**THE OFFICE OF THE CONTROLLER OF PATENTS, KOLKATA**

**IN THE MATTER OF:**

The Patents Act, 1970 as amended by the Patents (Amendment) Act 2005, and The Patents Rules, 2003, as amended by The Patents (Amendment) Rules, 2006

AND

**IN THE MATTER OF:**

An opposition by way of representation under Section 25(1) of The Patents, 1970, as amended by the Patents (Amendment) Act, 2005 read with Rule 55 of The Patents Rules, 2003, as amended by The Patents (Amendment) Rules, 2006 to the Indian National Phase Application No. **3140/KOLNP/2012** filed on **16/10/2012** by **VERTEX PHARMACEUTICALS INCORPORATED**

**IN THE MATTER OF:**

**DR. CHARANJIT KUMAR SEHGAL .....OPPONENT**

**VS.**

**VERTEX PHARMACEUTICALS INCORPORATED .....APPLICANT**

**STATEMENT OF CASE OF OPPONENT**

1. The Petitioner/Opponent has learnt that the Applicant has filed a PCT national phase application No. **3140/KOLNP/2012** (hereinafter "the Impugned Application") on **16/10/2012**. The Impugned application was published in the Official Journal of the patent office on June 21, 2013, which is currently pending before the Patent Office.

2. The Impugned application is entitled "solid forms of (R)-1(2,2-difluorobenzo[D][1,3]dioxol-5-yl)-N-(1-(2,3-dihyderoxypropyl)-6-

fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl) cyclopropane carboxamide". The Impugned Application No. **3140/KOLNP/2012** is national phase application of International application PCT/US2011/030032 dated March 25, 2011 which claims priority of March 25, 2010.

3. The opponent by way of present pre-grant opposition submits that the claims currently pending on record are not patentable under the provisions provided in this Act. The claims currently on record are annexed herewith as **Annexure-1** reproduced herein below for ready reference:

**GROUNDS OF OPPOSITION**

4. The Opponent submits its opposition by way of representation under Section 25(1) in respect of the said Indian Patent Application No. **3140/KOLNP/2012** on the following grounds below, which are without prejudice and in the alternative to each other.

   i) Section 25(1)(e)-Obviousness/Lack of Inventive step
   ii) Section 25(1)(f)-Not an invention
   iii) Section 25(1)(g)-Complete specification does not sufficiently and clearly describe the invention
   iv) Section 25(1)(h) - Failure to disclose information or furnishing false information relating to foreign filing

5. **Section 25(1) (e): Lack of inventive step -***The invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step, having regard to the matter published as mentioned in clause (b) or having regard to what was used in India before the priority date of the claim.*

6. The technical teaching of the impugned invention applies to limited knowledge which is well known in art without any inventiveness. It is submitted that the claims are obvious and lack

any inventive step in view of teachings, motivation and suggestion in various prior art documents listed herein below.

7. It is submitted that the impugned application is obvious in light of teachings of following:
   - US2009/131492, hereinafter referred to as US'492 (annexed herewith as **Annexure 2)**
   - Yamashita et al, "Establishment of new preparation method for solid dispersion formulation of Tacrolimus", published in 2003 (annexed herewith as **Annexure 3)**
   - Kennedy et al., "Enhanced bioavailability of a poorly soluble VR1 antagonist using an amorphous solid dispersion approach: A case study", published in 2008.(Annexed herewith as **Annexure 4**)

8. US492 discloses compounds 315 and 322 in Table 1 which is the compound of present invention i.e. (R)-1(2,2-difluorobenzo[D][1,3]dioxol-5-yl)-N-(1-(2,3-dihyderoxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropane carboxamide which is stated to be useful in treatment of cystic fibrosis.

9. US492 also discloses the preparation of said compound and it is stated that the compound is obtained as a foamy solid i.e. as an amorphous solid.

10. US492 also discloses that said compound can be used in solid dispersion comprising of a polymer, and which may further comprise of a surfactant.

11. Yamashita et al prepared solid dispersion formulations of a poorly soluble amorphous drug with different polymers namely PEG6000, PVP and HPMC. Yamashita and group found that the solid dispersion formulations prepared by using the polymer HPMC demonstrated highest increase in dissolution as well as bioavailability of the candidate drug. Yamashita states that HPMAC is considered one of the most suitable carriers for preparation of

solid dispersion formulations among the polymers commonly known and used at the time of invention.

12. Kennedy et al have explored solid dispersion formulation of a poorly soluble drug which is prepared by spray drying using a combination of HPMC, hydroxypropyl methylcellulose and HPMCAS, hydroxypropyl methylcellulose acetate succinate. Kennedy and group found that combination of HPMC polymer along with a variant of HPMC which is enteric polymer i.e. HPMCAS results in significant improvement in maintenance of the drug in amorphous form during stability as well as significantly enhanced bioavailability of the drug in solid dispersion as compared to the amorphous drug which is not present as a solid dispersion.

13. From the aforesaid data of the prior art documents it has been explicitly established that solid dispersion formulations of poorly soluble drugs comprising HPMC and HPMCAS polymers wherein the drug is in substantially amorphous form and which exhibit better dissolution and bioavailability as compared to the amorphous drug were already available in the field at the priority date of the impugned application.

14. Therefore, the claimed subject matter of impugned application lacks technical advancement and is obvious in view of what was already known in the field at the priority date of the impugned application.

15. Hence, in view of the disclosure of aforesaid prior art documents the invention claimed in the impugned application is obvious to a person skilled in the art.

16. Therefore, the claims of impugned invention should be rejected on this basis alone.

GROUND II

**SECTION 25(1) (F)-NOT AN INVENTION**

Claims of impugned application is not patentable as per section 2(1)(j)

17. As has been detailed in preceding paragraphs under the Ground of Lack of inventive step, the invention of impugned application is obvious in view of the disclosure of the prior art cited and discussed above. Further, the invention of impugned application lacks any technical advancement with respect to the similar formulations which known in the art at the time of the invention.

18. Thus, the claims of present invention are not an invention as defined in Section 2(1)(j) of the Act.

Claims of impugned application is not patentable as per section 3(d)

19. Section 3(d) states "*the mere discovery of a new form of a known substance which does not result in the enhancement of the known efficacy of that substance or the mere discovery of any new property or new use for a known substance or of the mere use of a known process, machine or apparatus unless such known process results in a new product or employs at least one new reactant.*

*Explanation—For the purposes of this clause, salts, esters, ethers, polymorphs, metabolites, pure form, particle size, isomers, mixtures of isomers, complexes, combinations and other derivatives of known substance shall be considered to be the same substance, unless they differ significantly in properties with regard to efficacy*"

20. The applicant has failed to give comparative data in the specification as filed establishing the enhanced therapeutic efficacy of the solid dispersion claimed in impugned application over solid dispersion formulation known in art at the time of invention.

21. A person skilled in the art knows that a solid dispersion of a drug is akin to a API which is then used to form a suitable pharmaceutical composition.

22. As discussed in preceding paragraphs under the Ground of Inventive step the solid dispersion of (R)-1(2,2-difluorobenzo[D][1,3]dioxol-5-yl)-N-(1-(2,3-dihyderoxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropane carboxamide was already disclosed in US492 before the priority date of the impugned application.

23. The Applicant has not given any comparative data in the specification as filed establishing enhanced therapeutic efficacy of the solid dispersion claimed in present invention as compared to the solid dispersion known in US492.

24. Hence, the claimed subject matter of impugned application is not patentable as per Section 3(d) of the Act and the claims should be rejected on this basis alone.

Claims of impugned application is not patentable as per section 3(e)

25. It is submitted that the impugned application claims a composition which is a mere admixture of various components without any synergistic effect. The specification as filed does not provide any synergistic effect of the claimed formulation. Thus, the claims of the impugned application are not patentable as per Section 3(e) of the Act and ought to be rejected on this basis alone.

GROUND III

**Section 25(1)(g): Complete specification does not sufficiently describe the invention.**

26. The opponent states that the complete specification of the alleged invention does not sufficiently and clearly describe the invention or the best method by which it is to be performed.

27. The opponent states that it is a well settled rule that the specification should clearly and fairly describe the invention and disclose the best mode of working the invention so that the person skilled in the art could perform the invention without any undue efforts and it is hereby stated that the applicant has failed to do so.

28. It is submitted that the specification as filed fails to recite the ratio /proportion of the components of the claimed solid dispersion as well as the claimed composition. In absence, these essential features a person skilled in the art has to undergo undue experimentation to arrive at the invention.

29. Thus, due to lack of proper enablement and best mode of working in the specification a person skilled in the art has to exercise undue experimentation in order to arrive at the claimed invention.

30. Therefore, the specification of the impugned application fails to sufficiently describe the invention and the impugned application should be rejected on this basis alone.

GROUND IV

**Section 25 (1) (h): The Applicant has failed to disclose to the Controller the information required under Section 8.**

31. The applicant has not filed the details of the prosecution of corresponding applications at the Patent Office and has, thus, failed to comply with the requirements of the provisions of Section 8 of the Act.

32. The opponents crave leave to file further submissions and evidence with respect to this ground.

33. Therefore, the impugned application should be rejected on this basis alone.

**P R A Y E R**

In the fact and circumstances of the case, the Opponent prays as follows:

i. that the Indian Patent Application No. **3140/KOLNP/2012** by **VERTEX PHARMACEUTICALS INCORPORATED**, be rejected under Section 25(1) of the Patents (Amendment) Act, 2005;

ii. the Opponent may be allowed to file further documents as evidence if necessary to support its averments;

iii. the Opponent may be granted an opportunity of being heard in the matter before any final orders are passed;

iv. the Opponent may be allowed to make further submissions in case the applicant makes any amendments in the claims;

v. Any other reliefs considering the facts and circumstances may be granted in favour of the Opponent in the interest of justice.

Dated this day 25th of March, 2021

for Dr. Charanjit Kumar Sehgal
(Opponent)

(Chirag Tanna, IN/PA-1785)
Authorized Patent Agent for the Opponent

To,
The Controller of Patents
The Patent Office, Kolkata

**We Claim:**

1. A solid dispersion comprising substantially amorphous (*R*)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (Compound 1) and a polymer selected from hydroxypropyl methylcellulose (HPMC) and hydroxypropyl methylcellulose acetate succinate (HPMCAS), wherein the solid substantially amorphous Compound 1 comprises less than 15% crystalline Compound 1.

2. The solid dispersion as claimed in claim 1, wherein the polymer is present in an amount from 10% by weight to 80% by weight.

3. The solid dispersion as claimed in claim 1 or 2, wherein the solid substantially amorphous Compound 1 is present in an amount from 10% by weight to 80% by weight.

4. The solid dispersion as claimed in any one of claims 1 to 3, wherein the polymer is hydroxypropyl methylcellulose (HPMC).

5. The solid dispersion as claimed in any one of claims 1 to 3, wherein the polymer is hydroxypropyl methylcellulose acetate succinate (HPMCAS).

6. The solid dispersion as claimed in any one of claims 1 to 5, wherein the polymer is present in the amount of 20% by weight and the solid substantially amorphous Compound 1 is present in the amount of 80% by weight.

7. A process for preparing a solid dispersion composition as claimed in claim 1 comprising the solid substantially amorphous Compound 1 and a suitable solvent and then spray drying the mixture to obtain the solid dispersion.

8. The process as claimed in claim 7, comprising combining the solid substantially amorphous Compound 1 and a suitable solvent and then spray drying the mixture to obtain the solid dispersion.

9. The process as claimed in claim 8, wherein the solvent is an alcohol.

10. The process as claimed in claim 8 or 9, wherein the solvent is methanol.

11. The process as claimed in any one of claims 7 to 10, comprising:

a) forming a mixture comprising solid substantially amorphous Compound 1, a polymer, and a solvent; and

b) spray drying the mixture to form a solid dispersion.

12. The process as claimed in claim 11, wherein the polymer is selected from hydroxypropyl methylcellulose (HPMC) and hydroxypropyl methylcellulose acetate succinate (HPMCAS).

13. The process as claimed in claim 11 or 12, wherein the polymer is present in an amount from 10% by weight to 80% by weight of the solid dispersion.

14. The process as claimed in any one of claims 11 to 13, wherein the solid substantially amorphous Compound 1 is present in an amount from 10% by weight to 80% by weight of the solid dispersion.

15. The process as claimed in any one of claims 11 to 14, wherein the solvent is methanol.

16. The process as claimed in any one of claims 11 to 15, wherein the solid substantially amorphous Compound 1 is present in the amount of 80% by weight of the solid dispersion, the polymer is HPMC and is present in the amount of 20% by weight of the solid dispersion, and the solvent is methanol.

ANNEXURE - 2

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(19) **United States**

(12) **Patent Application Publication**

**Ruah et al.**

(10) **Pub. No.: US 2009/0131492 A1**

(43) **Pub. Date: May 21, 2009**

(54) **INDOLE DERIVATIVES AS CFTR MODULATORS**

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(57) **ABSTRACT**

Compounds of the present invention and pharmaceutically acceptable compositions thereof, are useful as modulators of ATP-Binding Cassette ("ABC") transporters or fragments thereof, including Cystic Fibrosis Transmembrane Conductance Regulator ("CFTR"). The present invention also relates to methods of treating ABC transporter mediated diseases using compounds of the present invention.

# INDOLE DERIVATIVES AS CFTR MODULATORS

## CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** The present application claims the benefit under 35 U.S.C. § 120 of U.S. application Ser. No. 11/786,001, filed Apr. 9, 2007, which claims the benefit under 35 U.S.C. § 119 of U.S. Provisional Application No. 60/790,459, filed Apr. 7, 2006, the entire contents of both applications being incorporated herein by reference.

## TECHNICAL FIELD OF THE INVENTION

**[0002]** The present invention relates to modulators of ATP-Binding Cassette ("ABC") transporters or fragments thereof, including Cystic Fibrosis Transmembrane Conductance Regulator ("CFTR"), compositions thereof and methods therewith. The present invention also relates to methods of treating ABC transporter mediated diseases using such modulators.

## BACKGROUND OF THE INVENTION

**[0003]** ABC transporters are a family of membrane transporter proteins that regulate the transport of a wide variety of pharmacological agents, potentially toxic drugs, and xenobiotics, as well as anions. ABC transporters are homologous membrane proteins that bind and use cellular adenosine triphosphate (ATP) for their specific activities. Some of these transporters were discovered as multidrug resistance proteins (like the MDR1-P glycoprotein, or the multidrug resistance protein, MRP1), defending malignant cancer cells against chemotherapeutic agents. To date, 48 ABC Transporters have been identified and grouped into 7 families based on their sequence identity and function.

**[0004]** ABC transporters regulate a variety of important physiological roles within the body and provide defense against harmful environmental compounds. Because of this, they represent important potential drug targets for the treatment of diseases associated with defects in the transporter, prevention of drug transport out of the target cell, and intervention in other diseases in which modulation of ABC transporter activity may be beneficial.

**[0005]** One member of the ABC transporter family commonly associated with disease is the cAMP/ATP-mediated anion channel, CFTR. CFTR is expressed in a variety of cells types, including absorptive and secretory epithelia cells, where it regulates anion flux across the membrane, as well as the activity of other ion channels and proteins. In epithelia cells, normal functioning of CFTR is critical for the maintenance of electrolyte transport throughout the body, including respiratory and digestive tissue. CFTR is composed of approximately 1480 amino acids that encode a protein made up of a tandem repeat of transmembrane domains, each containing six transmembrane helices and a nucleotide binding domain. The two transmembrane domains are linked by a large, polar, regulatory (R)-domain with multiple phosphorylation sites that regulate channel activity and cellular trafficking.

**[0006]** The gene encoding CFTR has been identified and sequenced (See Gregory, R. J. et al. (1990) Nature 347:382-386; Rich, D. P. et al. (1990) Nature 347:358-362), (Riordan, J. R. et al. (1989) Science 245:1066-1073). A defect in this gene causes mutations in CFTR resulting in Cystic Fibrosis ("CF"), the most common fatal genetic disease in humans. Cystic Fibrosis affects approximately one in every 2,500 infants in the United States. Within the general United States population, up to 10 million people carry a single copy of the defective gene without apparent ill effects. In contrast, individuals with two copies of the CF associated gene suffer from the debilitating and fatal effects of CF, including chronic lung disease.

**[0007]** In patients with cystic fibrosis, mutations in CFTR endogenously expressed in respiratory epithelia leads to reduced apical anion secretion causing an imbalance in ion and fluid transport. The resulting decrease in anion transport contributes to enhanced mucus accumulation in the lung and the accompanying microbial infections that ultimately cause death in CF patients. In addition to respiratory disease, CF patients typically suffer from gastrointestinal problems and pancreatic insufficiency that, if left untreated, results in death. In addition, the majority of males with cystic fibrosis are infertile and fertility is decreased among females with cystic fibrosis. In contrast to the severe effects of two copies of the CF associated gene, individuals with a single copy of the CF associated gene exhibit increased resistance to cholera and to dehydration resulting from diarrhea—perhaps explaining the relatively high frequency of the CF gene within the population.

**[0008]** Sequence analysis of the CFTR gene of CF chromosomes has revealed a variety of disease causing mutations (Cutting, G. R. et al. (1990) Nature 346:366-369; Dean, M. et al. (1990) Cell 61:863:870; and Kerem, B-S. et al. (1989) Science 245:1073-1080; Kerem, B-S et al. (1990) Proc. Natl. Acad. Sci. USA 87:8447-8451). To date, >1000 disease causing mutations in the CF gene have been identified (http://www.genet.sickkids.on.ca/cftr/). The most prevalent mutation is a deletion of phenylalanine at position 508 of the CFTR amino acid sequence, and is commonly referred to as ΔF508-CFTR. This mutation occurs in approximately 70% of the cases of cystic fibrosis and is associated with a severe disease.

**[0009]** The deletion of residue 508 in ΔF508-CFTR prevents the nascent protein from folding correctly. This results in the inability of the mutant protein to exit the ER, and traffic to the plasma membrane. As a result, the number of channels present in the membrane is far less than observed in cells expressing wild-type CFTR. In addition to impaired trafficking, the mutation results in defective channel gating. Together, the reduced number of channels in the membrane and the defective gating lead to reduced anion transport across epithelia leading to defective ion and fluid transport. (Quinton, P. M. (1990), FASEB J. 4: 2709-2727). Studies have shown, however, that the reduced numbers of ΔF508-CFTR in the membrane are functional, albeit less than wild-type CFTR. (Dalemans et al. (1991), Nature Lond. 354: 526-528; Denning et al., supra; Pasyk and Foskett (1995), J. Cell. Biochem. 270: 12347-50). In addition to ΔF508-CFTR, other disease causing mutations in CFTR that result in defective trafficking, synthesis, and/or channel gating could be up- or down-regulated to alter anion secretion and modify disease progression and/or severity.

**[0010]** Although CFTR transports a variety of molecules in addition to anions, it is clear that this role (the transport of anions) represents one element in an important mechanism of transporting ions and water across the epithelium. The other elements include the epithelial $Na^+$ channel, ENaC, $Na^+/2Cl^-/K^+$ co-transporter, $Na^+$—$K^+$-ATPase pump and the

basolateral membrane $K^+$ channels, that are responsible for the uptake of chloride into the cell.

**[0011]** These elements work together to achieve directional transport across the epithelium via their selective expression and localization within the cell. Chloride absorption takes place by the coordinated activity of ENaC and CFTR present on the apical membrane and the $Na^+$—$K^+$-ATPase pump and Cl-channels expressed on the basolateral surface of the cell. Secondary active transport of chloride from the luminal side leads to the accumulation of intracellular chloride, which can then passively leave the cell via $Cl^-$ channels, resulting in a vectorial transport. Arrangement of $Na^+/2Cl^-/K^+$ co-transporter, $Na^+$—$K^+$-ATPase pump and the basolateral membrane $K^+$ channels on the basolateral surface and CFTR on the luminal side coordinate the secretion of chloride via CFTR on the luminal side. Because water is probably never actively transported itself, its flow across epithelia depends on tiny transepithelial osmotic gradients generated by the bulk flow of sodium and chloride.

**[0012]** In addition to Cystic Fibrosis, modulation of CFTR activity may be beneficial for other diseases not directly caused by mutations in CFTR, such as secretory diseases and other protein folding diseases mediated by CFTR. These include, but are not limited to, chronic obstructive pulmonary disease (COPD), dry eye disease, and Sjögren's Syndrome.

**[0013]** COPD is characterized by airflow limitation that is progressive and not fully reversible. The airflow limitation is due to mucus hypersecretion, emphysema, and bronchiolitis. Activators of mutant or wild-type CFTR offer a potential treatment of mucus hypersecretion and impaired mucociliary clearance that is common in COPD. Specifically, increasing anion secretion across CFTR may facilitate fluid transport into the airway surface liquid to hydrate the mucus and optimized periciliary fluid viscosity. This would lead to enhanced mucociliary clearance and a reduction in the symptoms associated with COPD. Dry eye disease is characterized by a decrease in tear aqueous production and abnormal tear film lipid, protein and mucin profiles. There are many causes of dry eye, some of which include age, Lasik eye surgery, arthritis, medications, chemical/thermal burns, allergies, and diseases, such as Cystic Fibrosis and Sjögrens's syndrome. Increasing anion secretion via CFTR would enhance fluid transport from the corneal endothelial cells and secretory glands surrounding the eye to increase corneal hydration. This would help to alleviate the symptoms associated with dry eye disease. Sjögrens's syndrome is an autoimmune disease in which the immune system attacks moisture-producing glands throughout the body, including the eye, mouth, skin, respiratory tissue, liver, vagina, and gut. Symptoms, include, dry eye, mouth, and vagina, as well as lung disease. The disease is also associated with rheumatoid arthritis, systemic lupus, systemic sclerosis, and polymypositis/dermatomyositis. Defective protein trafficking is believed to cause the disease, for which treatment options are limited. Modulators of CFTR activity may hydrate the various organs afflicted by the disease and help to elevate the associated symptoms.

**[0014]** As discussed above, it is believed that the deletion of residue 508 in ΔF508-CFTR prevents the nascent protein from folding correctly, resulting in the inability of this mutant protein to exit the ER, and traffic to the plasma membrane. As a result, insufficient amounts of the mature protein are present at the plasma membrane and chloride transport within epithelial tissues is significantly reduced. In fact, this cellular phenomenon of defective ER processing of ABC transporters by the ER machinery has been shown to be the underlying basis not only for CF disease, but for a wide range of other isolated and inherited diseases. The two ways that the ER machinery can malfunction is either by loss of coupling to ER export of the proteins leading to degradation, or by the ER accumulation of these defective/misfolded proteins [Aridor M, et al., Nature Med., 5(7), pp 745-751 (1999); Shastry, B. S., et al., Neurochem. International, 43, pp 1-7 (2003); Rutishauser, J., et al., Swiss Med Wkly, 132, pp 211-222 (2002); Morello, J P et al., TIPS, 21, pp. 466-469 (2000); Bross P., et al., Human Mut., 14, pp. 186-198 (1999)]. The diseases associated with the first class of ER malfunction are Cystic fibrosis (due to misfolded ΔF508-CFTR as discussed above), Hereditary emphysema (due to a1-antitrypsin; non Piz variants), Hereditary hemochromatosis, Coagulation-Fibrinolysis deficiencies, such as Protein C deficiency, Type 1 hereditary angioedema, Lipid processing deficiencies, such as Familial hypercholesterolemia, Type 1 chylomicronemia, Abetalipoproteinemia, Lysosomal storage diseases, such as I-cell disease/Pseudo-Hurler, Mucopolysaccharidoses (due to Lysosomal processing enzymes), Sandhof/Tay-Sachs (due to β-Hexosaminidase), Crigler-Najjar type II (due to UDP-glucuronyl-sialyc-transferase), Polyendocrinopathy/Hyperinsulemia, Diabetes mellitus (due to Insulin receptor), Laron dwarfism (due to Growth hormone receptor), Myleoperoxidase deficiency, Primary hypoparathyroidism (due to Preproparathyroid hormone), Melanoma (due to Tyrosinase). The diseases associated with the latter class of ER malfunction are Glycanosis CDG type 1, Hereditary emphysema (due to α1-Antitrypsin (PiZ variant), Congenital hyperthyroidism, Osteogenesis imperfecta (due to Type I, II, IV procollagen), Hereditary hypofibrinogenemia (due to Fibrinogen), ACT deficiency (due to α1-Antichymotrypsin), Diabetes insipidus (DI), Neurophyseal DI (due to Vasopvessin hormone/V2-receptor), Neprogenic DI (due to Aquaporin II), Charcot-Marie Tooth syndrome (due to Peripheral myelin protein 22), Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease (due to βAPP and presenilins), Parkinson's disease, Amyotrophic lateral sclerosis, Progressive supranuclear plasy, Pick's disease, several polyglutamine neurological disorders such as Huntington, Spinocerebullar ataxia type I, Spinal and bulbar muscular atrophy, Dentatorubal pallidoluysian, and Myotonic dystrophy, as well as Spongiform encephalopathies, such as Hereditary Creutzfeldt-Jakob disease (due to Prion protein processing defect), Fabry disease (due to lysosomal α-galactosidase A) and Straussler-Scheinker syndrome (due to Prp processing defect).

**[0015]** In addition to up-regulation of CFTR activity, reducing anion secretion by CFTR modulators may be beneficial for the treatment of secretory diarrheas, in which epithelial water transport is dramatically increased as a result of secretagogue activated chloride transport. The mechanism involves elevation of cAMP and stimulation of CFTR.

**[0016]** Although there are numerous causes of diarrhea, the major consequences of diarrheal diseases, resulting from excessive chloride transport are common to all, and include dehydration, acidosis, impaired growth and death.

**[0017]** Acute and chronic diarrheas represent a major medical problem in many areas of the world. Diarrhea is both a significant factor in malnutrition and the leading cause of death (5,000,000 deaths/year) in children less than five years old.

**[0018]** Secretory diarrheas are also a dangerous condition in patients of acquired immunodeficiency syndrome (AIDS) and chronic inflammatory bowel disease (IBD). 16 million travelers to developing countries from industrialized nations every year develop diarrhea, with the severity and number of cases of diarrhea varying depending on the country and area of travel.

**[0019]** Diarrhea in barn animals and pets such as cows, pigs and horses, sheep, goats, cats and dogs, also known as scours, is a major cause of death in these animals. Diarrhea can result from any major transition, such as weaning or physical movement, as well as in response to a variety of bacterial or viral infections and generally occurs within the first few hours of the animal's life.

**[0020]** The most common diarrhea causing bacteria is enterotoxogenic *E-coli* (ETEC) having the K99 pilus antigen. Common viral causes of diarrhea include rotavirus and coronavirus. Other infectious agents include *cryptosporidium, giardia lamblia*, and *salmonella*, among others.

**[0021]** Symptoms of rotaviral infection include excretion of watery feces, dehydration and weakness. Coronavirus causes a more severe illness in the newborn animals, and has a higher mortality rate than rotaviral infection. Often, however, a young animal may be infected with more than one virus or with a combination of viral and bacterial microorganisms at one time. This dramatically increases the severity of the disease.

**[0022]** Accordingly, there is a need for modulators of an ABC transporter activity, and compositions thereof, that can be used to modulate the activity of the ABC transporter in the cell membrane of a mammal.

**[0023]** There is a need for methods of treating ABC transporter mediated diseases using such modulators of ABC transporter activity.

**[0024]** There is a need for methods of modulating an ABC transporter activity in an ex vivo cell membrane of a mammal.

**[0025]** There is a need for modulators of CFTR activity that can be used to modulate the activity of CFTR in the cell membrane of a mammal.

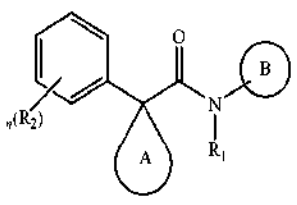
**[0026]** There is a need for methods of treating CFTR-mediated diseases using such modulators of CFTR activity.

**[0027]** There is a need for methods of modulating CFTR activity in an ex vivo cell membrane of a mammal.

## SUMMARY OF THE INVENTION

**[0028]** It has now been found that compounds of this invention, and pharmaceutically acceptable compositions thereof, are useful as modulators of ABC transporter activity, particularly CTFR activity. These compounds have the general formula I:

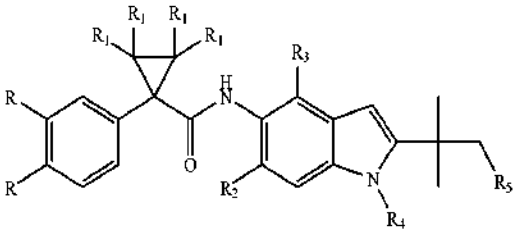
I




or a pharmaceutically acceptable salt thereof, wherein $R_1$, $R_2$, ring A, ring B, and n are defined below.

**[0029]** It has also now been found that compounds of this invention, and pharmaceutically acceptable compositions thereof, are useful as modulators of ABC transporter activity. These compounds have the general formula II:

II




or a pharmaceutically acceptable salt thereof, wherein R, $R_1$, $R_2$, $R_3$, $R_4$, and $R_5$ are defined below.

**[0030]** These compounds and pharmaceutically acceptable compositions are useful for treating or lessening the severity of a variety of diseases, disorders, or conditions, including, but not limited to, cystic fibrosis, hereditary emphysema, hereditary hemochromatosis, coagulation-fibrinolysis deficiencies, such as protein C deficiency, Type 1 hereditary angioedema, lipid processing deficiencies, such as familial hypercholesterolemia, Type 1 chylomicronemia, abetalipoproteinemia, lysosomal storage diseases, such as I-cell disease/pseudo-Hurler, mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, polyendocrinopathy/hyperinsulemia, diabetes mellitus, laron dwarfism, myeloperoxidase deficiency, primary hypoparathyroidism, melanoma, glycanosis CDG type 1, hereditary emphysema, congenital hyperthyroidism, osteogenesis imperfecta, hereditary hypofibrinogenemia, ACT deficiency, diabetes insipidus, neurophysiol, nephrogenic, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear plasy, Pick's disease, several polyglutamine neurological disorders such as Huntington, spinocerebullar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidoluysian, and myotonic dystrophy, as well as spongiform encephalopathies, such as hereditary Creutzfeldt-Jakob disease, Fabry disease, Straussler-Scheinker syndrome, COPD, dry-eye disease, and Sjögren's disease.

## DETAILED DESCRIPTION OF THE INVENTION

### I. Definitions

**[0031]** As used herein, the following definitions shall apply unless otherwise indicated.

**[0032]** The term "ABC-transporter" as used herein means an ABC-transporter protein or a fragment thereof comprising at least one binding domain, wherein said protein or fragment thereof is present in vivo or in vitro. The term "binding domain" as used herein means a domain on the ABC-transporter that can bind to a modulator. See, e.g., Hwang, T. C. et al., J. Gen. Physiol. (1998): 111(3), 477-90.

**[0033]** The term "CFTR" as used herein means cystic fibrosis transmembrane conductance regulator or a mutation thereof capable of regulator activity, including, but not limited to, ΔF508 CFTR and G551D CFTR (see, e.g., http://www.genet.sickkids.on.ca/cftr/, for CFTR mutations).

**[0034]** The term "modulating" as used herein means increasing or decreasing, e.g. activity, by a measurable amount. Compounds that modulate ABC Transporter activity, such as CFTR activity, by increasing the activity of the ABC Transporter, e.g., a CFTR anion channel, are called agonists. Compounds that modulate ABC Transporter activity, such as CFTR activity, by decreasing the activity of the ABC Transporter, e.g., CFTR anion channel, are called antagonists. An agonist interacts with an ABC Transporter, such as CFTR anion channel, to increase the ability of the receptor to transduce an intracellular signal in response to endogenous ligand binding. An antagonist interacts with an ABC Transporter, such as CFTR, and competes with the endogenous ligand(s) or substrate(s) for binding site(s) on the receptor to decrease the ability of the receptor to transduce an intracellular signal in response to endogenous ligand binding.

**[0035]** The phrase "treating or reducing the severity of an ABC Transporter mediated disease" refers both to treatments for diseases that are directly caused by ABC Transporter and/or CFTR activities and alleviation of symptoms of diseases not directly caused by ABC Transporter and/or CFTR anion channel activities. Examples of diseases whose symptoms may be affected by ABC Transporter and/or CFTR activity include, but are not limited to, Cystic fibrosis, Hereditary emphysema, Hereditary hemochromatosis, Coagulation-Fibrinolysis deficiencies, such as Protein C deficiency, Type 1 hereditary angioedema, Lipid processing deficiencies, such as Familial hypercholesterolemia. Type 1 chylomicronemia, Abetalipoproteinemia, Lysosomal storage diseases, such as I-cell disease/Pseudo-Hurler, Mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, Polyendocrinopathy/Hyperinsulemia, Diabetes mellitus, Laron dwarfism, Myleoperoxidase deficiency, Primary hypoparathyroidism, Melanoma, Glycanosis CDG type 1, Hereditary emphysema, Congenital hyperthyroidism, Osteogenesis imperfecta, Hereditary hypofibrinogenemia, ACT deficiency, Diabetes insipidus (DI), Neurophysiol DI, Nephrogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Progressive supranuclear plasy, Pick's disease, several polyglutamine neurological disorders such as Huntington, Spinocerebullar ataxia type I, Spinal and bulbar muscular atrophy, Dentatorubal pallidoluysian, and Myotonic dystrophy, as well as Spongiform encephalopathies, such as Hereditary Creutzfeldt-Jakob disease, Fabry disease, Straussler-Scheinker syndrome, COPD, dry-eye disease, and Sjogren's disease.

**[0036]** For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausolito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M. B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

**[0037]** As described herein, compounds of the invention may optionally be substituted with one or more substituents, such as are illustrated generally above, or as exemplified by particular classes, subclasses, and species of the invention.

**[0038]** As used herein the term "aliphatic" encompasses the terms alkyl, alkenyl, alkynyl, each of which being optionally substituted as set forth below.

**[0039]** As used herein, an "alkyl" group refers to a saturated aliphatic hydrocarbon group containing 1-12 (e.g., 1-8, 1-6, or 1-4) carbon atoms. An alkyl group can be straight or branched. Examples of alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, n-heptyl, or 2-ethylhexyl. An alkyl group can be substituted (i.e., optionally substituted) with one or more substituents such as halo, phospho, cycloaliphatic [e.g., cycloalkyl or cycloalkenyl], heterocycloaliphatic [e.g., heterocycloalkyl or heterocycloalkenyl], aryl, heteroaryl, alkoxy, aroyl, heteroaroyl, acyl [e.g., (aliphatic)carbonyl, (cycloaliphatic)carbonyl, or (heterocycloaliphatic)carbonyl], nitro, cyano, amido [e.g., (cycloalkylalkyl)carbonylamino, arylcarbonylamino, aralkylcarbonylamino, (heterocycloalkyl)carbonylamino, (heterocycloalkylalkyl)carbonylamino, heteroarylcarbonylamino, heteroaralkylcarbonylamino alkylaminocarbonyl, cycloalkylaminocarbonyl, heterocycloalkylaminocarbonyl, arylaminocarbonyl, or heteroarylaminocarbonyl], amino [e.g., aliphaticamino, cycloaliphaticamino, or heterocycloaliphaticamino], sulfonyl [e.g., aliphatic-$SO_2$—], sulfinyl, sulfanyl, sulfoxy, urea, thiourea, sulfamoyl, sulfamide, oxo, carboxy, carbamoyl, cycloaliphaticoxy, heterocycloaliphaticoxy, aryloxy, heteroaryloxy, aralkyloxy, heteroarylalkoxy, alkoxycarbonyl, alkylcarbonyloxy, or hydroxy. Without limitation, some examples of substituted alkyls include carboxyalkyl (such as HOOC-alkyl, alkoxycarbonylalkyl, and alkylcarbonyloxyalkyl), cyanoalkyl, hydroxyalkyl, alkoxyalkyl, acylalkyl, aralkyl, (alkoxyaryl)alkyl, (sulfonylamino)alkyl (such as (alkyl-$SO_2$-amino)alkyl), aminoalkyl, amidoalkyl, (cycloaliphatic)alkyl, or haloalkyl.

**[0040]** As used herein, an "alkenyl" group refers to an aliphatic carbon group that contains 2-8 (e.g., 2-12, 2-6, or 2-4) carbon atoms and at least one double bond. Like an alkyl group, an alkenyl group can be straight or branched. Examples of an alkenyl group include, but are not limited to allyl, isoprenyl, 2-butenyl, and 2-hexenyl. An alkenyl group can be optionally substituted with one or more substituents such as halo, phospho, cycloaliphatic [e.g., cycloalkyl or cycloalkenyl], heterocycloaliphatic [e.g., heterocycloalkyl or heterocycloalkenyl], aryl, heteroaryl, alkoxy, aroyl, heteroaroyl, acyl [e.g., (aliphatic)carbonyl, (cycloaliphatic)carbonyl, or (heterocycloaliphatic)carbonyl], nitro, cyano, amido [e.g., (cycloalkylalkyl)carbonylamino, arylcarbonylamino, aralkylcarbonylamino, (heterocycloalkyl)carbonylamino, (heterocycloalkylalkyl)carbonylamino, heteroarylcarbonylamino, heteroaralkylcarbonylamino alkylaminocarbonyl, cycloalkylaminocarbonyl, heterocycloalkylaminocarbonyl, arylaminocarbonyl, or heteroarylaminocarbonyl], amino [e.g., aliphaticamino, cycloaliphaticamino, heterocycloaliphaticamino, or aliphaticsulfonylamino], sulfonyl [e.g., alkyl-$SO_2$—, cycloaliphatic-$SO_2$—, or aryl-$SO_2$—], sulfinyl, sulfanyl, sulfoxy, urea, thiourea, sulfamoyl, sulfamide, oxo, carboxy, carbamoyl, cycloaliphaticoxy, heterocycloaliphaticoxy, aryloxy, heteroaryloxy, aralkyloxy, heteroaralkoxy, alkoxycarbonyl, alkylcarbonyloxy, or hydroxy. Without limitation, some examples of substituted alkenyls include cyanoalkenyl, alkoxyalkenyl, acylalkenyl, hydroxyalkenyl, aralkenyl, (alkoxyaryl)alkenyl, (sulfonylamino)alkenyl (such as (alkyl-

$SO_2$-amino)alkenyl), aminoalkenyl, amidoalkenyl, (cycloaliphatic)alkenyl, or haloalkenyl.

**[0041]** As used herein, an "alkynyl" group refers to an aliphatic carbon group that contains 2-8 (e.g., 2-12, 2-6, or 2-4) carbon atoms and has at least one triple bond. An alkynyl group can be straight or branched. Examples of an alkynyl group include, but are not limited to, propargyl and butynyl. An alkynyl group can be optionally substituted with one or more substituents such as aroyl, heteroaroyl, alkoxy, cycloalkyloxy, heterocycloalkyloxy, aryloxy, heteroaryloxy, aralkyloxy, nitro, carboxy, cyano, halo, hydroxy, sulfo, mercapto, sulfanyl [e.g., aliphaticsulfanyl or cycloaliphaticsulfanyl], sulfinyl [e.g., aliphaticsulfinyl or cycloaliphaticsulfinyl], sulfonyl [e.g., aliphatic-$SO_2$—, aliphaticamino-$SO_2$—, or cycloaliphatic-$SO_2$—], amido [e.g., aminocarbonyl, alkylaminocarbonyl, alkylcarbonylamino, cycloalkylaminocarbonyl, heterocycloalkylaminocarbonyl, cycloalkylcarbonylamino, arylaminocarbonyl, arylcarbonylamino, aralkylcarbonylamino, (heterocycloalkyl)carbonylamino, (cycloalkylalkyl)carbonylamino, heteroaralkylcarbonylamino, heteroarylcarbonylamino or heteroarylaminocarbonyl], urea, thiourea, sulfamoyl, sulfamide, alkoxycarbonyl, alkylcarbonyloxy, cycloaliphatic, heterocycloaliphatic, aryl, heteroaryl, acyl [e.g., (cycloaliphatic)carbonyl or (heterocycloaliphatic)carbonyl], amino [e.g., aliphaticamino], sulfoxy, oxo, carboxy, carbamoyl, (cycloaliphatic)oxy, (heterocycloaliphatic)oxy, or (heteroaryl)alkoxy.

**[0042]** As used herein, an "amido" encompasses both "aminocarbonyl" and "carbonylamino". These terms when used alone or in connection with another group refer to an amido group such as —$N(R^X)$—$C(O)$—$R^Y$ or —$C(O)$—$N(R^X)_2$, when used terminally, and —$C(O)$—$N(R^X)$— or —$N(R^X)$—$C(O)$— when used internally, wherein $R^X$ and $R^Y$ are defined below. Examples of amido groups include alkylamido (such as alkylcarbonylamino or alkylaminocarbonyl), (heterocycloaliphatic)amido, (heteroaralkyl)amido, (heteroaryl)amido, (heterocycloalkyl)alkylamido, arylamido, aralkylamido, (cycloalkyl)alkylamido, or cycloalkylamido.

**[0043]** As used herein, an "amino" group refers to —$NR^XR^Y$ wherein each of $R^X$ and $R^Y$ is independently hydrogen, aliphatic, cycloaliphatic, (cycloaliphatic)aliphatic, aryl, araliphatic, heterocycloaliphatic, (heterocycloaliphatic)aliphatic, heteroaryl, carboxy, sulfanyl, sulfinyl, sulfonyl, (aliphatic)carbonyl, (cycloaliphatic)carbonyl, ((cycloaliphatic)aliphatic)carbonyl, arylcarbonyl, (araliphatic)carbonyl, (heterocycloaliphatic)carbonyl, ((heterocycloaliphatic)aliphatic)carbonyl, (heteroaryl)carbonyl, or (heteroaraliphatic)carbonyl, each of which being defined herein and being optionally substituted. Examples of amino groups include alkylamino, dialkylamino, or arylamino. When the term "amino" is not the terminal group (e.g., alkylcarbonylamino), it is represented by —$NR^X$—. $R^X$ has the same meaning as defined above.

**[0044]** As used herein, an "aryl" group used alone or as part of a larger moiety as in "aralkyl", "aralkoxy", or "aryloxyalkyl" refers to monocyclic (e.g., phenyl); bicyclic (e.g., indenyl, naphthalenyl, tetrahydronaphthyl, tetrahydroindenyl); and tricyclic (e.g., fluorenyl tetrahydrofluorenyl, or tetrahydroanthracenyl, anthracenyl) ring systems in which the monocyclic ring system is aromatic or at least one of the rings in a bicyclic or tricyclic ring system is aromatic. The bicyclic and tricyclic groups include benzofused 2-3 membered carbocyclic rings. For example, a benzofused group includes phenyl fused with two or more $C_{4-8}$ carbocyclic moieties. An aryl is optionally substituted with one or more substituents including aliphatic [e.g., alkyl, alkenyl, or alkynyl]; cycloaliphatic; (cycloaliphatic)aliphatic; heterocycloaliphatic; (heterocycloaliphatic)aliphatic; aryl; heteroaryl; alkoxy; (cycloaliphatic)oxy; (heterocycloaliphatic)oxy; aryloxy; heteroaryloxy; (araliphatic)oxy; (heteroaraliphatic)oxy; aroyl; heteroaroyl; amino; oxo (on a non-aromatic carbocyclic ring of a benzofused bicyclic or tricyclic aryl); nitro; carboxy; amido; acyl [e.g., (aliphatic)carbonyl; (cycloaliphatic)carbonyl; ((cycloaliphatic)aliphatic)carbonyl; (araliphatic)carbonyl; (heterocycloaliphatic)carbonyl; ((heterocycloaliphatic)aliphatic)carbonyl; or (heteroaraliphatic)carbonyl]; sulfonyl [e.g., aliphatic-$SO_2$— or amino-$SO_2$—]; sulfinyl [e.g., aliphatic-S(O)— or cycloaliphatic-S(O)—]; sulfanyl [e.g., aliphatic-S—]; cyano; halo; hydroxy; mercapto; sulfoxy; urea; thiourea; sulfamoyl; sulfamide; or carbamoyl. Alternatively, an aryl can be unsubstituted.

**[0045]** Non-limiting examples of substituted aryls include haloaryl [e.g., mono-, di (such as p,m-dihaloaryl), and (trihalo)aryl]; (carboxy)aryl [e.g., (alkoxycarbonyl)aryl, ((aralkyl)carbonyloxy)aryl, and (alkoxycarbonyl)aryl]; (amido)aryl [e.g., (aminocarbonyl)aryl, (((alkylamino)alkyl)aminocarbonyl)aryl, (alkylcarbonyl)aminoaryl, (arylaminocarbonyl)aryl, and (((heteroaryl)amino)carbonyl)aryl]; aminoaryl [e.g., ((alkylsulfonyl)amino)aryl or ((dialkyl)amino)aryl]; (cyanoalkyl)aryl; (alkoxy)aryl; (sulfamoyl)aryl [e.g., (aminosulfonyl)aryl]; (alkylsulfonyl)aryl; (cyano)aryl; (hydroxyalkyl)aryl; ((alkoxy)alkyl)aryl; (hydroxy)aryl, ((carboxy)alkyl)aryl; (((dialkyl)amino)alkyl)aryl; (nitroalkyl)aryl; (((alkylsulfonyl)amino)alkyl)aryl; ((heterocycloaliphatic)carbonyl)aryl; ((alkylsulfonyl)alkyl)aryl; (cyanoalkyl)aryl; (hydroxyalkyl)aryl; (alkylcarbonyl)aryl; alkylaryl; (trihaloalkyl)aryl; p-amino-m-alkoxycarbonylaryl; p-amino-m-cyanoaryl; p-halo-m-aminoaryl; or (m-(heterocycloaliphatic)-o-(alkyl))aryl.

**[0046]** As used herein, an "araliphatic" such as an "aralkyl" group refers to an aliphatic group (e.g., a $C_{1-4}$ alkyl group) that is substituted with an aryl group. "Aliphatic," "alkyl," and "aryl" are defined herein. An example of an araliphatic such as an aralkyl group is benzyl.

**[0047]** As used herein, an "aralkyl" group refers to an alkyl group (e.g., a $C_{1-4}$ alkyl group) that is substituted with an aryl group. Both "alkyl" and "aryl" have been defined above. An example of an aralkyl group is benzyl. An aralkyl is optionally substituted with one or more substituents such as aliphatic [e.g., alkyl, alkenyl, or alkynyl, including carboxyalkyl, hydroxyalkyl, or haloalkyl such as trifluoromethyl], cycloaliphatic [e.g., cycloalkyl or cycloalkenyl], (cycloalkyl)alkyl, heterocycloalkyl, (heterocycloalkyl)alkyl, aryl, heteroaryl, alkoxy, cycloalkyloxy, heterocycloalkyloxy, aryloxy, heteroaryloxy, aralkyloxy, heteroaralkyloxy, aroyl, heteroaroyl, nitro, carboxy, alkoxycarbonyl, alkylcarbonyloxy, amido [e.g., aminocarbonyl, alkylcarbonylamino, cycloalkylcarbonylamino, (cycloalkylalkyl)carbonylamino, arylcarbonylamino, aralkylcarbonylamino, (heterocycloalkyl)carbonylamino, (heterocycloalkylalkyl)carbonylamino, heteroarylcarbonylamino, or heteroaralkylcarbonylamino], cyano, halo, hydroxy, acyl, mercapto, alkylsulfanyl, sulfoxy, urea, thiourea, sulfamoyl, sulfamide, oxo, or carbamoyl.

**[0048]** As used herein, a "bicyclic ring system" includes 8-12 (e.g., 9, 10, or 11) membered structures that form two rings, wherein the two rings have at least one atom in common (e.g., 2 atoms in common). Bicyclic ring systems include

bicycloaliphatics (e.g., bicycloalkyl or bicycloalkenyl), bicycloheteroaliphatics, bicyclic aryls, and bicyclic heteroaryls.

**[0049]** As used herein, a "carbocycle" or "cycloaliphatic" group encompasses a "cycloalkyl" group and a "cycloalkenyl" group, each of which being optionally substituted as set forth below.

**[0050]** As used herein, a "cycloalkyl" group refers to a saturated carbocyclic mono- or bicyclic (fused or bridged) ring of 3-10 (e.g., 5-10) carbon atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, adamantyl, norbornyl, cubyl, octahydro-indenyl, decahydro-naphthyl, bicyclo[3.2.1]octyl, bicyclo[2.2.2]octyl, bicyclo[3.3.1]nonyl, bicyclo[3.3.2.]decyl, bicyclo[2.2.2]octyl, adamantyl, or ((aminocarbonyl)cycloalkyl)cycloalkyl.

**[0051]** A "cycloalkenyl" group, as used herein, refers to a non-aromatic carbocyclic ring of 3-10 (e.g., 4-8) carbon atoms having one or more double bonds. Examples of cycloalkenyl groups include cyclopentenyl, 1,4-cyclohexadi-enyl, cycloheptenyl, cyclooctenyl, hexahydro-indenyl, octahydro-naphthyl, cyclohexenyl, cyclopentenyl, bicyclo[2.2.2]octenyl, or bicyclo[3.3.1]nonenyl.

**[0052]** A cycloalkyl or cycloalkenyl group can be optionally substituted with one or more substituents such as phosphor, aliphatic [e.g., alkyl, alkenyl, or alkynyl], cycloaliphatic, (cycloaliphatic) aliphatic, heterocycloaliphatic, (heterocycloaliphatic) aliphatic, aryl, heteroaryl, alkoxy, (cycloaliphatic)oxy, (heterocycloaliphatic) oxy, aryloxy, heteroaryloxy, (araliphatic)oxy, (heteroaraliphatic)oxy, aroyl, heteroaroyl, amino, amido [e.g., (aliphatic)carbonylamino, (cycloaliphatic)carbonylamino, ((cycloaliphatic)aliphatic)carbonylamino, (aryl)carbonylamino, (araliphatic)carbonylamino, (heterocycloaliphatic)carbonylamino, ((heterocycloaliphatic) aliphatic)carbonylamino, (heteroaryl)carbonylamino, or (heteroaraliphatic)carbonylamino], nitro, carboxy [e.g., HOOC—, alkoxycarbonyl, or alkylcarbonyloxy], acyl [e.g., (cycloaliphatic)carbonyl, ((cycloaliphatic) aliphatic)carbonyl, (araliphatic)carbonyl, (heterocycloaliphatic)carbonyl, ((heterocycloaliphatic)aliphatic) carbonyl, or (heteroaraliphatic)carbonyl], nitro, cyano, halo, hydroxy, mercapto, sulfonyl [e.g., alkylsulfonyl or arylsulfonyl], sulfinyl [e.g., alkylsulfinyl], sulfanyl [e.g., alkylsulfanyl], sulfoxy, urea, thiourea, sulfamoyl, sulfamide, oxo, or carbamoyl.

**[0053]** As used herein, the term "heterocycle" or "heterocycloaliphatic" encompasses a heterocycloalkyl group and a heterocycloalkenyl group, each of which being optionally substituted as set forth below.

**[0054]** As used herein, a "heterocycloalkyl" group refers to a 3-10 membered mono- or bicylic (fused or bridged) (e.g., 5- to 10-membered mono- or bicyclic) saturated ring structure, in which one or more of the ring atoms is a heteroatom (e.g., N, O, S, or combinations thereof). Examples of a heterocycloalkyl group include piperidyl, piperazyl, tetrahydropyranyl, tetrahydrofuryl, 1,4-dioxolanyl, 1,4-dithianyl, 1,3-dioxolanyl, oxazolidyl, isoxazolidyl, morpholinyl, thiomorpholyl, octahydrobenzofuryl, octahydrochromenyl, octahydrothiochromenyl, octahydroindolyl, octahydropyrindinyl, decahydroquinolinyl, octahydrobenzo[b]thiophenyl, 2-oxa-bicyclo[2.2.2]octyl, 1-aza-bicyclo[2.2.2]octyl, 3-aza-bicyclo[3.2.1]octyl, and 2,6-dioxa-tricyclo[3.3.1.$0^{3,7}$]nonyl. A monocyclic heterocycloalkyl group can be fused with a phenyl moiety to form structures, such as tetrahydroisoquinoline, which would be categorized as heteroaryls.

**[0055]** A "heterocycloalkenyl" group, as used herein, refers to a mono- or bicylic (e.g., 5- to 10-membered mono- or bicyclic) non-aromatic ring structure having one or more double bonds, and wherein one or more of the ring atoms is a heteroatom (e.g., N, O, or S). Monocyclic and bicyclic heterocycloaliphatics are numbered according to standard chemical nomenclature.

**[0056]** A heterocycloalkyl or heterocycloalkenyl group can be optionally substituted with one or more substituents such as phosphor, aliphatic [e.g., alkyl, alkenyl, or alkynyl], cycloaliphatic, (cycloaliphatic)aliphatic, heterocycloaliphatic, (heterocycloaliphatic)aliphatic, aryl, heteroaryl, alkoxy, (cycloaliphatic)oxy, (heterocycloaliphatic)oxy, aryloxy, heteroaryloxy, (araliphatic)oxy, (heteroaraliphatic)oxy, aroyl, heteroaroyl, amino, amido [e.g., (aliphatic)carbonylamino, (cycloaliphatic)carbonylamino, ((cycloaliphatic) aliphatic)carbonylamino, (aryl)carbonylamino, (araliphatic) carbonylamino, (heterocycloaliphatic)carbonylamino, ((heterocycloaliphatic) aliphatic)carbonylamino, (heteroaryl)carbonylamino, or (heteroaraliphatic)carbonylamino], nitro, carboxy [e.g., HOOC—, alkoxycarbonyl, or alkylcarbonyloxy], acyl [e.g., (cycloaliphatic)carbonyl, ((cycloaliphatic) aliphatic)carbonyl, (araliphatic)carbonyl, (heterocycloaliphatic)carbonyl, ((heterocycloaliphatic)aliphatic) carbonyl, or (heteroaraliphatic)carbonyl], nitro, cyano, halo, hydroxy, mercapto, sulfonyl [e.g., alkylsulfonyl or arylsulfonyl], sulfinyl [e.g., alkylsulfinyl], sulfanyl [e.g., alkylsulfanyl], sulfoxy, urea, thiourea, sulfamoyl, sulfamide, oxo, or carbamoyl.

**[0057]** A "heteroaryl" group, as used herein, refers to a monocyclic, bicyclic, or tricyclic ring system having 4 to 15 ring atoms wherein one or more of the ring atoms is a heteroatom (e.g., N, O, S, or combinations thereof) and in which the monocyclic ring system is aromatic or at least one of the rings in the bicyclic or tricyclic ring systems is aromatic. A heteroaryl group includes a benzofused ring system having 2 to 3 rings. For example, a benzofused group includes benzo fused with one or two 4 to 8 membered heterocycloaliphatic moieties (e.g., indolizyl, indolyl, isoindolyl, 3H-indolyl, indolinyl, benzo[b]furyl, benzo[b]thiophenyl, quinolinyl, or isoquinolinyl). Some examples of heteroaryl are azetidinyl, pyridyl, 1H-indazolyl, furyl, pyrrolyl, thienyl, thiazolyl, oxazolyl, imidazolyl, tetrazolyl, benzofuryl, isoquinolinyl, benzthiazolyl, xanthene, thioxanthene, phenothiazine, dihydroindole, benzo[1,3]dioxole, benzo[b]furyl, benzo[b] thiophenyl, indazolyl, benzimidazolyl, benzthiazolyl, puryl, cinnolyl, quinolyl, quinazolyl, cinnolyl, phthalazyl, quinazolyl, quinoxalyl, isoquinolyl, 4H-quinolizyl, benzo-1, 2,5-thiadiazolyl, or 1,8-naphthyridyl.

**[0058]** Without limitation, monocyclic heteroaryls include furyl, thiophenyl, 2H-pyrrolyl, pyrrolyl, oxazolyl, thazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, 1,3,4-thiadiazolyl, 2H-pyranyl, 4-H-pranyl, pyridyl, pyridazyl, pyrimidyl, pyrazolyl, pyrazyl, or 1,3,5-triazyl. Monocyclic heteroaryls are numbered according to standard chemical nomenclature.

**[0059]** Without limitation, bicyclic heteroaryls include indolizyl, indolyl, isoindolyl, 3H-indolyl, indolinyl, benzo[b] furyl, benzo[b]thiophenyl, quinolinyl, isoquinolinyl, indolizinyl, isoindolyl, indolyl, benzo[b]furyl, bexo[b]thiophenyl, indazolyl, benzimidazyl, benzthiazolyl, purinyl, 4H-quinolizyl, quinolyl, isoquinolyl, cinnolyl, phthalazyl, quinazolyl, quinoxalyl, 1,8-naphthyridyl, or pteridyl. Bicyclic heteroaryls are numbered according to standard chemical nomenclature.

**[0060]** A heteroaryl is optionally substituted with one or more substituents such as aliphatic [e.g., alkyl, alkenyl, or alkynyl]; cycloaliphatic; (cycloaliphatic)aliphatic; heterocycloaliphatic; (heterocycloaliphatic)aliphatic; aryl; heteroaryl; alkoxy; (cycloaliphatic)oxy; (heterocycloaliphatic) oxy; aryloxy; heteroaryloxy; (araliphatic)oxy; (heteroaraliphatic)oxy; aroyl; heteroaroyl; amino; oxo (on a non-aromatic carbocyclic or heterocyclic ring of a bicyclic or tricyclic heteroaryl); carboxy; amido; acyl [e.g., aliphaticcarbonyl; (cycloaliphatic)carbonyl; ((cycloaliphatic)aliphatic) carbonyl; (araliphatic)carbonyl; (heterocycloaliphatic)carbonyl; ((heterocycloaliphatic)aliphatic)carbonyl; or (heteroaraliphatic)carbonyl]; sulfonyl [e.g., aliphaticsulfonyl or aminosulfonyl]; sulfinyl [e.g., aliphaticsulfinyl]; sulfanyl [e.g., aliphaticsulfanyl]; nitro; cyano; halo; hydroxy; mercapto; sulfoxy; urea; thiourea; sulfamoyl; sulfamide; or carbamoyl. Alternatively, a heteroaryl can be unsubstituted.

**[0061]** Non-limiting examples of substituted heteroaryls include (halo)heteroaryl [e.g., mono- and di-(halo)heteroaryl]; (carboxy)heteroaryl [e.g., (alkoxycarbonyl)heteroaryl]; cyanoheteroaryl; aminoheteroaryl [e.g., ((alkylsulfonyl)amino)heteroaryl and ((dialkyl)amino)heteroaryl]; (amido)heteroaryl [e.g., aminocarbonylheteroaryl, ((alkylcarbonyl)amino)heteroaryl, ((((alkyl)amino)alkyl)aminocarbonyl)heteroaryl, (((heteroaryl)amino)carbonyl)heteroaryl, ((heterocycloaliphatic)carbonyl)heteroaryl, and ((alkylcarbonyl)amino)heteroaryl]; (cyanoalkyl)heteroaryl; (alkoxy) heteroaryl; (sulfamoyl)heteroaryl [e.g., (aminosulfonyl)heteroaryl]; (sulfonyl)heteroaryl [e.g., (alkylsulfonyl) heteroaryl]; (hydroxyalkyl)heteroaryl; (alkoxyalkyl) heteroaryl; (hydroxy)heteroaryl; ((carboxy)alkyl)heteroaryl; (((dialkyl)amino)alkyl]heteroaryl; (heterocycloaliphatic) heteroaryl; (cycloaliphatic)heteroaryl; (nitroalkyl)heteroaryl; (((alkylsulfonyl)amino)alkyl)heteroaryl; ((alkylsulfonyl)alkyl)heteroaryl; (cyanoalkyl)heteroaryl; (acyl) heteroaryl [e.g., (alkylcarbonyl)heteroaryl]; (alkyl) heteroaryl, and (haloalkyl)heteroaryl [e.g., trihaloalkylheteroaryl].

**[0062]** A "heteroaraliphatic" (such as a heteroaralkyl group) as used herein, refers to an aliphatic group (e.g., a $C_{1-4}$ alkyl group) that is substituted with a heteroaryl group. "Aliphatic," "alkyl," and "heteroaryl" have been defined above.

**[0063]** A "heteroaralkyl" group, as used herein, refers to an alkyl group (e.g., a $C_{1-4}$ alkyl group) that is substituted with a heteroaryl group. Both "alkyl" and "heteroaryl" have been defined above. A heteroaralkyl is optionally substituted with one or more substituents such as alkyl (including carboxyalkyl, hydroxyalkyl, and haloalkyl such as trifluoromethyl), alkenyl, alkynyl, cycloalkyl, (cycloalkyl)alkyl, heterocycloalkyl, (heterocycloalkyl)alkyl, aryl, heteroaryl, alkoxy, cycloalkyloxy, heterocycloalkyloxy, aryloxy, heteroaryloxy, aralkyloxy, heteroaralkyloxy, aroyl, heteroaroyl, nitro, carboxy, alkoxycarbonyl, alkylcarbonyloxy, aminocarbonyl, alkylcarbonylamino, cycloalkylcarbonylamino, (cycloalkylalkyl)carbonylamino, arylcarbonylamino, aralkylcarbonylamino, (heterocycloalkyl)carbonylamino, (heterocycloalkylalkyl)carbonylamino, heteroarylcarbonylamino, heteroaralkylcarbonylamino, cyano, halo, hydroxy, acyl, mercapto, alkylsulfanyl, sulfoxy, urea, thiourea, sulfamoyl, sulfamide, oxo, or carbamoyl.

**[0064]** As used herein, "cyclic moiety" and "cyclic group" refer to mono-, bi-, and tri-cyclic ring systems including cycloaliphatic, heterocycloaliphatic, aryl, or heteroaryl, each of which has been previously defined.

**[0065]** As used herein, a "bridged bicyclic ring system" refers to a bicyclic heterocyclicaliphatic ring system or bicyclic cycloaliphatic ring system in which the rings are bridged. Examples of bridged bicyclic ring systems include, but are not limited to, adamantanyl, norbornanyl, bicyclo[3.2.1]octyl, bicyclo[2.2.2]octyl, bicyclo[3.3.1]nonyl, bicyclo[3.2.3]nonyl, 2-oxabicyclo[2.2.2]octyl, 1-azabicyclo[2.2.2]octyl, 3-azabicyclo[3.2.1]octyl, and 2,6-dioxa-tricyclo[3.3.1.$0^{3,7}$]nonyl. A bridged bicyclic ring system can be optionally substituted with one or more substituents such as alkyl (including carboxyalkyl, hydroxyalkyl, and haloalkyl such as trifluoromethyl), alkenyl, alkynyl, cycloalkyl, (cycloalkyl)alkyl, heterocycloalkyl, (heterocycloalkyl)alkyl, aryl, heteroaryl, alkoxy, cycloalkyloxy, heterocycloalkyloxy, aryloxy, heteroaryloxy, aralkyloxy, heteroaralkyloxy, aroyl, heteroaroyl, nitro, carboxy, alkoxycarbonyl, alkylcarbonyloxy, aminocarbonyl, alkylcarbonylamino, cycloalkylcarbonylamino, (cycloalkylalkyl)carbonylamino, arylcarbonylamino, aralkylcarbonylamino, (heterocycloalkyl)carbonylamino, (heterocycloalkylalkyl)carbonylamino, heteroarylcarbonylamino, heteroaralkylcarbonylamino, cyano, halo, hydroxy, acyl, mercapto, alkylsulfanyl, sulfoxy, urea, thiourea, sulfamoyl, sulfamide, oxo, or carbamoyl.

**[0066]** As used herein, an "acyl" group refers to a formyl group or $R^X$—C(O)— (such as alkyl-C(O)—, also referred to as "alkylcarbonyl") where $R^X$ and "alkyl" have been defined previously. Acetyl and pivaloyl are examples of acyl groups.

**[0067]** As used herein, an "aroyl" or "heteroaroyl" refers to an aryl-C(O)— or a heteroaryl-C(O)—. The aryl and heteroaryl portion of the aroyl or heteroaroyl is optionally substituted as previously defined.

**[0068]** As used herein, an "alkoxy" group refers to an alkyl-O— group where "alkyl" has been defined previously.

**[0069]** As used herein, a "carbamoyl" group refers to a group having the structure —O—CO—$NR^XR^Y$ or —$NR^X$—CO—O—$R^Z$, wherein $R^X$ and $R^Y$ have been defined above and $R^Z$ can be aliphatic, aryl, araliphatic, heterocycloaliphatic, heteroaryl, or heteroaraliphatic.

**[0070]** As used herein, a "carboxy" group refers to —COOH, —$COOR^X$, —OC(O)H, —$OC(O)R^X$, when used as a terminal group; or —OC(O)— or —C(O)O— when used as an internal group.

**[0071]** As used herein, a "haloaliphatic" group refers to an aliphatic group substituted with 1-3 halogen. For instance, the term haloalkyl includes the group —$CF_3$.

**[0072]** As used herein, a "mercapto" group refers to —SH.

**[0073]** As used herein, a "sulfo" group refers to —$SO_3H$ or —$SO_3R^X$ when used terminally or —$S(O)_3$— when used internally.

**[0074]** As used herein, a "sulfamide" group refers to the structure —$NR^X$—$S(O)_2$—$NR^YR^Z$ when used terminally and —$NR^X$—$S(O)_2$—$NR^Y$— when used internally, wherein $R^X$, $R^Y$, and $R^Z$ have been defined above.

**[0075]** As used herein, a "sulfonamide" group refers to the structure —$S(O)_2$—$NR^XR^Y$ or —$NR^X$—$S(O)_2$—$R^Z$ when used terminally; or —$S(O)_2$—$NR^X$— or —$NR^X$—$S(O)_2$— when used internally, wherein $R^X$, $R^Y$, and $R^Z$ are defined above.

**[0076]** As used herein a "sulfanyl" group refers to —S—$R^X$ when used terminally and —S— when used internally, wherein $R^X$ has been defined above. Examples of sulfanyls include aliphatic-S—, cycloaliphatic-S—, aryl-S—, or the like.

**[0077]** As used herein a "sulfinyl" group refers to —S(O)—$R^X$ when used terminally and —S(O)— when used internally, wherein $R^X$ has been defined above. Exemplary sulfinyl groups include aliphatic-S(O)—, aryl-S(O)—, (cycloaliphatic(aliphatic))-S(O)—, cycloalkyl-S(O)—, heterocycloaliphatic-S(O)—, heteroaryl-S(O)—, or the like.

**[0078]** As used herein, a "sulfonyl" group refers to —S(O)$_2$—$R^X$ when used terminally and —S(O)$_2$— when used internally, wherein $R^X$ has been defined above. Exemplary sulfonyl groups include aliphatic-S(O)$_2$—, aryl-S(O)$_2$—, (cycloaliphatic(aliphatic))-S(O)$_2$—, cycloaliphatic-S(O)$_2$—, heterocycloaliphatic-S(O)$_2$—, heteroaryl-S(O)$_2$—, (cycloaliphatic(amido(aliphatic)))-S(O)$_2$— or the like.

**[0079]** As used herein, a "sulfoxy" group refers to —O—SO—$R^X$ or —SO—O—$R^X$, when used terminally and —O—S(O)— or —S(O)—O— when used internally, where $R^X$ has been defined above.

**[0080]** As used herein, a "halogen" or "halo" group refers to fluorine, chlorine, bromine or iodine.

**[0081]** As used herein, an "alkoxycarbonyl," which is encompassed by the term carboxy, used alone or in connection with another group refers to a group such as alkyl-O—C(O)—.

**[0082]** As used herein, an "alkoxyalkyl" refers to an alkyl group such as alkyl-O-alkyl-, wherein alkyl has been defined above.

**[0083]** As used herein, a "carbonyl" refer to —C(O)—.

**[0084]** As used herein, an "oxo" refers to =O.

**[0085]** As used herein, the term "phospho" refers to phosphinates and phosphonates. Examples of phosphinates and phosphonates include —P(O)($R^P$)$_2$, wherein $R^P$ is aliphatic, alkoxy, aryloxy, heteroaryloxy, (cycloaliphatic)oxy, (heterocycloaliphatic)oxy aryl, heteroaryl, cycloaliphatic or amino.

**[0086]** As used herein, an "aminoalkyl" refers to the structure ($R^X$)$_2$N-alkyl-.

**[0087]** As used herein, a "cyanoalkyl" refers to the structure (NC)-alkyl-.

**[0088]** As used herein, a "urea" group refers to the structure —$NR^X$—CO—$NR^YR^Z$ and a "thiourea" group refers to the structure —$NR^X$—CS—$NR^YR^Z$ when used terminally and —$NR^X$—CO—$NR^Y$— or —$NR^X$—CS—$NR^Y$— when used internally, wherein $R^X$, $R^Y$, and $R^Z$ have been defined above.

**[0089]** As used herein, a "guanidine" group refers to the structure —N=C(N($R^XR^Y$))N($R^XR^Y$) or —$NR^X$—C(=$NR^X$)$NR^XR^Y$ wherein $R^X$ and $R^Y$ have been defined above.

**[0090]** As used herein, the term "amidino" group refers to the structure —C=(N$R^X$)N($R^XR^Y$) wherein $R^X$ and $R^Y$ have been defined above.

**[0091]** In general, the term "vicinal" refers to the placement of substituents on a group that includes two or more carbon atoms, wherein the substituents are attached to adjacent carbon atoms.

**[0092]** In general, the term "geminal" refers to the placement of substituents on a group that includes two or more carbon atoms, wherein the substituents are attached to the same carbon atom.

**[0093]** The terms "terminally" and "internally" refer to the location of a group within a substituent. A group is terminal when the group is present at the end of the substituent not further bonded to the rest of the chemical structure. Carboxyalkyl, i.e., $R^X$O(O)C-alkyl is an example of a carboxy group used terminally. A group is internal when the group is present in the middle of a substituent of the chemical structure. Alkylcarboxy (e.g., alkyl-C(O)O— or alkyl-OC(O)—) and alkylcarboxyaryl (e.g., alkyl-C(O)O-aryl- or alkyl-O(CO)-aryl-) are examples of carboxy groups used internally.

**[0094]** As used herein, an "aliphatic chain" refers to a branched or straight aliphatic group (e.g., alkyl groups, alkenyl groups, or alkynyl groups). A straight aliphatic chain has the structure —[$CH_2$]$_v$—, where v is 1-12. A branched aliphatic chain is a straight aliphatic chain that is substituted with one or more aliphatic groups. A branched aliphatic chain has the structure —[CQQ]$_v$— where each Q is independently a hydrogen or an aliphatic group; however, Q shall be an aliphatic group in at least one instance. The term aliphatic chain includes alkyl chains, alkenyl chains, and alkynyl chains, where alkyl, alkenyl, and alkynyl are defined above.

**[0095]** The phrase "optionally substituted" is used interchangeably with the phrase "substituted or unsubstituted." As described herein, compounds of the invention can optionally be substituted with one or more substituents, such as are illustrated generally above, or as exemplified by particular classes, subclasses, and species of the invention. As described herein, the variables $R_1$, $R_2$, and $R_3$, and other variables contained in formulae described herein encompass specific groups, such as alkyl and aryl. Unless otherwise noted, each of the specific groups for the variables $R_1$, $R_2$, and $R_3$, and other variables contained therein can be optionally substituted with one or more substituents described herein. Each substituent of a specific group is further optionally substituted with one to three of halo, cyano, oxo, alkoxy, hydroxy, amino, nitro, aryl, cycloaliphatic, heterocycloaliphatic, heteroaryl, haloalkyl, and alkyl. For instance, an alkyl group can be substituted with alkylsulfanyl and the alkylsulfanyl can be optionally substituted with one to three of halo, cyano, oxo, alkoxy, hydroxy, amino, nitro, aryl, haloalkyl, and alkyl. As an additional example, the cycloalkyl portion of a (cycloalkyl)carbonylamino can be optionally substituted with one to three of halo, cyano, alkoxy, hydroxy, nitro, haloalkyl, and alkyl. When two alkoxy groups are bound to the same atom or adjacent atoms, the two alkoxy groups can form a ring together with the atom(s) to which they are bound.

**[0096]** In general, the term "substituted," whether preceded by the term "optionally" or not, refers to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. Specific substituents are described above in the definitions and below in the description of compounds and examples thereof. Unless otherwise indicated, an optionally substituted group can have a substituent at each substitutable position of the group, and when more than one position in any given structure can be substituted with more than one substituent selected from a specified group, the substituent can be either the same or different at every position. A ring substituent, such as a heterocycloalkyl, can be bound to another ring, such as a cycloalkyl, to form a spirobicyclic ring system, e.g., both rings share one common atom. As one of ordinary skill in the art will recognize, combinations of substituents envisioned by this invention are those combinations that result in the formation of stable or chemically feasible compounds.

**[0097]** The phrase "stable or chemically feasible," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and preferably their recovery, purification, and use for one or more of the purposes disclosed herein. In some

embodiments, a stable compound or chemically feasible compound is one that is not substantially altered when kept at a temperature of 40° C. or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

**[0098]** As used herein, an "effective amount" is defined as the amount required to confer a therapeutic effect on the treated patient, and is typically determined based on age, surface area, weight, and condition of the patient. The interrelationship of dosages for animals and humans (based on milligrams per meter squared of body surface) is described by Freireich et al., Cancer Chemother. Rep., 50: 219 (1966). Body surface area may be approximately determined from height and weight of the patient. See, e.g., Scientific Tables, Geigy Pharmaceuticals, Ardsley, N.Y., 537 (1970). As used herein, "patient" refers to a mammal, including a human.

**[0099]** Unless otherwise stated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, (Z) and (E) double bond isomers, and (Z) and (E) conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention. Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a $^{13}C$- or $^{14}C$-enriched carbon are within the scope of this invention. Such compounds are useful, for example, as analytical tools or probes in biological assays, or as therapeutic agents.

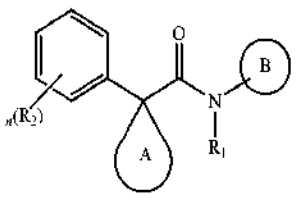
**[0100]** Compounds of the present invention are useful modulators of ABC transporters and are useful in the treatment of ABC transporter mediated diseases.

## II. Compounds

**[0101]** A. Generic Compounds

**[0102]** The present invention relates to compounds of formula I useful as modulators of ABC transporter activity:

I




or a pharmaceutically acceptable salt thereof.

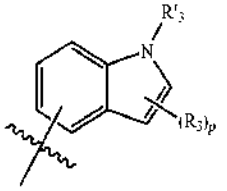
**[0103]** $R_1$ is $-Z^A R_4$, wherein each $Z^A$ is independently a bond or an optionally substituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^A$ are optionally and independently replaced by —CO—, —CS—, —$CONR^A$—, —$CONR^ANR^A$—, —$CO_2$—, —OCO—, —$NR^ACO_2$—, —O—, —$NR^ACONR^A$—, —$OCONR^A$—, —$NR^ANR^A$—, —$NR^ACO$—, —S—, —SO—, —$SO_2$—, —$NR^A$—, —$SO_2NR^A$—, —$NR^ASO_2$—, or —$NR^ASO_2NR^A$—. Each $R_4$ is independently $R^A$, halo, —OH, —$NH_2$, —$NO_2$, —CN, or —$OCF_3$. Each $R^A$ is independently hydrogen, an optionally substituted aliphatic, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted aryl, or an optionally substituted heteroaryl.

**[0104]** $R_2$ is $-Z^BR_5$, wherein each $Z^B$ is independently a bond or an optionally substituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^B$ are optionally and independently replaced by —CO—, —CS—, —$CONR^B$—, —$CONR^BNR^B$—, —$CO_2$—, —OCO—, —$NR^BCO_2$—, —O—, —$NR^BCONR^B$—, —$OCONR^B$—, —$NR^BNR^B$—, —$NR^BCO$—, —S—, —SO—, —$SO_2$—, —$NR^B$—, —$SO_2NR^B$—, —$NR^BSO_2$—, or —$NR^BSO_2NR^B$—. Each $R_5$ is independently $R^B$, halo, —OH, —$NH_2$, —$NO_2$, —CN, —$CF_3$, or —$OCF_3$. Each $R^B$ is independently hydrogen, an optionally substituted aliphatic, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted aryl, or an optionally substituted heteroaryl. Alternatively, any two adjacent $R_2$ groups together with the atoms to which they are attached form an optionally substituted carbocycle or an optionally substituted heterocycle.

**[0105]** Ring A is an optionally substituted 3-7 membered monocyclic ring having 0-3 heteroatoms selected from N, O, and S.

**[0106]** Ring B is a group having formula Ia:

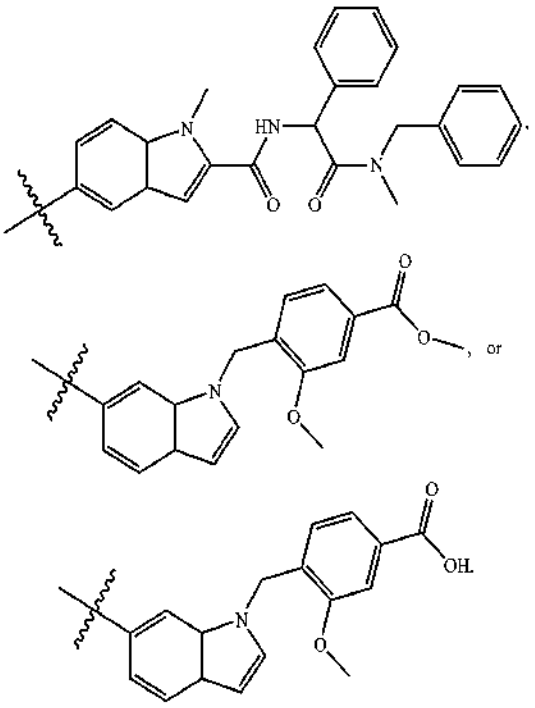
Ia




or a pharmaceutically acceptable salt thereof, wherein p is 0-3 and each $R_3$ and $R'_3$ is independently $-Z^CR_6$, where each $Z^C$ is independently a bond or an optionally substituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^C$ are optionally and independently replaced by —CO—, —CS—, —$CONR^C$—, —$CONR^CNR^C$—, —$CO_2$—, —OCO—, —$NR^CCO_2$—, —O—, —$NR^CCONR^C$—, —$OCONR^C$—, —$NR^CNR^C$—, —$NR^CCO$—, —S—, —SO—, —$SO_2$—, —$NR^C$—, —$SO_2NR^C$—, —$NR^CSO_2$—, or —$NR^CSO_2NR^C$—. Each $R_6$ is independently $R^C$, halo, —OH, —$NH_2$, —$NO_2$, —CN, or —$OCF_3$. Each $R^C$ is independently hydrogen, an optionally substituted aliphatic, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted aryl, or an optionally substituted heteroaryl. Alternatively, any two adjacent $R_3$ groups together with the atoms to which they are attached form an optionally substituted carbocycle or an optionally substituted heterocycle. Furthermore, $R'_3$ and an adjacent $R_3$ group, together with the atoms to which they are attached, form an optionally substituted heterocycle.

**[0107]** n is 1-3.

**[0108]** However, in several embodiments, when ring A is unsubstituted cyclopentyl, n is 1, $R_2$ is 4-chloro, and $R_1$ is hydrogen, then ring B is not 2-(tertbutyl)indol-5-yl, or (2,6-dichlorophenyl(carbonyl))-3-methyl-1H-indol-5-yl; and when ring A is unsubstituted cyclopentyl, n is 0, and $R_1$ is hydrogen, then ring B is not



**[0109]** B. Specific Compounds

**[0110]** 1. $R_1$ Group

**[0111]** $R_1$ is $-Z^A R_4$, wherein each $Z^A$ is independently a bond or an optionally substituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^A$ are optionally and independently replaced by —CO—, —CS—, —$CONR^A$—, —$CONR^ANR^A$—, —$CO_2$—, —OCO—, —$NR^ACO_2$—, —O—, —$NR^ACONR^A$—, —$OCONR^A$—, —$NR^ANR^A$—, —$NR^ACO$—, —S—, —SO—, —$SO_2$—, —$NR^A$—, —$SO_2NR^A$—, —$NR^ASO_2$—, or —$NR^ASO_2NR^A$—. Each $R_4$ is independently $R^A$, halo, —OH, —$NH_2$, —$NO_2$, —CN, or —$OCF_3$. Each $R^A$ is independently hydrogen, an optionally substituted aliphatic, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted aryl, or an optionally substituted heteroaryl.

**[0112]** In several embodiments, $R_1$ is $-Z^A R_4$, wherein each $Z^A$ is independently a bond or an optionally substituted branched or straight $C_{1-6}$ aliphatic chain and each $R_4$ is hydrogen.

**[0113]** In other embodiments, $R_1$ is $-Z^A R_4$, wherein each $Z^A$ is a bond and each $R_4$ is hydrogen.

**[0114]** 2. $R_2$ Group

**[0115]** Each $R_2$ is independently $-Z^B R_5$, wherein each $Z^B$ is independently a bond or an optionally substituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^B$ are optionally and independently replaced by —CO—, —CS—, —$CONR^B$—, —$CONR^BNR^B$—, —$CO_2$—, —OCO—, —$NR^BCO_2$—, —O—, —$NR^BCONR^B$—, —$OCONR^B$—, —$NR^BNR^B$—, —$NR^BCO$—, —S—, —SO—, —$SO_2$—, —$NR^B$—, —$SO_2NR^B$—, —$NR^BSO_2$—, or —$NR^BSO_2NR^B$—. Each $R_5$ is independently $R^B$, halo, —OH, —$NH_2$, —$NO_2$, —CN, —$CF_3$, or —$OCF_3$. Each $R^B$ is independently hydrogen, an optionally substituted aliphatic, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted aryl, or an optionally substituted heteroaryl. Alternatively, any two adjacent $R_2$ groups together with the atoms to which they are attached form an optionally substituted carbocycle or an optionally substituted heterocycle.

**[0116]** In several embodiments, $R_2$ is an optionally substituted aliphatic. For example, $R_2$ is an optionally substituted branched or straight $C_{1-6}$ aliphatic chain. In other examples, $R_2$ is an optionally substituted branched or straight $C_{1-6}$ alkyl chain, an optionally substituted branched or straight $C_{2-6}$ alkenyl chain, or an optionally substituted branched or straight $C_{2-6}$ alkynyl chain. In alternative embodiments, $R_2$ is a branched or straight $C_{1-6}$ aliphatic chain that is optionally substituted with 1-3 of halo, hydroxy, cyano, cycloaliphatic, heterocycloaliphatic, aryl, heteroaryl, or combinations thereof. For example, $R_2$ is a branched or straight $C_{1-6}$ alkyl that is optionally substituted with 1-3 of halo, hydroxy, cyano, cycloaliphatic, heterocycloaliphatic, aryl, heteroaryl, or combinations thereof. In still other examples, $R_2$ is a methyl, ethyl, propyl, butyl, isopropyl, or tert-butyl, each of which is optionally substituted with 1-3 of halo, hydroxy, cyano, aryl, heteroaryl, cycloaliphatic, or heterocycloaliphatic. In still other examples, $R_2$ is a methyl, ethyl, propyl, butyl, isopropyl, or tert-butyl, each of which is unsubstituted.

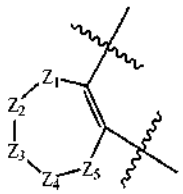
**[0117]** In several other embodiments, $R_2$ is an optionally substituted branched or straight $C_{1-5}$ alkoxy. For example, $R_2$ is a $C_{1-5}$ alkoxy that is optionally substituted with 1-3 of hydroxy, aryl, heteroaryl, cycloaliphatic, heterocycloaliphatic, or combinations thereof. In other examples, $R_2$ is a methoxy, ethoxy, propoxy, butoxy, or pentoxy, each of which is optionally substituted with 1-3 of hydroxy, aryl, heteroaryl, cycloaliphatic, heterocycloaliphatic, or combinations thereof.

**[0118]** In other embodiments, $R_2$ is hydroxy, halo, or cyano.

**[0119]** In several embodiments, $R_2$ is $-Z^B R_5$, and $Z^B$ is independently a bond or an optionally substituted branched or straight $C_{1-4}$ aliphatic chain wherein up to two carbon units of $Z^B$ are optionally and independently replaced by —C(O)—, —O—, —S—, —$S(O)_2$—, or —NH—, and $R_5$ is $R^B$, halo, —OH, —$NH_2$, —$NO_2$, —CN, —$CF_3$, or —$OCF_3$, and $R^B$ is hydrogen or aryl.

**[0120]** In several embodiments, two adjacent $R_2$ groups form an optionally substituted carbocycle or an optionally substituted heterocycle. For example, two adjacent $R_2$ groups form an optionally substituted carbocycle or an optionally substituted heterocycle, either of which is fused to the phenyl of formula I, wherein the carbocycle or heterocycle has formula Ib:

Ib



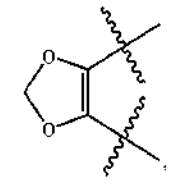
**[0121]** Each of $Z_1$, $Z_2$, $Z_3$, $Z_4$, and $Z_5$ is independently a bond, —$CR_7R'_7$—, —$NR_7$—, or —O—; each $R_7$ is independently $-Z^D R_8$, wherein each $Z^D$ is independently an optionally substituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^D$ are optionally and independently replaced by —CO—, —CS—, —$CONR^D$—, —$CO_2$—, —OCO—, —$NR^DCO_2$—, —O—, —$NR^D$—

$CONR^D$—, —$OCONR^D$—, —$NR^DNR^D$—, —$NR^DCO$—, —S—, —SO—, —$SO_2$—, —$NR^D$—, —$SO_2NR^D$—, —$NR^DSO_2$—, or —$NR^DSO_2NR^D$—. Each $R_8$ is independently $R^D$, halo. —OH, —$NH_2$, —$NO_2$, —CN, —$CF_3$, or —$OCF_3$. Each $R^D$ is independently hydrogen, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted aryl, or an optionally substituted heteroaryl. Each $R'_7$ is independently hydrogen, optionally substituted $C_{1-6}$ aliphatic, hydroxy, halo, cyano, nitro, or combinations thereof. Alternatively, any two adjacent $R_7$ groups together with the atoms to which they are attached form an optionally substituted 3-7 membered carbocyclic ring, such as an optionally substituted cyclobutyl ring, or any two $R_7$ and $R'_7$ groups together with the atom or atoms to which they are attached form an optionally substituted 3-7 membered carbocyclic ring or a heterocarbocyclic ring.

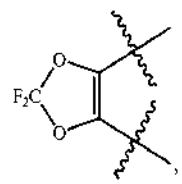
**[0122]** In several other examples, two adjacent $R_2$ groups form an optionally substituted carbocycle. For example, two adjacent $R_2$ groups form an optionally substituted 5-7 membered carbocycle that is optionally substituted with 1-3 of halo, hydroxy, cyano, oxo, cyano, alkoxy, alkyl, or combinations thereof. In another example, two adjacent $R_2$ groups form a 5-6 membered carbocycle that is optionally substituted with 1-3 of halo, hydroxy, cyano, oxo, cyano, alkoxy, alkyl, or combinations thereof. In still another example, two adjacent $R_2$ groups form an unsubstituted 5-7 membered carbocycle.

**[0123]** In alternative examples, two adjacent $R_2$ groups form an optionally substituted heterocycle. For instance, two adjacent $R_2$ groups form an optionally substituted 5-7 membered heterocycle having 1-3 heteroatoms independently selected from N, O, and S. In several examples, two adjacent $R_2$ groups form an optionally substituted 5-6 membered heterocycle having 1-2 oxygen atoms. In other examples, two adjacent $R_2$ groups form an unsubstituted 5-7 membered heterocycle having 1-2 oxygen atoms. In other embodiments, two adjacent $R_2$ groups form a heterocyclic ring selected from:

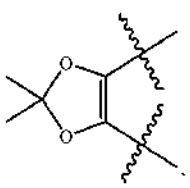
XA1



XA2



XA3



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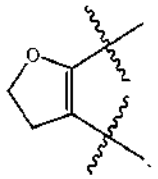
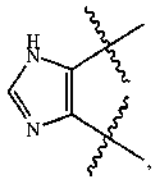
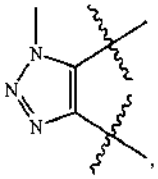
XA4



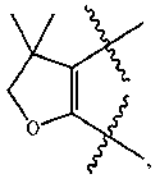
XA5



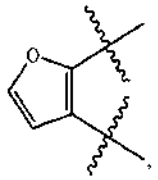
XA6



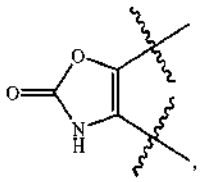
XA7



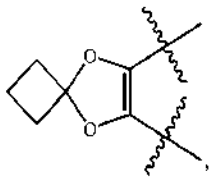
XA8



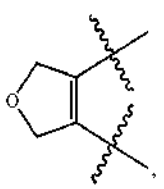
XA9



XA10



XA11



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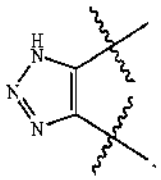
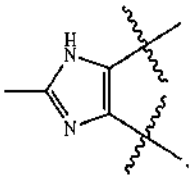
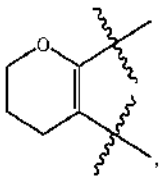
XA12



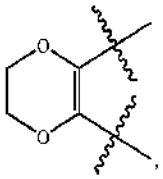
XA13



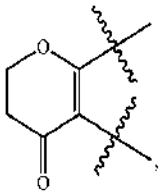
XA14



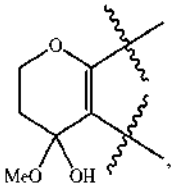
XA15



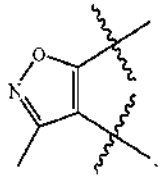
XA16



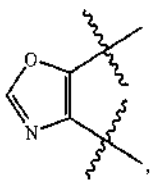
XA17



XA18



XA19



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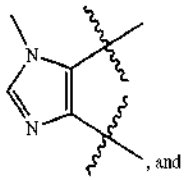
XA20



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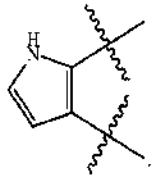
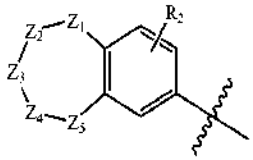
XA21



**[0124]** In alternative examples, two adjacent $R_2$ groups form an optionally substituted carbocycle or an optionally substituted heterocycle, and a third $R_2$ group is attached to any chemically feasible position on the phenyl of formula I. For instance, an optionally substituted carbocycle or an optionally substituted heterocycle, both of which is formed by two adjacent $R_2$ groups; a third $R_2$ group; and the phenyl of formula I form a group having formula Ic:

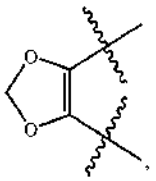
Ic



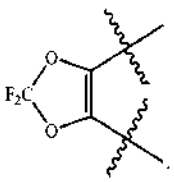
**[0125]** $Z_1$, $Z_2$, $Z_3$, $Z_4$, and $Z_5$ has been defined above in formula Ib, and $R_2$ has been defined above in formula I.

**[0126]** In several embodiments, each $R_2$ group is independently selected from hydrogen, halo, —$OCH_3$, —OH, —$CH_2OH$, —$CH_3$, and —$OCF_3$, and/or two adjacent $R_2$ groups together with the atoms to which they are attached form

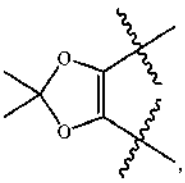
XA1



XA2



XA3



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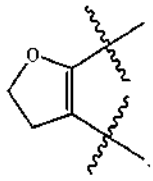
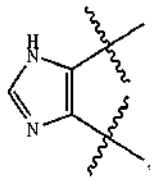
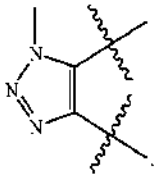
XA4



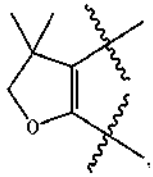
XA5



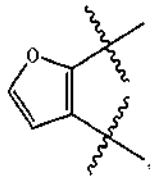
XA6



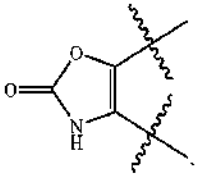
XA7



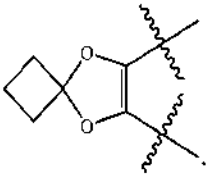
XA8



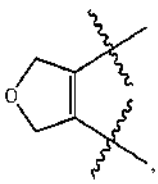
XA9



XA10



XA11



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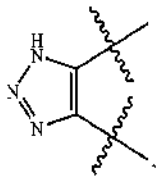
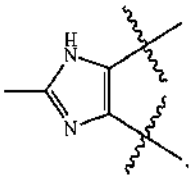
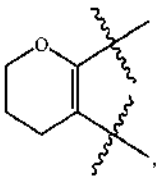
XA12



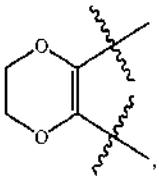
XA13



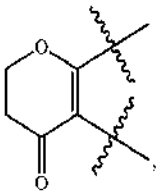
XA14



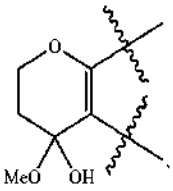
XA15



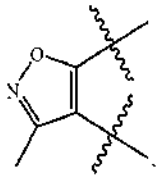
XA16



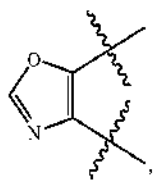
XA17




XA18



XA19



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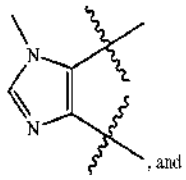
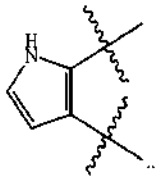
XA20



, and

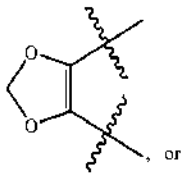
XA21



**[0127]** In other embodiments, $R_2$ is at least one selected from hydrogen, halo, methoxy, phenylmethoxy, hydroxy, hydroxymethyl, trifluoromethoxy, and methyl.

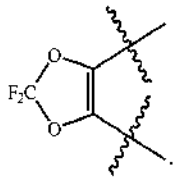
**[0128]** In some embodiments, two adjacent $R_2$ groups, together with the atoms to which they are attached, form

XA1



, or

XA2



**[0129]** 3. Ring A

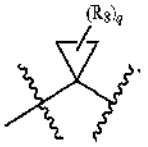
**[0130]** Ring A is an optionally substituted 3-7 membered monocyclic ring having 0-3 heteroatoms selected from N, O, and S.

**[0131]** In several embodiments, ring A is an optionally substituted 3-7 membered monocyclic cycloaliphatic. For example, ring A is a cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl, each of which is optionally substituted with 1-3 of halo, hydroxy, $C_{1-5}$ aliphatic, or combinations thereof.

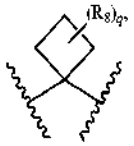
**[0132]** In other embodiments, ring A is an optionally substituted 3-7 membered monocyclic heterocycloaliphatic. For example, ring A is an optionally substituted 3-7 membered monocyclic heterocycloaliphatic having 1-2 heteroatoms independently selected from N, O, and S. In other examples, ring A is tetrahydrofuran-yl, tetrahydro-2H-pyran-yl, pyrrolidone-yl, or piperidine-yl, each of which is optionally substituted.

**[0133]** In still other examples, ring A is selected from

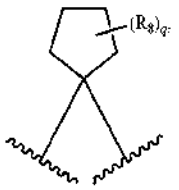
XB1



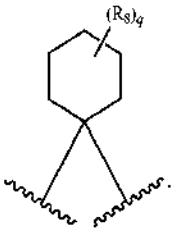
XB2



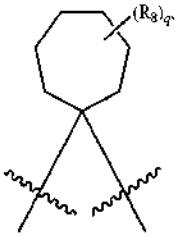
XB3



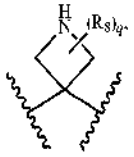
XB4



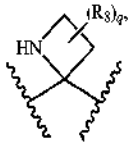
XB5



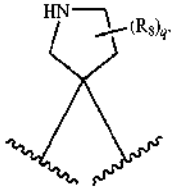
XB6



XB7



XB8



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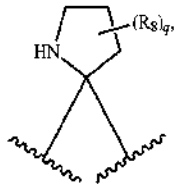
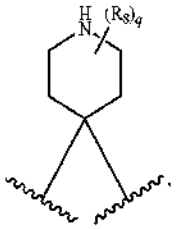
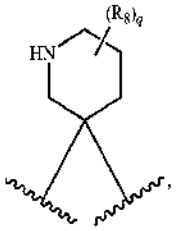
XB9



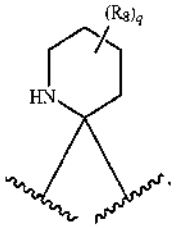
XB10



XB11



XB12



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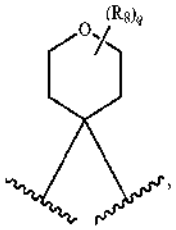
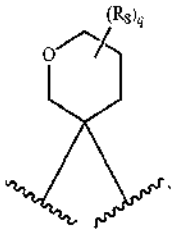
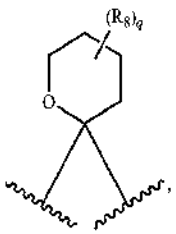


XB14



XB15



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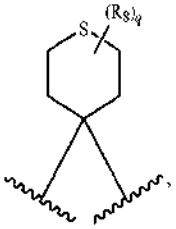
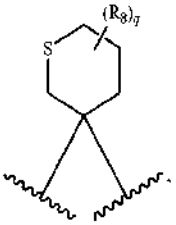
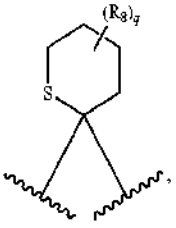
XB16



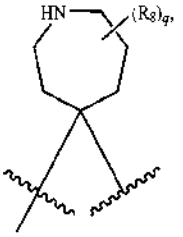
XB17



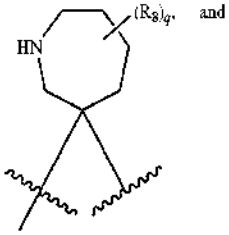
XB18



XB19

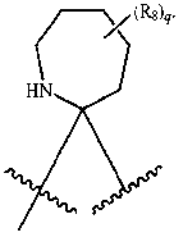


XB20



and

XB21



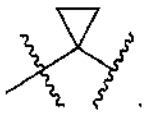
**[0134]** Each $R_8$ is independently $-Z^ER_9$, wherein each $Z^E$ is independently a bond or an optionally substituted branched or straight $C_{1-5}$ aliphatic chain wherein up to two carbon units of $Z^E$ are optionally and independently replaced by —CO—, —CS—, —CONR$^E$—, —CO$_2$—, —OCO—, —NR$^E$CO$_2$—, —O—, —NR$^E$CONR$^E$—, —OCONR$^E$—, —NR$^E$NR$^E$—, —NR$^E$CO—, —S—, —SO—, —SO$_2$—, —NR$^e$—, —SO$_2$NR$^E$—, —NR$^E$SO$_2$—, or —NR$^E$SO$_2$NR$^E$—, each $R_9$

is independently $R^E$, —OH, —$NH_2$, —$NO_2$, —CN, —$CF_3$, oxo, or —$OCF_3$. Each $R^E$ is independently hydrogen, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted aryl, or an optionally substituted heteroaryl.

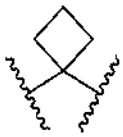
**[0135]** q is 0-5.

**[0136]** In other embodiments. ring A is one selected from

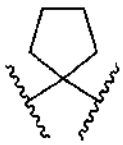
XC1



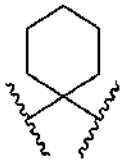
XC2



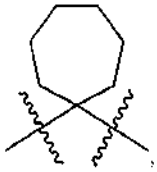
XC3



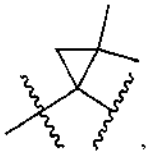
XC4



XC5

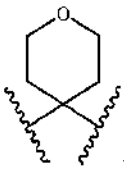


XC6

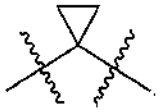


, and

XC4



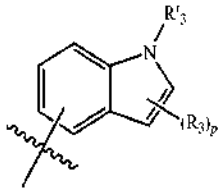
**[0137]** In several embodiments, ring A is



**[0138]** 4. Ring B

**[0139]** Ring B is a group having formula Ia:

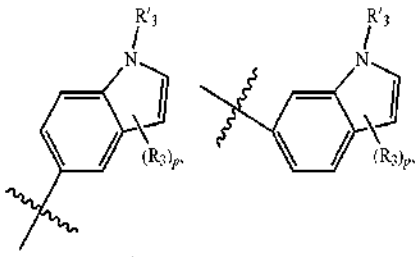
Ia

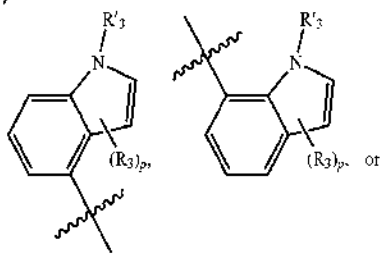


or a pharmaceutically acceptable salt thereof, wherein p is 0-3.

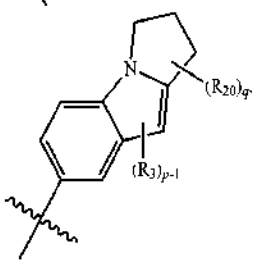
**[0140]** Each $R_3$ and $R'_3$ is independently -$Z^CR_6$, where each $Z^C$ is independently a bond or an optionally substituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^C$ are optionally and independently replaced by —CO—, —CS—, —$CONR^C$—, —$CONR^CNR^C$—, —$CO_2$—, —OCO—, —$NR^CCO_2$—, —O—, —$NR^CCO$-$NR^C$—, —$OCONR^C$—, —$NR^CNR^C$—, —$NR^CCO$—, —S—, —SO—, —$SO_2$—, —$NR^C$—, —$SO_2NR^C$—, —$NR^CSO_2$—, or —$NR^CSO_2NR^C$—. Each $R_6$ is independently $R^C$, halo, —OH, —$NH_2$, —$NO_2$, —CN, or —$OCF_3$. Each $R^C$ is independently hydrogen, an optionally substituted aliphatic, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted aryl, or an optionally substituted heteroaryl. Alternatively, any two adjacent $R_3$ groups together with the atoms to which they are attached form an optionally substituted carbocycle or an optionally substituted heterocycle, or $R'_3$ and an adjacent $R_3$, i.e., attached to the 2 position of the indole of formula Ia, together with the atoms to which they are attached form an optionally substituted heterocycle.

**[0141]** In several embodiments, ring B is



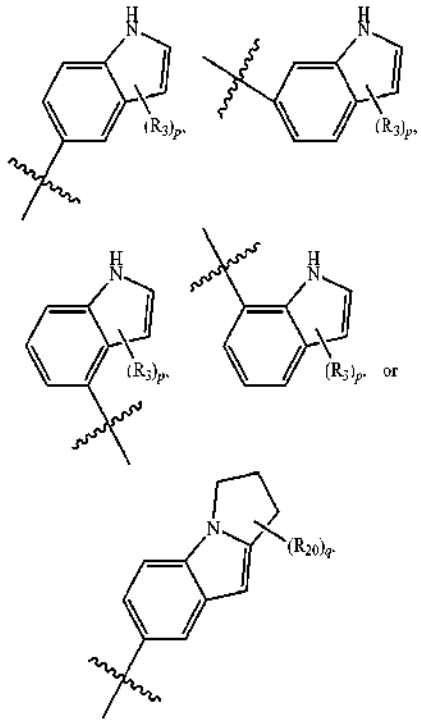


or



**[0142]** wherein q is 0-3 and each $R_{20}$ is $-Z^G R_{21}$, where each $Z^G$ is independently a bond or an optionally substituted branched or straight $C_{1-5}$ aliphatic chain wherein up to two carbon units of $Z^G$ are optionally and independently replaced by —CO—, —CS—, —$CONR^G$—, —CO2-, —OCO—, —$NR^GCO_2$—, —O—, —$OCONR^G$—, —$NR^GNR^G$—, —$NR^GCO$—, —S—, —SO—, —$SO_2$—, —$NR^G$—, —$SO_2NR^G$—, —$NR^GSO_2$—, or —$NR^GSO_2NR^G$—. Each $R_{21}$ is independently $R^G$, halo, —OH, —$NH_2$, —$NO_2$, —CN, or —$OCF_3$. Each $R^G$ is independently hydrogen, an optionally substituted aliphatic, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted aryl, or an optionally substituted heteroaryl.

**[0143]** For example, ring B is



**[0144]** In several embodiments, R'$_3$ is hydrogen and $R_3$ is attached to the 2, 3, 4, 6, or 7 position of the indole of formula Ia. In several other examples, $R_3$ is attached to the 2 or 3 position of the indole of formula Ia, and $R_3$ is independently an optionally substituted aliphatic. For instance, $R_3$ is an optionally substituted acyl group. In several instances, $R_3$ is an optionally substituted (alkoxy)carbonyl. In other instances, $R_3$ is (methoxy)carbonyl, (ethoxy)carbonyl, (propoxy)carbonyl, or (butoxy)carbonyl, each of which is optionally substituted with 1-3 of halo, hydroxy, or combinations thereof. In other instances, $R_3$ is an optionally substituted (aliphatic)carbonyl. For example, $R_3$ is an optionally substituted (alkyl)carbonyl that is optionally substituted with 1-3 of halo, hydroxy, or combinations thereof. In other examples, $R_3$ is (methyl)carbonyl, (ethyl)carbonyl, (propyl)carbonyl, or (butyl)carbonyl, each of which is optionally substituted with 1-3 of halo, hydroxy, or combinations thereof.

**[0145]** In several embodiments, $R_3$ is an optionally substituted (cycloaliphatic)carbonyl or an optionally substituted (heterocycloaliphatic)carbonyl. In several examples, $R_3$ is an optionally substituted ($C_{3-7}$ cycloaliphatic)carbonyl. For example, $R_3$ is a (cyclopropyl)carbonyl, (cyclobutyl)carbonyl, (cyclopentyl)carbonyl, (cyclohexyl)carbonyl, or (cycloheptyl)carbonyl, each of which is optionally substituted with aliphatic, halo, hydroxy, nitro, cyano, or combinations thereof. In several alternative examples, $R_3$ is an optionally substituted (heterocycloaliphatic)carbonyl. For example, $R_3$ is an optionally substituted (heterocycloaliphatic)carbonyl having 1-3 heteroatoms independently selected from N, O, and S. In other examples, $R_3$ is an optionally substituted (heterocycloaliphatic)carbonyl having 1-3 heteroatoms independently selected from N and O. In still other examples, $R_3$ is an optionally substituted 4-7 membered monocyclic (heterocycloaliphatic)carbonyl having 1-3 heteroatoms independently selected from N and O. Alternatively, $R_3$ is (piperidine-1-yl)carbonyl, (pyrrolidine-1-yl)carbonyl, or (morpholine-4-yl)carbonyl, (piperazine-1-yl)carbonyl, each of which is optionally substituted with 1-3 of halo, hydroxy, cyano, nitro, or aliphatic.

**[0146]** In still other instances, $R_3$ is optionally substituted (aliphatic)amido such as (aliphatic(amino(carbonyl)) that is attached to the 2 or 3 position on the indole ring of formula Ia. In some embodiments, $R_3$ is an optionally substituted (alkyl(amino))carbonyl that is attached to the 2 or 3 position on the indole ring of formula Ia. In other embodiments, $R_3$ is an optionally substituted straight or branched (aliphatic(amino))carbonyl that is attached to the 2 or 3 position on the indole ring of formula Ia. In several examples, $R_3$ is (N,N-dimethyl(amino))carbonyl, (methyl(amino))carbonyl, (ethyl(amino))carbonyl, (propyl(amino))carbonyl, (prop-2-yl(amino))carbonyl, (dimethyl(but-2-yl(amino)))carbonyl, (tertbutyl(amino))carbonyl, (butyl(amino))carbonyl, each of which is optionally substituted with 1-3 of halo, hydroxy, cycloaliphatic, heterocycloaliphatic, aryl, heteroaryl, or combinations thereof.

**[0147]** In other embodiments, $R_3$ is an optionally substituted (alkoxy)carbonyl. For example, $R_3$ is (methoxy)carbonyl, (ethoxy)carbonyl, (propoxy)carbonyl, or (butoxy)carbonyl, each of which is optionally substituted with 1-3 of halo, hydroxy, or combinations thereof. In several instances, $R_3$ is an optionally substituted straight or branched $C_{1-6}$ aliphatic. For example, $R_3$ is an optionally substituted straight or branched $C_{1-6}$ alkyl. In other examples, $R_3$ is independently an optionally substituted methyl, ethyl, propyl, butyl, isopropyl, or tertbutyl, each of which is optionally substituted with 1-3 of halo, hydroxy, cyano, nitro, or combination thereof. In other embodiments, $R_3$ is an optionally substituted $C_{3-6}$ cycloaliphatic. Exemplary embodiments include cyclopropyl, 1-methyl-cycloprop-1-yl, etc. In other examples, p is 2 and the two $R_3$ substituents are attached to the indole of formula Ia at the 2,4- or 2,6- or 2,7-positions. Exemplary embodiments include 6-F, 3-(optionally substituted $C_{1-6}$ aliphatic or $C_{3-6}$ cycloaliphatic); 7-F-2-(-(optionally substituted $C_{1-6}$ aliphatic or $C_{3-6}$ cycloaliphatic)), 4F-2-(optionally substituted $C_{1-6}$ aliphatic or $C_{3-6}$ cycloaliphatic); 7-CN-2-(optionally substituted $C_{1-6}$ aliphatic or $C_{3-6}$ cycloaliphatic); 7-Me-2-(optionally substituted $C_{14}$ aliphatic or $C_{3-6}$ cycloaliphatic) and 7-OMe-2-(optionally substituted $C_{1-6}$ aliphatic or $C_{3-6}$ cycloaliphatic).

**[0148]** In several embodiments, $R_3$ is hydrogen.

**[0149]** In several embodiments, $R_3$ is one selected from:

—H, —$CH_3$, —$CH_2OH$, —$CH_2CH_3$, —$CH_2CH_2OH$, —$CH_2CH_2CH_3$, —$NH_2$, halo, —$OCH_3$, —CN, —$CF_3$, —$C(O)OCH_2CH_3$, —$S(O)_2CH_3$, —$CH_2NH_2$, —$C(O)NH_2$,

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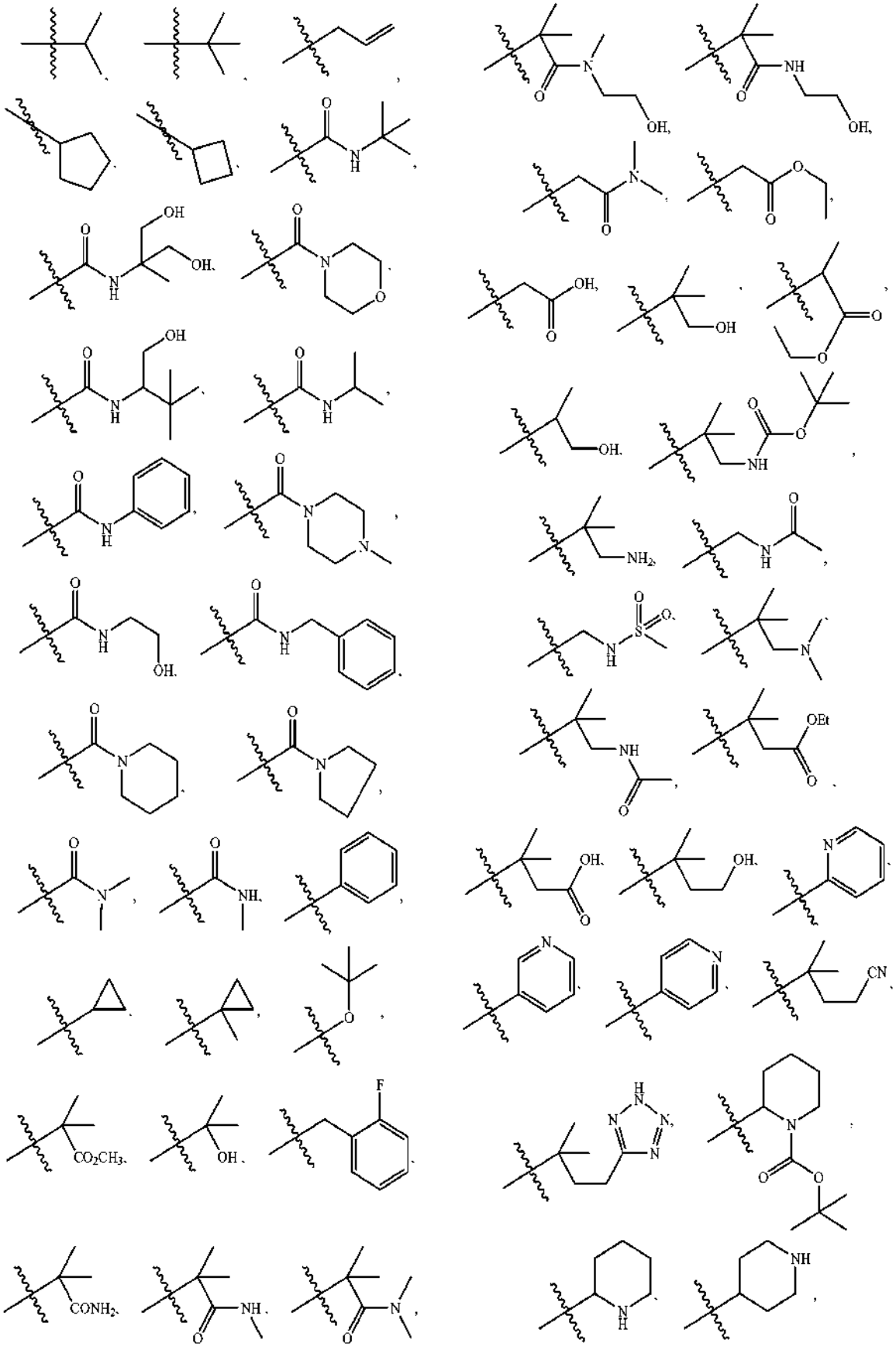

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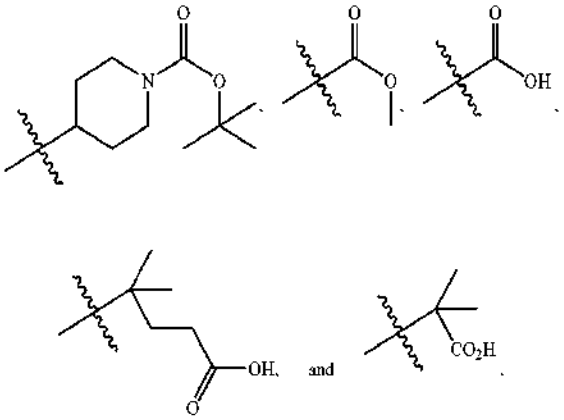
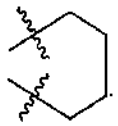


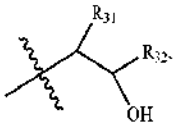
**[0150]** In another embodiment, two adjacent $R_3$ groups form



**[0151]** In several embodiments, $R'_3$ is independently $-Z^CR_6$, where each $Z^C$ is independently a bond or an optionally substituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^C$ are optionally and independently replaced by —CO—, —CS—, —$CONR^C$—, —$CONR^CNR^C$—, —$CO_2$—, —OCO—, —$NR^CCO_2$—, —O—, —$NR^CCONR^C$—, —$OCONR^C$—, —$NR^CNR^C$—, $NR^CCO$—, —S—, —SO—, —$SO_2$—, —$NR^C$—, —$SO_2NR^C$—, —$NR^CSO_2$—, or —$NR^CSO_2NR^C$—. Each $R_6$ is independently $R^C$, halo, —OH, —$NH_2$, —$NO_2$, —CN, or —$OCF_3$. Each $R^C$ is independently hydrogen, an optionally substituted aliphatic, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, or an optionally substituted heteroaryl. In one embodiment, each $R^C$ is hydrogen, $C_{1-6}$ aliphatic, or $C_{3-6}$ cycloaliphatic, wherein either of the aliphatic or cycloaliphatic is optionally substituted with up to 4 —OH substituents. In another embodiment, $R^C$ is hydrogen, or $C_{1-6}$ alkyl optionally substituted with up to 4 —OH substituents.

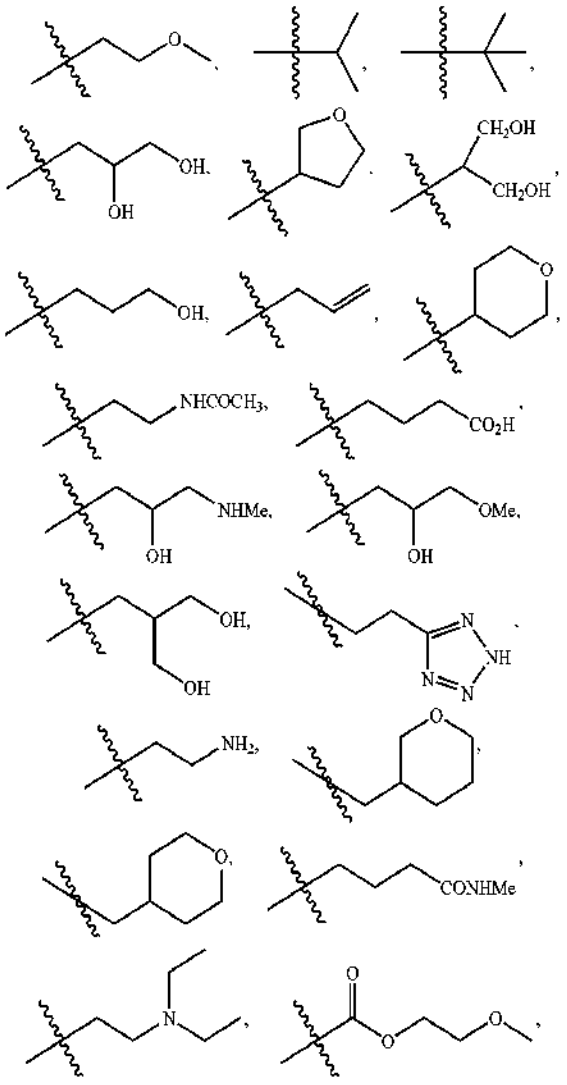
**[0152]** For example, in many embodiments, $R'_3$ is independently $-Z^CR_6$, where each $Z^C$ is independently a bond or an optionally substituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^C$ are optionally and independently replaced by —C(O)—, —C(O)$NR^C$—, —C(O)O—, —$NR^C$C(O)O—, —O—, —$NR^CS(O)_2$—, or —$NR^C$—. Each $R_6$ is independently $R^C$, —OH, or —$NH_2$. Each $R^C$ is independently hydrogen, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, or an optionally substituted heteroaryl. In one embodiment, each $R^C$ is hydrogen, $C_{1-6}$ aliphatic, or $C_{3-6}$ cycloaliphatic, wherein either of the aliphatic or cycloaliphatic is optionally substituted with up to 4 —OH substituents. In another embodiment, $R^C$ is hydrogen, or $C_{1-6}$ alkyl optionally substituted with up to 4 —OH substituents.

**[0153]** In other embodiments, $R'_3$ is hydrogen or



**[0154]** wherein $R_{31}$ is H or a $C_{1-2}$ aliphatic that is optionally substituted with 1-3 of halo, —OH, or combinations thereof. $R_{32}$ is -L-$R_{33}$, wherein L is a bond, —$CH_2$—, —$CH_2O$—, —$CH_2NHS(O)_2$—, —$CH_2C(O)$—, —$CH_2NHC(O)$—, or —$CH_2NH$—; and $R_{33}$ is hydrogen, or $C_{1-2}$ aliphatic, cycloaliphatic, heterocycloaliphatic, or heteroaryl, each of which is optionally substituted with 1 of —OH, —$NH_2$, or —CN. For example, in one embodiment, $R_{31}$ is hydrogen and $R_{32}$ is $C_{1-2}$ aliphatic optionally substituted with —OH, —$NH_2$, or —CN.

**[0155]** In several embodiments, $R'_3$ is independently selected from one of the following: —H, —$CH_3$, —$CH_2CH_3$, —$C(O)CH_3$, —$CH_2CH_2OH$, —$C(O)OCH_3$,



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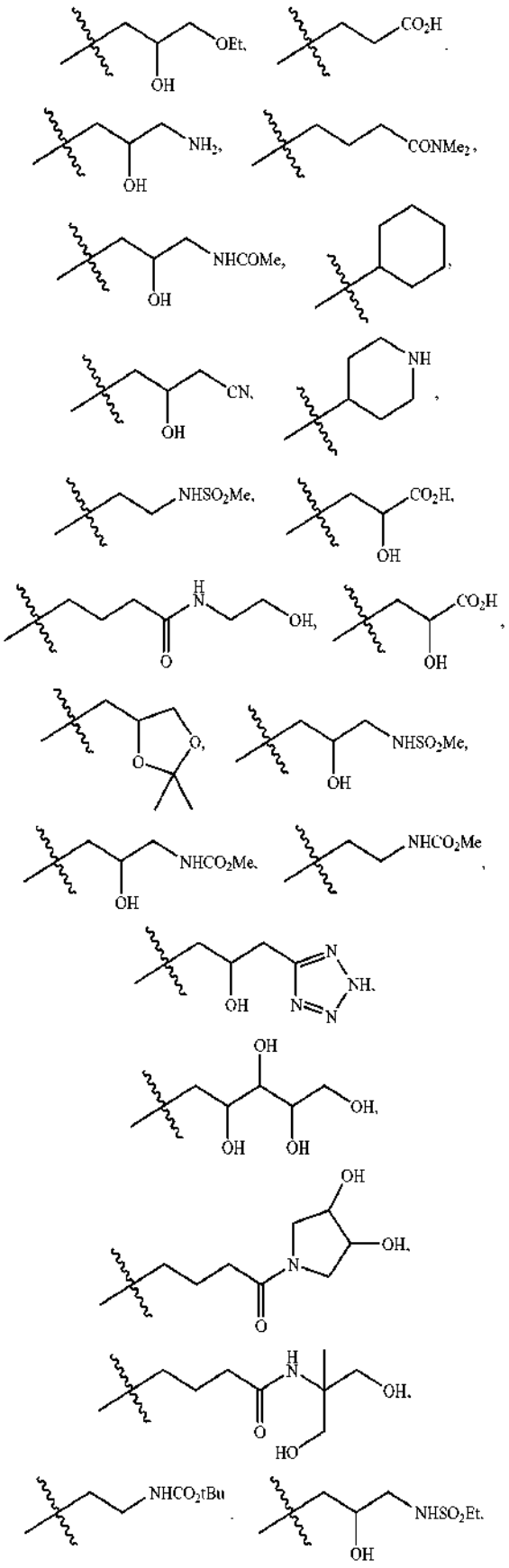

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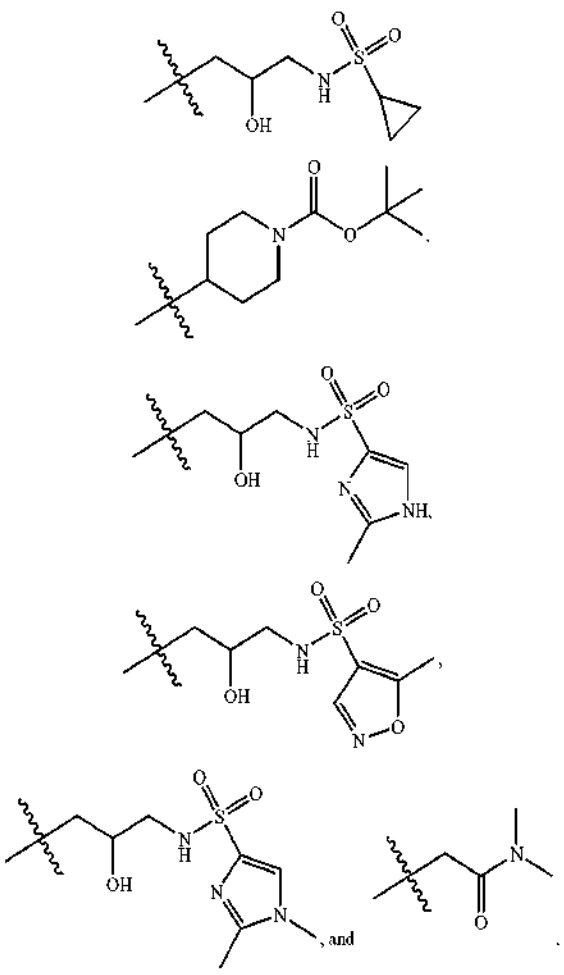
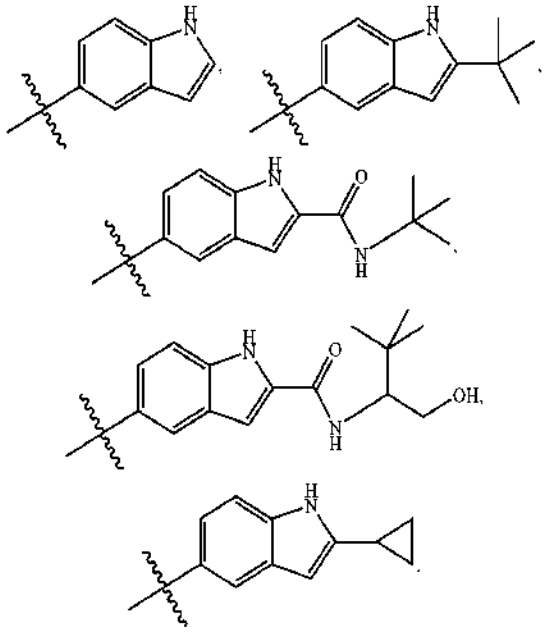


**[0156]** In several embodiments, ring B is one selected from:



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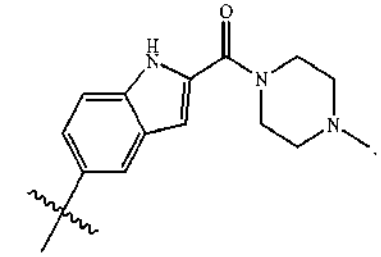

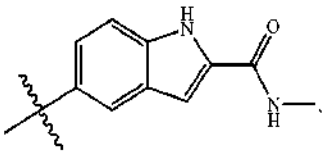

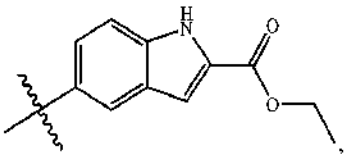

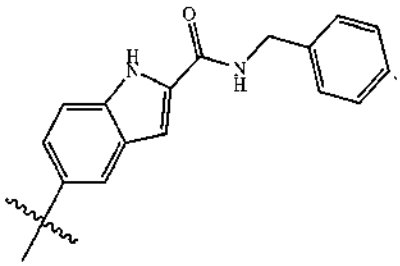

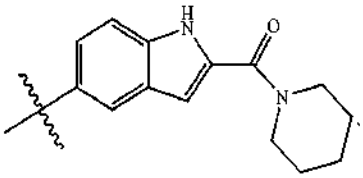

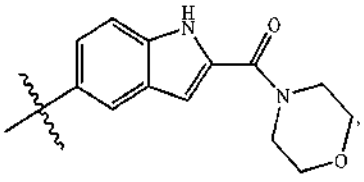

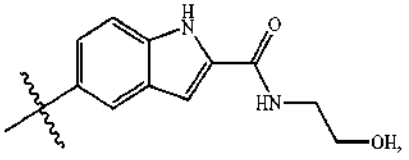

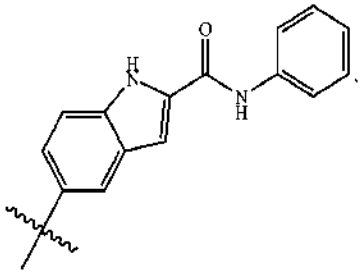

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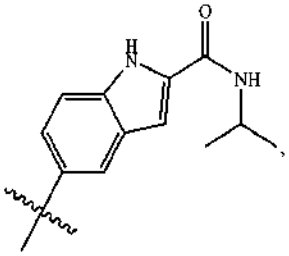

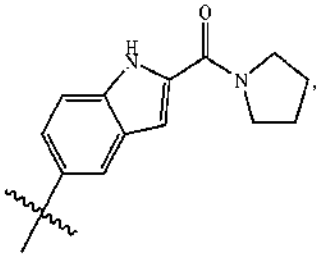

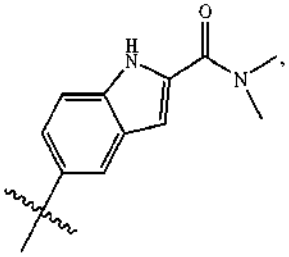

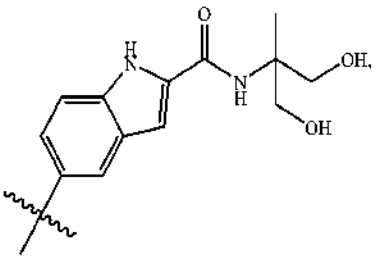

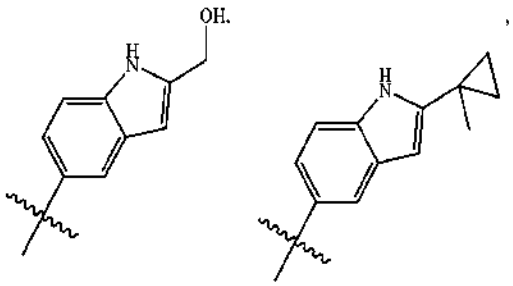

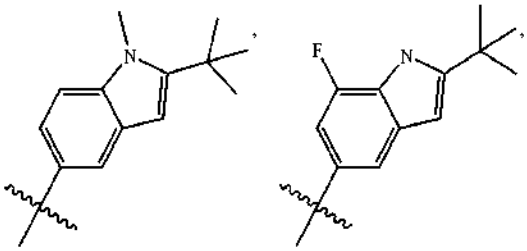

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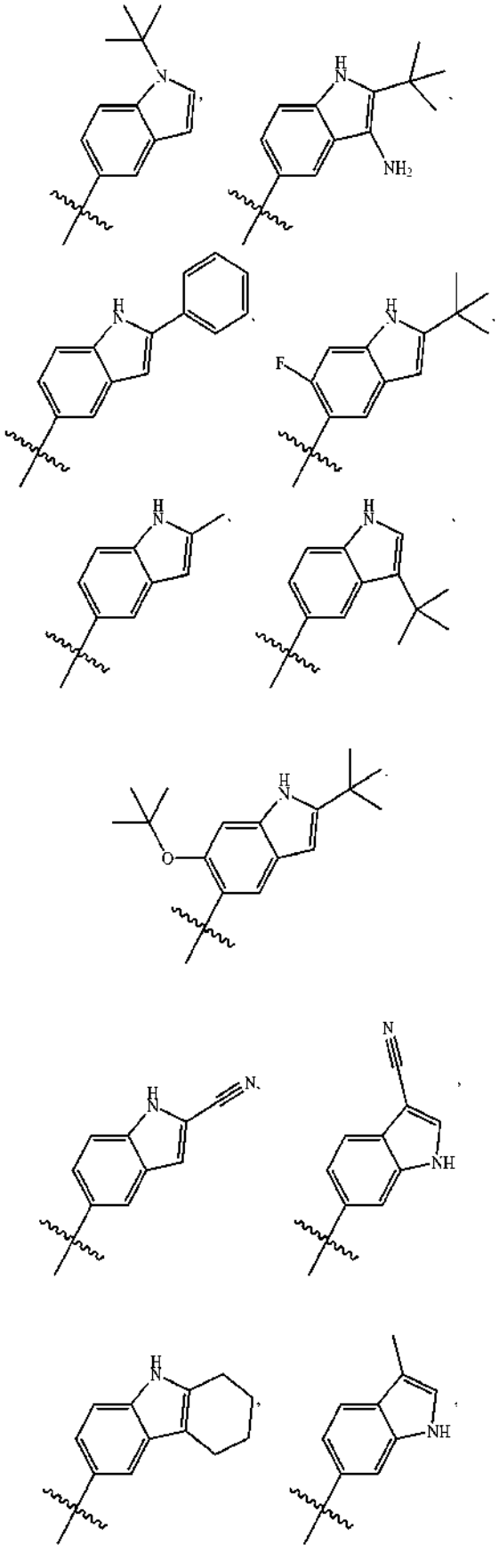

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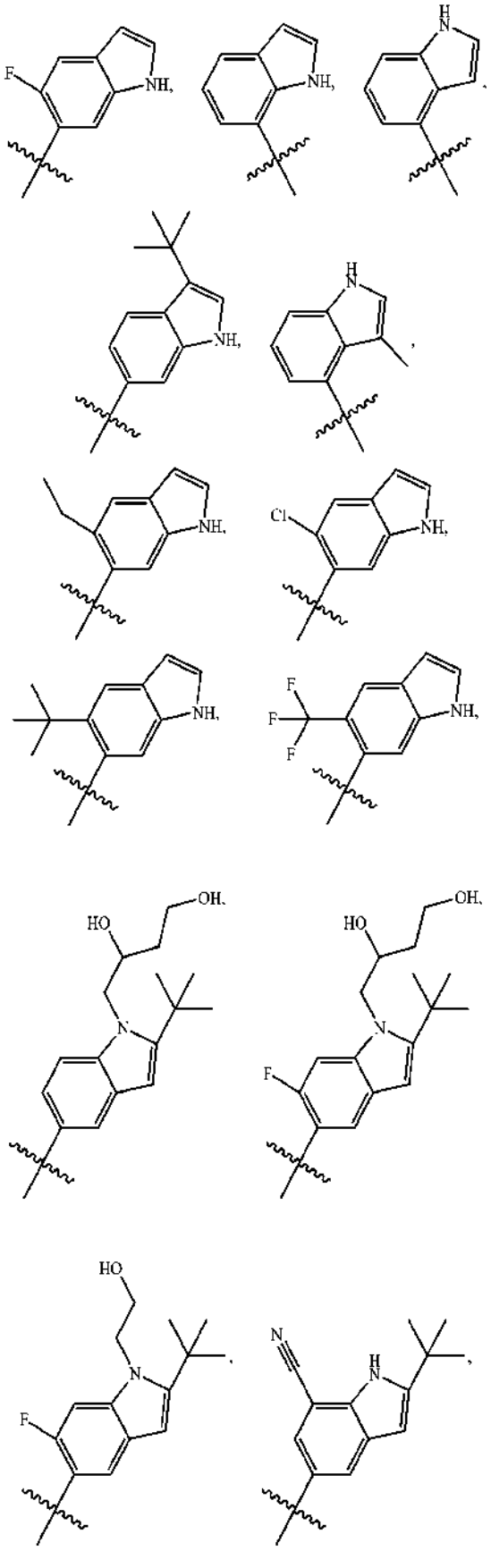

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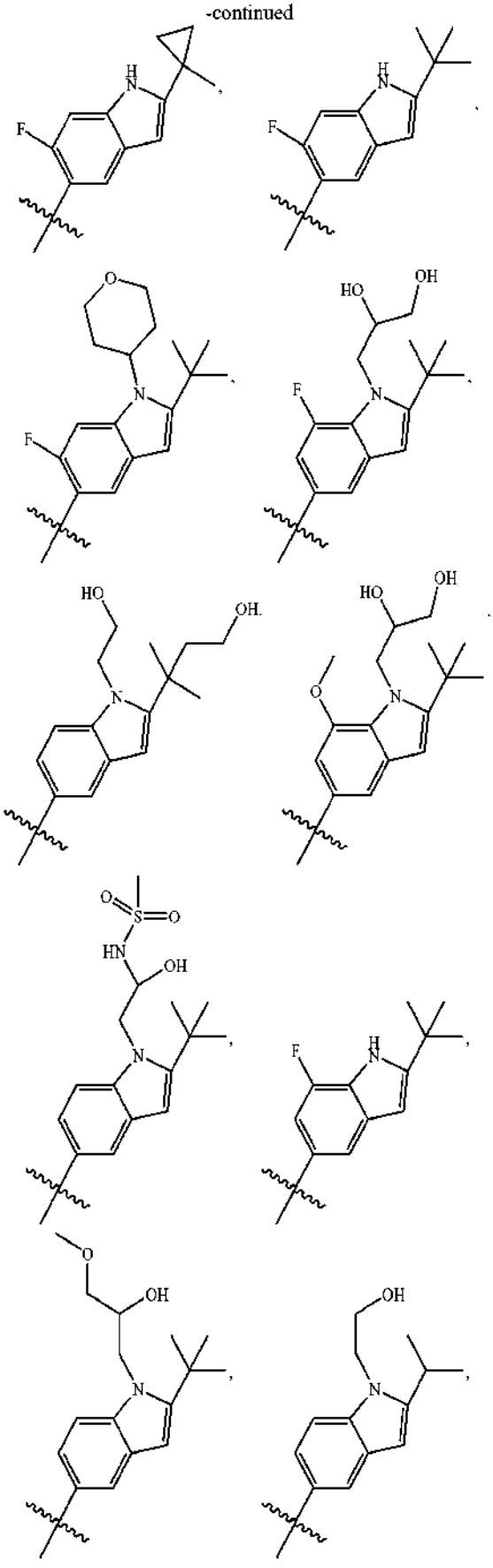

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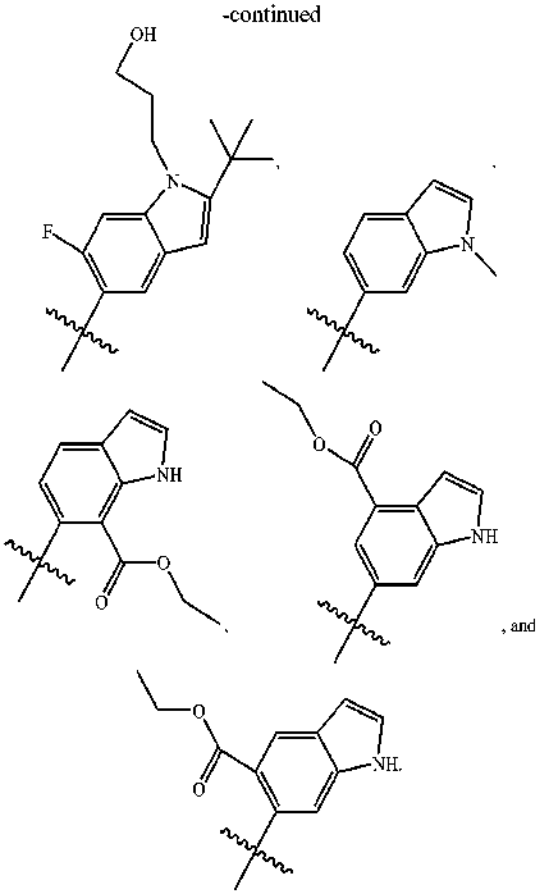


, and

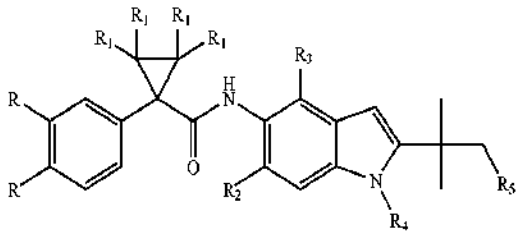
**[0157]** 5. n term

**[0158]** n is 1-3.

**[0159]** In several embodiments, n is 1. In other embodiments, n is 2. In still other embodiments, n is 3.

**[0160]** In one aspect, the present invention relates to compounds of formula II useful as modulators of ABC transporter activity:

II



**[0161]** or a pharmaceutically acceptable salt thereof, wherein independently for each occurrence:

**[0162]** R is H, OH, $OCH_3$ or two R taken together form $—OCH_2O—$ or $—OCF_2O—$;

**[0163]** $R_1$ is H or alkyl;

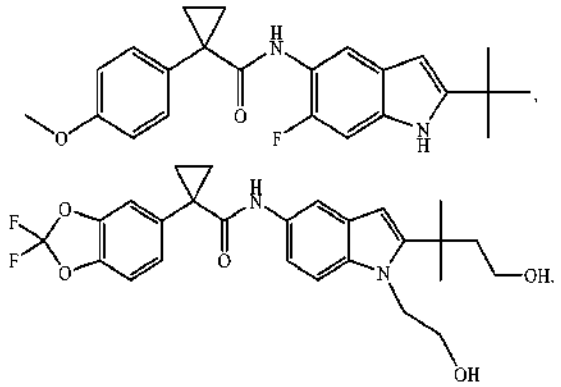
**[0164]** $R_2$ is H or F;

**[0165]** $R_5$ is H or CN;

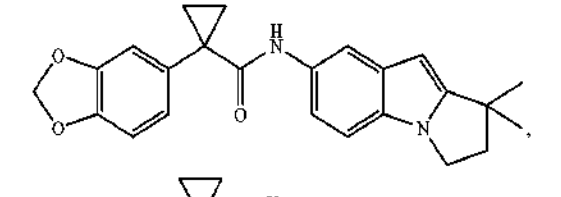
**[0166]** $R_4$ is H, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N(CH_3)_3$, or $—CH_2CH_2OH$;

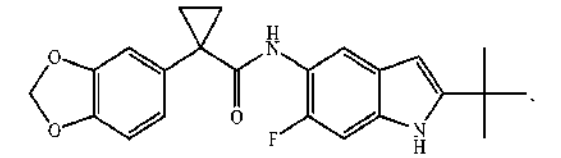
**[0167]** $R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a five membered ring.

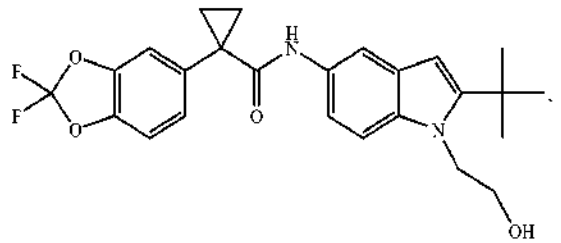
**[0168]** In one embodiment, the present invention provides compounds of formula II, wherein the compounds set forth below are excluded:

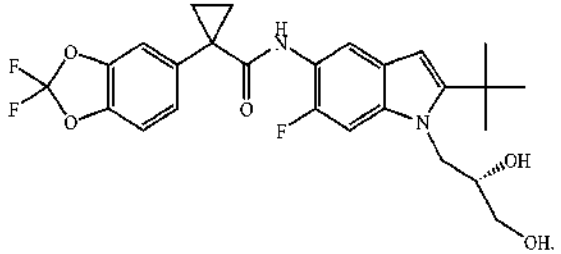


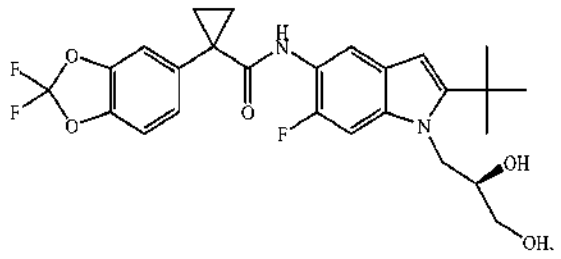












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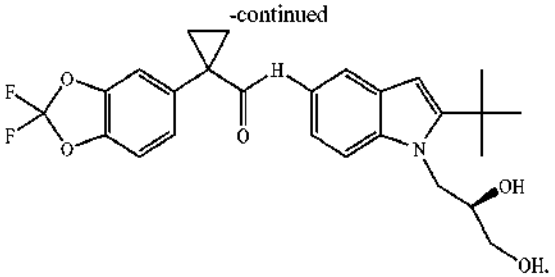

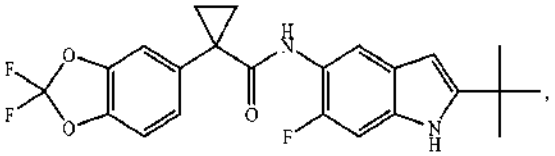

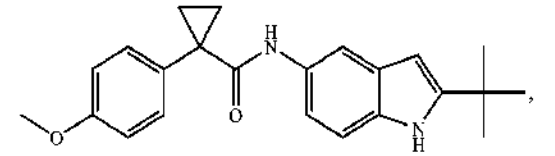

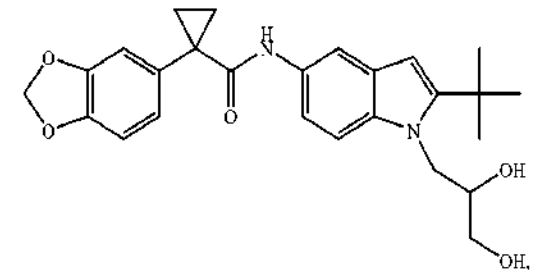

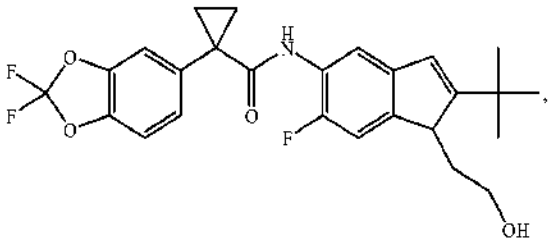

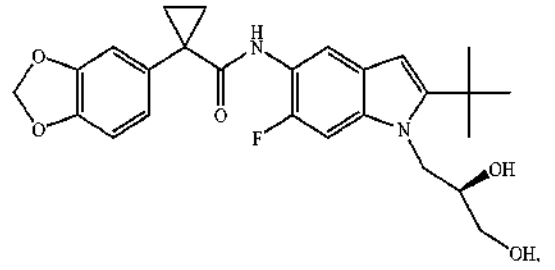

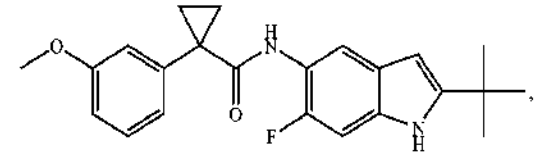

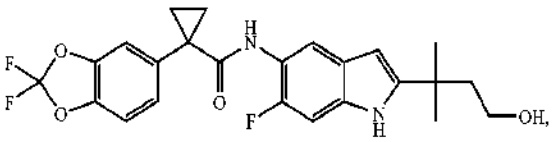

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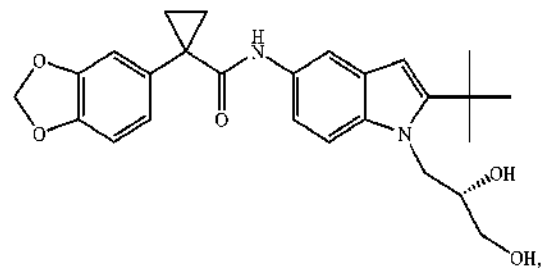

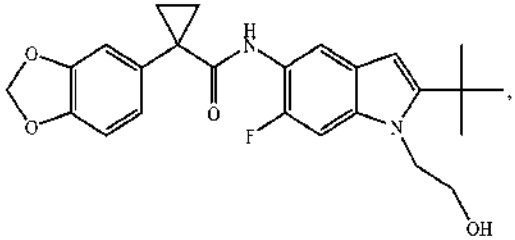

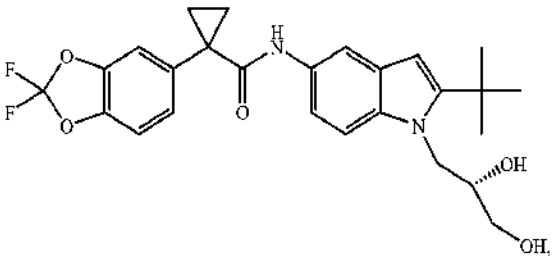

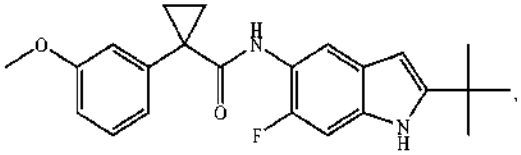

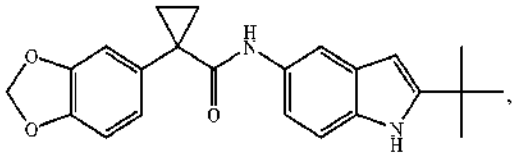

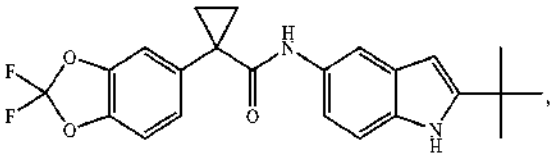

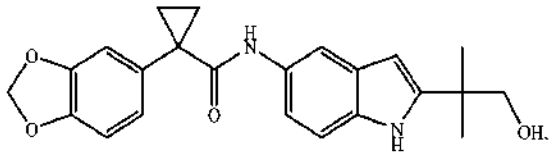

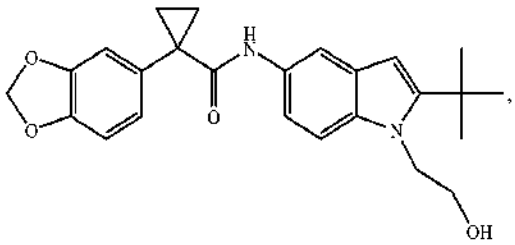

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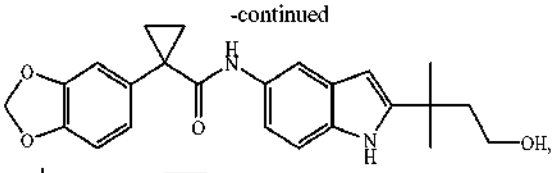
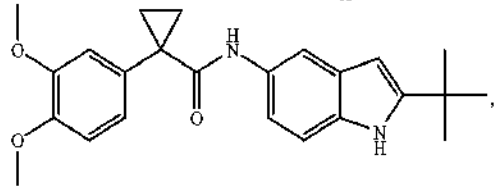
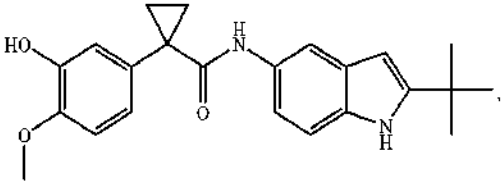
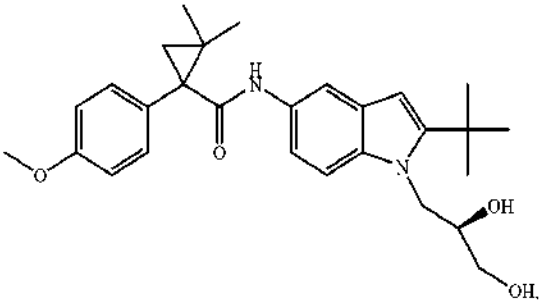
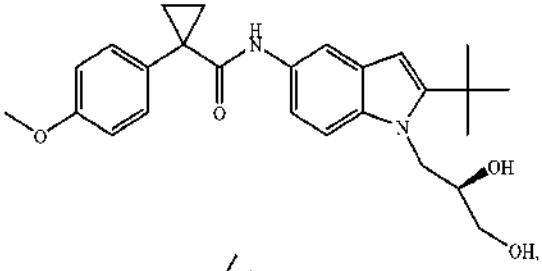
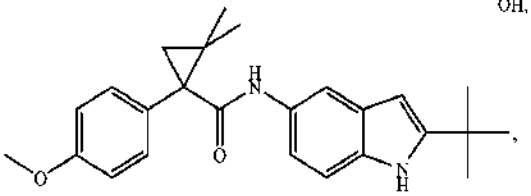


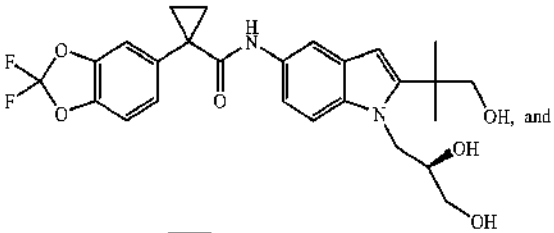


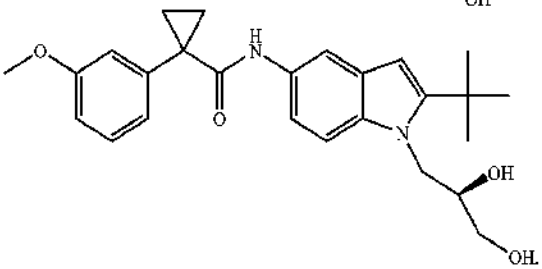












**[0169]** In one embodiment of the compounds, two R taken together form —$OCF_2O$—, $R_1$ is H, and $R_2$ is F. In another embodiment, two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, and $R_3$ is H. In another embodiment, two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is H. In another embodiment, two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH_2N^+(CH_3)_3$. In another embodiment, two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2OCH_2CH(OH)CH_2OH$. In another embodiment, two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ and $R_5$ taken together form a five membered ring.

**[0170]** In one embodiment of the compounds, two R taken together form —$OCH_2O$—, $R_1$ is H, and $R_2$ is F. In another embodiment, two R taken together form $CH_2O$—, $R_1$ is H, $R_2$ is F, and $R_3$ is H. In another embodiment, two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2OCH_2CH(OH)CH_2OH$.

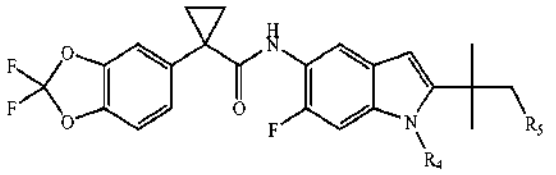
**[0171]** In one embodiment of the compounds, R is OH, $R_1$ is H, $R_2$ is H, $R_3$ is H, and $R_4$ is —$CH_2OCH_2CH(OH)CH_2OH$.

**[0172]** In one embodiment of the compounds, at least one R is $OCH_3$, at least two $R_1$ are methyl, $R_2$ is H, $R_3$ is H, and $R_4$ is H. In another embodiment, at least one R is $OCH_3$, at least two $R_1$ are methyl, $R_2$ is H, $R_3$ is H, and $R_4$ is —$CH_2OCH_2CH(OH)CH_2OH$.

**[0173]** In one embodiment of the compounds, two R taken together form —$CH_2CH_2CH_2$—, $R_1$ is H, $R_2$ is H, $R_3$ is H, and $R_4$ is —$CH_2OCH_2CH(OH)CH_2OH$.

**[0174]** In one embodiment, the compound is represented by formula IIa:

IIa



**[0175]** or a pharmaceutically acceptable salt thereof, wherein:

**[0176]** $R_4$ is H, —$CH_2OCH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and

**[0177]** $R_5$ is H, OH, —$CH_2OCH_2CH(OH)CH_2OH$, —$CH_2OH$, or $R_4$ and $R_5$ taken together form a five membered ring.

**[0178]** In one embodiment of the compounds, $R_4$ is —$CH_2OCH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$. In another embodiment, $R_5$ is OH, —$CH_2OCH_2CH(OH)CH_2OH$, or —$CH_2OH$. In another embodiment, $R_4$ is —$CH_2OCH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and $R_5$ is OH, —$CH_2OCH_2CH(OH)CH_2OH$, or —$CH_2OH$.

**[0179]** C. Exemplary Compounds of the Present Invention

**[0180]** Exemplary compounds of the present invention include, but are not limited to, those illustrated in Table 1 below.

TABLE 1

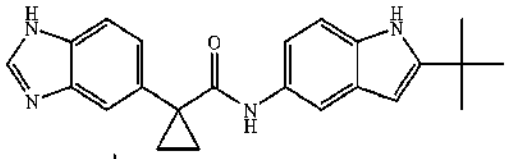
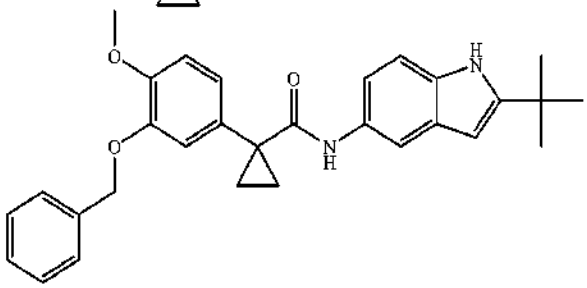
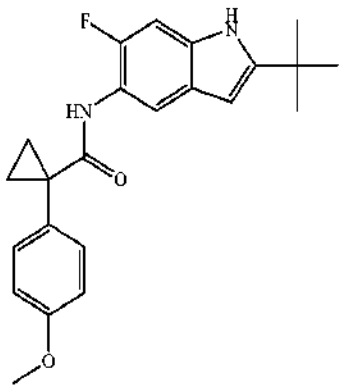
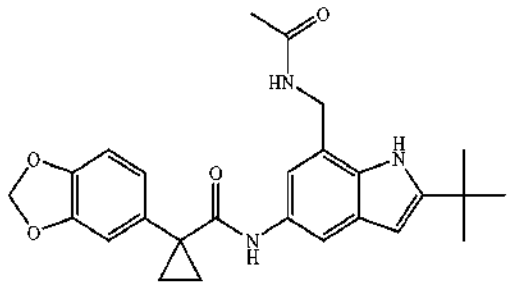
| Exemplary compounds of the present invention. |
|---|
| 1  |
| 2  |
| 3  |

TABLE 1-continued

Exemplary compounds of the present invention.

4



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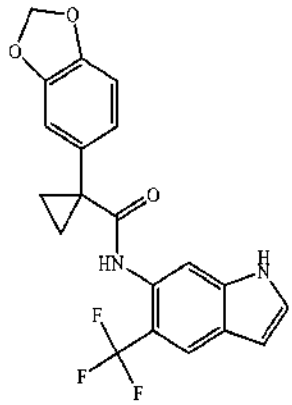

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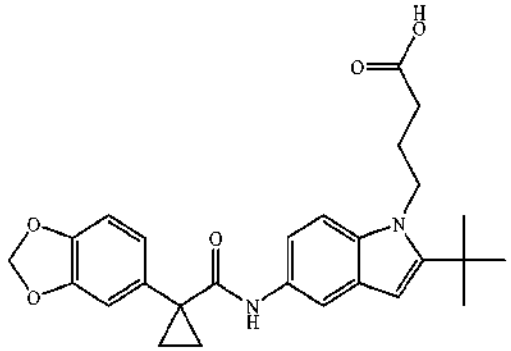

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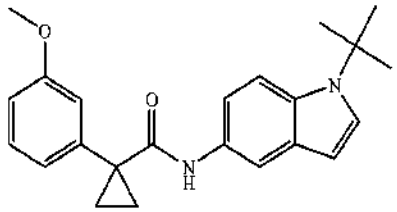

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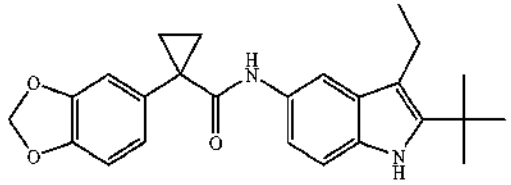

TABLE 1-continued

Exemplary compounds of the present invention.

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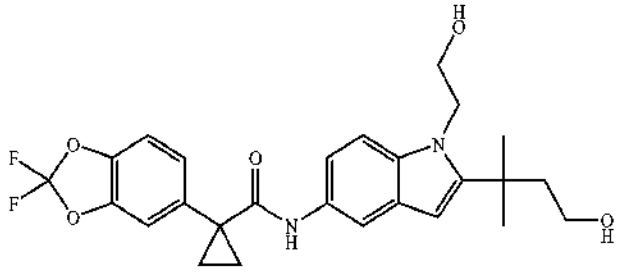

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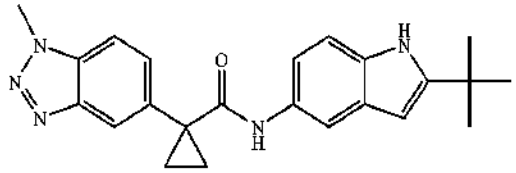

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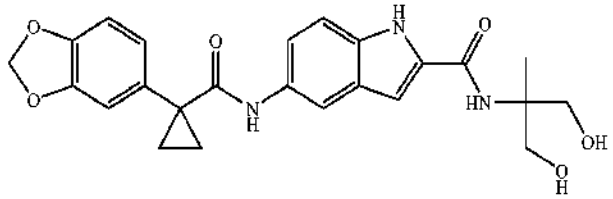

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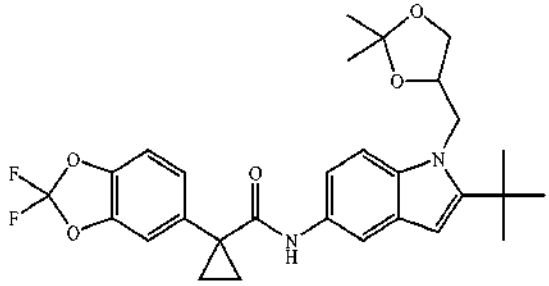

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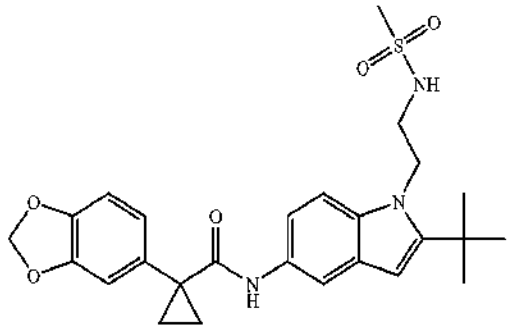

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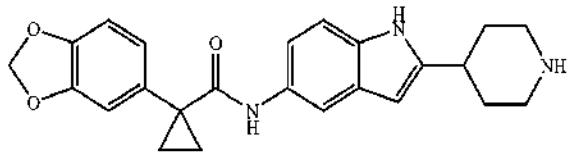

TABLE 1-continued

Exemplary compounds of the present invention.

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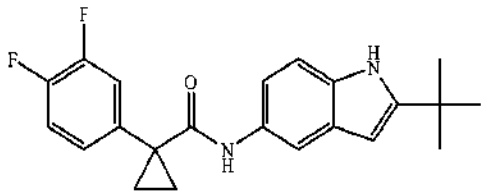

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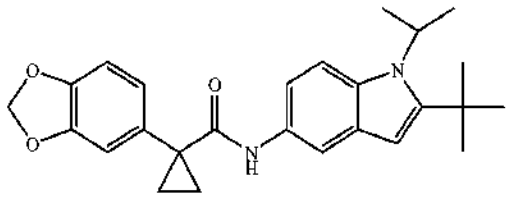

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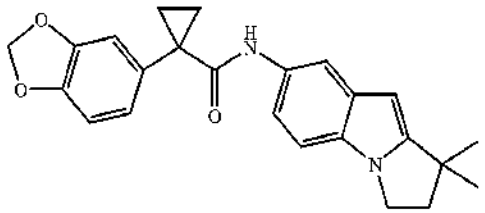

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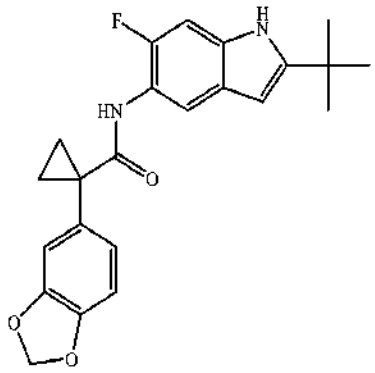

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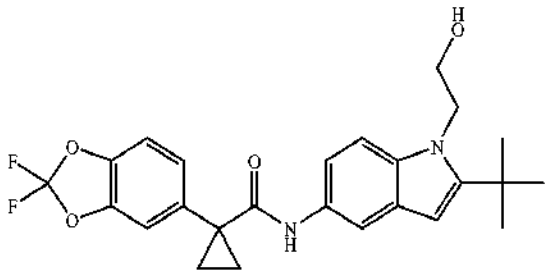

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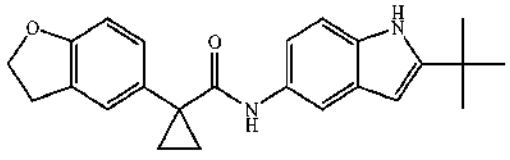


TABLE 1-continued

Exemplary compounds of the present invention.

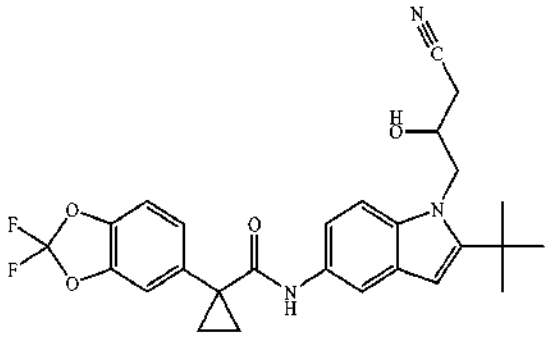
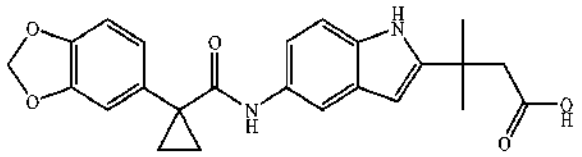
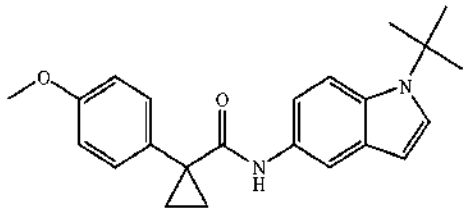
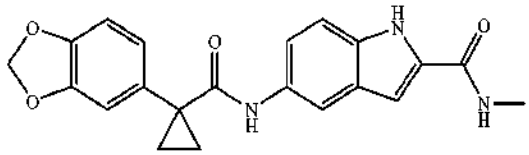
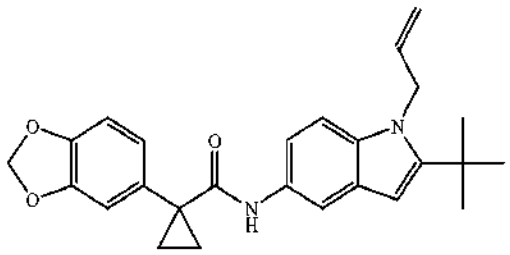
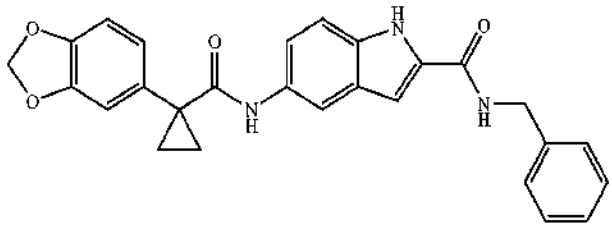
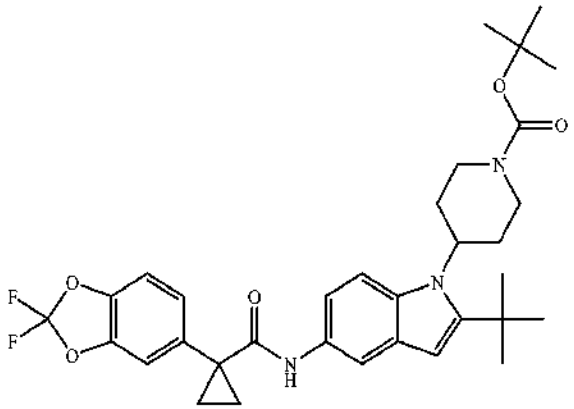
| | |
|---|---|
| 21 |  |
| 22 |  |
| 23 |  |
| 24 |  |
| 25 |  |
| 26 |  |

TABLE 1-continued

Exemplary compounds of the present invention.

