S. MAJUMDAR & CO.

PATENT & TRADEMARK ATTORNEYS

Undertakings : Intellectual Property Laws,
Patents, Trademarks, Designs, Copyrights,
Licencing, Investigations, Litigations
DOMESTIC AND INTERNATIONAL

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The Controller of Patents
The Patent Office
Delhi

June 6, 2013

D.No. 10058.
06/06/13

Dear Sir,

Re: Opposition under Section 25(1) against
Patent Application No. No. 9322/DELNP/2007
Applicant : **NOVARTIS AG**
Opponent: **NATCO PHARMA LTD.**
Our Ref :PII507

We submit herewith a Representation with Exhibit A1 to Exhibit A8 under Section 25(1) in duplicate.

Please take the documents on record and take necessary action. You are also requested to kindly acknowledge the receipt of the documents.

The Power of Attorney in our favour will follow.

Please grant a hearing in due course.

GOVT. OF INDIA PATENT OFFICE
D No. 10058
06 JUN 2013
BAUDHIK SAMPADA BHAWAN
NEW DELHI

Yours faithfully

Ganguli

Dr. Sanchita Ganguli
Of S. Majumdar & Co.
Opponent's Agent

Enclosures:
Representation with Exhibit A1 to Exhibit A8 under Section 25(1) in duplicate.

BEFORE THE CONTROLLER OF PATENTS,

PATENT OFFICE ,NEW DELHI

PRE-GRANT OPPOSITION UNDER SECTION 25(1) AGAINST APPLICATION PATENT APPLICATION NO. 9322/DELNP/2007 DATED December 3, 2007

NOVARTIS AG, of Lichtstrasse 35 CH-4056 Basel, Switzerland.

.....**Applicant**

- VS -

NATCO PHARMA LTD., of Natco House, Road No. 2, Banjara Hills, Hyderabad 500 033, India

..... **Opponent**

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EXHIBIT "A3": WO2004/005281 (D1) entitled "Inhibitors of Tyrosine Kinases" published on January 15, 2004	**70-149**
EXHIBIT "A4": Stephen M. Berger et al. Journal of Pharmaceutical Science,1977, 66,1-19 (D2) published in January 1977	**150-168**
EXHIBIT A5": Characterisation of Salts of Drug Substances, *D. Giron* Chemical and Analytical Development, Novartis Pharma, Basel, Switzerland, *Journal of Thermal Analysis and Calorimetry, Vol. 73 (2003) 441.457* (D3)	**169-185**

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Dr. Sanchita Ganguli
S. Majumdar & Co.
Opponent's Agent

BEFORE THE CONTROLLER OF PATENTS

PATENT OFFICE,

NEW DELHI.

In the matter of The Patents Act, 1970 *as amended by* The Patents (Amendment) Act 2005,

And

In the matter of The Patents Rules, 2003 as amended by the Patents Amendment Rules 2006

And

IN THE MATTER of Patent Application No. **9322/DELNP/2007** dated December 3, 2007 by Novartis Ag, Lichtstrasse 35 CH-4056 Basel, Switzerland.

..... Applicant

And

IN THE MATTER of opposition thereto by thereto by NATCO PHARMA Limited., NATCO HOUSE, Road No. 2, Banjara Hills, Hyderabad 500 033, India

..... Opponent

REPRESENTATION UNDER SECTION 25(1)

1. We, Natco Pharma Limited, having our registered office at NATCO HOUSE, Road No. 2, Banjara Hills, Hyderabad 500033, India (hereinafter called 'opponent') make the following opposition under Section 25(1) of the Act against grant of the patent application indicated in the cause title.

2. OPPONENT'S BUSINESS AND ACTIVITIES

The opponent, Natco Pharma Limited is a Company incorporated under the Companies Act, 1956 and having its principal office at NATCO HOUSE, Road No. 2, Banjara Hills, Hyderabad 500033, India. The opponent has access to the latest technologies relating to manufacture of the drugs/medicines. The opponent is the leading manufacturer of medicines in this country and the opponent's products are sold under different brands and the opponent enjoys considerable goodwill and reputation. The opponent is a very well known company and has been operating in this country for several decades. The opponent is also engaged in the research and development of medicines and pharmaceutical products and preparations.

3. LOCUS STANDI

The application under opposition relates to an alleged invention in the field of medicinal products. Though the locus standi of the opponent is not an issue for pre grant opposition, it is stated that the Opponent being engaged in the research and development as well as in the manufacture of drugs/medicinal compositions for many years has thus, a real, substantial and existing interest in this business and consequently in opposing the grant of Patent Application No. 9322/DELNP/2007.

4. GROUNDS OF OPPOSITION

The application is opposed on the following grounds:

a) **Section 25(1)(b) – Anticipation/ lack of novelty**

that the invention so far as claimed in any claim of the complete specification has been published before the priority date of the claim,

i) in any specification filed in pursuance of an application for a patent made in India on or after the 1st day of January, 1912; or

ii) in India or elsewhere, in any other document.

b) **Section 25(1) (d)-Prior Public Knowledge/Prior Public Use**

that the invention so far as claimed in any claim of the complete specification was publicly known or publicly used in India before the priority date of that claim;

c) **Section 25(1) (e)-Obviousness/lack of inventive step**

that 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 before the priority date of the claim,

in any specification filed in pursuance of an application for a patent made in India on or after the 1st day of January, 1912; or

in India or elsewhere, in any other document;

or having regard to what was used in India before the priority date of the applicant's claim.

d) **Section 25(1) (f)-Not an invention**

that the subject of any claim of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act;

e) **Section 25(1) (g)- Insufficiency**

that the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed;

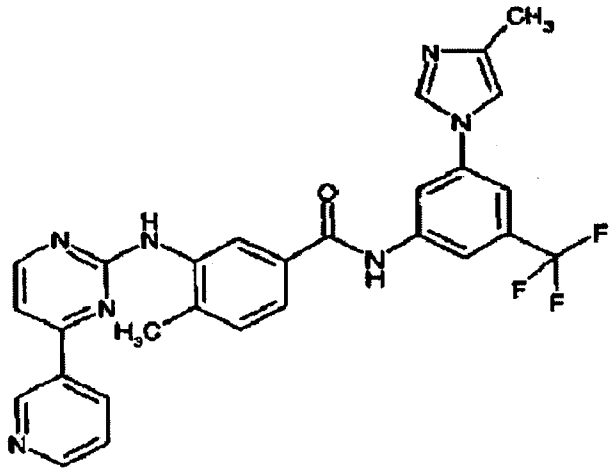
f) Section 25(1) (h)-Failure to disclose information or furnishing false information relating to foreign filing

that the applicant has failed to disclose to the Controller the information required by Section 8 or has furnished the information which in any material particular was false to his knowledge.

5. Analysis of the specification of 9322/DELNP/2007

5.1 The impugned patent application No. 9322/DELNP/2007 entitled "SALTS OF 4-METHYL-N-[3-(4-METHYL-IMIDAZOL-1-YL)-5-TRIFLUOROMETHYL-PHENYL]-3-(4-PYRIDIN-3-YL-PYRIMIDIN-2-YLAMINO)-BENZAMIDE" was nationalised on December 03, 2007 from the international application PCT/US2006/027878 dated July 18, 2006 and claims priority from US application 60/701,406 dated July 20, 2005 and 60/716213 dated September 12, 2005. The patent application was published on June 26, 2008, but has not yet been granted according to the status available at the website of the Indian Patent Office. Accordingly, the present written statement for pre-grant opposition is filed.

5.2 The impugned patent application annexed herein as **"EXHIBIT A1"** relates to salts e.g. salts of 4-methyl-n-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide of formula:



5.3 The said salts have role as a protein kinase inhibitor and are useful in therapy for diseases which respond to inhibition of protein kinase activity. The application is further directed to the process of preparation of such salts, pharmaceutical composition and method of treating a disease which responds to an inhibitor of protein kinase activity.

6. Prosecution History

6.1 The opponent caused to download the copies of all office actions and all examination reports issued by the Patent Office and responses filed by the applicant. The prosecution history downloaded was downloaded from the official website of the Indian Patent Office. First Examination Report is annexed herein as "**EXHIBIT A2**". The examiner has raised objections regarding Section 3 (i), 3(d) and 3 (e) and for unity of invention and insufficiency of the description. Further there is objection that the impugned invention lacks inventive step in light of WO 2004/005281 as in example 85, 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyrimidine-2-yl-amino)-benzamide is disclosed, therefore the examiner stated that the monohydrochloride salt, the monophosphate and the diphosphate salt is not inventive over WO 2004/005281.

7. Brief Review of the Applicant's Claims

7.1 The impugned patent application contains a statement of 9 claims. claims 1, 2, 3, 4, 5, 6 and 8 are independent and the claims 7 is dependent on Claim 6, claim 9 is dependent on claim 8. The claims are produced verbatim below for ready reference.

WHAT IS CLAIMED IS:

1. A salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide, which is 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate.

2. A salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide, which is a monophosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

3. A salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide, which is a diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

4. A method of preparing a salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide comprising the step of: reacting 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base with an acid of formula HB in a solvent, wherein the acid is phosphoric acid.

5. A method of preparing 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate comprising the steps of:

(a) combining 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and hydrochloric acid in methanol under a nitrogen atmosphere;

(b) heating the reaction mixture to a temperature ranging from about 42-50°C;

(c) stirring the reaction mixture;

(d) filtering the reaction mixture while maintaining the temperature above 40°C to obtain a clear solution;

(e) cooling the clear solution to about 30°C while stirring under nitrogen atmosphere;

(f) seeding the solution;

(g) cooling the seeded solution to about 23°C;

(h) stirring the solution to obtain a suspension;

(i) cooling the suspension to about -10°C;

(j) stirring the suspension;

(k) filtering solids;

(l) rinsing solids with cold methanol; and

(m) drying the solids at about 50-55°C and 10-20 torr to obtain 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate salt.

6. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of a salt according to any of claims 1 to 3; and

(b) at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient.

7. The pharmaceutical composition of Claim 6, wherein the salt is 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate.

8. A method of treating a disease which responds to an inhibition of protein kinase activity comprising the step of administering to a subject in need of such treatment a therapeutically effective amount of a salt according to any one of claims 1 to 3.

9. The method of Claim 8, wherein the salt is 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate.

7.2 The present opposition is framed on claims set out above. In the event the claims are amended and the present representation does not automatically address the subject matter of the amended claims, the opponent craves leave to file a fresh or supplementary representation, if the amendments warrant so.

7.3 The opponent has examined and carefully considered the complete specification of the application under opposition and wishes to draw the attention of the Ld. Controller to the most salient features therein under the grounds of opposition discussed hereinafter.

7.4 Having regard to the aforesaid discussions in relation to the alleged invention of the applicant the opponent now proceeds to deal with the various grounds of opposition.

8. Documents relied on in the present opposition

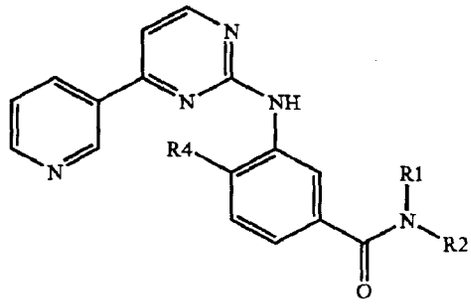
- **Exhibit A3**: WO2004/005281 (referred hereinafter as D1) entitled "Inhibitors of Tyrosine Kinases" published on January 15, 2004 and annexed herewith.
- **Exhibit A4**: Stephen M. Berger et al. Journal of Pharmaceutical Science,1977, 66,1-19 (referred hereinafter as D2) published in January 1977 and annexed herewith.
- **Exhibit A5**: Characterisation of Salts of Drug Substances, *D. Giron* Chemical and Analytical Development, Novartis Pharma, Basel, Switzerland, *Journal of Thermal Analysis and Calorimetry, Vol. 73 (2003) 441.457* (referred hereinafter as D3) and annexed herewith.
- **Exhibit A6**: An Encyclopedia of Chemicals, Drugs, and Biologicals, 14th ed. New Jersey: Merck, 2006 (referred hereinafter as D4) and annexed herewith.

9. Anticipation/ Lack of Novelty [Section 25(1)(b)]

9.1 It is stated that claims 1, 2 and 3 of the impugned invention claims **monohydrochloride monohydrate, monophosphate salt and diphosphate salt of** 4-methyl-N-(3-(4-methyl-imidazol-l-yl)-5-trifliiorornethyl-phenylJ-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide respectively.

9.2 The opponent states that the said claims are anticipated and lack novelty in view of the disclosures of D1, which will be evident from the following paragraphs.

9.3 The opponent states that WO2004/005281 (D1) relates to the compound of formula:



wherein R1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R2 represents hydrogen, lower alkyl, optionally substituted by one or more identical or different radicals R3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted;

and R3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted;

or wherein R1 and R2 together represent alkylene with four, five or six carbon atoms optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, oxo, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms; oxaalkylene with one oxygen and three or four carbon atoms; or azaalkylene with one nitrogen and three or four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl,

lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, carboxy, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R4 represents hydrogen, lower alkyl, or halogen;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

9.4 It is stated that **example 92 of D1** discloses the process of preparation of the compound **4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-N-[5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide.**

9.5 It is stated on Page 7 of D1 specifically mentions that salts are especially the pharmaceutically acceptable salts of compounds of Formula I. Further on Page 8 Para 1 it is mentioned that:

"Such salts are formed, for example, as acid addition salts, preferably with organic or inorganic salts, from compounds of formula I with a basic nitrogen atom, especially the pharmaceutically acceptable salts. Suitable inorganic acids are for example, halogen acids such as ***hydrochloric acid****, sulfuric acid, or* ***phosphoric acid****. Suitable organic acid are, for example carboxylic, phosphonic, sulfonic or sulfamic acids, for example acetic acid, propionic acid, octanoic acid, decanoic acid, dodecanoic acid, glycolic acid, lactic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, mallic acid, tartaric acid, citric acid, amino acid, such as glutamic acid or aspartic acid, maleic acid....."*

9.6 Further it is stated on Page 8 and 9 of D1 that: "Owing to the close relationship between the novel compounds in free form and in the form of their salts...hereinbefore and hereinafter any reference to the free compounds should be understood as including the corresponding salts, where appropriate and expedient." Notwithstanding the Applicant's attempt to distinguish between "embracing" and specific disclosure, these disclosures are sufficient to make out a finding of anticipation.

9.7 It is stated on Page 31 of D1 that:

> **The compounds of formula I, including their salts, are also obtainable in the form of hydrates, or their crystals can include for example the solvent used for crystallization (present as solvates).**

9.8 Further D1 discloses (Page 32, Para 4) " the present invention relates especially to pharmaceutical composition that comprise a compound of formula I, a tautomer, a N-oxide or a pharmaceutically acceptable salt, or a hydrate or solvate thereof, and at least one pharmaceutically acceptable carrier".

9.9 It is stated that nothing is stated in the application about any problem in making the salts. The impugned application also does not state that there was any problem in making the salts by usual techniques. Therefore, all the salts are already disclosed and claimed in D1. Therefore, the salts of the impugned invention as claimed in claims 1-3 are clearly anticipated by D1 and ought to be rejected in toto.

9.10 **Claim 4** claims a method of preparing a salt of 4-methyl-N-[3-(4-methyl-imidazol-l -yl)-5-trifluoromethyl-phenyl]-3-(4-pyriddn-3-yl-pyrimidin-2-ylamino)-benzamide comprising the step of: reacting 4-methyl-N-[3-(4-mcthyl-iinidazol-l-yl)-5-trifluororneihyl-phenylJ-3-(4-pyridin'3-yl-pyrimidin-2-ylamino)-benzamide free base with an acid of formula HB in a solvent, wherein the acid is phosphoric acid. It is stated that the process claimed in claim 4 of the impugned invention is the method generally known in the art and is not novel.

9.11 It is stated that D1 on Page 31 specifically states that:

"Salts of a compound of formula I with a salt forming group may be prepared in manner known per se. Acid addition salts of compounds of formula I may thus be obtained by treatment with an acid or with a suitable anion exchange reagent"

It is stated that claimed process appears to follow the standard process of making a salt by treating the free base with hydrochloric acid. This technique is well known in the art. Therefore Claim 4 adds nothing to the state of art and ought to be rejected in light of D1.

9.12 **Claim 5** claim a method of preparing 4-methyl-N-[3-(4-methyl-imidazol-l-yl)-5-trifluoromethyl-phenyl]-3-(4-pyriden-3-yl-pyrirnidin-2-ylamino)-benzamide monohydrochloride monohydrate comprising the steps of:

(a) combining 4-methyl-N-[3-(4-methyl-imidazol-l-yl)-5-trifluoromethyl-phetiyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and hydrochloric acid in mcthanol under a nitrogen atmosphere;

(b) heating the reaction mixture to a temperature ranging from about 42-50°C;

(c) stirring the reaction mixture;

(d) filtering the reaction mixture while maintaining the temperature above 40°C to obtain a clear solution;

(e) cooling the clear solution to about 30°C while stirring under nitrogen atmosphere;

(f) seeding the solution;

(g) cooling the seeded solution to about 230C;

(h) stirring the solution to obtain a suspension;

(i) cooling the suspension to about-10°C;

(j) stirring the suspension;

(k) filtering solids;

(1) rinsing solids with cold-methanol; and .

(m) drying the solids_at about 50-55°C and 10-20 torr to obtain 4-methyl-N-[3-(4-methyl-irnidazol-l-yl)-5-trifluoroinethyl-phenyJ]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate salt.7.10

9.13 It is stated that claim 5 of the impugned application is the method generally known in the art. It is further stated that claimed process appears to follow the standard process of making a salt by treating the free base with hydrochloric acid followed by recrystallization with seeding. Accordingly claim 5 is not novel and is well known in the state of art.

9.14 It is stated that on Page 30-31 of D1 it is stated that:

"All process steps described here can be carried out under known reaction conditions, preferably under those specifically mentioned, in the absence of or usually in the presence of solvents or diluents, preferably such as are inert to the reagents used and able to dissolve these, in the absence or presence of catalysts, condensing agents or neutralisiing agents, for example ion exchangers, typically cation exchangers, for example in the H^+ form, depending on the type of reaction and/or reactants at reduced, normal, or elevated temperature, for example in the range from -100°C to about 190°C, preferably from about -80°C to about 150°C, for example at -80 to -60°C, at room temperature, at - 20 to 40°C or at the boiling point of the solvent used, under atmospheric pressure or in a closed vessel, where appropriate under pressure, and/or in an inert atmosphere, for example under argon or nitrogen."

"The compounds of formula I, including their salts, are also obtainable in the form of hydrates, or their crystals can include for example the solvent used for crystallization (present as solvates)."

9.15 It is stated that claimed process appears to follow the standard process of making a salt by treating the free base with hydrochloric acid. This technique is well known in the art. Therefore Claim 5 adds nothing to the state of art and ought to be rejected.

9.16 **Claim 6** claims a pharmaceutical composition comprising:

(a) a therapeutically effective amount of a salt according to. any of claims 1 to 3; and

(b) at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient.

Claim 7 claims a pharmaceutical composition of Claim 6, wherein the salt is 4-methyl-N-[3-(4-methyl-imidazol-l-yl)-5-trifluoromethyl-phenyl3-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate.

9.17 It is stated that composition as claimed in claims 6 and 7 is not novel in light of D1. It is stated on Page 32 of D1 "The present invention relates also to pharmaceutical compositions that comprise a compound of formula I or a N-oxide thereof as active ingredient and that can be used especially in the treatment of the diseases mentioned at the beginning. Compositions for enteral administration, such as nasal, buccal, rectal or, especially, oral administration, and for parenteral administration, such as intravenous, intramuscular or subcutaneous administration, to warm-blooded animals, especially humans, are especially preferred. The compositions comprise the active ingredient alone or, preferably, together with a pharmaceutically acceptable carrier." Therefore Claim 6 and 7 of the impugned invention is not novel and ought to be rejected.

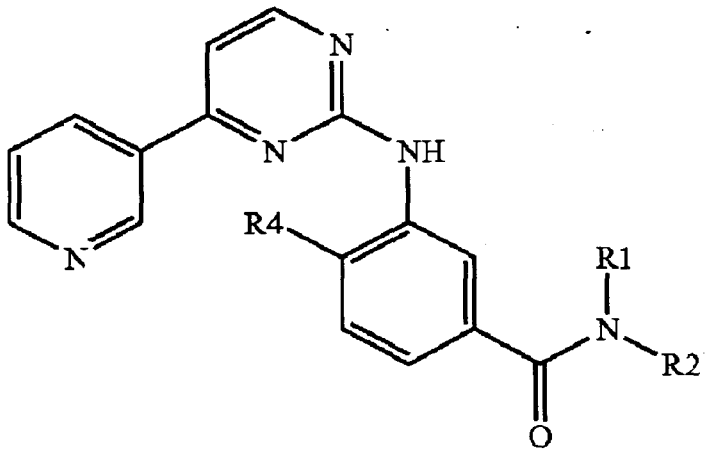
9.18 **Claim 8** claims a method of treating a disease which responds to an inhibition of protein kinase activity comprising the step of administering to a subject in need of such treatment a therapeutically effective amount of a salt according to any one of claims 1 to 3. Further **Claim 9** claims a method of Claim 8, wherein the salt is 4-methyl-N-[3-(4-methyl-imidazoI-l-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrinuoln-2-ylamino)-benzainide monohydrochloride monohydrate. Claim 8 and 9 are "**use**" type claims in that are dependent on claim 1. Use type claims are not allowable in Indian under Section 2(1)(j). Further since Claim 1 is not novel in light of D1 claims 8 and 9 shall also stand anticipated as they do not add any inventive feature to the invention, the said use in various cancers being a protein kinase inhibitor is known or similar compounds as already mentioned above.

10. Obviousness/Lack of Inventive Step [Section 25(1) (e)]

10.1 **Claim 1** claims 4-methyl-N-(3-(4-methyl-imidazol-l-yl)-5-trifliiorornethyl-phenylJ-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide **monohydrochloride monohydrate.** **Claim 2** claims a **monophosphate salt** of 4-methyl N-(3-(4-methyl-imidazol-1-yl)-5-tiifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

Claim 3 claims a **diphosphate salt** of 4-methyl-N-[3-(4-methyl-imidazol-l-yl)-5-trifluoroinethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylatnino)benzamide.

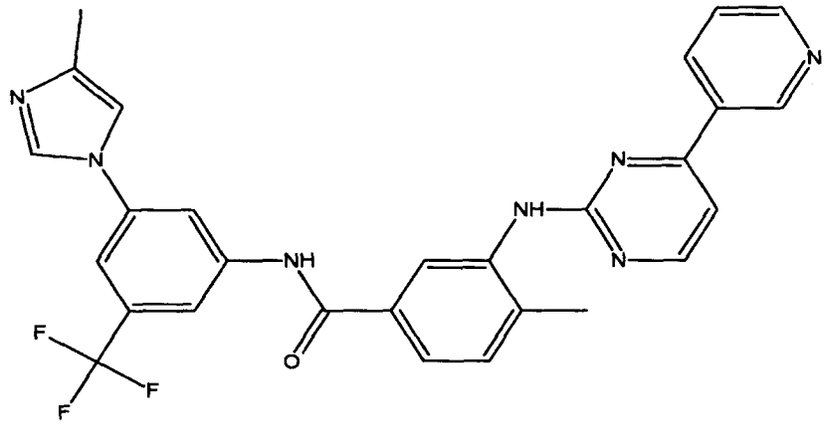
10.2 The opponent states that WO2004/005281 (**D1**) relates to the compound of formula:



10.3 It is stated that **example 92 of D1** discloses the process of preparation of the compound **4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-N-[5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide.** The process is

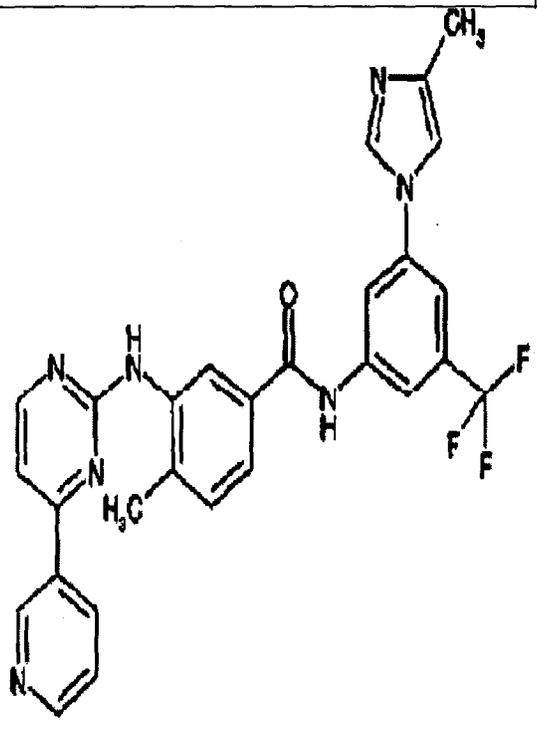
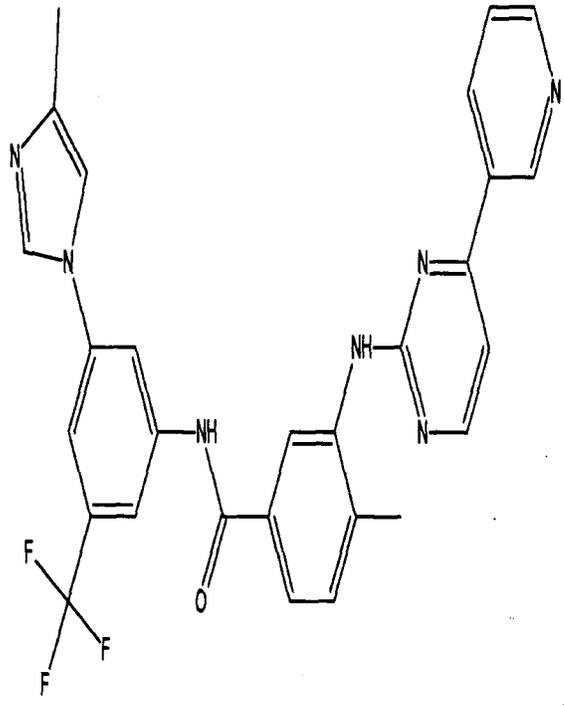
Diethylcyanophosphonate (Aldrich, Buchs, Switzerland; 0.50 mL, 3.0 mmol) is added to a stirred mixture of 3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid (438 mg, 1.5 mmol), 4-[(4-methyl-1-piperazinyl)methyl]benzeneamine (308 mg, 1.5 mmol) and triethylamine (840 .mu.L, 3.0 mmol) in 10 mL N,N-dimethylformamide at 10.degree. C. After stirring for 12 hours at 60.degree. C., the mixture is treated with an aqueous solution of sodium hydrogen carbonate and extracted three times with ethyl acetate. The combined extracts are washed with water, and the solvent is evaporated off under reduced pressure to give a residue. The residue is resuspended in water and filtered to afford the crude product which is recrystallised from tetrahydrofuran-ethyl acetate to give N-[3-[[4-(3-Pyridinyl)-2-pyrimidinyl]amino]-N-[(4-methyl-1-piperazinyl)-m- ethyl]benzamide as a crystalline solid, m.p. 220 224° C.