27



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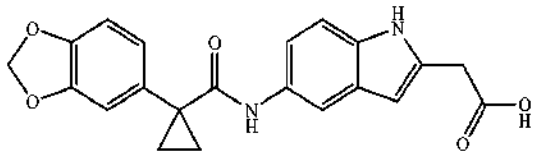

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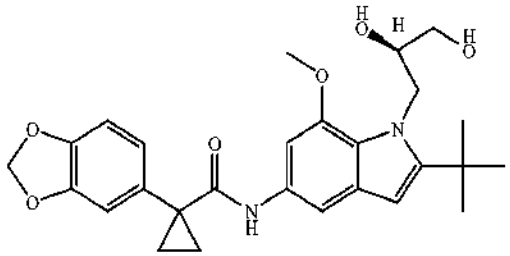

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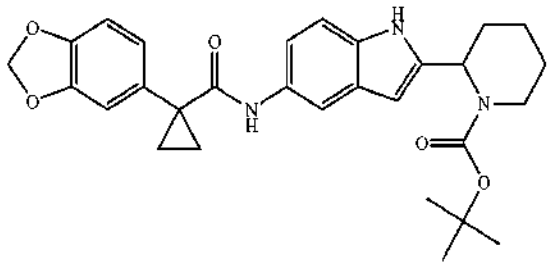

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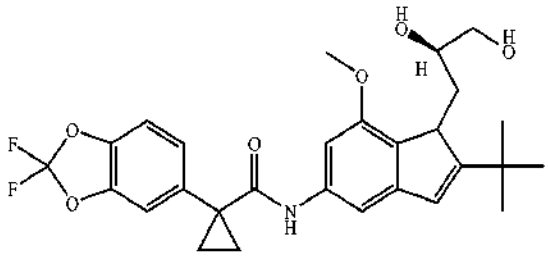

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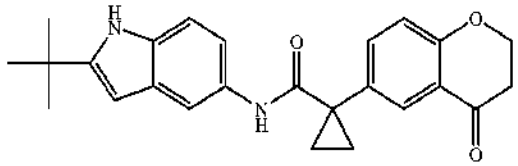


TABLE 1-continued

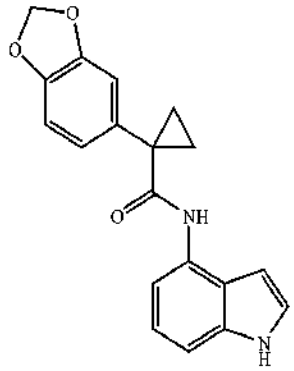
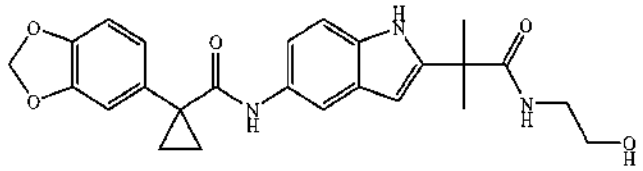
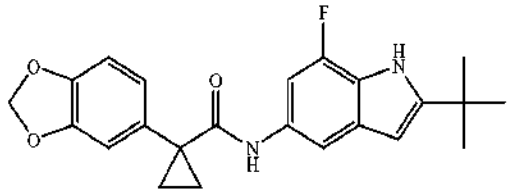
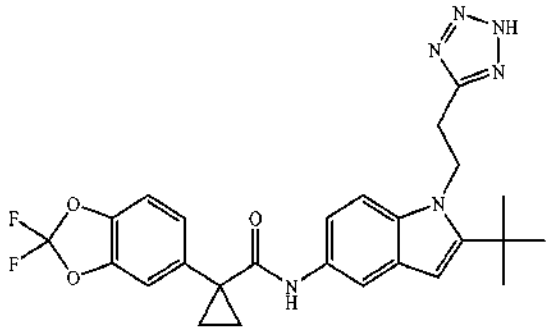
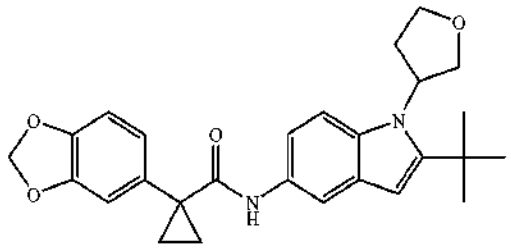
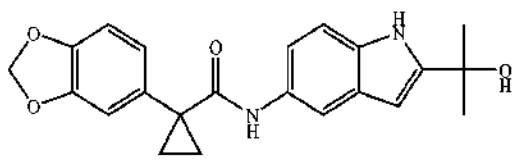
| Exemplary compounds of the present invention. | |
|---|---|
| 33 |  |
| 34 |  |
| 35 |  |
| 36 |  |
| 37 |  |
| 38 |  |

TABLE 1-continued

Exemplary compounds of the present invention.

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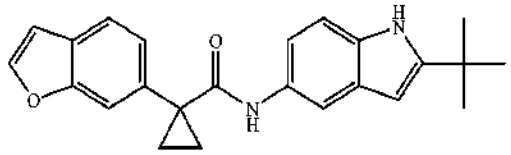

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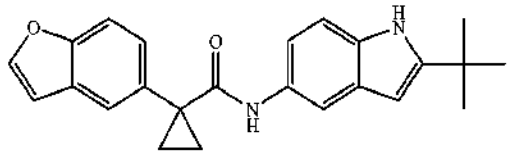

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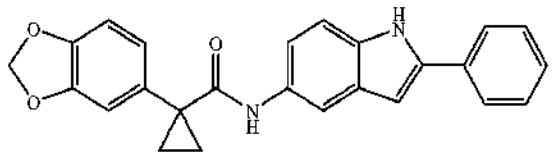

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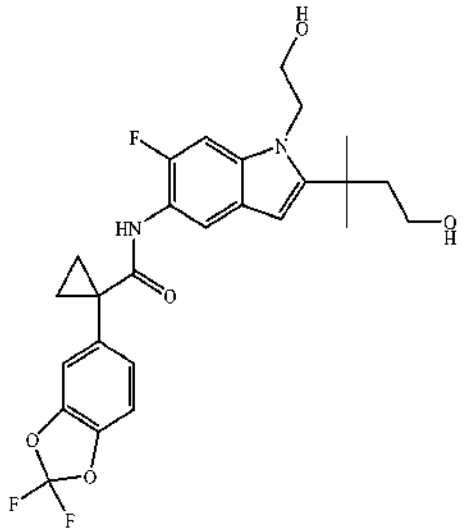

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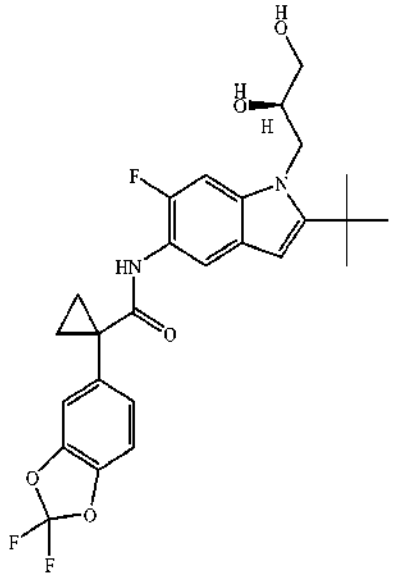


TABLE 1-continued

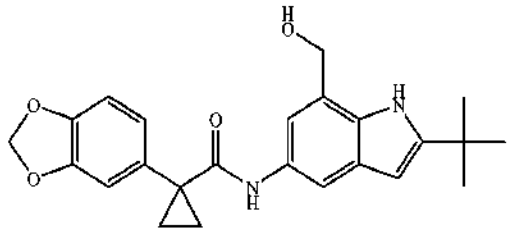
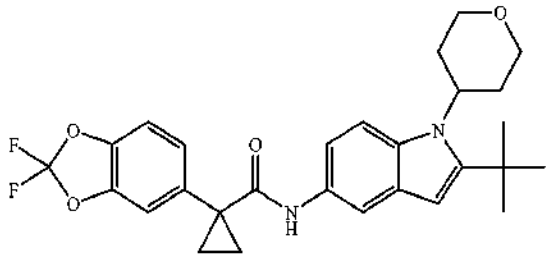
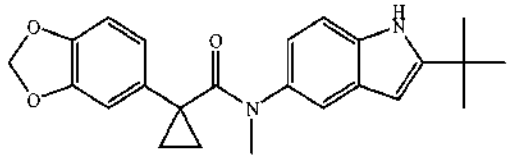
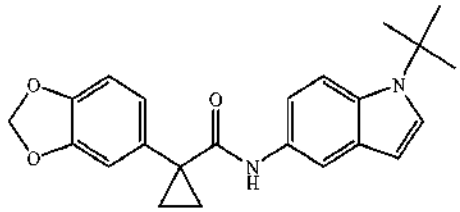
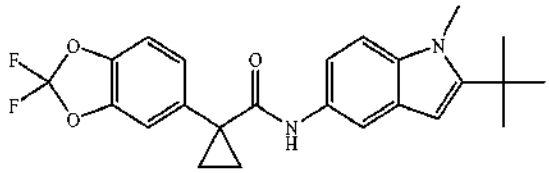
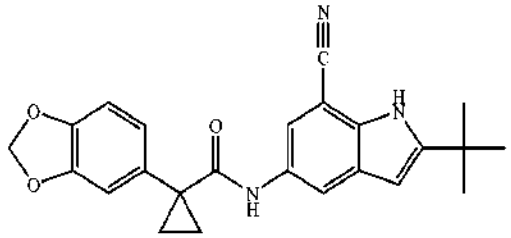
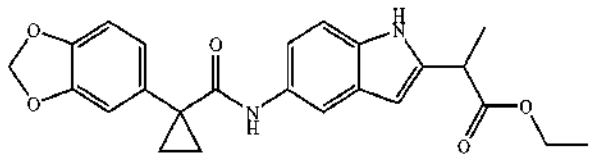
| Exemplary compounds of the present invention. | |
|---|---|
| 44 |  |
| 45 |  |
| 46 |  |
| 47 |  |
| 48 |  |
| 49 |  |
| 50 |  |

TABLE 1-continued

Exemplary compounds of the present invention.

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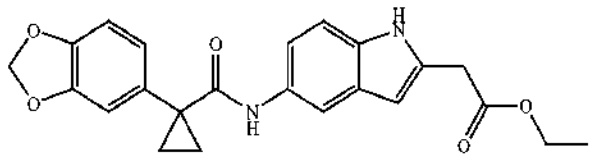

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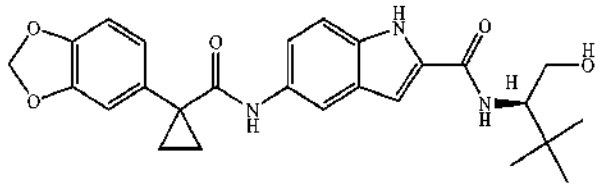

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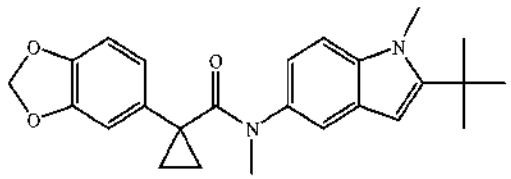

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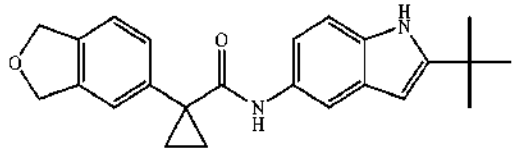

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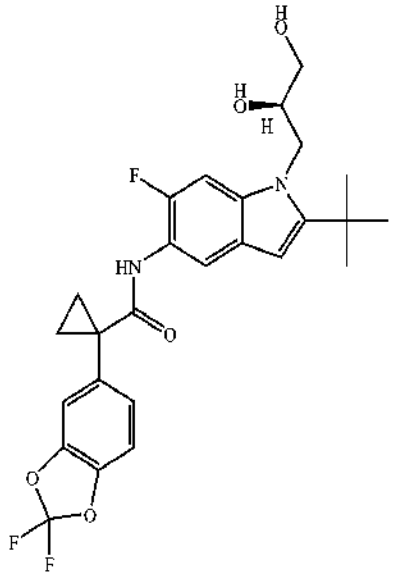

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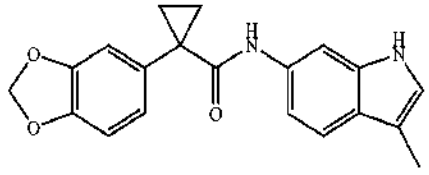

TABLE 1-continued

Exemplary compounds of the present invention.

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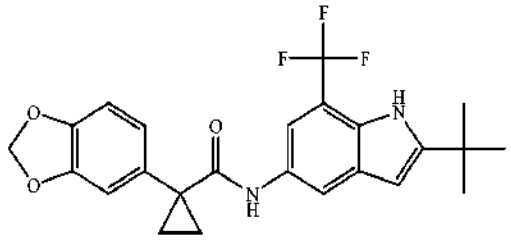

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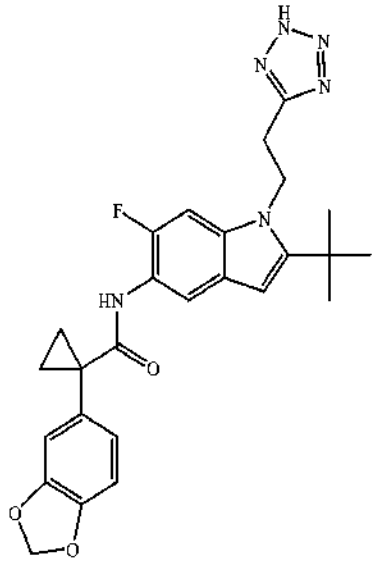

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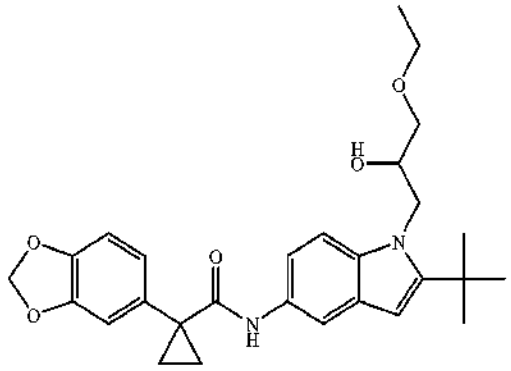

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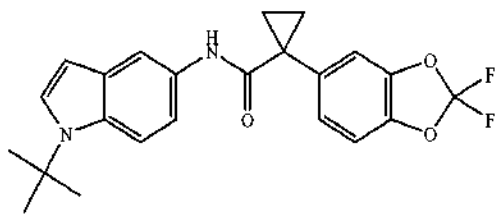

TABLE 1-continued

Exemplary compounds of the present invention.

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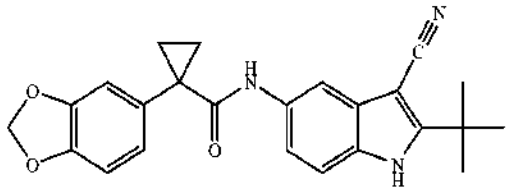

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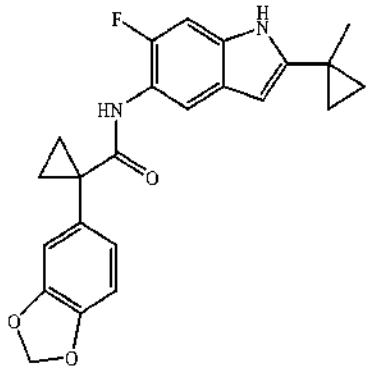

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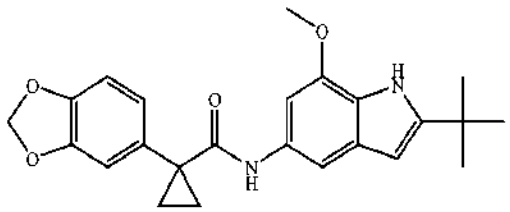

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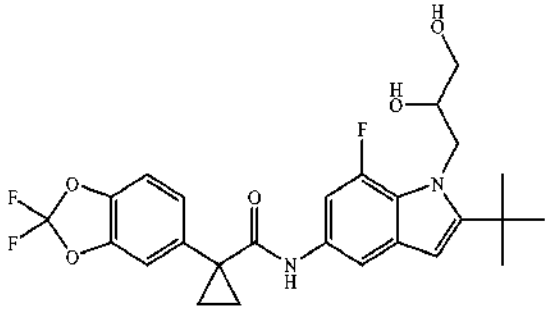

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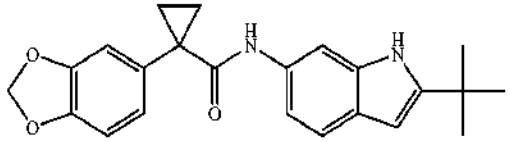

TABLE 1-continued

Exemplary compounds of the present invention.

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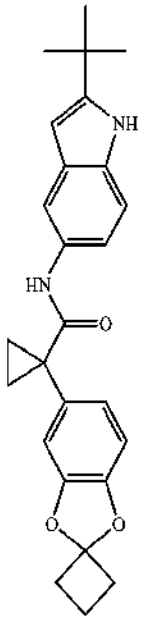

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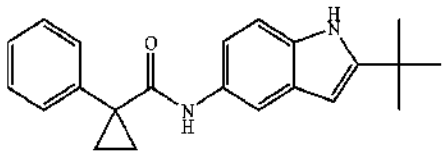

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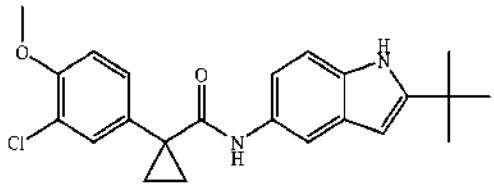

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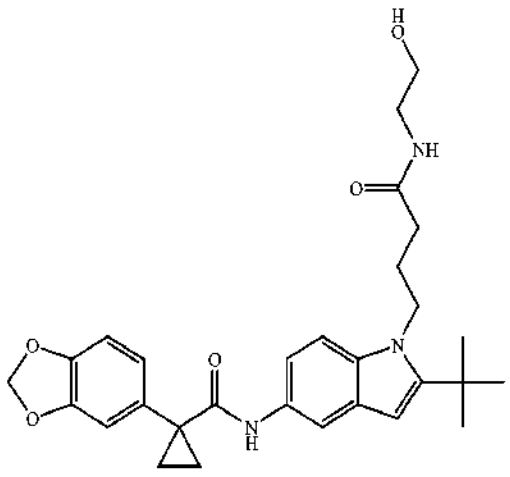


TABLE 1-continued

Exemplary compounds of the present invention.

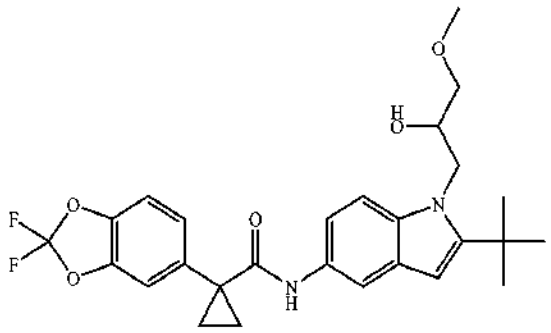
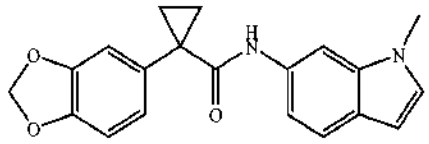
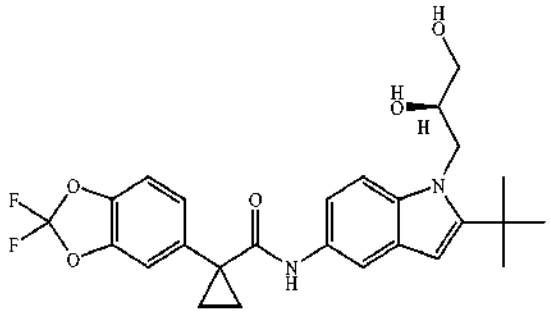
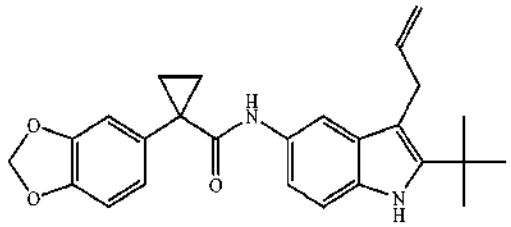
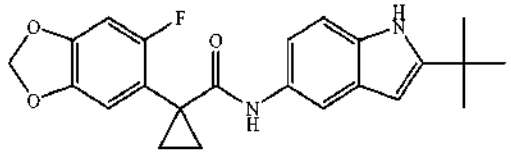
| | |
|---|---|
| 70 |  |
| 71 |  |
| 72 |  |
| 73 |  |
| 74 |  |

TABLE 1-continued

Exemplary compounds of the present invention.

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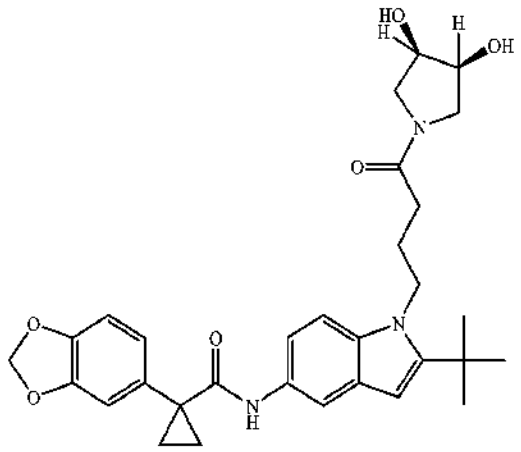

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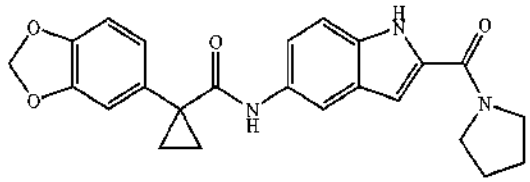

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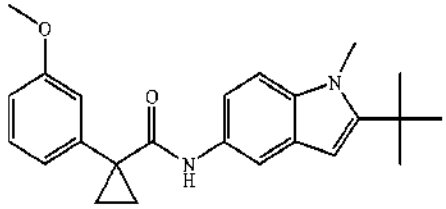

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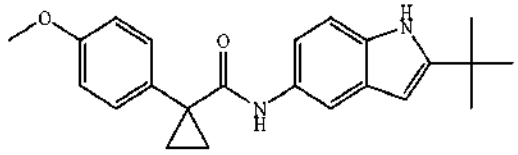

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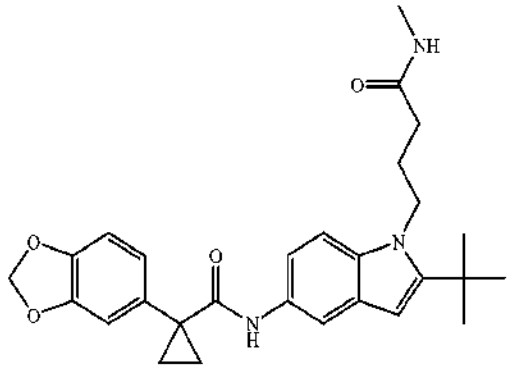

TABLE 1-continued

Exemplary compounds of the present invention.

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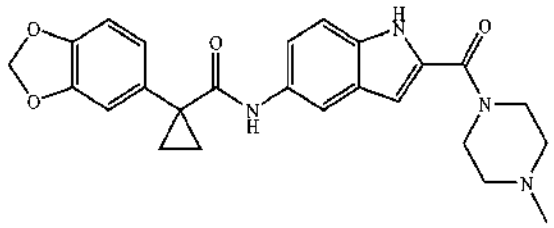

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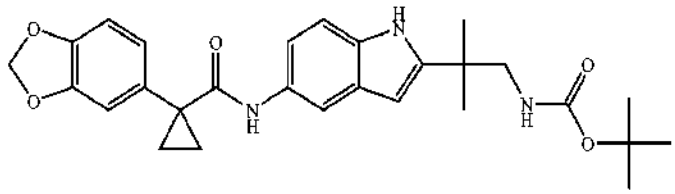

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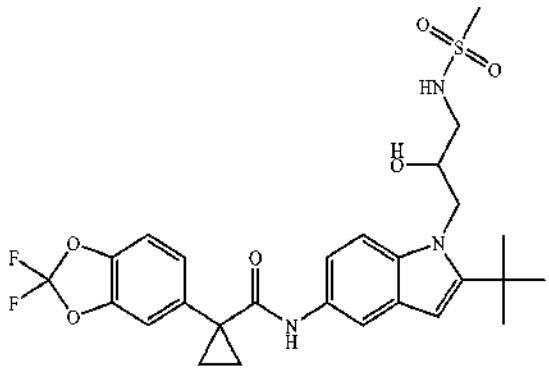

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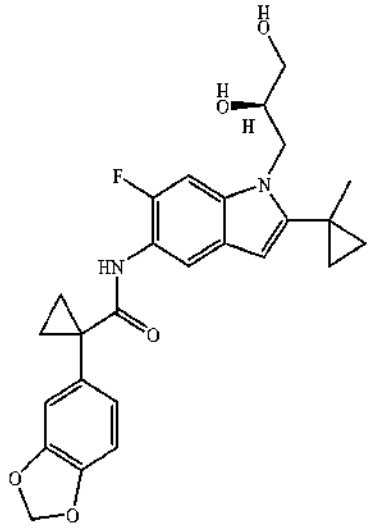

TABLE 1-continued

Exemplary compounds of the present invention.

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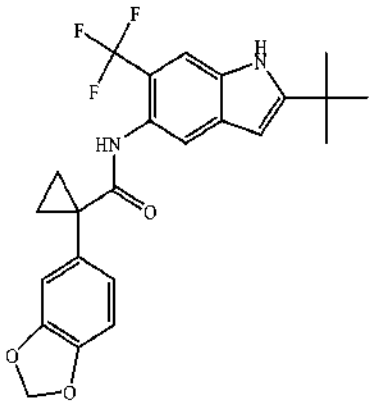

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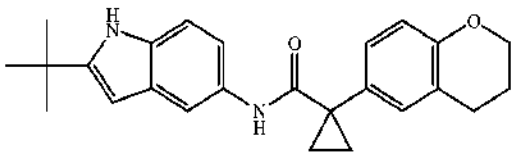

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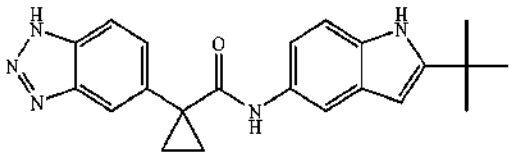

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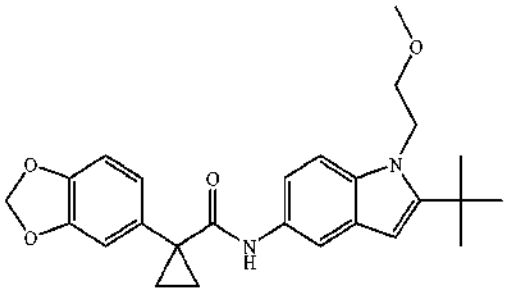

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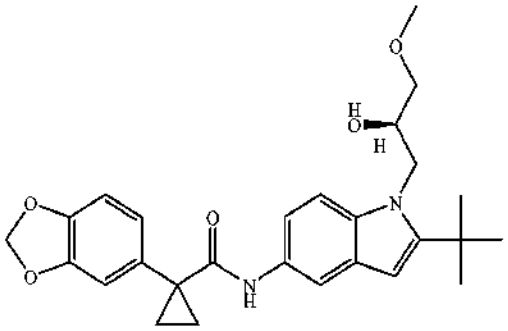

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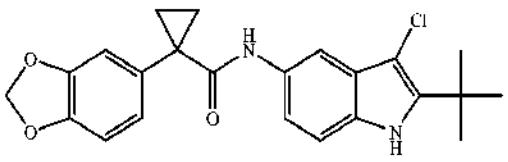

TABLE 1-continued

Exemplary compounds of the present invention.

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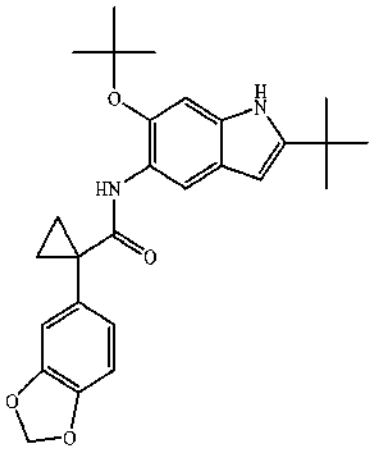

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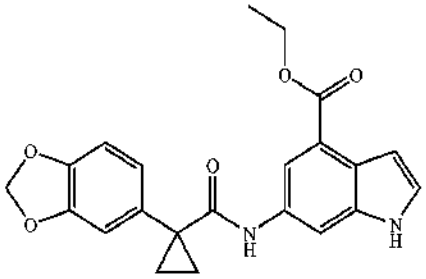

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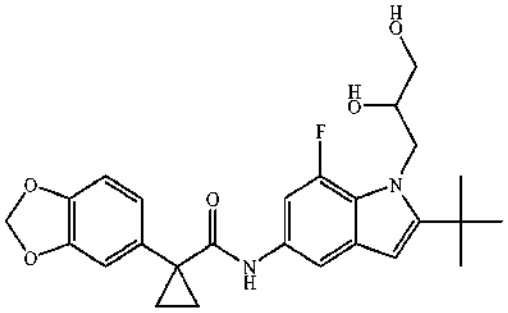

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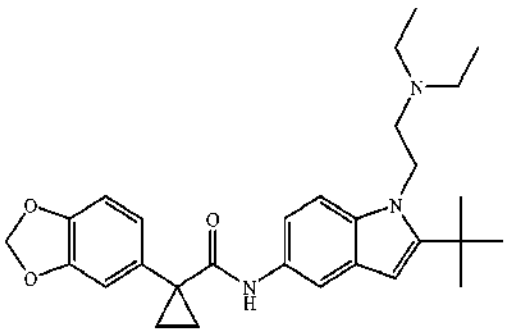

TABLE 1-continued

Exemplary compounds of the present invention.

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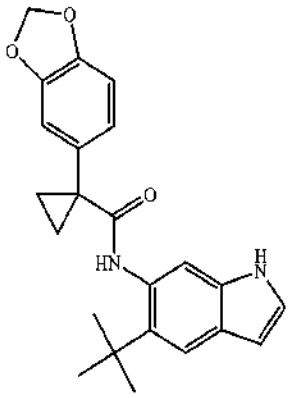

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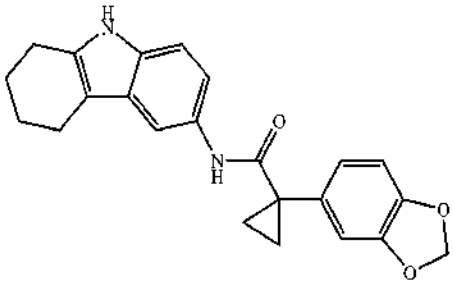

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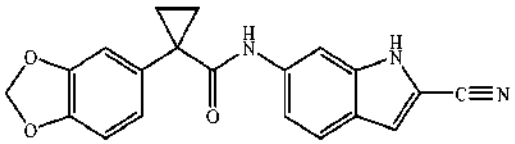

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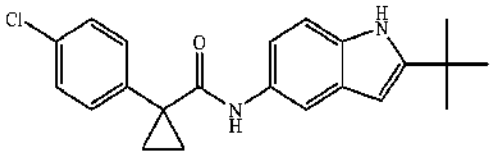

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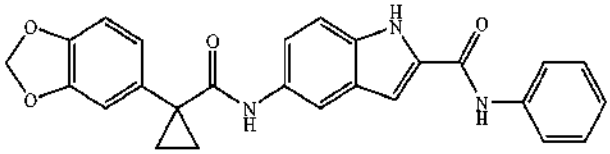

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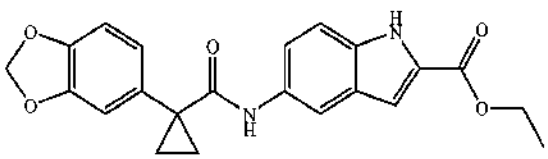

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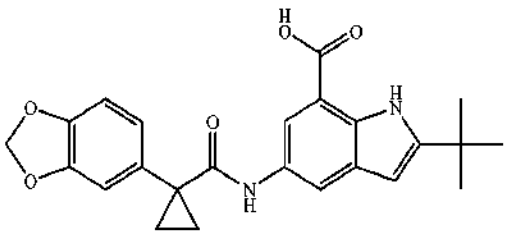

TABLE 1-continued

Exemplary compounds of the present invention.

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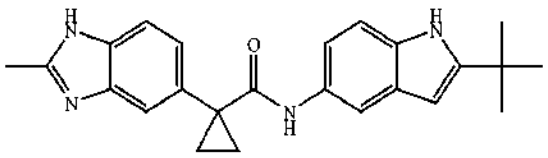

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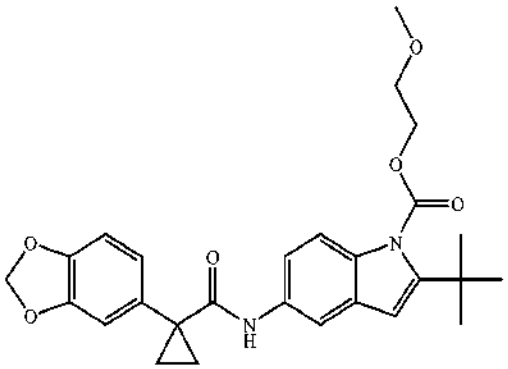
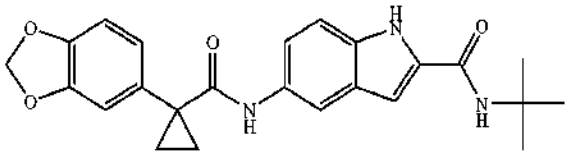


103



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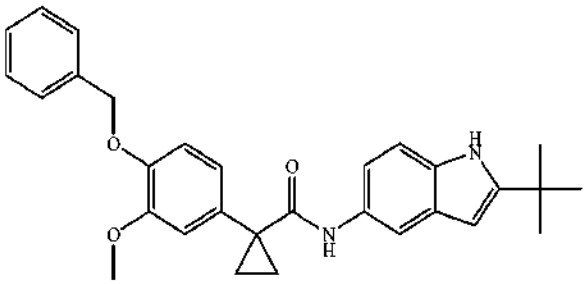

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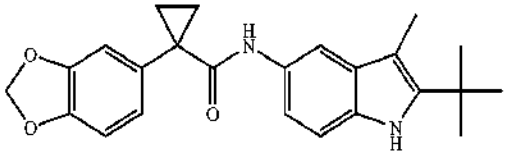

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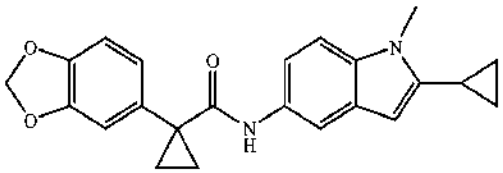

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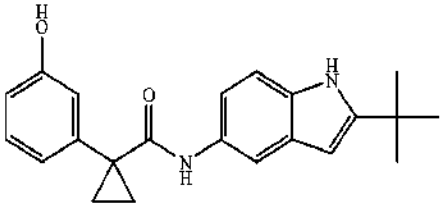

TABLE 1-continued

Exemplary compounds of the present invention.

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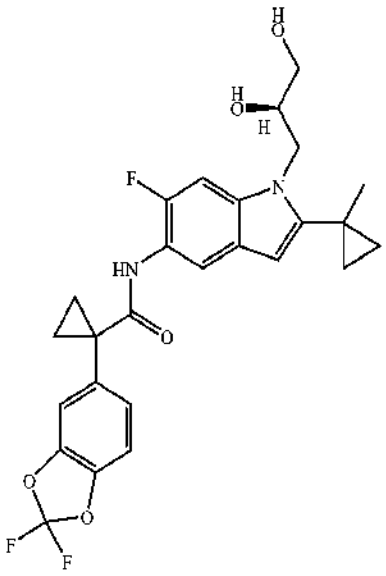

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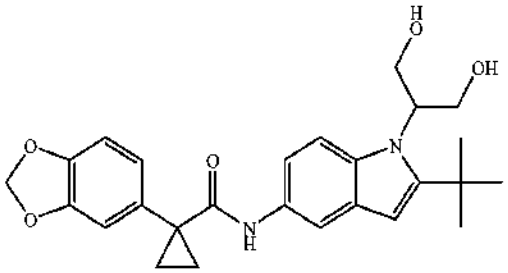

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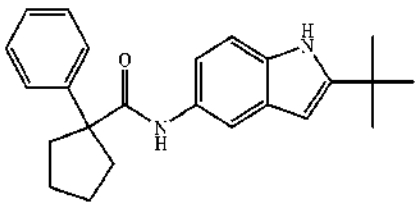

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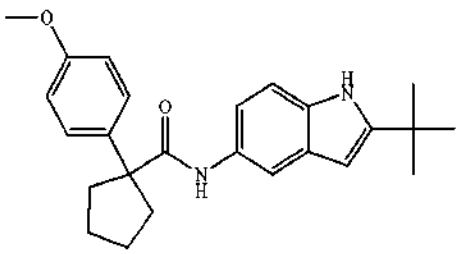

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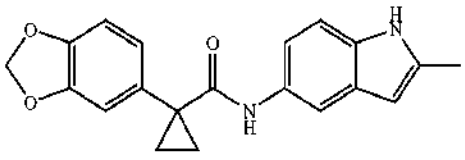
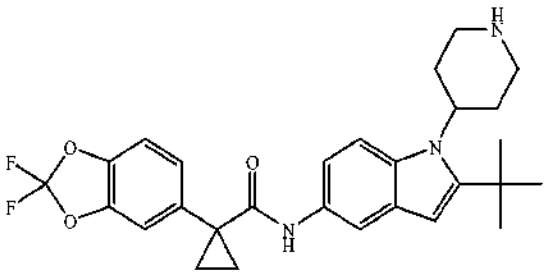


TABLE 1-continued

Exemplary compounds of the present invention.

113



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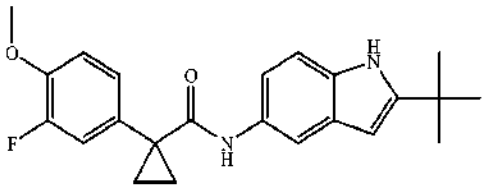

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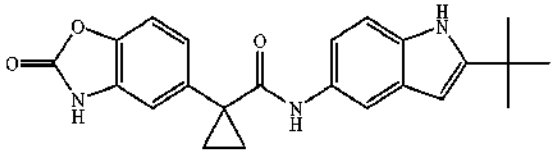

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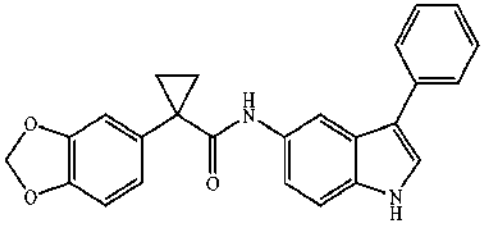

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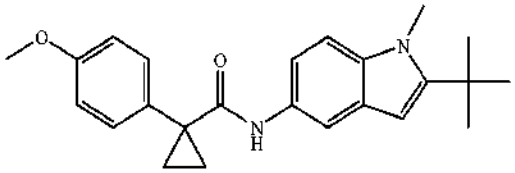

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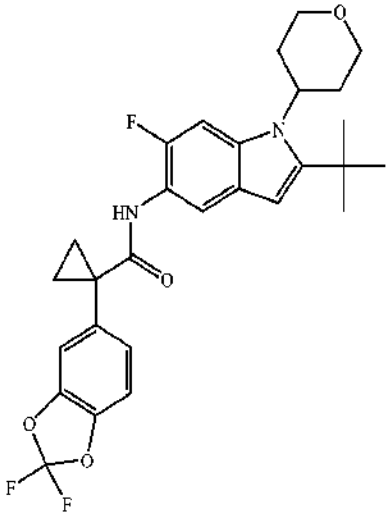

TABLE 1-continued

Exemplary compounds of the present invention.

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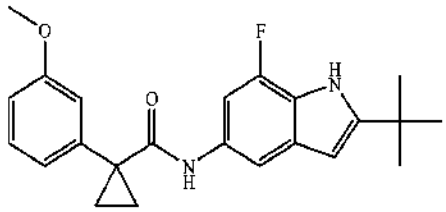

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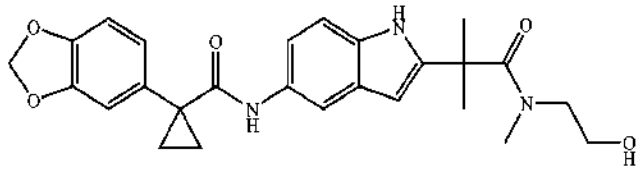

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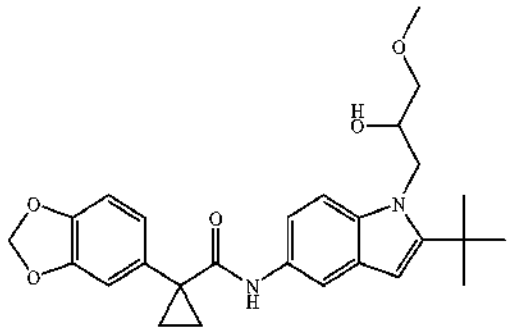

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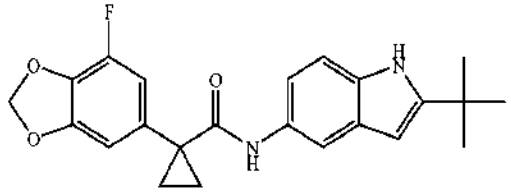

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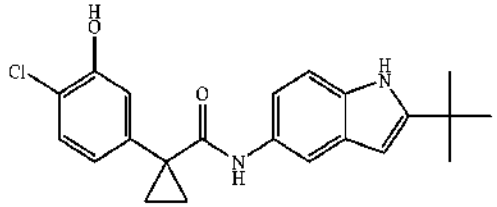

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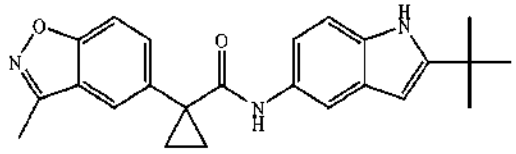

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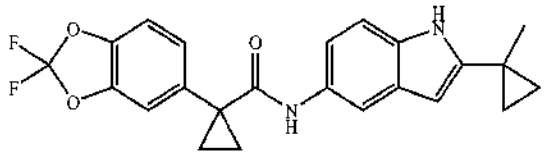
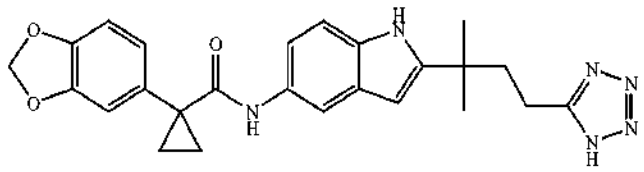


TABLE 1-continued

Exemplary compounds of the present invention.

126



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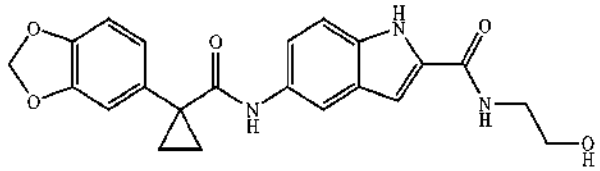

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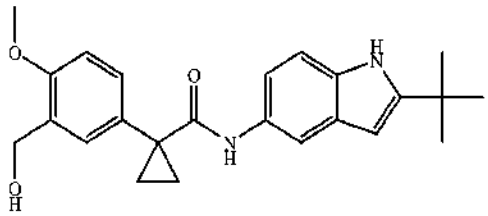

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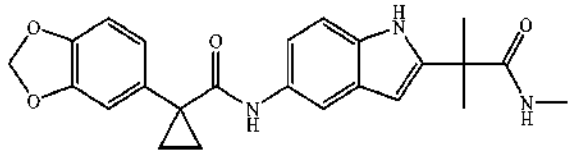

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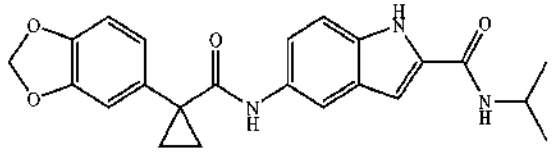

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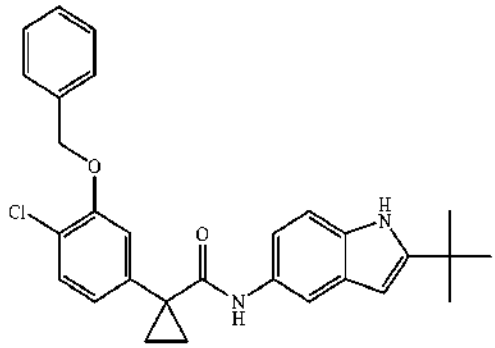

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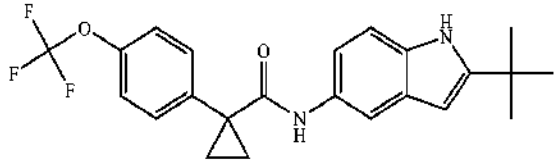


TABLE 1-continued

Exemplary compounds of the present invention.

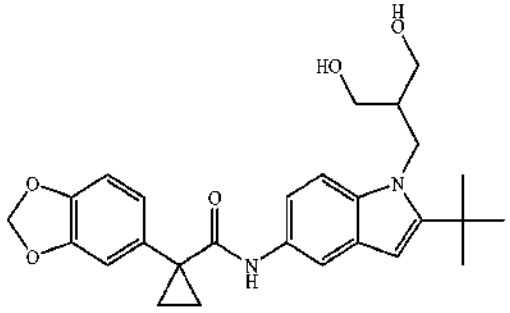
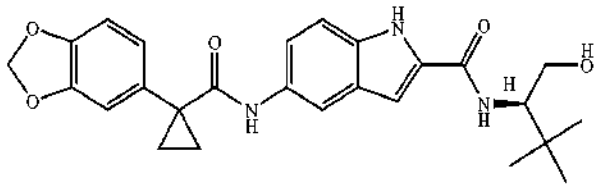
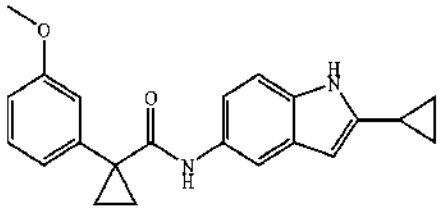
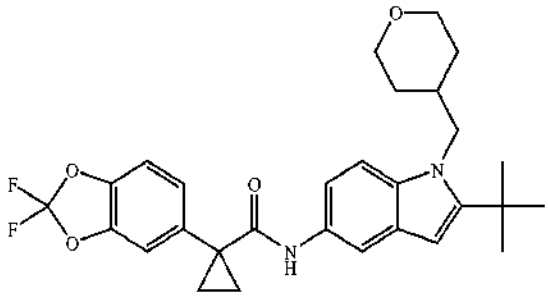
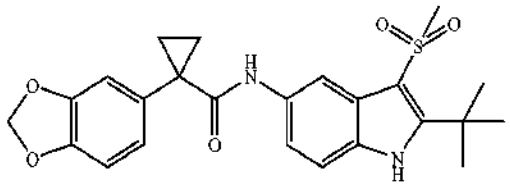
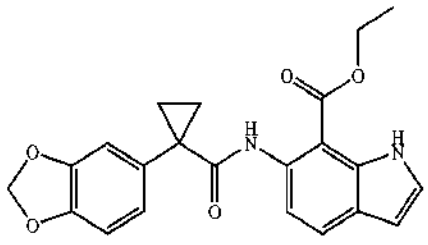
| | |
|---|---|
| 133 |  |
| 134 |  |
| 135 |  |
| 136 |  |
| 137 |  |
| 138 |  |

TABLE 1-continued

Exemplary compounds of the present invention.

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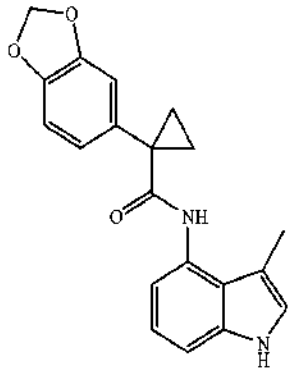

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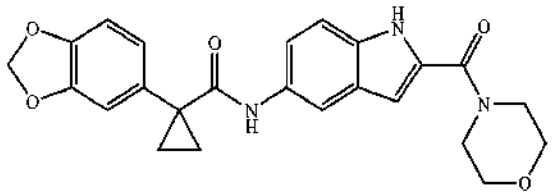

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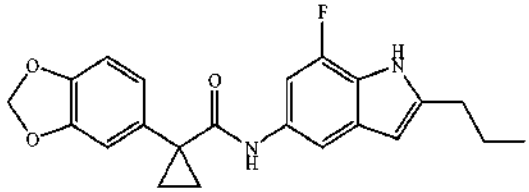

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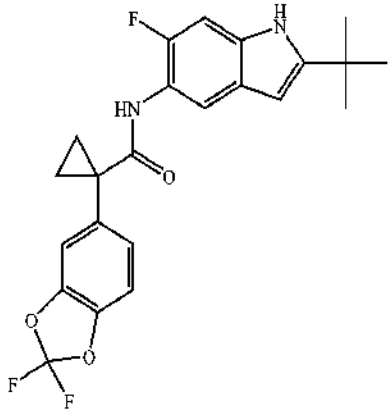

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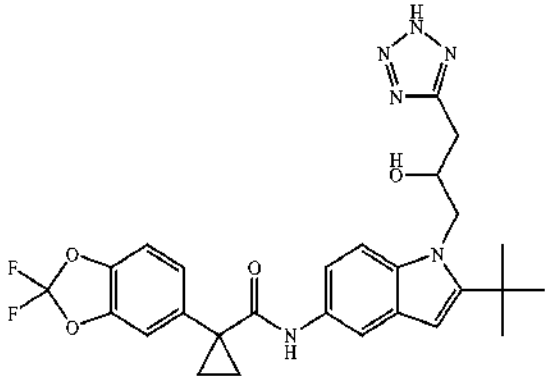
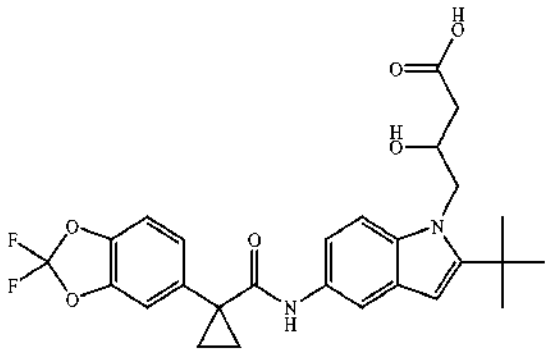
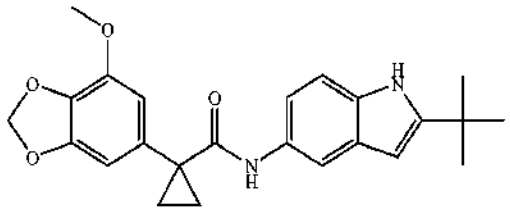
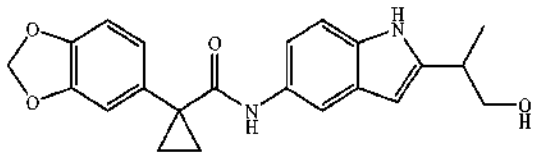


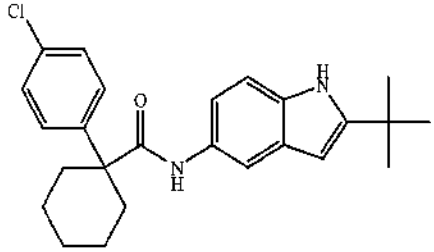
TABLE 1-continued

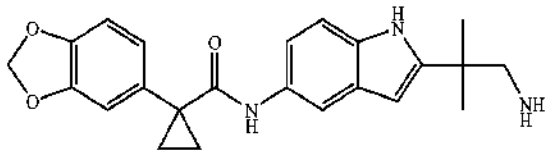
Exemplary compounds of the present invention.

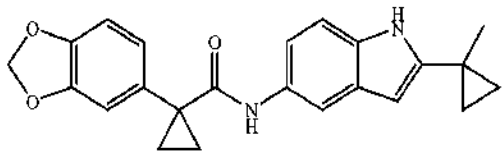
144 

145 

146 

147 

148 

149 

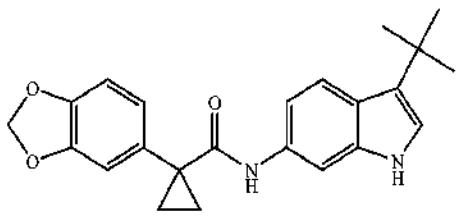
150 

TABLE 1-continued

Exemplary compounds of the present invention.

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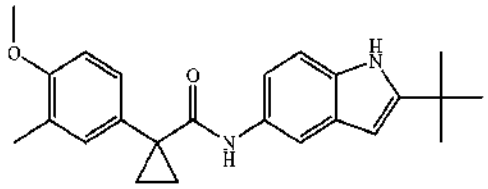

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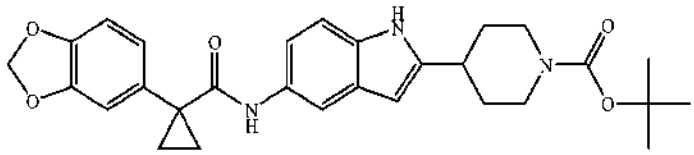

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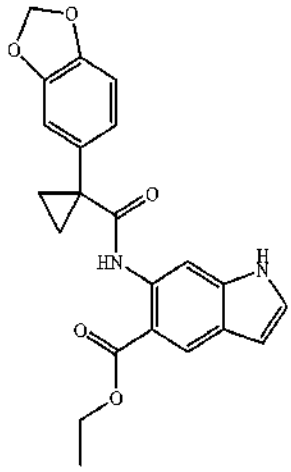

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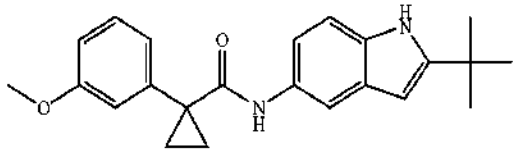

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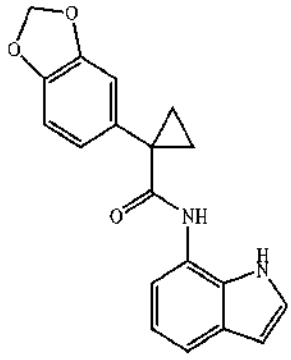
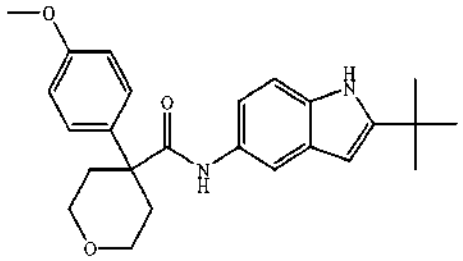
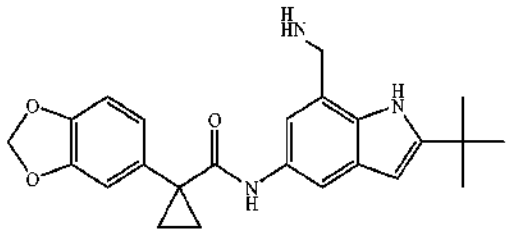
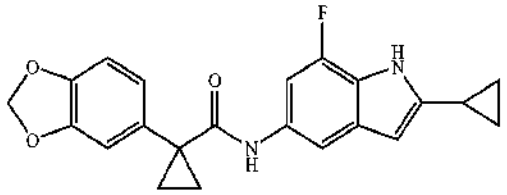


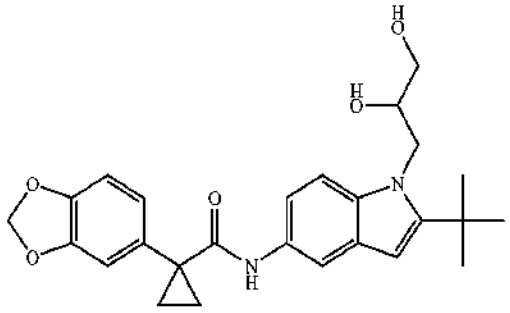
TABLE 1-continued

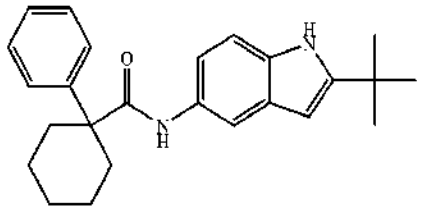
Exemplary compounds of the present invention.

156 

157 

158 

159 

160 

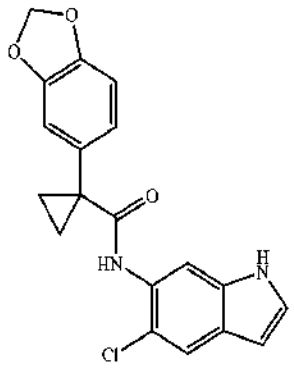
161 

TABLE 1-continued

Exemplary compounds of the present invention.

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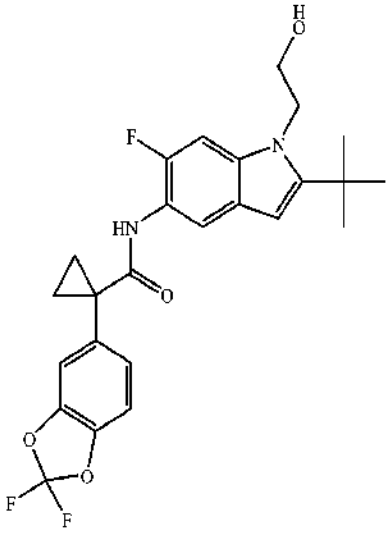

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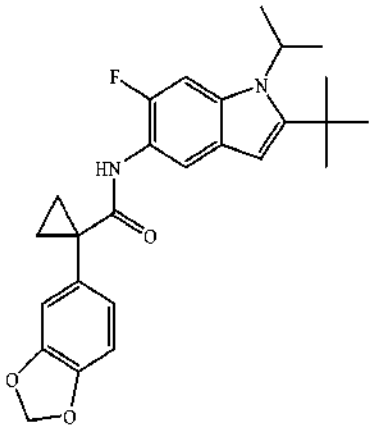

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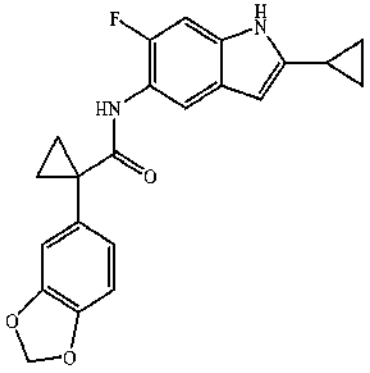

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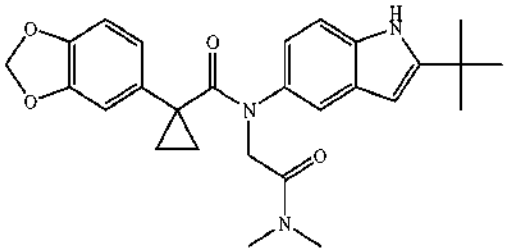

TABLE 1-continued

Exemplary compounds of the present invention.

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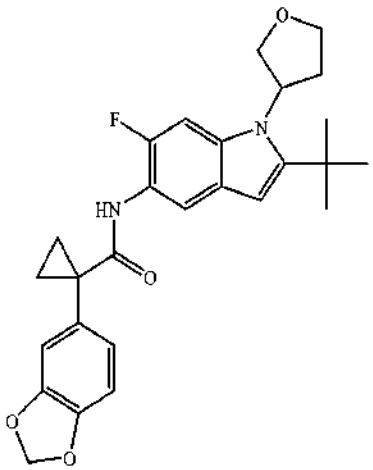

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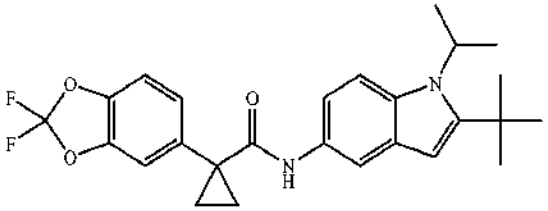

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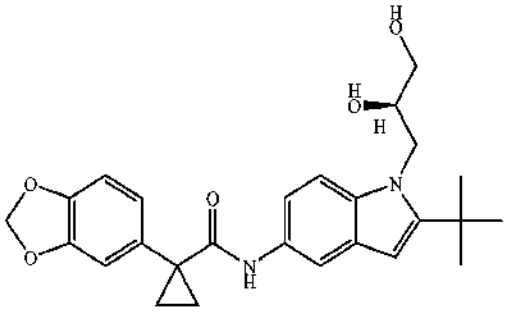

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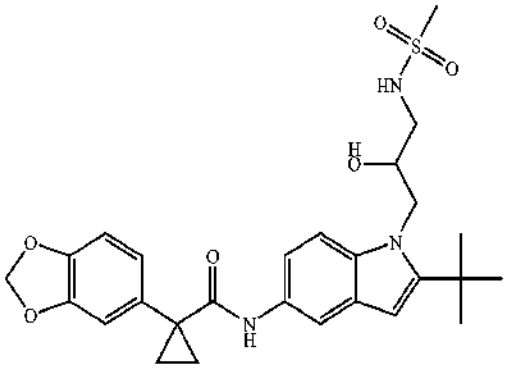

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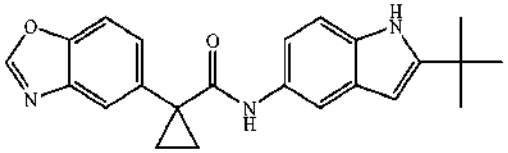

TABLE 1-continued

Exemplary compounds of the present invention.

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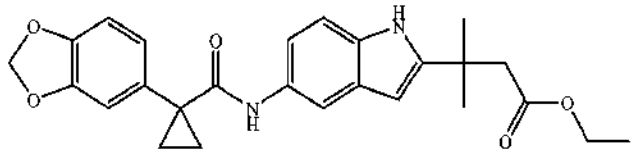

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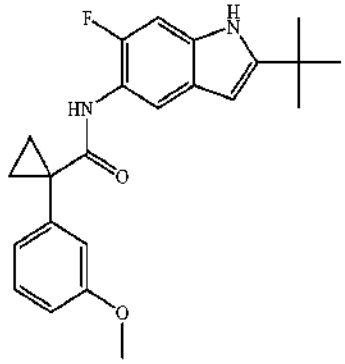

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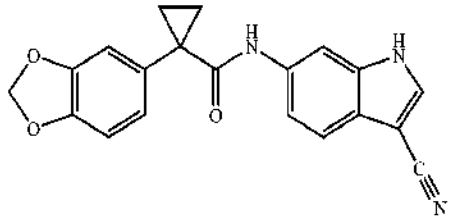

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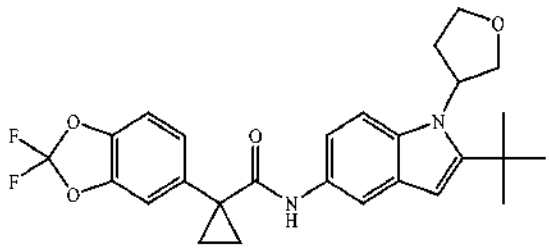

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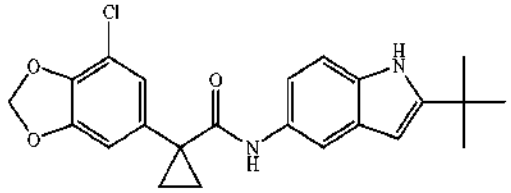

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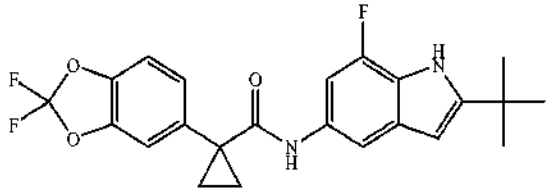

TABLE 1-continued

Exemplary compounds of the present invention.

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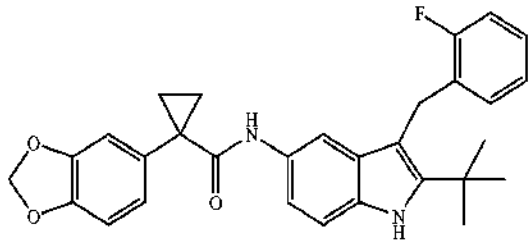

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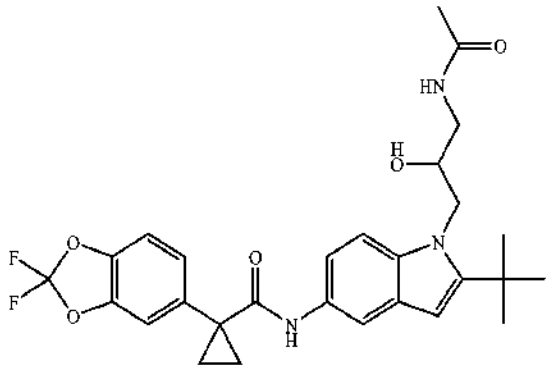

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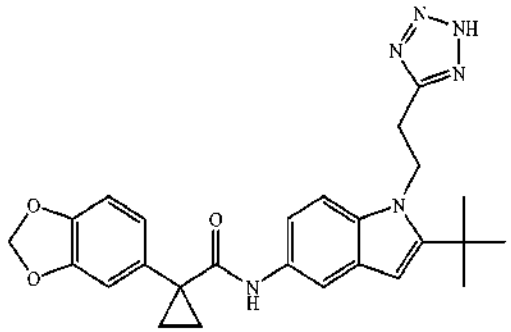

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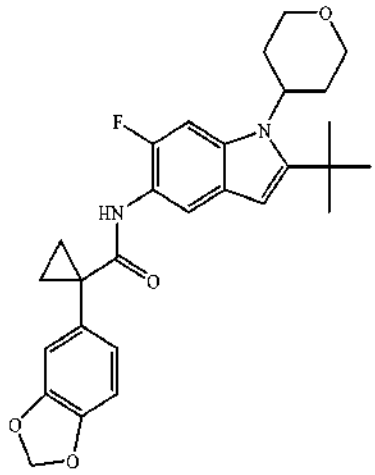

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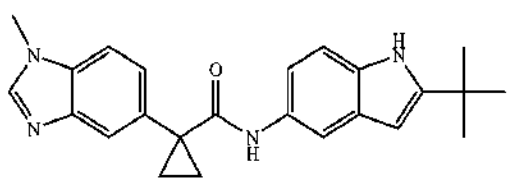

TABLE 1-continued

Exemplary compounds of the present invention.

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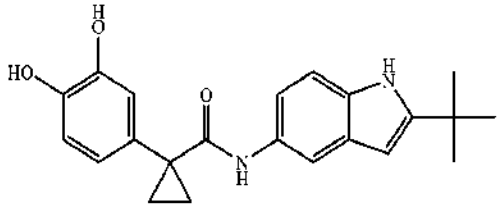

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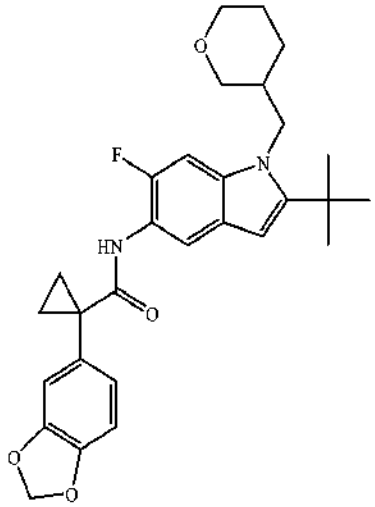

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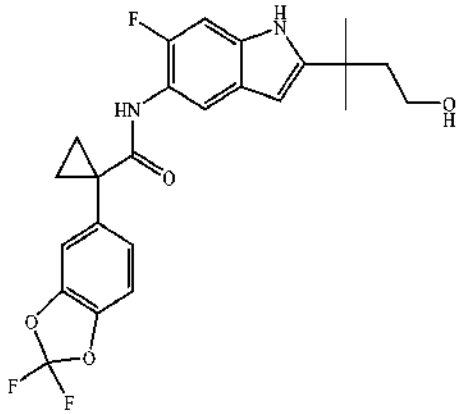

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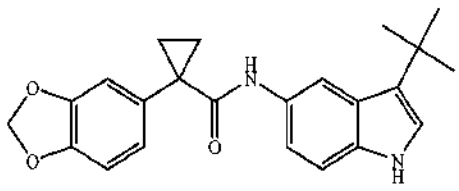

TABLE 1-continued

Exemplary compounds of the present invention.

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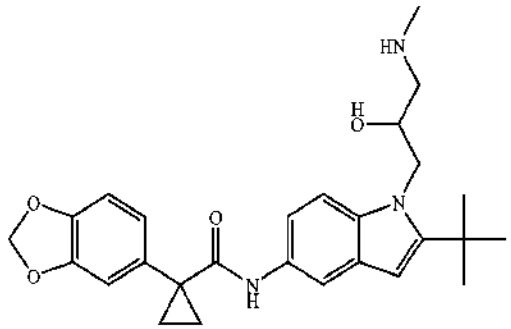

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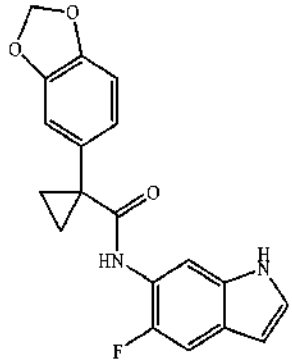

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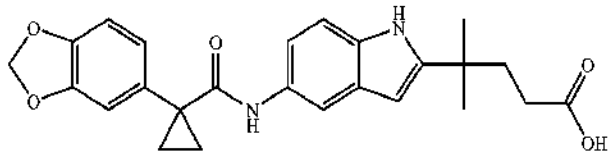

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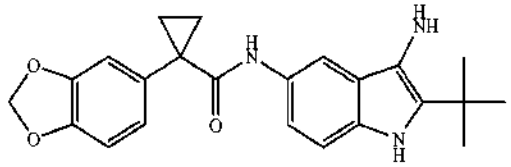

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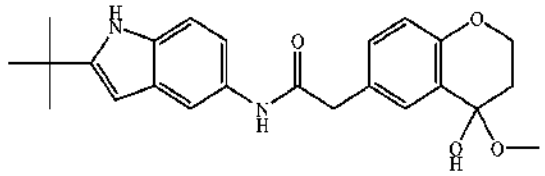

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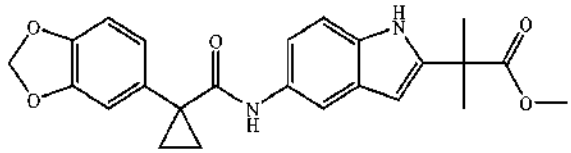

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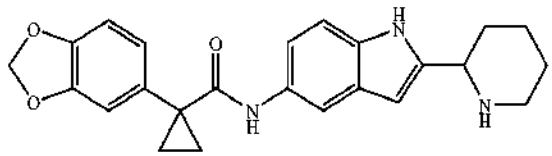

TABLE 1-continued

Exemplary compounds of the present invention.

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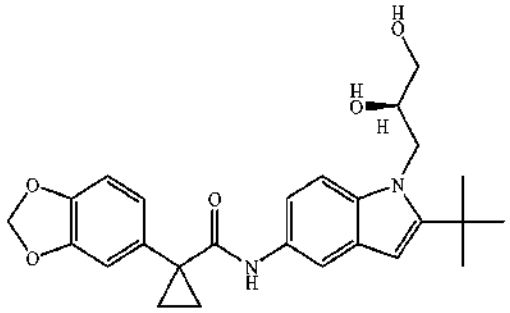

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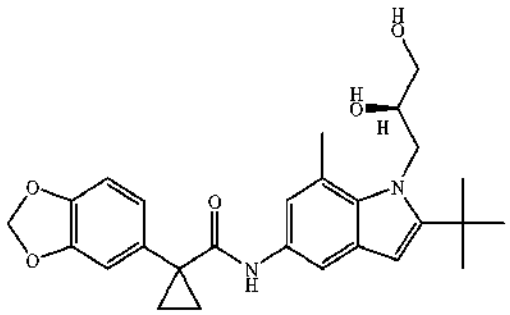

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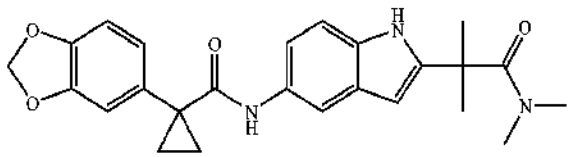

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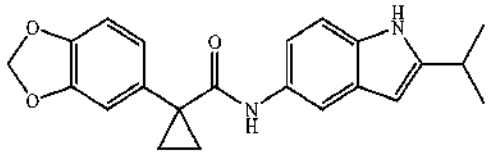

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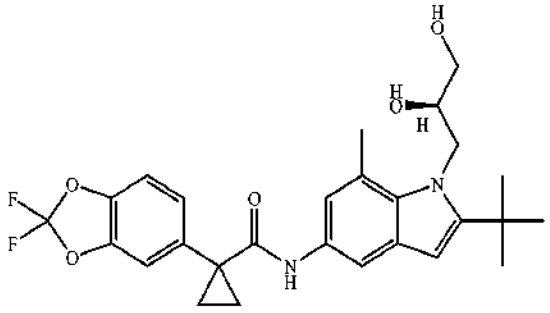

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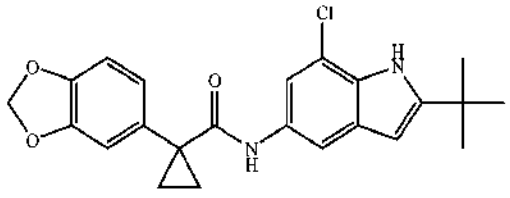

TABLE 1-continued

Exemplary compounds of the present invention.

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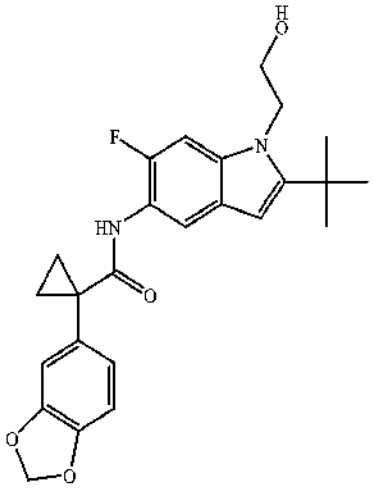

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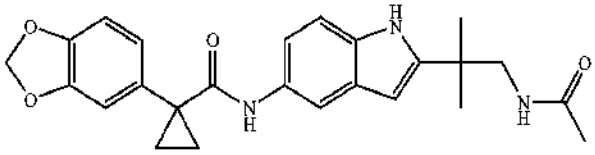

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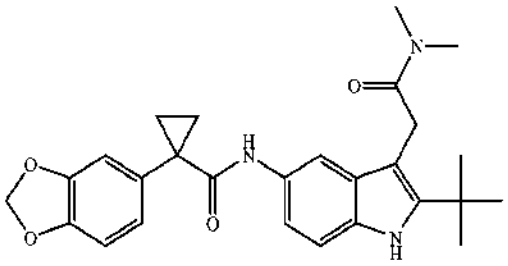

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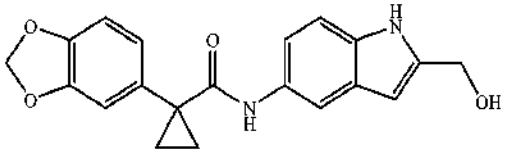

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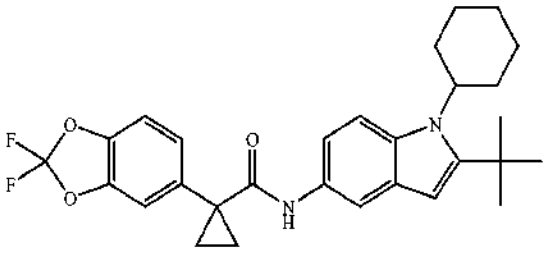

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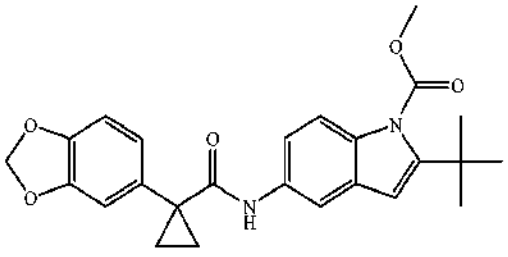

TABLE 1-continued

Exemplary compounds of the present invention.

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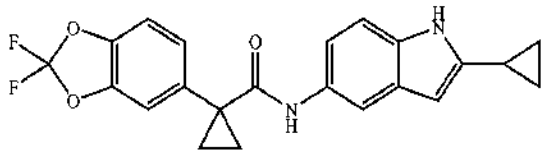

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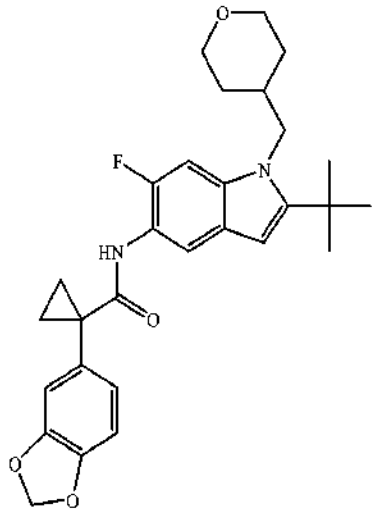

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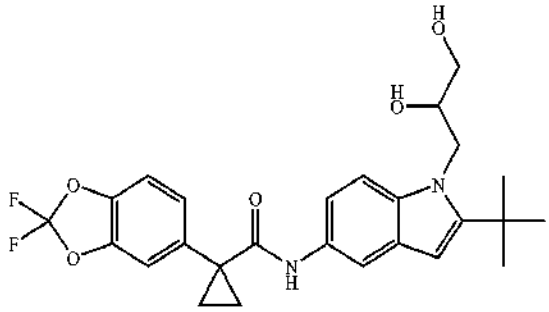

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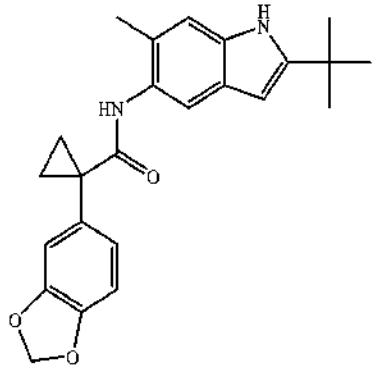

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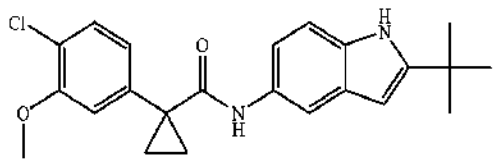

TABLE 1-continued

Exemplary compounds of the present invention.

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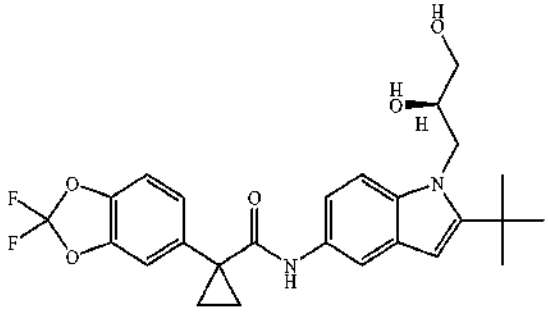

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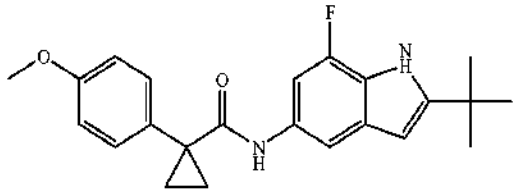

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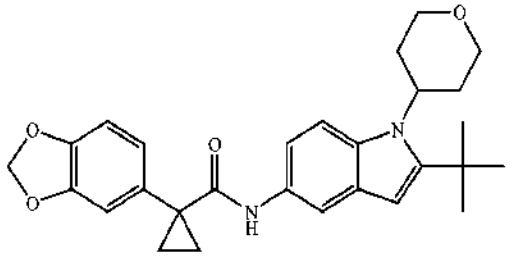

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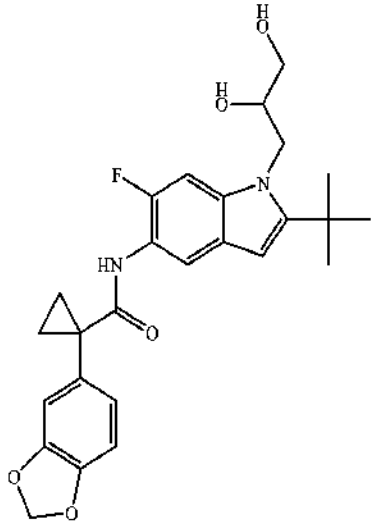

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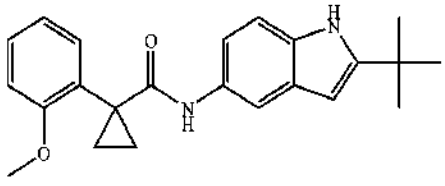

TABLE 1-continued

Exemplary compounds of the present invention.

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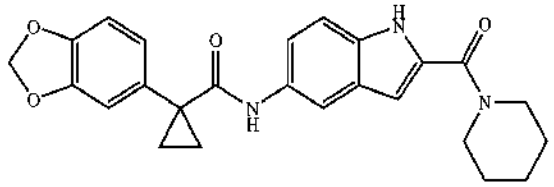

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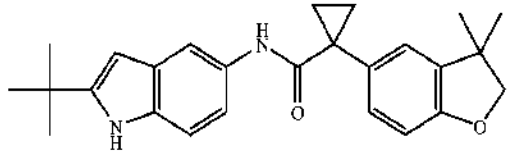

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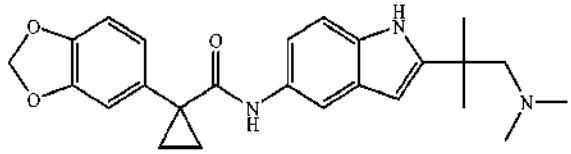

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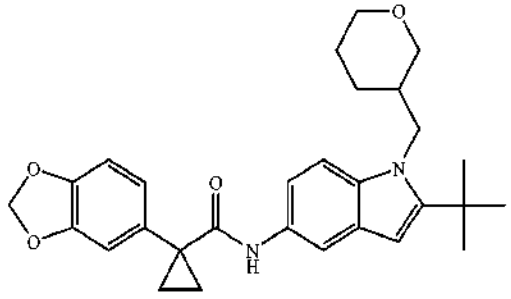

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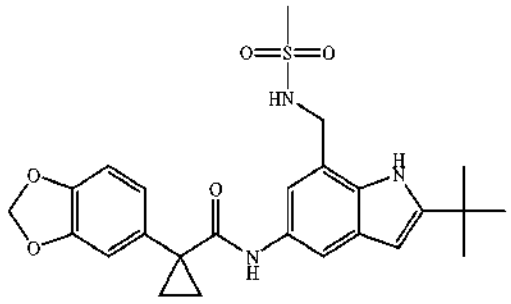

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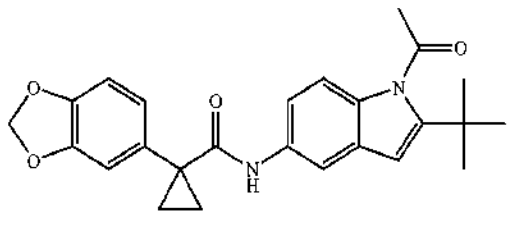

TABLE 1-continued

Exemplary compounds of the present invention.

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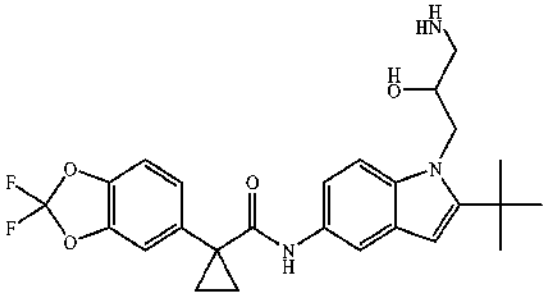

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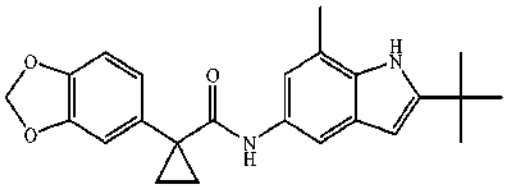

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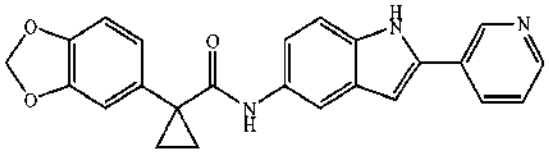

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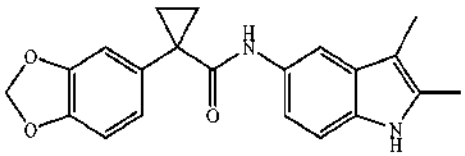

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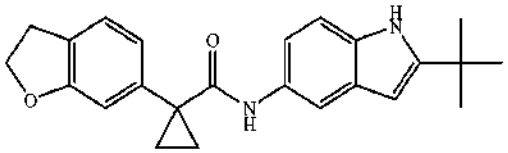

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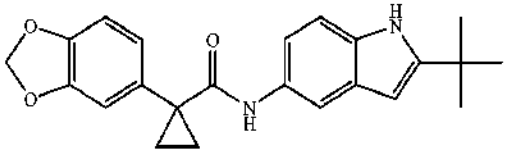

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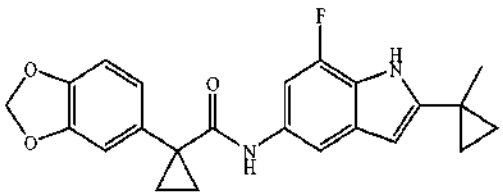

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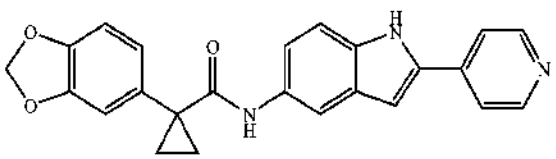

TABLE 1-continued

Exemplary compounds of the present invention.

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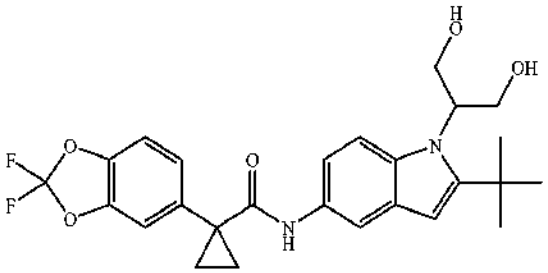

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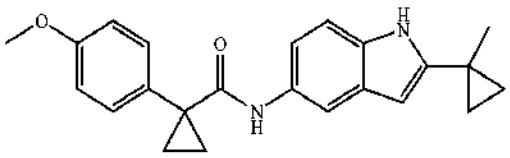

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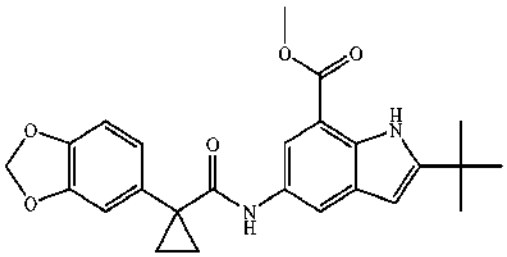

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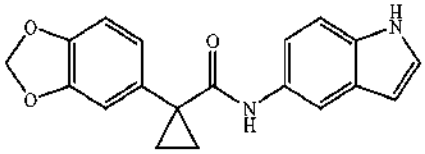

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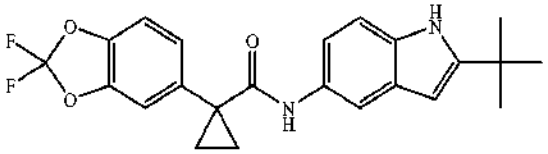

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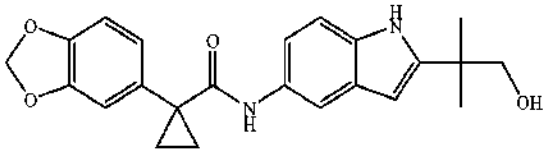

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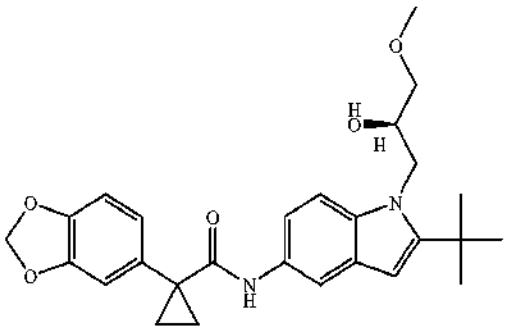

TABLE 1-continued

Exemplary compounds of the present invention.

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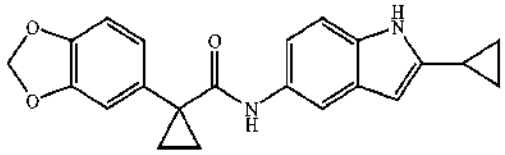

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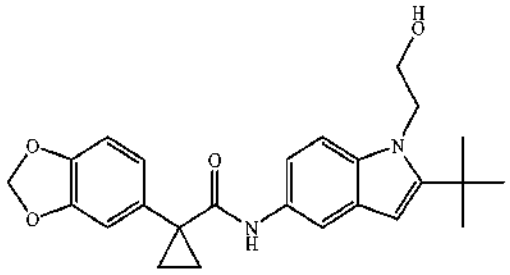

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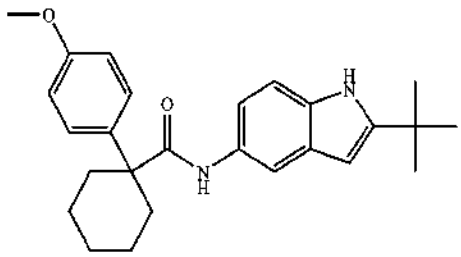

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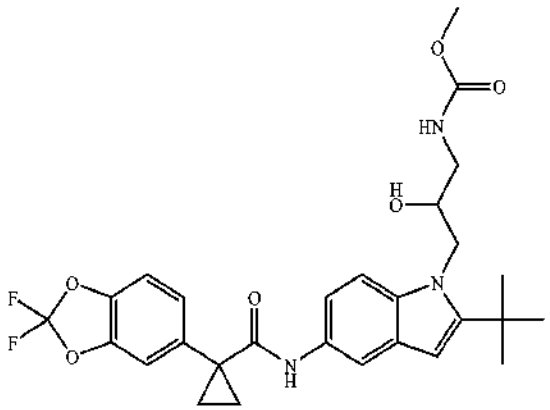

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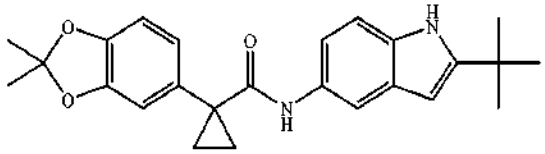

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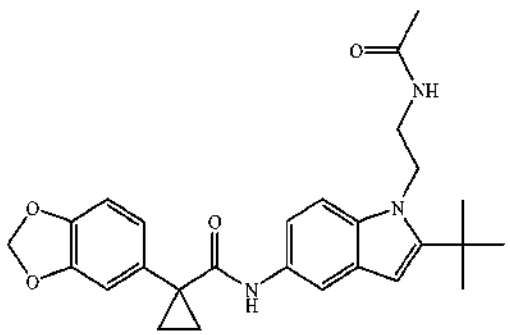

TABLE 1-continued

Exemplary compounds of the present invention.

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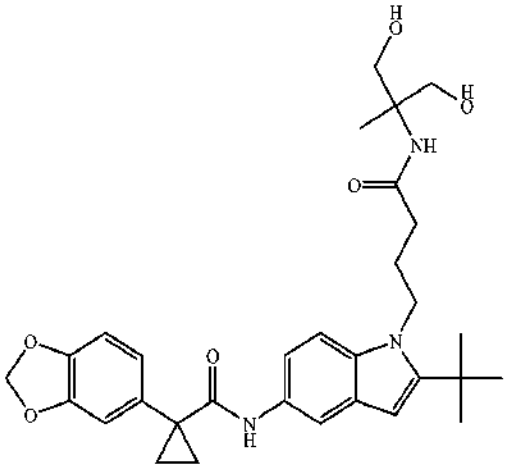

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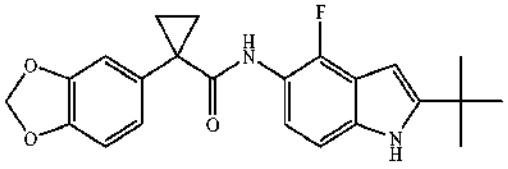

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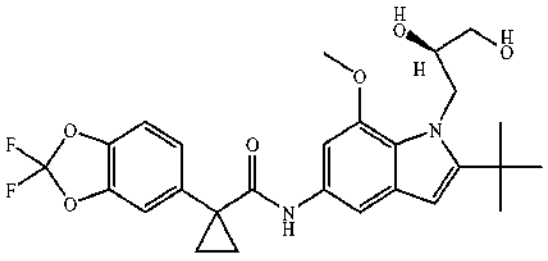

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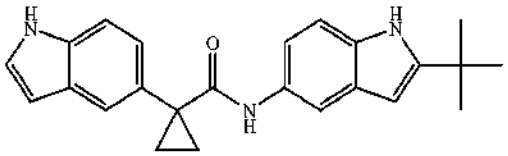

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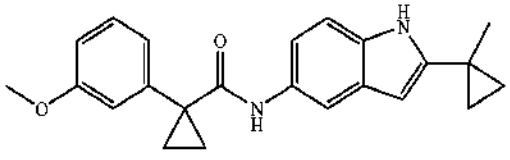

TABLE 1-continued

Exemplary compounds of the present invention.

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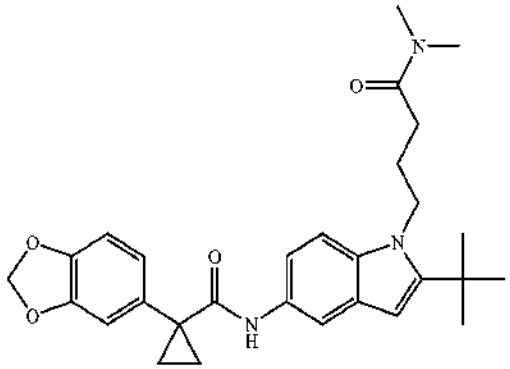

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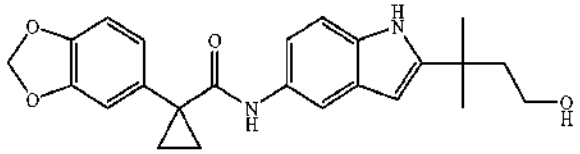

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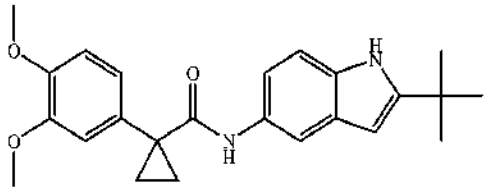

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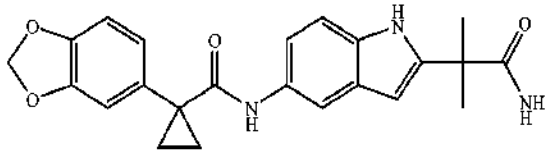

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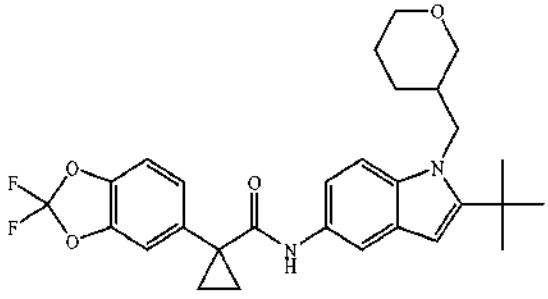

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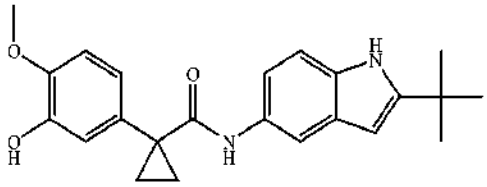

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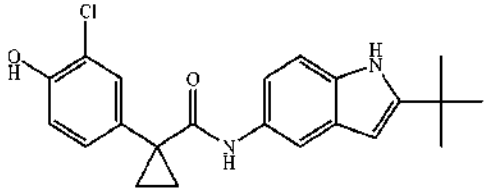
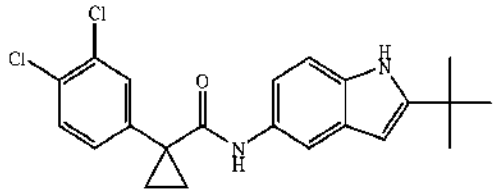
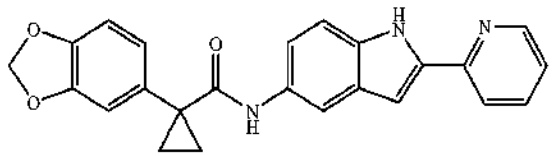
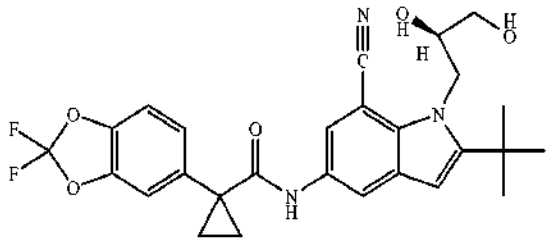


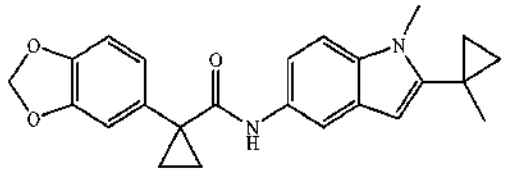
TABLE 1-continued

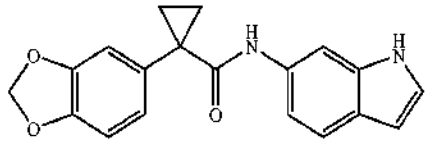
Exemplary compounds of the present invention.

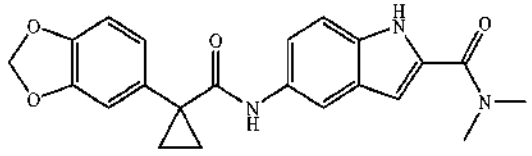
254 

255 

256 

257 

258 

259 

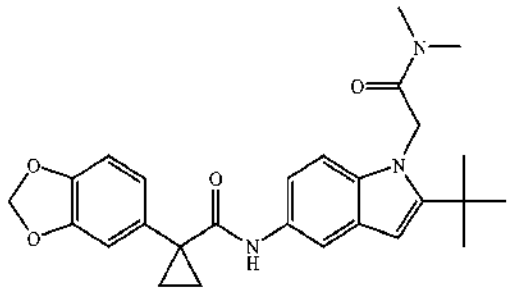
260 

TABLE 1-continued

Exemplary compounds of the present invention.

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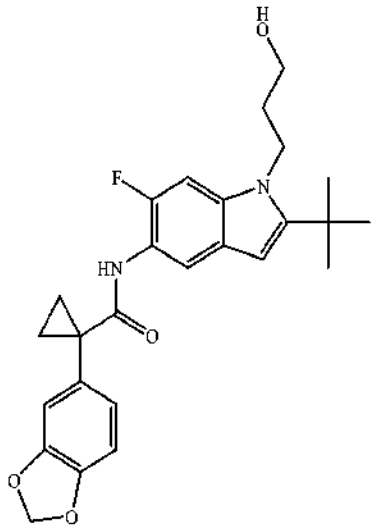

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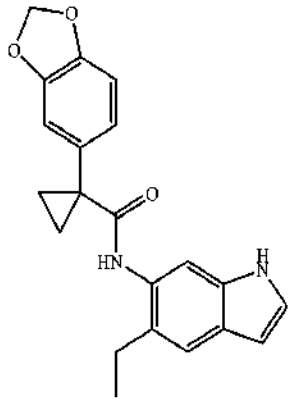

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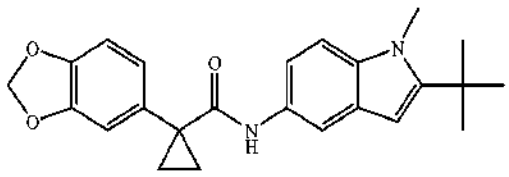

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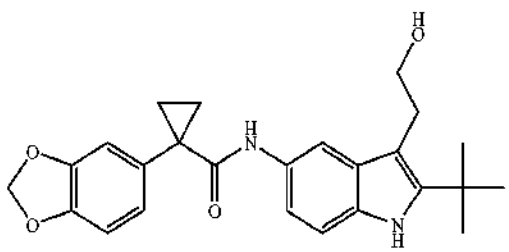

TABLE 1-continued

Exemplary compounds of the present invention.

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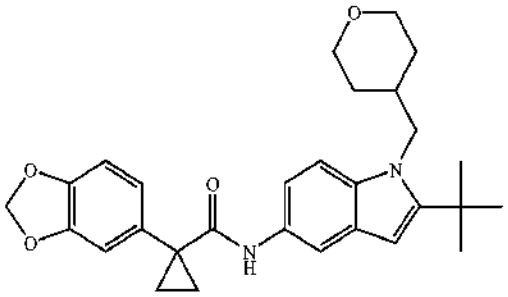

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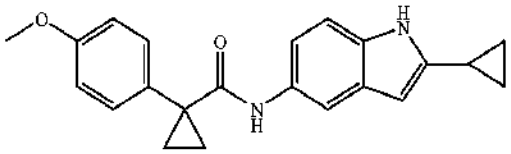

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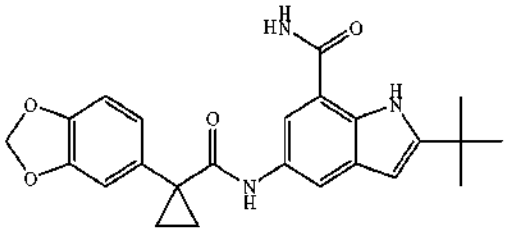

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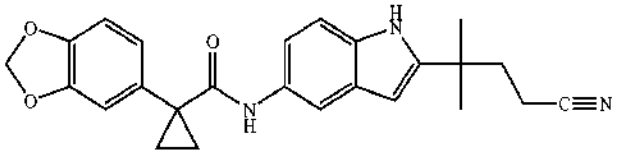

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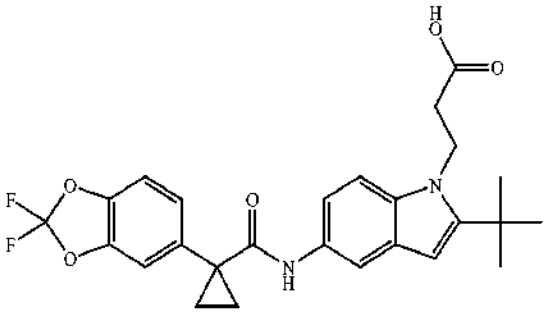

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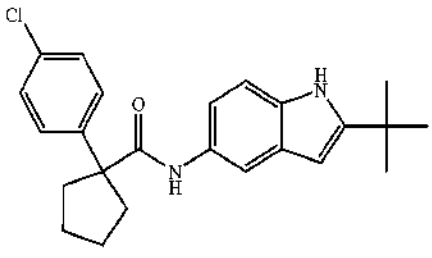

TABLE 1-continued

Exemplary compounds of the present invention.

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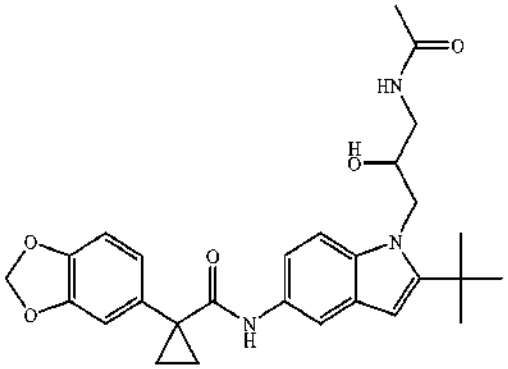

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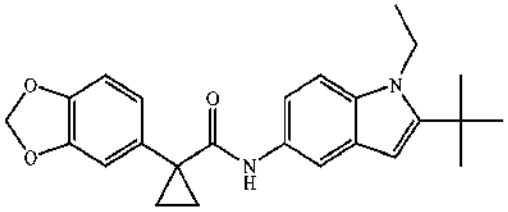

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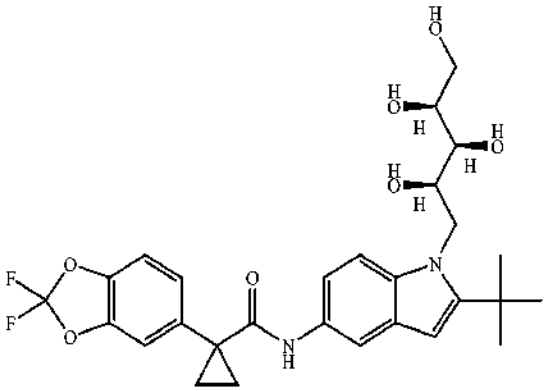

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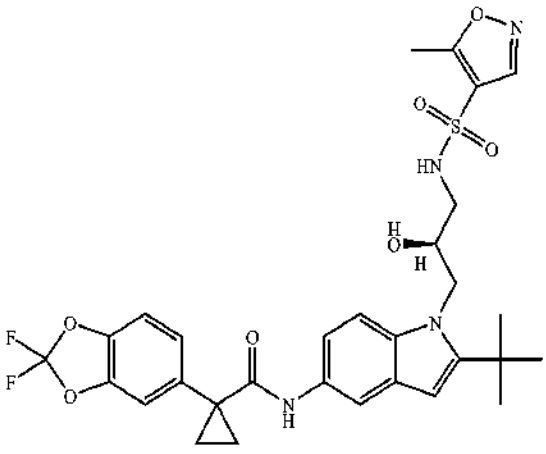

TABLE 1-continued

Exemplary compounds of the present invention.

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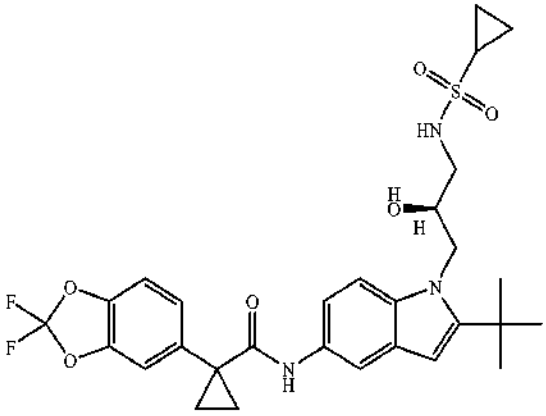

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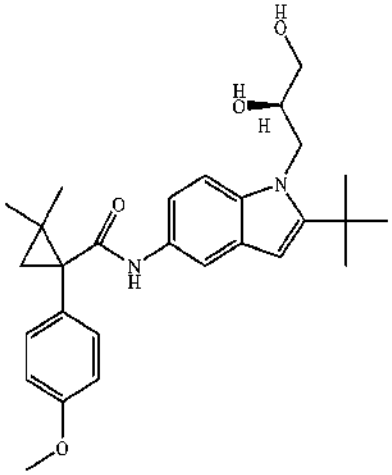

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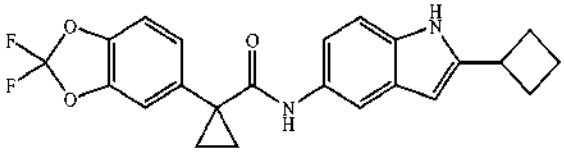

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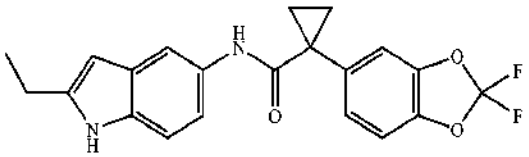

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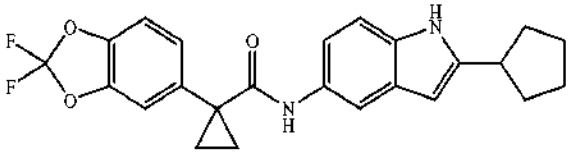

TABLE 1-continued

Exemplary compounds of the present invention.

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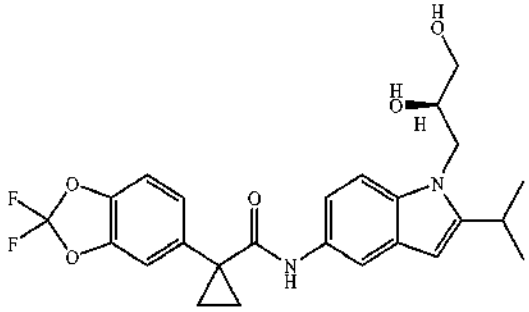

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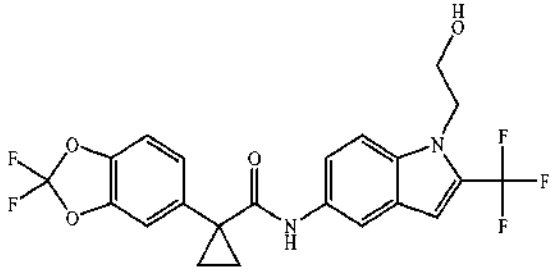

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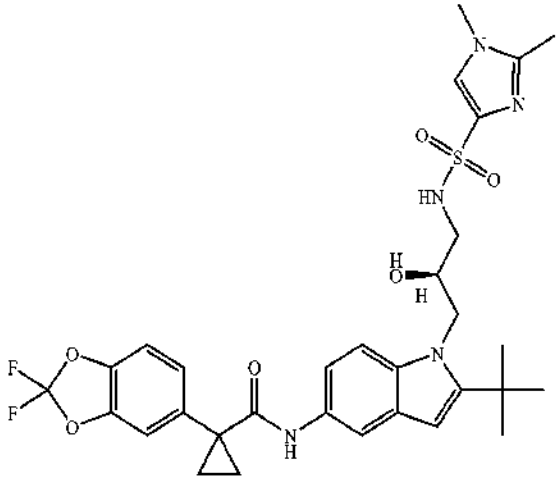

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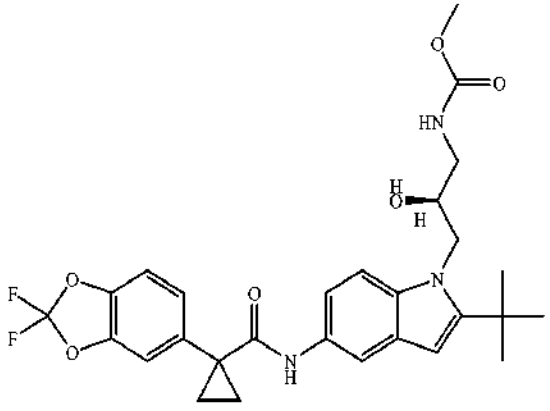

TABLE 1-continued

Exemplary compounds of the present invention.

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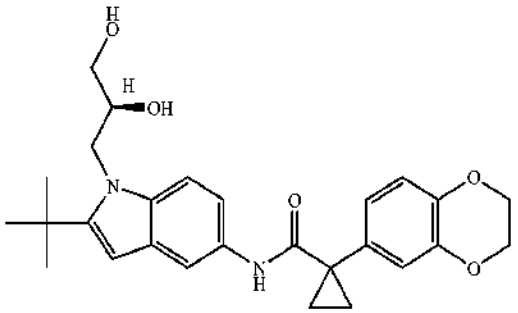

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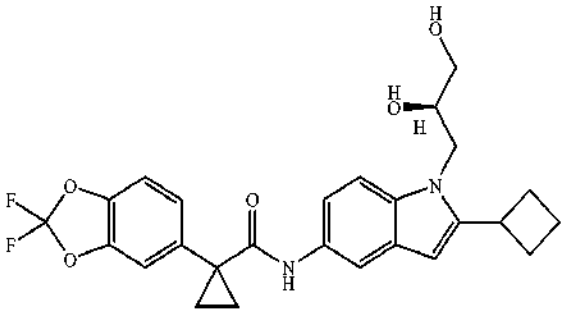

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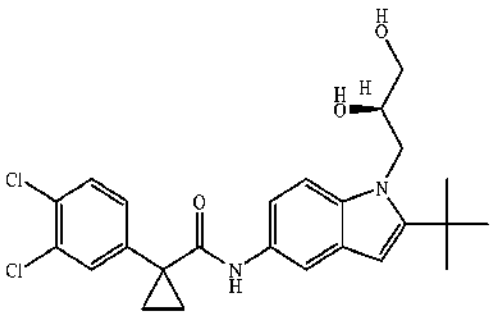

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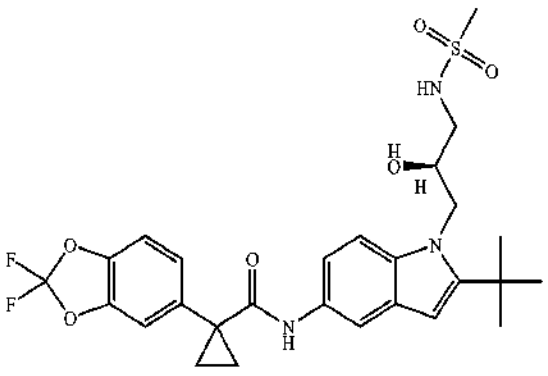

TABLE 1-continued

Exemplary compounds of the present invention.

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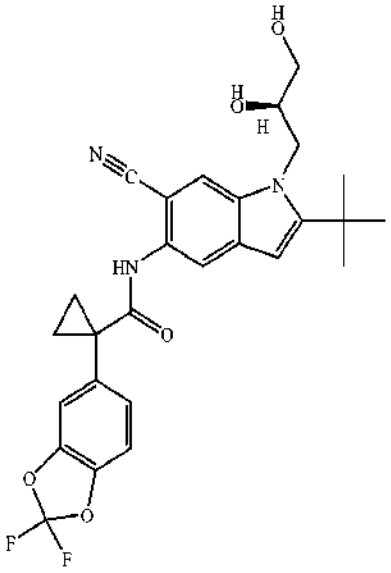

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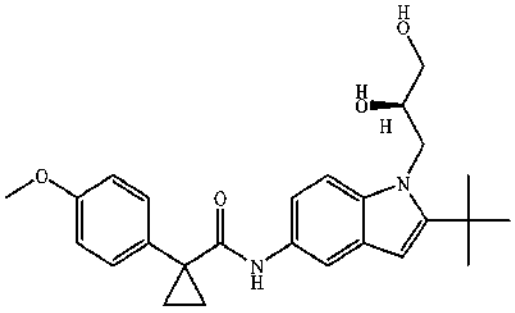

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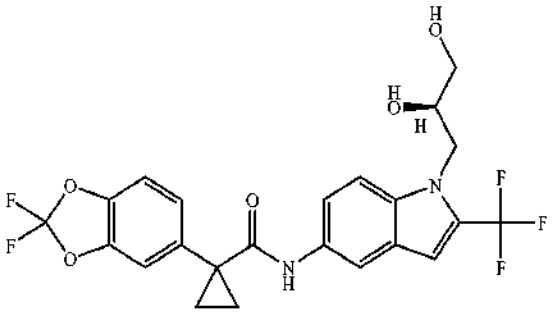

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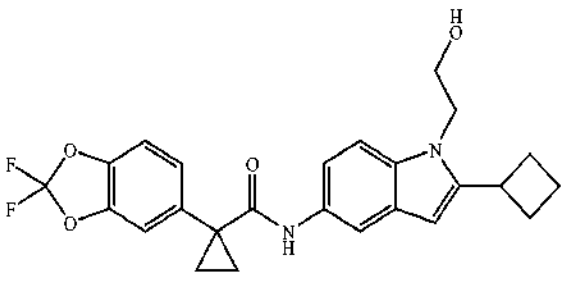

TABLE 1-continued

Exemplary compounds of the present invention.

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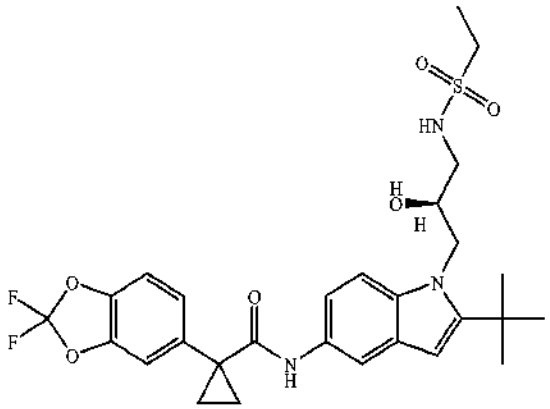

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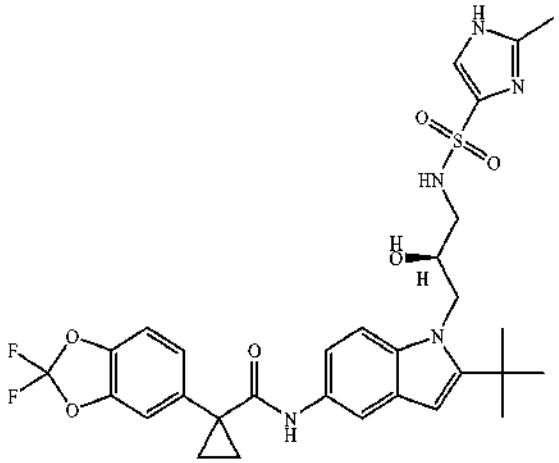

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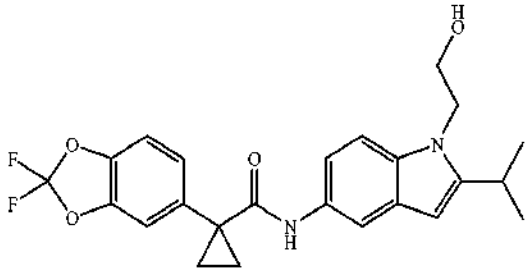

TABLE 1-continued

Exemplary compounds of the present invention.

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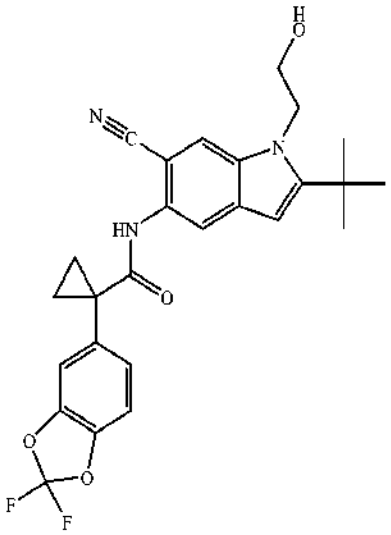

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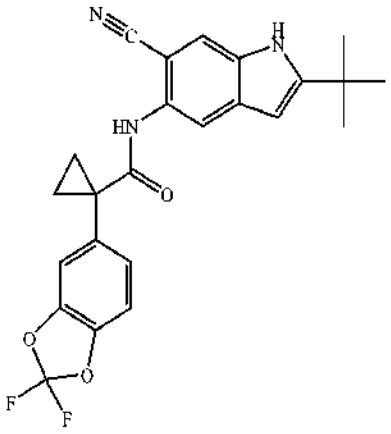

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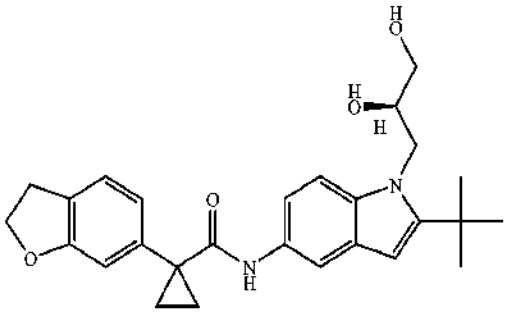

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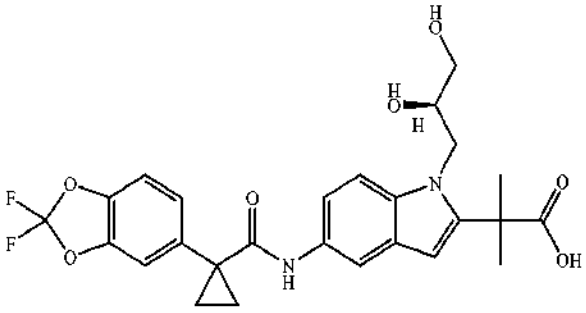

TABLE 1-continued

Exemplary compounds of the present invention.

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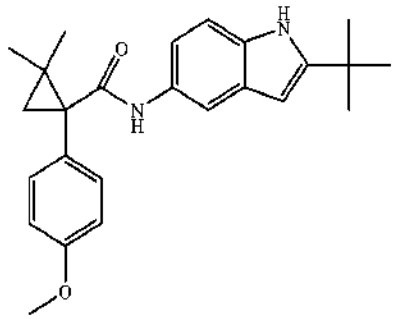

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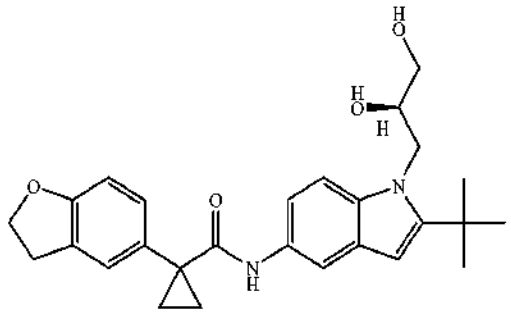

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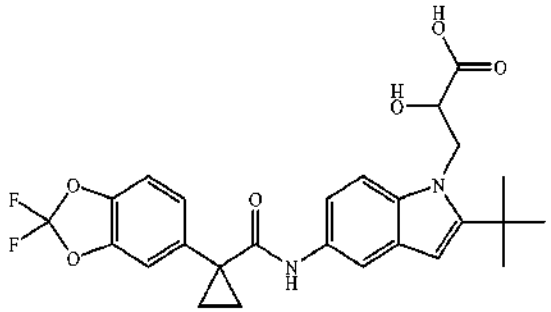

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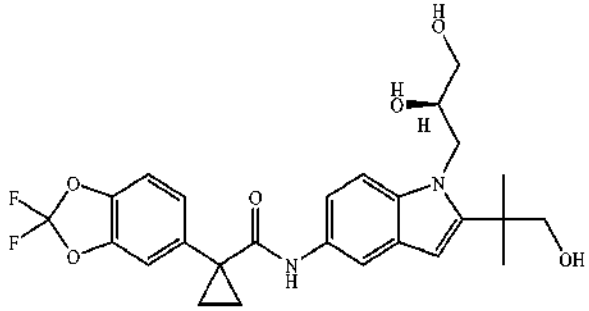

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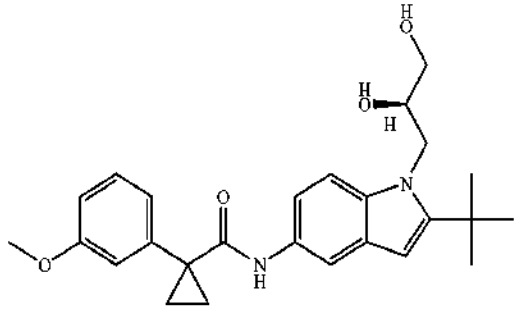

TABLE 1-continued

Exemplary compounds of the present invention.

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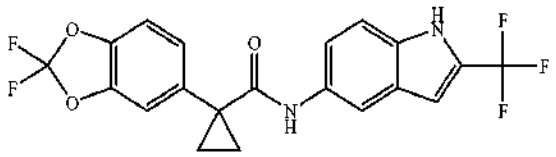

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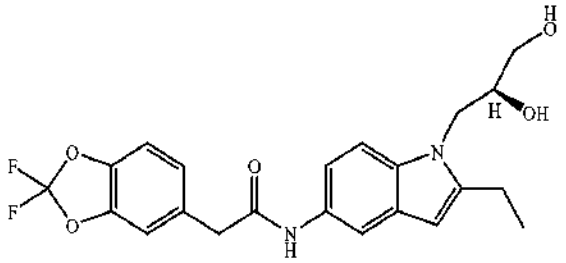

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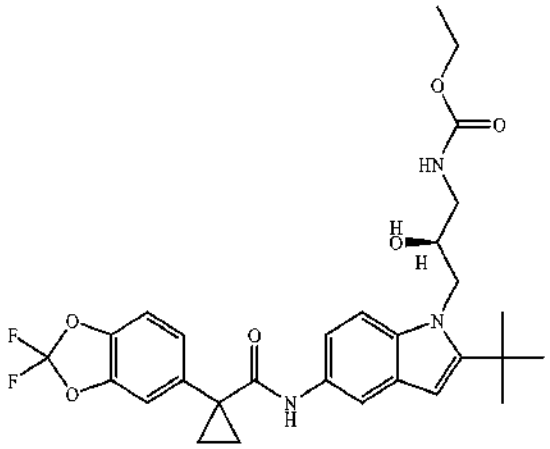

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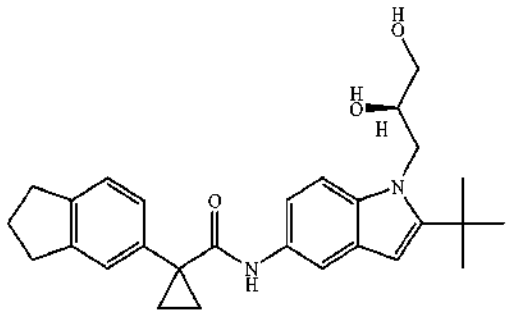

TABLE 1-continued

Exemplary compounds of the present invention.

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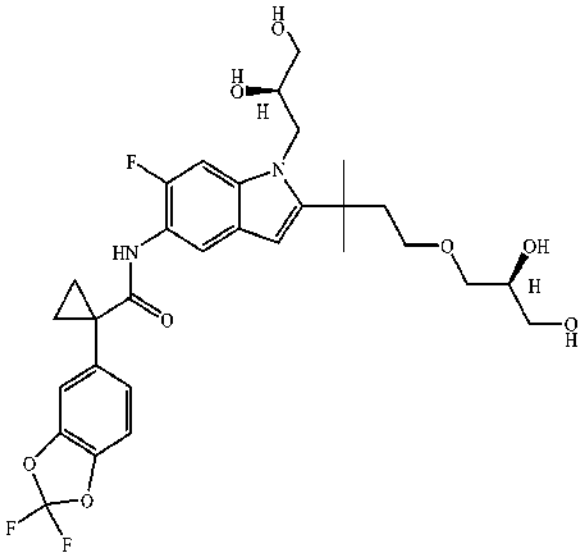

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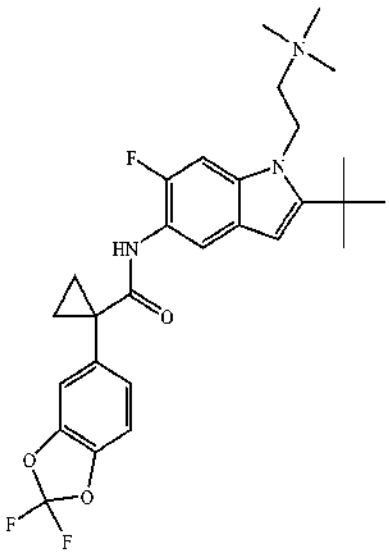

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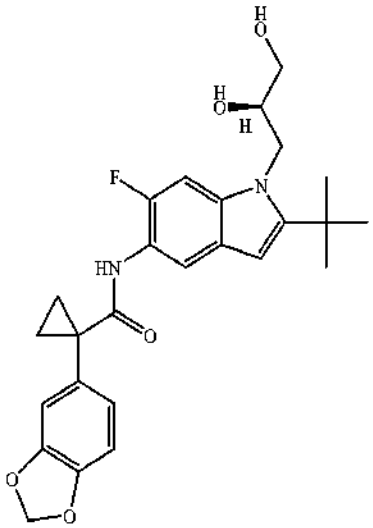

TABLE 1-continued

Exemplary compounds of the present invention.

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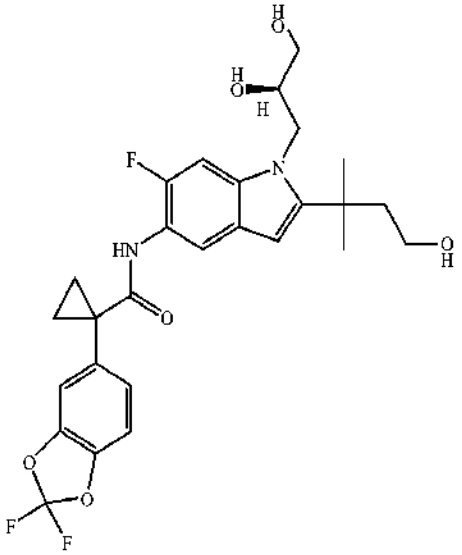

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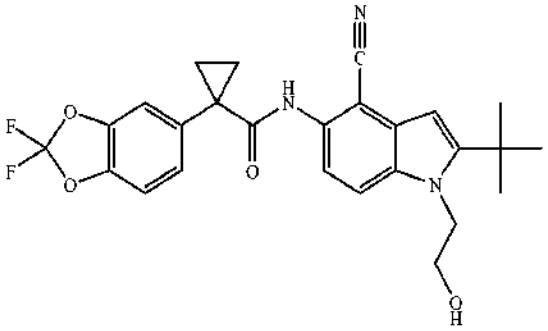

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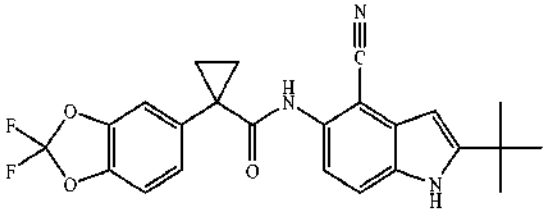

TABLE 1-continued

Exemplary compounds of the present invention.

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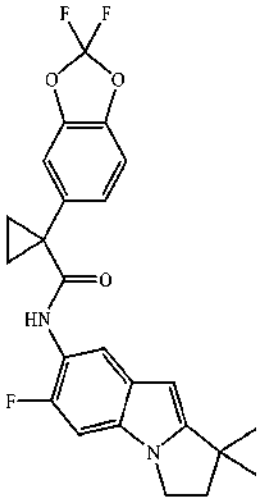

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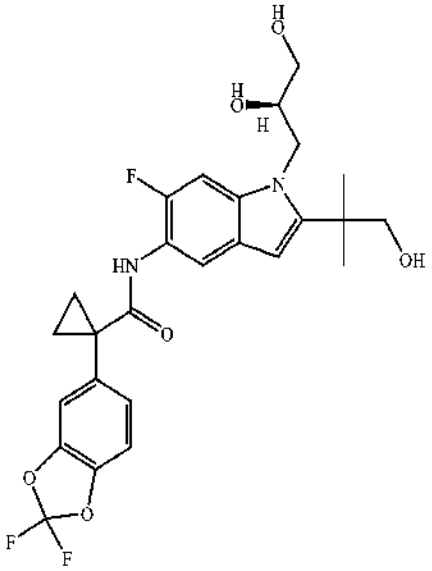

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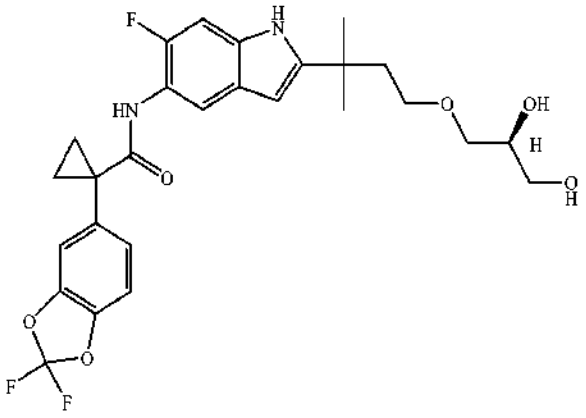

TABLE 1-continued

Exemplary compounds of the present invention.

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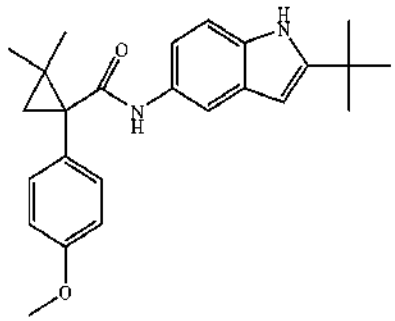

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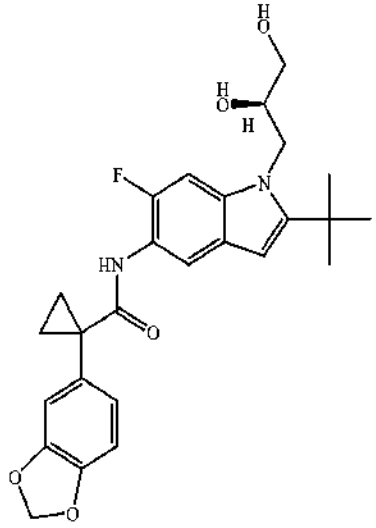

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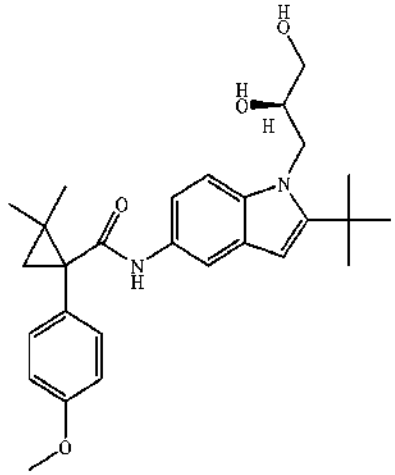
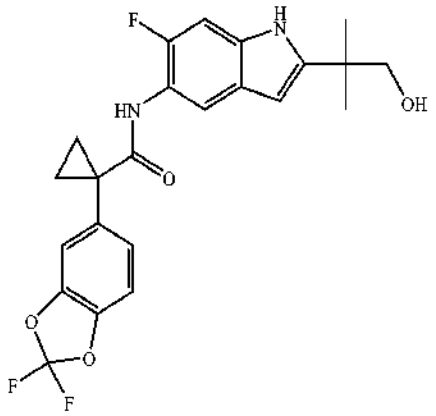
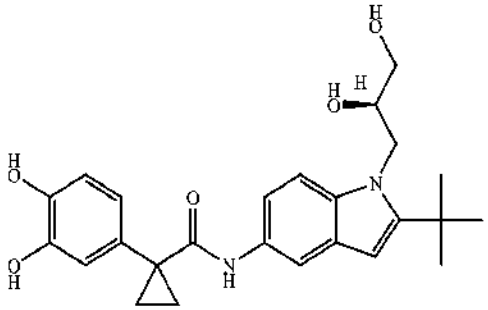
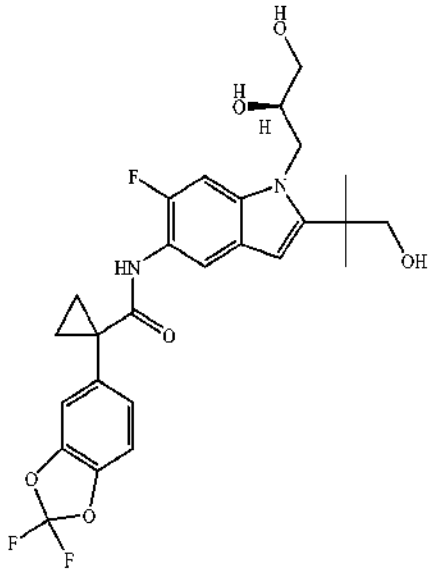


TABLE 1-continued

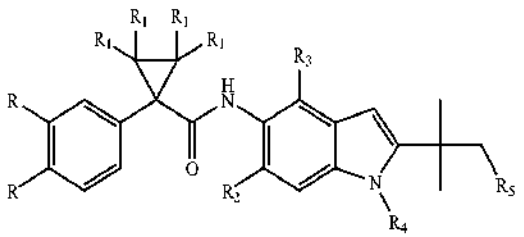
Exemplary compounds of the present invention.

| | |
|---|---|
| 320 |  |
| 321 |  |
| 322 |  |

**[0181]** In another aspect, the present invention relates to a pharmaceutical composition comprising (i) a compound of the present invention; and (ii) a pharmaceutically acceptable carrier. In another embodiment, the composition further comprises an additional agent selected from a mucolytic agent, bronchodialator, an anti-biotic. an anti-infective agent, an anti-inflammatory agent, CFTR corrector, or a nutritional agent. In another embodiment, the composition further comprises an additional agent selected from compounds disclosed in U.S. patent application Ser. No. 11/165,818, published as U.S. Published Patent Application No. 2006/0074075, filed Jun. 24, 2005, and hereby incorporated by reference in its entirety. In another embodiment, the composition further comprises N-(5-hydroxy-2,4-ditert-butyl-phenyl)-4-oxo-1H-quinoline-3-carboxamide. These compositions are useful for treating the diseases described below including cystic fibrosis. These compositions are also useful in the kits described below.

**[0182]** In another aspect, the present invention relates to a method of increasing the number of functional ABC transporters in a membrane of a cell, comprising the step of contacting said cell with a compound of formula II:

II



**[0183]** wherein independently for each occurrence:

**[0184]** R is H, OH, $OCH_3$ or two R taken together form $—CH_2CH_2CH_2—$, $—OCH_2O—$ or $—OCF_2O—$;

**[0185]** $R_1$ is H or alkyl;

**[0186]** $R_2$ is H or F;

**[0187]** $R_3$ is H or CN;

**[0188]** $R_4$ is H, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and

**[0189]** $R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a five membered ring.

**[0190]** In one embodiment of this method, the ABC transporter is CFTR.

**[0191]** In one embodiment of this method, two R taken together form $—OCF_2O—$, $R_1$ is H, and $R_2$ is F. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, and $R_3$ is H. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is H. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2CH_2N^+(CH_3)_3$. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ and $R_5$ taken together form a five membered ring.

**[0192]** In one embodiment of this method, two R taken together form $—OCH_2O—$, $R_1$ is H, and $R_2$ is F. In another embodiment, two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, and $R_3$ is H. In another embodiment, two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$.

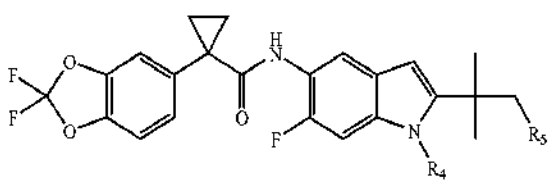
**[0193]** In one embodiment of this method, R is OH, $R_1$ is H, $R_2$ is H, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$.

**[0194]** In one embodiment of this method, at least one R is $OCH_3$, at least two $R_1$ are methyl, $R_2$ is H, $R_3$ is H, and $R_4$ is H. In another embodiment, at least one R is $OCH_3$, at least two $R_1$ are methyl, $R_2$ is H, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$.

**[0195]** In one embodiment of this method, two R taken together form $—CH_2CH_2CH_2—$, $R_1$ is H, $R_2$ is H, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$.

**[0196]** In one embodiment of this method, the compound is represented by formula IIa:

IIa



**[0197]** or a pharmaceutically acceptable salt thereof, wherein:

**[0198]** $R_4$ is H, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and

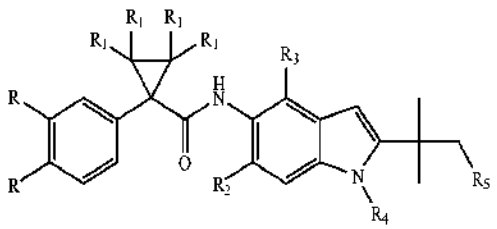
**[0199]** $R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a five membered ring.

**[0200]** In one embodiment of this method, $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$. In another embodiment, $R_5$ is OH, $—CH_2OCH_2CH(OH)CH_2OH$, or $—CH_2OH$. In another embodiment, $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and $R_5$ is OH, $—CH_2OCH_2CH(OH)CH_2OH$, or $—CH_2OH$.

**[0201]** In one embodiment of this method, the compound is selected from Table 1.

**[0202]** In another aspect, the present invention relates to a method of treating a condition, disease, or disorder in a patient implicated by ABC transporter activity, comprising the step of administering to said patient a compound having formula II:

II



**[0203]** or a pharmaceutically acceptable salt thereof, wherein independently for each occurrence:

**[0204]** R is H, OH, $OCH_3$ or two R taken together form $—CH_2CH_2CH_2—$, $—OCH_2O—$ or $—OCF_2O—$;

**[0205]** $R_1$ is H or alkyl;

**[0206]** $R_2$ is H or F;

**[0207]** $R_3$ is H or CN;

**[0208]** $R_4$ is H, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and

**[0209]** $R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a five membered ring.

**[0210]** In one embodiment of this method, two R taken together form $—OCF_2O—$, $R_1$ is H, and $R_2$ is F. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, and $R_3$ is H. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is H. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2CH_2N^+(CH_3)_3$. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ and $R_5$ taken together form a five membered ring.

**[0211]** In one embodiment of this method, two R taken together form $—OCH_2O—$, $R_1$ is H, and $R_2$ is F. In another embodiment, two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, and $R_3$ is H. In another embodiment, two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$.

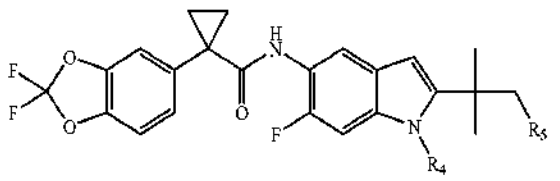
**[0212]** In one embodiment of this method, R is OH, $R_1$ is H, $R_2$ is H, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$.

**[0213]** In one embodiment of this method, at least one R is $OCH_3$, at least two $R_1$ are methyl, $R_2$ is H, $R_3$ is H, and $R_4$ is H. In another embodiment, at least one R is $OCH_3$, at least two $R_1$ are methyl, $R_2$ is H, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$.

**[0214]** In one embodiment of this method, two R taken together form $—CH_2CH_2CH_2—$, $R_1$ is H, $R_2$ is H, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$.

**[0215]** In one embodiment of this method, the compound is represented by formula IIa:

IIa



**[0216]** or a pharmaceutically acceptable salt thereof, wherein:

**[0217]** $R_4$ is H, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and

**[0218]** $R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a five membered ring.

**[0219]** In one embodiment of this method, $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$. In another embodiment, $R_5$ is OH, $—CH_2OCH_2CH(OH)CH_2OH$, or $—CH_2OH$. In another embodiment, $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and $R_5$ is OH, $—CH_2OCH_2CH(OH)CH_2OH$, or $—CH_2OH$.

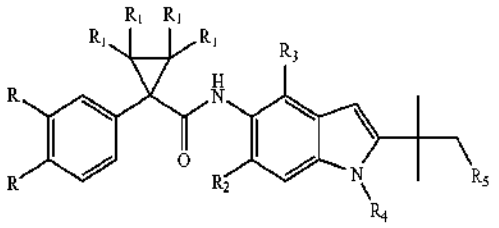
**[0220]** In one embodiment of this method, the compound is selected from Table 1.

**[0221]** In one embodiment of this method, said condition, disease, or disorder is selected from cystic fibrosis, hereditary emphysema, hereditary hemochromatosis, coagulation-fibrinolysis deficiencies, such as protein C deficiency, Type 1 hereditary angioedema, lipid processing deficiencies, such as familial hypercholesterolemia, Type 1 chylomicronemia, abetalipoproteinemia, lysosomal storage diseases, such as I-cell disease/pseudo-Hurler, mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, polyendocrinopathy/hyperinsulemia, diabetes mellitus, laron dwarfism, myeloperoxidase deficiency, primary hypoparathyroidism, melanoma, glycanosis CDG type 1, hereditary emphysema, congenital hyperthyroidism, osteogenesis imperfecta, hereditary hypofibrinogenemia, ACT deficiency, diabetes insipidus (di), neurophyseal di, neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear plasy, Pick's disease, several polyglutamine neurological disorders such as Huntington, spinocerebullar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidoluysian, and myotonic dystrophy, as well as spongiform encephalopathies, such as hereditary Creutzfeldt-Jakob disease, Fabry disease, Straussler-Scheinker syndrome, COPD, dry-eye disease, and Sjögren's disease.

**[0222]** In another aspect, the present invention relates to a kit for use in measuring the activity of a ABC transporter or a fragment thereof in a biological sample in vitro or in vivo, comprising:

**[0223]** (i) a first composition comprising a compound of formula II:

II



**[0224]** wherein independently for each occurrence:

**[0225]** R is H, OH, $OCH_3$ or two R taken together form $—CH_2CH_2CH_2—$, $—OCH_2O—$ or $—OCF_2O—$;

**[0226]** $R_1$ is H or alkyl;

**[0227]** $R_2$ is H or F;

**[0228]** $R_3$ is H or CN;

**[0229]** $R_4$ is H, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and

**[0230]** $R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a five membered ring; and (ii) instructions for: a) contacting the composition with the biological sample; and b) measuring activity of said ABC transporter or a fragment thereof.

**[0231]** In one embodiment, the kit further comprises instructions for a) contacting an additional composition with the biological sample; b) measuring the activity of said ABC transporter or a fragment thereof in the presence of said additional compound, and c) comparing the activity of the ABC transporter in the presence of the additional compound with the density of the ABC transporter in the presence of said first composition.

**[0232]** In one embodiment, the kit is used to measure the density of CFTR.

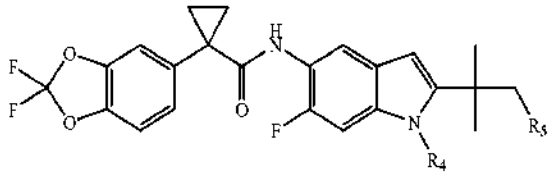
**[0233]** In one embodiment of this kit, two R taken together form $—OCF_2O—$, $R_1$ is H, and $R_2$ is F. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, and $R_3$ is H. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is H. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2CH_2N^+(CH_3)_3$. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$. In another embodiment, two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ and $R_5$ taken together form a five membered ring.

**[0234]** In one embodiment of this kit, two R taken together form $—OCH_2O—$, $R_1$ is H, and $R_2$ is F. In another embodiment, two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, and $R_3$ is H. In another embodiment, two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$. In another embodiment, R is OH, $R_1$ is H, $R_2$ is H, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)$

$CH_2OH$. In another embodiment, at least one R is $OCH_3$, at least two $R_1$ are methyl, $R_2$ is H, $R_3$ is H, and $R_4$ is H. In another embodiment, at least one R is $OCH_3$, at least two $R_1$ are methyl, $R_2$ is H, $R_3$ is H, and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$. In another embodiment, two R taken together form $—CH_2CH_2CH_2—$, $R_1$ is H, $R_2$ is H, $R_3$ is H. and $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$.

**[0235]** In one embodiment of this kit, the compound is represented by formula IIa:

IIa



**[0236]** or a pharmaceutically acceptable salt thereof, wherein:

**[0237]** $R_4$ is H, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and

**[0238]** $R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a five membered ring.

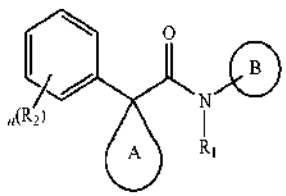
**[0239]** In one embodiment of this kit, $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$. In another embodiment, $R_5$ is OH, $—CH_2OCH_2CH(OH)CH_2OH$, or $—CH_2OH$. In another embodiment, $R_4$ is $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and $R_5$ is OH, $—CH_2OCH_2CH(OH)CH_2OH$, or $—CH_2OH$.

**[0240]** In one embodiment of this kit, the compound is selected from Table 1.

III. Subgeneric Compounds of the Present Invention

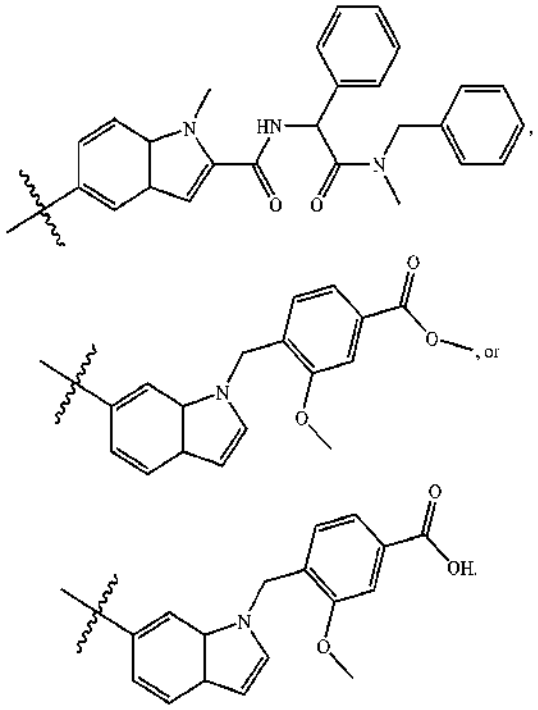
**[0241]** Another aspect of the present invention provides a compound that is useful for modulating ABC transporter activity. The compound has formula Id:

Ic



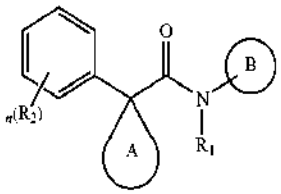
or a pharmaceutically acceptable salt thereof.

**[0242]** $R_1$, $R_2$, and ring A are defined above in formula I, and ring B, $R_3$ and p are defined in formula Ia. Furthermore, when ring A is unsubstituted cyclopentyl, n is 1, $R_2$ is 4-chloro, and $R_1$ is hydrogen, then ring B is not 2-(tertbutyl) indol-5-yl, or (2,6-dichlorophenyl(carbonyl))-3-methyl-1H-indol-5-yl; and when ring A is unsubstituted cyclopentyl, n is 0, and $R_1$ is hydrogen, then ring B is not



**[0243]** Another aspect of the present invention provides a compound that is useful for modulating ABC transporter activity. The compound has formula Id:

Id



or a pharmaceutically acceptable salt thereof.

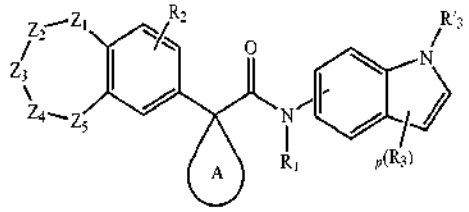
**[0244]** $R_1$, $R_2$, and ring A are defined above in formula I, and ring B, $R_3$ and p are defined in formula Ia.

**[0245]** However, when $R_1$ is H, n is 0, ring A is an unsubstituted cyclopentyl, and ring B is an indole-5-yl substituted with 1-2 of $R_3$, then each $R_3$ is independently $-Z^GR_{12}$, where each $Z^G$ is independently a bond or an unsubstituted branched or straight $C_{1-6}$ aliphatic chain wherein up to two carbon units of $Z^G$ are optionally and independently replaced by $—CS—$, $—CONR^GNR^G—$, $—CO_2—$, $—OCO—$, $—NR^GCO_2—$, $—O—$, $—NR^GCONR^G—$, $—OCONR^G$, $—NR^GNR^G—$, $—S—$, $—SO—$, $—SO_2—$, $—NR^G—$, $—SO_2NR^G$, $—NR^GSO_2—$, or $—NR^GSO_2NR^C—$, each $R_{12}$ is independently $R^G$, halo, $—OH$, $—NH_2$, $—NO_2$, $—CN$, or $—OCF_3$, and each $R^G$ is independently hydrogen, an unsubstituted aliphatic, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an unsubstituted aryl, or an optionally substituted heteroaryl; or any two adjacent $R_3$ groups together with the atoms to which they are attached form an optionally substituted heterocycle. Furthermore, when $R_1$ is H, n is 1, $R_2$ is 4-chloro, ring A is an unsubstituted cyclopentyl, and ring B is an indole-5-yl substituted with 1-2

of $R_3$, then each $R_3$ is independently $-Z^HR_{22}$, where each $Z^H$ is independently a bond or an unsubstituted branched or straight $C_{1-3}$ aliphatic chain wherein up to two carbon units of $Z^H$ are optionally and independently replaced by —CS—, $—CONR^HNR^H$, $—CO_2—$, —OCO—, $—NR^HCO_2—$, —O—, $—NR^HCONR^H—$, $—OCONR^H—$, $—NR^HNR^H—$, —S—, —SO—, $—SO_2—$, $—NR^H—$, $—SO_2NR^H—$, $—NR^HSO_2—$, or $—NR^HSO_2NR^H—$, each $R_{22}$ is independently $R^H$, halo, —OH, $—NH_2$, $—NO_2$, —CN, or $—OCF_3$, and each $R^H$ is independently hydrogen, a substituted $C_4$ alkyl, an optionally substituted $C_{2-6}$ alkenyl, an optionally substituted $C_{2-6}$ alkynyl, an optionally substituted $C_4$ alkenyl, an optionally substituted $C_4$ alkynyl, an optionally substituted cycloaliphatic, an optionally substituted heterocycloaliphatic, an optionally substituted heteroaryl, an unsubstituted phenyl, or a mono-substituted phenyl, or any two adjacent $R_3$ groups together with the atoms to which they are attached form an optionally substituted heterocycle.

**[0246]** Another aspect of the present invention provides a compound that is useful for modulating ABC transporter activity. The compound has formula II:

II

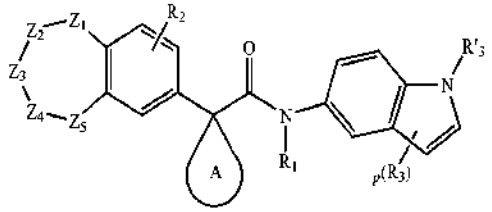


or a pharmaceutically acceptable salt thereof.

**[0247]** $R_1$, $R_2$, and ring A are defined above in formula I; $R_3$, $R'_3$, and p are defined above in formula Ia; and $Z_1$, $Z_2$, $Z_3$, $Z_4$, and $Z_5$ are defined above in formula Ib.

**[0248]** Another aspect of the present invention provides a compound that is useful for modulating ABC transporter activity. The compound has formula IIa:

IIa

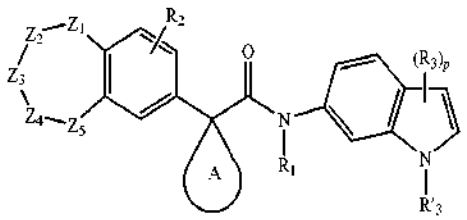


or a pharmaceutically acceptable salt thereof.

**[0249]** $R_1$, $R_2$, and ring A are defined above in formula I; $R_3$, $R'_3$, and p are defined above in formula Ia; and $Z_1$, $Z_2$, $Z_3$, $Z_4$, and $Z_5$ are defined above in formula Ib.

**[0250]** Another aspect of the present invention provides a compound that is useful for modulating ABC transporter activity. The compound has formula IIb:

IIb

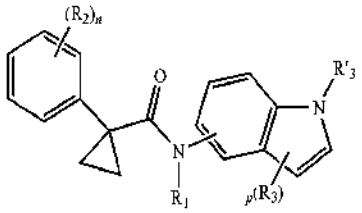


or a pharmaceutically acceptable salt thereof.

**[0251]** $R_1$, $R_2$, and ring A, are defined above in formula I; $R_3$, $R'_3$, and p are defined above in formula Ia; and $Z_1$, $Z_2$, $Z_3$, $Z_4$, and $Z_5$ are defined above in formula Ib.

**[0252]** Another aspect of the present invention provides a compound that is useful for modulating ABC transporter activity. The compound has formula IIc:

IIc

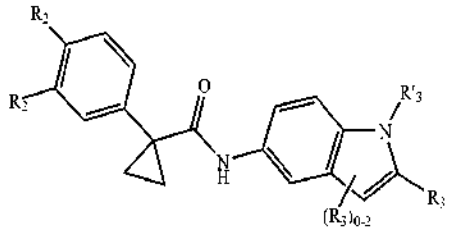


or a pharmaceutically acceptable salt thereof.

**[0253]** $R_1$, $R_2$ and n are defined above in formula I; and $R_3$, $R'_3$, and p are defined in formula Ia.

**[0254]** Another aspect of the present invention provides a compound that is useful for modulating ABC transporter activity. The compound has formula IId:

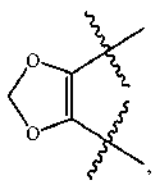
IId



or a pharmaceutically acceptable salt thereof.

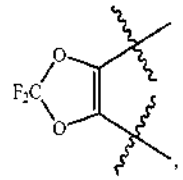
**[0255]** Both $R_2$ groups, together with the atoms to which they are attached form a group selected from:

XA1

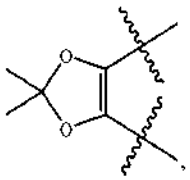


-continued

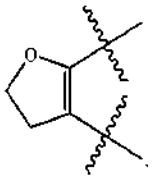
XA2



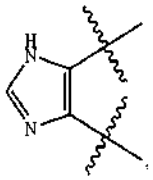
XA3



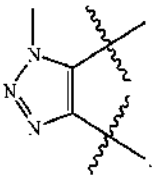
XA4



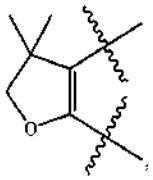
XA5



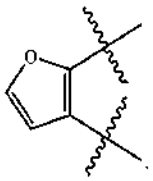
XA6



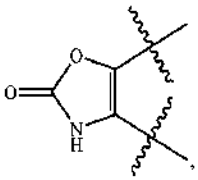
XA7



XA8



XA9



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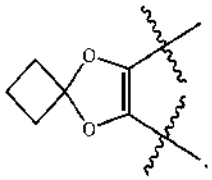
XA10



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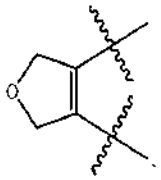
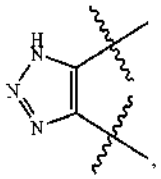


XA12



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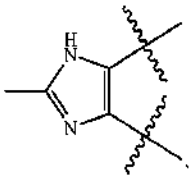

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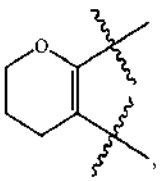

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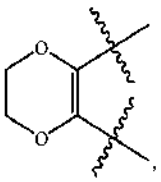

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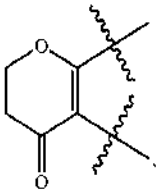

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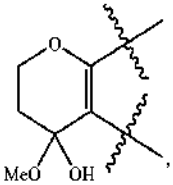

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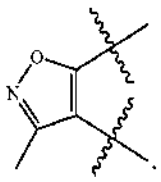

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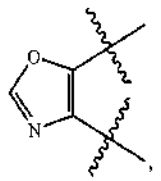

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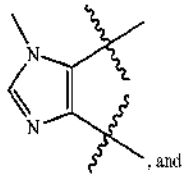

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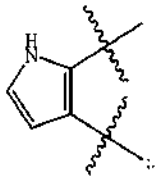
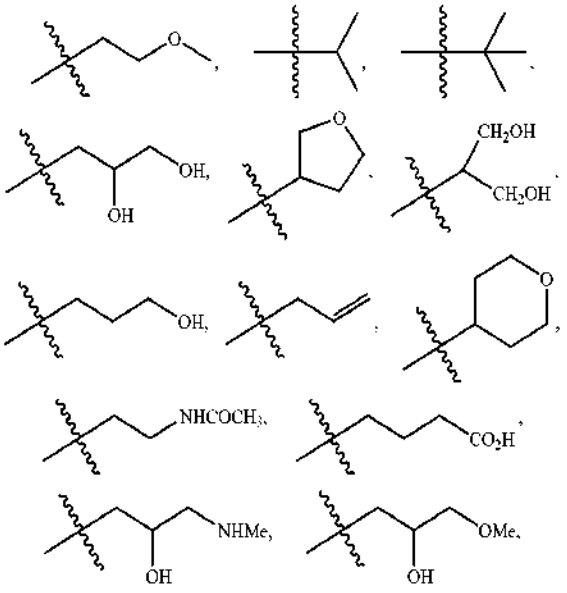


**[0256]** R'$_3$ is independently selected from one of the following:

—H, —$CH_3$, —$CH_2CH_3$, —C(O)$CH_3$, —$CH_2CH_2OH$, —C(O)$OCH_3$,

**[0257]**



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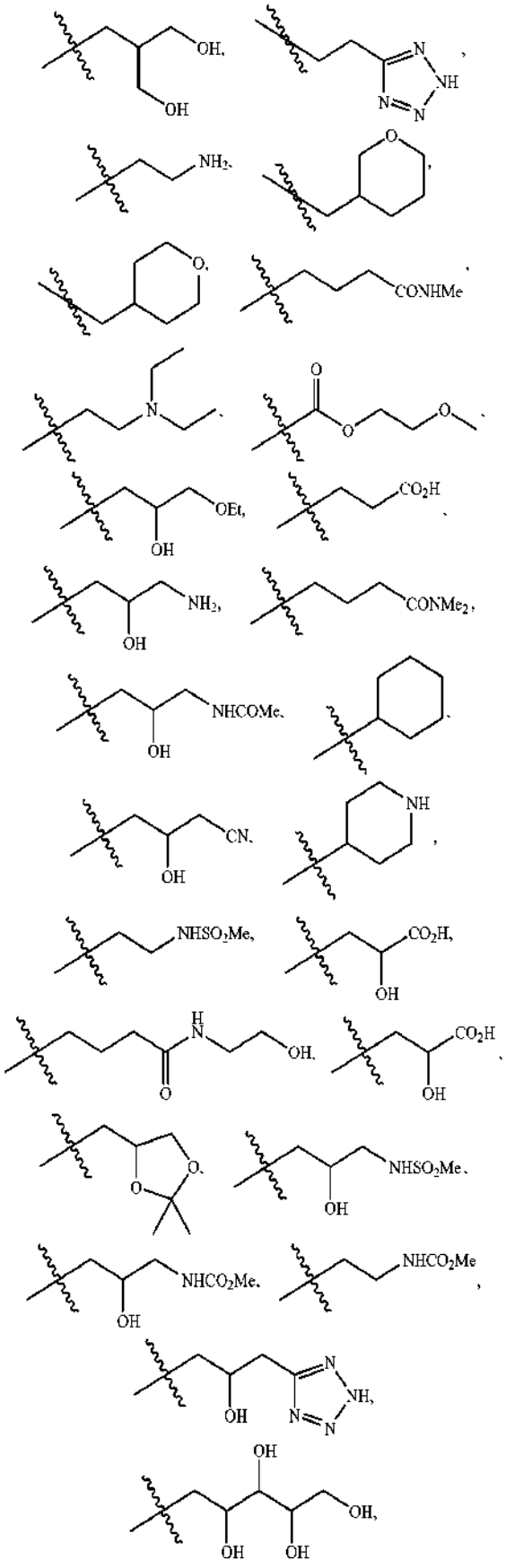

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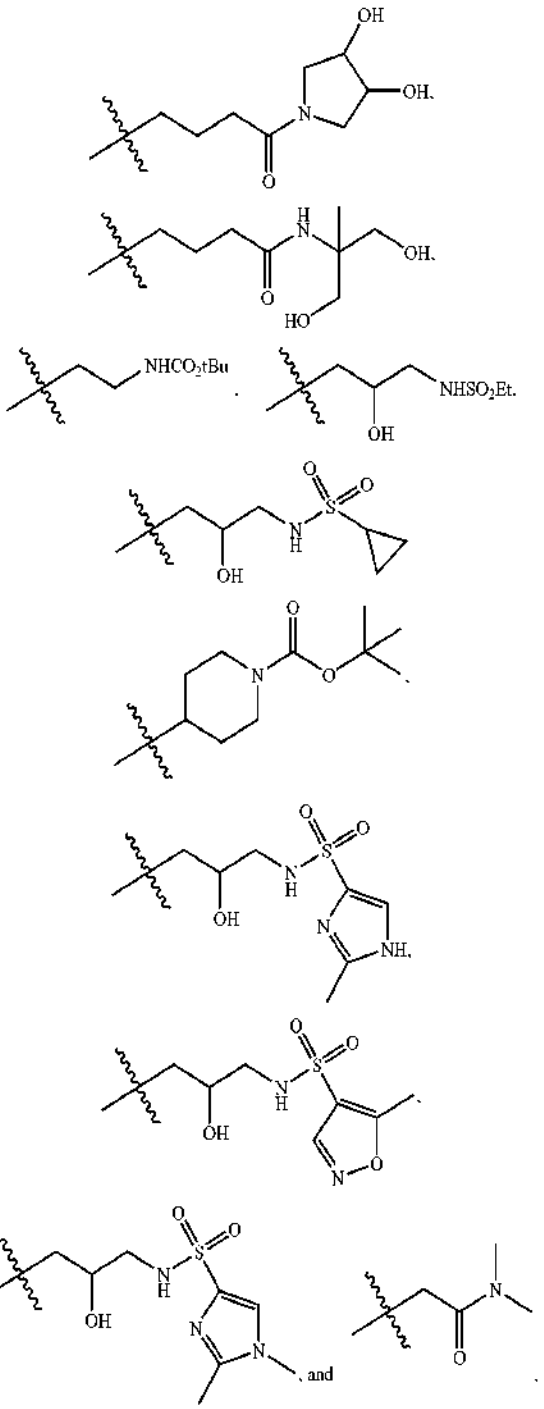
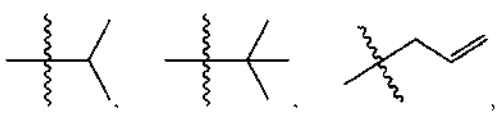


and each $R_3$ is independently selected from —H, —$CH_3$, —$CH_2OH$, —$CH_2CH_3$, —$CH_2CH_2OH$, —$CH_2CH_2CH_3$, —$NH_2$, halo, —$OCH_3$, —CN, —$CF_3$, —$C(O)OCH_2CH_3$, —$S(O)_2CH_3$, —$CH_2NH_2$, —$C(O)NH_2$,



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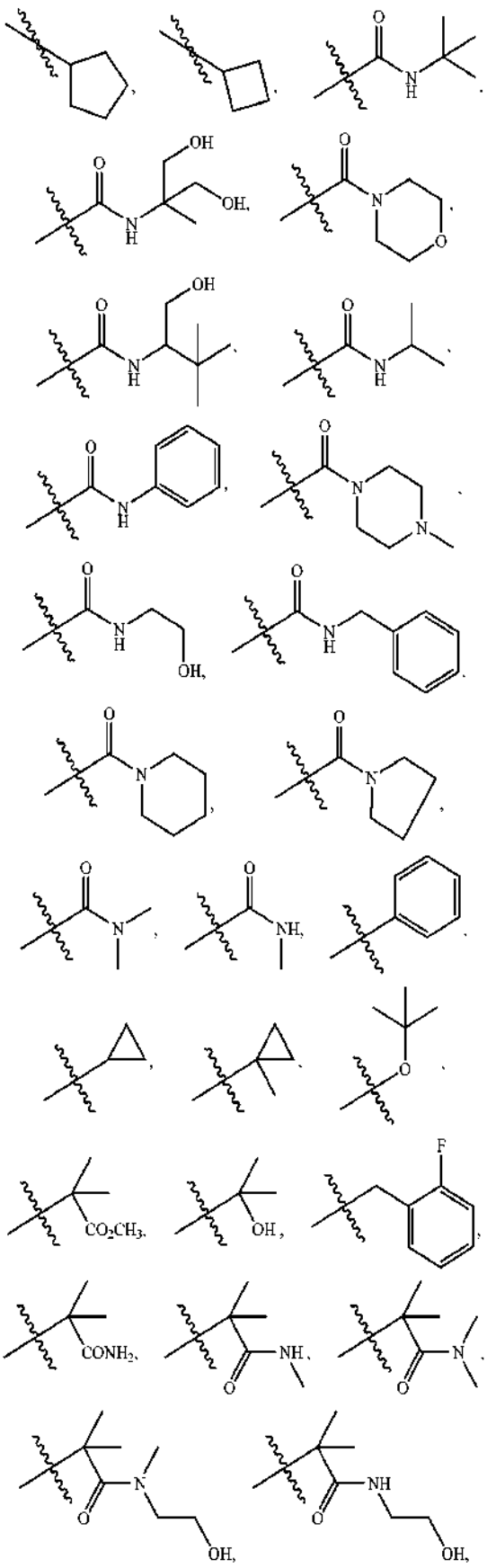

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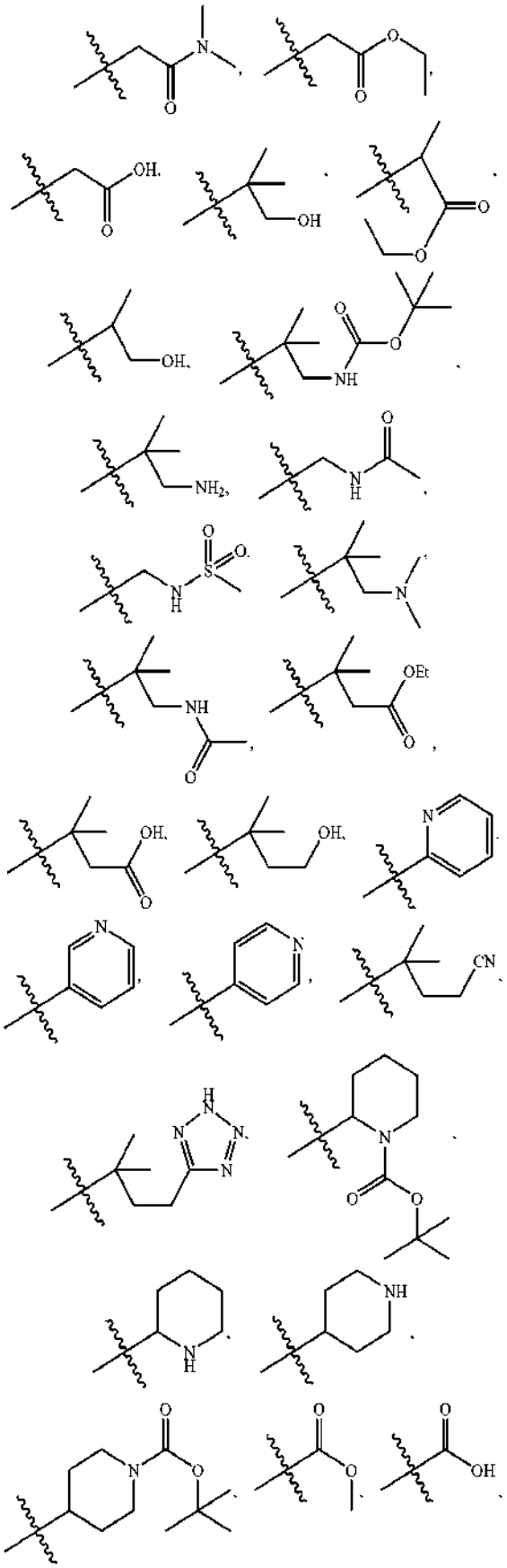

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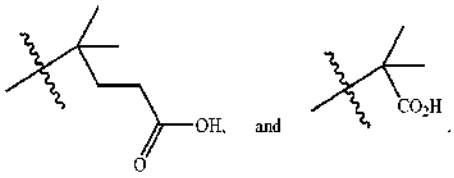
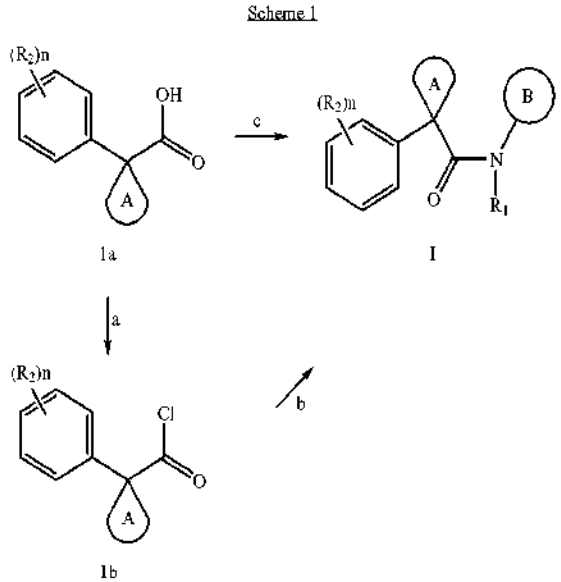


IV. Generic Synthetic Schemes

**[0258]** The compounds of formulae (I, Ic, Id, II, IIa, IIb, IIc, and IId) may be readily synthesized from commercially available or known starting materials by known methods. Exemplary synthetic routes to produce compounds of formulae (I, Ic, Id, II, IIa, IIb, IIc, and IId) are provided below in Schemes 1-22 below.