10.4 This compound is structurally represented as:



4-methyl-3[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-N-[5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide

Table 1: Illustration of Similarities between the structures of claim 1 of the impugned invention and Example 92 of D1

Structure as taught by impugned invention	Structure as taught in Example 92 of D1	Comments
		Impugned invention teaches the monohydrochloride monohydrate form of the compound taught in D1 in claim 1 Monophosphate salt of the compound of D1 in claim 2 Diphosphate salt of the compound of D1 in claim 3

10.5 It is stated that D1 discloses the same compound with the same use as the compounds of the present application i.e. a protein kinase inhibitor. In claim 1 of D1, pharmaceutically acceptable salts are also claimed. On page 3 of the description, it is clearly mentioned that the salts claimed in D1 refer to the pharmaceutically acceptable salts of the compound of D1. Mentioned on the said page are amongst others, hydrochloride acid and phosphoric acid. It is prudent to mention that among inorganic acid to be used to form salts with the compound of D1 only three specific acids are mentioned. The impugned patent application uses two of the said three salts namely hydrochloric acid and phosphoric acid salts. Thus even the applicant did not have many options to choose from so as to verify their results. D1 teaches compound of the impugned application with salts and out of three the applicant chose two and filed the present application. It is stated that that at column 6 lines 37 to 44 it is clearly mentioned that whenever the compound is mentioned the salts are also meant to be included. Thus D1 already describes the salts as well as its working. It is also mentioned at col 20 lines 61-65 that the salts of the compound of formula 1 can be prepared by treatment with an acid or with suitable anion exchange agent. Hence even preparation of the salt is taught. Applicant does not mention any problem which this invention tends to solve.

10.6 The opponent states that Pharmaceutical Salts, *Journal of Pharmaceutical Sciences,* January 1977, Volume 66 Number 1, Berge et al., (herein referred to as **D2**) relates to preparation of salt which is simply an acid-base reaction and every such compound that exhibits acid or base characteristics can participate in salt formation. Further it is stated that the chemical, biological, physical and economic characteristics of medicinal agents can be manipulated and often optimized by conversion to a salt form. D2 lists the commercially marketed FDA approved and non-approved salt forms as shown in Table 1. It is further stated that Hydrochloride, sulfate and phosphate salts are three of the most commonly used salts of organic bases used conventionally for therapeutic applications. Accordingly there is motivation for the person skilled in the art from the teachings of D2 to develop the pharmaceutically acceptable salts of a known compound.

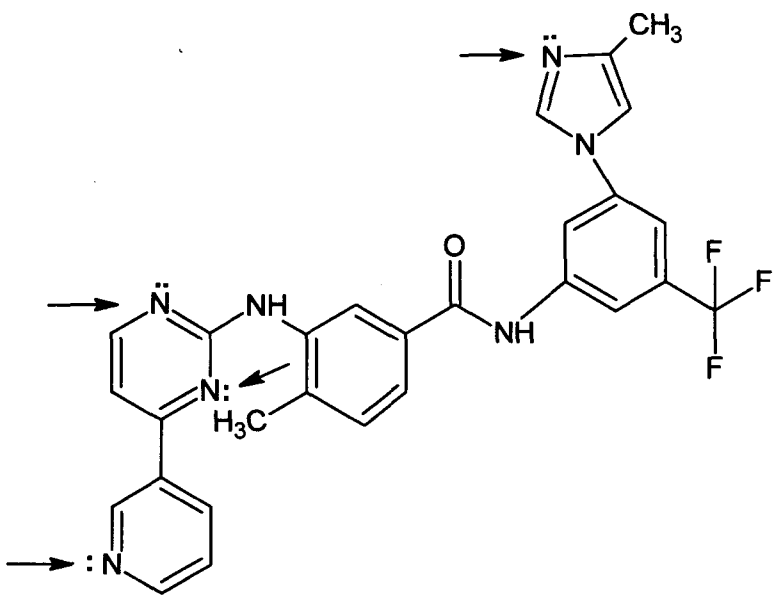
10.7 The opponent states that the aforesaid line of reasoning is strengthened given the fact that the genus of FDA approved anions and non-approved anions known at the priority date of the alleged invention which were tabulated in D2 was small and limited. It is therefore stated that it would have been a matter of routine testing for a person skilled in the art to prepare salts of the freebase compound known from D1 using the anions listed in D2 to arrive at the best salt form. This constitutes mere trial and error experimentation devoid of any application of inventive ingenuity. The opponent states that it is evident from the document D2 that salt formation is known to influence a number of physicochemical properties of the parent compound including dissolution rate, solubility, stability and hygroscopicity. It is stated that D2 page 9, column 2 teaches that *penicillin G potassium is much less hygroscopic than penicillin G sodium and is the preferred form for marketing in dry state*. It is therefore quiet evident that choosing an appropriate salt and further modifying its physicochemical properties to make it the suitable candidate is a matter of trial and error. The petitioner understands that there may be unpredictability in the findings that whether the salt form would meet up with all the desired properties, but it is known jurisprudence that in the assessment of inventive step it is not necessary to establish that the success of an envisaged solution of a technical problem was predictable with certainty. On the contrary, in order to demonstrate that a skilled person would have combined the technical teaching of two documents, it is sufficient to establish that he would have done so with a reasonable expectation of success. Moreover it would be obvious for skilled person to try and find/verify that salt would be an appropriate choice in view of D1 read with D2.

10.8 The opponent states that Characterisation of Salts of Drug Substances, *D. Giron* Chemical and Analytical Development, Novartis Pharma, Basel, Switzerland, *Journal of Thermal Analysis and Calorimetry, Vol. 73 (2003) 441.457* (herein referred to as **D3**) relates to a study wherein it is stated that the properties of the solid-state of drug substances are critical factors that determine the choice of an appropriate salt form for the development of the pharmaceutical formulation. The most relevant properties may affect the therapeutic efficacy, toxicity, bioavailability, pharmaceutical processing and stability.

It is also stated that an estimated half of all the drugs molecules used in medicinal therapy are administrated as salts, therefore the choice of an appropriate salt form is an important task of the development. D3 is the study undertaken by the assignee of the impugned invention, he is well aware that the drug molecules i.e. the free base are used as salts in the medical therapy, further with the teachings of D1 deriving at the present invention is merely a routine laboratory experimentation with verification of results.

10.9 Starting from D1 a skilled person would try a pharmaceutically acceptable salt like hydrochloride acid or phosphate salt of the compound of example 92 from the motivation provided in D2 and D3 and would come to the solution of claims 1-3 without any inventive skill and ingenuity of thought. Accordingly the monohydrochloride monohydrate salt, the monophosphate and the diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyrimidine-2-yl-amino)-benzamide i.e. claims 1, 2 and 3 are not inventive over the prior art D1 read with D2 and D3.

10.10 The opponent states that An Encyclopedia of Chemicals, Drugs, and Biologicals, 14th ed. New Jersey: Merck, 2006. (herein referred to as **D4**) is a compilation of chemicals, drugs and biologicals with over 10,000 monographs on single substances or groups of related compounds. It also includes an appendix with monographs on organic name reactions. According to D4 formation of hydrates is an inherent property of certain salts like hydrochlorides provided at Pages 95, 373, 397, 1444, 1577, 1706, 1742, 1814. According to the structure of the impugned application there are at least four basic N-atoms capable of salt formation.



Thus, it is clear that the free base can form multiple addition salts like diphosphate etc depending on mole ratio of the corresponding mineral acid used. Therefore discovering the existence of specific monohydrochloride monohydrate cannot be regarded to as having an inventive merit over D1. Therefore D1 read along with D2 or D3 and D4 provides teachings for the person skilled in the art to arrive at monohydrochloride monohydrate salt, the monophosphate and the diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyrimidine-2-yl-amino)-benzamide. Accordingly claims 1-3 have no inventive merit and ought to be rejected on this ground alone.

10.11 **Claim 4** claims a method of preparing a salt of 4-methyl-N-[3-(4-methyl-imidazol-l -yl)-5-trifluoromethyl-phenyl]-3-(4-pyriddn-3-yl-pyrimidin-2-ylamino)-benzamide comprising the step of: reacting 4-methyl-N-[3-(4-mcthyl-iinidazol-l-yl)-5-trifluororneihyl-phenylJ-3-(4-pyridin'3-yl-pyrimidin-2-ylamino)-benzamide free base with an acid of formula HB in a solvent, wherein the acid is phosphoric acid. It is stated that the process claimed in claim 4 of the impugned invention is the method generally known in the art and has no inventive step. It is stated that D1 on Page 31 specifically states that:

Salts of a compound of formula I with a salt-forming group may be prepared in a manner known per se. Acid addition salts of compounds of formula I may thus be obtained by treatment with an acid or with a suitable anion exchange reagent.

10.12 It is stated that claimed process appears to follow the standard process of making a salt by treating the free base with hydrochloric acid. This technique is well known in the art. A person skilled in the art is aware of the compound of Example 92 from D1 will try to find an alternative compound of acceptable salts and in that venture will react the free base with acid to obtain an alternative salt. D2 lists the commercially marketed FDA approved and non-approved salt forms as shown in Table 1 wherein Hydrochloride, sulfate and phosphate salts are three of the most commonly used salts of organic bases used conventionally for therapeutic applications. Accordingly there is motivation for the person skilled in the art from the teachings of D1 read with D2 or D3 and D4 to develop the pharmaceutically acceptable salts of a known compound. Therefore Claim 4 is obvious to person skilled in the art and adds nothing to the state of art and ought to be rejected.

10.13 **Claim 5** claim a method of preparing 4-methyl-N-[3-(4-methyl-imidazol-l-yl)-5-trifluoromethyl-phenyl]-3-(4-pyriden-3-yl-pyrirnidin-2-ylamino)-benzamide monohydrochloride monohydrate comprising the steps of:

(a) combining 4-methyl-N-[3-(4-methyl-imidazol-l-yl)-5-trifluoromethyl-phetiyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and hydrochloric acid in mcthanol under a nitrogen atmosphere;

(b) heating the reaction mixture to a temperature ranging from about 42-50°C;

(c) stirring the reaction mixture;

(d) filtering the reaction mixture while maintaining the temperature above 40°C to obtain a clear solution;

(e) cooling the clear solution to about 30°C while stirring under nitrogen atmosphere;

(f) seeding the solution;

(g) cooling the seeded solution to about 230C;

(h) stirring the solution to obtain a suspension;

(i) cooling the suspension to about-10°C;

(j) stirring the suspension;

(k) filtering solids;

(1) rinsing solids with cold-methanol; and .

(m) drying the solids_at about 50-55°C and 10-20 torr to obtain 4-methyl-N-[3-(4-methyl-irnidazol-l-yl)-5-trifluoroinethyl-phenyJ]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate salt.7.10

10.14 It is stated that claim 5 of the impugned invention is the method generally known in the art and has no inventive step. It is further stated that claimed process appears to follow the standard process of making a salt by treating the free base with hydrochloric acid, this is provided in D2 followed by recrystallization with seeding which is also described on Page 30 of D1. Accordingly claim 5 is not inventive and is obvious to ordinary persons skilled in the art.

10.15 **Claim 6** claims a pharmaceutical composition comprising:

(a) a therapeutically effective amount of a salt according to any of claims 1 to 3; and

(b) at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient.

It is stated that composition as claimed in claim 6 is indirectly dependent on claim 1-3, as it claims a composition which uses the therapeutically effective amount of salt as claimed in claim 1-3. Further as mentioned above claims 1-3 are not inventive, accordingly claim 6 is obvious as it does not add anything to the state of art. **Claim 7** claims a pharmaceutical composition of Claim 6, wherein the salt is 4-methyl-N-[3-(4-methyl-imidazol-l-yl)-5-trifluoromethyl-phenyl3-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate. It is stated that claim 7 indirectly claims the composition with compound of claim 1, as claims 1 is not inventive claim 7 is obvious and not inventive as it does not add anything to the state of art.

10.16 **Claim 8** claims a method of treating a disease which responds to an inhibition of protein kinase activity comprising the step of administering to a subject in need of such treatment a therapeutically effective amount of a salt according to any one of claims 1 to

3. Further **Claim 9** claims a method of Claim 8, wherein the salt is 4-methyl-N-[3-(4-methyl-imidazoI-l-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrinuoln-2-ylamino)-benzainide monohydrochloride monohydrate. Claim 8 and 9 are method of treatment claims which are not allowable in Indian under Section 2(1)(j). Further since Claim 1 is obvious in light of D1-D4 claims 8 and 9 shall also stand obvious as they do not add any inventive feature to the invention.

10.17 The opponent states that the applicant has tried to claim the compound already known in the art. Further he has used the methods in the prior art to derive the impugned compound and there is nothing inventive about the compound or the process as such. The opponent further states that this is a conventional process that falls within the purview of a skilled artisan and there is no technical contribution in the claims. Hence the claims are obvious and lack inventive step and ought to be rejected in toto.

10.18 It is stated that Applicant has merely claimed an alternative compound, without even mentioning any problems faced in the prior art. Further there is no demonstration of technical advancement or surprising effect by the applicant with respect to the said compound when the prior art showing close compounds having similar activity and hence is not entitled to a patent. It is a known jurisprudence that where there is a prima facie obviousness case based upon closeness of structure, then it is incumbent upon the applicant to demonstrate that there are actual differences between the claimed compound and the prior art such that the invention as a whole is non-obvious. A claim for unexpected results require to be supported by data based evidence. Since the applicant has failed to demonstrate such result and comparative data with the closest prior art therefore the patent application ought to be rejected in toto.

11. Prior Public Knowledge/ Prior Public Use [Section 25(1) (d)]

It is stated that the impugned application as claimed in claims 1-9 publicly known in view of the disclosure in prior art document D1.Accordingly the alleged invention as claimed in these claims were in public domain and was publicly known. Thus the claims ought to be rejected on this ground alone.

12. Not an Invention / Not Patentable within the meaning of the Act [Section 25(1)(f)]

12.1 Section 2(1)(j)/Section 2(1) (ja)

12.1.1 The opponent states that the claimed invention falls under the mischief of section 2(1)(ja) by virtue of failing the requirements of an 'invention' and also being devoid of novelty and inventive step. The opponent states that the invention should be novel, be a technical advancement over the prior art or it should show economical significance or both and should not be obvious to a person skilled in the art.

12.1.2 It is stated the compound in the prior art i.e. D1 claims exactly the same structure as claimed in the impugned application. Further the applicant has not mentioned any problems faced in the prior art, let alone a solution for the same. The Opponent states that the applicant is trying to claim an already known compound and therefore is not entitled to a patent and ought to be rejected in toto.

12.2 Claims an invention not patentable as per section 3 (d)

12.2.1 The opponent states that the invention claimed by the applicant is not an invention within the meaning of Section 3(d) of the Act because the claimed invention falls within the mischief of Section 3(d) which clearly states that "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 derivatives of known substance shall be considered to be the same

substance, **unless they differ significantly in properties with regard to efficacy**."

12.2.2 The opponent states that Section 3(d), declares that the very discovery of a new form of a known substance which does not result in enhancement of know efficacy of that substance will not be treated as an invention. Thus if the discovery of a new form must be treated as an invention, then the Patent applicant should show that the substance so discovered has a better therapeutic effect. For the purpose efficacy is defined as therapeutic efficacy for compounds intended as use in medicine. It is stated that *Efficacy means "the ability to produce a desired or intended result"*. Hence, the test of efficacy in the context of section 3(d) would depend on the intended use of the product. Thus the product which is intended to be used as a medicine to cure diseases it needs to be shown whether there is therapeutic efficacy to qualify the test of efficacy under Section 3(d). Thus the parameter of therapeutic efficacy and the advantages and benefits that may be taken into account for determining the enhancement of therapeutic efficacy with respect to Section 3(d), and particularly the "therapeutic efficacy" of a medicine must be judged strictly and narrowly. Therefore it may be said that not all advantageous or beneficial properties are relevant, but only such properties that directly relate to efficacy, which in case of medicine, is its therapeutic efficacy are relevant. It is to be kept in mind that each of the different forms mentioned in the explanation have some properties inherent to that form, solubility to a salt and hygroscopicity to a polymorph. Therefore, unless they differ significantly in property with regard to efficacy, they are expressly excluded from the definition of "invention". Hence, the mere change of form with properties inherent to that form would not qualify as "enhancement of efficacy" of a known substance. Thus the salts of the impugned invention with only change in solubility or stability properties falls within the explanation of Section 3(d) which is meant to indicate what is not to be considered as therapeutic efficacy and hence not patentable.

12.2.3 The opponent states that the claimed invention falls within the mischief of Section 3 (d) read with its explanation. It is stated that the impugned application claims the salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4 pyrimidine-2-yl-amino)-benzamide. D1 discloses the same compound with the same use as the compounds of the present application. In claim 1 of D1, pharmaceutically acceptable salts are claimed. On page 3 of the description, it is clearly mentioned that the salts claimed in D1 refer to the pharmaceutically acceptable salts of the compound of D1. Mentioned on the said page are amongst others, hydrochloride acid and phosphoric acid. The problem to be solved by the applicant, if any, was to provide alternative compounds as tyrosine kinase inhibitors. Starting from D1 a skilled person would try a pharmaceutically acceptable salt like hydrochloride acid or phosphate salt of the compound of example 92 and come to the solution of claims 1-3 without any inventive skill. Accordingly the monohydrochloride monohydrate salt, the monophosphate and the diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyrimidine-2-yl-amino)-benzamide are merely a new form i.e. Pharmaceutically acceptable salt of a known substance. As comparative data the applicant has provided Solubility Decomposition and other Chemical and Physicochemical data. That a salt specially hydrochloride salt has a better solubility is known to person skilled in the art and is an inherent property. Hence finding a solubility of the salt cannot be regarded as invention and falls square under Section 3(d).According to Section 3(d) it is incumbent upon the applicant to provide data of enhancement of known efficacy, the applicant has lacked in providing any therapeutic efficacy data let alone to show and enhancement.

12.2.4 Applicant has failed miserably to show enhancement of efficacy of the salts vis-à-vis the admittedly known prior art and hence ought to be rejected under Section 3(d). There is no data in the impugned specification which goes to show that the claimed salts differ significantly in properties with regard to efficacy. The only data provided is with respect to the solubility and stability which cannot and

ought not to be regarded as properties to satisfy enhanced efficacy for compounds used for medicine. The applicant has failed to show such in invivo data to establish an enhanced efficacy, and is not patentable under Section 3(d).

12.3 Claims an invention not patentable as per section 3 (e)

12.3.1 The opponent states that the claims 6 and 7 of the impugned invention, insofar as it claims a mere mixture falls under the mischief of Section 3 (e) which clearly states that a substance obtained by a mere admixture resulting only in the aggregation of properties of components thereof is not patentable.

12.3.2 It is stated that the composition claimed in the impugned patent application claims 6 and 7 is a mere admixture of a therapeutically effective amount of a salt according to any of claims 1 to 3; and at least one pharmaceutically acceptable carrier, diluents, vehicle or excipient without any improvement over the prior art as evident from the arguments advanced hereinabove. It is stated that there is no demonstrated synergy expected or unexpected provided in the impugned application. It is not clear if the combined agents act to gather to provide a technical effect that is greater than just sum of the two or more agents alone, or whether the combination is in fact a mere juxtaposition with no interaction of the agents. Accordingly it is stated that the mere presentation of a salt of a known substance along with known excipient clearly falls within the mischief of section 3(e) and thus ought to be rejected in view of this section itself.

12.4. Claims an invention not patentable as per section 3 (i)

12.4.1. The opponent states that the claims 8 and 9 of the impugned invention, fall directly under section 3(i) of The patents Act, which states that any process for the medicinal, surgical, curative, prophylactic or other treatment of human beings or any process for a similar treatment of animals to render them free of disease or to increase their economic value are not patentable.

12.4.2 Therefore claims 8 and 9 are not patentable as the said claims are directed to method of treatment of disorders associated with inhibition of protein kinase activity in human being and/or animals.

13. Insufficiency [Section 25(1) (g)]

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

13.2 It is stated that the diphosphate salt of 4-methyl-N-(3-(4-methyl-imidazol-l-yl)-5-trifliiorornethyl-phenylJ-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide as claimed in claim 3 is not illustrated by way of examples. The applicant has failed to demonstrate the working of the impugned compound in treatment of any of the diseases. It is apparent from this that applicant is himself not sure of the potential functional activity of the impugned compound. Further the description is not enablement due lack of such workable effects.

13.3 The opponent state that there is no comparative data provided demonstrating improved efficacy or any unexpected advantage of the present compound over the prior art. The application is liable to be rejected on this ground alone.

14. Breach of Section 8 [Section 25(1) (h)]

14.1 The opponent states that the applicant has not discharged its burden under Section 8 since it failed to comply with the provisions of Section 8(1) and (2). The details with respect to, *inter alia,* the following foreign applications in respect of the same/substantially same invention were not disclosed completely in accordance with the requirement of the Act. The

opponent states that the INPADOC data found from the data base of the European Patent Office reflects a large number of family patents i.e. 36 applications including their status and it is stated that the applicant has failed to inform the Patent Office of such information. The list of such applications as found in the *espacenet* online site which was present with the applicant at the time of filing of the impugned application is listed below for ready reference:

AR057467
AU2006276205
AU2010241419
AU2012203844
CA2615669
EP1910336
EP2186808
ECSP088118
GT200600316
PE02412007
PT1910336
US2008200487
US8389537
UY29683
WO2007015871

14.2 It is stated that form 3 dated December 3, 2007 annexed herein as "**EXHIBIT "A7**" shows that the applicant has only informed that patent office of their three pending applications i.e. US 60/701406, US 60/716213 and PCT/US2006/027878.

14.3 In paragraph 10 of the FER the examiner had objected:

"Details regarding the search and/or examination report including claims of the application allowed, as referred to in Rule 12(3) of the Patent Rule, 2003, in respect of

same or substantially the same invention filed in all the major Patent offices along with appropriate translation where applicable, that should be submitted within a period of Six months from the date of receipt of this communication as provided under section 8(2) of the Indian Patents Act."

It is stated that the applicant has slacked in providing the office actions issued during EP prosecution and a copy of IPER annexed herein as "**EXHIBIT "A8**", applicant has intentionally tried to hide information from the examiner as there report were negative. There has been a default on the part of the applicant in providing the said details and accordingly the application ought to be rejected on this ground alone.

The opponent craves leave to produce further documents in this regard if required.

15. RELIEF SOUGHT

The opponent states that it has established and made out a case on each of the aforesaid grounds of opposition and pray to the Ld. Controller for the following relief(s):

1) Take on record the present representation;
2) Leave to file evidence;
3) Forward copy of reply of applicant and evidence if any and any amendments filed;
4) Leave to file a replication to the reply of the applicant and evidence;
5) Grant of hearing;
6) Refusal of the application *in toto*;
7) Such other relief or reliefs as the Controller may deem appropriate.

Dated this the 6th day of June 2013

Dr. Sanchita Ganguli
Of S. Majumdar & Co.
Opponent's Agent

To

The Controller of Patents

The Patent Office Branch

New Delhi.

Enclosures:

- Exhibit A1;
- Exhibit A2;
- Exhibit A3;
- Exhibit A4;
- Exhibit A5;
- Exhibit A6;
- Exhibit A7;
- Exhibit A8.

Exhibit A1

SALTS OF 4-METHYL-N-[3-(4-METHYL-IMIDAZOL-1-YL)-5-TRIFLUOROMETHYL-PHENYL]-3-(4-PYRIDIN-3-YL-PYRIMIDIN-2-YLAMINO)-BENZAMIDE

This application claims the benefit of U.S. Provisional Patent Application No. 60/701,406, filed July 20, 2005, the entire disclosure of which is incorporated by reference herein.

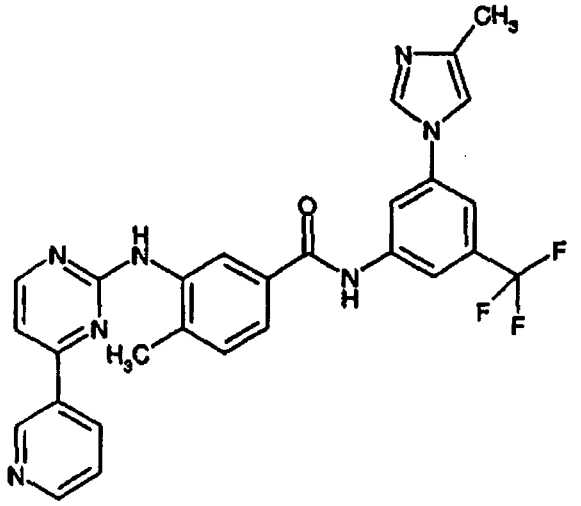
Background of the Invention

Field of the Invention

[0001] This invention relates to salts of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide, as well as to methods of making the same, pharmaceutical compositions comprising the same and methods of treatment using the same.