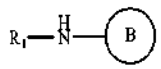
**[0259]** Preparation of the Compounds of the Invention is Achieved by the Coupling of a Ring B amine with a ring A carboxylic acid as illustrated in Scheme 1.

Scheme 1



**[0260]** Referring to Scheme 1, the acid 1a may be converted to the corresponding acid chloride 1b using thionyl chloride in the presence of a catalystic amount of dimethylformamide.

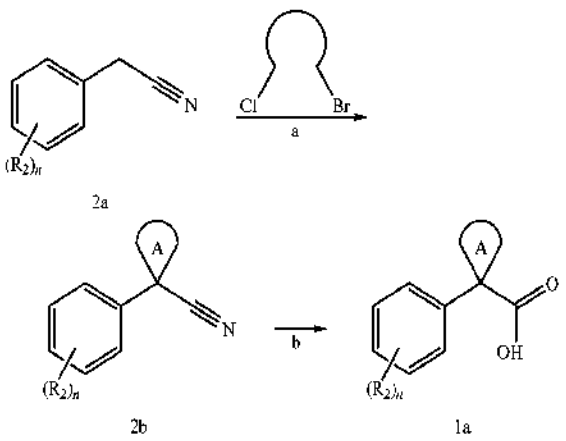
**[0261]** Reaction of the acid chloride with the amine



provides compounds of the invention I. Alternatively, the acid 1a may be directly coupled to the amine using known coupling reagents such as, for example, HATU in the presence of triethylamine.

**[0262]** Preparation of the acids 1a may be achieved as illustrated in Scheme 2.

Scheme 2

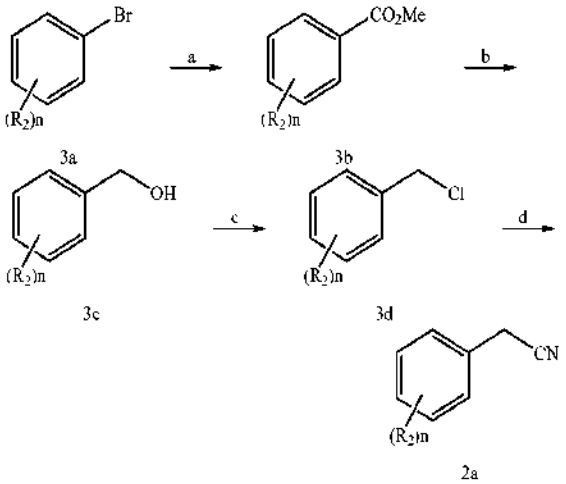



a) NaOH, BTEAC;
b) NaOH, Δ

**[0263]** Referring to Scheme 2, the nitrile 2a reacts with a suitable bromochloroalkane in the presence of sodium hydroxide and a phase transfer catalyst such as butyltriethylammonium chloride to provide the intermediate 2b. Hydrolysis of the nitrile of 2b provides the acid 1a. In some instances, isolation of the intermediate 2b is unnecessary.

**[0264]** The phenylacetonitriles 2a are commercially available or may be prepared as illustrated in Scheme 3.

Scheme 3

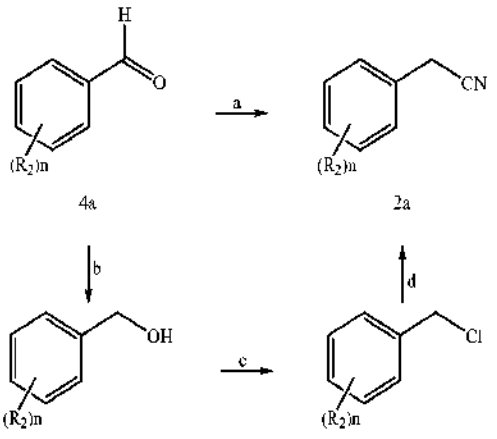



a) $Pd(PPh_3)_4$, CO, MeOH;
b) $LiAlH_4$, THF;
c) $SOCl_2$;
d) NaCN

**[0265]** Referring to Scheme 3, reaction of an aryl bromide 3a with carbon monoxide in the presence of methanol and tetrakis(triphenylphosphine)palladium (0) provides the ester 3b. Reduction of 3b with lithium aluminum hydride provides the alcohol 3c which is converted to the halide 3d with thionyl chloride. Reaction of 3d with sodium cyanide provides the nitrile 2a.

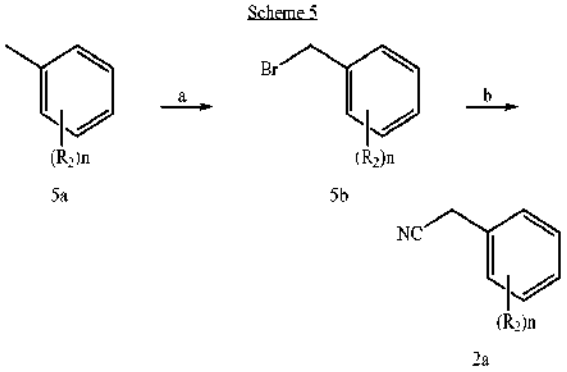
**[0266]** Other methods of producing the nitrile 2a are illustrated in schemes 4 and 5 below.

Scheme 4



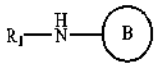

a) TosMIC;
b) $NaBH_4$, THF;
c) $SOCl_2$;
d) NaCN

Scheme 5



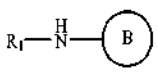

a) NBS, AIBN, $CCl_4$;
b) NaCN, EtOH

**[0267]** Preparation of



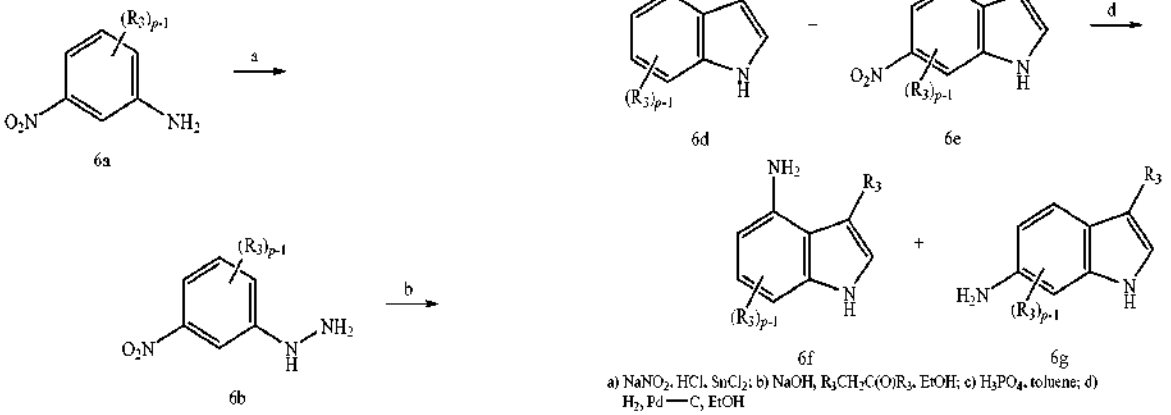

components is illustrated in the schemes that follow. A number of methods for preparing ring B compounds wherein ring B is an indole have been reported. See for example Angew. Chem. 2005,44, 606; J. Am. Chem. Soc. 2005, 127, 5342); J. Comb. Chem. 2005, 7, 130; Tetrahedron 2006, 62, 3439; J. Chem. Soc. Perkin Trans. 1, 2000, 1045.

**[0268]** One method for preparing



is illustrated in Scheme 6.

Scheme 6

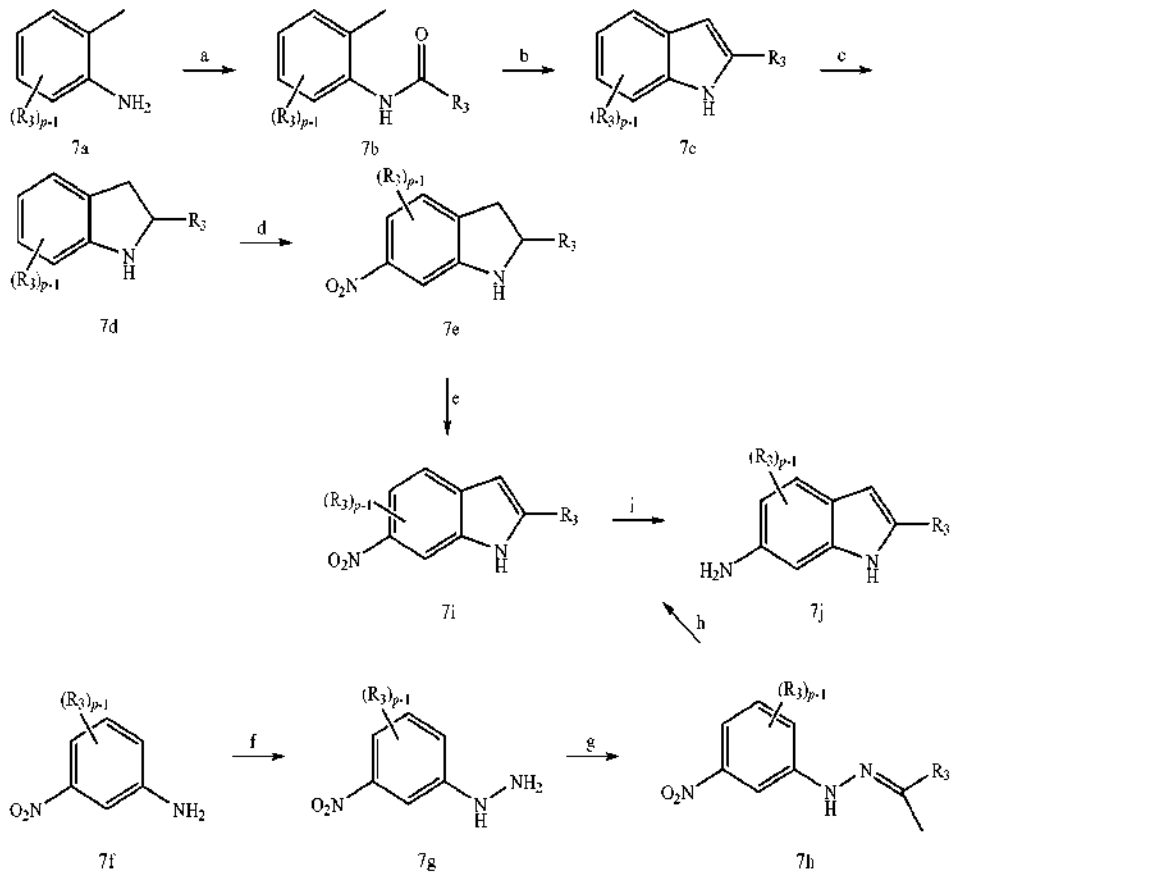



a) $NaNO_2$, HCl, $SnCl_2$; b) NaOH, $R_3CH_2C(O)R_3$, EtOH; c) $H_3PO_4$, toluene; d) $H_2$, Pd—C, EtOH

**[0269]** Referring to Scheme 6, a nitroaniline 6a is converted to the hydrazine 6b using nitrous acid in the presence of HCl and stannous chloride. Reaction of 6b with an aldehyde or ketone $CH_3C(O)R_3$ provides the hydrazone 6c which on treatment with phosphoric acid in toluene leads to a mixture of nitro indoles 6d and 6e. Catalytic hydrogenation in the presence of palladium on carbon provides a mixture of the amino indoles 6f and 6g which may be separated using know methods such as, for example, chromatography.

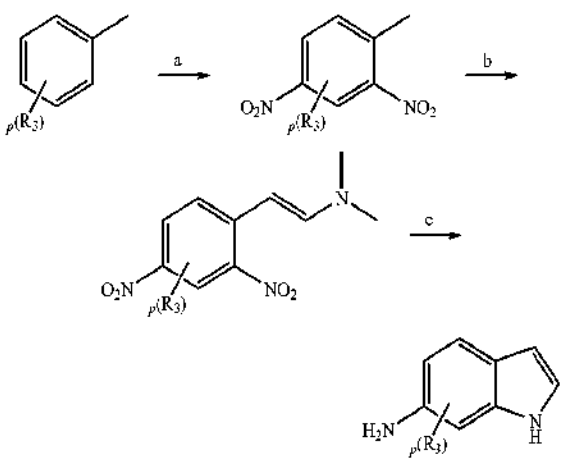
**[0270]** An alternative method is illustrated in scheme 7.

Scheme 7



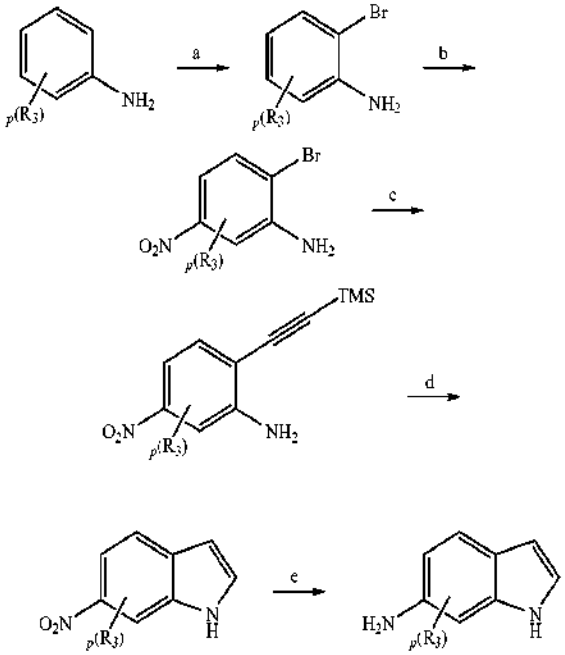

a) $R_3aCOCl$, $Et_3N$, $CH_2Cl_2$; b) n-BuLi, THF; c) $NaBH_4$, AcOH; d) $KNO_3$, $H_2SO_4$; e) DDQ, 1,4-dioxane; f) $NaNO_2$, HCl, $SnCl_2{\cdot}2H_2O$, $H_2O$; g) $MeCOR_3$, EtOH; h) PPA; i) Pd/C, EtOH or $H_2$, Raney Ni, EtOH or MeOH

Scheme 8



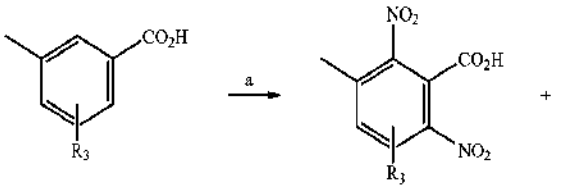
a) $HNO_3$, $H_2SO_4$; b) $Me_2NCH(OMe)_2$, DMF; c) $H_2$, Raney Ni, EtOH

Scheme 9



a) NBS, DMF; b) $KNO_3$, $H_2SO_4$; c) HC≡C-TMS, $Pd(PPh_3)_2Cl_2$, CuI, $Et_3N$, toluene, $H_2O$; d) CuI, DMF; e) $H_2$, Raney Ni, MeOH

Scheme 10



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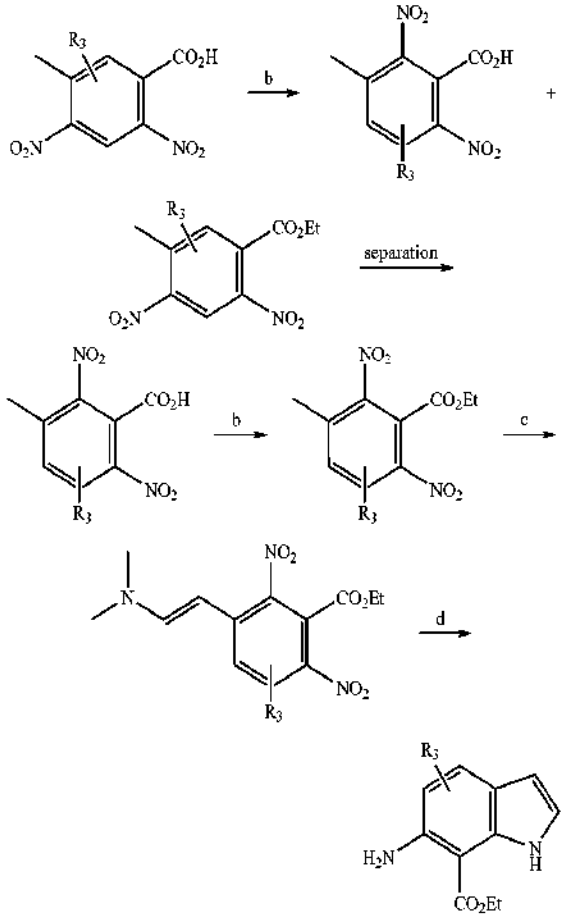
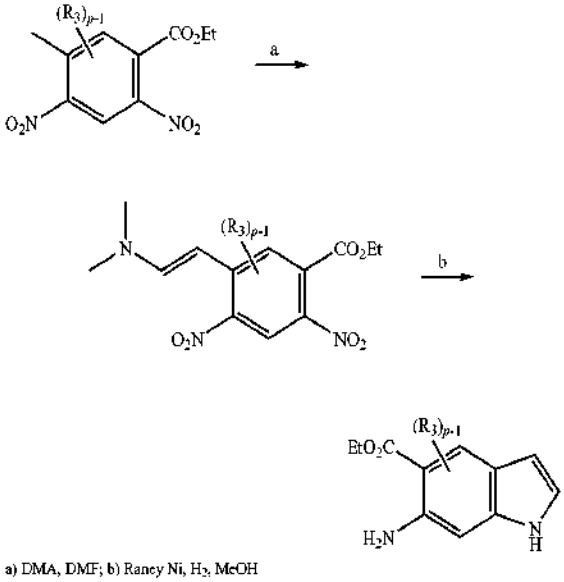


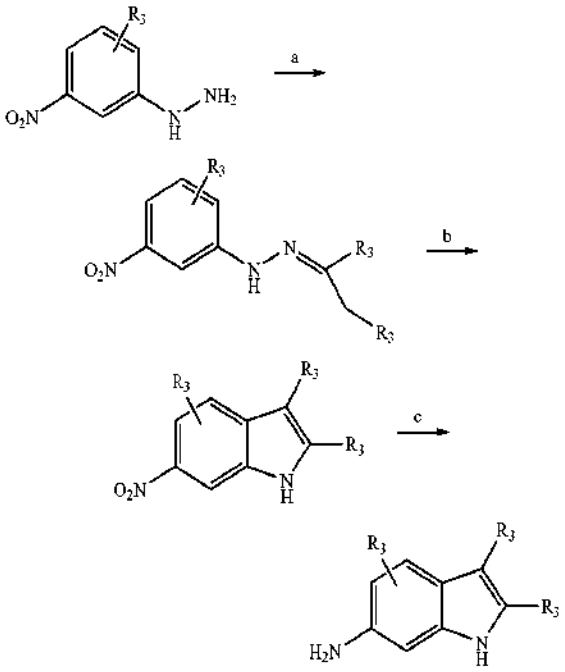
a) $HNO_3$, $H_2SO_4$; b) $SOCl_2$; EtOH; c) DMA, DMF; d) Raney Ni, $H_2$, MeOH

Scheme 11



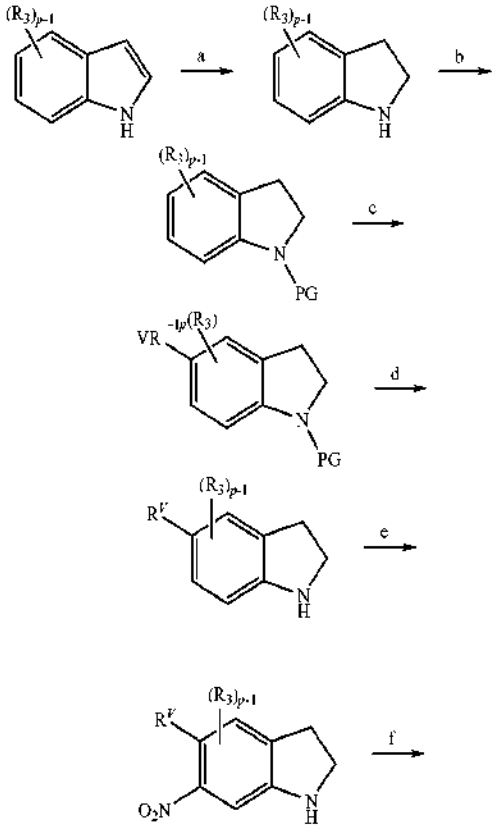
a) DMA, DMF; b) Raney Ni, $H_2$, MeOH

Scheme 12



a) $R_3CH_2COR_3$, AcOH, EtOH; b) $H_3PO_4$, toluene; c) $H_2$, Pd/C, EtOH

Scheme 14



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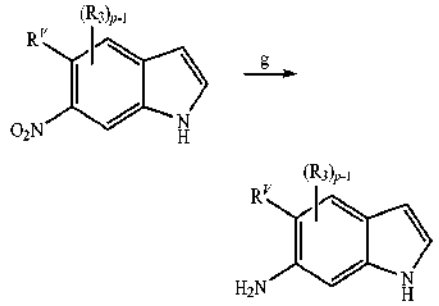
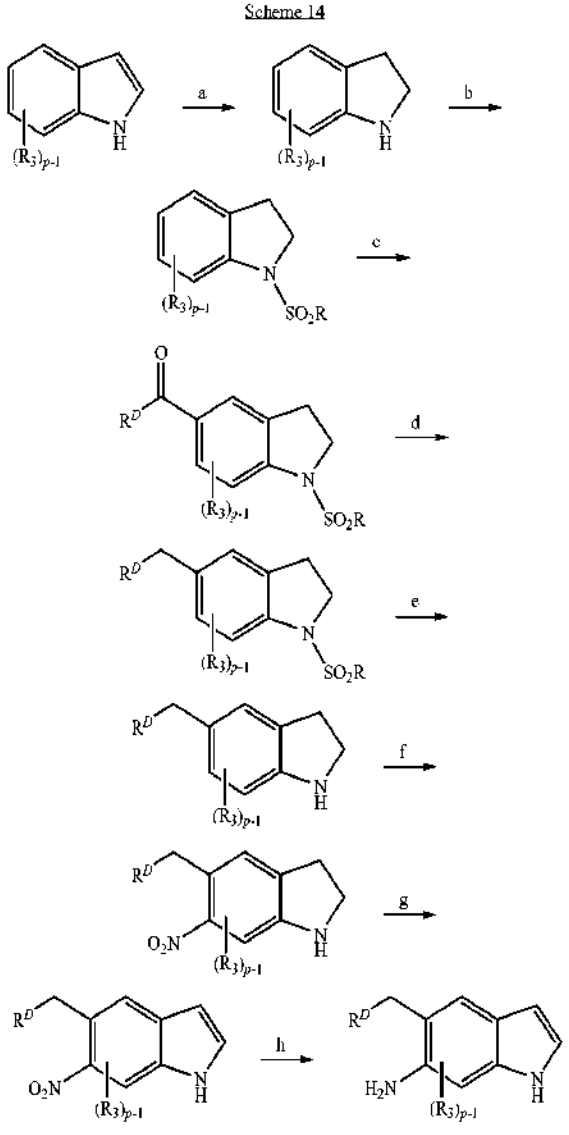


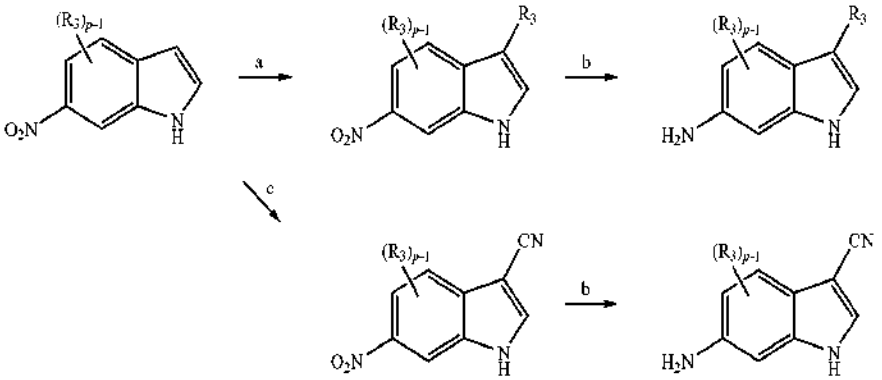
a) $NaBH_3CN$; b) When PG = $SO_2Ph$: $PhSO_2Cl$, $Et_3N$, DMAP, $CH_2Cl_2$; When PG = Ac: AcCl, $NaHCO_3$, $CH_2Cl_2$; c) When $R^V$ = RCO: $(RCO)_2O$, $AlCl_3$, $CH_2Cl_2$; When $R^V$ = Br: $Br_2$, AcOH; d) HBr or HCl; e) $KNO_3$, $H_2SO_4$; f) $MnO_2$, $CH_2Cl_2$ or DDQ, 1,4-dioxane; g) $H_2$, Raney Ni, EtOH.

Scheme 14



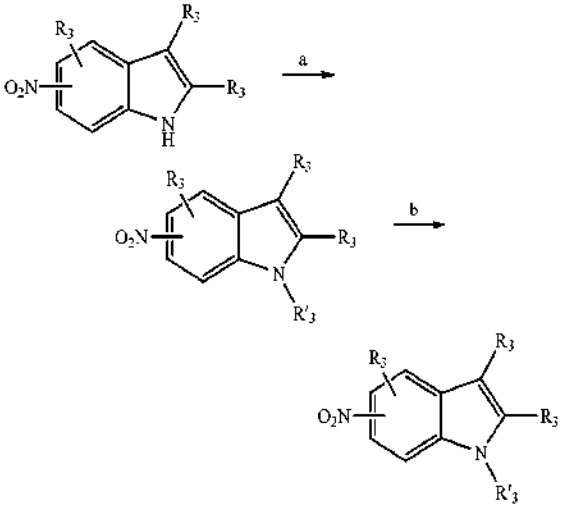
a) $NaBH_3CN$; b) $RSO_2Cl$, DMAP, $Et_3N$, $CH_2Cl_2$; c) $R^DC(O)Cl$, $AlCl_3$, $CH_2Cl_2$; d) $NaBH_4$, THF; e) HBr; f) $KNO_3$, $H_2SO_4$; g) $MnO_2$; g) Raney Ni, $H_2$, EtOH

Scheme 15



a) $R_3X$ (X = Br, I), zinc triflate, TBAI, DIEA, toluene; b) $H_2$, Raney Ni, EtOH or $H_2$, Pd/C, EtOH or $SnCl_2 \cdot 2H_2O$, EtOH; c) $ClSO_2NCO$, DMF, $CH_3CN$

Scheme 16



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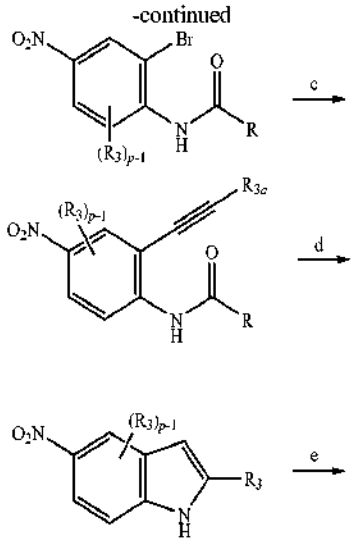
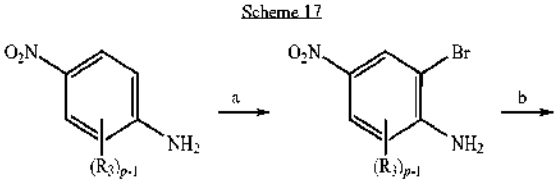
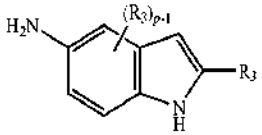


a) when X = Cl, Br, I, or OTs: $R'_3X$, $K_2CO_3$, DMF or $CH_3CN$; b) $H_2$, Pd/C, EtOH or $SnCl_2 \cdot 2H_2O$, EtOH or $SnCl_2 \cdot 2H_2O$, DIEA, EtOH

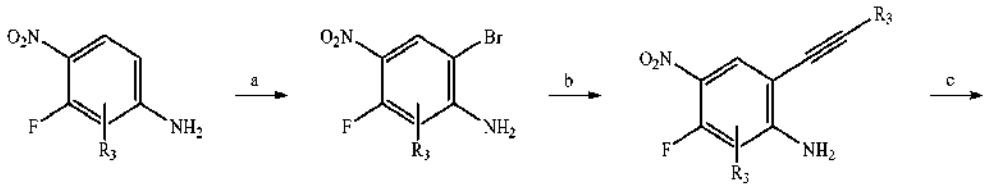
Scheme 17





a) $Br_2$, AcOH; b) RC(O)Cl, $Et_3N$, $CH_2Cl_2$; c) $HC{\equiv}CR_{3a}$, $Pd(PPh_3)_2Cl_2$, CuI, $Et_3N$; d) TBAF, THF or tBuOK, DMF or $Pd(PPh_3)_2Cl_2$, CuI, DMF; e) $H_2$, Pd/C, EtOH or $SnCl_2$, MeOH or $HCO_2NH_4$, Pd/C, EtOH

Scheme 18



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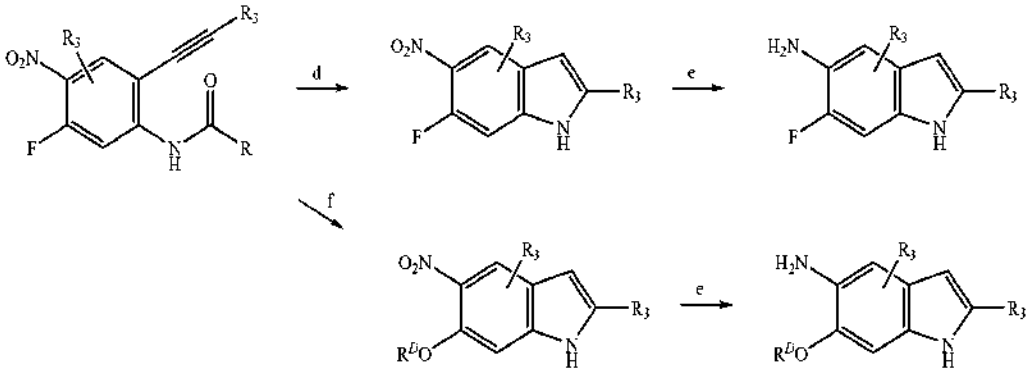
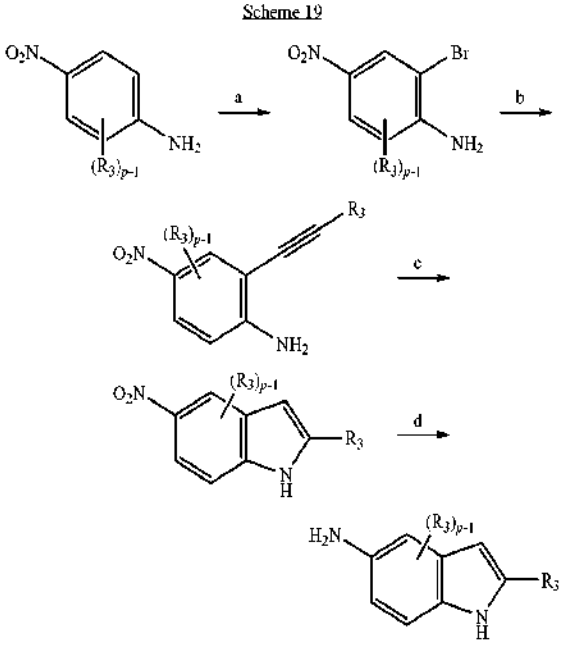


a) $Br_2$, AcOH, $CHCl_3$; b) $R_3C{\equiv}CH$, CuI, $Et_3N$, $Pd(PPh_3)_2Cl_2$; c) RCOCl, $Et_3N$, $CH_2Cl_2$; d) TBAF, DMF; e) Raney Ni, $H_2$, MeOH; f) ROK, DMF

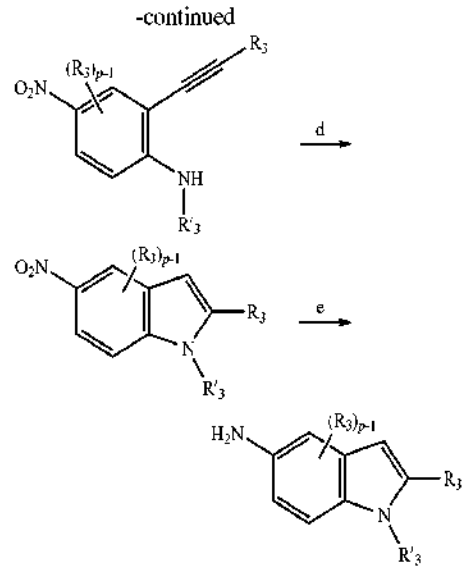
Scheme 19



a) $Br_2$, AcOH; b) $HC{\equiv}CR_3$,

$Pd(PPh_3)_2Cl_2$, CuI, $Et_3N$; c) $Pd(PPh_3)_2Cl_2$, CuI, DMF; d) $H_2$, Pd/C, EtOH or $SnCl_2$, MeOH or $HCO_2NH_4$, Pd/C, EtOH

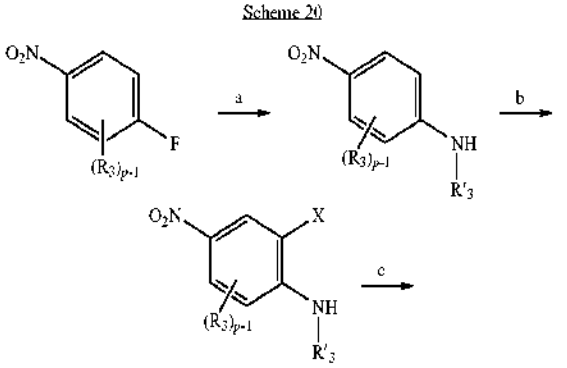
-continued



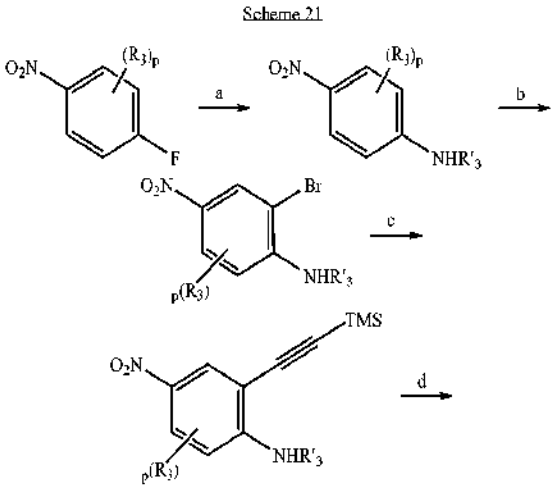
a) $H_2NR'_3$; b) X = Br: $Br_2$, HOAc; X = I: NIS; c) $HC{\equiv}CR_3$,

$Pd(PPh_3)_2Cl_2$, CuI, $Et_3N$; d) CuI, DMF or TBAF, THF; e) $H_2$, Pd/C, EtOH or $SnCl_2$, MeOH or $HCO_2NH_4$, Pd/C, EtOH

Scheme 20



Scheme 21



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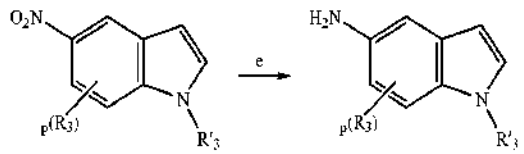
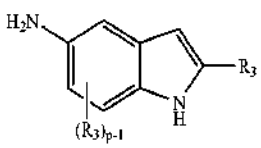


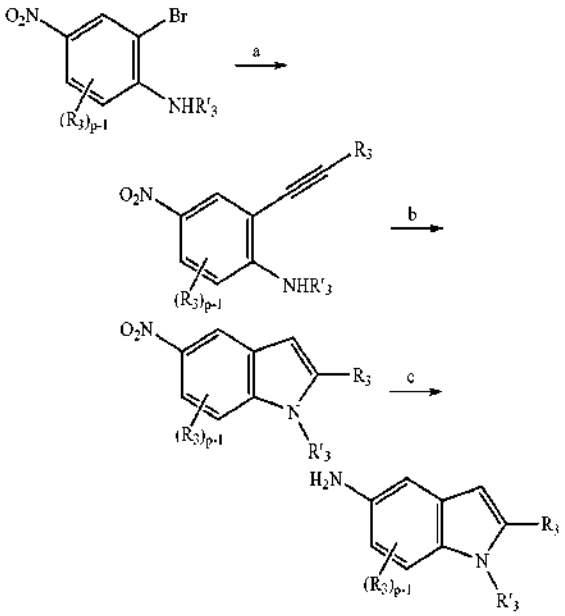
a) $R'_3NH_2$, DMSO; b) $Br_2$, AcOH; c) TMS-C≡CH, CuI, TEA, $Pd(PPh_3)_2Cl_2$; d) CuI, DMSO; e) Raney Ni, $H_2$, MeOH

-continued



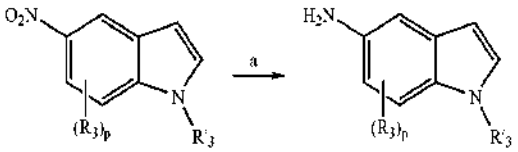
a) $NaBH_4$, $NiCl_2$, MeOH; b) RC(O)Cl; c) $Pd(PPh_3)Cl_2$, HC≡C−$R_3$, CuI, $Et_3N$; d) tBuOK, DMF; e) $KNO_3$, $H_2SO_4$; f) $NaBH_4$, $NiCl_2$, MeOH

Scheme 22



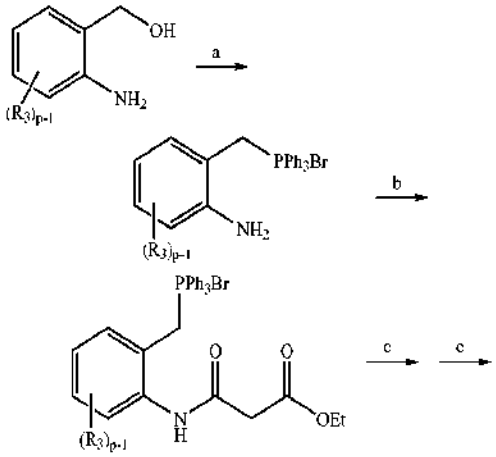
a) $R_3C$≡CH, CuI, TEA, $Pd(PPh_3)_2Cl_2$; b) TBAF, THF; c) Raney Ni, MeOH

Scheme 24

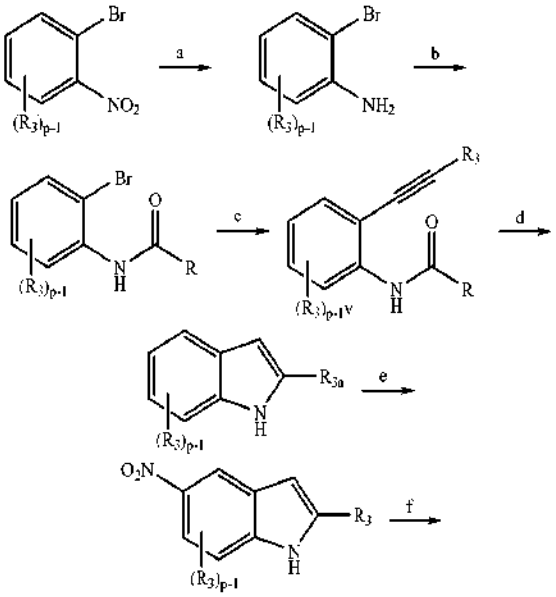


a) $SnCl_2$, EtOH or Pd/C, $HCO_2NH_4$ or $H_2$, Pd/C, EtOH or Raney Ni, $H_2$, EtOH

Scheme 25



Scheme 23



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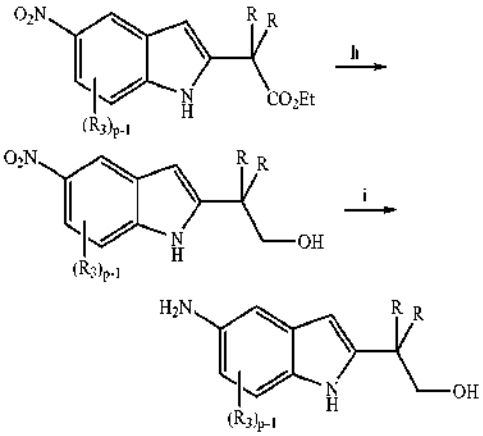
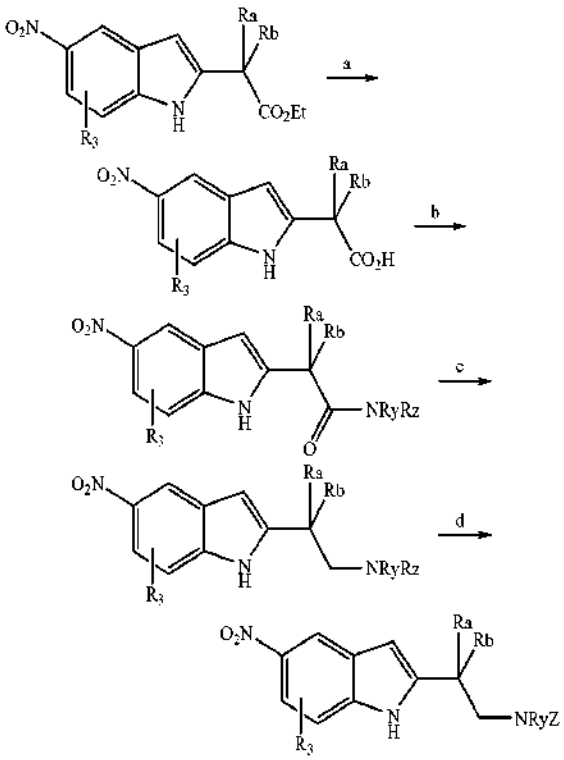


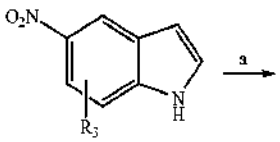
a) $PPh_3$, HBr; b) $Cl(O)CCH_2CO_2Et$; c) tBuOK; d) $(Boc)_2O$, DMAP; e) KHMDS, R-X; KHMDS, R-X; f) TFA; g) $NaNO_2$, $H_2SO_4$; h) $LiAlH_4$, THF; i) $SnCl_2$, EtOH

Scheme 26



a) LiOH; b) EDC, HOBt, $Et_3N$, HNRyRz; c) $BH_3$-THF; d) if Rz = H, RC(O)Cl (Z = RC(O)-) or $RSO_2Cl$ (Z = $RSO_2$-) or RO(CO)Cl (Z = RO(CO)-) or $(RO(CO))_2O$ (Z = Z = RO(CO)-), $Et_3N$, $CH_2Cl_2$

Scheme 27



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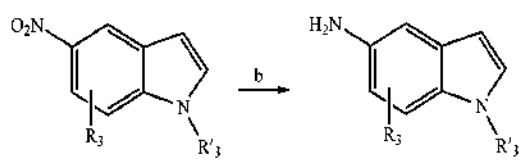
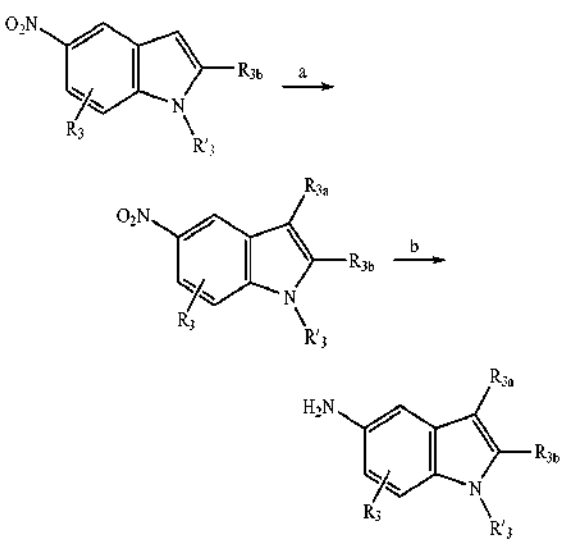


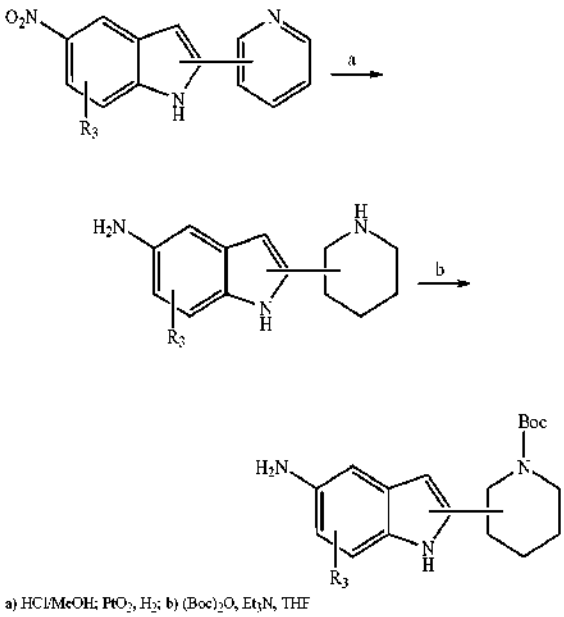
a) R'3-X (X = Br, I, or OTs), base ($K_2CO_3$ or $Cs_2CO_3$), DMF or $CH_3CN$; b) $H_2$, Pd/C, EtOH or Pd/C, $HCO_2NH_4$

Scheme 28



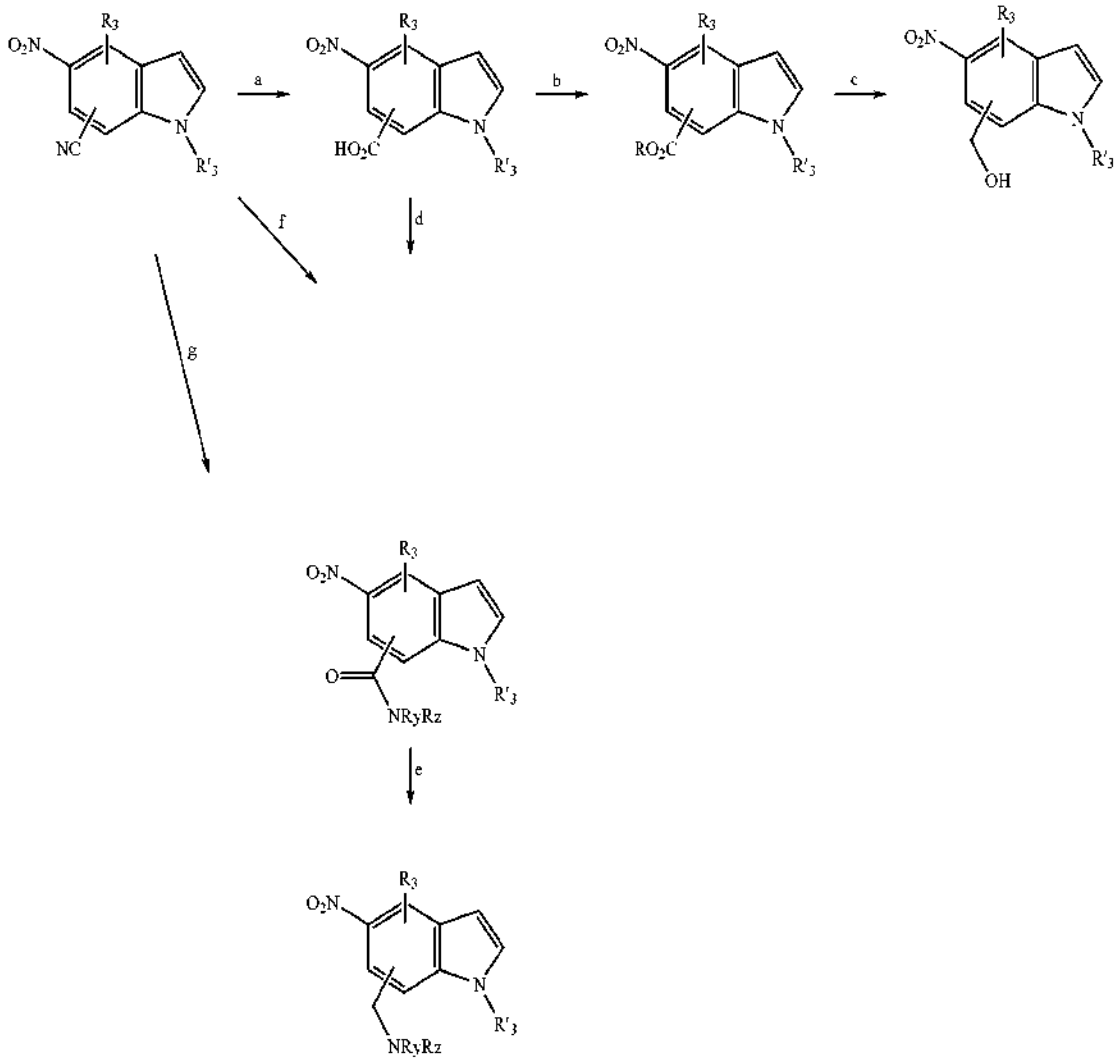
a) $R_{3a}X$ (X = Cl, Br, I), $AlCl_3$, $CH_2Cl_2$; b) Raney Ni, $H_2$, MeOH

Scheme 29



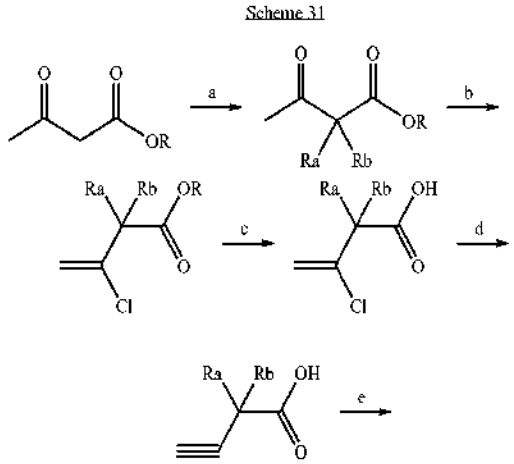
a) HCl/MeOH; $PtO_2$, $H_2$; b) $(Boc)_2O$, $Et_3N$, THF

Scheme 30



a) NaOH or LiOH; b) ROH, HCl; c) $NaBH_4$ or $LiAlH_4$ or DIBAL-H, THF; d) HNRyRz, HATU, $Et_3N$, EtOH or DMF; e) $LiAlH_4$, THF or $BH_3$•THF; f) $H_2O_2$, $H_2O$ (Ry = Rz = H); g) $H_2$, Pd/C

Scheme 31



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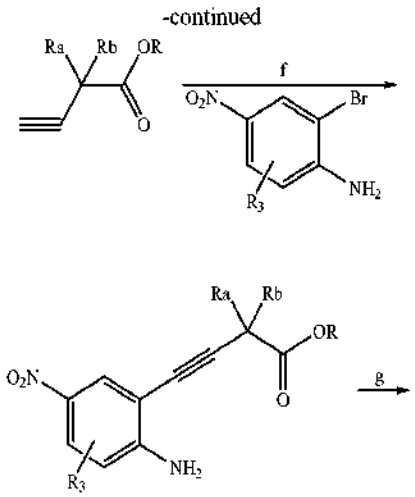

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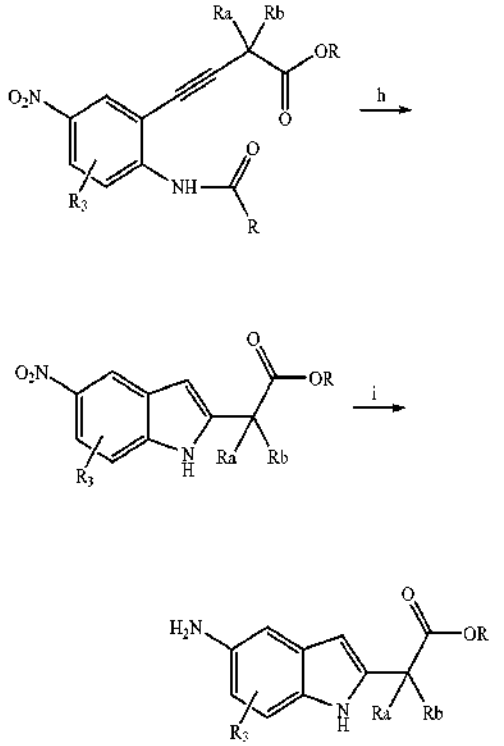
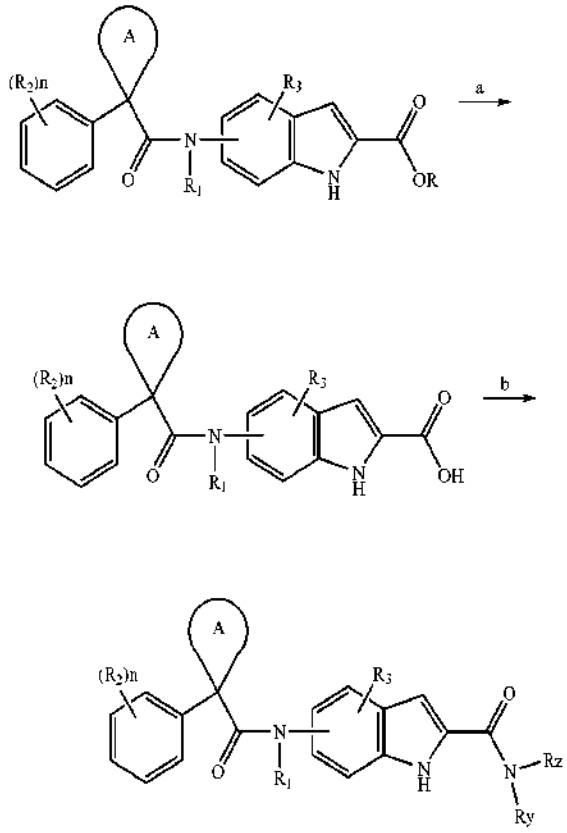


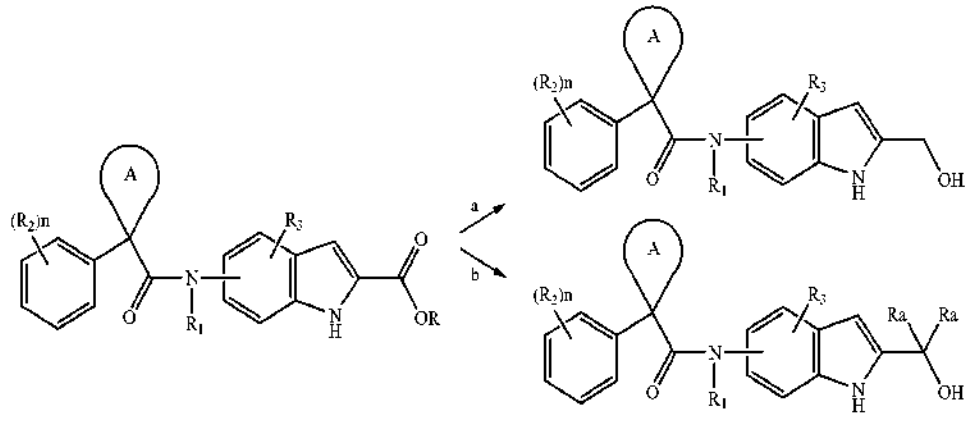
a) $R_a$-X, NaH; $R_b$-X, NaH; b) $PCl_5$, $CH_2Cl_2$; c) NaOH; d) $NaNH_2$, DMSO; e) $CH_2N_2$; f) $Pd(PPh_3)_4$, CuI, $Et_3N$; g) RC(O)Cl, pyr, $CH_2Cl_2$; h) $Pd(CH_3CN)_2Cl_2$, $CH_3CN$; i) Raney Ni, $H_2$, MeOH

Scheme 32



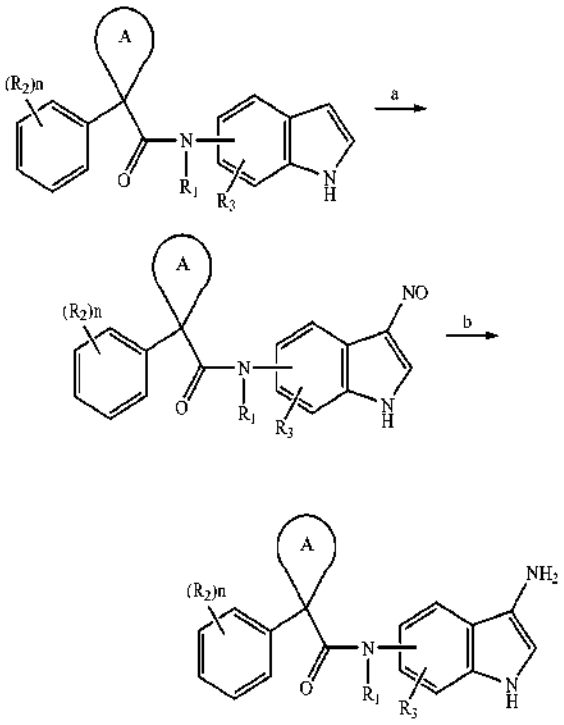
a) LiOH, $THF/H_2O$; b) $HNR_yR_z$, HATU, TEA, $DMF/CH_2Cl_2$

Scheme 33



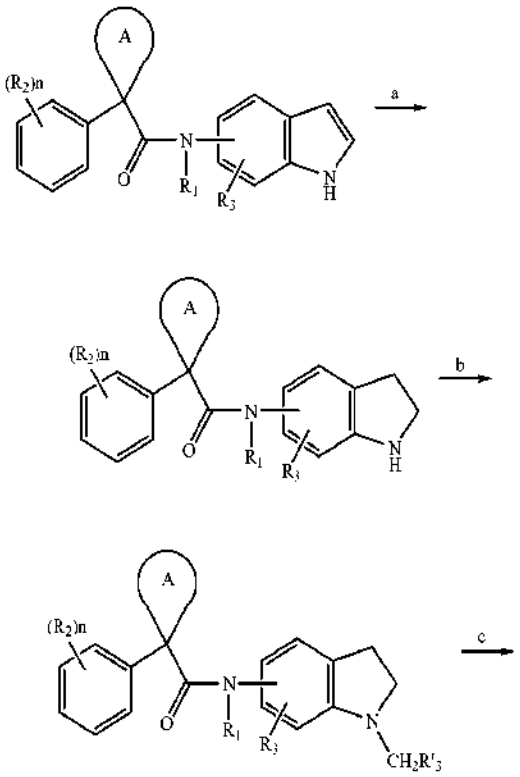
a) $LiBH_4$, $THF/H_2O$ or $LiAlH_4$, THF; b) $R_a$—Li, THF

Scheme 34



a) $NaNO_2$, $AcOH$-$H_2O$; b) Zn, AcOH

Scheme 35



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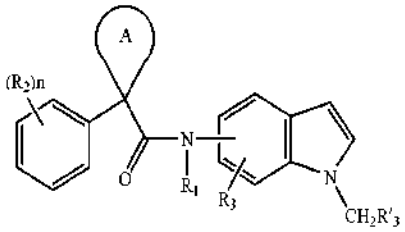
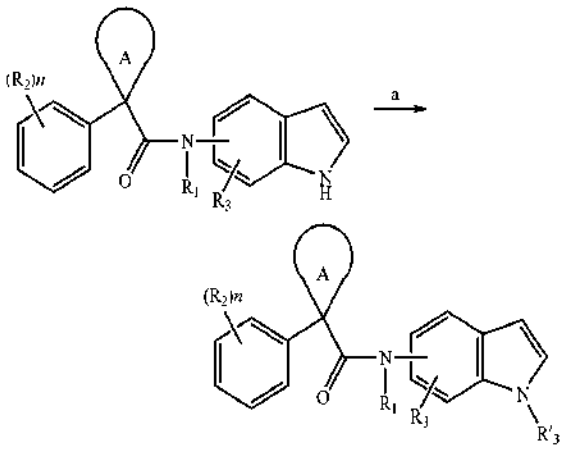


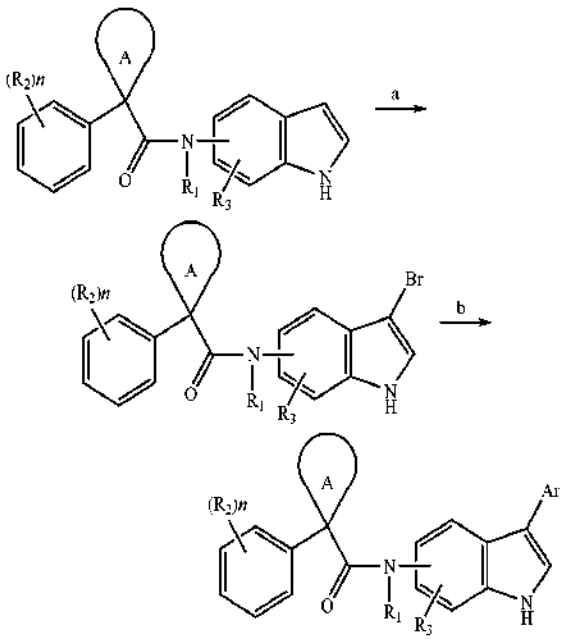
a) $NaBH_3CN$; b) $R'_3CHO$, $NaHB(OAc)_3$, TFA, DCE; c) chloranil or $CDCl_3$, light or DDQ

Scheme 36



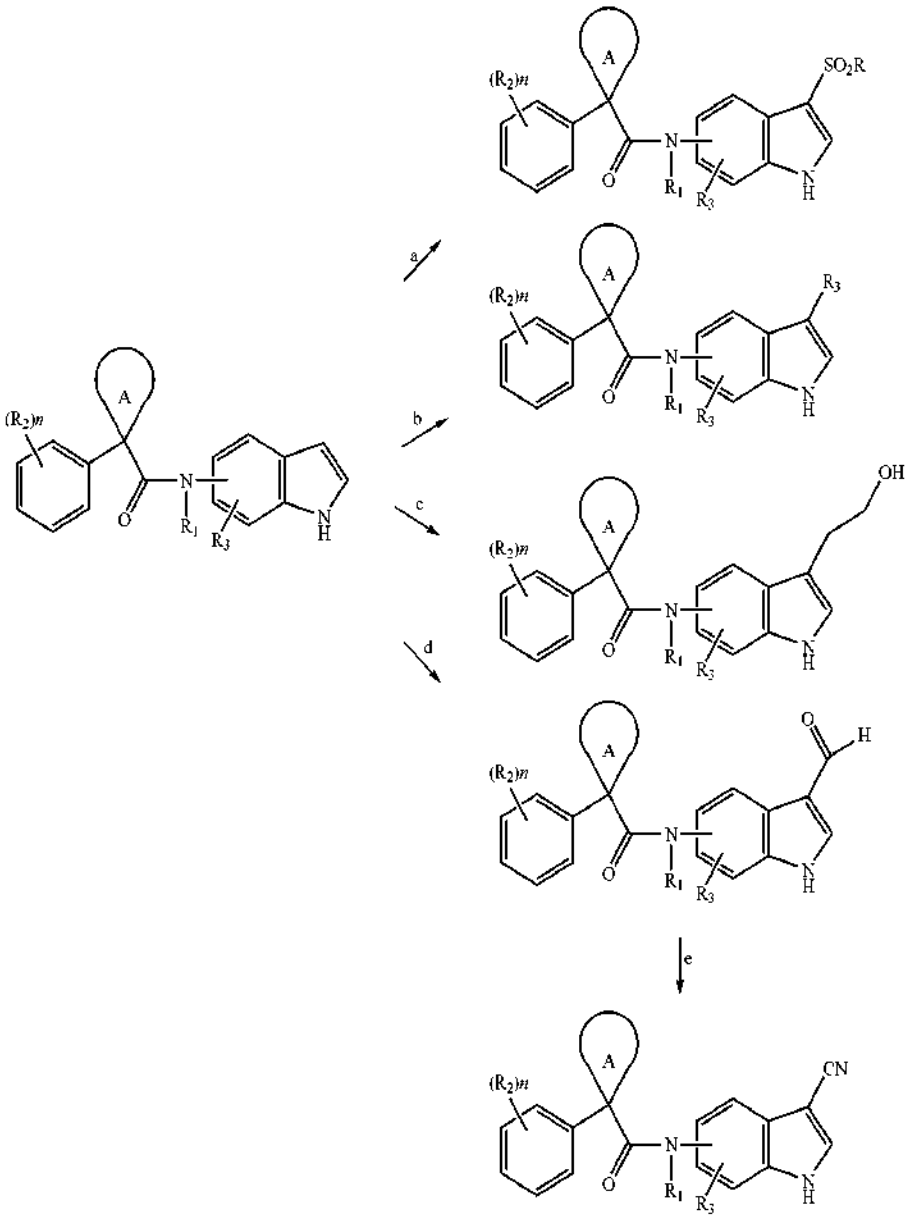
a) NaH, DMF-THF; $R_3$—X (X = Cl, Br, I, or OTs)

Scheme 37



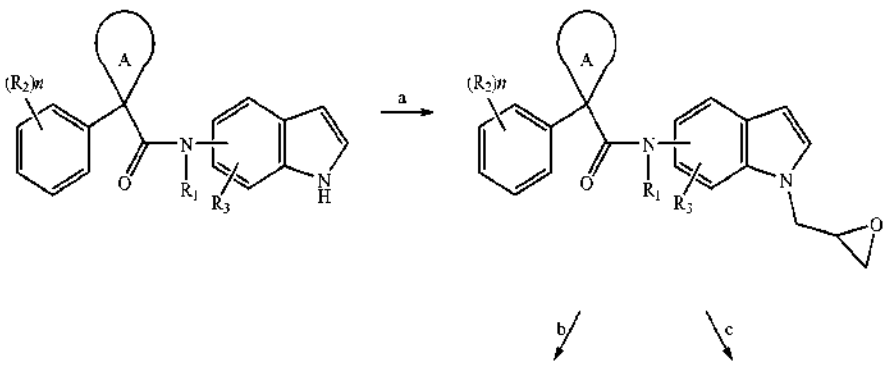
a) NBS; b) Ar—$B(OR)_2$, Pd-FibreCat 1007, $K_2CO_3$, EtOH

Scheme 38



a) $RSO_2Cl$, NaH, THF-DMF; b) $R_3-X$ (X = Br, I, or OTs), NaH. THF-DMF; c) ethylene dioxide, $InCl_3$; d) $POCl_3$, DMF; e) $H_2N-OH$, $CH_2Cl_2$; $Ac_2O$

Scheme 39



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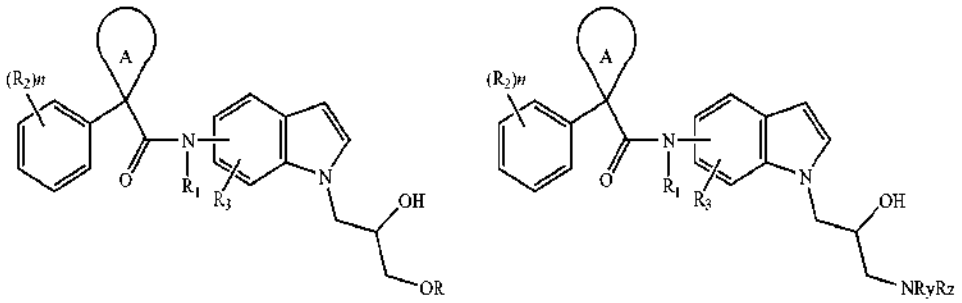
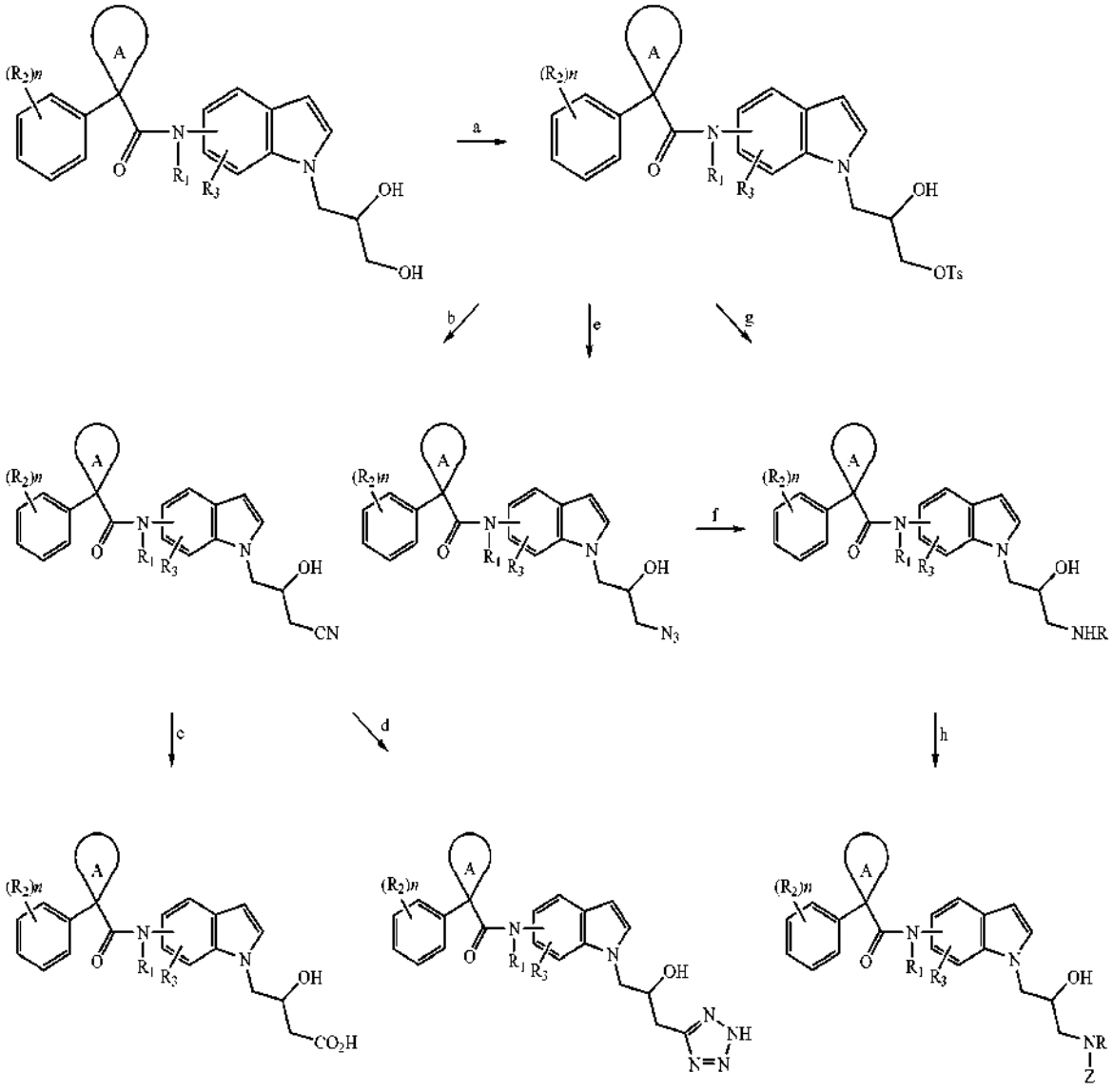


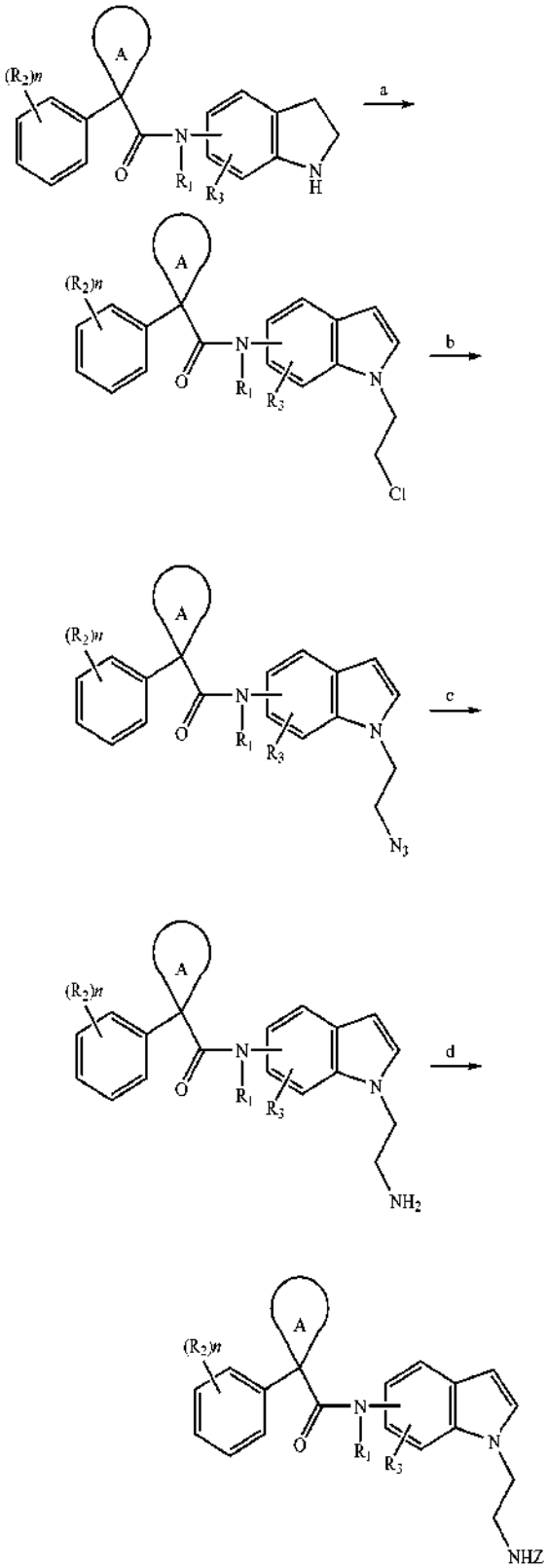
a) NaH, THF-DMF; epichlorohydrin; b) ROH; c) HNRyRz

Scheme 40



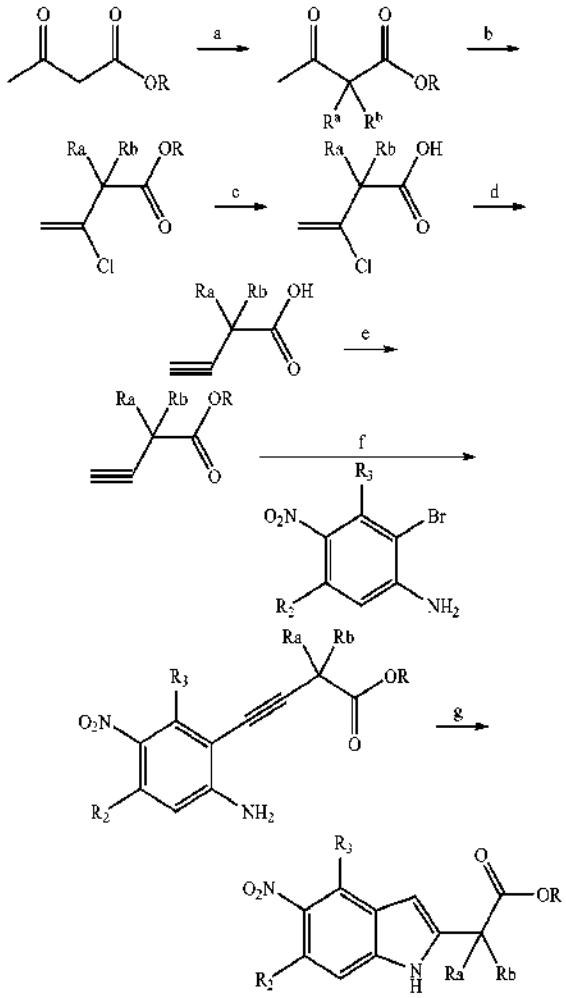
a) TsCl, $Et_3N$, $CH_2Cl_2$; b) NaCN, DMF; c) NaOH, MeOH; d) $NaN_3$, $NH_4Cl$; e) $NaN_3$, DMF; f) Pd/C, $H_2$, MeOH (R = H); h) $R^xC(O)Cl$ ($Z = R^xC(O)-$) or $R^xSO_2Cl$ ($Z = R^xSO_2-$) or $R^xO(CO)Cl$ ($Z = R^xO(CO)-$) or $(R^xO(CO))_2O$ ($Z = R^xO(CO)-$), $Et_3N$, $CH_2Cl_2$

Scheme 41



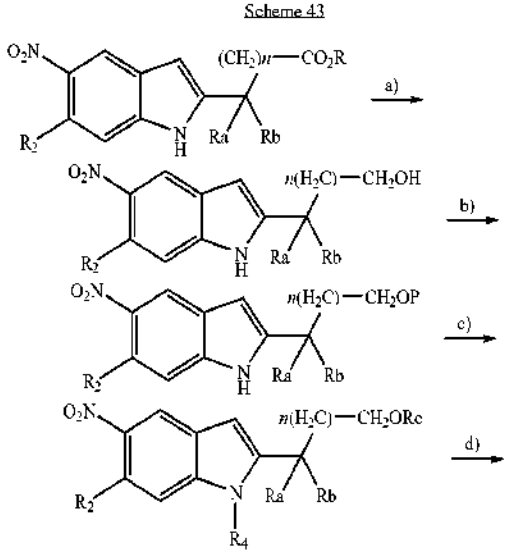
a) $ClCH_2CHO$, $NaHB(OAc)_3$, $CH_2Cl_2$; $CDCl_3$, light; b) $NaN_3$, NaI, DMF; c) $H_2$, Pd/C, MeOH, AcOH; d) RC(O)Cl (Z = RC(O)—) or $RSO_2Cl$ (Z = $RSO_2$—) or RO(CO)Cl (Z = RO(CO)—) or $(RO(CO))_2O$ (Z = RO(CO)—), $Et_3N$, $CH_2Cl_2$.

Scheme 42



b) $R_a$—X, NaH; $R_b$—X, NaH; b) $PCl_5$, $CH_2Cl_2$; c) NaOH; d) $NaNH_2$, DMSO; e) R—OH, DCC, f) $Pd(PPh_3)_2Cl_2$, CuI, $Et_3N$; g) $PdCl_2$, $CH_3CN$

Scheme 43



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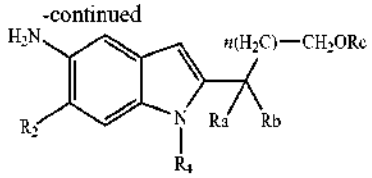


n = 0 or 1

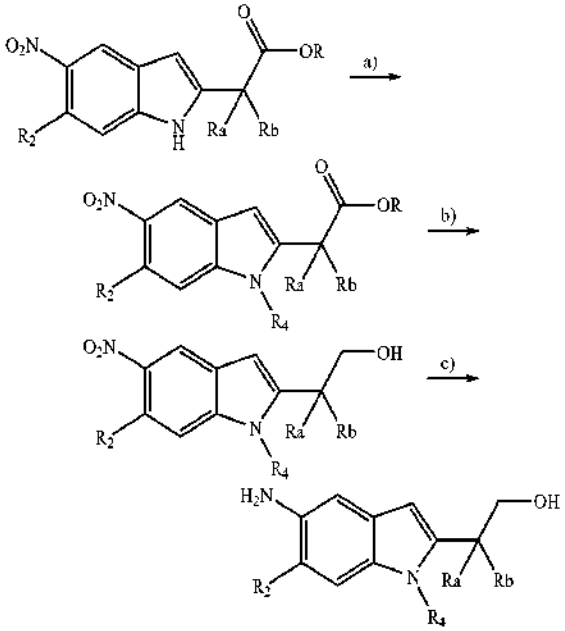
a) DIBAL-H;

b) P-LG; P = protecting group like TBDMS and LG = leaving group like Cl;

c) R4-LG, base like $Cs_2CO_3$; $R_4$ is alkyl and LG is tosylate, Rc = H or $R_4$;

d) reducing conditions like Pd/C, $H_2$ or ammonium formate.

Scheme 43



**[0271]** $R_4$-LG, base like $Cs_2CO_3$; $R_4$ is alkyl and LG is tosylate; b) $LiAlH_4$; c) reducing conditions like Pd/C, $H_2$ or ammonium formate.

**[0272]** In the schemes above, the radical R employed therein is a substituent, e.g., RW as defined hereinabove. One of skill in the art will readily appreciate that synthetic routes suitable for various substituents of the present invention are such that the reaction conditions and steps employed do not modify the intended substituents.

## V. Formulations, Administrations, and Uses

**[0273]** Accordingly, in another aspect of the present invention, pharmaceutically acceptable compositions are provided, wherein these compositions comprise any of the compounds as described herein, and optionally comprise a pharmaceutically acceptable carrier, adjuvant or vehicle. In certain embodiments, these compositions optionally further comprise one or more additional therapeutic agents.

**[0274]** It will also be appreciated that certain of the compounds of present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative or a prodrug thereof. According to the present invention, a pharmaceutically acceptable derivative or a prodrug includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or any other adduct or derivative which upon administration to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

**[0275]** As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. A "pharmaceutically acceptable salt" means any non-toxic salt or salt of an ester of a compound of this invention that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an inhibitorily active metabolite or residue thereof.

**[0276]** Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66, 1-19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

**[0277]** As described above, the pharmaceutically acceptable compositions of the present invention additionally comprise a pharmaceutically acceptable carrier, adjuvant, or vehicle, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) discloses

various carriers used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component (s) of the pharmaceutically acceptable composition, its use is contemplated to be within the scope of this invention. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, or potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, wool fat, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol or polyethylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

**[0278]** In yet another aspect, the present invention provides a method of treating a condition, disease, or disorder implicated by ABC transporter activity. In certain embodiments, the present invention provides a method of treating a condition, disease, or disorder implicated by a deficiency of ABC transporter activity, the method comprising administering a composition comprising a compound of formulae (I, Ic, Id, II, IIa, IIb, IIc, and IId) to a subject, preferably a mammal, in need thereof.

**[0279]** In certain preferred embodiments, the present invention provides a method of treating Cystic fibrosis, Hereditary emphysema, Hereditary hemochromatosis, Coagulation-Fibrinolysis deficiencies, such as Protein C deficiency, Type 1 hereditary angioedema, Lipid processing deficiencies, such as Familial hypercholesterolemia, Type 1 chylomicronemia, Abetalipoproteinemia, Lysosomal storage diseases, such as I-cell disease/Pseudo-Hurler, Mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, Polyendocrinopathy/Hyperinsulemia, Diabetes mellitus, Laron dwarfism, Myleoperoxidase deficiency, Primary hypoparathyroidism, Melanoma, Glycanosis CDG type 1, Hereditary emphysema, Congenital hyperthyroidism, Osteogenesis imperfecta, Hereditary hypofibrinogenemia, ACT deficiency, Diabetes insipidus (DI), Neurophyseal DI, Neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Progressive supranuclear plasy, Pick's disease, several polyglutamine neurological disorders such as Huntington, Spinocerebullar ataxia type I, Spinal and bulbar muscular atrophy, Dentatorubal pallidoluysian, and Myotonic dystrophy, as well as Spongiform encephalopathies, such as Hereditary Creutzfeldt-Jakob disease (due to Prion protein processing defect), Fabry disease, Straussler-Scheinker disease, secretory diarrhea, polycystic kidney disease, chronic obstructive pulmonary disease (COPD), dry eye disease, and Sjögren's Syndrome, comprising the step of administering to said mammal an effective amount of a composition comprising a compound of formulae (I, Ic, Id, II, IIa, IIb, IIc, and IId), or a preferred embodiment thereof as set forth above.

**[0280]** According to an alternative preferred embodiment, the present invention provides a method of treating cystic fibrosis comprising the step of administering to said mammal a composition comprising the step of administering to said mammal an effective amount of a composition comprising a compound of formulae (I, Ic, Id, II, IIa, IIb, IIc, and IId), or a preferred embodiment thereof as set forth above.

**[0281]** According to the invention an "effective amount" of the compound or pharmaceutically acceptable composition is that amount effective for treating or lessening the severity of one or more of Cystic fibrosis, Hereditary emphysema, Hereditary hemochromatosis, Coagulation-Fibrinolysis deficiencies, such as Protein C deficiency, Type 1 hereditary angioedema, Lipid processing deficiencies, such as Familial hypercholesterolemia, Type 1 chylomicronemia, Abetalipoproteinemia, Lysosomal storage diseases, such as I-cell disease/Pseudo-Hurler, Mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, Polyendocrinopathy/Hyperinsulemia, Diabetes mellitus, Laron dwarfism, Myleoperoxidase deficiency, Primary hypoparathyroidism, Melanoma, Glycanosis CDG type 1, Hereditary emphysema, Congenital hyperthyroidism, Osteogenesis imperfecta, Hereditary hypofibrinogenemia, ACT deficiency, Diabetes insipidus (DI), Neurophyseal DI, Neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Progressive supranuclear plasy, Pick's disease, several polyglutamine neurological disorders such as Huntington, Spinocerebullar ataxia type I, Spinal and bulbar muscular atrophy, Dentatorubal pallidoluysian, and Myotonic dystrophy, as well as Spongiform encephalopathies, such as Hereditary Creutzfeldt-Jakob disease, Fabry disease, Straussler-Scheinker disease, secretory diarrhea, polycystic kidney disease, chronic obstructive pulmonary disease (COPD), dry eye disease, and Sjögren's Syndrome.

**[0282]** The compounds and compositions, according to the method of the present invention, may be administered using any amount and any route of administration effective for treating or lessening the severity of one or more of Cystic fibrosis, Hereditary emphysema, Hereditary hemochromatosis, Coagulation-Fibrinolysis deficiencies, such as Protein C deficiency, Type 1 hereditary angioedema, Lipid processing deficiencies, such as Familial hypercholesterolemia, Type 1 chylomicronemia, Abetalipoproteinemia, Lysosomal storage diseases, such as I-cell disease/Pseudo-Hurler, Mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, Polyendocrinopathy/Hyperinsulemia, Diabetes mellitus, Laron dwarfism, Myleoperoxidase deficiency, Primary hypoparathyroidism, Melanoma, Glycanosis CDG type 1, Hereditary emphysema, Congenital hyperthyroidism, Osteogenesis imperfecta, Hereditary hypofibrinogenemia, ACT deficiency,

Diabetes insipidus (DI), Neurophyseal DI, Neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease. Amyotrophic lateral sclerosis. Progressive supranuclear plasy, Pick's disease, several polyglutamine neurological disorders such as Huntington, Spinocerebullar ataxia type I, Spinal and bulbar muscular atrophy. Dentatorubal pallidoluysian, and Myotonic dystrophy, as well as Spongiform encephalopathies. such as Hereditary Creutzfeldt-Jakob disease, Fabry disease, Straussler-Scheinker disease, secretory diarrhea, polycystic kidney disease, chronic obstructive pulmonary disease (COPD), dry eye disease, and Sjögren's Syndrome.

**[0283]** The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the infection, the particular agent, its mode of administration, and the like. The compounds of the invention are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of agent appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective dose level for any particular patient or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed, and like factors well known in the medical arts. The term "patient", as used herein, means an animal, preferably a mammal, and most preferably a human.

**[0284]** The pharmaceutically acceptable compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated. In certain embodiments, the compounds of the invention may be administered orally or parenterally at dosage levels of about 0.01 mg/kg to about 50 mg/kg and preferably from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect.

**[0285]** Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

**[0286]** Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

**[0287]** The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

**[0288]** In order to prolong the effect of a compound of the present invention, it is often desirable to slow the absorption of the compound from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the compound then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered compound form is accomplished by dissolving or suspending the compound in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the compound in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of compound to polymer and the nature of the particular polymer employed, the rate of compound release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the compound in liposomes or microemulsions that are compatible with body tissues.

**[0289]** Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

**[0290]** Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene gly-

cols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

**[0291]** Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

**[0292]** The active compounds can also be in microencapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

**[0293]** Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, eardrops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms are prepared by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

**[0294]** As described generally above, the compounds of the invention are useful as modulators of ABC transporters. Thus, without wishing to be bound by any particular theory, the compounds and compositions are particularly useful for treating or lessening the severity of a disease, condition, or disorder where hyperactivity or inactivity of ABC transporters is implicated in the disease, condition, or disorder. When hyperactivity or inactivity of an ABC transporter is implicated in a particular disease, condition, or disorder, the disease, condition, or disorder may also be referred to as a "ABC transporter-mediated disease, condition or disorder". Accordingly, in another aspect, the present invention provides a method for treating or lessening the severity of a disease, condition, or disorder where hyperactivity or inactivity of an ABC transporter is implicated in the disease state.

**[0295]** The activity of a compound utilized in this invention as a modulator of an ABC transporter may be assayed according to methods described generally in the art and in the Examples herein.

**[0296]** It will also be appreciated that the compounds and pharmaceutically acceptable compositions of the present invention can be employed in combination therapies, that is, the compounds and pharmaceutically acceptable compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an inventive compound may be administered concurrently with another agent used to treat the same disorder), or they may achieve different effects (e.g., control of any adverse effects). As used herein, additional therapeutic agents that are normally administered to treat or prevent a particular disease, or condition, are known as "appropriate for the disease, or condition, being treated".

**[0297]** The amount of additional therapeutic agent present in the compositions of this invention will be no more than the amount that would normally be administered in a composition comprising that therapeutic agent as the only active agent. Preferably the amount of additional therapeutic agent in the presently disclosed compositions will range from about 50% to 100% of the amount normally present in a composition comprising that agent as the only therapeutically active agent.

**[0298]** The compounds of this invention or pharmaceutically acceptable compositions thereof may also be incorporated into compositions for coating an implantable medical device, such as prostheses, artificial valves, vascular grafts, stents and catheters. Accordingly, the present invention, in another aspect, includes a composition for coating an implantable device comprising a compound of the present invention as described generally above, and in classes and subclasses herein, and a carrier suitable for coating said implantable device. In still another aspect, the present invention includes an implantable device coated with a composition comprising a compound of the present invention as described generally above, and in classes and subclasses herein, and a carrier suitable for coating said implantable device. Suitable coatings and the general preparation of coated implantable devices are described in U.S. Pat. Nos. 6,099,562; 5,886,026; and 5,304,121. The coatings are typically biocompatible polymeric materials such as a hydrogel polymer, polymethyldisiloxane, polycaprolactone, polyethylene glycol, polylactic acid, ethylene vinyl acetate, and mixtures thereof. The coatings may optionally be further covered by a suitable topcoat of fluorosilicone, polysaccarides, polyethylene glycol, phospholipids or combinations thereof to impart controlled release characteristics in the composition.

**[0299]** Another aspect of the invention relates to modulating ABC transporter activity in a biological sample or a patient (e.g., in vitro or in vivo), which method comprises administering to the patient, or contacting said biological sample with a compound of formula I or a composition comprising said compound. The term "biological sample", as used herein, includes, without limitation, cell cultures or extracts thereof; biopsied material obtained from a mammal or extracts thereof; and blood, saliva, urine, feces, semen, tears, or other body fluids or extracts thereof.

**[0300]** Modulation of ABC transporter activity in a biological sample is useful for a variety of purposes that are known to one of skill in the art. Examples of such purposes include, but are not limited to, the study of ABC transporters in biological and pathological phenomena; and the comparative evaluation of new modulators of ABC transporters.

**[0301]** In yet another embodiment, a method of modulating activity of an anion channel in vitro or in vivo, is provided comprising the step of contacting said channel with a compound of formulae (I, Ic, Id, II, IIa, IIb, IIc, and IId). In preferred embodiments, the anion channel is a chloride channel or a bicarbonate channel. In other preferred embodiments, the anion channel is a chloride channel.

**[0302]** According to an alternative embodiment, the present invention provides a method of increasing the number of functional ABC transporters in a membrane of a cell, comprising the step of contacting said cell with a compound of formulae (I, Ic, Id, II, IIa, IIb, IIc, and IId). The term "functional ABC transporter" as used herein means an ABC transporter that is capable of transport activity. In preferred embodiments, said functional ABC transporter is CFTR.

**[0303]** According to another preferred embodiment, the activity of the ABC transporter is measured by measuring the transmembrane voltage potential. Means for measuring the voltage potential across a membrane in the biological sample may employ any of the known methods in the art, such as optical membrane potential assay or other electrophysiological methods.

**[0304]** The optical membrane potential assay utilizes voltage-sensitive FRET sensors described by Gonzalez and Tsien (See, Gonzalez, J. E. and R. Y. Tsien (1995) "Voltage sensing by fluorescence resonance energy transfer in single cells" Biophys J 69(4): 1272-80, and Gonzalez, J. E. and R. Y. Tsien (1997) "Improved indicators of cell membrane potential that use fluorescence resonance energy transfer" Chem Biol 4(4): 269-77) in combination with instrumentation for measuring fluorescence changes such as the Voltage/Ion Probe Reader (VIPR) (See, Gonzalez, J. E., K. Oades, et al. (1999) "Cell-based assays and instrumentation for screening ion-channel targets" Drug Discov Today 4(9): 431-439).

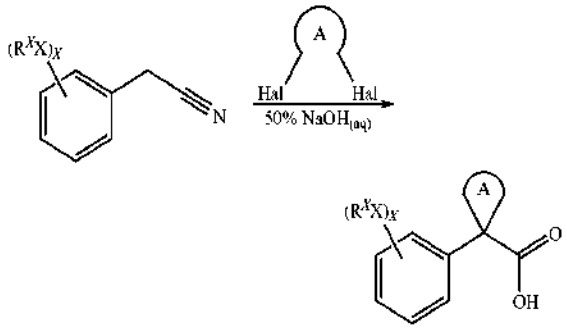
**[0305]** These voltage sensitive assays are based on the change in fluorescence resonant energy transfer (FRET) between the membrane-soluble, voltage-sensitive dye, $DiSBAC_2(3)$, and a fluorescent phospholipid, CC2-DMPE, which is attached to the outer leaflet of the plasma membrane and acts as a FRET donor. Changes in membrane potential ($V_m$) cause the negatively charged $DiSBAC_2(3)$ to redistribute across the plasma membrane and the amount of energy transfer from CC2-DMPE changes accordingly. The changes in fluorescence emission can be monitored using VIPR™ II, which is an integrated liquid handler and fluorescent detector designed to conduct cell-based screens in 96- or 384-well microtiter plates.

**[0306]** In another aspect the present invention provides a kit for use in measuring the activity of a ABC transporter or a fragment thereof in a biological sample in vitro or in vivo comprising (i) a composition comprising a compound of formulae (I, Ic, Id, II, IIa, IIb, IIc, and IId) or any of the above embodiments; and (ii) instructions for a.) contacting the composition with the biological sample and b.) measuring activity of said ABC transporter or a fragment thereof. In one embodiment, the kit further comprises instructions for a.) contacting an additional composition with the biological sample; b.) measuring the activity of said ABC transporter or a fragment thereof in the presence of said additional compound, and c.) comparing the activity of the ABC transporter in the presence of the additional compound with the density of the ABC transporter in the presence of a composition of formulae (I, Ic, Id, II, IIa, IIb, IIc, and IId). In preferred embodiments, the kit is used to measure the density of CFTR.

**[0307]** In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

## VI. Preparations and Examples

**[0308]** General Procedure I: Carboxylic Acid Building Block



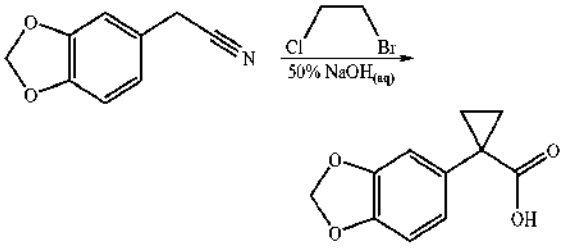

Hal = Cl, Br, I

**[0309]** Benzyltriethylammonium chloride (0.025 equivalents) and the appropriate dihalo compound (2.5 equivalents) were added to a substituted phenyl acetonitrile. The mixture was heated at 70° C. and then 50% sodium hydroxide (10 equivalents) was slowly added to the mixture. The reaction was stirred at 70° C. for 12-24 hours to ensure complete formation of the cycloalkyl moiety and then heated at 130° C. for 24-48 hours to ensure complete conversion from the nitrile to the carboxylic acid. The dark brown/black reaction mixture was diluted with water and extracted with dichloromethane three times to remove side products. The basic aqueous solution was acidified with concentrated hydrochloric acid to pH less than one and the precipitate which began to form at pH 4 was filtered and washed with 1 M hydrochloric acid two times. The solid material was dissolved in dichloromethane and extracted two times with 1 M hydrochloric acid and one time with a saturated aqueous solution of sodium chloride. The organic solution was dried over sodium sulfate and evaporated to dryness to give the cycloalkylcarboxylic acid. Yields and purities were typically greater than 90%.

Example 1

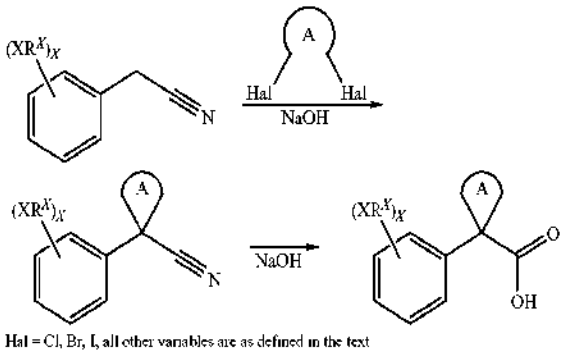
1-Benzo[1,3]dioxol-5-yl-cyclopropanecarboxylic acid

**[0310]**



**[0311]** A mixture of 2-(benzo[d][1,3]dioxol-5-yl)acetonitrile (5.10 g 31.7 mmol), 1-bromo-2-chloro-ethane (9.00 mL 109 mmol), and benzyltriethylammonium chloride (0.181 g, 0.795 mmol) was heated at 70° C. and then 50% (wt./wt.) aqueous sodium hydroxide (26 mL) was slowly added to the mixture. The reaction was stirred at 70° C. for 24 hours and then heated at 130° C. for 48 hours. The dark brown reaction mixture was diluted with water (400 mL) and extracted once with an equal volume of ethyl acetate and once with an equal volume of dichloromethane. The basic aqueous solution was acidified with concentrated hydrochloric acid to pH less than one and the precipitate filtered and washed with 1 M hydrochloric acid. The solid material was dissolved in dichloromethane (400 mL) and extracted twice with equal volumes of 1 M hydrochloric acid and once with a saturated aqueous solution of sodium chloride. The organic solution was dried over sodium sulfate and evaporated to dryness to give a white to slightly off-white solid (5.23 g, 80%) ESI-MS m/z calc. 206.1, found 207.1 $(M+1)^+$. Retention time 2.37 minutes. $^1H$ NMR (400 MHz, DMSO-$d_6$) δ 1.07-1.11 (m, 2H), 1.38-1.42 (m, 2H), 5.98 (s, 2H), 6.79 (m, 2H), 6.88 (m, 1H), 12.26 (s, 1H).

**[0312]** General Procedure II: Carboxylic Acid Building Block

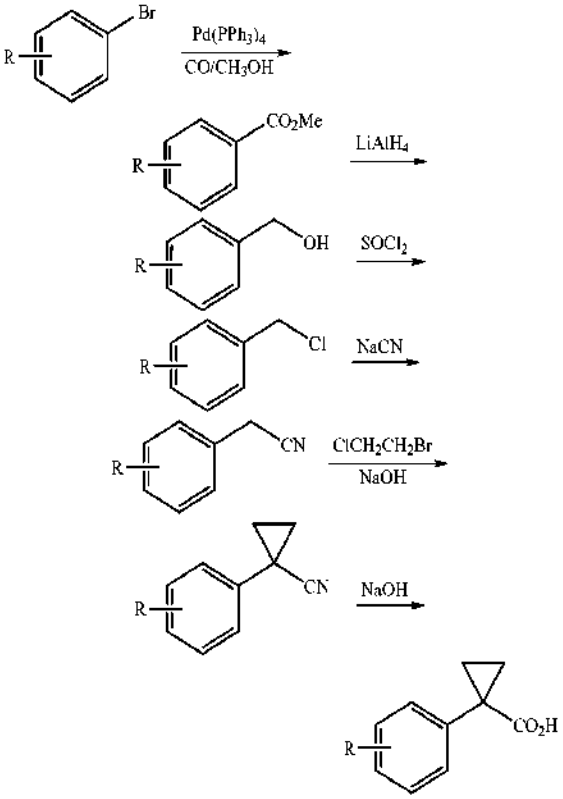


Hal = Cl, Br, I, all other variables are as defined in the text

**[0313]** Sodium hydroxide (50% aqueous solution, 7.4 equivalents) was slowly added to a mixture of the appropriate phenyl acetonitrile, benzyltriethylammonium chloride (1.1 equivalents), and the appropriate dihalo compound (2.3 equivalents) at 70° C. The mixture was stirred overnight at 70° C. and the reaction mixture was diluted with water (30 mL) and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and evaporated to dryness to give the crude cyclopropanecarbonitrile, which was used directly in the next step.

**[0314]** The crude cyclopropanecarbonitrile was refluxed in 10% aqueous sodium hydroxide (7.4 equivalents) for 2.5 hours. The cooled reaction mixture was washed with ether (100 mL) and the aqueous phase was acidified to pH 2 with 2M hydrochloric acid. The precipitated solid was filtered to give the cyclopropanecarboxylic acid as a white solid.

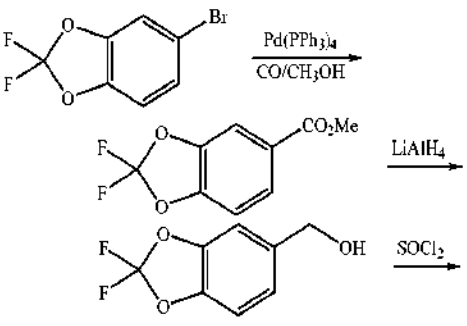
**[0315]** General Procedure III: Carboxylic Acid Building Block

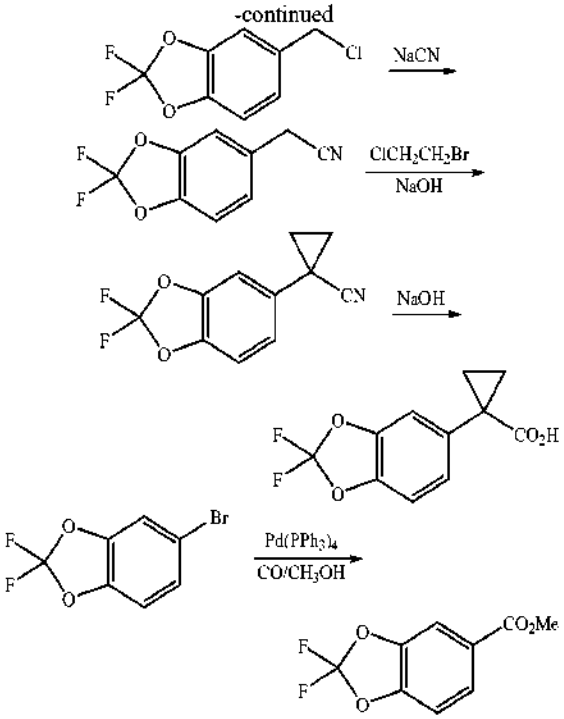


Example 2

1-(2,2-Difluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarboxylic acid

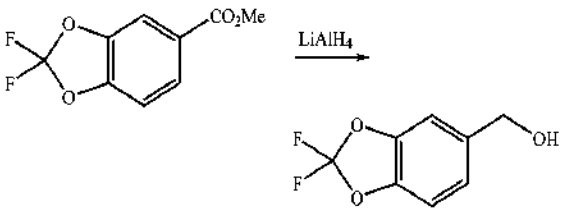
**[0316]**





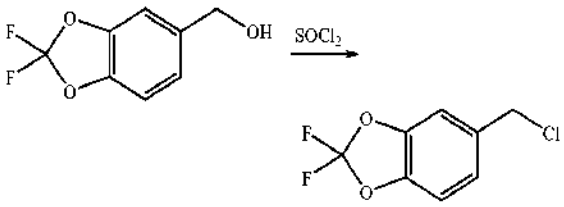

2,2-Difluoro-benzo[1,3]dioxole-5-carboxylic acid methyl ester

**[0317]** A solution of 5-bromo-2,2-difluoro-benzo[1,3]dioxole (11.8 g, 50.0 mmol) and tetrakis(triphenylphosphine) palladium (0) [$Pd(PPh_3)_4$, 5.78 g, 5.00 mmol] in methanol (20 mL) containing acetonitrile (30 mL) and triethylamine (10 mL) was stirred under a carbon monoxide atmosphere (55 PSI) at 75° C. (oil bath temperature) for 15 hours. The cooled reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography to give crude 2,2-difluoro-benzo[1,3]dioxole-5-carboxylic acid methyl ester (11.5 g), which was used directly in the next step.



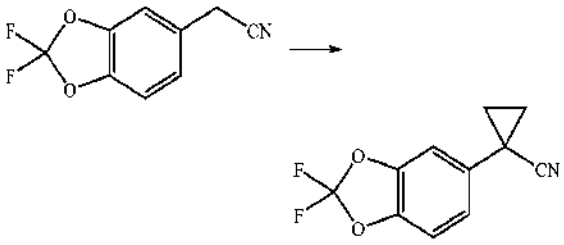

(2,2-Difluoro-benzo[1,3]dioxol-5-yl)-methanol

**[0318]** Crude 2,2-difluoro-benzo[1,3]dioxole-5-carboxylic acid methyl ester (11.5 g) dissolved in 20 mL of anhydrous tetrahydrofuran (THF) was slowly added to a suspension of lithium aluminum hydride (4.10 g, 106 mmol) in anhydrous THF (100 mL) at 0° C. The mixture was then warmed to room temperature. After being stirred at room temperature for 1 hour, the reaction mixture was cooled to 0° C. and treated with water (4.1 g), followed by sodium hydroxide (10% aqueous solution, 4.1 mL). The resulting slurry was filtered and washed with THF. The combined filtrate was evaporated to dryness and the residue was purified by silica gel column chromatography to give (2,2-difluoro-benzo[1,3]dioxol-5-yl)-methanol (7.2 g, 38 mmol, 76% over two steps) as a colorless oil.



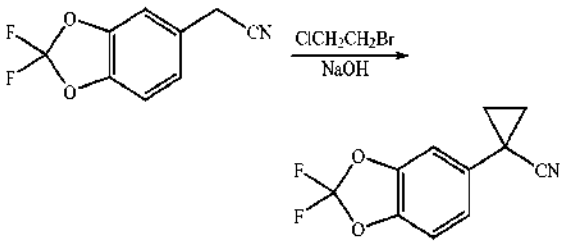

5-Chloromethyl-2,2-difluoro-benzo[1,3]dioxole

**[0319]** Thionyl chloride (45 g, 38 mmol) was slowly added to a solution of (2,2-difluoro-benzo[1,3]dioxol-5-yl)-methanol (7.2 g, 38 mmol) in dichloromethane (200 mL) at 0° C. The resulting mixture was stirred overnight at room temperature and then evaporated to dryness. The residue was partitioned between an aqueous solution of saturated sodium bicarbonate (100 mL) and dichloromethane (100 mL). The separated aqueous layer was extracted with dichloromethane (150 mL) and the organic layer was dried over sodium sulfate, filtrated, and evaporated to dryness to give crude 5-chloromethyl-2,2-difluoro-benzo[1,3]dioxole (4.4 g) which was used directly in the next step.



(2,2-Difluoro-benzo[1,3]dioxol-5-yl)-acetonitrile

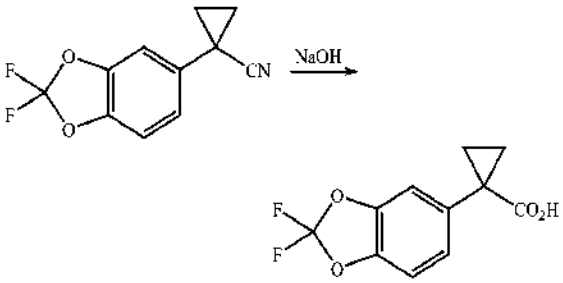
**[0320]** A mixture of crude 5-chloromethyl-2,2-difluoro-benzo[1,3]dioxole (4.4 g) and sodium cyanide (1.36 g, 27.8 mmol) in dimethylsulfoxide (50 mL) was stirred at room temperature overnight. The reaction mixture was poured into ice and extracted with ethyl acetate (300 mL). The organic layer was dried over sodium sulfate and evaporated to dryness to give crude (2,2-difluoro-benzo[1,3]dioxol-5-yl)-acetonitrile (3.3 g) which was used directly in the next step.



1-(2,2-Difluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarbonitrile

**[0321]** Sodium hydroxide (50% aqueous solution, 10 mL) was slowly added to a mixture of crude (2,2-difluoro-benzo[1,3]dioxol-5-yl)-acetonitrile, benzyltriethylammonium chloride (3.00 g, 15.3 mmol), and 1-bromo-2-chloroethane (4.9 g, 38 mmol) at 70° C.

**[0322]** The mixture was stirred overnight at 70° C. before the reaction mixture was diluted with water (30 mL) and extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and evaporated to dryness to give crude 1-(2,2-difluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarbonitrile, which was used directly in the next step.



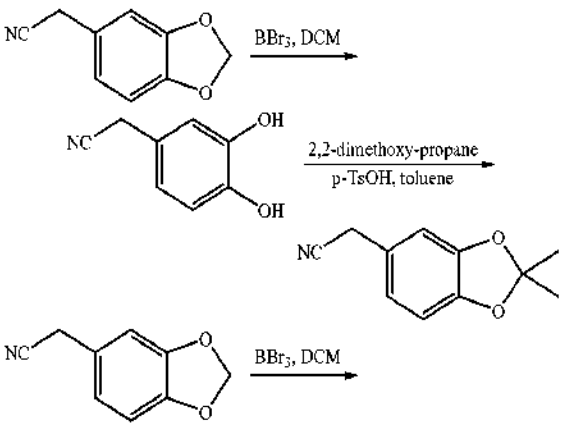

1-(2,2-Difluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarboxylic acid

**[0323]** 1-(2,2-Difluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarbonitrile (crude from the last step) was refluxed in 10% aqueous sodium hydroxide (50 mL) for 2.5 hours. The cooled reaction mixture was washed with ether (100 mL) and the aqueous phase was acidified to pH 2 with 2M hydrochloric acid. The precipitated solid was filtered to give 1-(2,2-difluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarboxylic acid as a white solid (0.15 g, 1.6% over four steps). ESI-MS m/z calc. 242.04, found 241.58 $(M+1)^+$; $^1$H NMR ($CDCl_3$) δ 7.14-7.04 (m, 2H), 6.98-6.96 (m, 1H), 1.74-1.64 (m, 2H), 1.26-1.08 (m, 2H).

Example 3

2-(2,2-Dimethylbenzo[d][1,3]dioxol-5-yl)acetonitrile

**[0324]**

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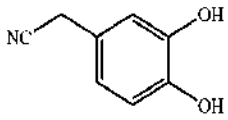
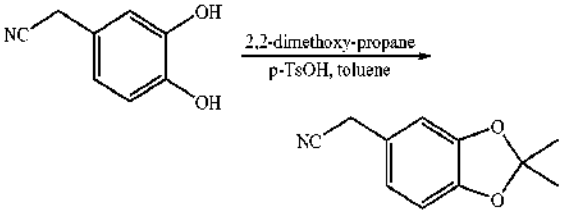



(3,4-Dihydroxy-phenyl)-acetonitrile

**[0325]** To a solution of benzo[1,3]dioxol-5-yl-acetonitrile (0.50 g, 3.1 mmol) in $CH_2Cl_2$ (15 mL) was added dropwise $BBr_3$ (0.78 g, 3.1 mmol) at −78° C. under $N_2$. The mixture was slowly warmed to room temperature and stirred overnight. $H_2O$ (10 mL) was added to quench the reaction and the $CH_2Cl_2$ layer was separated. The aqueous phase was extracted with $CH_2Cl_2$ (2×7 mL). The combined organics were washed with brine, dried over $Na_2SO_4$ and purified by column chromatography on silica gel (petroleum ether/ethyl acetate 5:1) to give (3,4-dihydroxy-phenyl)-acetonitrile (0.25 g, 54%) as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 9.07 (s, 1H), 8.95 (s, 1H), 6.68-6.70 (m, 2H), 6.55 (dd, J=8.0, 2.0 Hz, 1H), 3.32 (s, 2H).



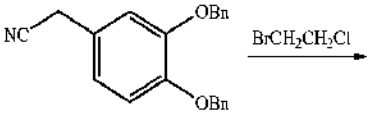

2-(2,2-Dimethylbenzo[d][1,3]dioxol-5-yl)acetonitrile

**[0326]** To a solution of (3,4-dihydroxy-phenyl)-acetonitrile (0.20 g, 1.3 mmol) in toluene (4 mL) was added 2,2-dimethoxy-propane (0.28 g, 2.6 mmol) and TsOH (0.010 g, 0.065 mmol). The mixture was heated at reflux overnight. The reaction mixture was evaporated to remove the solvent and the residue was dissolved in ethyl acetate. The organic layer was washed with $NaHCO_3$ solution, $H_2O$, brine, and dried over $Na_2SO_4$. The solvent was evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 10:1) to give 2-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)acetonitrile (40 mg, 20%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.68-6.71 (m, 3H), 3.64 (s, 2H), 1.67 (s, 6H).

Example 4

1-(3,4-Dihydroxy-phenyl)-cyclopropanecarboxylic acid

**[0327]**



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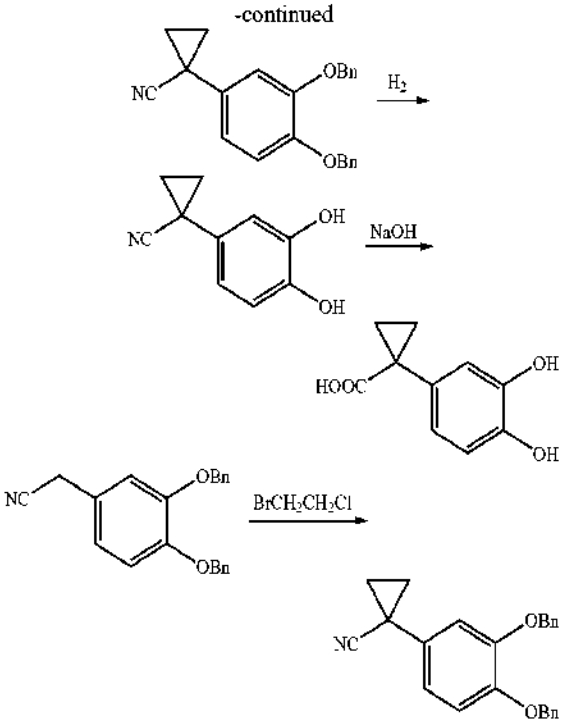
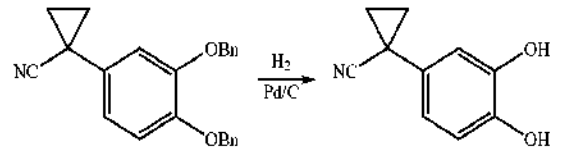


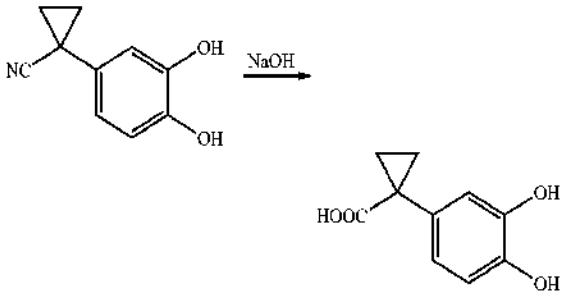
1-(3,4-Bis-benzyloxy-phenyl)-cyclopropanecarbonitrile

**[0328]** To a mixture of $(n\text{-}C_4H_9)_4NBr$ (0.50 g, 1.5 mmol), toluene (7 mL) and (3,4-bis-benzyloxy-phenyl)-acetonitrile (14 g, 42 mmol) in NaOH (50 g) and $H_2O$ (50 mL) was added $BrCH_2CH_2Cl$ (30 g, 0.21 mol). The reaction mixture was stirred at 50° C. for 5 h before being cooled to room temperature. Toluene (30 mL) was added and the organic layer was separated and washed with $H_2O$, brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by column on silica gel (petroleum ether/ethyl acetate 10:1) to give 1-(3,4-bis-benzyloxy-phenyl)-cyclopropanecarbonitrile (10 g, 66%). $^1H$ NMR (DMSO 300 MHz) δ 7.46-7.30 (m, 10H), 7.03 (d, J=8.4 Hz, 1H), 6.94 (d, J=2.4 Hz, 1H), 6.89 (dd, J=2.4, 8.4 Hz, 1H), 5.12 (d, J=7.5 Hz, 4H), 1.66-1.62 (m, 2H), 1.42-1.37 (m, 2H).



1-(3,4-Dihydroxy-phenyl)-cyclopropanecarbonitrile

**[0329]** To a solution of 1-(3,4-bis-benzyloxy-phenyl)-cyclopropanecarbonitrile (10 g, 28 mmol) in MeOH (50 mL) was added Pd/C (0.5 g) under nitrogen atmosphere. The mixture was stirred under hydrogen atmosphere (1 atm) at room temperature for 4 h. The catalyst was filtered off through a celite pad and the filtrate was evaporated under vacuum to give 1-(3,4-dihydroxy-phenyl)-cyclopropanecarbonitrile (4.5 g, 92%). $^1H$ NMR (DMSO 400 MHz) δ 9.06 (br s, 2H), 6.67-6.71 (m, 2H), 6.54 (dd, J=2.4, 8.4 Hz, 1H), 1.60-1.57 (m, 2H), 1.30-1.27 (m, 2H).



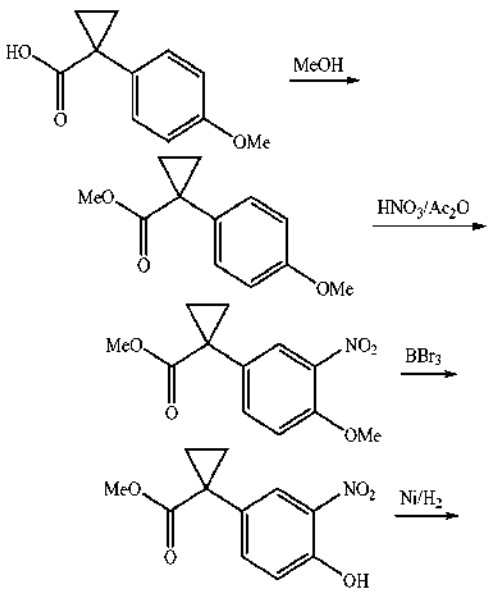
1-(3,4-Dihydroxy-phenyl)-cyclopropanecarboxylic acid

**[0330]** To a solution of NaOH (20 g, 0.50 mol) in $H_2O$ (20 mL) was added 1-(3,4-dihydroxy-phenyl)-cyclopropanecarbonitrile (4.4 g, 25 mmol). The mixture was heated at reflux for 3 h before being cooled to room temperature. The mixture was neutralized with HCl (0.5 N) to pH 3-4 and extracted with ethyl acetate (20 mL×3). The combined organic layers were washed with water, brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum to obtain 1-(3,4-dihydroxy-phenyl)-cyclopropanecarboxylic acid (4.5 g crude). From 900 mg crude, 500 mg pure 1-(3,4-dihydroxy-phenyl)-cyclopropanecarboxylic acid was obtained by preparatory HPLC. $^1H$ NMR (DMSO, 300 MHz) δ 12.09 (br s, 1H), 8.75 (br s, 2H), 6.50-6.67 (m, 3H), 1.35-1.31 (m, 2H), 1.01-0.97 (m, 2H).

Example 5

1-(2-Oxo-2,3-dihydrobenzo[d]oxazol-5-yl)cyclopropane-carboxylic acid

**[0331]**



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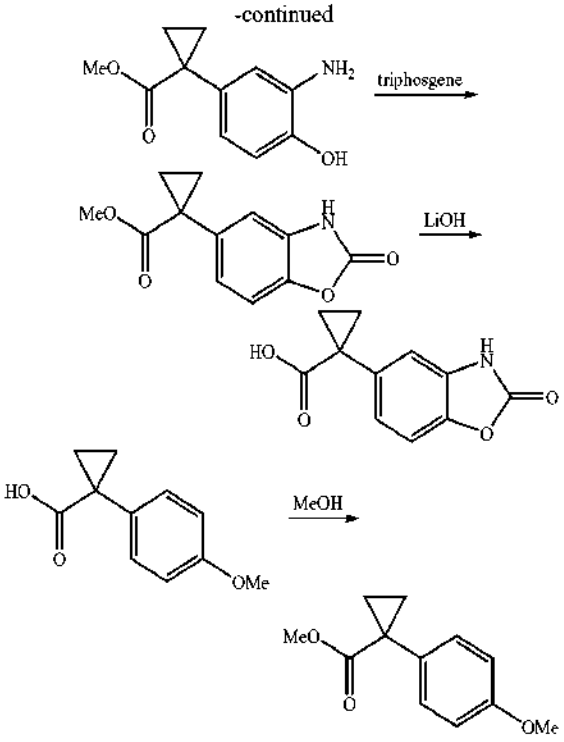
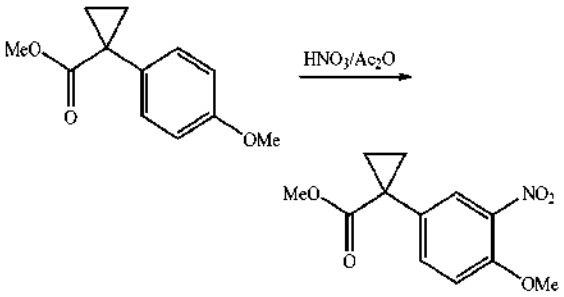


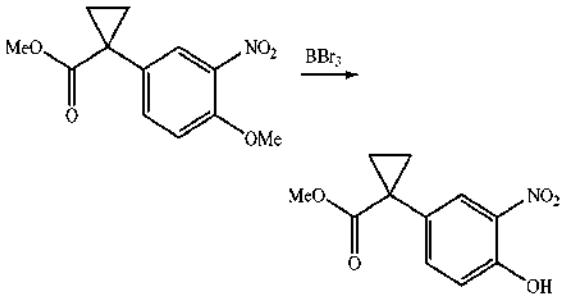

1-(4-Methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester

**[0332]** To a solution of 1-(4-methoxy-phenyl)-cyclopropanecarboxylic acid (50 g, 0.26 mol) in MeOH (500 mL) was added toluene-4-sulfonic acid monohydrate (2.5 g, 13 mmol) at room temperature. The reaction mixture was heated at reflux for 20 hours. MeOH was removed by evaporation under vacuum and EtOAc (200 mL) was added. The organic layer was washed with sat. aq. $NaHCO_3$ (100 mL) and brine, dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-(4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester (53 g, 99%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.25-7.27 (m, 2H), 6.85 (d, J=8.8 Hz, 2H), 3.80 (s, 3H), 3.62 (s, 3H), 1.58 (q, J=3.6 Hz, 2H), 1.15 (q, J=3.6 Hz, 2H).



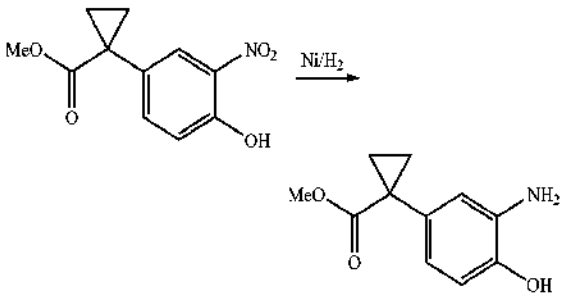

1-(4-Methoxy-3-nitro-phenyl)-cyclopropanecarboxylic acid methyl ester

**[0333]** To a solution of 1-(4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester (30.0 g, 146 mmol) in $Ac_2O$ (300 mL) was added a solution of $HNO_3$ (14.1 g, 146 mmol, 65%) in AcOH (75 mL) at 0° C. The reaction mixture was stirred at 0-5° C. for 3 h before aq. HCl (20%) was added dropwise at 0° C. The resulting mixture was extracted with EtOAc (200 mL×3). The organic layer was washed with sat. aq. $NaHCO_3$ then brine, dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-(4-methoxy-3-nitro-phenyl)-cyclopropanecarboxylic acid methyl ester (36.0 g, 98%), which was directly used in the next step. $^1H$ NMR ($CDCl_3$, 300 MHz) δ 7.84 (d, J=2.1 Hz, 1H), 7.54 (dd, J=2.1, 8.7 Hz, 1H), 7.05 (d, J=8.7 Hz, 1H), 3.97 (s, 3H), 3.65 (s, 3H), 1.68-1.64 (m, 2H), 1.22-1.18 (m, 2H).




1-(4-Hydroxy-3-nitro-phenyl)-cyclopropanecarboxylic acid methyl ester

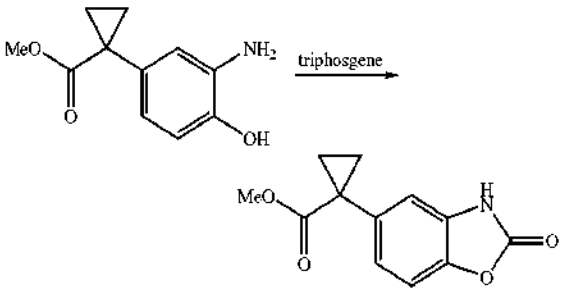
**[0334]** To a solution of 1-(4-methoxy-3-nitro-phenyl)-cyclopropane-carboxylic acid methyl ester (10.0 g, 39.8 mmol) in $CH_2Cl_2$ (100 mL) was added $BBr_3$ (12.0 g, 47.8 mmol) at −70° C. The mixture was stirred at −70° C. for 1 hour, then allowed to warm to −30° C. and stirred at this temperature for 3 hours. Water (50 mL) was added dropwise at −20° C., and the resulting mixture was allowed to warm room temperature before it was extracted with EtOAc (200 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 15:1) to afford 1-(4-hydroxy-3-nitro-phenyl)-cyclopropanecarboxylic acid methyl ester (8.3 g, 78%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 10.5 (s, 1H), 8.05 (d, J=2.4 Hz, 1H), 7.59 (dd, J=2.0, 8.8 Hz, 1H), 7.11 (d, J=8.4 Hz, 1H), 3.64 (s, 3H), 1.68-1.64 (m, 2H), 1.20-1.15 (m, 2H).




1-(3-Amino-4-hydroxy-phenyl)-cyclopropanecarboxylic acid methyl ester

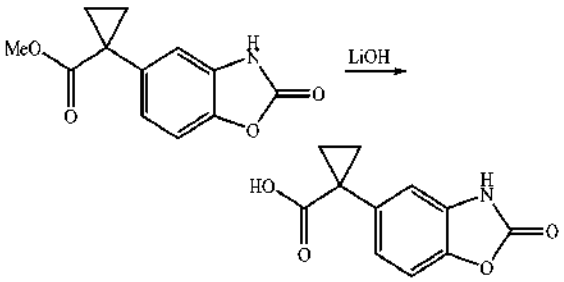
**[0335]** To a solution of 1-(4-hydroxy-3-nitro-phenyl)-cyclopropanecarboxylic acid methyl ester (8.3 g, 35 mmol) in MeOH (100 mL) was added Raney Nickel (0.8 g) under

nitrogen atmosphere. The mixture was stirred under hydrogen atmosphere (1 atm) at 35° C. for 8 hours. The catalyst was filtered off through a Celite pad and the filtrate was evaporated under vacuum to give crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 1:1) to give 1-(3-amino-4-hydroxy-phenyl)-cyclopropanecarboxylic acid methyl ester (5.3 g, 74%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.77 (s, 1H), 6.64 (d, J=2.0 Hz, 2H), 3.64 (s, 3H), 1.55-1.52 (m, 2H), 1.15-1.12 (m, 2H).




1-(2-Oxo-2,3-dihydro-benzooxazol-5-yl)-cyclopropanecarboxylic acid methyl ester

**[0336]** To a solution of 1-(3-amino-4-hydroxy-phenyl)-cyclopropanecarboxylic acid methyl ester (2.0 g, 9.6 mmol) in THF (40 mL) was added triphosgene (4.2 g, 14 mmol) at room temperature. The mixture was stirred for 20 minutes at this temperature before water (20 mL) was added dropwise at 0° C. The resulting mixture was extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-(2-oxo-2,3-dihydro-benzooxazol-5-yl)-cyclopropanecarboxylic acid methyl ester (2.0 g, 91%), which was directly used in the next step. $^1$H NMR ($CDCl_3$, 300 MHz) δ 8.66 (s, 1H), 7.13-7.12 (m, 2H), 7.07 (s, 1H), 3.66 (s, 3H), 1.68-1.65 (m, 2H), 1.24-1.20 (m, 2H).



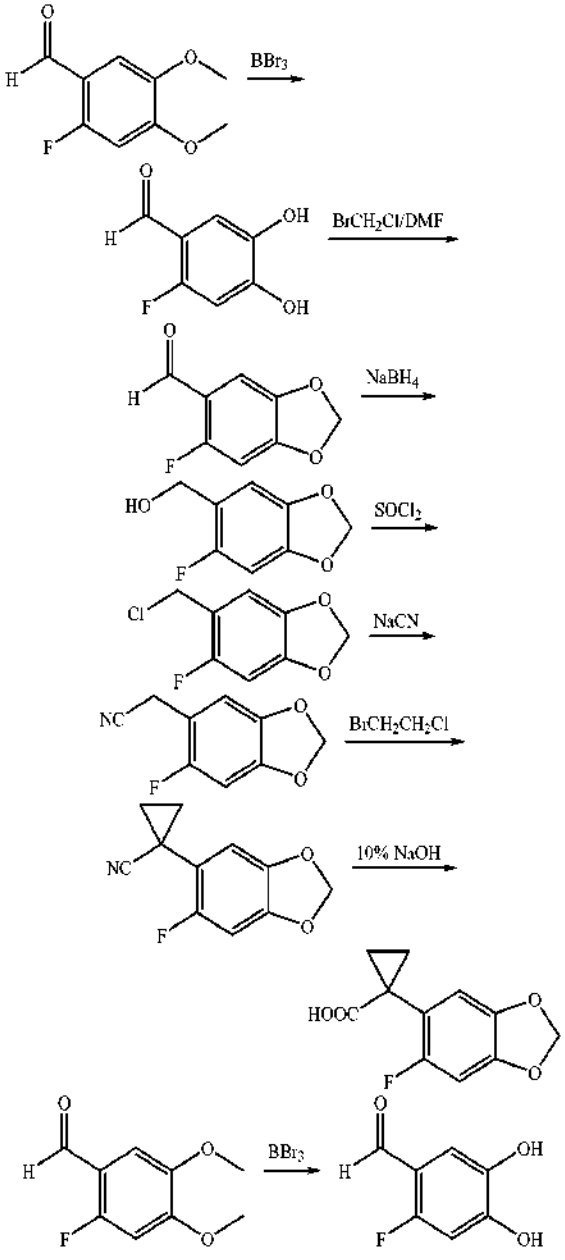

1-(2-Oxo-2,3-dihydrobenzo[d]oxazol-5-yl)cyclopropanecarboxylic acid

**[0337]** To a solution of 1-(2-oxo-2,3-dihydro-benzooxazol-5-yl)-cyclopropanecarboxylic acid methyl ester (1.9 g, 8.1 mmol) in MeOH (20 mL) and water (2 mL) was added $LiOH.H_2O$ (1.7 g, 41 mmol) in portions at room temperature. The reaction mixture was stirred for 20 hours at 50° C. MeOH was removed by evaporation under vacuum before water (100 mL) and EtOAc (50 mL) were added. The aqueous layer was separated, acidified with HCl (3 mol/L) and extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-(2-oxo-2,3-dihydrobenzo[d]oxazol-5-yl)cyclopropanecarboxylic acid (1.5 g, 84%). $^1$H NMR (DMSO, 400 MHz) δ 12.32 (brs, 1H), 11.59 (brs, 1H), 7.16 (d, J=8.4 Hz. 1H), 7.00 (d, J=8.0 Hz, 1H), 1.44-1.41 (m, 2H), 1.13-1.10 (m, 2H). MS (ESI) m/e ($M+H^+$) 218.1.

Example 6

1-(6-Fluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarboxylic acid

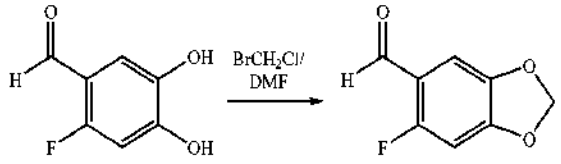
**[0338]**



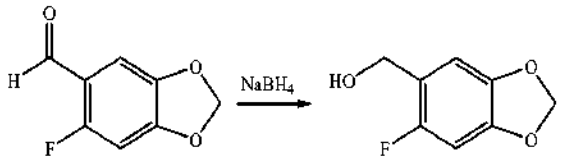

2-Fluoro-4,5-dihydroxy-benzaldehyde

**[0339]** To a stirred suspension of 2-fluoro-4,5-dimethoxy-benzaldehyde (3.00 g, 16.3 mmol) in dichloromethane (100

mL) was added $BBr_3$ (12.2 mL, 130 mmol) dropwise at −78° C. under nitrogen atmosphere. After addition, the mixture was warmed to −30° C. and stirred at this temperature for 5 h. The reaction mixture was poured into ice water and the precipitated solid was collected by filtration and washed with dichloromethane to afford 2-fluoro-4,5-dihydroxy-benzaldehyde (8.0 g), which was used directly in the next step.

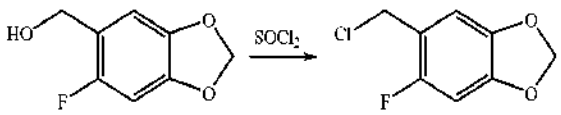



**[0340]** 6-Fluoro-benzo[1.3]dioxole-5-carbaldehyde To a stirred solution of 2-fluoro-4,5-dihydroxy-benzaldehyde (8.0 g) and $BrClCH_2$ (24.8 g, 190 mmol) in dry DMF (50 mL) was added $Cs_2CO_3$ (62.0 g, 190 mmol) in portions. The resulting mixture was stirred at 60° C. overnight and then poured into water. The mixture was extracted with EtOAc (200 mL×3). The combined organic layers were washed with brine (200 mL), dried over $Na_2SO_4$, and evaporated in vacuo to give crude product, which was purified by column chromatography on silica gel (5-20% ethyl acetate/petroleum ether) to afford 6-fluoro-benzo[1,3]dioxole-5-carbaldehyde (700 mg, two steps yield: 24%). $^1$H-NMR (400 MHz, $CDCl_3$) δ 10.19 (s, 1H), 7.23 (d, J=5.6, 1H), 6.63 (d, J=9.6, 1H), 6.08 (s, 2H).



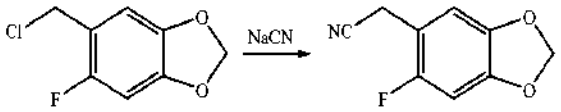

(6-Fluoro-benzo[1,3]dioxol-5-yl)-methanol

**[0341]** To a stirred solution of 6-fluoro-benzo[1,3]dioxole-5-carbaldehyde (700 mg, 4.2 mmol) in MeOH (50 mL) was added $NaBH_4$ (320 mg, 8.4 mmol) in portions at 0° C. The mixture was stirred at this temperature for 30 min and was then concentrated in vacuo to give a residue. The residue was dissolved in EtOAc and the organic layer was washed with water, dried over $Na_2SO_4$, and concentrated in vacuo to afford (6-fluoro-benzo[1,3]dioxol-5-yl)-methanol (650 mg, 92%), which was directly used in the next step.



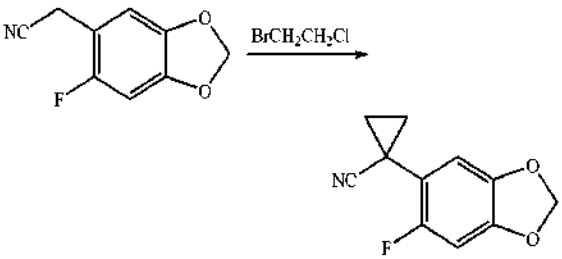

5-Chloromethyl-6-fluoro-benzo[1,3]dioxole

**[0342]** (6-Fluoro-benzo[1,3]dioxol-5-yl)-methanol (650 mg, 3.8 mmol) was added to $SOCl_2$ (20 mL) in portions at 0° C. The mixture was warmed to room temperature for 1 h and then heated at reflux for 1 h. The excess $SOCl_2$ was evaporated under reduced pressure to give the crude product, which was basified with sat. $NaHCO_3$ solution to pH ~7. The aqueous phase was extracted with EtOAc (50 mL×3). The combined organic layers were dried over $Na_2SO_4$ and evaporated under reduced pressure to give 5-chloromethyl-6-fluoro-benzo[1,3]dioxole (640 mg, 90%), which was directly used in the next step.



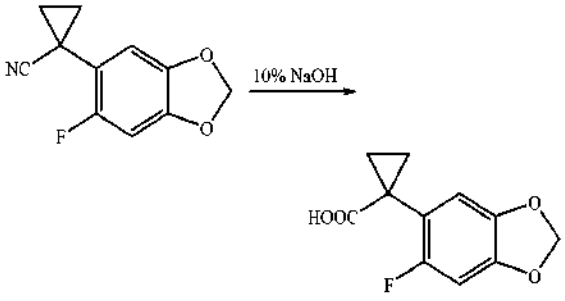

(6-Fluoro-benzo[1,3]dioxol-5-yl)-acetonitrile

**[0343]** A mixture of 5-chloromethyl-6-fluoro-benzo[1,3]dioxole (640 mg, 3.4 mmol) and NaCN (340 mg, 6.8 mmol) in DMSO (20 mL) was stirred at 30° C. for 1 h and then poured into water. The mixture was extracted with EtOAc (50 mL×3). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried over $Na_2SO_4$, and evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (5-10% ethyl acetate/petroleum ether) to afford (6-fluoro-benzo[1,3]dioxol-5-yl)-acetonitrile (530 mg, 70%). $^1$H-NMR (300 MHz, $CDCl_3$) δ 6.82 (d, J=4.8, 1H), 6.62 (d, J=5.4, 1H), 5.99 (s, 2H), 3.65 (s, 2H).




1-(6-Fluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarbonitrile

**[0344]** A flask was charged with water (10 mL), followed by a rapid addition of NaOH (10 g, 0.25 mol) in three portions over a 5 min period. The mixture was allowed to cool to room temperature. Subsequently, the flask was charged with toluene (6 mL), tetrabutyl-ammonium bromide (50 mg, 0.12 mmol), (6-fluoro-benzo[1,3]dioxol-5-yl)-acetonitrile (600 mg, 3.4 mmol) and 1-bromo-2-chloroethane (1.7 g, 12 mmol). The mixture stirred vigorously at 50° C. overnight. The cooled flask was charged with additional toluene (20 mL). The organic layer was separated and washed with water (30 mL) and brine (30 mL). The organic layer was removed in vacuo to give the crude product, which was purified by column chromatography on silica gel (5-10% ethyl acetate/petroleum ether) to give 1-(6-fluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarbonitrile (400 mg, 60%). $^1$H NMR (300 MHz, $CDCl_3$) δ 6.73 (d, J=3.0 Hz, 1H), 6.61 (d, J=9.3 Hz, 1H), 5.98 (s, 2H), 1.67-1.62 (m, 2H), 1.31-1.27 (m, 2H).



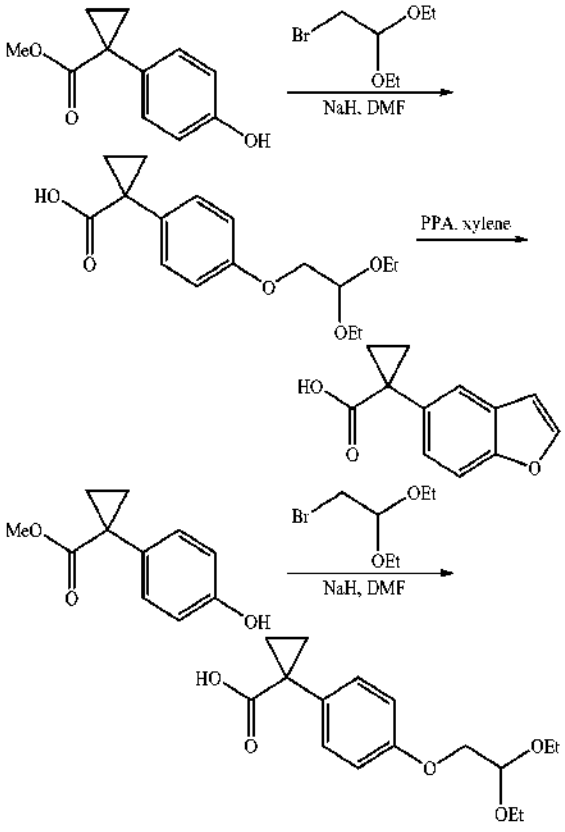

1-(6-Fluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarboxylic acid

**[0345]** A mixture of 1-(6-fluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarbonitrile (400 mg, 0.196 mmol) and 10% NaOH (10 mL) was stirred at 100° C. overnight. After the reaction was cooled, 5% HCl was added until the pH<5 and then EtOAc (30 mL) was added to the reaction mixture. The layers were separated and combined organic layers were evaporated in vacuo to afford 1-(6-fluoro-benzo[1,3]dioxol-5-yl)-cyclopropanecarboxylic acid (330 mg, 76%). $^1$H NMR (400 MHz, DMSO) δ 12.2 (s, 1H), 6.87-6.85 (m, 2H), 6.00 (s, 1H), 1.42-1.40 (m, 2H), 1.14-1.07 (m. 2H).

Example 7

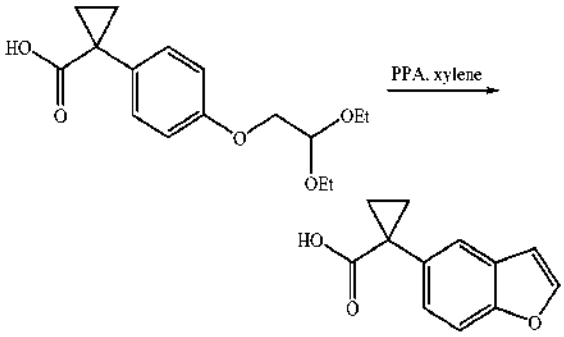
1-(Benzofuran-5-yl)cyclopropanecarboxylic acid

**[0346]**




1-[4-(2,2-Diethoxy-ethoxy)-phenyl]-cyclopropanecarboxylic acid

**[0347]** To a stirred solution of 1-(4-hydroxy-phenyl)-cyclopropanecarboxylic acid methyl ester (15.0 g, 84.3 mmol) in DMF (50 mL) was added sodium hydride (6.7 g, 170 mmol, 60% in mineral oil) at 0° C. After hydrogen evolution ceased, 2-bromo-1,1-diethoxy-ethane (16.5 g, 84.3 mmol) was added dropwise to the reaction mixture. The reaction was stirred at 160° C. for 15 hours. The reaction mixture was poured onto ice (100 g) and was extracted with $CH_2Cl_2$. The combined organics were dried over $Na_2SO_4$. The solvent was evaporated under vacuum to give 1-[4-(2,2-diethoxy-ethoxy)-phenyl]-cyclopropanecarboxylic acid (10 g), which was used directly in the next step without purification.



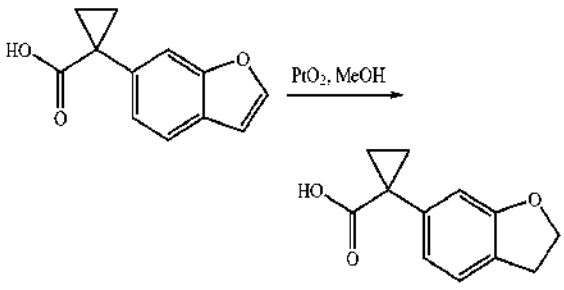

1-Benzofuran-5-yl-cyclopropanecarboxylic acid

**[0348]** To a suspension of 1-[4-(2,2-diethoxy-ethoxy)-phenyl]-cyclopropanecarboxylic acid (20 g, ~65 mmol) in xylene (100 mL) was added PPA (22.2 g, 64.9 mmol) at room temperature. The mixture was heated at reflux (140° C.) for 1 hour before it was cooled to room temperature and decanted from the PPA. The solvent was evaporated under vacuum to obtain the crude product, which was purified by preparative HPLC to provide 1-(benzofuran-5-yl)cyclopropanecarboxylic acid (1.5 g, 5%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.25 (br s, 1H), 7.95 (d, J=2.8 Hz, 1H), 7.56 (d, J=2.0 Hz, 1H), 7.47 (d, J=11.6 Hz, 1H), 7.25 (dd, J=2.4, 11.2 Hz, 1H), 6.89 (d, J=1.6 Hz, 1H), 1.47-1.44 (m, 2H), 1.17-1.14 (m, 2H).

Example 8

1-(2,3-Dihydrobenzofuran-6-yl)cyclopropanecarboxylic acid

**[0349]**



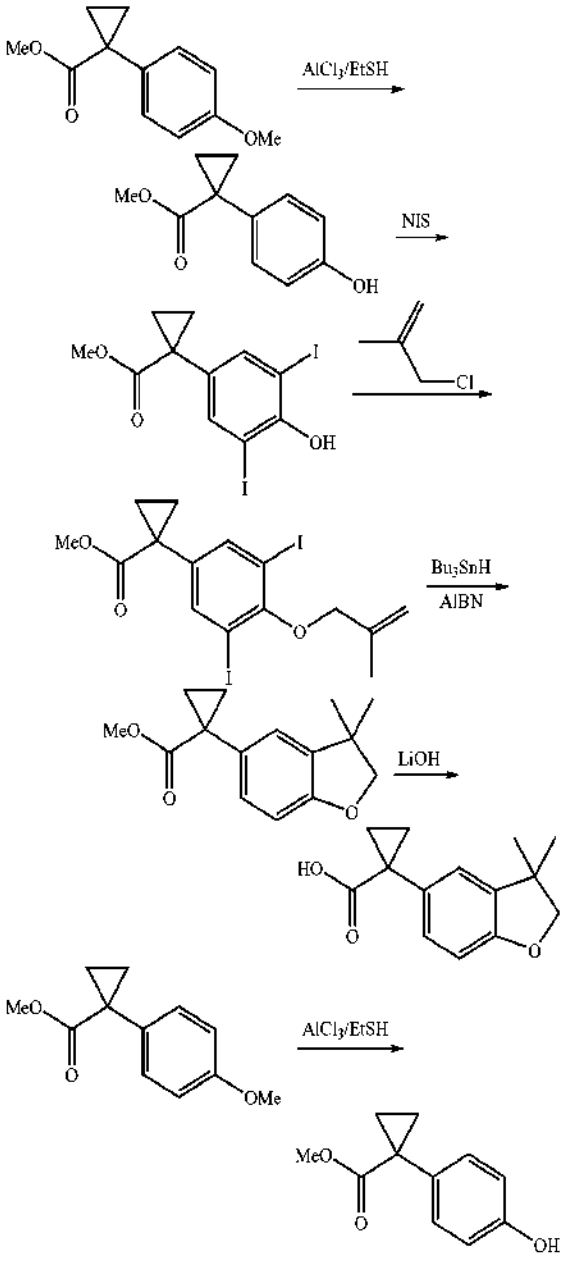

**[0350]** To a solution of 1-(benzofuran-6-yl)cyclopropanecarboxylic acid (370 mg, 1.8 mmol) in MeOH (50 mL) was added $PtO_2$ (75 mg, 20%) at room temperature. The reaction mixture was stirred under hydrogen atmosphere (1 atm) at

20° C. for 3 d. The reaction mixture was filtered and the solvent was evaporated in vacuo to afford the crude product, which was purified by prepared HPLC to give 1-(2,3-dihydrobenzofuran-6-yl)cyclopropanecarboxylic acid (155 mg, 42%). $^1$H NMR (300 MHz, MeOD) δ 7.13 (d, J=7.5 Hz, 1H), 6.83 (d, J=7.8 Hz, 1H), 6.74 (s, 1H), 4.55 (t, J=8.7 Hz, 2H), 3.18 (t, J=8.7 Hz, 2H), 1.56-1.53 (m, 2H), 1.19-1.15 (m, 2H).

Example 9

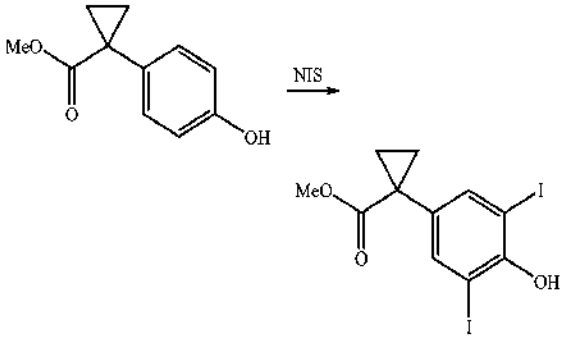
1-(3,3-Dimethyl-2,3-dihydrobenzofuran-5-yl)cyclopropanecarboxylic acid

**[0351]**



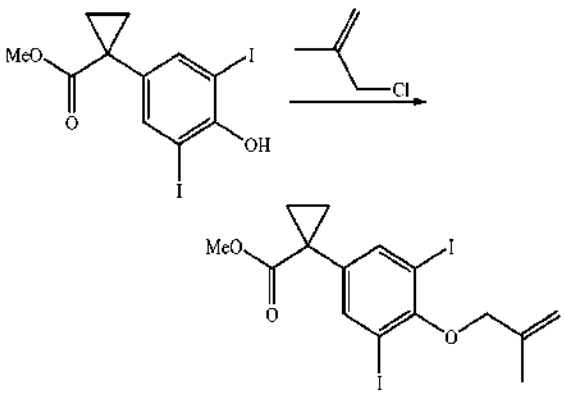

1-(4-Hydroxy-phenyl)-cyclopropanecarboxylic acid methyl ester

**[0352]** To a solution of methyl 1-(4-methoxyphenyl)cyclopropanecarboxylate (10.0 g, 48.5 mmol) in dichloromethane (80 mL) was added EtSH (16 mL) under ice-water bath. The mixture was stirred at 0° C. for 20 min before $AlCl_3$ (19.5 g, 0.15 mmol) was added slowly at 0° C. The mixture was stirred at 0° C. for 30 min. The reaction mixture was poured into ice-water, the organic layer was separated, and the aqueous phase was extracted with dichloromethane (50 mL×3). The combined organic layers were washed with $H_2O$, brine, dried over $Na_2SO_4$ and evaporated under vacuum to give 1-(4-hydroxy-phenyl)-cyclopropanecarboxylic acid methyl ester (8.9 g, 95%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.20-7.17 (m, 2H), 6.75-6.72 (m, 2H), 5.56 (s, 1H), 3.63 (s, 3H), 1.60-1.57 (m, 2H), 1.17-1.15 (m, 2H).




1-(4-Hydroxy-3,5-diiodo-phenyl)-cyclopropanecarboxylic acid methyl ester

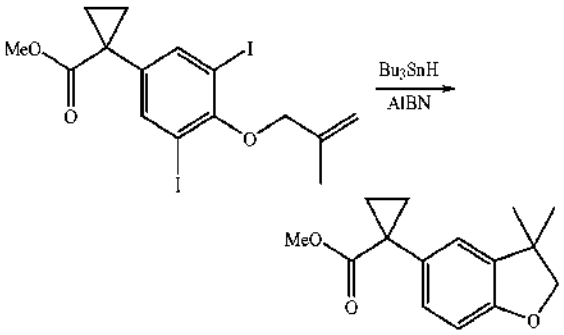
**[0353]** To a solution of 1-(4-hydroxy-phenyl)-cyclopropanecarboxylic acid methyl ester (8.9 g, 46 mmol) in $CH_3CN$ (80 mL) was added NIS (15.6 g, 69 mmol). The mixture was stirred at room temperature for 1 hour. The reaction mixture was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 10:1) to give 1-(4-hydroxy-3,5-diiodo-phenyl)-cyclopropanecarboxylic acid methyl ester (3.5 g, 18%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 2H), 5.71 (s, 1H), 3.63 (s, 3H), 1.59-1.56 (m, 2H), 1.15-1.12 (m, 2H).




1-[3,5-Diiodo-4-(2-methyl-allyloxy)-phenyl]-cyclopropanecarboxylic acid methyl ester

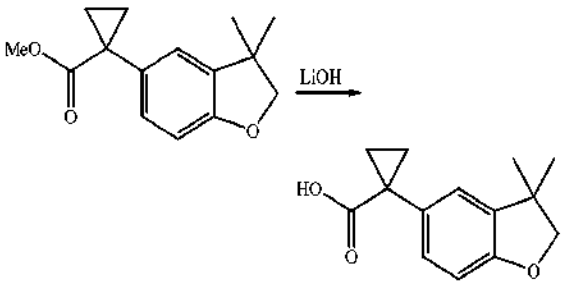
**[0354]** A mixture of 1-(4-hydroxy-3,5-diiodo-phenyl)-cyclopropanecarboxylic acid methyl ester (3.2 g, 7.2 mmol), 3-chloro-2-methyl-propene (1.0 g, 11 mmol), $K_2CO_3$ (1.2 g,

8.6 mmol), NaI (0.1 g, 0.7 mmol) in acetone (20 mL) was stirred at 20° C. overnight. The solid was filtered off and the filtrate was concentrated under vacuum to give 1-[3,5-diiodo-4-(2-methyl-allyloxy)-phenyl]-cyclopropane-carboxylic acid methyl ester (3.5 g, 97%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.75 (s, 2H), 5.26 (s, 1H), 5.06 (s, 1H), 4.38 (s, 2H), 3.65 (s, 3H), 1.98 (s, 3H), 1.62-1.58 (m, 2H), 1.18-1.15 (m, 2H).




1-(3,3-Dimethyl-2,3-dihydro-benzofuran-5-yl)-cyclopropanecarboxylic acid methyl ester

**[0355]** To a solution of 1-[3,5-diiodo-4-(2-methyl-allyloxy)-phenyl]-cyclopropane-carboxylic acid methyl ester (3.5 g, 7.0 mmol) in toluene (15 mL) was added $Bu_3SnH$ (2.4 g, 8.4 mmol) and AIBN (0.1 g, 0.7 mmol). The mixture was heated at reflux overnight. The reaction mixture was concentrated under vacuum and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 20:1) to give 1-(3,3-dimethyl-2,3-dihydro-benzofuran-5-yl)-cyclopropanecarboxylic acid methyl ester (1.05 g, 62%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.10-7.07 (m, 2H), 6.71 (d, J=8 Hz, 1H), 4.23 (s, 2H), 3.62 (s, 3H), 1.58-1.54 (m, 2H), 1.34 (s, 6H), 1.17-1.12 (m, 2H).



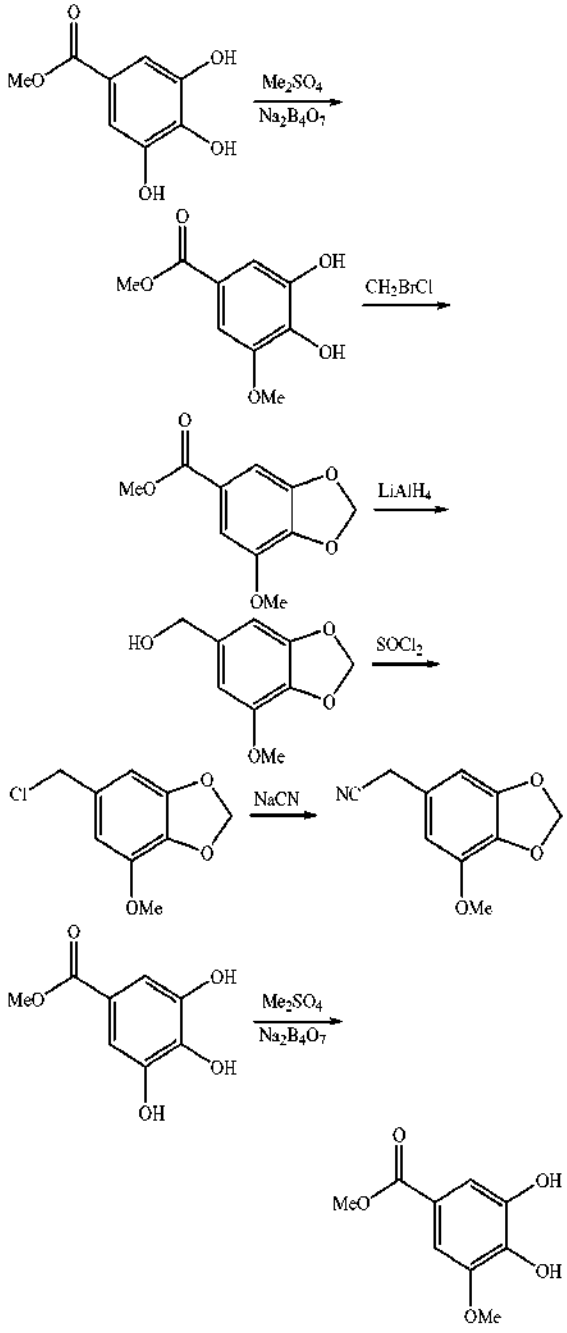

1-(3,3-Dimethyl-2,3-dihydrobenzofuran-5-yl)cyclopropanecarboxylic acid

**[0356]** To a solution of 1-(3,3-dimethyl-2,3-dihydro-benzofuran-5-yl)-cyclopropanecarboxylic acid methyl ester (1.0 g, 4.0 mmol) in MeOH (10 mL) was added LiOH (0.40 g, 9.5 mmol). The mixture was stirred at 40° C. overnight. HCl (10%) was added slowly to adjust the pH to 5. The resulting mixture was extracted with ethyl acetate (10 mL×3). The extracts were washed with brine and dried over $Na_2SO_4$. The solvent was removed under vacuum and the crude product was purified by preparative HPLC to give 1-(3,3-dimethyl-2,3-dihydrobenzofuran-5-yl)cyclopropanecarboxylic acid (0.37 g, 41%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.11-7.07 (m, 2H), 6.71 (d, J=8 Hz, 1H), 4.23 (s, 2H), 1.66-1.63 (m, 2H), 1.32 (s, 6H), 1.26-1.23 (m, 2H).