Related Background Art

[0002] The compound 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide of the formula



is described in WO 2004/005281 A1. Valuable pharmacological properties are attributed to this compound; thus, it can be used, for example, as a protein kinase inhibitor useful in therapy for diseases which respond to inhibition of protein kinase activity. WO 2004/005281 A1 does not disclose any specific salts or salt hydrates or solvates of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

Summary of the Invention

[0003] The present invention is directed to salts of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. Preferred embodiments of the present invention are directed to the hydrochloride, monophosphate, diphosphate, sulfate, methane sulfonate, ethane sulfonate, benzene sulfonate and p-toluene sulfonate salts of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

[0004] The present invention is further directed to a method of preparing a variety of crystalline salts of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide comprising the step of: reacting 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base with an acid of formula HB in a solvent.

[0005] The invention is further directed to pharmaceutical compositions comprising:

(a) a therapeutically effective amount of a salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide; and

(b) at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient.

[0006] The present invention is also directed to a method of treating a disease which responds to an inhibition of protein kinase activity comprising the step of administering to a subject in need of such treatment a therapeutically effective amount of a salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

Brief Description of the Drawings

[0007] Figure 1 shows the x-ray powder diffraction patterns (XRPDs) for forms A and B of the hydrochloride salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

[0008] Figure 2 shows the x-ray powder diffraction pattern (XRPD) for the monophosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

[0009] Figure 3 shows the x-ray powder diffraction pattern for the diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

[0010] Figure 4 shows the x-ray powder diffraction patterns for forms A and B of the sulfate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

[0011] Figure 5 shows the x-ray powder diffraction pattern for the methane sulfonate (mesylate) salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

[0012] Figure 6 shows the x-ray powder diffraction pattern for the ethane sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

[0013] Figure 7 shows the x-ray powder diffraction pattern for the benzene sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

[0014] Figure 8 shows the x-ray powder diffraction pattern for the p-toluene sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

Detailed Description of the Invention

[0015] The present invention is directed to salts of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide; preferred embodiments of those salts are described below. Generally, as used herein, "salt" refers to a compound prepared by the reaction of an organic acid or base drug with a pharmaceutically acceptable mineral or organic acid or base; as used herein, "salt" includes hydrates and solvates of salts made in accordance with this invention. Exemplary pharmaceutically acceptable mineral or organic acids or bases are as listed in Tables 1-8 in *Handbook of Pharmaceutical Salts*, P.H. Stahl and C.G. Wermuth (eds.), VHCA, Zurich, pp. 334-345 (2002). As used herein, "polymorph" refers to a distinct "crystal modification" or "polymorphic form" or "crystalline form", which differs from another with respect to x-ray powder diffraction pattern, physicochemical and/or pharmacokinetic properties, and thermodynamic stability. Co-pending U.S. Patent Application No. 60/701,405 (Attorney Docket No. 4-34384), filed concurrently herewith, addresses the various polymorphic forms of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide and salts thereof; the disclosure of that co-pending application is incorporated in its entirety by reference herein.

[0016] The first embodiment of the present invention is directed to the hydrochloride salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. The hydrochloride salt (form B, monohydrate) is reproducibly produced from methanol when one equivalent hydrochloric acid is used. It is hygroscopic (when first tested, moisture uptake was up to 2% at 60% relative humidity and up to 2.7% at 95% relative humidity, though subsequent testing has shown even greater moisture uptake). It is very slightly soluble in water and slightly soluble in 0.1 N HCl, ethanol and 2-propanol. When tested with thermogravimetric analysis (TGA), two weight loss stages occur. The first stage (onset at about 80°C) represents dehydration, and the second stage weight loss (at about 173°C) represents the loss of HCl (decomposition). Its crystal structure ranges from good to excellent, it becomes amorphous upon grinding and it can withstand compression. The hydrochloride salt is stable at room temperature in standard equilibration tests. Other polymorphic forms of the hydrochloride salt, i.e., forms A, A', A'', B', S_B, S_B',

C, C', S_C, D, and S_E, were also isolated. The XRPD pattern for forms A and B of the hydrochloride salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide are shown in Figure 1.

[0017] The second embodiment of the present invention is directed to the monophosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. The H_3PO_4 mono-salt is reproducibly produced from methanol when one equivalent phosphoric acid is used. The weight loss (room temperature to 200°C) is about 0.29%, and the sample melts at about 208°C and decomposes at about 212°C. Its crystal structure is excellent. The XRPD pattern for the monophosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide is shown in Figure 2.

[0018] The third embodiment of the present invention is directed to the diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. The H_3PO_4 di-salt can be produced from methanol when two equivalents phosphoric acid are used. The weight loss (room temperature to 200°C) is about 0.2%, and the sample decomposes at about 210°C. The XRPD pattern for the diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide is shown in Figure 3.

[0019] The fourth embodiment of the present invention is directed to the sulfate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. The H_2SO_4 salt (form B) is reproducibly produced from methanol when one equivalent sulfuric acid is used. The weight loss (room temperature to 200°C) is about 0.15%, and the sample melts with decomposition at about 206°C. Its crystal structure ranges from poor to good. One other form (form A) and an amorphous form were isolated. The XRPD patterns for forms A and B of the sulfate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide are shown in Figure 4.

[0020] The fifth embodiment of the present invention is directed to the methane sulfonate (mesylate) salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. This salt is reproducibly produced from ethyl acetate when one equivalent methane sulfonic acid is used. The weight loss (room temperature to 150°C) is about 0.44%, and the sample melts at about 160°C and decomposes at about 260°C. Its crystal structure is poor. The XRPD pattern for the methane sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide is shown in Figure 5.

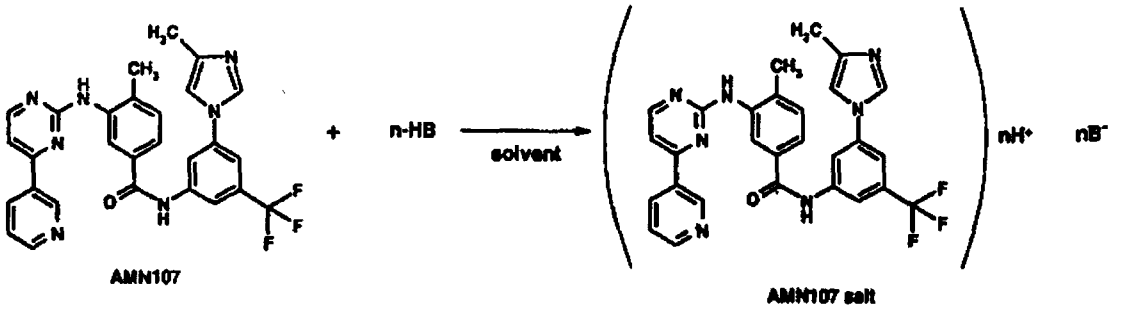
[0021] The sixth embodiment of the present invention is directed to the ethane sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. This salt is reproducibly produced from ethyl acetate when one equivalent ethane sulfonic acid is used. The weight loss (room temperature to 150°C) is about 0.74%, and the sample melts at about 259°C and decomposes at about 220°C. Its crystal structure is poor. The XRPD pattern for the ethane sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide is shown in Figure 6.

[0022] The seventh embodiment of the present invention is directed to the benzene sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. This salt is reproducibly produced from ethyl acetate when one equivalent benzene sulfonic acid is used. The weight loss (room temperature to 250°C) is about 0.63%, and the sample melts with decomposition at about 260°C. Its crystal structure ranges from poor to good. The XRPD pattern for the benzene sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide is shown in Figure 7.

[0023] The eighth embodiment of the present invention is directed to the p-toluene sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. This salt is reproducibly produced from ethyl acetate when one equivalent p-toluene sulfonic acid is used. The weight loss (room temperature to 150°C) is about 0.26%, and the sample melts at about 187°C and decomposes at about 256°C. Its

crystal structure ranges from good to excellent. The XRPD pattern for the p-toluene sulfonate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide is shown in Figure 8.

[0024] Another embodiment of the present invention is directed to a method of preparing a variety of crystalline salts of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide according to the following scheme:



[0025] More specifically, 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide salts are made by reacting 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base with an acid of formula HB in a solvent. Such reaction is typically conducted in two steps, though it is within the scope of this invention to simply combine both the free base and the acid in the solvent at the same time.

[0026] In a first step, 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base is dissolved or suspended in an appropriate amount of solvent at an appropriate temperature. Solvents suitable for use in the present invention include, without limitation, methanol, ethanol, 2-propanol, acetone, ethyl acetate, acetonitrile, tetrahydrofuran and combinations thereof. It is within the skill of one of ordinary skill in the art to determine suitable amounts of base to be used, as well as suitable reaction temperatures.

[0027] In a second step of the present inventive method, the 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base is treated with an appropriate acid of the formula HB. Given the pKa values for

4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base of 5.1 and 3.9, salt forming acids with a pKa of ≤3.1 have the potential to form stable crystalline salts therewith. Suitable acids include, without limitation, inorganic acids such as hydrochloric acid, phosphoric acid, sulfuric acid, and sulfonic acid and organic acids such as methane sulfonic acid, ethane sulfonic acid, benzene sulfonic acid, p-toluene sulfonic acid, citric acid, fumaric acid, gentisic acid, malonic acid, maleic acid, and tartaric acid.

[0028] In optional steps of the present inventive method, the 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide salt is isolated by filtration or some other suitable means and the isolated salt is dried to remove residual solvent. In a preferred embodiment of this invention, the hydrochloride salt is first obtained as a methanol solvate which must be exposed to moisture in order to convert to the monohydrate hydrochloride salt.

[0029] A particularly preferred embodiment of the present invention is directed to a method of preparing 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate comprising the steps of:

(a) combining 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and hydrochloric acid in methanol under a nitrogen atmosphere;

(b) heating the reaction mixture to a temperature ranging from about 42-50°C;

(c) stirring the reaction mixture;

(d) filtering the reaction mixture while maintaining the temperature above 40°C to obtain a clear solution;

(e) cooling the clear solution to about 30°C while stirring under nitrogen atmosphere;

(f) seeding the solution;

(g) cooling the seeded solution to about 23°C;

(h) stirring the solution to obtain a suspension;

(i) cooling the suspension to about -10°C;

(j) stirring the suspension;

(k) filtering solids;

(l) rinsing solids with cold methanol; and

(m) drying the solids at about 50-55°C and 10-20 torr to obtain 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate salt.

In more preferred embodiments, stirring is conducted for about 15 minutes in step (c), cooling is accomplished over a period of about 30 minutes in step (e), cooling is accomplished over a period of about 45 minutes in step (g), stirring is conducted for about 3 hours in step (h), cooling is accomplished over a period of about 1.5 hours in step (i), stirring is conducted for about 30 minutes in step (j), the cold methanol of step (l) has a temperature of about -10°C, and/or drying is accomplished over a period of about 8-16 hours.

[0030] The tenth embodiment of the present invention is directed to a pharmaceutical composition comprising:

(a) a therapeutically effective amount of a salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide; and

(b) at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient.

[0031] A "therapeutically effective amount" is intended to mean the amount of the inventive salt that, when administered to a subject in need thereof, is sufficient to effect treatment for disease conditions alleviated by the inhibition of protein kinase activity. The amount of a given compound of the invention that will be therapeutically effective will vary depending upon factors such as the disease condition and the severity thereof, the identity of the subject in need thereof, etc., which amount may be routinely determined by artisans of ordinary skill in the art.

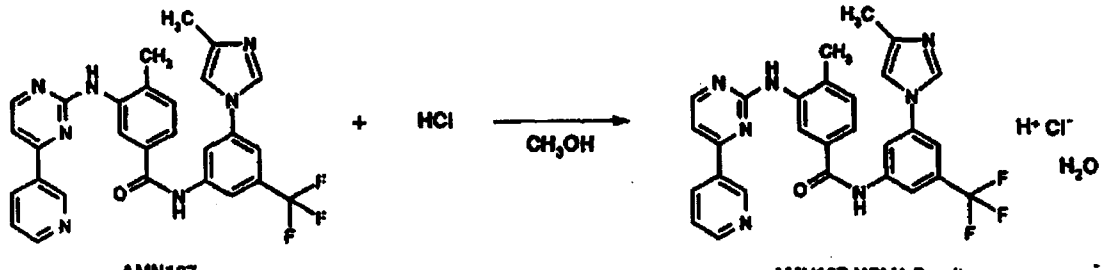
[0032] The at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient can readily be selected by one of ordinary skill in the art and will be determined by the desired mode of administration. Illustrative examples of suitable modes of administration include oral, nasal, parenteral, topical, transdermal, and rectal. The pharmaceutical compositions of

this invention may take any pharmaceutical form recognizable to the skilled artisan as being suitable. Suitable pharmaceutical forms include solid, semisolid, liquid, or lyophilized formulations, such as tablets, powders, capsules, suppositories, suspensions, liposomes, and aerosols.

[0033] The eleventh embodiment of the present invention is directed to a method of treating a disease which responds to an inhibition of protein kinase activity comprising the step of administering to a subject in need of such treatment a therapeutically effective amount of a salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide. As noted above, illustrative modes of administration include oral, nasal, parenteral, topical, transdermal, and rectal. Administration of the crystalline form may be accomplished by administration of a pharmaceutical composition of the ninth embodiment of the invention or via any other effective means.

[0034] Specific embodiments of the invention will now be demonstrated by reference to the following examples. It should be understood that these examples are disclosed solely by way of illustrating the invention and should not be taken in any way to limit the scope of the present invention.

Example 1 **Preparation of Monohydrochloride Monohydrate Salt**



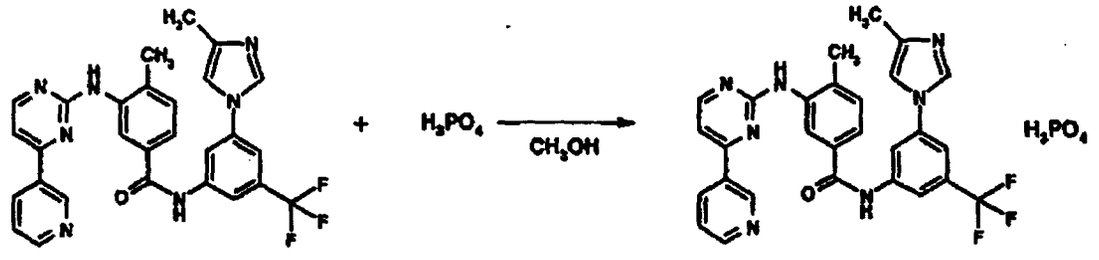
AMN107 AMN107 HCl H_2O salt

[0035] A 1 L, 4-neck, round-bottom flask equipped with a mechanical stirrer, a thermometer, heating/cooling capacity, and an addition funnel was charged in sequence with 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base (10 g), methanol (250 mL), and 37% hydrochloric acid (1.85 g) under nitrogen purge. The mixture was heated to 42-50°C and stirred for an additional 15 minutes. The resulting solution was filtered through a polypropylene pad, while maintaining the batch temperature above 40°C. The clear solution was transferred under nitrogen

atmosphere to another 1 L, 4-neck, and round-bottom flask equipped with a mechanical stirrer, a thermometer, and heating/cooling capacity. The batch was stirred and cooled to 30°C over a period of 30 minutes. Seeds (20 mg) were added at this temperature, and the batch was cooled to 23°C over a period of 45 minutes. The batch was stirred for an additional 3 hours to obtain a thick white suspension. The suspension was cooled to -10°C over a period of 1.5 hours and stirred for an additional 30 minutes. Any solid was collected by filtration and rinsed with cold (-10°C) methanol (20 mL). The solid was dried at 50-55°C/10-20 torr for 8-16 hours to obtain 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate salt (9.8 g) as a white solid.

[0036] ^{1}H NMR 300 MHz, DMSO-d_6), δ 10.9 (s, 1H), 9.58 (s, 1H), 9.29 (s, 1H), 9.20 (s, 1H), 8.70 (d, 1H), 8.63 (s, 1H), 8.55 (d, 1H), 8.49 (d, 1H), 8.32 (d, 2H), 8.00 (s, 1H), 7.91 (s, 1H), 7.84 (d, 1H), 7.56-7.44 (m, 3H), 2.50 (s, 3H), 2.35 (s, 3H); x-ray diffraction pattern showing maxima at 2θ = 7.4°, 9.4°, 11.6°, 12.1°, 15.8°, 19.3°, 19.6°, 22.1°, 24.1°, 25.7°.

Example 2 **Preparation of Monophosphate Salt**

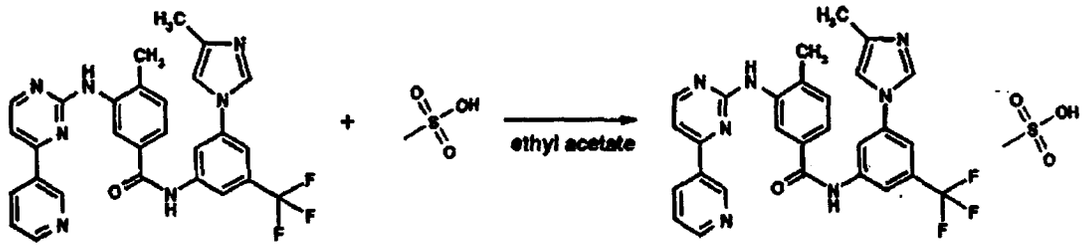


AMN107 AMN107 H_3PO_4 salt

[0037] To a 1 L round-bottom flask equipped with a mechanical stirrer, a thermometer, and a condenser, 4 g of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and 500 mL of methanol were charged. The slurry was stirred and heated to 64°C and held at that temperature for ~30 minutes. To the resulting clear solution, 7.5 mL of 1 M phosphorous acid solution (in methanol) was added. The mixture was stirred at 64°C for one hour, cooled down to room temperature by natural cooling (cooling rate ~0.5°C/min) and held at room temperature for 3-4 hours. The solid was collected by filtration and was dried at 50-55°C/10-20 torr for 8-16 hours to obtain 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-

pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monophosphate salt (3.25 g) as a white solid. Melting point = ~208°C (dec.); x-ray diffraction pattern showing maxima at 2θ = 6.1°, 7.5°, 9.1°, 15.8°, 17.5°, 18.3°, 21.8°, 23.1°, 24.9°, 26.6°.

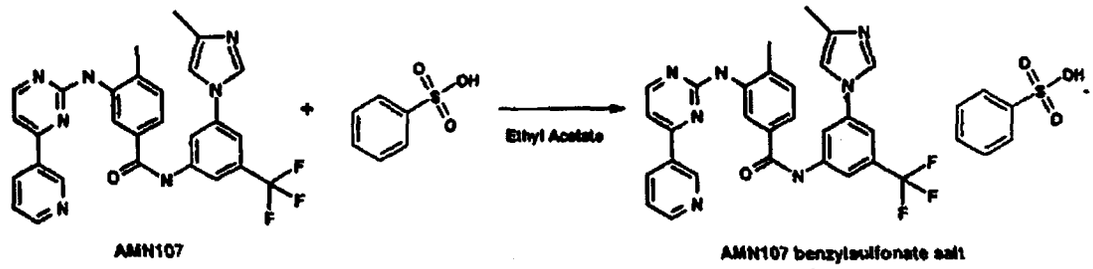
Example 3 **Preparation of Methane Sulfonate Salt**



AMN107 AMN107 mesylate salt

[0038] To a 75 mL reactor equipped with a temperature probe and a condenser, 307 mg of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and 30 mL of ethyl acetate were charged. The slurry was stirred and heated to 76°C. To the solution, 580 μL of 1 M methane sulfonic acid solution (in ethyl acetate) was added. The mixture was stirred at 76°C for six hours, cooled to 25°C at a rate of 0.5°C/minute and held at 25°C overnight. The solid was collected by filtration and was dried at 50-55°C/10-20 torr for 8-16 hours to obtain 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide mesylate salt (~250 mg) as a yellowish solid. X-ray diffraction pattern showing maxima at 2θ = 7.7°, 10.1°, 20.3°, 26.2°.

Example 4 **Preparation of Benzylsulfonate Salt**

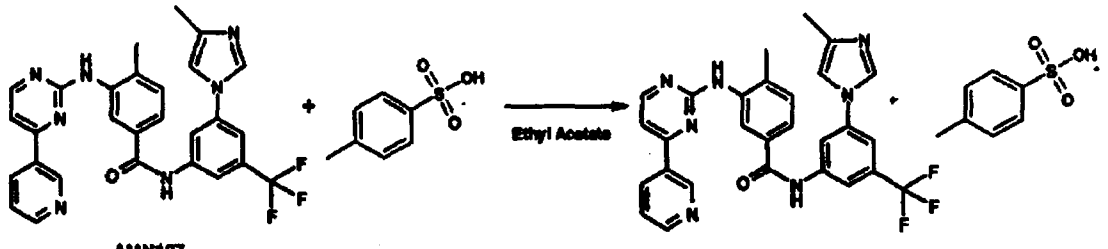


AMN107 AMN107 benzylsulfonate salt

[0039] To a 1 L round-bottom flask equipped with a mechanical stirrer, a thermometer, and a condenser, 4 g of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and 500 mL of ethyl acetate were charged. The slurry was stirred and heated to 76°C (reflux) and held at that temperature for

40 minutes. To the resulting clear solution, 7.5 mL of 1 M benzene sulfonic acid solution (in ethyl acetate) was added. The mixture was stirred at 76°C for 5 hours, cooled down to room temperature by natural cooling (cooling rate ~0.5°C/min) and held at room temperature for ~1 hour. The solid was collected by filtration and was dried at 50-55°C/10-20 torr for 8-16 hours to obtain 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide mono benzyl sulfonate salt as a yellowish solid. Melting point = ~260°C; x-ray diffraction pattern showing maxima at 2θ = 6.5°, 7.8°, 9.4°, 10.4°, 13.7°, 17.0°, 17.5°, 17.9°, 18.8°, 21.2°.

Example 5 **Preparation of p-Toluene Sulfonate Salt**



AMN107 AMN107 p-tolysulfonate salt

[0040] To a 75 mL reactor equipped with a temperature probe and a condenser, 305.6 mg of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and 30 mL of ethyl acetate were charged. The slurry was stirred and heated to 76°C. To the solution, 580 μL of 1 M p-toluene sulfonic acid solution (in ethyl acetate) was added. The mixture was stirred at 76°C for six hours, cooled to 25°C at a rate of 0.5°C/minute and held at 25°C overnight. The solid was collected by filtration and was dried at 50-55°C/10-20 torr for 8-16 hours to obtain 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide p-toluene sulfonate salt (~250 mg) as a white solid. Melting point = ~187°C; x-ray diffraction pattern showing maxima at 2θ = 7.3°, 15.4°, 16.1°, 17.5°, 18.3°, 19.0°, 19.7°, 22.5°.

Example 6 **Hydrochloride Salt**

[0041] 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and about 400 mL methanol are charged into a flask. While stirring, 744.4 mg of 37% HCl solution is added dropwise. The slurry becomes clear. The solution is stirred for 30 minutes. The solution is concentrated to 100 mL. The

solution is then stirred for 2 hours; a slurry is obtained. The slurry is filtered and dried under house vacuum overnight at 50°C. Polymorphic form B is obtained with a yield of about 72.6%.

Example 7

[0042] About 50-60 mg of form A of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base was suspended in 0.75 mL of a listed solvent. The stoichiometric amount of a noted acid was subsequently added to the suspension. For inorganic acids, the mixture was stirred at ambient temperature for about 5 hours, and for sulfonic acids, it was stirred at 50°C overnight. Solids were collected by filtration and analyzed by XRPD and NMR.

Table 1. Formation of Hydrochloride Salt

Solvent	Comments	Results	
		Crystallinity*	^{1}H-NMR
Methanol	Slurry becomes thinner after HCl addition.	Good; form B	No solvent peak
Ethanol	Slurry becomes thinner after HCl addition.	Good; forms A & B	No solvent peak
2-Propanol	Slurry becomes thinner after HCl addition.	Good; form A	No solvent peak
Acetone	Slurry becomes thinner after HCl addition.	Excellent; form A	---
Ethyl acetate	Slurry becomes thinner after HCl addition.	Good; forms A & B	---
Tetrahydrofuran	Slurry becomes thinner after HCl addition.	Excellent; form A	---
Acetonitrile	Slurry becomes thinner after HCl addition.	Excellent; forms A & B	---

* excellent = when main peaks are sharp and their intensities above 70 counts
good = when main peaks are sharp and their intensities within 30-70 counts

Table 2. Formation of Sulfate Salt

Solvent	Comments	Results	
		Crystallinity*	^{1}H-NMR
Methanol	Slurry becomes thinner after H_2SO_4 addition.	Good; forms A & B	No solvent peak
Ethanol	Slurry becomes thinner after H_2SO_4 addition.	Good; form B	No solvent peak
2-Propanol	Slurry becomes thinner after H_2SO_4 addition.	Poor	---
Acetone	Slurry becomes thinner after H_2SO_4 addition.	Poor	---
Ethyl acetate	Slurry becomes thinner after H_2SO_4 addition.	Poor	---
Tetrahydrofuran	Slurry becomes thinner after H_2SO_4 addition.	Poor	---
Acetonitrile	Slurry becomes thinner after H_2SO_4 addition.	Poor	---

* good = when main peaks are sharp and their intensities within 30-70 counts
poor = when main peaks are broad and their intensities below 30 counts; could be amorphous salt and free base form A

Table 3. Formation of Methane Sulfonate Salt

Solvent	Comments	Results	
		Crystallinity*	^{1}H-NMR
Acetone	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Poor	1) 1.3% (w) acetone 2) acid:base = 1.2:1.0
Tetrahydrofuran	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Amorphous	---

* poor = when main peaks are broad and their intensities below 30 counts

Table 4. Formation of Ethane Sulfonate Salt

Solvent	Comments	Results	
		Crystallinity*	^{1}H-NMR
Acetone	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Good	1) 0.9% (w) acetone 2) acid:base = 1.4:1.0
Tetrahydrofuran	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Poor	---

* good = when main peaks are sharp and their intensities within 30-70 counts
poor = when main peaks are broad and their intensities below 30 counts

[0043] The ethane sulfonate salt from acetone has an x-ray diffraction pattern showing maxima at $2\theta = 6.6°, 7.9°, 9.5°, 14.2°, 17.8°$.