Example 10

2-(7-Methoxybenzo[d][1,3]dioxol-5-yl)acetonitrile

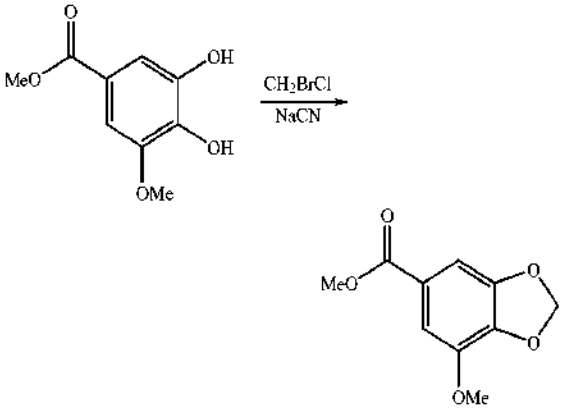
**[0357]**




3,4-Dihydroxy-5-methoxybenzoate

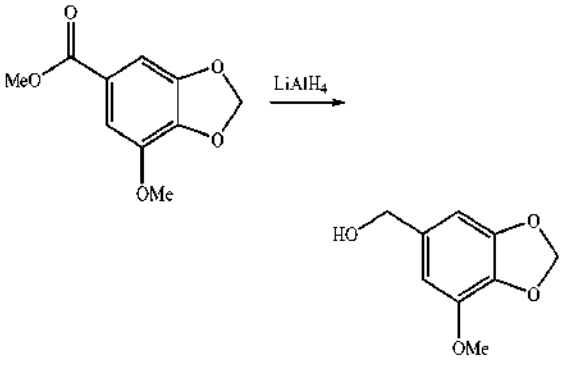
**[0358]** To a solution of 3,4,5-trihydroxy-benzoic acid methyl ester (50 g, 0.27 mol) and $Na_2B_4O_7$ (50 g) in water (1000 mL) was added $Me_2SO_4$ (120 mL) and aqueous NaOH

solution (25%, 200 mL) successively at room temperature. The mixture was stirred at room temperature for 6 h before it was cooled to 0° C. The mixture was acidified to pH ~2 by adding conc. $H_2SO_4$ and then filtered. The filtrate was extracted with EtOAc (500 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure to give methyl 3,4-dihydroxy-5-methoxybenzoate (15.3 g 47%), which was used in the next step without further purification.



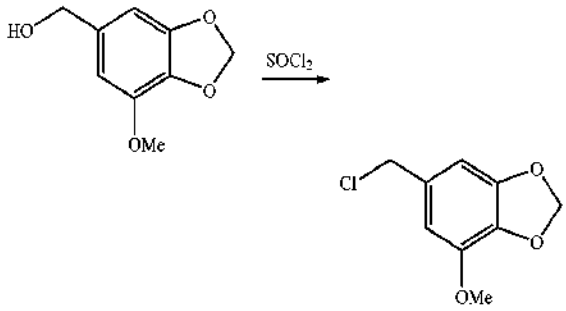

Methyl
7-methoxybenzo[d][1,3]dioxole-5-carboxylate

**[0359]** To a solution of methyl 3,4-dihydroxy-5-methoxybenzoate (15.3 g, 0.0780 mol) in acetone (500 mL) was added $CH_2BrCl$ (34.4 g, 0.270 mol) and $K_2CO_3$ (75.0 g, 0.540 mol) at 80° C. The resulting mixture was heated at reflux for 4 h. The mixture was cooled to room temperature and solid $K_2CO_3$ was filtered off. The filtrate was concentrated under reduced pressure, and the residue was dissolved in EtOAc (100 mL). The organic layer was washed with water, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10:1) to afford methyl 7-methoxybenzo[d][1,3]dioxole-5-carboxylate (12.6 g, 80%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.21 (s, 1H), 6.05 (s, 2H), 3.93 (s, 3H), 3.88 (s, 3H).



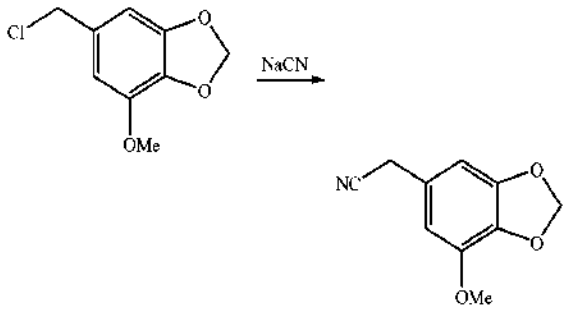

(7-Methoxybenzo[d][1,3]dioxol-5-yl)methanol

**[0360]** To a solution of methyl 7-methoxybenzo[d][1,3]dioxole-5-carboxylate (14 g, 0.040 mol) in THF (100 mL) was added $LiAlH_4$ (3.1 g, 0.080 mol) in portions at room temperature. The mixture was stirred for 3 h at room temperature. The reaction mixture was cooled to 0° C. and treated with water (3.1 g) and NaOH (10%, 3.1 mL) successively. The slurry was filtered off and washed with THF. The combined filtrates were evaporated under reduced pressure to give (7-methoxy-benzo[d][1,3]dioxol-5-yl)methanol (7.2 g, 52%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.55 (s, 1H), 6.54 (s, 1H), 5.96 (s, 2H), 4.57 (s, 2H), 3.90 (s, 3H).




6-(Chloromethyl)-4-methoxybenzo[d][1,3]dioxole

**[0361]** To a solution of $SOCl_2$ (150 mL) was added (7-methoxybenzo[d][1,3]dioxol-5-yl)methanol (9.0 g, 54 mmol) in portions at 0° C. The mixture was stirred for 0.5 h. The excess $SOCl_2$ was evaporated under reduced pressure to give the crude product, which was basified with sat. aq. $NaHCO_3$ to pH ~7. The aqueous phase was extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated to give 6-(chloromethyl)-4-methoxybenzo[d][1,3]dioxole (10 g 94%), which was used in the next step without further purification. $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.58 (s, 1H), 6.57 (s, 1H), 5.98 (s, 2H), 4.51 (s, 2H), 3.90 (s, 3H).




2-(7-Methoxybenzo[d][1,3]dioxol-5-yl)acetonitrile

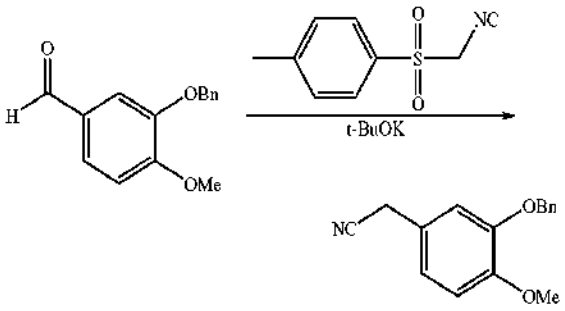
**[0362]** To a solution of 6-(chloromethyl)-4-methoxybenzo[d][1,3]dioxole (10 g, 40 mmol) in DMSO (100 mL) was added NaCN (2.4 g, 50 mmol) at room temperature. The mixture was stirred for 3 h and poured into water (500 mL). The aqueous phase was extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated to give the crude product, which was washed with ether to afford 2-(7-methoxybenzo[d][1,3]dioxol-5-yl)acetonitrile (4.6 g, 45%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.49 (s, 2H), 5.98 (s, 2H), 3.91 (s, 3H), 3.65 (s, 2H).

$^{13}C$ NMR (400 MHz, $CDCl_3$) δ 148.9, 143.4, 134.6, 123.4, 117.3, 107.2, 101.8, 101.3, 56.3, 23.1.

Example 11

2-(3-(Benzyloxy)-4-methoxyphenyl)acetonitrile

**[0363]**

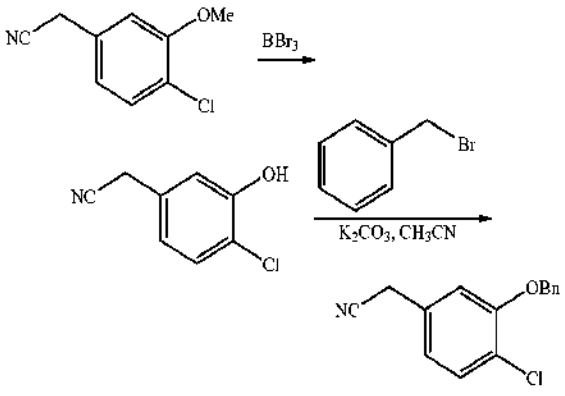



**[0364]** To a suspension of t-BuOK (20.2 g, 0.165 mol) in THF (250 mL) was added a solution of TosMIC (16.1 g, 82.6 mmol) in THF (100 mL) at −78° C. The mixture was stirred for 15 minutes, treated with a solution of 3-benzyloxy-4-methoxy-benzaldehyde (10.0 g, 51.9 mmol) in THF (50 mL) dropwise, and continued to stir for 1.5 hours at −78° C. To the cooled reaction mixture was added methanol (50 mL). The mixture was heated at reflux for 30 minutes. Solvent was removed to give a crude product, which was dissolved in water (300 mL). The aqueous phase was extracted with EtOAc (100 mL×3). The combined organic layers were dried and evaporated under reduced pressure to give crude product, which was purified by column chromatography (petroleum ether/ethyl acetate 10:1) to afford 2-(3-(benzyloxy)-4-methoxyphenyl)-acetonitrile (5.0 g, 48%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.48-7.33 (m, 5H), 6.89-6.86 (m, 3H), 5.17 (s, 2H), 3.90 (s, 3H), 3.66 (s, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 149.6, 148.6, 136.8, 128.8, 128.8, 128.2, 127.5, 127.5, 122.1, 120.9, 118.2, 113.8, 112.2, 71.2, 56.2, 23.3.

Example 12

2-(3-(Benzyloxy)-4-chlorophenyl)acetonitrile

**[0365]**

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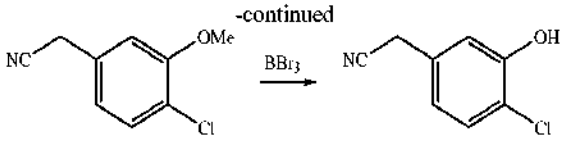
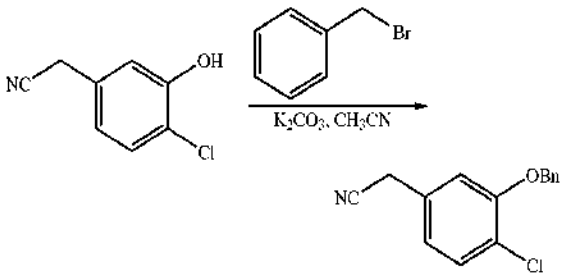



(4-Chloro-3-hydroxy-phenyl)acetonitrile

**[0366]** $BBr_3$ (17 g, 66 mmol) was slowly added to a solution of 2-(4-chloro-3-methoxyphenyl)acetonitrile (12 g, 66 mmol) in dichloromethane (120 mL) at −78° C. under $N_2$. The reaction temperature was slowly increased to room temperature. The reaction mixture was stirred overnight and then poured into ice and water. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (40 mL×3). The combined organic layers were washed with water, brine, dried over $Na_2SO_4$, and concentrated under vacuum to give (4-chloro-3-hydroxy-phenyl)-acetonitrile (9.3 g, 85%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.34 (d, J=8.4 Hz, 1H), 7.02 (d, J=2.1 Hz, 1H), 6.87 (dd, J=2.1, 8.4 Hz, 1H), 5.15 (brs, 1H), 3.72 (s, 2H).



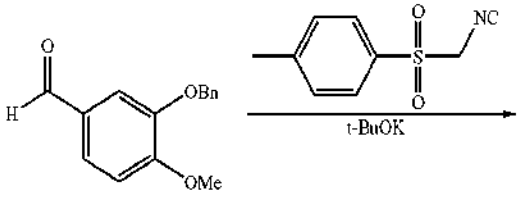

2-(3-(Benzyloxy)-4-chlorophenyl)acetonitrile

**[0367]** To a solution of (4-chloro-3-hydroxy-phenyl)acetonitrile (6.2 g, 37 mmol) in $CH_3CN$ (80 mL) was added $K_2CO_3$ (10 g, 74 mmol) and BnBr (7.6 g, 44 mmol). The mixture was stirred at room temperature overnight. The solids were filtered off and the filtrate was evaporated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 50:1) to give 2-(3-(benzyloxy)-4-chlorophenyl)-acetonitrile (5.6 g, 60%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.48-7.32 (m, 6H), 6.94 (d, J=2 Hz, 2H), 6.86 (dd, J=2.0, 8.4 Hz, 1H), 5.18 (s, 2H), 3.71 (s, 2H).

Example 13

2-(3-(Benzyloxy)-4-methoxyphenyl)acetonitrile

**[0368]**



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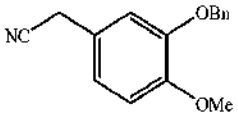
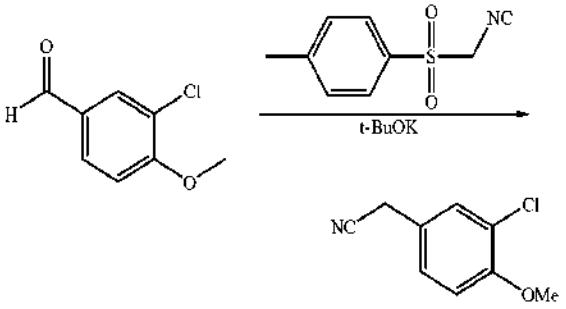



**[0369]** To a suspension of t-BuOK (20.2 g, 0.165 mol) in THF (250 mL) was added a solution of TosMIC (16.1 g, 82.6 mmol) in THF (100 mL) at −78° C. The mixture was stirred for 15 minutes, treated with a solution of 3-benzyloxy-4-methoxy-benzaldehyde (10.0 g, 51.9 mmol) in THF (50 mL) dropwise, and continued to stir for 1.5 hours at −78° C. To the cooled reaction mixture was added methanol (50 mL). The mixture was heated at reflux for 30 minutes. Solvent of the reaction mixture was removed to give a crude product, which was dissolved in water (300 mL). The aqueous phase was extracted with EtOAc (100 mL×3). The combined organic layers were dried and evaporated under reduced pressure to give crude product, which was purified by column chromatography (petroleum ether/ethyl acetate 10:1) to afford 2-(3-(benzyloxy)-4-methoxyphenyl)acetonitril (5.0 g, 48%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.48-7.33 (m, 5H), 6.89-6.86 (m, 3H), 5.17 (s, 2H), 3.90 (s, 3H), 3.66 (s, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$) 149.6, 148.6, 136.8, 128.8, 128.8, 128.2, 127.5, 127.5, 122.1, 120.9, 118.2, 113.8, 112.2, 71.2, 56.2, 23.3.

Example 14

2-(3-Chloro-4-methoxyphenyl)acetonitrile

**[0370]**

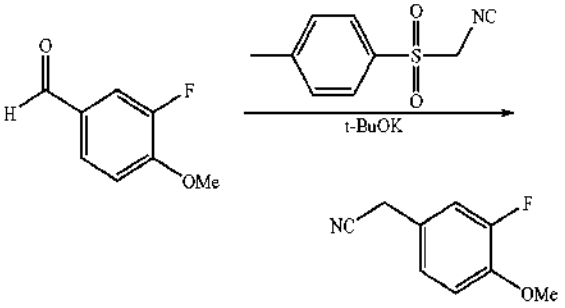



**[0371]** To a suspension of t-BuOK (4.8 g, 40 mmol) in THF (30 mL) was added a solution of TosMIC (3.9 g, 20 mmol) in THF (10 mL) at −78° C. The mixture was stirred for 10 minutes, treated with a solution of 3-chloro-4-methoxy-benzaldehyde (1.7 g, 10 mmol) in THF (10 mL) dropwise, and continued to stir for 1.5 hours at −78° C. To the cooled reaction mixture was added methanol (10 mL). The mixture was heated at reflux for 30 minutes. Solvent of the reaction mixture was removed to give a crude product, which was dissolved in water (20 mL). The aqueous phase was extracted with EtOAc (20 mL×3). The combined organic layers were dried and evaporated under reduced pressure to give crude product, which was purified by column chromatography (petroleum ether/ethyl acetate 10:1) to afford 2-(3-chloro-4-methoxyphenyl)acetonitrile (1.5 g, 83%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.33 (d, J=2.4 Hz, 1H), 7.20 (dd, J=2.4, 8.4 Hz, 1H), 6.92 (d, J=8.4 Hz, 1H), 3.91 (s, 3H), 3.68 (s, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 154.8, 129.8, 127.3, 123.0, 122.7, 117.60, 112.4, 56.2, 22.4.

Example 15

2-(3-Fluoro-4-methoxyphenyl)acetonitrile

**[0372]**

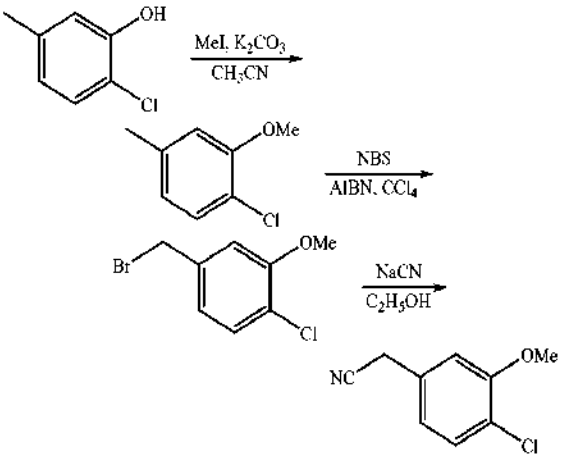



**[0373]** To a suspension of t-BuOK (25.3 g, 0.207 mol) in THF (150 mL) was added a solution of TosMIC (20.3 g, 0.104 mol) in THF (50 mL) at −78° C. The mixture was stirred for 15 minutes, treated with a solution of 3-fluoro-4-methoxy-benzaldehyde (8.00 g, 51.9 mmol) in THF (50 mL) dropwise, and continued to stir for 1.5 hours at −78° C. To the cooled reaction mixture was added methanol (50 mL). The mixture was heated at reflux for 30 minutes. Solvent of the reaction mixture was removed to give a crude product, which was dissolved in water (200 mL). The aqueous phase was extracted with EtOAc (100 mL×3). The combined organic layers were dried and evaporated under reduced pressure to give crude product, which was purified by column chromatography (petroleum ether/ethyl acetate 10:1) to afford 2-(3-fluoro-4-methoxyphenyl)acetonitrile (5.0 g, 58%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.02-7.05 (m, 2H), 6.94 (t, J=8.4 Hz, 1H), 3.88 (s, 3H), 3.67 (s, 2H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 152.3, 147.5, 123.7, 122.5, 117.7, 115.8, 113.8, 56.3, 22.6.

Example 16

2-(4-Chloro-3-methoxyphenyl)acetonitrile

**[0374]**



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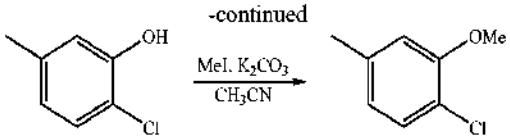
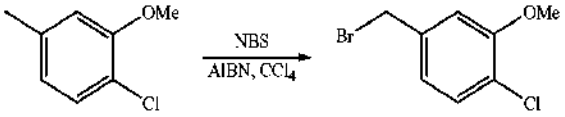


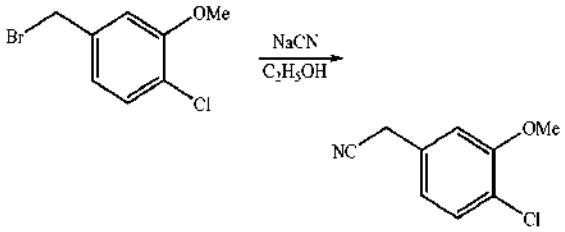

Chloro-2-methoxy-4-methyl-benzene

**[0375]** To a solution of 2-chloro-5-methyl-phenol (93 g, 0.65 mol) in $CH_3CN$ (700 mL) was added $CH_3I$ (110 g, 0.78 mol) and $K_2CO_3$ (180 g, 1.3 mol). The mixture was stirred at 25° C. overnight. The solid was filtered off and the filtrate was evaporated under vacuum to give 1-chloro-2-methoxy-4-methyl-benzene (90 g, 89%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.22 (d, J=7.8 Hz, 1H), 6.74-6.69 (m, 2H), 3.88 (s, 3H), 2.33 (s, 3H).




4-Bromomethyl-1-chloro-2-methoxy-benzene

**[0376]** To a solution of 1-chloro-2-methoxy-4-methyl-benzene (50 g, 0.32 mol) in $CCl_4$ (350 mL) was added NBS (57 g, 0.32 mol) and AIBN (10 g, 60 mmol). The mixture was heated at reflux for 3 hours. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=20:1) to give 4-bromomethyl-1-chloro-2-methoxy-benzene (69 g, 92%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.33-7.31 (m, 1H), 6.95-6.91 (m, 2H), 4.46 (s, 2H), 3.92 (s, 3H).



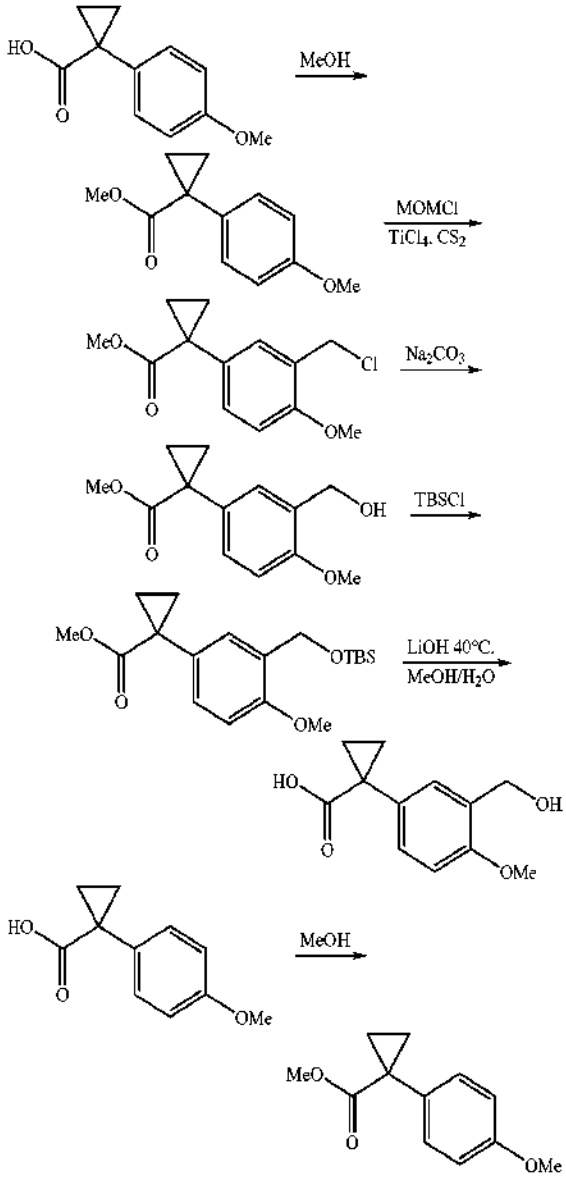

2-(4-Chloro-3-methoxyphenyl)acetonitrile

**[0377]** To a solution of 4-bromomethyl-1-chloro-2-methoxy-benzene (68.5 g, 0.290 mol) in $C_2H_5OH$ (90%, 500 mL) was added NaCN (28.5 g, 0.580 mol). The mixture was stirred at 60° C. overnight. Ethanol was evaporated and the residue was dissolved in $H_2O$. The mixture was extracted with ethyl acetate (300 mL×3). The combined organic layers were washed with brine, dried over $Na_2SO_4$ and purified by column chromatography on silica gel (petroleum ether/ethyl acetate 30:1) to give 2-(4-chloro-3-methoxyphenyl)acetonitrile (25 g, 48%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J=8 Hz, 1H), 6.88-6.84 (m, 2H), 3.92 (s, 3H), 3.74 (s, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 155.4, 130.8, 129.7, 122.4, 120.7, 117.5, 111.5, 56.2, 23.5.

Example 17

1-(3-(Hydroxymethyl)-4-methoxyphenyl)cyclopropanecarboxylic acid

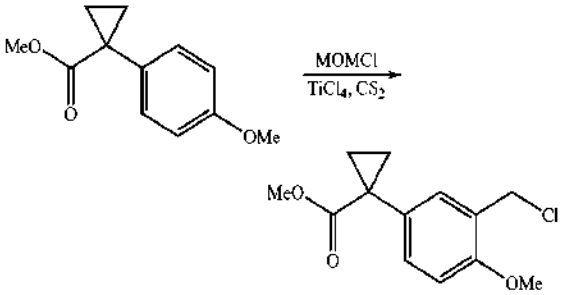
**[0378]**




1-(4-Methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester

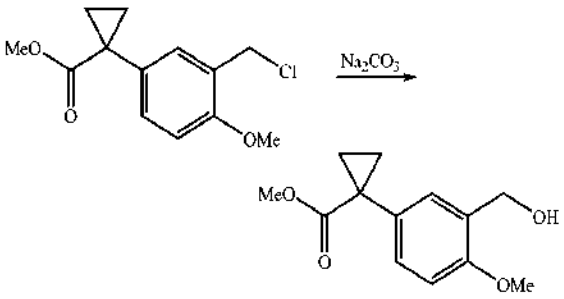
**[0379]** To a solution of 1-(4-methoxy-phenyl)-cyclopropanecarboxylic acid (50 g, 0.26 mol) in MeOH (500 mL) was added toluene-4-sulfonic acid monohydrate (2.5 g, 13 mmol) at room temperature. The reaction mixture was heated at reflux for 20 hours. MeOH was removed by evaporation under vacuum and EtOAc (200 mL) was added. The organic layer was washed with sat. aq. $NaHCO_3$ (100 mL) and brine, dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-(4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester (53 g, 99%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ

7.25-7.27 (m, 2H), 6.85 (d, J=8.8 Hz, 2H), 3.80 (s, 3H), 3.62 (s, 3H), 1.58 (m, 2H), 1.15 (m, 2H).



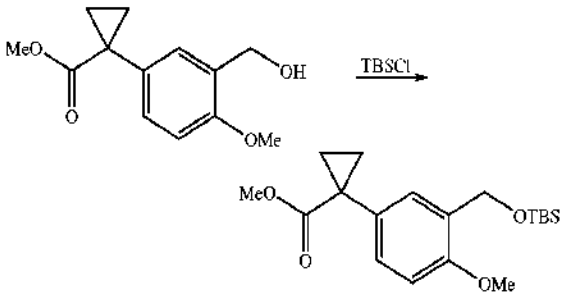

1-(3-Chloromethyl-4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester

**[0380]** To a solution of 1-(4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester (30.0 g, 146 mmol) and MOMCl (29.1 g, 364 mmol) in $CS_2$ (300 mL) was added $TiCl_4$ (8.30 g, 43.5 mmol) at 5° C. The reaction mixture was heated at 30° C. for 1 d and poured into ice-water. The mixture was extracted with $CH_2Cl_2$ (150 mL×3). The combined organic extracts were evaporated under vacuum to give 1-(3-chloromethyl-4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester (38.0 g), which was used in the next step without further purification.



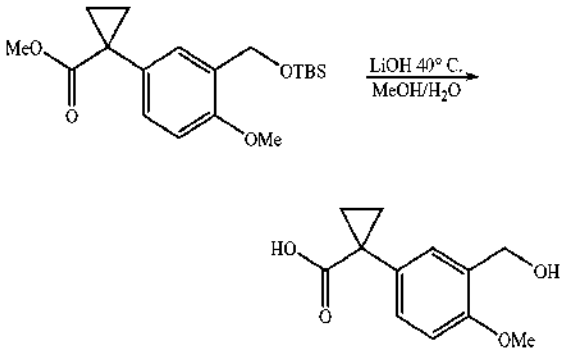

1-(3-Hydroxymethyl-4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester

**[0381]** To a suspension of 1-(3-chloromethyl-4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester (20 g) in water (350 mL) was added $Bu_4NBr$ (4.0 g) and $Na_2CO_3$ (90 g, 0.85 mol) at room temperature. The reaction mixture was heated at 65° C. overnight. The resulting solution was acidified with aq. HCl (2 mol/L) and extracted with EtOAc (200 mL×3). The organic layer was washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give crude product, which was purified by column (petroleum ether/ethyl acetate 15:1) to give 1-(3-hydroxymethyl-4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester (8.0 g, 39%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.23-7.26 (m, 2H), 6.83 (d, J=8.0 Hz, 1H), 4.67 (s, 2H), 3.86 (s, 3H), 3.62 (s, 3H), 1.58 (q, J=3.6 Hz, 2H), 1.14-1.17 (m, 2H).




1-[3-(tert-Butyl-dimethyl-silanyloxymethyl)-4-methoxy-phenyl]cyclopropane carboxylic acid methyl ester

**[0382]** To a solution of 1-(3-hydroxymethyl-4-methoxy-phenyl)-cyclopropanecarboxylic acid methyl ester (8.0 g, 34 mmol) in $CH_2Cl_2$ (100 mL) were added imidazole (5.8 g, 85 mmol) and TBSCl (7.6 g, 51 mmol) at room temperature. The mixture was stirred overnight at room temperature. The mixture was washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give crude product, which was purified by column (petroleum ether/ethyl acetate 30:1) to give 1-[3-(tert-butyl-dimethyl-silanyloxymethyl)-4-methoxy-phenyl]-cyclopropanecarboxylic acid methyl ester (6.7 g, 56%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.44-7.45 (m, 1H), 7.19 (dd, J=2.0, 8.4 Hz, 1H), 6.76 (d, J=8.4 Hz, 1H), 4.75 (s, 2H), 3.81 (s, 3H), 3.62 (s, 3H), 1.57-1.60 (m, 2H), 1.15-1.18 (m, 2H), 0.96 (s, 9H), 0.11 (s, 6H).



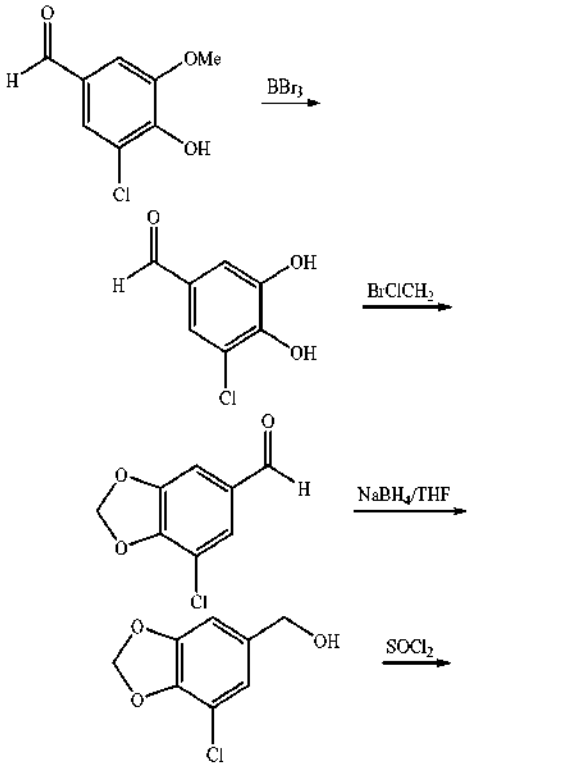

1-(3-Hydroxymethyl-4-methoxy-phenyl)-cyclopropanecarboxylic acid

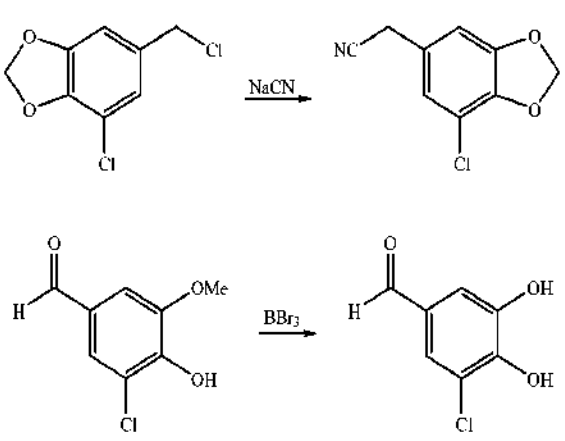
**[0383]** To a solution of 1-[3-(tert-butyl-dimethyl-silanyloxymethyl)-4-methoxy-phenyl]-cyclopropane carboxylic acid methyl ester (6.2 g, 18 mmol) in MeOH (75 mL) was added a solution of $LiOH.H_2O$ (1.5 g, 36 mmol) in water (10 mL) at 0° C. The reaction mixture was stirred overnight at 40° C. MeOH was removed by evaporation under vacuum. AcOH (1 mol/L, 40 mL) and EtOAc (200 mL) were added. The organic layer was separated, washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to provide 1-(3-hydroxymethyl-4-methoxy-phenyl)-cyclopropanecarboxylic acid (5.3 g).

Example 18

2-(7-Chlorobenzo[d][1,3]dioxol-5-yl)acetonitrile

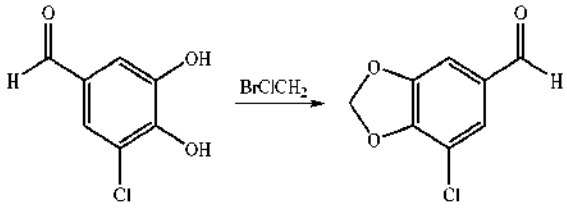
**[0384]**





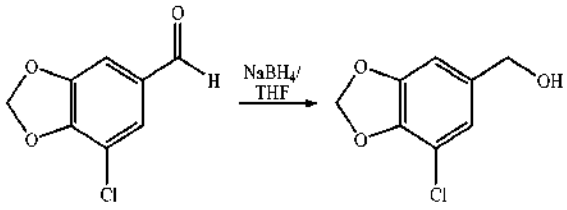
3-Chloro-4,5-dihydroxybenzaldehyde

**[0385]** To a suspension of 3-chloro-4-hydroxy-5-methoxybenzaldehyde (10 g, 54 mmol) in dichloromethane (300 mL) was added $BBr_3$ (26.7 g, 107 mmol) dropwise at −40° C. under $N_2$. After addition, the mixture was stirred at this temperature for 5 h and then was poured into ice water. The precipitated solid was filtered and washed with petroleum ether. The filtrate was evaporated under reduced pressure to afford 3-chloro-4,5-dihydroxybenzaldehyde (9.8 g, 89%), which was directly used in the next step.



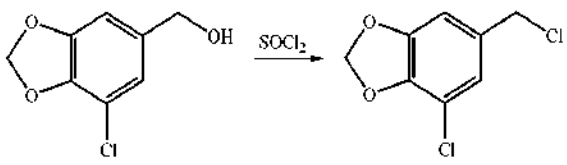
7-Chlorobenzo[d][1,3]dioxole-5-carbaldehyde

**[0386]** To a solution of 3-chloro-4,5-dihydroxybenzaldehyde (8.0 g, 46 mmol) and $BrClCH_2$ (23.9 g, 185 mmol) in dry DMF (100 mL) was added $Cs_2CO_3$ (25 g, 190 mmol). The mixture was stirred at 60° C. overnight and was then poured into water. The resulting mixture was extracted with EtOAc (50 mL×3). The combined extracts were washed with brine (100 mL), dried over $Na_2SO_4$ and concentrated under reduced pressure to afford 7-chlorobenzo[d][1,3]dioxole-5-carbaldehyde (6.0 g, 70%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H), 7.42 (d, J=0.4 Hz, 1H), 7.26 (d, J=3.6 Hz, 1H), 6.15 (s, 2H).



(7-Chlorobenzo[d][1,3]dioxol-5-yl)methanol

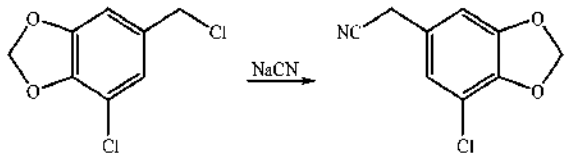
**[0387]** To a solution of 7-chlorobenzo[d][1,3]dioxole-5-carbaldehyde (6.0 g, 33 mmol) in THF (50 mL) was added $NaBH_4$ (2.5 g, 64 mmol)) in portions at 0° C. The mixture was stirred at this temperature for 30 min and then poured into aqueous $NH_4Cl$ solution. The organic layer was separated, and the aqueous phase was extracted with EtOAc (50 mL×3). The combined extracts were dried over $Na_2SO_4$ and evaporated under reduced pressure to afford (7-chlorobenzo[d][1,3]dioxol-5-yl)methanol, which was directly used in the next step.



4-Chloro-6-(chloromethyl)benzo[d][1,3]dioxole

**[0388]** A mixture of (7-chlorobenzo[d][1,3]-dioxol-5-yl) methanol (5.5 g, 30 mmol) and $SOCl_2$ (5.0 mL, 67 mmol) in dichloromethane (20 mL) was stirred at room temperature for 1 h and was then poured into ice water. The organic layer was separated and the aqueous phase was extracted with dichloromethane (50 mL×3). The combined extracts were washed

with water and aqueous $NaHCO_3$ solution, dried over $Na_2SO_4$ and evaporated under reduced pressure to afford 4-chloro-6-(chloromethyl)benzo[d][1,3]dioxole, which was directly used in the next step.



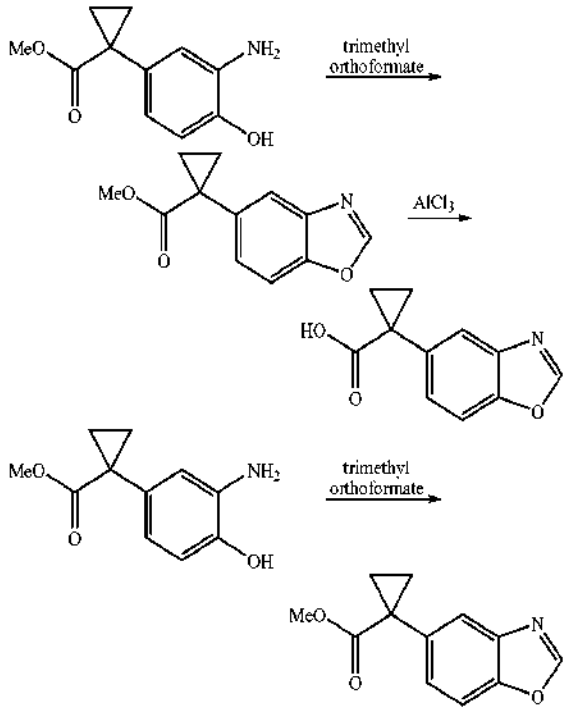

2-(7-Chlorobenzo[d][1,3]dioxol-5-yl)acetonitrile

**[0389]** A mixture of 4-chloro-6-(chloromethyl)benzo[d][1,3]dioxole (6.0 g, 29 mmol) and NaCN (1.6 g, 32 mmol) in DMSO (20 mL) was stirred at 40° C. for 1 h and was then poured into water. The mixture was extracted with EtOAc (30 mL×3). The combined organic layers were washed with water and brine, dried over $Na_2SO_4$ and evaporated under reduced pressure to afford 2-(7-chlorobenzo[d][1,3]dioxol-5-yl)acetonitrile (3.4 g, 58%). $^1H$ NMR δ 6.81 (s, 1H), 6.71 (s, 1H), 6.07 (s, 2H), 3.64 (s, 2H). $^{13}C$-NMR 149.2, 144.3, 124.4, 122.0, 117.4, 114.3, 107.0, 102.3, 23.1.

Example 19

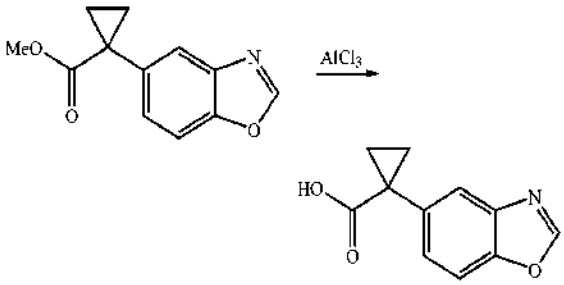
1-(Benzo[d]oxazol-5-yl)cyclopropanecarboxylic acid

**[0390]**




1-Benzooxazol-5-yl-cyclopropanecarboxylic acid methyl ester

**[0391]** To a solution of 1-(3-amino-4-hydroxyphenyl)cyclopropanecarboxylic acid methyl ester (3.00 g, 14.5 mmol) in DMF were added trimethyl orthoformate (5.30 g, 14.5 mmol) and a catalytic amount of p-toluenesulfonic acid monohydrate (0.3 g) at room temperature. The mixture was stirred for 3 hours at room temperature. The mixture was diluted with water and extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-benzooxazol-5-yl-cyclopropanecarboxylic acid methyl ester (3.1 g), which was directly used in the next step. $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.09 (s, 1), 7.75 (d, J=1.2 Hz, 1H), 7.53-7.51 (m, 1H), 7.42-7.40 (m, 1H), 3.66 (s, 3H), 1.69-1.67 (m, 2H), 1.27-1.24 (m, 2H).



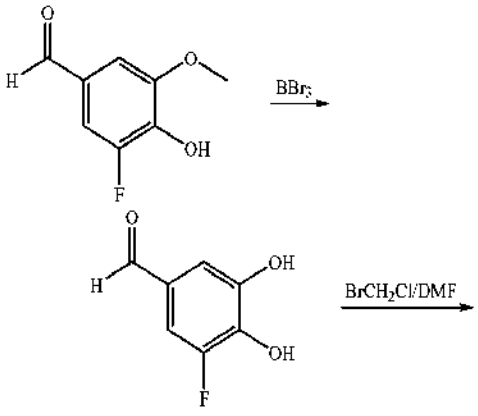

1-(Benzo[d]oxazol-5-yl)cyclopropanecarboxylic acid

**[0392]** To a solution of 1-benzooxazol-5-yl-cyclopropanecarboxylic acid methyl ester (2.9 g) in EtSH (30 mL) was added $AlCl_3$ (5.3 g, 40 mmol) in portions at 0° C. The reaction mixture was stirred for 18 hours at room temperature. Water (20 mL) was added dropwise at 0° C. The resulting mixture was extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 1:2) to give 1-(benzo[d]oxazol-5-yl)cyclopropanecarboxylic acid (280 mg, 11% over two steps). $^1H$ NMR (DMSO, 400 MHz) δ 12.25 (brs, 1H), 8.71 (s, 1H), 7.70-7.64 (m, 2H), 7.40 (dd, J=1.6, 8.4 Hz, 1H), 1.49-1.46 (m, 2H), 1.21-1.18 (m, 2H). MS (ESI) m/e ($M+H^+$) 204.4.

Example 20

2-(7-Fluorobenzo[d][1,3]dioxol-5-yl)acetonitrile

**[0393]**



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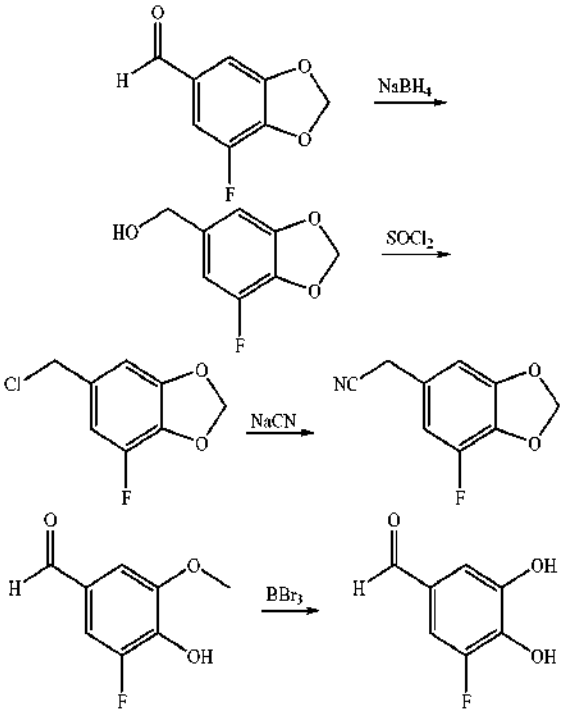
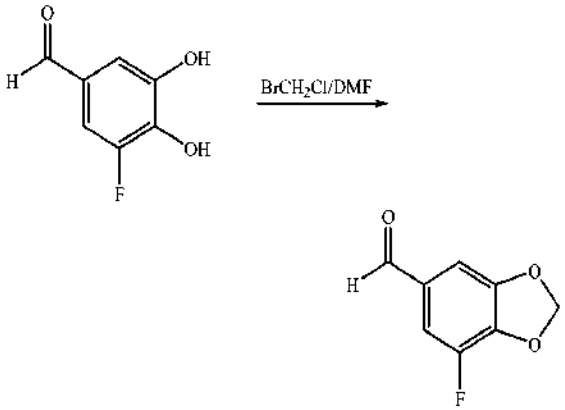


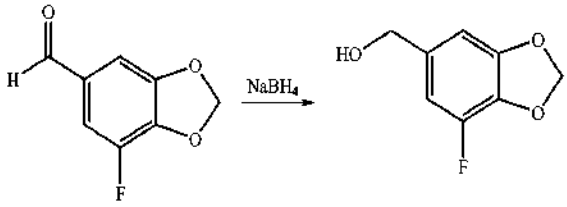
3-Fluoro-4,5-dihydroxy-benzaldehyde

**[0394]** To a suspension of 3-fluoro-4-hydroxy-5-methoxy-benzaldehyde (1.35 g, 7.94 mmol) in dichloromethane (100 mL) was added $BBr_3$ (1.5 mL, 16 mmol) dropwise at −78° C. under $N_2$ After addition, the mixture was warmed to −30° C. and it was stirred at this temperature for 5 h. The reaction mixture was poured into ice water. The precipitated solid was collected by filtration and washed with dichloromethane to afford 3-fluoro-4,5-dihydroxy-benzaldehyde (1.1 g, 89%), which was directly used in the next step.



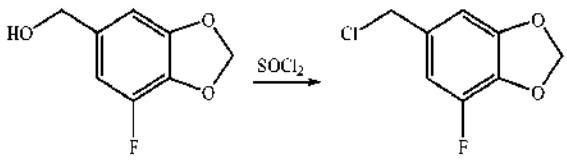
7-Fluoro-benzo[1,3]dioxole-5-carbaldehyde

**[0395]** To a solution of 3-fluoro-4,5-dihydroxy-benzaldehyde (1.5 g, 9.6 mmol) and $BrClCH_2$ (4.9 g, 38.5 mmol) in dry DMF (50 mL) was added $Cs_2CO_3$ (12.6 g, 39 mmol). The mixture was stirred at 60° C. overnight and was then poured into water. The resulting mixture was extracted with EtOAc (50 mL×3). The combined organic layers were washed with brine (100 mL), dried over $Na_2SO_4$ and evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to afford 7-fluoro-benzo[1,3]dioxole-5-carbaldehyde (0.80 g, 49%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 9.78 (d, J=0.9 Hz, 1H), 7.26 (dd, J=1.5, 9.3 Hz, 1H), 7.19 (d, J=1.2 Hz, 1H), 6.16 (s, 2H).



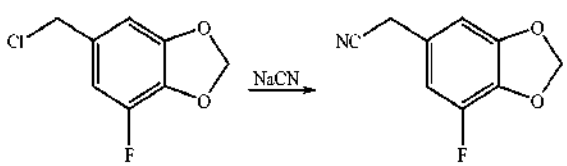
(7-Fluoro-benzo[1,3]dioxol-5-yl)-methanol

**[0396]** To a solution of 7-fluoro-benzo[1,3]dioxole-5-carbaldehyde (0.80 g, 4.7 mmol) in MeOH (50 mL) was added $NaBH_4$ (0.36 g, 9.4 mmol) in portions at 0° C. The mixture was stirred at this temperature for 30 min and was then concentrated to dryness. The residue was dissolved in EtOAc. The EtOAc layer was washed with water, dried over $Na_2SO_4$ and concentrated to dryness to afford (7-fluoro-benzo[1,3]dioxol-5-yl)-methanol (0.80 g, 98%), which was directly used in the next step.



6-Chloromethyl-4-fluoro-benzo[1,3]dioxole

**[0397]** To $SOCl_2$ (20 mL) was added (7-fluoro-benzo[1,3]dioxol-5-yl)-methanol (0.80 g, 4.7 mmol) in portions at 0° C. The mixture was warmed to room temperature over 1 h and then was heated at reflux for 1 h. The excess $SOCl_2$ was evaporated under reduced pressure to give the crude product, which was basified with saturated aqueous $NaHCO_3$ to pH ~7. The aqueous phase was extracted with EtOAc (50 mL×3). The combined organic layers were dried over $Na_2SO_4$ and evaporated under reduced pressure to give 6-chloromethyl-4-fluoro-benzo[1,3]dioxole (0.80 g, 92%), which was directly used in the next step.



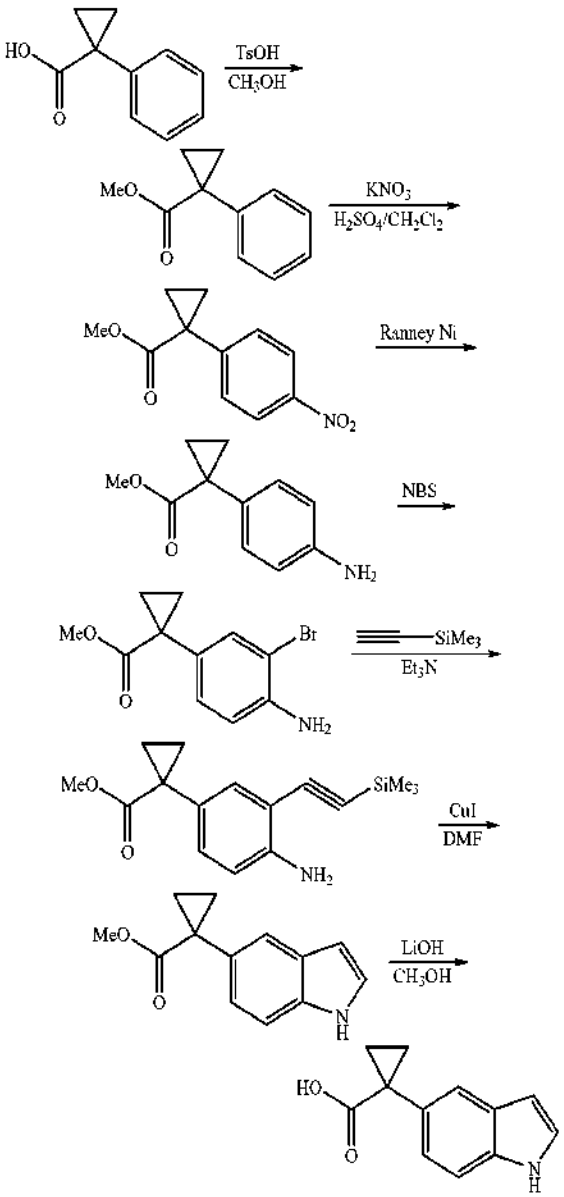
2-(7-Fluorobenzo[d][1,3]dioxol-5-yl)acetonitrile

**[0398]** A mixture of 6-chloromethyl-4-fluoro-benzo[1,3] dioxole (0.80 g, 4.3 mmol) and NaCN (417 mg, 8.51 mmol) in DMSO (20 mL) was stirred at 30° C. for 1 h and was then poured into water. The mixture was extracted with EtOAc (50 mL×3). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried over $Na_2SO_4$ and evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to afford 2-(7-fluorobenzo[d] [1,3]dioxol-5-yl)acetonitrile (530 mg, 70%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 6.68-6.64 (m, 2H), 6.05 (s, 2H), 3.65 (s, 2H). $^{13}C$-NMR δ151.1, 146.2. 134.1, 124.2, 117.5, 110.4, 104.8, 102.8, 23.3.

Example 21

1-(1H-Indol-5-yl)cyclopropanecarboxylic acid

**[0399]**

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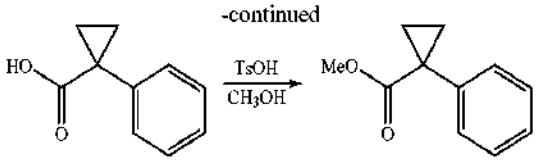
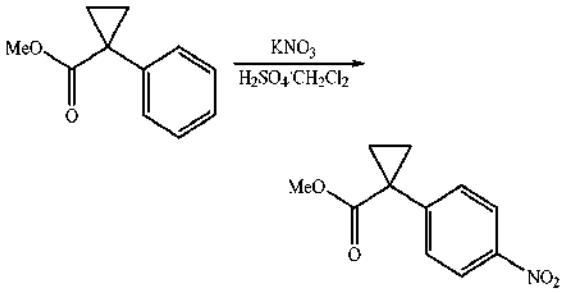


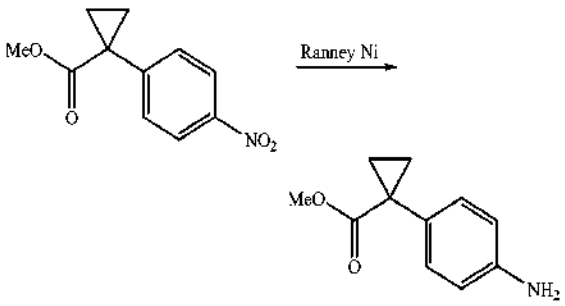

Methyl 1-phenylcyclopropanecarboxylate

**[0400]** To a solution of 1-phenylcyclopropanecarboxylic acid (25 g, 0.15 mol) in $CH_3OH$ (200 mL) was added TsOH (3 g, 0.1 mol) at room temperature. The mixture was refluxed overnight. The solvent was evaporated under reduced pressure to give crude product, which was dissolved into EtOAc. The EtOAc layer was washed with aq. sat. $NaHCO_3$. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure to give methyl 1-phenylcyclopropanecarboxylate (26 g, 96%), which was used directly in the next step. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.37-7.26 (m, 5H), 3.63 (s, 3H), 1.63-1.60 (m, 2H), 1.22-1.19 (m, 2H).



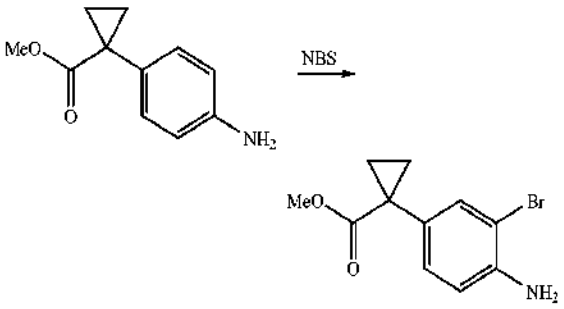

Methyl 1-(4-nitrophenyl)cyclopropanecarboxylate

**[0401]** To a solution of 1-phenylcyclopropanecarboxylate (20.62 g, 0.14 mol) in $H_2SO_4/CH_2Cl_2$ (40 mL/40 mL) was added $KNO_3$ (12.8 g, 0.13 mol) in portion at 0° C. The mixture was stirred for 0.5 hr at 0° C. Ice water was added and the mixture was extracted with EtOAc (100 mL×3). The organic layers were dried with anhydrous $Na_2SO_4$ and evaporated to give methyl 1-(4-nitrophenyl)cyclopropanecarboxylate (21 g, 68%), which was used directly in the next step. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.18 (dd, J=2.1, 6.9 Hz, 2H), 7.51 (dd, J=2.1, 6.9 Hz, 2H), 3.64 (s, 3H), 1.72-1.69 (m, 2H), 1.25-1.22 (m, 2H).



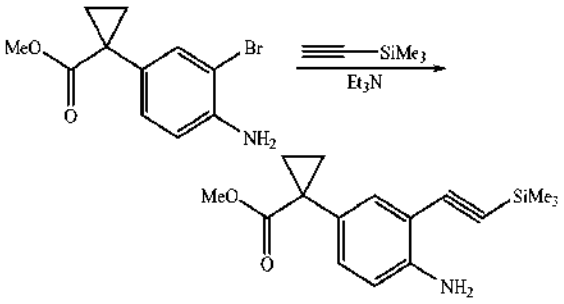
Methyl 1-(4-aminophenyl)cyclopropanecarboxylate

**[0402]** To a solution of methyl 1-(4-nitrophenyl)cyclopropanecarboxylate (20 g, 0.09 mol) in MeOH (400 mL) was added Ni (2 g) under nitrogen atmosphere. The mixture was stirred under hydrogen atmosphere (1 atm) at room temperature overnight. The catalyst was filtered off through a pad of Celite and the filtrate was evaporated under vacuum to give crude product, which was purified by chromatography column on silica gel (petroleum ether/ethyl acetate=10:1) to give methyl 1-(4-aminophenyl)cyclopropanecarboxylate (11.38 g, 66%). $^1$H NMR (300 MHz, $CDCl_3$) δ7.16 (d, J=8.1 Hz, 2H), 6.86 (d, J=7.8 Hz, 2H), 4.31 (br, 2H), 3.61 (s, 3H), 1.55-1.50 (m, 2H), 1.30-1.12 (m, 2H).



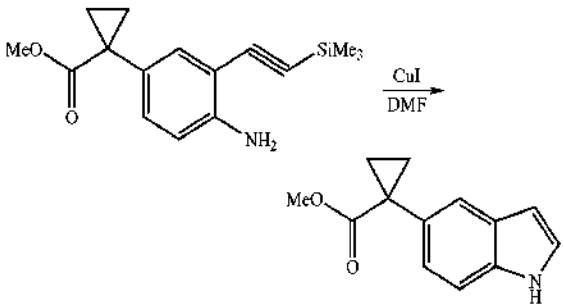

Methyl
1-(4-amino-3-bromophenyl)cyclopropanecarboxylate

**[0403]** To a solution of methyl 1-(4-aminophenyl)cyclopropanecarboxylate (10.38 g, 0.05 mol) in acetonitrile (200 mL) was added NBS (9.3 g, 0.05 mol) at room temperature. The mixture was stirred overnight. Water (200 mL) was added. The organic layer was separated and the aqueous phase was extracted with EtOAc (80 mL×3). The organic layers were dried with anhydrous $Na_2SO_4$ and evaporated to give methyl 1-(4-amino-3-bromophenyl)cyclopropanecarboxylate (10.6 g, 78%), which was used directly in the next step. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J=2.0 Hz, 1H), 7.08 (dd, J=1.6, 8.4 Hz, 1H), 6.70 (d, J=8.4 Hz, 1H), 3.62 (s, 3H), 1.56-1.54 (m, 2H), 1.14-1.11 (m, 2H).



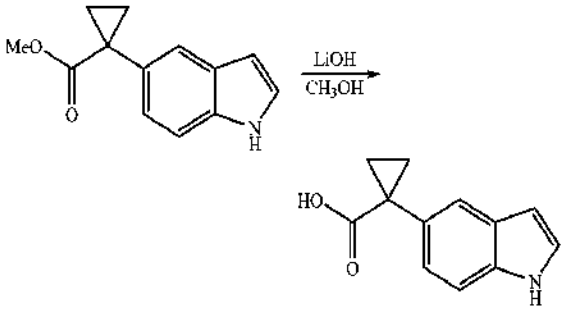

Methyl 1-(4-amino-3-((trimethylsilyl)ethynyl)phenyl)cyclopropane carboxylate

**[0404]** To a degassed solution of methyl 1-(4-amino-3-bromophenyl)cyclopropane carboxylate (8 g, 0.03 mol) in $Et_3N$ (100 mL) was added ethynyl-trimethyl-silane (30 g, 0.3 mol), DMAP (5% mol) and $Pd(PPh_3)_2Cl_2$ (5% mol) under $N_2$. The mixture was refluxed at 70° C. overnight. The insoluble solid was filtered off and washed with EtOAc (100 mL×3). The filtrate was evaporated under reduced pressure to give a residue, which was purified by chromatography column on silica gel (petroleum ether/ethyl acetate=20:1) to give methyl 1-(4-amino-3-((trimethylsilyl)ethynyl)phenyl)cyclopropanecarboxylate (4.8 g, 56%). $^1$H NMR (300 MHz, $CDCl_3$) δ7.27 (s, 1H), 7.10 (dd, J=2.1, 8.4 Hz, 1H), 6.64 (d, J=8.4 Hz, 1H), 3.60 (s, 3H), 1.55-1.51 (m, 2H), 1.12-1.09 (m, 2H), 0.24 (s, 9H).




Methyl 1-(1H-indol-5-yl)cyclopropanecarboxylate

**[0405]** To a degassed solution of methyl 1-(4-amino-3-((trimethylsilyl)ethynyl)phenyl)cyclopropanecarboxylate (4.69 g, 0.02 mol) in DMF (20 mL) was added CuI (1.5 g, 0.008 mol) under $N_2$ at room temperature. The mixture was stirred for 3 hr at room temperature. The insoluble solid was filtered off and washed with EtOAc (50 mL×3). The filtrate was evaporated under reduced pressure to give a residue, which was purified by chromatography column on silica gel (petroleum ether/ethyl acetate=20:1) to give methyl 1-(1H-indol-5-yl)cyclopropanecarboxylate (2.2 g, 51%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.33 (d, J=8.4 Hz, 1H), 7.23-7.18 (m, 2H), 6.52-6.51 (m, 1H) 3.62 (s, 3H), 1.65-1.62 (m, 2H), 1.29-1.23 (m, 2H).




1-(1H-Indol-5-yl)cyclopropanecarboxylic acid

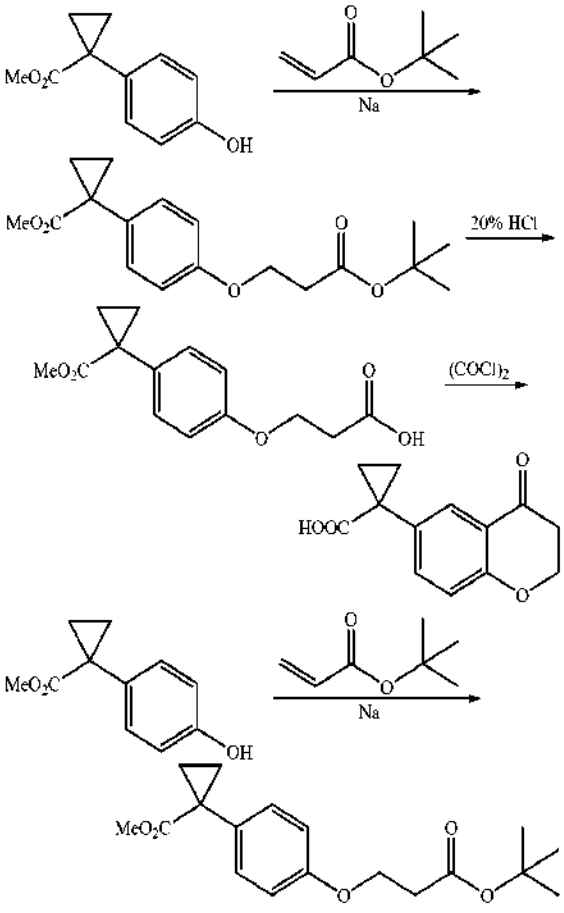
**[0406]** To a solution of methyl 1-(1H-indol-5-yl)cyclopropanecarboxylate (1.74 g, 8 mmol) in $CH_3OH$ (50 m L) and water (20 mL) was added LiOH (1.7 g, 0.04 mol). The mixture was heated at 45° C. for 3 hr. Water was added and the mixture was acidified with concentrated HCl to pH ~3 before being extracted with EtOAc (20 mL×3). The organic layers were dried over anhydrous $Na_2SO_4$ and evaporated to give 1-(1H-indol-5-yl)cyclopropanecarboxylic acid (1.4 g, 87%).

$^1$H NMR (300 MHz, DMSO-$d_6$) 7.43 (s, 1H), 7.30-7.26 (m, 2H), 7.04 (dd, J=1.5, 8.4 Hz, 1H), 6.35 (s, 1H); 1.45-1.41 (m, 2H), 1.14-1.10 (m, 2H).

Example 22

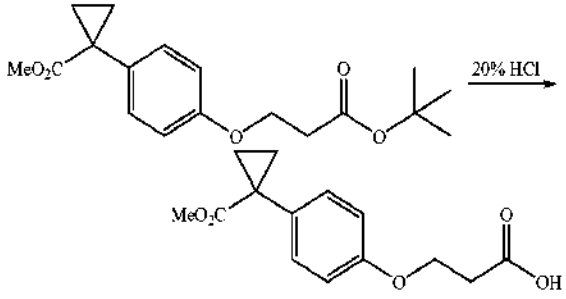
1-(4-Oxochroman-6-yl)cyclopropanecarboxylic acid

**[0407]**



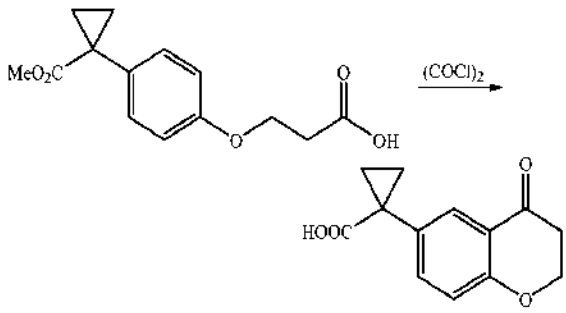

1-[4-(2-tert-Butoxycarbonyl-ethoxy)-phenyl]-cyclopropanecarboxylic methyl ester

**[0408]** To a solution of 1-(4-hydroxy-phenyl)-cyclopropanecarboxylic methyl ester (7.0 g, 3.6 mmol) in acrylic tert-butyl ester (50 mL) was added Na (42 mg, 1.8 mmol) at room temperature. The mixture was heated at 110° C. for 1 h. After cooling to room temperature, the resulting mixture was quenched with water and extracted with EtOAc (100 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 20:1) to give 1-[4-(2-tert-butoxycarbonyl-ethoxy)-phenyl]-cyclopropanecarboxylic methyl ester (6.3 g, 54%) and unreacted start material (3.0 g). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.24 (d, J=8.7 Hz, 2H), 6.84 (d, J=8.7 Hz, 2H), 4.20 (t, J=6.6 Hz, 2H), 3.62 (s, 3H), 2.69 (t, J=6.6 Hz, 2H), 1.59-1.56 (m, 2H), 1.47 (s, 9H), 1.17-1.42 (m, 2H).




1-[4-(2-Carboxy-ethoxy)-phenyl]-cyclopropanecarboxylic methyl ester

**[0409]** A solution of 1-[4-(2-tert-butoxycarbonyl-ethoxy)-phenyl]-cyclopropanecarboxylic methyl ester (6.3 g, 20 mmol) in HCl (20%, 200 mL) was heated at 110° C. for 1 h. After cooling to room temperature, the resulting mixture was filtered. The solid was washed with water and dried under vacuum to give 1-[4-(2-carboxy-ethoxy)-phenyl]-cyclopropanecarboxylic methyl ester (5.0 g, 96%). $^1$H NMR (300 MHz, DMSO) δ 7.23-7.19 (m, 2H), 6.85-6.81 (m, 2H), 4.13 (t, J=6.0 Hz, 2H), 3.51 (s, 3H), 2.66 (t, J=6.0 Hz, 2H), 1.43-1.39 (m, 2H), 1.14-1.10 (m, 2H).




1-(4-Oxochroman-6-yl)cyclopropanecarboxylic acid

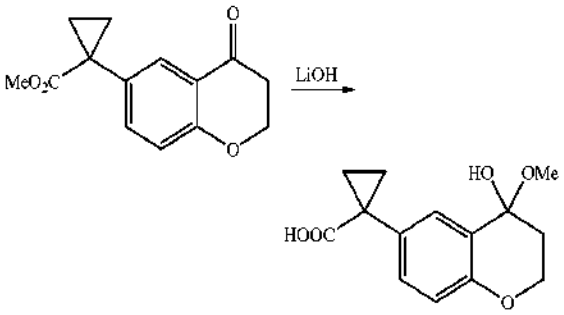
**[0410]** To a solution of 1-[4-(2-carboxy-ethoxy)-phenyl]-cyclopropanecarboxylic methyl ester (5.0 g, 20 mmol) in $CH_2Cl_2$ (50 mL) were added oxalyl chloride (4.8 g, 38 mmol) and two drops of DMF at 0° C. The mixture was stirred at 0~5° C. for 1 h and then evaporated under vacuum. To the resulting mixture was added $CH_2Cl_2$ (50 mL) at 0° C. and stirring was continued at 0~5° C. for 1 h. The reaction was slowly quenched with water and was extracted with EtOAc (50 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 20:1-2:1) to give 1-(4-oxochroman-6-yl)cyclopropanecarboxylic acid (830 mg, 19%) and methyl 1-(4-oxochroman-6-yl)cyclopropanecarboxylate (1.8 g, 38%). 1-(4-Oxochroman-6-yl)cyclopropane-carboxylic acid: $^1$H NMR (400 MHz, DMSO) δ 12.33 (br s, 1H), 7.62 (d, J=2.0 Hz, 1H), 7.50 (dd, J=2.4, 8.4 Hz, 1H), 6.95 (d, J=8.4 Hz, 1H), 4.50 (t, J=6.4 Hz, 2H), 2.75 (t, J=6.4 Hz, 2H), 1.44-1.38 (m, 2H), 1.10-1.07 (m, 2H). MS (ESI) m/z ($M+H^+$) 231.4. 1-(4-Oxochroman-6-yl)cyclopro-

panecarboxylate: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J=2.4 Hz, 1H), 7.48 (dd, J=2.4, 8.4 Hz, 1H), 6.93 (d, J=8.4 Hz, 1H), 4.55-4.52 (m, 2H), 3.62 (s, 3H), 2.80 (t, J=6.4 Hz, 2H), 1.62-1.56 (m, 2H), 1.18-1.15 (m, 2H).

Example 23

1-(4-Hydroxy-4-methoxychroman-6-yl)cyclopropanecarboxylic acid

**[0411]**



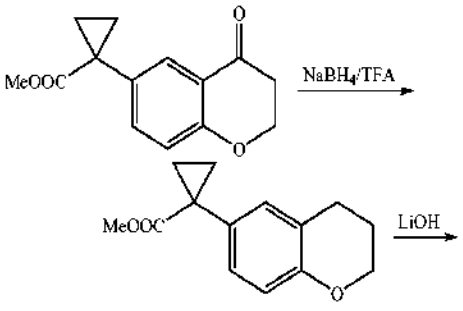

1-(4-Hydroxy-4-methoxychroman-6-yl)cyclopropanecarboxylic acid

**[0412]** To a solution of methyl 1-(4-oxochroman-6-yl)cyclopropanecarboxylate (1.0 g, 4.1 mmol) in MeOH (20 mL) and water (20 mL) was added $LiOH.H_2O$ (0.70 g, 16 mmol) in portions at room temperature. The mixture was stirred overnight at room temperature before the MeOH was removed by evaporation under vacuum. Water and $Et_2O$ were added to the residue and the aqueous layer was separated, acidified with HCl and extracted with EtOAc (50 mL×3). The combined organic extracts dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-(4-hydroxy-4-methoxychroman-6-yl)cyclopropanecarboxylic acid (480 mg, 44%). $^1$H NMR (400 MHz, $CDCl_3$) δ 12.16 (s, 1H), 7.73 (d, J=2.0 Hz, 1H), 7.47 (dd, J=2.0, 8.4 Hz, 1H), 6.93 (d, J=8.8 Hz, 1H), 3.83-3.80 (m, 2H), 3.39 (s, 3H), 3.28-3.25 (m, 2H), 1.71-1.68 (m, 2H), 1.25-1.22 (m, 2H). MS (ESI) m/z ($M+H^+$) 263.1.

Example 24

1-(4-Hydroxy-4-methoxychroman-6-yl)cyclopropanecarboxylic acid

**[0413]**

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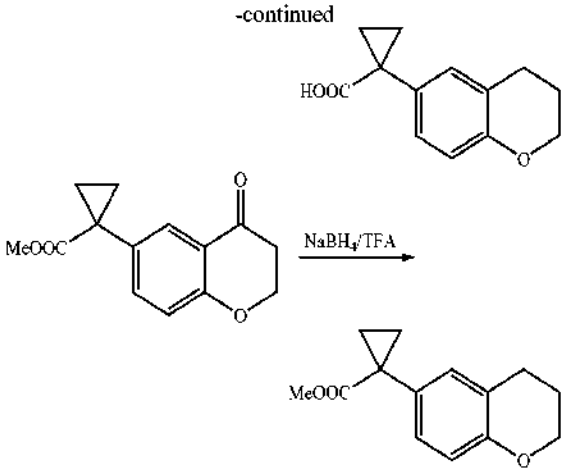
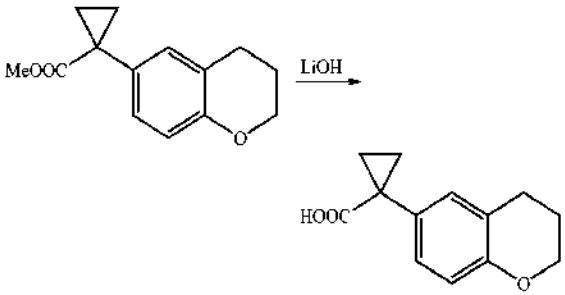



1-Chroman-6-yl-cyclopropanecarboxylic methyl ester

**[0414]** To trifluoroacetic acid (20 mL) was added $NaBH_4$ (0.70 g, 130 mmol) in portions at 0° C. under $N_2$ atmosphere. After stirring for 5 min, a solution of 1-(4-oxo-chroman-6-yl)-cyclopropanecarboxylic methyl ester (1.6 g, 6.5 mmol) was added at 15° C. The reaction mixture was stirred for 1 h at room temperature before being slowly quenched with water. The resulting mixture was extracted with EtOAc (50 mL×3). The combined organic extracts dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-chroman-6-yl-cyclopropanecarboxylic methyl ester (1.4 g, 92%), which was used directly in the next step. $^1$H NMR (300 MHz, $CDCl_3$) δ 7.07-7.00 (m, 2H), 6.73 (d, J=8.4 Hz, 1H), 4.17 (t, J=5.1 Hz, 2H), 3.62 (s, 3H), 2.79-2.75 (m, 2H), 2.05-1.96 (m, 2H), 1.57-1.54 (m, 2H), 1.16-1.13 (m, 2H).




1-(4-Hydroxy-4-methoxychroman-6-yl)cyclopropanecarboxylic acid

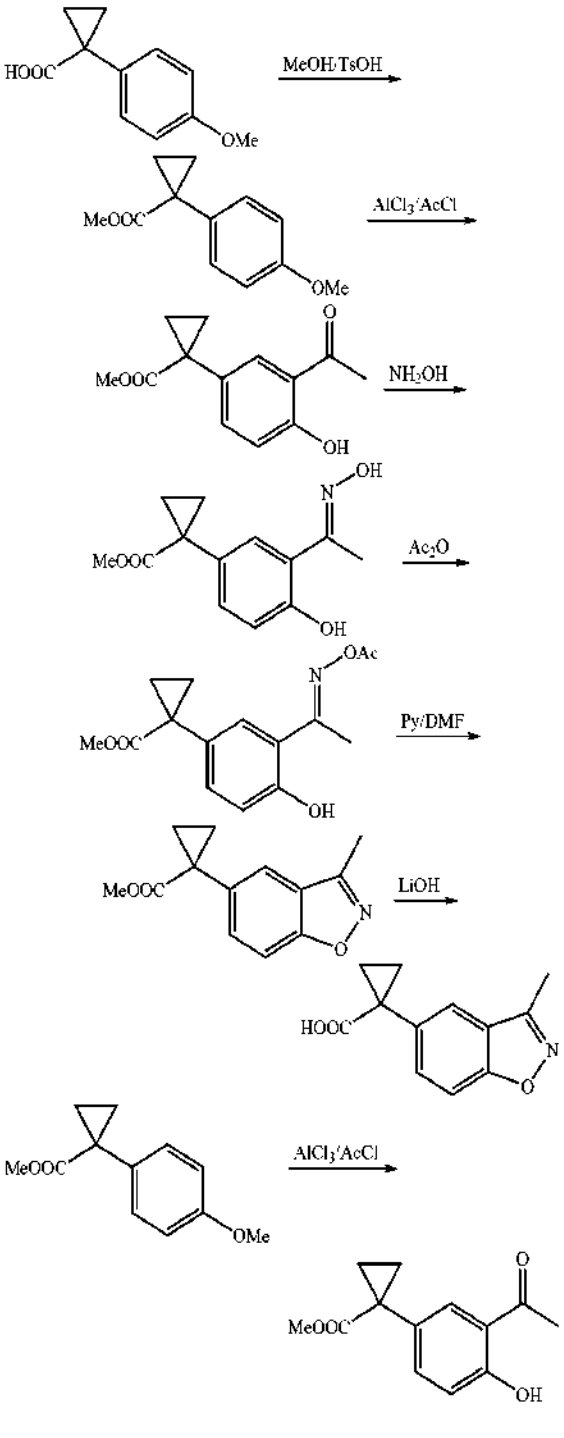
**[0415]** To a solution of 1-chroman-6-yl-cyclopropanecarboxylic methyl ester (1.4 g, 60 mmol) in MeOH (20 mL) and water (20 mL) was added $LiOH.H_2O$ (1.0 g, 240 mmol) in portions at room temperature. The mixture was stirred overnight at room temperature before the MeOH was removed by evaporation under vacuum. Water and $Et_2O$ were added and the aqueous layer was separated, acidified with HCl and extracted with EtOAc (50 mL×3). The combined organic extracts dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-(4-Hydroxy-4-methoxychroman-6-yl)cy-

clopropanecarboxylic acid (1.0 g, 76%). $^1$H NMR (400 MHz, DMSO) δ 12.10 (br s, 1H), 6.95 (d, J=2.4 Hz, 2H), 6.61-6.59 (m, 1H), 4.09-4.06 (m, 2H), 2.70-2.67 (m, 2H), 1.88-1.86 (m, 2H), 1.37-1.35 (m, 2H), 1.04-1.01 (m, 2H). MS (ESI) m/z ($M+H^+$) 217.4.

Example 25

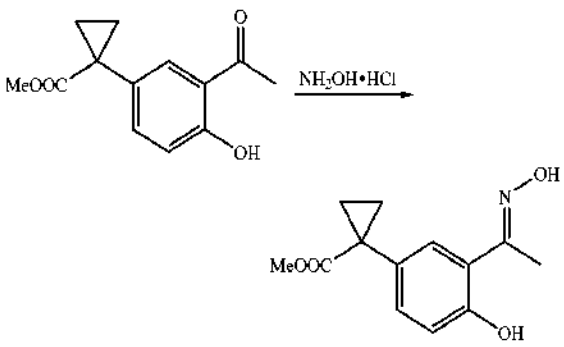
1-(3-Methylbenzo[d]isoxazol-5-yl)cyclopropanecarboxylic acid

**[0416]**



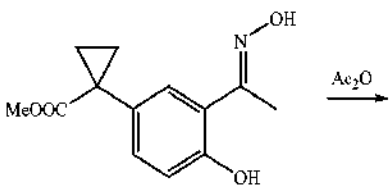

1-(3-Acetyl-4-hydroxy-phenyl)-cyclopropanecarboxylic methyl ester

**[0417]** To a stirred suspension of $AlCl_3$ (58 g, 440 mmol) in $CS_2$ (500 mL) was added acetyl chloride (7.4 g, 95 mmol) at room temperature. After stirring for 5 min, methyl 1-(4-methoxyphenyl)cyclopropanecarboxylate (15 g, 73 mmol) was added. The reaction mixture was heated at reflux for 2 h before ice water was added carefully to the mixture at room temperature. The resulting mixture was extracted with EtOAc (150 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure to give 1-(3-acetyl-4-hydroxy-phenyl)-cyclopropanecarboxylic methyl ester (15 g, 81%), which was used in the next step without further purification. $^1$H NMR ($CDCl_3$, 400 MHz) δ 12.28 (s, 1H), 7.67 (d, J=2.0 Hz, 1H), 7.47 (dd, J=2.0, 8.4 Hz, 1H), 6.94 (d, J=8.4 Hz, 1H), 3.64 (s, 3H), 2.64 (s, 3H), 1.65-1.62 (m, 2H), 1.18-1.16 (m, 2H).




1-[4-Hydroxy-3-(1-hydroxyimino-ethyl)-phenyl]-cyclopropanecarboxylic methyl ester

**[0418]** To a stirred solution of 1-(3-acetyl-4-hydroxy-phenyl)-cyclopropanecarboxylic methyl ester (14.6 g, 58.8 mmol) in EtOH (500 mL) were added hydroxylamine hydrochloride (9.00 g, 129 mmol) and sodium acetate (11.6 g, 141 mmol) at room temperature. The resulting mixture was heated at reflux overnight. After removal of EtOH under vacuum, water (200 mL) and EtOAc (200 mL) were added. The organic layer was separated and the aqueous layer was extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-[4-hydroxy-3-(1-hydroxyimino-ethyl)-phenyl]-cyclopropanecarboxylic methyl ester (14.5 g, 98%), which was used in the next step without further purification. $^1$H NMR ($CDCl_3$, 400 MHz) δ 11.09 (s, 1H), 7.39 (d, J=2.0 Hz, 1H), 7.23 (d, J=2.0 Hz, 1H), 7.14 (s, 1H), 6.91 (d, J=8.4 Hz, 1H), 3.63 (s, 3H), 2.36 (s, 3H), 1.62-1.59 (m, 2H), 1.18-1.15 (m, 2H).



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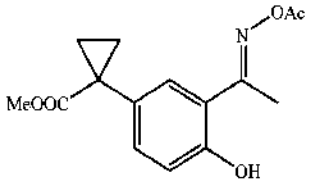
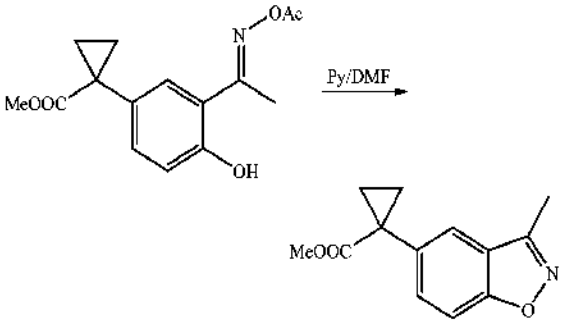


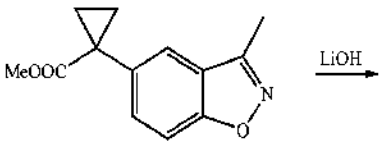
(E)-Methyl 1-(3-(1-(acetoxyimino)ethyl)-4-hydroxyphenyl)cyclopropanecarboxylate

**[0419]** The solution of 1-[4-hydroxy-3-(1-hydroxyiminoethyl)-phenyl]-cyclopropanecarboxylic methyl ester (10.0 g, 40.1 mmol) in $Ac_2O$ (250 mL) was heated at 45° C. for 4 h. The $Ac_2O$ was removed by evaporation under vacuum before water (100 mL) and EtOAc (100 mL) were added. The organic layer was separated and the aqueous layer was extracted with EtOAc (100 mL×2). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give (E)-methyl 1-(3-(1-(acetoxyimino) ethyl)-4-hydroxyphenyl)cyclopropanecarboxylate (10.5 g, 99%), which was used in the next step without further purification.



Methyl 1-(3-methylbenzo[d]isoxazol-5-yl)cyclopropanecarboxylate

**[0420]** A solution of (E)-methyl 1-(3-(1-(acetoxyimino) ethyl)-4-hydroxyphenyl)cyclopropane carboxylate (10.5 g, 39.6 mmol) and pyridine (31.3 g, 396 mmol) in DMF (150 mL) was heated at 125° C. for 10 h. The cooled reaction mixture was poured into water (250 mL) and was extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 50:1) to give methyl 1-(3-methylbenzo[d]isoxazol-5-yl)cyclopropanecarboxylate (7.5 g, 82%). $^1H$ NMR ($CDCl_3$, 300 MHz) δ 7.58-7.54 (m, 2H), 7.48 (dd, J=1.5, 8.1 Hz, 1H), 3.63 (s, 3H), 2.58 (s, 3H), 1.71-1.68 (m, 2H) 1.27-1.23 (m, 2H).



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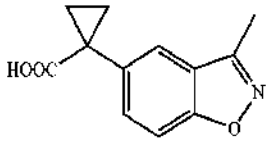


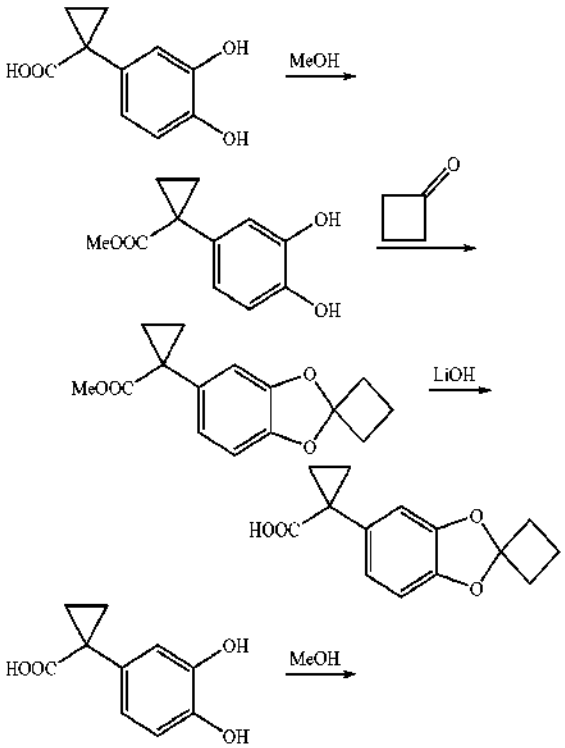
1-(3-Methylbenzo[d]isoxazol-5-yl)cyclopropanecarboxylic acid

**[0421]** To a solution of methyl 1-(3-methylbenzo[d]isoxazol-5-yl)cyclopropanecarboxylate (1.5 g, 6.5 mmol) in MeOH (20 mL) and water (2 mL) was added $LiOH.H_2O$ (0.80 g, 19 mmol) in portions at room temperature. The reaction mixture was stirred at room temperature overnight before the MeOH was removed by evaporation under vacuum. Water and $Et_2O$ were added and the aqueous layer was separated, acidified with HCl and extracted with EtOAc (50 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 1-(3-methylbenzo[d]isoxazol-5-yl)cyclopropanecarboxylic acid (455 mg, 32%). $^1H$ NMR (400 MHz, DMSO) δ 12.40 (br s, 1H), 7.76 (s, 1H), 7.60-7.57 (m, 2H), 2.63 (s, 3H), 1.52-1.48 (m, 2H), 1.23-1.19 (m, 2H). MS (ESI) m/z ($M+H^+$) 218.1.

Example 26

1-(Spiro[benzo[d][1,3]dioxole-2,1'-cyclobutane]-5-yl)cyclopropane carboxylic acid

**[0422]**



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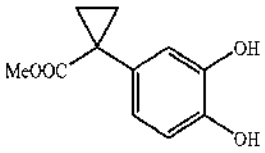
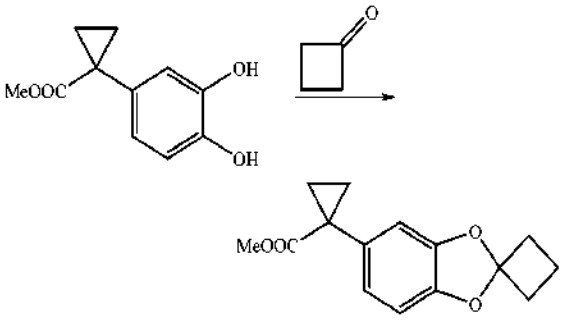


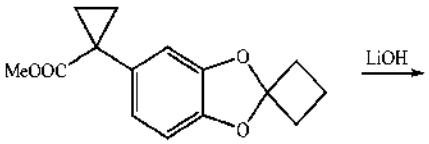
1-(3,4-Dihydroxy-phenyl)-cyclopropanecarboxylic methyl ester

**[0423]** To a solution of 1-(3,4-dihydroxyphenyl)cyclopropanecarboxylic acid (4.5 g) in MeOH (30 mL) was added TsOH (0.25 g, 1.3 mmol). The stirring was continued at 50° C. overnight before the mixture was cooled to room temperature. The mixture was concentrated under vacuum and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 3:1) to give 1-(3,4-dihydroxyphenyl)-cyclopropanecarboxylic methyl ester (2.1 g). $^1$H NMR (DMSO 300 MHz) δ 8.81 (brs, 2H), 6.66 (d, J=2.1 Hz, 1H), 6.61 (d, J=8.1 Hz, 1H), 6.53 (dd, J=2.1, 8.1 Hz, 1H), 3.51 (s, 3H), 1.38-1.35 (m, 2H), 1.07-1.03 (m, 2H).



Methyl 1-(spiro[benzo[d][1,3]dioxole-2,1'-cyclobutane]-5-yl)cyclopropane carboxylate

**[0424]** To a solution of 1-(3,4-dihydroxy-phenyl)-cyclopropanecarboxylic methyl ester (1.0 g, 4.8 mmol) in toluene (30 mL) was added TsOH (0.10 g, 0.50 mmol) and cyclobutanone (0.70 g, 10 mmol). The reaction mixture was heated at reflux for 2 h before being concentrated under vacuum. The residue was purified by chromatography on silica gel (petroleum ether/ethyl acetate 15:1) to give methyl 1-(spiro[benzo[d][1,3]dioxole-2,1'-cyclobutane]-5-yl)cyclopropanecarboxylate (0.6 g, 50%). $^1$H NMR ($CDCl_3$ 300 MHz) δ 6.78-6.65 (m, 3H), 3.62 (s, 3H), 2.64-2.58 (m, 4H), 1.89-1.78 (m, 2H), 1.56-1.54 (m, 2H), 1.53-1.12 (m, 2H).



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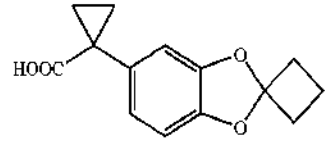


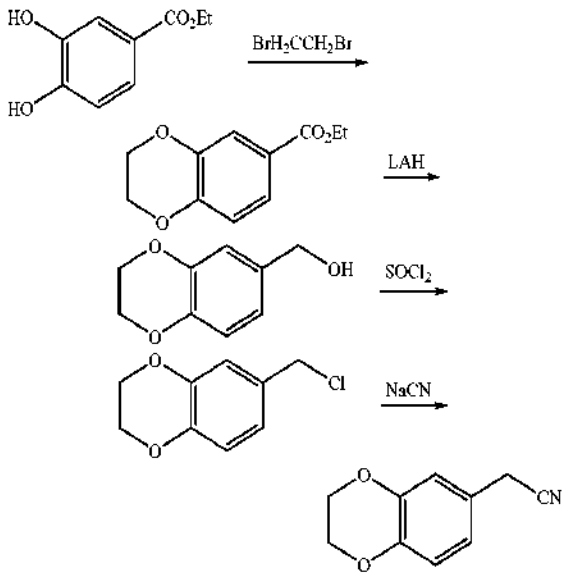
1-(Spiro[benzo[d][1,3]dioxole-2,1'-cyclobutane]-5-yl)cyclopropane carboxylic acid

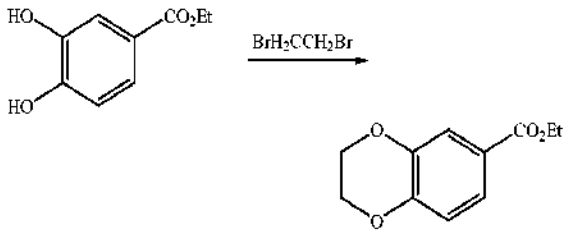
**[0425]** To a mixture of methyl 1-(spiro[benzo[d][1,3]dioxole-2,1'-cyclobutane]-5-yl)cyclopropanecarboxylate (0.60 g, 2.3 mmol) in THF/$H_2O$ (4:1, 10 mL) was added LiOH (0.30 g, 6.9 mmol). The mixture was stirred at 60° C. for 24 h. HCl (0.5 N) was added slowly to the mixture at 0° C. until pH 2-3. The mixture was extracted with EtOAc (10 mL×3). The combined organic phases were washed with brine, dried over anhydrous $MgSO_4$, and washed with petroleum ether to give 1-(spiro[benzo[d][1,3]-dioxole-2,1'-cyclobutane]-5-yl)cyclopropane carboxylic acid (330 mg, 59%). $^1$HNMR (400 MHz, $CDCl_3$) δ 6.78-6.65 (m, 3H), 2.65-2.58 (m, 4H), 1.86-1.78 (m, 2H), 1.63-1.60 (m, 2H), 1.26-1.19 (m, 2H).

Example 27

2-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)acetonitrile

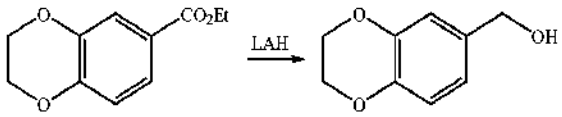
**[0426]**





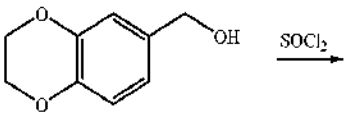
2,3-Dihydro-benzo[1,4]dioxine-6-carboxylic acid ethyl ester

**[0427]** To a suspension of $Cs_2CO_3$ (270 g, 1.49 mol) in DMF (1000 mL) were added 3,4-dihydroxybenzoic acid ethyl ester (54.6 g, 0.3 mol) and 1,2-dibromoethane (54.3 g, 0.29 mol) at room temperature. The resulting mixture was stirred at 80° C. overnight and then poured into ice-water. The mixture was extracted with EtOAc (200 mL×3). The combined organic layers were washed with water (200 mL×3) and brine (100 mL), dried over $Na_2SO_4$ and concentrated to dryness. The residue was purified by column (petroleum ether/ethyl acetate 50:1) on silica gel to obtain 2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid ethyl ester (18 g, 29%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.53 (dd, J=1.8, 7.2 Hz, 2H), 6.84-6.87 (m, 1H), 4.22-4.34 (m, 6H), 1.35 (t, J=7.2 Hz, 3H).




(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-methanol

**[0428]** To a suspension of $LiAlH_4$ (2.8 g, 74 mmol) in THF (20 mL) was added dropwise a solution of 2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid ethyl ester (15 g, 72 mmol) in THF (10 mL) at 0° C. under $N_2$. The mixture was stirred at room temperature for 1 h and then quenched carefully with addition of water (2.8 mL) and NaOH (10%, 28 mL) with cooling. The precipitated solid was filtered off and the filtrate was evaporated to dryness to obtain (2,3-dihydro-benzo[1,4]dioxin-6-yl)-methanol (10.6 g). $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 6.73-6.78 (m, 3H), 5.02 (t, J=5.7 Hz, 1H), 4.34 (d, J=6.0 Hz, 2H), 4.17-4.20 (m, 4H).

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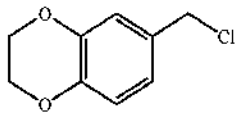
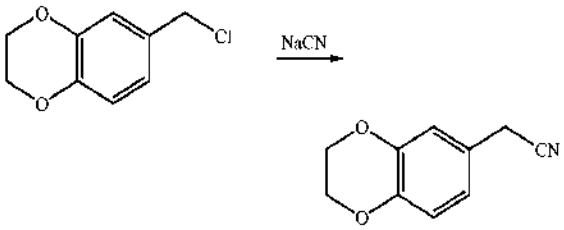



6-Chloromethyl-2,3-dihydro-benzo[1,4]dioxine

**[0429]** A mixture of (2,3-dihydro-benzo[1,4]dioxin-6-yl) methanol (10.6 g) in $SOCl_2$ (10 mL) was stirred at room temperature for 10 min and then poured into ice-water. The organic layer was separated and the aqueous phase was extracted with dichloromethane (50 mL×3). The combined organic layers were washed with $NaHCO_3$ (sat solution), water and brine, dried over $Na_2SO_4$ and concentrated to dryness to obtain 6-chloromethyl-2,3-dihydro-benzo[1,4]dioxine (12 g, 88% over two steps), which was used directly in next step.




2-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)acetonitrile

**[0430]** A mixture of 6-chloromethyl-2,3-dihydro-benzo[1,4]dioxine (12.5 g, 67.7 mmol) and NaCN (4.30 g, 87.8 mmol) in DMSO (50 mL) was stirred at rt for 1 h. The mixture was poured into water (150 mL) and then extracted with dichloromethane (50 mL×4). The combined organic layers were washed with water (50 mL×2) and brine (50 mL), dried over $Na_2SO_4$ and concentrated to dryness. The residue was purified by column (petroleum ether/ethyl acetate 50:1) on silica gel to obtain 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acetonitrile as a yellow oil (10.2 g, 86%). $^1H$-NMR (300 MHz, $CDCl_3$) δ 6.78-6.86 (m, 3H), 4.25 (s, 4H), 3.63 (s, 2H).

**[0431]** The following Table 2 contains a list of carboxylic acid building blocks that were commercially available, or prepared by one of the three methods described above:

TABLE 2

Carboxylic acid building blocks.

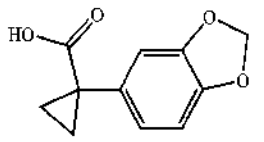
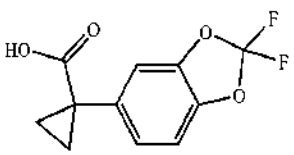
| Name | Structure |
|---|---|
| 1-benzo[1,3]dioxol-5-ylcyclopropane-1-carboxylic acid |  |
| 1-(2,2-difluorobenzo[1,3]dioxol-5-yl)cyclopropane-1-carboxylic acid |  |

TABLE 2-continued

Carboxylic acid building blocks.

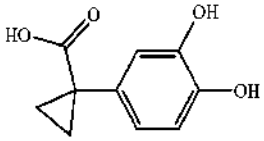
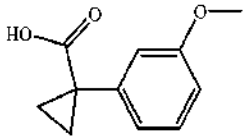
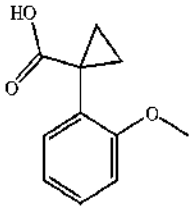
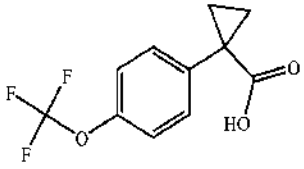
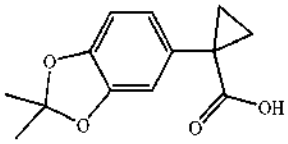
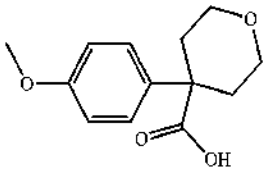
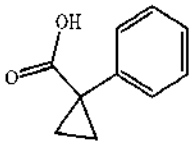
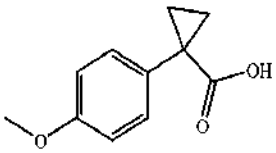
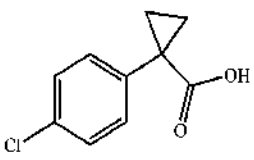
| Name | Structure |
|---|---|
| 1-(3,4-dihydroxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(3-methoxyphenyl)cyclopropane-1-carboxylic acid |  |
| 1-(2-methoxyphenyl)cyclopropane-1-carboxylic acid |  |
| 1-[4-(trifluoromethoxy)phenyl]cyclopropane-1-carboxylic acid |  |
| 1-(2,2-dimethylbenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid |  |
| tetrahydro-4-(4-methoxyphenyl)-2H-pyran-4-carboxylic acid |  |
| 1-phenylcyclopropane-1-carboxylic acid |  |
| 1-(4-methoxyphenyl)cyclopropane-1-carboxylic acid |  |
| 1-(4-chlorophenyl)cyclopropane-1-carboxylic acid |  |

TABLE 2-continued

Carboxylic acid building blocks.

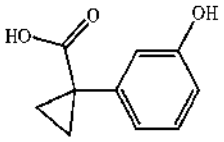
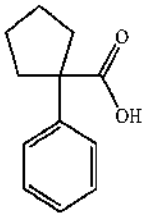
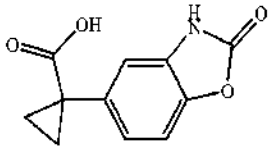
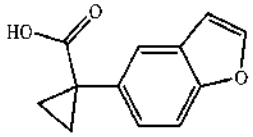
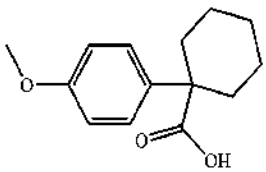
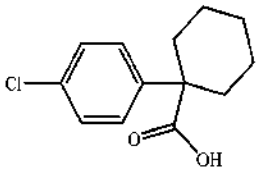
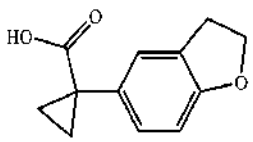
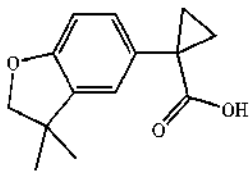
| Name | Structure |
|---|---|
| 1-(3-hydroxyphenyl)cyclopropanecarboxylic acid |  |
| 1-phenylcyclopentanecarboxylic acid |  |
| 1-(2-oxo-2,3-dihydrobenzo[d]oxazol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(benzofuran-5-yl)cyclopropanecarboxylic acid |  |
| 1-(4-methoxyphenyl)cyclohexanecarboxylic acid |  |
| 1-(4-chlorophenyl)cyclohexanecarboxylic acid |  |
| 1-(2,3-dihydrobenzofuran-5-yl)cyclopropanecarboxylic acid |  |
| 1-(3,3-dimethyl-2,3-dihydrobenzofuran-5-yl)cyclopropanecarboxylic acid |  |

TABLE 2-continued

Carboxylic acid building blocks.

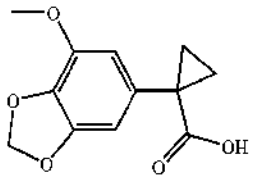
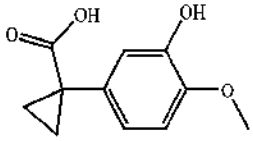
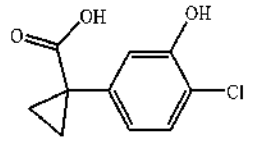
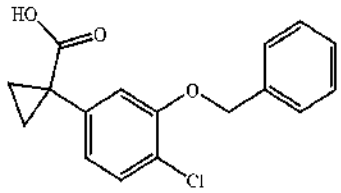
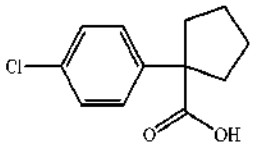
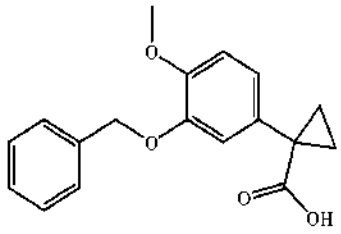
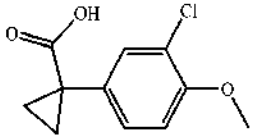
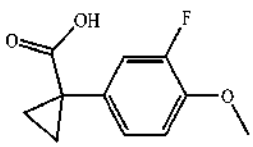
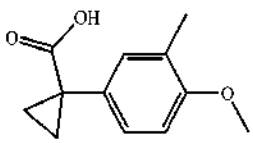
| Name | Structure |
|---|---|
| 1-(7-methoxybenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(3-hydroxy-4-methoxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(4-chloro-3-hydroxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(3-(benzyloxy)-4-chlorophenyl)cyclopropanecarboxylic acid |  |
| 1-(4-chlorophenyl)cyclopentanecarboxylic acid |  |
| 1-(3-(benzyloxy)-4-methoxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(3-chloro-4-methoxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(3-fluoro-4-methoxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(4-methoxy-3-methylphenyl)cyclopropanecarboxylic acid |  |

TABLE 2-continued

Carboxylic acid building blocks.

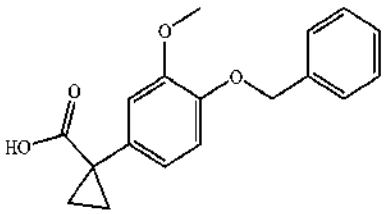
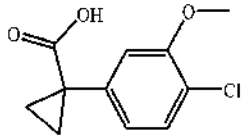
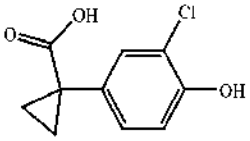
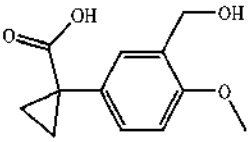
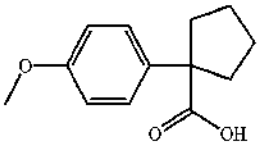
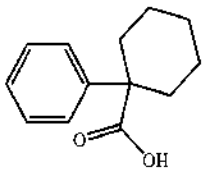
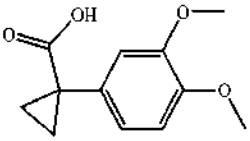
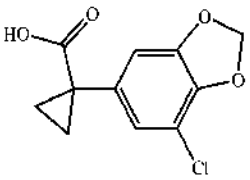
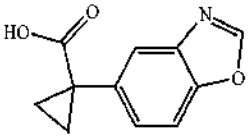
| Name | Structure |
|---|---|
| 1-(4-(benzyloxy)-3-methoxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(4-chloro-3-methoxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(3-chloro-4-hydroxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(3-(hydroxymethyl)-4-methoxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(4-methoxyphenyl)cyclopentanecarboxylic acid |  |
| 1-phenylcyclohexanecarboxylic acid |  |
| 1-(3,4-dimethoxyphenyl)cyclopropanecarboxylic acid |  |
| 1-(7-chlorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(benzo[d]oxazol-5-yl)cyclopropanecarboxylic acid |  |

TABLE 2-continued

Carboxylic acid building blocks.

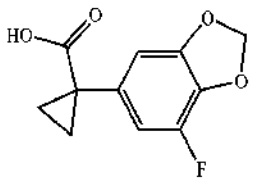
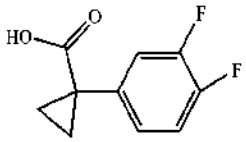
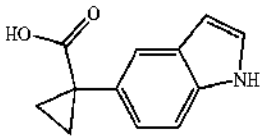
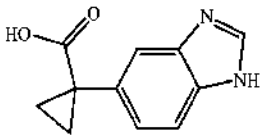
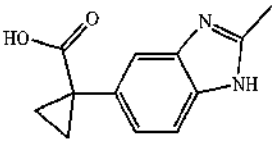
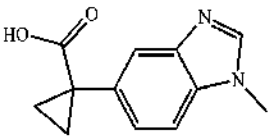
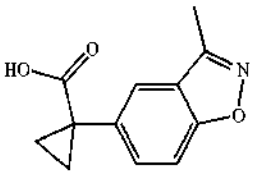
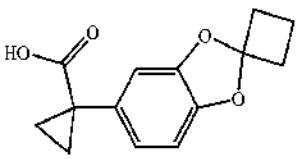
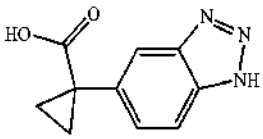
| Name | Structure |
|---|---|
| 1-(7-fluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(3,4-difluorophenyl)cyclopropanecarboxylic acid |  |
| 1-(1H-indol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(1H-benzo[d]imidazol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(2-methyl-1H-benzo[d]imidazol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(1-methyl-1H-benzo[d]imidazol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(3-methylbenzo[d]isoxazol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(spiro[benzo[d][1,3]dioxole-2,1'-cyclobutane]-5-yl)cyclopropanecarboxylic acid |  |
| 1-(1H-benzo[d][1,2,3]triazol-5-yl)cyclopropanecarboxylic acid |  |

TABLE 2-continued

Carboxylic acid building blocks.

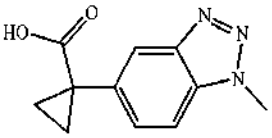
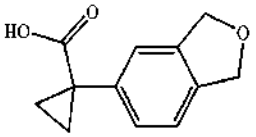
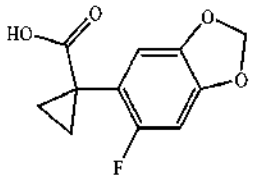
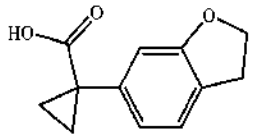
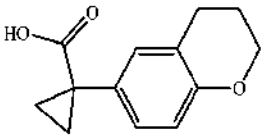
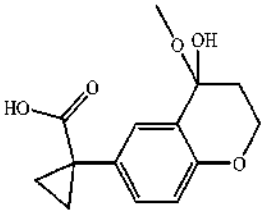
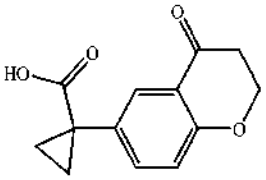
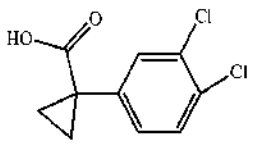
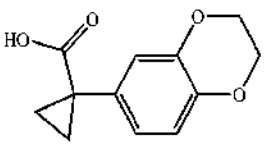
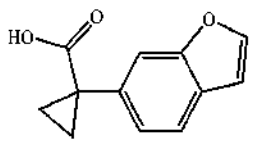
| Name | Structure |
|---|---|
| 1-(1-methyl-1H-benzo[d][1,2,3]triazol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(1,3-dihydroisobenzofuran-5-yl)cyclopropanecarboxylic acid |  |
| 1-(6-fluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid |  |
| 1-(2,3-dihydrobenzofuran-6-yl)cyclopropanecarboxylic acid |  |
| 1-(chroman-6-yl)cyclopropanecarboxylic acid |  |
| 1-(4-hydroxy-4-methoxychroman-6-yl)cyclopropanecarboxylic acid |  |
| 1-(4-oxochroman-6-yl)cyclopropanecarboxylic acid |  |
| 1-(3,4-dichlorophenyl)cyclopropanecarboxylic acid |  |
| 1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)cyclopropanecarboxylic acid |  |

TABLE 2-continued

Carboxylic acid building blocks.

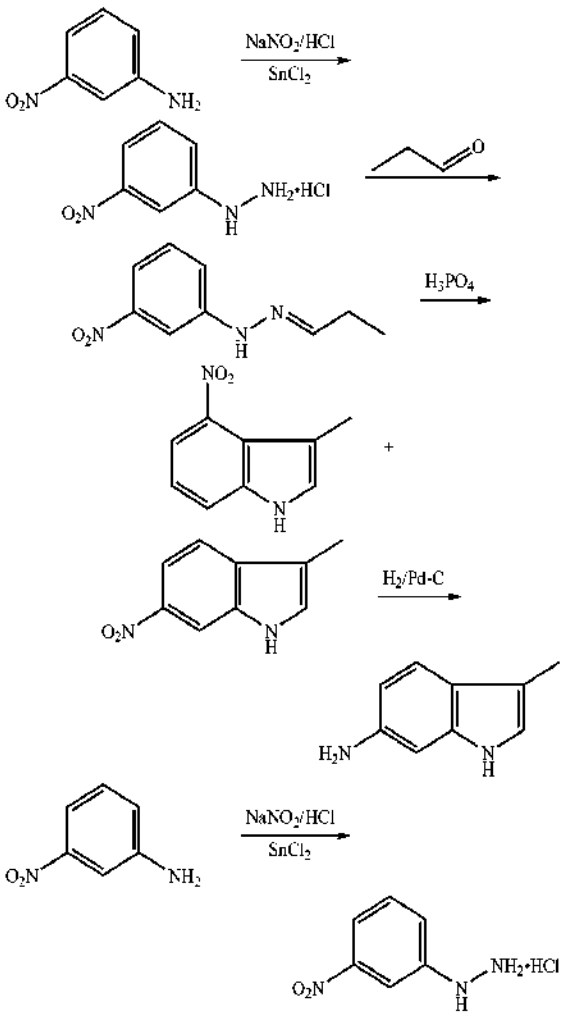
| Name | Structure |
|---|---|
| 1-(benzofuran-6-yl)cyclopropanecarboxylic acid |  |

**[0432]** Specific Procedures: Synthesis of Aminoindole Building Blocks

Example 28

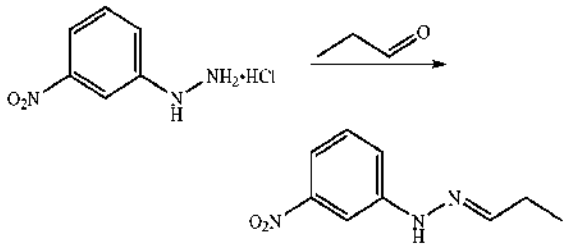
3-Methyl-1H-indol-6-amine

**[0433]**



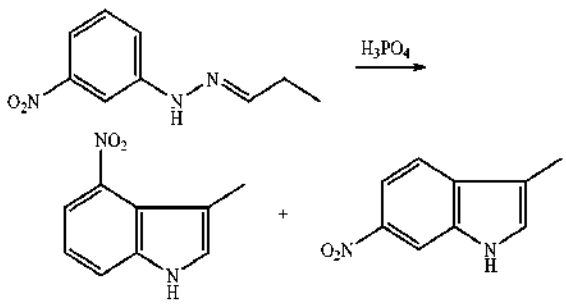
(3-Nitro-phenyl)-hydrazine hydrochloride salt

**[0434]** 3-Nitro-phenylamine (27.6 g, 0.2 mol) was dissolved in the mixture of $H_2O$ (40 mL) and 37% HCl (40 mL). A solution of $NaNO_2$ (13.8 g, 0.2 mol) in $H_2O$ (60 mL) was added to the mixture at 0° C., and then a solution of $SnCl_2 \cdot H_2O$ (135.5 g, 0.6 mol) in 37% HCl (100 mL) was added at that temperature. After stirring at 0° C. for 0.5 h, the insoluble material was isolated by filtration and was washed with water to give (3-nitrophenyl)hydrazine hydrochloride (27.6 g, 73%).



N-(3-Nitro-phenyl)-N'-propylidene-hydrazine

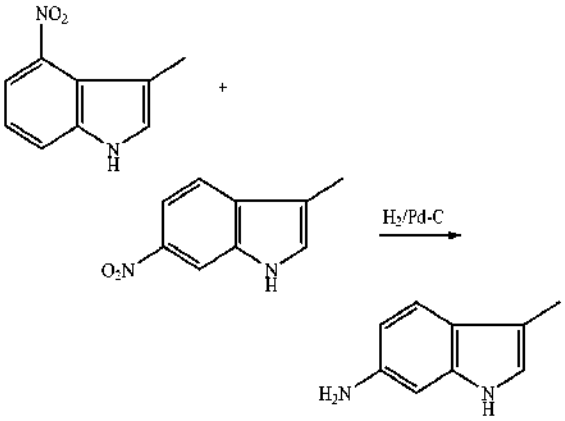
**[0435]** Sodium hydroxide solution (10%, 15 mL) was added slowly to a stirred suspension of (3-nitrophenyl)hydrazine hydrochloride (1.89 g, 10 mmol) in ethanol (20 mL) until pH 6. Acetic acid (5 mL) was added to the mixture followed by propionaldehyde (0.7 g, 12 mmol). After stirring for 3 h at room temperature, the mixture was poured into ice-water and the resulting precipitate was isolated by filtration, washed with water and dried in air to obtain (E)-1-(3-nitrophenyl)-2-propylidenehydrazine, which was used directly in the next step.



3-Methyl-4-nitro-1H-indole 3 and
3-methyl-6-nitro-1H-indole

**[0436]** A mixture of (E)-1-(3-nitrophenyl)-2-propylidenehydrazine dissolved in 85% $H_3PO_4$ (20 mL) and toluene (20 mL) was heated at 90-100° C. for 2 h. After cooling, toluene

was removed under reduced pressure. The resultant oil was basified to pH 8 with 10% NaOH. The aqueous layer was extracted with EtOAc (100 mL×3). The combined organic layers were dried, filtered and concentrated under reduced pressure to afford the mixture of 3-methyl-4-nitro-1H-indole and 3-methyl-6-nitro-1H-indole [1.5 g in total, 86%, two steps from (3-nitrophenyl)hydrazine hydrochloride] which was used to the next step without further purification.



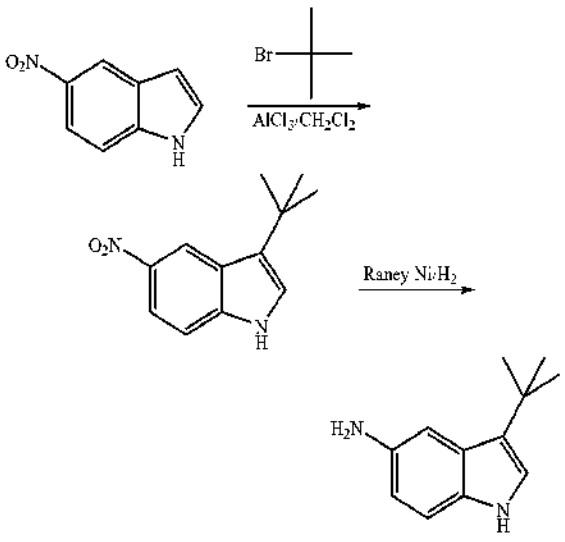

3-Methyl-1H-indol-6-amine

**[0437]** The crude mixture from previous steps (3 g, 17 mmol) and 10% Pd—C (0.5 g) in ethanol (30 mL) was stirred overnight under $H_2$ (1 atm) at room temperature. Pd—C was filtered off and the filtrate was concentrated under reduced pressure. The solid residue was purified by column to give 3-methyl-1H-indol-6-amine (0.6 g, 24%). $^1$H NMR ($CDCl_3$) δ 7.59 (br s. 1H), 7.34 (d, J=8.0 Hz, 1H), 6.77 (s, 1H), 6.64 (s, 1H), 6.57 (m, 1H), 3.57 (brs, 2H), 2.28 (s, 3H); MS (ESI) m/e ($M+H^+$) 147.2.

Example 29

3-tert-Butyl-1H-indol-5-amine

**[0438]**

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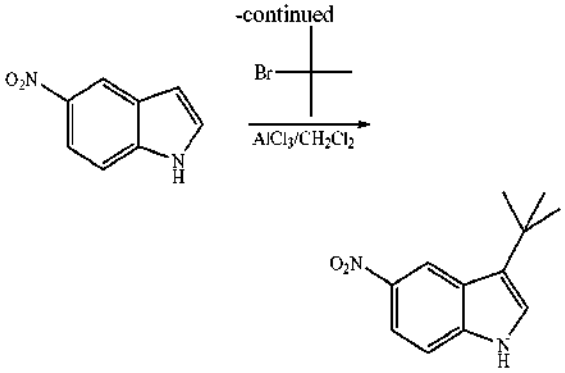
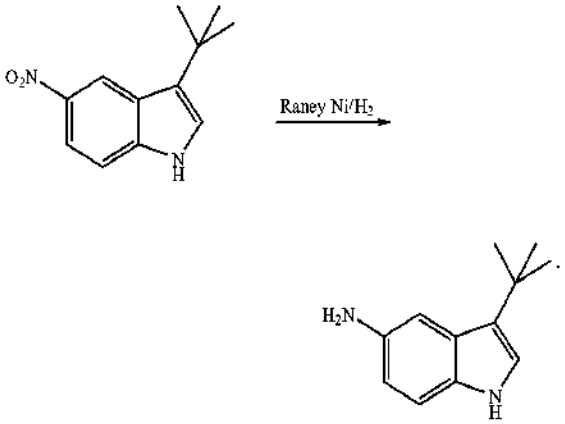



3-tert-Butyl-5-nitro-1H-indole

**[0439]** To a mixture of 5-nitro-1H-indole (6.0 g, 37 mmol) and $AlCl_3$ (24 g, 0.18 mol) in $CH_2Cl_2$ (100 mL) at 0° C. was added 2-bromo-2-methyl-propane (8.1 g, 37 mmol) dropwise. After being stirred at 15° C. overnight, the mixture was poured into ice (100 mL). The precipitated salts were removed by filtration and the aqueous layer was extracted with $CH_2Cl_2$ (30 mL×3). The combined organic layers were washed with water, brine, dried over $Na_2SO_4$ and concentrated under vacuum to obtain the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=20:1) to give 3-tert-butyl-5-nitro-1H-indole (2.5 g, 31%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 8.49 (d, J=1.6 Hz, 1H), 8.31 (brs, 1H), 8.05 (dd, J=2.0, 8.8 Hz, 1H), 7.33 (d, J=8.8 Hz, 1H), 6.42 (d, J=1.6 Hz, 1H), 1.42 (s, 9H).



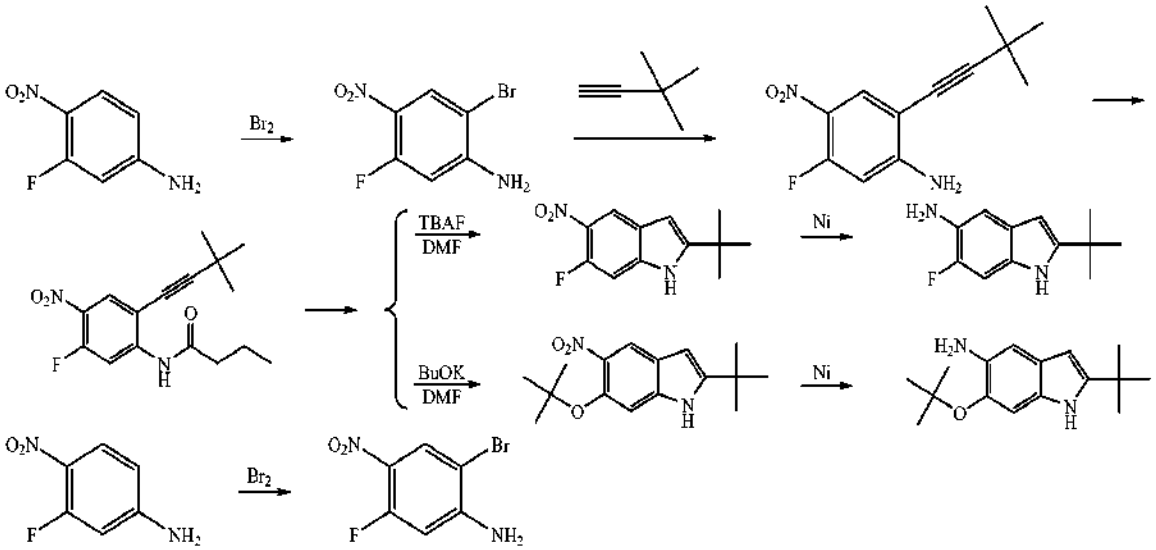

3-tert-Butyl-1H-indol-5-amine

**[0440]** To a solution of 3-tert-butyl-5-nitro-1H-indole (2.5 g, 12 mmol) in MeOH (30 mL) was added Raney Nickel (0.2 g) under $N_2$ protection. The mixture was stirred under hydrogen atmosphere (1 atm) at 15° C. for 1 h. The catalyst was filtered off and the filtrate was concentrated to dryness under vacuum. The residue was purified by preparative HLPC to afford 3-tert-butyl-1H-indol-5-amine (0.43 g, 19%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.72 (br.s, 1H), 7.11 (d, J=8.4 Hz, 1H), 6.86 (d, J=2.0 Hz, 1H), 6.59 (dd, J=2.0, 8.4 Hz, 1H), 6.09 (d, J=1.6 Hz, 1H), 1.37 (s, 9H); MS (ESI) m/e ($M+H^+$) 189.1.

Example 30

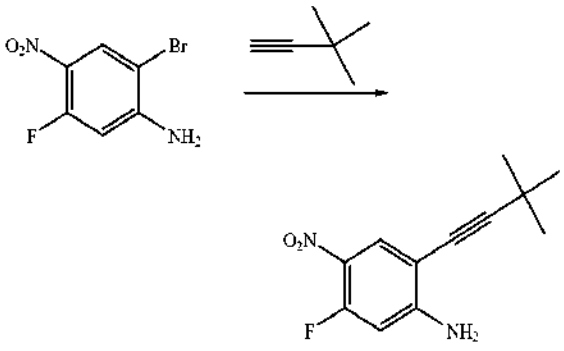
2-tert-Butyl-6-fluoro-1H-indol-5-amine and 6-tert-butoxy-2-tert-butyl-1H-indol-5-amine

**[0441]**



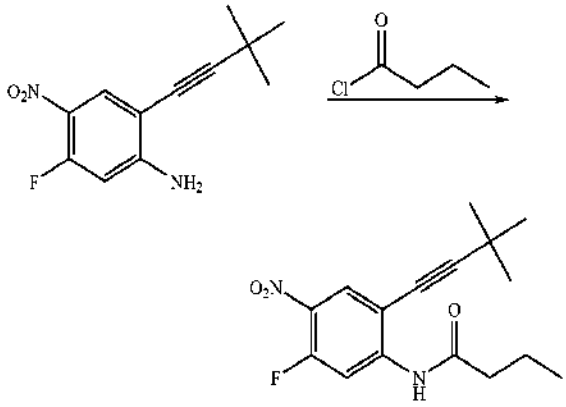
2-Bromo-5-fluoro-4-nitroaniline

**[0442]** To a mixture of 3-fluoro-4-nitroaniline (6.5 g, 42.2 mmol) in AcOH (80 mL) and chloroform (25 mL) was added dropwise $Br_2$ (2.15 mL, 42.2 mmol) at 0° C. After addition, the resulting mixture was stirred at room temperature for 2 h and then poured into ice water. The mixture was basified with aqueous NaOH (10%) to pH ~8.0-9.0 under cooling and then extracted with EtOAc (50 mL×3). The combined organic layers were washed with water (80 mL×2) and brine (100 mL), dried over $Na_2SO_4$ and concentrated under reduced pressure to give 2-bromo-5-fluoro-4-nitroaniline (9 g, 90%). $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 8.26 (d, J=8.0, Hz, 1H), 7.07 (brs, 2H), 6.62 (d, J=9.6 Hz, 1H).



2-(3,3-Dimethylbut-1-ynyl)-5-fluoro-4-nitroaniline

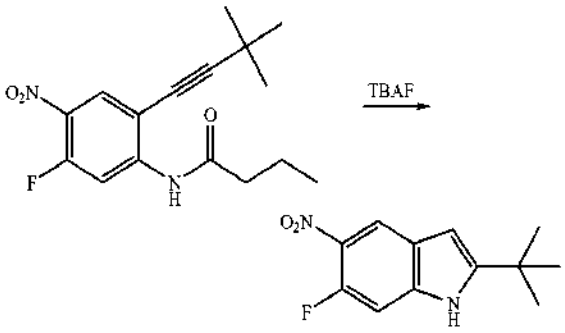
**[0443]** A mixture of 2-bromo-5-fluoro-4-nitroaniline (9.0 g, 38.4 mmol), 3,3-dimethyl-but-1-yne (9.95 g, 121 mmol), CuI (0.5 g 2.6 mmol), $Pd(PPh_3)_2Cl_2$ (3.4 g, 4.86 mmol) and $Et_3N$ (14 mL, 6.9 mmol) in toluene (100 mL) and water (50 mL) was heated at 70° C. for 4 h. The aqueous layer was separated and the organic layer was washed with water (80 mL×2) and brine (100 mL), dried over $Na_2SO_4$ and concentrated under reduced pressure to dryness. The residue was recrystallized with ether to afford 2-(3,3-dimethylbut-1-ynyl)-5-fluoro-4-nitroaniline (4.2 g, 46%). $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 7.84 (d, J=8.4 Hz, 1H), 6.84 (brs, 2H), 6.54 (d, J=14.4 Hz, 1H). 1.29 (s, 9H).



N-(2-(3,3-Dimethylbut-1-ynyl)-5-fluoro-4-nitrophenyl)butyramide

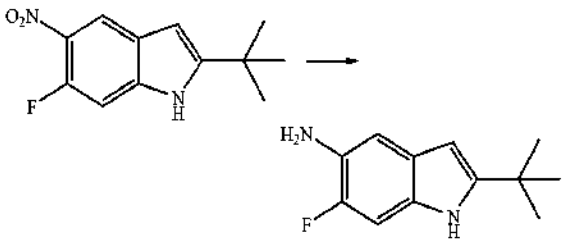
**[0444]** To a solution of 2-(3,3-dimethylbut-1-ynyl)-5-fluoro-4-nitroaniline (4.2 g, 17.8 mmol) in dichloromethane (50 mL) and $Et_3N$ (10.3 mL, 71.2 mmol) was added butyryl chloride (1.9 g, 17.8 mmol) at 0° C. The mixture was stirred at room temperature for 1 h and then poured into water. The aqueous phase was separated and the organic layer was washed with water (50 mL×2) and brine (100 mL), dried over $Na_2SO_4$ and concentrated under reduced pressure to dryness. The residue was washed with ether to give N-(2-(3,3-dimeth-

ylbut-1-ynyl)-5-fluoro-4-nitrophenyl)butyramide (3.5 g, 67%), which was used in the next step without further purification.



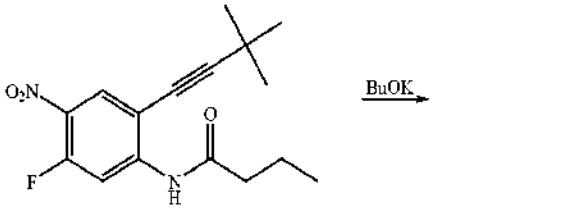

2-tert-Butyl-6-fluoro-5-nitro-1H-indole

**[0445]** A solution of N-(2-(3,3-dimethylbut-1-ynyl)-5-fluoro-4-nitrophenyl)butyramide (3.0 g, 9.8 mmol) and TBAF (4.5 g, 17.2 mmol) in DMF (25 mL) was heated at 100° C. overnight. The mixture was poured into water and then extracted with EtOAc (80 mL×3). The combined extracts were washed with water (50 mL) and brine (50 mL), dried over $Na_2SO_4$ and concentrated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 20:1) to give compound 2-tert-butyl-6-fluoro-5-nitro-1H-indole (1.5 g, 65%). $^1$H-NMR (400 MHz, $CDCl_3$) δ 8.30 (d, J=7.2 Hz, 1H), 7.12 (d, J=11.6 Hz, 1H), 6.35 (d, J=1.2 Hz, 1H), 1.40 (s, 9H).



2-tert-Butyl-6-fluoro-1H-indol-5-amine

**[0446]** A suspension of 2-tert-butyl-6-fluoro-5-nitro-1H-indole (1.5 g, 6.36 mmol) and Ni (0.5 g) in MeOH (20 mL) was stirred under $H_2$ atmosphere (1 atm) at the room temperature for 3 h. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to dryness. The residue was recrystallized in ether to give 2-tert-butyl-6-fluoro-1H-indol-5-amine (520 mg, 38%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 10.46 (brs, 1H), 6.90 (d, J=8.7 Hz, 1H), 6.75 (d, J=9.0 Hz, 1H), 5.86 (s, 1H), 4.37 (brs, 2H), 1.29 (s, 9H); MS (ESI) m/e 206.6.

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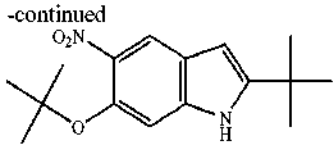
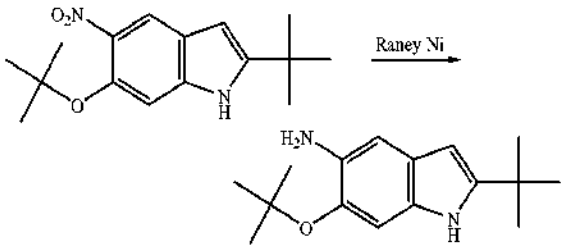


6-tert-Butoxy-2-tert-butyl-5-nitro-1H-indole

**[0447]** A solution of N-(2-(3,3-dimethylbut-1-ynyl)-5-fluoro-4-nitrophenyl)butyramide (500 mg, 1.63 mmol) and t-BuOK (0.37 g, 3.26 mmol) in DMF (10 mL) was heated at 70° C. for 2 h. The mixture was poured into water and then extracted with EtOAc (50 mL×3). The combined extracts were washed with water (50 mL) and brine (50 mL), dried over $Na_2SO_4$ and concentrated under reduced pressure to give 6-tert-butoxy-2-tert-butyl-5-nitro-1H-indole (100 mg, 21%). $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 11.35 (brs, 1H), 7.99 (s, 1H), 7.08 (s, 1H), 6.25 (s, 1H), 1.34 (s, 9H), 1.30 (s, 9H).



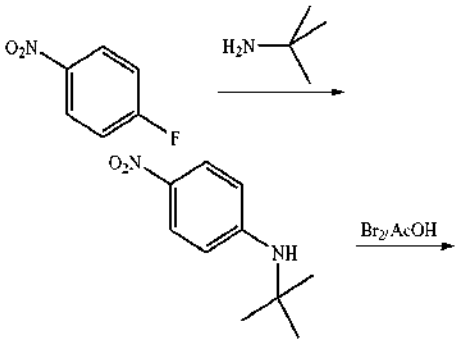

6-tert-Butoxy-2-tert-butyl-1H-indol-5-amine

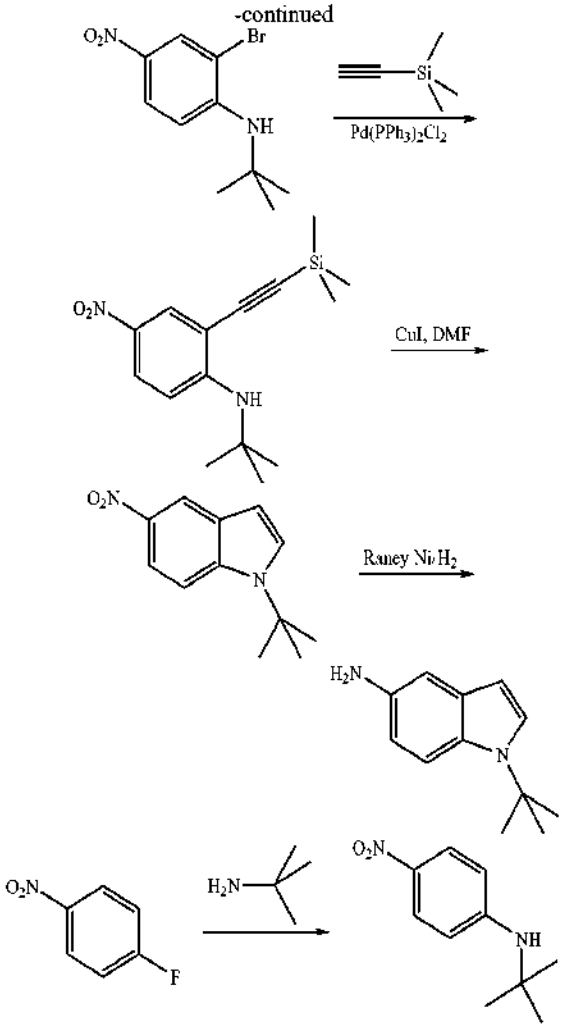
**[0448]** A suspension of 6-tert-butoxy-2-tert-butyl-5-nitro-1H-indole (100 mg, 0.36 mmol) and Raney Ni (0.5 g) in MeOH (15 mL) was stirred under $H_2$ atmosphere (1 atm) at the room temperature for 2.5 h. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to dryness. The residue was recrystallized in ether to give 6-tert-butoxy-2-tert-butyl-1H-indol-5-amine (30 mg, 32%). $^1$H-NMR (300 MHz, MeOD) 6.98 (s, 1H), 6.90 (s, 1H), 5.94 (d, J=0.6 Hz, 1H), 1.42 (s, 9H), 1.36 (s, 9H); MS (ESI) m/e 205.0.

Example 31

1-tert-Butyl-1H-indol-5-amine

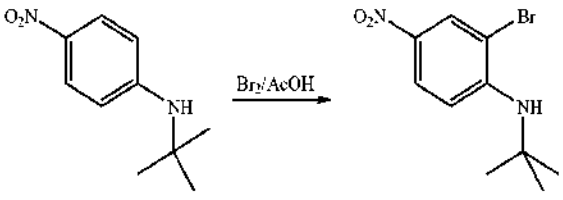
**[0449]**





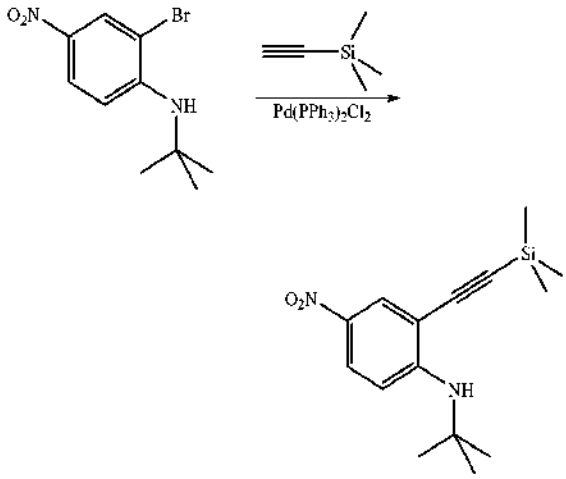

N-tert-Butyl-4-nitroaniline

**[0450]** A solution of 1-fluoro-4-nitro-benzene (1 g, 7.1 mmol) and tert-butylamine (1.5 g, 21 mmol) in DMSO (5 mL) was stirred at 75° C. overnight. The mixture was poured into water (10 mL) and extracted with EtOAc (7 mL×3). The combined organic layers were washed with water, brine, dried over $Na_2SO_4$ and concentrated under vacuum to dryness. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 30:1) to afford N-tert-butyl-4-nitroaniline (1 g, 73%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 8.03-8.00 (m, 2H), 6.61-6.57 (m, 2H), 4.67 (brs, 1H), 1.42 (s, 9H).



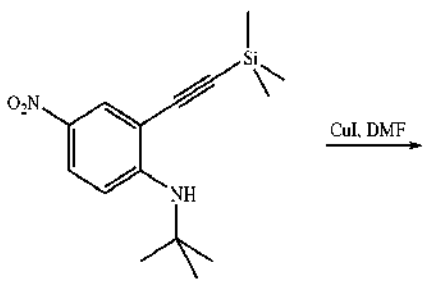

(2-Bromo-4-nitro-phenyl)-tert-butyl-amine

**[0451]** To a solution of N-tert-butyl-4-nitroaniline (1 g, 5.1 mmol) in AcOH (5 mL) was added $Br_2$ (0.86 g, 54 mmol) dropwise at 15° C. After addition, the mixture was stirred at 30° C. for 30 min and then filtered. The filter cake was basified to pH 8-9 with aqueous $NaHCO_3$. The aqueous layer was extracted with EtOAc (10 mL×3). The combined organic layers were washed with water, brine, dried over $Na_2SO_4$ and concentrated under vacuum to give (2-bromo-4-nitro-phenyl)-tert-butyl-amine (0.6 g, 43%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ 8.37 (dd, J=2.4 Hz, 1H), 8.07 (dd, J=2.4, 9.2 Hz, 1H), 6.86 (d, J=9.2 Hz, 1H), 5.19 (brs, 1H), 1.48 (s, 9H).




tert-Butyl-(4-nitro-2-trimethylsilanylethynyl-phenyl)-amine

**[0452]** To a solution of (2-bromo-4-nitro-phenyl)-tert-butyl-amine (0.6 g, 2.2 mmol) in $Et_3N$ (10 mL) was added $Pd(PPh_3)_2Cl_2$ (70 mg, 0.1 mmol), CuI (20.9 mg, 0.1 mmol) and ethynyl-trimethyl-silane (0.32 g, 3.3 mmol) successively under $N_2$ protection. The reaction mixture was heated at 70° C. overnight. The solvent was removed under vacuum and the residue was washed with EtOAc (10 mL×3). The combined organic layers were washed with water, brine, dried over $Na_2SO_4$ and concentrated under vacuum to dryness. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 20:1) to afford tert-butyl-(4-nitro-2-trimethylsilanylethynyl-phenyl)-amine (100 mg, 16%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ 8.20 (d, J=2.4, Hz, 1H), 8.04 (dd, J=2.4, 9.2 Hz, 1H), 6.79 (d, J=9.6 Hz, 1H), 5.62 (brs, 1H), 1.41 (s, 9H), 0.28 (s, 9H).



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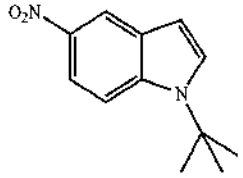
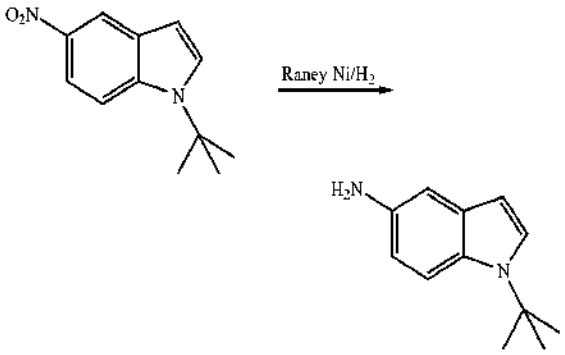


1-tert-Butyl-5-nitro-1H-indole

**[0453]** To a solution of tert-butyl-(4-nitro-2-trimethylsilanylethynyl-phenyl)-amine (10 mg, 0.035 mmol) in DMF (2 mL), was added CuI (13 mg, 0.07 mmol) under $N_2$ protection. The reaction mixture was stirred at 100° C. overnight. At this time, EtOAc (4 mL) was added to the mixture. The mixture was filtered and the filtrate was washed with water, brine, dried over $Na_2SO_4$ and concentrated under vacuum to obtain 1-tert-butyl-5-nitro-1H-indole (7 mg, 93%). $^1$H-NMR ($CDCl_3$, 300 MHz) δ 8.57 (d, J=2.1 Hz, 1H), 8.06 (dd, J=2.4, 9.3 Hz, 1H), 7.65 (d, J=9.3 Hz, 1H), 7.43 (d, J=3.3 Hz, 1H), 6.63 (d, J=3.3 Hz, 1H), 1.76 (s, 9H).



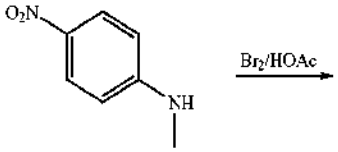

1-tert-Butyl-1H-indol-5-amine

**[0454]** To a solution of 1-tert-butyl-5-nitro-1H-indole (6.5 g, 0.030 mol) in MeOH (100 mL) was added Raney Nickel (0.65 g, 10%) under $N_2$ protection. The mixture was stirred under hydrogen atmosphere (1 atm) at 30° C. for 1 h. The catalyst was filtered off and the filtrate was concentrated under vacuum to dryness. The residue was purified by column chromatography on silica gel (PE/EtOAc 1:2) to give 1-tert-butyl-1H-indol-5-amine (2.5 g, 45%). $^1$H-NMR ($CDCl_3$, 400 MHz) δ 7.44 (d, J=8.8 Hz, 1H), 7.19 (dd, J=3.2 Hz, 1H), 6.96 (d, J=2.0 Hz, 1H), 6.66 (d, J=2.0, 8.8 Hz, 1H), 6.26 (d, J=3.2 Hz, 1H), 1.67 (s, 9H). MS (ESI) m/e ($M+H^+$) 189.2.

Example 32

2-tert-Butyl-1-methyl-1H-indol-5-anine

**[0455]**

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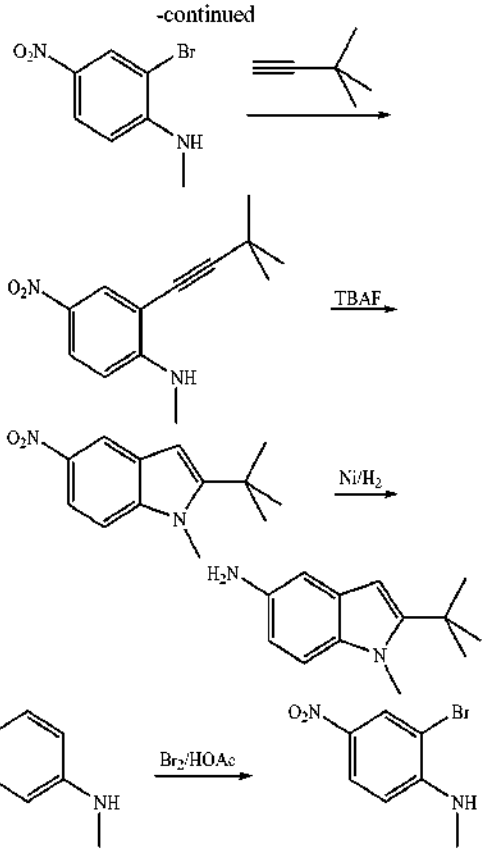
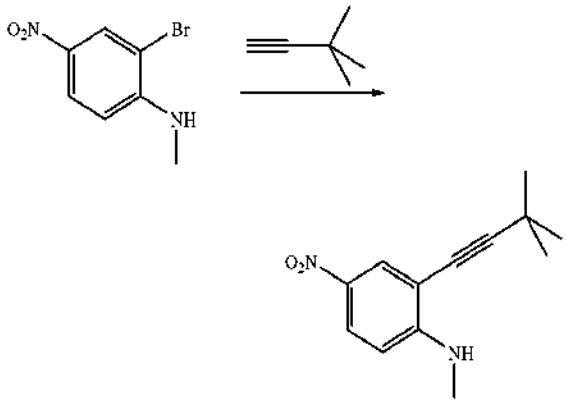


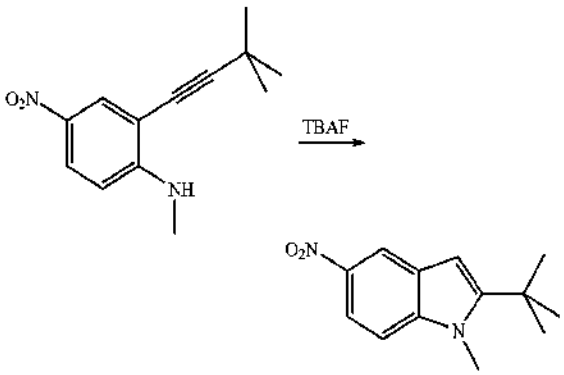

(2-Bromo-4-nitro-phenyl)-methyl-amine

**[0456]** To a solution of methyl-(4-nitro-phenyl)-amine (15.2 g, 0.1 mol) in AcOH (150 mL) and $CHCl_3$ (50 mL) was added $Br_2$ (16.0 g, 0.1 mol) dropwise at 5° C. The mixture was stirred at 10° C. for 1 h and then basified with sat. aq. $NaHCO_3$. The resulting mixture was extracted with EtOAc (100 mL×3), and the combined organics were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give (2-bromo-4-nitro-phenyl)-methyl-amine (2-bromo-4-nitro-phenyl)-methyl-amine (23.0 g, 99%), which was used in the next step without further purification. $^1$H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, J=2.4 Hz, 1H), 8.13 (dd, J=2.4, 9.0 Hz, 1H), 6.58 (d, J=9.0 Hz, 1H), 5.17 (brs, 1H), 3.01 (d, J=5.4 Hz, 3H).



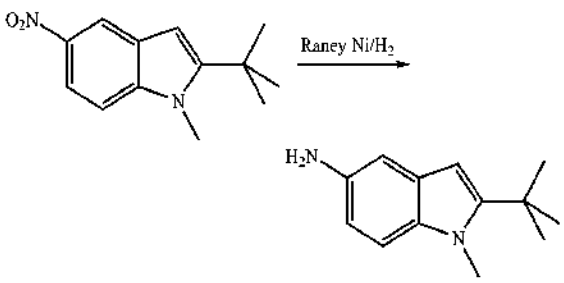
[2-(3,3-Dimethyl-but-1-ynyl)-4-nitro-phenyl]-methyl-amine

**[0457]** To a solution of (2-bromo-4-nitro-phenyl)-methyl-amine (22.5 g, 97.4 mmol) in toluene (200 mL) and water (100 mL) were added $Et_3N$ (19.7 g, 195 mmol), $Pd(PPh_3)_2Cl_2$ (6.8 g, 9.7 mmol), CuI (0.7 g, 3.9 mmol) and 3,3-dimethyl-but-1-yne (16.0 μg, 195 mmol) successively under $N_2$ protection. The mixture was heated at 70° C. for 3 hours and then cooled down to room temperature. The resulting mixture was extracted with EtOAc (100 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give [2-(3,3-dimethyl-but-1-ynyl)-4-nitro-phenyl]-methyl-amine (20.1 g, 94%), which was used in the next step without further purification. $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J=2.4 Hz, 1H), 8.08 (dd, J=2.8, 9.2 Hz, 1H), 6.50 (d, J=9.2 Hz, 1H), 5.30 (brs, 1H), 3.00 (s, 3H), 1.35 (s, 9H).




2-tert-Butyl-1-methyl-5-nitro-1H-indole

**[0458]** A solution of [2-(3,3-dimethyl-but-1-ynyl)-4-nitro-phenyl]-methyl-amine (5.0 g, 22.9 mmol) and TBAF (23.9 g, 91.6 mmol) in THF (50 mL) was heated at reflux overnight. The solvent was removed by evaporation under vacuum and the residue was dissolved in brine (100 mL) and EtOAc (100 mL). The organic phase was separated, dried over $Na_2SO_4$ and evaporated under vacuum to give 2-tert-butyl-1-methyl-5-nitro-1H-indole (5.0 g, 99%), which was used in the next step without further purification. $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.47 (d, J=2.4 Hz, 1H), 8.07 (dd, J=2.4, 9.2 Hz, 1H), 7.26-7.28 (m, 1H), 6.47 (s, 1H), 3.94 (s, 3H), 1.50 (s, 9H).



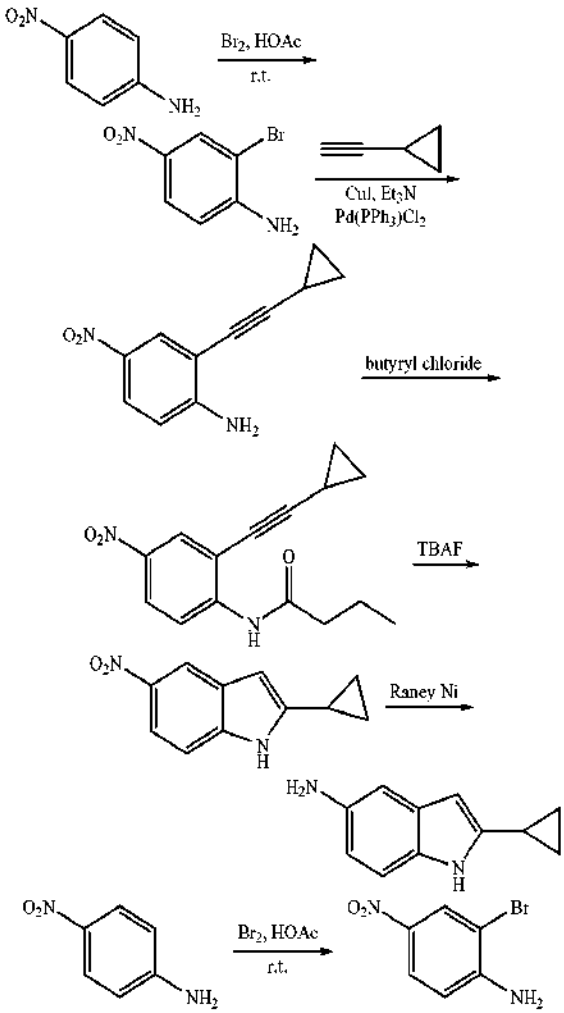

2-tert-Butyl-1-methyl-1H-indol-5-amine

**[0459]** To a solution of 2-tert-butyl-1-methyl-5-nitro-1H-indole (3.00 g, 13.7 mmol) in MeOH (30 mL) was added Raney Ni (0.3 g) under nitrogen atmosphere. The mixture was stirred under hydrogen atmosphere (1 atm) at room temperature overnight. The mixture was filtered through a Celite pad and the filtrate was evaporated under vacuum. The crude residue was purified by column chromatography on silica gel (P.E/EtOAc 20:1) to give 2-tert-butyl-1-methyl-1H-indol-5-amine (1.7 g, 66%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.09 (d, J=8.4 Hz, 1H), 6.89-6.9 (m, 1H), 6.66 (dd, J=2.4, 8.7 Hz, 1H), 6.14 (d, J=0.6 Hz, 1H), 3.83 (s, 3H), 3.40 (brs, 2H), 1.45 (s, 9H); MS (ESI) m/e ($M+H^+$) 203.1.

Example 33

2-Cyclopropyl-1H-indol-5-amine

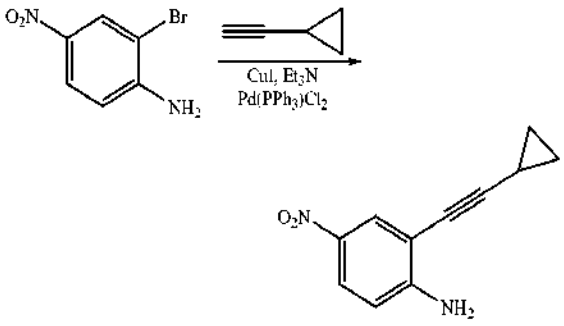
**[0460]**




2-Bromo-4-nitroaniline

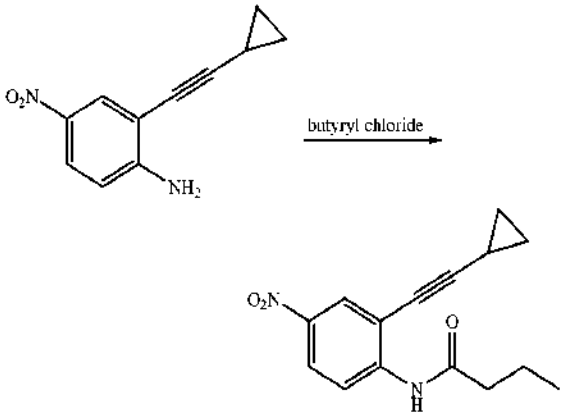
**[0461]** To a solution of 4-nitro-aniline (25 g, 0.18 mol) in HOAc (150 mL) was added liquid $Br_2$ (30 g, 0.19 mol) dropwise at room temperature. The mixture was stirred for 2 hours. The solid was collected by filtration and poured into water (100 mL), which was basified with sat. aq. $NaHCO_3$ to pH 7 and extracted with EtOAc (300 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evapo-

rated under reduced pressure to give 2-bromo-4-nitroaniline (30 g, 80%), which was directly used in the next step.



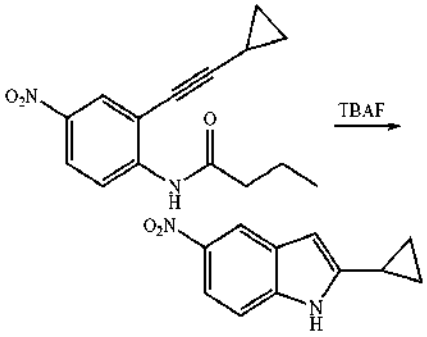

2-(Cyclopropylethynyl)-4-nitroaniline

**[0462]** To a deoxygenated solution of 2-bromo-4-nitroaniline (2.17 g, 0.01 mmol), ethynyl-pane cyclopropane (1 g, 15 mmol) and CuI (10 mg, 0.05 mmol) in triethylamine (20 mL) was added $Pd(Ph_3)_2Cl_2$ (210 mg, 0.3 mmol) under $N_2$. The mixture was heated at 70° C. and stirred for 24 hours. The solid was filtered off and washed with EtOAc (50 mL×3). The filtrate was evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to give 2-(cyclopropylethynyl)-4-nitroaniline (470 mg, 23%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.14 (d, J=2.7 Hz, 1H), 7.97 (dd, J=2.7, 9.0 Hz, 1H), 6.63 (d, J=9.0 Hz, 1H), 4.81 (brs, 2H), 1.55-1.46 (m, 1H), 0.98-0.90 (m, 2H), 0.89-0.84 (m, 2H).



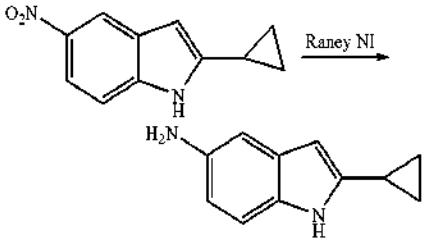

N-(2-(Cyclopropylethynyl)phenyl)-4-nitrobutyramide

**[0463]** To a solution of 2-(cyclopropylethynyl)-4-nitroaniline (3.2 g, 15.8 mmol) and pyridine (2.47 g, 31.7 mmol) in $CH_2Cl_2$ (60 mL) was added butyryl chloride (2.54 g, 23.8 mmol) at 0° C. The mixture was warmed to room temperature and stirred for 3 hours. The resulting mixture was poured into ice-water. The organic layer was separated. The aqueous phase was extracted with $CH_2Cl_2$ (30 m L×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to give N-(2-(cyclopropylethynyl)phenyl)-4-nitrobutyramide (3.3 g, 76%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.61 (d, J=9.2 Hz, 1H), 8.22 (d, J=2.8 Hz, 1H), 8.18 (brs, 1H), 8.13 (dd, J=2.4, 9.2 Hz, 1H), 2.46 (t, J=7.2 Hz, 2H), 1.83-1.76 (m, 2H), 1.59-1.53 (m, 1H), 1.06 (t, J=7.2 Hz, 3H), 1.03-1.01 (m, 2H), 0.91-0.87 (m, 2H).




2-Cyclopropyl-5-nitro-1H-indole

**[0464]** A mixture of N-(2-(cyclopropylethynyl)phenyl)-4-nitrobutyramide (3.3 g, 0.01 mol) and TBAF (9.5 g, 0.04 mol) in THF (100 mL) was heated at reflux for 24 hours. The mixture was cooled to the room temperature and poured into ice water. The mixture was extracted with $CH_2Cl_2$ (50 m Lx3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to give 2-cyclopropyl-5-nitro-1H-indole (1.3 g, 64%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J=2.0 Hz, 1H), 8.40 (brs, 1H), 8.03 (dd, J=2.0, 8.8 Hz, 1H), 7.30 (d, J=8.8 Hz, 1H), 6.29 (d, J=0.8 Hz, 1H), 2.02-1.96 (m, 1H) 1.07-1.02 (m, 2H), 0.85-0.81 (m, 2H).



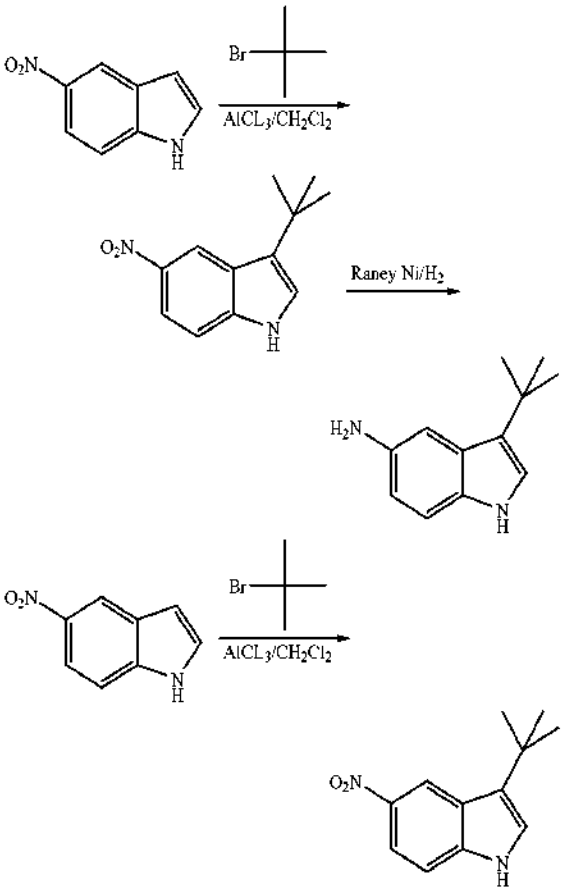

2-Cyclopropyl-1H-indol-5-amine

**[0465]** To a solution of 2-cyclopropyl-5-nitro-1H-indole (1.3 g, 6.4 mmol) in MeOH (30 mL) was added Raney Nickel (0.3 g) under nitrogen atmosphere. The mixture was stirred under hydrogen atmosphere (1 atm) at room temperature overnight. The catalyst was filtered through a Celite pad and the filtrate was evaporated under vacuum to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=5/1) to give 2-cyclopropyl-1H-indol-5-amine (510 mg, 56%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.89 (d, J=8.4 Hz, 1H), 6.50 (d, J=1.6 Hz, 1H), 6.33 (dd, J=2.0, 8.4 Hz, 1H), 5.76 (s, 1H), 4.33 (brs, 2H), 1.91-1.87 (m, 1H), 0.90-0.85 (m, 2H), 0.70-0.66 (m, 2H); MS (ESI) m/e ($M+H^+$) 173.2.

Example 34

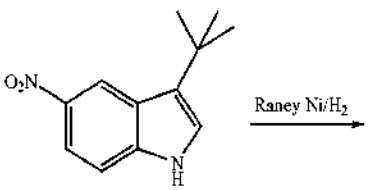
3-tert-Butyl-1H-indol-5-amine

**[0466]**



3-tert-Butyl-5-nitro-1H-indole

**[0467]** To a mixture of 5-nitro-1H-indole (6 g, 36.8 mmol) and $AlCl_3$ (24 g, 0.18 mol) in $CH_2Cl_2$ (100 mL) was added 2-bromo-2-methyl-propane (8.1 g, 36.8 mmol) dropwise at 0° C. After being stirred at 15° C. overnight, the reaction mixture was poured into ice (100 mL). The precipitated salts were removed by filtration and the aqueous layer was extracted with $CH_2Cl_2$ (30 mL×3). The combined organic layers were washed with water, brine, dried over $Na_2SO_4$ and concentrated under vacuum to obtain the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 20:1) to give 3-tert-butyl-5-nitro-1H-indole (2.5 g, 31%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.49 (d, J=1.6 Hz, 1H), 8.31 (brs, 1H), 8.05 (dd, J=2.0, 8.8 Hz, 1H), 7.33 (d, J=8.8 Hz, 1H), 6.42 (d, J=1.6 Hz, 1H), 1.42 (s, 9H).



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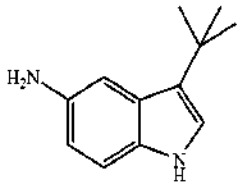


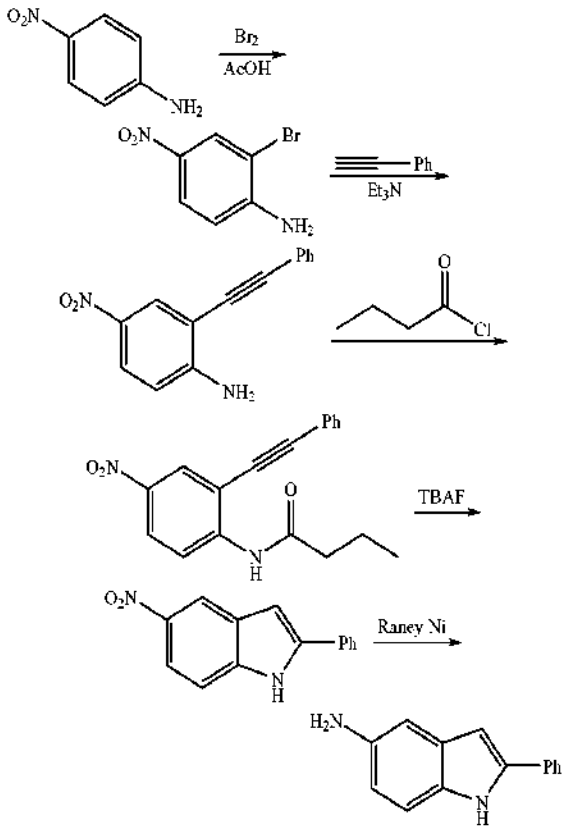
3-tert-Butyl-1H-indol-5-amine

**[0468]** To a solution of 3-tert-butyl-5-nitro-1H-indole (2.5 g, 11.6 mmol) in MeOH (30 mL) was added Raney Nickel (0.2 g) under $N_2$ protection. The mixture was stirred under hydrogen atmosphere (1 atm) at 15° C. for 1 hr. The catalyst was filtered off and the filtrate was concentrated under vacuum to dryness. The residue was purified by preparative HLPC to afford 3-tert-butyl-1H-indol-5-amine (0.43 g, 19%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.72 (brs, 1H), 7.11 (d, J=8.4 Hz, 1H), 6.86 (d, J=2.0 Hz, 1H), 6.59 (dd, J=2.0, 8.4 Hz, 1H), 6.09 (d, J=1.6 Hz, 1H), 1.37 (s, 9H); MS (ESI) m/e ($M+H^+$) 189.1.

Example 35

2-Phenyl-1H-indol-5-amine

**[0469]**



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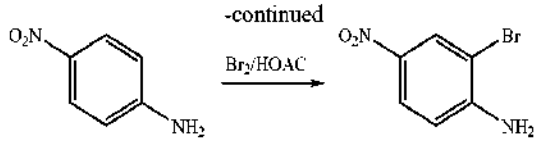
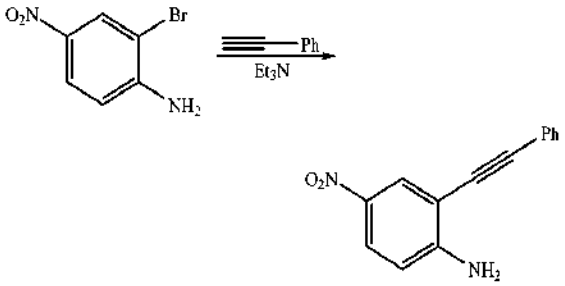


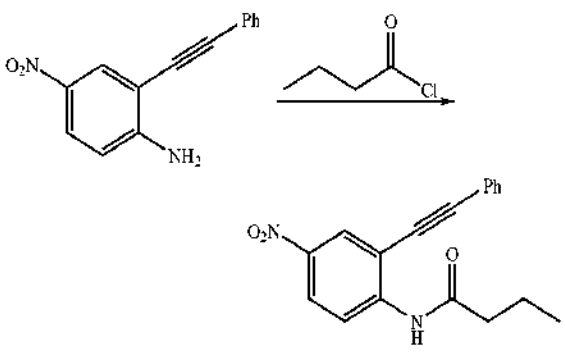
2-Bromo-4-nitroaniline

**[0470]** To a solution of 4-nitroaniline (50 g, 0.36 mol) in AcOH (500 mL) was added liquid $Br_2$ (60 g, 0.38 mol) dropwise at 5° C. The mixture was stirred for 30 min at that temperature. The insoluble solid was collected by filtration and poured into EtOAc (200 mL). The mixture was basified with saturated aqueous $NaHCO_3$ to pH 7. The organic layer was separated. The aqueous phase was extracted with EtOAc (300 mL×3). The combined organic layers were dried and evaporated under reduced pressure to give 2-bromo-4-nitroaniline (56 g, 72%), which was directly used in the next step.