Table 5. Formation of Benzene Sulfonate Salt

Solvent	Comments	Results	
		Crystallinity*	^{1}H-NMR
Tetrahydrofuran	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Poor	1) 1.2% (w) THF 2) acid:base = 1.4:1.0
Acetone	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Poor	---

* poor = when main peaks are broad and their intensities below 30 counts

Table 6. Formation of p-Toluene Sulfonate Salt

Solvent	Comments	Results	
		Crystallinity*	^{1}H-NMR
Tetrahydrofuran	Slurry became thinner after acid addition. It did not become clear at 50°C. White solid was obtained by filtration.	Good	1) 4.6% (w) THF 2) acid:base = 1.2:1.0
Acetone	Slurry became thinner after acid addition. It did not become clear at 50°C. White solid was obtained by filtration.	Good	---

* good = when main peaks are sharp and their intensities within 30-70 counts

Example 8

[0044] About 300-310 mg of form B of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base was suspended in 9 mL of 2-propanol for HCl and 15 mL acetone for the sulfonic acids. The stoichiometric amount of the noted acid was subsequently added to the suspension. For HCl, the mixture was stirred at ambient temperature for 5 hours, and for sulfonic acids, it was stirred at 50°C overnight. Then, the mixture was cooled to ambient temperature, collected by filtration and analyzed by XRPD and NMR.

Table 7.

Acid	Comments	Results	
		Crystallinity	^{1}H-NMR
HCl	After HCl addition, the slurry became yellow, then off-white. After 4 hours of holding, the slurry was like paste, difficult to pour and filter.	1) good 2) form A	1) shifts changed 2) no solvent peak
Methane sulfonic acid	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Poor	1) shifts changed 2) 0.67% (w) acetone
Ethane sulfonic acid	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Poor	1) shifts changed 2) no solvent peak
p-Toluene sulfonic acid	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C. White solid was obtained by filtration.	Good	1) shifts changed 2) no solvent peak

Example 9

[0045] About 100 mg of form B of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl}-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base was suspended in 15 mL of methanol for the inorganic acids and in 15 mL THF for the sulfonic acids noted below. The stoichiometric amount of the listed acid was subsequently added to the suspension, except for H_3PO_4, for which two equivalents were added. The solution was stirred at 50°C for about 5 hours and then cooled to ambient temperature. Solids were collected by filtration if slurry formed; otherwise, a slow N_2 flow was applied to evaporate

some solvent to yield thicker slurry for filtration. The solids were analyzed by XRPD and NMR.

Table 8.

Acid	Comments	Results	
		Crystallinity	^{1}H-NMR
HCl	The slurry became clear while heating and remained so. Slow N_2 flow was used to evaporate some solvent.	1) good 2) Form B	1) shifts changed 2) no solvent peak
H_2SO_4	The slurry became clear after heating. It became slurry during cooling.	1) good 2) form A + B	1) shifts changed 2) <2% methanol
H_3PO_4 (diphosphate)	Slurry becomes thicker after acid addition.	1) excellent 2) different from free base and mono-salt	1) no shift change 2) no solvent peak
Methane sulfonic acid	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Poor	1) shifts changed 2) no solvent peak
Benzene sulfonic acid	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C.	Good	1) shifts changed 2) no solvent peak
p-Toluene sulfonic acid	Slurry became thinner and turned yellow after acid addition. It did not become clear at 50°C. White solid was obtained by filtration.	Excellent	1) shifts changed 2) no solvent peak

[0046] Elemental analysis was used to check salt formation for the diphosphate salt. The results are as follows:

Table 9.

	C	H	N	P
Theoretical	45.91	3.83	13.39	8.47
H_3PO_4 above	45.86	3.81	13.32	9.01

Example 10

[0047] About 100 mg of form B of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base was suspended in 15 mL of methanol for HCl and H_2SO_4 and in 15 mL of ethyl acetate for methane sulfonic acid. The listed amount of the listed acid was subsequently added to the

suspension. The solution was stirred at ambient temperature (HCl) or 50°C (H_2SO_4 and methane sulfonic acid). The solids were obtained by evaporating solvent to dryness using a slow N_2 flow and analyzed by XRPD and NMR.

Table 10.

Acid	Comments	Results	
		Crystallinity	^{1}H-NMR
1 equivalent HCl	The slurry became clear while heating and remained so.	1) good 2) form B	1) shifts changed 2) no solvent peak
2 equivalents HCl	The slurry became clear while heating and remained so.	Amorphous	---
0.5 equivalents H_2SO_4	The slurry became clear while heating and remained so.	1) good 2) form A & free base form B	1) shifts changed 2) small solvent peak
1 equivalent H_2SO_4	The slurry became clear after acid addition and remained so.	1) good 2) form A	1) shifts changed 2) no solvent peak
1 equivalent methane sulfonic acid	Slurry became clear after acid addition and remained so after 4 hours holding.	Poor	1) acid:base = 1.3:1.0 2) no solvent peak
2 equivalents methane sulfonic acid	Slurry became clear after acid addition and remained so after 4 hours holding.	Poor	1) acid:base = 1.9:1.0 2) no solvent peak

Example 11

[0048] About 300 mg of form B of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base was suspended in 30 mL of methanol for the inorganic acids and in 30 mL ethyl acetate for the sulfonic acids. The suspension was heated to reflux temperature -64°C for methanol and 76°C for ethyl acetate. The stoichiometric amount of the listed acid, dissolved in the corresponding solvent, was subsequently added to the solution. The solution was stirred under reflux for 5 hours and then cooled to ambient temperature. The solid was collected by filtration and analyzed by XRPD.

Table 11.

Acid	Comments	Form
H_2SO_4	The slurry became clear under reflux. Solid precipitated out after holding.	1) sulfate 2) form B
H_3PO_4	The slurry became clear under reflux. Solid precipitated out after holding.	Monophosphate
Methane sulfonic acid	The solution remained slurry under reflux. It became thinner and turned yellow after acid addition.	Methane sulfonate
Benzene sulfonic acid	The solution remained slurry under reflux. It became thinner and turned yellow after acid addition.	Benzene sulfonate
p-Toluene sulfonic acid	The solution remained slurry under reflux. It became clear after acid addition.	p-Toluene sulfonate

Thermal Behavior

[0049] The LOD and decomposition temperature of the salts of the invention were determined by TGA, and the melting point was determined by DSC.

Table 12.

Salt	LOD	Decomposition temperature (°C)*	Melting point (°C)
Hydrochloride (form B)	2.60% (RT-150°C) 4.87% (150-250°C)		
Monophosphate	0.29% (RT-200°C)	212	~208
Sulfate (form B)	0.15% (RT-200°C)	201	1) 126.5 2) 206.2
Methane sulfonate	0.44% (RT-150°C)	260	160.1
Ethane sulfonate	0.74% (RT-150°C)	220	1) 259.2 2) 261.3
Benzene sulfonate	0.63% (RT-250°C)	260	>258.7
p-Toluene sulfonate	0.26% (RT-150°C)	256	1) 187 2) 232

* The decomposition temperature was determined by the onset of the first derivative of the sample weight loss v. temperature of TGA data

Hygroscopicity

[0050] The hygroscopicity of the salts of the invention was determined by TGA after one day at ambient temperature and 93% relative humidity.

Table 13.

Salt	% moisture gain
Hydrochloride (form B)	0.20
Monophosphate	1.33
Sulfate (form B)	0.22
Methane sulfonate	0.22
Ethane sulfonate	1.11
Benzene sulfonate	0.11
p-Toluene sulfonate	1.02
Control – free base form B	0.08

[0051] It should be noted that, upon further testing, hygroscopicity results have varied. At least with regard to the hydrochloride salt, moisture is lost too quickly upon testing to capture the true value; such may be true for the other salts as well.

Solubility

[0052] The solubility of the salts of the invention was determined in pH 6.8, pH 3.0 and pH 1.0 buffers by suspending 1-5 mg of each salt in 10 mL of corresponding aqueous solution. The samples were allowed to equilibrate at ambient temperature for at least 20 hours for pH 6.8 and 3.0 or about 5 hours for pH 1.0. The supernatant was filtered and used for the solubility determination by UV-VIS spectroscopy. The solid residue was analyzed by XRPD.

Table 14.

Solute	Solubility at pH 6.8 (μg/mL)	Solubility at pH 3.0 (μg/mL)	Solubility at pH 1.0 (μg/mL)
Hydrochloride salt (form B)	0.3	0.9	1040
Monophosphate salt	----	----	1160
Sulfate salt (form B)	0.1	6.5	1380
Methane sulfonate salt	0.4	5.2	1330
Ethane sulfonate salt	0.4	2.8	----
Benzene sulfonate salt	<3.0	----	1420
p-Toluene sulfonate salt	<8.0	<10.0	1340
Control – free base form B	0.2	2.8	839

Comparative Testing

[0053] The stability of both 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base (form B) and 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrate hydrochloride salt (form B) were evaluated as described below.

Table 15.

Test conditions	Salt form			
	Free base (form B)		Hydrochloride monohydrate (form B)	
	Degradation products Assay [% area]	Appearance	Degradation products Assay [% area]	Appearance
Unstressed	0.00 100.99 [100.00]	----	0.00 99.10 [100.00]	----
0.1% solutions or suspensions, 1 week at 80°C				
pH 1 (pH measured: 1.26) PH 1; 1 week @ 50°C	60.61 50.22 [45.31] 6.58 94.01 [93.44]	A* A*	62.06 46.68 [42.93] 6.86 94.14 [93.21]	A* A*
pH 2 (pH measured: 2.00)	5.20 96.00 [94.86]	B↓	8.41 91.77 [91.61]	B↓
pH 3 (pH measured: 2.94)	0.00 102.19 [100.00]	A↓	0.00 98.84 [100.00]	B↓
pH 5 (pH measured: 5.01)	0.00 100.80 [100.00]	A↓	0.00 100.02 [100.00]	A↓
pH 7 (pH measured: 6.02)	0.00 100.14 [100.00]	A↓	0.00 99.56 [100.00]	B↓
pH 9 (pH measured: 8.92)	0.00 99.19 [100.00]	A↓	0.00 101.19 [100.00]	B↓
pH 11 (pH measured: 10.86)	0.00 100.50 [100.00]	A↓	0.00 102.19 [100.00]	B↓
Water (pH measured: 4.74)(pH measured for HCl salt: 4.22)	0.00 101.93 [100.00]	A↓	0.00 101.43 [100.00]	A↓
Ethanol	0.04 99.85 [99.96]	A*	0.06 100.41 [100.00]	A*
Acetonitrile	0.00 100.16 [100.00]	A*	0.00 100.33 [100.00]	B↓
Methanol	1.06 98.04 [98.90]	A*	1.29 99.169 [98.72]	A*

Test conditions	Salt form			
	Free base (form B)		Hydrochloride monohydrate (form B)	
	Degradation products Assay [% area]	Appearance	Degradation products Assay [% area]	Appearance
2% solutions or suspensions, 1 day at room temperature				
0.5% CMC	0.00 98.28 [100.00]	A↓	0.00 103.06 [100.00]	A↓
0.5% HPMC cellulose 4000	0.00 98.27 [100.00]	A↓	0.00 100.44 [100.00]	A↓
0.8% Tween 80	0.00 98.78 [100.00]	A↓	0.00 102.42 [100.00]	A↓
5% solutions in DMSO, 1 day at room temperature				
1:100 dilution in pH 6.8 buffer	0.00 96.98 [100.00]	A↓	0.00 101.85 [100.00]	A↓
Solid state, 1 week 80°C, tight container				
Bulk (HPLC)	0.00 99.77 [100.00]	A	0.00 100.77 [100.00]	A
Bulk (XRPD)	No change		No change	
30% in mixture 1	0.00 100.11 [100.00]	A	0.00 101.23 [100.00]	A
30% in mixture 2	2.17 94.28 [97.75]	A	2.08 93.43 [97.82]	A
Solid state, 1 week 80°C, 75% relative humidity				
Bulk (HPLC)	0.00 99.97 [100.00]	A	0.00 100.71 [100.00]	A
Bulk (XRPD)	No change		No change	
30% in mixture 1	0.00 99.38 [100.00]	B	0.00 100.88 [100.00]	B
30% in mixture 2	3.71 89.37 [96.02]	B	1.89 92.17 [97.99]	B
Xenon light (approximately 1200 kLuxh)				
Bulk (HPLC)	0.00 96.03 [100.00]	A	0.00 99.73 [100.00]	A
Bulk (XRPD)	No change		No change	
Bulk corrosivity				
2 day, 80% relative humidity with steel coupon	N/A		No change	

↓ suspension
A no change of color
* clear solution after stress test
B slight discoloration

Mixture 1: 30% 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide (free base or salt), 63% lactose 100 mesh/lactose 200 mesh (50:50), 5% crosprovidone, 1% Aerosil 200, 1% magnesium stearate

Mixture 2: 30% 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide (free base or salt), 34% mannitol 60, 34% Avicel PH102, 1% Aerosil 200, 1% magnesium stearate (% by weight of free base or salt)

Table 16. Forced Decomposition Test

Test condition	Appearance	Degradation products	Assay [% area]
Unstressed		0.00 (0)	99.22 [100.00]
Bulk 3 days/100°C	A	0.00 (0)	99.02 [100.00]
10 mg/1.5 mL DMSO + 0.5 mL water 3 days/100°C	A*	0.75 (4)	97.04 [99.24]
10 mg/1.5 mL DMSO + 0.5 mL 0.1 N HCl 3 days/50°C	A* A*	11.64 (7) 0.00 (0)	89.15 [88.45] 100.04 [100.00]
10 mg/1.5 mL DMSO + 0.5 mL 0.1 N NaOH 3 days/50°C	A*	6.79 (3)	94.64 [93.30]
10 mg/1.5 mL DMSO + 0.5 mL water containing 200 ppm Fe^{3+}, Ni^{2+} and Cu^{2+} saturated with O_2 3 days/100°C	A*	1.66 (5)	96.89 [98.32]
10 mg/1.5 mL DMSO + 0.5 mL water saturated with O_2 3 days/100°C	A*	0.58 (2)	99.37 [99.42]
10 mg/1.5 mL DMSO + 0.5 mL 10% H_2O_2 3 days/100°C	B*	0.34 (2)	98.85 [99.66]
10 mg/1.5 mL DMSO + 0.5 mL water xenon light (1200 kLux)	B*	2.74 (5)	96.10 [97.23]

[0054] The chemical, physicochemical and morphic characteristics of both 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base (form B) and 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrate hydrochloride salt (form B) were evaluated as described below.

[0055] Determination of Approximate Solubility: A weighted amount (20-50 mg) of sample was charged into 2 mL of the solvent. The obtained slurry was allowed to equilibrate for 24 hours at room temperature and then filtered. The concentration of DS in saturated filtrate was measured by either UV or HPLC.

[0056] Intrinsic Dissolution Rate (IDR): Dissolution rate measurements were performed at 37°C using the rotating disk method (VanKell Instrument). A single rotation speed of 200 rpm was used. For IDR in 0.1 N HCl, an 800 mL volume, and for IDR in water, a 200 mL volume were used. The solution was continuously pumped through a UV measuring cell and recycled to the dissolution vessel.

[0057] Hygroscopicity: Sorption/desorption isotherms were collected using a Surface Measurements Systems dynamic vapor sorption device (DVS-1). The measurements were carried out at 25°C.

Table 17. Chemical and Physicochemical Characteristics

Parameter	Salt form			
	Free base form B		Hydrochloride monohydrate (form B)	
Elementary analysis	Calculated	Found	Calculated	Found
%C	63.46	63.58	57.58	57.66
%H	4.15	3.97	4.29	4.25
%F	10.76	10.22	9.77	9.83
%N	18.51	18.57	16.80	16.58
%O	3.02	3.56	5.48	5.68
%Cl	N/A	N/A	6.08	6.00
DSC purity (mol %) (10°C/minute)	98.65		N/A due to decomposition prior to melting	
HPLC purity (area %)	100.00		100.00	
DSC melting point (°C) (10°C/minute)	249.0		N/A due to decomposition prior to melting	
Melting enthalpy (J/g)	153.9		N/A due to decomposition prior to melting	
pH of 1% solution or suspension in water	7.99		2.53	
Solubility (approximately at 25°C, mg/mL)				
0.1 N HCl	0.60		0.94	
0.01 N HCl	0.0014		0.08	
Phosphate buffer, pH 6.8	0.0002		Below detection	
Water	Below detection		0.17	
Ethanol	0.63		3.69	
Isopropanol	0.33		1.93	
Thermogravimetry (weight loss %) (10°C/minute)	0.026 (RT to 200°C)		0.91 (RT to 80°C)	
Residual solvents (%)	0.2		0.0	
Intrinsic dissolution rate (mg $min^{-1}cm^{-2}$)				
pH 1 (0.1 N HCl)	0.17		0.17	
Water	0.0013		0.0024	

[0058] While the invention has been described above with reference to specific embodiments thereof, it is apparent that many changes, modifications, and variations can be made without departing from the inventive concept disclosed herein. Accordingly, it is intended to embrace all such changes, modifications, and variations that fall within the spirit and broad scope of the appended claims. All patent applications, patents, and other publications cited herein are incorporated by reference in their entirety.