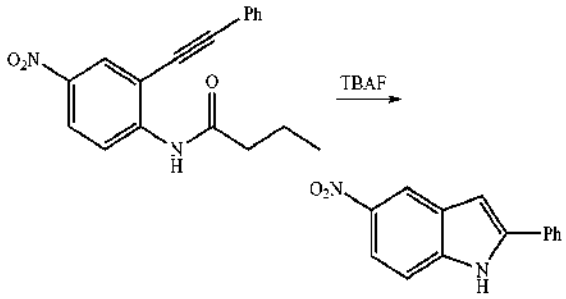
4-Nitro-2-(phenylethynyl)aniline

**[0471]** To a deoxygenated solution of 2-bromo-4-nitroaniline (2.17 g, 0.01 mmol), ethynyl-benzene (1.53 g, 0.015 mol) and CuI (10 mg, 0.05 mmol) in triethylamine (20 mL) was added $Pd(PPh_3)_2Cl_2$ (210 mg, 0.2 mmol) under $N_2$. The mixture was heated at 70° C. and stirred for 24 hours. The solid was filtered off and washed with EtOAc (50 mL×3). The filtrate was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to give 4-nitro-2-(phenylethynyl)aniline (340 mg, 14%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.37-8.29 (m, 1H), 8.08-8.00 (m, 1H), 7.56-7.51 (m, 2H), 7.41-7.37 (m, 3H), 6.72 (m, 1H), 4.95 (brs, 2H).



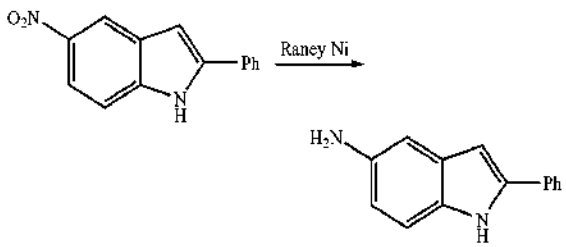
N-(2-(Phenylethynyl)phenyl)-4-nitrobutyramide

**[0472]** To a solution of 4-nitro-2-(phenylethynyl)aniline (17 g, 0.07 mmol) and pyridine (11.1 g, 0.14 mol) in $CH_2Cl_2$ (100 mL) was added butyryl chloride (11.5 g, 0.1 mol) at 0° C. The mixture was warmed to room temperature and stirred for 3 hours. The resulting mixture was poured into ice-water. The organic layer was separated. The aqueous phase was extracted with $CH_2Cl_2$ (30 m L×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to give N-(2-(phenylethynyl)phenyl)-4-nitrobutyramide (12 g, 55%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.69 (d, J=9.2 Hz, 1H), 8.39 (d, J=2.8 Hz, 1H), 8.25-8.20 (m, 2H), 7.58-7.55 (m, 2H), 7.45-7.42 (m, 3H), 2.49 (t, J=7.2 Hz, 2H), 1.85-1.79 (m, 2H), 1.06 (t, J=7.2 Hz, 3H).



5-Nitro-2-phenyl-1H-indole

**[0473]** A mixture of N-(2-(phenylethynyl)phenyl)-4-nitrobutyramide (5.0 g, 0.020 mol) and TBAF (12.7 g, 0.050 mol) in THF (30 mL) was heated at reflux for 24 h. The mixture was cooled to room temperature and poured into ice water. The mixture was extracted with $CH_2Cl_2$ (50 m L×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to give 5-nitro-2-phenyl-1H-indole (3.3 g, 69%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.06 (dd, J=2.0, 8.8 Hz, 1H), 7.75 (d, J=7.6 Hz, 2H), 7.54 (d, J=8.8 Hz, 1H), 7.45 (t, J=7.6 Hz, 2H), 7.36 (t, J=7.6 Hz, 1H), 6.95 (s, 1H).



2-Phenyl-1H-indol-5-amine

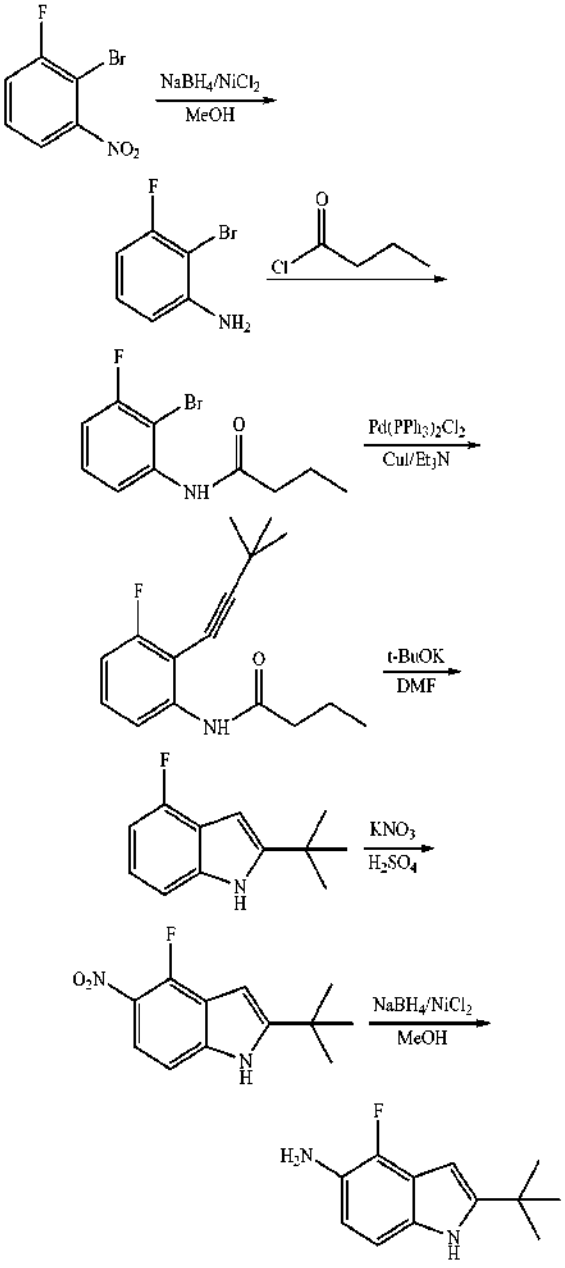
**[0474]** To a solution of 5-nitro-2-phenyl-1H-indole (2.83 g, 0.01 mol) in MeOH (30 mL) was added Raney Ni (510 mg) under nitrogen atmosphere. The mixture was stirred under hydrogen atmosphere (1 atm) at room temperature overnight.

The catalyst was filtered through a Celite pad and the filtrate was evaporated under vacuum to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=5/1) to give 2-phenyl-1H-indol-5-amine (1.6 g, 77%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J=7.6 Hz, 2H), 7.39 (t, J=7.6 Hz, 2H), 7.24 (t, J=7.6 Hz, 1H), 7.07 (d, J=8.4 Hz, 1H), 6.64 (d, J=1.6 Hz, 1H), 6.60 (d, J=1.2 Hz, 1H), 6.48 (dd, J=2.0, 8.4 Hz, 1H), 4.48 (brs, 2H); MS (ESI) m/e ($M+H^+$) 209.0.

Example 36

2-tert-Butyl-4-fluoro-1H-indol-5-amine

**[0475]**

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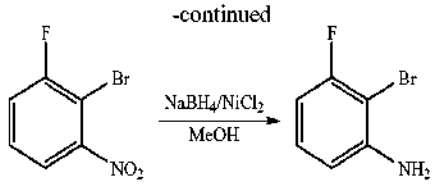
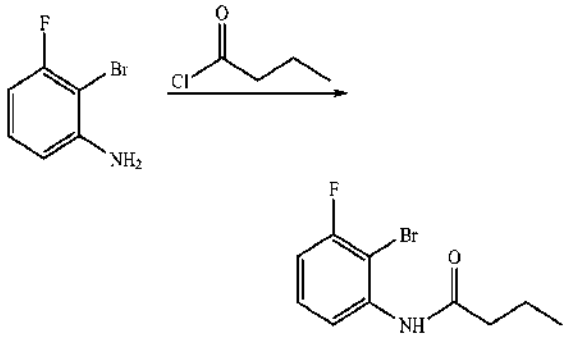


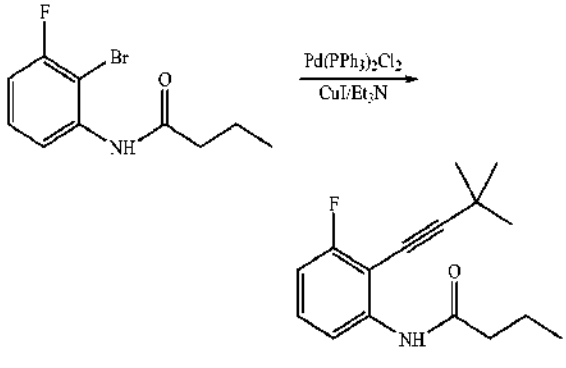

2-Bromo-3-fluoroaniline

**[0476]** To a solution of 2-bromo-1-fluoro-3-nitrobenzene (1.0 g, 5.0 mmol) in $CH_3OH$ (50 mL) was added $NiCl_2$ (2.2 g 10 mmol) and $NaBH_4$ (0.50 g 14 mmol) at 0° C. After the addition, the mixture was stirred for 5 min. Water (20 mL) was added and the mixture was extracted with EtOAc (20 mL×3). The organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 2-bromo-3-fluoroaniline (600 mg, 70%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.07-7.02 (m, 1H), 6.55-6.49 (m, 1H), 4.22 (br s, 2H).



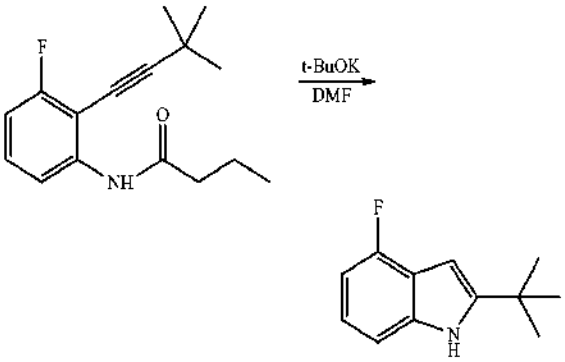

N-(2-Bromo-3-fluorophenyl)butyramide

**[0477]** To a solution of 2-bromo-3-fluoroaniline (2.0 g, 11 mmol) in $CH_2Cl_2$ (50 mL) was added butyryl chloride (1.3 g, 13 mmol) and pyridine (1.7 g, 21 mmol) at 0° C. The mixture was stirred at room temperature for 24 h. Water (20 mL) was added and the mixture was extracted with $CH_2Cl_2$ (50 mL×3). The organic layers were dried anhydrous over $Na_2SO_4$ and evaporated under vacuum to give N-(2-bromo-3-fluorophenyl)butyramide (2.0 g, 73%), which was directly used in the next step.



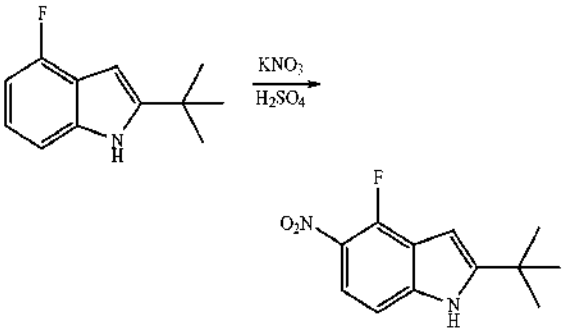
N-(2-(3,3-Dimethylbut-1-ynyl)-3-fluorophenyl)butyramide

**[0478]** To a solution of N-(2-bromo-3-fluorophenyl)butyramide (2.0 g, 7.0 mmol) in $Et_3N$ (100 mL) was added 4,4-dimethylpent-2-yne (6.0 g, 60 mmol), CuI (70 mg, 3.8 mmol), and $Pd(PPh_3)_2Cl_2$ (500 mg) successively at room temperature under $N_2$. The mixture was heated at 80° C. overnight. The cooled mixture was filtered and the filtrate was extracted with EtOAc (40 mL×3). The organic layers were washed with sat. NaCl, dried over anhydrous $Na_2SO_4$, and evaporated under vacuum. The crude compound was purified by column chromatography on silica gel (10% EtOAc in petroleum ether) to give N-(2-(3,3-dimethylbut-1-ynyl)-3-fluorophenyl)butyramide (1.1 g, 55%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J=7.6, 1H), 7.95 (s, 1H), 7.21 (m, 1H), 6.77 (t, J=7.6 Hz, 1H), 2.39 (t, J=7.6 Hz, 2H), 1.82-1.75 (m, 2H), 1.40 (s, 9H), 1.12 (t, J=7.2 Hz, 3H).



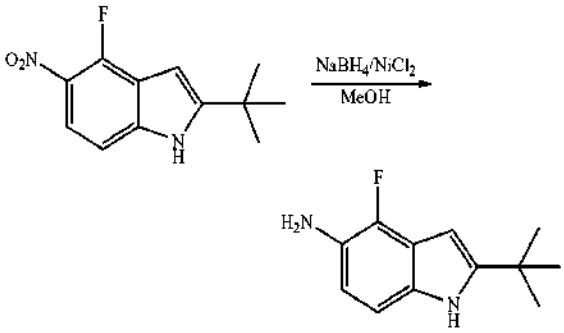

2-tert-Butyl-4-fluoro-1H-indole

**[0479]** To a solution of N-(2-(3,3-dimethylbut-1-ynyl)-3-fluorophenyl)butyramide (6.0 g, 20 mmol) in DMF (100 mL) was added t-BuOK (5.0 g, 50 mmol) at room temperature. The mixture was heated at 90° C. overnight before it was poured into water and extracted with EtOAc (100 mL×3). The organic layers were washed with sat. NaCl and water, dried over anhydrous $Na_2SO_4$, and evaporated under vacuum to give 2-tert-butyl-4-fluoro-1H-indole (5.8 g, 97%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.17 (br s, 1H), 7.11 (d, J=7.2 Hz, 1H), 7.05-6.99 (m, 1H), 6.76-6.71 (m, 1H), 6.34 (m, 1H), 1.41 (s, 9H).




2-tert-Butyl-4-fluoro-5-nitro-1H-indole

**[0480]** To a solution of 2-tert-butyl-4-fluoro-1H-indole (2.5 g, 10 mmol) in $H_2SO_4$ (30 mL) was added $KNO_3$ (1.3 g, 10 mmol) at 0° C. The mixture was stirred for 0.5 h at −10° C. The mixture was poured into water and extracted with EtOAc (100 mL×3). The organic layers were washed with sat. NaCl and water, dried over anhydrous $Na_2SO_4$, and evaporated under vacuum. The crude compound was purified by column chromatography on silica gel (10% EtOAc in petroleum ether) to give 2-tert-butyl-4-fluoro-5-nitro-1H-indole (900 mg, 73%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.50 (br s, 1H), 7.86 (dd, J=7.6, 8.8 Hz, 1H), 7.13 (d, J=8.8 Hz, 1H), 6.52 (dd, J=0.4, 2.0 Hz, 1H), 1.40 (s, 9H).



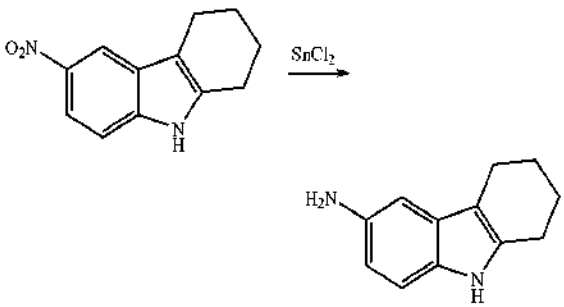

2-tert-Butyl-fluoro-1H-indol-5-amine

**[0481]** To a solution of 2-tert-butyl-4-fluoro-5-nitro-1H-indole (2.1 g, 9.0 mmol) in methanol (50 mL) was added $NiCl_2$ (4.2 g, 18 mmol) and $NaBH_4$ (1.0 g, 27 mmol) at 0° C. After the addition, the mixture was stirred for 5 min. Water (20 mL) was added and the mixture was extracted with EtOAc (30 mL×3). The organic layers were washed with sat. NaCl and water, dried over anhydrous $Na_2SO_4$, evaporated under vacuum to give 2-tert-butyl-4-fluoro-1H-indol-5-amine (900 mg, 50%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.80 (brs, 1H), 6.91 (d, J=8.4 Hz, 1H), 6.64 (dd, J=0.9, 2.4 Hz, 1H), 6.23 (s, 1H), 1.38 (s, 9H).

Example 37

2,3,4,9-Tetrahydro-1H-carbazol-6-amine

**[0482]**



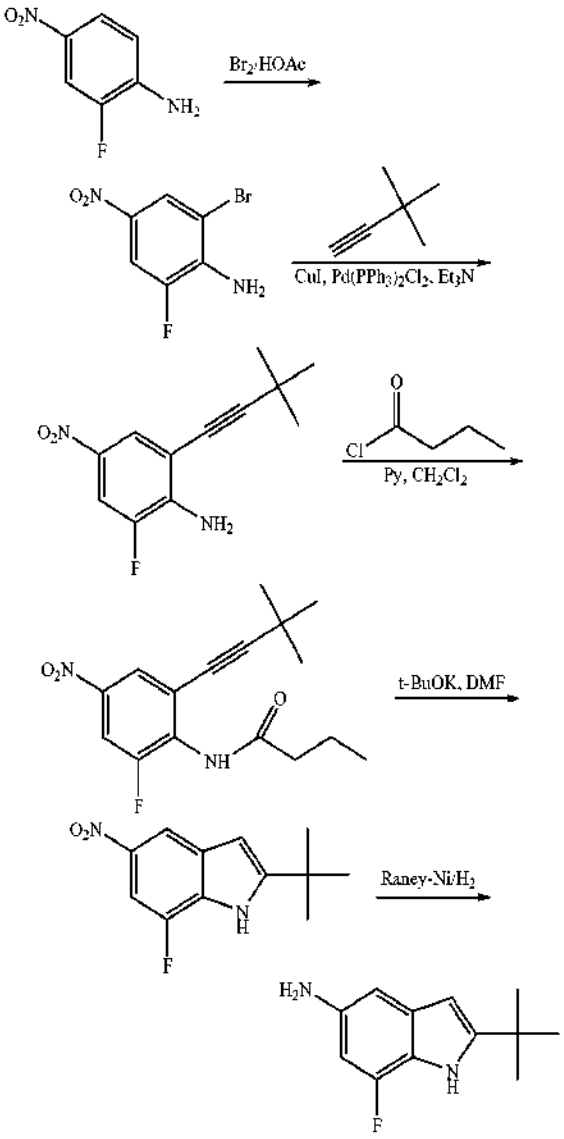
2,3,4,9-Tetrahydro-1H-carbazol-6-amine

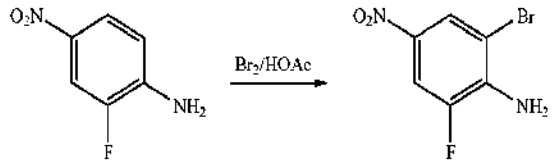
**[0483]** 6-Nitro-2,3,4,9-tetrahydro-1H-carbazole (0.100 g, 0.462 mmol) was dissolved in a 40 mL scintillation vial containing a magnetic stir bar and 2 mL of ethanol. Tin(II) chloride dihydrate (1.04 g, 4.62 mmol) was added to the reaction mixture and the resulting suspension was heated at 70° C. for 16 h. The crude reaction mixture was then diluted with 15 mL of a saturated aqueous solution of sodium bicarbonate and extracted three times with an equivalent volume of ethyl acetate. The ethyl acetate extracts were combined, dried over sodium sulfate, and evaporated to dryness to yield 2,3,4,9-tetrahydro-1H-carbazol-6-amine (82 mg, 95%) which was used without further purification.

Example 38

2-tert-Butyl-7-fluoro-1H-indol-5-amine

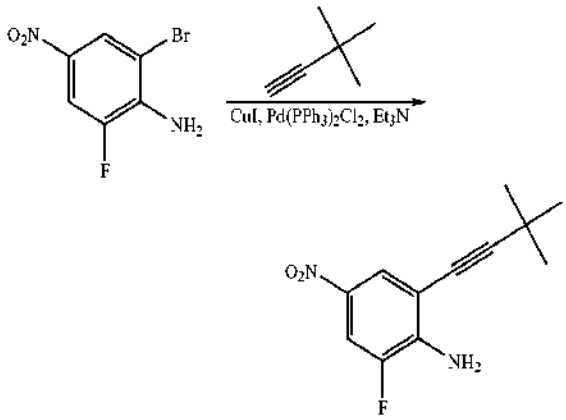
**[0484]**



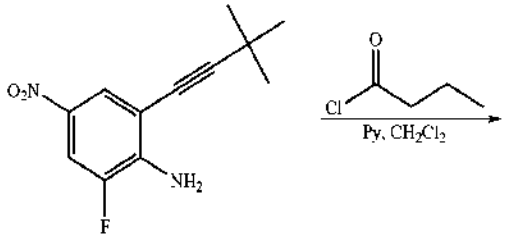

2-Bromo-6-fluoro-4-nitro-phenylamine

**[0485]** To a solution of 2-fluoro-4-nitro-phenylamine (12 g, 77 mmol) in AcOH (50 mL) was added $Br_2$ (3.9 mL, 77 mmol) dropwise at 0° C. The mixture was stirred at 20° C. for 3 h. The reaction mixture was basified with sat. aq. $NaHCO_3$, and extracted with EtOAc (100 mL×3). The combined organics were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 2-bromo-6-fluoro-4-nitro-phenylamine (18 g, 97%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.22 (m, 1H), 7.90 (dd, J=2.4, 10.8 Hz, 1H), 4.88 (brs, 2H).




2-(3,3-Dimethyl-but-1-ynyl)-6-fluoro-4-nitro-phenylamine

**[0486]** To a solution of 2-bromo-6-fluoro-4-nitro-phenylamine (11 g, 47 mmol) in dry $Et_3N$ (100 mL) was added CuI (445 mg, 5% mol). $Pd(PPh_3)_2Cl_2$ (550 mg, 5% mol) and 3,3-dimethyl-but-1-yne (9.6 g, 120 mmol) under $N_2$ protection. The mixture was stirred at 80° C. for 10 h. The reaction mixture was filtered, poured into ice (100 g), and extracted with EtOAc (50 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give the crude product, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 50:1) to give 2-(3,3-dimethyl-but-1-ynyl)-6-fluoro-4-nitro-phenylamine (4.0 g, 36%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J=1.2 Hz, 1H), 7.84 (dd, J=2.4, 10.8 Hz, 1H), 4.85 (brs, 2H), 1.36 (s, 9H).



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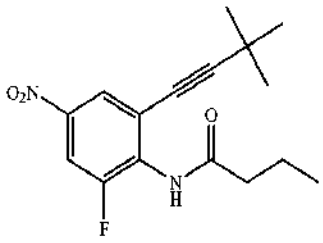
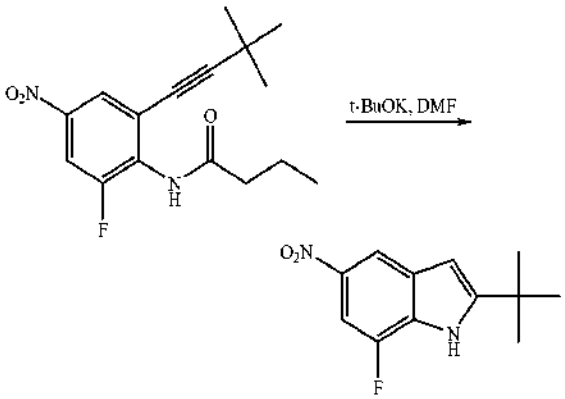


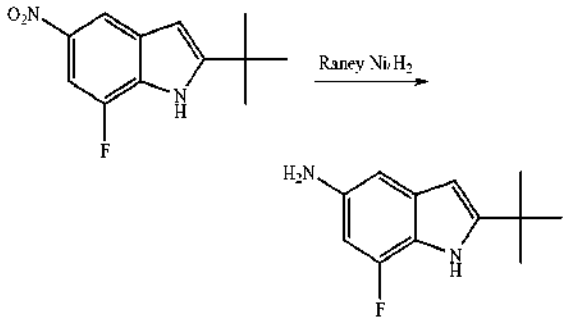
N-[2-(3,3-Dimethyl-but-1-ynyl)-6-fluoro-4-nitro-phenyl]-butyramide

**[0487]** To a solution of 2-(3,3-dimethyl-but-1-ynyl)-6-fluoro-4-nitro-phenylamine (4.0 g, 17 mmol) and pyridine (2.7 g, 34 mmol) in anhydrous $CH_2Cl_2$ (30 mL) was added and butyryl chloride (1.8 g, 17 mmol) dropwise at 0° C. After stirring for 5 h at 0° C., the reaction mixture was poured into ice (50 g) and extracted with $CH_2Cl_2$ (30 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give N-[2-(3,3-dimethyl-but-1-ynyl)-6-fluoro-4-nitro-phenyl]-butyramide (3.2 g, 62%), which was used in the next step without further purification. $^1H$ NMR (300 MHz, DMSO) δ 8.10 (dd, J=1.5, 2.7 Hz, 1H), 7.95 (dd, J=2.4, 9.6 Hz, 1H), 7.22 (brs, 1H), 2.45 (t, J=7.5 Hz, 2H), 1.82 (m, 2H), 1.36 (s, 9H), 1.06 (t, J=7.5 Hz, 3H).



2-tert-Butyl-7-fluoro-5-nitro-1H-indole

**[0488]** To a solution of N-[2-(3,3-dimethyl-but-1-ynyl)-6-fluoro-4-nitro-phenyl]-butyramide (3.2 g, 10 mmol) in DMF (20 mL) was added t-BuOK (2.3 g, 21 mmol) at room temperature. The mixture was heated at 120° C. for 2 g before being cooled down to room temperature. Water (50 mL) was added to the reaction mixture and the resulting mixture was extracted with $CH_2Cl_2$ (30 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 2-tert-butyl-7-fluoro-5-nitro-1H-indole (2.0 g, 81%), which was used in the next step without further purification. $^1H$ NMR (300 MHz, $CDCl_3$) δ 9.95 (brs, 1H), 8.30 (d, J=2.1 Hz, 1H), 7.74 (dd, J=1.8, 11.1 Hz, 1H), 6.43 (dd, J=2.4, 3.3 Hz, 1H), 1.43 (s, 9H).



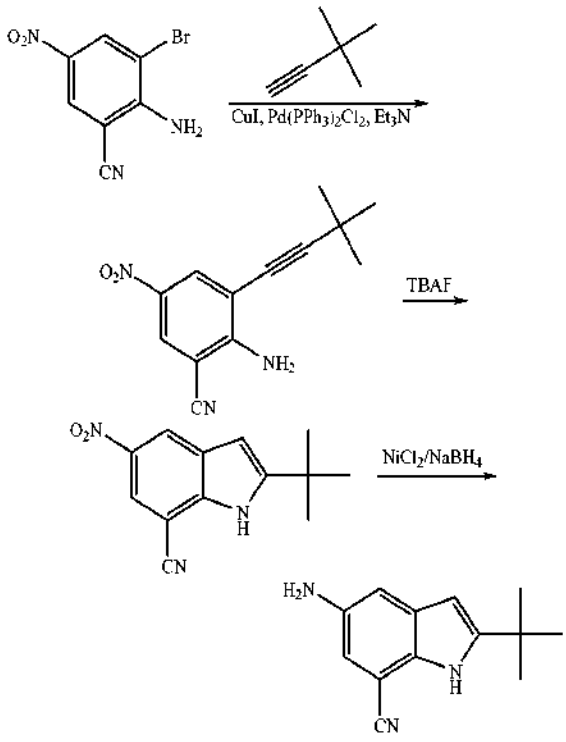
2-tert-Butyl-7-fluoro-1H-indol-5-amine

**[0489]** To a solution of 2-tert-butyl-7-fluoro-5-nitro-1H-indole (2.0 g, 8.5 mmol) in MeOH (20 mL) was added Ni (0.3 g) under nitrogen atmosphere. The reaction mixture was stirred under hydrogen atmosphere (1 atm) at room temperature overnight. The catalyst was filtered off through the celite pad and the filtrate was evaporated under vacuum. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 100:1) to give 2-tert-butyl-7-fluoro-1H-indol-5-amine (550 mg, 24%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.87 (brs, 1H), 6.64 (d, J=1.5 Hz, 1H), 6.37 (dd, J=1.8, 12.3 Hz, 1H), 6.11 (dd, J=2.4, 3.6 Hz, 1H), 1.39 (s, 9H). MS (ESI) m/z ($M+H^+$) 207.

Example 39

5-Amino-2-tert-butyl-1H-indole-7-carbonitrile

**[0490]**



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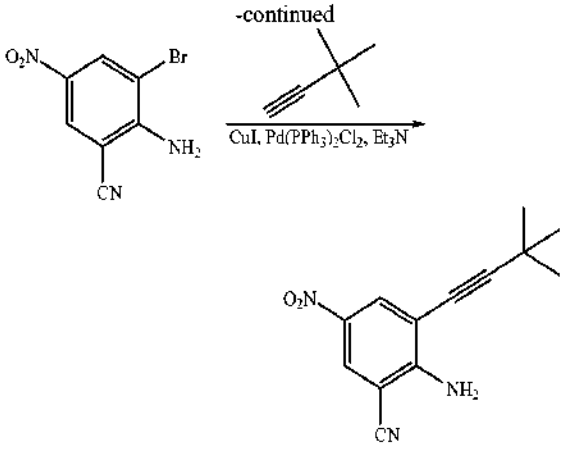
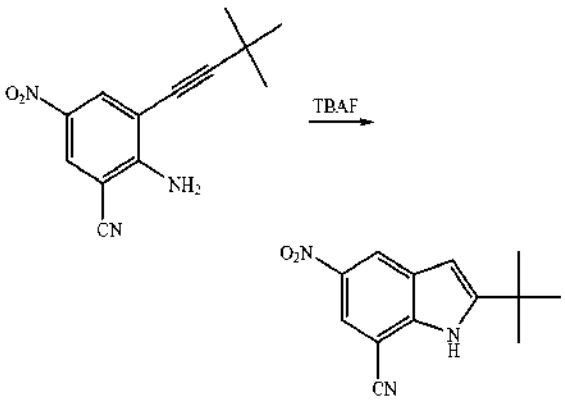


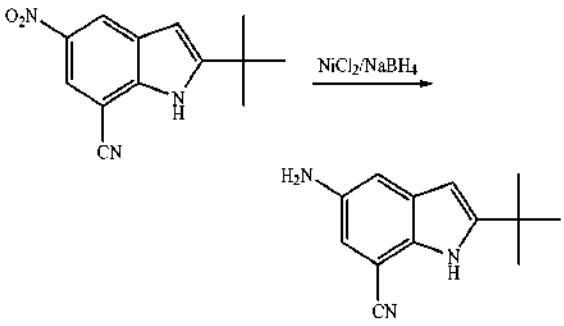

2-Amino-3-(3,3-dimethylbut-1-ynyl)-5-nitrobenzonitrile

**[0491]** To a stirred solution of 2-amino-3-bromo-5-nitrobenzonitrile (2.4 g, 10 mmol) in dry $Et_3N$ (60 mL) was added CuI (380 mg, 5% mol) and $Pd(PPh_3)_2Cl_2$ (470 mg, 5% mol) at room temperature. 3,3-dimethyl-but-1-yne (2.1 g, 25 mmol) was added dropwise to the mixture at room temperature. The reaction mixture was stirred at 80° C. for 10 h. The reaction mixture was filtered and the filtrate was poured into ice (60 g), extracted with ethyl acetate. The phases were separated and the organic phase was dried over $Na_2SO_4$. The solvent was removed under vacuum to obtain the crude product, which was purified by column chromatography (2-10% EtOAc in petroleum ether) to obtain 2-amino-3-(3,3-dimethylbut-1-ynyl)-5-nitrobenzonitrile (1.7 g, 71%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J=2.7 Hz, 1H), 8.27 (d, J=2.7 Hz, 1H), 5.56 (br s, 2H), 1.37 (s, 9H).




2-tert-Butyl-5-nitro-1H-indole-7-carbonitrile

**[0492]** To a solution of 2-amino-3-(3,3-dimethylbut-1-ynyl)-5-nitrobenzonitrile (1.7 g, 7.0 mmol) in THF (35 mL) was added TBAF (9.5 g, 28 mmol) at room temperature. The mixture was heated at reflux overnight. The reaction mixture was cooled and the THF was removed under reduced pressure. Water (50 ml) was added to the residue and the mixture was extracted with EtOAc. The organics were dried over $Na_2SO_4$ and the solvent was evaporated under vacuum to obtain 0.87 g of crude product 2-tert-butyl-5-nitro-1H-indole-7-carbonitrile which was used directly in the next step without purification.



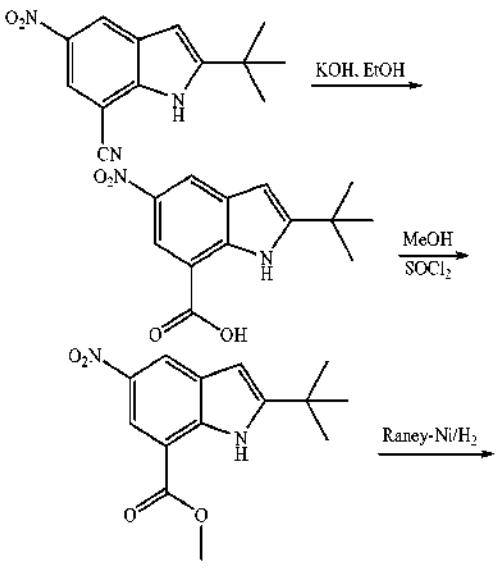

5-Amino-2-tert-butyl-1H-indol-7-carbonitrile

**[0493]** To a solution of crude product 2-tert-butyl-5-nitro-1H-indole-7-carbonitrile (0.87 g, 3.6 mmol) in MeOH (10 mL) was added $NiCl_2.6H_2O$ (1.8 g, 7.2 mmol) at −5° C. The reaction mixture was stirred for 30 min, then $NaBH_4$ (0.48 g, 14.32 mmol) was added to the reaction mixture at 0° C. After 5 min, the reaction mixture was quenched with water, filtered and extracted with EtOAc. The combined organic layers were dried over $Na_2SO_4$ and concentrated under vacuum to obtain the crude product, which was purified by column chromatography (5-20% EtOAc in petroleum ether) to obtain 5-amino-2-tert-butyl-1H-indol-7-carbonitrile (470 mg, 32% over two steps). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.06 (d, J=2.4 Hz, 1H), 6.84 (d, J=2.4 Hz, 1H), 6.14 (d, J=2.4 Hz, 1H), 3.57 (br s, 2H), 1.38 (s, 9H). MS (ESI) m/z: 214 ($M+H^+$).

Example 40

Methyl 5-amino-2-tert-butyl-1H-indole-7-carboxylate

**[0494]**



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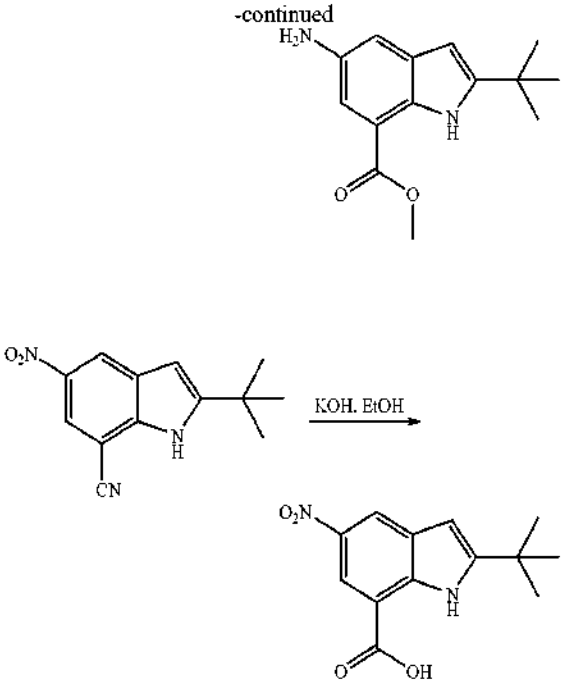
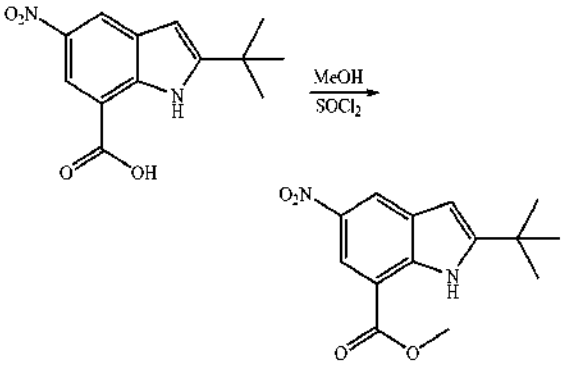


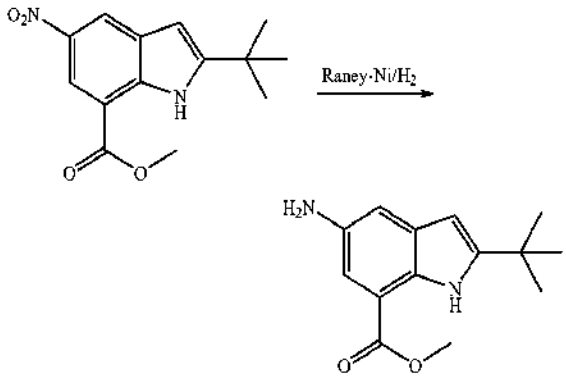
2-tert-Butyl-5-nitro-1H-indole-7-carboxylic acid

**[0495]** 2-tert-Butyl-5-nitro-1H-indole-7-carbonitrile (4.6 g, 19 mmol) was added to a solution of KOH in EtOH (10%, 100 mL) and the mixture was heated at reflux overnight. The solution was evaporated to remove alcohol, a small amount of water was added, and then the mixture was acidified with dilute hydrochloric acid. Upon standing in the refrigerator, an orange-yellow solid precipitated, which was purified by chromatography on silica gel (15% EtOAc in petroleum ether) to afford 2-tert-butyl-5-nitro-1H-indole-7-carboxylic acid (4.0 g, 77%). $^1$H NMR ($CDCl_3$, 300 MHz) δ 10.79 (brs, 1H), 8.66 (s, 1H), 8.45 (s, 1H), 6.57 (s, 1H), 1.39 (s, 9H).



Methyl 2-tert-butyl-5-nitro-1H-indole-7-carboxylate

**[0496]** $SOCl_2$ (3.6 g, 30 mol) was added dropwise to a solution of 2-tert-butyl-5-nitro-1H-indole-7-carboxylic acid (4.0 g, 15 mol) and methanol (30 mL) at 0° C. The mixture was stirred at 80° C. for 12 h. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica gel (5% EtOAc in petroleum ether) to afford methyl 2-tert-butyl-5-nitro-1H-indole-7-carboxylate (2.95 g, 70%). $^1$H NMR ($CDCl_3$, 300 MHz) δ 9.99 (brs, 1H), 8.70 (d, J=2.1 Hz, 1H), 8.65 (d, J=2.1 Hz, 1H), 6.50 (d, J=2.4 Hz, 1H), 4.04 (s, 3H), 1.44 (s, 9H).



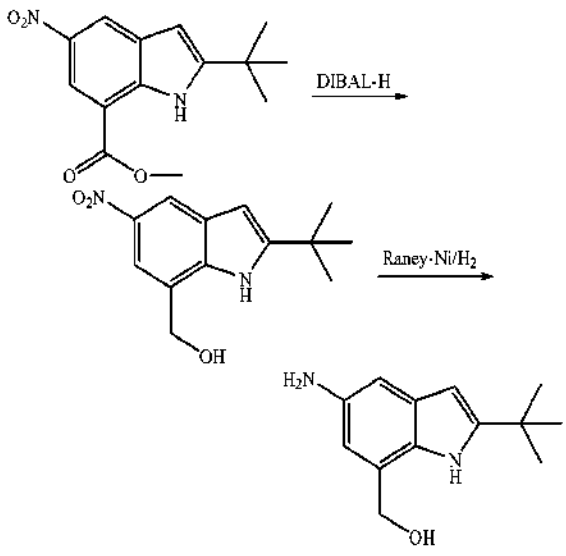
Methyl
5-amino-2-tert-butyl-1H-indole-7-carboxylate

**[0497]** A solution of 2-tert-butyl-5-nitro-1H-indole-7-carboxylate (2.0 g, 7.2 mmol) and Raney Nickel (200 mg) in $CH_3OH$ (50 mL) was stirred for 5 h at the room temperature under $H_2$ atmosphere. The catalyst was filtered off through a celite pad and the filtrate was evaporated under vacuum to give methyl 5-amino-2-tert-butyl-1H-indole-7-carboxylate (1.2 g, 68%) $^1$H NMR ($CDCl_3$, 400 MHz) δ 9.34 (brs, 1H), 7.24 (d, J=1.6 Hz, 1H), 7.10 (s, 1H), 6.12 (d, J=1.6 Hz, 1H), 3.88 (s, 3H), 1.45 (s, 9H).

Example 41

(5-Amino-2-tert-butyl-1H-indol-7-yl)methanol

**[0498]**



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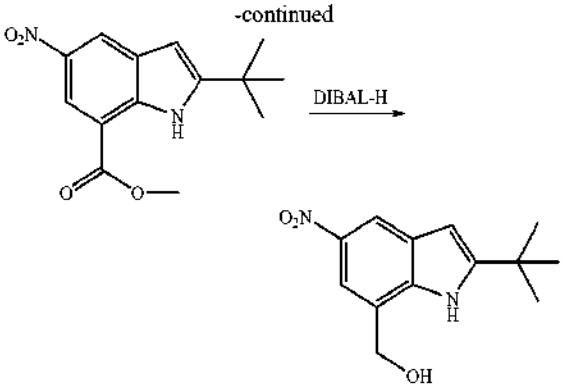
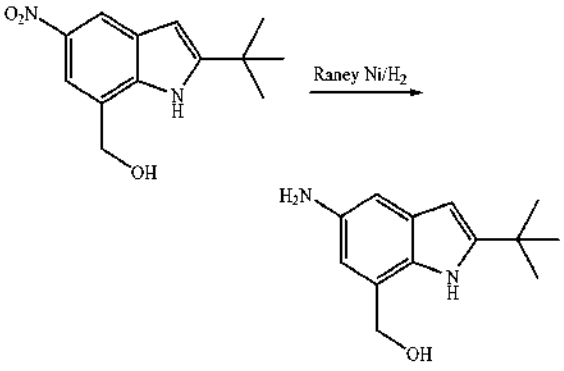


(2-tert-Butyl-5-nitro-1H-indol-7-yl)methanol

**[0499]** To a solution of methyl 2-tert-butyl-5-nitro-1H-indole-7-carboxylate (6.15 g, 22.3 mmol) and dichloromethane (30 ml) was added DIBAL-H (1.0 M, 20 mL, 20 mmol) at 78° C. The mixture was stirred for 1 h before water (10 mL) was added slowly. The resulting mixture was extracted with EtOAc (120 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give (2-tert-butyl-5-nitro-1H-indol-7-yl)methanol (4.0 g, 73%), which was used in the next step directly.



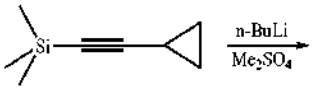
(5-Amino-2-tert-butyl-1H-indol-7-yl)methanol

**[0500]** A mixture of (2-tert-butyl-5-nitro-1H-indol-7-yl) methanol (4.0 g, 16 mmol) and Raney Nickel (400 mg) in $CH_3OH$ (100 mL) was stirred for 5 g at room temperature under $H_2$. The catalyst was filtered off through a celite pad and the filtrate was evaporated under vacuum to give (5-amino-2-tert-butyl-1H-indol-7-yl)methanol (3.4 g, 80%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 8.53 (br s, 1H), 6.80 (d, J=2.0 Hz, 1H), 6.38 (d, J=1.6 Hz, 1H), 4.89 (s, 2H), 1.37 (s, 9H).

Example 42

2-(1-Methylcyclopropyl)-1H-indol-5-amine

**[0501]**



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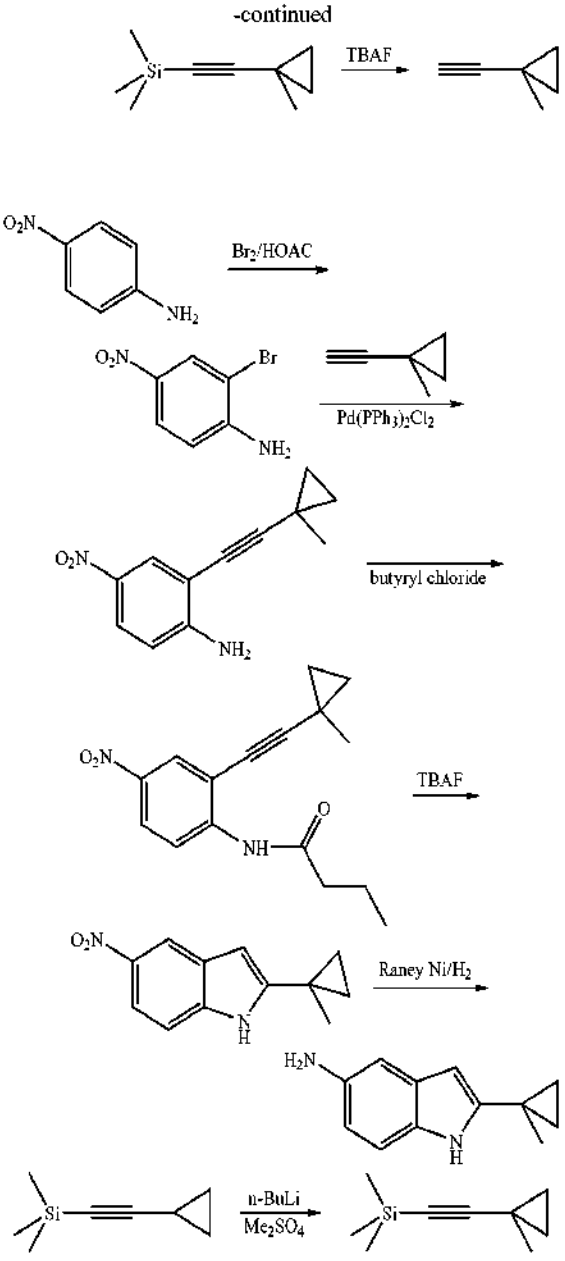
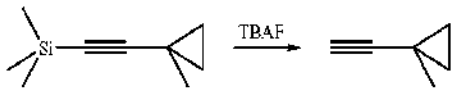


Trimethyl-(1-methyl-cyclopropylethynyl)-silane

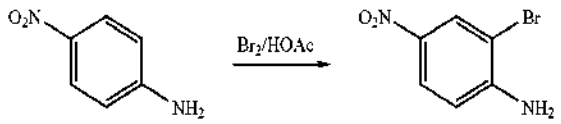
**[0502]** To a solution of cyclopropylethynyl-trimethyl-silane (3.0 g, 22 mmol) in ether (20 mL) was added dropwise n-BuLi (8.6 mL, 21.7 mol, 2.5 M solution in hexane) at 0° C. The reaction mixture was stirred at ambient temperature for 24 h before dimethyl sulfate (6.85 g, 54.3 mmol) was added dropwise at −10° C. The resulting solution was stirred at 10° C. and then at 20° C. for 30 min each. The reaction was quenched by adding a mixture of sat. aq. $NH_4Cl$ and 25% aq. ammonia (1:3, 100 mL). The mixture was then stirred at ambient temperature for 1 h. The aqueous phase was extracted with diethyl ether (3×50 mL) and the combined organic layers were washed successively with 5% aqueous hydrochloric acid (100 mL), 5% aq. $NaHCO_3$ solution (100 mL), and water (100 mL). The organics were dried over anhydrous $NaSO_4$ and concentrated at ambient pressure.

After fractional distillation under reduced pressure, trimethyl-(1-methyl-cyclopropylethynyl)-silane (1.7 g, 52%) was obtained as a colorless liquid. $^1$H NMR (400 MHz, $CDCl_3$) δ 1.25 (s, 3H), 0.92-0.86 (m, 2H), 0.58-0.56 (m. 2H), 0.15 (s, 9H).



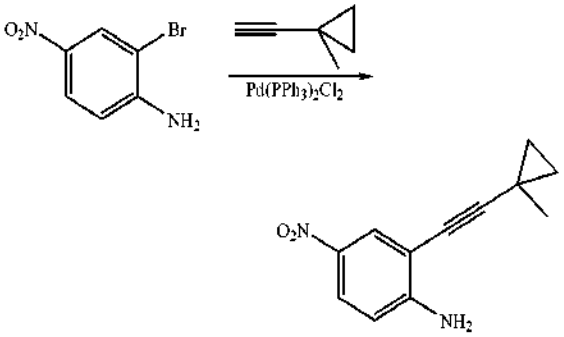

1-Ethynyl-1-methyl-cyclopropane

**[0503]** To a solution of trimethyl-(1-methyl-cyclopropylethynyl)-silane (20 g, 0.13 mol) in THF (250 mL) was added TBAF (69 g, 0.26 mol). The mixture was stirred overnight at 20° C. The mixture was poured into water and the organic layer was separated. The aqueous phase was extracted with THF (50 mL). The combined organic layers were dried over anhydrous $Na_2SO_4$ and distilled under atmospheric pressure to obtain 1-ethynyl-1-methyl-cyclopropane (7.0 g, contained 1/2 THF, 34%). $^1$H NMR (400 MHz, $CDCl_3$) δ 1.82 (s, 1H), 1.26 (s, 3H), 0.90-0.88 (m, 2H), 0.57-0.55 (m, 2H).



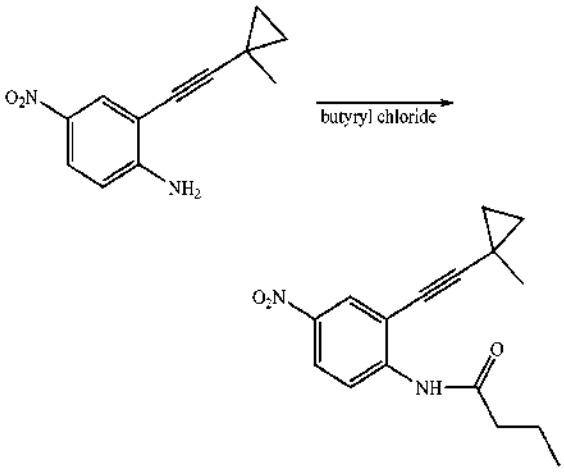

2-Bromo-4-nitroaniline

**[0504]** To a solution of 4-nitro-phenylamine (50 g, 0.36 mol) in AcOH (500 mL) was added $Br_2$ (60 g, 0.38 mol) dropwise at 5° C. The mixture was stirred for 30 min at that temperature. The insoluble solid was collected by filtration and basified with saturated aqueous $NaHCO_3$ to pH 7. The aqueous phase was extracted with EtOAc (300 mL×3). The combined organic layers were dried and evaporated under reduced pressure to obtain compound 2-bromo-4-nitroaniline (56 g, 72%), which was directly used in the next step.



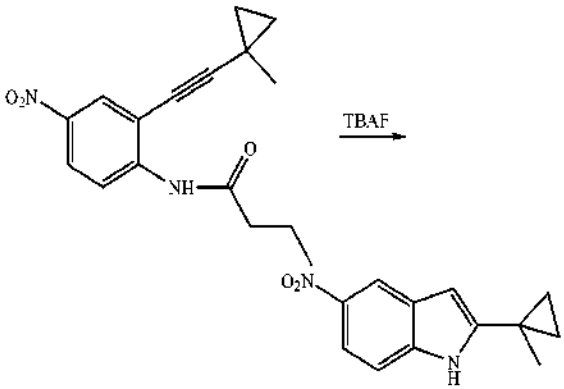

2-((1-Methylcyclopropyl)ethynyl)-4-nitroaniline

**[0505]** To a deoxygenated solution of 2-bromo-4-nitroaniline (430 mg, 2.0 mmol) and 1-ethynyl-1-methyl-cyclopropane (630 mg, 8.0 mmol) in triethylamine (20 mL) was added CuI (76 mg, 0.40 mmol) and $Pd(PPh_3)_2Cl_2$ (140 mg, 0.20 mmol) under $N_2$. The mixture was heated at 70° C. and stirred for 24 h. The solid was filtered off and washed with EtOAc (50 mL×3). The filtrate was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to give 2-((1-methylcyclopropyl)ethynyl)-4-nitroaniline (340 mg, 79%). $^1$H NMR (300 MHz, $CDCl_3$) δ 8.15-8.14 (m, 1H), 7.98-7.95 (m, 1H), 6.63 (d, J=6.9 Hz, 1H), 4.80 (brs, 2H), 1.38 (s, 3H), 1.04-1.01 (m, 2H), 0.76-0.73 (m, 2H).




N-[2-(1-Methyl-cyclopropylethynyl)-4-nitro-phenyl]-butyramide

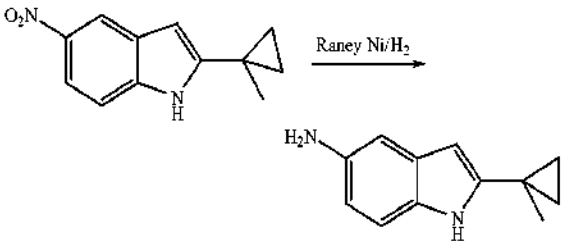
**[0506]** To a solution of 2-((1-methylcyclopropyl)ethynyl)-4-nitroaniline (220 mg, 1.0 mmol) and pyridine (160 mg, 2.0 mol) in $CH_2Cl_2$ (20 mL) was added butyryl chloride (140 mg, 1.3 mmol) at 0° C. The mixture was warmed to room temperature and stirred for 3 h. The mixture was poured into ice-water. The organic layer was separated and the aqueous phase was extracted with $CH_2Cl_2$ (30 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure to obtain N-[2-(1-methyl-cyclopropyl-ethynyl)-4-nitro-phenyl]-butyramide (230 mg, 82%), which was directly used in the next step.




2-(1-Methylcyclopropyl)-5-nitro-1H-indole

**[0507]** A mixture of N-[2-(1-methyl-cyclopropylethynyl)-4-nitro-phenyl]-butyramide (1.3 g, 4.6 mmol) and TBAF (2.4

g, 9.2 mmol) in THF (20 mL) was heated at reflux for 24 h. The mixture was cooled to room temperature and poured into ice water. The mixture was extracted with $CH_2Cl_2$ (30 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to afford 2-(1-methylcyclopropyl)-5-nitro-1H-indole (0.70 g, 71%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.56 (brs, 1H), 8.44 (d, J=2.0 Hz, 1H), 8.01 (dd, J=2.4, 8.8 Hz, 1H), 7.30 (d, J=8.8 Hz, 1H), 6.34 (d, J=1.6 Hz, 1H), 1.52 (s, 3H), 1.03-0.97 (m, 2H), 0.89-0.83 (m, 2H).



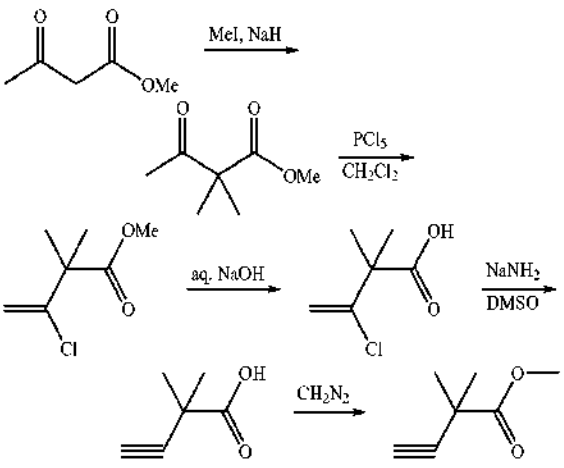

2-(1-Methyl-cyclopropyl)-1H-indol-5-ylamine

**[0508]** To a solution of 2-(1-methylcyclopropyl)-5-nitro-1H-indole (0.70 g, 3.2 mmol) in EtOH (20 mL) was added Raney Nickel (100 mg) under nitrogen atmosphere. The mixture was stirred under hydrogen atmosphere (1 atm) at room temperature overnight. The catalyst was filtered off through a celite pad and the filtrate was evaporated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=5/1) to afford 2-(1-methylcyclopropyl)-1H-indol-5-ylamine (170 mg, 28%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.65 (brs, 1H), 7.08 (d, J=8.4 Hz, 1H), 6.82 (s, 1H), 6.57 (d, J=8.4 Hz, 1H), 6.14 (s, 1H), 3.45 (brs, 2H), 1.47 (s, 3H), 0.82-0.78 (m, 2H), 0.68-0.63 (m, 2H).

Example 43

Methyl 2-(5-amino-1H-indol-2-yl)-2-methylpropanoate

**[0509]**

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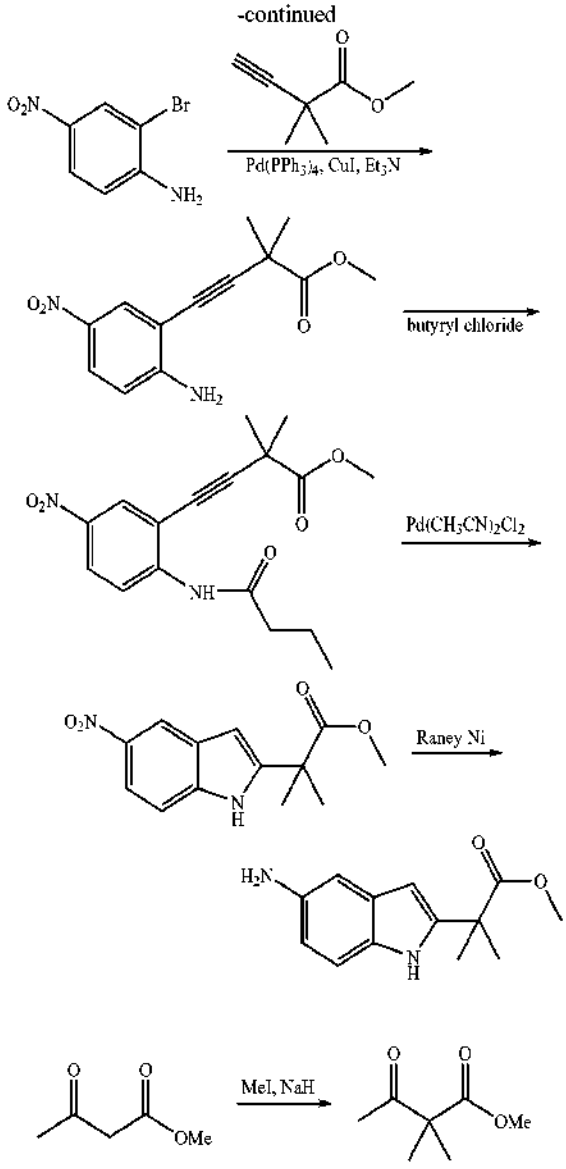
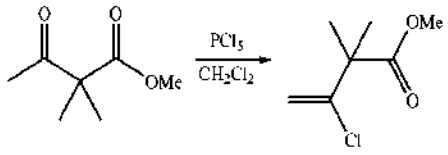






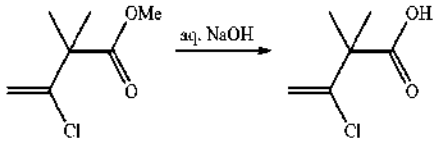
Methyl 2,2-dimethyl-3-oxobutanoate

**[0510]** To a suspension of NaH (42 g, 1.1 mol, 60%) in THF (400 mL) was added dropwise a solution of methyl 3-oxobutanoate (116 g, 1.00 mol) in THF (100 mL) at 0° C. The mixture was stirred for 0.5 h at that temperature before MeI (146 g, 1.1 mol) was added dropwise at 0° C. The resultant mixture was warmed to room temperature and stirred for 1 h. NaH (42 g, 1.05 mol, 60%) was added in portions at 0° C. and the resulting mixture was continued to stir for 0.5 h at this temperature. MeI (146 g, 1.05 mol) was added dropwise at 0° C. The reaction mixture was warmed to room temperature and stirred overnight. The mixture was poured into ice water and the organic layer was separated. The aqueous phase was extracted with EtOAc (500 mL×3). The combined organic layers were dried and evaporated under reduced pressure to give methyl 2,2-dimethyl-3-oxobutanoate (85 g), which was used directly in the next step.



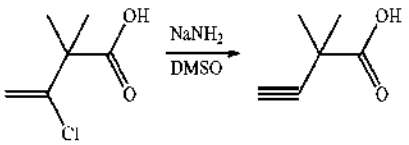
Methyl 3-chloro-2,2-dimethylbut-3-enoate

**[0511]** To a suspension of $PCl_5$ (270 g, 1.3 mol) in $CH_2Cl_2$ (1000 mL) was added dropwise methyl 2,2-dimethyl-3-oxobutanoate (85 g) at 0° C., following by addition of approximately 30 drops of dry DMF. The mixture was heated at reflux overnight. The reaction mixture was cooled to ambient temperature and slowly poured into ice water. The organic layer was separated and the aqueous phase was extracted with $CH_2Cl_2$ (500 mL×3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the residue was distilled under reduced pressure to give methyl 3-chloro-2,2-dimethylbut-3-enoate (37 g, 23%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.33 (s, 1H), 3.73 (s, 3H), 1.44 (s, 6H).



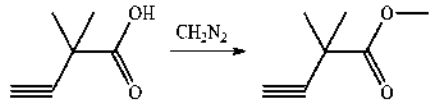
3-Chloro-2,2-dimethylbut-3-enoic acid

**[0512]** A mixture of methyl 3-chloro-2,2-dimethylbut-3-enoate (33 g, 0.2 mol) and NaOH (9.6 g, 0.24 mol) in water (200 mL) was heated at reflux for 5 h. The mixture was cooled to ambient temperature and extracted with ether. The organic layer was discarded. The aqueous layer was acidified with cold 20% HCl solution and extracted ether (200 mL×3). The combined organic layers were dried and evaporated under reduced pressure to give 3-chloro-2,2-dimethyl-but-3-enoic acid (21 g, 70%), which was used directly in the next step. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.90 (brs, 1H), 5.37 (dd, J=2.4, 6.8 Hz, 2H), 1.47 (s, 6H).



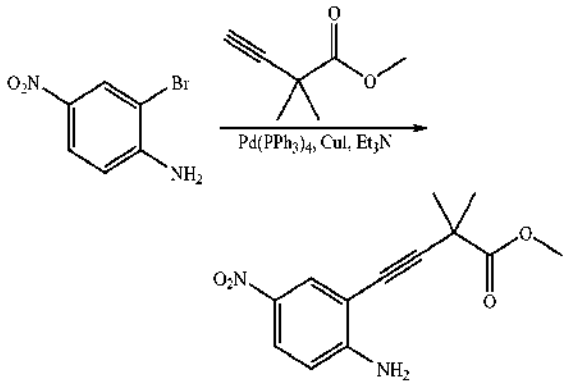
2,2-Dimethyl-but-3-ynoic acid

**[0513]** Liquid $NH_3$ was condensed in a 3-neck, 250 mL round bottom flask at −78° C. Na (3.98 g, 0.173 mol) was added to the flask in portions. The mixture was stirred for 2 h at −78° C. before anhydrous DMSO (20 mL) was added dropwise at −78° C. The mixture was stirred at room temperature until no more $NH_3$ was given off. A solution of 3-chloro-2,2-dimethyl-but-3-enoic acid (6.5 g, 43 mmol) in DMSO (10 mL) was added dropwise at −40° C. The mixture was warmed and stirred at 50° C. for 5 h, then stirred at room temperature overnight. The cloudy, olive green solution was poured into cold 20% HCl solution and then extracted three times with ether. The ether extracts were dried over anhydrous $Na_2SO_4$ and concentrated to give crude 2,2-dimethylbut-3-ynoic acid (2 g), which was used directly in the next step. $^1H$ NMR (400 MHz, $CDCl_3$) δ 2.30 (s, 1H), 1.52 (s, 6H).



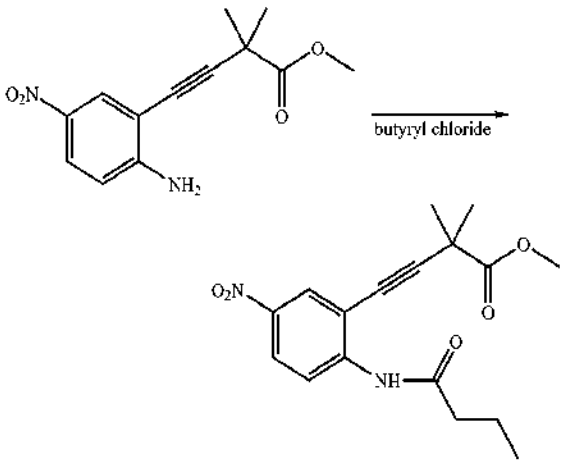
Methyl 2,2-dimethylbut-3-ynoate

**[0514]** To a solution of diazomethane (~10 g) in ether (400 mL) was added dropwise 2,2-dimethyl-but-3-ynoic acid (10.5 g, 93.7 mmol) at 0° C. The mixture was warmed to room temperature and stirred overnight. The mixture was distilled under atmospheric pressure to give crude methyl 2,2-dimethylbut-3-ynoate (14 g), which was used directly in the next step. $^1H$ NMR (400 MHz, $CDCl_3$) δ 3.76 (s, 3H), 2.28 (s, 1H), 1.50 (s, 6H).



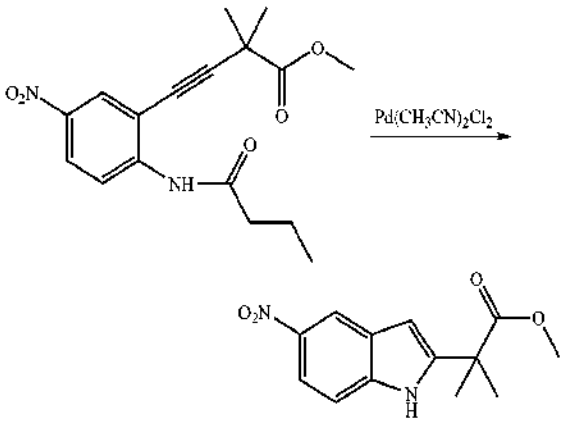
Methyl
4-(2-amino-5-nitrophenyl)-2,2-dimethylbut-3-ynoate

**[0515]** To a deoxygenated solution of compound 2-bromo-4-nitroaniline (9.43 g, 43.7 mmol), methyl 2,2-dimethylbut-3-ynoate (5.00 g, 39.7 mmol), CuI (754 mg, 3.97 mmol) and triethylamine (8.03 g, 79.4 mmol) in toluene/$H_2O$ (100/30 mL) was added $Pd(PPh_3)_4$ (6.17 g, 3.97 mmol) under $N_2$. The mixture was heated at 70° C. and stirred for 24 h. After cooling, the solid was filtered off and washed with EtOAc (50 mL×3). The organic layer was separated and the aqueous phase was washed with EtOAc (50 mL×3). The combined organic layers were dried and evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=10/1) to obtain methyl 4-(2-amino-5-nitrophenyl)-2,2-dimethylbut-3-ynoate (900 mg, 9%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J=2.8 Hz, 1H), 8.01 (dd, J=2.8, 9.2 Hz, 1H), 6.65 (d, J=9.2 Hz, 1H), 5.10 (brs, 2H), 3.80 (s, 3H), 1.60 (s, 6H).



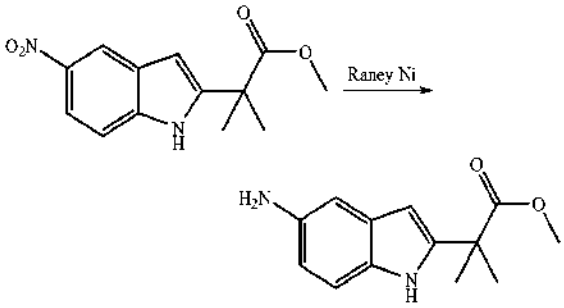

Methyl 4-(2-butyramido-5-nitrophenyl)-2,2-dimethylbut-3-ynoate

**[0516]** To a solution of methyl 4-(2-amino-5-nitrophenyl)-2.2-dimethylbut-3-ynoate (260 mg, 1.0 mmol) and pyridine (160 mg, 2.0 mol) in $CH_2Cl_2$ (20 mL) was added butyryl chloride (140 mg, 1.3 mmol) at 0° C. The reaction mixture was warmed to room temperature and stirred for 3 h before the mixture was poured into ice-water. The organic layer was separated and the aqueous phase was extracted with $CH_2Cl_2$ (30 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure to obtain methyl 4-(2-butyramido-5-nitrophenyl)-2,2-dimethylbut-3-ynoate (150 mg, 45%), which was used directly in the next step. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.79 (brs, 1H), 8.71 (d, J=9.2 Hz, 1H), 8.24 (d, J=2.8 Hz, 1H), 8.17 (dd, J=2.8, 9.2 Hz, 1H), 3.82 (s, 3H), 2.55 (t, J=7.2 Hz, 2H), 1.85-1.75 (m, 2H), 1.63 (s, 6H), 1.06 (t, J=6.8 Hz, 3H).




Methyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propanoate

**[0517]** To a deoxygenated solution of methyl 4-(2-butyramido-5-nitrophenyl)-2,2-dimethylbut-3-ynoate (1.8 g, 5.4 mmol) in acetonitrile (30 mL) was added $Pd(CH_3CN)_2Cl_2$ (0.42 g, 1.6=mmol) under $N_2$. The mixture was heated at reflux for 24 h. After cooling the mixture to ambient temperature, the solid was filtered off and washed with EtOAc (50 mL×3). The filtrate was evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=30/1) to give methyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propanoate (320 mg, 23%). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.05 (brs, 1H), 8.52 (d, J=2.0 Hz, 1H), 8.09 (dd, J=2.0, 8.8 Hz, 1H), 7.37 (d, J=8.8 Hz, 1H), 6.54 (d, J=1.6 Hz, 1H), 3.78 (d, J=9.6 Hz, 3H), 1.70 (s, 6H).



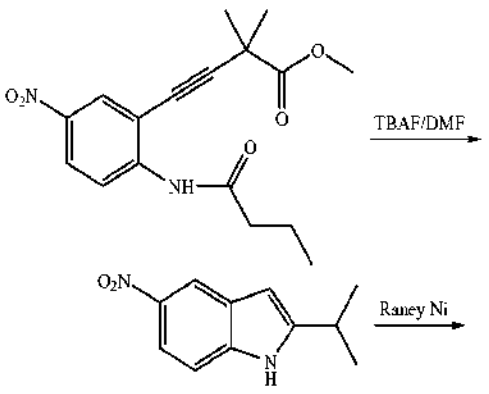

Methyl 2-(5-amino-1H-indol-2-yl)-2-methylpropanoate

**[0518]** A suspension of methyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propanoate (60 mg, 0.23 mmol) and Raney Nickel (10 mg) in MeOH (5 mL) was hydrogenated under hydrogen (1 atm) at room temperature overnight. The catalyst was filtered off through a celite pad and the filtrate was evaporated under vacuum to give a residue, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=5/1) to give methyl 2-(5-amino-1H-indol-2-yl)-2-methylpropanoate (20 mg, 38%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.37 (br s, 1H), 7.13 (d, J=8.4 Hz, 1H), 6.87 (d, J=2.0 Hz, 1H), 6.63 (dd, J=2.0, 8.4 Hz, 1H), 6.20 (d, J=1.2 Hz, 1H), 3.72 (d, J=7.6 Hz, 3H), 3.43 (br s, 1H), 1.65 (s, 6H); MS (ESI) m/e ($M+H^+$) 233.2.

Example 44

2-Isopropyl-1H-indol-5-amine

**[0519]**



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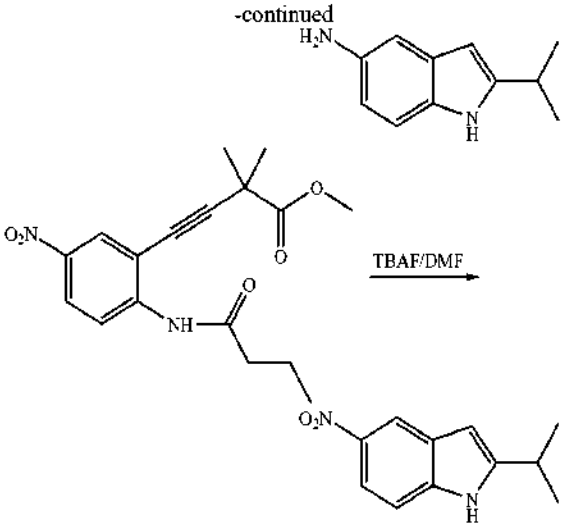
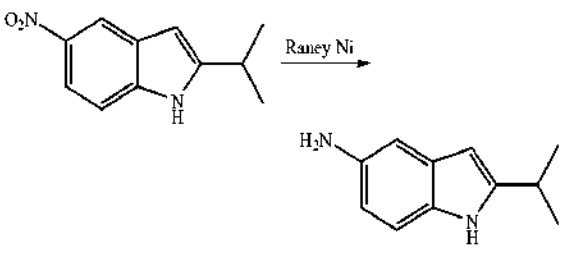


2-Isopropyl-5-nitro-1H-indole

**[0520]** A mixture of methyl 4-(2-butyramido-5-nitrophenyl)-2,2-dimethylbut-3-ynoate (0.50 g, 1.5 mmol) and TBAF (790 mg, 3.0 mmol) in DMF (20 mL) was heated at 70° C. for 24 h. The reaction mixture was cooled to room temperature and poured into ice water. The mixture was extracted with ether (30 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=20/1) to give 2-isopropyl-5-nitro-1H-indole (100 mg, 33%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.25 (br s, 1H), 8.21 (dd, J=2.4, 10.0 Hz, 1H), 7.32 (d, J=8.8 Hz, 1H), 6.41 (s, 1H), 3.07-3.14 (m, 1H), 1.39 (d, J=6.8 Hz, 6H).



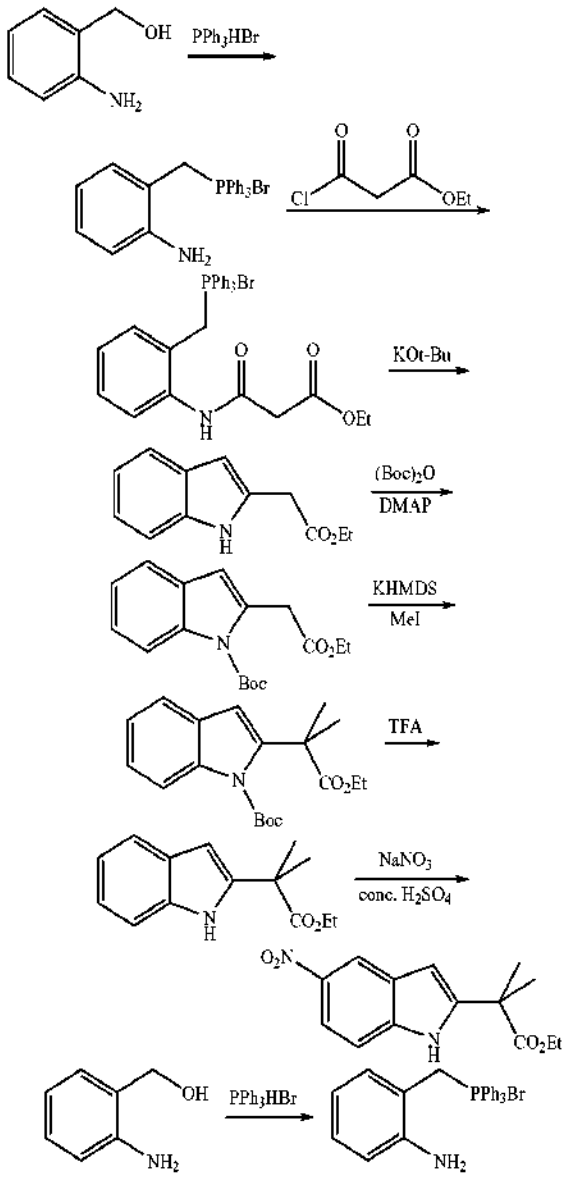
2-Isopropyl-1H-indol-5-amine

**[0521]** A suspension of 2-isopropyl-5-nitro-1H-indole (100 mg, 0.49 mmol) and Raney Nickel (10 mg) in MeOH (10 mL) was hydrogenated under hydrogen (1 atm) at the room temperature overnight. The catalyst was filtered off through a celite pad and the filtrate was evaporated under vacuum to give a residue, which was purified by column (petroleum ether/ethyl acetate=5/1) to give 2-isopropyl-1H-indol-5-amine (35 mg, 41%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.69 (br s, 1H), 7.10 (d, J=8.4 Hz, 1H), 6.86 (d, J=2.4 Hz, 1H), 6.58 (dd, J=2.4, 8.8 Hz, 1H), 6.07 (t, J=1.2 Hz, 1H), 3.55 (br s, 2H), 3.06-2.99 (m, 1H), 1.33 (d, J=7.2 Hz, 6H); MS (ESI) m/e ($M+H^+$) 175.4.

Example 45

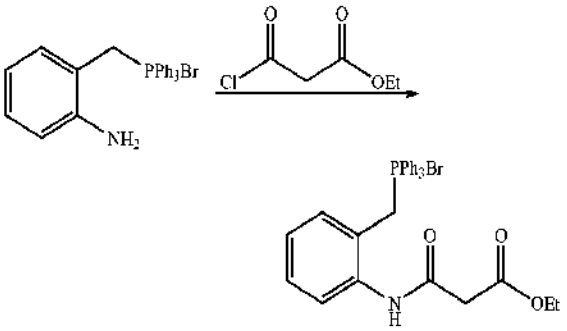
1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(1-hydroxy-2-methylpropan-2-yl)-1-5-yl)cyclopropanecarboxamide

**[0522]**



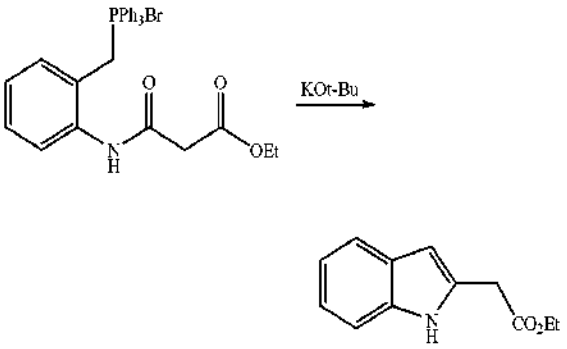
Triphenyl(2-aminobenzyl)phosphonium bromide

**[0523]** 2-Aminobenzyl alcohol (60.0 g, 0.487 mol) was dissolved in acetonitrile (2.5 L) and brought to reflux. Triphenylphosphine hydrobromide (167 g, 0.487 mol) was added and the mixture was heated at reflux for 3 h. The reaction mixture was concentrated to approximately 500 mL and left at room temperature for 1 h. The precipitate was filtered and washed with cold acetonitrile followed by hexane. The solid was dried overnight at 40° C. under vacuum to give triphenyl (2-aminobenzyl)phosphonium bromide (193 g, 88%).



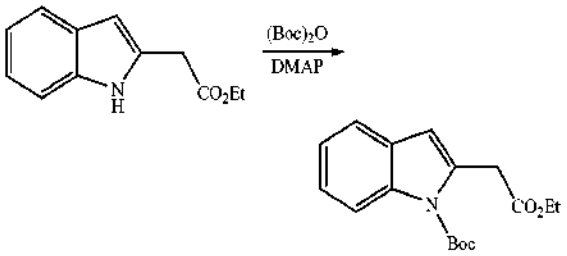
Triphenyl((ethyl(2-carbamoyl)acetate)-2-benzyl) phosphonium bromide

**[0524]** To a suspension of triphenyl(2-aminobenzyl)phosphonium bromide (190 g, 0.43 mol) in anhydrous dichloromethane (1 L) was added ethyl malonyl chloride (55 ml, 0.43 mol). The reaction was stirred for 3 h at room temperature. The mixture was evaporated to dryness before ethanol (400 mL) was added. The mixture was heated at reflux until a clear solution was obtained. The solution was left at room temperature for 3 h. The precipitate was filtered, washed with cold ethanol followed by hexane and dried. A second crop was obtained from the mother liquor in the same way. In order to remove residual ethanol both crops were combined and dissolved in dichloromethane (approximately 700 mL) under heating and evaporated. The solid was dried overnight at 50° C. under vacuum to give triphenyl((ethyl(2-carbamoyl)acetate)-2-benzyl)-phosphonium bromide (139 g, 58%).



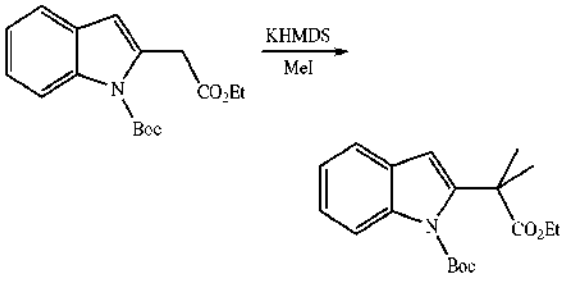
Ethyl 2-(1H-indol-2-yl)acetate

**[0525]** Triphenyl((ethyl(2-carbamoyl)acetate)-2-benzyl) phosphonium bromide (32.2 g, 57.3 mmol) added to anhydrous toluene (150 mL) and the mixture was heated at reflux. Fresh potassium tert-butoxide (7.08 g, 63.1 mmol) was added in portions over 15 minutes. Reflux was continued for another 30 minutes. The mixture was filtered hot through a plug of celite and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (0-30% ethyl acetate in hexane over 45 min) to give ethyl 2-(1H-indol-2-yl)acetate (9.12 g, 78%).



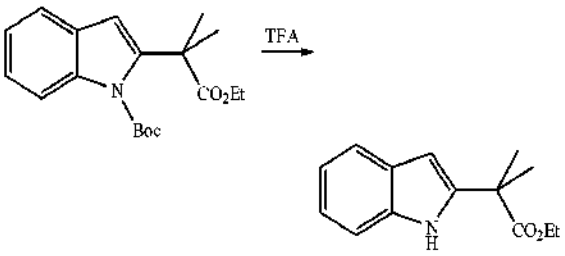
tert-Butyl 2-((ethoxycarbonyl)methyl)-1H-indole-1-carboxylate

**[0526]** To a solution of ethyl 2-(1H-indol-2-yl)acetate (14.7 g, 72.2 mmol) in dichloromethane (150 mL) was added 4-dimethylaminopyridine (8.83 g, 72.2 mmol) and di-tert-butyl carbonate (23.7 g, 108 mmol) in portions. After stirring for 2 h at room temperature, the mixture was diluted with dichloromethane, washed with water, dried over magnesium sulfate and purified by silica gel chromatography (0 to 20% EtOAc in hexane) to give tert-butyl 2-((ethoxycarbonyl)methyl)-1H-indole-1-carboxylate (20.0 g, 91%).



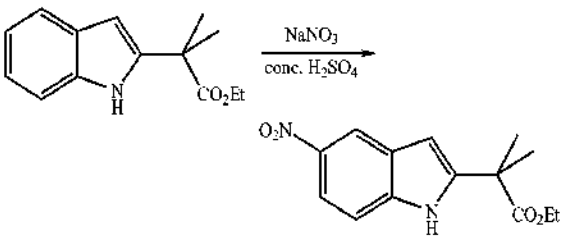
tert-Butyl 2-(2-(ethoxycarbonyl)propan-2-yl)-1H-indole-1-carboxylate

**[0527]** tert-Butyl 2-((ethoxycarbonyl)methyl)-1H-indole-1-carboxylate (16.7 g, 54.9 mmol) was added to anhydrous THF (100 mL) and cooled to −78° C. A 0.5M solution of potassium hexamethyldisilazane (165 mL, 82 mmol) was added slowly such that the internal temperature stayed below −60° C. Stirring was continued for 30 minutes at −78° C. To this mixture, methyl iodide (5.64 mL, 91 mmol) was added. The mixture was stirred for 30 min at room temperature and then cooled to −78° C. A 0.5M solution of potassium hexamethyldisilazane (210 mL, 104 mmol) was added slowly and the mixture was stirred for another 30 minutes at −78° C. More methyl iodide (8.6 mL, 137 mmol) was added and the mixture was stirred for 1.5 h at room temperature. The reaction was quenched with sat. aq. ammonium chloride and partitioned between water and dichloromethane. The aqueous phase was extracted with dichloromethane and the combined organic phases were dried over magnesium sulfate and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (0 to 20% ethylacetate in hexane) to give tert-butyl 2-(2-(ethoxycarbonyl)propan-2-yl)-1H-indole-1-carboxylate (17.1 g, 94%).



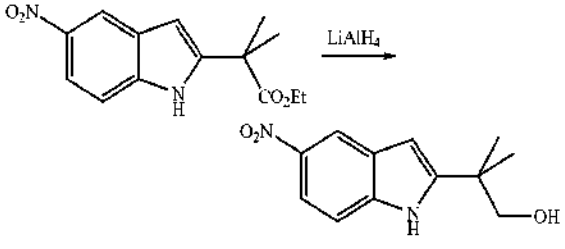

Ethyl 2-(1H-indol-2-yl)-2-methylpropanoate

**[0528]** tert-Butyl 2-(2-(ethoxycarbonyl)propan-2-yl)-1H-indole-1-carboxylate (22.9 g, 69.1 mmol) was dissolved in dichloromethane (200 mL) before TFA (70 mL) was added. The mixture was stirred for 5 h at room temperature. The mixture was evaporated to dryness, taken up in dichloromethane and washed with saturated sodium bicarbonate solution, water, and brine. The product was purified by column chromatography on silica gel (0-20% EtOAc in hexane) to give ethyl 2-(1H-indol-2-yl)-2-methylpropanoate (12.5 g, 78%).



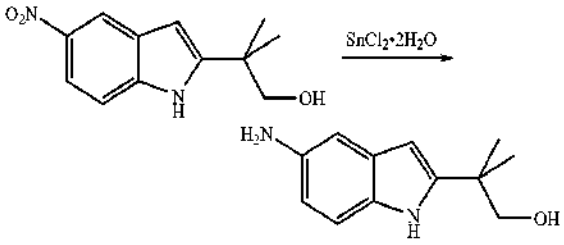

Ethyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propanoate

**[0529]** Ethyl 2-(1H-indol-2-yl)-2-methylpropanoate (1.0 g, 4.3 mmol) was dissolved in concentrated sulfuric acid (6 mL) and cooled to −10° C. (salt/ice-mixture). A solution of sodium nitrate (370 mg, 4.33 mmol) in concentrated sulfuric acid (3 mL) was added dropwise over 30 min. Stirring was continued for another 30 min at −10° C. The mixture was poured into ice and the product was extracted with dichloromethane. The combined organic phases were washed with a small amount of sat. aq. sodium bicarbonate. The product was purified by column chromatography on silica gel (5-30% EtOAc in hexane) to give ethyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propanoate (0.68 g, 57%).




2-Methyl-2-(5-nitro-1H-indol-2-yl)propan-1-ol

**[0530]** To a cooled solution of $LiAlH_4$ (1.0 M in THF, 1.1 mL, 1.1 mmol) in THF (5 mL) at 0° C. was added a solution of ethyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propanoate (0.20 g, 0.72 mmol) in THF (3.4 mL) dropwise. After addition, the mixture was allowed to warm up to room temperature and was stirred for 3 h. The mixture was cooled to 0° C. before water (2 mL) was slowly added followed by careful addition of 15% NaOH (2 mL) and water (4 mL). The mixture was stirred at room temperature for 0.5 h and was filtered through a short plug of celite using ethyl acetate. The organic layer was separated from the aqueous layer, dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane=1/1) to give 2-methyl-2-(5-nitro-1H-indol-2-yl)propan-1-ol (0.098 g, 58%).



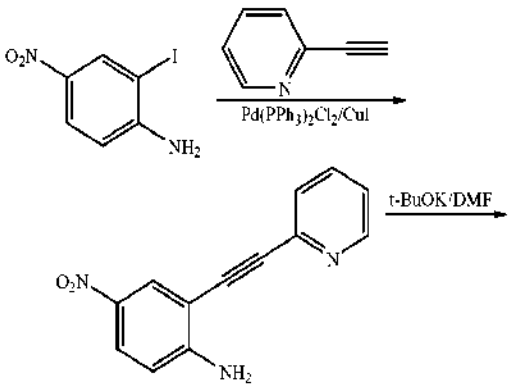

2-(5-Amino-1H-indol-2-yl)-2-methylpropan-1-ol

**[0531]** To a solution of 2-methyl-2-(5-nitro-1H-indol-2-yl) propan-1-ol (0.094 g, 0.40 mmol) in ethanol (4 mL) was added tin chloride dihydrate (0.451 g, 2.0 mmol). The mixture was heated in the microwave at 120° C. for 1 h. The mixture was diluted with ethyl acetate and water before being quenched with saturated aqueous $NaHCO_3$. The reaction mixture was filtered through a plug of celite using ethyl acetate. The organic layer was separated from the aqueous layer, dried over $Na_2SO_4$, filtered and evaporated under reduced pressure to give 2-(5-amino-1H-indol-2-yl)-2-methylpropan-1-ol (0.080 g, 98%).

Example 46

2-(Pyridin-2-yl)-1H-indol-5-amine

**[0532]**



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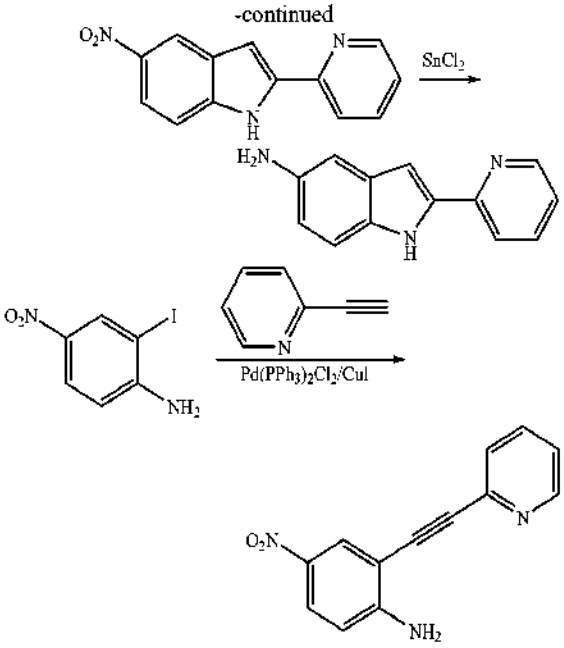
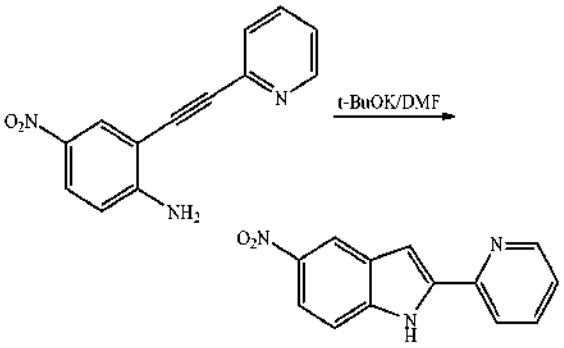


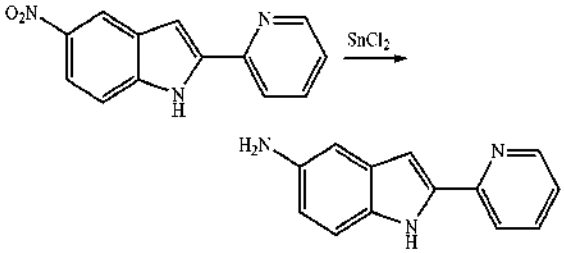
4-Nitro-2-(pyridin-2-ylethynyl)aniline

**[0533]** To the solution of 2-iodo-4-nitroaniline (3.0 g, 11 mmol) in DMF (60 mL) and $Et_3N$ (60 mL) was added 2-ethynylpyridine (3.0 g, 45 mmol), $Pd(PPh_3)_2Cl_2$ (600 mg) and CuI (200 mg) under $N_2$. The reaction mixture was stirred at 60° C. for 12 h. The mixture was diluted with water and extracted with dichloromethane (3×100 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated in vacuum. The residue was purified by chromatography on silica gel (5-10% ethyl acetate/petroleum ether) to afford 4-nitro-2-(pyridin-2-ylethynyl)aniline (1.5 g, 60%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.13 (d, J=2.1 Hz, 1H), 7.98 (d, J=1.8, 6.9 Hz, 1H), 7.87-7.80 (m, 2H), 7.42-7.39 (m, 1H), 7.05 (brs, 2H), 6.80 (d, J=6.9 Hz, 1H).



5-Nitro-2-(pyridin-2-yl)-1H-indole

**[0534]** To the solution of 4-nitro-2-(pyridin-2-ylethynyl) aniline (1.5 g, 6.3 mmol) in DMF (50 mL) was added t-BuOK (1.5 g, 13 mmol). The reaction mixture was stirred at 90° C. for 2 h. The mixture was diluted with water and extracted with dichloromethane (3×50 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated in vacuum. The residue was purified by chromatography on silica gel (5-10% ethyl acetate/petroleum ether) to afford 5-nitro-2-(pyridin-2-yl)-1H-indole (1.0 g, 67% yield). $^1H$ NMR (300 MHz, d-DMSO) δ 12.40 (s, 1H), 8.66 (d, J=2.1 Hz, 1H), 8.58 (d, J=1.8 Hz, 1H), 8.07-7.91 (m, 3H), 7.59 (d, J=6.6 Hz, 1H), 7.42-7.37 (m, 2H).



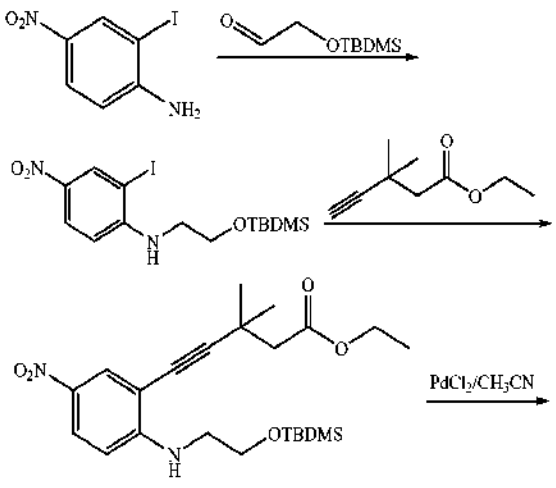
2-(Pyridin-2-yl)-1H-indol-5-amine

**[0535]** To a solution of 5-nitro-2-(pyridin-2-yl)-1H-indole (700 mg, 2.9 mmol) in EtOH (20 mL) was added $SnCl_2$ (2.6 g, 12 mmol). The mixture was heated at reflux for 10 h. Water was added and the mixture was extracted with EtOAc (50 mL×3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated in vacuum. The residue was purified by chromatography on silica gel (5-10% ethyl acetate/petroleum ether) to afford 2-(pyridin-2-yl)-1H-indol-5-amine (120 mg, 20%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 9.33 (brs, 1H), 8.55 (dd, J=1.2, 3.6 Hz, 1H), 7.76-7.67 (m, 2H), 7.23 (d, J=6.4 Hz, 1H), 7.16-7.12 (m, 1H), 6.94 (d, J=2.0 Hz, 1H), 6.84 (d, J=2.4 Hz, 1H), 6.71-6.69 (dd, J=2.0, 8.4 Hz, 1H).

Example 47

2-(Pyridin-2-yl)-1H-indol-5-amine

**[0536]**



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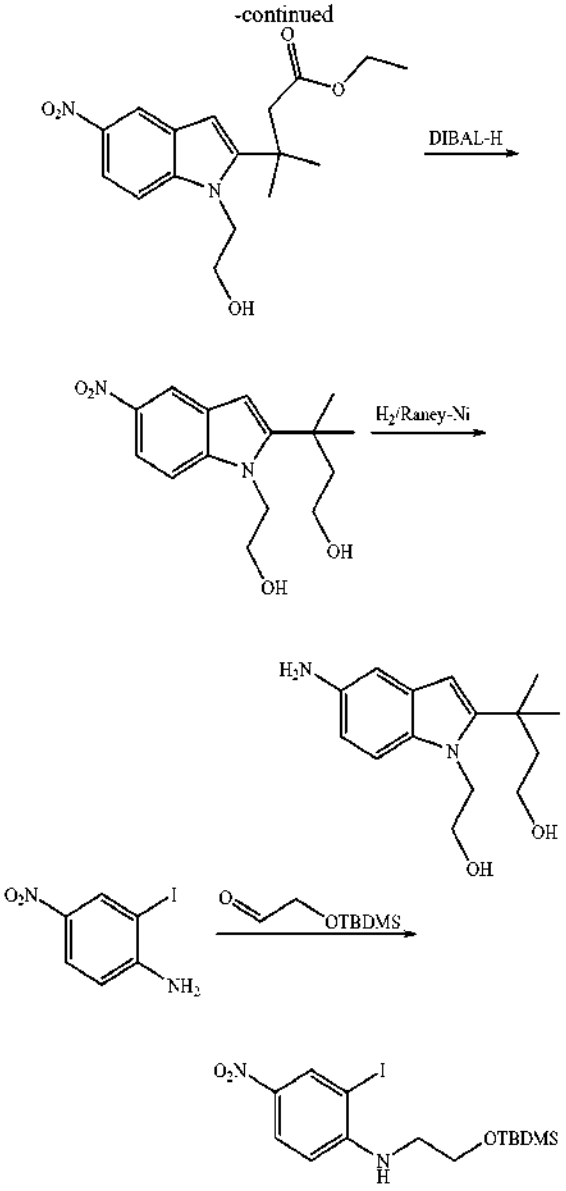
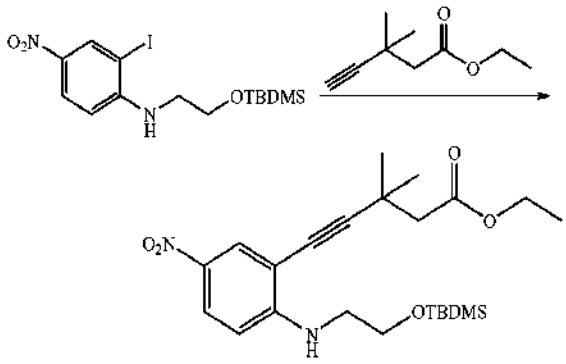


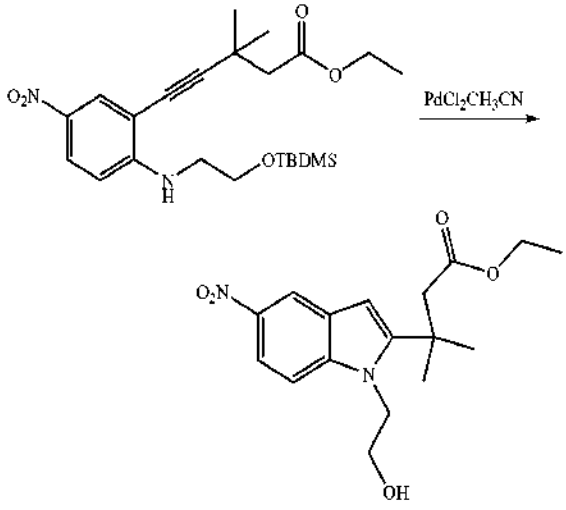
[2-(tert-Butyl-dimethyl-silanyloxy)-ethyl]-(2-iodo-4-nitro-phenyl)-amine

**[0537]** To a solution of 2-iodo-4-nitroaniline (2.0 g, 7.6 mmol) and 2-(tert-butyldimethylsilyloxy)-acetaldehyde (3.5 g, 75% purity, 15 mmol) in methanol (30 mL) was added TFA (1.5 mL) at 0° C. The reaction mixture was stirred at this temperature for 30 min before $NaCNBH_3$ (900 mg, 15 mmol) was added in portions. The mixture was stirred for 2 h and was then quenched with water. The resulting mixture was extracted with EtOAc (30 mL×3), the combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum, and the residue was purified by chromatography on silica gel (5% ethyl acetate/petroleum) to afford [2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-(2-iodo-4-nitro-phenyl)-amine (800 mg, 25%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J=2.7 Hz, 1H), 8.12 (dd, J=2.4, 9.0 Hz, 1H), 6.49 (d, J=9.3 Hz, 1H), 5.46 (br s, 1H), 3.89 (t, J=5.4 Hz, 2H), 3.35 (q, J=5.4 Hz, 2H), 0.93 (s, 9H), 0.10 (s, 6H).



5-{2-[2-(tert-Butyl-dimethyl-silanyloxy)-ethylamino]-5-nitro-phenyl}-3,3-dimethyl-pent-4-ynoic acid ethyl ester

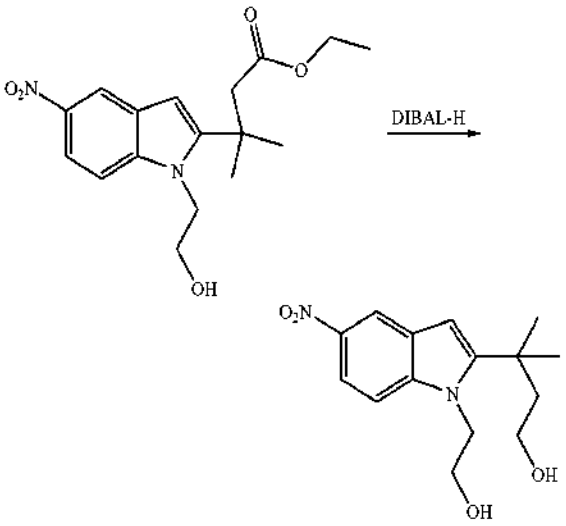
**[0538]** To a solution of [2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-(2-iodo-4-nitro-phenyl)-amine (800 mg, 1.9 mmol) in $Et_3N$ (20 mL) was added $Pd(PPh_3)_2Cl_2$ (300 mg, 0.040 mmol), CuI (76 mg, 0.040 mmol) and 3,3-dimethyl-but-1-yne (880 mg, 5.7 mmol) successively under $N_2$ protection. The reaction mixture was heated at 80° C. for 6 h and allowed to cool down to room temperature. The resulting mixture was extracted with EtOAc (30 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum to give 5-{2-[2-(tert-butyl-dimethyl-silanyloxy)-ethylamino]-5-nitro-phenyl}-3,3-dimethyl-pent-4-ynoic acid ethyl ester (700 mg, 82%), which was used in the next step without further purification. $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 8.00 (d, J=9.2 Hz, 1H), 6.54 (d, J=9.2 Hz, 1H), 6.45 (brs, 1H), 4.17-4.10 (m, 4H), 3.82 (t, J=5.6 Hz, 2H), 3.43 (q, J=5.6 Hz, 2H), 2.49 (s, 2H), 1.38 (s, 6H), 1.28 (t, J=7.2 Hz, 3H), 0.84 (s, 9H), 0.00 (s, 6H).



3-[1-(2-Hydroxy-ethyl)-5-nitro-1H-indol-2-yl]-3-methyl-butyric acid ethyl ester

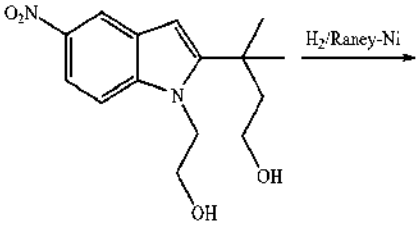
**[0539]** A solution of 5-{2-[2-(tert-butyl-dimethyl-silanyloxy)-ethylamino]-5-nitro-phenyl}-3,3-dimethyl-pent-4-

ynoic acid ethyl ester (600 mg, 1.34 mmol) and $PdCl_2$(650 mg) in $CH_3CN$ (30 mL) was heated at reflux overnight. The resulting mixture was extracted with EtOAc (30 mL×3). The combined organic extracts were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum. The residue was dissolved in THF (20 mL) and TBAF (780 mg, 3.0 mmol) was added. The mixture was stirred at room temperature for 1 h, the solvent was removed under vacuum, and the residue was purified by chromatography on silica gel (10% ethyl acetate/petroleum) to afford 3-[1-(2-hydroxy-ethyl)-5-nitro-1H-indol-2-yl]-3-methyl-butyric acid ethyl ester (270 mg, 60%). $^1$H NMR (300 MHz, $CDCl_3$) δ 8.45 (d, J=2.1 Hz, 1H), 8.05 (dd, J=2.1, 9.0 Hz, 1H), 6.36 (d, J=9.0 Hz, 1H), 6.48 (s, 1H), 4.46 (t, J=6.6 Hz, 2H), 4.00-3.91 (m, 4H), 2.76 (s, 2H), 1.61 (s, 6H), 0.99 (t, J=7.2 Hz, 1H), 0.85 (s, 9H), 0.03 (s, 6H).




3-[1-(2-Hydroxy-ethyl)-5-nitro-1H-indol-2-yl]-3-methyl-butan-1-ol

**[0540]** To a solution of 3-[1-(2-hydroxy-ethyl)-5-nitro-1H-indol-2-yl]-3-methyl-butyric acid ethyl ester (700 mg, 2.1 mmol) in THF (25 mL) was added DIBAL-H (1.0 M, 4.2 mL, 4.2 mmol) at −78° C. The mixture was stirred at room temperature for 1 h. Water (2 mL) was added and the resulting mixture was extracted with EtOAc (15 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$ and evaporated under vacuum. The residue was purified by chromatography on silica gel (15% ethyl acetate/petroleum) to afford 3-[1-(2-hydroxy-ethyl)-5-nitro-1H-indol-2-yl]-3-methyl-butan-1-ol (300 mg, 49%). $^1$H NMR (300 MHz, d-DMSO) δ 8.42 (d, J=1.5 Hz, 1H), 7.95 (dd, J=1.2, 8.7 Hz, 1H), 6.36 (d, J=9.3 Hz, 1H), 6.50 (s, 1H), 5.25 (br s, 1H), 4.46-4.42 (m, 4H), 3.69-3.66 (m, 2H), 3.24-3.21 (m, 2H), 1.42 (s, 6H).

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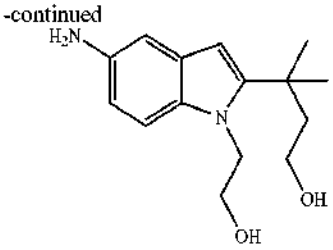


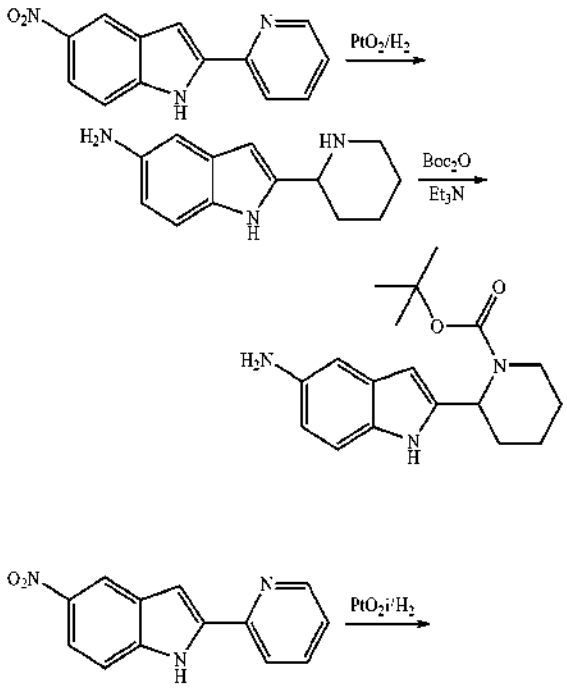

3-[5-Amino-1-(2-hydroxy-ethyl)-1H-indol-2-yl]-3-methyl-butan-1-ol

**[0541]** A solution of 3-[1-(2-hydroxy-ethyl)-5-nitro-1H-indol-2-yl]-3-methyl-butan-1-ol (300 mg, 1.03 mmol) and Raney Nickel (200 mg) in $CH_3OH$ (30 mL) was stirred for 5 h at room temperature under a $H_2$ atmosphere. The catalyst was filtered through a celite pad and the filtrate was evaporated under vacuum to give a residue, which was purified by preparative TLC to afford 3-[5-amino-1-(2-hydroxy-ethyl)-1H-indol-2-yl]-3-methyl-butan-1-ol (70 mg, 26%). $^1$H NMR (300 MHz, $CDCl_3$) δ 7.07 (d, J=8.7 Hz, 1H), 6.83 (d, J=2.1 Hz, 1H), 6.62 (dd, J=2.1, 8.4 Hz, 1H), 6.15 (s, 1H), 4.47 (t, J=5.4 Hz, 2H), 4.07 (t, J=5.4 Hz, 2H), 3.68 (t, J=5.7 Hz, 2H), 2.16 (t, J=5.7 Hz, 2H), 4.00-3.91 (m, 4H), 2.76 (s, 2H), 1.61 (s, 6H), 1.42 (s, 6H).

Example 48

tert-Butyl 2-(5-amino-1H-indol-2-yl)piperidine-1-carboxylate

**[0542]**



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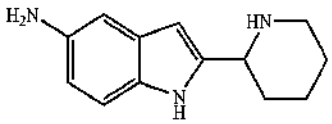
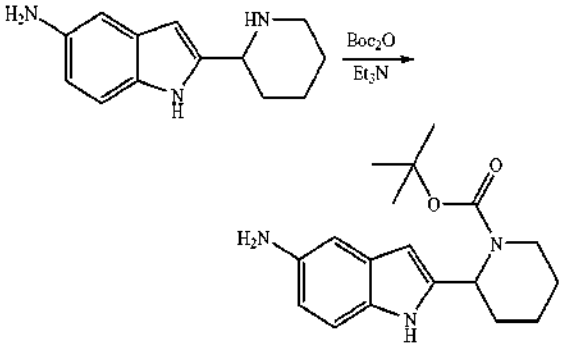


2-(Piperidin-2-yl)-1H-indol-5-amine

**[0543]** 5-Nitro-2-(pyridin-2-yl)-1H-indole (1.0 g, 4.2 mmol) was added to HCl/MeOH (2 M, 50 mL). The reaction mixture was stirred at room temperature for 1 h and the solvent was evaporated under vacuum. $PtO_2$ (200 mg) was added to a solution of the residue in MeOH (50 mL) and the reaction mixture was stirred under hydrogen atmosphere (1 atm) at room temperature for 2 h. The catalyst was filtered through a celite pad and the solvent was evaporated under vacuum to afford 2-(piperidin-2-yl)-1H-indol-5-amine (1.0 g), which was directly used in the next step.



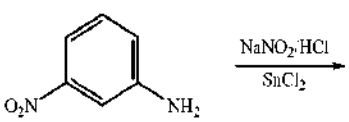
tert-Butyl 2-(5-amino-1H-indol-2-yl)piperidine-1-carboxylate

**[0544]** To a solution of 2-(piperidin-2-yl)-1H-indol-5-amine (1.0 g) in $Et_3N$ (25 mL) and THF (25 mL) was added $Boc_2O$ (640 mg, 2.9 mmol). The reaction mixture was stirred at room temperature overnight. The mixture was diluted with water and extracted with dichloromethane (3×25 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated in vacuum. The residue was purified by chromatography on silica gel (5-10% ethyl acetate/petroleum ether) followed by preparative HPLC to afford tert-butyl 2-(5-amino-1H-indol-2-yl)piperidine-1-carboxylate (15 mg, 1% over 2 steps). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.58 (s, 1H), 7.22 (d, J=8.8 Hz, 1H), 7.02 (d, J=1.6, 8.0 Hz, 1H), 6.42 (s, 1H), 6.25 (s, 1H), 3.91-3.88 (m, 1H), 3.12-3.10 (m, 1H), 2.81-2.76 (m, 1H), 2.06-1.97 (m, 4H), 1.70-1.58 (m, 2H), 1.53 (s, 9H).

Example 49

6-amino-1H-indole-2-carbonitrile

**[0545]**



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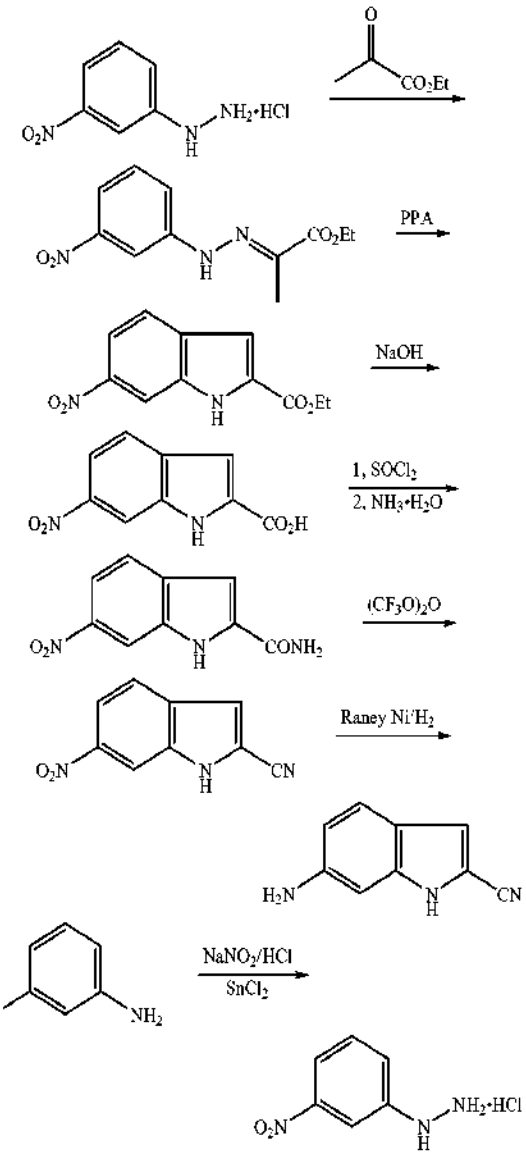
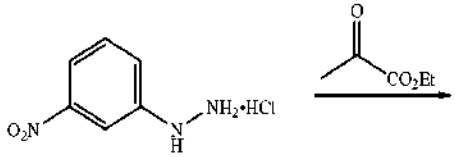


(3-Nitrophenyl)hydrazine hydrochloride

**[0546]** 3-Nitroaniline (28 g, 0.20 mol) was dissolved in a mixture of $H_2O$ (40 mL) and 37% HCl (40 mL). A solution of $NaNO_2$ (14 g, 0.20 mol) in $H_2O$ (60 mL) was added to the mixture at 0° C., and then a solution of $SnCl_2.H_2O$ (140 g, 0.60 mol) in 37% HCl (100 mL) was added. After stirring at 0° C. for 0.5 h, the insoluble material was isolated by filtration and was washed with water to give (3-nitrophenyl)hydrazine hydrochloride (28 g, 73%).



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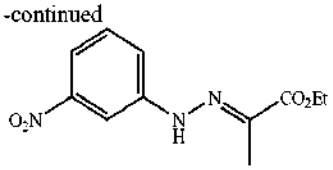
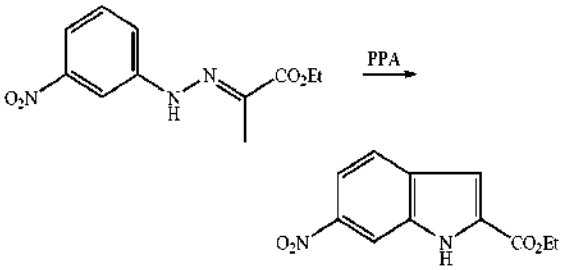


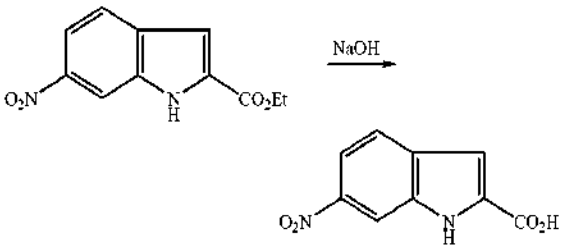
(E)-Ethyl 2-(2-(3-nitrophenyl)hydrazono)propanoate

**[0547]** (3-Nitrophenyl)hydrazine hydrochloride (30 g, 0.16 mol) and 2-oxo-propionic acid ethyl ester (22 g, 0.19 mol) were dissolved in ethanol (300 mL). The mixture was stirred at room temperature for 4 h before the solvent was evaporated under reduced pressure to give (E)-ethyl 2-(2-(3-nitrophenyl) hydrazono)propanoate, which was used directly in the next step.



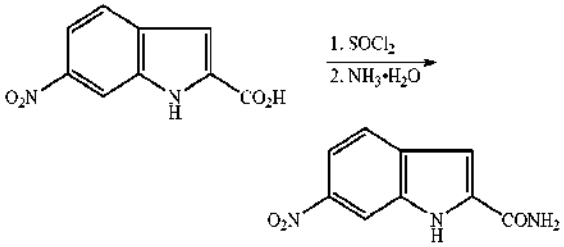
Ethyl 4-nitro-1H-indole-2-carboxylate and ethyl 6-nitro-1H-indole-2-carboxylate

**[0548]** (E)-Ethyl 2-(2-(3-nitrophenyl)hydrazono)propanoate was dissolved in toluene (300 mL) and PPA (30 g) was added. The mixture was heated at reflux overnight and then was cooled to room temperature. The solvent was decanted and evaporated to obtain a crude mixture that was taken on to the next step without purification (15 g, 40%).



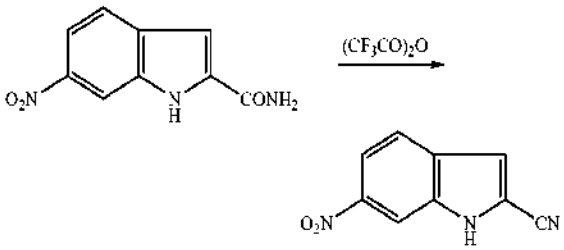
4-Nitro-1H-indole-2-carboxylic acid and 6-nitro-1H-indole-2-carboxylic acid

**[0549]** A mixture of ethyl 6-nitro-1H-indole-2-carboxylate (0.5 g) and 10% NaOH (20 mL) was heated at reflux overnight and then was cooled to room temperature. The mixture was extracted with ether and the aqueous phase was acidified with HCl to pH 1~2. The insoluble solid was isolated by filtration to give a crude mixture that was taken on to the next step without purification (0.3 g, 68%).



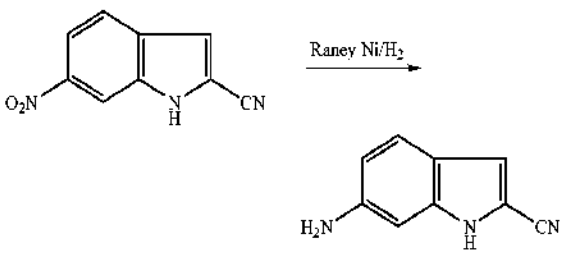
4-Nitro-1H-indole-2-carboxamide and 6-nitro-1H-indole-2-carboxamide

**[0550]** A mixture of 6-nitro-1H-indole-2-carboxylic acid (12 g, 58 mmol) and $SOCl_2$ (50 mL, 64 mmol) in benzene (150 mL) was heated at reflux for 2 h. The benzene and excess $SOCl_2$ was removed under reduced pressure. The residue was dissolved in anhydrous $CH_2Cl_2$ (250 mL) and $NH_3.H_2O$ (22 g, 0.32 mol) was added dropwise at 0° C. The mixture was stirred at room temperature for 1 h. The insoluble solid was isolated by filtration to obtain crude mixture (9.0 g, 68%), which was used directly in the next step.



4-Nitro-1H-indole-2-carbonitrile and 6-nitro-1H-indole-2-carbonitrile

**[0551]** 6-Nitro-1H-indole-2-carboxamide (5.0 g, 24 mmol) was dissolved in $CH_2Cl_2$ (200 mL). $Et_3N$ (24 g, 0.24 mol) and $(CF_3CO)_2O$ (51 g, 0.24 mol) were added dropwise to the mixture at room temperature. The mixture was continued to stir for 1 h and was then poured into water (100 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (100 mL×3). The combined organic layers were dried over $Na_2SO_4$, filtered and concentrated under reduced pressure to obtain crude product which was purified by column chromatography on silica gel to give a impure sample of 4-nitro-1H-indole-2-carbonitrile (2.5 g, 55%).



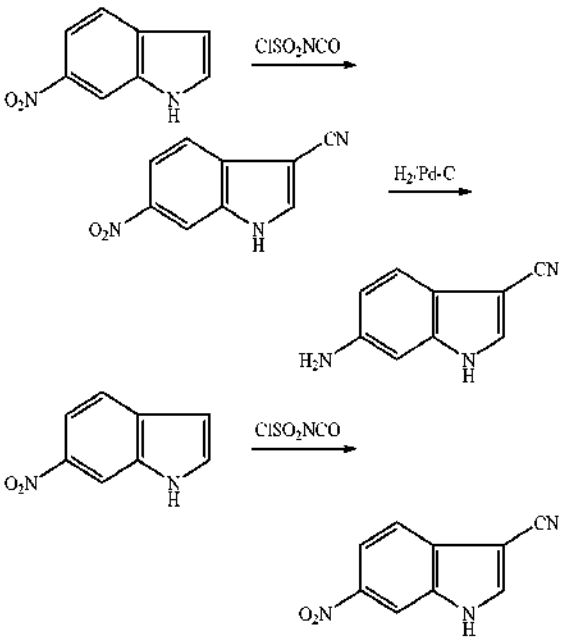
6-Amino-1H-indole-2-carbonitrile

**[0552]** A mixture of 6-nitro-1H-indole-2-carbonitrile (2.5 g, 13 mmol) and Raney Nickel (500 mg) in EtOH (50 mL) was stirred at room temperature under $H_2$ (1 atm) for 1 h. Raney Nickel was removed via filtration and the filtrate was evaporated under reduced pressure to give a residue, which was purified by column chromatography on silica get to give 6-amino-1H-indole-2-carbonitrile (1.0 g, 49%). $^1$H NMR (DMSO-$d_6$) δ 12.75 (br s, 1H), 7.82 (d, J=8 Hz, 1H), 7.57 (s, 1H), 7.42 (s, 1H), 7.15 (d, J=8 Hz, 1H); MS (ESI) m/e ($M+H^+$) 158.2.

Example 50

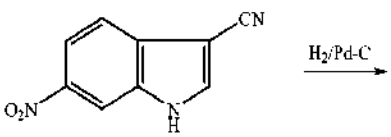
6-Amino-1H-indole-3-carbonitrile

**[0553]**




6-Nitro-1H-indole-3-carbonitrile

**[0554]** To a solution of 6-nitroindole (4.9 g 30 mmol) in DMF (24 mL) and $CH_3CN$ (240 mL) was added dropwise a solution of $ClSO_2NCO$ (5.0 mL) in $CH_3CN$ (39 mL) at 0° C. After addition, the reaction was allowed to warm to room temperature and was stirred for 2 h. The mixture was then poured into ice-water and basified with sat. $NaHCO_3$ solution to pH 7~8. The mixture was extracted with ethyl acetate. The organics were washed with brine, dried over $Na_2SO_4$ and concentrated to give 6-nitro-1H-indole-3-carbonitrile (4.6 g, 82%).

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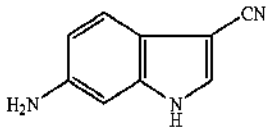


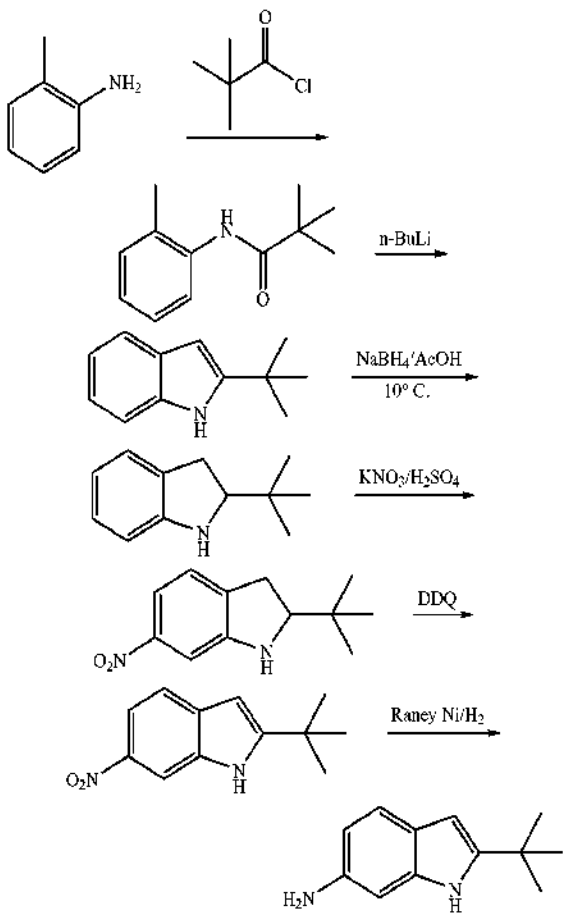
6-Amino-1H-indole-3-carbonitrile

**[0555]** A suspension of 6-nitro-1H-indole-3-carbonitrile (4.6 g, 25 mmol) and 10% Pd—C (0.46 g) in EtOH (50 mL) was stirred under $H_2$ (1 atm) at room temperature overnight. After filtration, the filtrate was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=3/1) to give 6-amino-1H-indole-3-carbonitrile (1.0 g, 98%) as a pink solid. $^1$H NMR (DMSO-$d_6$) δ 11.51 (s, 1H), 7.84 (d, J=2.4 Hz, 1H), 7.22 (d, J=8.4 Hz, 1H), 6.62 (s, 1H), 6.56 (d, J=8.4 Hz, 1H), 5.0 (s, 2H); MS (ESI) m/e ($M+H^+$) 157.1

Example 51

2-tert-Butyl-1H-indol-6-amine

**[0556]**



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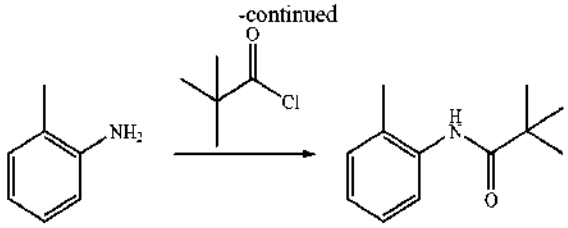
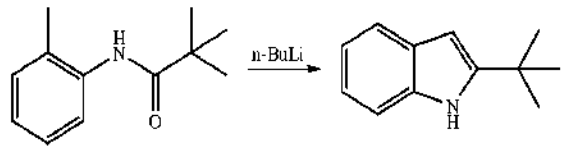


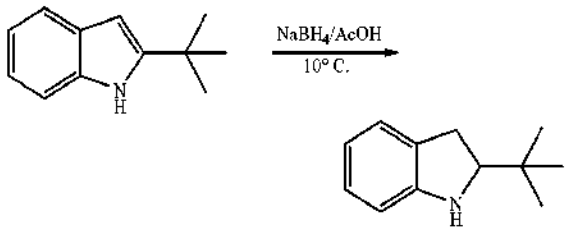
N-o-Tolylpivalamide

**[0557]** To a solution of o-tolylamine (21 g, 0.20 mol) and $Et_3N$ (22 g, 0.22 mol) in $CH_2Cl_2$ was added 2,2-dimethyl-propionyl chloride (25 g, 0.21 mol) at 10° C. After addition, the mixture was stirred overnight at room temperature. The mixture was washed with aq. HCl (5%, 80 mL), saturated aq. $NaHCO_3$ and brine. The organic layer was dried over $Na_2SO_4$ and concentrated under vacuum to give N-o-tolylpivalamide (35 g, 91%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.88 (d, J=7.2 Hz, 1H), 7.15-7.25 (m, 2H), 7.05 (t, J=7.2 Hz, 1H), 2.26 (s, 3H), 1.34 (s, 9H).



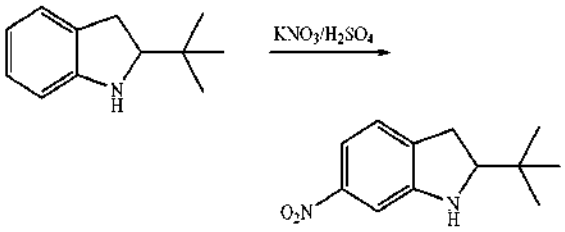
2-tert-Butyl-1H-indole

**[0558]** To a solution of N-o-tolylpivalamide (30.0 g, 159 mmol) in dry THF (100 mL) was added dropwise n-BuLi (2.5 M in hexane, 190 mL) at 15° C. After addition, the mixture was stirred overnight at 15° C. The mixture was cooled in an ice-water bath and treated with saturated $NH_4Cl$. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated in vacuum. The residue was purified by column chromatography on silica gel to give 2-tert-butyl-1H-indole (24 g, 88%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 7.99 (br. s, 1H), 7.54 (d, J=7.2 Hz, 1H), 7.05 (d, J=7.8 Hz, 1H), 7.06-7.13 (m, 2H), 6.26 (s, 1H), 1.39 (s, 9H).



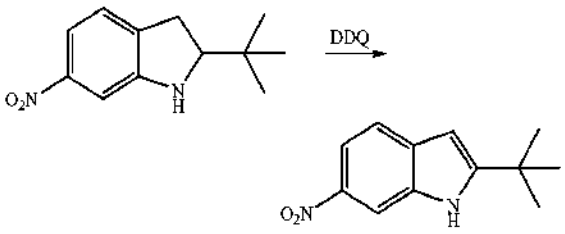
2-tert-Butylindoline

**[0559]** To a solution of 2-tert-butyl-1H-indole (10 g, 48 mmol) in AcOH (40 mL) was added $NaBH_4$ at 10° C. The mixture was stirred for 20 minutes at 10° C. before being treated dropwise with $H_2O$ under ice cooling. The mixture was extracted with ethyl acetate. The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, and concentrated under vacuum to give 2-tert-butylindoline (9.8 g), which was used directly in the next step.



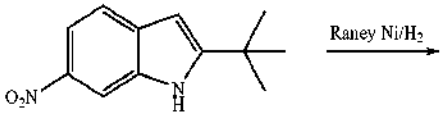
2-tert-butyl-6-nitroindoline and
2-tert-butyl-5-nitro-1H-indole

**[0560]** To a solution of 2-tert-butylindoline (9.7 g) in $H_2SO_4$ (98%, 80 mL) was slowly added $KNO_3$ (5.6 g, 56 mmol) at 0° C. After addition, the reaction mixture was stirred at room temperature for 1 h. The mixture was carefully poured into cracked ice, basified with $Na_2CO_3$ to pH 8 and extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under vacuum. The residue was purified by column chromatography to give 2-tert-butyl-6-nitroindoline (4.0 g, 31% over two steps). $^1H$ NMR (300 MHz, $CDCl_3$) δ7.52 (dd, J=1.8, 8.1 Hz, 1H), 7.30 (s, 1H), 7.08 (d, J=7.8 Hz, 1H), 3.76 (t, J=9.6 Hz, 1H), 2.98-3.07 (m, 1H), 2.82-2.91 (m, 1H), 0.91 (s, 9H).



2-tert-Butyl-6-nitro-1H-indole

**[0561]** To a solution of 2-tert-butyl-6-nitroindoline (2.0 g, 9.1 mmol) in 1,4-dioxane (20 mL) was added DDQ (6.9 g, 30 mmol) at room temperature. The mixture was heated at reflux for 2.5 h before being filtered and concentrated under vacuum. The residue was purified by column chromatography to give 2-tert-butyl-6-nitro-1H-indole (1.6 g, 80%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.30 (br. s, 1H), 8.29 (s, 1H), 8.00 (dd, J=2.1, 8.7 Hz, 1H), 7.53 (d, J=9.3 Hz, 1H), 6.38 (s, 1H), 1.43 (s, 9H).



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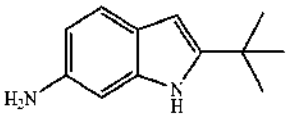


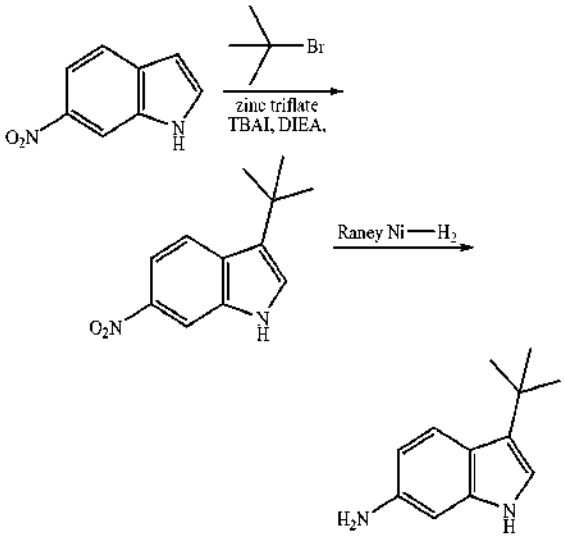
2-tert-Butyl-1H-indol-6-amine

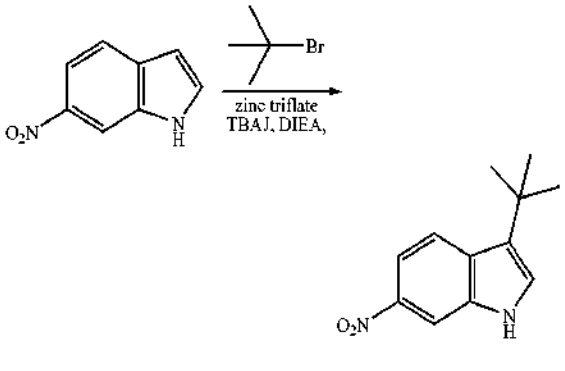
**[0562]** To a solution of 2-tert-butyl-6-nitro-1H-indole (1.3 g, 6.0 mmol) in MeOH (10 mL) was added Raney Nickel (0.2 g). The mixture was hydrogenated under 1 atm of hydrogen at room temperature for 3 h. The reaction mixture was filtered and the filtrate was concentrated. The residue was washed with petroleum ether to give 2-tert-butyl-1H-indol-6-amine (1.0 g, 89%). $^1$H NMR (300 MHz, DMSO-$d_6$) δ 10.19 (s, 1H), 6.99 (d, J=8.1 Hz, 1H), 6.46 (s, 1H), 6.25 (dd, J=1.8, 8.1 Hz, 1H), 5.79 (d, J=1.8 Hz, 1H), 4.52 (s, 2H), 1.24 (s, 9H); MS (ESI) m/e (M+H$^+$) 189.1.

Example 52

3-tert-Butyl-1H-indol-6-amine

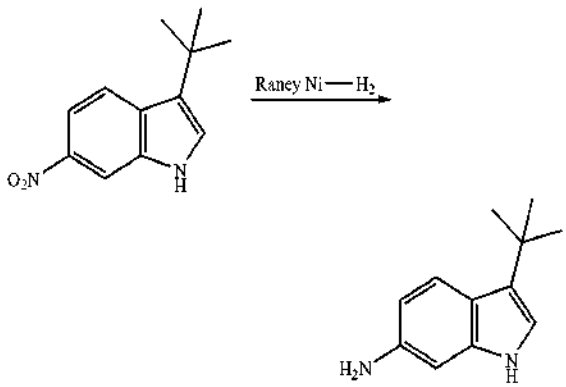
**[0563]**




3-tert-Butyl-6-nitro-1H-indole

**[0564]** To a mixture of 6-nitroindole (1.0 g, 6.2 mmol), zinc triflate (2.1 g, 5.7 mmol), and TBAI (1.7 g, 5.2 mmol) in anhydrous toluene (11 mL) was added DIEA (1.5 g, 11 mmol) at room temperature under nitrogen. The reaction mixture was stirred for 10 min at 120° C., followed by the addition of t-butyl bromide (0.71 g, 5.2 mmol). The resulting mixture was stirred for 45 min at 120° C. The solid was filtered off and the filtrate was concentrated to dryness. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate=20:1) to give 3-tert-butyl-6-nitro-1H-indole (0.25 g, 19%) as a yellow solid. $^1$H-NMR ($CDCl_3$) δ 8.32 (d, J=2.1 Hz, 1H), 8.00 (dd, J=2.1, 14.4 Hz, 1H), 7.85 (d, J=8.7 Hz, 1H), 7.25 (s, 1H), 1.46 (s, 9H).



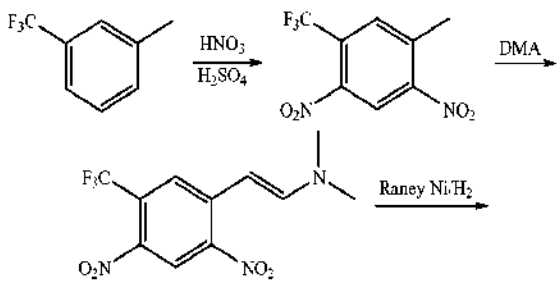

3-tert-Butyl-1H-indol-6-amine

**[0565]** A suspension of 3-tert-butyl-6-nitro-1H-indole (3.0 g, 14 mmol) and Raney Nickel (0.5 g) was hydrogenated under $H_2$ (1 atm) at room temperature for 3 h. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was purified by column on silica gel (petroleum ether/ethyl acetate=4:1) to give 3-tert-butyl-1H-indol-6-amine (2.0 g, 77%) as a gray solid. $^1$HNMR ($CDCl_3$) δ 7.58 (m, 2H), 6.73 (d, J=1.2 Hz, 1H), 6.66 (s, 1H), 6.57 (dd, J=0.8, 8.6 Hz, 1H), 3.60 (br, 2H), 1.42 (s, 9H).

Example 53

5-(Trifluoromethyl)-1H-indol-6-amine

**[0566]**



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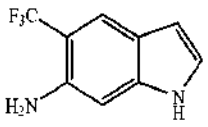
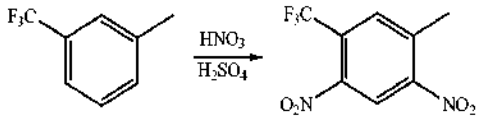
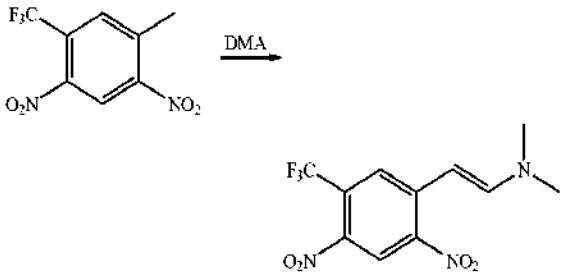




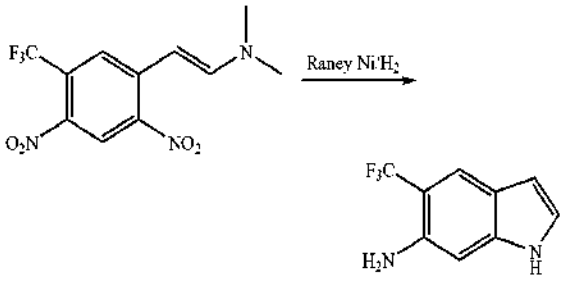
1-Methyl-2,4-dinitro-5-(trifluoromethyl)benzene

**[0567]** To a mixture of $HNO_3$ (98%, 30 mL) and $H_2SO_4$ (98%, 30 mL) was added dropwise 1-methyl-3-trifluoromethyl-benzene (10 g, 63 mmol) at 0° C. After addition, the mixture was stirred at rt for 30 min and was then poured into ice-water. The precipitate was filtered and washed with water to give 1-methyl-2,4-dinitro-5-trifluoromethyl-benzene (2.0 g, 13%).



(E)-2-(2,4-Dinitro-5-(trifluoromethyl)phenyl)-N,N-dimethylethenamine

**[0568]** A mixture of 1-methyl-2,4-dinitro-5-trifluoromethyl-benzene (2.0 g, 8.0 mmol) and DMA (1.0 g, 8.2 mmol) in DMF (20 mL) was stirred at 100° C. for 30 min. The mixture was poured into ice-water and stirred for 1 h. The precipitate was filtered and washed with water to give (E)-2-(2,4-dinitro-5-(trifluoromethyl)phenyl)-N,N-dimethylethenamine (2.1 g, 86%).



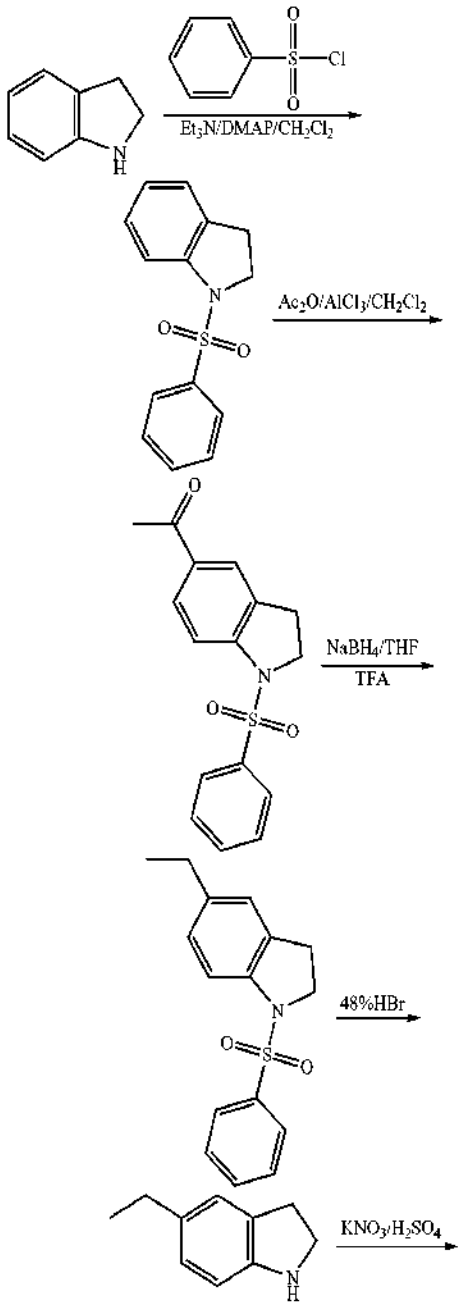
5-(Trifluoromethyl)-1H-indol-6-amine

**[0569]** A suspension of (E)-2-(2,4-dinitro-5-(trifluoromethyl)phenyl)-N,N-dimethylethenamine (2.1 g, 6.9 mmol) and Raney Nickel (1 g) in ethanol (80 mL) was stirred under $H_2$ (1 atm) at room temperature for 5 h. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was purified by column on silica gel to give 5-(trifluoromethyl)-1H-indol-6-amine (200 mg, 14%). $^1H$ NMR (DMSO-$d_6$) δ 10.79 (br s, 1H), 7.55 (s, 1H), 7.12 (s, 1H), 6.78 (s, 1H), 6.27 (s, 1H), 4.92 (s, 2H); MS (ESI) m/e ($M+H^+$): 200.8.

Example 54

5-Ethyl-1H-indol-6-amine

**[0570]**



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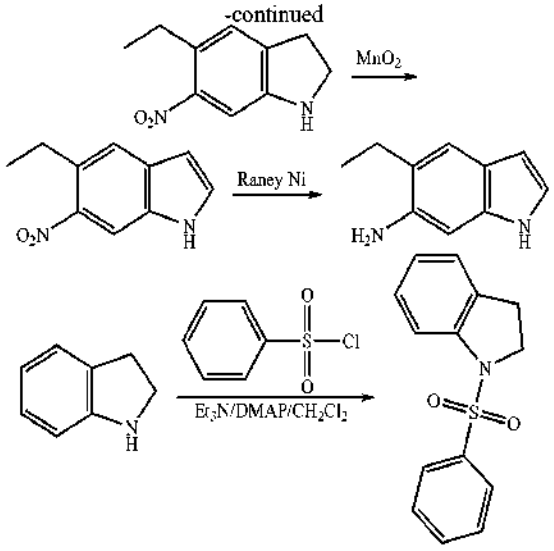
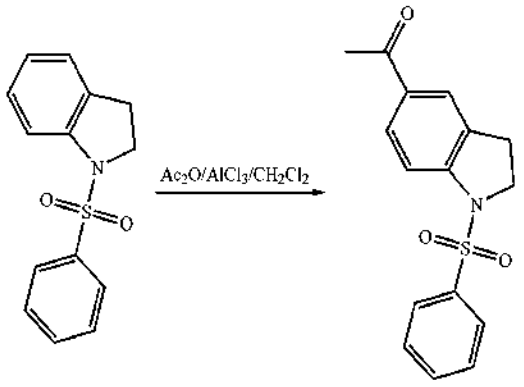


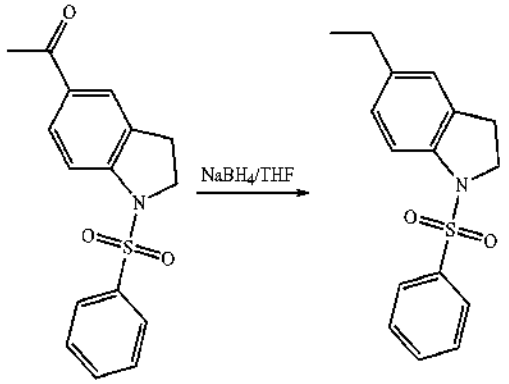

1-(Phenylsulfonyl)indoline

**[0571]** To a mixture of DMAP (1.5 g), benzenesulfonyl chloride (24.0 g, 136 mmol) and indoline (14.7 g, 124 mmol) in $CH_2Cl_2$ (200 mL) was added dropwise $Et_3N$ (19.0 g, 186 mmol) at 0° C. The mixture was stirred at room temperature overnight. The organic layer was washed with water (2×), dried over $Na_2SO_4$ and concentrated to dryness under reduced pressure to obtain 1-(phenylsulfonyl)indoline (30.9 g, 96%).



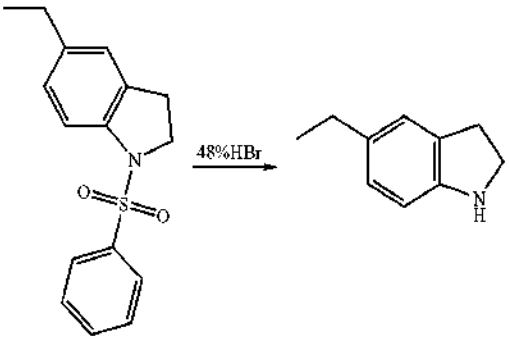

1-(1-(Phenylsulfonyl)indolin-5-yl)ethanone

**[0572]** To a suspension of $AlCl_3$ (144 g, 1.08 mol) in $CH_2Cl_2$ (1070 mL) was added acetic anhydride (54 mL). The mixture was stirred for 15 minutes before a solution of 1-(phenylsulfonyl)indoline (46.9 g, 0.180 mol) in $CH_2Cl_2$ (1070 mL) was added dropwise. The mixture was stirred for 5 h and was quenched by the slow addition of crushed ice. The organic layer was separated and the aqueous layer was extracted with $CH_2Cl_2$. The combined organics were washed with saturated aqueous $NaHCO_3$ and brine, dried over $Na_2SO_4$, and concentrated under vacuum to obtain 1-(1-(phenylsulfonyl)indolin-5-yl)ethanone (42.6 g).



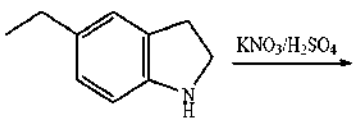

5-Ethyl-1-(phenylsulfonyl)indoline

**[0573]** To TFA (1600 mL) at 0° C. was added sodium borohydride (64.0 g, 1.69 mol) over 1 h. To this mixture was added dropwise a solution of 1-(1-(phenylsulfonyl)indolin-5-yl)ethanone (40.0 g, 0.133 mol) in TFA (700 mL) over 1 h. The mixture was then stirred overnight at 25° C. After dilution with $H_2O$ (1600 mL), the mixture was made basic by the addition of sodium hydroxide pellets at 0° C. The organic layer was separated and the aqueous layer was extracted with $CH_2Cl_2$. The combined organic layers were washed with brine, dried over $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by silica column to give 5-ethyl-1-(phenylsulfonyl)indoline (16.2 g, 47% over two steps).




5-Ethylindoline

**[0574]** A mixture of 5-ethyl-1-(phenylsulfonyl)indoline (15 g, 0.050 mol) in HBr (48%, 162 mL) was heated at reflux for 6 h. The mixture was basified with sat. NaOH to pH 9 and then it was extracted with ethyl acetate. The organic layer was washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified by silica column to give 5-ethylindoline (2.5 g, 32%).



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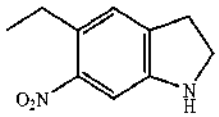
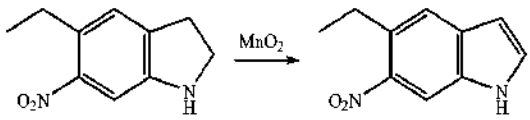


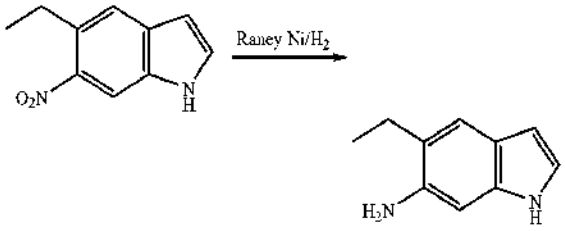
5-Ethyl-6-nitroindoline

**[0575]** To a solution of 5-ethylindoline (2.5 g, 17 mmol) in $H_2SO_4$ (98%, 20 mL) was slowly added $KNO_3$ (1.7 g, 17 mmol) at 0° C. The mixture was stirred at 0-10° C. for 10 minutes. The mixture was then carefully poured into ice, basified with NaOH solution to pH 9, and extracted with ethyl acetate. The combined extracts were washed with brine, dried over $Na_2SO_4$ and concentrated to dryness. The residue was purified by silica column to give 5-ethyl-6-nitroindoline (1.9 g, 58%).



5-Ethyl-6-nitro-1H-indole

**[0576]** To a solution of 5-ethyl-6-nitroindoline (1.9 g, 9.9 mmol) in $CH_2Cl_2$ (30 mL) was added $MnO_2$ (4.0 g, 46 mmol). The mixture was stirred at ambient temperature for 8 h. The solid was filtered off and the filtrate was concentrated to dryness to give 5-ethyl-6-nitro-1H-indole (1.9 g).



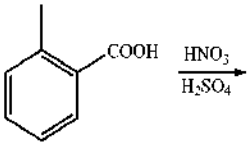
5-Ethyl-1H-indol-6-amine

**[0577]** A suspension of 5-ethyl-6-nitro-1H-indole (1.9 g, 10 mmol) and Raney Nickel (1 g) was hydrogenated under $H_2$ (1 atm) at room temperature for 2 h. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was purified by silica gel column to give 5-ethyl-1H-indol-6-amine (760 mg, 48% over two steps). $^1H$ NMR ($CDCl_3$) δ 7.90 (br s, 1H), 7.41 (s, 1H), 7.00 (s, 1H), 6.78 (s, 2H), 6.39 (s, 1H), 3.39 (br s, 2H), 2.63 (q, J=7.2 Hz, 2H), 1.29 (t, J=6.9 Hz, 3H); MS (ESI) m/e ($M+H^+$) 161.1.

Example 55

Ethyl 6-amino-1H-indole-4-carboxylate

**[0578]**



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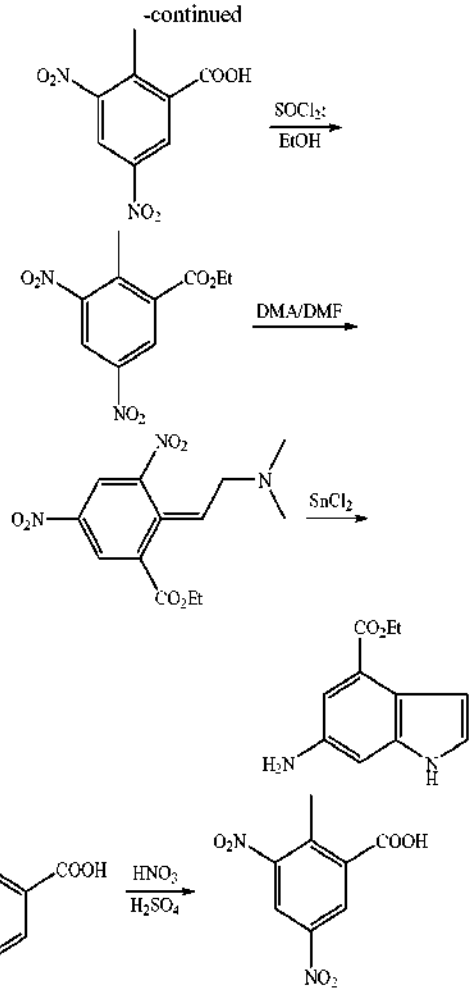
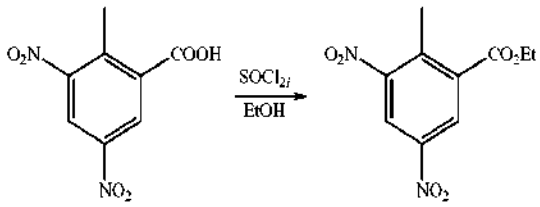


2-Methyl-3,5-dinitrobenzoic acid

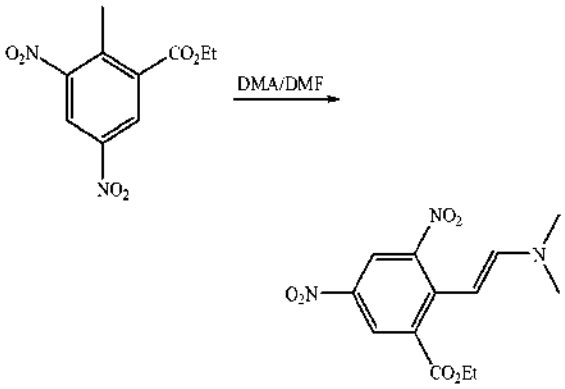
**[0579]** To a mixture of $HNO_3$ (95%, 80 mL) and $H_2SO_4$ (98%, 80 mL) was slowly added 2-methylbenzoic acid (50 g, 0.37 mol) at 0° C. After addition, the reaction mixture was stirred below 30° C. for 1.5 h. The mixture then was poured into ice-water and stirred for 15 min. The precipitate was filtered and washed with water to give 2-methyl-3,5-dinitrobenzoic acid (70 g, 84%).



Ethyl 2-methyl-3,5-dinitrobenzoate

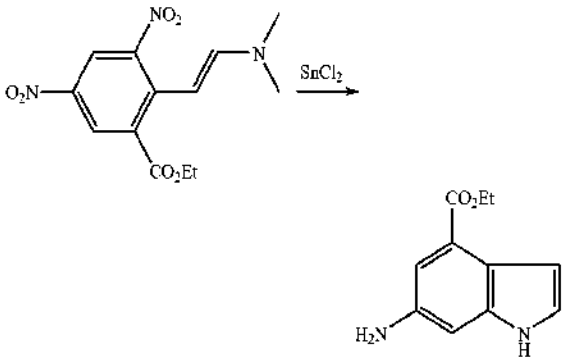
**[0580]** A mixture of 2-methyl-3,5-dinitrobenzoic acid (50 g, 0.22 mol) in $SOCl_2$ (80 mL) was heated at reflux for 4 h and then was concentrated to dryness. The residue was dissolved in $CH_2Cl_2$ (50 mL), to which EtOH (80 mL) was added and

the mixture was stirred at room temperature for 1 h. The mixture was poured into ice-water and extracted with EtOAc (3×100 mL). The combined extracts were washed sat. $Na_2CO_3$ (80 mL), water (2×100 mL) and brine (100 mL), dried over $Na_2SO_4$ and concentrated to dryness to give ethyl 2-methyl-3,5-dinitrobenzoate (50 g, 88%)




(E)-Ethyl 2-(2-(dimethylamino)vinyl)-3,5-dinitrobenzoate

**[0581]** A mixture of ethyl 2-methyl-3,5-dinitrobenzoate (35 g, 0.14 mol) and DMA (32 g, 0.27 mol) in DMF (200 mL) was heated at 100° C. for 5 h. The mixture was poured into ice-water and the precipitated solid was filtered and washed with water to give (E)-ethyl 2-(2-(dimethylamino)vinyl)-3,5-dinitrobenzoate (11 g, 48%)



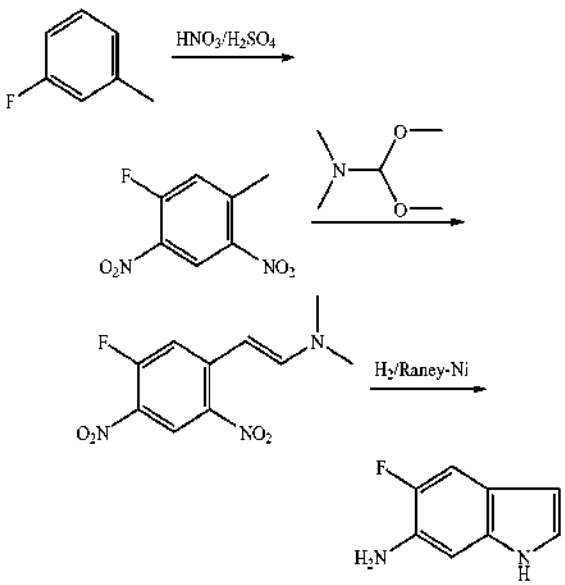

Ethyl 6-amino-1H-indole-4-carboxylate

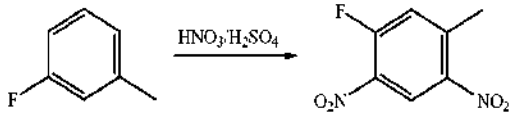
**[0582]** A mixture of (E)-ethyl 2-(2-(dimethylamino)vinyl)-3,5-dinitrobenzoate (11 g, 0.037 mol) and $SnCl_2$ (83 g, 0.37 mol) in ethanol was heated at reflux for 4 h. The mixture was concentrated to dryness and the residue was poured into water and basified using sat. aq. $Na_2CO_3$ to pH 8. The precipitated solid was filtered and the filtrate was extracted with ethyl acetate (3×100 mL). The combined extracts were washed with water (2×100 mL) and brine (150 mL), dried over $Na_2SO_4$, and concentrated to dryness. The residue was purified by column on silica gel to give ethyl 6-amino-1H-indole-4-carboxylate (3.0 g, 40%). $^1$HNMR (DMSO-$d_6$) δ 10.76 (br s, 1H), 7.11-7.14 (m, 2H), 6.81-6.82 (m, 1H), 6.67-6.68 (m, 1H), 4.94 (br s, 2H), 4.32-4.25 (q, J=7.2 Hz, 2H), 1.35-1.31 (t, J=7.2, 3H); MS (ESI) m/e ($M+H^+$) 205.0.

Example 56

5-Fluoro-1H-indol-6-amine

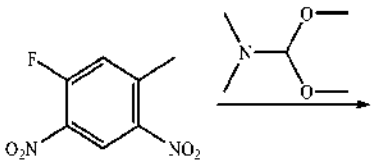
**[0583]**




1-Fluoro-5-methyl-2,4-dinitrobenzene

**[0584]** To a stirred solution of $HNO_3$ (60 mL) and $H_2SO_4$ (80 mL) was added dropwise 1-fluoro-3-methylbenzene (28 g, 25 mmol) under ice-cooling at such a rate that the temperature did not rise above 35° C. The mixture was allowed to stir for 30 min at rt and was then poured into ice water (500 mL). The resulting precipitate (a mixture of 1-fluoro-5-methyl-2,4-dinitrobenzene and 1-fluoro-3-methyl-2,4-dinitrobenzene, 32 g, ca. 7:3 ratio) was collected by filtration and purified by recrystallization from 50 mL isopropyl ether to give pure 1-fluoro-5-methyl-2,4-dinitro-benzene as a white solid (18 g, 36%).



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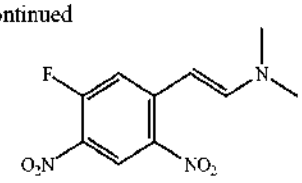
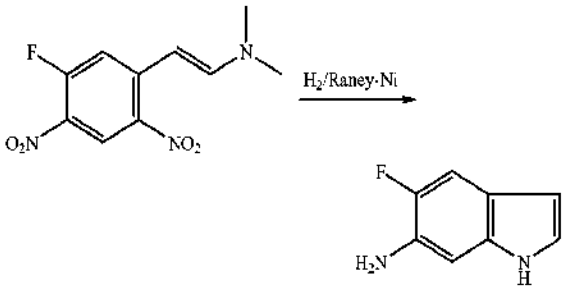


(E)-2-(5-Fluoro-2,4-dinitrophenyl)-N,N-dimethyl-ethenamine

**[0585]** A mixture of 1-fluoro-5-methyl-2,4-dinitro-benzene (10 g, 50 mmol), DMA (12 g, 100 mmol) and DMF (50 mL) was heated at 100° C. for 4 h. The solution was cooled and poured into water. The precipitated red solid was collected, washed with water, and dried to give (E)-2-(5-fluoro-2,4-dinitrophenyl)-N,N-dimethylethenamine (8.0 g, 63%).



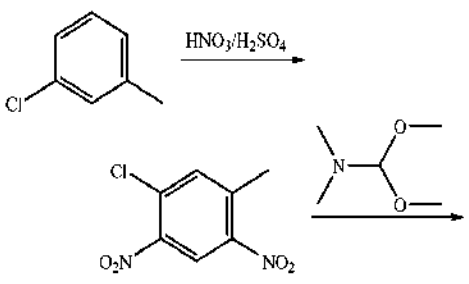
5-Fluoro-1H-indol-6-amine

**[0586]** A suspension of (E)-2-(5-fluoro-2,4-dinitrophenyl)-N,N-dimethylethenamine (8.0 g, 31 mmol) and Raney Nickel (8 g) in EtOH (80 mL) was stirred under $H_2$ (40 psi) at room temperature for 1 h. After filtration, the filtrate was concentrated and the residue was purified by column chromatography (petroleum ether/ethyl acetate=5/1) to give 5-fluoro-1H-indol-6-amine (1.0 g, 16%) as a brown solid. $^1$HNMR (DMSO-$d_6$) δ 10.56 (br s, 1H), 7.07 (d, J=12 Hz, 1H), 7.02 (m, 1H), 6.71 (d, J=8 Hz, 1H), 6.17 (s, 1H), 3.91 (br s, 2H); MS (ESI) m/e (M+H$^+$) 150.1.

Example 57

5-Chloro-1H-indol-6-amine

**[0587]**



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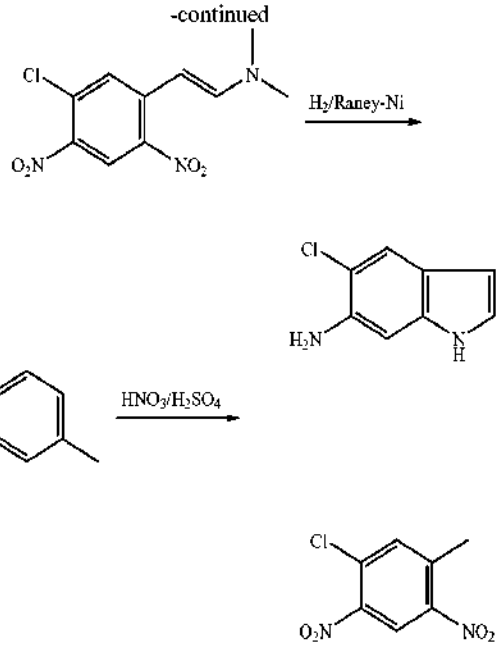
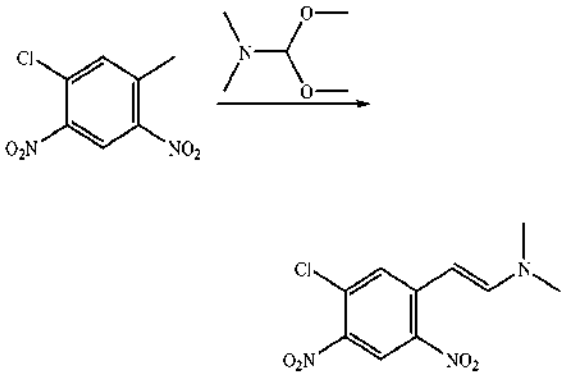


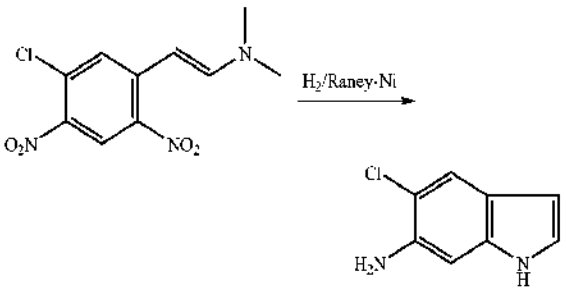
1-Chloro-5-methyl-2,4-dinitrobenzene

**[0588]** To a stirred solution of $HNO_3$ (55 mL) and $H_2SO_4$ (79 mL) was added dropwise 1-chloro-3-methylbenzene (25.3 g, 200 mmol) under ice-cooling at such a rate that the temperature did not rise above 35° C. The mixture was allowed to stir for 30 min at ambient temperature and was then poured into ice water (500 mL). The resulting precipitate was collected by filtration and purified by recrystallization to give 1-chloro-5-methyl-2,4-dinitrobenzene (26 g, 60%).



(E)-2-(5-Chloro-2,4-dinitrophenyl)-N,N-dimethyl-ethenamine

**[0589]** A mixture of 1-chloro-5-methyl-2,4-dinitro-benzene (11.6 g, 50.0 mmol), DMA (11.9 g, 100 mmol) in DMF (50 mL) was heated at 100° C. for 4 h. The solution was cooled and poured into water. The precipitated red solid was collected by filtration, washed with water, and dried to give (E)-2-(5-chloro-2,4-dinitrophenyl)-N,N-dimethyleth-enamine (9.84 g, 72%).



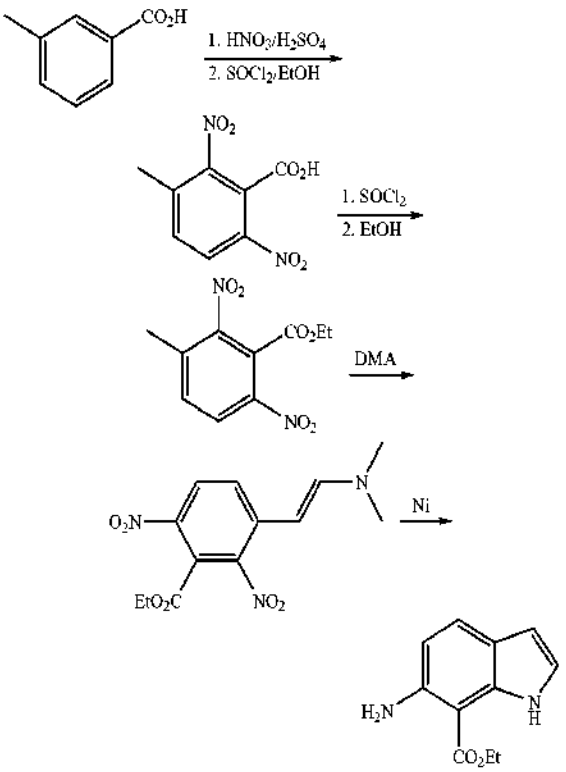
5-Chloro-1H-indol-6-amine

**[0590]** A suspension of (E)-2-(5-chloro-2,4-dinitrophenyl)-N,N-dimethylethenamine (9.8 g, 36 mmol) and Raney Nickel (9.8 g) in EtOH (140 mL) was stirred under $H_2$ (1 atm) at room temperature for 4 h. After filtration, the filtrate was concentrated and the residue was purified by column chromatograph (petroleum ether/ethyl acetate=10:1) to give 5-chloro-1H-indol-6-amine (0.97 g, 16%) as a gray powder. $^1$HNMR ($CDCl_3$) δ 7.85 (br s, 1H), 7.52 (s, 1H), 7.03 (s, 1H), 6.79 (s, 1H), 6.34 (s, 1H), 3.91 (br s, 1H); MS (ESI) m/e ($M+H^+$) 166.0.

Example 58

Ethyl 6-amino-1H-indole-7-carboxylate

**[0591]**



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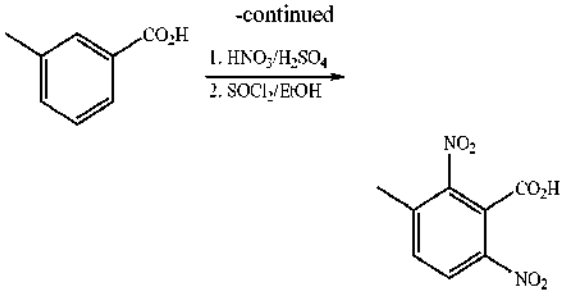
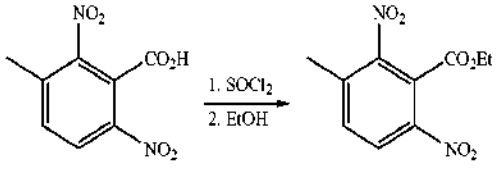


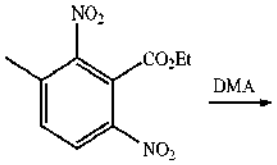
3-Methyl-2,6-dinitrobenzoic acid

**[0592]** To a mixture of $HNO_3$ (95%, 80 mL) and $H_2SO_4$ (98%, 80 mL) was slowly added 3-methylbenzoic acid (50 g, 0.37 mol) at 0° C. After addition, the mixture was stirred below 30° C. for 1.5 hours. The mixture was then poured into ice-water and stirred for 15 min. The precipitate solid was filtered and washed with water to give a mixture of 3-methyl-2,6-dinitro-benzoic acid and 5-methyl-2,4-dinitrobenzoic acid (70 g, 84%). To a solution of this mixture (70 g, 0.31 mol) in EtOH (150 mL) was added dropwise $SOCl_2$ (54 g, 0.45 mol). The mixture was heated at reflux for 2 h before being concentrated to dryness under reduced pressure. The residue was partitioned between EtOAc (100 mL) and aq. $Na_2CO_3$ (10%, 120 mL). The organic layer was washed with brine (50 mL), dried over $Na_2SO_4$, and concentrated to dryness to obtain ethyl 5-methyl-2,4-dinitrobenzoate (20 g), which was placed aside. The aqueous layer was acidified by HCl to pH 2~3 and the precipitated solid was filtered, washed with water, and dried in air to give 3-methyl-2,6-dinitrobenzoic acid (39 g, 47%).



Ethyl 3-methyl-2,6-dinitrobenzoate

**[0593]** A mixture of 3-methyl-2,6-dinitrobenzoic acid (39 g, 0.15 mol) and $SOCl_2$ (80 mL) was heated at reflux 4 h. The excess $SOCl_2$ was evaporated off under reduced pressure and the residue was added dropwise to a solution of EtOH (100 mL) and $Et_3N$ (50 mL). The mixture was stirred at 20° C. for 1 h and then concentrated to dryness. The residue was dissolved in EtOAc (100 mL), washed with $Na_2CO_3$ (10%, 40 mL×2), water (50 mL×2) and brine (50 mL), dried over $Na_2SO_4$ and concentrated to give ethyl 3-methyl-2,6-dinitrobenzoate (20 g, 53%).



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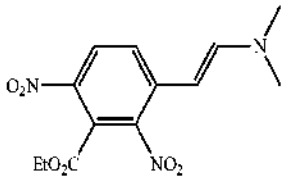
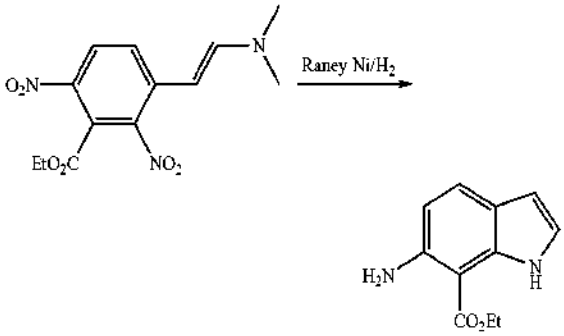


(E)-Ethyl 3-(2-(dimethylamino)vinyl)-2,6-dinitrobenzoate

**[0594]** A mixture of ethyl 3-methyl-2,6-dinitrobenzoate (35 g, 0.14 mol) and DMA (32 g, 0.27 mol) in DMF (200 mL) was heated at 100° C. for 5 h. The mixture was poured into ice water. The precipitated solid was filtered and washed with water to give (E)-ethyl 3-(2-(dimethylamino)vinyl)-2,6-dinitrobenzoate (25 g, 58%).



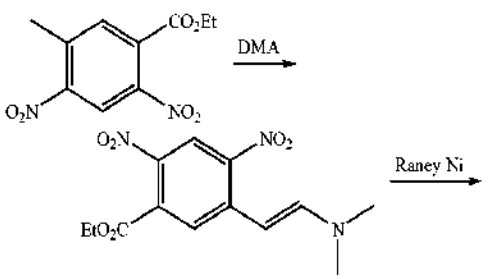
Ethyl 6-amino-1H-indole-7-carboxylate

**[0595]** A mixture of (E)-ethyl 3-(2-(dimethylamino)vinyl)-2,6-dinitrobenzoate (30 g, 0.097 mol) and Raney Nickel (10 g) in EtOH (1000 mL) was hydrogenated at room temperature under 50 psi for 2 h. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was purified by column on silica gel to give ethyl 6-amino-1H-indole-7-carboxylate as an off-white solid (3.2 g, 16%). $^1$H NMR (DMSO-$d_6$) δ 10.38 (s, 1H), 7.42 (d, J=8.7 Hz, 1H), 6.98 (t, J=3.0 Hz, 1H), 6.65 (s, 2H), 6.48 (d, J=8.7 Hz, 1H), 6.27-6.26 (m, 1H), 4.38 (q, J=7.2 Hz, 2H), 1.35 (t, J=7.2 Hz, 3H).

Example 59

Ethyl 6-amino-1H-indole-5-carboxylate

**[0596]**



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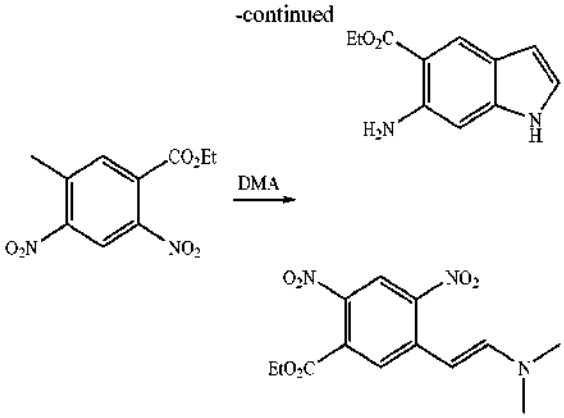
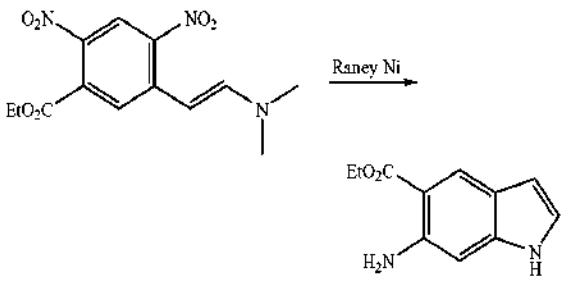


(E)-Ethyl 5-(2-(dimethylamino)vinyl)-2,4-dinitrobenzoate

**[0597]** A mixture of ethyl 5-methyl-2,4-dinitrobenzoate (39 g, 0.15 mol) and DMA (32 g, 0.27 mol) in DMF (200 mL) was heated at 100° C. for 5 h. The mixture was poured into ice water and the precipitated solid was filtered and washed with water to afford (E)-ethyl 5-(2-(dimethylamino)vinyl)-2,4-dinitrobenzoate (15 g, 28%).



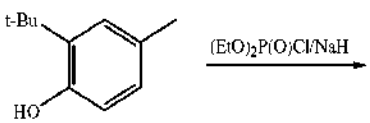
Ethyl 6-amino-1H-indole-5-carboxylate

**[0598]** A mixture of (E)-ethyl 5-(2-(dimethylamino)vinyl)-2,4-dinitrobenzoate (15 g, 0.050 mol) and Raney Nickel (5 g) in EtOH (500 mL) was hydrogenated at room temperature under 50 psi of hydrogen for 2 h. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was purified by column on silica gel to give ethyl 6-amino-1H-indole-5-carboxylate (3.0 g, 30%). $^1$H NMR (DMSO-$d_6$) δ 10.68 (s, 1H), 7.99 (s, 1H), 7.01-7.06 (m, 1H), 6.62 (s, 1H), 6.27-6.28 (m, 1H), 6.16 (s, 2H), 4.22 (q, J=7.2 Hz, 2H), 1.32-1.27 (t, J=7.2 Hz, 3H).

Example 60

5-tert-Butyl-1H-indol-6-amine

**[0599]**



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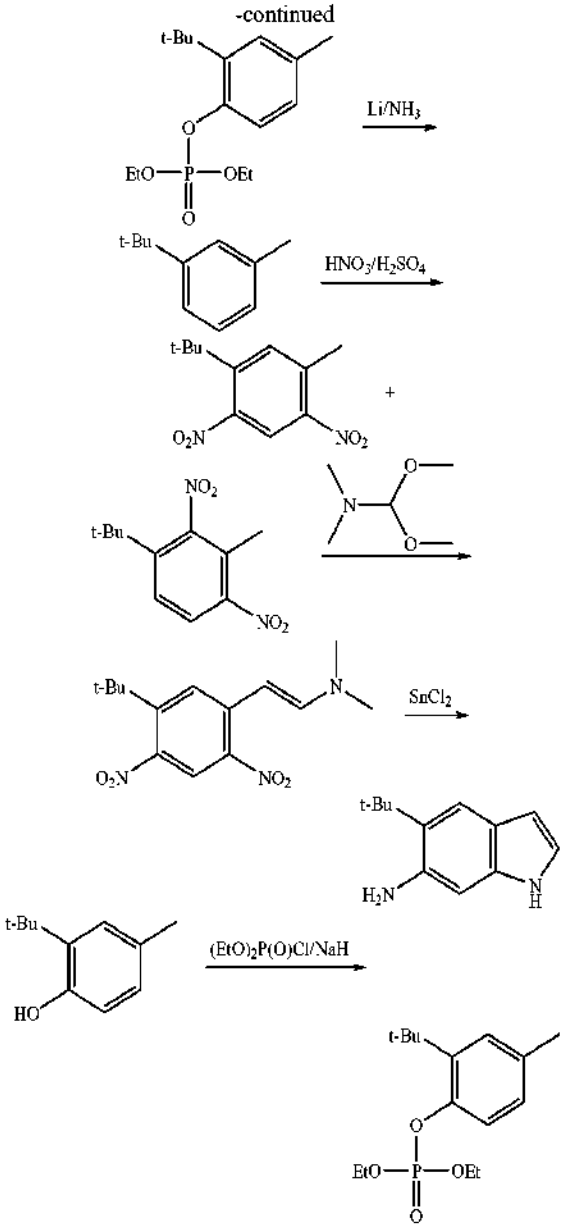
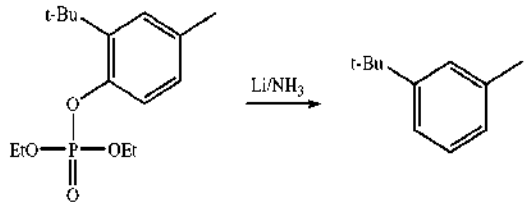


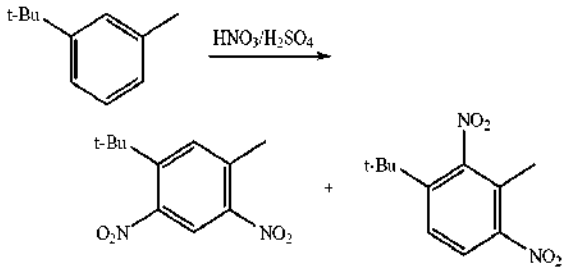
2-tert-Butyl-4-methylphenyl diethyl phosphate

**[0600]** To a suspension of NaH (60% in mineral oil, 8.4 g, 0.21 mol) in THF (200 mL) was added dropwise a solution of 2-tert-butyl-4-methylphenol (33 g, 0.20 mol) in THF (100 mL) at 0° C. The mixture was stirred at 0° C. for 15 min and then phosphorochloridic acid diethyl ester (37 g, 0.21 mol) was added dropwise at 0° C. After addition, the mixture was stirred at ambient temperature for 30 min. The reaction was quenched with sat. $NH_4Cl$ (300 mL) and then extracted with $Et_2O$ (350 mL×2). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, and then evaporated under vacuum to give 2-tert-butyl-4-methylphenyl diethyl phosphate (contaminated with mineral oil) as a colorless oil (60 g, 100%), which was used directly in the next step.



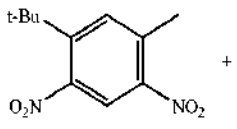
1-tert-Butyl-3-methylbenzene

**[0601]** To $NH_3$ (liquid, 1000 mL) was added a solution of 2-tert-butyl-4-methylphenyl diethyl phosphate (60 g, crude from last step, about 0.2 mol) in $Et_2O$ (anhydrous, 500 mL) at −78° C. under $N_2$ atmosphere. Lithium metal was added to the solution in small pieces until the blue color persisted. The reaction mixture was stirred at −78° C. for 15 min and then was quenched with sat. $NH_4Cl$ until the mixture turned colorless. Liquid $NH_3$ was evaporated and the residue was dissolved in water. The mixture was extracted with $Et_2O$ (400 mL×2). The combined organics were dried over $Na_2SO_4$ and evaporated to give 1-tert-butyl-3-methylbenzene (contaminated with mineral oil) as a colorless oil (27 g, 91%), which was used directly in next step.



1-tert-Butyl-5-methyl-2,4-dinitrobenzene and 1-tert-butyl-3-methyl-2,4-dinitro-benzene

**[0602]** To $HNO_3$ (95%, 14 mL) was added $H_2SO_4$ (98%, 20 mL) at 0° C. and then 1-tert-butyl-3-methylbenzene (7.4 g, ~50 mmol, crude from last step) dropwise to the with the temperature being kept below 30° C. The mixture was stirred at ambient temperature for 30 min, poured onto crushed ice (100 g), and extracted with EtOAc (50 mL×3). The combined organic layers were washed with water and brine, before being evaporated to give a brown oil, which was purified by column chromatography to give a mixture of 1-tert-butyl-5-methyl-2,4-dinitrobenzene and 1-tert-butyl-3-methyl-2,4-dinitrobenzene (2:1 by NMR) as a yellow oil (9.0 g, 61%).



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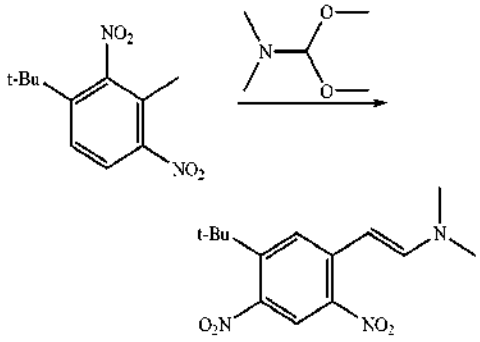
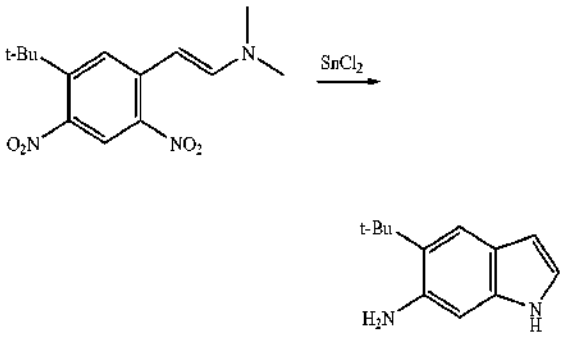


(E)-2-(5-tert-Butyl-2,4-dinitrophenyl)-N,N-dimethylethenamine

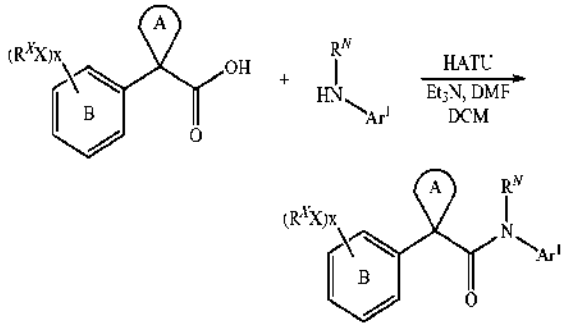
**[0603]** A mixture of 1-tert-butyl-5-methyl-2,4-dinitrobenzene and 1-tert-butyl-3-methyl-2,4-dinitrobenzene (9.0 g, 38 mmol, 2:1 by NMR) and DMA (5.4 g, 45 mmol) in DMF (50 mL) was heated at reflux for 2 h before being cooled to room temperature. The reaction mixture was poured into water-ice and extracted with EtOAc (50 mL×3). The combined organic layers were washed with water and brine, before being evaporated to give a brown oil, which was purified by column to give (E)-2-(5-tert-butyl-2,4-dinitrophenyl)-N,N-dimethylethen-amine (5.0 g, 68%).



5-tert-Butyl-1H-indol-6-amine

**[0604]** A solution of (E)-2-(5-tert-butyl-2,4-dinitrophenyl)-N,N-dimethylethen-amine (5.3 g, 18 mmol) and tin (II) chloride dihydrate (37 g, 0.18 mol) in ethanol (200 mL) was heated at reflux overnight. The mixture was cooled to room temperature and the solvent was removed under vacuum. The residual slurry was diluted with water (500 mL) and was basifed with 10% aq. $Na_2CO_3$ to pH 8. The resulting suspension was extracted with ethyl acetate (3×100 mL). The ethyl acetate extract was washed with water and brine, dried over $Na_2SO_4$, and concentrated. The residual solid was washed with $CH_2Cl_2$ to afford a yellow powder, which was purified by column chromatography to give 5-tert-butyl-1H-indol-6-amine (0.40 g, 12%). $^1H$ NMR (DMSO. $d_6$) δ 10.34 (br s, 1H), 7.23 (s, 1H), 6.92 (s, 1H), 6.65 (s, 1H), 6.14 (s, 1H), 4.43 (br s, 2H), 2.48 (s, 9H); MS (ESI) m/e ($M+H^+$) 189.1.

**[0605]** General Procedure IV: Synthesis of Acylaminoindoles

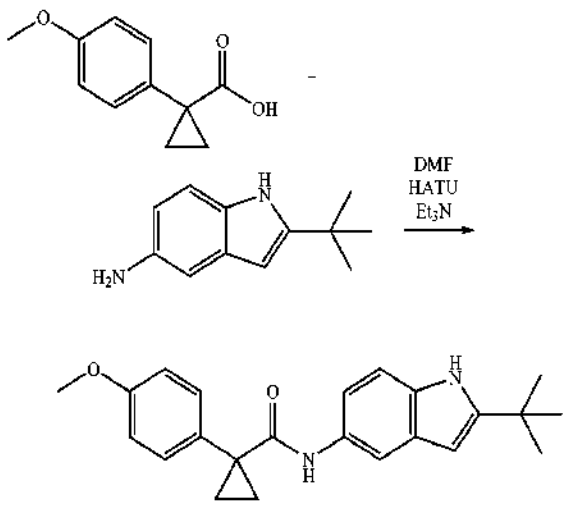


**[0606]** One equivalent of the appropriate carboxylic acid and one equivalent of the appropriate amine were dissolved in N,N-dimethylformamide (DMF) containing triethylamine (3 equivalents). O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) was added and the solution was allowed to stir. The crude product was purified by reverse-phase preparative liquid chromatography to yield the pure product.

Example 61

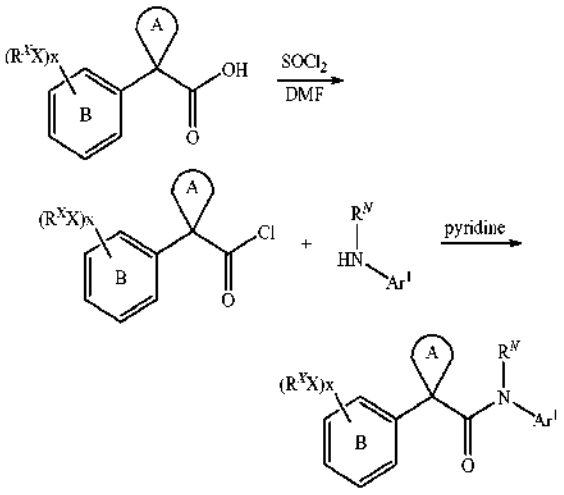
N-(2-tert-Butyl-1H-indol-5-yl)-1-(4-methoxyphenyl)-cyclopropanecarboxamide

**[0607]**



**[0608]** 2-tert-Butyl-1H-indol-5-amine (19 mg, 0.10 mmol) and 1-(4-methoxyphenyl)-cyclopropanecarboxylic acid (19 mg, 0.10 mmol) were dissolved in N,N-dimethylformamide (1.00 mL) containing triethylamine (28 μL, 0.20 mmol). O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (42 mg, 0.11 mmol) was added to the mixture and the resulting solution was allowed to stir for 3 hours. The crude reaction mixture was filtered and purified by reverse phase HPLC. ESI-MS m/z calc. 362.2, found 363.3 $(M+1)^+$; Retention time 3.48 minutes.

**[0609]** General Procedure V: Synthesis of Acylaminoindoles

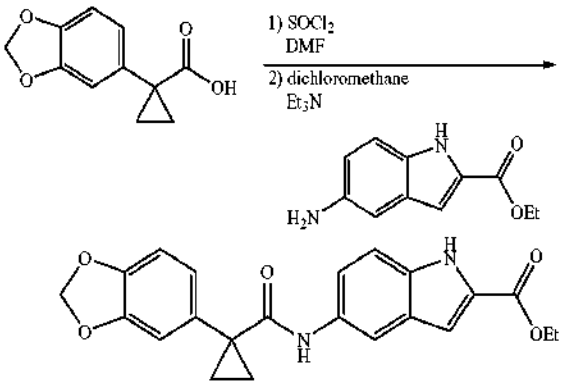


**[0610]** One equivalent of the appropriate carboxylic acid was placed in an oven-dried flask under nitrogen. A minimum (3 equivalents) of thionyl chloride and a catalytic amount of and N,N-dimethylformamide were added and the solution was allowed to stir for 20 minutes at 60° C. The excess thionyl chloride was removed under vacuum and the resulting solid was suspended in a minimum of anhydrous pyridine. This solution was slowly added to a stirred solution of one equivalent the appropriate amine dissolved in a minimum of anhydrous pyridine. The resulting mixture was allowed to stir for 15 hours at 110° C. The mixture was evaporated to dryness, suspended in dichloromethane, and then extracted three times with 1N HCl. The organic layer was then dried over sodium sulfate, evaporated to dryness, and then purified by column chromatography.

Example 62

Ethyl 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indole-2-carboxylate (Compd. 28)

**[0611]**

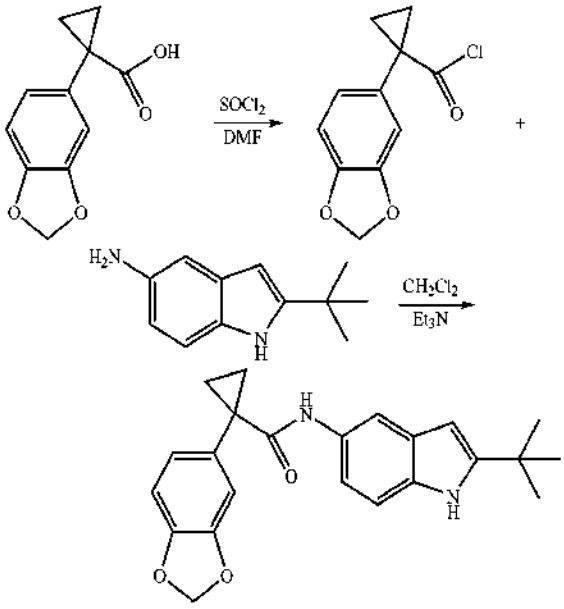


**[0612]** 1-Benzo[1,3]dioxol-5-yl-cyclopropanecarboxylic acid (2.07 g, 10.0 mmol) was dissolved in thionyl chloride (2.2 mL) under $N_2$. N,N-dimethylformamide (0.3 mL) was added and the solution was allowed to stir for 30 minutes. The excess thionyl chloride was removed under vacuum and the resulting solid was dissolved in anhydrous dichloromethane (15 mL) containing triethylamine (2.8 mL, 20.0 mmol). Ethyl 5-amino-1H-indole-2-carboxylate (2.04 g, 10.0 mmol) in 15 mL of anhydrous dichloromethane was slowly added to the reaction. The resulting solution was allowed to stir for 1 hour. The reaction mixture was diluted to 50 mL with dichloromethane and washed three times with 50 mL of 1N HCl, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The organic layer was dried over sodium sulfate and evaporated to dryness to yield ethyl 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indole-2-carboxylate as a gray solid (3.44 g, 88%). ESI-MS m/z calc. 392.4; found 393.1 $(M+1)^+$ Retention time 3.17 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 11.80 (s, 1H), 8.64 (s, 1H), 7.83 (m, 1H), 7.33-7.26 (m, 2H), 7.07 (m, 1H), 7.02 (m, 1H), 6.96-6.89 (m, 2H), 6.02 (s, 2H), 4.33 (q, J=7.1 Hz, 2H), 1.42-1.39 (m, 2H), 1.33 (t, J=7.1 Hz, 3H), 1.06-1.03 (m, 2H).

Example 63

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclopropanecarboxamide

**[0613]**



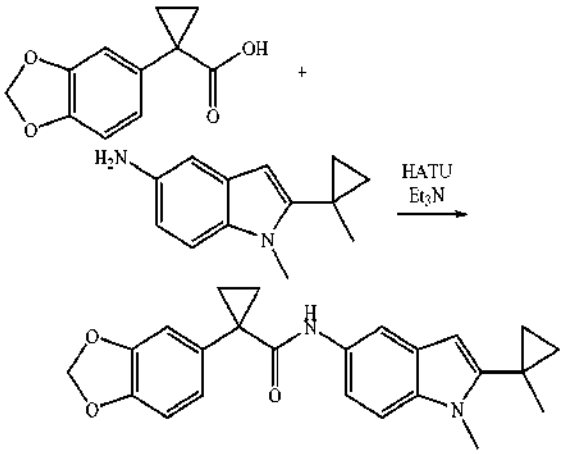
**[0614]** 1-Benzo[1,3]dioxol-5-yl-cyclopropanecarboxylic acid (1.09 g, 5.30 mmol) was dissolved in 2 mL of thionyl chloride under nitrogen. A catalytic amount (0.3 mL) of N,N-dimethylformamide (DMF) was added and the reaction mixture was stirred for 30 minutes. The excess thionyl chloride was evaporated and the resulting residue was dissolved in 15 mL of dichloromethane. This solution was slowly added to a solution of 2-tert-butyl-1H-indol-5-amine (1.0 g, 5.3 mmol) in 10 mL of dichloromethane containing triethylamine (1.69 mL, 12.1 mmol). The resulting solution was allowed to stir for 10 minutes. The solvent was evaporated to dryness and the crude reaction mixture was purified by silica gel column chromatography using a gradient of 5-50% ethyl acetate in hexanes. The pure fractions were combined and evaporated to

dryness to yield a pale pink powder (1.24 g 62%). ESI-MS m/z calc. 376.18. found 377.3 $(M+1)^+$. Retention time of 3.47 minutes. $^1$H NMR (400 MHz, DMSO) δ 10.77 (s, 1H), 8.39 (s, 1H), 7.56 (d, J=1.4 Hz, 1H), 7.15 (d, J=8.6 Hz, 1H), 7.05-6.87 (m, 4H), 6.03 (s, 3H), 1.44-1.37 (m, 2H), 1.33 (s, 9H), 1.05-1.00 (m, 2H).

Example 64

1-(Benzo[d][1,3]dioxol-5-yl)-N-(1-methyl-2-(1-methylcyclopropyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0615]**

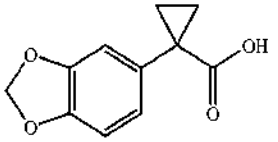



**[0616]** 1-Methyl-2-(1-methylcyclopropyl)-1H-indol-5-amine (20.0 mg, 0.100 mmol) and 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (20.6 mg, 0.100 mmol) were dissolved in N,N-dimethylformamide (1 mL) containing triethylamine (42.1 μL, 0.300 mmol) and a magnetic stir bar. O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (42 mg, 0.11 mmol) was added to the mixture and the resulting solution was allowed to stir for 6 h at 80° C. The crude product was then purified by preparative HPLC utilizing a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(1-methyl-2-(1-methylcyclopropyl)-1H-indol-5-yl)cyclopropanecarboxamide. ESI-MS m/z calc. 388.2, found 389.2 $(M+1)^+$. Retention time of 3.05 minutes.

Example 65

1-(Benzo[d][1,3]dioxol-5-yl)-N-(1,1-dimethyl-2,3-dihydro-1H-pyrrolo[1,2-a]indol-7-yl)cyclopropanecarboxamide

**[0617]**

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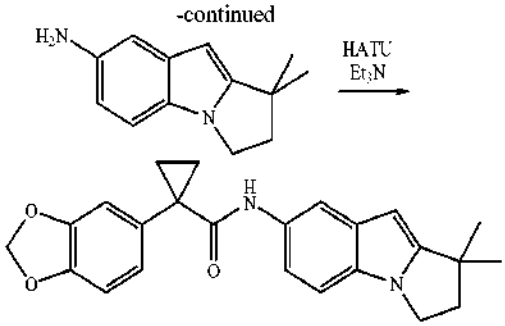
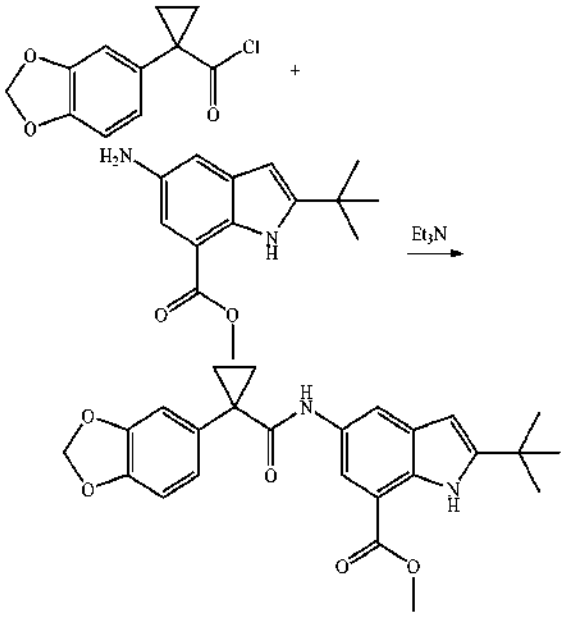



**[0618]** 1,1-Dimethyl-2,3-dihydro-1H-pyrrolo[1,2-a]indol-7-amine (40.0 mg, 0.200 mmol) and 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (41.2 mg, 0.200 mmol) were dissolved in N,N-dimethylformamide (1 mL) containing triethylamine (84.2 μL, 0.600 mmol) and a magnetic stir bar. O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (84 mg, 0.22 mmol) was added to the mixture and the resulting solution was allowed to stir for 5 minutes at room temperature. The crude product was then purified by preparative HPLC utilizing a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(1,1-dimethyl-2,3-dihydro-1H-pyrrolo[1,2-a]-indol-7-yl)cyclopropanecarboxamide. ESI-MS nm/z calc. 388.2, found 389.2 $(M+1)^+$. Retention time of 2.02 minutes. $^1$H NMR (400 MHz, DMSO-d6) δ 8.41 (s, 1H), 7.59 (d, J=1.8 Hz, 1H), 7.15 (d, J=8.6 Hz, 1H), 7.06-7.02 (m, 2H), 6.96-6.90 (m, 2H), 6.03 (s, 2H), 5.98 (d, J=0.7 Hz, 1H), 4.06 (t, J=6.8 Hz, 2H), 2.35 (t, J=6.8 Hz, 2H), 1.42-1.38 (m, 2H), 1.34 (s, 6H), 1.05-1.01 (m, 2H).

Example 66

Methyl 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butyl-1H-indole-7-carboxylate

**[0619]**

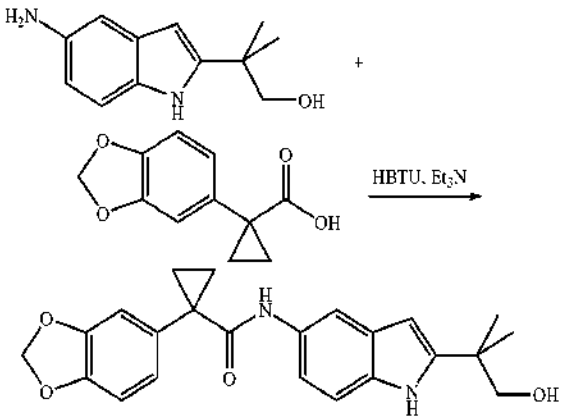


**[0620]** 1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarbonyl chloride (45 mg, 0.20 mmol) and methyl 15-amino-2-tert-butyl-1H-indole-7-carboxylate (49.3 mg, 0.200 mmol) were dissolved in N,N-dimethylformamide (2 mL) containing a magnetic stir bar and triethylamine (0.084 mL, 0.60 mmol). The resulting solution was allowed to stir for 10 minutes at room temperature. The crude product was then purified by preparative HPLC using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield methyl 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butyl-1H-indole-7-carboxylate. ESI-MS m/z calc. 434.2, found 435.5. $(M+1)^+$. Retention time of 2.12 minutes.

Example 67

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0621]**

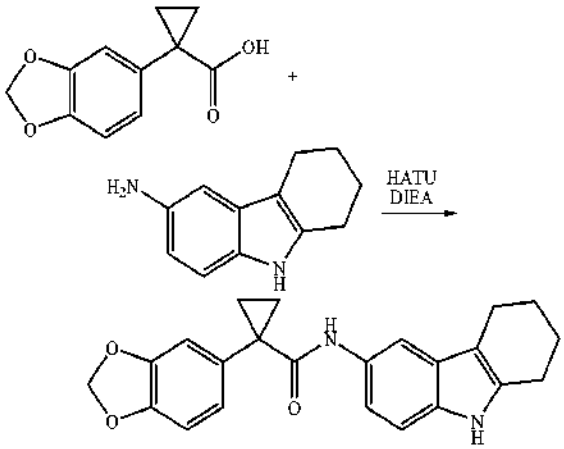



**[0622]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (0.075 g, 0.36 mmol) in acetonitrile (1.5 mL) were added HBTU (0.138 g, 0.36 mmol) and $Et_3N$ (152 μL, 1.09 mmol) at room temperature. The mixture was stirred at room temperature for 10 minutes before a solution of 2-(5-amino-1H-indol-2-yl)-2-methylpropan-1-ol (0.074 g, 0.36 mmol) in acetonitrile (1.94 mL) was added. After addition, the reaction mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure and the residue was dissolved in dichloromethane. The organic layer was washed with 1 N HCl (1×3 mL) and saturated aqueous $NaHCO_3$ (1×3 mL). The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The crude material was purified by column chromatography on silica gel (ethyl acetate/hexane=1/1) to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (0.11 g, 75%). $^1H$ NMR (400 MHz, DMSO-d6) δ 10.64 (s, 1H), 8.38 (s, 1H), 7.55 (s, 1H), 7.15 (d, J=8.6 Hz, 1H), 7.04-6.90 (m, 4H), 6.06 (s, 1H), 6.03 (s, 2H), 4.79 (t, J=2.7 Hz. 1H), 3.46 (d, J=0.0 Hz, 2H), 1.41-1.39 (m, 2H), 1.26 (s, 6H), 1.05-1.02 (m, 2H).

Example 67

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2,3,4,9-tetrahydro-1H-carbazol-6-yl)cyclopropanecarboxamide

**[0623]**

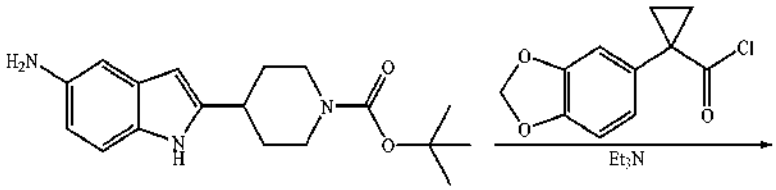



**[0624]** 2,3,4,9-Tetrahydro-1H-carbazol-6-amine (81.8 mg, 0.439 mmol) and 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (90.4 mg, 0.439 mmol) were dissolved in acetonitrile (3 mL) containing diisopropylethylamine (0.230 mL, 1.32 mmol) and a magnetic stir bar. O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (183 mg, 0.482 mmol) was added to the mixture and the resulting solution was allowed to stir for 16 h at 70° C. The solvent was evaporated and the crude product was then purified on 40 g of silica gel utilizing a gradient of 5-50% ethyl acetate in hexanes to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2,3,4,9-tetrahydro-1H-carbazol-6-yl)cyclopropanecarboxamide as a beige powder (0.115 g, 70%) after drying. ESI-MS m/z calc. 374.2. found 375.3 $(M+1)^+$. Retention time of 3.43 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 10.52 (s, 1H), 8.39 (s. 1H), 7.46 (d, J=1.8 Hz. 1H), 7.10-6.89 (m. 5H), 6.03 (s, 2H), 2.68-2.65 (m, 2H), 2.56-2.54 (m, 2H), 1.82-1.77 (m, 4H), 1.41-1.34 (m, 2H), 1.04-0.97 (m, 2H).

Example 69

tert-Butyl-4-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)piperidine-1-carboxylate

**[0625]**



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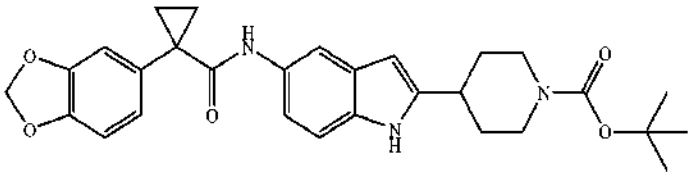
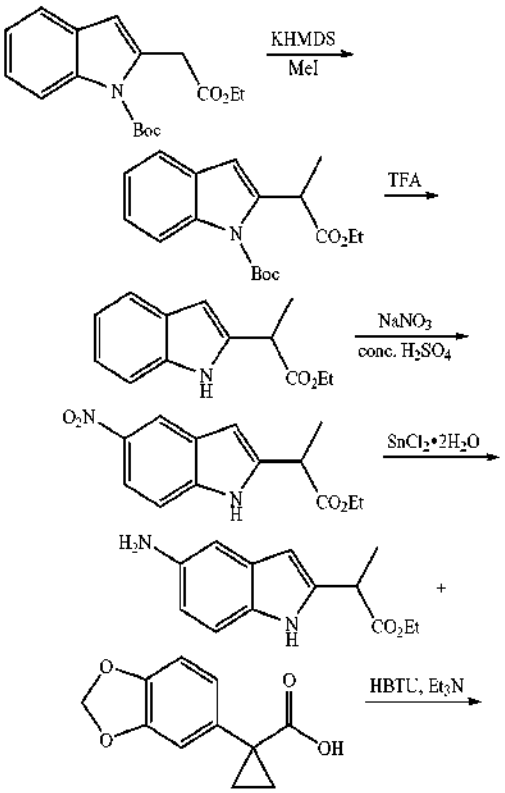


**[0626]** 1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarbonyl chloride (43 mg, 0.19 mmol) and tert-butyl 4-(5-amino-1H-indol-2-yl)piperidine-1-carboxylate (60 mg, 0.19 mmol) were dissolved in dichloromethane (1 mL) containing a magnetic stir bar and triethylamine (0.056 mL, 0.40 mmol). The resulting solution was allowed to stir for two days at room temperature. The crude product was then evaporated to dryness, dissolved in a minimum of N,N-dimethylformamide, and then purified by preparative HPLC using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield tert-butyl 4-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)piperidine-1-carboxylate. ESI-MS m/z calc. 503.2, found 504.5. $(M+1)^+$. Retention time of 1.99 minutes.

Example 70

Ethyl 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)propanoate

**[0627]**

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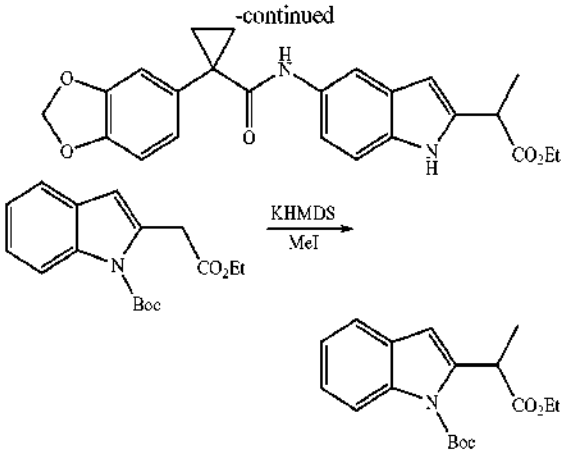
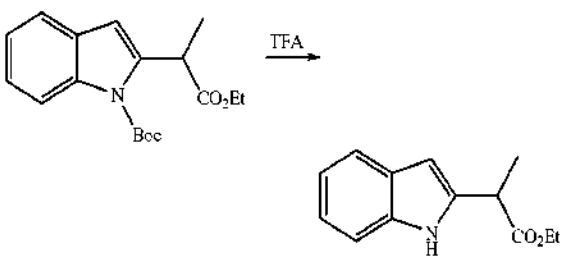



tert-Butyl 2-(1-ethoxy-1-oxopropan-2-yl)-1H-indole-1-carboxylate

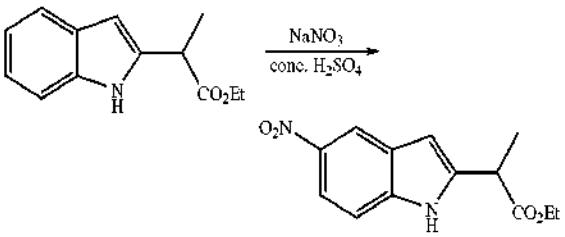
**[0628]** tert-Butyl 2-(2-ethoxy-2-oxoethyl)-1H-indole-1-carboxylate (3.0 g, 9.9 mmol) was added to anhydrous THF (29 mL) and cooled to −78° C. A 0.5M solution of potassium hexamethyldisilazane (20 mL, 9.9 mmol) was added slowly such that the internal temperature stayed below −60° C. Stirring was continued for 1 h at −78° C. Methyl iodide (727 μL, 11.7 mmol) was added to the mixture. The mixture was stirred for 30 minutes at room temperature. The mixture was quenched with sat. aq. ammonium chloride and partitioned between water and dichloromethane. The aqueous phase was extracted with dichloromethane and the combined organic phases were dried over $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (ethylacetate/hexane=1/9) to give tert-butyl 2-(1-ethoxy-1-oxopropan-2-yl)-1H-indole-1-carboxylate (2.8 g, 88%).




Ethyl 2-(1H-indol-2-yl)propanoate

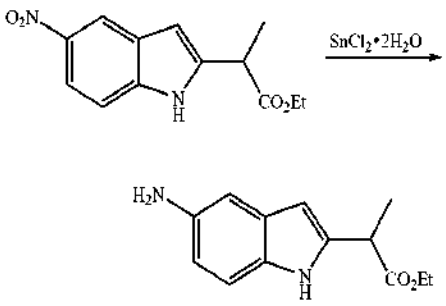
**[0629]** tert-Butyl 2-(1-ethoxy-1-oxopropan-2-yl)-1H-indole-1-carboxylate (2.77 g, 8.74 mmol) was dissolved in

dichloromethane (25 mL) before TFA (9.8 mL) was added. The mixture was stirred for 1.5 h at room temperature. The mixture was evaporated to dryness, taken up in dichloromethane and washed with sat. aq. sodium bicarbonate, water, and brine. The product was purified by column chromatography on silica gel (0-20% EtOAc in hexane) to give ethyl 2-(1H-indol-2-yl)propanoate (0.92 g, 50%).



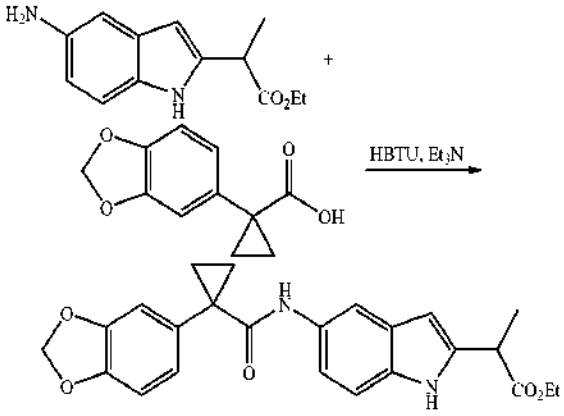

Ethyl 2-(5-nitro-1H-indol-2-yl)propanoate

**[0630]** Ethyl 2-(1H-indol-2-yl)propanoate (0.91 g, 4.2 mmol) was dissolved in concentrated sulfuric acid (3.9 mL) and cooled to −10° C. (salt/ice-mixture). A solution of sodium nitrate (0.36 g, 4.2 mmol) in concentrated sulfuric acid (7.8 mL) was added dropwise over 35 min. Stirring was continued for another 30 min at −10° C. The mixture was poured into ice and the product was extracted with ethyl acetate. The combined organic phases were washed with a small amount of sat. aq. sodium bicarbonate. The product was purified by column chromatography on silica gel (5-30% EtOAc in hexane) to give ethyl 2-(5-nitro-1H-indol-2-yl)propanoate (0.34 g, 31%).




Ethyl 2-(5-amino-1H-indol-2-yl)propanoate

**[0631]** To a solution of ethyl 2-(5-nitro-1H-indol-2-yl)propanoate (0.10 g, 0.38 mmol) in ethanol (4 mL) was added tin chloride dihydrate (0.431 g, 1.91 mmol). The mixture was heated in the microwave at 120° C. for 1 h. The mixture was diluted with ethyl acetate before water and saturated aqueous $NaHCO_3$ were added. The reaction mixture was filtered through a plug of celite using ethyl acetate. The organic layer was separated from the aqueous layer. The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure to give ethyl 2-(5-amino-1H-indol-2-yl)propanoate (0.088 g, 99%).



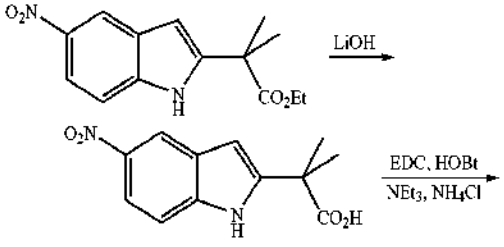

Ethyl 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)propanoate

**[0632]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (0.079 g, 0.384 mmol) in acetonitrile (1.5 mL) were added HBTU (0.146 g, 0.384 mmol) and $Et_3N$ (160 μL, 1.15 mmol) at room temperature. The mixture was allowed to stir at room temperature for 10 min before a solution of ethyl 2-(5-amino-1H-indol-2-yl)propanoate (0.089 g, 0.384 mmol) in acetonitrile (2.16 mL) was added. After addition, the reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated under reduced pressure and the residue was dissolved in dichloromethane. The organic layer was washed with 1 N HCl (1×3 mL) and then saturated aqueous $NaHCO_3$ (1×3 mL). The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The crude material was purified by column chromatography on silica gel (ethyl acetate/hexane=1/1) to give ethyl 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)propanoate (0.081 g, 50%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.67 (s, 1H), 7.23-7.19 (m, 2H), 7.04-7.01 (m, 3H), 6.89 (d, J=0.0 Hz, 1H), 6.28 (s, 1H), 6.06 (s, 2H), 4.25-4.17 (m, 2H), 3.91 (q, J=7.2 Hz, 1H), 1.72-1.70 (m, 2H), 1.61 (s, 2H), 1.29 (t, J=7.1 Hz, 4H), 1.13-1.11 (m, 2H).

Example 71

tert-Butyl 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)-2-methylpropylcarbamate

**[0633]**



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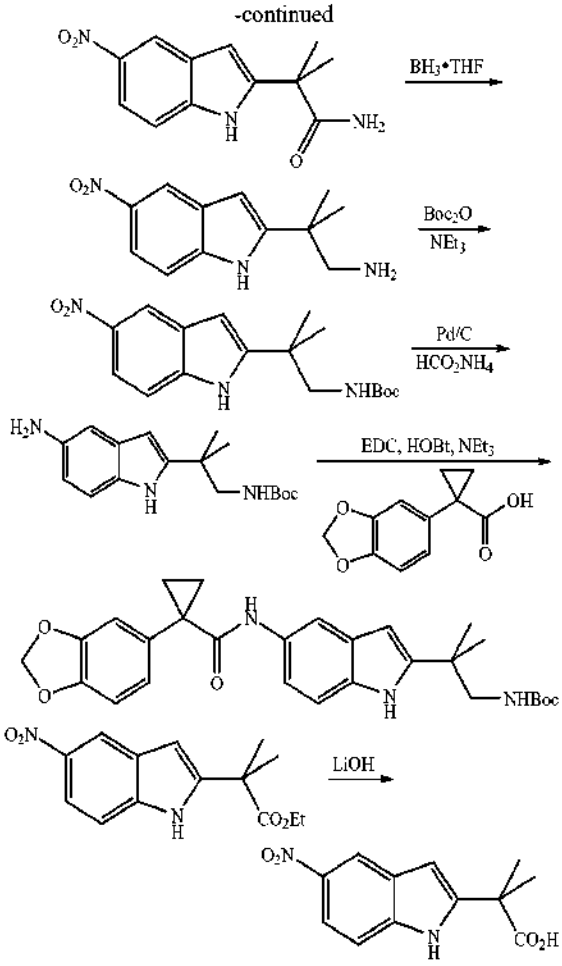
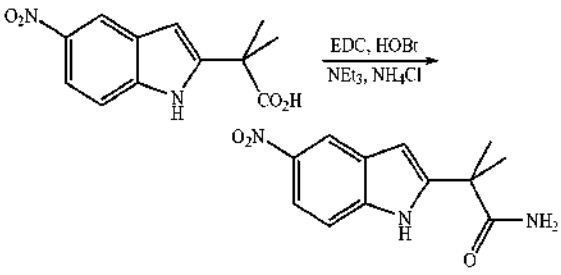


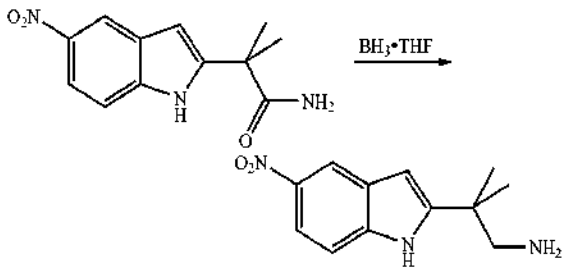
2-Methyl-2-(5-nitro-1H-indol-2-yl)propanoic acid

**[0634]** Ethyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propanoate (4.60 g, 16.7 mmol) was dissolved in THF/water (2:1, 30 mL). $LiOH.H_2O$ (1.40 g, 33.3 mmol) was added and the mixture was stirred at 50° C. for 3 h. The mixture was made acidic by the careful addition of 3N HCl. The product was extracted with ethylacetate and the combined organic phases were washed with brine and dried over magnesium sulfate to give 2-methyl-2-(5-nitro-1H-indol-2-yl)propanoic acid (4.15 g, 99%).



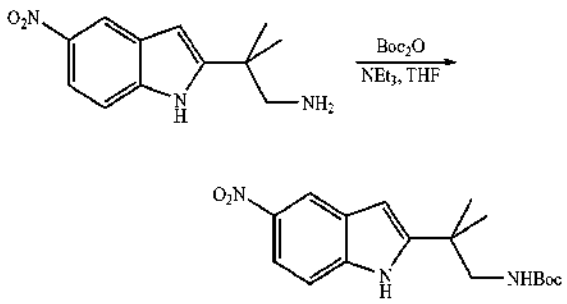
2-Methyl-2-(5-nitro-1H-indol-2-yl)propanamide

**[0635]** 2-Methyl-2-(5-nitro-1H-indol-2-yl)-propanoic acid (4.12 g, 16.6 mmol) was dissolved in acetonitrile (80 mL). EDC (3.80 g, 0.020 mmol), HOBt (2.70 g, 0.020 mmol), $Et_3N$ (6.9 mL, 0.05 mmol) and ammonium chloride (1.34 g, 0.025 mmol) were added and the mixture was stirred overnight at room temperature. Water was added and the mixture was extracted with ethylacetate. Combined organic phases were washed with brine, dried over magnesium sulfate and dried to give 2-methyl-2-(5-nitro-1H-indol-2-yl)propanamide (4.3 g, 99%).



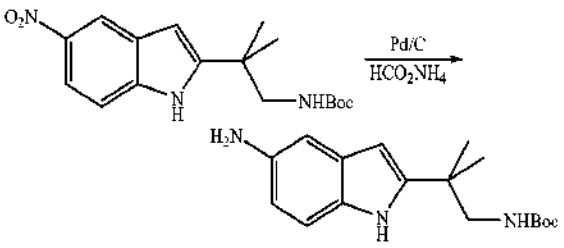
2-Methyl-2-(5-nitro-1H-indol-2-yl)propan-1-amine

**[0636]** 2-Methyl-2-(5-nitro-1H-indol-2-yl)propanamide (200 mg, 0.81 mmol) was suspended in THF (5 ml) and cooled to 0° C. Borane-THF complex solution (1.0 M, 2.4 mL, 2.4 mmol) was added slowly and the mixture was allowed to stir overnight at room temperature. The mixture was cooled to 0° C. and carefully acidified with 3 N HCl. THF was evaporated off, water was added and the mixture was washed with ethylacetate. The aqueous layer was made alkaline with 50% NaOH and the mixture was extracted with ethylacetate. The combined organic layers were dried over magnesium sulfate, filtered and evaporated to give 2-methyl-2-(5-nitro-1H-indol-2-yl)propan-1-amine (82 mg, 43%).



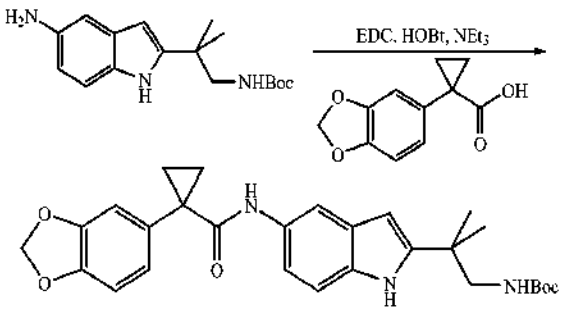
tert-Butyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propylcarbamate

**[0637]** 2-Methyl-2-(5-nitro-1H-indol-2-yl)propan-1-amine (137 mg, 0.587 mmol) was dissolved in THF (5 mL) and cooled to 0° C. $Et_3N$ (82 μL, 0.59 mmol) and di-tert-butyl dicarbonate (129 mg, 0.587 mmol) were added and the mixture was stirred at room temperature overnight. Water was added and the mixture was extracted with ethylacetate. The residue was purified by silica gel chromatography (10-40% ethylacetate in hexane) to give tert-butyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propylcarbamate (131 mg, 67%).




tert-Butyl 2-(5-amino-1H-indol-2-yl)-2-methylpropylcarbamate

**[0638]** To a solution of tert-butyl 2-methyl-2-(5-nitro-1H-indol-2-yl)propylcarbamate (80 mg, 0.24 mmol) in THF (9 mL) and water (2 mL) was added ammonium formate (60 mg, 0.96 mmol) followed by 10% Pd/C (50 mg). The mixture was stirred at room temperature for 45 minutes. Pd/C was filtered off and the organic solvent was removed by evaporation. The remaining aqueous phase was extracted with dichloromethane. The combined organic phases were dried over magnesium sulfate and evaporated to give tert-butyl 2-(5-amino-1H-indol-2-yl)-2-methylpropylcarbamate (58 mg, 80%).



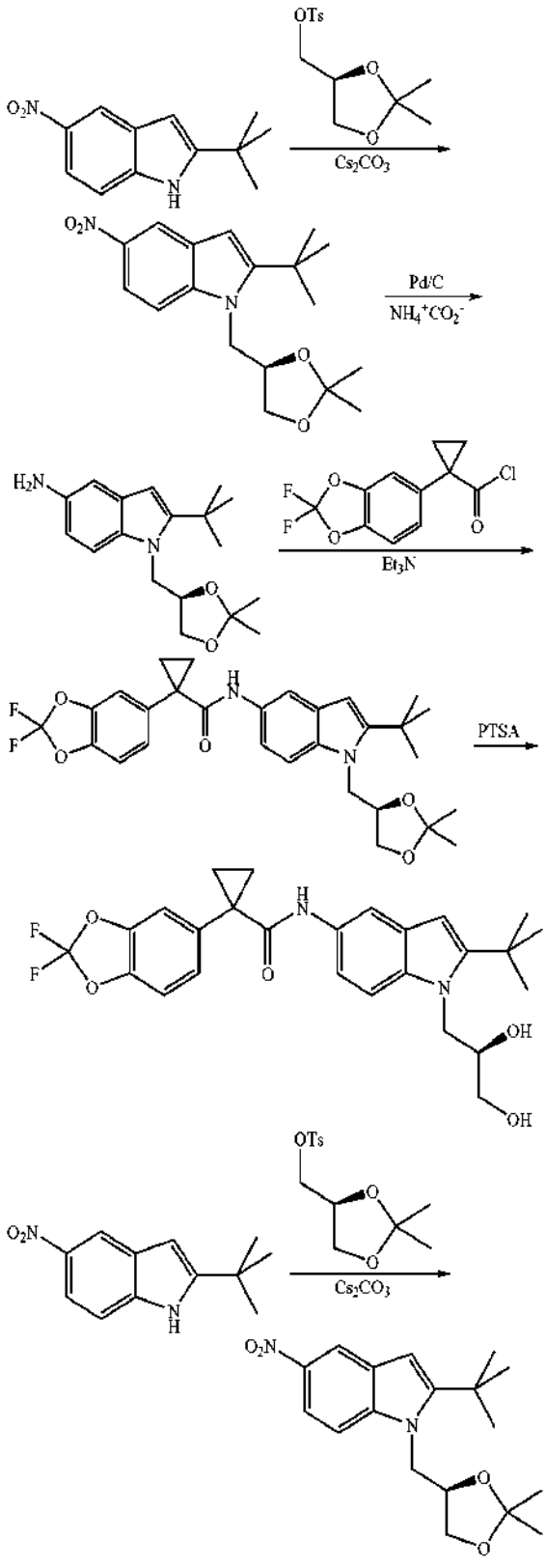

tert-Butyl 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)-2-methylpropylcarbamate

**[0639]** tert-Butyl 2-(5-amino-1H-indol-2-yl)-2-methylpropylcarbamate (58 mg, 0.19 mmol), 1-(benzo[d][1.3]dioxol-6-yl)cyclopropanecarboxylic acid (47 mg, 0.23 mmol), EDC (45 mg, 0.23 mmol), HOBt (31 mg, 0.23 mmol) and $Et_3N$ (80 μL, 0.57 mmol) were dissolved in DMF (4 mL) and stirred overnight at room temperature. The mixture was diluted with water and extracted with ethylacetate. The combined organic phases were dried over magnesium sulfate and evaporated to dryness. The residue was purified by silica gel chromatography (10-30% ethylacetate in hexane) to give tert-butyl 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)-2-methylpropyl-carbamate (88 mg, 94%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.62 (d, J=1.5 Hz, 1H), 7.18-7.16 (m, 2H), 7.02-6.94 (m, 3H). 6.85 (d, J=7.8 Hz, 1H), 6.19 (d, J=1.5 Hz, 1H), 6.02 (s, 2H), 4.54 (m, 1H), 3.33 (d, J=6.2 Hz, 2H), 1.68 (dd, J=3.7, 6.8 Hz, 2H), 1.36 (s, 9H), 1.35 (s, 6H), 1.09 (dd, J=3.7, 6.8 Hz, 2H).

Example 72

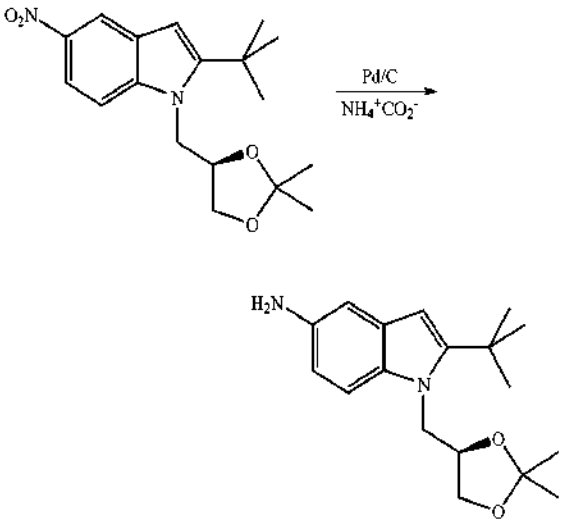
(R)—N-(2-tert-Butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamide

**[0640]**



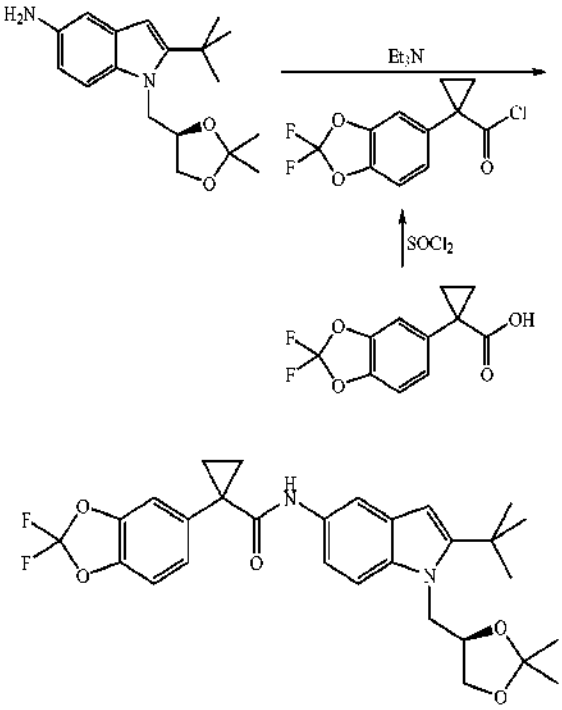
(R)-2-tert-Butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl) methyl)-5-nitro-1H-indole

**[0641]** To a stirred solution of (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (1.58 g, 5.50 mmol) in anhydrous DMF (10 mL) under nitrogen gas was added 2-tert-butyl-5-nitro-1H-indole (1.00 g, 4.58 mmol) followed by $Cs_2CO_3$ (2.99 g, 9.16 mol). The mixture was stirred and heated at 80° C. under nitrogen gas. After 20 hours, 50% conversion was observed by LCMS. The reaction mixture was re-treated with $Cs_2CO_3$ (2.99 g, 9.16 mol) and (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (1.58 g, 5.50 mmol) and heated at 80° C. for 24 hours. The reaction mixture was cooled to room temperature. The solids were filtered and washed with ethyl acetate and hexane (1:1). The layers were separated and the organic layer was washed with water (2×10 mL) and brine (2×10 mL). The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (dichloromethane/hexane=1.5/1) to give (R)-2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-5-nitro-1H-indole (1.0 g, 66%). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, J=2.2 Hz, 1H), 8.08 (dd, J=2.2, 9.1 Hz, 1H), 7.49 (d, J=9.1 Hz, 1H), 6.00 (s, 1H), 4.52-4.45 (m, 3H), 4.12 (dd, J=6.0, 8.6 Hz, 1H), 3.78 (dd, J=6.0, 8.6 Hz, 1H), 1.53 (s, 3H), 1.51 (s, 9H), 1.33 (s, 3H).



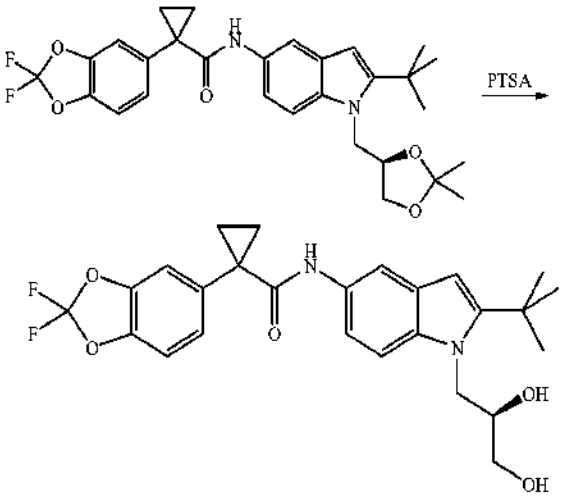

(R)-2-tert-Butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl) methyl-1H-indol-5-amine

**[0642]** To a stirred solution of (R)-2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-5-nitro-1H-indole (1.0 g, 3.0 mmol) in ethanol (20 mL) and water (5 mL) was added ammonium formate (0.76 g, 12 mmol) followed by slow addition of 10% palladium on carbon (0.4 g). The mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through a plug of celite and rinsed with ethyl acetate. The filtrate was evaporated under reduced pressure and the crude product was dissolved in ethyl acetate. The organic layer was washed with water (2×5 mL) and brine (2×5 mL). The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure to give (R)-2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl-1H-indol-5-amine (0.89 g, 98%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.04 (d, J=4 Hz, 1H), 6.70 (d, J=2.2 Hz, 1H), 6.48 (dd, J=2.2, 8.6 Hz, 1H), 6.05 (s, 1H), 4.38-4.1 (m, 2H), 4.21 (dd, J=7.5, 16.5 Hz, 1H), 3.87 (dd, J=6.0, 8.6 Hz, 1H), 3.66 (dd, J=6.0, 8.6 Hz, 1H), 3.33 (br s, 2H), 1.40 (s, 3H), 1.34 (s, 9H), 1.25 (s, 3H).




N—((R)-2-tert-Butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d] [1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0643]** To 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (0.73 g, 3.0 mmol) was added thionyl chloride (660 μL, 9.0 mmol) and DMF (20 μL) at room temperature. The mixture was stirred for 30 minutes before the excess thionyl chloride was evaporated under reduced pressure. To the resulting acid chloride, dichloromethane (6.0 mL) and $Et_3N$ (2.1 mL, 15 mmol) were added. A solution of (R)-2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl-1H-indol-5-amine (3.0 mmol) in dichloromethane (3.0 mL) was added to the cooled acid chloride solution. After addition, the reaction mixture was stirred at room temperature for 45 minutes. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane=3/7) to give N—((R)-2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo [d][1,3]dioxol-5-yl)cyclopropanecarboxamide (1.33 g, 84%). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J=2 Hz, 1H,), 7.31 (dd, J=2, 8 Hz, 1H), 7.27 (dd, J=2, 8 Hz, 1H), 7.23 (d, J=8 Hz, 1H), 7.14 (d, J=8 Hz, 1H), 7.02 (dd, J=2, 8 Hz, 1H), 6.92 (br s, 1H), 6.22 (s, 1H), 4.38-4.05 (m, 3H), 3.91 (dd, J=5, 8 Hz, 1H), 3.75 (dd, J=5, 8 Hz, 1H), 2.33 (q, J=8 Hz, 2H), 1.42 (s, 3H), 1.37 (s, 9H), 1.22 (s, 3H), 1.10 (q, J=8 Hz, 2H).



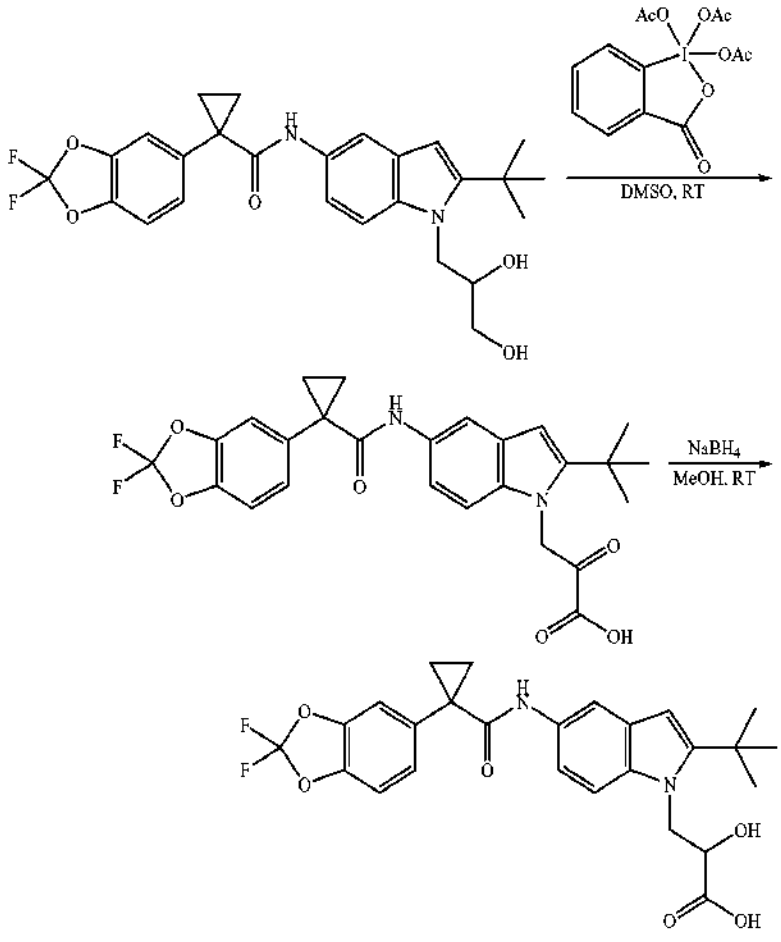

N—((R)-2-tert-Butyl-1-((2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo-[d][1,3]dioxol-5-yl) cyclopropanecarboxamide

**[0644]** To a stirred solution of N-(2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (1.28 g, 2.43 mmol) in methanol (34 mL) and water (3.7 mL) was added para-toluenesulfonic acid-hydrate (1.87 g, 9.83 mmol). The reaction mixture was stirred and heated at 80° C. for 25 minutes. The solvent was evaporated under reduced pressure. The crude product was dissolved in ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ (2×10 mL) and brine (2×10 mL). The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane=13/7) to give N—((R)-2-tert-butyl-1-((2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (0.96 g, 81%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J=2 Hz, 1H), 7.31 (dd, J=2, 8 Hz, 1H), 7.27 (dd, J=2, 8 Hz, 1H), 7.23 (d, J=8 Hz, 1H), 7.14 (d, J=8 Hz, 1H), 7.02 (br s, 1H,), 6.96 (dd, J=2, 8 Hz, 1H), 6.23 (s, 1H), 4.35 (dd, J=8, 15 Hz, 1H), 4.26 (dd, J=4, 15 Hz, 1H,), 4.02-3.95 (m, 1H), 3.60 (dd, J=4, 11 Hz, 1H), 3.50 (dd, J=5, 11 Hz, 1H), 1.75 (q, J=8 Hz, 3H), 1.43 (s, 9H), 1.14 (q, J=8 Hz, 3H).

Example 73

3-(2-tert-Butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-hydroxypropanoic acid

**[0645]**



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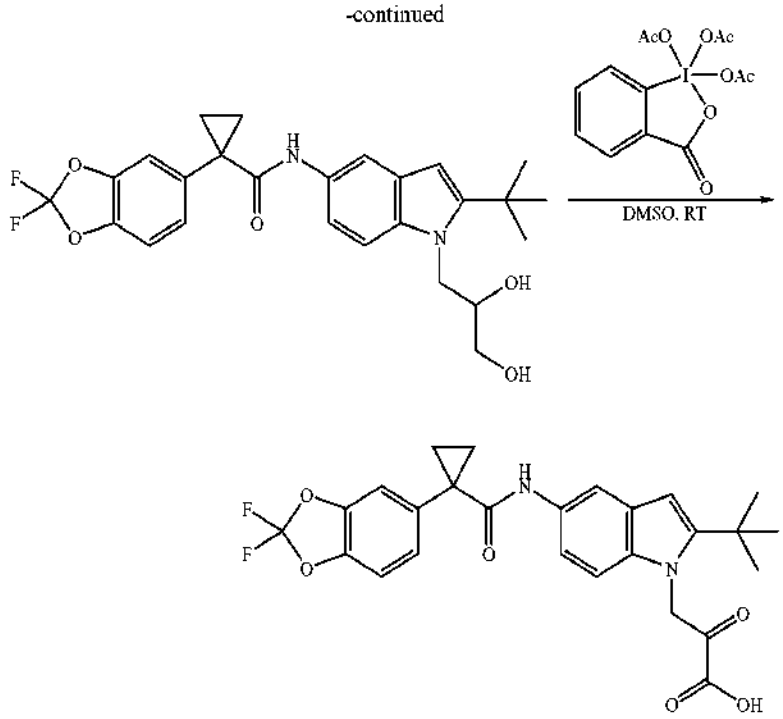
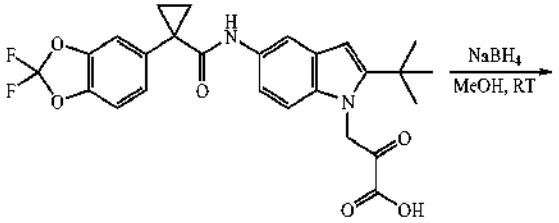



3-(2-tert-Butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-oxopropanoic acid

**[0646]** To a solution of N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-carboxamide (97 mg, 0.20 mmol) in DMSO (1 mL) was added Dess-Martin periodinane (130 mg, 0.30 mmol). The mixture was stirred at room temperature for 3 h. The solid was filtered off and washed with EtOAc. The filtrate was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc twice and the combined organic layers were washed with brine and dried over $MgSO_4$. After the removal of solvent, the residue was purified by preparative TLC to yield 3-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-oxopropanoic acid that was used without further purification.

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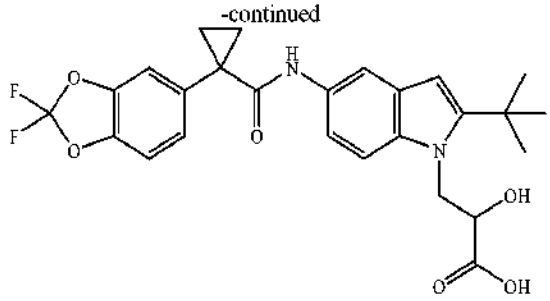



3-(2-tert-Butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-hydroxypropanoic acid

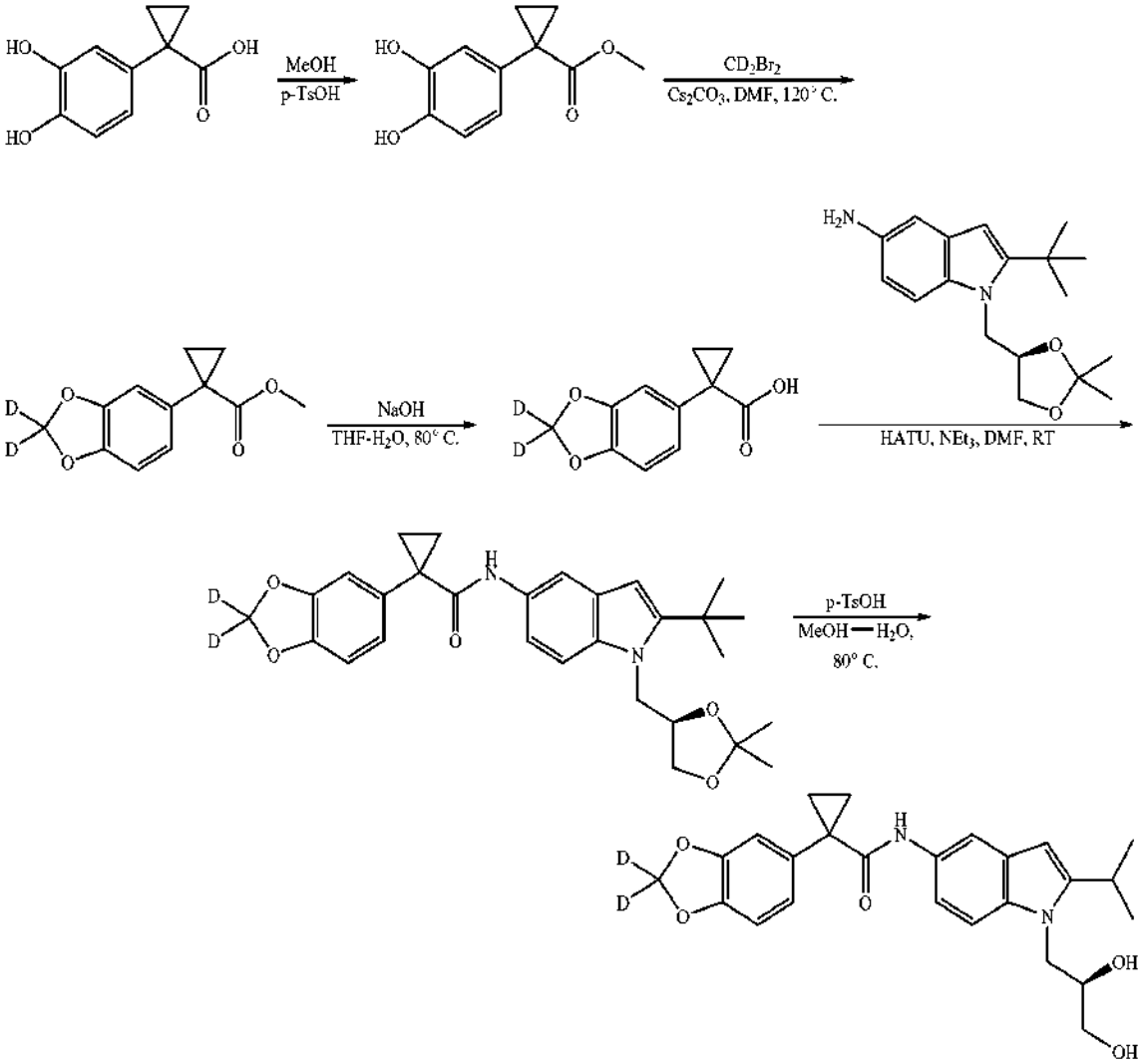
**[0647]** To a solution of 3-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-oxopropanoic acid (50 mg, 0.10 mmol) in MeOH (1 mL) was added $NaBH_4$ (19 mg, 0.50 mmol) at 0° C. The mixture was stirred at room temperature for 15 min. The resulting mixture was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc twice and the combined organic layers were washed with brine and dried over anhydrous $MgSO_4$. After the removal of the solvent, the residue was taken up in DMSO and purified by preparative LC/MS to give 3-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-hydroxypropanoic acid. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.36 (s), 7.27-7.23 (m, 2H), 7.15-7.11 (m, 2H), 6.94 (d, J=8.5 Hz, 1H), 6.23 (s, 1H), 4.71 (s, 3H), 4.59 (q, J=10.3 Hz, 1H), 4.40-4.33 (m, 2H), 1.70 (d, J=1.9 Hz, 2H), 1.15 (q, J=4.0 Hz, 2H). $^{13}C$ NMR (400 MHz, $CDCl_3$) δ 173.6, 173.1, 150.7, 144.1, 143.6, 136.2, 135.4, 134.3, 131.7, 129.2, 129.0, 127.6,

126.7, 116.6, 114.2, 112.4, 110.4, 110.1, 99.7, 70.3, 48.5, 32.6, 30.9, 30.7, 16.8. MS (ESI) m/e ($M+H^+$) 501.2.

Example 74

(R)—N-(2-tert-Butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,2-dideuteriumbenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0648]**




Methyl 1-(3,4-dihydroxyphenyl)cyclopropanecarboxylate

**[0649]** To a solution of 1-(3,4-dihydroxyphenyl)cyclopropanecarboxylic acid (190 mg, 1.0 (mmol) in MeOH (3 mL) was added 4-methylbenzenesulfonic acid (19 mg, 0.10 mmol). The mixture was heated at 80° C. overnight. The reaction mixture was concentrated in vacuo and partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc twice and the combined organic layers were washed with sat. $NaHCO_3$ and brine and dried over $MgSO_4$. After the removal of solvent, the residue was dried in vacuo to yield methyl 1-(3,4-dihydroxyphenyl)cyclopropanecarboxylate (190 mg, 91%) that was used without further purification. $^1H$ NMR (400 MHz, DMSO-$d^6$) δ 6.76-6.71 (m, 2H), 6.66 (d, J=7.9 Hz, 1H), 3.56 (s, 3H), 1.50 (q, J=3.6 Hz, 2H), 1.08 (q, J=3.6 Hz, 2H).

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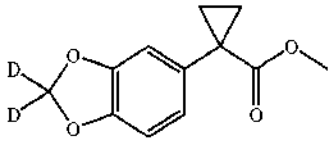
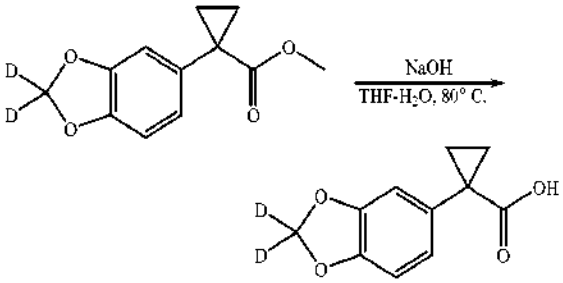


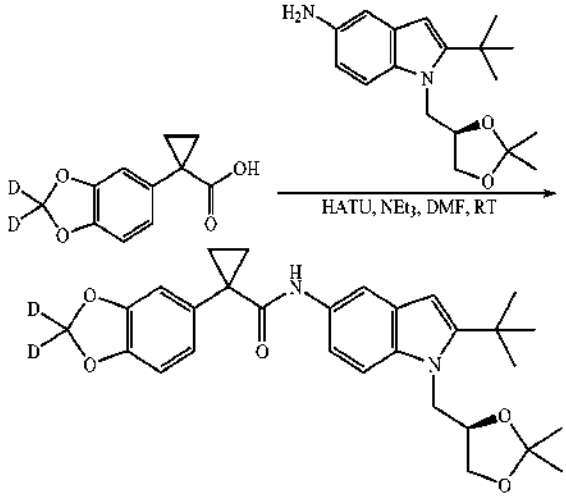
Methyl 1-(2,2-dideuteriumbenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxylate

**[0650]** To a solution of methyl 1-(3,4-dihydroxyphenyl) cyclopropanecarboxylate (21 mg, 0.10 mmol) and $CD_2Br_2$ (35 mg, 0.20 mmol) in DMF (0.5 mL) was added $Cs_2CO_3$ (19 mg, 0.10 mmol). The mixture was heated at 120° C. for 30 min. The reaction mixture was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc twice and the combined organic layers were washed with 1N NaOH and brine before being dried over $MgSO_4$. After the removal of solvent, the residue was dried in vacuo to yield methyl 1-(2,2-dideuteriumbenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylate (22 mg) that was used without further purification. $^1H$ NMR (400 MHz, $CDCl_3$) δ 6.76-6.71 (m, 2H), 6.66 (d, J=7.9 Hz, 1H), 3.56 (s, 3H), 1.50 (q, J=3.6 Hz, 2H), 1.08 (q, J=3.6 Hz, 2H).



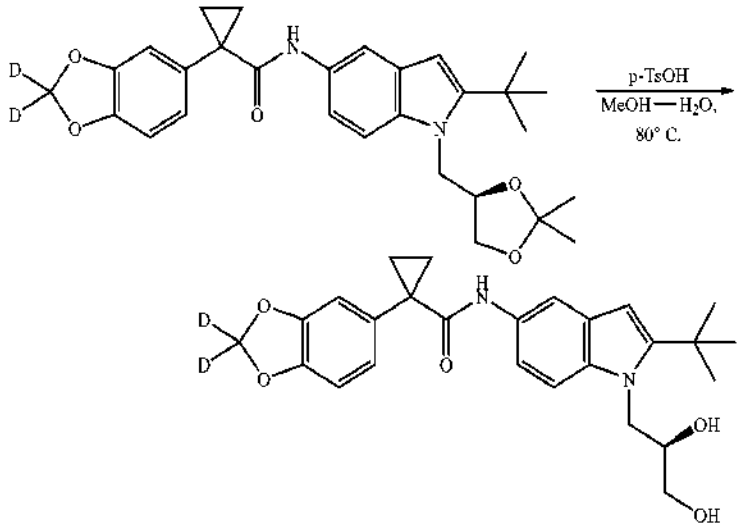

1-(2,2-Dideuteriumbenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid

**[0651]** To a solution of methyl 1-(2,2-dideuteriumbenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylate (22 mg, 0.10 mmol) in THF (0.5 mL) was added NaOH (1N, 0.25 mL, 0.25 mmol). The mixture was heated at 80° C. for 2 h. The reaction mixture was partitioned between EtOAc and 1N NaOH. The aqueous layer was extracted with EtOAc twice, neutralized with 1N HCl and extracted with EtOAc twice. The combined organic layers were washed with brine and dried over $MgSO_4$. After the removal of solvent, the residue was dried in vacuo to yield 1-(2,2-dideuteriumbenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxylic acid (21 mg) that was used without further purification.




(R)—N-(2-tert-Butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1H-indol-5-yl)-1-(2,2-dideuteriumbenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0652]** To a solution of 1-(2,2-dideuteriumbenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (21 mg, 0.10 mmol), (R)-2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1H-indol-5-amine (30 mg, 0.10 mmol), HATU (42 mg, 0.11 mol) in DMF (1 mL) was added triethylamine (0.030 mL, 0.22 mmol). The mixture was heated at room temperature for 5 min. The reaction mixture was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc twice and the combined organic layers were washed with 1N NaOH, 1N HCl, and brine before being dried over $MgSO_4$. After the removal of solvent, the residue was purified by column chromatography (20-40% ethyl acetate/hexane) to yield (R)—N-(2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1H-indol-5-yl)-1-(2,2-dideuteriumbenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (24 mg, 49% from methyl 1-(3,4-dihydroxyphenyl)cyclopropanecarboxylate). MS (ESI) m/e ($M+H^+$) 493.5.



(R)—N-(2-tert-Butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,2-dideuterium-benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

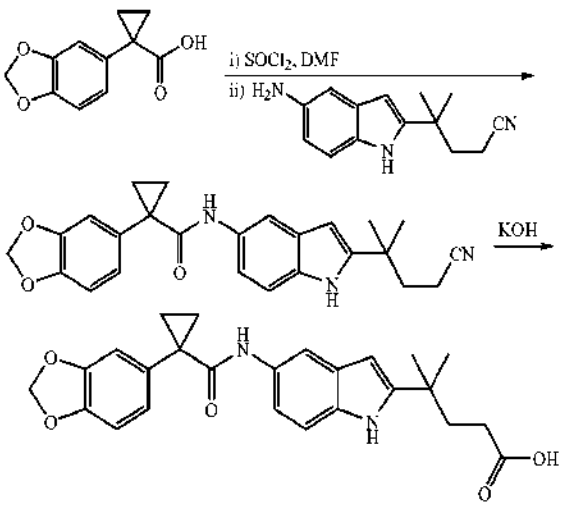
**[0653]** To a solution of (R)—N-(2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1H-indol-5-yl)-1-(2,2-dideuterium-benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (24 mg, 0.050 mmol), in methanol (0.5 mL) and water (0.05 mL) was added 4-methylbenzenesulfonic acid (2.0 mg, 0.010 mmol). The mixture was heated at 80° C. for 30 min. The reaction mixture was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc twice and the combined organic layers were washed with sat. $NaHCO_3$ and brine before being dried over $MgSO_4$. After the removal of solvent, the residue was purified by preparative HPLC to yield (R)—N-(2-tert-butyl-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1H-indol-5-yl)-1-(2,2-dideuteriumbenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (12 mg, 52%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J=2.0 Hz, 1H), 7.14 (dd, J=22.8, 14.0 Hz, 2H), 6.95-6.89 (m, 2H), 6.78 (d, J=7.8 Hz, 1H), 6.14 (s, 1H), 4.28 (dd, J=15.1, 8.3 Hz, 1H), 4.19 (dd, J=15.1, 4.5 Hz, 1H), 4.05 (q, J=7.1 Hz, 1H), 3.55 (dd, J=11.3, 4.0 Hz, 1H), 3.45 (dd, J=11.3, 5.4 Hz, 1H), 1.60 (q, J=3.5 Hz, 2H), 1.35 (s, 9H), 1.02 (q, J=3.5 Hz, 2H). $^{13}C$ NMR (400 MHz, $CDCl_3$) δ 171.4, 149.3, 147.1, 146.5, 134.8, 132.3, 129.2, 126.5, 123.6, 114.3, 111.4, 110.4, 109.0, 107.8, 98.5, 70.4, 63.1, 46.6, 31.6, 30.0, 29.8, 15.3. MS (ESI) m/e ($M+H^+$) 453.5.

**[0654]** It is further noted that the mono-deuterated analogue for this compound can be synthesized by substitution the reagent $CHDBR_2$ for $CD_2BR_2$ and following the procedures described in example 74. Furthermore, mono-deuterated analogues of other compounds of the present invention can be synthesized by substituting the reagent $CHDBR_2$ for $CD_2BR_2$ and following the steps described herein.

Example 75

4-(5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)-4-methylpentanoic acid

**[0655]**

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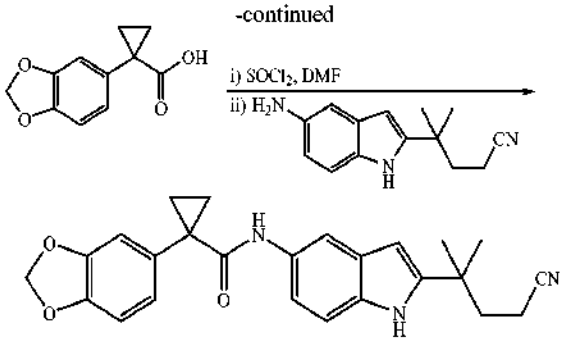
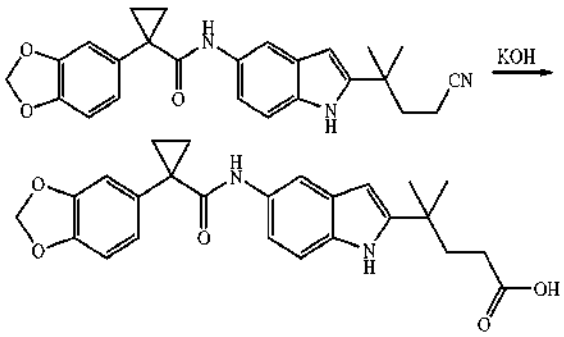



1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(4-cyano-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0656]** To 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (0.068 g, 0.33 mmol) was added thionyl chloride (72. IL, 0.99 mmol) and DMF (20 μL) at room temperature. The mixture was stirred for 30 minutes before the excess thionyl chloride was evaporated under reduced pressure. To the resulting acid chloride, dichloromethane (0.5 mL) and $Et_3N$ (230 μL, 1.7 mmol) were added. A solution of 4-(5-amino-1H-indol-2-yl)-4-methylpentanenitrile (0.33 mmol) in dichloromethane (0.5 mL) was added to the acid chloride solution and the mixture was stirred at room temperature for 1.5 h. The resulting mixture was diluted with dichloromethane and washed with 1 N HCl (2×2 mL), saturated aqueous $NaHCO_3$ (2×2 mL) and brine (2×2 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-(4-cyano-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide.




4-(5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)-4-methylpentanoic acid

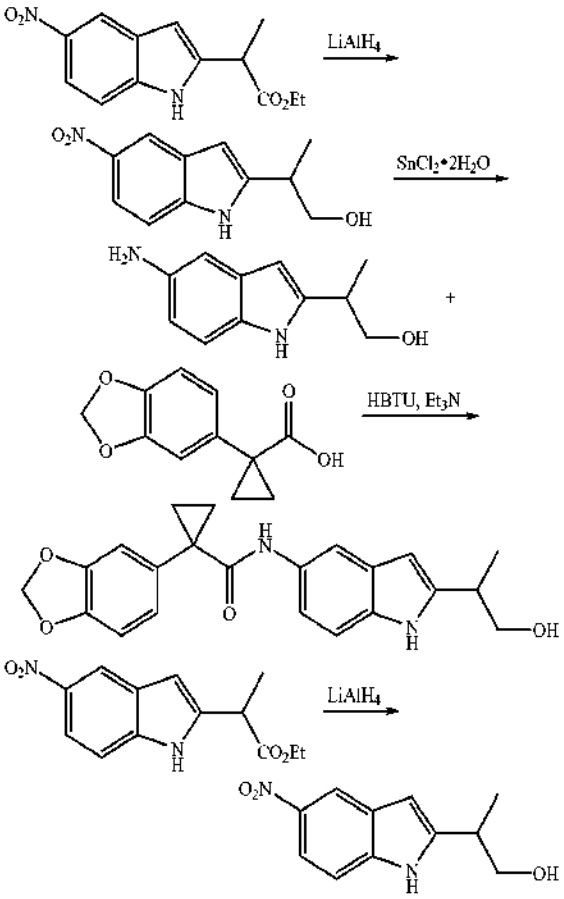
**[0657]** A mixture of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-(4-cyano-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (0.060 g, 0.15 mmol) and KOH (0.081 g, 1.5 mmol) in 50% EtOH/water (2 mL) was heated in the microwave at 100° C. for 1 h. The solvent was evaporated under reduced pressure. The crude product was dissolved in DMSO (1 mL), filtered, and purified by reverse phase preparative HPLC to give 4-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)-4-methylpentanoic acid. $^1H$ NMR (400 MHz, DMSO-d6) δ 11.98 (s, 1H), 10.79 (s,

1H), 8.44 (s, 1H), 7.56 (s, 1H), 7.15 (d, J=8.6 Hz, 1H), 7.03-6.90 (m, 4H), 6.05 (s, 1H), 6.02 (s, 2H), 1.97-1.87 (m, 4H), 1.41-1.38 (m, 2H), 1.30 (s, 6H), 1.04-1.02 (m, 2H).

Example 76

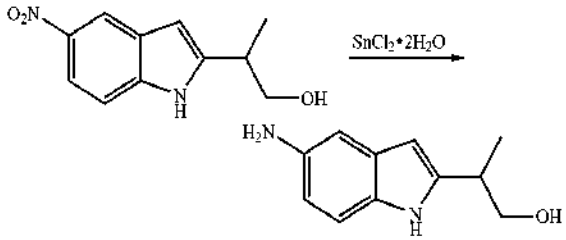
1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(1-hydroxypropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0658]**



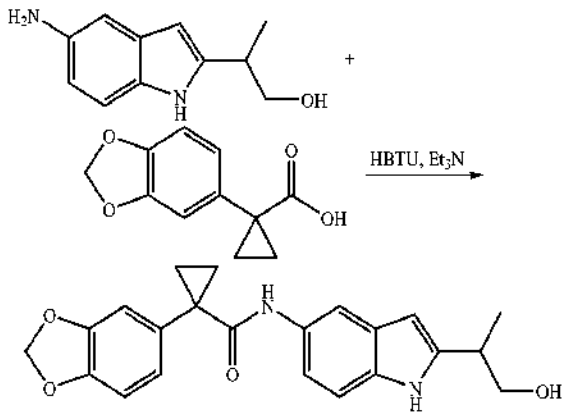

2-(5-Nitro-1H-indol-2-yl)propan-1-ol

**[0659]** To a cooled solution of $LiAlH_4$ (1.0 M in THF, 1.2 mL, 1.2 mmol) in THF (5.3 mL) at 0° C. was added a solution of ethyl 2-(5-nitro-1H-indol-2-yl)propanoate (0.20 g, 0.76 mmol) in THF (3.66 mL) dropwise. After addition, the mixture was allowed to warm up to room temperature and was stirred at room temperature for 3 h. The mixture was cooled to 0° C. Water (2 mL) was slowly added followed by careful addition of 15% NaOH (2 mL) and water (4 mL). The mixture was stirred at room temperature for 0.5 h and was then filtered through a short plug of celite using ethyl acetate. The organic layer was separated from the aqueous layer, dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane=1/1) to give 2-(5-nitro-1H-indol-2-yl)propan-1-ol (0.14 g, 81%).




2-(5-Amino-1H-indol-2-yl)propan-1-ol

**[0660]** To a solution of 2-(5-nitro-1H-indol-2-yl)propan-1-ol (0.13 g, 0.60 mmol) in ethanol (5 mL) was added tin chloride dihydrate (0.67 g, 3.0 mmol). The mixture was heated in the microwave at 120° C. for 1 h. The mixture was diluted with ethyl acetate before water and saturated aqueous $NaHCO_3$ were added. The reaction mixture was filtered through a plug of celite using ethyl acetate. The organic layer was separated from the aqueous layer, dried over $Na_2SO_4$, filtered and evaporated under reduced pressure to give 2-(5-amino-1H-indol-2-yl)propan-1-ol (0.093 g, 82%).




1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(1-hydroxypropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

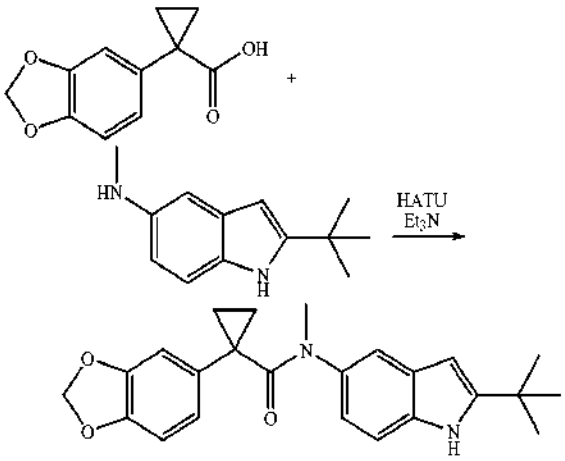
**[0661]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (0.10 g, 0.49 mmol) in acetonitrile (2.0 mL) were added HBTU (0.185 g, 0.49 mmol) and $Et_3N$ (205 μL, 1.47 mmol) at room temperature. The mixture was allowed to stir at room temperature for 10 minutes before a slurry of 2-(5-amino-1H-indol-2-yl)propan-1-ol (0.093 g, 0.49 mmol) in acetonitrile (2.7 mL) was added. After addition, the reaction mixture was stirred at room temperature for 5.5 h. The solvent was evaporated under reduced pressure and the residue was dissolved in dichloromethane. The organic layer was washed with 1 N HCl (1×3 mL) and saturated aqueous $NaHCO_3$ (1×3 mL). The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The crude material was purified by column chromatography on silica gel (ethyl acetate/hexane=13/7) to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-(1-hydroxypropan-2-yl)-1H-indol-5-yl) cyclopropanecarboxamide (0.095 g, 51%). $^1H$ NMR (400 MHz, DMSO-d6) δ 10.74 (s, 1H), 8.38 (s, 1H), 7.55 (s, 1H), 7.14 (d, J=8.6 Hz, 1H), 7.02-6.90 (m, 4H), 6.06 (s, 1H), 6.02

(s, 2H), 4.76 (t, J=5.3 Hz, 1H), 3.68-3.63 (m, 1H), 3.50-3.44 (m, 1H), 2.99-2.90 (m, 1H), 1.41-1.38 (m, 2H), 1.26 (d, J=7.0 Hz, 3H), 1.05-1.02 (m, 2H).

Example 77

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)-N-methylcyclopropanecarboxamide

**[0662]**



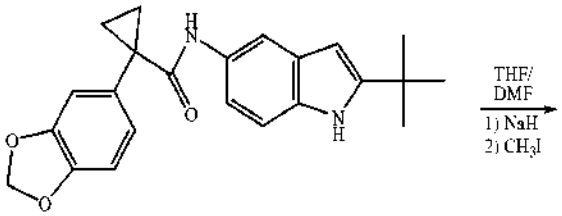

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)-N-methylcyclopropanecarboxamide

**[0663]** 2-tert-Butyl-N-methyl-1H-indol-5-amine (20.2 mg, 0.100 mmol) and 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (20.6 mg, 0.100 mmol) were dissolved in N,N-dimethylformamide (1 mL) containing triethylamine (42.1 μL, 0.300 mmol) and a magnetic stir bar. O-(7-Azabenzotriazol-1-yl)-N,N',N'-tetramethyluronium hexafluorophosphate (42 mg, 0.11 mmol) was added to the mixture and the resulting solution was allowed to stir for 16 h at 80° C. The crude product was then purified by preparative HPLC utilizing a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)-N-methylcyclopropanecarboxamide. ESI-MS m/z calc. 390.2, found 391.3 $(M+1)^+$. Retention time of 3.41 minutes.

Example 78

N-(2-tert-Butyl-1-methyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-6-yl)-N-methylcyclopropanecarboxamide

**[0664]**

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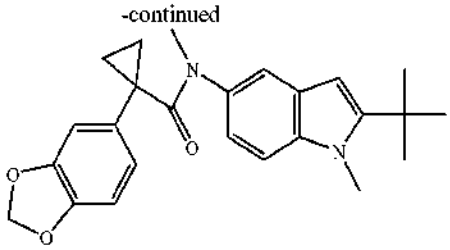
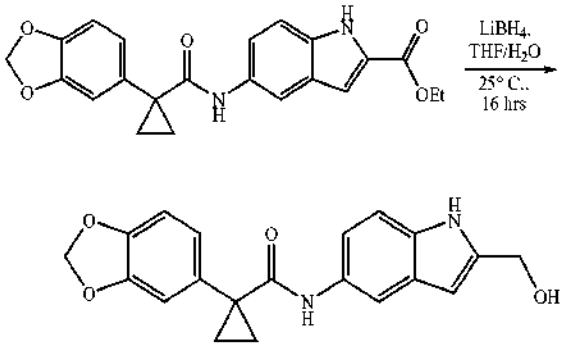


**[0665]** Sodium hydride (0.028 g, 0.70 mmol, 60% by weight dispersion in oil) was slowly added to a stirred solution of N-(2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-6-yl)cyclopropanecarboxamide (0.250 g, 0.664 mmol) in a mixture of 4.5 mL of anhydrous tetrahydrofuran (THF) and 0.5 mL of anhydrous N,N-dimethylformamide (DMF). The resulting suspension was allowed to stir for 2 minutes and then iodomethane (0.062 mL, 1.0 mmol) was added to the reaction mixture. Two additional aliquots of sodium hydride and iodomethane were required to consume all of the starting material which was monitored by LC/MS. The crude reaction product was evaporated to dryness, redissolved in a minimum of DMF and purified by preparative LC/MS chromatography to yield the pure product (0.0343 g, 13%) ESI-MS m/z calc. 404.2, found 405.3 $(M+1)^+$. Retention time of 3.65 minutes.

Example 79

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(hydroxymethyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0666]**

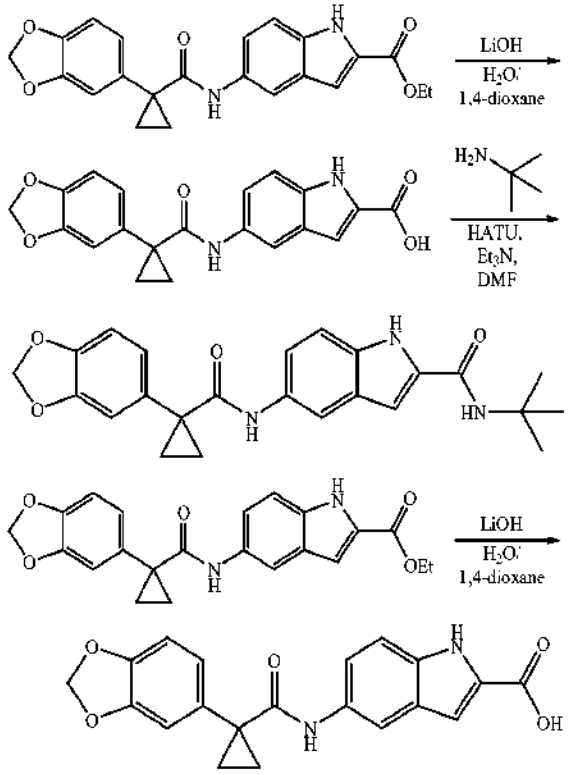



**[0667]** Ethyl 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indole-2-carboxylate (1.18 g, 3.0 mmol) was added to a solution of $LiBH_4$ (132 mg, 6.0 mmol) in THF (10 mL) and water (0.1 mL). The mixture was allowed to stir for 16 h at 25° C. before it was quenched with water (10 mL) and slowly made acidic by addition of 1 N HCl. The mixture was extracted with three 50-mL portions of ethyl acetate. The organic extracts were dried over $Na_2SO_4$ and evaporated to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-(hydroxymethyl)-1H-indol-5-yl)cyclopropanecarboxamide (770 mg, 73%). A small amount was further purified by reverse phase HPLC. ESI-MS m/z calc. 350.4, found 351.3 $(M+1)^+$; retention time 2.59 minutes.

Example 80

5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-N-tert-butyl-1H-indole-2-carboxamide

**[0668]**




5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indole-2-carboxylic acid

**[0669]** Ethyl 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indole-2-carboxylate (392 mg, 1.0 mmol) and LiOH (126 mg, 3 mmol) were dissolved in $H_2O$ (5 mL) and 1,4-dioxane (3 mL). The mixture was heated in an oil bath at 100° C. for 24 hours before it was cooled to room temperature. The mixture was acidified with 1N HCl and it was extracted with three 20 mL portions of dichloromethane. The organic extracts were dried over $Na_2SO_4$ and evaporated to yield 5-(1-(benzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamido)-1H-indole-2-carboxylic acid (302 mg, 83%). A small amount was further purified by reverse phase HPLC. ESI-MS m/z calc. 364.1, found 365.1 $(M+1)^+$; retention time 2.70 minutes.

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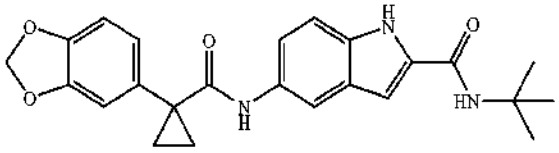


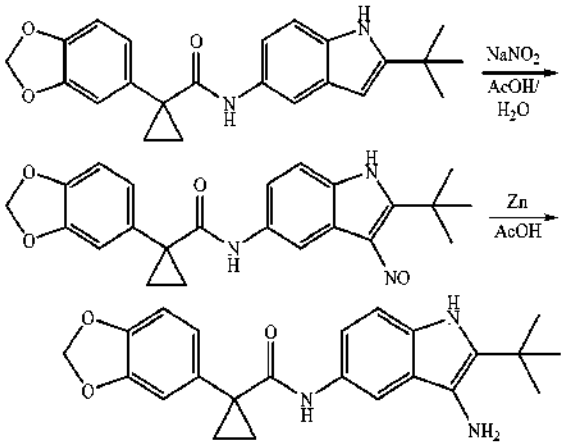
5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-N-tert-butyl-1H-indole-2-carboxamide

**[0670]** 5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropane-carboxamido)-1H-indole-2-carboxylic acid (36 mg, 0.10 mmol) and 2-methylpropan-2-amine (8.8 mg, 0.12 mmol) were dissolved in N,N-dimethylformamide (1.0 mL) containing triethylamine (28 μL, 0.20 mmol). O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (46 mg, 0.12 mmol) was added to the mixture and the resulting solution was allowed to stir for 3 hours. The mixture was filtered and purified by reverse phase HPLC to yield 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-N-tert-butyl-1H-indole-2-carboxamide. ESI-MS m/z calc. 419.2, found 420.3 $(M+1)^+$; retention time 3.12 minutes.

Example 81

N-(3-Amino-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0671]**



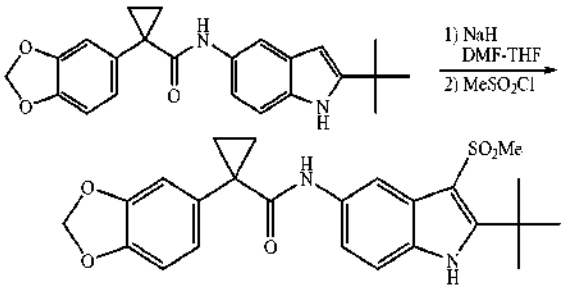

**[0672]** A solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclopropane carboxamide (50 mg, 0.13 mmol) was dissolved in AcOH (2 mL) and warmed to 45° C. To the mixture was added a solution of $NaNO_2$ (9 mg) in $H_2O$ (0.03 mL). The mixture was allowed to stir for 30 min at 45° C. before the precipitate was collected and washed with $Et_2O$. This material was used in the next step without further purification. To the crude material, 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-nitroso-1H-indol-5-yl)cyclopropanecarboxamide, was added AcOH (2 mL) and Zn dust (5 mg). The mixture was allowed to stir for 1 h at ambient temperature. EtOAc and $H_2O$ were added to the mixture. The layers were separated and the organic layer was washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. The

residue was taken up in DMF (1 mL) and was purified using prep-HPLC. LCMS: m/z 392.3: retention time of 2.18 min.

Example 82

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-(methylsulfonyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0673]**



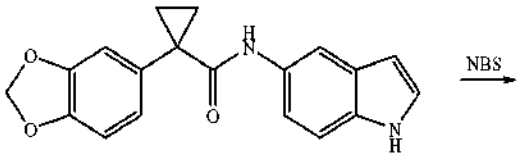

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-(methylsulfonyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0674]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclopropanecarboxamide (120 mg, 0.31 mmol) in anhydrous DMF-THF (3.3 mL, 1:9) was added NaH (60% in mineral oil, 49 mg, 1.2 mmol) at room temperature. After 30 min under $N_2$, the suspension was cooled down to −15° C. and a solution of methanesulfonyl chloride (1.1 eq.) in DMF (0.5 mL) was added dropwise. The reaction mixture was stirred for 30 min at −15° C. then for 6 h at room temperature. Water (0.5 mL) was added at 0° C., solvent was removed, and the residue was diluted with MeOH, filtrated and purified by preparative HPLC to give 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-(methylsulfonyl)-1H-indol-5-yl)cyclopropanecarboxamide. $^1$H NMR (400 MHz, DMSO) δ 11.6 (s, 1H), 8.7 (s, 1H), 7.94 (d, J=1.7 Hz, 1H), 7.38 (d. J=8.7 Hz, 1H), 7.33 (dd, J1=1.9 Hz, J2=8.7 Hz, 1H), 7.03 (d. J=1.7 Hz, 1H), 6.95 (dd, J1=1.7 Hz, J2=8.0 Hz, 1H), 6.90 (d, J=8.0 Hz, 1H), 6.02 (s, 2H), 3.07 (s, 3H), 1.56-1.40 (m, 9H), 1.41 (dd, J1=4.0 Hz, J2=6.7 Hz, 2H), 1.03 (dd, J1=4.0 Hz, J2=6.7 Hz, 2H). MS (ESI) m/e (M+H$^+$) 455.5.

Example 83

1-(Benzo[d][1,3]dioxol-5-yl)-N-(3-phenyl-1H-indol-5-yl)cyclopropane carboxamide

**[0675]**

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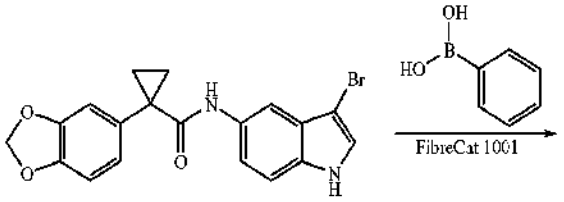
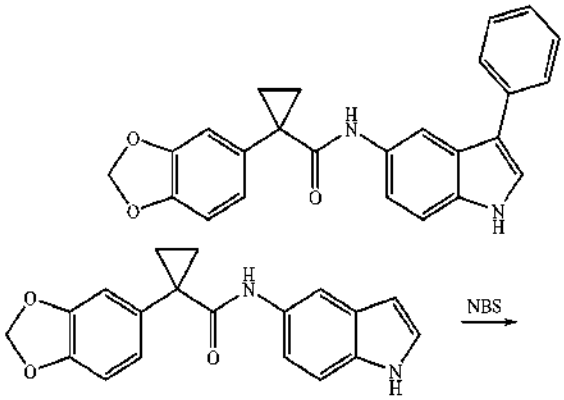
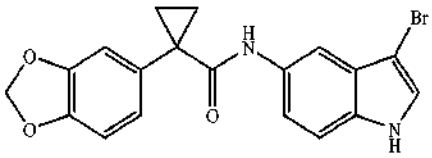
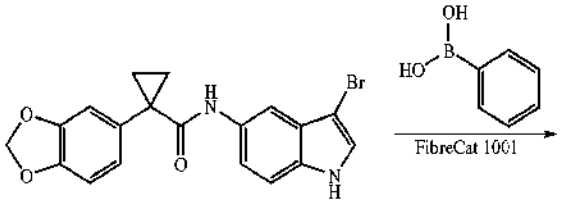



1-(Benzo[d][1,3]dioxol-5-yl)-N-(3-bromo-1H-indol-5-yl)cyclopropanecarboxamide

**[0676]** Freshly recrystallized N-bromosuccinimide (0.278 g, 1.56 mmol) was added portionwise to a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(1H-indol-5-yl)cyclopropanecarboxamide (0.500 g, 1.56 mmol) in N,N-dimethylformamide (2 mL) over 2 minutes. The reaction mixture was protected from light and was stirred bar for 5 minutes. The resulting green solution was poured into 40 mL of water. The grey precipitate which formed was filtered and washed with water to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(3-bromo-1H-indol-5-yl)cyclopropanecarboxamide (0.564 g, 91%). ESI-MS m/z calc. 398.0, found 399.3 (M+1)$^+$. Retention time of 3.38 minutes. $^1$H NMR (400 MHz, DMSO-d6) 11.37 (s, 1H), 8.71 (s, 1H), 7.67 (d, J=1.8 Hz, 1H), 7.50 (d, J=2.6 Hz, 1H), 7.29 (d, J=8.8 Hz, 1H), 7.22 (dd, J=2.0, 8.8 Hz, 1H), 7.02 (d, J=1.6 Hz, 1H), 6.96-6.88 (m, 2H), 6.03 (s, 2H), 1.43-1.40 (m, 2H), 1.09-1.04 (m, 2H).



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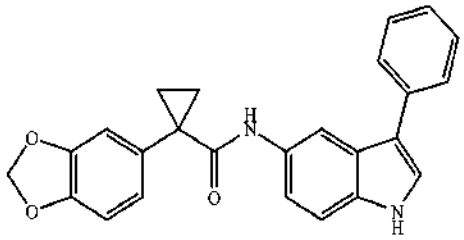


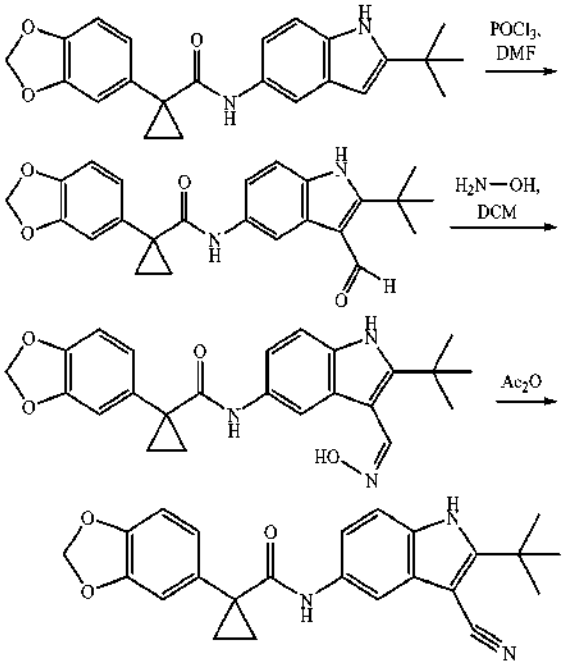
1-(Benzo[d][1,3]dioxol-5-yl)-N-(3-phenyl-1H-indol-5-yl)cyclopropanecarboxamide

**[0677]** Phenyl boronic acid (24.6 mg, 0.204 mmol) was added to a solution of 1-(benzo[d][1,3]-dioxol-5-yl)-N-(3-bromo-1H-indol-5-yl)cyclopropanecarboxamide (39.9 mg, 0.100 mmol) in ethanol (1 mL) containing FibreCat 1001 (6 mg) and 1M aqueous potassium carbonate (0.260 mL). The reaction mixture was then heated at 130° C. in a microwave reactor for 20 minutes. The crude product was then purified by preparative HPLC utilizing a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(3-phenyl-1H-indol-5-yl) cyclopropane carboxamide. ESI-MS m/z calc. 396.2, found 397.3 $(M+1)^+$. Retention time of 3.52 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 11.27 (d, J=1.9 Hz, 1H), 8.66 (s, 1H), 8.08 (d, J=1.6 Hz, 1H), 7.65-7.61 (m, 3H), 7.46-7.40 (m, 2H), 7.31 (d, J=8.7 Hz, 1H), 7.25-7.17 (m, 2H), 7.03 (d, J=1.6 Hz, 1H), 6.98-6.87 (m, 2H), 6.02 (s, 2H), 1.43-1.39 (m, 2H), 1.06-1.02 (m, 2H).

Example 84

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-cyano-1H-indol-5-yl)cyclopropanecarboxamide

**[0678]**

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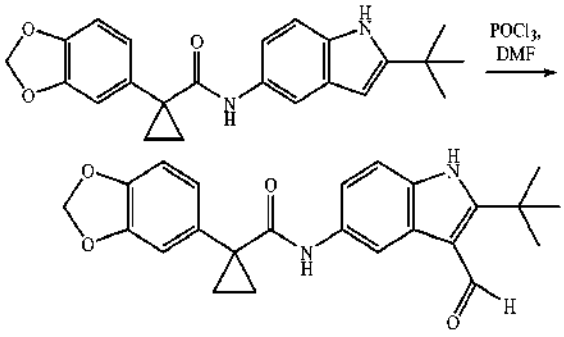
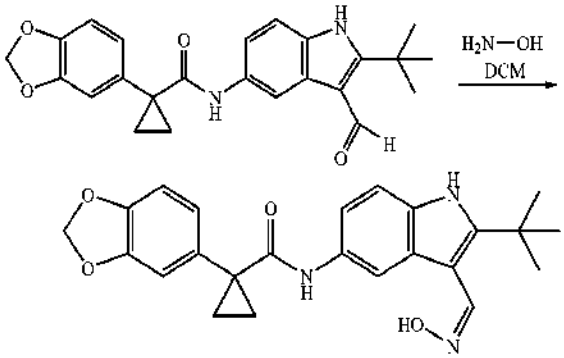



1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-formyl-1H-indol-5-yl)cyclopropanecarboxamide

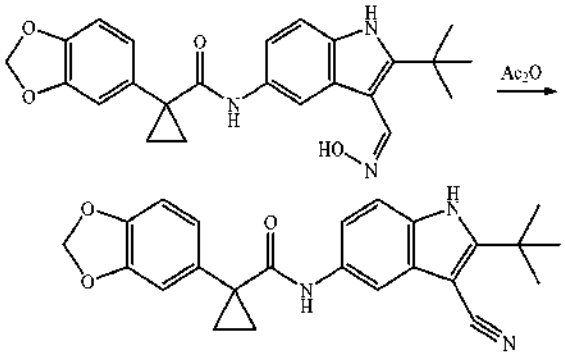
**[0679]** $POCl_3$ (12 g, 80 mmol) was added dropwise to DMF (40 mL) held at −20° C. After the addition was complete, the reaction mixture was allowed to warm to 0° C. and was stirred for 1 h. 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclopropanecarboxamide (3.0 g, 8.0 mmol) was added and the mixture was warmed to 25° C. After stirring for 30 minutes the reaction mixture was poured over ice and stirred for 2 h. The mixture was then heated at 100° C. for 30 min. The mixture was cooled and the solid precipitate was collected and washed with water. The solid was then dissolved in 200 mL dichloromethane and washed with 200 mL of a saturated aq. $NaHCO_3$. The organics were dried over $Na_2SO_4$ and evaporated to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-formyl-1H-indol-5-yl)cyclopropanecarboxamide (2.0 g, 61%). ESI-MS m/z calc. 404.5, found 405.5 $(M+1)^+$; retention time 3.30 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 11.48 (s, 1H), 10.39 (s, 1H), 8.72 (s, 1H), 8.21 (s, 1H), 7.35-7.31 (m, 2H), 7.04-7.03 (m, 1H), 6.97-6.90 (m, 2H), 6.03 (s, 2H), 1.53 (s, 9H), 1.42-1.39 (m, 2H), 1.05-1.03 (m, 2H).




(Z)-1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-((hydroxyimino)methyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0680]** To a solution of 1-(benzo[d][1.3]dioxol-5-yl)-N-(2-tert-butyl-3-formyl-1H-indol-5-yl)cyclopropanecarboxamide (100 mg, 0.25 mmol) in dichloromethane (5 mL) was added hydroxylamine hydrochloride (21 mg, 0.30 mmol). After stirring for 48 h, the mixture was evaporated to dryness

and purified by column chromatography (0-100% ethyl acetate/hexanes) to yield (Z)-1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-((hydroxyimino)methyl)-1H-indol-5-yl) cyclopropanecarboxamide (81 mg, 77%). ESI-MS m/z calc. 419.5, found 420.5 $(M+1)^+$; retention time 3.42 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 10.86 (s, 0.5H), 10.55 (s, 0.5H), 8.56-8.50 (m, 2H), 8.02 (m, 1H), 7.24-7.22 (m, 1H), 7.12-7.10 (m, 1H), 7.03 (m, 1H), 6.96-6.90 (m, 2H), 6.03 (s, 2H), 1.43 (s, 9H), 1.40-1.38 (m, 2H), 1.04-1.01 (m, 2H).



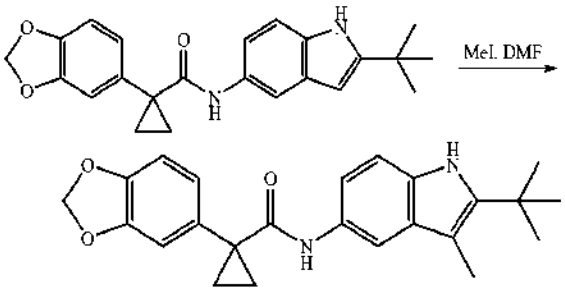

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-cyano-1H-indol-5-yl)cyclopropanecarboxamide

**[0681]** (Z)-1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-((hydroxyimino)-methyl)-1H-indol-5-yl)cyclopropanecarboxamide (39 mg, 0.090 mmol) was dissolved in acetic anhydride (1 mL) and heated at reflux for 3 h. The mixture was cooled in an ice bath and the precipitate was collected and washed with water. The solid was further dried under high vacuum to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-cyano-1H-indol-5-yl)cyclopropanecarboxamide. ESI-MS m/z calc. 401.5, found 402.5 $(M+1)^+$; retention time 3.70 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 11.72 (s, 1H), 8.79 (s, 1H), 7.79 (s, 1H), 7.32 (m, 2H), 7.03-7.02 (m, 1H), 6.95-6.89 (m, 2H), 6.03 (s, 2H), 1.47 (s, 9H), 1.43-1.41 (m, 2H), 1.06-1.04 (m, 2H).

Example 85

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-methyl-1H-indol-5-yl)cyclopropanecarboxamide

**[0682]**

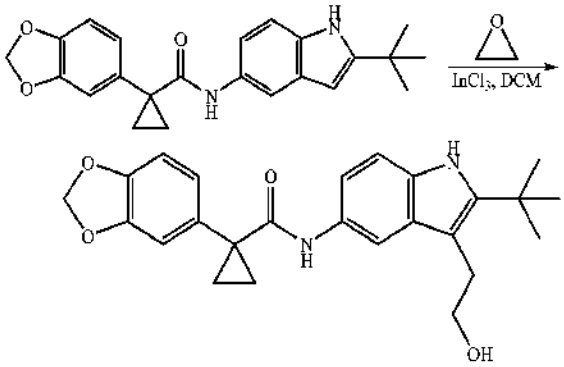



**[0683]** A solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclopropanecarboxamide (75 mg, 0.20 mmol) and iodomethane (125 μL, 2.0 mmol) in N,N-dimethylformamide (1 mL) was heated at 120° C. in a sealed tube for 24 h. The reaction was filtered and purified by reverse phase HPLC. ESI-MS m/z calc. 390.5, found 391.3 $(M+1)^+$; retention time 2.04 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 10.30 (s, 1H), 8.39 (s, 1H), 7.51 (m, 1H), 7.13-7.11 (m, 1H), 7.03-6.90 (m, 4H), 6.03 (s, 2H), 2.25 (s, 3H), 1.40-1.38 (m, 11H), 1.03-1.01 (m, 2H).

Example 86

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-(2-hydroxyethyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0684]**

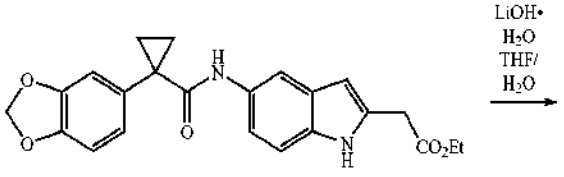



**[0685]** Approximately 100 μL of ethylene dioxide was condensed in a reaction tube at −78° C. A solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclopropanecarboxamide (200 mg, 0.50 mmol) and indium trichloride (20 mg, 0.10 mmol) in dichloromethane (2 mL) was added and the reaction mixture was irradiated in the microwave for 20 min at 100° C. The volatiles were removed and the residue was purified by column chromatography (0-100% ethyl acetate/hexanes) to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-3-(2-hydroxyethyl)-1H-indol-5-yl)cyclopropanecarboxamide (5 mg, 3%). ESI-MS m/z calc. 420.5, found 421.3 $(M+1)^+$; retention time 1.67 minutes. $^1H$ NMR (400 MHz, $CD_3CN$) δ 8.78 (s, 1H), 7.40 (m, 1H), 7.33 (s, 1H), 7.08 (m, 1H), 6.95-6.87 (m, 3H), 6.79 (m, 1H), 5.91 (s, 2H), 3.51 (dd, J=5.9, 7.8 Hz, 2H), 2.92-2.88 (m, 2H), 2.64 (t, J=5.8 Hz, 1H), 1.50 (m, 2H), 1.41 (s, 9H), 1.06 (m, 2H).

Example 87

2-(5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)acetic acid

**[0686]**



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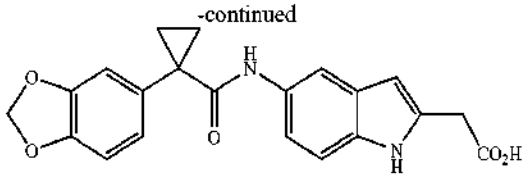
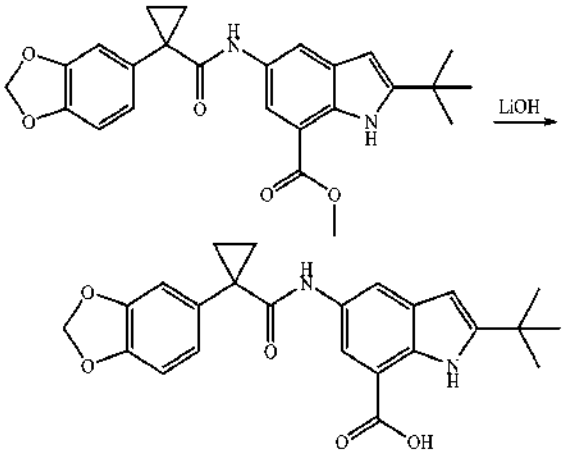


**[0687]** To a solution of ethyl 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)acetate (0.010 g, 0.025 mmol) in THF (0.3 mL) were added LiOH.$H_2O$ (0.002 g, 0.05 mmol) and water (0.15 mL) were added. The mixture was stirred at room temperature for 2 h. dichloromethane (3 mL) was added to the reaction mixture and the organic layer was washed with 1 N HCl (2×1.5 mL) and water (2×1.5 mL). The organic layer was dried over $Na_2SO_4$ and filtered. The filtrate was evaporated under reduced pressure to give 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)-acetic acid. $^1$H NMR (400 MHz, DMSO-d6) δ 12.53 (s, 1H), 10.90 (s, 1H), 8.42 (s, 1H), 7.57 (s, 1H), 7.17 (d, J=8.6 Hz, 1H), 7.05-6.90 (m, 4H), 6.17 (s, 1H), 6.02 (s, 2H), 3.69 (s, 2H), 1.41-1.39 (m, 2H), 1.04-1.02 (m, 2H).

Example 88

5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butyl-1H-indole-7-carboxylic acid

**[0688]**

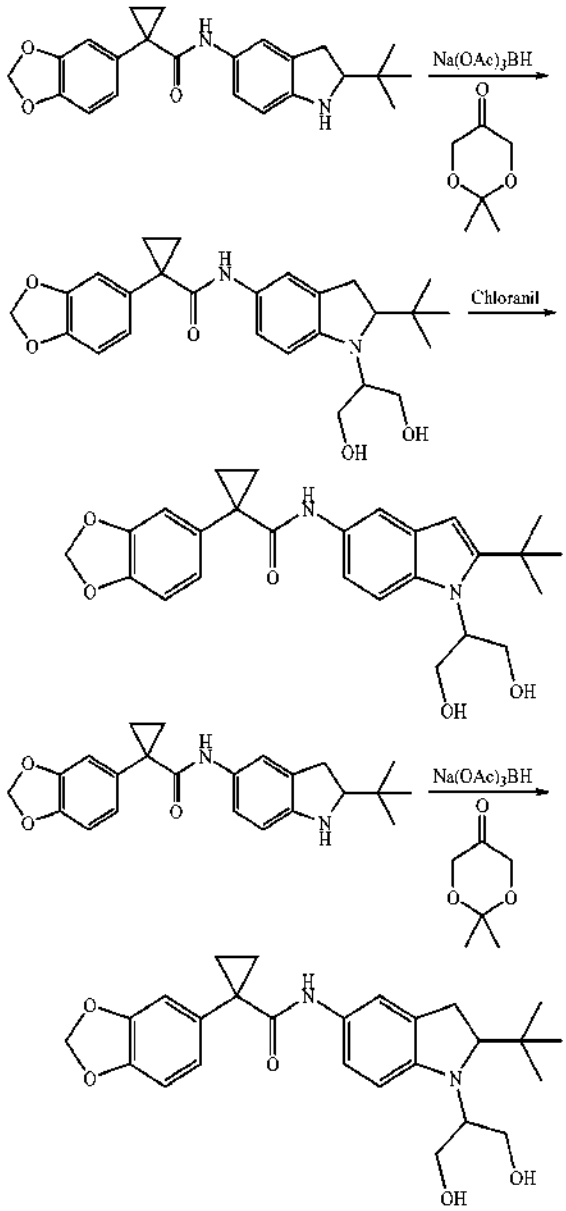



**[0689]** Methyl 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butyl-1H-indole-7-carboxylate (30 mg, 0.069 mmol) was dissolved in a mixture of 1,4-dioxane (1.5 mL) and water (2 mL) containing a magnetic star bar and lithium hydroxide (30 mg, 0.71 mmol). The resulting solution was stirred at 70° C. for 45 minutes. The crude product was then acidified with 2.6 M hydrochloric acid and extracted three times with an equivalent volume of dichloromethane. The dichloromethane extracts were combined, dried over sodium sulfate, filtered, and evaporated to dryness. The residue was dissolved in a minimum of N,N-dimethylformamide and then purified by preparative HPLC using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 5-(1-(benzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-2-tert-butyl-1H-indole-7-carboxylic acid. ESI-MS m/z calc. 434.2, found 435.5. Retention time of 1.85 minutes. $^1$H NMR (400 MHz, DMSO-d6) δ 13.05 (s, 1H), 9.96 (d, J=1.6 Hz, 1H), 7.89 (d, J=1.9 Hz, 1H), 7.74 (d, J=2.0 Hz, 1H), 7.02 (d, J=1.6 Hz, 1H), 6.96-6.88 (m, 2H), 6.22 (d, J=2.3 Hz, 1H), 6.02 (s, 2H), 1.43-1.40 (m, 2H), 1.37 (s, 9H), 1.06-1.02 (m, 2H).

Example 89

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(1,3-dihydroxypropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

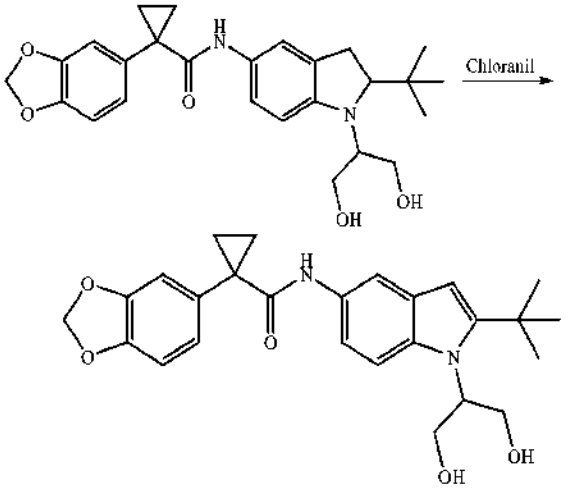
**[0690]**




1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(1,3-dihydroxypropan-2-yl)indolin-5-yl)cyclopropanecarboxamide

**[0691]** 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butylindolin-5-yl)cyclopropanecarboxamide (50 mg, 0.13 mmol) was

dissolved in dichloroethane (0.20 mL) and 2,2-dimethyl-1,3-dioxan-5-one (0.20 mL). Trifluoroacetic acid was added (0.039 mL) and the resulting solution was allowed to stir for 20 minutes. Sodium triacetoxyborohydride was added (55 mg, 0.26 mmol) and the reaction mixture was stirred for 30 minutes. The crude reaction mixture was then evaporated to dryness, dissolved in N,N-dimethylformamide and purified by preparative HPLC using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid.



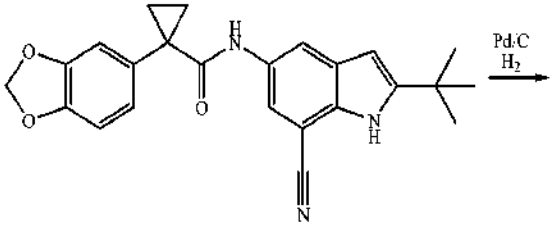

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(1,3-dihydroxypropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0692]** 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(1,3-dihydroxypropan-2-yl)indolin-5-yl)cyclopropanecarboxamide (40.3 mg, 0.0711 mmol as the trifluoracetic acid salt) was dissolved in toluene (1 mL). To the resulting solution was added 2,3,5,6-tetrachlorocyclohexa-2,5-diene-1,4-dione (35 mg, 0.14 mmol). The resulting suspension was heated at 100° C. in an oil bath for 10 minutes. The crude product was then evaporated to dryness, dissolved in a 1 mL of N,N-dimethylformamide and purified by purified by preparative HPLC using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(1,3-dihydroxypropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide. ESI-MS m/z calc. 450.2, found 451.5 $(M+1)^+$. Retention time of 1.59 minutes.

Example 90

N-(7-(Aminomethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamide

**[0693]**

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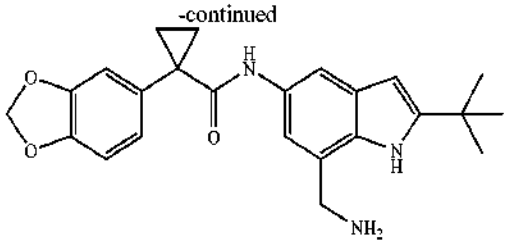


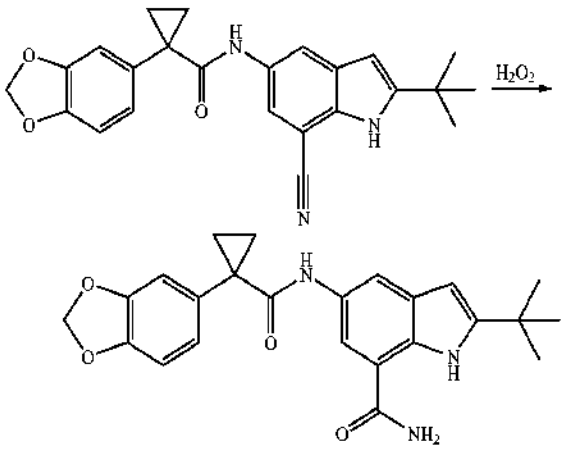

N-(7-(Aminomethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0694]** 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-7-cyano-1H-indol-5-yl)cyclopropanecarboxamide (375 mg, 0.934 mmol) was dissolved in 35 mL of ethyl acetate. The solution was recirculated through a continuous flow hydrogenation reactor containing 10% palladium on carbon at 100° C. under 100 bar of hydrogen for 8 h. The crude product was then evaporated to dryness and purified on 12 g of silica gel utilizing a gradient of 0-100% ethyl acetate (containing 0.5% triethylamine) in hexanes to yield N-(7-(aminomethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]-dioxol-5-yl)-cyclopropanecarboxamide (121 mg, 32%). ESI-MS m/z calc. 405.2, found 406.5 $(M+1)^+$. Retention time of 1.48 minutes.

Example 91

5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butyl-1H-indole-7-carboxamide

**[0695]**




5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butyl-1H-indole-7-carboxamide

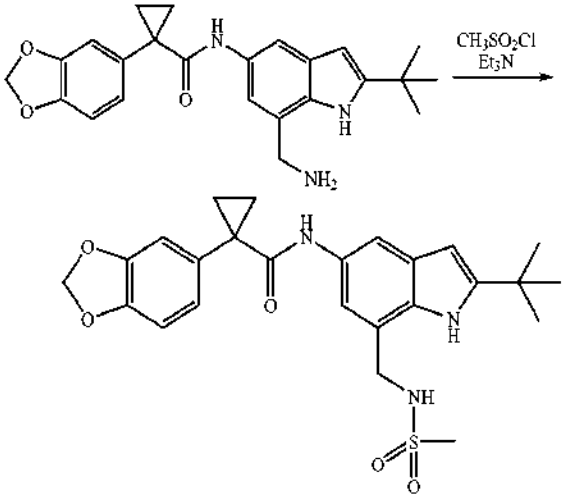
**[0696]** 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-7-cyano-1H-indol-5-yl)-cyclopropanecarboxamide (45 mg, 0.11 mmol) was suspended in a mixture of methanol (1.8 mL), 30% aqueous hydrogen peroxide (0.14 mL, 4.4 mmol) and 10% aqueous sodium hydroxide (0.150 mL). The resulting suspension was stirred for 72 h at room temperature. The hydrogen peroxide was then quenched with sodium sulfite. The reaction mixture was diluted with 0.5 mL of N,N-dimethylformamide, filtered, and purified by preparative HPLC

using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropane-carboxamido)-2-tert-butyl-1H-indole-7-carboxamide. ESI-MS m/z calc. 419.2, found 420.3 $(M+1)^+$. Retention time of 1.74 minutes.

Example 92

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-7-(methylsulfonamido-methyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0697]**



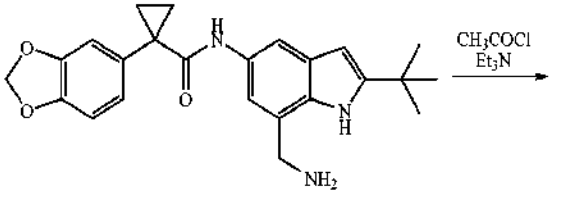

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-7-(methylsulfonamidomethyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0698]** N-(7-(Aminomethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (20 mg, 0.049 mmol) was dissolved in DMF (0.5 mL) containing triethylamine (20.6 μL, 0.147 mmol) and a magnetic stir bar. Methanesulfonyl chloride (4.2 μL, 0.054 mmol) was then added to the reaction mixture. The reaction mixture was allowed to stir for 12 h at room temperature. The crude product was purified by preparative HPLC using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-7-(methylsulfonamidomethyl)-1H-indol-5-yl)cyclopropanecarboxamide. ESI-MS m/z calc. 483.2, found 484.3 $(M+1)^+$. Retention time of 1.84 minutes.

Example 93

N-(7-(Acetamidomethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamide

**[0699]**

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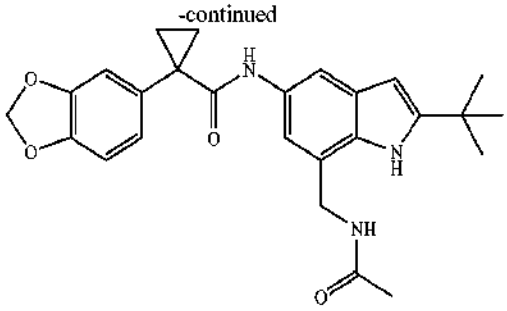
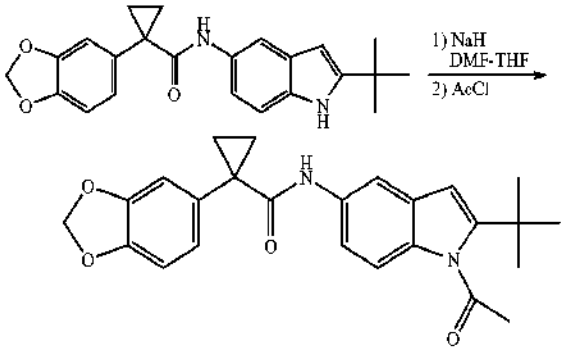



**[0700]** N-(7-(Aminomethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (20 mg, 0.049 mmol) was dissolved in DMF (0.5 mL) containing triethylamine (20.6 μL, 0.147 mmol) and a magnetic stir bar. Acetyl chloride (4.2 μL, 0.054 mmol) was then added to the reaction mixture. The reaction mixture was allowed to stir for 16 h at room temperature. The crude product was purified by preparative HPLC using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield N-(7-(acetamidomethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide. ESI-MS m/z calc. 447.2, found 448.3 $(M+1)^+$. Retention time of 1.76 minutes.

Example 94

N-(1-Acetyl-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)-cyclopropanecarboxamide

**[0701]**



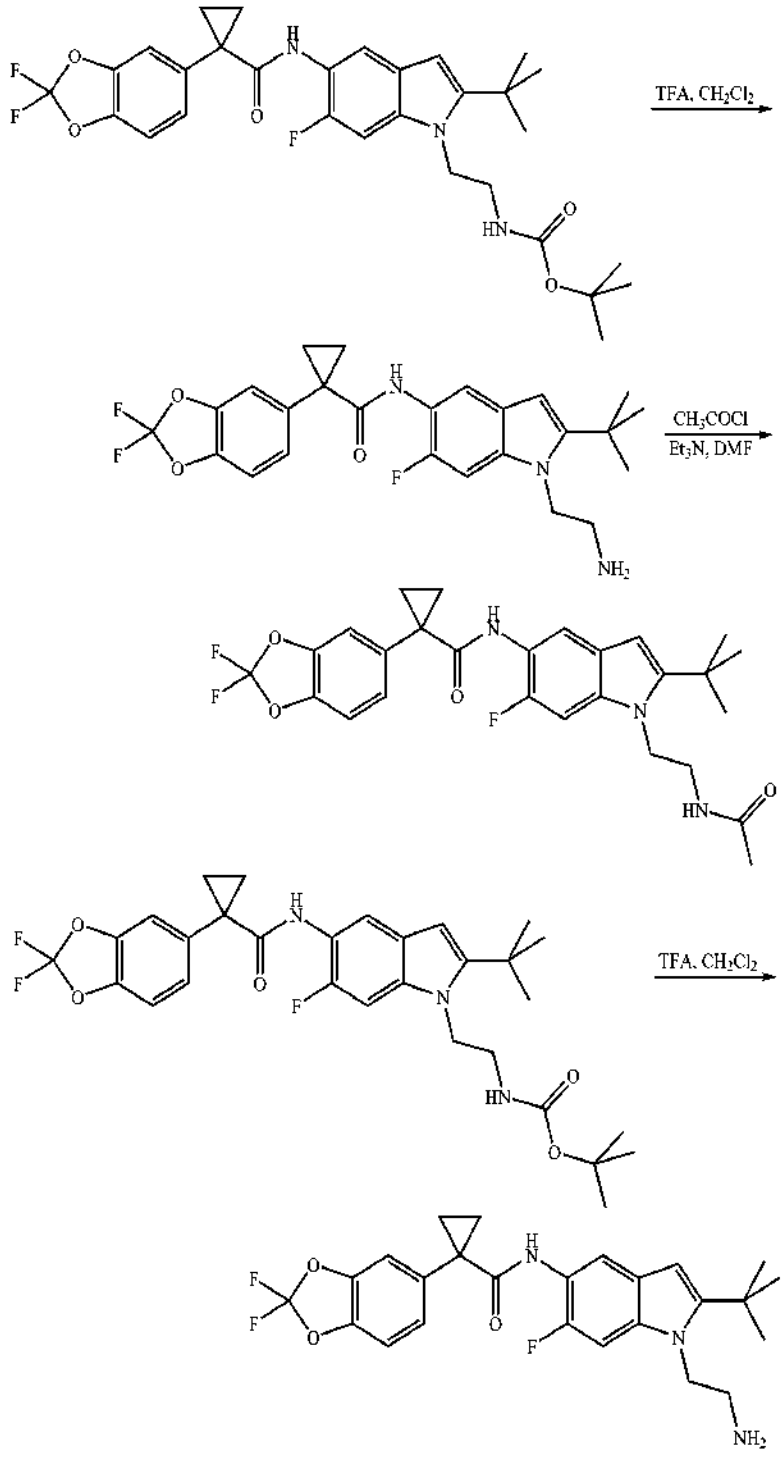

**[0702]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclopropanecarboxamide (120 mg, 0.31 mmol) in anhydrous DMF-THF (3.3 mL, 1:9) was added NaH (60% in mineral oil, 49 mg, 1.2 mmol) at room temperature. After 30 min under $N_2$, the suspension was cooled down to −15° C. and a solution of acetyl chloride (1.1 eq.) in DMF (0.5 mL) was added dropwise. The reaction mixture was stirred for 30 min at −15° C. then for 6 h at room temperature. Water (0.5 mL) was added at 0° C., solvent was removed, and the residue was diluted with MeOH, filtrated and purified by preparative HPLC to give N-(1-acetyl-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide. $^1$H NMR (400 MHz, DMSO) δ 8.9 (s, 1H), 7.74 (d, J=2.1 Hz, 1H), 7.54 (d, J=9.0 Hz, 1H), 7.28 (dd, J1=2.1 Hz, J2=9.0 Hz, 1H), 7.01 (d, J=1.5 Hz, 1H), 6.93 (dd, J1=1.7 Hz, J2=8.0 Hz, 1H), 6.89 (d, J=8.0 Hz, 1H), 6.54 (bs,

1H), 6.02 (s, 2H), 2.80 (s, 3H), 1.42-1.40 (m, 11H), 1.06-1.05 (m, 2H). MS (ESI) m/e ($M+H^+$) 419.3.

Example 95

N-(1-(2-Acetamidoethyl)-2-tert-butyl-6-fluoro-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamide

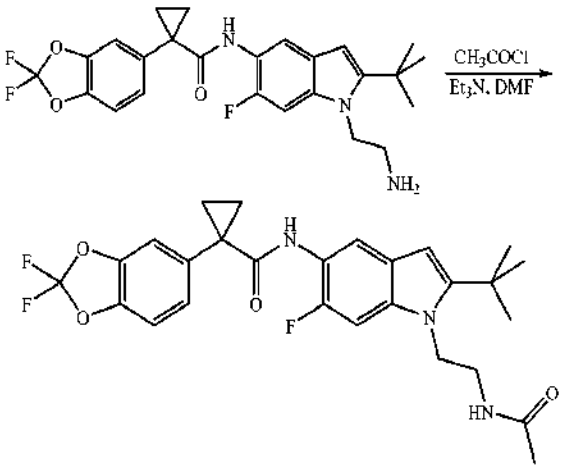
**[0703]**




N-(1-(2-Aminoethyl)-2-tert-butyl-6-fluoro-1H-indol-5-yl)-1-(2,2-difluorobenzo-[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0704]** To a solution of tert-butyl 2-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-6-fluoro-1H-indol-1-yl)ethylcarbamate (620 mg, 1.08 mmol) in $CH_2Cl_2$ (8 mL) was added TFA (2 mL). The reaction was stirred at room temperature for 1.5 h before being

neutralized with solid $NaHCO_3$. The solution was partitioned between $H_2O$ and $CH_2Cl_2$. The organic layer was dried over $MgSO_4$, filtered and concentrated to yield the product as a cream colored solid (365 mg, 71%). $^1H$ NMR (400 MHz, DMSO-d6) δ 8.38 (s, 1H), 7.87 (br s, 3H, $NH_3^+$), 7.52 (s, 1H), 7.45-7.38 (m, 3H), 7.32 (dd, J=8.3, 1.5 Hz, 1H), 6.21 (s, 1H), 4.46 (m, 2H), 3.02 (m, 2H), 1.46 (m, 2H), 1.41 (s, 9H), 1.14 (m, 2H). HPLC ret. time 1.66 min, 10-99% $CH_3CN$, 3 min run; ESI-MS 474.4 m/z ($M+H^+$).



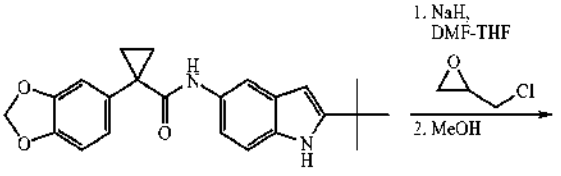

N-(1-(2-Acetamidoethyl)-2-tert-butyl-6-fluoro-1H-indol-5-yl)-1-(2,2-difluorobenzo [d][1,3]dioxol-5-yl) cyclopropanecarboxamide

**[0705]** To a solution of N-(1-(2-aminoethyl)-2-tert-butyl-6-fluoro-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-carboxamide (47 mg, 0.10 mmol) and $Et_3N$ (28 μL, 0.20 mmol) in DMF (1 mL) was added acetyl chloride (7.1 μL, 0.10 mmol). The mixture was stirred at room temperature for 1 h before being filtered and purified by reverse phase HPLC (10-99% $CH_3CN/H_2O$) to yield N-(1-(2-acetamidoethyl)-2-tert-butyl-6-fluoro-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.35 (s, 1H), 8.15 (t, J=5.9 Hz, 1H), 7.53 (s, 1H), 7.43-7.31 (m, 4H), 6.17 (s, 1H), 4.22 (m, 2H), 3.30 (m, 2H), 1.85 (s, 3H), 1.47 (m, 2H), 1.41 (s, 9H), 1.13 (m, 2H). HPLC ret. time 2.06 min, 10-99% $CH_3CN$, 3 min run; ESI-MS 516.4 m/z ($M+H^+$).

Example 96

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-hydroxy-3-methoxy-propyl)-1H-indol-5-yl)cyclopropenecarboxamide

**[0706]**

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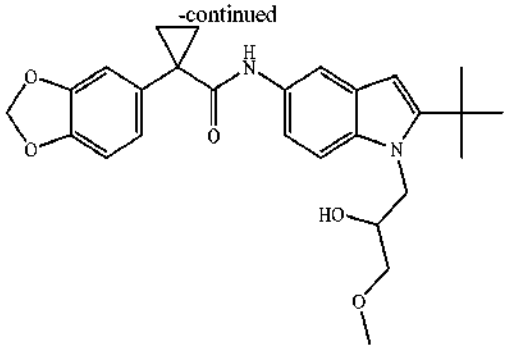
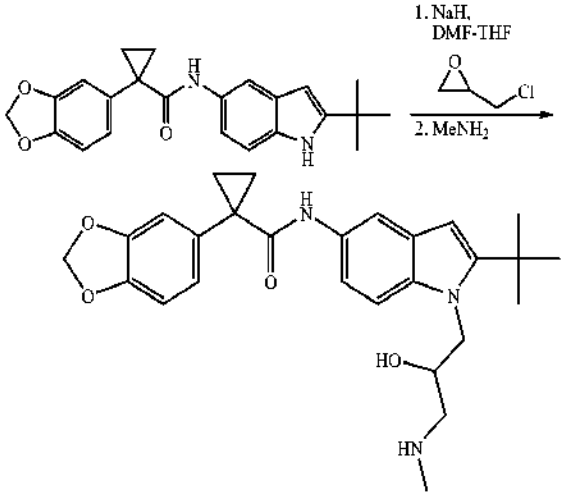


**[0707]** 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclopropanecarboxamide (320 mg, 0.84 mmol) was dissolved in a mixture composed of anhydrous DMF (0.5 mL) and anhydrous THF (5 mL) under $N_2$. NaH (60% in mineral oil, 120 mg, 3.0 mmol) was added at room temperature. After 30 min of stirring, the reaction mixture was cooled to −15° C. before a solution of epichlorohydrin (79 μL, 1.0 mmol) in anhydrous DMF (1 mL) was added dropwise. The reaction mixture was stirred for 15 min at −15° C., then for 8 h at room temperature. MeOH (1 mL) was added and the mixture was heated for 10 min at 105° C. in the microwave oven. The mixture was cooled, filtered and purified by preparative HPLC to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-hydroxy-3-methoxy-propyl)-1H-indol-5-yl) cyclopropanecarboxamide. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.44 (s, 1H), 7.59 (d, J=1.9 Hz, 1H), 7.31 (d, J=8.9 Hz, 1H), 7.03 (dd, J=8.7, 1.9 Hz, 2H), 6.95 (dd, J=8.0, 1.7 Hz, 1H), 6.90 (d, J=8.0 Hz, 1H), 6.16 (s, 1H), 6.03 (s, 2H), 4.33 (dd, J=15.0, 4.0 Hz, 1H), 4.19 (dd, J=15.0, 8.1 Hz, 1H), 4.02 (ddd, J=8.7, 4.8 Hz, 1H), 3.41-3.32 (m, 2H), 3.30 (s, 3H), 1.41 (s, 9H), 1.41-1.38 (m, 2H), 1.03 (dd, J=6.7, 4.0 Hz, 2H). MS (ESI) m/e ($M+H^+$) 465.0.

Example 97

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-hydroxy-3-(methylamino)propyl)-1H-indol-5-yl) cyclopropanecarboxamide

**[0708]**



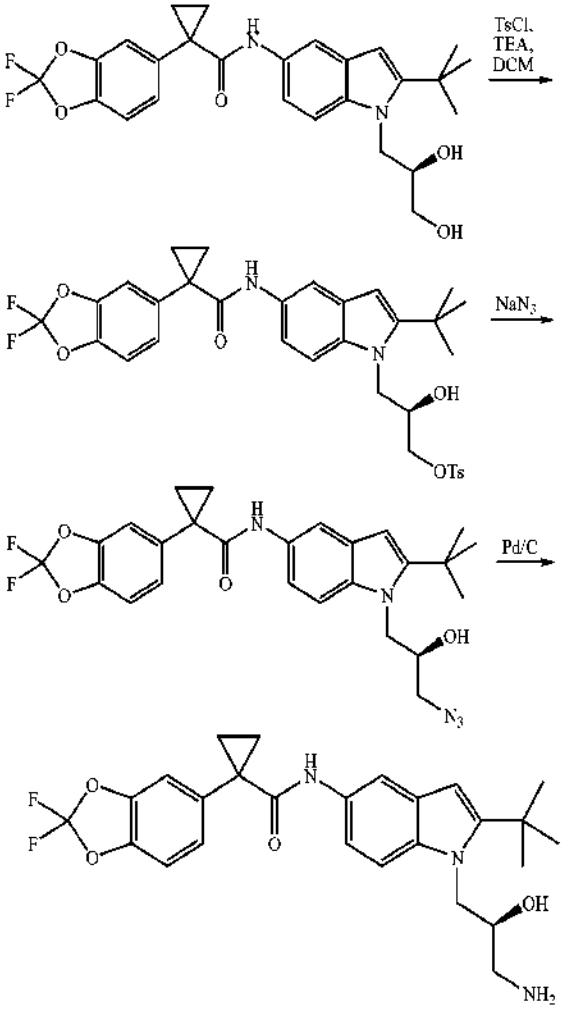

**[0709]** 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclopropanecarboxamide (320 mg, 0.84 mmol)

was dissolved in a mixture composed of anhydrous DMF (0.5 mL) and anhydrous THF (5 mL) under $N_2$. NaH (60% in mineral oil, 120 mg, 3.0 mmol) was added at room temperature. After 30 min of stirring, the reaction mixture was cooled to −15° C. before a solution of epichlorohydrin (79 μL, 1.0 mmol) in anhydrous DMF (1 mL) was added dropwise. The reaction mixture was stirred for 15 min at −15° C., then for 8 h at room temperature. $MeNH_2$ (2.0 M in MeOH, 1.0 mL) was added and the mixture was heated for 10 min at 105° C. in the microwave oven. The mixture was cooled, filtered and purified by preparative HPLC to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-hydroxy-3-(methylamino)propyl)-1H-indol-5-yl)cyclopropanecarboxamide. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.50 (s, 1H), 7.60-7.59 (m, 1H), 7.35 (dd, J=14.3, 8.9 Hz, 1H), 7.10 (d, J=8.8 Hz, 1H), 1H), 6.94 (dd, J=8.0, 1.6 Hz, 1H), 6.91 (d, J=7.9 Hz, 1H), 6.20 (d, J=2.3 Hz, 1H), 6.03 (s, 2H), 2.82 (d, J=4.7 Hz, 1H), 2.72 (d, J=4.7 Hz, 1H), 2.55 (dd, J=5.2, 5.2 Hz, 1H), 2.50 (s, 3H), 1.43 (s, 9H), 1.39 (dd, J=6.4, 3.7 Hz, 2H), 1.04 (dd, J=6.5, 3.9 Hz, 2H). MS (ESI) m/e ($M+H^+$) 464.0.

Example 98

(S)—N-(1-(3-Amino-2-hydroxypropyl)-2-tert-butyl-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0710]**

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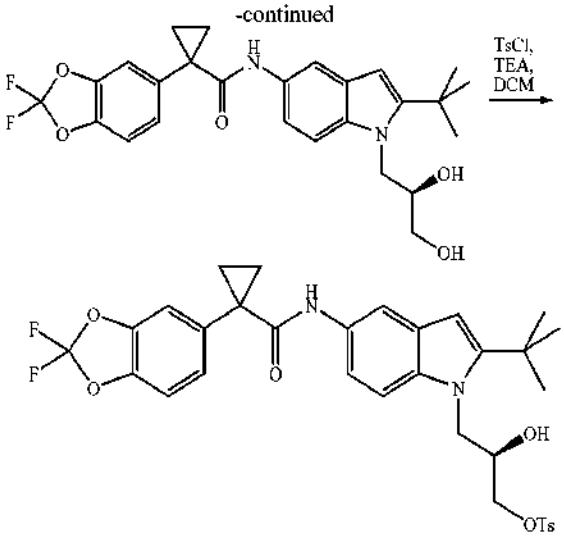
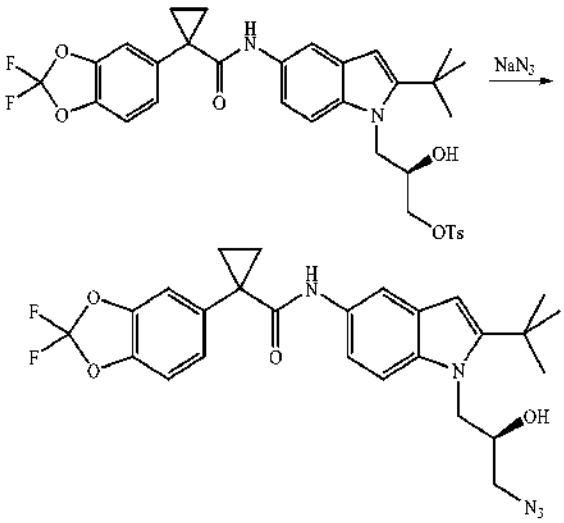


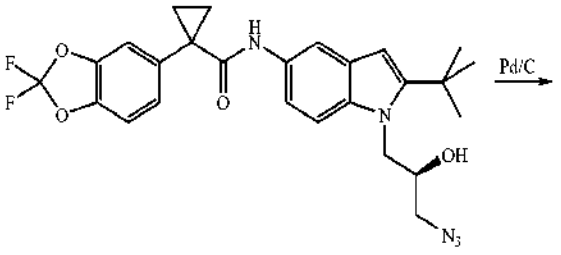

(R)-3-(2-tert-Butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbox-amido)-1H-indol-1-yl)-2-hydroxypropyl-4-methylbenzenesulfonate

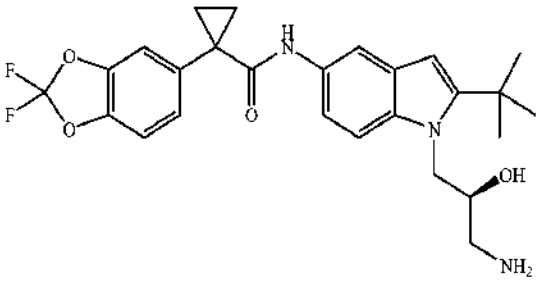
**[0711]** To a stirred solution of (R)—N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluoro-benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (3.0 g, 6.1 mmol) in dichloromethane (20 mL) was added triethylamine (2 mL) and para-toluenesulfonylchloride (1.3 g, 7.0 mmol). After 18 hours, the reaction mixture was partitioned between 10 mL of water and 10 mL of ethyl acetate. The organic layer was dried over magnesium sulfate, filtered and evaporated. The residue was purified using column chromatography on silica gel (0-60% ethyl acetate/hexane) providing (R)-3-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-hydroxypropyl-4-methyl-benzenesulfonate (3.21 g, 86%). LC/MS (M+1) =641.2. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 2H, J=16 Hz), 7.55 (d, 1H, J=2 Hz), 7.35 (d, 2H, J=16 Hz), 7.31 (m, 3H), 6.96 (s, 1H), 6.94 (dd, 1H, J=2, 8 Hz), 6.22 (s, 1H), 4.33 (m, 1H), 4.31 (dd, 1H, J=6, 15 Hz), 4.28 (dd, 1H, J=11, 15 Hz), 4.18 (m, 1H), 3.40 (dd, 1H, J=3, 6 Hz), 3.36 (dd, 1H, J=3, 6 Hz), 2.46 (s, 3H), 2.40 (brs, 1H), 1.74 (m, 2H), 1.40 (s, 9H), 1.11 (m, 2H).



(R)—N-(1-(3-Azido-2-hydroxypropyl)-2-tert-butyl-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0712]** To a stirred solution (R)-3-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-hydroxypropyl-4-methylbenzenesulfonate (3.2 g, 5.0 mmol) in DMF (6 mL) was added sodium azide (2.0 g, 30 mmol). The reaction was heated at 80° C. for 2 h. The mixture was partitioned between 20 mL ethyl acetate and 20 mL water. The layers were separated and the organic layer was evaporated. The residue was purified using column chromatography (0-85% ethyl acetate/hexane) to give (R)—N-(1-(3-azido-2-hydroxypropyl)-2-tert-butyl-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-cyclopropanecarboxamide (2.48 g). LC/MS (M+1)=512.5. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, 1H, J=2 Hz), 7.31 (m, 3H), 6.96 (s, 1H), 6.94 (dd, 1H, J=2, 8 Hz), 6.22 (s, 1H), 4.33 (m, 1H), 4.31 (dd, 1H, J=6, 15 Hz), 4.28 (dd, 1H, J=11, 15 Hz), 4.18 (m, 1H), 3.40 (dd, 1H, J=3, 6 Hz), 3.36 (dd, 1H, J=3, 6 Hz), 2.40 (br s, 1H), 1.74 (m, 2H), 1.40 (s, 9H), 1.11 (m, 2H).



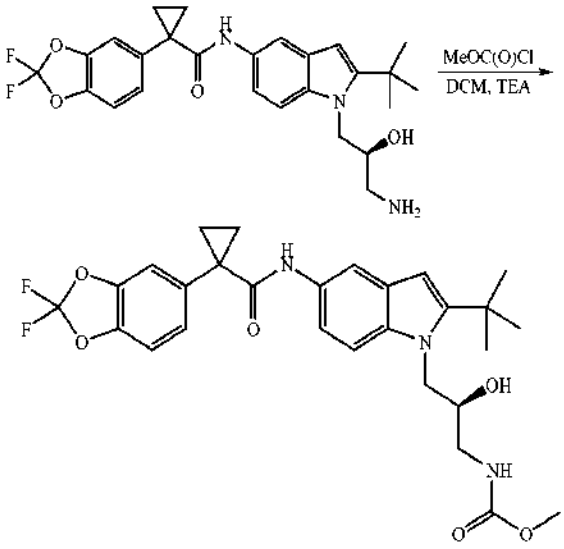

(S)—N-(1-(3-Amino-2-hydroxypropyl)-2-tert-butyl-1H-indol-5-yl)-1-(2,2-difluoro-benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0713]** To a stirred solution (R)—N-(1-(3-azido-2-hydroxypropyl)-2-tert-butyl-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (2.4 g, 4.0 mmol) in MeOH (25 mL) was added 5% Pd/C (2.4 g) under a Hydrogen gas filled balloon. After 18 h, the reaction mixture was filtered through celite and rinsed with 300 mL ethyl acetate. The organic layer was washed with 1 N HCl and evaporated to give (S)—N-(1-(3-amino-2-hydroxypropyl)-2-tert-butyl-1H-indol-5-yl)-1-(2,2-difluoro-benzo[d][1,3]-dioxol-5-yl)cyclopropane-carboxamide (1.37 g). MS (M+1) =486.5.