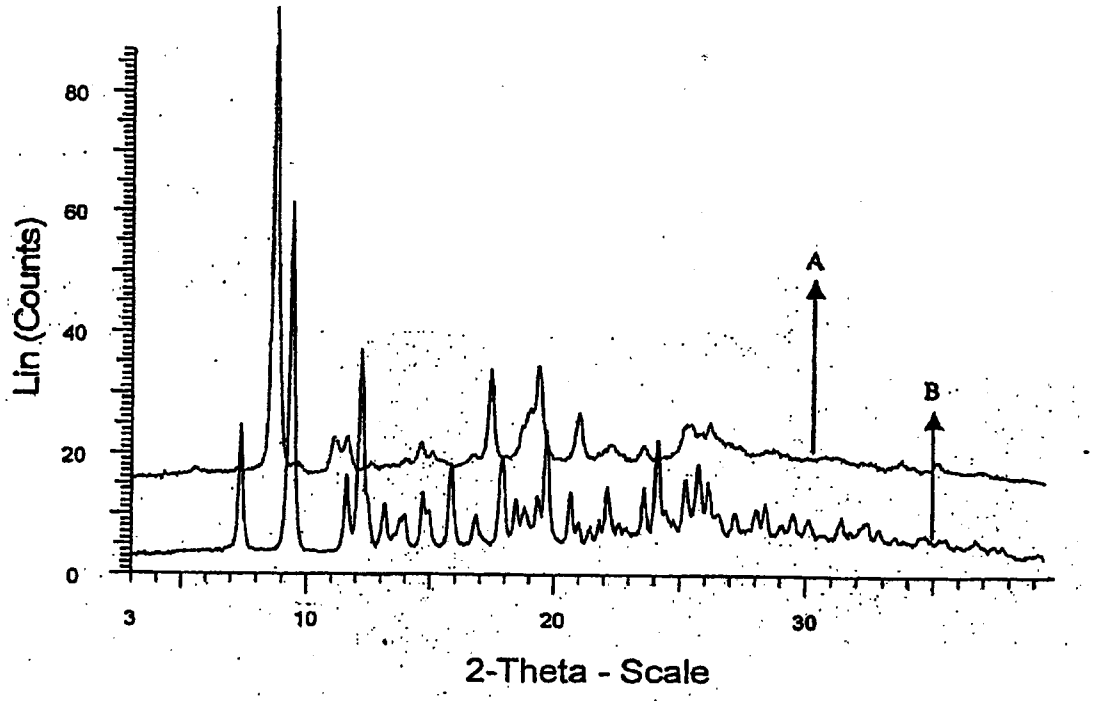


Figure 1

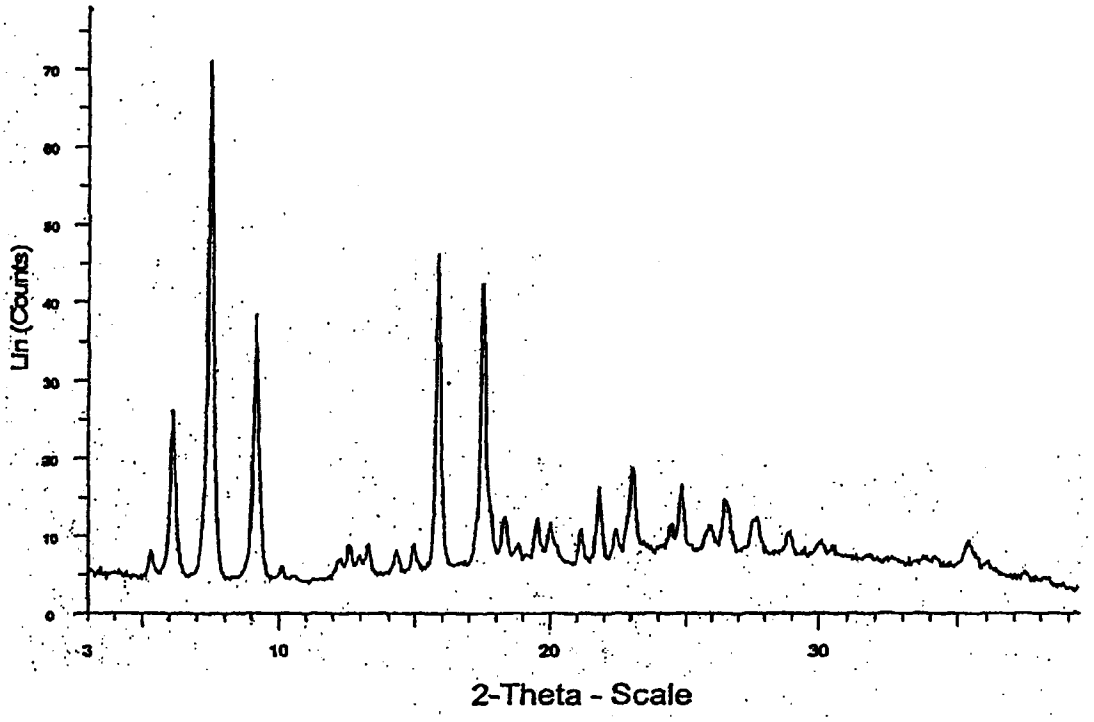


Figure 2

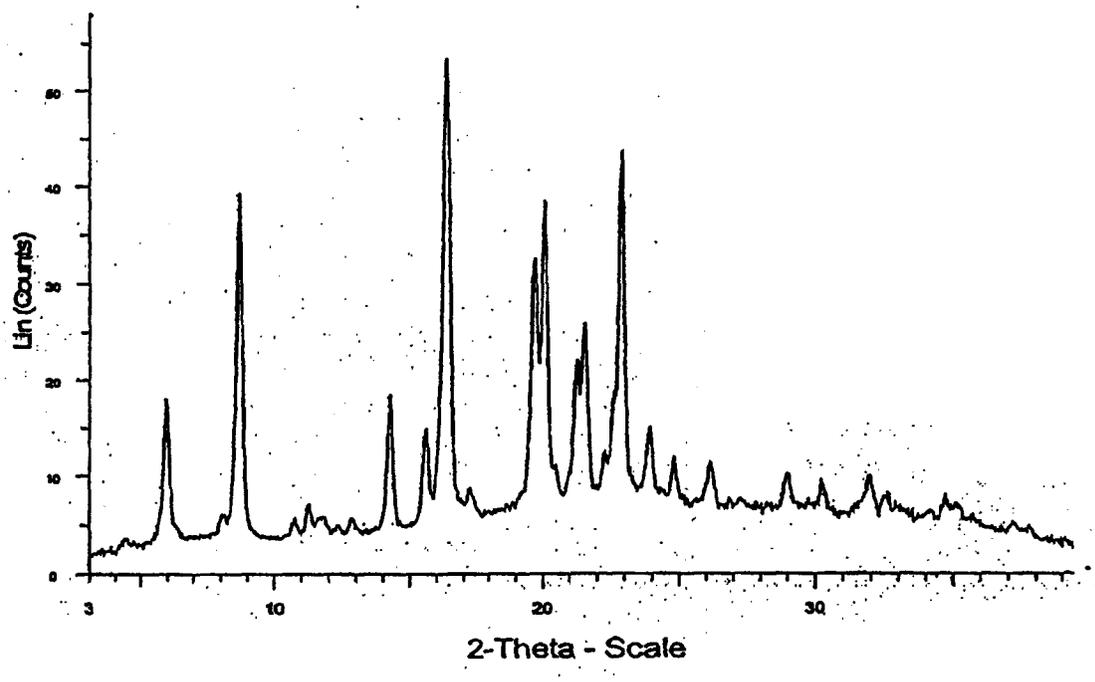


Figure 3

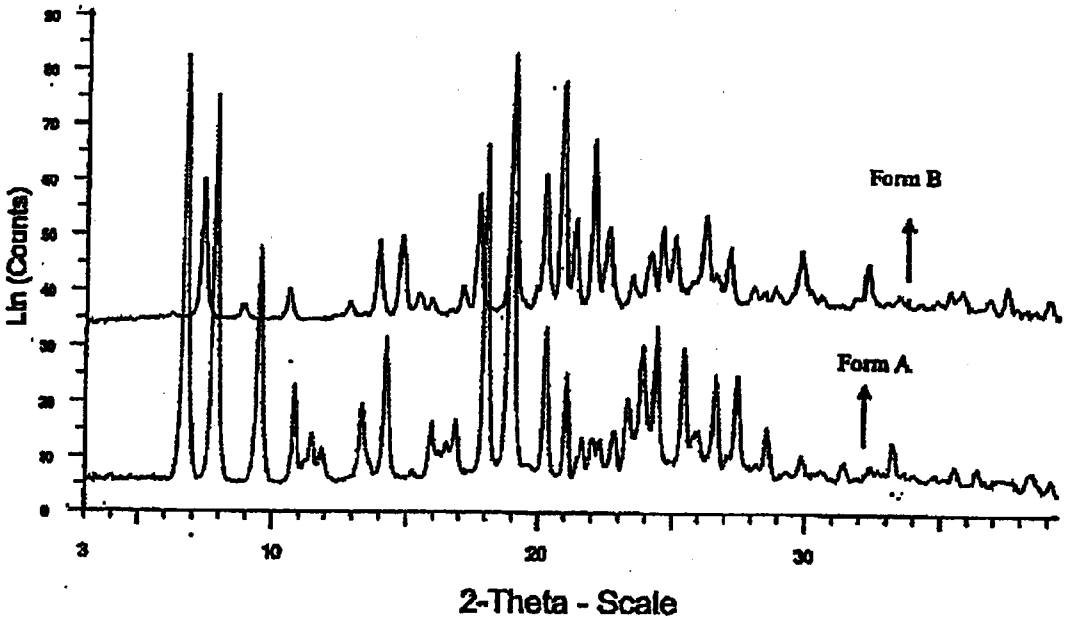


Figure 4

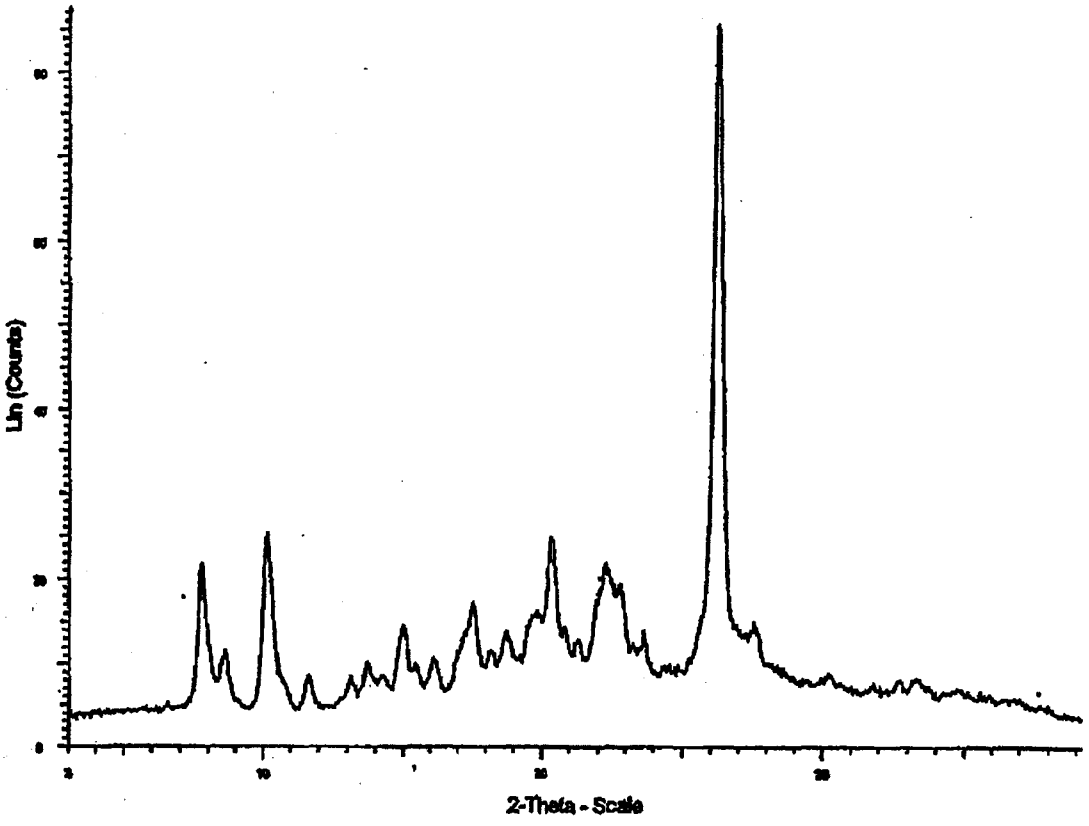


Figure 5

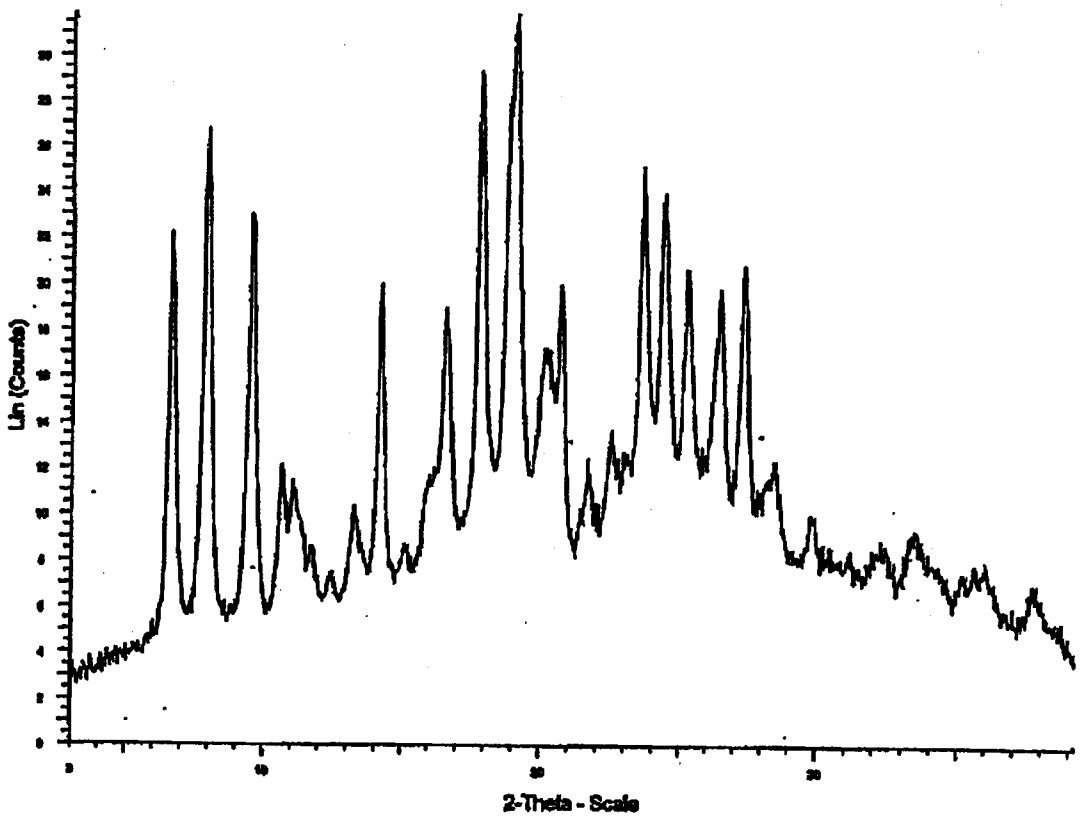


Figure 6

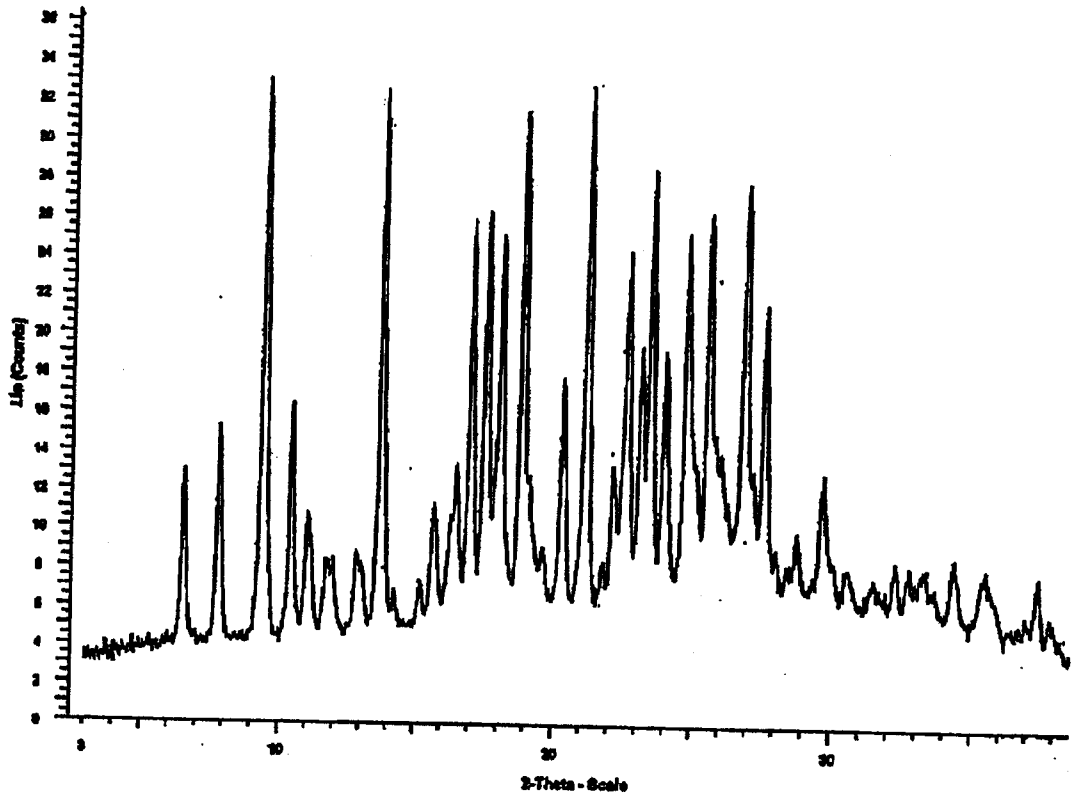


Figure 7

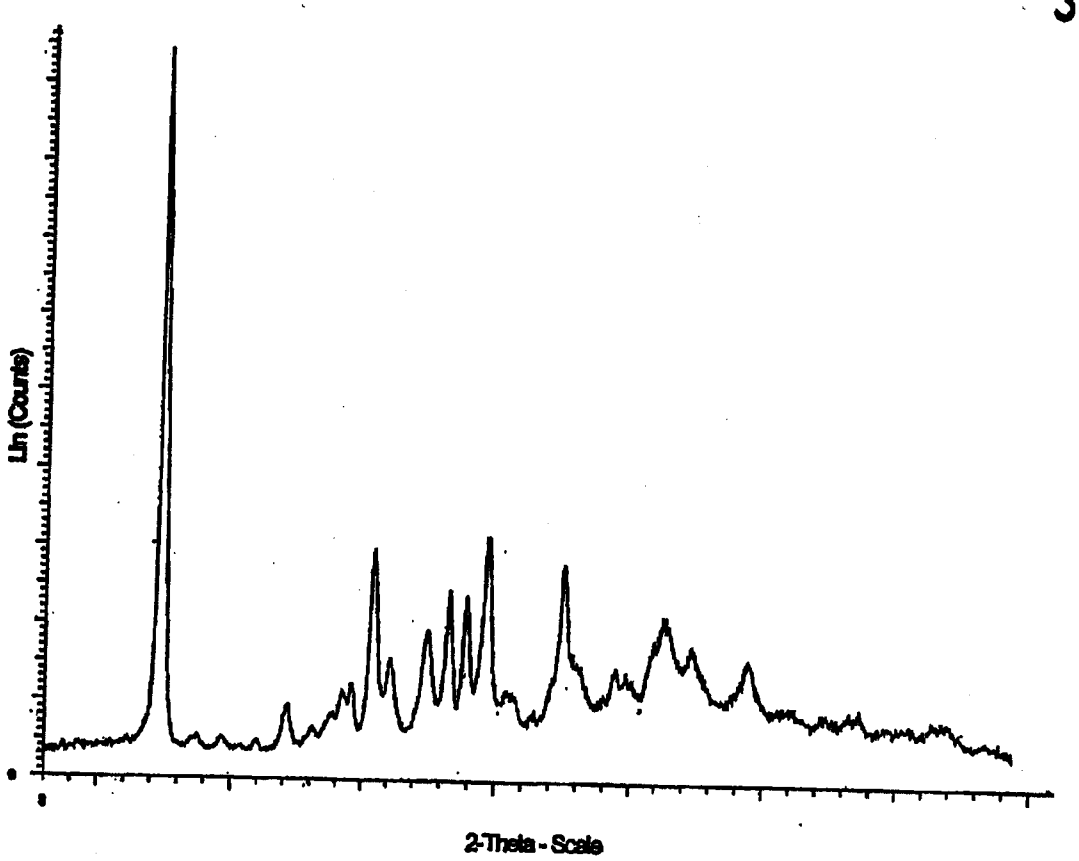


Figure 8

WHAT IS CLAIMED IS:

1. A salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide, which is 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate.

2. A salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide, which is a monophosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

3. A salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide, which is a diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide.

4. A method of preparing a salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide comprising the step of: reacting 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base with an acid of formula HB in a solvent, wherein the acid is phosphoric acid.

5. A method of preparing 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate comprising the steps of:

(a) combining 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide free base and hydrochloric acid in methanol under a nitrogen atmosphere;

(b) heating the reaction mixture to a temperature ranging from about 42-50°C;

(c) stirring the reaction mixture;

(d) filtering the reaction mixture while maintaining the temperature above 40°C to obtain a clear solution;

(e) cooling the clear solution to about 30°C while stirring under nitrogen atmosphere;

(f) seeding the solution;

(g) cooling the seeded solution to about 23°C;

(h) stirring the solution to obtain a suspension;

(i) cooling the suspension to about -10°C;

(j) stirring the suspension;

(k) filtering solids;

(l) rinsing solids with cold methanol; and

(m) drying the solids at about 50-55°C and 10-20 torr to obtain 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate salt.

6. A pharmaceutical composition comprising:

(a) a therapeutically effective amount of a salt according to any of claims 1 to 3; and

(b) at least one pharmaceutically acceptable carrier, diluent, vehicle or excipient.

7. The pharmaceutical composition of Claim 6, wherein the salt is 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate.

8. A method of treating a disease which responds to an inhibition of protein kinase activity comprising the step of administering to a subject in need of such treatment a therapeutically effective amount of a salt according to any one of claims 1 to 3.

9. The method of Claim 8, wherein the salt is 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate.

Dated this 03rd day of December 2007

Snigdha Rani Jay

Of Anand And Anand Advocates
Attorney for the Applicant

67

Exhibit A2

सत्यमेव जयते

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Date : 20/03/2012

Letter No.:-CHEM/2013/

To,
SNIGDHA RANI DAS
Anand & Anand Advocates
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New Delhi-110013, India.

SUB : **Examination Report**

APPLICATION NUMBER	:	9322/DELNP/2007
DATE OF FILING	:	03/12/2007
DATE OF REQUEST FOR EXAMINATION	:	24/07/2008
DATE OF PUBLICATION	:	27/06/2008

With reference to the RQ No. 7263/RQ-DEL/2008 Dated 24/07/2008 in the above mentioned

a) application for Grant of Patent , Examination has been conducted under Section 12 and 13 of the Patents Act 1970 , The following objections are hereby communicated

b) Objections :

Claims 1 to 3, 6 and 7 fall u/s 3(d) of the Patents (Amended) Act, 2005 as the said claims defines new use and/or new form of the known compound (as cited by the prior art documents as described in the report). In the absence of experimental data, it is not clear if the substituted derivatives of the said compound and the composition thereof act to provide an enhancement of the known efficacy i.e., demonstrate a greater technical effect and/or differ significantly in properties w.r.t the known compound .

Claims 4 and 5 appears to be a mere use of a known process which neither results in a known product nor employs any new reactant (please refer the prior art documents as cited in the report). Therefore, the said claims fall u/s 3(d) of the Patents (Amendment) Act,

1 2005.

Claims 6 and 7 fall u/s 3(e) of the Patents (Amended) Act, 2005 as the said claims defines a mere admixture resulting only in the aggregation of the properties of the components thereof. It is not clear if the combined agents act together to provide a technical effect that is greater than just the sum of the two or more agents alone, or whether the combination is in fact a mere juxtaposition with no interaction of the agents.

Claims 8 and 9 fall u/s 3(i) of the Patents (Amended) Act, 2005 as the said claims are directed to method of treatment of disorders associated with modulation of peripheral cannabinoid receptors of human beings and/or animals.

Each set of claims relates to an independent invention.

1 & 5 (relate to 4-methyl-N-(3-(4-methyl-imidazol-l-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-benzamide monohydrochloride monohydrate and process of preparation thereof);

2 (a monophosphate salt of 4-methyl-N-(3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-"3-(4-pyridin-3-yl-

2 pyrimidin-2-ylamino)-benzamide);

3(a diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-l-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-ylalmino)-benzamide) and

4(a method of preparing a salt of 4-methyl-N-[3-(4-methyl-imidazol-l -yl)-5-trifluoromethyl-phenyl]-3-(4-pyriddn-3-yl-pyrimidin-2-ylamino)-benzamide)

Subject matter of the claims does not constitute an invention u/s 2(1) (j) as the claims lack inventive step in view of **WO 2004/005281 A1** which discloses compounds of Formula I with the same use as the compounds of the present application. In claim 1 of the said document, pharmaceutically acceptable salts are claimed, which includes

3 hydrochloric acid and phosphoric acid (page 8). In example 85, 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4- pyridin-3-yl-pyrimidin-2-yl-amino)-benzamide is disclosed. It is therefore considered, that the monohydrochloride monohydrate salt, the monophosphate and the diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5- trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-yl-amino)-benzamide are not inventive over the prior art.

4 Extraneous matter like title from page 2, PCT application details on pages of complete specification etc. should be deleted and fresh retyped pages should be filed.

5 Pages of complete specification should be renumbered.

6 The claim part of the Complete Specification should commence with phrase; "We claim ".

7 Full name of inventors should be given in form-1 and form-5.

8 Form-26 pr power of attorney should be filed.

9 The Drawings referred to in the specification should be prepared inaccordance with the instructions contained in the Rule 15 of the Patent Rules, 2003(as amended in 2006).

Details regarding the search and/or examination report including claims of the application allowed, as referred to in Rule 12(3) of the Patent Rule, 2003, in respect of same or substantially the same

10 invention filed in all the major Patent offices along with appropriate translation where applicable, should be submitted within a period of Six months from the date of receipt of this communication as provided under section 8(2) of the Indian Patents Act.

Details regarding application for Patents which may be filed outside India from time to time for the

11 same or substantially the same invention should be furnished within Six months from the date of filing of the said application under clause(b) of sub section(1) of secton 8 and rule 12(1) of Indian Patent Act.

You are requested to comply with the objections by filing your reply by way of explanation and/or

c) amendments within 12 months from the date of issue of FER failing which you application will be treated as "Deemed to have been abandoned" under section 21(1) of the Act. The last Date is

20/03/2013.

d) You are advised to file your reply at the earliest so that the office can further proceed with application and complete the process within the prescribed period.

(Dr. N. Mukherjee)

Asst. Controller of Patents & Designs

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70 Exhibit A3

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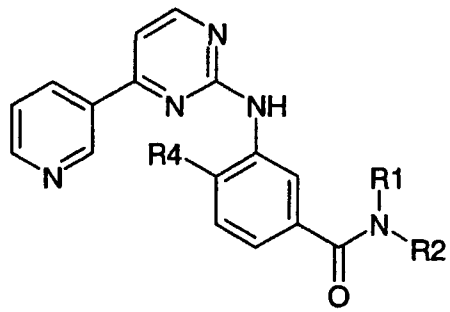
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/005281 A1

(54) Title: INHIBITORS OF TYROSINE KINASES



(I)

(57) Abstract: The invention relates to compounds of formula (I) Wherein the substituents R1, R2 and R4 have the meaning as set forth and explained in the description of the invention, to processes for the preparation of these compounds, pharmaceutical compositions containing same, the use thereof optionally in combination with one or more other pharmaceutically active compounds for the therapy of a disease which responds to an inhibition of protein kinase activity, especially a neoplastic disease, in particular leukaemia, and a method for the treatment of such disease.

Inhibitors of Tyrosine Kinases

The invention relates to novel substituted pyrimidinylaminobenzamides, processes for the preparation thereof, pharmaceutical compositions containing same, the use thereof optionally in combination with one or more other pharmaceutically active compounds for the therapy of a disease which responds to an inhibition of protein kinase activity, especially a neoplastic disease, in particular leukaemia, and a method for the treatment of such a disease.

Background of the invention

Protein kinases (PKs) are enzymes which catalyze the phosphorylation of specific serine, threonine or tyrosine residues in cellular proteins. These post-translational modifications of substrate proteins act as molecular switches regulating cell proliferation, activation and/or differentiation. Aberrant or excessive PK activity has been observed in many disease states including benign and malignant proliferative disorders. In a number of cases, it has been possible to treat diseases, such as proliferative disorders, by making use of PK inhibitors *in vitro* and *in vivo*.

In view of the large number of protein kinase inhibitors and the multitude of proliferative and other PK-related diseases, there is an ever-existing need to provide novel classes of compounds that are useful as PK inhibitors and thus in the treatment of these PTK related diseases. What is required are new classes of pharmaceutically advantageous PK inhibiting compounds.

The Philadelphia Chromosome is a hallmark for chronic myelogenous leukaemia (CML) and carries a hybrid gene that contains N-terminal exons of the bcr gene and the major C-terminal part (exons 2–11) of the c-abl gene. The gene product is a 210 kD protein (p210 Bcr-Abl). The Abl-part of the Bcr-Abl protein contains the abl-tyrosine kinase which is tightly regulated in the wild type c-abl, but constitutively activated in the Bcr-Abl fusion protein. This deregulated tyrosine kinase interacts with multiple cellular signalling pathways leading to transformation and deregulated proliferation of the cells (Lugo *et al.*, Science 247, 1079 [1990]).

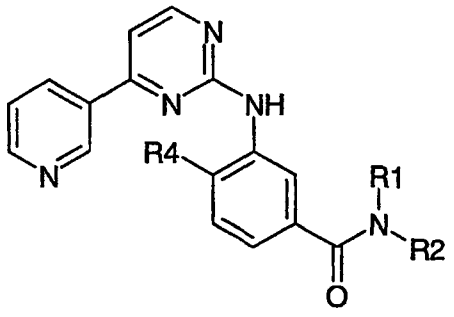
General description of the invention

It has now been found that various compounds of the pyrimidinylaminobenzamide class show inhibition of protein kinase activity. The compounds of formula I, described below in

more detail, especially show inhibition of one or more tyrosine kinases, such as c-Abl, Bcr-Abl, the receptor tyrosine kinases PDGF-R, Flt3, VEGF-R, EGF-R, and c-Kit, as well as combinations of two or more of these; in the case of novel pyrimidinylaminobenzamides according to the invention, the compounds are appropriate for the inhibition of these and/or other protein kinases, especially those mentioned above and/or for the inhibition of mutants of these enzymes, especially of Bcr-Abl, for example the Glu255 -> Valine mutant. In view of these activities, the compounds can be used for the treatment of diseases related to especially aberrant or excessive activity of such types of kinases, especially those mentioned.

Detailed description of the invention

The invention relates to a compound of formula I,



(I)

wherein

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or more identical or different radicals R_3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted;
and R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two

or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted;

or wherein R_1 and R_2 together represent alkylene with four, five or six carbon atoms optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, oxo, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms; oxaalkylene with one oxygen and three or four carbon atoms; or azaalkylene with one nitrogen and three or four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, carboxy, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

The general terms used hereinbefore and hereinafter preferably have within the context of this disclosure the following meanings, unless otherwise indicated:

The prefix "lower" denotes a radical having up to and including a maximum of 7, especially up to and including a maximum of 4 carbon atoms, the radicals in question being either linear or branched with single or multiple branching.

Where the plural form is used for compounds, salts, and the like, this is taken to mean also a single compound, salt, or the like.

Any asymmetric carbon atoms may be present in the (R)-, (S)- or (R,S)-configuration, preferably in the (R)- or (S)-configuration. The compounds may thus be present as mixtures of isomers or as pure isomers, preferably as enantiomer-pure diastereomers.

The invention relates also to possible tautomers of the compounds of formula I.

Lower alkyl is preferably alkyl with from and including 1 up to and including 7, preferably from

and including 1 to and including 4, and is linear or branched; preferably, lower alkyl is butyl, such as n-butyl, sec-butyl, isobutyl, tert-butyl, propyl, such as n-propyl or isopropyl, ethyl or methyl. Preferably lower alkyl is methyl, propyl or tert-butyl.

Lower acyl is preferably formyl or lower alkylcarbonyl, in particular acetyl.

An aryl group is an aromatic radical which is bound to the molecule via a bond located at an aromatic ring carbon atom of the radical. In a preferred embodiment, aryl is an aromatic radical having 6 to 14 carbon atoms, especially phenyl, naphthyl, tetrahydronaphthyl, fluorenyl or phenanthrenyl, and is unsubstituted or substituted by one or more, preferably up to three, especially one or two substituents, especially selected from amino, mono- or disubstituted amino, halogen, lower alkyl, substituted lower alkyl, lower alkenyl, lower alkynyl, phenyl, hydroxy, etherified or esterified hydroxy, nitro, cyano, carboxy, esterified carboxy, alkanoyl, benzoyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amidino, guanidino, ureido, mercapto, sulfo, lower alkylthio, phenylthio, phenyl-lower alkylthio, lower alkylphenylthio, lower alkylsulfinyl, phenylsulfinyl, phenyl-lower alkylsulfinyl, lower alkylphenylsulfinyl, lower alkylsulfonyl, phenylsulfonyl, phenyl-lower alkylsulfonyl, lower alkylphenylsulfonyl, halogen-lower alkylmercapto, halogen-lower alkylsulfonyl, such as especially trifluoromethanesulfonyl, dihydroxybora (-$B(OH)_2$), heterocyclyl, a mono- or bicyclic heteroaryl group and lower alkylene dioxy bound at adjacent C-atoms of the ring, such as methylene dioxy. Aryl is more preferably phenyl, naphthyl or tetrahydronaphthyl, which in each case is either unsubstituted or independently substituted by one or two substituents selected from the group comprising halogen, especially fluorine, chlorine, or bromine; hydroxy; hydroxy etherified by lower alkyl, e.g. by methyl, by halogen-lower alkyl, e.g. trifluoromethyl, or by phenyl; lower alkylene dioxy bound to two adjacent C-atoms, e.g. methylenedioxy, lower alkyl, e.g. methyl or propyl; halogen-lower alkyl, e.g. trifluoromethyl; hydroxy-lower alkyl, e.g. hydroxymethyl or 2-hydroxy-2-propyl; lower alkoxy-lower alkyl; e.g. methoxymethyl or 2-methoxyethyl; lower alkoxycarbonyl-lower alkyl, e.g. methoxy-carbonylmethyl; lower alkynyl, such as 1-propynyl; esterified carboxy, especially lower alkoxycarbonyl, e.g. methoxycarbonyl, n-propoxy carbonyl or iso-propoxy carbonyl; N-mono-substituted carbamoyl, in particular carbamoyl monosubstituted by lower alkyl, e.g. methyl, n-propyl or iso-propyl; amino; lower alkylamino, e.g. methylamino; di-lower alkylamino, e.g. dimethylamino or diethylamino; lower alkylene-amino, e.g. pyrrolidino or piperidino; lower oxaalkylene-amino, e.g. morpholino, lower azaalkylene-amino, e.g. piperazino, acylamino,

e.g. acetylamino or benzoylamino; lower alkylsulfonyl, e.g. methylsulfonyl; sulfamoyl; or phenylsulfonyl.

A cycloalkyl group is preferably cyclopropyl, cyclopentyl, cyclohexyl or cycloheptyl, and may be unsubstituted or substituted by one or more, especially one or two, substitutents selected from the group defined above as substitutents for aryl, most preferably by lower alkyl, such as methyl, lower alkoxy, such as methoxy or ethoxy, or hydroxy, and further by oxo or fused to a benzo ring, such as in benzcyclopentyl or benzcyclohexyl.

Substituted alkyl is alkyl as last defined, especially lower alkyl, preferably methyl; where one or more, especially up to three, substituents may be present, primarily from the group selected from halogen, especially fluorine, amino, N-lower alkylamino, N,N-di-lower alkylamino, N-lower alkanoylamino, hydroxy, cyano, carboxy, lower alkoxycarbonyl, and phenyl-lower alkoxycarbonyl. Trifluoromethyl is especially preferred.

Mono- or disubstituted amino is especially amino substituted by one or two radicals selected independently of one another from lower alkyl, such as methyl; hydroxy-lower alkyl, such as 2-hydroxyethyl; lower alkoxy lower alkyl, such as methoxy ethyl; phenyl-lower alkyl, such as benzyl or 2-phenylethyl; lower alkanoyl, such as acetyl; benzoyl; substituted benzoyl, wherein the phenyl radical is especially substituted by one or more, preferably one or two, substituents selected from nitro, amino, halogen, N-lower alkylamino, N,N-di-lower alkylamino, hydroxy, cyano, carboxy, lower alkoxycarbonyl, lower alkanoyl, and carbamoyl; and phenyl-lower alkoxycarbonyl, wherein the phenyl radical is unsubstituted or especially substituted by one or more, preferably one or two, substituents selected from nitro, amino, halogen, N-lower alkylamino, N,N-di-lower alkylamino, hydroxy, cyano, carboxy, lower alkoxycarbonyl, lower alkanoyl, and carbamoyl; and is preferably N-lower alkylamino, such as N-methylamino, hydroxy-lower alkylamino, such as 2-hydroxyethylamino or 2-hydroxypropyl, lower alkoxy lower alkyl, such as methoxy ethyl, phenyl-lower alkylamino, such as benzylamino, N,N-di-lower alkylamino, N-phenyl-lower alkyl-N-lower alkylamino, N,N-di-lower alkylphenylamino, lower alkanoylamino, such as acetylamino, or a substituent selected from the group comprising benzoylamino and phenyl-lower alkoxycarbonylamino, wherein the phenyl radical in each case is unsubstituted or especially substituted by nitro or amino, or also by halogen, amino, N-lower alkylamino, N,N-di-lower alkylamino, hydroxy, cyano, carboxy, lower alkoxycarbonyl, lower alkanoyl, carbamoyl or aminocarbonylamino.

Disubstituted amino is also lower alkylene-amino, e.g. pyrrolidino, 2-oxopyrrolidino or piperidino; lower oxaalkylene-amino, e.g. morpholino, or lower azaalkylene-amino, e.g. piperazino or N-substituted piperazino, such as N-methylpiperazino or N-methoxycarbonylpiperazino.

Halogen is especially fluorine, chlorine, bromine, or iodine, especially fluorine, chlorine, or bromine.

Etherified hydroxy is especially C_8-C_{20}alkyloxy, such as n-decyloxy, lower alkoxy (preferred), such as methoxy, ethoxy, isopropyloxy, or tert-butyloxy, phenyl-lower alkoxy, such as benzyloxy, phenyloxy, halogen-lower alkoxy, such as trifluoromethoxy, 2,2,2-trifluoroethoxy or 1,1,2,2-tetrafluoroethoxy, or lower alkoxy which is substituted by mono- or bicyclic heteroaryl comprising one or two nitrogen atoms, preferably lower alkoxy which is substituted by imidazolyl, such as 1H-imidazol-1-yl, pyrrolyl, benzimidazolyl, such as 1-benzimidazolyl, pyridyl, especially 2-, 3- or 4-pyridyl, pyrimidinyl, especially 2-pyrimidinyl, pyrazinyl, isoquinolinyl, especially 3-isoquinolinyl, quinolinyl, indolyl or thiazolyl.

Esterified hydroxy is especially lower alkanoyloxy, benzoyloxy, lower alkoxycarbonyloxy, such as tert-butoxycarbonyloxy, or phenyl-lower alkoxycarbonyloxy, such as benzyloxycarbonyloxy.

Esterified carboxy is especially lower alkoxycarbonyl, such as tert-butoxycarbonyl, isopropoxycarbonyl, methoxycarbonyl or ethoxycarbonyl, phenyl-lower alkoxycarbonyl, or phenyloxycarbonyl.

Alkanoyl is primarily alkylcarbonyl, especially lower alkanoyl, e.g. acetyl.

N-Mono- or N,N-disubstituted carbamoyl is especially substituted by one or two substituents independently selected from lower alkyl, phenyl-lower alkyl and hydroxy-lower alkyl, or lower alkylene, oxa-lower alkylene or aza-lower alkylene optionally substituted at the terminal nitrogen atom.

A mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are

unsubstituted or mono- or polysubstituted, refers to a heterocyclic moiety that is unsaturated in the ring binding the heteroaryl radical to the rest of the molecule in formula I and is preferably a ring, where in the binding ring, but optionally also in any annealed ring, at least one carbon atom is replaced by a heteroatom selected from the group consisting of nitrogen, oxygen and sulfur; where the binding ring preferably has 5 to 12, more preferably 5 or 6 ring atoms; and which may be unsubstituted or substituted by one or more, especially one or two, substitutents selected from the group defined above as substitutents for aryl, most preferably by lower alkyl, such as methyl, lower alkoxy, such as methoxy or ethoxy, or hydroxy. Preferably the mono- or bicyclic heteroaryl group is selected from 2H-pyrrolyl, pyrrolyl, imidazolyl, benzimidazolyl, pyrazolyl, indazolyl, purinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, 4H-quinolizinyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalyl, quinazolinyl, quinnolinyl, pteridinyl, indolizinyl, 3H-indolyl, indolyl, isoindolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, tetrazolyl, furazanyl, benzo[d]pyrazolyl, thienyl and furanyl. More preferably the mono- or bicyclic heteroaryl group is selected from the group consisting of pyrrolyl, imidazolyl, such as 1H-imidazol-1-yl, benzimidazolyl, such as 1-benzimidazolyl, indazolyl, especially 5-indazolyl, pyridyl, especially 2-, 3- or 4-pyridyl, pyrimidinyl, especially 2-pyrimidinyl, pyrazinyl, isoquinolinyl, especially 3-isoquinolinyl, quinolinyl, especially 4- or 8-quinolinyl, indolyl, especially 3-indolyl, thiazolyl, benzo[d]pyrazolyl, thienyl, and furanyl. In one preferred embodiment of the invention the pyridyl radical is substituted by hydroxy in ortho position to the nitrogen atom and hence exists at least partially in the form of the corresponding tautomer which is pyridin-(1H)2-one. In another preferred embodiment, the pyrimidinyl radical is substituted by hydroxy both in position 2 and 4 and hence exists in several tautomeric forms, e.g. as pyrimidine-(1H, 3H)2,4-dione.

Heterocyclyl is especially a five, six or seven-membered heterocyclic system with one or two heteroatoms selected from the group comprising nitrogen, oxygen, and sulfur, which may be unsaturated or wholly or partly saturated, and is unsubstituted or substituted especially by lower alkyl, such as methyl, phenyl-lower alkyl, such as benzyl, oxo, or heteroaryl, such as 2-piperazinyl; heterocyclyl is especially 2- or 3-pyrrolidinyl, 2-oxo-5-pyrrolidinyl, piperidinyl, N-benzyl-4-piperidinyl, N-lower alkyl-4-piperidinyl, N-lower alkyl-piperazinyl, morpholinyl, e.g. 2- or 3-morpholinyl, 2-oxo-1H-azepin-3-yl, 2-tetrahydrofuranyl, or 2-methyl-1,3-dioxolan-2-yl.

Salts are especially the pharmaceutically acceptable salts of compounds of formula I.

Such salts are formed, for example, as acid addition salts, preferably with organic or inorganic acids, from compounds of formula I with a basic nitrogen atom, especially the pharmaceutically acceptable salts. Suitable inorganic acids are, for example, halogen acids, such as hydrochloric acid, sulfuric acid, or phosphoric acid. Suitable organic acids are, for example, carboxylic, phosphonic, sulfonic or sulfamic acids, for example acetic acid, propionic acid, octanoic acid, decanoic acid, dodecanoic acid, glycolic acid, lactic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, malic acid, tartaric acid, citric acid, amino acids, such as glutamic acid or aspartic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, cyclohexanecarboxylic acid, adamantanecarboxylic acid, benzoic acid, salicylic acid, 4-aminosalicylic acid, phthalic acid, phenylacetic acid, mandelic acid, cinnamic acid, methane- or ethane-sulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 2-naphthalenesulfonic acid, 1,5-naphthalene-disulfonic acid, 2-, 3- or 4-methylbenzenesulfonic acid, methylsulfuric acid, ethylsulfuric acid, dodecylsulfuric acid, N-cyclohexylsulfamic acid, N-methyl-, N-ethyl- or N-propyl-sulfamic acid, or other organic protonic acids, such as ascorbic acid.

In the presence of negatively charged radicals, such as carboxy or sulfo, salts may also be formed with bases, e.g. metal or ammonium salts, such as alkali metal or alkaline earth metal salts, for example sodium, potassium, magnesium or calcium salts, or ammonium salts with ammonia or suitable organic amines, such as tertiary monoamines, for example triethylamine or tri(2-hydroxyethyl)amine, or heterocyclic bases, for example N-ethyl-piperidine or N,N'-dimethylpiperazine.

When a basic group and an acid group are present in the same molecule, a compound of formula I may also form internal salts.

For isolation or purification purposes it is also possible to use pharmaceutically unacceptable salts, for example picrates or perchlorates. For therapeutic use, only pharmaceutically acceptable salts or free compounds are employed (where applicable in the form of pharmaceutical preparations), and these are therefore preferred.

In view of the close relationship between the novel compounds in free form and those in the form of their salts, including those salts that can be used as intermediates, for example in the

purification or identification of the novel compounds, any reference to the free compounds hereinbefore and hereinafter is to be understood as referring also to the corresponding salts, as appropriate and expedient.

The compounds of formula I and N-oxides thereof have valuable pharmacological properties, as described hereinbefore and hereinafter.

The efficacy of the compounds of the invention as inhibitors of c-Abl, Bcr-Abl, and VEGF-receptor tyrosine kinase activity can be demonstrated as follows:

Test for activity against c-Abl protein tyrosine kinase. The test is conducted as a filter binding assay as follows: The His-tagged kinase domain of c-Abl is cloned and expressed in the baculovirus/Sf9 system as described by Bhat *et al.*, J Biol Chem. 272, 16170-5 (1997). A protein of 37 kD (c-Abl kinase) is purified by a two-step procedure over a Cobalt metal chelate column followed by an anion exchange column with a yield of 1-2 mg/L of Sf9 cells. The purity of the c-Abl kinase is >90% as judged by SDS-PAGE after Coomassie blue staining. The assay contains: c-Abl kinase (50 ng), 20 mM Tris·HCl, pH 7.5, 10 mM $MgCl_2$, 10 μM Na_3VO_4, 1 mM DTT and 0.06 μCi/assay [γ^{33} P]-ATP (5 μM ATP) using 30 μg/mL poly-Ala,Glu,Lys,Tyr-6:2:5:1 (Poly-AEKY, Sigma P1152) in the presence of 1% DMSO, total volume of 30 μL. Reactions are terminated by adding 10 μL of 250 mM EDTA, and 30 μL of the reaction mixture is transferred onto Immobilon-PVDF membrane (Millipore, Bedford, MA, USA) previously soaked for 5 min with methanol, rinsed with water, then soaked for 5 min with 0.5% H_3PO_4 and mounted on vacuum manifold with disconnected vacuum source. After spotting all samples, vacuum is connected and each well rinsed with 200 μL 0.5 % H_3PO_4. Membranes are removed and washed on a shaker with 0.5% H_3PO_4 (4 times) and once with ethanol. Membranes are counted after drying at ambient temperature, mounting in Packard TopCount 96-well frame, and addition of 10 μL/well of Microscint TM (Packard).

Test for activity against Bcr-Abl. The murine myeloid progenitor cell line 32Dcl3 transfected with the p210 Bcr-Abl expression vector pGDp210Bcr/Abl (32D-bcr/abl) was obtained from J. Griffin (Dana Faber Cancer Institue, Bosten, MA, USA). The cells express the fusion Bcr-Abl protein with a constitutively active abl kinase and proliferate growth factor independent. The cells are expanded in RPMI 1640 (AMIMED), 10% fetal calf serum, 2 mM glutamine (Gibco) („complete medium"), and a working stock is prepared by freezing aliquots of 2 x 10^6 cells

per vial in freezing medium (95% FCS, 5% DMSO (SIGMA)). After thawing, the cells are used during maximally 10 –12 passages for the experiments.

For cellular assays, compounds are dissolved in DMSO and diluted with complete medium to yield a starting concentration of 10 μM followed by preparation of serial 3-fold dilutions in complete medium. 200'000 32D-Bcr/Abl cells in 50 μL complete medium are seeded per well in 96 well round bottom tissue culture plates. 50 μL per well of serial 3-fold dilutions of the test compound are added to the cells in triplicates. Untreated cells are used as control. The compound is incubated together with the cells for 90 min at 37°C, 5% CO_2, followed by centrifugation of the tissue culture plates at 1300 rpm (Beckmann GPR centrifuge) and removal of the supernatants by careful aspiration taking care not to remove any of the pelleted cells. The cell pellets are lysed by addition of 150 μL lysis buffer (50 mM Tris/HCl, pH 7.4, 150 mM sodium chloride, 5 mM EDTA, 1 mM EGTA, 1% NP-40, 2 mM sodium ortho-vanadate, 1 mM PMSF, 50 μg/mL aprotinin and 80 μg/mL leupeptin) and either used immediatedly for the ELISA or stored frozen in the plates at –20°C until usage.

Black ELISA plates (Packard HTRF-96 black plates) are precoated over night at 4°C with 50 ng/well of the rabbit polyclonal anti-abl-SH3 domain Ab 06-466 from Upstate in 50 μL PBS. After washing 3 times with 200 μL/well PBS containing 0.05% Tween20 (PBST) and 0.5% TopBlock (Juro), residual protein binding sites are blocked with 200 μL/well PBST, 3% TopBlock for 4 h at room temperature followed by incubation with 50 μL lysates of untreated or compound-treated cells (20 μg total protein per well) for 3-4 h at 4°C. After 3 washings, 50 μL/well anti-phosphotyrosine Ab PY20(AP) labeled with alkaline phosphatase (Zymed) diluted to 0.2 μg/mL in blocking buffer is added and incubated over night (4°C). For all incubation steps the plates are covered with plate sealers (Costar). Finally, the plates are washed another three times with washing buffer and once with deionized water before addition of 90 μL/well of the AP-substrate CDPStar RTU with Emerald II. The plates, now sealed with Packard TopSeal™-A plate sealers, are incubated for 45 min at room temperature in the dark and luminescence is quantified by measuring counts per second (CPS) with a Packard Top Count Microplate Scintillation Counter (Top Count).

The difference between the ELISA-readout (CPS) obtained for with the lysates of the untreated 32D-Bcr/Abl cells and the readout for the assay-background (all components, but without cell lysate) is calculated and taken as 100% reflecting the constitutively phosphorylated Bcr-Abl protein present in these cells. The activity of the compound on the Bcr-Abl kinase activity is expressed as percent reduction of the Bcr-Abl phosphorylation. The

values for the IC_{50} and IC_{90} are determined from the dose response curves by graphical extrapolation.

Test for activity against VEGF-receptor tyrosine kinase. The test is conducted using Flt-1 VEGF-receptor tyrosine kinase. The detailed procedure is as follows: 30 µL kinase solution (10 ng of the kinase domain of Flt-1, Shibuya *et al.*, Oncogene 5, 519-24 [1990]) in 20 mM Tris•HCl pH 7.5, 3 mM manganese dichloride ($MnCl_2$), 3 mM magnesium chloride ($MgCl_2$), 10 µM sodium vanadate, 0.25 mg/mL polyethylenglycol (PEG) 20000, 1mM dithiothreitol and 3 µg/µL poly(Glu,Tyr) 4:1 (Sigma, Buchs, Switzerland), 8 µM [^{33}P]-ATP (0.2 µCi) , 1% DMSO, and 0 to 100 µM of the compound to be tested are incubated together for 10 minutes at room temperature. The reaction is then terminated by the addition of 10 µL 0.25 M ethylenediaminetetraacetate (EDTA) pH 7. Using a multichannel dispenser (LAB SYSTEMS, USA), an aliquot of 20 µL is applied to a PVDF (= polyvinyl difluoride) Immobilon P membrane (Millipore, Bedford, USA), through a Gibco-BRL microtiter filter manifold and connected to a vacuum. Following complete elimination of the liquid, the membrane is washed 4 times successively in a bath containing 0.5% phosphoric acid (H_3PO_4) and once with ethanol, incubated for 10 minutes each time while shaking, then mounted in a Hewlett Packard TopCount Manifold and the radioactivity measured after the addition of 10 µL Microscint® (ß-scintillation counter liquid). IC_{50}-values are determined by linear regression analysis of the percentages for the inhibition of each compound in at least four concentrations (as a rule 0.01, 0.1, 1.0 and 10 µmol). The IC_{50}-values that can be found with compounds of formula I are in the range of 1 to 10'000 nM, preferably in the range of 1 to 100 nM.

The inhibition of VEGF-induced KDR-receptor autophosphorylation can be confirmed with a further in vitro experiment in cells: transfected CHO cells, which permanently express human VEGF receptor (KDR), are seeded in complete culture medium with 10% fetal calf serum (FCS) in 6-well cell-culture plates and incubated at 37°C under 5% CO_2 until they show about 80% confluency. The compounds to be tested are then diluted in culture medium (without FCS, with 0.1% bovine serum albumin) and added to the cells. (Controls comprise medium without test compounds). After two hours of incubation at 37°C, recombinant VEGF is added; the final VEGF concentration is 20 ng/mL). After a further five minute incubation at 37°C, the cells are washed twice with ice-cold PBS (phosphate-buffered saline) and immediately lysed in 100 µL

lysis buffer per well. The lysates are then centrifuged to remove the cell nuclei, and the protein concentrations of the supernatants are determined using a commercial protein assay (BIORAD). The lysates can then either be immediately used or, if necessary, stored at –20°C.

A sandwich ELISA is carried out to measure the KDR-receptor phosphorylation: a monoclonal antibody to KDR (for example Mab 1495.12.14) is immobilized on black ELISA plates (OptiPlate™ HTRF-96 from Packard). The plates are then washed and the remaining free protein-binding sites are saturated with 1% BSA in PBS. The cell lysates (20 µg protein per well) are then incubated in these plates overnight at 4°C together with an anti-phosphotyrosine antibody coupled with alkaline phosphatase (PY20:AP from Transduction Laboratories). The plates are washed again and the binding of the antiphosphotyrosine antibody to the captured phosphorylated receptor is then demonstrated using a luminescent AP substrate (CDP-Star, ready to use, with Emerald II; TROPIX). The luminescence is measured in a Packard Top Count Microplate Scintillation Counter (Top Count). The difference between the signal of the positive control (stimulated with VEGF) and that of the negative control (not stimulated with VEGF) corresponds to VEGF-induced KDR-receptor phosphorylation (= 100 %). The activity of the tested substances is calculated as % inhibition of VEGF-induced KDR-receptor phosphorylation, wherein the concentration of substance that induces half the maximum inhibition is defined as the ED50 (effective dose for 50% inhibition). Compounds of formula I here preferably show ED50 values in the range of 0.25 nM to 1000 nM, preferably 0.25 to 250 nM.

A compound of formula I or a N-oxide thereof inhibits to varying degrees also other tyrosine kinases involved in signal transduction which are mediated by trophic factors, for example Bcr-Abl and Abl kinase, Arg, kinases from the Src family, especially c-Src kinase, Lck, and Fyn; also kinases of the EGF family, for example, c-erbB2 kinase (HER-2), c-erbB3 kinase, c-erbB4 kinase; insulin-like growth factor receptor kinase (IGF-1 kinase), especially members of the PDGF-receptor tyrosine kinase family, such as PDGF-receptor kinase, CSF-1-receptor kinase, Kit-receptor kinase and VEGF-receptor kinase; and also serine/threonine kinases, all of which play a role in growth regulation and transformation in mammalian cells, including human cells.

The inhibition of c-erbB2 tyrosine kinase (HER-2) can be measured, for example, in the same way as the inhibition of EGF-R protein kinase, using known procedures.

On the basis of these studies, a compound of formula I according to the invention shows therapeutic efficacy especially against disorders dependent on protein kinase, especially proliferative diseases.

On the basis of their efficacy as inhibitors of VEGF-receptor tyrosine kinase activity, the compounds of the formula I primarily inhibit the growth of blood vessels and are thus, for example, effective against a number of diseases associated with deregulated angiogenesis, especially diseases caused by ocular neovascularisation, especially retinopathies, such as diabetic retinopathy or age-related macula degeneration, psoriasis, haemangioblastoma, such as haemangioma, mesangial cell proliferative disorders, such as chronic or acute renal diseases, e.g. diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes or transplant rejection, or especially inflammatory renal disease, such as glomerulonephritis, especially mesangioproliferative glomerulonephritis, haemolytic-uraemic syndrome, diabetic nephropathy, hypertensive nephrosclerosis, atheroma, arterial restenosis, autoimmune diseases, diabetes, endometriosis, chronic asthma, and especially neoplastic diseases (solid tumors, but also leukemias and other "liquid tumors", especially those expressing c-kit, KDR, Flt-1 or Flt-3), such as especially breast cancer, cancer of the colon, lung cancer (especially small-cell lung cancer), cancer of the prostate or Kaposi's sarcoma. A compound of formula I (or an N-oxide thereof) inhibits the growth of tumours and is especially suited to preventing the metastatic spread of tumors and the growth of micrometastases.

A compound of formula I can be administered alone or in combination with one or more other therapeutic agents, possible combination therapy taking the form of fixed combinations or the administration of a compound of the invention and one or more other therapeutic agents being staggered or given independently of one another, or the combined administration of fixed combinations and one or more other therapeutic agents. A compound of formula I can besides or in addition be administered especially for tumor therapy, such as leukaemia therapy, in combination with chemotherapy, radiotherapy, immunotherapy, surgical intervention, or a combination of these. Long-term therapy is equally possible as is adjuvant therapy in the context of other treatment strategies, as described above. Other possible treatments are therapy to maintain the patient's status after tumor regression, or even chemopreventive therapy, for example in patients at risk.

Therapeutic agents for possible combination are especially one or more cytostatic or cytotoxic compounds, for example a chemotherapeutic agent or several selected from the group comprising indarubicin, cytarabine, interferon, hydroxyurea, bisulfan, or an inhibitor of polyamine biosynthesis, an inhibitor of protein kinase, especially of serine/threonine protein kinase, such as protein kinase C, or of tyrosine protein kinase, such as epidermal growth factor receptor tyrosine kinase, a cytokine, a negative growth regulator, such as TGF-ß or IFN-ß, an aromatase inhibitor, a classical cytostatic, and an inhibitor of the interaction of an SH2 domain with a phosphorylated protein.

A compound according to the invention is not only for the (prophylactic and preferably therapeutic) management of humans, but also for the treatment of other warm-blooded animals, for example of commercially useful animals, for example rodents, such as mice, rabbits or rats, or guinea-pigs. Such a compound may also be used as a reference standard in the test systems described above to permit a comparison with other compounds.

In general, the invention relates also to the use of a compound of formula I or a N-oxide thereof for the inhibition of tyrosine kinase activity, either in vitro or in vivo.

With the groups of preferred compounds of formula I and N-oxides thereof mentioned hereinafter, definitions of substituents from the general definitions mentioned hereinbefore may reasonably be used, for example, to replace more general definitions with more specific definitions or especially with definitions characterized as being preferred.