Example 99

(S)-Methyl 3-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-hydroxypropylcarbamate

**[0714]**

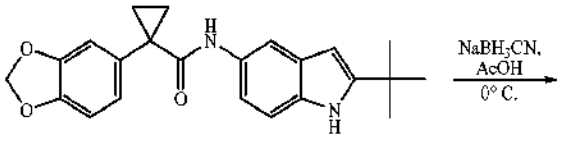



**[0715]** To a stirred solution (R)—N-(1-(3-amino-2-hydroxypropyl)-2-tert-butyl-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (0.10 g, 0.20 mmol) in methanol (1 mL) was added 2 drops of triethylamine and methylchloroformyl chloride (0.020 mL, 0.25 mmol). After 30 min. the reaction mixture was filtered and purified using reverse phase HPLC providing (S)-methyl 3-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclo-propanecarboxamido)-1H-indol-1-yl)-2-hydroxypropylcarbamate. The retention time on a three minute run is 1.40 min. LC/MS (M+1)=544.3. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, 1H, J=2 Hz), 7.30 (dd, 1H, J=2, 8 Hz), 7.28 (m, 1H), 7.22 (d, 1H, J=8 Hz), 7.14 (d, 1H, J=8 Hz), 7.04 (br s, 1H), 6.97 (dd, 1H, J=2, 8 Hz), 6.24 (s, 1H), 5.19 (1H, br s), 4.31 (dd, 1H, J=6, 15 Hz), 4.28 (dd, 1H, J=11, 15 Hz), 4.18 (m, 1H), 3.70 (s, 3H), 3.40 (dd, 1H, J=3, 6 Hz), 3.36 (dd, 1H, J=3, 6 Hz), 3.26 (m, 1H), 1.74 (m, 2H), 1.40 (s, 9H), 1.11 (m, 2H).

Example 100

4-(5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butyl-1H-indol-1-yl)butanoic acid

**[0716]**



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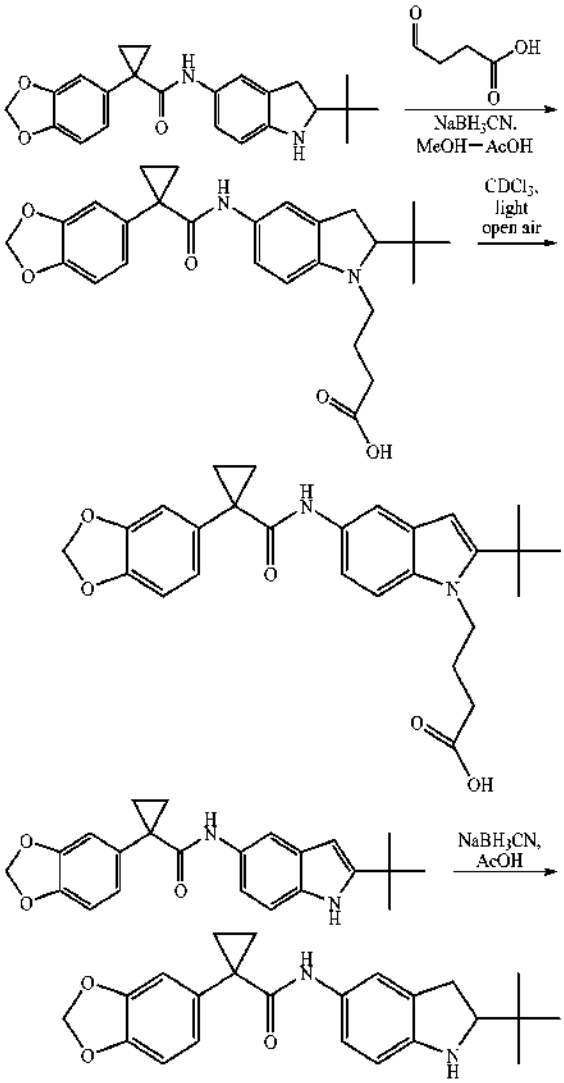
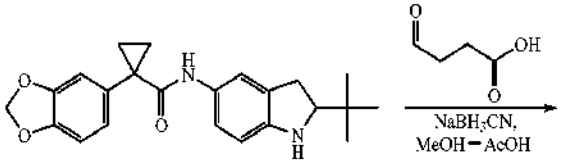



1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butylindolin-5-yl)cyclopropanecarboxamide

**[0717]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1H-indol-5-yl)cyclo-propanecarboxamide (851 mg, 2.26 mmol) in acetic acid (60 mL) was added $NaBH_3CN$ (309 mg, 4.91 mmol) at 0° C. The reaction mixture was stirred for 5 min at room temperature after which no starting material could be detected by LCMS. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (5-40% ethyl acetate/hexanes) to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butylindolin-5-yl)cyclopropanecarboxamide (760 mg, 89%).

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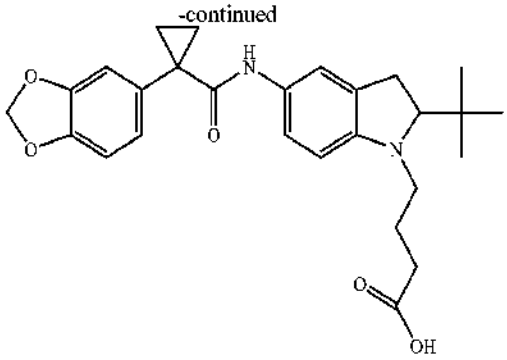
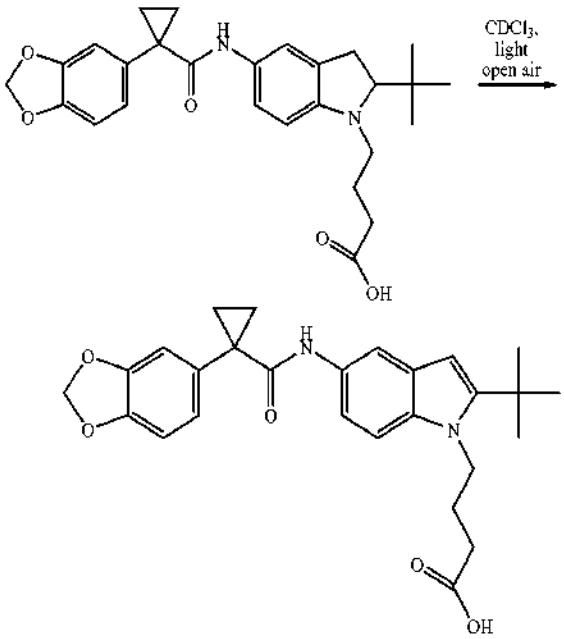


4-(5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butylindolin-1-yl)butanoic acid

**[0718]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butylindolin-5-yl)cyclopropanecarboxamide (350 mg, 0.93 mmol, 1 eq) in anhydrous methanol (6.5 mL) and AcOH (65 μL) was added 4-oxobutanoic acid (15% in water, 710 mg, 1.0 mmol) at room temperature. After 20 min of stirring, $NaBH_3CN$ (130 mg, 2.0 mmol) was added in one portion and the reaction mixture was stirred for another 4 h at room temperature. The reaction mixture was quenched by addition of AcOH (0.5 mL) at 0° C. and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (5-75% ethyl acetate/hexanes) to give 4-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butylindolin-1-yl)butanoic acid (130 mg, 30%).




4-(5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butyl-1H-indol-1-yl)butanoic acid

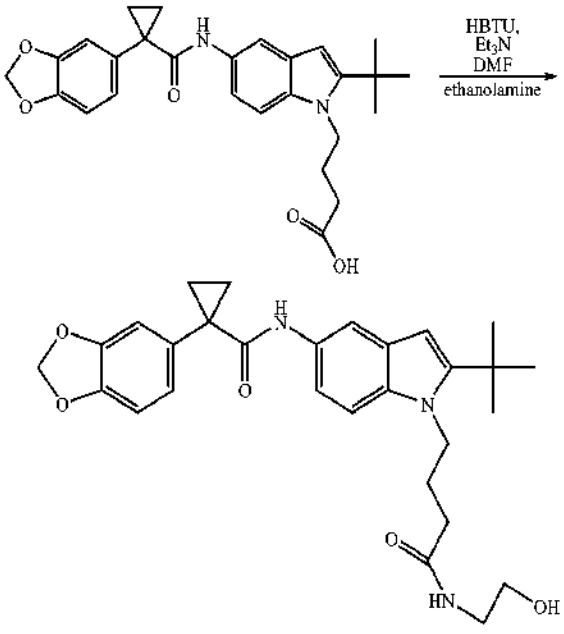
**[0719]** 4-(5-(1-(Benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butylindolin-1-yl)butanoic acid (130

mg, 0.28 mmol) was taken up in a mixture of acetonitrile-$H_2O$-TFA. The solvent was removed under reduced pressure and the residue obtained was dissolved in $CDCl_3$. After a brief exposition to daylight (5-10 min), the solution turned purple. The mixture was stirred open to the atmosphere at room temperature until complete disappearance of the starting material (8 h). Solvent was removed under reduced pressure and the residue was purified by reverse phase HPLC to give 4-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-2-tert-butyl-1H-indol-1-yl)butanoic acid. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J=1.9 Hz, 1H), 7.18 (d, J=2.1 Hz, 1H), 7.16 (s, 1H), 7.03 (dd, J=9.4, 1.9 Hz, 1H), 7.00-6.98 (m, 2H), 6.85 (d, J=7.9 Hz, 1H), 6.16 (s, 1H), 6.02 (s, 2H), 4.29-4.24 (m, 2H), 2.48 (dd, J=6.9, 6.9 Hz, 2H), 2.12-2.04 (m, 2H), 1.69 (dd, J=6.8, 3.7 Hz, 2H), 1.43 (s, 9H), 1.09 (dd, J=6.8, 3.7 Hz, 2H). MS (ESI) m/e (M+H$^+$) 463.0.

Example 101

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(4-(2-hydroxyethyl-amino)-4-oxobutyl)-1H-indol-5-yl) cyclopropanecarboxamide

**[0720]**

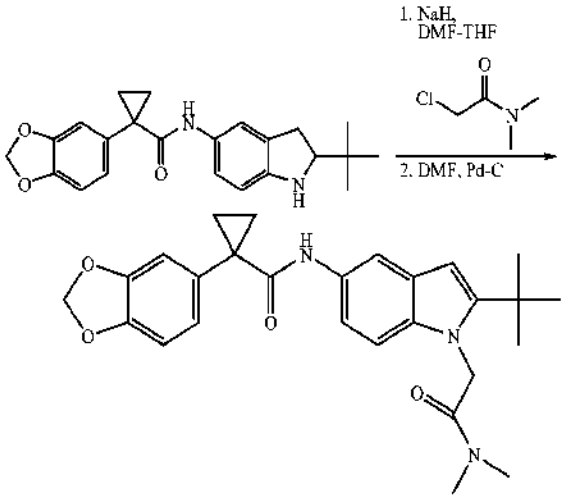



**[0721]** To a solution of 4-(5-(1-(benzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamido)-2-tert-butyl-1H-indol-1-yl)butanoic acid (10 mg) in anhydrous DMF (0.25 mL) were successively added $Et_3N$ (9.5 mL, 0.069 mmol) and HBTU (8.2 mg, 0.022 mmol). After stirring for 10 min at 60° C., ethanolamine (1.3 μL, 0.022 mmol) was added, and the mixture was stirred for another 4 h at 60° C. 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(4-(2-hydroxyethyl-amino)-4-oxobutyl)-1H-indol-5-yl)cyclopropanecarboxamide (5.8 mg, 64%) was obtained after purification by preparative HPLC. MS (ESI) m/e (M+H$^+$) 506.0.

Example 102

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-(dimethylamino)-2-oxoethyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0722]**

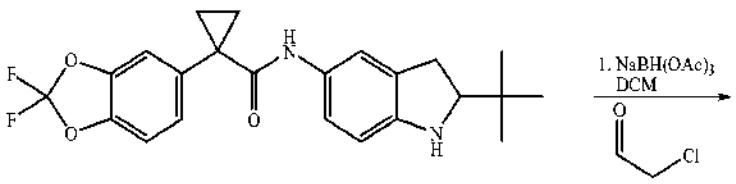



**[0723]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butylindolin-5-yl)cyclopropanecarboxamide (62 mg, 0.16 mmol) in anhydrous DMF (0.11 mL) and THF (1 mL) was added NaH (60% in mineral oil, 21 mg, 0.51 mmol) at room temperature under $N_2$. After 30 min of stirring, the reaction mixture was cooled to 0° C. and 2-chloro-N,N-dimethylacetamide (11 mL, 0.14 mmol) was added. The reaction mixture was stirred for 5 min at 0° C. and then for 10 h at room temperature. The mixture was purified by preparative HPLC and the resultant solid was dissolved in DMF (0.6 mL) in the presence of Pd—C (10 mg). The mixture was stirred open to the atmosphere overnight at room temperature. The reaction mixture was filtrated and purified by preparative HPLC providing 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-(dimethylamino)-2-oxoethyl)-1H-indol-5-yl)cyclopropanecarboxamide. MS (ESI) m/e (M+H$^+$) 462.0.

Example 103

3-(2-tert-Butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclo-propanecarboxamido)-1H-indol-1-yl)propanoic acid

**[0724]**



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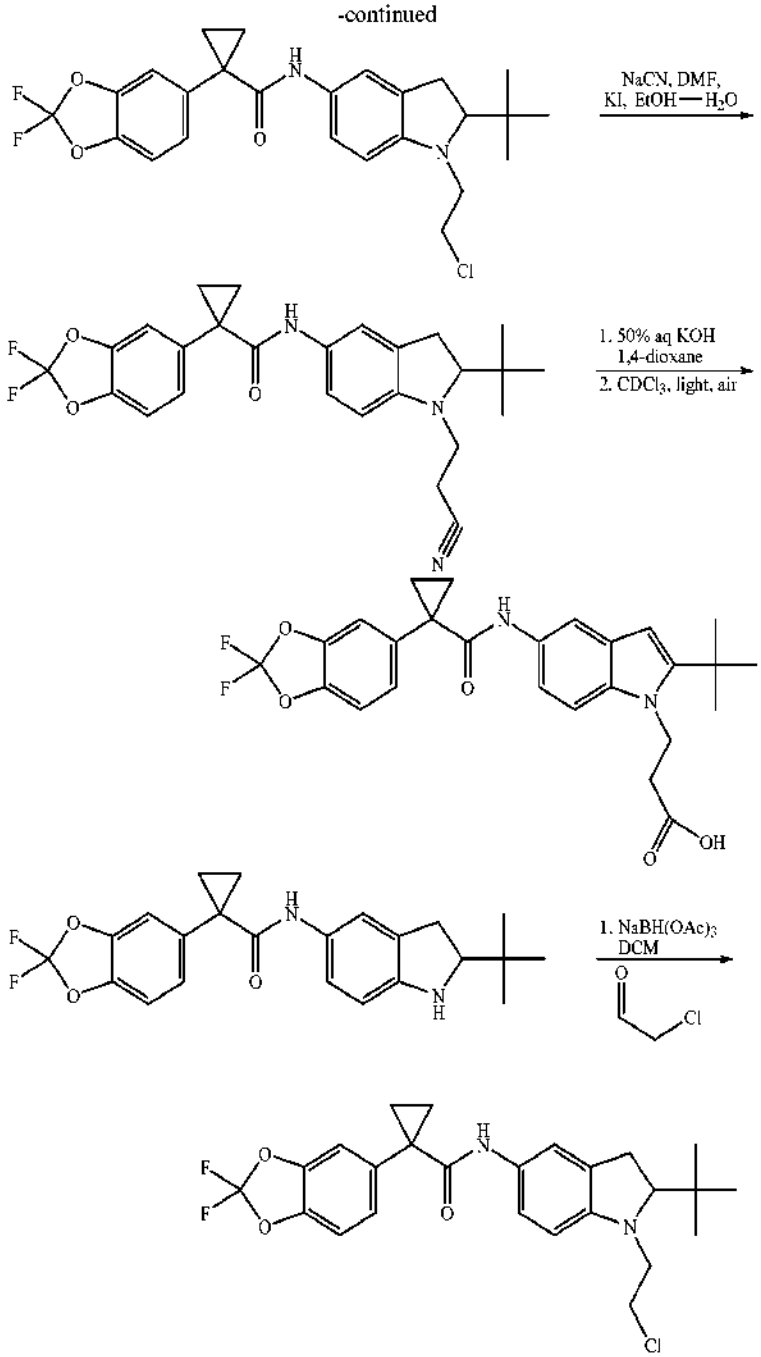
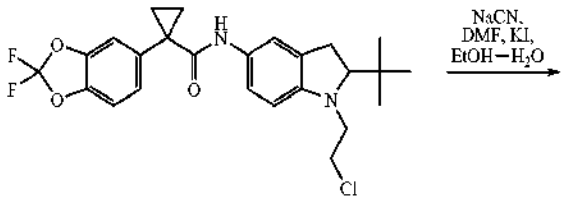



N-(2-tert-Butyl-1-(2-chloroethyl)indolin-5-yl)-1-(2, 2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0725]** To a solution of N-(2-tert-butyl-1-(2-cyanoethyl)indolin-5-yl)-1-(2,2-difluorobenzo[d][1.3]dioxol-5-yl)cyclopropanecarboxamide (71 mg, 0.17 mmol) in anhydrous dichloromethane (1 mL) was added chloroacetaldehyde (53 μL, 0.41 mmol) at room temperature under $N_2$. After 20 min of stirring, $NaBH(OAc)_3$ (90 mg, 0.42 mmol) was added in two portions. The reaction mixture was stirred overnight at room temperature. The product was purified by column chromatography on silica gel (2-15% ethyl acetate/hexanes) providing N-(2-tert-butyl-1-(2-chloroethyl)indolin-5-yl)-1-(2, 2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamide (51 mg, 63%).



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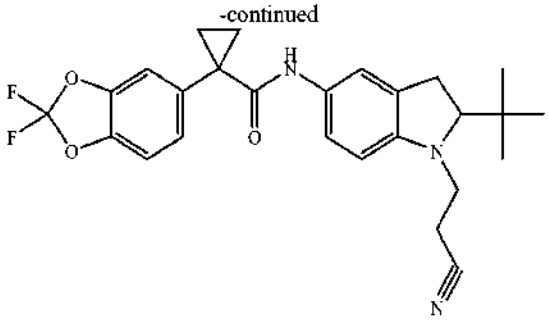
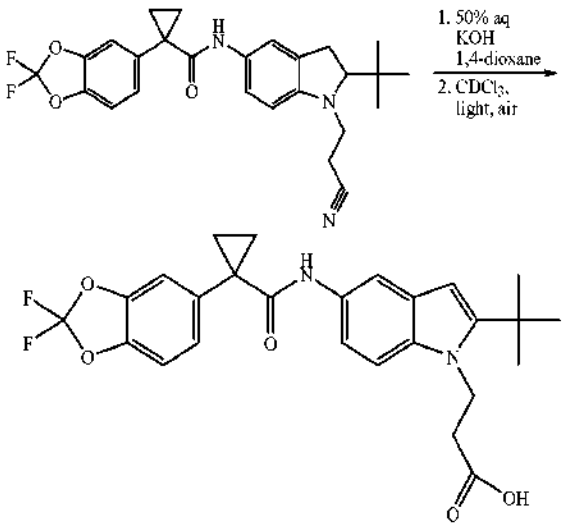


N-(2-tert-Butyl-1-(2-cyanoethyl)indolin-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0726]** N-(2-tert-butyl-1-(2-chloroethyl)indolin-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (51 mg), NaCN (16 mg, 0.32 mmol) and KI (cat) in EtOH (0.6 mL) and water (0.3 mL) were combined and heated at 110° C. for 30 min in the microwave. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (2-15% ethyl acetate/hexanes) providing N-(2-tert-butyl-1-(2-cyanoethyl)indolin-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (24 mg, 48%).



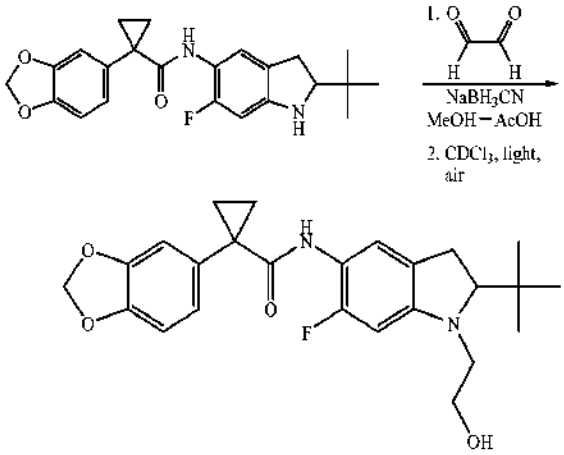

3-(2-tert-Butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclo-propanecarboxamido)-1H-indol-1-yl)propanoic acid

**[0727]** N-(2-tert-butyl-1-(2-cyanoethyl)indolin-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropane-carboxamide (24 mg, 0.050 mmol) was taken up in 50% aq. KOH (0.5 mL) and 1,4-dioxane (1 mL). The mixture was heated at 125° C. for 2 h. The solvent was removed and the residue was purified by preparative HPLC. The residue was dissolved in $CDCl_3$ (1 mL) then briefly exposed to daylight. The purple solution that formed was stirred until complete disappearance of the starting material (1 h). The solvent was removed under reduced pressure and the residue was purified by preparative HPLC providing 3-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclo-propanecarboxamido)-1H-indol-1-yl)propanoic acid. MS (ESI) m/e ($M+H^+$) 485.0.

Example 104

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoro-1-(2-hydroxyethyl)-1H-indol-5-yl)cyclopropenecarboxamide

**[0728]**

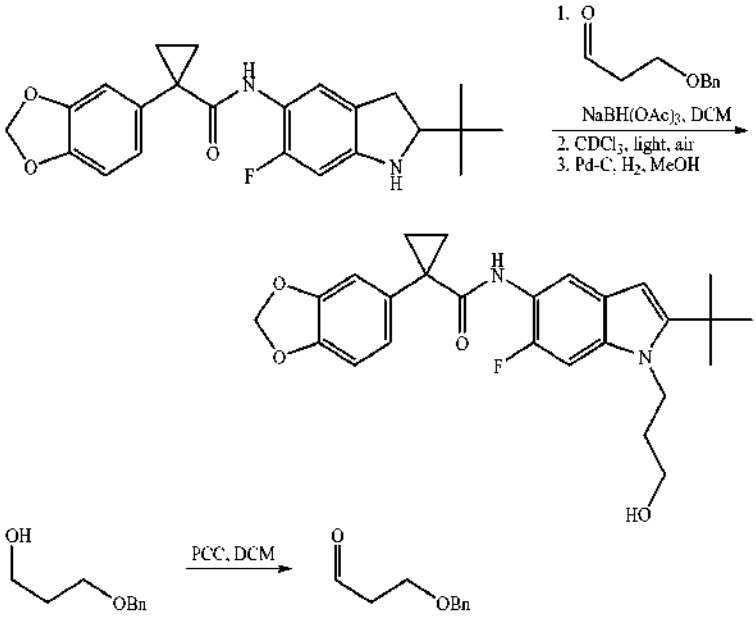



**[0729]** To a solution of 1-(benzo[d][1.3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoroindolin-5-yl)cyclopropanecarboxamide (340 mg, 0.86 mmol) in anhydrous MeOH (5.7 mL) containing 1% of acetic acid was added glyoxal 40% in water (0.60 mL, 5.2 mmol) at room temperature under $N_2$. After 20 min of stirring, $NaBH_3CN$ (120 mg, 1.9 mmol) was added in one portion and the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue obtained was purified by column chromatography on silica gel (10-40% ethyl acetate/hexanes) providing a pale yellow oil which was treated with 50/50 $CH_3CN$—$H_2O$ containing 0.05% TFA and $CDCl_3$. Solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (20-35% ethyl acetate/hexanes) to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoro-1-(2-hydroxyethyl)-1H-indol-5-yl) cyclopropanecarboxamide. $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J=7.7 Hz, 1H), 7.30 (d, J=2.1 Hz, 1H), 6.93 (dd, J=1.6, 7.9 Hz, 1H), 6.90 (d, J=1.6 Hz, 1H), 6.90 (d, J=1.6 Hz, 1H), 6.78 (d, J=7.9 Hz, 1H), 6.08 (s, 1H), 5.92 (s, 2H), 4.21 (dd, J=6.9, 6.9 Hz, 2H), 3.68 (m, 2H), 2.28 (s, 1H), 1.60 (dd, J=3.7, 6.7 Hz, 2H), 1.35-1.32 (m, 9H), 1.04 (dd, J=3.7, 6.8 Hz, 2H). MS (ESI) m/e ($M+H^+$) 439.0.

Example 105

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoro-1-(3-hydroxypropyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0730]**

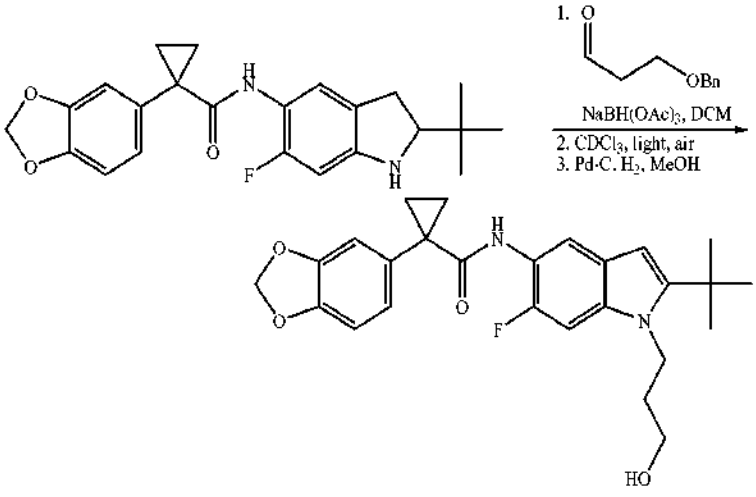



3-(Benzyloxy)propanal

**[0731]** To a suspension of PCC (606 mg, 2.82 mmol) in anhydrous dichloromethane (8 mL) at room temperature under $N_2$ was added a solution of 3-benzyloxy-1-propanol (310 mg, 1.88 mmol) in anhydrous dichloromethane. The reaction mixture was stirred overnight at room temperature, filtrated through Celite, and concentrated. The residue was purified by column chromatography on silica gel (1-10% ethyl acetate/hexanes) to give 3-(benzyloxy)propanal (243 mg, 79%).

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoro-1-(3-hydroxypropyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0732]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoroindolin-5-yl)cyclopropanecarboxamide (160 mg, 0.50 mmol) in anhydrous dichloromethane (3.4 mL) was added 3-(benzyloxy)propanal (160 mg, 0.98 mmol) at room temperature. After 10 min of stirring, $NaBH(OAc)_3$ (140 mg, 0.65 mmol) was added in one portion and the reaction mixture was stirred for 4 h at room temperature. The solvent was removed under reduced pressure and the residue was taken-up in a mixture of 50/50 $CH_3CN$—$H_2O$ containing 0.05% TFA. The mixture was concentrated to dryness and the residue was dissolved in $CDCl_3$ (5 mL) and briefly exposed to daylight. The purple solution was stirred open to the atmosphere at room temperature for 2 h. The solvent was removed

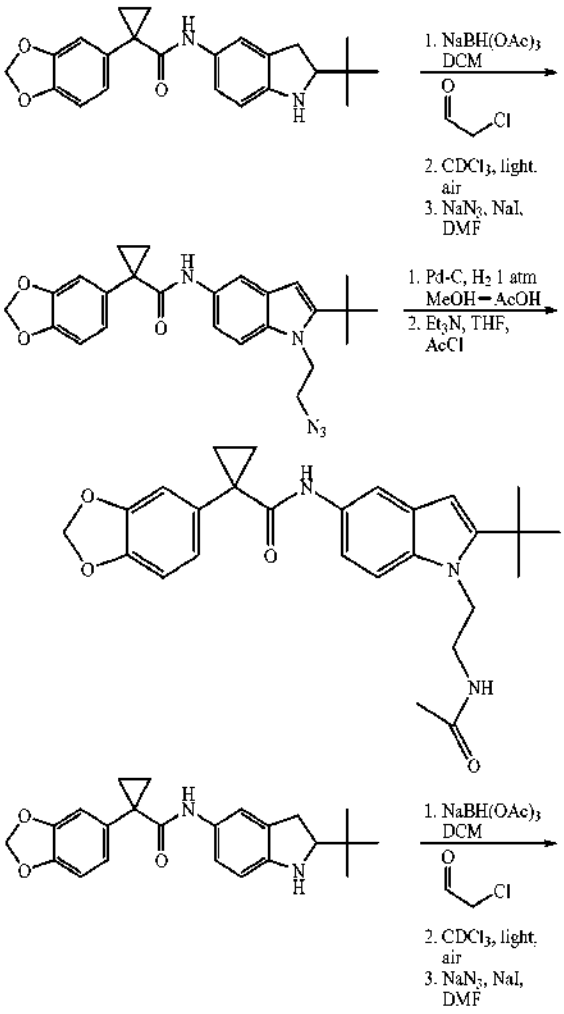


under reduced pressure and the residue was treated with Pd—C (10 mg) in MeOH (2 mL) under 1 atm of $H_2$ for 2 h. The catalyst was filtered through Celite and the solvent was removed under reduced pressure. The residue was purified by preparative TLC 30% ethyl acetate/hexanes to provide 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoro-1-(3-hydroxypropyl)-1H-indol-5-yl)cyclopropanecarboxamide (18 mg, 8% from 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoroindolin-5-yl)cyclopropane-carboxamide). $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J=7.8 Hz, 1H), 7.31 (d, J=2.2 Hz, 1H), 6.94 (dd, J=7.9, 1.7 Hz, 1H), 6.91 (d, J=1.6 Hz, 1H), 6.85 (d, J=11.7 Hz, 1H), 6.79 (d, J=7.9 Hz, 1H), 6.10 (s, 1H), 5.94 (s, 2H), 4.25-4.21 (m, 2H), 3.70 (dd, J=5.7, 5.7 Hz, 2H), 1.93-1.86 (m, 2H), 1.61 (dd, J=6.8, 3.7 Hz, 2H), 1.35 (s, 9H), 1.04 (dd, J=6.8, 3.7 Hz, 2H). MS (ESI) m/e ($M+H^+$) 453.0.

Example 106

N-(1-(2-Acetamidoethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamide

**[0733]**

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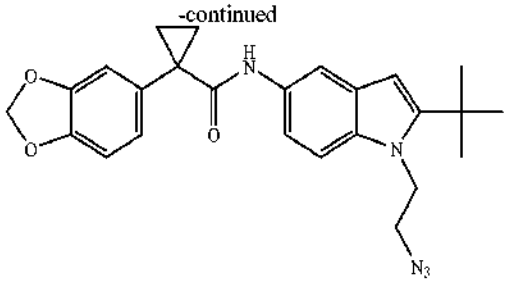
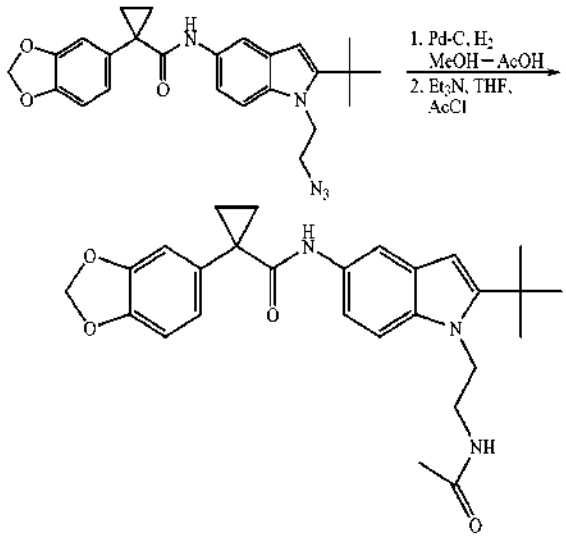


N-(1-(2-azidoethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)-cyclopropanecarboxamide

**[0734]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butylindolin-5-yl)cyclopropane-carboxamide (73 mg, 0.19 mmol) in anhydrous dichloromethane (1.2 mL) was added chloroacetaldehyde (60 μL, 0.24 mmol) at room temperature. After 10 min of stirring, $NaBH(OAc)_3$ (52 mg, 0.24 mmol) was added in one portion and the reaction mixture was stirred for another 30 min at room temperature. The solvent was removed under reduced pressure and the residue was purified by preparative HPLC to give the indoline, which oxidized to the corresponding indole when taken-up in $CDCl_3$. The resulting indole was treated with $NaN_3$ (58 mg, 0.89 mmol) and NaI (cat) in anhydrous DMF (0.8 mL) for 2 h at 85° C. The reaction mixture was purified by preparative HPLC providing N-(1-(2-azidoethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (15 mg, 18% from 1-(benzo[d][1.3]dioxol-5-yl)-N-(2-tert-butylindolin-5-yl)cyclopropane-carboxamide).




N-(1-(2-Acetamidoethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamide

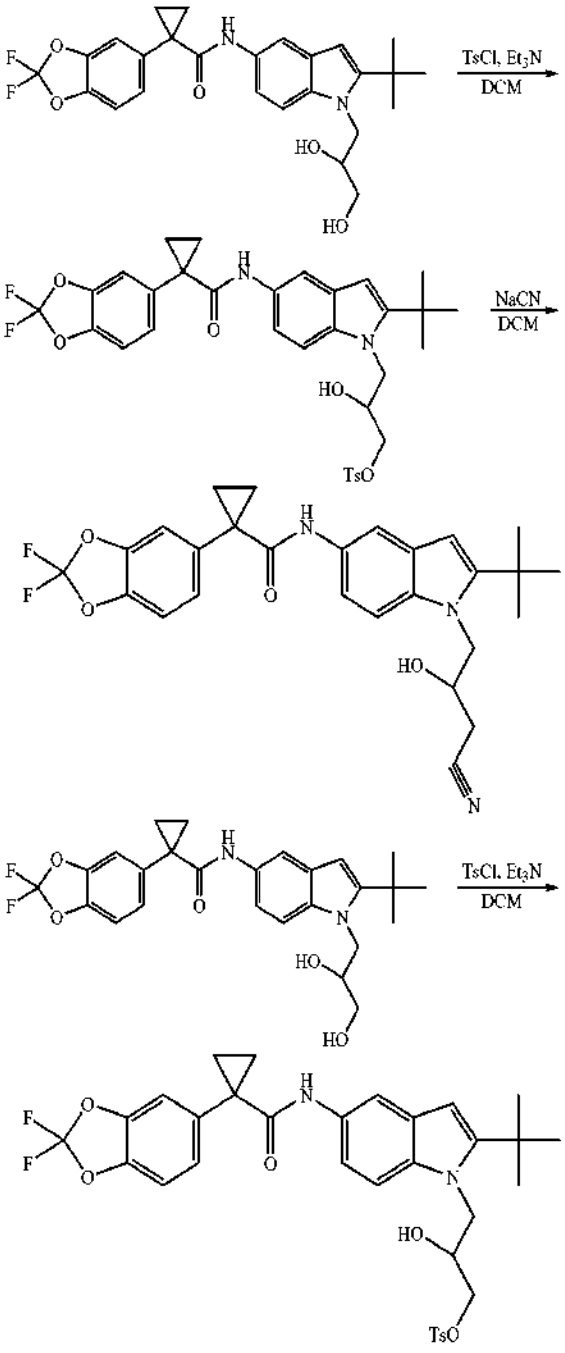
**[0735]** A solution of N-(1-(2-azidoethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (13 mg, 0.029 mmol) in MeOH-AcOH (0.2 mL, 99:1) in the presence of Pd—C (2 mg) was stirred at room

temperature under 1 atm of $H_2$ for 2 h, filtered through Celite, and concentrated under reduced pressure. The crude product was treated with AcCl (0.05 mL) and $Et_3N$ (0.05 mL) in anhydrous THF (0.2 mL) at 0° C. for 30 min and then 1 h at room temperature. The mixture was purified by preparative HPLC providing N-(1-(2-acetamidoethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamide. MS (ESI) m/e ($M+H^+$) 462.0.

Example 107

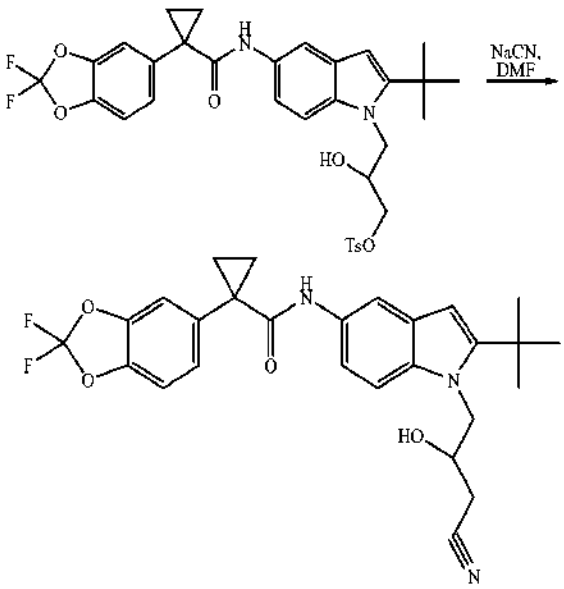
N-(2-tert-Butyl-1-(3-cyano-2-hydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamide

**[0736]**




3-(2-tert-Butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbox-amido)-1H-indol-1-yl)-2-hydroxypropyl-4-methylbenzenesulfonate

**[0737]** To a solution of N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamide (172 mg, 0.35 mmol) in anhydrous dichloromethane (1.4 mL) at 0° C. in the presence of $Et_3N$ (56 μL, 0.40 mmol) was added TsCl (71 mg, 0.37 mmol). The reaction mixture was stirred for 2 h at room temperature before being cooled to 0° C. and another portion of TsCl (71 mg, 0.37 mmol) was added. After 1 h of stirring at room temperature, the mixture was purified by column chromatography on silica gel (10-30% ethyl acetate/hexanes) providing 3-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-2-hydroxypropyl-4-methylbenzene-sulfonate (146 mg, 64%).




N-(2-tert-Butyl-1-(3-cyano-2-hydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamide

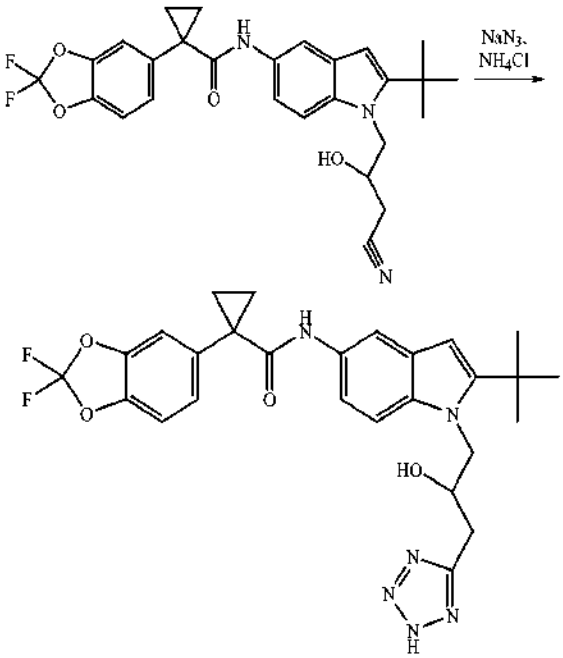
**[0738]** N-(2-tert-Butyl-1-(3-cyano-2-hydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-cyclopropanecarboxamide (145 mg, 0.226 mmol) was treated with powdered NaCN (34 mg, 0.69 mmol) in anhydrous DMF (1.5 mL) at 85° C. for 2 h. The reaction mixture was cooled down to room temperature before it was diluted with dichloromethane (10 mL) and aq. sat. $NaHCO_3$ (10 mL). The organic phase was separated and the aqueous phase was extracted with dichloromethane (2×10 mL). The organic phases were combined, washed with brine, dried with sodium sulfate, filtered then concentrated. The residue was purified by column chromatography on silica gel (25-55% ethyl acetate/hexanes) providing N-(2-tert-butyl-1-(3-cyano-2-hydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3] dioxol-5-yl)cyclopropanecarboxamide (89 mg, 79%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J=1.9 Hz, 1H), 7.20-7.16 (m, 2H), 7.08 (d, J=8.8 Hz, 1H), 7.04 (d, J=8.2 Hz, 1H), 6.94 (s, 1H), 6.88 (dd, J=8.7, 2.0 Hz, 1H), 6.16 (s, 1H), 4.32-4.19

(m, 3H), 2.83 (s, 1H), 2.40 (dd, J=5.2, 5.2 Hz, 2H), 1.62 (dd, J=6.6, 3.6 Hz, 2H), 1.35 (s, 9H), 1.04 (dd, J=6.9, 3.9 Hz, 2H). MS (ESI) m/e ($M+H^+$) 496.0.

Example 108

N-(2-tert-Butyl-1-(2-hydroxy-3-(2H-tetrazol-5-yl) propyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3] dioxol-5-yl)cyclopropanecarboxamide

**[0739]**

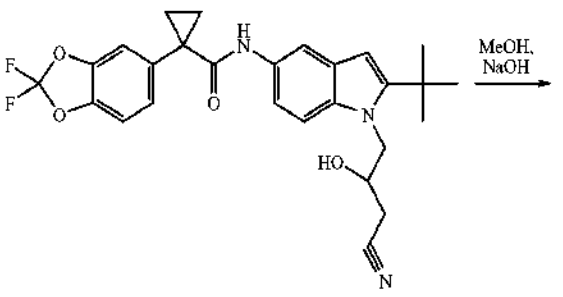



**[0740]** To a solution of N-(2-tert-butyl-1-(3-cyano-2-hydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3] dioxol-5-yl)cyclopropanecarboxamide (27 mg, 0.054 mmol) in anhydrous DMF (1.2 mL) were successively added $NH_4Cl$ (35 mg, 0.65 mmol) and $NaN_3$ (43 mg, 0.65 mmol) at room temperature. The reaction mixture was stirred for 4 h at 110° C. in the microwave, at which stage 50% of the starting material was converted to the desired product. The reaction mixture was purified by preparative HPLC to provide N-(2-tert-butyl-1-(2-hydroxy-3-(2H-tetrazol-5-yl)propyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo-[d][1,3]dioxol-5-yl)cyclopropanecarboxamide. MS (ESI) m/e ($M+H^+$) 539.0.

Example 109

4-(2-tert-Butyl-5-(1-(2,2-difluorobenzo[d][1,3]di-oxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-3-hydroxybutanoic acid

**[0741]**

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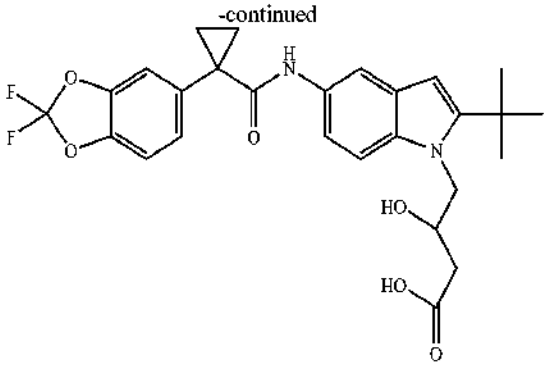
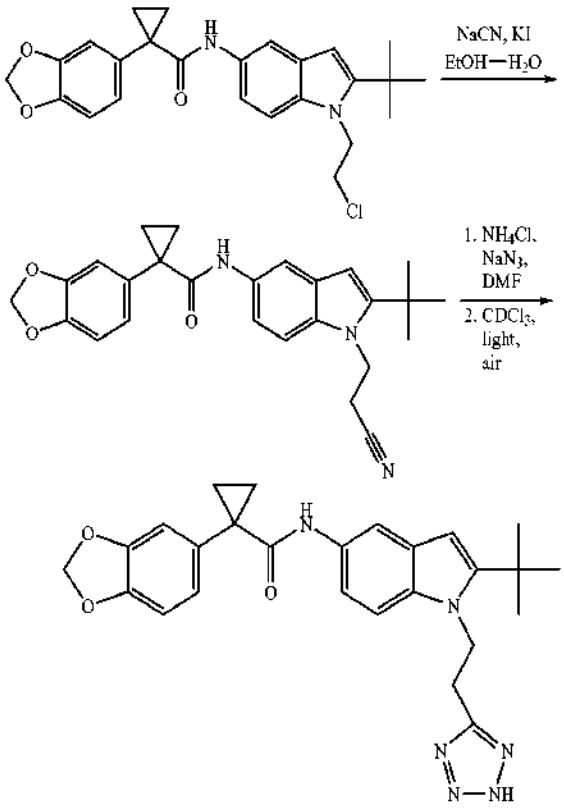


**[0742]** A solution of N-(2-tert-butyl-1-(3-cyano-2-hydroxypropyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (14 mg, 0.028 mmol) in methanol (0.8 mL) and 4 M NaOH (0.8 mL) was stirred at 60° C. for 4 h. The reaction mixture was neutralized with 4 M HCl and concentrated. The residue was purified by preparative HPLC to provide 4-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d] [1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-1-yl)-3-hydroxybutanoic acid. MS (ESI) m/e ($M+H^+$) 515.0.

Example 110

N-(1-(2-(2H-Tetrazol-5-yl)ethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopro-panecarboxamide

**[0743]**



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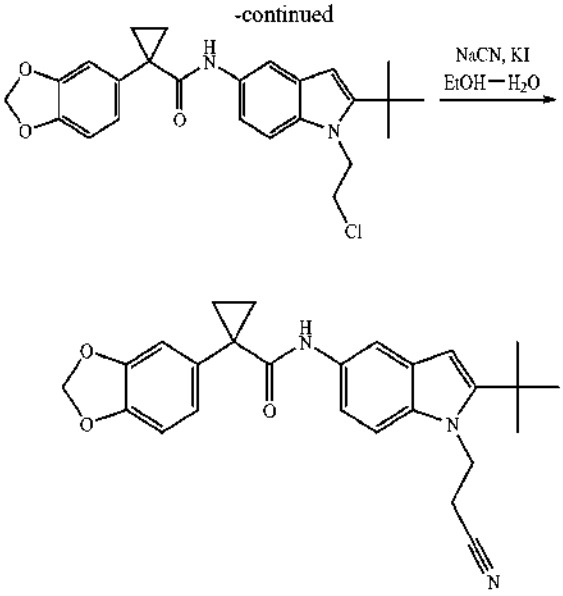
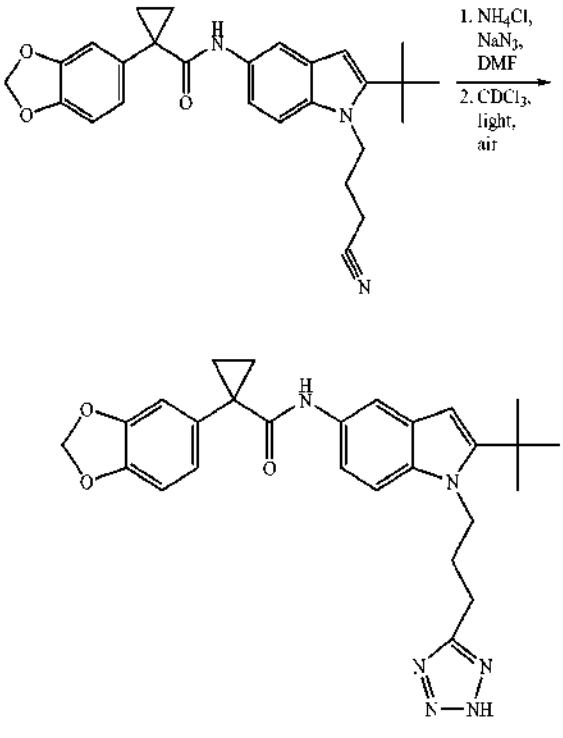



**[0744]** 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-cyanoethyl)indolin-5-yl)-cyclopropanecarboxamide

**[0745]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-chloroethyl)indolin-5-yl)cyclopropanecarboxamide (66 mg, 0.15 mmol) in ethanol (0.8 mL) and water (0.4 mL) were added NaCN (22 mg, 0.45 mmol) and KI (cat) at room temperature. The reaction mixture was stirred for 30 min at 110° C. in the microwave before being purified by column chromatography on silica gel (5-15% ethyl acetate/hexanes) to provide 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-cyano-ethyl)indolin-5-yl)cyclopropanecarboxamide (50 mg, 77%).



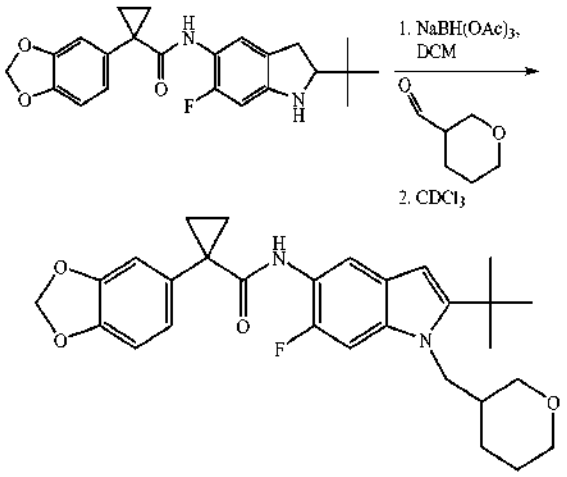

N-(1-(2-(2H-Tetrazol-5-yl)ethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0746]** To a solution of 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2-cyano-ethyl)indolin-5-yl)cyclopropanecarboxamide (50 mg, 0.12 mmol) in anhydrous DMF (2.6 mL) was added $NH_4Cl$ (230 mg, 4.3 mmol) and $NaN_3$ (280 mg, 4.3 mmol). The reaction mixture was stirred for 30 min at 110° C. in the microwave, filtrated, and purified by preparative HPLC. The solid residue was dissolved in $CDCl_3$ (3 mL) and briefly (2 to 4 min) exposed to daylight, which initiated a color change (purple). After 2 h of stirring open to the atmosphere at room temperature, the solvent was removed and the residue was purified by preparative HPLC to give N-(1-(2-(2H-tetrazol-5-yl)ethyl)-2-tert-butyl-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide. MS (ESI) m/e ($M+H^+$) 473.0.

Example 111

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoro-1-((tetrahydro-2H-pyran-3-yl)methyl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0747]**

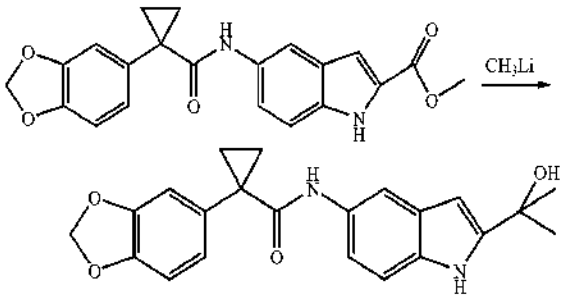



**[0748]** To a solution of 1-(benzo[d][1.3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoroindolin-5-yl)cyclopropane-carboxamide (150 mg, 0.38 mmol) in anhydrous dichloromethane (2.3 mL) at room temperature under $N_2$ was added tetrahydropyran-3-carbaldehyde (54 mg, 0.47 mmol). After 20 min of stirring, $NaBH(OAc)_3$ (110 mg, 0.51 mmol) was added in one portion at room temperature. The reaction mixture was stirred for 6 h at room temperature before being purified by column chromatography on silica gel (5-20% ethyl acetate/hexanes) to provide 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoro-1-((tetrahydro-2H-pyran-3-yl)methyl)indolin-5-yl) cyclopropanecarboxamide (95 mg, 50%). $CDCl_3$ was added to the indoline and the solution was allowed to stir overnight at ambient temperature. The solution was concentrated to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-6-fluoro-1-((tetrahydro-2H-pyran-3-yl)methyl)-1H-indol-5-yl)cyclopropanecarboxamide. MS (ESI) m/e ($M+H^+$) 493.0.

Example 112

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(2-hydroxypropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0749]**

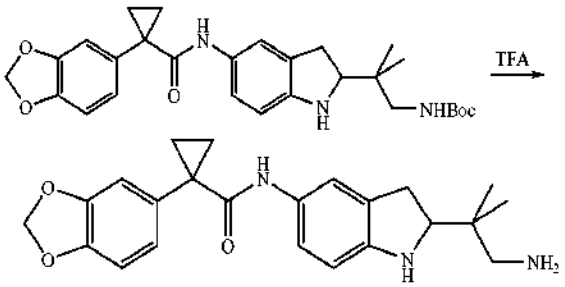



**[0750]** Methyl 5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropane-carboxamido)-1H-indole-2-carboxylate (100 mg, 0.255 mmol) was dissolved in anhydrous tetrahydrofuran (2 mL) under an argon atmosphere. The solution was cooled to 0° C. in an ice water bath before methyllithium (0.85 mL, 1.6 M in diethyl ether) was added by syringe. The mixture was allowed to warm to room temperature. The crude product was then partitioned between a saturated aqueous solution of sodium chloride (5 mL) and dichloromethane (5 mL). The organic layers were combined, dried over sodium sulfate, filtered, evaporated to dryness, and purified on 12 g of silica gel utilizing a gradient of 20-80% ethyl acetate in hexanes to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-(2-hydroxypropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (35 mg, 36%) as a white solid. ESI-MS m/z calc. 378.2, found 379.1 $(M+1)^+$. Retention time of 2.18 minutes. $^1$H NMR (400 MHz, DMSO-d6) δ 10.78 (s, 1H), 8.39 (s, 1H), 7.57 (d, J=1.7 Hz, 1H), 7.17 (d, J=8.6 Hz, 1H), 7.03-6.90 (m, 4H), 6.12 (d, J=1.5 Hz, 1H), 6.03 (s, 2H), 5.18 (s, 1H), 1.50 (s, 6H), 1.41-1.38 (m, 2H), 1.05-0.97 (m, 2H).

Example 113

N-(2-(1-Amino-2-methylpropan-2-yl)-1H-indol-5-yl)-1-(benzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamide

**[0751]**

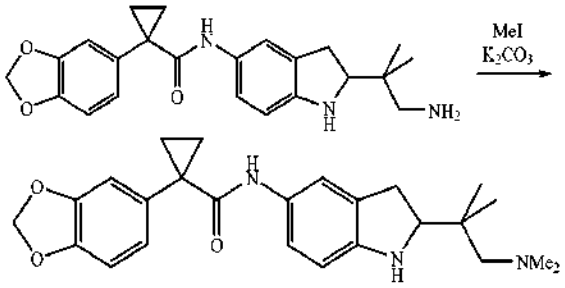



**[0752]** Trifluoroacetic acid (0.75 mL) was added to a solution of tert-butyl 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)-2-methylpropylcarbamate (77 mg, 0.16 mmol) in dichloromethane (3 mL) and the mixture was stirred at room temperature for 1.5 h. The mixture was evaporated, dissolved in dichloromethane, washed with saturated sodium bicarbonate solution, dried over magnesium sulfate and evaporated to dryness to give N-(2-(1-amino-2-methylpropan-2-yl)-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (53 mg, 86%). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.58 (s, 1H), 7.60. (d, J=1.6 Hz, 1H), 7.18-7.15 (m, 2H), 7.02-6.94 (m, 3H), 6.85 (d, J=7.8 Hz, 1H). 6.14 (d, J=1.2 Hz, 1H), 6.02 (s, 2H), 2.84 (s, 2H), 1.68 (dd, J=3.6, 6.7 Hz, 2H), 1.32 (s, 6H), 1.08 (dd, J=3.7, 6.8 Hz, 2H).

Example 114

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(1-(dimethylamino)-2-methyl-propan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0753]**

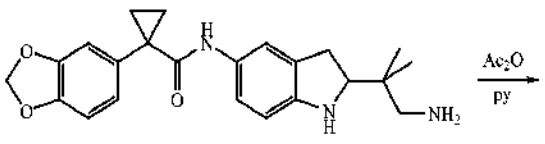



**[0754]** To a solution of N-(2-(1-amino-2-methylpropan-2-yl)-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (20 mg, 0.051 mmol) in DMF (1 mL) was added potassium carbonate (35 mg, 0.26 mmol) and iodomethane (7.0 μL, 0.11 mmol). The mixture was stirred for 2 h. Water was added and the mixture was extracted with dichloromethane. Combined organic phases were dried over magnesium sulfate, evaporated, coevaporated with toluene (3×) and purified by silica gel chromatography (0-30% EtOAc in hexane) to give 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-(1-(dimethylamino)-2-methylpropan-2-yl)-1H-indol-5-yl) cyclopropanecarboxamide (7 mg, 33%). $^1$H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H), 7.58 (d, J=1.9 Hz, 1H), 7.20 (d, J=8.6 Hz, 1H), 7.15 (s, 1H), 7.01-6.95 (m, 3H), 6.85 (d, J=7.9 Hz, 1H), 6.10 (d, J=0.9 Hz, 1H), 6.02 (s, 2H), 2.43 (s, 2H). 2.24 (s, 6H), 1.68 (dd, J=3.7, 6.7 Hz, 2H), 1.33 (s, 6H), 1.08 (dd, J=3.7, 6.8 Hz, 2H).

Example 115

N-(2-(1-Acetamido-2-methylpropan-2-yl)-1H-indol-5-yl)-1-(benzo[d][1,3]-dioxol-5-yl)cyclopropanecarboxamide

**[0755]**



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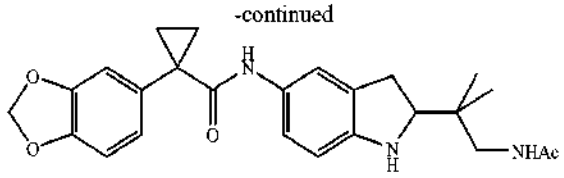
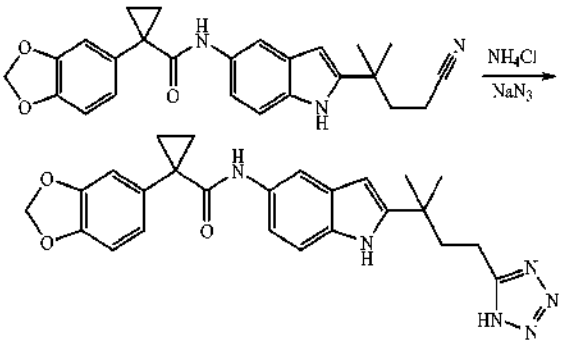


**[0756]** To a solution of N-(2-(1-amino-2-methylpropan-2-yl)-1H-indol-5-yl)-1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (21 mg, 0.054 mmol) in dichloromethane (1 mL) was added pyridine (14 μL, 0.16 mmol) followed by acetic anhydride (6.0 μL, 0.059 mmol). The mixture was stirred for 2 h. Water was added and the mixture was extracted with dichloromethane, evaporated, coevaporated with toluene (3×) and purified by silica gel chromatography (60-100% ethylacetate in hexane) to give N-(2-(1-acetamido-2-methylpropan-2-yl)-1H-indol-5-yl)-1-(benzo[d][1,3]-dioxol-5-yl) cyclopropanecarboxamide (17 mg, 73%). $^1$H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 8.39 (s, 1H), 7.66 (t, J=6.2 Hz, 1H), 7.56 (d, J=1.7 Hz, 1H), 7.18-7.14 (m, 1H), 7.02-6.89 (m, 4H), 6.08 (d, J=1.5 Hz, 1H), 6.03 (s, 2H), 3.31 (d, J=6.2 Hz, 2H), 1.80 (s, 3H), 1.41-1.38 (m, 2H), 1.26 (s, 6H), 1.04-1.01 (m, 2H).

Example 116

1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(2-methyl-4-(1H-tetrazol-5-yl)butan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0757]**

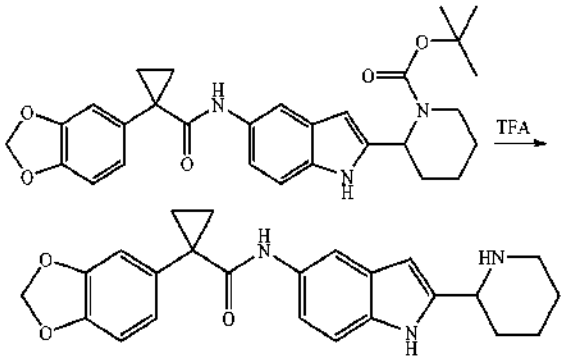


**[0758]** 1-(Benzo[d][1,3]dioxol-5-yl)-N-(2-(4-cyano-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (83 mg, 0.20 mmol) was dissolved in N,N-dimethylformamide (1 mL) containing ammonium chloride (128 mg, 2.41 mmol), sodium azide (156 mg, 2.40 mmol), and a magnetic stir bar. The reaction mixture was heated at 110° C. for 40 minutes in a microwave reactor. The crude product was filtered and then purified by preparative HPLC using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-(2-methyl-4-(1H-tetrazol-5-yl)butan-2-yl)-1H-indol-5-yl) cyclopropanecarboxamide. ESI-MS m/z calc. 458.2, found 459.2 (M+1)$^+$. Retention time of 1.53 minutes. $^1$H NMR (400 MHz, $CD_3CN$) 9.23 (s, 1H), 7.51-7.48 (m, 2H), 7.19 (d, J=8.6 Hz, 1H), 7.06-7.03 (m, 2H), 6.95-6.89 (m, 2H), 6.17 (dd, J=0.7, 2.2 Hz, 1H), 6.02 (s, 2H), 2.61-2.57 (m, 2H), 2.07-2.03 (m, 2H), 1.55-1.51 (m, 2H), 1.39 (s, 6H), 1.12-1.09 (m, 2H).

Example 117

1-(Benzo[d][1,3]-dioxol-5-yl)-N-(2-(piperidin-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0759]**

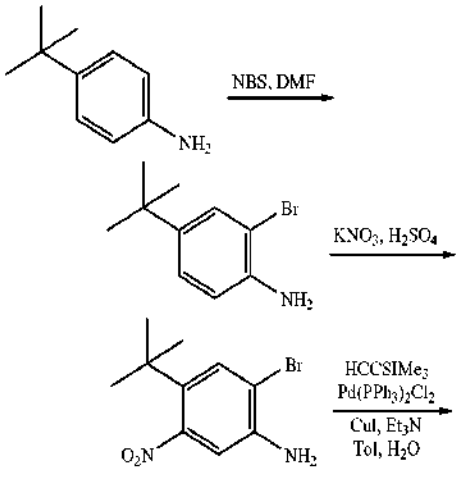


**[0760]** tert-Butyl 2-(5-(1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-1H-indol-2-yl)piperidine-1-carboxylate (55 mg, 0.11 mmol) was dissolved in dichloromethane (2.5 mL) containing trifluoroacetic acid (1 mL). The reaction mixture was stirred for 6 h at room temperature. The crude product was purified by preparative HPLC using a gradient of 0-99% acetonitrile in water containing 0.05% trifluoroacetic acid to yield 1-(benzo[d][1,3]dioxol-5-yl)-N-(2-(piperidin-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide. ESI-MS m/z calc. 403.2, found 404.4 (M+1)$^+$. Retention time of 0.95 minutes.

Example 118

5-tert-Butyl-1H-indol-6-ylamine

**[0761]**



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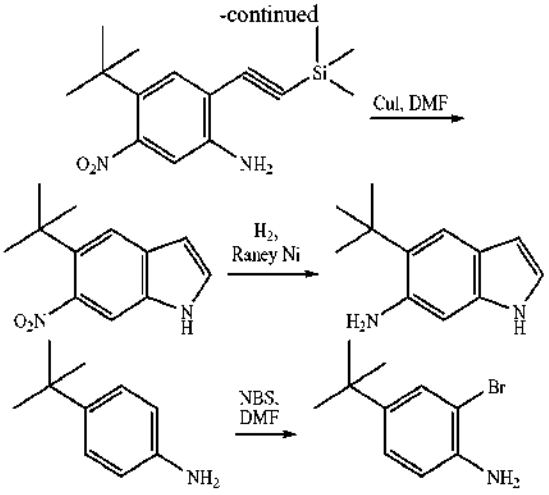
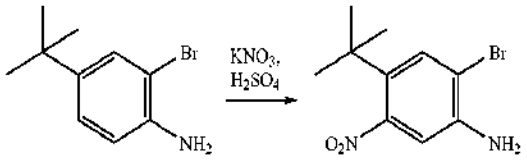


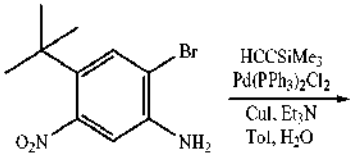

2-Bromo-4-tert-butyl-phenylamine

**[0762]** To a solution of 4-tert-Butyl-phenylamine (447 g, 3.00 mol) in DMF (500 mL) was added dropwise NBS (531 g, 3.00 mol) in DMF (500 mL) at room temperature. Upon completion, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with water, brine, dried over $Na_2SO_4$ and concentrated. The crude product was directly used in the next step without further purification.




2-Bromo-4-tert-butyl-5-nitro-phenylamine

**[0763]** 2-Bromo-4-tert-butyl-phenylamine (160 g, 0.71 mol) was added dropwise to $H_2SO_4$ (410 mL) at room temperature to yield a clear solution. This clear solution was then cooled down to −5 to −10° C. A solution of $KNO_3$ (83 g, 0.82 mol) in $H_2SO_4$ (410 mL) was added dropwise while the temperature was maintained between −5 to −10° C. Upon completion, the reaction mixture was poured into ice/water and extracted with EtOAc. The combined organic layers were washed with 5% $Na_2CO_3$ and brine, dried over $Na_2SO_4$ and concentrated. The residue was purified by a column chromatography (ethyl acetate/petroleum ether 1:10) to give 2-bromo-4-tert-butyl-5-nitro-phenylamine as a yellow solid (150 g, 78%).

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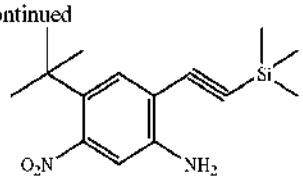
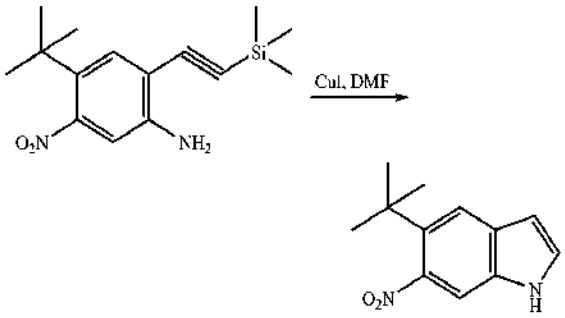


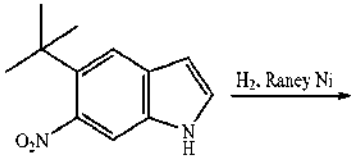
4-tert-Butyl-5-nitro-2-trimethylsilanylethynyl-phenylamine

**[0764]** To a mixture of 2-bromo-4-tert-butyl-5-nitro-phenylamine (27.3 g, 100 mmol) in toluene (200 mL) and water (100 mL) was added $Et_3N$ (27.9 mL, 200 mmol), $Pd(PPh_3)_2Cl_2$ (2.11 g, 3.00 mmol). CuI (950 mg, 0.500 mmol) and trimethylsilyl acetylene (21.2 mL, 150 mmol) under a nitrogen atmosphere. The reaction mixture was heated at 70° C. in a sealed pressure flask for 2.5 h., cooled down to room temperature and filtered through a short plug of Celite. The filter cake was washed with EtOAc. The combined filtrate was washed with 5% $NH_4OH$ solution and water, dried over $Na_2SO_4$ and concentrated. The crude product was purified by column chromatography (0-10% ethyl acetate/petroleum ether) to provide 4-tert-butyl-5-nitro-2-trimethylsilanylethynyl-phenylamine as a brown viscous liquid (25 g, 81%).




5-tert-Butyl-6-nitro-1H-indole

**[0765]** To a solution of 4-tert-butyl-5-nitro-2-trimethylsilanylethynyl-phenylamine (25 g, 86 mmol) in DMF (100 mL) was added CuI (8.2 g, 43 mmol) under a nitrogen atmosphere. The mixture was heated at 135° C. in a sealed pressure flask overnight, cooled down to room temperature and filtered through a short plug of Celite. The filter cake was washed with EtOAc. The combined filtrate was washed with water, dried over $Na_2SO_4$ and concentrated. The crude product was purified by column chromatography (10-20% ethyl acetate/hexane) to provide 5-tert-butyl-6-nitro-1H-indole as a yellow solid (13 g, 69%).



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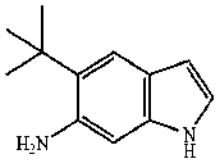
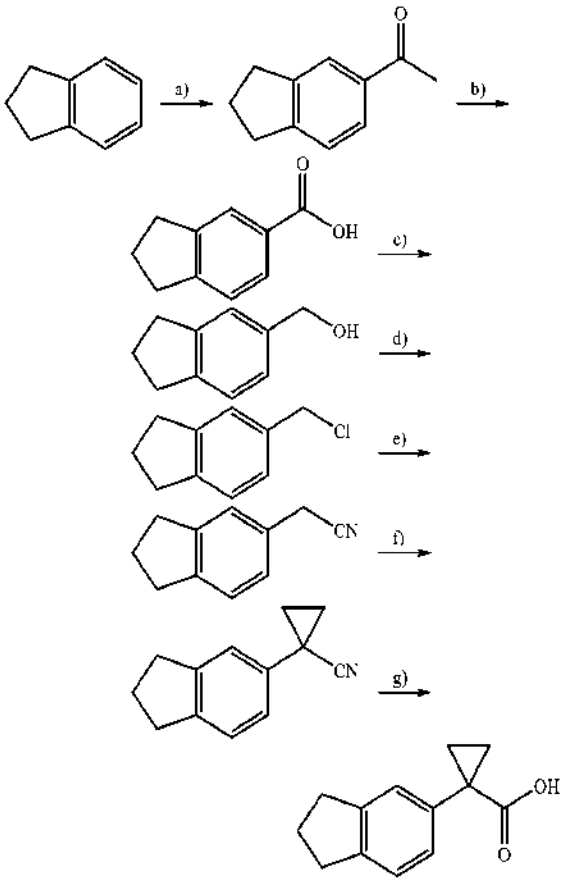


5-tert-Butyl-1H-indol-6-ylamine

**[0766]** Raney Nickel (3 g) was added to 5-tert-butyl-6-nitro-1H-indole (15 g, 67 mmol) in methanol (100 mL). The mixture was stirred under hydrogen (1 atm) at 30° C. for 3 h. The catalyst was filtered off. The filtrate was dried over $Na_2SO_4$ and concentrated. The crude dark brown viscous oil was purified by column chromatography (10-20% ethyl acetate/petroleum ether) to give 5-tert-butyl-1H-indol-6-ylamine as a gray solid (11 g, 87%). $^1H$ NMR (300 MHz, DMSO-d6) δ 10.3 (br s, 1H), 7.2 (s, 1H), 6.9 (m, 1H), 6.6 (s, 1H), 6.1 (m, 1H), 4.4 (br s, 2H), 1.3 (s, 9H).

1-(2,3-Dihydro-1H-inden-5-yl)cyclopropanecarboxylic acid

**[0767]**




Step a: 1-(2,3-Dihydro-1H-inden-6-yl)ethanone

**[0768]** A mixture of 2,3-dihydro-1H-indene (100.0 g, 0.85 mol) and acetic anhydride (104.2 g, 1.35 mol) was added drop-wise to a slurry of $AlCl_3$ (272.0 g, 2.04 mol) in $CH_2Cl_2$ (1000 ml) at 0° C. over a period of 3 h. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 15 h. Then the reaction mixture was poured into ice water (500 mL) and extracted with ethyl acetate (500 mL×3). The combined organic layers were washed with brine (500 mL), dried over $Na_2SO_4$ and evaporated in vacuo. The residue that was purified by column chromatography (petroleum ether: ethyl acetate=20:1) to give the product (120.0 g, 88%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 2.08-2.15 (m, 2H), 2.58 (s, 3H), 2.95 (t, J=7.2, 4H), 7.28 (d, J=8.0, 1H), 7.75 (d, J=8.0, 1H) 7.82 (s, 1H).

Step b: 2,3-dihydro-1H-indene-5-carboxylic acid

**[0769]** To a stirred aqueous sodium hypochlorite solution (2230 ml, 1.80 mmol, 6%) at 55° C. was added 1-(2,3-dihydro-1H-inden-6-yl)ethanone (120.0 g, 0.75 mol) and the mixture was stirred at 55° C. for 2 h. After cooling to room temperature, saturated $NaHCO_3$ solution was added until the solution became clear. The produced precipitate was filtered, washed several times with water and dried to afford the desired product (120.0 g, 99%). $^1H$ NMR ($CDCl_3$, 300 MHz) δ 2.07-2.17 (m, 2H), 2.96 (t, J=7.5 Hz, 4H), 7.30 (d, J=7.8, 1H), 7.91 (d, J=7.8, 1H), 7.96 (s, 1H).

Step c: (2,3-dihydro-1H-inden-5-yl)methanol

**[0770]** To a stirred solution of LAH (72.8 g, 1.92 mol) in THF (2.5 L) at 0° C. was slowly added 2,3-dihydro-1H-indene-5-carboxylic acid (100.0 g, 0.62 mol). The reaction mixture was stirred at 0° C. for 1 h. Then the reaction was quenched with $H_2O$ (72 ml) and NaOH (68 ml, 20%). The mixture was filtered and the organic layer was dried over $Na_2SO_4$, evaporated in vacuo and the residue was purified by column chromatography (petroleum ether:ethyl acetate=10:1) to give the desired product (82.0 g, 90%). $^1H$ NMR ($CDCl_3$, 300 MHz): δ 2.03-2.13 (m, 2H), 2.91 (t, J=7.5 Hz, 4H), 4.64 (s, 2H), 7.13 (d, J=7.5, 1H), 7.18-7.24 (m, 2H).

Step d: 5-(chloromethyl)-2,3-dihydro-1H-indene

**[0771]** Thionyl chloride (120 ml, 1.65 mol) was added drop-wise to a rapidly stirred mixture of (2,3-dihydro-1H-inden-5-yl)methanol (81.4 g, 0.55 mol) in chloroform (500 ml) at 0° C. After the addition was complete, the resulting mixture was allowed to warm to room temperature and the stirring was continued for an additional 12 h. The chloroform was evaporated under reduced pressure to give a residue, that was purified by column chromatography (petroleum ether: ethyl acetate=15:1) to afford 5-(chloromethyl)-2,3-dihydro-1H-indene (90.5 g, 99%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 2.06-2.19 (m, 4H), 2.93 (t, J=7.5, 4H), 4.54 (s, 2H), 7.15-7.31 (m, 3H).

Step e: 2-(2,3-dihydro-1H-inden-5-yl)acetonitrile

**[0772]** To a stirred solution of 5-(chloromethyl)-2,3-dihydro-1H-indene (90.0 g, 0.54 mol) in DMSO (500 ml) was added sodium cyanide (54.0 g, 1.08 mol) at 0° C. portion wise. The reaction mixture was then stirred at room temperature for 3 hours. The reaction was quenched with water (1000 ml), extracted with ethyl acetate (3×250 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$

and evaporated in vacuo to afford 2-(2,3-dihydro-1H-inden-5-yl)acetonitrile (82.2 g, 97%), that was used in the next step without further purification.

Step f: 1-(2,3-dihydro-1H-inden-6-yl)cyclopropanecarbonitrile

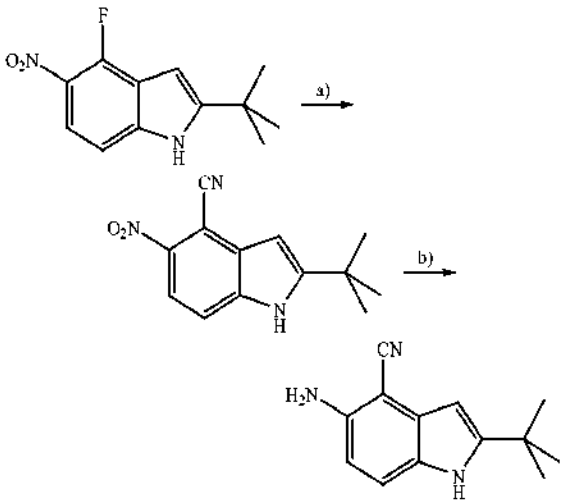
**[0773]** To a stirred solution of 2-(2,3-dihydro-1H-inden-5-yl)acetonitrile (50.0 g, 0.32 mol) in toluene (150 mL) was added sodium hydroxide (300 mL, 50 percent in water W/W), 1-bromo-2-chloroethane (92.6 ml, 1.12 mol) and $(n\text{-}Bu)_4NBr$ (5 g, 15.51 mmol). The mixture was heated at 60° C. overnight. After cooling to room temperature, the reaction mixture was diluted with water (400 mL) and extracted with EtOAc (3×200 mL). The combined organic extracts were washed with brine, dried over $Na_2SO_4$, filtered and concentrated under vacuum and purified by column chromatography (petroleum ether:ethyl acetate=10:1) to yield 1-(2,3-dihydro-1H-inden-6-yl)cyclopropanecarbonitrile (9.3 g, 16%). "H" NMR ($CDCl_3$, 300 MHz) δ 1.35-1.38 (m, 2H), 1.66-1.69 (m, 2H), 2.05-2.13 (m, 2H), 2.87-294 (m, 4H), 7.07-7.22 (m, 3H).

Step g: 1-(2,3-dihydro-1H-inden-6-yl)cyclopropanecarboxylic acid

**[0774]** To a stirred 1-(2,3-dihydro-1H-inden-6-yl)cyclopropanecarbonitrile (9.3 g, 50.8 mmol) in methanol (40 mL) was added a solution of 150 mL of sodium hydroxide (25% NaOH w/w in water). The mixture was heated at 100° C. for 8 hours. After cooling to room temperature, the reaction mixture was poured over ice-water (0° C.), the pH was adjusted to pH=4 with hydrogen chloride (1 N) and the mixture was extracted with dichloromethane (3×100 mL). The combined organic layers were dried over $Na_2SO_4$ and evaporated under vacuum. The residue that was purified by column chromatography (petroleum ether:ethyl acetate=5:1) to give 1-(2,3-dihydro-1H-inden-6-yl)cyclopropanecarboxylic acid (4.8 g, 47%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 1.23-1.26 (m, 2H), 1.62-1.65 (m, 2H), 2.03-210 (m, 2H), 2.81-2.91 (m, 4H), 7.11-7.21 (m, 3H).

5-Amino-2-tert-butyl-1H-indole-4-carbonitrile

**[0775]**




a) KCN, DMSO; b) Pd/C, EtOAc

Step a: 2-tert-butyl-5-nitro-1H-indole-4-carbonitrile

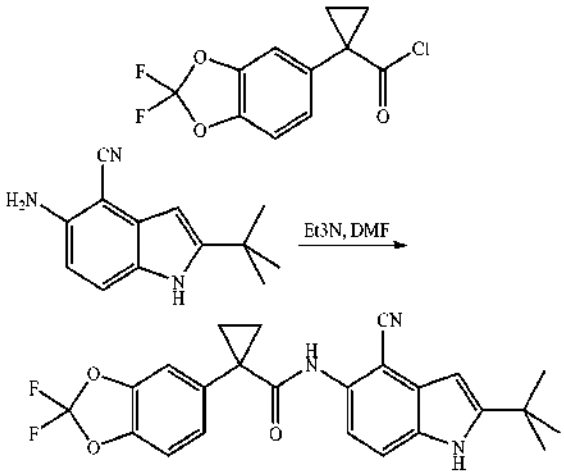
**[0776]** To a solution of 2-tert-butyl-4-fluoro-5-nitro-1H-indole (4.0 g, 17 mmol) in DMSO (30 mL) was added KCN (3.4 g, 51 mmol). The mixture was stirred at 70° C. for 3 hours, and poured into water (80 mL) and extracted with ethyl acetate (50 mL×3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under vacuum. The residue was purified by column chromatography on silica gel (7% EtOAc in petroleum ether) to afford 2-tert-butyl-5-nitro-1H-indole-4-carbonitrile (2.2 g, 53%). $^1H$ NMR (DMSO, 300 MHz) δ 12.23 (br s, 1H), 8.09 (d, J=9.0 Hz, 1H), 7.75 (d, J=9.0 Hz, 1H), 6.50 (s, 1H), 1.38 (s, 9H). MS (ESI) m/z: 244.2 [M+H$^+$].

Step b: 5-amino-2-tert-butyl-1H-indole-4-carbonitrile

**[0777]** To a solution of 2-tert-butyl-5-nitro-1H-indole-4-carbonitrile (550 mg, 2.3 mmol) in EtOAc (10 mL) was added Raney Ni (0.1 g) under a nitrogen atmosphere. The mixture was stirred under hydrogen atmosphere (1 atm) at room temperature for 1 h. The catalyst was filtered over Celite and the filtrate was evaporated in vacuo to afford 5-amino-2-tert-butyl-1H-indole-4-carbonitrile (250 mg, 51%). $^1H$ NMR (DMSO, 300 MHz) δ 10.93 (br s, 1H), 7.25 (d, J=8.7 Hz, 1H), 6.49 (d, J=8.7 Hz, 1H), 5.94 (d, J=2.1 Hz, 1H), 5.40 (br s, 2H), 1.30 (s, 9H). MS (ESI) m/z: 214.0 [M+H$^+$].

N-(2-tert-butyl-4-cyano-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0778]**



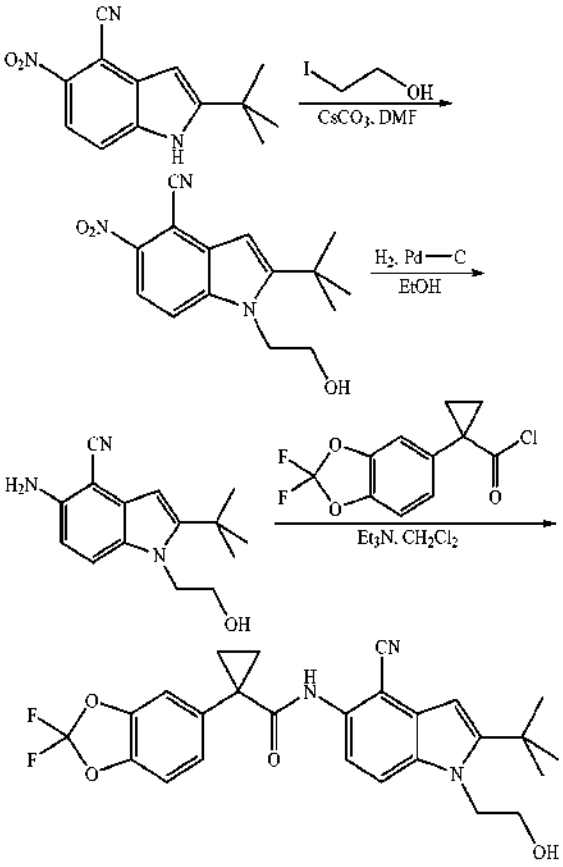

Step a: N-(2-tert-butyl-4-cyano-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0779]** 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbonyl chloride (26 mg, 0.1 mmol) was added to a solution of 5-amino-2-tert-butyl-1H-indole-4-carbonitrile (21 mg, 0.1 μmol) and triethylamine (41.7 μL, 0.3 mmol) in DMF (1 mL). The reaction was stirred at room temperature overnight, then filtered and purified by reverse-phase HPLC to yield the product, N-(2-tert-butyl-4-cyano-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide. ESI-MS m/z calc. 437.2, found 438.7 (M+1)$^+$. Retention time 2.10 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 11.48 (s, 1H), 8.88 (s, 1H), 7.52 (d, J=8.5 Hz, 2H), 7.41

(d, J=8.3 Hz, 1H), 7.32 (dd, J=1.5, 8.3 Hz, 1H), 7.03 (d, J=8.6 Hz, 1H), 6.21 (d, J=1.8 Hz, 1H), 1.51-1.49 (m, 2H), 1.36 (s, 9H), 1.18-1.16 (m, 2H).

N-(2-tert-butyl-4-cyano-1-(2-hydroxyethyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl) cyclopropanecarboxamide

**[0780]**




Step a: 2-tert-butyl-1-(2-hydroxyethyl)-5-nitro-1H-indole-4-carbonitrile

**[0781]** A mixture of 2-tert-butyl-5-nitro-1H-indole-4-carbonitrile (200 mg, 0.82 mmol), 2-iodoethanol (77 μL, 0.98 mmol), cesium carbonate (534 mg, 1.64 mmol) and DMF (1.3 mL) was heated to 90° C. overnight. Then more 2-iodoethanol (77 μL, 0.98 mmol) was added and the reaction was stirred at 90° C. for 3 days. The reaction mixture was partitioned between ethyl acetate and water. The aqueous layer was washed with ethyl acetate and then the combined ethyl acetate layers were washed with water (×3) and brine, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (50-100% $CH_2Cl_2$—Hexanes) to yield the product as a yellow solid (180 mg, ~25% purity by NMR, product co-elutes with the indole starting material). ESI-MS m/z calc. 287.1, found 288.5 $(M+1)^+$. Retention time 1.59 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 12.23 (s, 1H), 8.14 (d, J=9.1 Hz, 1H), 8.02 (d, J=9.1 Hz, 1H), 6.60 (s, 1H), 5.10 (t, J=5.5 Hz, 1H), 4.55 (t, J=6.3 Hz, 2H), 3.78-3.73 (m, 2H) and 1.49 (s, 9H) ppm.

Step b: 5-amino-2-tert-butyl-1-(2-hydroxyethyl)-1H-indole-4-carbonitrile

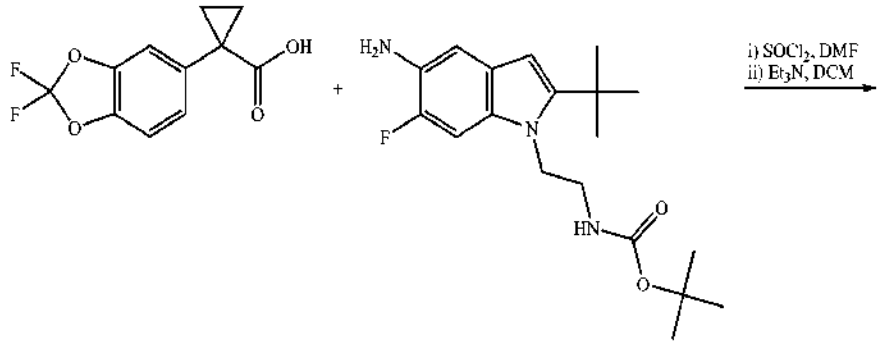
**[0782]** To a solution of 2-tert-butyl-1-(2-hydroxyethyl)-5-nitro-1H-indole-4-carbonitrile (180 mg, 0.63 mmol) in ethanol (6 mL) under $N_2$ atmosphere was added Pd—C (5% wt, 18 mg). The reaction was flushed with $N_2$ (g) and then with $H_2$ (g) and stirred under $H_2$ (atm) at room temperature for 1.5 hours. The reaction was filtered over Celite and concentrated to yield the product (150 mg, 93%). ESI-MS m/z calc. 257.2, found 258.5 $(M+1)^+$. Retention time 1.26 minutes.

Step c: N-(2-tert-butyl-4-cyano-1-(2-hydroxyethyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0783]** 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarbonyl chloride (196 mg, 0.75 mmol) was added to a solution of 5-amino-2-tert-butyl-1-(2-hydroxyethyl)-1H-indole-4-carbonitrile (150 mg, 0.58 mmol) and triethylamine (242 μL, 1.74 mmol) in dichloromethane (2 mL). The reaction was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane and extracted with 1N HCl solution (×2), saturated $NaHCO_3$ solution (×2), brine, dried over $MgSO_4$, filtered and concentrated. The residue was dissolved in DMSO and purified by reverse-phase HPLC to yield the product, N-(2-tert-butyl-4-cyano-1-(2-hydroxyethyl)-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide. ESI-MS m/z calc. 481.2, found 482.5 $(M+1)^+$. Retention time 1.99 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.93 (s, 1H), 7.71 (d, J=8.8 Hz, 1H), 7.51 (s, 1H), 7.42 (d, J=8.3 Hz, 1H), 7.33 (d, J=1.6 Hz, 1H), 7.08 (d, J=8.8 Hz, 1H), 6.28 (s, 1H), 5.05 (t, J=5.6 Hz, 1H), 4.42 (t, J=6.8 Hz, 2H), 3.70-3.65 (m, 2H), 1.51-1.48 (m, 2H), 1.44 (s, 9H), 1.19-1.16 (m, 2H).

2-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-6-fluoro-1H-indol-1-yl)-N,N,N-trimethylethanaminium chloride

**[0784]**



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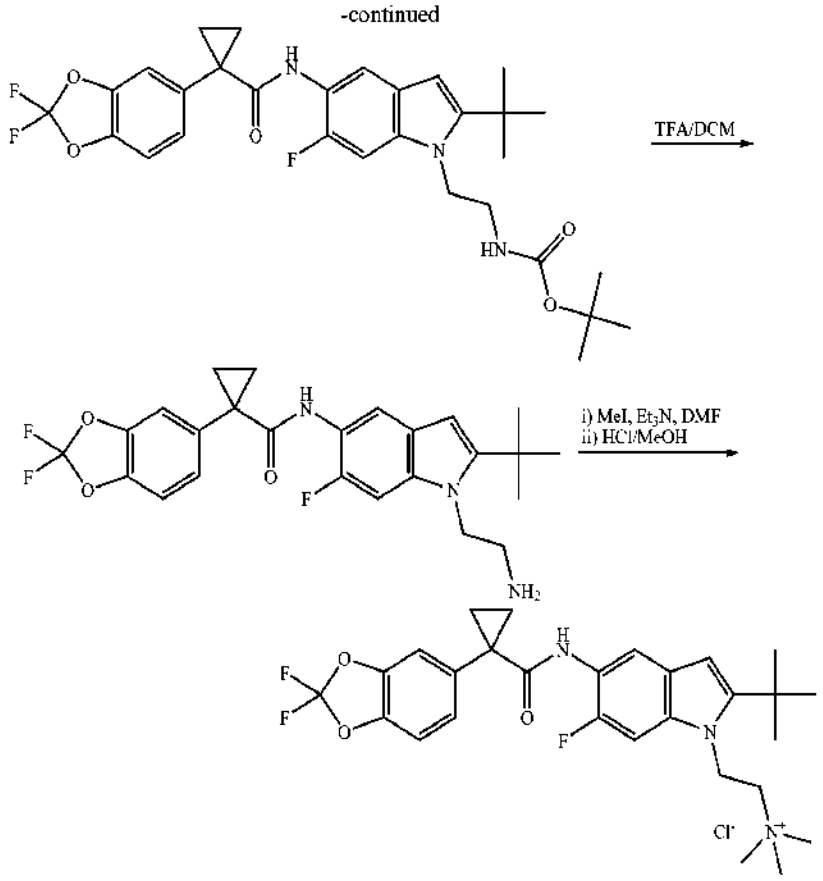



Step a: tert-Butyl 2-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-6-fluoro-1H-indol-1-yl)ethylcarbamate

**[0785]** To 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (90.14 mg, 0.3722 mmol) in thionyl chloride (81.28 μL, 1.117 mmol) was added N,N-dimethyl formamide (8.204 μL, 0.1064 mmol). The reaction mixture was stirred at room temperature for 30 minutes before excess thionyl chloride and N,N-dimethyl formamide were removed in vacuo to yield the acid chloride. The acid chloride was then dissolved in dichloromethane (1.5 mL) and added slowly to a solution of tert-butyl 2-(5-amino-2-tert-butyl-6-fluoro-1H-indol-1-yl)ethylcarbamate (156.1 mg, 0.4467 mmol) and triethylamine (155.6 μL, 1.117 mmol) in dichloromethane (1.5 mL). The resulting reaction mixture was stirred at room temperature for 21 hours. The reaction mixture was diluted with dichloromethane (5 mL) and washed with 1N aqueous HCl (5 mL) and a saturated aqueous $NaHCO_3$ solution (5 mL). The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (0-30% ethyl acetate in hexane) to yield tert-butyl 2-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-6-fluoro-1H-indol-1-yl)ethylcarbamate as a white solid (140 mg, 66%). ESI-MS m/z calc. 573.2, found 574.7 $(M+1)^+$. Retention time 2.41 minutes. $^1H$ NMR (400.0 MHz, DMSO) d 8.35 (s, 1H), 7.53 (s, 1H), 7.44-7.41 (m, 2H), 7.34-7.29 (m, 2H), 7.13-7.10 (m, 1H), 6.17 (s, 1H), 4.24-4.20 (m, 2H), 3.20-3.17 (m, 2H), 1.48-1.45 (m, 2H), 1.41 (s, 18H) and 1.15-1.12 (m, 2H) ppm.

Step b: N-(1-(2-aminoethyl)-2-tert-butyl-6-fluoro-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide

**[0786]** To a solution of tert-butyl 2-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-6-fluoro-1H-indol-1-yl)ethylcarbamate (137.5 mg, 0.24 mmol) in dichloromethane (1.8 mL) was added trifluoroacetic acid (444 μL, 5.8 mmol) and the mixture was stirred at room temperature for 1 hour. The reaction was diluted with dichloromethane and washed with saturated aqueous $NaHCO_3$ solution (3 mL) and brine (3 mL). The organic layer was dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (0-10% methanol in dichloromethane) to yield N-(1-(2-aminoethyl)-2-tert-butyl-6-fluoro-1H-indol-5-yl)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide as a white solid (93.7 mg, 82%). ESI-MS m/z calc. 473.19, found 474.5 $(M+1)^+$. Retention time 1.61 minutes.

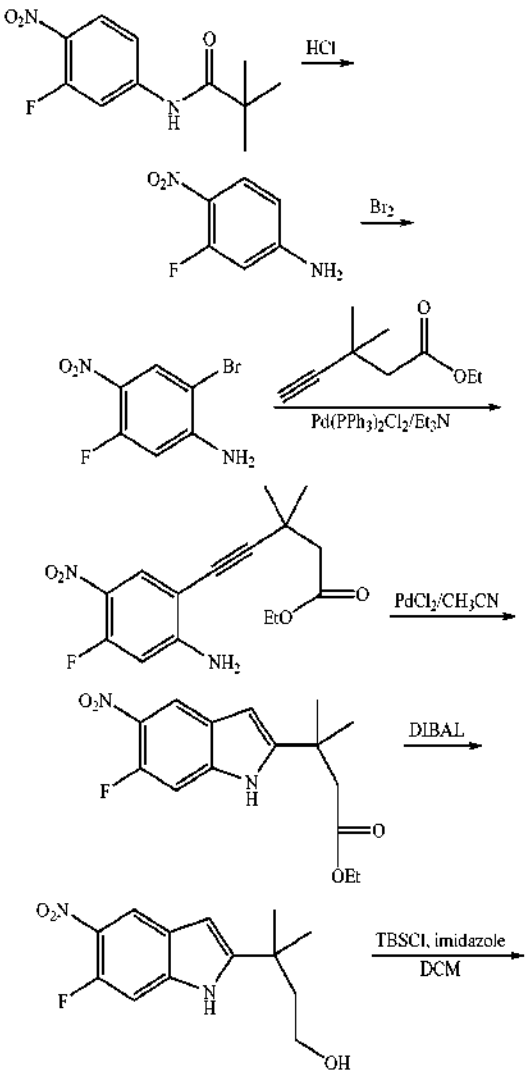
Step c: 2-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-6-fluoro-1H-indol-1-yl)-N,N,N-trimethylethanaminium chloride

**[0787]** To a clear solution of N-(1-(2-aminoethyl)-2-tert-butyl-6-fluoro-1H-indol-5-yl)-1-(2.2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamide (50 mg, 0.1056 mmol) in N,N-dimethyl formamide (1 mL), methyl iodide (336.8 mg, 147.7 μL, 2.37 mmol) and triethylamine (106.9

mg, 147.2 μL, 1.05 mmol) were added and the mixture was heated at 80° C. for 2 hours. The crude product was purified by reverse phase preparative HPLC. 22 mg of this product were dissolved in 1.25 M HCl in methanol (112 μL, 0.14 mmol) and heated at 60° C. for 1 hour. The reaction was cooled to room temperature. The product was first dried and then dissolved in dichloromethane and dried again. This procedure was repeated four times to yield 2-(2-tert-butyl-5-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxamido)-6-fluoro-1H-indol-1-yl)-N,N,N-trimethylethanaminium chloride. ESI-MS m/z calc. 516.25, found 516.7 $(M+1)^+$. Retention time 1.69 minutes. $^1$H NMR (400.0 MHz, DMSO) d 8.43 (s, 1H), 7.53 (s, 1H), 7.45-7.41 (m, 2H), 7.36-7.31 (m, 2H), 6.27 (s, 1H), 4.74-4.70 (m, 2H), 3.57-3.53 (m, 2H), 3.29 (s, 9H), 1.48-1.42 (m, 11H), and 1.15 (dd, J=3.9, 6.8 Hz, 2H) ppm.

2-(4-(Tert-butyldimethylsilyloxy)-2-methylbutan-2-yl)-6-fluoro-5-nitro-1H-indole

**[0788]**

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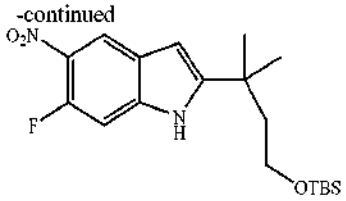



Step a: 3-fluoro-4-nitroaniline

**[0789]** A mixture of N-(3-fluoro-4-nitro-phenyl)-2,2-dimethyl-propionamide (87.0 g, 0.36 mol) in $CH_2Cl_2$ (400 mL) and 6N hydrochloric acid (800 mL) was heated to reflux for 2 hours. The reaction mixture was cooled to room temperature. The reaction mixture was diluted with 1000 mL of ethyl acetate and potassium carbonate (500.0 g) was added portion wise. The aqueous solution was separated and the organic layer was washed with brine and dried over anhydrous $Na_2SO_4$. The solvent was removed by evaporation under reduced pressure; the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 30:1) to afford 3-fluoro-4-nitroaniline (56.0 g, 99%). $^1$H NMR (300 MHz, $CDCl_3$) δ 8.07 (t, J=8.7 Hz, 1H), 7.86 (dd, J=2.1, 13.2 Hz 1H), 7.59 (brs, 2H), 7.22 (s, 1H).

Step b: 2-bromo-5-fluoro-4-nitroaniline

**[0790]** To a solution of 3-fluoro-4-nitroaniline (56 g, 0.36 mol) in acetic acid (500 mL) was added drop-wise bromine (17.7 mL, 0.36 mol) over 1 hour. The reaction mixture was stirred for 1 hour at 0-5° C. in an ice bath. The reaction mixture was basified with saturated $Na_2CO_3$ and extracted with ethyl acetate (200 mL×3). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, filtered and concentrated under reduced pressure to yield a residue that was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 10:1) to give the 2-bromo-5-fluoro-4-nitroaniline (45.6 g, 84%) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, J=7.6 Hz, 1H), 653 (d, J=12.4 Hz, 1H), 4.94 (br s, 2H).

Step c: ethyl 5-(2-amino-4-fluoro-5-nitrophenyl)-3,3-dimethylpent-4-ynoate

**[0791]** To a solution of 2-bromo-5-fluoro-4-nitroaniline (45.7 g, 0.19 mol) and ethyl 3,3-dimethylpent-4-ynoate (88.3 g, 0.57 mol) in $Et_3N$ (700 mL) was added $Pd(PPh_3)_2Cl_2$ (13.8 g, 0.02 mol) and CuI (3.6 g, 0.02 mol) under $N_2$. The reaction mixture was stirred at 70° C. for 8 hours. The reaction mixture was diluted with 500 mL of ethyl acetate and 1500 mL of water. The organic layer was separated and the aqueous phase was extracted with ethyl acetate (500 mL×3), the combined organic layers were washed with brine and dried over anhydrous $Na_2SO_4$, filtered and evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 10:1) to give ethyl-5-(2-amino-4-fluoro-5-nitrophenyl)-3,3-dimethylpent-4-ynoate (34.5 g, 57%). $^1$H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, J=8.1 Hz, 1H), 6.36 (d, J=13.2 Hz, 1H), 5.60 (brs, 2H), 4.16 (q, J=7.2 Hz, 2H), 2.51 (s, 2H), 1.40 (s, 6H), 1.28 (t, J=7.2 Hz, 3H).

Step d: ethyl 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutanoate

**[0792]** To a mixture of ethyl 5-(2-amino-4-fluoro-5-nitrophenyl)-3,3-dimethylpent-4-ynoate (34.5 g, 0.11 mol) and

$PdCl_2$ (10.4 g, 58.6 nmol) in $CH_3CN$ (350 mL) was heated to reflux for 1.5 hours. The reaction mixture was cooled down to room temperature. Ethyl acetate (300 mL) was added, the precipitate was filtered off and washed with methanol. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 40:1) to give ethyl 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutanoate (34.0 g, 98%) as a deep yellow solid. $^1H$ NMR (300 MHz, $CDCl_3$) δ 10.11 (brs, 1H), 8.30 (d, J=7.2 Hz, 1H), 7.14 (d, J=11.7 Hz, 1H), 6.35 (d, J=1.5 Hz, 1H), 4.17 (q, J=7.2 Hz, 2H), 2.69 (s, 2H), 1.51 (s, 6H), 1.25 (t, J=7.2 Hz, 3H).

Step e: 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol

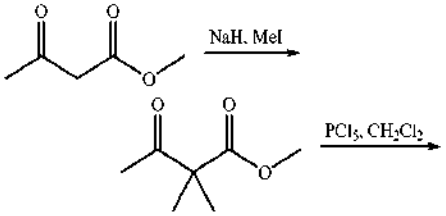
**[0793]** To a solution of ethyl 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutanoate (34 g, 0.11 mol) in dry $CH_2Cl_2$ (400 mL) was added drop-wise DIBAL-H (283.4 mL, 0.27 mol) over 2 hours at −78° C. The reaction mixture was stirred for 10 hours at −78° C. and then quenched by adding water (200 mL). The precipitate was filtered off and washed with methanol. The filtrate was extracted with $CH_2Cl_2$ (200 mL×3), the combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 50:1) to give 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol (6.6 g, 22%). $^1H$ NMR (400 MHz, $CDCl_3$) δ 9.35 (brs, 1H), 8.30 (d, J=7.6 Hz, 1H), 7.11 (d, J=12.0 Hz, 1H), 6.35 (d, J=1.2 Hz, 1H), 3.74 (t, J=6.4 Hz, 2H), 1.9 (t, J=6.4 Hz, 2H), 1.4 (s, 6H).

Step f: 2-(4-(tert-butyldimethylsilyloxy)-2-methylbutan-2-yl)-6-fluoro-5-nitro-1H-indole

**[0794]** To a solution of 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol (6.6 g, 25 mmol) in $CH_2Cl_2$ (80 mL) was added TBSCl (3.7 g, 25 mmol) and imidazole (4.2 g, 62 mmol) at 0° C. The reaction mixture was stirred at room temperature for 12 hours. The precipitate was filtered off and washed with the methanol. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 10:1) to give the desired product as a brown solid (5.0 g, 53%). $^1H$ NMR (300 MHz, $CDCl_3$) δ 9.80 (brs, 1H), 8.30 (d, J=7.2 Hz, 1H), 7.05 (d, J=11.7 Hz, 1H), 6.33 (t, J=1.2 Hz, 1H), 3.7 (t, J=6.0 Hz, 2H), 1.91 (t, J=6.0 Hz, 2H), 1.42 (s, 6H), 0.94 (s, 9H), 0.12 (s, 6H). MS (ESI) m/z ($M+H^+$): 381.1.

Benzyl 2,2-dimethylbut-3-ynoate

**[0795]**

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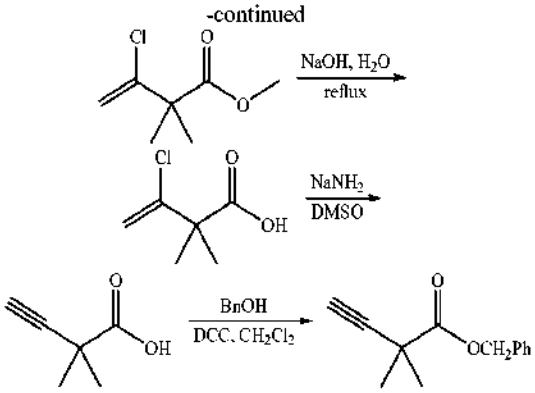



Step a: methyl 2,2-dimethyl-3-oxobutanoate

**[0796]** To a suspension of NaH (28.5 g, 0.718 mol, 60%) in THF (270 mL) was added dropwise a solution of 3-oxobutyric acid methyl ester (78.6 g, 0.677 mol) in THF (70 mL) at 0° C. The mixture was stirred for 0.5 hours at 0° C. MeI (99.0 g, 0.698 mol) was added dropwise at 0° C. The resultant mixture was warmed to room temperature and stirred for 1 hour. NaH (28.5 g, 0.718 mol, 60%) was added in portions at 0° C. and the resulting mixture was continued to stir for 0.5 h at 0° C. MeI (99.0 g, 0.698 mol) was then added dropwise at 0° C. The reaction mixture was warmed to room temperature and stirred overnight. The mixture was poured into ice water. The organic layer was separated. The aqueous phase was extracted with EtOAc (300 mL×3). The combined organic layers were dried and evaporated under reduced pressure to give methyl 2,2-dimethyl-3-oxobutanoate (52 g, 53%), which was used directly in the next step.

Step b: methyl 3-chloro-2,2-dimethylbut-3-enoate

**[0797]** To a suspension of $PCl_5$ (161 g, 0.772 mol) in dichloromethane (600 mL) was added dropwise methyl 2,2-dimethyl-3-oxobutanoate (52 g, 0.361 mol. crude from last step) at 0° C.. followed by the addition of approximately 20 drops of dry DMF. The mixture was heated at reflux overnight. After cooling, the reaction mixture was slowly poured into ice water. The organic layer was separated and the aqueous phase was extracted with dichloromethane (300 mL×3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and dried over anhydrous $Na_2SO_4$. The solvent was evaporated to give the product, methyl 3-chloro-2,2-dimethylbut-3-enoate which was used without further purification (47 g, 82%).

Step c: 3-chloro-2,2-dimethylbut-3-enoic acid

**[0798]** A mixture of methyl 3-chloro-2,2-dimethylbut-3-enoate (42.0 g, 0.26 mol) and NaOH (12.4 g, 0.31 mol) in water (300 mL) was heated at reflux overnight. After cooling, the reaction mixture was extracted with ether. The organic layer contained 20 g of methyl 3-chloro-2,2-dimethylbut-3-enoate (48% recovered). The aqueous layer was acidified with cold 20% HCl solution and was extracted with ether (250 mL×3). The combined organic layers were dried and evapo-

rated under reduced pressure to give 3-chloro-2,2-dimethylbut-3-enoic acid (17 g, 44%), which was used directly in the next step.

Step d: 2,2-dimethylbut-3-ynoic acid

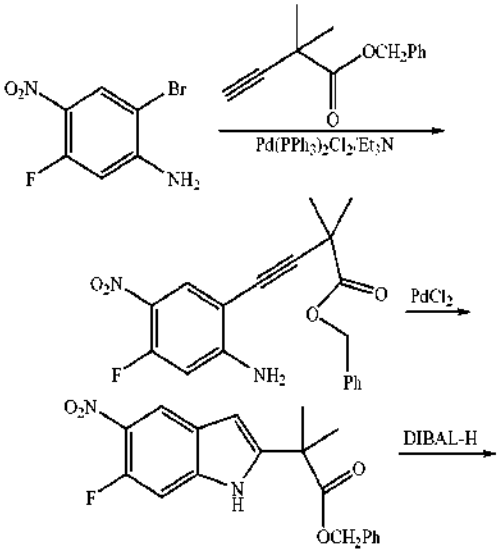
**[0799]** To a three-neck flask (500 mL) was added $NaNH_2$ (17.8 g, 0.458 mmol, pellets) and DMSO (50 mL). The mixture was stirred at room temperature until no more $NH_3$ (g) was given off. A solution of 3-chloro-2,2-dimethylbut-3-enoic acid (17.0 g, 114 mmol) in DMSO (50 mL) was added dropwise at 0° C. The mixture was warmed and stirred at 50° C. for 5 hours, then stirred at room temperature overnight. The mixture was poured into cold 20% HCl solution, and then extracted three times with ether. The ether extracts were dried over anhydrous $Na_2SO_4$ and concentrated to give a 6:1 ratio of starting material and alkyne product. The residue was re-dried using ether and $Na_2SO_4$ and re-subjected to the reaction conditions above. The reaction mixture was worked up in the same manner to provide 2,2-dimethylbut-3-ynoic acid (12.0 g, 94%).

benzyl 2,2-dimethylbut-3-ynoate

**[0800]** To a stirred solution of 2,2-dimethylbut-3-ynoic acid (87.7 g, 0.782 mmol) and benzyl alcohol (114.6 g, 0.938 mol) in dichloromethane (800 mL) was added DCC (193.5 g, 0.938 mmol) at −20° C. The reaction mixture was stirred at room temperature overnight and then the solvent was evaporated in vacuo. The residue was purified by chromatography on silica gel (2% ethyl acetate in petroleum ether as eluant) to afford benzyl 2,2-dimethylbut-3-ynoate (100 g, 59% yield). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.37-7.36 (m, 5H), 5.19 (s, 2H), 2.28 (s, 1H), 1.52 (s, 6H).

2-(1-(Tert-butyldimethylsilyloxy)-2-methylpropan-2-yl)-6-fluoro-5-nitro-1H-indole

**[0801]**

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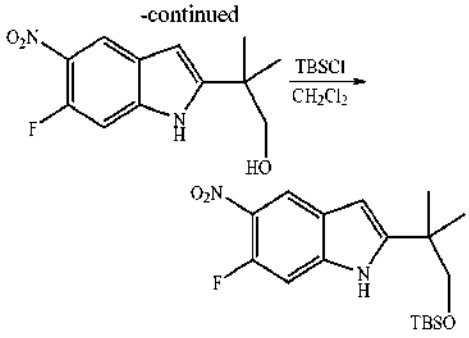



Step a: benzyl 4-(2-amino-4-fluoro-5-nitrophenyl)-2,2-dimethylbut-3-ynoate

**[0802]** To a solution of 2-bromo-5-fluoro-4-nitroaniline (23.0 g, 0.1 mol) in $Et_3N$ (250 mL) was added benzoic 2,2-dimethylbut-3-ynoic anhydride (59.0 g, 0.29 mol), CuI (1.85 g) and $Pd(PPh_3)_2Cl_2$ (2.3 g) at room temperature. The mixture was stirred at 80° C. overnight. After cooling to room temperature, the reaction was quenched with water and the aqueous layer was extracted with ethyl acetate (100 mL×3). The combined organic layer was dried over anhydrous $Na_2SO_4$, the solvent was evaporated in vacuo. The residue was purified by chromatography on silica gel (10% ethyl acetate in petroleum ether) to give benzyl 4-(2-amino-4-fluoro-5-nitrophenyl)-2,2-dimethylbut-3-ynoate (20.0 g, 56%). $^1H$ NMR (400 MHz, $CDCl_3$) 8.05 (d, J=8.4 Hz, 1H), 7.39-7.38 (m, 5H), 6.33 (d, J=13.2 Hz, 1H), 5.20 (s, 2H), 4.89 (br s, 2H), 1.61 (s, 6H).

Step b: benzyl 2-(6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate

**[0803]** To a solution of benzyl 4-(2-amino-4-fluoro-5-nitrophenyl)-2,2-dimethylbut-3-ynoate (20.0 g, 56 mmol) in acetonitrile (100 mL) was added $PdCl_2$ (5.0 g, 28 mmol) at room temperature. The mixture was stirred at 80° C. overnight. The mixture was filtered off and the solvent was evaporated in vacuo, the residue was purified by chromatography on silica gel (10% EtOAc in petroleum ether) to give benzyl 2-(6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate (18.0 g, 90%). $^1H$ NMR (300 MHz, $CDCl_3$) 8.96 (br s, 1H), 8.33 (d, J=7.2 Hz, 1H) 7.35-7.28 (m, 5H) 7.08 (d, J=11.7 Hz, 1H), 6.47 (s, 1H), 5.18 (s, 2H) 1.69 (s, 6H).

Step c: 2-(6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol

**[0804]** To a solution of benzyl 2-(6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate (18.0 g, 0.05 mol) in $CH_2Cl_2$ (100 mL) was added DIBAL-H (12 mL) at −78° C. The mixture was stirred for 1 h at that temperature and was warmed to room temperature. The reaction was quenched with water and the aqueous layer was extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous $Na_2SO_4$, the solvent was evaporated in vacuo. The residue was purified by chromatography on silica gel (10% EtOAc in petroleum ether) to give 2-(6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol (10.0 g, 77%). $^1H$ NMR (300 MHz, $CDCl_3$) 9.37 (s, 1H), 8.32 (d, J=7.2 Hz, 1H), 7.11

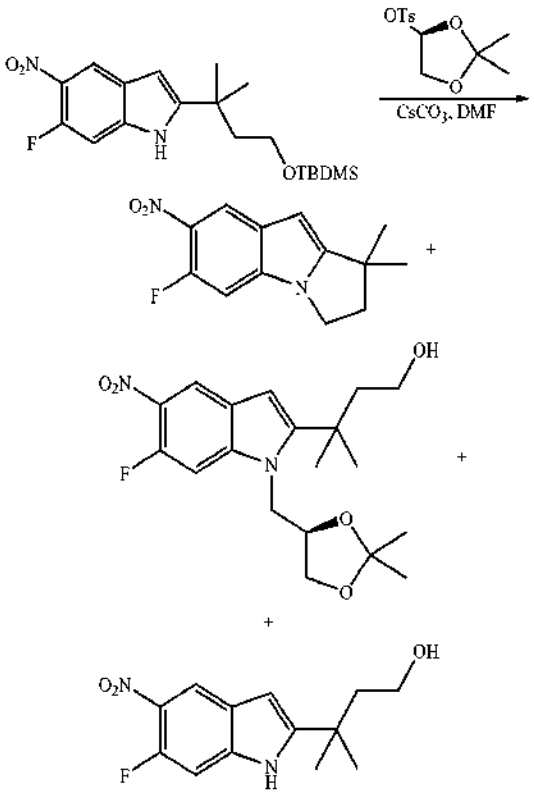
(d, J=11.7 Hz, 1H), 6.36 (s, 1H), 3.73 (d, J=5.1 Hz 2H), 1.97 (t, J=5.1 Hz. 1H), 1.39 (s, 6H).

Step d: 2-(1-(tert-butyldimethylsilyloxy)-2-methylpropan-2-yl)-6-fluoro-5-nitro-1H-indole

**[0805]** To a stirred solution of 2-(6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol (10.0 g) in $CH_2Cl_2$ was added TBSCl (8.9 g), imidazole (8.1 g, 0.12 mol) at room temperature. The mixture was stirred overnight. The solvent was evaporated in vacuo and the residue was purified by chromatography on silica gel (10% EtOAc in petroleum ether) to give 2-(1-(tert-butyldimethylsilyloxy)-2-methylpropan-2-yl)-6-fluoro-5-nitro-1H-indole (5.3 g, 38%). $^1$H NMR (300 MHz, $CDCl_3$) 9.51 (s, 1H), 8.31 (d, J=7.5 Hz, 1H), 7.02 (d, J=11.7 Hz, 1H), 6.32 (s, 1H), 3.63 (s, 2H), 1.35 (s, 6H), 0.99 (s, 9H), 0.11 (s, 6H).

6-fluoro-1,1-dimethyl-7-nitro-2,3-dihydro-1H-pyrrolo[1,2-a]indole, (R)-3-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol, 2-(4-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-methylbutan-2-yl)-1-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indole, 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol and (R)-2-(4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-methylbutan-2-yl)-6-fluoro-5-nitro-1H-indole

**[0806]**

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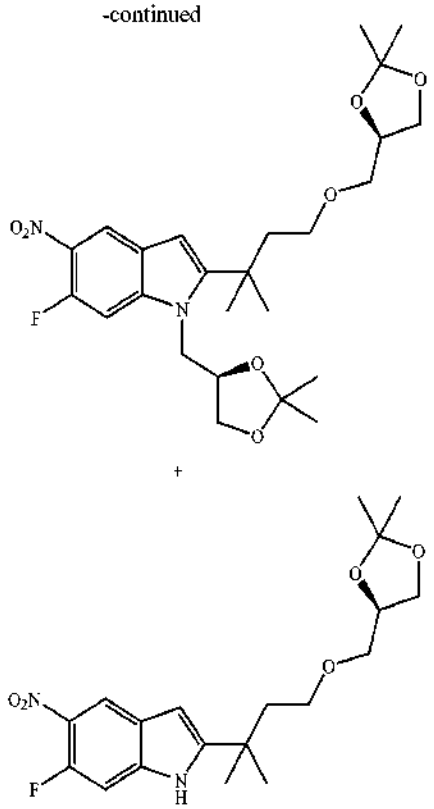


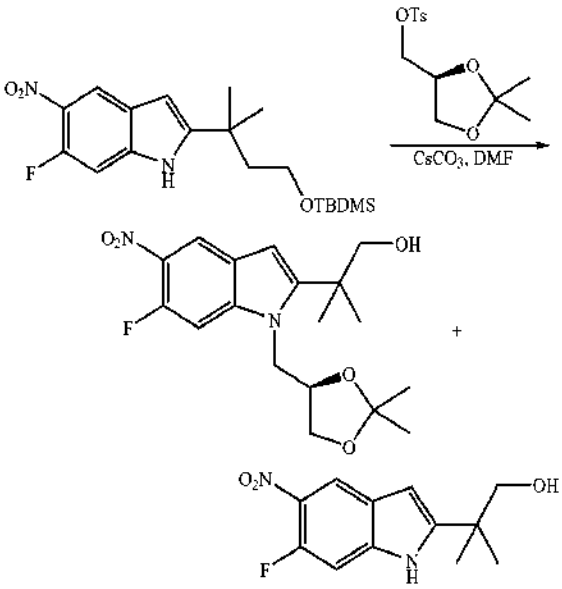
Step a: 6-fluoro-1,1-dimethyl-7-nitro-2,3-dihydro-1H-pyrrolo[1,2-a]indole, (R)-3-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol, 2-(4-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-methylbutan-2-yl)-1-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indole, 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol and (R)-2-(4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-methylbutan-2-yl)-6-fluoro-5-nitro-1H-indole

**[0807]** To a solution of 2-(4-(tert-butyldimethylsilyloxy)-2-methylbutan-2-yl)-6-fluoro-5-nitro-1H-indole (1.9 g, 5.0 mmol) and (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (2.86 g, 10.0 mmol) in DMF (10 mL) was added $Cs_2CO_3$ (4.88 g, 15.0 mmol). The mixture was heated at 90° C. for 24 hours. The reaction was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine and dried over $MgSO_4$. After the removal of solvent, the residue was purified by column chromatography (10-50% ethyl acetate-hexane) to afford 6-fluoro-1,1-dimethyl-7-nitro-2,3-dihydro-1H-pyrrolo[1,2-a]indole (600 mg, 48%). ESI-MS m/z calc. 248.1, found 249.2 $(M+1)^+$. Retention time 2.00 minutes; 2-(4-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-methylbutan-2-yl)-1-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indole (270 mg, containing some (R)-2-(4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-methylbutan-2-yl)-6-fluoro-5-nitro-1H-indole). ESI-MS m/z calc. 494.2 and 380.2, found 495.4 and 381.4 $(M+1)^+$. Retention time 2.12 and 1.92 minutes; (R)-3-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-

1-ol (1.0 g, containing some 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol). ESI-MS m/z calc. 380.2 and 266.1, found 381.2 and 267.2 $(M+1)^+$. Retention time 1.74 and 1.48 minutes.

(R)-2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol and 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol

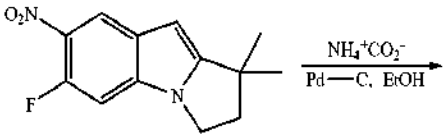
**[0808]**




**[0809]** A mixture containing (R)-2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol and 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol was obtained following the procedure shown above starting from 2-(1-(tert-butyldimethylsilyloxy)-2-methylpropan-2-yl)-6-fluoro-5-nitro-1H-indole. (R)-2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol, ESI-MS m/z calc. 366.2, found 367.2 $(M+1)^+$. Retention time 1.71 minutes; 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol, ESI-MS m/z calc. 252.1, found 253.4 $(M+1)^+$. Retention time 1.42 minutes.

1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(6-fluoro-1,1-dimethyl-2,3-dihydro-1H-pyrrolo[1,2-a]indol-7-yl)cyclopropanecarboxamide

**[0810]**

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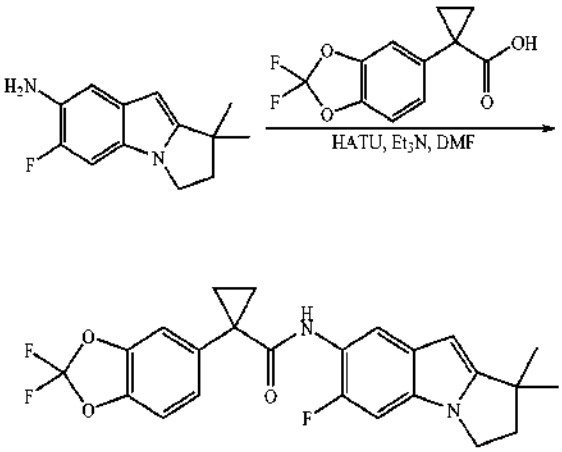



Step a: 6-fluoro-1,1-dimethyl-2,3-dihydro-1H-pyrrolo[1,2-a]indol-7-amine

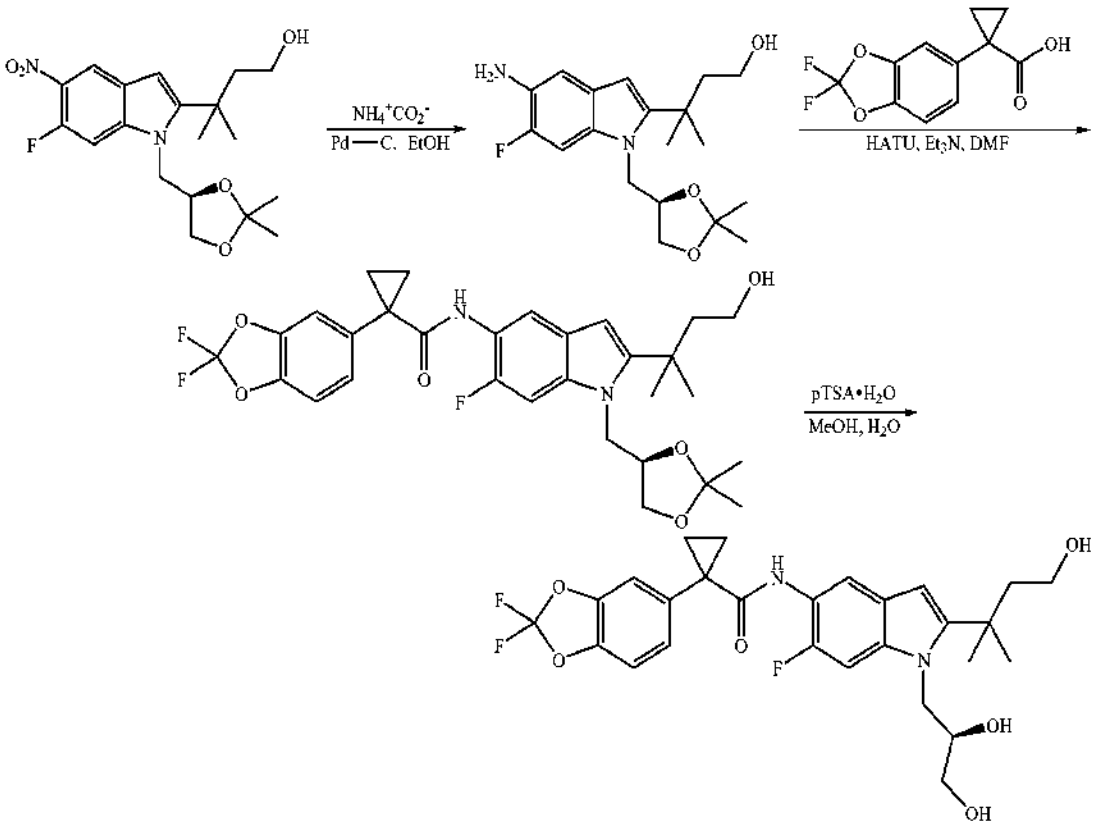
**[0811]** To a solution of 6-fluoro-1,1-dimethyl-7-nitro-2,3-dihydro-1H-pyrrolo[1,2-a]indole (600 mg, 2.4 mmol) in ethanol (15 mL) was added ammonium formate (600 mg, 9.5 mmol) and Pd/C (10%, 129 mg. 0.12 mmol). The mixture was refluxed for 10 min. The Pd catalyst was removed via filtration through Celite and washed with ethanol. The filtrate was concentrated and purified by column chromatography (20-40% ethyl acetate-hexanes) to provide 6-fluoro-1,1-dimethyl-2,3-dihydro-1H-pyrrolo[1,2-a]indol-7-amine (260 mg, 49%). ESI-MS m/z calc. 218.1, found 219.2 $(M+1)^+$. Retention time 1.01 minutes.

Step b: 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(6-fluoro-1,1-dimethyl-2,3-dihydro-1H-pyrrolo[1,2-a]indol-7-yl)cyclopropanecarboxamide

**[0812]** To a mixture of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (346 mg, 1.4 mmol), 6-fluoro-1,1-dimethyl-2,3-dihydro-1H-pyrrolo[1,2-a]indol-7-amine (260 mg, 1.2 mmol) and HATU (543 mg, 1.4 mmol) in DMF (5 mL) was added triethylamine (0.40 mL, 2.9 mmol). The reaction was stirred at room temperature overnight and then partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine and dried over $MgSO_4$. After the removal of solvent, the residue was purified by column chromatography (10-20% ethyl acetate-hexanes) to afford 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(6-fluoro-1,1-dimethyl-2,3-dihydro-1H-pyrrolo[1,2-a]indol-7-yl)cyclopropanecarboxamide (342 mg, 65%). ESI-MS m/z calc. 442.2, found 443.5 $(M+1)^+$. Retention time 2.30 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.20 (d, J=7.6 Hz, 1H), 7.30-7.25 (m, 3H), 7.20 (m, 1H), 7.12 (d, J=8.2 Hz, 1H), 6.84 (d, J=11.1 Hz, 1H), 6.01 (d, J=0.5 Hz, 1H), 3.98 (t, J=6.8 Hz, 2H), 2.37 (t, J=6.8 Hz, 2H), 1.75 (dd, J=3.8, 6.9 Hz, 2H), 1.37 (s, 6H) and 1.14 (dd, J=3.9, 6.9 Hz, 2H) ppm.

(R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(4-hydroxy-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0813]**




Step a: (R)-3-(5-amino-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-H-indol-2-yl)-3-methylbutan-1-ol

**[0814]** To a solution of (R)-3-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol containing some 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol (500 mg, 1.3 mmol) in ethanol (10 mL) was added ammonium formate (500 mg, 7.9 mmol) and Pd/C (10%, 139 mg, 0.13 mmol). The mixture was refluxed for 5 min. The Pd catalyst was removed via filtration through Celite and washed with ethanol. The filtrate was evaporated to dryness and purified by column chromatography (30-50% ethyl acetate-hexanes) to provide (R)-3-(5-amino-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-1H-indol-2-yl)-3-methylbutan-1-ol (220 mg, 48%, contains some 3-(5-amino-6-fluoro-1H-indol-2-yl)-3-methylbutan-1-ol). ESI-MS m/z calc. 350.2 found 351.4 $(M+1)^+$. Retention time 0.94 minutes.

Step b: (R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-2-(4-hydroxy-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0815]** To a mixture of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (183 mg, 0.75 mmol), (R)-3-(5-amino-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-1H-indol-2-yl)-3-methylbutan-1-ol containing some 3-(5-amino-6-fluoro-1H-indol-2-yl)-3-methylbutan-1-ol (220 mg, 0.63 mmol) and HATU (287 mg, 0.75 mmol) in DMF (3.0 mL) was added triethylamine (0.21 mL, 1.5 mmol). The reaction was stirred at room temperature overnight and then partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine and dried over $MgSO_4$. After the removal of solvent, the residue was purified by column chromatography (20-40% ethyl acetate-hexanes) to afford (R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-2-(4-hydroxy-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (315 mg, 87%, contains some 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(6-fluoro-2-(4-hydroxy-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide). ESI-MS m/z calc. 574.2 found 575.7 $(M+1)^+$. Retention time 2.08 minutes.

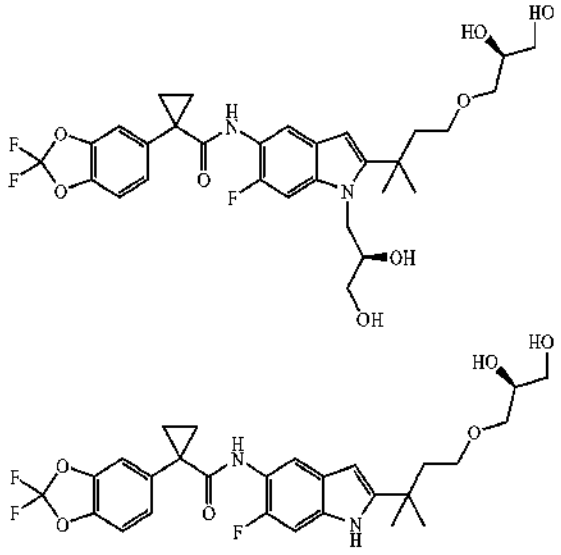
Step c: (R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(4-hydroxy-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0816]** To a solution of (R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-2-(4-hydroxy-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide containing some 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(6-fluoro-2-(4-hydroxy-2-methylbutan-2-yl)-1H-indol-5-yl)

cyclopropanecarboxamide (315 mg, 0.55 mmol) in methanol (3 mL) and water (0.3 mL) was added p-TsOH.$H_2O$ (21 mg, 0.11 mmol). The mixture was heated at 80° C. for 30 minutes. The reaction was partitioned between ethyl acetate and water and the aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with saturated. $NaHCO_3$ solution and brine and dried over $MgSO_4$. After the removal of solvent, the residue was purified by column chromatography (20-80% ethyl acetate-hexanes) to provide (R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(4-hydroxy-2-methylbutan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (92 mg, 31%). ESI-MS m/z calc. 534.2, found 535.5 $(M+1)^+$. Retention time 1.72 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.32 (s, 1H), 7.53 (d, J=1.0 Hz, 1H), 7.43-7.31 (m, 4H), 6.17 (s, 1H), 4.97-4.92 (m, 2H), 4.41 (dd, J=2.4, 15.0 Hz, 1H), 4.23 (t, J=5.0 Hz, 1H), 4.08 (dd, J=8.6, 15.1 Hz, 1H), 3.87 (s, 1H), 3.48-3.44 (m, 1H), 3.41-3.33 (m, 1H), 3.20 (dd, J=7.4, 12.7 Hz, 2H), 1.94-1.90 (m, 2H), 1.48-1.45 (m, 2H), 1.42 (s, 3H), 1.41 (s, 3H) and 1.15-1.12 (m, 2H) ppm.

1-(2,2-difluorobenzo[d][1,3]-dioxol-5-yl)-N-(2-(4-((S)-2,3-dihydroxypropoxy)-2-methylbutan-2-yl)-1-((R)-2,3-dihydroxypropyl)-6-fluoro-1H-indol-5-yl) cyclopropanecarboxamide and (S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(2-(4-(2,3-dihydroxypropoxy)-2-methylbutan-2-yl)-6-fluoro-1H-indol-5-yl)cyclopropanecarboxamide

**[0817]**

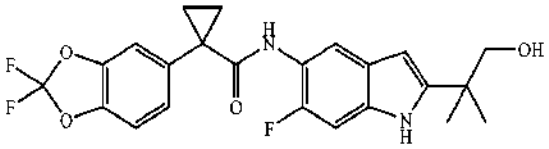


1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(2-(4-((S)-2,3-dihydroxypropoxy)-2-methylbutan-2-yl)-1-((R)-2,3-dihydroxypropyl)-6-fluoro-1H-indol-5-yl) cyclopropanecarboxamide and (S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(2-(4-(2,3-dihydroxypropoxy)-2-methylbutan-2-yl)-6-fluoro-1H-indol-5-yl)cyclopropanecarboxamide

**[0818]** 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(2-(4-((S)-2,3-dihydroxypropoxy)-2-methylbutan-2-yl)-1-((R)-2,3-dihydroxypropyl)-6-fluoro-1H-indol-5-yl)cyclopropanecarboxamide and (S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(2-(4-(2,3-dihydroxypropoxy)-2-methylbutan-2-yl)-6-fluoro-1H-indol-5-yl)cyclopropanecarboxamide were made following a scheme similar as shown above starting from 2-(4-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-methylbutan-2-yl)-1-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indole containing some (R)-2-(4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-2-methylbutan-2-yl)-6-fluoro-5-nitro-1H-indole). 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(2-(4-((S)-2,3-dihydroxypropoxy)-2-methylbutan-2-yl)-1-((R)-2,3-dihydroxypropyl)-6-fluoro-1H-indol-5-yl) cyclopropanecarboxamide, ESI-MS m/z calc. 608.2, found 609.5 $(M+1)^+$. Retention time 1.67 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.32 (s, 1H), 7.53 (s, 1H), 7.43-7.31 (m, 4H), 6.19 (s, 1H), 4.95-4.93 (m, 2H), 4.51 (d, J=5.0 Hz, 1H), 4.42-4.39 (m, 2H), 4.10-4.04 (m, 1H), 3.86 (s, 1H), 3.49-3.43 (m, 2H), 3.41-3.33 (m, 1H), 3.30-3.10 (m, 6H), 2.02-1.97 (m, 2H), 1.48-1.42 (m, 8H) and 1.13 (dd, J=4.0, 6.7 Hz, 2H) ppm; (S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(2-(4-(2,3-dihydroxypropoxy)-2-methylbutan-2-yl)-6-fluoro-1H-indol-5-yl)cyclopropanecarboxamide, ESI-MS m/z calc. 534.2, found 535.5 $(M+1)^+$. Retention time 1.81 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 10.91 (d, J=1.5 Hz, 1H), 8.30 (s, 1H), 7.53 (s, 1H), 7.42-7.33 (m, 3H), 7.03 (d, J=10.9 Hz, 1H), 6.07 (d, J=1.6 Hz, 1H), 4.56 (d, J=5.0 Hz, 1H), 4.43 (t, J=5.7 Hz, 1H), 3.51-3.46 (m, 1H), 3.31-3.13 (m, 6H), 1.88 (t, J=7.3 Hz, 2H), 1.48-1.45 (m, 2H), 1.31 (s, 6H) and 1.15-1.12 (m, 2H) ppm.

1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0819]**

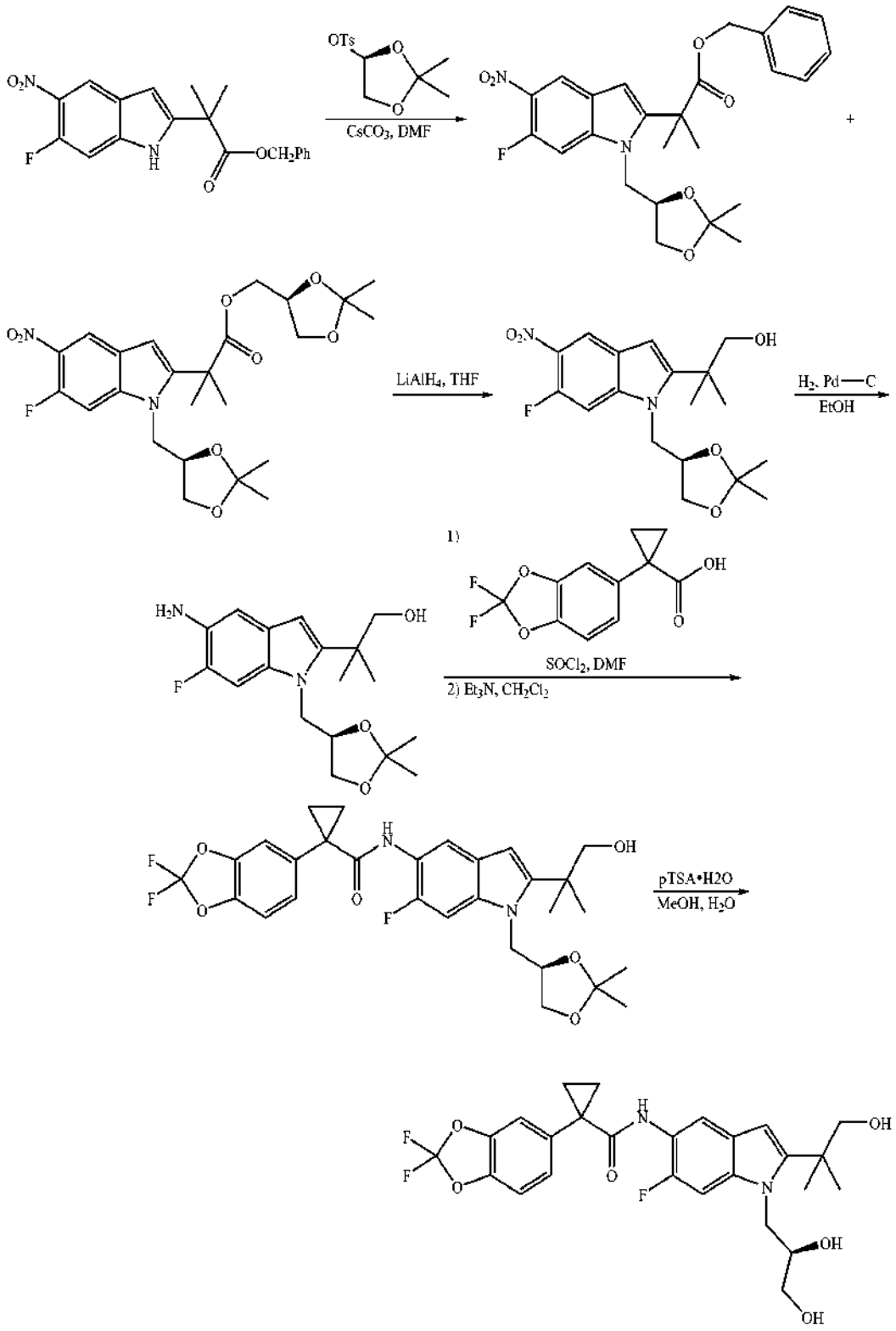


1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0820]** 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl) cyclopropanecarboxamide was made following the scheme shown above starting from a mixture containing (R)-2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol and 3-(6-fluoro-5-nitro-1H-indol-2-yl)-3-methylbutan-1-ol. ESI-MS m/z calc. 446.2, found 447.5 $(M+1)^+$. Retention time 1.88 minutes. $^1H$ NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.20 (d, J=7.7 Hz, 1H), 7.30-7.21 (m, 3H), 7.12 (d, J=8.2 Hz, 1H), 6.94 (d, J=11.2 Hz, 1H), 6.18 (s, 1H), 3.64 (s, 2H), 1.75 (dd, J=3.8, 6.8 Hz, 2H), 1.34 (s, 6H) and 1.14 (dd, J=3.9, 6.9 Hz, 2H) ppm.

(R)-1-(2,2-Difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0821]**



Step a: (R)-Benzyl 2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate and ((S)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-(1-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate

**[0822]** Cesium carbonate (8.23 g, 25.3 mmol) was added to a mixture of benzyl 2-(6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate (3.0 g, 8.4 mmol) and (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (7.23 g, 25.3 mmol) in DMF (17 mL). The reaction was stirred at 80° C. for 46 hours under nitrogen atmosphere. The mixture was then partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate. The combined ethyl acetate layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product, a viscous brown oil which contains both of the products shown above, was taken directly to the next step without further purification. (R)-Benzyl 2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate, ESI-MS m/z calc. 470.2, found 471.5 $(M+1)^+$. Retention time 2.20 minutes. ((S)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-(1-(((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate, ESI-MS m/z calc. 494.5, found 495.7 $(M+1)^+$. Retention time 2.01 minutes.

Step b: (R)-2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol

**[0823]** To the crude reaction mixture obtained in step (a) was dissolved in THF (42 mL) and cooled in an ice-water bath. $LiAlH_4$ (16.8 mL of 1 M solution, 16.8 mmol) was added drop-wise. After the addition was complete, the reaction was stirred for an additional 5 minutes. The reaction was quenched by adding water (1 mL), 1-5% NaOH solution (1 mL) and then water (3 mL). The mixture was filtered over Celite, and the solids were washed with THF and ethyl acetate. The filtrate was concentrated and purified by column chromatography (30-60% ethyl acetate-hexanes) to obtain the product as a brown oil (2.68 g, 87% over 2 steps). ESI-MS m/z calc. 366.4, found 367.3 $(M+1)^+$. Retention time 1.68 minutes. $^1$H NMR (400 MHz, DMSO-d6) δ 8.34 (d, J=7.6 Hz, 1H), 7.65 (d, J=13.4 Hz, 1H), 6.57 (s, 1H), 4.94 (t, J=5.4 Hz, 1H), 4.64-4.60 (m, 1H), 4.52-4.42 (m, 2H), 4.16-4.14 (m, 1H), 3.76-3.74 (m, 1H), 3.63-3.53 (m, 2H), 1.42 (s, 3H), 1.38-1.36 (m, 6H) and 1.19 (s, 3H) ppm

Step c: (R)-2-(5-amino-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-1H-indol-2-yl)-2-methylpropan-1-ol

**[0824]** (R)-2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol (2.5 g, 6.82 mmol) was dissolved ethanol (70 mL) and the reaction was flushed with $N_2$. Then Pd—C (250 mg, 5% wt) was added. The reaction was flushed with nitrogen again and then stirred under $H_2$ (atm). After 2.5 hours only partial conversion to the product was observed by LCMS. The reaction was filtered through Celite and concentrated. The residue was re-subjected to the conditions above. After 2 hours LCMS indicated complete conversion to product. The reaction mixture was filtered through Celite. The filtrate was concentrated to yield the product as a black solid (1.82 g, 79%). ESI-MS m/z calc. 336.2, found 337.5 $(M+1)^+$. Retention time 0.86 minutes. $^1$H NMR (400 MHz, DMSO-d6) δ 7.17 (d, J=12.6 Hz, 1H), 6.76 (d, J=9.0 Hz, 1H), 6.03 (s, 1H), 4.79-4.76 (m, 1H), 4.46 (s, 2H), 4.37-4.31 (m, 3H), 4.06 (dd, J=6.1, 8.3 Hz, 1H), 3.70-3.67 (m, 1H), 3.55-3.52 (m, 2H), 1.41 (s, 3H), 1.32 (s, 6H) and 1.21 (s, 3H) ppm.

Step d: (R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

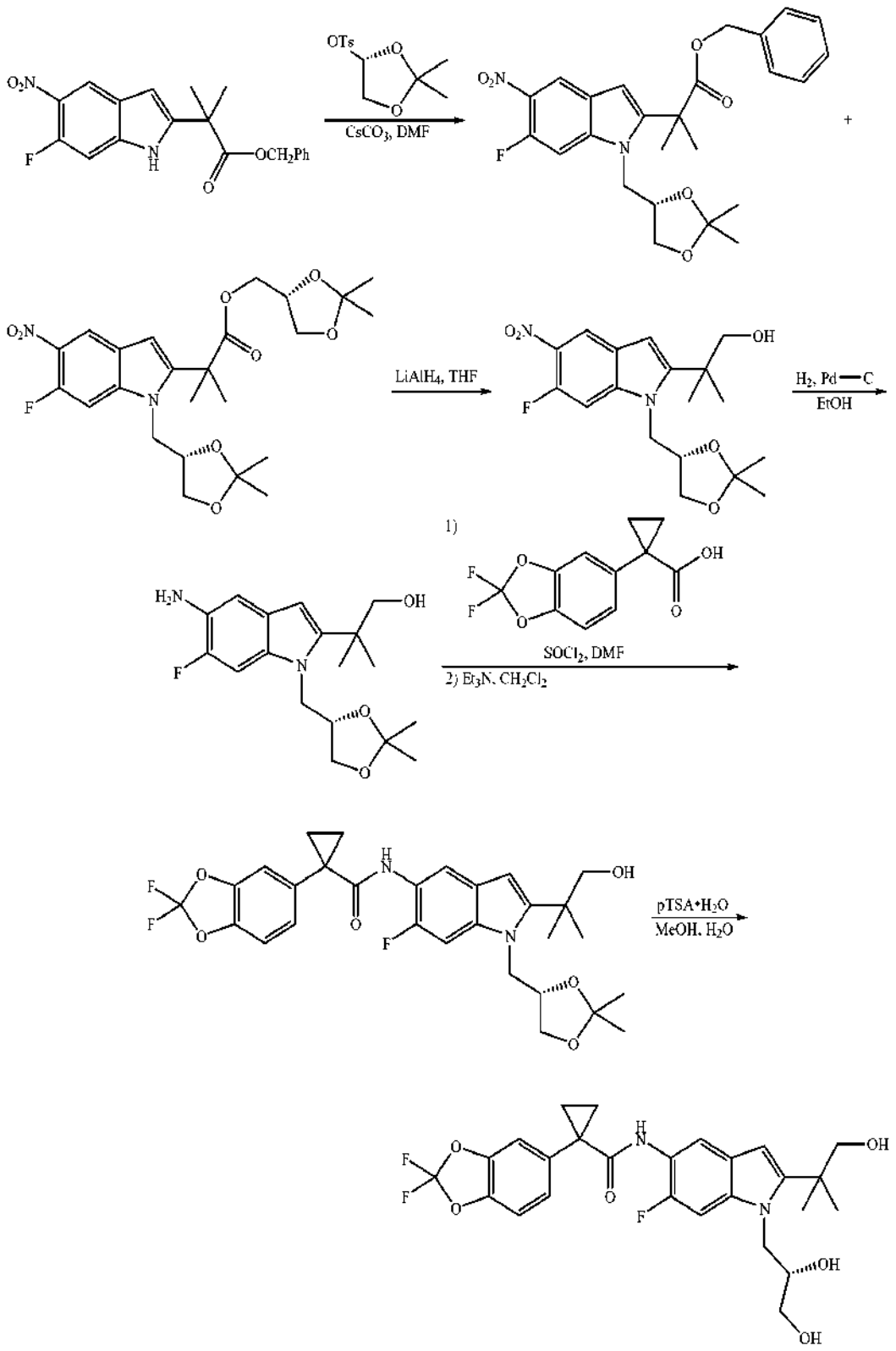
**[0825]** DMF (3 drops) was added to a stirring mixture of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (1.87 g, 7.7 mmol) and thionyl chloride (1.30 mL, 17.9 mmol). After 1 hour a clear solution had formed. The solution was concentrated under vacuum and then toluene (3 mL) was added and the mixture was concentrated again. The toluene step was repeated once more and the residue was placed on high vacuum for 10 minutes. The acid chloride was then dissolved in dichloromethane (10 mL) and added to a mixture of (R)-2-(5-amino-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-1H-indol-2-yl)-2-methylpropan-1-ol (1.8 g, 5.4 mmol) and triethylamine (2.24 mL, 16.1 mmol) in dichloromethane (45 mL). The reaction was stirred at room temperature for 1 hour. The reaction was washed with 1N HCl solution, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and concentrated to yield the product as a black foamy solid (3 g, 100%). ESI-MS m/z calc. 560.6, found 561.7 $(M+1)^+$. Retention time 2.05 minutes. $^1$H NMR (400 MHz, DMSO-d6) δ 8.31 (s, 1H), 7.53 (s, 1H), 7.42-7.40 (m, 2H), 7.34-7.30 (m, 3H), 6.24 (s, 1H), 4.51-4.48 (m, 1H), 4.39-4.34 (m, 2H), 4.08 (dd, J=6.0, 8.3 Hz, 1H), 3.69 (t, J=7.6 Hz, 1H), 3.58-3.51 (m, 2H), 1.48-1.45 (m, 2H), 1.39 (s, 3H), 1.34-1.33 (m, 6H), 1.18 (s, 3H) and 1.14-1.12 (m, 2H) ppm

Step e: (R)-1 (2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0826]** (R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (3.0 g, 5.4 mmol) was dissolved in methanol (52 mL). Water (5.2 mL) was added followed by p-TsOH. $H_2O$ (204 mg, 1.1 mmol). The reaction was heated at 80° C. for 45 minutes. The solution was concentrated and then partitioned between ethyl acetate and saturated $NaHCO_3$ solution. The ethyl acetate layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (50-100% ethyl acetate-hexanes) to yield the product as a cream colored foamy solid. (1.3 g, 47%, ee>98% by SFC). ESI-MS m/z calc. 520.5, found 521.7 $(M+1)^+$. Retention time 1.69 minutes. $^1$H NMR (400 MHz, DMSO-d6) δ 8.31 (s, 1H), 7.53 (s, 1H), 7.42-7.38 (m, 2H), 7.33-7.30 (m, 2H), 6.22 (s, 1H), 5.01 (d, J=5.2 Hz, 1H), 4.90 (t, J=5.5 Hz, 1H), 4.75 (t, J=5.8 Hz, 1H), 4.40 (dd, J=2.6, 15.1 Hz, 1H), 4.10 (dd, J=8.7, 15.1 Hz, 1H), 3.90 (s, 1H), 3.65-3.54 (m, 2H), 3.48-3.33 (m, 2H), 1.48-1.45 (m, 2H), 1.35 (s, 3H), 1.32 (s, 3H) and 1.14-1.11 (m, 2H) ppm.

(S)-1-(2,2-Difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0827]**



Step a: (S)-Benzyl 2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate and ((R)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-(1-(((S)-2,2-dimethyl-1.3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate

**[0828]** Cesium carbonate (2.74 g, 8.4 mmol) was added to a mixture of benzyl 2-(6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate (1.0 g, 2.8 mmol) and (S)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (3.21 g, 11.2 mmol) in DMF (5.6 mL). The reaction was stirred at 80° C. for 64 hours under nitrogen atmosphere. The mixture was then partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate. The combined ethyl acetate layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product, a viscous brown oil which contains both of the products shown above, was taken directly to the next step without further purification. (S)-Benzyl 2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate, ESI-MS m/z calc. 470.2, found 471.5 $(M+1)^+$. Retention time 2.22 minutes. ((R)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-(1-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate, ESI-MS m/z calc. 494.5, found 495.5 $(M+1)^+$. Retention time 2.03 minutes.

Step b: (S)-2-(1-((2,2-Dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol

**[0829]** The mixture of crude reaction mixture of (S)-benzyl 2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate and ((R)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl 2-(1-(((S)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropanoate was dissolved in THF (15 mL) and cooled in an ice-water bath. $LiAlH_4$ (2.8 mL of 1 M solution, 2.8 mmol) was added dropwise. After addition was complete the reaction was stirred for 5 minutes. The reaction was quenched by adding water (0.5 mL), 15% NaOH solution (0.5 mL) and then water (1.5 mL). The mixture was filtered over Celite, and the solids were washed with THF and ethyl acetate. The filtrate was concentrated and purified by column chromatography (30-60% ethyl acetate-hexanes) to obtain the product as a brown oil (505 mg, 49% over 2 steps). ESI-MS m/z calc. 366.4, found 367.3 $(M+1)^+$. Retention time 1.68 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.34 (d, J=7.6 Hz, 1H), 7.65 (d, J=13.5 Hz, 1H), 6.57 (s, 1H), 4.94 (t, J=5.4 Hz, 1H), 4.64-4.60 (m, 1H), 4.52-4.42 (m, 2H), 4.14 (dd, J=6.2, 8.4 Hz, 1H), 3.74 (dd, J=7.0, 8.3 Hz, 1H), 3.63-3.53 (m, 2H), 1.42 (s, 3H), 1.37 (m, 6H) and 1.19 (s, 3H) ppm.

Step c: (S)-2-(5-amino-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-1H-indol-2-yl)-2-methylpropan-1-ol

**[0830]** (S)-2-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-5-nitro-1H-indol-2-yl)-2-methylpropan-1-ol (500 mg, 1.4 mmol) was dissolved ethanol (15 mL) and the reaction was flushed with $N_2$. Then Pd—C (50 mg, 5% wt) was added. The reaction was flushed with nitrogen again and then stirred under $H_2$ (atm). After 1 hour only partial conversion to the product was observed by LCMS. The reaction was filtered through Celite and concentrated. The residue was resubjected to the conditions above. After 1 hour LCMS indicated complete conversion to product. The reaction mixture was filtered through Celite. The filtrate was concentrated to yield the product as a black solid (420 mg, 91%). ESI-MS m/z calc. 336.2, found 337.5 $(M+1)^+$. Retention time 0.90 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 7.17 (d, J=12.6 Hz, 1H), 6.76 (d, J=9.0 Hz, 1H), 6.03 (s, 1H), 4.78 (br s, 1H), 4.46 (s, 2H), 4.41-4.27 (m, 3H), 4.06 (dd, J=6.1, 8.3 Hz, 1H), 3.70-3.67 (m, 1H), 3.53 (dd, J=10.7, 17.2 Hz, 2H), 1.40 (s, 3H), 1.32 (s, 6H) and 1.21 (s, 3H) ppm.

Step d: (S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0831]** DMF (3 drops) was added to a stirring mixture of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid (187 mg, 0.8 mmol) and thionyl chloride (0.13 mL, 1.8 mmol). After 30 minutes a clear solution had formed. A small amount was mixed piperidine to test that the acid chloride had been formed. The solution was concentrated on the rotovap and then toluene (1 mL) was added and the mixture was concentrated again. The toluene step was repeated once more and the residue was placed on high vacuum for 10 minutes. The acid chloride was then dissolved in dichloromethane (2 mL) and added to a mixture of (S)-2-(5-amino-1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-1H-indol-2-yl)-2-methylpropan-1-ol (200 mg, 0.6 mmol) and triethylamine (0.25 mL, 1.8 mmol) in dichloromethane (4 mL). The reaction was stirred at room temperature for 45 minutes. The reaction was washed with 1N HCl solution, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and concentrated to yield the product as a black foamy solid (320 mg, 96%). ESI-MS m/z calc. 560.6, found 561.5 $(M+1)^+$. Retention time 2.05 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.31 (s, 1H), 7.53 (s, 1H), 7.42-7.40 (m, 2H), 7.34-7.30 (m, 3H), 6.24 (s, 1H), 4.84 (t, J=5.5 Hz, 1H), 4.51-4.46 (m, 1H), 4.41-4.32 (m, 2H), 4.08 (dd, J=6.0, 8.3 Hz, 1H), 3.71-3.67 (m, 1H), 3.58-3.50 (m, 2H), 1.48-1.45 (m, 2H), 1.40 (s, 3H), 1.34-1.33 (m, 6H), 1.18 (s, 3H) and 1.14-1.12 (m, 2H) ppm.

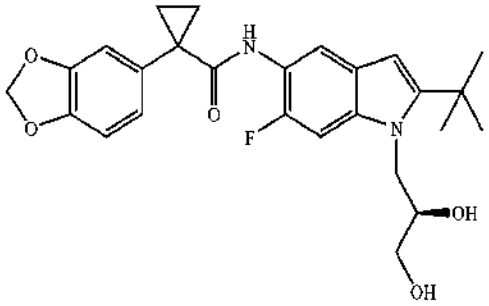
Step e: (S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide

**[0832]** (S)-1-(2,2-difluorobenzo[d][1.3]dioxol-5-yl)-N-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (290 g, 0.5 mmol) was dissolved in methanol (5 mL). Water (0.5 mL) was added followed by p-TsOH.$H_2O$ (20 mg, 0.1 mmol). The reaction was heated at 80° C. for 45 minutes. The solution was then partitioned between ethyl acetate and saturated $NaHCO_3$ solution. The ethyl acetate layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (50-100% ethyl acetate-hexanes) to yield the product as a cream colored foamy solid. (146 mg, 54%, ee>97% by SFC). ESI-MS m/z calc. 520.5, found 521.5 $(M+1)^+$. Retention time 1.67 minutes. $^1H$ NMR (400 MHz, DMSO-d6) δ 8.31 (s, 1H), 7.53 (d, J=1.1 Hz, 1H), 7.42-7.37 (m, 2H), 7.33-7.30 (m, 2H), 6.22 (s, 1H), 5.01 (d, J=5.0 Hz, 1H), 4.91 (t, J=5.5 Hz, 1H), 4.75 (t, J=5.8 Hz, 1H), 4.42-4.38 (m, 1H), 4.10 (dd, J=8.8, 15.1 Hz,

1H), 3.90 (s, 1H), 3.64-3.54 (m, 2H), 3.48-3.33 (m, 2H), 1.48-1.45 (m, 2H). 1.35 (s, 3H), 1.32 (s, 3H) and 1.14-1.11 (m, 2H) ppm.

(R)-1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-6-fluoro-1H-indol-5-yl)cyclopropanecarboxamide

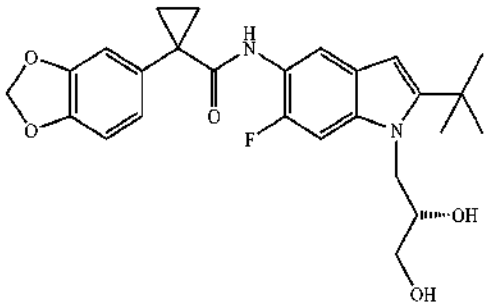
**[0833]**



**[0834]** (R)-1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-6-fluoro-1H-indol-5-yl)cyclopropanecarboxamide was prepared using an experimental procedure similar to example 72 from 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid and 2-tert-butyl-6-fluoro-5-nitro-1H-indole.

(S)-1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-6-fluoro-1H-indol-5-yl)cyclopropanecarboxamide

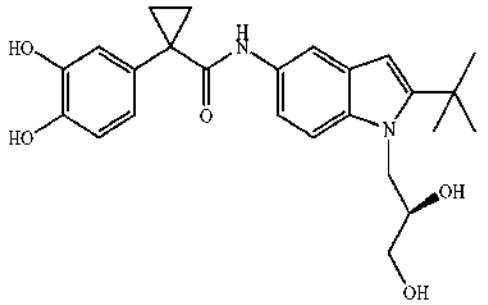
**[0835]**



**[0836]** (S)-1-(benzo[d][1,3]dioxol-5-yl)-N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-6-fluoro-1H-indol-5-yl)cyclopropanecarboxamide was prepared using an experimental procedure similar to Example 72 from 1-(benzo[d][1,3]dioxol-5-yl)cyclopropanecarboxylic acid and 2-tert-butyl-6-fluoro-5-nitro-1H-indole.

(R) —N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(3,4-dihydroxyphenyl)cyclopropanecarboxamide

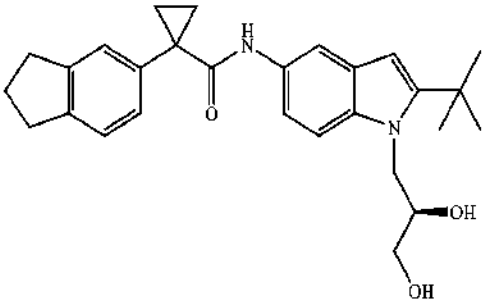
**[0837]**



**[0838]** (R)—N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(3,4-dihydroxyphenyl)cyclopropanecarboxamide was prepared using an experimental procedure similar to Example 72 from 1-(3,4-dihydroxyphenyl)cyclopropanecarboxylic acid and 2-tert-butyl-5-nitro-1H-indole.

(R) —N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,3-dihydro-1H-inden-5-yl)cyclopropanecarboxamide

**[0839]**



**[0840]** (R)—N-(2-tert-butyl-1-(2,3-dihydroxypropyl)-1H-indol-5-yl)-1-(2,3-dihydro-1H-inden-5-yl)cyclopropanecarboxamide was prepared using an experimental procedure similar to Example 72 from 1-(2,3-dihydro-1H-inden-5-yl) cyclopropanecarboxylic acid and 2-tert-butyl-5-nitro-1H-indole.

**[0841]** A person skilled in the chemical arts can use the examples and schemes along with known synthetic methodologies to synthesize compounds of the present invention, including the compounds in Table 3, below.