In particular, the invention relates to compounds of formula I, wherein

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or two identical or different radicals R_3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising one, two or three nitrogen atoms or one sulfur atom, which aryl and heteroaryl groups in each case are unsubstituted or mono- or polysubstituted;

and R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, furanoyl, thienoyl, or a mono- or bicyclic heteroaryl group comprising one, two or three ring nitrogen atoms, zero or one ring oxygen atom and zero or one ring sulphur atom, which aryl and heteroaryl groups in each case are unsubstituted or mono- or polysubstituted;

or wherein R_1 and R_2 together represent alkylene with four or five carbon atoms, optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms in the alkylene group; oxaalkylene with one oxygen and three or four carbon atoms, or azaalkylene with one nitrogen and three or four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

More particular, the invention relates to compounds of formula I, wherein

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or two identical or different radicals R_3, cyclopentyl, benzcyclopentyl, cylcohexyl, pyrrolidinyl, oxazolinyl, piperidinyl, N-substituted piperidinyl, morpholinyl, azepinyl, oxo-azepinyl, oxazepinyl, phenyl, naphthalinyl, tetrahydronaphthalinyl or a mono- or bicyclic heteroaryl group comprising one or two nitrogen atoms, which phenyl, naphthalinyl and heteroaryl groups in each case are unsubstituted or mono- or polysubstituted, thienyl, or lower alkoxycarbonyl-lower alkylthienyl;

and R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, lower alkylamino, di-lower alkylamino, phenylamino, N-lower alkyl-N-phenylamino, pyrrolidino, oxopyrrolidino, piperidino, morpholino, imidazolino, oxoimidazolino, cycloalkyl, heterocyclyl, furyl, phenyl, naphthalinyl, tetrahydronaphthalinyl, or a mono- or bicyclic heteroaryl group comprising one or two nitrogen atoms, which phenyl, naphthalinyl and heteroaryl group are unsubstituted or mono- or polysubstituted;

or wherein R_1 and R_2 together represent alkylene with four or five carbon atoms, optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms in the alkylene group; oxaalkylene with one oxygen and four carbon atoms; or azaalkylene with one nitrogen and four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

More particular, the invention relates to compounds of formula I, wherein

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen; lower alkyl, optionally substituted by one radical R_3, by two phenyl groups, by two lower alkoxycarbonyl groups, by phenyl and lower alkoxycarbonyl, or by hydroxyphenyl and lower alkoxycarbonyl; cyclopentyl; benzcyclopentyl; cylcohexyl; pyrrolidinyl; oxazolinyl; piperidinyl; N-lower alkylpiperidinyl; N-benzylpiperidinyl; N-pyrimidinylpiperidinyl; morpholinyl; azepinyl; oxo-azepinyl; oxazepinyl; phenyl, naphthalinyl, tetrahydronaphthalinyl or a mono- or bicyclic heteroaryl group comprising one or two nitrogen atoms, which phenyl, naphthalinyl and heteroaryl groups in each case are unsubstituted or

substituted by one or two substituents selected from the group consisting of lower alkyl, trifluoro-lower alkyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl, amino-lower alkyl, lower alkylamino-lower alkyl, di-lower alkylamino-lower alkyl, N-cyclohexyl-N-lower alkylamino-lower alkyl, lower alkoxycarbonylpiperidino-lower alkyl, N-lower alkylpiperazino-lower alkyl, lower alkoxycarbonyl-lower alkyl, hydroxy, lower alkoxy, trifluoro-lower alkoxy, 1H-imidazolyl-lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, lower alkyl carbamoyl, amino, lower alkanoylamino, benzoylamino, amino mono- or disubstituted by lower alkyl, by hydroxy-lower alkyl or by lower alkoxy-lower alkyl, 1H-imidazolyl, mono- or di-lower alkyl-1H-imidazolyl, pyrrolidino, piperidino, piperazino, N-lower alkylpiperazino, morpholino, sulfamoyl, lower alkylsulfonyl, phenylsulfonyl, lower alkylsulfinyl, phenylsulfinyl, lower alkylthio, phenylthio, phenyl, pyridyl, halogenyl, or benzoyl; thienyl; or lower alkoxycarbonyl-lower alkylthienyl; and

R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, carbamoyl mono-or disubstituted by lower alkyl, phenyl or lower alkylene, amino, lower alkylamino, di-lower alkylamino, phenylamino, N-lower alkyl-N-phenylamino, pyrrolidino, oxopyrrolidino, piperidino, morpholino, imidazolino, oxoimidazolino, cycloalkyl, heterocyclyl, furyl; phenyl, naphthalinyl, tetrahydronaphthalinyl, or a mono- or bicyclic heteroaryl group comprising one or two nitrogen atoms, which phenyl, naphthalinyl and heteroaryl group is unsubstituted or substituted by one or two substituents selected from the group consisting of lower alkyl, trifluoro-lower alkyl, lower alkoxycarbonyl-lower alkyl, hydroxy, lower alkoxy, trifluoro-lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, amino, lower alkanoylamino, benzoylamino, amino mono- or disubstituted by lower alkyl, by hydroxy-lower alkyl or by loweralkoxy-lower alkyl, pyrrolidino, piperidino, morpholino, piperazino, N-lower alkylpiperazino, N-lower alkoxycarbonylpiperazino, phenyl, pyridyl, 1H-imidazolyl, lower alkyl-1H-imidazolyl, sulfamoyl, lower alkylsulfonyl, phenylsulfonyl, lower alkylsulfinyl, phenylsulfinyl, lower alkylthio, phenylthio, halogenyl, or benzoyl;

or wherein R_1 and R_2 together represent alkylene with four or five carbon atoms, optionally mono- or disubstituted by lower alkyl, cycloalkyl, phenyl, hydroxy, lower alkoxy, amino, benzoylamino, piperidino, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms in the alkylene group; oxaalkylene with one oxygen and four carbon atoms; or azaalkylene with one nitrogen and four carbon atoms wherein nitrogen is unsubstituted or

substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, carbamoyl-lower alkyl N-mono- or N,N-disubstituted by lower alkyl, phenyl, lower alkylene or oxa-lower alkylene, cycloalkyl, lower alkoxycarbonyl, phenyl, methoxyphenyl, trifluoromethylphenyl, trifluoromethoxyphenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen or lower alkyl;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

In a preferred group of compounds of formula I,

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, or benzyl;

R_2 represents lower alkyl, optionally substituted by one radical R_3, by two phenyl groups, by two lower alkoycarbonyl groups, by phenyl and lower alkoxycarbonyl, or by hydroxyphenyl and lower alkoxycarbonyl; cyclopentyl; benzcyclopentyl; cylcohexyl; pyrrolidinyl; piperidinyl; N-lower alkylpiperidinyl; N-benzylpiperidinyl; N-pyrimidinylpiperidinyl; morpholinyl; azepinyl; oxoazepinyl; phenyl; naphthalinyl; tetrahydronaphthalinyl; pyridyl; lower alkyl-pyridyl; quinolinyl; thienyl; lower alkoxycarbonylmethylthienyl; or phenyl substituted by one or two substituents selected from the group consisting of lower alkyl, trifluoro-lower alkyl, hydroxy-lower alkyl, amino-lower alkyl, lower alkylamino-lower alkyl, di-lower alkylamino-lower alkyl, N-cyclohexyl-N-lower alkylamino-lower alkyl, lower alkoxycarbonylpiperidino-lower alkyl, N-lower alkylpiperazino-lower alkyl, lower alkoxycarbonyl-lower alkyl, hydroxy, lower alkoxy, trifluoro-lower alkoxy, 1H-imidazolyl-lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, lower alkylcarbamoyl, amino, lower alkanoylamino, benzoylamino, amino mono- or disubstituted by lower alkyl, by hydroxy-lower alkyl or by loweralkoxy-lower alkyl, 1H-imidazolyl, lower alkyl-1H-imidazolyl, pyrrolidino, piperidino, piperazino, N-lower alkylpiperazino, morpholino, sulfamoyl, lower alkylsulfonyl, phenyl, pyridyl, halogenyl, or benzoyl;

and R_3 represents hydroxy, lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, amino, lower alkylamino, di-lower alkylamino, phenylamino, N-lower alkyl-N-phenylamino, pyrrolidino, oxopyrrolidino, piperidino, morpholino, imidazolino,

oxoimidazolino, cyclopropyl, cyclopentyl, cyclohexyl, tetrahydrofuranyl, phenyl, naphthalinyl, tetrahydronaphthalinyl, furyl, a mono- or bicyclic heteroaryl group comprising one or two nitrogen atoms, which heteroaryl group is unsubstituted or mono- or disubstituted by lower alkyl, hydroxy and lower alkoxy, or phenyl substituted by one or two substituents selected from the group consisting of lower alkyl, trifluoro-lower alkyl, lower alkoxycarbonyl-lower alkyl, hydroxy, lower alkoxy, trifluoro-lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, amino, lower alkanoylamino, benzoylamino, amino mono- or disubstituted by lower alkyl, by hydroxy-lower alkyl or by loweralkoxy-lower alkyl, pyrrolidino, piperidino, morpholino, piperazino, N-lower alkylpiperazino, N-lower alkoxycarbonylpiperazino, phenyl, pyridyl, 1H-imidazolyl, lower alkyl-1H-imidazolyl, sulfamoyl, lower alkylsulfonyl, halogenyl, or benzoyl;

or wherein R_1 and R_2 together represent alkylene with four or five carbon atoms, optionally mono- or disubstituted by phenyl, hydroxy, amino, benzoylamino, or piperidino; benzalkylene with four or five carbon atoms in the alkylene group; oxaalkylene with one oxygen and four carbon atoms; or azaalkylene with one nitrogen and four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carbamoyl-lower alkyl, pyrrolidinocarbonyl-lower alkyl, morpholinocarbonyl-lower alkyl, cyclopentyl, lower alkoxycarbonyl, phenyl, methoxyphenyl, trifluoromethylphenyl, pyridinyl; pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen or methyl;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

A specially preferred group of compounds comprises compounds of formula I wherein
R_1 represents hydrogen, and
R_2 represents phenyl substituted by trifluoromethyl, especially 3-trifluoromethylphenyl, and optionally a further substituent selected from the group consisting of hydroxy-lower alkyl, e.g. 1-hydroxy-1-methylethyl, lower alkylamino, e.g. methyl- or ethylamino, hydroxy-lower alkylamino, e.g. 2-hydroxy-1-propylamino or 2-hydroxy-2-propylamino, di-lower alkylamino, e.g. diethylamino, 1H-imidazolyl, lower alkyl-1H-imidazolyl, e.g. 2- or 4-methyl-1H-imidazolyl, carbamoyl, lower alkylcarbamoyl, e.g. methylcarbamoyl, pyrrolidino, piperidino, piperazino, lower alkylpiperazino, e.g. 4-methylpiperazino, morpholino, lower alkoxy, e.g. methoxy,

fluoro-lower alkoxy, e.g. trifluoromethoxy or 2,2,2-trifluoroethoxy, phenyl, pyridyl, e.g. 2-, 3- or 4-pyridyl, and halogenyl, e.g.chloro or fluoro;
R_4 represents methyl;
and a N-oxide or a pharmaceutically acceptable salt of such a compound.

One preferred embodiment of the invention relates to compounds of formula I wherein
R_1 is hydrogen,
R_2 represents phenyl which is mono- or disubstituted by imidazol-lower alkoxy, lower alkyl amino, trifluoromethyl, hydroxy lower alkyl amino, bis-(lower alkoxy lower alkyl) amino, lower alkyl piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, phenyl, pyridyl, imidazolyl which is unsubstituted or mono- or disubstituted by lower alkyl or N-lower alkyl carbamoyl;
R_4 is lower alkyl;
and to the N-oxides and pharmaceutically acceptable salts of such compounds.

Particularly preferred are the compounds of the Examples.

Other compounds which are particularly preferred are:
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzanilide,
4-Methyl-*N*-(3-pyridinyl)-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
N-(4-Chlorophenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
2(R)- and 2(S)-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoylamino]propanoic acid,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(8-quinolinyl)benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(3-[trifluoromethoxy]phenyl)benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(2-pyrrolidinoethyl)benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(3-pyrrolidinophenyl)benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(1-[2-pyrimidinyl]-4-piperidinyl)benzamide,
N-(4-Di-[2-methoxyethyl]amino-3-trifluoromethylphenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
N-(4-[1H-Imidazolyl]-3-trifluoromethylphenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(2-pyrrolidino-5-trifluoromethylphenyl)benzamide,
N-(3,4-difluorophenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,

4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(3-trifluoromethylbenzyl)benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(3-trifluoromethylphenyl)benzamide,
N-(3-Chloro-5-trifluoromethylphenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
N-(4-Dimethylaminobutyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-*N*-[4-(4-methyl-1-piperazinyl)-3-trifluoromethylphenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(2,2,2-trifluoroethoxy)-3-trifluoromethylphenyl]benzamide,
4-Methyl-*N*-[4-(2-methyl-1H-imidazolyl)-3-trifluoromethylphenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-*N*-(4-phenyl-3-trifluoromethylphenyl)-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-*N*-[4-(4-methyl-1H-imidazolyl)-3-trifluoromethylphenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
Methyl 2(R)- and 2(S)-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoylamio]-3-[4-hydroxyphenyl]propanoate,
N-[2-(*N*-Cyclohexyl-*N*-methylaminomethyl)phenyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
N-[3-[2-(1H-Imidazolyl)ethoxy]phenyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-*N*-[3-morpholino-5-trifluoromethylphenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(4-pyrrolidino-3-trifluoromethylphenyl)benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(4-piperidino-3-trifluoromethylphenyl)benzamide,
4-Methyl-*N*-[4-morpholino-3-trifluoromethylphenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
N-(4-Ethylamino-3-trifluoromethylphenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-(3-trifluoromethoxyphenyl)benzamide,
N-[4-(2-Hydroxypropylamino)-3-trifluoromethylphenyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,

N-(4-Diethylamino-3-trifluoromethylphenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-(3-pyridinyl)-5-trifluorophenyl]benzamide,
N-[3-[3-(1H-Imidazolyl)propoxy]phenyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(3-pyridinyl)-3-trifluorophenyl]benzamide,
4-Methyl-*N*-[3-(4-methyl-1-piperazinyl)-5-trifluorophenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-*N*-[3-methylcarbamoyl-5-trifluorophenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide,
4-Methyl-*N*-[3-methylcarbamoyl-5-morpholino]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide.

Further compounds which are particularly preferred are:
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-[3-(1H-imidazol-1-yl)propoxy]-phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-[2-(1H-imidazol-1-yl)ethoxy]phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(ethylamino)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(diethylamino)-3-(trifluoromethyl)phenyl]benzamide,
(±)-4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-[(2-hydroxypropyl)amino]-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-[bis(2-methoxyethyl)amino]-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(4-methyl-1-piperazinyl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(1-piperidinyl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(1-pyrrolidinyl)-3-(trifluoromethyl)phenyl]benzamide,

4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(4-morpholinyl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-phenyl-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-N-[3-[4-(3-pyridinyl)-3-(trifluoromethyl)phenyl]methyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(2,4-dimethyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(2-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-(4-morpholinyl)-5-[(methylamino)carbonyl]phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-[(methylamino)carbonyl]-5-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(3-pyridinyl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(4-morpholinyl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(2-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(5-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide,
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-(4-methyl-1-piperazinyl)-5-(trifluoromethyl)phenyl]benzamide, and
4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[2-(1-pyrrolidinyl)-5-(trifluoromethyl)phenyl]benzamide.

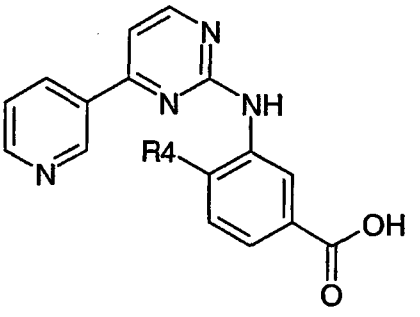
The invention relates also to 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoic acid and to 3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoic acid; intermediates for the formation of the preferred amides of the invention.

Especially, the invention relates to the use of a compound of formula I or of a N-oxide or a possible tautomer thereof or of a pharmaceutically acceptable salt of such a compound for the preparation of a pharmaceutical composition for the treatment of a disease which responds to an inhibition of protein kinase activity, wherein the disease is a neoplastic disease.

More particularly, the invention relates to the use of a compound of the formula I or of a N-oxide or a possible tautomer thereof; or of a pharmaceutically acceptable salt of such a compound for the preparation of a pharmaceutical composition for the treatment of leukaemia which responds to an inhibition of the Abl tyrosine kinase activity.

Furthermore, the invention provides a method for the treatment of a disease which responds to an inhibition of protein kinase activity, which comprises administering a compound of formula I or a N-oxide or a pharmaceutically acceptable salt thereof, wherein the radicals and symbols have the meanings as defined above, in a quantity effective against said disease, to a warm-blooded animal requiring such treatment.

A compound of the invention may be prepared by processes that, though not applied hitherto for the new compounds of the present invention, are known per se, especially a process characterized in that for the synthesis of a compound of the formula I wherein the symbols R_1, R_2 and R_4 are as defined for a compound of the formula I, a 4- R_4-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid of formula II



(II)

wherein R_4 is as defined for a compound of formula I, or a derivative thereof wherein the carboxy group –COOH is in activated form, is reacted with an amine of the formula III

$$R_1\text{-NH-}R_2 \qquad \text{(III)}$$

wherein R_1 and R_2 are as defined for a compound of the formula I, optionally in the presence of a dehydrating agent and an inert base and/or a suitable catalyst, and optionally in the presence of an inert solvent;

where the above starting compounds II and III may also be present with functional groups in protected form if necessary and/or in the form of salts, provided a salt-forming group is present and the reaction in salt form is possible;

any protecting groups in a protected derivative of a compound of the formula I are removed;

and, if so desired, an obtainable compound of formula I is converted into another compound of formula I or a N-oxide thereof, a free compound of formula I is converted into a salt, an obtainable salt of a compound of formula I is converted into the free compound or another salt, and/or a mixture of isomeric compounds of formula I is separated into the individual isomers.

Detailed description of the process:

A derivative of the compound of formula II wherein the carboxy group is in activated form is especially a reactive ester, a reactive anhydride or a reactive cyclic amide.

Reactive esters of the acid of formula II are especially esters unsaturated at the linking carbon atom of the esterifying radical, for example esters of the vinyl ester type, such as actual vinyl esters (obtainable, for example, by transesterification of a corresponding ester with vinyl acetate; activated vinyl ester method), carbamoylvinyl esters (obtainable, for example, by treatment of the corresponding acid with an isoxazolium reagent; 1,2-oxazolium or Woodward method), or 1-lower alkoxyvinyl esters (obtainable, for example, by treatment of the corresponding acid with a lower alkoxyacetylene; ethoxyacetylene method), or esters of the amidino type, such as N,N'-disubstituted amidino esters (obtainable, for example, by

treatment of the corresponding acid with a suitable N,N'-disubstituted carbodiimide, for example N,N'-dicyclohexylcarbodiimide; carbodiimide method), or N,N-disubstituted amidino esters (obtainable, for example, by treatment of the corresponding acid with an N,N-disubstituted cyanamide; cyanamide method), suitable aryl esters, especially phenyl esters suitably substituted by electron-attracting substituents (obtainable, for example, by treatment of the corresponding acid with a suitably substituted phenol, for example 4-nitrophenol, 4-methylsulfonyl-phenol, 2,4,5-trichlorophenol, 2,3,4,5,6-pentachloro-phenol or 4-phenyldiazophenol, in the presence of a condensation agent, such as N,N'-dicyclohexyl-carbodiimide; activated aryl esters method), cyanomethyl esters (obtainable, for example, by treatment of the corresponding acid with chloroacetonitrile in the presence of a base; cyanomethyl esters method), thio esters, especially unsubstituted or substituted, for example nitro-substituted, phenylthio esters (obtainable, for example, by treatment of the corresponding acid with unsubstituted or substituted, for example nitro-substituted, thiophenols, inter alia by the anhydride or carbodiimide method; activated thiol esters method), amino or amido esters (obtainable, for example, by treatment of the corresponding acid with an N-hydroxy-amino or N-hydroxy-amido compound, for example N-hydroxy-succinimide, N-hydroxy-piperidine, N-hydroxy-phthalimide or 1-hydroxy-benzotriazole, for example by the anhydride or carbodiimide method; activated N-hydroxy esters method), or silyl esters (which are obtainable, for example, by treatment of the corresponding acid with a silylating agent, for example hexamethyl disilazane, and react readily with hydroxy groups but not with amino groups).

Anhydrides of the acid of formula II may be symmetric or preferably mixed anhydrides of that acid, for example anhydrides with inorganic acids, such as acid halides, especially acid chlorides (obtainable, for example, by treatment of the corresponding acid with thionyl chloride, phosphorus pentachloride or oxalyl chloride; acid chloride method), azides (obtainable, for example, from a corresponding acid ester via the corresponding hydrazide and treatment thereof with nitrous acid; azide method), anhydrides with carbonic acid semiderivatives, such as corresponding esters, for example carbonic acid lower alkyl semiesters (obtainable, for example, by treatment of the corresponding acid with haloformic, such as chloroformic, acid lower alkyl esters or with a 1-lower alkoxycarbonyl-2-lower alkoxy-1,2-dihydroquinoline, for example 1-lower alkoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; mixed O-alkylcarbonic acid anhydrides method), or anhydrides with dihalogenated, especially dichlorinated, phosphoric acid (obtainable, for example, by treatment of the

corresponding acid with phosphorus oxychloride; phosphorus oxychloride method), or anhydrides with organic acids, such as mixed anhydrides with organic carboxylic acids (obtainable, for example, by treatment of the corresponding acid with an unsubstituted or substituted lower alkane- or phenylalkane-carboxylic acid halide, for example phenylacetic acid chloride, pivalic acid chloride or trifluoroacetic acid chloride; mixed carboxylic acid anhydrides method), with organic sulfonic acids (obtainable, for example, by treatment of a salt, such as an alkali metal salt, of the corresponding acid, with a suitable organic sulfonic acid halide, such as lower alkane- or aryl-, for example methane- or p-toluene-sulfonic acid chloride; mixed sulfonic acid anhydrides method), or with organic phosphonic acids (obtainable, for example, by treatment of the corresponding acid with a suitable organic phosphonic anhydride or phosphonic cyanide; mixed phosphonic acid anhydrides methhod), and symmetric anhydrides (obtainable, for example, by condensation of the corresponding acid in the presence of a carbodiimide or of 1-diethylaminopropyne; symmetric anhydrides method).

Suitable cyclic amides are especially amides with five-membered diazacycles of aromatic character, such as amides with imidazoles, for example imidazole (obtainable, for example, by treatment of the corresponding acid with N,N'-carbonyldiimidazole; imidazolide method), or pyrazoles, for example 3,5-dimethyl-pyrazole (obtainable, for example, by way of the acid hydrazide by treatment with acetylacetone; pyrazolide method).

Derivatives of the acid of formula II wherein the carboxy group is in activated form are preferably formed in situ. For example, N,N'-disubstituted amidino esters can be formed in situ by reacting a mixture of the acid of formula II and the amine of formula III in the presence of a suitable N,N-disubstituted carbodiimide, for example N,N'-dicyclohexylcarbodiimide. Reactive mixed anhydrides of the acid of formula II with an organic phosphonic acid may be formed in situ by reaction with e.g. propylphosphonic anhydride or diethylcyanophosphonate in the presence of suitable base, preferably a tertiary amine, e.g. triethylamine or dimethylaminopyridine.

The reaction can be carried out in a manner known per se, the reaction conditions being dependent especially on whether, and if so how, the carboxy group of the carboxylic acid of formula II has been activated, usually in the presence of a suitable solvent or diluent or of a mixture thereof and, if necessary, in the presence of a condensation agent, which, for

example when the carboxy group participating in the reaction is in the form of an anhydride, may also be an acid-binding agent, with cooling or heating, for example in a temperature range from approximately -30 °C to approximately +150 °C, especially approximately from 0 °C to +100 °C, preferably from room temperature (approx. +20 °C) to +70 °C, in an open or closed reaction vessel and/or in the atmosphere of an inert gas, for example nitrogen. Customary condensation agents are, for example, carbodiimides, for example N,N'-diethyl-, N,N'-dipropyl-, N,N'-dicyclohexyl- or N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide, suitable carbonyl compounds, for example carbonyldiimidazole, or 1,2-oxazolium compounds, for example 2-ethyl-5-phenyl-1,2-oxazolium 3'-sulfonate and 2-tert-butyl-5-methyl-isoxazolium perchlorate, or a suitable acylamino compound, for example 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline. Customary acid-binding condensation agents are, for example, alkali metal carbonates or hydrogen carbonates, for example sodium or potassium carbonate or hydrogen carbonate (customarily together with a sulfate), or organic bases, such as, customarily, pyridine or triethylamine, or sterically hindered tri-lower alkylamines, for example N,N-diisopropyl-N-ethyl-amine.

In a preferred variant, the carboxylic acid of formula II is reacted with an amine of formula III in a suitable solvent, such as e.g. N,N-dimethylformamide, in the presence of propyl-phosphonic anhydride or diethylcyanophosphanate and triethylamine, between 1 and 48 hours at between 0°C and around 50°C, preferably at room temperature.

Protecting groups

If one or more other functional groups, for example carboxy, hydroxy, amino, or mercapto, are or need to be protected in a compound of formula III, because they should not take part in the reaction, these are such groups as are usually used in the synthesis of amides, in particular peptide compounds, and also of cephalosporins and penicillins, as well as nucleic acid derivatives and sugars.

The protecting groups may already be present in precursors and should protect the functional groups concerned against unwanted secondary reactions, such as acylations, etherifications, esterifications, oxidations, solvolysis, and similar reactions. It is a characteristic of protecting groups that they lend themselves readily, i.e. without undesired secondary reactions, to removal, typically by solvolysis, reduction, photolysis or also by

enzyme activity, for example under conditions analogous to physiological conditions, and that they are not present in the end-products.The specialist knows, or can easily establish, which protecting groups are suitable with the reactions mentioned hereinabove and hereinafter.

The protection of such functional groups by such protecting groups, the protecting groups themselves, and their removal reactions are described for example in standard reference books for peptide synthesis as cited hereinbefore, and in special books on protective groups such as J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London and New York 1973, in "Methoden der organischen Chemie" (Methods of organic chemistry), Houben-Weyl, 4th edition, Volume 15/I, Georg Thieme Verlag, Stuttgart 1974, and in T. W. Greene, "Protective Groups in Organic Synthesis", Wiley, New York.

Additional process steps

In the additional process steps, carried out as desired, functional groups of the starting compounds which should not take part in the reaction may be present in unprotected form or may be protected for example by one or more of the protecting groups mentioned hereinabove under "protecting groups". The protecting groups are then wholly or partly removed according to one of the methods described there.

Salts of a compound of formula I with a salt-forming group may be prepared in a manner known per se. Acid addition salts of compounds of formula I may thus be obtained by treatment with an acid or with a suitable anion exchange reagent.

Salts can usually be converted to free compounds, e.g. by treating with suitable basic agents, for example with alkali metal carbonates, alkali metal hydrogencarbonates, or alkali metal hydroxides, typically potassium carbonate or sodium hydroxide.