TABLE 3

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| 1 | 373.3 | 2.49 | |
| 2 | 469.4 | 3.99 | |
| 3 | 381.3 | 3.69 | |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| 4 | 448.3 | 1.75 | |
| 5 | 389.3 | 3.3 | |
| 6 | 463 | 1.87 | |
| 7 | 363.3 | 3.7 | |
| 8 | 405.5 | 3.87 | |
| 9 | 487.3 | 2.12 | H NMR (400 MHz, DMSO-d6) 8.65 (s, 1H), 7.55 (d, J = 1.7 Hz, 1H), 7.49 (d, J = 1.4 Hz, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.30-7.25 (m, 2H), 7.08 (dd, J = 8.8, 1.9 Hz, 1H), 6.11 (s, 1H), 4.31 (t, J = 7.4 Hz, 2H), 3.64 (t, J = 7.3 Hz, 2H), 3.20 (t, J = 7.6 Hz, 2H), 1.92 (t, J = 7.6 Hz, 2H), 1.45 (m, 2H), 1.39 (s, 6H), 1.10 (m, 2H) |
| 10 | 388 | 3.34 | |
| 11 | 452.3 | 2.51 | |
| 12 | 527 | 2.36 | |
| 13 | 498 | 1.85 | |
| 14 | 404.5 | 1.18 | |
| 15 | 369.2 | 3.81 | |
| 16 | 419.2 | 2.24 | |
| 17 | 389.2 | 2.02 | H NMR (400 MHz, DMSO) 8.41 (s, 1H), 7.59 (d, J = 1.8 Hz, 1H), 7.15 (d, J = 8.6 Hz, 1H), 7.06-7.02 (m, 2H), 6.96-6.90 (m, 2H), 6.03 (s, 2H), 5.98 (d, J = 0.7 Hz, 1H), 4.06 (t, J = 6.8 Hz, 2H), 2.35 (t, J = 6.8 Hz, 2H), 1.42-1.38 (m, 2H), 1.34 (s, 6H), 1.05-1.01 (m, 2H) |
| 18 | 395.3 | 3.6 | H NMR (400 MHz, DMSO) 10.91 (s, 1H), 7.99 (s, 1H), 7.67 (d, J = 7.7 Hz, 1H), 7.08-6.92 (m, 4H), 6.09-6.03 (m, 3H), 1.47-1.42 (m, 2H), 1.31 (d, J = 7.3 Hz, 9H), 1.09-1.05 (m, 2H) |
| 19 | 457.2 | 1.97 | H NMR (400 MHz, CD3CN) 7.50 (d, J = 1.9 Hz, 1H), 7.41 (d, J = 1.6 Hz, 2H), 7.36 (dd, J = 1.7, 8.3 Hz, 1H), 7.29-7.24 (m, 2H), 7.02 (dd, J = 2.1, 8.8 Hz, 1H), 6.24 (s, 1H), 4.40 (t, J = 7.1 Hz, 2H), 3.80 (t, J = 7.1 Hz, 2H), 1.59-1.55 (m, 2H), 1.50 (s, 9H), 1.15-1.12 (m, 2H) |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| 20 | 375.5 | 3.71 | |
| 21 | 496 | 206 | |
| 22 | 421.14 | 1.53 | |
| 23 | 363.3 | 3.62 | |
| 24 | 378.5 | 2.66 | |
| 25 | 417.5 | 3.53 | |
| 26 | 454.3 | 3.18 | |
| 27 | 596.2 | 2.58 | |
| 28 | 379.3 | 2.92 | |
| 29 | 481 | 1.69 | |
| 30 | 504.2 | 1.95 | |
| 31 | 517 | 1.92 | |
| 32 | 403.5 | 3.5 | H NMR (400 MHz, DMSO) 10.76 (s, 1H), 8.72 (s, 1H), 7.79 (d, J = 2.3 Hz, 1H), 7.62 (dd, J = 2.4, 8.6 Hz, 1H), 7.55 (d, J = 1.5 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H), 7.05-7.01 (m, 2H), 6.03 (d, J = 1.6 Hz, 1H), 4.54 (t, J = 6.4 Hz, 2H), 2.79 (t, J = 6.4 Hz, 2H), 1.44 (m, 2H), 1.32 (s, 9H), 1.03 (m, 2H) |
| 33 | 321.3 | 2.98 | |
| 34 | 450.2 | 2.02 | |
| 35 | 395.1 | 3.59 | |
| 36 | 509 | 2.01 | |
| 37 | 447.2 | 2.02 | |
| 38 | 379.1 | 2.16 | H NMR (400 MHz, DMSO) 10.78 (s, 1H), 8.39 (s, 1H), 7.57 (d, J = 1.7 Hz, 1H), 7.17 (d, J = 8.6 Hz, 1H), 7.03-6.90 (m, 4H), 6.12 (d, J = 1.5 Hz, 1H), 6.03 (s, 2H), 5.18 (s, 1H), 1.50 (s, 6H), 1.41-1.38 (m, 2H), 1.05-0.97 (m, 2H) |
| 39 | 373.3 | 3.74 | |
| 40 | 372.8 | 3.8 | |
| 41 | 397.3 | 3.41 | H NMR (400 MHz, DMSO) 11.44 (s, 1H), 8.52 (s, 1H), 7.85 (d, J = 1.2 Hz, 2H), 7.71 (d, J = 1.7 Hz, 1H), 7.47-7.43 (m, 2H), 7.32-7.26 (m, 2H), 7.12 (dd, J = 2.0, 8.7 Hz, 1H), 7.04 (d, J = 1.6 Hz, 1H), 6.97-6.90 (m, 2H), 6.84 (d, J = 1.3 Hz, 1H), 6.03 (s, 2H), 1.43-1.40 (m, 2H), 1.07-1.03 (m, 2H) |
| 42 | 505.3 | 2.23 | H NMR (400 MHz, DMSO-d6) 8.33 (s, 1H), 7.52 (s, 1H), 7.42-7.39 (m, 2H), 7.33-7.25 (m, 2H), |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| | | | 6.14 (s, 1H),<br>4.99 (s, 1H),<br>4.31-4.27 (m, 3H), 3.64 (t, J = 7.0 Hz,<br>2H),<br>3.20 (t, J = 7.6 Hz,<br>2H), 1.91 (t, J = 7.6 Hz, 2H),<br>1.46 (m, 2H),<br>1.39 (s, 6H),<br>1.13 (m, 2H) |
| 43 | 505.4 | 1.97 | |
| 44 | 407.7 | 1.76 | H NMR (400 MHz,<br>DMSO)<br>10.31 (s, 1H),<br>8.34 (s, 1H),<br>7.53 (d, J = 1.8 Hz,<br>1H), 7.03 (d, J = 1.6 Hz, 1H),<br>6.97-6.90 (m, 3H),<br>6.05-6.03 (m,<br>3H), 4.72 (s, 2H),<br>1.40-1.38 (m,<br>2H), 1.34 (s, 9H),<br>1.04-1.00 (m, 2H) |
| 45 | 497.2 | 2.26 | |
| 46 | 391.3 | 3.41 | |
| 47 | 377.5 | 3.48 | |
| 48 | 427.5 | 4.09 | |
| 49 | 402.2 | 3.06 | |
| 50 | 421.1 | 1.81 | |
| 51 | 407.5 | 3.34 | |
| 52 | 464.3 | 2.87 | |
| 53 | 405.3 | 3.65 | |
| 54 | 375 | 1.84 | |
| 55 | 505.4 | 1.96 | |
| 56 | 335.3 | 3.18 | |
| 57 | 445.2 | 3.27 | |
| 58 | 491 | 1.88 | |
| 59 | 478 | 1.98 | |
| 60 | 413.3 | 3.95 | |
| 61 | 402.5 | 3.71 | |
| 62 | 393.3 | 1.98 | |
| 63 | 407.2 | 2.91 | |
| 64 | 505.4 | 1.98 | |
| 65 | 377.5 | 3.53 | |
| 66 | 417.5 | 4.06 | |
| 67 | 333.3 | 3.53 | |
| 68 | 397.3 | 3.86 | |
| 69 | 506 | 1.67 | |
| 70 | 501 | 2.1 | |
| 71 | 335.3 | 3.22 | |
| 72 | 487 | 1.93 | |
| 73 | 417.5 | 3.88 | |
| 74 | 395 | 1.95 | |
| 75 | 548 | 1.64 | |
| 76 | 418.3 | 2.9 | |
| 77 | 377.3 | 3.87 | |
| 78 | 363.3 | 3.48 | |
| 79 | 476 | 1.8 | |
| 80 | 447.3 | 2.18 | |
| 81 | 492.4 | 2 | |
| 82 | 564.3 | 1.35 | |
| 83 | 467.3 | 1.72 | |
| 84 | 445.2 | 3.08 | |
| 85 | 389.5 | 3.86 | |
| 86 | 374.3 | 3.11 | |
| 87 | 435 | 3.87 | |
| 88 | 465 | 1.89 | |
| 89 | 411.3 | 3.89 | |
| 90 | 449.3 | 3.92 | |
| 91 | 393.3 | 3.12 | |
| 92 | 469.6 | 1.75 | |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| 93 | 476.5 | 2.88 | |
| 94 | 377.5 | 3.41 | |
| 95 | 375.3 | 3.43 | H NMR (400 MHz, DMSO) 10.52 (s, 1H), 8.39 (s, 1H), 7.46 (d, J = 1.8 Hz, 1H), 7.10-6.89 (m, 5H), 6.03 (s, 2H), 2.68-2.65 (m, 2H), 2.56-2.54 (m, 2H), 1.82-1.77 (m, 4H), 1.41-1.34 (m, 2H), 1.04-0.97 (m, 2H) |
| 96 | 346.1 | 3.1 | |
| 97 | 367.3 | 3.72 | |
| 98 | 440.3 | 3.26 | |
| 99 | 393.1 | 3.18 | H NMR (400 MHz, DMSO-d6) 11.80 (s, 1H), 8.64 (s, 1H), 7.83 (m, 1H), 7.33-7.26 (m, 2H), 7.07 (m, 1H), 7.02 (m, 1H), 6.96-6.89 (m, 2H), 6.02 (s, 2H), 4.33 (q, J = 7.1 Hz, 2H), 1.42-1.39 (m, 2H), 1.33 (t, J = 7.1 Hz, 3H), 1.06-1.03 (m, 2H) |
| 100 | 421.3 | 1.85 | H NMR (400 MHz, DMSO) 13.05 (s, 1H), 9.96 (d, J = 1.6 Hz, 1H), 7.89 (d, J = 1.9 Hz, 1H), 7.74 (d, J = 2.0 Hz, 1H), 7.02 (d, J = 1.6 Hz, 1H), 6.96-6.88 (m, 2H), 6.22 (d, J = 2.3 Hz, 1H), 6.02 (s, 2H), 1.43-1.40 (m, 2H), 1.37 (s, 9H), 1.06-1.02 (m, 2H) |
| 101 | 387.5 | 2.51 | |
| 102 | 479 | 3.95 | |
| 103 | 420.3 | 3.12 | |
| 104 | 469.5 | 3.97 | |
| 105 | 391.3 | 2.04 | |
| 106 | 375.2 | 2.82 | |
| 107 | 349.3 | 3.33 | |
| 108 | 503.3 | 1.88 | |
| 109 | 451.5 | 1.59 | |
| 110 | 361.5 | 3.7 | |
| 111 | 391.3 | 3.65 | |
| 112 | 335.3 | 3.03 | |
| 113 | 496.5 | 1.68 | |
| 114 | 381.5 | 3.72 | |
| 115 | 390.3 | 3.22 | |
| 116 | 397.3 | 3.52 | H NMR (400 MHz, DMSO-d6) 11.27 (d, J = 1.9 Hz, 1H), 8.66 (s, 1H), 8.08 (d, J = 1.6 Hz, 1H), 7.65-7.61 (m, 3H), 7.46-7.40 (m, 2H), 7.31 (d, J = 8.7 Hz, |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| | | | 1H),<br>7.25-7.17 (m, 2H),<br>7.03 (d, J = 1.6 Hz,<br>1H),<br>6.98-6.87 (m, 2H),<br>6.02 (s, 2H),<br>1.43-1.39 (m, 2H),<br>1.06-1.02 (m, 2H) |
| 117 | 377.5 | 3.77 | |
| 118 | 515.3 | 2.3 | |
| 119 | 381.3 | 3.8 | |
| 120 | 464.2 | 2.1 | |
| 121 | 465 | 1.74 | |
| 122 | 395.2 | 3.74 | |
| 123 | 383.3 | 3.52 | |
| 124 | 388.5 | 3.56 | |
| 125 | 411.3 | 3.85 | |
| 126 | 459.2 | 1.53 | H NMR (400 MHz,<br>CD3CN)<br>9.23 (s, 1H),<br>7.51-7.48 (m, 2H),<br>7.19 (d, J = 8.6 Hz,<br>1H),<br>7.06-7.03 (m, 2H),<br>6.95-6.89 (m,<br>2H), 6.17 (dd, J = 0.7,<br>2.2 Hz, 1H),<br>6.02 (s, 2H),<br>2.61-2.57 (m, 2H),<br>2.07-2.03 (m,<br>2H),<br>1.55-1.51 (m, 2H), 1.39 (s,<br>6H),<br>1.12-1.09 (m, 2H) |
| 127 | 408.5 | 2.48 | |
| 128 | 393 | 3.26 | |
| 129 | 420.2 | 2.16 | |
| 130 | 406.3 | 2.88 | |
| 131 | 473.3 | 4.22 | |
| 132 | 417.3 | 3.8 | |
| 133 | 465 | 1.74 | |
| 134 | 464.3 | 2.91 | |
| 135 | 347.3 | 3.42 | |
| 136 | 511 | 2.35 | |
| 137 | 455.5 | 3.29 | |
| 138 | 393.3 | 3.54 | |
| 139 | 335.1 | 3.08 | |
| 140 | 434.5 | 2.74 | |
| 141 | 381.3 | 2.91 | |
| 142 | 431.5 | 3.97 | |
| 143 | 539 | 1.89 | |
| 144 | 515 | 1.89 | |
| 145 | 407.5 | 3.6 | |
| 146 | 379.5 | 1.51 | |
| 147 | 409.3 | 4 | |
| 148 | 392.2 | 1.22 | |
| 149 | 375.3 | 3.37 | |
| 150 | 377.3 | 3.61 | |
| 151 | 377.22 | 3.96 | |
| 152 | 504.5 | 1.99 | |
| 153 | 393.1 | 3.47 | |
| 154 | 363.3 | 3.52 | |
| 155 | 321.3 | 3.13 | |
| 156 | 407.5 | 3.2 | |
| 157 | 406.3 | 1.43 | |
| 158 | 379.3 | 1.89 | |
| 159 | 451 | 3.34 | |
| 160 | 375.3 | 3.82 | |
| 161 | 355.1 | 3.32 | |
| 162 | 475 | 2.06 | |
| 163 | 437.2 | 2.35 | |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| 164 | 379.2 | 2.76 | |
| 165 | 462 | 3.44 | |
| 166 | 465.2 | 2.15 | |
| 167 | 455.2 | 2.45 | |
| 168 | 451 | 1.65 | |
| 169 | 528 | 1.71 | |
| 170 | 374.3 | 3.4 | |
| 171 | 449.5 | 1.95 | |
| 172 | 381.3 | 3.8 | |
| 173 | 346.3 | 2.93 | |
| 174 | 483.1 | 2.25 | |
| 175 | 411.2 | 3.85 | |
| 176 | 431.5 | 4.02 | |
| 177 | 485.5 | 4.02 | |
| 178 | 528.5 | 1.18 | |
| 179 | 473 | 1.79 | |
| 180 | 479 | 2.15 | |
| 181 | 387.5 | 2.56 | |
| 182 | 365.3 | 3.13 | |
| 183 | 493 | 2.3 | |
| 184 | 461.3 | 2.4 | H NMR (400 MHz, DMSO-d6) 10.89 (s, 1H), 8.29 (s, 1H), 7.52 (s, 1H), 7.42-7.37 (m, 2H), 7.32 (dd, J = 8.3, 1.4 Hz, 1H), 7.01 (d, J = 10.9 Hz, 1H), 6.05 (d, J = 1.7 Hz, 1H), 4.29 (t, J = 5.0 Hz, 1H), 3.23 (m, 2H), 1.81 (t, J = 7.7 Hz, 2H), 1.46 (m, 2H), 1.29 (s, 6H), 1.13 (m, 2H) |
| 185 | 377.5 | 3.63 | |
| 186 | 464 | 1.46 | |
| 187 | 339.1 | 3.2 | |
| 188 | 435.5 | 1.64 | |
| 189 | 392.3 | 2.18 | |
| 190 | 435.5 | 3.67 | H NMR (400 MHz, DMSO) 11.83 (s, 1H), 10.76 (s, 1H), 8.53 (s, 1H), 7.93 (d, J = 1.8 Hz, 1H), 7.60 (dd, J = 2.3, 8.5 Hz, 1H), 7.53 (d, J = 1.4 Hz, 1H), 7.14 (d, J = 8.6 Hz, 1H), 7.02-6.97 (m, 2H), 6.02 (d, J = 1.5 Hz, 1H), 3.71 (t, J = 6.2 Hz, 2H), 3.37 (t, J = 6.2 Hz, 2H), 3.25 (s, 3H), 1.44 (m, 2H), 1.32 (s, 9H), 1.08 (m, 2H) |
| 191 | 421.3 | 3.32 | |
| 192 | 404.4 | 0.95 | |
| 193 | 451 | 1.71 | |
| 194 | 465 | 1.69 | |
| 195 | 434.2 | 2.29 | |
| 196 | 363.3 | 3.4 | |
| 197 | 501 | 1.91 | |
| 198 | 411.2 | 3.14 | |
| 199 | 439 | 1.89 | |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| 200 | 434.4 | 1.53 | |
| 201 | 462 | 3.22 | |
| 202 | 351.3 | 2.59 | |
| 203 | 495.2 | 2.71 | |
| 204 | 435 | 3.94 | |
| 205 | 397.3 | 3.69 | |
| 206 | 493 | 2.26 | |
| 207 | 487 | 1.87 | |
| 208 | 391.3 | 2.94 | |
| 209 | 397.2 | 3.3 | |
| 210 | 487.2 | 1.85 | H NMR (400 MHz, CD3CN) 7.50 (d, J = 2.0 Hz, 1H), 7.41 (d, J = 1.6 Hz, 2H), 7.37-7.32 (m, 2H), 7.25 (d, J = 8.3 Hz, 1H), 6.98 (dd, J = 2.1, 8.8 Hz, 1H), 6.27 (d, J = 0.6 Hz, 1H), 4.40-4.28 (m, 2H), 4.12-4.06 (m, 1H), 3.59-3.51 (m, 2H), 1.59-1.50 (m, 2H), 1.47 (s, 9H), 1.15-1.12 (m, 2H) |
| 211 | 381.3 | 3.69 | |
| 212 | 461 | 2.04 | |
| 213 | 469 | 1.72 | |
| 214 | 363.3 | 3.48 | |
| 215 | 432.3 | 3.07 | |
| 216 | 403.5 | 3.94 | |
| 217 | 420.4 | 1.27 | |
| 218 | 475 | 2.2 | |
| 219 | 484.3 | 1.84 | |
| 220 | 419.3 | 3.87 | |
| 221 | 486.3 | 0.91 | |
| 222 | 391.3 | 3.01 | |
| 223 | 398.3 | 1.3 | |
| 224 | 349.2 | 2.54 | |
| 225 | 375.5 | 3.74 | |
| 226 | 377.5 | 3.47 | H NMR (400 MHz, DMSO-d6) 10.76 (s, 1H), 8.39 (s, 1H), 7.55 (s, 1H), 7.15-7.13 (m, 1H), 7.03-6.89 (m, 4H), 6.03 (m, 3H), 1.41-1.38 (m, 2H), 1.32 (s, 9H), 1.04-1.01 (m, 2H) |
| 227 | 393.3 | 2.03 | |
| 228 | 398.3 | 1.24 | |
| 229 | 487.2 | 1.78 | |
| 230 | 361.1 | 3.47 | |
| 231 | 435.5 | 2.12 | |
| 232 | 321.3 | 2.91 | |
| 233 | 413.3 | 3.77 | |
| 234 | 393.3 | 1.58 | |
| 235 | 465 | 1.92 | |
| 236 | 361.3 | 3.18 | |
| 237 | 421 | 1.8 | |
| 238 | 405.5 | 3.79 | |
| 239 | 544.3 | 1.4 | |
| 240 | 405.3 | 3.9 | |
| 241 | 462 | 1.74 | |
| 242 | 550 | 1.68 | |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| 243 | 395.2 | 1.98 | |
| 244 | 517.3 | 1.94 | |
| 245 | 372.2 | 3.59 | |
| 246 | 361.3 | 3.58 | |
| 247 | 490 | 1.95 | |
| 248 | 407.3 | 1.52 | H NMR (400 MHz, DMSO) 10.74 (d, J = 1.2 Hz, 1H), 8.40 (s, 1H), 7.54 (d, J = 1.8 Hz, 1H), 7.15 (d, J = 8.6 Hz, 1H), 7.03-6.90 (m, 4H), 6.03-6.00 (m, 3H), 3.26-3.22 (m, 2H), 1.85-1.80 (m, 2H), 1.41-1.38 (m, 2H), 1.31 (s, 6H), 1.05-1.01 (m, 2H) |
| 249 | 393.3 | 3.32 | |
| 250 | 406.2 | 2.08 | |
| 251 | 511 | 2.39 | |
| 252 | 379.3 | 3.3 | |
| 253 | 383 | 3.46 | |
| 254 | 401.2 | 3.26 | |
| 255 | 398.3 | 1.38 | |
| 256 | 512.5 | 1.96 | |
| 257 | 389.2 | 3.05 | |
| 258 | 321.3 | 3.02 | |
| 259 | 392.1 | 2.74 | |
| 260 | 462 | 1.81 | |
| 261 | 453 | 1.91 | |
| 262 | 349.3 | 3.22 | |
| 263 | 391.1 | 3.67 | H NMR (400 MHz, DMSO) 1.01-1.05 (dd, J = 4.0, 6.7 Hz, 2H), 1.41-1.39 (m, 11H), 3.81 (s, 3H), 6.03 (s, 2H), 6.15 (s, 1H), 6.96-6.90 (m, 2H), 7.02 (d, J = 1.6 Hz, 1H), 7.09 (dd, J = 2.0, 8.8 Hz, 1H), 7.25 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 1.9 Hz, 1H), 8.46 (s, 1H) |
| 264 | 421.3 | 1.66 | H NMR (400 MHz, CD3CN) 8.78 (s, 1H), 7.40 (m, 1H), 7.33 (s, 1H), 7.08 (m, 1H), 6.95-6.87 (m, 3H), 6.79 (m, 1H), 5.91 (s, 2H), 3.51 (dd, J = 5.9, 7.8 Hz, 2H), 2.92-2.88 (m, 2H), 2.64 (t, J = 5.8 Hz, 1H), 1.50 (m, 2H), 1.41 (s, 9H), 1.06 (m, 2H) |
| 265 | 475 | 2.15 | |
| 266 | 347.3 | 3.32 | |
| 267 | 420.5 | 1.81 | |
| 268 | 416.2 | 1.76 | |
| 269 | 485 | 2.06 | |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| 270 | 395.3 | 3.89 | |
| 271 | 492 | 1.59 | |
| 272 | 405.5 | 3.96 | |
| 273 | 547.2 | 1.65 | |
| 274 | 631.6 | 1.91 | |
| 275 | 590.4 | 2.02 | |
| 276 | 465.7 | 1.79 | |
| 277 | 411.3 | 2.14 | |
| 278 | 385.3 | 1.99 | |
| 279 | 425.3 | 2.19 | |
| 280 | 473.2 | 1.74 | |
| 281 | 469.4 | 2.02 | H NMR (400 MHz, DMSO) 8.82 (s, 1H), 7.84 (d, J = 1.7 Hz, 1H), 7.55-7.51 (m, 2H), 7.40-7.35 (m, 2H), 7.29 (dd, J = 1.7, 8.3 Hz, 1H), 7.04 (s, 1H), 4.98 (t, J = 5.6 Hz, 1H), 4.27 (t, J = 6.1 Hz, 2H), 3.67 (q, J = 6.0 Hz, 2H), 1.48 (dd, J = 4.0, 6.7 Hz, 2H), 1.13 (dd, J = 4.1, 6.8 Hz, 2H) |
| 282 | 644.4 | 1.83 | |
| 283 | 544.6 | 1.97 | |
| 284 | 465.4 | 1.56 | |
| 285 | 485.2 | 1.8 | |
| 286 | 475.2 | 1.87 | |
| 287 | 564.2 | 1.95 | |
| 288 | 512.5 | 1.89 | H NMR (400 MHz, DMSO) 8.77 (s, 1H), 7.97 (s, 1H), 7.51 (s, 1H), 7.43-7.40 (m, 2H), 7.33 (d, J = 8.2 Hz, 1H), 6.36 (s, 1H), 4.99-4.97 (m, 2H), 4.52 (d, J = 13.1 Hz, 1H), 4.21 (dd, J = 9.2, 15.2 Hz, 1H), 3.86 (m, 1H), 3.51-3.36 (m, 2H), 1.51-1.48 (m, 2H), 1.43 (s, 9H), 1.17-1.15 (m, 2H) |
| 289 | 437.3 | 1.6 | |
| 290 | 499.5 | 1.81 | H NMR (400 MHz, DMSO) 8.82 (s, 1H), 7.83 (d, J = 1.7 Hz, 1H), 7.55-7.50 (m, 2H), 7.39-7.28 (m, 3H), 7.03 (s, 1H), 4.97 (d, J = 5.6 Hz, 1H), 4.83 (t, J = 5.6 Hz, 1H), 4.33 (dd, J = 3.4, 15.1 Hz, 1H), 4.09 (dd, J = 8.7, 15.1 Hz, 1H), 3.80-3.78 (m, 1H), 3.43-3.38 (m, 1H), |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| | | | 3.35-3.30 (m, 1H), 1.49-1.46 (m, 2H), 1.14-1.11 (m, 2H) |
| 291 | 455.4 | 2.02 | H NMR (400 MHz, DMSO) 8.62 (s, 1H), 7.56 (s, 1H), 7.50 (s, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.29 (dd, J = 1.5, 8.3 Hz, 1H), 7.23 (d, J = 8.7 Hz, 1H), 7.06 (dd, J = 1.7, 8.7 Hz, 1H), 6.19 (s, 1H), 4.86 (t, J = 5.4 Hz, 1H), 4.03 (t, J = 6.1 Hz, 2H), 3.73 (qn, J = 8.5 Hz, 1H), 3.57 (q, J = 5.9 Hz, 2H), 2.39-2.33 (m, 2H), 2.18-1.98 (m, 3H), 1.88-1.81 (m, 1H), 1.47-1.44 (m, 2H), 1.11-1.09 (m, 2H) |
| 292 | 578.4 | 1.99 | |
| 293 | 630.4 | 1.8 | |
| 294 | 443.4 | 1.98 | H NMR (400 MHz, DMSO) 8.62 (s, 1H), 7.55 (d, J = 1.8 Hz, 1H), 7.50 (d, J = 1.5 Hz, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.30-7.24 (m, 2H), 7.05 (dd, J = 2.0, 8.8 Hz, 1H), 6.13 (s, 1H), 4.88 (t, J = 5.5 Hz, 1H), 4.14 (t, J = 6.1 Hz, 2H), 3.61 (m, 2H), 3.21 (septet, J = 6.8 Hz, 1H), 1.47-1.44 (m, 2H), 1.26 (d, J = 6.8 Hz, 6H), 1.11-1.08 (m, 2H) |
| 295 | 482.3 | 2 | H NMR (400 MHz, DMSO) 8.78 (s, 1H), 7.92 (s, 1H), 7.51 (s, 1H), 7.45 (s, 1H), 7.41 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 6.34 (s, 1H), 5.01 (t, J = 5.7 Hz, 1H), 4.41 (t, J = 6.6 Hz, 2H), 3.68 (m, 2H), 1.51-1.47 (m, 2H), 1.42 (s, 9H), 1.19-1.15 (m, 2H) |
| 296 | 438.7 | 2.12 | H NMR (400 MHz, DMSO) 11.43 (s, 1H), |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| | | | 8.74 (s, 1H), 7.63 (s, 1H), 7.51 (s, 1H), 7.45-7.40 (m, 2H), 7.33 (dd, J = 1.4, 8.3 Hz, 1H), 6.25 (d, J = 1.5 Hz, 1H), 1.51-1.48 (m, 2H), 1.34 (s, 9H), 1.17-1.14 (m, 2H) |
| 297 | 449.3 | 1.6 | |
| 298 | 517.5 | 1.64 | |
| 299 | 391.5 | 2.05 | |
| 300 | 449.3 | 1.59 | |
| 301 | 501.2 | 1.93 | |
| 302 | 503.5 | 1.63 | |
| 303 | 437.3 | 1.6 | |
| 304 | 425.1 | 2.04 | H NMR (400 MHz, DMSO) 12.16 (s, 1H), 8.80 (s, 1H), 7.83 (s, 1H), 7.51 (d, J = 1.4 Hz, 1H), 7.39-7.28 (m, 4H), 6.95 (s, 1H), 1.48 (dd, J = 4.0, 6.6 Hz, 2H), 1.13 (dd, J = 4.0, 6.7 Hz, 2H) |
| 305 | 459.2 | 1.67 | |
| 306 | 558.4 | 2.05 | |
| 307 | 447.5 | 1.93 | |
| 308 | 516.7 | 1.69 | $^1$H NMR (400 MHz, DMSO-d6) δ 8.32 (s, 1H), 7.53 (s, 1H), 7.43-7.31 (m, 4H), 6.19 (s, 1H), 4.95-4.93 (m, 2H), 4.51 (d, J = 5.0 Hz, 1H), 4.42-4.39 (m, 2H), 4.10-4.04 (m, 1H), 3.86 (s, 1H), 3.49-3.43 (m, 2H), 3.41-3.33 (m, 1H), 3.30-3.10 (m, 6H), 2.02-1.97 (m, 2H), 1.48-1.42 (m, 8H) and 1.13 (dd, J = 4.0, 6.7 Hz, 2H) ppm |
| 309 | 535.7 | 1.79 | 1H NMR (400.0 MHz, DMSO) d 8.43 (s, 1H), 7.53 (s, 1H), 7.45-7.41 (m, 2H), 7.36-7.31 (m, 2H), 6.27 (s, 1H), 4.74-4.70 (m, 2H), 3.57-3.53 (m, 2H), 3.29 (s, 9H), 1.48-1.42 (m, 11H), and 1.15 (dd, J = 3.9, 6.8 Hz, 2H) ppm. |
| 310 | 609.5 | 1.64 | |
| 311 | 535.7 | 1.7 | $^1$H NMR (400 MHz, DMSO-d6) δ 8.32 (s, 1H), 7.53 (d, J = 1.0 Hz, 1H), 7.43-7.31 (m, 4H), 6.17 (s, 1H), 4.97-4.92 (m, 2H), 4.41 (dd, J = 2.4, 15.0 Hz, 1H), 4.23 (t, J = 5.0 Hz, 1H), 4.08 (dd, J = 8.6, 15.1 Hz, 1H), 3.87 (s, 1H), 3.48-3.44 (m, 1H), 3.41-3.33 (m, 1H), 3.20 (dd, J = 7.4, 12.7 Hz, 2H), 1.94-1.90 (m, 2H), 1.48-1.45 (m, 2H), 1.42 (s, 3H), 1.41 (s, 3H) and 1.15-1.12 (m, 2H) ppm. |
| 312 | 443 | 2.31 | $^1$H NMR (400 MHz, DMSO-d6) δ 8.93 (s, 1H), 7.71 (d, J = 8.8 Hz, 1H), 7.51 (s, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 1.6 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 6.28 (s, 1H), 5.05 (t, J = 5.6 Hz, 1H), 4.42 (t, J = 6.8 Hz, 2H), 3.70-3.65 (m, 2H), 1.51-1.48 (m, 2H), 1.44 (s, 9H), 1.19-1.16 (m, 2H) ppm. |
| 313 | 521.5 | 1.69 | 1H NMR (400.0 MHz, CD3CN) d 7.69 (d, J = 7.7 Hz, 1H), 7.44 (d, J = 1.6 Hz, 1H), 7.39 (dd, J = 1.7, 8.3 Hz, 1H), 7.31 (s, 1H), 7.27 (d, J = 8.3 Hz, 1H), 7.20 (d, J = 12.0 Hz, 1H), 6.34 (s, 1H), 4.32 (d, J = 6.8 Hz, 2H), 4.15-4.09 (m, 1H), 3.89 (dd, J = 6.0, 11.5 Hz, 1H), 3.63-3.52 (m, 3H), 3.42 (d, J = 4.6 Hz, 1H), 3.21 (dd, J = 6.2, 7.2 Hz, 1H), 3.04 (t, J = 5.8 Hz, 1H), 1.59 (dd, J = 3.8, 6.8 Hz, 2H), 1.44 (s, 3H), 1.33 (s, 3H) and 1.18 (dd, J = 3.7, 6.8 Hz, 2H) ppm |
| 314 | 447.5 | 1.86 | $^1$H NMR (400 MHz, DMSO-d6) δ 8.20 (d, J = 7.6 Hz, 1H), 7.30-7.25 (m, 3H), 7.20 (m, 1H), 7.12 (d, J = 8.2 Hz, 1H), 6.84 (d, J = 11.1 Hz, 1H), 6.01 (d, J = 0.5 Hz, |

TABLE 3-continued

Physical data of exemplary compounds.

| Compound No. | LC/MS M + 1 | LC/RT Min | NMR |
|---|---|---|---|
| | | | 1H), 3.98 (t, J = 6.8 Hz, 2H), 2.37 (t, J = 6.8 Hz, 2H), 1.75 (dd, J = 3.8, 6.9 Hz, 2H), 1.37 (s, 6H) and 1.14 (dd, J = 3.9, 6.9 Hz, 2H) ppm. |
| 315 | 482.5 | 1.99 | H NMR (400 MHz, DMSO) 8.93 (s, 1H), 7.71 (d, J = 8.8 Hz, 1H), 7.51 (s, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 1.6 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 6.28 (s, 1H), 5.05 (t, J = 5.6 Hz, 1H), 4.42 (t, J = 6.8 Hz, 2H), 3.70-3.65 (m, 2H), 1.51-1.48 (m, 2H), 1.44 (s, 9H), 1.19-1.16 (m, 2H) |
| 316 | 438.7 | 2.1 | H NMR (400 MHz, DMSO) 11.48 (s, 1H), 8.88 (s, 1H), 7.52 (d, J = 8.5 Hz, 2H), 7.41 (d, J = 8.3 Hz, 1H), 7.32 (dd, J = 1.5, 8.3 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 6.21 (d, J = 1.8 Hz, 1H), 1.51-1.49 (m, 2H), 1.36 (s, 9H), 1.18-1.16 (m, 2H) ppm. |
| 317 | 439.4 | 1.36 | |
| 318 | 469.016 | 1.66 | |
| 319 | 469.016 | 1.66 | |
| 320 | 465.7 | 1.79 | H NMR (400 MHz, DMSO) 9.26 (s, 1H), 7.65 (d, J = 1.9 Hz, 1H), 7.49 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 8.9 Hz, 1H), 7.11 (dd, J = 1.9, 8.9 Hz, 1H), 6.89 (d, J = 8.8 Hz, 2H), 6.14 (s, 1H), 4.42-4.37 (m, 1H), 4.16-4.10 (m, 1H), 3.90-3.88 (m, 1H), 3.73 (s, 3H), 3.46-3.42 (m, 2H), 1.41 (s, 9H), 1.36 (d, J = 5.0 Hz, 1H), 1.21 (s, 3H), 0.99 (d, J = 5.0 Hz, 1H), 0.84 (s, 3H) |
| 321 | 391.5 | 2.05 | H NMR (400 MHz, DMSO) 10.73 (s, 1H), 9.23 (s, 1H), 7.61 (d, J = 1.5 Hz, 1H), 7.49 (d, J = 8.8 Hz, 2H), 7.13 (s, 1H), 7.10 (d, J = 1.9 Hz, 1H), 6.88 (d, J = 8.8 Hz, 2H), 6.02 (d, J = 1.8 Hz, 1H), 3.73 (s, 3H), 1.36 (d, J = 5.0 Hz, 1H), 1.31 (s, 9H), 1.22 (s, 3H), 0.98 (d, J = 5.0 Hz, 1H), 0.84 (s, 3H) |
| 322 | 521.5 | 1.67 | 1H NMR (400.0 MHz, DMSO) d 8.31 (s, 1H), 7.53 (d, J = 1.1 Hz, 1H), 7.42-7.37 (m, 2H), 7.33-7.30 (m, 2H), 6.22 (s, 1H), 5.01 (d, J = 5.0 Hz, 1H), 4.91 (t, J = 5.5 Hz, 1H), 4.75 (t, J = 5.8 Hz, 1H), 4.42-4.38 (m, 1H), 4.10 (dd, J = 8.8, 15.1 Hz, 1H), 3.90 (s, 1H), 3.64-3.54 (m, 2H), 3.48-3.33 (m, 2H), 1.48-1.45 (m, 2H), 1.35 (s, 3H), 1.32 (s, 3H) and 1.14-1.11 (m, 2H) ppm |

**[0842]** Assays for Detecting and Measuring ΔF508-CFTR Correction Properties of Compounds

**[0843]** Membrane potential optical methods for assaying ΔF508-CFTR modulation properties of compounds.

**[0844]** The assay utilizes fluorescent voltage sensing dyes to measure changes in membrane potential using a fluorescent plate reader (e.g., FLIPR III, Molecular Devices, Inc.) as a readout for increase in functional ΔF508-CFTR in NIH 3T3 cells. The driving force for the response is the creation of a chloride ion gradient in conjunction with channel activation by a single liquid addition step after the cells have previously been treated with compounds and subsequently loaded with a voltage sensing dye.

**[0845]** Identification of Correction Compounds

**[0846]** To identify small molecules that correct the trafficking defect associated with ΔF508-CFTR; a single-addition HTS assay format was developed. Assay Plates containing cells are incubated for ~2-4 hours in tissue culture incubator at 37° C., 5% $CO_2$, 90% humidity. Cells are then ready for compound exposure after adhering to the bottom of the assay plates.

**[0847]** The cells were incubated in serum-free medium for 16-24 hrs in tissue culture incubator at 37° C., 5% $CO_2$, 90% humidity in the presence or absence (negative control) of test compound. The cells were subsequently rinsed 3× with Krebs Ringers solution and loaded with a voltage sensing redistribution dye. To activate ΔF508-CFTR, 10 μM forskolin and the CFTR potentiator, genistein (20 μM). were added along with $Cl^-$-free medium to each well. The addition of $Cl^-$-free medium promoted $Cl^-$ efflux in response to ΔF508-CFTR activation and the resulting membrane depolarization was optically monitored using voltage sensor dyes.

**[0848]** Identification of Potentiator Compounds

**[0849]** To identify potentiators of ΔF508-CFTR, a double-addition HTS assay format was developed. This HTS assay utilizes fluorescent voltage sensing dyes to measure changes in membrane potential on the FLIPR III as a measurement for increase in gating (conductance) of ΔF508 CFTR in temperature-corrected ΔF508 CFTR NIH 3T3 cells. The driving force for the response is a $Cl^-$ ion gradient in conjunction with channel activation with forskolin in a single liquid addition step using a fluorescent plate reader such as FLIPR III after the cells have previously been treated with potentiator compounds (or DMSO vehicle control) and subsequently loaded with a redistribution dye.

**[0850]** Solutions:

**[0851]** Bath Solution #1: (in mM) NaCl 160, KCl 4.5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, pH 7.4 with NaOH.

**[0852]** Chloride-free bath solution: Chloride salts in Bath Solution #1 are substituted with gluconate salts.

**[0853]** Cell Culture

**[0854]** NIH3T3 mouse fibroblasts stably expressing ΔF508-CFTR are used for optical measurements of membrane potential. The cells are maintained at 37° C. in 5% $CO_2$ and 90% humidity in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine, 10% fetal bovine serum, 1×NEAA, β-ME, 1× pen/strep, and 25 mM HEPES in 175 $cm^2$ culture flasks. For all optical assays, the cells were seeded at ~20,000/well in 384-well matrigel-coated plates and cultured for 2 hrs at 37° C. before culturing at 27° C. for 24 hrs. for the potentiator assay. For the correction assays, the cells are cultured at 27° C. or 37° C. with and without compounds for 16-24 hours.

**[0855]** Electrophysiological Assays for assaying ΔF508-CFTR modulation properties of compounds.

**[0856]** 1. Ussing Chamber Assay

**[0857]** Ussing chamber experiments were performed on polarized airway epithelial cells expressing ΔF508-CFTR to further characterize the ΔF508-CFTR modulators identified in the optical assays. Non-CF and CF airway epithelia were isolated from bronchial tissue, cultured as previously described (Galietta, L. J. V., Lantero, S., Gazzolo, A., Sacco, O., Romano, L., Rossi, G. A., & Zegarra-Moran, o. (1998) *In Vitro Cell. Dev. Biol.* 34, 478-481), and plated onto Costar® Snapwel™ filters that were precoated with NIH3T3-conditioned media. After four days the apical media was removed and the cells were grown at an air liquid interface for >14 days prior to use. This resulted in a monolayer of fully differentiated columnar cells that were ciliated, features that are characteristic of airway epithelia. Non-CF HBE were isolated from non-smokers that did not have any known lung disease. CF-HBE were isolated from patients homozygous for ΔF508-CFTR.

**[0858]** HBE grown on Costar® Snapwell™ cell culture inserts were mounted in an Ussing chamber (Physiologic Instruments, Inc., San Diego, Calif.), and the transepithelial resistance and short-circuit current in the presence of a basolateral to apical $Cl^-$ gradient ($I_{SC}$) were measured using a voltage-clamp system (Department of Bioengineering, University of Iowa, IA). Briefly, HBE were examined under voltage-clamp recording conditions ($V_{hold}$=0 mV) at 37° C. The basolateral solution contained (in mM) 145 NaCl, 0.83 $K_2HPO_4$, 3.3 $KH_2PO_4$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 10 Glucose, 10 HEPES (pH adjusted to 7.35 with NaOH) and the apical solution contained (in mM) 145 NaGluconate, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 10 glucose, 10 HEPES (pH adjusted to 7.35 with NaOH).

**[0859]** Identification of Correction Compounds

**[0860]** Typical protocol utilized a basolateral to apical membrane $Cl^-$ concentration gradient. To set up this gradient, normal ringer was used on the basolateral membrane, whereas apical NaCl was replaced by equimolar sodium gluconate (titrated to pH 7.4 with NaOH) to give a large $Cl^-$ concentration gradient across the epithelium. All experiments were performed with intact monolayers. To fully activate ΔF508-CFTR, forskolin (10 μM), PDE inhibitor, IBMX (100 μM) and CFTR potentiator, genistein (50 μM) were added to the apical side.

**[0861]** As observed in other cell types, incubation at low temperatures of FRT cells and human bronchial epithelial cells isolated from diseased CF patients (CF-HBE) expressing ΔF508-CFTR increases the functional density of CFTR in the plasma membrane. To determine the activity of correction compounds, the cells were incubated with test compound for 24-48 hours at 37° C. and were subsequently washed 3× prior to recording. The cAMP- and genistein-mediated $I_{SC}$ in compound-treated cells was normalized to 37° C. controls and expressed as percentage activity of CFTR activity in wt-HBE. Preincubation of the cells with the correction compound significantly increased the cAMP- and genistein-mediated $I_{SC}$ compared to the 37° C. controls.

**[0862]** Identification of Potentiator Compounds

**[0863]** Typical protocol utilized a basolateral to apical membrane $Cl^-$ concentration gradient. To set up this gradient, normal ringers was used on the basolateral membrane, whereas apical NaCl was replaced by equimolar sodium gluconate (titrated to pH 7.4 with NaOH) to give a large $Cl^-$ concentration gradient across the epithelium. Forskolin (10 μM) and all test compounds were added to the apical side of the cell culture inserts. The efficacy of the putative ΔF508-CFTR potentiators was compared to that of the known potentiator, genistein.

**[0864]** 2. Patch-Clamp Recordings

**[0865]** Total $Cl^-$ current in ΔF508-NIH3T3 cells was monitored using the perforated-patch recording configuration as previously described (Rae, J., Cooper, K., Gates, P., & Watsky, M. (1991) *J. Neurosci. Methods* 37, 15-26). Voltage-clamp recordings were performed at 22° C. using an Axopatch 200B patch-clamp amplifier (Axon Instruments Inc., Foster City, Calif.). The pipette solution contained (in mM) 150 N-methyl-D-glucamine (NMDG)-Cl, 2 $MgCl_2$, 2 $CaCl_2$, 10 EGTA, 10 HEPES, and 240 μg/ml amphotericin-B (pH adjusted to 7.35 with HCl). The extracellular medium contained (in mM) 150 NMDG-Cl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES (pH adjusted to 7.35 with HCl). Pulse generation, data acquisition, and analysis were performed using a PC equipped with a Digidata 1320 A/D interface in conjunction with Clampex 8 (Axon Instruments Inc.). To activate ΔF508-CFTR, 10 μM forskolin and 20 μM genistein were added to the bath and the current-voltage relation was monitored every 30 sec.

**[0866]** Identification of Correction Compounds

**[0867]** To determine the activity of correction compounds for increasing the density of functional ΔF508-CFTR in the plasma membrane, we used the above-described perforated-patch-recording techniques to measure the current density following 24-hr treatment with the correction compounds. To fully activate ΔF508-CFTR, 10 μM forskolin and 20 μM genistein were added to the cells. Under our recording conditions, the current density following 24-hr incubation at 27° C. was higher than that observed following 24-hr incubation at 37° C. These results are consistent with the known effects of low-temperature incubation on the density of ΔF508-CFTR in the plasma membrane. To determine the effects of correction compounds on CFTR current density, the cells were incubated with 10 μM of the test compound for 24 hours at 37° C. and the current density was compared to the 27° C. and 37° C. controls (% activity). Prior to recording, the cells were washed 3× with extracellular recording medium to remove any remaining test compound. Preincubation with 10 μM of correction compounds significantly increased the cAMP- and genistein-dependent current compared to the 37° C. controls.

**[0868]** Identification of Potentiator Compounds

**[0869]** The ability of ΔF508-CFTR potentiators to increase the macroscopic ΔF508-CFTR $Cl^-$ current ($I_{\Delta F508}$) in NIH3T3 cells stably expressing ΔF508-CFTR was also investigated using perforated-patch-recording techniques. The potentiators identified from the optical assays evoked a dose-

dependent increase in $I_{\Delta F508}$ with similar potency and efficacy observed in the optical assays. In all cells examined, the reversal potential before and during potentiator application was around −30 mV, which is the calculated $E_{Cl}$ (−28 mV).

**[0870]** Cell Culture

**[0871]** NIH3T3 mouse fibroblasts stably expressing ΔF508-CFTR are used for whole-cell recordings. The cells are maintained at 37° C. in 5% $CO_2$ and 90% humidity in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine, 10% fetal bovine serum, 1×NEAA, β-ME, 1× pen/strep, and 25 mM HEPES in 175 $cm^2$ culture flasks. For whole-cell recordings, 2,500-5,000 cells were seeded on poly-L-lysine-coated glass coverslips and cultured for 24-48 hrs at 27° C. before use to test the activity of potentiators; and incubated with or without the correction compound at 37° C. for measuring the activity of correctors.

**[0872]** 3. Single-Channel Recordings

**[0873]** Gating activity of wt-CFTR and temperature-corrected ΔF508-CFTR expressed in NIH3T3 cells was observed using excised inside-out membrane patch recordings as previously described (Dalemans, W., Barbry, P., Champigny, G., Jallat, S., Dott, K., Dreyer, D., Crystal, R. G., Pavirani, A., Lecocq, J-P., Lazdunski, M. (1991) *Nature* 354, 526-528) using an Axopatch 200B patch-clamp amplifier (Axon Instruments Inc.). The pipette contained (in mM): 150 NMDG, 150 aspartic acid, 5 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES (pH adjusted to 7.35 with Tris base). The bath contained (in mM): 150 NMDG-Cl, 2 $MgCl_2$, 5 EGTA, 10 TES, and 14 Tris base (pH adjusted to 7.35 with HCl). After excision, both wt- and ΔF508-CFTR were activated by adding 1 mM Mg-ATP, 75 nM of the catalytic subunit of cAMP-dependent protein kinase (PKA; Promega Corp. Madison, Wis.), and 10 mM NaF to inhibit protein phosphatases, which prevented current rundown. The pipette potential was maintained at 80 mV. Channel activity was analyzed from membrane patches containing ≦2 active channels. The maximum number of simultaneous openings determined the number of active channels during the course of an experiment. To determine the single-channel current amplitude, the data recorded from 120 sec of ΔF508-CFTR activity was filtered "off-line" at 100 Hz and then used to construct all-point amplitude histograms that were fitted with multigaussian functions using Bio-Patch Analysis software (Bio-Logic Comp. France). The total microscopic current and open probability ($P_o$) were determined from 120 sec of channel activity. The PO was determined using the Bio-Patch software or from the relationship $P_o=I/i(N)$, where I=mean current, i=single-channel current amplitude, and N=number of active channels in patch.

**[0874]** Cell Culture

**[0875]** NIH3T3 mouse fibroblasts stably expressing ΔF508-CFTR are used for excised-membrane patch-clamp recordings. The cells are maintained at 37° C. in 5% $CO_2$ and 90% humidity in Dulbecco's modified Eagle's medium supplemented with 2 mM glutamine, 10% fetal bovine serum, 1×NEAA, β-ME, 1× pen/strep, and 25 mM HEPES in 175 $cm^2$ culture flasks. For single channel recordings, 2,500-5,000 cells were seeded on poly-L-lysine-coated glass coverslips and cultured for 24-48 hrs at 27° C. before use.

**[0876]** The compounds of Table 1 were found to exhibit Correction activity as measured in the assay described above.

**[0877]** Compounds of the invention are useful as modulators of ATP binding cassette transporters. Using the procedures described above, the activities, i.e., EC50s, of compounds of the present invention have been measured to be from about 3.8 nM to about 13.5 μM. Furthermore, using those methods described above, the efficacies of compounds of the present invention have been measured to be from about 35% to about 110%.

**[0878]** In Table 4, the following meanings apply:

**[0879]** EC50: "+++" means <2 uM; "++" means between 2 uM to 5 uM; "+" means between 5 uM to 25 uM.

**[0880]** % Efficacy: "+" means <25%; "++" means between 25% and 100%; "+++" means >100%.

TABLE 4

| Cmpd. No. | EC50 | Binned EC50 | Max Efficacy | Binned Max Efficacy |
|---|---|---|---|---|
| 307 | 0.981 | +++ | 160 | +++ |
| 308 | 3.095 | ++ | 100 | +++ |
| 309 | 0.0381 | +++ | 122 | +++ |
| 310 | 0.1595 | +++ | 120.5 | +++ |
| 311 | 0.08175 | +++ | 126 | +++ |
| 312 | 0.181 | +++ | 117.5 | +++ |
| 313 | 0.2835 | +++ | 102 | +++ |
| 314 | 0.2285 | +++ | 124.5 | +++ |
| 315 | 0.272 | +++ | 106 | +++ |
| 316 | 0.285 | +++ | 126.5 | +++ |
| 317 | 4.525 | ++ | 65.5 | ++ |
| 318 | 0.06595 | +++ | 132 | +++ |
| 319 | 0.03905 | +++ | 125.5 | +++ |
| 320 | 4.315 | ++ | 94 | ++ |
| 321 | 1.81 | +++ | 76 | ++ |
| 322 | | | | None |

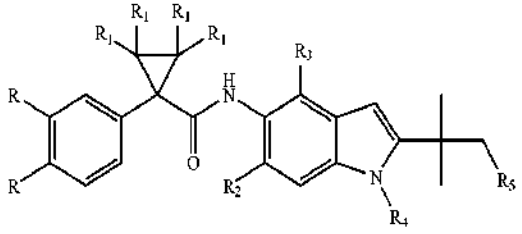
## OTHER EMBODIMENTS

**[0881]** It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

We claim:

**1**. A compound of formula II:

II



or a pharmaceutically acceptable salt thereof, wherein independently for each occurrence:

R is H, OH, $OCH_3$ or two R taken together form $—CH_2CH_2CH_2—$, $—OCH_2O—$ or $—OCF_2O—$;

$R_1$ is H or up to two C1-C6 alkyl;

$R_2$ is H or F;

$R_3$ is H or CN;

$R_4$ is H, $—CH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and

$R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**2**. The compound of claim 1, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, and $R_2$ is F.

**3**. The compound of claim 1, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, and $R_3$ is H.

**4**. The compound of claim 1, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is H.

**5**. The compound of claim 1, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH_2N^+(CH_3)_3$.

**6**. The compound of claim 1, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH(OH)CH_2OH$.

**7**. The compound of claim 1, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (R)—$CH_2CH(OH)CH_2OH$.

**8**. The compound of claim 1, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (S)—$CH_2CH(OH)CH_2OH$

**9**. The compound of claim 1, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**10**. The compound of claim **1**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, and $R_2$ is F.

**11**. The compound of claim **1**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, and $R_3$ is H.

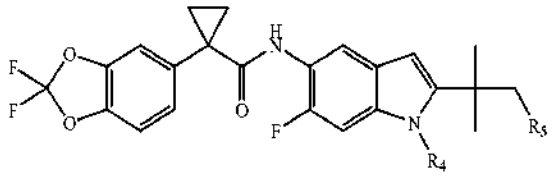
**12**. The compound of claim **1**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH(OH)CH_2OH$.

**13**. The compound of claim **1**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (R)—$CH_2CH(OH)CH_2OH$.

**14**. The compound of claim **1**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (S)—$CH_2CH(OH)CH_2OH$.

**15**. The compound of claim **1**, having formula IIa:

IIa



or a pharmaceutically acceptable salt thereof, wherein:

$R_4$ is H, —$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and

$R_5$ is H, OH, —$CH_2OCH_2CH(OH)CH_2OH$, —$CH_2OH$, or $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**16**. The compound of claim **15**, wherein $R_4$ is (R)—$CH_2CH(OH)CH_2OH$, (S)—$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$.

**17**. The compound of claim **15**, wherein $R_5$ is OH, —$CH_2OCH_2CH(OH)CH_2OH$, or —$CH_2OH$.

**18**. The compound of claim **15**, wherein $R_4$ is (R)—$CH_2CH(OH)CH_2OH$, (S)—$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and $R_5$ is OH, —$CH_2OCH_2CH(OH)CH_2OH$, or —$CH_2OH$.

**19**. A compound is selected from Table 1.

**20**. A pharmaceutical composition comprising

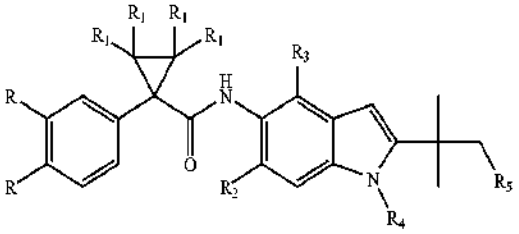
(i) a compound according to claim **1**; and

(ii) a pharmaceutically acceptable carrier.

**21**. The composition of claim **20**, further comprising an additional agent selected from a mucolytic agent, bronchodialator, an anti-biotic, an anti-infective agent, an anti-inflammatory agent, CFTR corrector, CFTR potentiator, or a nutritional agent.

**22**. A method of increasing the number of functional ABC transporters in a membrane of a cell, comprising the step of contacting said cell with a compound of formula II:

II



wherein independently for each occurrence:

R is H, OH, $OCH_3$ or two R taken together form —$CH_2CH_2CH_2$—, —$OCH_2O$— or —$OCF_2O$—;

$R_1$ is H or up to two C1-C6 alkyl;

$R_2$ is H or F;

$R_3$ is H or CN;

$R_4$ is H, —$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and

$R_5$ is H, OH, —$CH_2OCH_2CH(OH)CH_2OH$, —$CH_2OH$, or $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**23**. The method of claim **22**, wherein the ABC transporter is CFTR.

**24**. The method of claim **22**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, and $R_2$ is F.

**25**. The method of claim **22**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, and $R_3$ is H.

**26**. The method of claim **22**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is H.

**27**. The method of claim **22**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH_2N^+(CH_3)_3$.

**28**. The method of claim **22**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH(OH)CH_2OH$.

**29**. The method of claim **22**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (R)—$CH_2CH(OH)CH_2OH$.

**30**. The method of claim **22**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (S)—$CH_2CH(OH)CH_2OH$.

**31**. The method of claim **22**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**32**. The method of claim **22**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, and $R_2$ is F.

**33**. The method of claim **22**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, and $R_3$ is H.

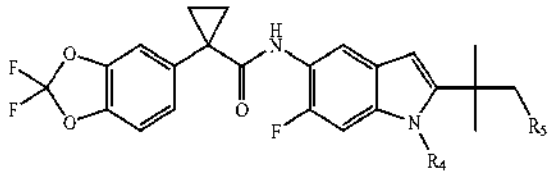
**34**. The method of claim **22**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH(OH)CH_2OH$.

**35**. The method of claim **22**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (R)—$CH_2CH(OH)CH_2OH$.

**36**. The method of claim **22**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (S)—$CH_2CH(OH)CH_2OH$.

**37**. The method of claim **22**, wherein the compound is represented by formula IIa:

IIa



or a pharmaceutically acceptable salt thereof, wherein:

$R_4$ is H, —$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and

$R_5$ is H, OH, —$CH_2OCH_2CH(OH)CH_2OH$, —$CH_2OH$, or $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**38**. The method of claim **37**, wherein $R_4$ is (R)—$CH_2CH(OH)CH_2OH$, (S)—$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$.

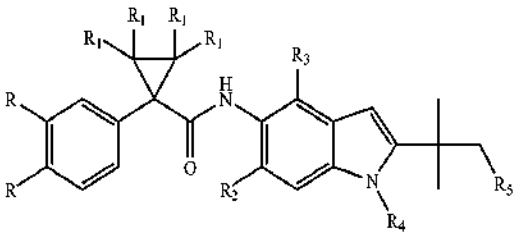
**39**. The method of claim **37**, wherein $R_5$ is OH, —$CH_2OCH_2CH(OH)CH_2OH$, or —$CH_2OH$.

**40**. The method of claim **37**, wherein $R_4$ is R)—$CH_2CH(OH)CH_2OH$, (S)—$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and $R_5$ is OH, —$CH_2OCH_2CH(OH)CH_2OH$, or —$CH_2OH$.

**41**. The method of claim **22**, wherein the compound is selected from Table 1.

**42**. A method of treating a condition, disease, or disorder in a patient implicated by ABC transporter activity, comprising the step of administering to said patient a compound having formula II:

II



or a pharmaceutically acceptable salt thereof, wherein independently for each occurrence:

R is H, OH, $OCH_3$ or two R taken together form —$CH_2CH_2CH_2$—, —$OCH_2O$— or —$OCF_2O$—;

$R_1$ is H or up to two C1-C6 alkyl;

$R_2$ is H or F;

$R_3$ is H or CN;

$R_4$ is H, —$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and

$R_5$ is H, OH, —$CH_2OCH_2CH(OH)CH_2OH$, —$CH_2OH$, or $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**43**. The method of claim **42**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, and $R_2$ is F.

**44**. The method of claim **42**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, and $R_3$ is H.

**45**. The method of claim **42**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is H.

**46**. The method of claim **42**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH_2N^+(CH_3)_3$.

**47**. The method of claim **42**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH(OH)CH_2OH$.

**48**. The method of claim **42**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (R)—$CH_2CH(OH)CH_2OH$.

**49**. The method of claim **42**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (S)—$CH_2CH(OH)CH_2OH$.

**50**. The method of claim **42**, wherein two R taken together form —$OCF_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**51**. The method of claim **42**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, and $R_2$ is F.

**52**. The method of claim **42**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, and $R_3$ is H.

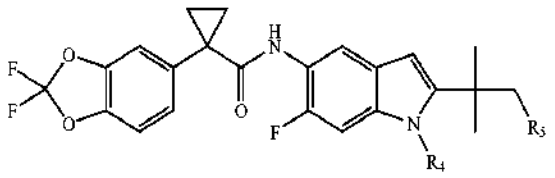
**53**. The method of claim **42**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is —$CH_2CH(OH)CH_2OH$.

**54**. The method of claim **42**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (R)—$CH_2CH(OH)CH_2OH$.

**55**. The method of claim **42**, wherein two R taken together form —$OCH_2O$—, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (S)—$CH_2CH(OH)CH_2OH$.

**56**. The method of claim **42**, wherein the compound is represented by formula IIa:

IIa



or a pharmaceutically acceptable salt thereof, wherein:

$R_4$ is H, —$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and

$R_5$ is H, OH, —$CH_2OCH_2CH(OH)CH_2OH$, —$CH_2OH$, or $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**57**. The method of claim **56**, wherein $R_4$ is (R)—$CH_2CH(OH)CH_2OH$, (S)—$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$.

**58**. The method of claim **56**, wherein $R_5$ is OH, —$CH_2OCH_2CH(OH)CH_2OH$, or —$CH_2OH$.

**59**. The method of claim **56**, wherein $R_4$ is (R)—$CH_2CH(OH)CH_2OH$, (S)—$CH_2CH(OH)CH_2OH$, —$CH_2CH_2N^+(CH_3)_3$, or —$CH_2CH_2OH$; and $R_5$ is OH, —$CH_2OCH_2CH(OH)CH_2OH$, or —$CH_2OH$.

**60**. The method of claim **42**, wherein the compound is selected from Table 1.

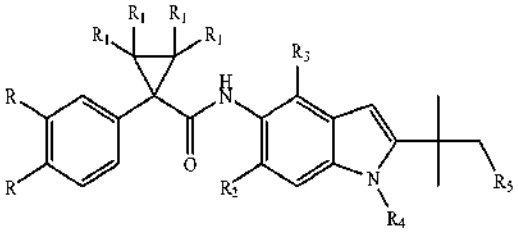
**61**. The method according to claim **42**, wherein said condition, disease, or disorder is selected from cystic fibrosis, hereditary emphysema, hereditary hemochromatosis, coagulation-fibrinolysis deficiencies, such as protein C deficiency, Type 1 hereditary angioedema, lipid processing deficiencies, such as familial hypercholesterolemia, Type 1 chylomicronemia, abetalipoproteinemia, lysosomal storage diseases, such as I-cell disease/pseudo-Hurler, mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, polyendocrinopa-

thy/hyperinsulemia, diabetes mellitus, laron dwarfism, myleoperoxidase deficiency, primary hypoparathyroidism, melanoma, glycanosis CDG type 1, hereditary emphysema, congenital hyperthyroidism, osteogenesis imperfecta, hereditary hypofibrinogenemia, ACT deficiency, diabetes insipidus (di), neurophyseal di, neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear plasy, Pick's disease, several polyglutamine neurological disorders such as Huntington, spinocerebullar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidoluysian, and myotonic dystrophy, as well as spongiform encephalopathies, such as hereditary Creutzfeldt-Jakob disease, Fabry disease, Straussler-Scheinker syndrome, COPD, dry-eye disease, and Sjögren's disease.

**62**. A kit for use in measuring the activity of a ABC transporter or a fragment thereof in a biological sample in vitro or in vivo, comprising:

(i) a first composition comprising a compound of formula II:

II



wherein independently for each occurrence:

R is H, OH, $OCH_3$ or two R taken together form $—CH_2CH_2CH_2—$, $—OCH_2O—$ or $—OCF_2O—$;

$R_1$ is H or up to two C1-C6 alkyl;

$R_2$ is H or F;

$R_3$ is H or CN;

$R_4$ is H, $—CH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and

$R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a fused pyrrolidine ring; and

(ii) instructions for:

a) contacting the composition with the biological sample;

b) measuring activity of said ABC transporter or a fragment thereof.

**63**. The kit according to claim **62**, further comprising instructions for

a) contacting an additional composition with the biological sample;

b) measuring the activity of said ABC transporter or a fragment thereof in the presence of said additional compound, and

c) comparing the activity of the ABC transporter in the presence of the additional compound with the density of the ABC transporter in the presence of said first composition.

**64**. The kit of claim **62**, wherein the kit is used to measure the density of CFTR.

**65**. The kit of claim **62**, wherein two R taken together form $—OCF_2O—$, $R_1$ is H, and $R_2$ is F.

**66**. The kit of claim **62**, wherein two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, and $R_3$ is H.

**67**. The kit of claim **62**, wherein two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is H.

**68**. The kit of claim **62**, wherein two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2CH_2N^+(CH_3)_3$.

**69**. The kit of claim **62**, wherein two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2CH(OH)CH_2OH$.

**70**. The kit of claim **62**, wherein two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (R)$—CH_2CH(OH)CH_2OH$.

**71**. The kit of claim **62**, wherein two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (S)$—CH_2CH(OH)CH_2OH$.

**72**. The kit of claim **62**, wherein two R taken together form $—OCF_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**73**. The kit of claim **62**, wherein two R taken together form $—OCH_2O—$, $R_1$ is H, and $R_2$ is F.

**74**. The kit of claim **62**, wherein two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, and $R_3$ is H.

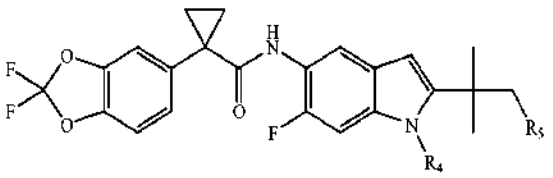
**75**. The kit of claim **62**, wherein two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is $—CH_2CH(OH)CH_2OH$.

**76**. The kit of claim **62**, wherein two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (R)$—CH_2CH(OH)CH_2OH$.

**77**. The kit of claim **62**, wherein two R taken together form $—OCH_2O—$, $R_1$ is H, $R_2$ is F, $R_3$ is H, and $R_4$ is (S)$—CH_2CH(OH)CH_2OH$.

**78**. The kit of claim **62**, wherein the compound is represented by formula IIa:

IIa



or a pharmaceutically acceptable salt thereof, wherein:

$R_4$ is H, $—CH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and

$R_5$ is H, OH, $—CH_2OCH_2CH(OH)CH_2OH$, $—CH_2OH$, or $R_4$ and $R_5$ taken together form a fused pyrrolidine ring.

**79**. The kit of claim **78**, wherein $R_4$ is (R)$—CH_2CH(OH)CH_2OH$, (S)$—CH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$.

**80**. The kit of claim **78**, wherein $R_5$ is OH, $—CH_2OCH_2CH(OH)CH_2OH$, or $—CH_2OH$.

**81**. The kit of claim **78**, wherein $R_4$ is (R)$—CH_2CH(OH)CH_2OH$, (S)$—CH_2CH(OH)CH_2OH$, $—CH_2CH_2N^+(CH_3)_3$, or $—CH_2CH_2OH$; and $R_5$ is OH, $—CH_2OCH_2CH(OH)CH_2OH$, or $—CH_2OH$.

**82**. The kit of claim **62**, wherein the compound is selected from Table 1.

* * * * *

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# Establishment of new preparation method for solid dispersion formulation of tacrolimus

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**Abstract**

The aim of this study was to establish a new preparation method for solid dispersion formulation (SDF) of tacrolimus, a poorly water-soluble drug, without dichloromethane, because no use of dichloromethane is recommended by ICH harmonized tripartite guideline. To select the appropriate carrier, three different SDFs with polyethylene glycol 6000 (PEG 6000), polyvinylpyrrolidone (PVP) and hydroxypropylmethylcellulose (HPMC) were prepared by the conventional solvent method, in which tacrolimus and the carrier were completely dissolved in the mixture of dichloromethane and ethanol. Powder X-ray diffraction (XRD) and differential scanning calorimetry (DSC) patterns indicated that tacrolimus exists in an amorphous state in all three SDFs. The supersaturated dissolution profiles of tacrolimus were observed in all SDFs, and the highest level of supersaturation for tacrolimus was obtained and maintained for 24 h from SDF with HPMC. On the other hand, the supersaturated level from SDF with PEG 6000 or PVP decreased rapidly. The in vivo oral absorption study in dogs showed that bioavailability of tacrolimus from SDF with HPMC was remarkably improved compared with the crystalline powder. It was clarified that HPMC is the most appropriate carrier for SDF of tacrolimus. Then, SDF of tacrolimus was prepared by the new method, which allows us to make SDF of tacrolimus by swelling HPMC with ethanol, in which tacrolimus was completely dissolved. This new method does not need dichloromethane. The physicochemical properties of SDF with HPMC prepared by the new method were the same as those of SDF prepared by the conventional solvent method. Furthermore, SDF with HPMC prepared by the new method was still stable after stored at 40 °C for 3 months. The pharmacokinetic parameters after oral administration in monkeys showed no significant difference ($P > 0.01$) between SDFs with HPMC prepared by the two methods. In conclusion, we have established the new preparation method for SDF of tacrolimus with HPMC and the new method makes it possible to prepare SDF of tacroliumus without dichloromethane.


*Keywords:* Tacrolimus; Solid dispersion formulation; Hydroxypropylmethylcellulose; Powder X-ray diffraction; Differential scanning calorimetry; Supersaturation; Oral administration

## 1. Introduction

Tacrolimus (Fig. 1), which is a 23-member macrolide lactone with potent immunosuppressive activity, was isolated from *Streptomyces tsukubaensis* in 1984 (Kino et al., 1987a,b). Tacrolimus is a poorly water-soluble compound, which has the low solubility in water, about 1–2 μg/ml (Hane et al., 1992) and showed relatively low bioavailability (Honbo et al., 1987). In order to enhance the oral absorption of tacrolimus, Honbo et al. (1987), reported that oily ethanol formulation and solid dispersion formulation

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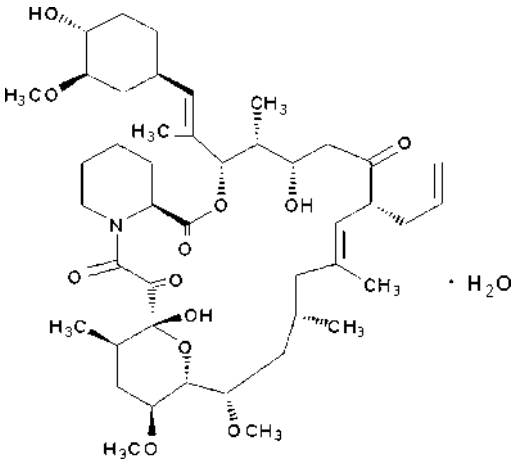

Fig. 1. Chemical structure of tacrolimus.

(SDF) are most potent among many different formulations of tacrolimus examined.

SDF, which was developed by Chiou and Riegelman (1971), is the formulation that possibly enhances the dissolution rate, solubility and oral absorption of a poorly water-soluble drug. Swarbrick (1990), Shargel (1993) and Craig (2001) discussed the increase in drug dissolution rate from SDF and they concluded that the dissolution rate was increased by the following factors: (1) the reduction of the drug particle size to molecular level, (2) the solubilizing effect on the drug by the water-soluble carrier, and (3) the enhancement of the wettability and dispersibility of the drug by the carrier material. According to the definition of SDF, both a given drug and carrier should be completely dissolved with organic solvents (solvent method) or fused by the heating (melting method) in order to prepare SDF. With regard to carriers for SDF, many carriers such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, gelucires, eudragits and chitosans have been reported to improve the solubility and bioavailability of poorly water-soluble drugs (Okimoto et al., 1997; Portero et al., 1998; Jung et al., 1999; Kohri et al., 1999; Trapani et al., 1999; Damian et al., 2002; Tantishaiyakul et al., 1999; Yamada et al., 2000; Cilurzo et al., 2002; Kushida et al., 2002; Nakamichi et al., 2002), but among them HPMC is considered as one of the most suitable carriers for SDF (Kohri et al., 1999; Kushida et al., 2002). HPMC remarkably enhances the water-solubility of drugs compared with other water-soluble carriers and/or prevents drugs from re-crystallizing in the dissolution medium (Sugimoto et al., 1982; Suzuki and Sunada, 1998; Kohri et al., 1999; Kushida et al., 2002).

HPMC is a water-soluble polymer and cannot be dissolved in alcohol alone, while HPMC can be easily dissolved in water, and the mixtures such as water and alcohol or alcohol and chlorohydrocarbon. Any method, in which a single solvent such as ethanol alone is employed, has not been reported for the preparation of SDF with HPMC by the solvent method. Usually, the mixed solvents with dichloromethane have been utilized for the preparation of SDF with HPMC by the solvent method (Ho et al., 1996; Yano et al., 1997; Jung et al., 1999; Kobayashi et al., 2001; Kushida et al., 2002). SDF of tacrolimus with HPMC was also prepared using the mixture of ethanol and dichloromethane (Honbo et al., 1987). Dichloromethane, commonly used in the solvent method for HPMC, is classified in Class 2 solvents of ICH harmonized tripartite guideline. Therefore, it is really expected that a preparation method for SDF without dichloromethane is established from the environmental point of view.

In this study, physicochemical properties of SDFs prepared with different water-soluble carriers, PEG 6000, PVP and HPMC, were evaluated to select an appropriate carrier for SDF of tacrolimus. Then, to establish the new preparation method for SDF of tacrolimus with HPMC, we tried to use a single solvent, ethanol, and prepared SDF by swelling HPMC with ethanol solution of tacrolimus. Furthermore, SDF prepared by the new method was compared with that prepared by the conventional solvent method in terms of the physicochemical and biopharmaceutical properties.

## 2. Materials and methods

### 2.1. Materials

Tacrolimus was provided by Fujisawa Pharmaceutical Co., Ltd. (Osaka and Tokyo, Japan). HPMC, PVP and PEG 6000 were purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo), BASF Japan, Ltd. (Tokyo) and

Sanyo Chemical Industries, Ltd. (Kyoto, Japan), respectively. All other materials were of analytical regent grade.

### 2.2. Animals

Male beagle dogs (Japan Laboratory Animals, Inc., Tokyo) and male cynomolgus monkeys (Charles River Japan, Kanagawa, Japan), maintained at 23 °C and 55% humidity, were allowed free access to standard laboratory chow (Maruha Pet Food Co., Ltd., Tokyo; Oriental Yeast Co., Ltd., Tokyo) and water prior to the experiments. Dogs weighing 11.5–15.9 kg and monkeys weighing 6.2–7.0 kg were assigned randomly to each experimental group. Our investigations were performed after approval by our local ethical committee at Fujisawa Pharmaceutical Co., Ltd. and Okayama University.

### 2.3. Preparation of SDF

#### 2.3.1. Solvent method

Three different water-soluble polymers, PEG 6000, PVP and HPMC, were used as the carrier of SDF. SDF was prepared by the solvent evaporation method (Chiou and Riegelman, 1971). Briefly, 5 g of tacrolimus and 5 g of each water-soluble polymer were accurately weighed and dissolved in the mixture of 50 ml of ethanol and 25 ml of dichloromethane. Then, the mixed solvent was evaporated under reduced pressure using a vacuum dryer at 40 °C. After drying, SDF was pulverized using an agate mortar and pestle. The pulverized powder was classified using the sieves (size: 60 and 330 mesh), and the particle size fraction of 45–250 μm was used for the study. A physical mixture of tacrolimus and each water-soluble polymer (1:1, w/w) was prepared by the pulverization with an agate mortar and pestle. The mixtures through the sieve (size: 60 mesh) were used for the study.

#### 2.3.2. New preparation method

Five grams of tacrolimus and 5 g of HPMC were accurately weighed and tacrolimus was completely dissolved into 15 ml of ethanol. Then, HPMC was swollen by ethanol, in which tacrolimus is dissolved. Ethanol was evaporated under reduced pressure using a vacuum dryer at 40 °C. After drying, SDF was prepared and obtained by the same manner of the conventional solvent method described above.

### 2.4. Scanning electron microscope (SEM) study

The SEM pictures were obtained by a scanning electron microscope (Type S-800, Hitachi, Tokyo). The accelerating voltage is 10 kV at 100×.

### 2.5. Powder X-ray diffraction (XRD) measurement

XRD measurement was performed using a powder X-ray diffractometer (Multiflex, Rigaku, Tokyo) with Ni-filtered, Cu Kα radiation, a voltage of 40 kV and a current of 40 mA. The scanning rate was 4°/min over a $2\theta$ range of 2.5–40° and with a sampling interval of 0.02°.

### 2.6. Differential scanning calorimetry (DSC) measurement

DSC curves were measured with a DSC instrument (SSC/560S, Seiko Instruments, Chiba, Japan). An aliquot of each sample corresponding to 2 mg as tacrolimus was placed in an aluminum pan. The heating rate was 10 °C/min and the heating ranging was 30–215 °C.

### 2.7. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analyses were performed using a FTIR spectrometer (Type FT-720, Horiba, Kyoto, Japan). Data were collected over a spectral region from 4000 to 400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$.

### 2.8. Dissolution studies

The dissolution test was carried out in accordance with Japanese Pharmacopoeia (JP) 14 paddle method. The dissolution medium was 900 ml of JP first medium, pH 1.2, which was maintained at 37 ± 0.5 °C. The paddle rotation speed was 200 rpm. The excess amount of SDF was applied to the dissolution medium. Aliquots were withdrawn through the G4 glass filter at appropriate times, and equal volumes of fresh dissolution medium were replaced. The concentration of tacrolimus dissolved in the medium was

analyzed by high performance liquid chromatography (HPLC).

### 2.9. HPLC analysis

The HPLC analyses were performed using Waters HPLC system (Model 515 pump, Model 717 plus auto sampler, Model 486 UV detector) equipped with a 4.6 mm × 150 mm ODS column (TSK-Gel ODS 80TM, Tosoh, Tokyo). The mobile phase consisted of water, isopropyl alcohol and tetrahydrofuran (5:2:2, v/v/v). The flow rate was 1.0 ml/min, and the detection wavelength was 220 nm.

### 2.10. In vivo absorption studies

#### 2.10.1. Comparison of oral absorption between crystalline powder and SDF with HPMC

The in vivo absorption studies of tacrolimus crystalline powder or SDF with HPMC prepared by the conventional solvent method were carried out using male beagle dogs ($n = 6$). Each dog was fasted overnight. The crystalline powder or SDF with HPMC containing 1 mg as tacrolimus was suspended with 20 ml of water, and then each suspension was orally administered. The blood samples were withdrawn into a heparinized syringe at 0.167, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 8 h after oral administration. Those blood samples were kept under −20 °C until assayed. The concentrations of tacrolimus in the blood samples were analyzed by the enzyme immunoassay (EIA) method (Tamura et al., 1987).

#### 2.10.2. Comparison of oral absorption between SDFs with two preparation method

The in vivo oral absorption study of SDFs prepared by the conventional solvent method and the new preparation method was performed using male cynomolgus monkeys ($n = 3$). Each monkey was fasted overnight. The SDF containing 5 mg as tacrolimus was filled into a hard gelatin capsule and then the capsule was orally administered with 20 ml of water. The blood samples were withdrawn into a heparinized syringe at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h after oral administration. Those blood samples were kept under −20 °C until assayed. The concentrations of tacrolimus in the blood samples were analyzed by the EIA method (Tamura et al., 1987).

### 2.11. Pharmacokinetic analysis

The area under the blood concentration of tacrolimus versus time curve (AUC) and mean residence time (MRT) were calculated by the trapezoidal method.

### 2.12. Stability studies

The SDF of tacrolimus prepared by the new method was stored at 40 °C for 3 months. The content of tacrolimus in the SDF was measured by HPLC. XRD, DSC and the supersaturated dissolution studies were also performed.

### 2.13. Statistic analysis

Results are expressed as mean ± S.E. ANOVA was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was determined by Student's *t*-test.

## 3. Results and discussion

### 3.1. Selection of the appropriate water-soluble carrier for SDF

#### 3.1.1. Solid state characterization

In general, it is well known that a drug in SDF often exists as an amorphous form. The amorphous form of a drug has a higher thermodynamic activity than its crystalline form. The higher thermodynamic energy level of the drug leads to the rapid dissolution property (Betageri and Makarla, 1995; Jung et al., 1999). In order to investigate the crystallinity of tacrolimus in SDF with PEG 6000, PVP or HPMC prepared by the conventional solvent method (tacrolimus:carrier = 1:1, w/w), XRD and DSC studies were carried out.

Fig. 2 shows the XRD patterns of tacrolimus, each water-soluble polymer, the physical mixture of tacrolimus with each carrier, and SDF of tacrolimus with each carrier. The XRD pattern of the physical mixture of tacrolimus with each water-soluble polymer was similar to the XRD pattern of tacrolimus crystalline powder alone. It was confirmed that the crystallinity of tacrolimus does not change in the physical mixtures with each carrier. On the other

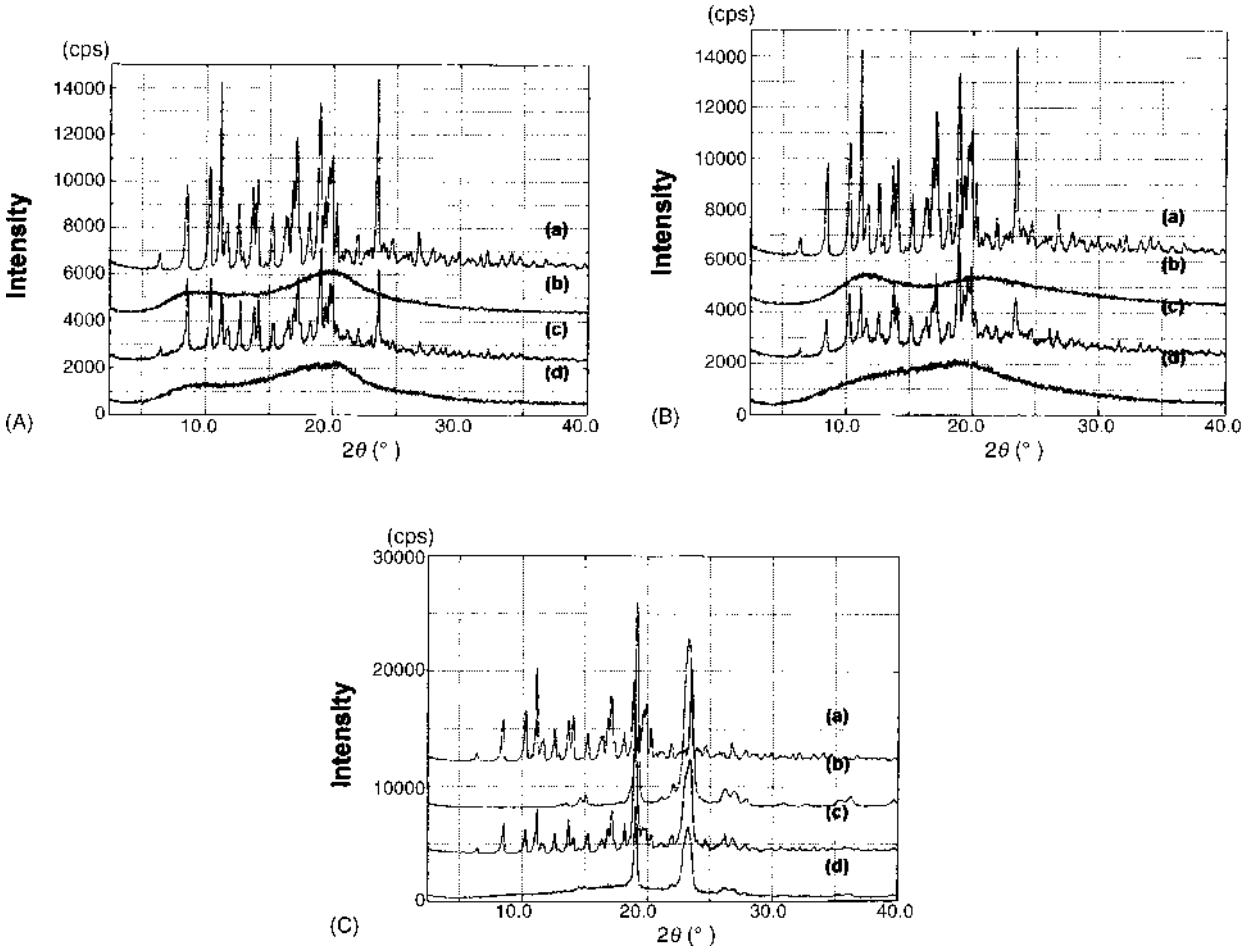


Fig. 2. XRD patterns for SDF of tacrolimus with HPMC (A), PVP (B) or PEG 6000 (C). (a) Tacrolimus crystalline powders; (b) carrier alone; (c) physical mixture of tacrolimus and a carrier; (d) SDF of tacrolimus with a carrier.

hand, no diffraction peak from tacrolimus was observed in all the SDFs investigated here, and the XRD pattern of each SDF was similar to that of the carrier itself used for each SDF. This result suggests that tacrolimus exists in an amorphous state in the SDF.

Fig. 3 shows the DSC thermograms of the physical mixture of tacrolimus and each carrier, and SDF of tacrolimus with each carrier. The physical mixture of tacrolimus and HPMC or PVP exhibited an endothermic peak at around 130 °C, which corresponds to the melting of tacrolimus. The physical mixture of tacrolimus and PEG 6000 showed no endothermic peak of tacrolimus, even though the peaks derived from tacrolimus were observed in XRD (Fig. 2C). It is speculated that tacrolimus is dissolved in melted PEG 6000 during DSC measurement, and only one endothermic peak at around 60 °C, which corresponds to the melting of PEG 6000, is observed. On the other hand, SDF of tacrolimus with each carrier exhibited no endothermic peak corresponding to tacrolimus, suggesting no crystalline of tacrolimus in each SDF. From the results of XRD and DSC studies, it was confirmed that tacrolimus exists in an amorphous state in every SDF with each of the three carriers investigated.

### *3.1.2. Dissolution study*

To increase the oral absorption of poorly water-soluble drugs, it is very important to improve the

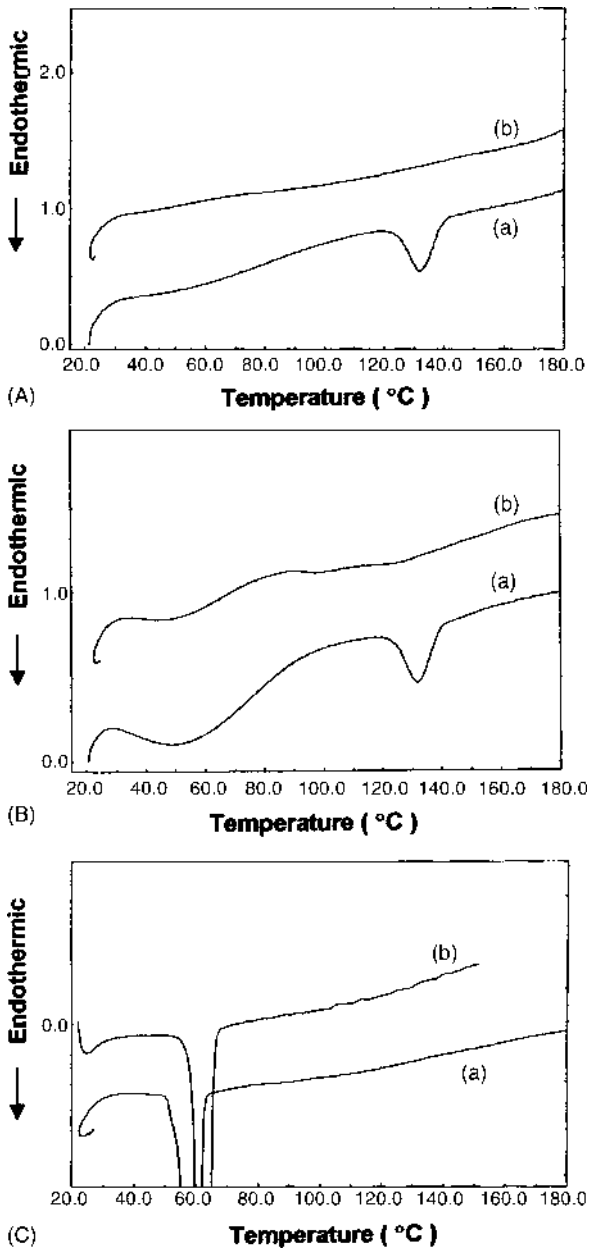


Fig. 3. DSC thermograms of SDF of tacrolimus with HPMC (A), PVP (B) or PEG 6000 (C). (a) Physical mixture of tacrolimus and a carrier; (b) SDF of tacrolimus with a carrier.

drug solubility in the gastrointestinal tract. Therefore, the dissolution of tacrolimus from SDF with each carrier, which was prepared by the conventional solvent method, was examined. Fig. 4 shows the dissolution profile of tacrolimus from SDF with each carrier together with that for tacrolimus crystalline powder. The supersaturation of tacrolimus for all SDFs was observed just after starting the dissolution test. The maximum supersaturated concentrations of tacrolimus from the three SDFs were almost similar and were about 25-fold higher (about 50 μg/ml) than the solubility of tacrolimus in JP first solution. However, the dissolution profile of tacrolimus from SDF with PEG 6000 showed that the supersaturated level of tacrolimus rapidly decreased and was only about 6 μg/ml after 24 h. Moreover, the supersaturated level of tacrolimus from SDF with PVP also decreased gradually to about 30 μg/ml after 24 h. The decrease of tacrolimus concentration in a supersaturated state could be due to the re-crystallization of tacrolimus as reported with regard to many other compounds (Sugimoto et al., 1980; Okimoto et al., 1997; Kohri et al., 1999). On the other hand, the extremely high concentration of tacrolimus in a supersaturated state was maintained up to 24 h in the case of SDF with HPMC, suggesting that the usage of HPMC as a carrier can prevent supersaturated tacrolimus from re-crystallizing. These results of the dissolution study clearly indicated that HPMC is the most appropriate

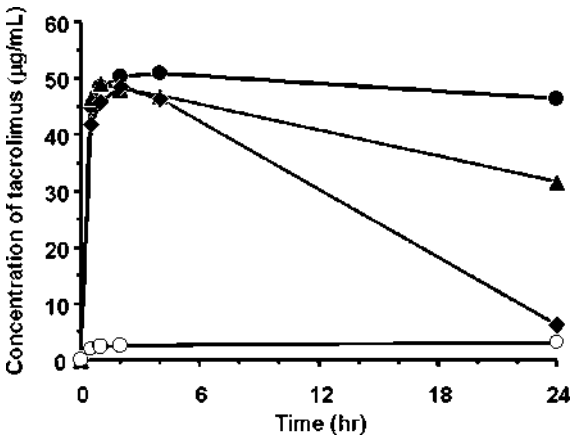


Fig. 4. Dissolution profiles of tacrolimus from SDFs. (●) SDF of tacrolimus with HPMC; (▲) SDF of tacrolimus with PVP; (◆) SDF of tacrolimus with PEG 6000; (○) tacrolimus crystalline powder. Each SDF corresponding to 70 mg as tacrolimus was dissolved in JP14 first fluid (pH 1.2). The test method was in accordance with JP14 paddle method; the rotation speed of paddle was 200 rpm and the dissolution medium was maintained at 37 ± 0.5 °C. Results are the mean of duplicate experiments.

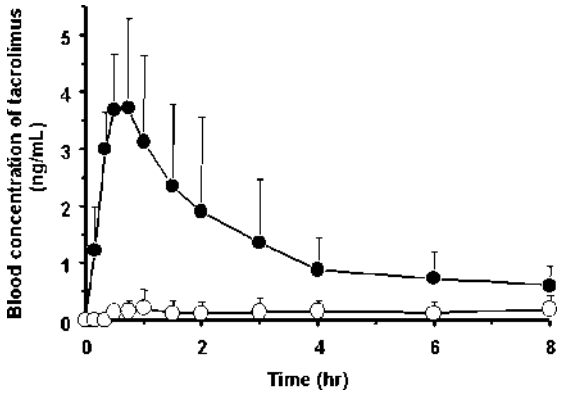


Fig. 5. Blood concentration of tacrolimus after oral administration of SDF with HPMC to beagle dogs. (●) SDF of tacrolimus with HPMC; (○) tacrolimus crystalline powders. Values are expressed as the mean with a vertical bar showing S.E. of six animals. Each dosage form was administered at the dose of 1 mg as tacrolimus.

carrier for SDF of tacrolimus among carriers examined in the present study.

### 3.1.3. *In vivo oral absorption studies*

To investigate whether the improved dissolution profile of tacrolimus by SDF with HPMC can be reflected in the in vivo oral absorption, the SDF was administered to dogs. Fig. 5 shows blood concentration–time profile of tacrolimus after oral administration of SDF with HPMC or crystalline powder of tacrolimus at the dose of 1 mg as tacrolimus. The pharmacokinetic parameters of tacrolimus are listed in Table 1. As shown in Fig. 5, when tacrolimus crystalline powder was administered orally, the tacrolimus levels in the blood were detected very low, and the $C_{max}$ and $AUC_{0-8\,h}$ values were 0.4 ng/ml and 1.1 ng h/ml, respectively (Table 1). On the other hand, when the SDF with HPMC was administered orally, tacrolimus levels in the blood were markedly increased in comparison with the crystalline powder. The $C_{max}$ and $AUC_{0-8\,h}$ values were 4.0 ng/ml and 10.9 ng h/ml, respectively (Table 1). Both AUC and $C_{max}$ values for the SDF with HPMC were about 10 times higher than those of the crystalline powder, indicating that oral absorption of tacrolimus is extremely enhanced by its administration as SDF with HPMC. From the results of the physicochemical and pharmacokinetic studies, HPMC was selected as the most suitable water-soluble carrier for SDF of tacrolimus.

## 3.2. *Establishment of new preparation method of SDF*

To prepare SDF of tacrolimus with HPMC by the conventional solvent method, the mixed solvent systems including dichloromethane have been used (Ho et al., 1996; Yano et al., 1997; Jung et al., 1999; Kobayashi et al., 2001; Kushida et al., 2002; Honbo et al., 1987). However, since dichloromethane is classified in Class 2 solvents of ICH harmonized tripartite guideline, a new preparation method for SDF that does not need dichloromethane is really expected from the environmental point of view. Therefore, we tried to develop a new preparation method for SDF of tacrolimus with HPMC, in which dichloromethane is not used.

### 3.2.1. *Observation of SDF by SEM*

Fig. 6 shows SEM pictures of tacrolimus crystalline powder, HPMC, SDF of tacrolimus with HPMC prepared by the conventional solvent method and the SDF prepared by the new preparation method. Tacrolimus crystalline powder (Fig. 6A) and HPMC (Fig. 6B) showed the prismatic shape and the fibrous shape, respectively. The appearance of SDF prepared by the conventional solvent method was like a uniformed and homogeneously mixed mass (Fig. 6C). On the contrary, the appearance of SDF prepared by the new method showed the fibrous shape, and the unit shape of SDF is almost similar to that of HPMC (Fig. 6D). These results suggest that tacrolimus in SDF prepared by the new method is adsorbed into

Table 1
Pharmacokinetic parameters of tacrolimus after its oral administration to dogs as crystalline powders or SDF of tacrolimus with HPMC

| Sample | $AUC_{0-8\,h}$ (ng h/ml) | $C_{max}$ (ng/ml) | $T_{max}$ (h) | MRT (h) |
|---|---|---|---|---|
| Crystalline powders | 1.1 ± 1.4 | 0.4 ± 0.3 | 3.1 ± 3.0 | 3.3 ± 0.9 |
| SDF with HPMC | 10.9 ± 6.1* | 4.0 ± 1.2* | 0.6 ± 0.2 | 2.7 ± 0.1 |

Each value represents the mean ± S.E. of six animals. Each dosage form was administered at the dose of 1 mg as tacrolimus.
* $P < 0.05$, compared to the corresponding parameter of crystalline powder.

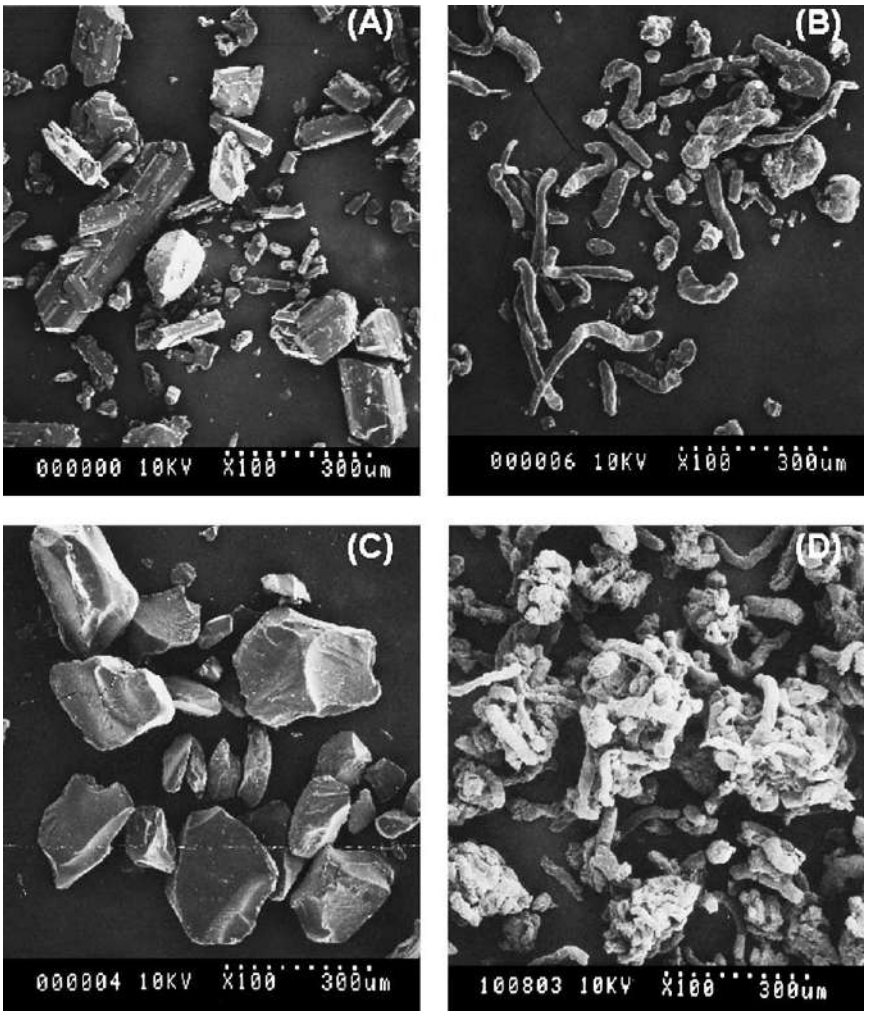


Fig. 6. SEM pictures of (A) tacrolimus crystalline powder, (B) HPMC, (C) SDF prepared by the conventional solvent method and (D) SDF of tacrolimus prepared by the new method.

HPMC swollen with ethanol and is homogeneously dispersed into HPMC at the molecular level.

*3.2.2. Solid state characterization of SDF prepared by the new method*

The crystallinity of tacrolimus in SDF with HPMC prepared by the new method was checked by XRD and DSC measurements. The XRD pattern and DSC thermogram are shown in Fig. 7A and B, respectively. No diffraction peaks were observed in the XRD pattern (Fig. 7A) and no endothermic peak corresponding to the melting of tacrolimus was observed in the DSC thermogram (Fig. 7B) for SDF prepared by the new method. These results suggest that tacrolimus exists in an amorphous state in SDF with HPMC prepared by the new method.

The FTIR studies were carried out to investigate the interaction between tacrolimus and HPMC. Fig. 8 shows the FTIR spectra of tacrolimus crystalline, HPMC, the physical mixture of tacrolimus and HPMC, and SDF with HPMC prepared by the new method. In the FTIR spectra of tacrolimus crystalline (Fig. 8A),

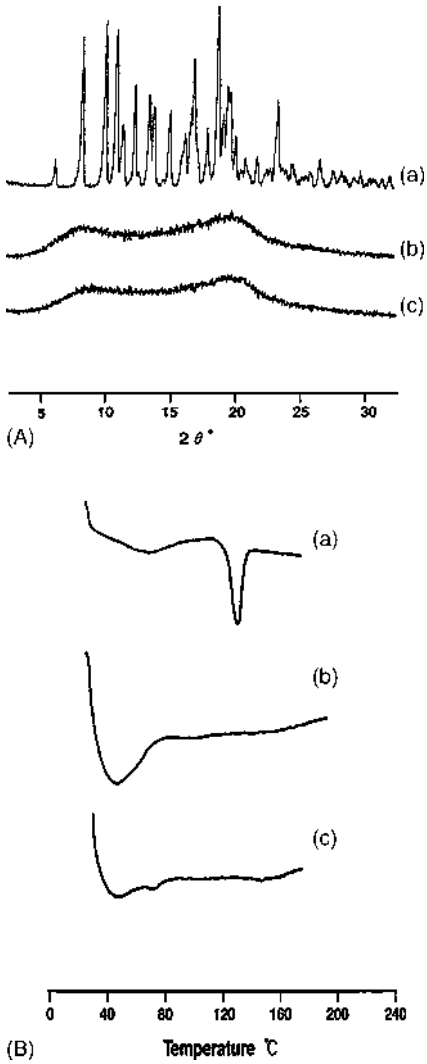


Fig. 7. Comparison of XRD patterns (A) and DSC thermograms (B) between SDFs of tacrolimus prepared by two different methods. (a) Tacrolimus crystalline powder; (b) SDF of tacrolimus prepared by the conventional solvent method; (c) SDF of tacrolimus prepared by the new method.

absorption bands of O–H stretching vibration at 3450 $cm^{-1}$, C=O (ester and ketone) stretching vibrations at 1740, 1725 and 1693 $cm^{-1}$, C=O (keto-amide) and C=C stretching vibration at 1637 $cm^{-1}$, C–O (ester) stretching vibration at 1194 $cm^{-1}$, C–O–C (ether) stretching vibrations at 1176 and 1094 $cm^{-1}$ were observed (Hane et al., 1992). These bands were also observed for the physical mixture of tacrolimus and HPMC with the same absorbance (Fig. 8C). From these results, it was confirmed that there is no interaction between tacrolimus and HPMC in the physical mixture. In contrast, the bands due to the C=O stretching vibration of tacrolimus in SDF were different from those of physical mixtures, whereas the other stretching vibrations of tacrolimus were not affected. The absorption band attributed to C=O groups at 1725 $cm^{-1}$ was disappeared, and the absorption bands at 1693 and 1637 $cm^{-1}$ were shifted up to 1708 and 1647 $cm^{-1}$, respectively (Fig. 8D). These results suggest that the C=O functional groups and O–H of tacrolimus are interacted with the functional group of HPMC at the molecular level in SDF prepared by the new method (Cherng-Yih et al., 1998; Tantishaiyakul et al., 1999; Hirasawa et al., 1999; Kushida et al., 2002).

### *3.2.3. Dissolution property of tacrolimus from SDF prepared by the new method*

As shown in Fig. 4, SDFs prepared by the conventional solvent method have a great difference in the dissolution profile of tacrolimus, even though they show almost the same maximal value of supersaturated concentration of tacrolimus. Therefore, it is very important to check the supersaturated dissolution profiles for the assessment of SDF potency. Fig. 9 shows the dissolution profiles of tacrolimus from SDFs prepared by the conventional solvent method and the new preparation method at four different amounts of SDF. Both SDFs showed the same supersaturated dissolution profiles and levels at each amount of SDF. The maximal value of the concentration of tacrolimus for both SDFs tended to increase as the amount of tacrolimus examined increased. The reason for this phenomenon remains to be clarified, but HPMC, of which the amount also increased, may improve the dissolution of tacrolimus a little bit more. Fig. 9C shows the statistically significant linear relationship in the equilibrium concentrations of tacrolimus between SDFs with HPCM prepared by the conventional and new methods ($r^2 = 0.99$). The slope of regression line was 1.024, indicating that the equilibrium (maximum) concentrations of tacrolimus from both SDFs are almost the same each other. From these results, it was confirmed that the supersaturated dissolution properties of tacrolimus from SDF with HPMC prepared by

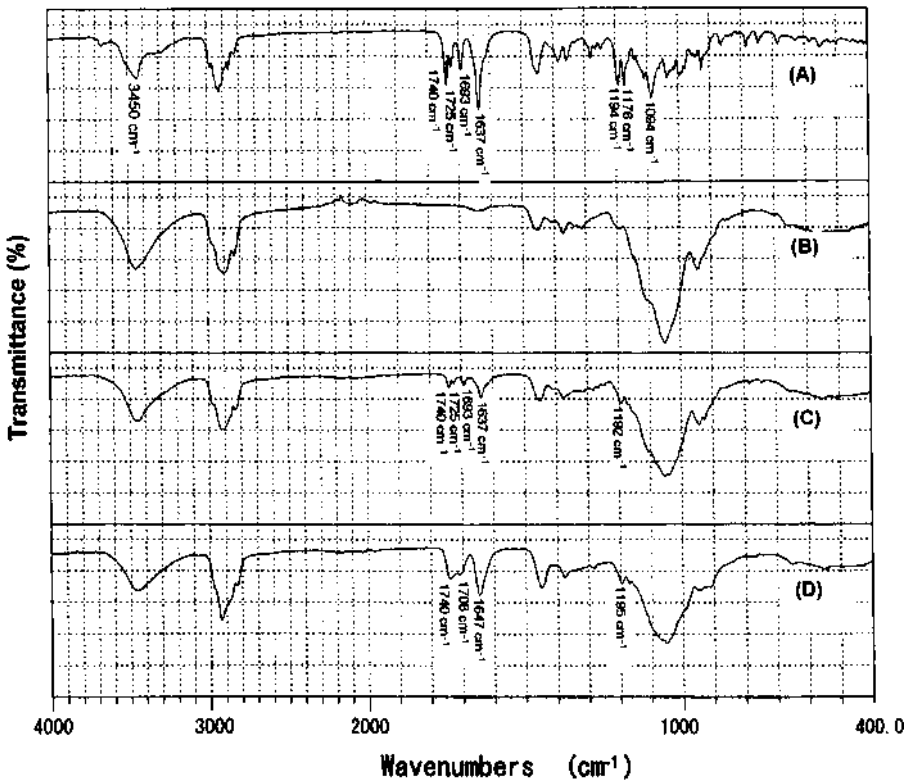


Fig. 8. FTIR spectra of (A) tacrolimus crystalline powder, (B) HPMC alone, (C) physical mixture of tacrolimus and HPMC and (D) SDF of tacrolimus prepared by the new method.

the new method were similar to that prepared by the conventional solvent method.

### 3.2.4. In vivo absorption study

Since SDF of tacrolimus with HPMC prepared by the new method showed the similar physicochemical properties to SDF prepared by the conventional solvent method, the in vivo oral absorption was compared between the two SDFs in monkeys (Fig. 10). The blood concentration profile of tacrolimus after dosing each of SDF was quite similar and the pharmacokinetic parameters such as AUC, $C_{max}$, $T_{max}$ and MRT were not significantly different ($P > 0.01$) between the two SDFs (Table 2). These results of physicochemical and biopharmaceutical studies clearly show that an excellent SDF of tacrolimus with HPMC could be prepared not only by the conventional solvent method, but also the new preparing method.

### 3.2.5. Stability study

One of the problems that must be overcome for the commercial application of SDF is the stability is-

Table 2
Pharmacokinetic parameters of tacrolimus after oral administration of SDF prepared by the conventional solvent method or the new preparation method to monkeys

| Sample | $AUC_{0-72\,h}$ (ng h/ml) | $C_{max}$ (ng/ml) | $T_{max}$ (h) | MRT (h) |
|---|---|---|---|---|
| Conventional solvent method | 551.7 ± 88.6 | 31.7 ± 2.3 | 2.7 ± 0.8 | 21.6 ± 1.4 |
| New preparation method | 578.1 ± 57.2 | 38.7 ± 10.6 | 3.3 ± 0.8 | 20.5 ± 3.0 |

Each value represents the mean ± S.E. of three animals. Each dosage form was administered at the dose of 5 mg as tacrolimus.

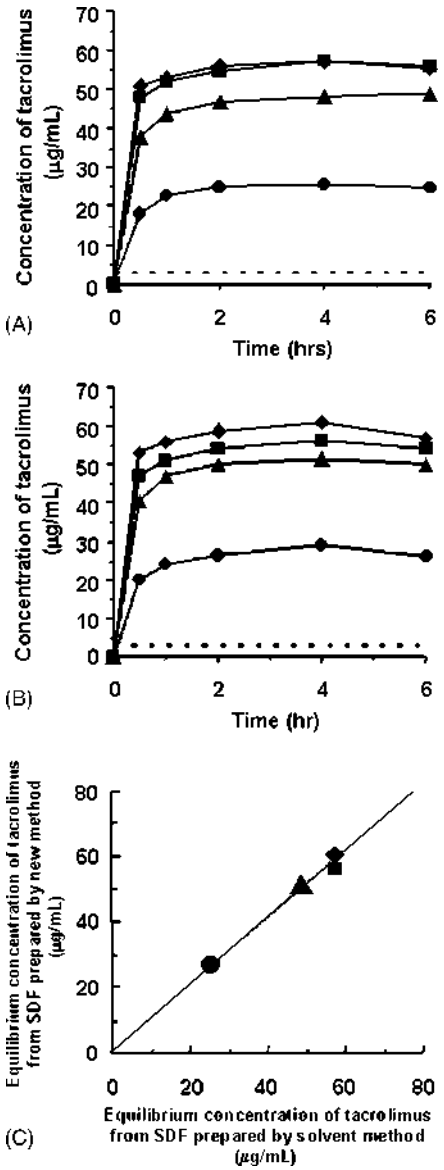


Fig. 9. Comparison of dissolution profiles between SDFs of tacrolimus prepared by two different methods. (A) and (B) represent dissolution profiles of SDFs of tacrolimus prepared by the conventional solvent method and the new method, respectively, at four different amounts applied, and the relationship between the equilibrium concentrations from the two SDFs are shown in (C). Applied amounts of SDF: (●) 25 mg as tacrolimus; (▲) 70 mg as tacrolimus; (■) 100 mg as tacrolimus; (◆) 200 mg as tacrolimus. The dotted line represents the solubility of tacrolimus in the dissolution medium. The test method was in accordance with JP14 paddle rotation method; the rotation speed of paddle was 200 rpm, and the dissolution medium (JP14 first fluid) was maintained at $37 \pm 0.5\,^{\circ}C$. Results are the mean of duplicate experiments.

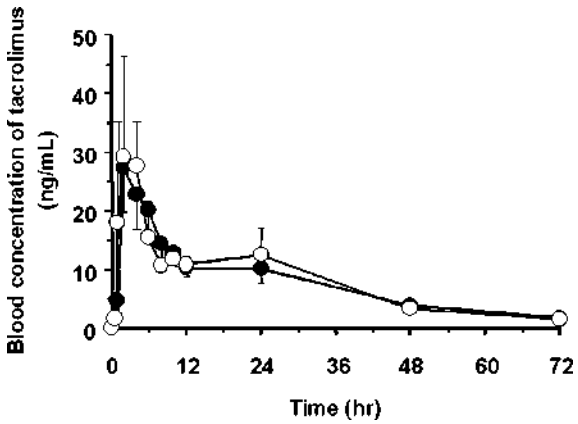


Fig. 10. Blood concentration of tacrolimus after oral administration of SDFs of tacrolimus prepared by two different methods to cynomolgus monkeys. (●) SDF prepared by the conventional solvent method; (○) SDF prepared by new method. Values are expressed as the mean with a vertical bar showing S.E. of three animals. Each dosage form was administered at the dose of 5 mg as tacrolimus.

sue of SDF, that is to say, the amorphous form may re-crystallize out on aging (Serajuddin, 1999). The re-crystallization of the drug results in the decrease of the supersaturated level. In fact, it was reported that griseofulvin was precipitated out from SDF with PEG 6000 during the storage (Chiou, 1977). Damian et al. (2002) reported that the dissolution rate of UC-781 from SDF with PEG 6000, Gelucire® 44/14 and PVP K30 decreased with time of the storage, which could be also attributed to the re-crystallization during the storage.

The stability study of SDF prepared by the new method was performed after the SDF was stored at 40 °C for 3 months. The amount of tacrolimus in the SDF was not decreased (101.5%) after the storage. The equilibrium concentration of tacrolimus in the supersaturated dissolution profile for the stored SDF was kept at 52.0 μg/ml, which is almost the same as that of SDF soon after the preparation (51.6 μg/ml). The supersaturated concentration of tacrolimus after 24 h for the stored SDF was the same as that of initial SDF. Furthermore, no diffraction peak of XRD and no endothermic peak of DSC were observed in SDF stored (data not shown). Thus, the amorphous state of tacrolimus in SDF prepared by the new method, was kept by storing at the accelerate condition. From these results, it

was confirmed that the SDF of tacrolimus with HPMC prepared by the new method is very stable.

## 4. Conclusion

From the physicochemical, biopharmaceutical and stability studies, it was clarified that SDF of tacrolimus with HPMC prepared by the new method, in which dichloromethane is not used and HPMC is just swollen with ethanol, has such an excellent property as SDF prepared by the conventional solvent method. Therefore, the new method for the preparation of SDF with HPMC could be very useful from the view-point of the environmental issue.

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# Enhanced Bioavailability of a Poorly Soluble VR1 Antagonist Using an Amorphous Solid Dispersion Approach: A Case Study

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**Abstract:** Amorphous solid dispersions (ASD) of a poorly soluble water-soluble VR1 antagonist (AMG 517) were explored for improving physical stability and in vivo exposure. AMG 517 was incorporated at 15 or 50 wt % into polymeric microparticles of hydroxypropyl methylcellulose acetate succinate (HPMCAS) and hydroxypropyl methylcellulose (HPMC) by spray-drying. Solid particles having a collapsed, corrugated structure were observed by SEM. Median particle size ranged from 29 to 40 $\mu$m by laser light scattering, and residual solvent levels were below 2% by thermal gravimetric analysis. ASD powders exhibited single glass transition temperatures ($T_g$) in the range of 98−117 °C by modulated DSC and were amorphous by XRPD. Amorphous stability, characterized at 40 °C/75% RH (open dish) by XRPD, was at least six months for ASD formulations. Drug dissolution and supersaturation testing in a USP-2 apparatus indicated superior performance of ASD formulations over micronized AMG 517. PK of an ASD formulation in capsule (15 wt % AMG 517 in HPMCAS blended with 5 wt % SDS) in cynomolgus monkeys ($n = 6$, crossover) increased AUC 163% and $C_{max}$ 145% in comparison to an OraPlus suspension control. The study demonstrates the ASD approach provides improved amorphous physical stability and oral bioavailability for a poorly soluble development-stage molecule.

**Keywords:** Solid dispersion; amorphous stability; HPMC-AS; spray-drying; improved bioavailability

## Introduction

Development of potentially efficacious drug compounds can be hindered by a compound's poor solubility and, as a result, its poor oral bioavailability. Solid dispersion technology is applicable to structurally diverse, poorly water soluble compounds with a wide range of physicochemical properties, and offers the potential of improved bioavailability for these compounds via improved dissolution behavior. Dissolution and solubility enhancement may be achieved by dispersing the poorly soluble drug in a solid matrix carrier, either as a eutectic or phase separated mixture, or as an amorphous solid dispersion (ASD). The matrix carrier may be a hydrophilic polymer, such as polyvinylpyrroliddone (PVP),[1−7] polyeth-

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ylene glycol (PEG),[8,9] or the various cellulose derivatives with broad NF acceptance.[10−25] Application of solid dispersions to improve oral bioavailability of poorly soluble molecules dates back over 40 years, and has been the basis of several review articles.[26−29] Ideally in an amorphous solid dispersion, the drug is molecularly dispersed within the matrix carrier. Thus, upon exposure to aqueous media, dissolution is believed to generate a supersaturated state due to enhanced dissolution rate of the amorphous, molecularly dispersed drug. The matrix polymer is also believed to have a role in trapping of the drug in a metastable form to prevent precipitation or crystallization from the supersaturated state, either through formation of drug−polymer assemblies or by preventing or retarding nucleation and crystal growth.[29,30]

Over the past two decades, the use of amorphous drugs in solid dispersions has been investigated with practical consideration of product development within the pharmaceutical industry.[27−29,31,32] The primary challenges in developing amorphous drug based solid dispersion formulations relate to assuring long-term physical stability of the drug in the amorphous state, as well as the methods for preparing the formulation on a large scale. In solid dispersions, the matrix material provides benefit in meeting both challenges. Matrix polymers can enhance the storage stability of the solid dispersion by acting as an antiplasticizer, so long as the glass transition temperature ($T_g$) of the polymer is equal to or


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greater than the $T_g$ of the amorphous pure drug.[7,27,31,33,34] The physical presence of the polymer can also retard the rate of drug crystallization during preparation of the solid dispersion and in storage, as drug molecules are disrupted from interacting within the polymer matrix.[18,34] In some cases, intermolecular interactions between drug and polymer may exist within the polymer phase, further stabilizing the blend.[35]

Hot melt extrusion and spray-drying from organic solvents have emerged as the predominant technologies for preparation of amorphous solid dispersions of poorly soluble drugs at both the laboratory and large scale.[2,4,9,12,17,19,23,36−40] The spray-drying technique is useful for rapidly removing the organic solvent used to dissolve both the drug and matrix polymer, resulting in an amorphous phase. Use of volatile solvents minimizes the processing temperatures used for production of the dry product, which can be of concern for temperature-sensitive compounds in the hot melt extrusion technique. Spray-drying also has the benefits of providing a somewhat uniform particle size, based on the atomization nozzle selection and dryer design.

The vanilloid receptor VR1, identified as a result of its activation by capsaicin, is a cation channel protein expressed by primary sensory neurons termed nociceptors.[41] AMG 517 has been investigated as a potent and selective VR1 antagonist for the treatment of acute and chronic pain. The structure of AMG 517 free base is depicted in Figure 1. The free base is characterized as having very low aqueous solubility (≤7.0 μg/mL over pH 2 to 7; <0.3 μg/mL in PBS at pH 7.1) and a high melting point (232 °C). Due to the low p$K_a$ of the free base, forming of salts having improved solubility proved difficult.

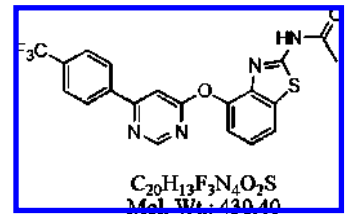


***Figure 1.*** Structure of AMG 517.

The poor solubility of AMG 517 free base makes it a challenge to develop as a solid dosage form. Ora-Plus vehicle, in combination with a dispersing agent, such as Pluronic, is typically used for preclinical and clinical phase I pharmacokinetic evaluations of small molecule compounds. In early animal exposure studies, improved exposure was attained from a suspension of the micronized free base in 10% w/v Pluronic F108 in OraPlus at lower doses (<10 mg/kg). Investigation by Bak et al. revealed the improved exposure in OraPlus was actually due to the formation of a cocrystal between AMG 517 and sorbic acid, a preservative in OraPlus.[42] At higher doses (>30 mg/kg), solubility-limited absorption was still observed with the OraPlus suspension.

In the present study, we investigate application of an amorphous solid dispersion (ASD) formulation approach to provide a solid dosage form with improved in vivo exposure of AMG 517 over its free base suspended in an OraPlus vehicle. ASD particles are formed from organic solvent solutions of drug and codissolved hydrophilic carrier polymers using a spray-drying technique. Hydroxypropyl methylcellulose (HPMC-E5) and hydroxypropyl methylcellulose acetate succinate (HPMCAS-MF) were selected as matrix polymers. Each polymer has been studied previously for use in solid dispersions, and each has particular advantage both for processing by spray-drying and in promoting stability due to their high glass transition temperatures ($T_g$).[17,18,22−25]

HPMCAS is an enteric polymer, its acetyl and succinoyl groups affording control over its dissolution behavior at different pH. This feature could have advantage in delaying gelation and dissolution of the ASD matrix until the formulation reaches the small intestine, thus better maintaining a high degree of drug supersaturation within the absorptive region of the GI tract. HPMCAS also has a lower degree of moisture uptake compared to HPMC, which may impact storage stability of the ASD by decreasing the plasticization effect of water. The variation in functional groups between HPMC and HPMCAS are also likely to affect the drug's ability to interact with the polymer via hydrogen bonding or hydrophobic interactions, with a

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potential impact on both solid-state stability and in vivo performance. Amorphous solid dispersions were formed at low (15 wt %) and high (50 wt %) drug loads and initially characterized to verify formation of amorphous, dry powders. Practical consideration was given toward exploring compositions that could ultimately provide a solid dosage form at a strength relevant to the range explored in recent human clinical trials.[43] ASD powders were then subjected to stability testing under open conditions at elevated temperature and humidity (40 °C/75% RH) for up to six months. In vitro dissolution testing screened the concentration-enhancement features of ASD powders in a supersaturated system, and intrinsic dissolution characteristics were determined for the highest ranking ASD powder. Pharmacokinetics of an AMG 517 formulation, prepared in a capsule using the highest ranking ASD powder, was evaluated in cynomolgus monkeys and compared with AMG 517 free base suspended in OraPlus.

## Experimental Section

**Materials.** AMG 517 micronized free base (Lot # 9902174) was synthesized in-house. Hydroxypropyl methylcellulose acetate succinate, "AQOAT AS-MF" (HPMCAS-MF), was obtained from Shin Etsu Chemical Company (Tokyo, Japan). Hydroxypropyl methylcellulose, "Methocel E5 Premium LV", (HPMC-E5) was obtained from Dow Chemical Company (Midland, MI). Pluronic F108 (Lot # WPAY635B was obtained from BASF (Florham Park, NJ). Ora-Plus (Lot # 6267276) was obtained from Paddock Laboratories Inc. (Minneapolis, MN).

Methyl acetate (minimum 99%) was obtained from Alfa Aesar (Ward Hill, MA). Methanol (minimum 99.5%) and ethyl acetate (minimum 99.5%) were obtained from Mallinckrodt Baker Inc. (Phillipsburg, NJ). All other excipients and chemicals from vendors were either pharmaceutical NF or reagent grade, and were used as received.

**Preparation of Spray-Dried Amorphous Solid Dispersions.** Polymers HPMCAS-MF and HPMC-E5 were dissolved in ethyl acetate (HPMCAS-MF) or 50:50 v/v methyl acetate:methanol (HPMC-E5) at 20 mg/mL. Polymer solutions were added to AMG 517 micronized free base to form solutions containing 50:50 or 85:15 w/w of polymer to drug. AMG 517 solubility in ethyl acetate and 50:50 methyl acetate:methanol were previously determined as ~30 mg/mL and >20 mg/mL, respectively. Solutions were spray-dried at 0.75 mL/min using a Büchi 290 spray-dryer (Brinkmann Instruments, Inc., Westbury, NY) specially equipped with a 48 KHz ultrasonic atomizing nozzle (Sono-Tek Corp., Milton, NY). The spray-dryer was configured with an inert $N_2$ gas feed in open cycle mode and the aspirator was bypassed; the drying gas flow rate was 300 slpm. Exhaust was routed to a vented exhaust manifold. For ethyl acetate, the inlet $N_2$ temperature was 75 °C; for methyl acetate/methanol the inlet temperature was either 45 or 50 °C. The atomizing nozzle was supplied with focusing $N_2$ at 30 slph; power to the nozzle was approximately 2 W.

Following spray-drying, the amorphous solid dispersion powder was collected into vials, sealed, and stored under desiccant at 4 °C until analysis or use. The effect of secondary drying in a vacuum oven (80 °C/84 h) was assessed for one lot of the amorphous solid dispersion formulations (HPMCAS, 15 wt % AMG 517, Lot 2); this secondary dried material was subsequently used in the pharmacokinetic study.

**Initial Characterization. (a) Scanning Electron Microscopy (SEM).** A thin layer of dry particles were placed on the adhesive surface of a carbon conductive tab adhered to a pin stub SEM mount (Ted Pella, Redding, CA) and sputter-coated under argon with a thin layer of gold−palladium using a Pelco SC-7 (Ted Pella, Redding, CA) sputter coater unit. For freeze-fracture SEM analysis of internal particle morphology, particles were sandwiched between two adhesive stubs, chilled in liquid nitrogen, and rapidly separated to produce two fracture surfaces for sputter-coating. Samples were imaged using a Philips XL30 ESEM TMP scanning electron microscope (Philips, FEI Company, Hillsboro, OR), operating at an acceleration voltage of 5 kV. Images were captured with analysis XL Docu software (Soft Imaging Systems, Lakewood, CO).

**(b) Particle Size Analysis.** Particle size was measured using a Malvern Mastersizer 2000 equipped with a Hydro 2000 $\mu$P wet dispersion cell (Malvern Instruments Ltd., Worcestershire, U.K.). Approximately 10 mg of particles was dispersed in 0.5 mL of Sedisperse A-12 (Micromeritics Corp., Norcross, GA), and added to the dispersion cell containing hexane. Particle size distribution was calculated using the Mie theory optical diffraction model[44](particle refractive index and absorption estimated at 1.500 and 0.001, respectively) to yield the volume based diameter parameters at 10%, 50%, and 90% cumulative volume percent: $d[v,0.1]$, $d[v,0.5]$, and $d[v,0.9]$.

**(c) Determination of Free Base Content.** AMG 517 content within HPMCAS and HPMC-E5 solid dispersion microparticles was quantified by high-performance liquid chromatography (HPLC). Samples were prepared in acetonitrile/water (50/50, v/v), with AMG 517 reference standard lot 0502L740, prepared at 0.125 mg/mL, as the calibration standard for quantitation. A Phenomenex Luna 5 $\mu$ C18(2), 150 mm × 4.6 mm, 5 $\mu$m, 100A (Phenomenex, P/N 00F-4252-E0) analytical column was used, with a mobile phase consisting of acetonitrile, water and trifluoroacetic acid (80:20:0.1). A gradient elution method at 1 mL/min was employed, with detection at 254 nm via a HP1100 diode

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array detector (Agilent HP1100 G1315B). Loading efficiency (LE %) within the amorphous solid dispersion was defined as: LE % = [(determined wt % AMG 517) ÷ (theoretical wt % AMG 517)] × 100%.

**(d) Thermal Analysis.** Mass-loss properties were characterized using a TGA Q500 (TA Instruments, New Castle, DE). Modulated DSC (MDSC) was performed on a DSC Q1000 by TA Instruments. Data analysis utilized Universal Analysis 2000 thermal analysis software by TA Instruments. Samples were allowed to equilibrate to room temperature in sealed vials prior to sample preparation. For TGA, samples of 2−6 mg were heated at 10 °C/min over a temperature range of 10 to 300 °C. For MDSC, samples of 2−3 mg were weighed and placed in aluminum crimped pans. MDSC parameters were modulated at ±1.00 °C every 60 s with heating rates of 3.00 °C/min from −10 °C to 200−250 °C. Measurements were performed under nitrogen purge.

**(e) X-ray Powder Diffraction (XRPD).** X-ray diffraction patterns were obtained using a Phillips automated X-ray powder diffractometer, X'Pert PRO (PANalytical, Almelo, Netherlands). A Cu Kα X-ray tube (PW337310 LFF, 1.54 Å) was used with voltage and current of 45 kV and 40 mA, respectively. The incident path was set with a 0.04 rad solar slit, 15 mm fixed mask, 1/2° fixed antiscatter slit, and a 1/4° fixed divergence slit. The diffracted beam path detected by the RTMS detector/X'Cellerator, was set with a 0.04 rad solar slit, 0.09° parallel plate collimator, and 0.02 mm nickel filter. Samples of 5−10 mg were prepared on the sample holder and the stage rotated over the range of $2\theta$ from 3° to 40°. High resolution scans of about 6 h were collected.

**Stability Study.** The stability of ASD formulations was monitored up to 6 months at elevated temperature and relative humidity. Samples were placed in the humidity chamber at 40 °C/75% RH (open dish). Periodically samples were removed and characterized by XRPD.

**Dissolution Studies.** Dissolution screening was performed on Vankel VK7000 dissolution station equipped with a USP 2 (paddle) apparatus and 200 mL Varian mini-vessels (P/N 12-5050). Samples were manually drawn through 10 μm filters (Varian 10 μm cannula filters, UHMW polyethylene 17-4000, FIL010-01-100)) using a 3 mL syringe attached with a sampling cannula (CAN475-HR, 4.75", Hansen Research).

The dissolution profiles of AMG 517 amorphous solid dispersion samples (powder and capsules) were studied in 100 mL of pH 6.8 phosphate buffer at a paddle rotation of 100 rpm, with a constant temperature bath at 37 ± 0.5 °C. Powder samples were weighed into a paper boat, while capsule samples (as prepared below) were placed into Sortax sinkers. Each test system used 12.5 mg of drug. Samples were then dropped into the dissolution vessel to start the dissolution experiment. 1.5 mL samples were drawn at 5, 10, 15, 30, 45, 60, 90 and 120 min with no dissolution medium replacement, and the sample was transferred to a 2 mL HPLC autosampler vial for analysis. AMG 517 was quantified by HPLC, using a method similar to that described above.

The intrinsic dissolution rate was determined using a stationary disk with the USP 2 apparatus equilibrated at 37 °C, with a paddle speed of 100 rpm. 0.8 cm disks were produced by compression using a benchtop press at ∼2000 psi for 1 min. The dissolution medium was 900 mL of pH 6.8 phosphate buffer with 2% SDS.

**Pharmacokinetic Study. (a) Preparation.** All investigations using experiment animals adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised in 1985). The pharmacokinetic study of AMG 517 was conducted in fasted male cynomolgus monkeys ($n = 6$) in a crossover study design comparing an AMG 517 Ora-Plus aqueous suspension to an ASD formulation dosed within a capsule. The body weight of the monkeys ranged from 3 to 4 kg; a fixed dose of AMG 517 of 12.5 mg was given to individual animals. Wash-out time between each dosing phase of the study was 13 days.

Preparation of the Ora-Plus aqueous suspension began by combining 20 g of Pluronic F108 to 200 mL of OraPlus and stirring overnight at room temperature. 150.0 g of the 10% Pluronic F108 in OraPlus vehicle was added to a bottle containing 150.1 mg of AMG 517 micronized free base in a 250 mL Pyrex bottle. The components were mixed using a homogenizer probe for 3 min until the suspension appeared uniform. Final suspension concentration was approximately 1 mg/mL. For the ASD formulation, 950 mg of powder was added to a vial with 50 mg of sodium dodecyl sulfate, and the contents intimately mixed using a vortex mixer. Approximately 85 mg of the mixture was filled into size 1 gelatin capsules.

AMG 517 aqueous suspension in the 10% pluronic F108/Ora-Plus vehicle was administered via oral gavage intubation, followed by a flush of approximately 10 mL of air to clear the gavage tube. The ASD formulation blend in capsule (HPMCAS, 15 wt % AMG 517, Lot 2 + 5 wt % SDS) was administered orally, and 15 mL of water was provided to each monkey as flush liquid after capsule administration.

For each phase of the study, approximately 1 mL of blood was collected from each animal via the femoral vein into 3 mL tubes containing lithium heparin anticoagulant at predose, and following oral dose administration at 0.5, 1, 2, 4, 8, 12, 24, 30, 48, 72, 96, 120, 192, 216, and 240 h postdose. Blood tubes were inverted 10−15 times and stored on ice prior to centrifugation (14000*g* for 2 min) to obtain plasma. Plasma (approximately 500 μL) was transferred to separate tubes and stored at −10 °C or below until analysis.

**(b) Bioanalytical Method for Pharmacokinetic Studies.** Lithium heparinized male cynomolgus monkey plasma samples (50 μL) were extracted using 96-well liquid/liquid extraction (LLE) to isolate the analyte and internal standard. Samples were separated by reversed-phase liquid chromatography on a Thermo Electron Hypersil BDS C18, 50 × 2.1 mm (5 μm) analytical column. The mobile phase was 75:25 MeOH:10 mM ammonium acetate in water (mobile phase B). An isocratic of mobile phase B at a flow rate of 450 μL/min was utilized, with a total run time of 4.0 min.

***Table 1.*** Characterization Summary for ASD Formulations of AMG 517 Prepared from HPMCAS-MF and HPMC-E5 by Spray-Drying

| formulation ID | polymer | drug (wt %) | yield (%) | LE[a] (%) | $d_{50}$[b] ($\mu$m) | % wt loss by TGA | $T_g$ (°C) by MDSC |
|---|---|---|---|---|---|---|---|
| A, Lot 1 | HPMCAS-MF | 15 | 76 | | 34.2 | 2.39 | 106 |
| A, Lot 2 | | 15 | 85 | 98.8 | 35.3[c] | 0.32[c] | 101[c] |
| B | | 50 | 55 | 97.5 | 40.7 | 2.84 | 98 |
| C | HPMC-E5 | 15 | 61 | 99.1 | 34.6 | 1.94 | 117 |
| D, Lot 1 | | 50 | 12[d] | 99.0 | 34.6 | 1.88 | 106 |
| D, Lot 2 | | 50 | 60 | | 28.7 | 1.44 | 107 |

[a] Load efficiency (LE). [b] Particle size diameter at 50% cumulative volume %. [c] For "A, Lot 2", results are provided for material after secondary drying in a vacuum oven. [d] Inlet drying temperature was 45 °C.

A related molecule, AMG1664, was used as the internal standard. Plasma calibration curve standards were prepared at concentrations of 0.500, 1.00, 2.50, 5.00, 10.0, 25.0, 50.0, 100, 250, 500 and 1000 ng of AMG 517/mL of lithium heparinized male cynomolgus monkey plasma.

AMG 517 concentrations were determined in lithium heparinized male cynomolgus monkey plasma samples by LC−MS/MS using ion atmospheric pressure chemical ionization (APCI) with multiple reaction monitoring (MRM) in the positive ion mode. Peak areas were integrated by the Sciex program Analyst, version 1.4.1, residing on a Windows 2003 computer. Following peak area integration, the data was exported to the software Watson (PROD) (version 7.0.0.01, InnaPhase Corp., Philadelphia, PA) where concentrations were determined by a weighted ($1/x^2$) linear regression of peak area ratios (peak area of AMG 517/peak area of AMG1664) versus the theoretical concentrations of the plasma calibration standards. Calculations were performed on unrounded numbers. Overall precision and accuracy for the calibration standards and QC samples were determined by Watson (PROD). Study sample concentrations were rounded to three decimal places before reporting.

**(c) Pharmacokinetic Analysis.** Individual plasma concentration−time data were analyzed by noncompartmental methods using WinNonlinTM v. 4.1e Build 200408051632 (Pharsight Corporation, Mountain View, CA). Nominal sampling times were used in the pharmacokinetic analysis; actual times were all ±10% of the respective nominal time. The area under the concentration−time curve from time zero to infinity ($AUC_{0-inf}$) was calculated using the linear−log trapezoidal method. The maximum plasma concentration ($C_{max}$) observed and the time at which it was observed ($T_{max}$) were determined directly from the individual plasma concentration−time profiles. Relative bioavailability ($F_{rel}$) was calculated by comparing exposures from the capsule formulation to the standard Ora-Plus suspension formulation.

## Results

**Preparation of ASD Material by Spray-Drying.** Formulations produced by spray-drying are summarized in Table 1. Formulations produced from HPMCAS appeared as a white, dry, fine powder in the product collector. Collection yield values were high based on the quantities produced. For formulation A, batch sizes of 8 g (Lot 1) and 6 g (Lot 2) gave yields of 76% and 85%, respectively. For formulation B, a batch size of only 2 g gave a yield of 55%. Generally, yields will be impacted at lower batch sizes due to fixed losses within the dryer. The yield values obtained are in line with typical production yields from easily dried materials, based on our experience with the laboratory-scale dryer.

As described, the effect of secondary drying in a vacuum oven (80 °C/84 h) was assessed for formulation A, Lot 2. After drying, the secondary-dried material still appeared as a white, fine powder. The secondary dried form of A, Lot 2 was subsequently characterized as described below.

Spray-drying of formulations with HPMC-E5 began chronologically with formulation D, Lot 1, for which the inlet temperature was 45 °C and the outlet temperature of the dryer was 32 °C. Collection yield for this initial lot was only 12% for a 3 g batch size, and observations suggested heavy accumulation of sprayed material within the dryer column. Thus, inlet temperature was increased to 50 °C for subsequent formulations of HPMC-E5. Yield values, as a result, improved to 60%, for a 3 g batch size (D, Lot 2). Spray-drying of formulation C, at a 2 g batch size, produced a yield of 61%, in line with the aforementioned yields obtainable in the dryer.

The drug load efficiency in spray-dried ASD formulations of AMG 517 are indicated in Table 1. As expected, loading efficiency was near 100% for all formulations. Values were not adjusted for the minor levels of residual solvents within the particles (see TGA results, below).

**Initial Characterization. (a) SEM.** Representative SEM micrographs of the 15 wt % AMG 517 solid dispersions prepared from HPMCAS are presented in Figures 2A and 2B, while a micrograph of the 15 wt % AMG 517 solid dispersions prepared from HPMC-E5 is presented in Figure 2C. The low resolution images (Figure 2A) shows the particles to be predominantly disperse and irregularly shaped, with sizes ranging over about 10−60 $\mu$m. Higher resolution images (Figure 2B, Figure 2C) suggest the irregular shape is a result of a dense skin forming at the surface of an evaporating droplet and subsequently collapsing upon itself during drying, much like a raisin. Electron micrographs for 50 wt % AMG 517 solid dispersions (not shown) appeared similar.

A freeze-fracture SEM micrograph of HPMCAS formulations containing 15 wt % AMG 517 is presented in Figure 2D (50 wt %, not shown, appeared similar). Internal morphology of the collapsed structures appears as a smooth,

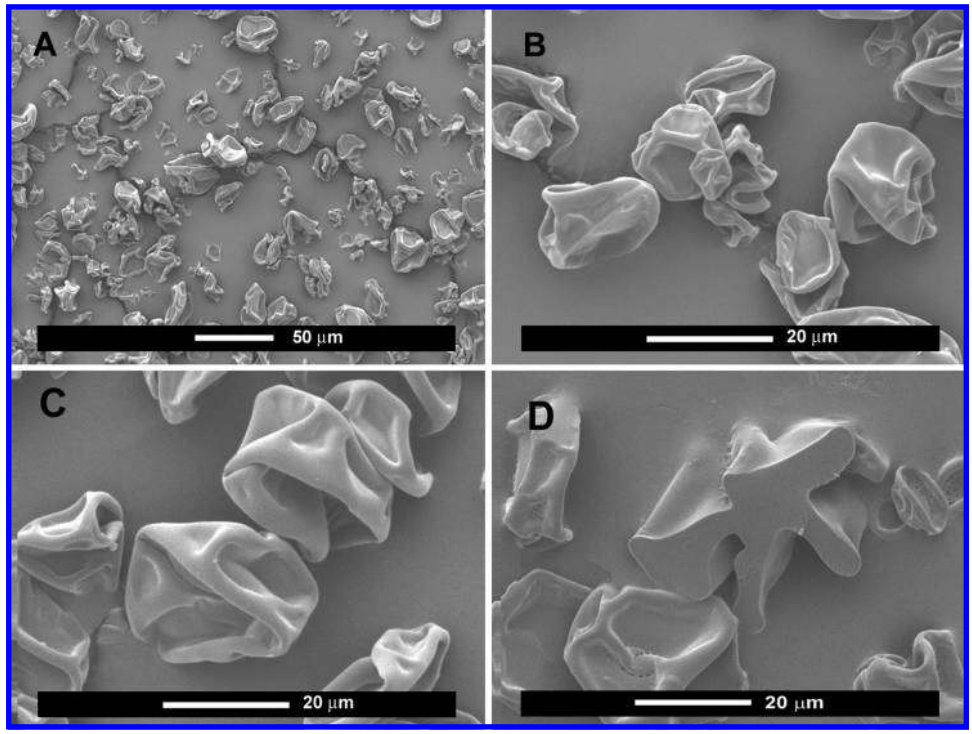


***Figure 2.*** SEM micrographs of ASD formulations: (A) formulation "A, Lot 2" (15 wt % AMG 517 in HPMCAS), 402×; (B) formulation "A, Lot 2" (15 wt % AMG 517 in HPMCAS), 1609×; (C) formulation "C" (15 wt % AMG 517 in HPMC-E5), 1609×; (D) freeze-fracture SEM micrograph of formulation "A, Lot 2" (15 wt % AMG 517 in HPMCAS), 1508×.

solid monolithic structure, devoid of any pores or macrovoids. The lack of any observable crystalline structure in the surface or internal SEM micrographs supports the presence of an amorphous form of the drug.

**(b) Particle Size.** Median particle size (volume based diameter parameter, $d[v,0.5]$) for formulations produced by spray-drying are summarized in Table 1. The results agree with the qualitative assessment of the SEM micrographs. The median size, $d[v,0.5]$, was similar across the formulations, with $d[v,0.5]$ ranging from 29 to 41 $\mu$m. The 10% cumulative volume diameter ($d[v,0.1]$) and 90% cumulative volume parameter ($d[v,0.9]$) ranged from 15 to 21 $\mu$m and 52 to 75 $\mu$m, respectively.

For formulation A, Lot 2, the distribution parameters following secondary drying were similar to the particle size of the same formulation (A, Lot 1) measured immediately after spray-drying. Thus, secondary drying at 80 °C did not lead to significant particle agglomeration.

**(c) Thermal Analysis.** Thermogravametric analysis (TGA) was performed on initially formed spray-dried ASD formulations to gauge residual solvent content (Table 1). The method was not distinguishing between the residual polymer solvent and moisture uptake within the as-prepared formulations. Manufacturer literature for HPMC-AS suggests moisture can be on the order of 2%−5% for pure polymers after storage at 40−60% RH; moisture levels for HPMC-E5 over the same % RH range are on the order of 4%−8%. Values from Table 1 indicate weight loss values on the order of 1% to 3% across formulations, within or slightly below the limits of typical moisture absorption for pure polymers. Although particles were formed from an organic-solvent solution in dry nitrogen, it is expected that at least a portion of the TGA weight loss is a result of moisture. The presence of the hydrophobic drug may also decrease moisture uptake over the pure-polymer values. The values in Table 1 thus set an upper bound for polymer solvent content. Headspace-GC analysis for HPMCAS formulations, produced previously, indicated ethyl acetate solvent content was approximately 3%, consistent with TGA weight loss values (data not shown). Formulation A, Lot 2, had lower weight loss by TGA following secondary drying in a vacuum oven at 80 °C, likely indicating the removal of residual ethyl acetate (and/or moisture). Spray-drying at a higher dryer inlet temperature had an impact on weight loss by TGA: formulation D, Lot 1, exhibited higher weight loss by TGA compared with formulation D, Lot 2.

TGA weight loss curves over the range 25−225 °C are presented in Figure 3 for HPMCAS formulations (A, Lot 1 and B), and HPMC-E5 formulations (C and D, Lot 2). Weight loss by solvent/moisture loss occurs primarily over the range from 25 °C to about 150 °C. Secondary drying conditions (80 °C) were based on the observed loss curves to maximize the rate of solvent removal, yet maintain physical stability by drying at least 20 °C below the $T_g$ of the formulation (see MDSC results, below).

MDSC measurements for all formulations indicated single glass transitions over the entire range of the measurements (Figure 3). The results suggest the drug and polymers formed uniform solid dispersions at the compositions studied. Melting point of AMG 517 free base (form A) is reported as 229 °C; MDSC analysis of pure amorphous AMG 517

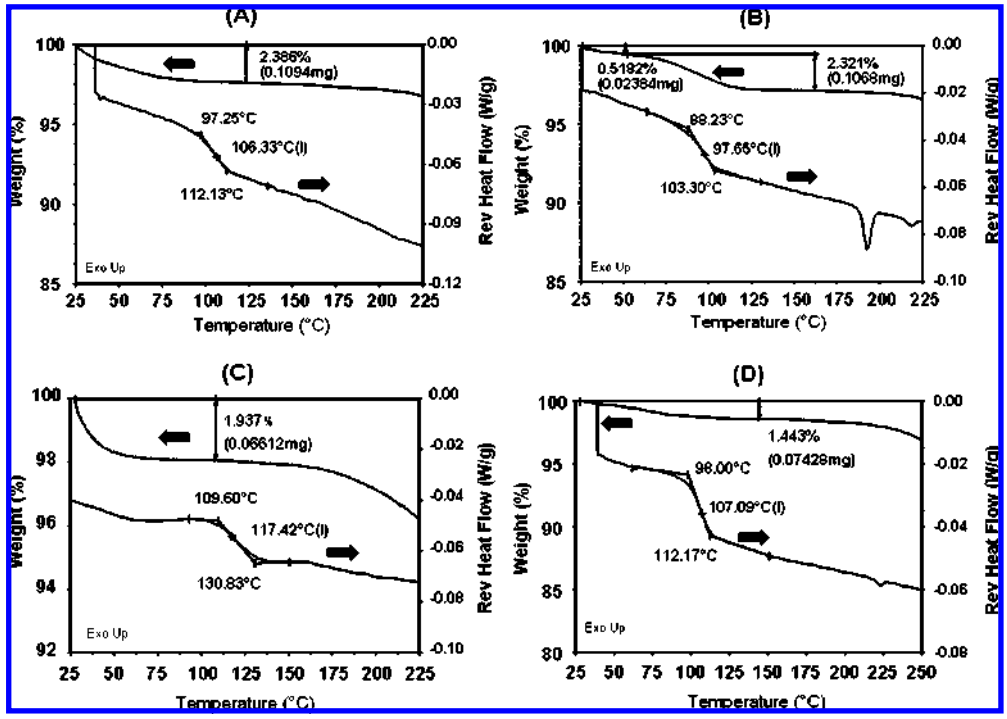


***Figure 3.*** Thermal analysis (TGA and MDSC) of amorphous solid dispersions, as initially formed: (A) formulation "A, Lot 1", 15 wt % AMG 517, HPMCAS; (B) formulation "B", 50 wt % AMG 517, HPMCAS; (C) formulation "C", 15 wt % AMG 517, HPMC-E5; (D) formulation "D, Lot 2", 50 wt % AMG 517, HPMC-E5.

produced by spray-drying (not shown), gave a single $T_g$ at 103 °C ($\Delta C_p$ = 0.42 J/g/°C). The endotherm in the MDSC plots for the 50 wt % HPMCAS formulation at ~190 °C (Figure 3B) corresponds to melting of a polymorph, as a broad recrystallization exotherm in the total heat flow curve was observed between ~160 °C and ~180 °C (not shown). Similar endothermic transitions were observed for the 50% HPMC-E5 formulations near 220 °C, suggesting both formulations containing 50% drug experienced devitrification upon heating beyond their respective glass transition temperatures. Numerical values for the $T_g$ of each lot produced are summarized in Table 1.

**(d) X-ray Powder Diffraction.** X-ray powder diffraction (XRPD) profiles of initially formed spray-dried formulations from HPMCAS and HPMC-E5 are presented in Figure 4. All formulations, analyzed over 6 h, show a halo in XRPD characteristic to the amorphous form. The small peak at $2\theta \sim 27°$ within HPMC-E5 formulations at 50 wt % AMG 517 (Figure 4B) are likely anomalies caused by foreign material and do not correspond to any peaks observed within the XRPD profile of AMG 517 free base (Figure 5, I). Secondary drying of formulation A, Lot 2, under vacuum at 80 °C for greater than 3 days (Figure 4A, II), did not change the XRPD profile.

**(e) Stability Study.** Physical stability of ASD formulations, under open conditions at 40 °C/75% RH, was assessed by XRPD for 6 months (Figure 6). XRPD profiles for HPMCAS formulations at both 15 wt % and 50 wt % AMG 517 show a halo characteristic to the amorphous form after long-term storage (Figure 6 I, II). For HPMC-E5, the 50 wt % AMG 517 formulation was also predominantly X-ray amorphous (Figure 6, III); a small peak at $2\theta \sim 33°$ correlates to a minor peak within the AMG 517 free base profile (Figure 5, I). In contrast, highly amorphous AMG 517 produced by spray-drying (Figure 5, II) shows a tendency toward crystallization after only 15 days storage at 40 °C/75% RH (Figure 5, III).

Physical and chemical assay of A, Lot 1 was assessed after 23 months storage at ambient temperature under desiccant. The sample was still X-ray amorphous. Assay value of A, Lot 1 (15% AMG 517 in HPMCAS), previously not measured at time 0 (Table 1), was determined to have a load efficiency (LE) of 100.1%. Percent main peak area was 98.0%, as compared to an AMG 517 standard with percent main peak of 98.5%. Thus, only minor (0.5%) impurities were seen to develop over 23 months at ambient conditions for the formulations.

**Dissolution Study.** Cellulose ethers such as hydroxypropyl methylcellulose (HPMC) and hydroxypropyl methylcellulose acetate succinate (HPMCAS) of the type used in our experiments are typically used in aqueous-soluble film coating applications. Each has been extensively evaluated as carriers for amorphous solid dispersions.[18,19,22,23,25,36,37] HPMC-E5, a nonenteric polymer, is generally soluble in water, is nonionic, and is not expected to have a pH-dependent solubility. The acetyl and succinoyl moieties on HPMCAS render it insoluble in pure water and acidic

(45) Streubel, A.; Siepmann, J.; Peppas, N. A.; Bodmeier, R. Bimodal drug release achieved with multi-layer matrix tablets: transport mechanisms and device design. *J. Controlled Release* **2000**, *69* (3), 455–468.

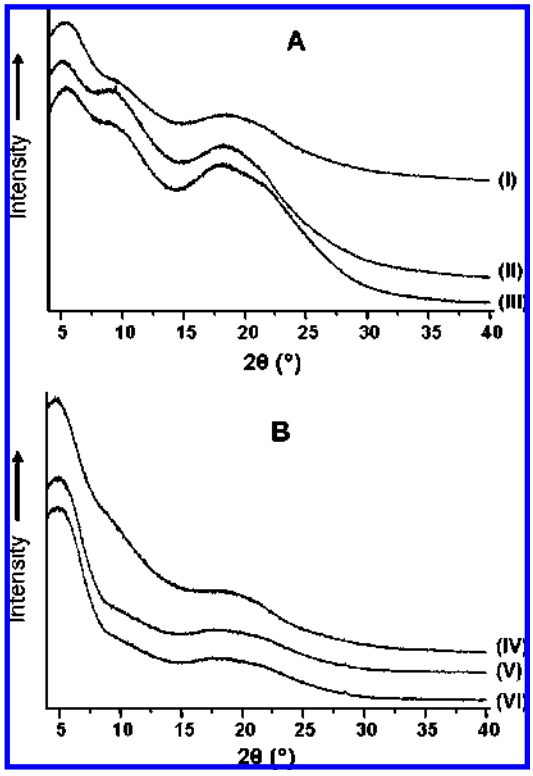


***Figure 4.*** XRPD profiles of spray-dried formulations as initially characterized: (A, I) formulation "A, Lot 1", 15 wt % AMG 517, HPMCAS; (A, II) formulation "A, Lot 2", 15 wt % AMG 517, HPMCAS, post secondary drying; (A, III) formulation "B", 50 wt % AMG 517, HPMCAS; (B, IV) formulation "C", 15 wt % AMG 517, HPMC-E5; (B, V) formulation "D, Lot 1", 50 wt % AMG 517, HPMC-E5; (B, VI) formulation "D, Lot 2", 50 wt % AMG 517, HPMC-E5.

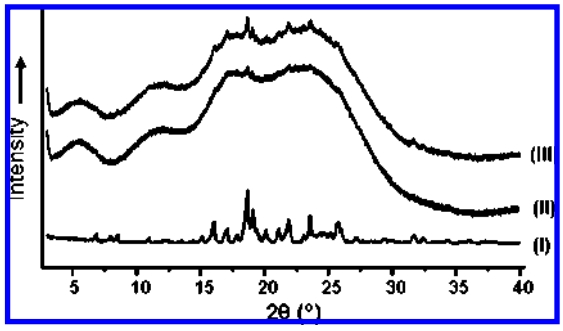


***Figure 5.*** XRPD profiles of AMG 517 free base: (I) micronized free base ("form A"); (II) substantially amorphous free base, as initially formed by spray-drying from ethyl acetate; (III) substantially amorphous free-base, after storage at 40 °C/75% RH for 15 days under open conditions.

environments, enabling its use as an enteric coating polymer.[45] HPMCAS-MF is characterized as having increased solubility above pH 6.5. Kai has demonstrated relative differences in the dissolution behavior at low pH (1.2) and neutral pH (6.8) for solid dispersions of HPMC and a similar enteric polymer, HPMCP.[17] Kai's results suggest attenuated drug dissolution in low pH for the enteric solid dispersions,

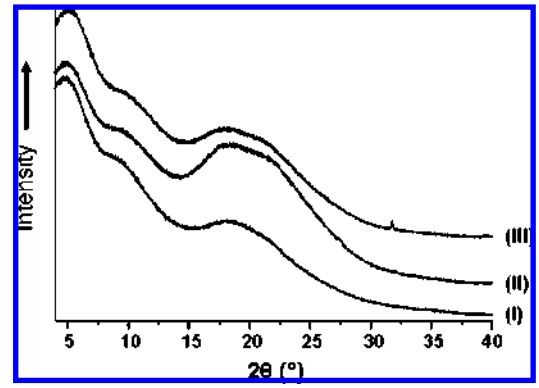


***Figure 6.*** XRPD profiles of spray-dried formulations after 6 months under open conditions at 40 °C/75% RH: (I) formulation "A, Lot 1", 15 wt % AMG 517, HPMCAS; (II) formulation "B", 50 wt % AMG 517, HPMCAS; (III) formulation "D, Lot 1", 50 wt % AMG 517, HPMC-E5.

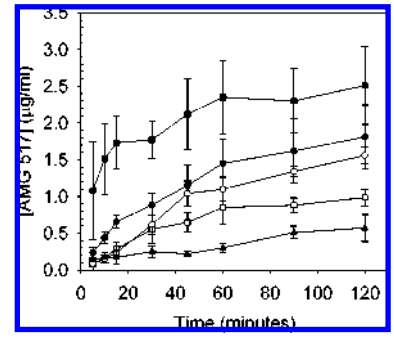


***Figure 7.*** Powder dissolution profiles of AMG 517 under saturation conditions in 100 mL of phosphate buffer, pH 6.8 (12.5 mg drug): (■) ASD formulation A, Lot 2 (15 wt % AMG 517 in HPMCAS); (□) ASD formulation B (50% AMG 517 in HPMCAS); (●) ASD formulation C (15% AMG 517 in HPMC-E5); (○) ASD formulation D, Lot 1 (50% AMG 517 in HPMC-E5); (▲) AMG 517 free base, micronized.

while the HPMC dissolution profile was pH-invariant. During our method development, HPMCAS formulations exhibited poor wettability and reduced dissolution in pH 1.2 and water. Thus, to gauge potential performance in vivo, pH 6.8 phosphate buffer was selected as the dissolution medium for the ASD formulations produced by HPMCAS and HPMC.[22]

Dissolution profiles of ASD formulations as powders, over 120 min, under saturation conditions, are shown in Figure 7. For the ASD powders, dissolution concentration over two hours increased 2- to 5-fold over micronized AMG 517 free base. At 15 wt % AMG 517, the ASD powder containing HPMCAS (A, Lot 2) showed greater dissolution than that made in HPMC-E5 (C). For each polymer, ASD formulations show a clear trend of better dissolution with low drug load, e.g. 15% drug loading showed better dissolution than that of 50%. A higher amount of swellable polymers in the 15% wt % AMG 517 formulations will increase the amount of water uptake in the ASD sample, and therefore increases the wetting of the drug, leading to a greater dissolution compared to formulations with lower polymer content. There was a wider disparity between results for HPMCAS,

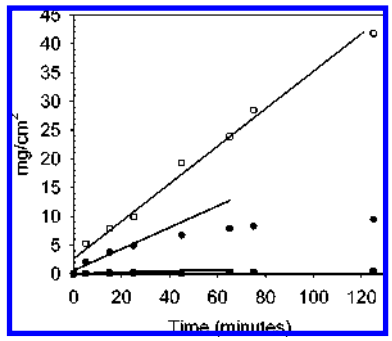


***Figure 8.*** Intrinsic dissolution profiles fit by linear regression for slope ($m$) and intercept ($b$): (□) ASD formulation A, Lot 2 (15 wt % AMG 517 in HPMCAS) with correction for load (fit: $m = 0.33$, $b = 2.62$, $R^2 = 0.98$); (■) AMG 517 free base (fit: $m = 0.0115$, $b = 0.013$, $R^2 = 0.96$); (●) amorphous AMG 517 (fit: $m = 0.187$, $b = 0.583$, $R^2 = 0.94$).

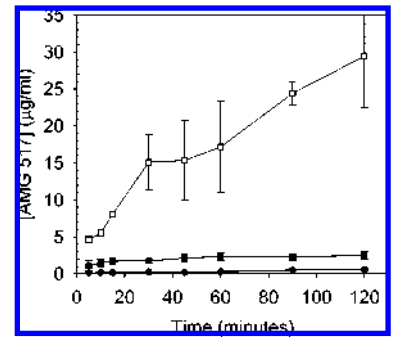


***Figure 9.*** Powder dissolution profiles in 100 mL phosphate buffer, pH 6.8 (12.5 mg drug): (●) AMG 517 free base, micronized; (■) ASD formulation A, Lot 2 (15 wt % AMG 517 in HPMCAS) without SDS surfactant; (□) ASD formulation A, Lot 2 (15 wt % AMG 517 in HPMCAS) blended with 5 wt % SDS surfactant.

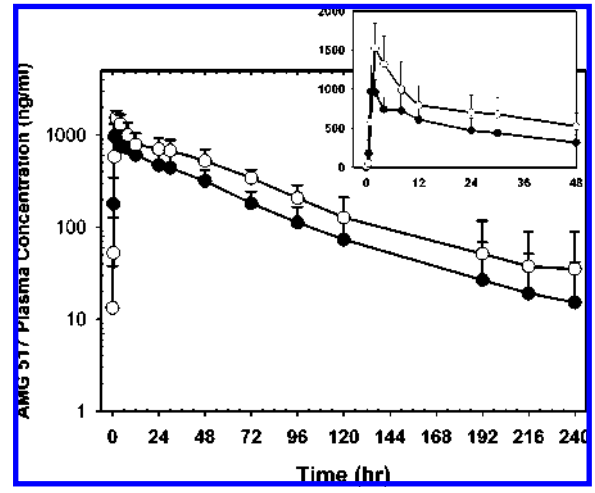


***Figure 10.*** AMG 517 plasma concentration vs time profiles following oral administration at 12.5 mg AMG 517/animal to male cynomolgus monkeys ($n = 6$) by (●) AMG 517 aqueous suspension in the 10% pluronic F108 Ora-Plus vehicle; (○) 15 wt % AMG 517 as an ASD in HPMCAS, blended with 5 wt % SDS and administered by capsule.

compared with HPMC. Analysis of the suspended solid by XRD, following dissolution, was not performed. Nevertheless, the profiles in Figure 7 indicate increasing drug concentrations over the study time.

To further understand the enhancement effect of the polymer on dissolution rate, the highest ranking ASD formulation (A, Lot 2), as shown in Figure 7, was selected for intrinsic dissolution studies. XRPD analysis of ASD disks produced by compression did not indicate devitrification of the samples. Figure 8 shows an initial 28-fold increase in intrinsic dissolution rate for the ASD sample compared to micronized AMG 517 crystalline material, and nearly a 2-fold initial increase compared with pure amorphous material. Results were based on determining the maximum slope of the dissolution curves for the crystalline and pure amorphous material, both of which were nonlinear beyond 15−25 min. In contrast, the load-corrected intrinsic dissolution rate (IDR) for the ASD formulation, measured to be 0.32 mg/cm$^2$-min, was constant over the 120 min experiment. The results indicate the ability of the hydrophilic polymeric carriers to significantly improve the in vitro dissolution, and thus possibly enhance the oral bioavailability of poorly soluble drugs. Dissolution rate in the ASD formulation was maintained at a higher relative rate throughout the experiment, whereas crystalline and pure amorphous rates diminished relatively rapidly.

Surfactants are well-known for the enhancement of wettability of drug products, and thus their frequent use in poorly soluble drug formulations. Poor wettability of ASD powders in aqueous medium was also observed from the various dissolution experiments. Poor wetting results in the ASD particles gelling into a mass rather than dispersing, slowing the dissolution rate. Therefore, to enhance the dissolution performance of ASD powders and powders in capsules, the effect of blending small quantities of a surfactant with the ASD powder was investigated in creating a prototype solid dosage form. Powder dissolution rate of micronized AMG 517 free base and ASD formulation A, Lot 2 (with and without 5 wt % sodium dodecyl sulfate) was carried out in 100 mL of pH 6.8 phosphate buffer as described. Overall resulting concentration of SDS in the dissolution medium was low (∼0.15 mM), well below the CMC of SDS (8.1 mM). As shown in Figure 9, the ASD powder with surfactant increased in vitro dissolution 12-fold over two hours, as compared to ASD powder without surfactant. Based on above studies, the 15 wt % AMG 517 in HPMCAS formulation (A, Lot 2, ∼83 mg) coblended with 5 wt % SDS (∼4.2 mg) was selected as a prototype solid dosage form for PK evaluation.

**Pharmacokinetic Study.** An oral bioavailability study was conducted in fasted cynomolgus monkeys ($n = 6$, crossover) with the highest ranking ASD formulation from in vitro dissolution and the OraPlus suspension (as control reference). The mean plasma concentration−time profiles of AMG 517 are plotted against time in Figure 10. Plasma concentrations

***Table 2.*** AMG 517 PK Parameters[a] and Summary Statistics Following Oral Administration at 12.5 mg/Animal to Male Cynomolgus Monkeys

| formulation | | $T_{max}$ (h) | $C_{max}$ (ng/mL) | $AUC_{0-inf}$ (ng h/mL) | $F_{rel}$ (%) |
|---|---|---|---|---|---|
| AMG 517 Ora-Plus suspenion | | 1.5 (1.0−2.0) | 1020 (189) | 40800 (10300) | 100 |
| | % CV | 36.5 | 18.5 | 25.3 | |
| AMG 517 ASD in capsule | | 2.0 (1.0−2.0) | 1480 (309) | 66600 (13200) | 163 |
| | % CV | 22.3 | 20.9 | 19.8 | |

[a] $T_{max}$: Time at which $C_{max}$ was observed, presented as a median. $C_{max}$: Maximum observed plasma concentration; $AUC_{0\text{-}inf}$ = Area under the plasma concentration−time curve from time zero to infinity. $F_{rel}$ = bioavailabilty, relative to the suspension. The range of $T_{max}$ and standard deviation of other parameters are presented in parentheses.

of AMG 517 in Figure 10 are on the log scale and extended to 10 days; for clarity, the plasma concentrations in the inset plot are on a linear scale and extended to 48 h. The error bars observed at each point as determined from 6 monkeys were also shown in both plots. The PK parameters ($C_{max}$, $T_{max}$, AUC, CV, etc.) of both formulations are summarized in Table 2.

Although both formulations showed rapid absorption with a short $T_{max}$ around 1−2 h, ASD formulation showed a significantly higher mean $C_{max}$ of 1480 ng/mL as compared to that (1020 ng/mL) of the OraPlus suspension (Table 2). Consistently, the area-under-the-curve (AUC) value of ASD formulation was approximately 163% of the OraPlus formulation, indicating a significant improvement in exposure. The variabilities of $C_{max}$ and AUC between the two formulations were comparable, as seen by the % CV in Table 2.

## Discussion

ASD powders of controlled size were prepared at low (15 wt %) and high (50 wt %) AMG 517 content by spray-drying HPMCAS and HPMC formulations with a laboratory-scale dryer. TGA weight loss of spray-dried particles indicated residual solvent levels of 2−3%, which was reduced by an additional vacuum drying operation. The film-forming tendency of HPMCAS and HPMC, enabling each to gel on removal of solvent, likely contributes to the observed corrugated morphology by SEM. It remains to be determined if the particles, as formed, would present considerable downstream challenges for efficient processing into a solid dosage form. Presently, the enlarged surface area of the particles and skinlike thickness of the polymer film could well have advantage in speeding the rate of swelling of the particles in use. In addition, removal of residual polymer solvent within the spray-drying process and secondary drying operation may likely be more efficient due to the shorter diffusional path lengths created by the corrugated structure.

ASD particles had a single $T_g$ observed at 106 and 98 °C in HPMCAS matrices containing 15 wt % and 50 wt % AMG 517, respectively, and at 117 and 107 °C in HPMC-E5 matrices containing 15 wt % and 50 wt % AMG 517. The single $T_g$ of the ASD matrices observed is significant, implying formation of solid dispersions; 15 wt % formulations did not devitrify on heating above the $T_g$. Actual glass transition temperature is dependent on the moisture content of the sample, and hence the storage conditions.[46,47] During MDSC experiments, water (or solvent) loss from the sample is expected. The modulated DSC method employed resolves via the reversible heat-flow plots (Figure 3) the $T_g$ transition from the nonreversible broad endothermic transition associated with moisture or solvent loss. The measured $T_g$ value is thus likely greater than the $T_g$ of the stored sample due to this loss of water or solvent during the DSC heating cycle. During method development, repeat scans through the $T_g$ region did not show great variability in the measured $T_g$, suggesting most water or solvent was removed on first pass. $T_g$ values thus presented are believed to be of samples devoid of most water and/or solvents. Secondary drying following spray-drying was an effective means for reducing moisture and solvent content of the ASD powders.

The $T_g$ of a mixture ($T_{g,mix}$) can be related to the $T_g$ and mass fraction of the individual components within the mixture by the Gordon−Taylor/Kelly−Beuche relationship,[48,49] which assumes the two components are miscible and the free volumes of the components are additive. Assuming no specific interaction between the two components in the mixture:

$$T_{g,mix} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2} \quad (1)$$

where $w_1$ and $w_2$ represent the weight fractions for the two components, $T_{g1}$ and $T_{g2}$ are their respective glass transition temperatures, and $K$ is the ratio of free volumes calculated using the relationship of Simha−Boyer:[50]

$$K = \frac{\rho_1 T_{g1}}{\rho_2 T_{g2}} \quad (2)$$

In eq 2, $\rho_1$ and $\rho_2$ are the respective densities of the amorphous materials.

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The experimental $T_g$ values can be compared with estimated values ($T_{g,mix}$) according to the relationship in eq 1. The $T_g$ of pure HPMCAS, HPMC-E5 and amorphous AMG 517 are approximately 119.5 °C, 154 °C,[51] and 103 °C, respectively. True density of HPMCAS is approximately 1.3 g/mL, as reported within the manufacture literature; true density of HPMC was reported as 1.19 g/mL.[47] True density of micronized AMG 517 (1.56 g/mL) was used as a surrogate for the true density of amorphous AMG 517. Varying the density value used in the Gordon−Taylor equation for AMG 517 by ±0.3 g/mL did not change the outcome of our analysis significantly.

Experimental (Table 1) and theoretical (eq 1) $T_{g,mix}$'s deviated significantly, with the experimental values falling below those computed by the available parameters by 11 and 14 °C for HPMCAS at 15% and 50% drug load, and 20 and 29 °C for HPMC at 15% and 50% drug load. Within the literature, negative deviations from ideality have been observed when studying HPMC and HPMCAS.[18,19] The negative deviation from ideality suggests the free volume in the homogeneous phase is larger than in the ideal mixture, and relative weakness in the heteromolecular interactions within the solid.[19] As described above, the presence of residual solvent and moisture could also depress the determined $T_g$ in these systems,[7] although for formulation A, Lot 2 the secondary dried material had a comparably low $T_g$ as well. Formulations grouped by polymer trended toward lower $T_g$ with increasing drug content, as expected by the theory, given the $T_g$ of pure amorphous AMG 517 was less than the $T_g$ of the polymers.

The physical stability evaluation of the HPMCAS ASD powders with a drug load of 15% and 50% indicated that AMG 517 remained at the amorphous state without detectable crystalline drug by XRPD at 40 °C/75% RH (open dish) for at least 6 months (evaluation limit). Hancock has asserted that molecular mobility becomes insignificant with respect to shelf life stability when amorphous materials are stored at 50 K below the $T_g$.[27,31,52] In the studied ASD formulations, $T_g$'s were all approximately 100 °C or greater, and would thus be expected to be storage stable at 40 °C. Maintaining the amorphous characteristics of the formulations for 6 months at elevated humidity indicates water uptake and $T_g$ suppression in the AMG 517 amorphous system were not significant enough to impact physical stability. The presence of the polymer vastly improved storage stability under these conditions over the pure amorphous drug, suggesting the possibility of either an antiplasticization effect of the polymer under humid conditions or specific drug−carrier interaction within the amorphous state of the dispersion.[27,53] Certainly, dispersing the drug in a carrier also contributed to improving storage stability.

Polymer type and concentration have been known to affect the dissolution rate and oral bioavailability of lipophilic drugs.[54] Although there was a clear trend with high and low drug load for each polymer, a polymer effect in the dissolution profile was not as apparent. ASD samples containing HPMCAS showed superior dissolution profile with higher supersaturation compared to HPMC-E5 at 15%, but the trend was reversed for 50%.

At low drug load (15%), enhanced dissolution rate for HPMCAS over HPMC-E5 may result from better wetting and water uptake of HPMCAS, on account of the higher number of hydrophilic carboxyl groups contained in the HPMCAS polymer. However, at higher drug loading (50%), the diminished dissolution profile for both polymers may not only be caused by the reduced polymer load, and therefore less water uptake, but may also be impacted by the reduced drug−polymer compatibility. Poorly compatible components, lacking specific drug−polymer interactions or miscibility, may phase demix at the surface of the solid dispersion during dissolution, potentially lowering dissolution rate. Observation of a constant intrinsic dissolution rate over 2 h for the lead ASD powder, 15% AMG 517 in HPMCAS-MF, suggests it had adequate drug−polymer compatibility; additional intrinsic dissolution data is required to assess the corresponding 50% ASD formulation for potential demixing.

Yu has recently proposed classifying compounds having intrinisic dissolution rates (IDRs) exceeding 0.1 mg/cm$^2$/min as "high IDR" for purposes using IDR, rather than solubility, to classify drugs within the biopharmaceutics classification system (BCS).[55,56] Interestingly, the load-adjusted IDR obtained for the 15% AMG 517 in HPMCAS formulation, at 0.32 mg/cm$^2$/min, effectively transitions AMG 517 from a "low IDR" compound as a crystal (0.011 mg/cm$^2$/min) to a "high IDR" compound as an amorphous solid dispersion.

A comparative pharmacokinetic evaluation of the lead ASD formulation as a blend in a capsule (15 wt % AMG 517 in HPMCAS + 5% SDS) and an OraPlus suspension (as control reference) was conducted in cynomolgus monkeys ($n = 6$, crossover) at an AMG 517 dose of 12.5 mg. Such a dose was midrange of the 1−25 mg doses recently tested in humans.[43] PK results suggest that the ASD formulation

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showed 163% of AUC, 145% $C_{max}$ and comparable $T_{max}$ (~1−2 h) as compared to the OraPlus formulation. It is worth emphasizing that OraPlus suspension was the best performer with the highest exposure and $C_{max}$ values in previous preclinical in vivo evaluations and a phase I clinical trial. The performance of the OraPlus suspension was attributed to formation of a more highly soluble cocrystal between AMG 517 and sorbic acid in the suspension.[42] However, the OraPlus suspension was not feasible for commercialization due to a low drug load (~0.75 mg/mL AMG 517) and large volume required for oral administration. Had the comparator in the monkey PK study been strictly micronized AMG 517 free base, the relative performance of the ASD formulation would have been even greater.

Collectively, in vitro dissolution and a comparative PK evaluation against an OraPlus suspension suggests rapid dissolution and a more complete absorption of AMG 517 via the amorphous solid dispersion approach. The ASD approach thus significantly improved oral bioavailability for a poorly soluble VR1 antagonist, while stabilizing the amorphous form of the molecule. Preparing ASD materials at laboratory scale is a material-sparing operation which may be best applied at the discovery-development interface, in situations when low exposure is observed during high-dose toxicology evaluation due to a compound's poor solubility. In this setting, particle yield and quality of powders produced by spray-drying are adequate for rapidly evaluating the ASD approach.

## Abbreviations Used

ASD, amorphous solid dispersion; SD, spray-drying; SDS, sodium dodecyl sulfate; slpm, standard liters per minute; slph, standard liters per hour; XRPD, X-ray powder diffraction.

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