Stereoisomeric mixtures, e.g. mixtures of diastereomers, can be separated into their corresponding isomers in a manner known per se by means of suitable separation methods. Diastereomeric mixtures for example may be separated into their individual diastereomers by means of fractionated crystallization, chromatography, solvent distribution, and similar procedures. This separation may take place either at the level of a starting compound or in a compound of formula I itself. Enantiomers may be separated through the formation of

diastereomeric salts, for example by salt formation with an enantiomer-pure chiral acid, or by means of chromatography, for example by HPLC, using chromatographic substrates with chiral ligands.

A compound of the formula I wherein R_1 is hydrogen can be converted to the respective compound wherein R_1 is lower alkyl by reaction e.g. with a diazo lower alkyl compound, especially diazomethane, in an inert solvent, preferably in the presence of a noble metal catalyst, especially in dispersed form, e.g. copper, or a noble metal salt, e.g. copper(I)-chloride or copper(II)-sulfate. Also reaction with lower alkylhalogenides is possible, or with other leaving group carrying lower alkanes, e.g. lower alkyl alcohols esterified by a strong organic sulfonic acid, such as a lower alkanesulfonic acid (optionally substituted by halogen, such as fluoro), an aromatic sulfonic acid, for example unsubstituted or substituted benzenesulfonic acid, the substituents preferably being selected from lower alkyl, such as methyl, halogen, such as bromo, and/or nitro, e.g. esterified by methanesulfonic acid, or p-toluene sulfonic acid. The alkylation takes place under usual conditions for alkylation of amides, especially in aqueous solution and/or in the presence of polar solvents, typically alcohols, for example methanol, ethanol, isopropanol, or ethylene glycol, or dipolar aprotic solvents, e.g. tetrahydrofuran, dioxane, or dimethylformamide, where applicable in the presence of acidic or basic catalysts, generally at temperatures from about 0°C to the boiling temperature of the corresponding reaction mixture, preferably between 20°C and reflux temperature, if necessary under increased pressure, e.g. in a sealed tube, and/or under inert gas, typically nitrogen or argon.

It should be emphasized that reactions analogous to the conversions mentioned in this chapter may also take place at the level of appropriate intermediates.

General process conditions

All process steps described here can be carried out under known reaction conditions, preferably under those specifically mentioned, in the absence of or usually in the presence of solvents or diluents, preferably such as are inert to the reagents used and able to dissolve these, in the absence or presence of catalysts, condensing agents or neutralising agents, for example ion exchangers, typically cation exchangers, for example in the H^+ form, depending on the type of reaction and/or reactants at reduced, normal, or elevated temperature, for

example in the range from -100°C to about 190°C, preferably from about -80°C to about 150°C, for example at -80 to -60°C, at room temperature, at - 20 to 40°C or at the boiling point of the solvent used, under atmospheric pressure or in a closed vessel, where appropriate under pressure, and/or in an inert atmosphere, for example under argon or nitrogen.

Salts may be present in all starting compounds and transients, if these contain salt-forming groups. Salts may also be present during the reaction of such compounds, provided the reaction is not thereby disturbed.

At all reaction stages, isomeric mixtures that occur can be separated into their individual isomers, e.g. diastereomers or enantiomers, or into any mixtures of isomers, e.g. racemates or diastereomeric mixtures.

The invention relates also to those forms of the process in which one starts from a compound obtainable at any stage as a transient and carries out the missing steps, or breaks off the process at any stage, or forms a starting material under the reaction conditions, or uses said starting material in the form of a reactive derivative or salt, or produces a compound obtainable by means of the process according to the invention and processes the said compound in situ. In the preferred embodiment, one starts from those starting materials which lead to the compounds described hereinabove as preferred, particularly as especially preferred, primarily preferred, and/or preferred above all.

In the preferred embodiment, a compound of formula I is prepared according to or in analogy to the processes and process steps defined in the Examples.

The compounds of formula I, including their salts, are also obtainable in the form of hydrates, or their crystals can include for example the solvent used for crystallization (present as solvates).

Pharmaceutical preparations, methods, and uses

The present invention relates furthermore to a method for the treatment of a neoplastic disease which responds to an inhibition of a protein kinase activity, which comprises

administering a compound of formula I or a N-oxide or a pharmaceutically acceptable salt thereof, wherein the radicals and symbols have the meanings as defined above for formula I, in a quantity effective against said disease, to a warm-blooded animal requiring such treatment.

In particular the invention relates to a method for the treatment of leukaemia which responds to an inhibition of the Abl tyrosine kinase activity, which comprises administering a compound of formula I or a N-oxide or a pharmaceutically acceptable salt thereof, wherein the radicals and symbols have the meanings as defined above for formula I, in a quantity effective against said leukaemia, to a warm-blooded animal requiring such treatment.

The present invention relates also to pharmaceutical compositions that comprise a compound of formula I or a N-oxide thereof as active ingredient and that can be used especially in the treatment of the diseases mentioned at the beginning. Compositions for enteral administration, such as nasal, buccal, rectal or, especially, oral administration, and for parenteral administration, such as intravenous, intramuscular or subcutaneous administration, to warm-blooded animals, especially humans, are especially preferred. The compositions comprise the active ingredient alone or, preferably, together with a pharmaceutically acceptable carrier. The dosage of the active ingredient depends upon the disease to be treated and upon the species, its age, weight, and individual condition, the individual pharmacokinetic data, and the mode of administration.

The present invention relates especially to pharmaceutical compositions that comprise a compound of formula I, a tautomer, a N-oxide or a pharmaceutically acceptable salt, or a hydrate or solvate thereof, and at least one pharmaceutically acceptable carrier.

The invention relates also to pharmaceutical compositions for use in a method for the prophylactic or especially therapeutic management of the human or animal body, to a process for the preparation thereof (especially in the form of compositions for the treatment of tumors) and to a method of treating tumor diseases, especially those mentioned hereinabove.

The invention relates also to processes and to the use of compounds of formula I or N-oxides thereof for the preparation of pharmaceutical preparations which comprise compounds of formula I or N-oxides thereof as active component (active ingredient).

In the preferred embodiment, a pharmaceutical preparation is suitable for administration to a warm-blooded animal, especially humans or commercially useful mammals suffering from a disease responsive to an inhibition of the Abl tyrosine kinase, for example chronic myelogenous leukaemia (CML), and comprises an effective quantity of a compound of formula I or N-oxides thereof for the inhibition of the Bcr-Abl fusion protein, or a pharmaceutically acceptable salt thereof, if salt-forming groups are present, together with at least one pharmaceutically acceptable carrier.

A pharmaceutical composition for the prophylactic or especially therapeutic management of neoplastic and other proliferative diseases of a warm-blooded animal, especially a human or a commercially useful mammal requiring such treatment, especially suffering from such a disease, comprising as active ingredient in a quantity that is prophylactically or especially therapeutically active against the said diseases a novel compound of formula I or N-oxides thereof, is likewise preferred.

The pharmaceutical compositions comprise from approximately 1% to approximately 95% active ingredient, single-dose administration forms comprising in the preferred embodiment from approximately 20% to approximately 90% active ingredient and forms that are not of single-dose type comprising in the preferred embodiment from approximately 5% to approximately 20% active ingredient. Unit dose forms are, for example, coated and uncoated tablets, ampoules, vials, suppositories, or capsules. Further dosage forms are, for example, ointments, creams, pastes, foams, tinctures, sprays, etc. Examples are capsules containing from about 0.05 g to about 1.0 g active ingredient.

The pharmaceutical compositions of the present invention are prepared in a manner known per se, for example by means of conventional mixing, granulating, coating, dissolving or lyophilizing processes.

Preference is given to the use of solutions of the active ingredient, and also suspensions or dispersions, especially isotonic aqueous solutions, dispersions or suspensions which, for example in the case of lyophilized compositions comprising the active ingredient alone or together with a carrier can be made up before use. The pharmaceutical compositions may be sterilized and/or may comprise excipients, for example preservatives, stabilizers, wetting

agents and/or emulsifiers, solubilizers, salts for regulating osmotic pressure and/or buffers and are prepared in a manner known per se, for example by means of conventional dissolving and lyophilizing processes. The said solutions or suspensions may comprise viscosity-increasing agents or solubilizers.

Suspensions in oil comprise as the oil component the vegetable, synthetic, or semi-synthetic oils customary for injection purposes. In respect of such, special mention may be made of liquid fatty acid esters that contain as the acid component a long-chained fatty acid having from 8 to 22 carbon atoms. The alcohol component of these fatty acid esters has a maximum of 6 carbon atoms and is a monovalent or polyvalent, for example a mono-, di- or trivalent, alcohol, especially glycol and glycerol.

Pharmaceutical compositions for oral administration can be obtained, for example, by combining the active ingredient with one or more solid carriers, if desired granulating a resulting mixture, and processing the mixture or granules, if desired or necessary, by the inclusion of additional excipients, to form tablets or tablet cores.

Suitable carriers are especially fillers, such as sugars, cellulose preparations, and/or calcium phosphates, and also binders, such as starches, and/or polyvinylpyrrolidone, and/or, if desired, disintegrators. Additional excipients are especially flow conditioners and lubricants.

Tablet cores can be provided with suitable, optionally enteric, coatings through the use of, inter alia, concentrated sugar solutions which may comprise gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, or coating solutions in suitable organic solvents or solvent mixtures, or, for the preparation of enteric coatings, solutions of suitable cellulose preparations.

Pharmaceutical compositions for oral administration also include hard capsules consisting of gelatin, and also soft, sealed capsules consisting of gelatin and a plasticizer. The hard capsules may contain the active ingredient in the form of granules, for example in admixture with fillers, binders, and/or glidants, and optionally stabilizers. In soft capsules, the active ingredient is preferably dissolved or suspended in suitable liquid excipients, to which stabilizers and detergents may also be added.

Pharmaceutical compositions suitable for rectal administration are, for example, suppositories that consist of a combination of the active ingredient and a suppository base.

For parenteral administration, aqueous solutions of an active ingredient in water-soluble form, for example of a water-soluble salt, or aqueous injection suspensions that contain viscosity-increasing substances, for example sodium carboxymethylcellulose, sorbitol and/or dextran, and, if desired, stabilizers, are especially suitable. The active ingredient, optionally together with excipients, can also be in the form of a lyophilizate and can be made into a solution before parenteral administration by the addition of suitable solvents.

Solutions such as are used, for example, for parenteral administration can also be employed as infusion solutions.

Preferred preservatives are, for example, antioxidants, such as ascorbic acid, or microbicides, such as sorbic acid or benzoic acid.

The invention relates likewise to a process or a method for the treatment of one of the pathological conditions mentioned hereinabove, especially a disease which responds to an inhibition of a tyrosine kinase, especially a corresponding neoplastic disease. The compounds of formula I or N-oxides thereof can be administered as such or especially in the form of pharmaceutical compositions, prophylactically or therapeutically, preferably in an amount effective against the said diseases, to a warm-blooded animal, for example a human, requiring such treatment. In the case of an individual having a bodyweight of about 70 kg the daily dose administered is from approximately 0.05 g to approximately 5 g, preferably from approximately 0.25 g to approximately 1.5 g, of a compound of the present invention.

The present invention relates especially also to the use of a compound of formula I or N-oxides thereof, or a pharmaceutically acceptable salt thereof, especially a compound of formula I which is said to be preferred, or a pharmaceutically acceptable salt thereof, as such or in the form of a pharmaceutical formulation with at least one pharmaceutically acceptable carrier for the therapeutic and also prophylactic management of one or more of the diseases mentioned hereinabove, preferably a disease which responds to an inhibition of a protein kinase, especially a neoplastic disease, more especially leukaemia which responds to an inhibition of the Abl tyrosine kinase.

The preferred dose quantity, composition, and preparation of pharmaceutical formulations (medicines) which are to be used in each case are described above.

Starting materials

New starting materials and/or intermediates, as well as processes for the preparation thereof, are likewise the subject of this invention. In the preferred embodiment, such starting materials are used and reaction conditions so selected as to enable the preferred compounds to be obtained.

The substituted aminobenzoic acid of formula II, for example, can be obtained by reaction of an ester of 3-amino-4-R_4-benzoic acid, e.g. 3-amino-4-methylbenzoic acid, with cyanamide and condensing the obtainable guanidine with 3-(dimethylamino)-1-(3-pyridinyl)-2-propen-1-one, and finally hyrolysing the ester function.

Starting materials of the formula III are known, commercially available, or can be synthesized in analogy to or according to methods that are known in the art.

The following Examples serve to illustrate the invention without limiting the invention in its scope.

Abbreviations

DMSO	dimethylsulfoxide
HPLC/MS-MS	high-pressure liquid chromatography/ tandem mass spectrometry
min	minutes
m.p.	melting point
NMP	N-methyl-pyrrolidone
NMR	nuclear magnetic resonance
PEG	polyethylen glycol
THF	tetrahydrofuran

Examples

Example 1: *N*-(2-Furanylmethyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide

A solution containing ~50% of propylphosphonic anhydride in *N,N*-dimethylformamide (Fluka, Buchs, Switzerland; 674 µL, ~1 mmol) is added within 20 minutes to a stirred mixture of 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid (214.4 mg, 0.7 mmol), furfurylamine (Aldrich, Buchs, Switzerland; 61.8 µL, 0.7 mmol) and triethylamine (776 µL, 5.6 mmol) in 2 mL *N,N*-dimethylformamide. After stirring for 24 hours at room temperature, the mixture is treated with a half-saturated aqueous solution of sodium hydrogen carbonate and extracted three times with ethyl acetate. The solvent is evaporated off under reduced pressure and the residue dried *in vacuo*. The crude product is crystallised from dichloromethane to give the title compound as a crystalline solid.

^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.28 (s, 3H); 4.43 (d, 2H); 6.23 (m, 1H); 6.33-6.37 (m, 1H); 7.30 (d,1H); 7.42 (d, 1H); 7.49 (ddd, 1H); 7.53 (m, 1H); 7.59 (dd, 1H); 8.11 (d, 1H); 8.38 (m, 1H); 8.49 (d, 1H); 8.66 (dd, 1H); 8.87 (t, 1H); 9.05 (s, 1H); 9.22 (m, 1H).

The starting material is prepared as follows:

Example 1a: 3-[(Aminoiminomethyl)amino]-4-methyl-benzoic acid ethyl ester mononitrate

Cyanamide (Fluka, Buchs, Switzerland; 77.4 g, 1.842 mol) is added to a solution of 3-amino-4-methylbenzoic acid ethyl ester (J. Med. Chem. 16, 118-122, 1973; 150 g, 0.837 mol) in 850 mL of ethanol. Hydrochloric acid (Fluka, Buchs, Switzerland; 108 mL of 12M, 1.27 mol) is then added dropwise over 15 min and the reaction mixture is then stirred at 90°C (bath temperature) for 15 hours. The solvent is evaporated off under reduced pressure to give a residue which is treated with water (1000 mL) and stirred with cooling at 5-10°C. A solution of ammonium nitrate (Merck, Darmstadt, Germany; 134.8 g, 1.68 mol) in water (400 mL) is added dropwise over 30 min. followed by ice-water (1200 mL). After stirring for an additional 30 min. the product is filtered off, washed with ice-water (3 x 1000 mL) and air-dried. The residue is washed with diethyl ether (2 x 2000 m L) and dried *in vacuo* at 50° to give the title compound as a crystalline solid, m.p.195-197°C.

Example 1b: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid ethyl ester

A stirred mixture of the intermediate Example 1a (164 g, 0.577 mol), 3-(dimethylamino)-1-(3-pyridinyl)-2-propen-1-one (113.8 g, 0.646 mol) and powdered NaOH (99%; Merck,

Darmstadt, Germany; 26.6 g, 0.658 mol) in ethanol (2200 mL) is heated under reflux for 68 h. The reaction solvent is evaporated off under reduced pressure and the residue partitioned between ethyl acetate and water. The organic layer is separated and the aqueous phase extracted twice with ethyl acetate. The combined organic extracts are washed with water and brine, dried (Na_2SO_4) and the solvent is evaporated off under reduced pressure to give a residue, which is crystallised from diethyl ether to give the title compound as a crystalline solid, m.p. 95-96°C.

Example 1c: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid

Aqueous sodium hydroxide (500 mL of 2M) is added dropwise to a stirred suspension of the intermediate Example 1b (132.8 g, 0.397 mol) in ethanol (1200 m L) and water (1200 mL). The reaction mixture is stirred at 45°C for 2.5 h and then treated dropwise with aqueous HCl (1000 mL of 1M) over 1.5 hours. After addition of water (1000 mL) the precipitate is filtered off, washed with water (4 x 500 mL) and dried at room temperature. Residual water present in the air-dried product is removed by azeotropic distillation with toluene under reduced pressure. The dried toluene suspension is diluted with diethyl ether and filtered. The solid residue is washed with diethyl ether and dried *in vacuo* at 80°C to give the title compound, m.p. 277-278°C.

Example 2: *N*-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-4-[(4-methyl-1-piperazinyl)methyl]benzeneamine

A solution containing ~50% of propylphosphonic anhydride in *N,N*-dimethylformamide (Fluka, Buchs, Switzerland; 875 µL, ~1.5 mmol) is added within 20 minutes to a stirred mixture of 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid (306 mg, 1.0 mmol), 4-[(4-methyl-1-piperazinyl)methyl]benzeneamine (Chem. Abstr. Reg. Number: 70261-82-4; 205 mg, 1.0 mmol) and triethylamine (830 µL, 6.0 mmol) in 8 mL *N,N*-dimethylformamide. After stirring for 24 hours at room temperature, the mixture is treated with a saturated aqueous ammonium chloride and extracted three times with ethyl acetate. The solvent is evaporated off under reduced pressure and the residue dried *in vacuo*. The crude product is crystallised from ethanol-ethyl acetate to give the title compound as a crystalline solid, m.p. 153-155°C.

Example 3: 1-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-4-(2-pyridinyl)-piperazine

A solution containing ~50% of propylphosphonic anhydride in *N,N*-dimethylformamide (Fluka, Buchs, Switzerland; 674 µL, ~1 mmol) is added within 20 minutes to a stirred mixture of 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid (214.4 mg, 0.7 mmol), 1-(2-pyridyl)piperazine (Aldrich, Buchs, Switzerland; 114.3 mg, 0.7 mmol) and triethylamine (776 µL, 5.6 mmol) in 2 mL *N,N*-dimethylformamide. After stirring for 24 hours at room temperature, the mixture is treated with a half-saturated aqueous solution of sodium hydrogen carbonate and extracted three times with ethyl acetate. The solvent is evaporated off under reduced pressure and the residue dried *in vacuo*. The crude product is purified by column chromatography on silica gel, eluent 5-10% methanol in dichloromethane, to give the title compound as a solid. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.31 (s, 3H); 3.35-3.74 (m, 8H); 6.65 (ddd, 1H); 6.79 (d, 1H); 7.13 (dd, 1H); 7.32 (d, 1H); 7.44 (d, 1H); 7.49-7.56 (m, 2H); 7.69 (m, 1H); 8.11 (m, 1H); 8.40 (m, 1H); 8.52 (d, 1H); 8.66 (dd, 1H); 9.06 (s, 1H); 9.24 (m, 1H).

The following compounds are prepared analogously by utilising the appropriate amine (supplier in parenthesis):

Example 4: 4-Methyl-*N*-[2-(2-pyridinyl)ethyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 2-(2-aminoethyl)pyridine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.27 (s, 3H); 2.97 (t, 2H); 3.58 (m, 2H); 7.18 (ddd, 1H); 7.25 (m, 1H); 7.29 (d,1H); 7.42 (d, 1H); 7.47-7.56 (m, 2H); 7.65 (m, 1H); 8.06 (d, 1H); 8.39 (m, 1H); 8.44-8.51 (m, 3H); 8.66 (dd, 1H); 9.04 (s, 1H); 9.22 (m, 1H).

Example 5: 4-Methyl-*N*-[1-(phenylmethyl)-4-piperidinyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzamide utilising 4-amino-1-benzylpiperidine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.47-1.63 (m, 2H); 1.69-1.80 (m, 2H); 1.92-2.05 (m, 2H); 2.27 (s, 3H); 2.73-2.83 (m, 2H); 3.43 (s, 2H); 3.68-3.83 (m, 1H); 7.18-7.33 (m ,6H); 7.42 (d, 1H); 7.49 (ddd, 1H); 7.55 (dd, 1H); 8.10 (m, 1H); 8.14 (d, 1H); 8.37 (m, 1H); 8.49 (d, 1H); 8.65 (dd, 1H); 9.04 (s, 1H); 9.21 (m, 1H).

Example 6: 4-Methyl-*N*-(4-pyridinylmethyl)-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising 4-(aminomethyl)pyridine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.30 (s, 3H); 4.46 (d, 2H); 7.26 (m, 2H); 7.33 (d, 1H); 7.43 (d, 1H); 7.47 (ddd, 1H);7.62 (dd, 1H); 8.16 (d, 1H); 8.38 (m, 1H); 8.45 (m, 2H); 8.50 (d, 1H); 8.66 (dd, 1H); 9.03 (t, 1H); 9.08 (s, 1H); 9.23 (m, 1H).

Example 7: 4-Methyl-*N*-[2-(1-methyl-1H-pyrrol-2-yl)ethyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzamide utilising 2-(2-aminoethyl)-1-methylpyrrol [Chem. Abstr. Reg. Number: 83732-75-6]. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.28 (s, 3H); 2.75 (t, 2H); 3.42 (m, 2H); 3.51 (s, 3H); 5.76-5.85 (m, 2H); 6.57 (m,1H); 7.30 (d,1H); 7.43 (d, 1H); 7.46-7.58 (m, 2H); 8.10 (br. 1H); 8.40 (m, 1H); 8.48-8.55 (m, 2H); 8.64-8.69 (m, 1H); 9.05 (s, 1H); 9.23 (m, 1H).

Example 8: *N*-[(4-Methoxyphenyl)methyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 4-methoxybenzylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.28 (s, 3H); 3.69 (s, 3H); 4.37 (d, 2H); 6.80-6.87 (m, 2H); 7.17-7.23 (m, 2H); 7.31 (d,1H); 7.42 (d, 1H); 7.47 (ddd, 1H); 7.59 (dd, 1H); 8.11 (d, 1H); 8.38 (m, 1H); 8.49 (d, 1H); 8.66 (dd, 1H); 8.87 (t, 1H); 9.05 (s, 1H); 9.23 (m, 1H).

Example 9: 4-Methyl-*N*-(2-methylpropyl)-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising isobutylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 0.85 (d, 6H); 1.81 (m, 1H); 2.27 (s, 3H); 3.04 (m, 2H); 7.29 (d,1H); 7.42 (d, 1H); 7.48 (dd, 1H); 7.55 (dd, 1H); 8.07 (d, 1H); 8.31-8.41 (m, 2H); 8.49 (d, 1H); 8.65 (dd, 1H); 9.05 (s, 1H); 9.22 (m, 1H).

Example 10: 4-Methyl-*N*-(2-morpholinoethyl)-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 4-(2-aminoethyl)morpholine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.28 (s, 3H); 2.33-2.46 (m, 6H); 3.30-3.40 (m, 2H); 3.53 (m, 4H); 7.30 (d,1H); 7.42 (d, 1H); 7.46-7.57 (m, 2H); 8.06 (d, 1H); 8.30 (m, 1H); 8.38 (m, 1H); 8.49 (d, 1H); 8.66 (dd, 1H); 9.05 (s, 1H); 9.22 (m, 1H).

Example 11: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[(tetrahydro-2-furanyl)-methyl]benzamide utilising tetrahydrofurfurylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.49-1.63 (m, 1H); 1.70-1.93 (m, 3H); 2.27 (s, 3H); 3.27 (m, 2H); 3.58 (m, 1H); 3.72 (m, 1H); 3.94 (m, 1H); 7.29 (d,1H); 7.42 (d, 1H); 7.49 (ddd, 1H); 7.56 (dd, 1H); 8.08 (d, 1H); 8.35-8.45 (m, 2H); 8.49 (d, 1H); 8.66 (dd, 1H); 9.04 (s, 1H); 9.21 (m, 1H).

Example 12: *N*-[2-(2,4-Dihydroxy-5-pyrimidinyl)ethyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising 5-(2-aminoethyl)-2,4(1H,3H)-pyrimidinedione [Chem. Abstr. Reg. Number: 221170-25-8]. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.27 (s, 3H); 2.40 (t,

2H); 3.34 (m, 2H); 7.15 (m,1H); 7.29 (d,1H); 7.42 (d, 1H); 7.47-7.55 (m, 2H); 8.07 (d, 1H); 8.35-8.42 (m, 2H); 8.49 (d, 1H); 8.66 (dd, 1H); 9.04 (s, 1H); 9.22 (m, 1H); 10.59 (s, 1H); 11.01 (s, 1H).

Example 13: *N*-Cyclohexyl-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising cyclohexylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.00-1.16 (m, 1H); 1.18-1.36 (m, 4H); 1.52-1.85 (m, 5H); 2.27 (s, 3H); 3.66-3.82 (m, 1H); 7.28 (d, 1H); 7.41 (d, 1H); 7.48 (m, 1H); 7.55 (dd, 1H); 8.06-8.12 (m, 2H); 8.37 (m, 1H); 8.49 (d, 1H); 8.66 (dd, 1H); 9.04 (s, 1H); 9.21 (m, 1H).

Example 14: *N*-[(3*S*)-Hexahydro-2-oxo-1H-azepin-3-yl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising L(-)-alpha-amino-epsilon-caprolactam [Chem. Abstr. Reg. Number: 21568-87-6]. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.11-1.31 (m, 1H); 1.37-1.82 (m, 3H); 1.83-1.96 (m, 2H); 2.28 (s, 3H); 3.00-3.13 (m, 1H); 3.15-3.30 (m, 1H); 4.58 (m, 1H); 7.32 (d,1H); 7.43 (d, 1H); 7.51 (ddd, 1H); 7.55 (dd, 1H); 7.84 (m, 1H); 8.08 (d, 1H); 8.13 (d, 1H); 8.40 (m, 1H); 8.50 (d, 1H); 8.66 (dd, 1H); 9.06 (s, 1H); 9.22 (m, 1H).

Example 15: *N*-[2-(3,4-Dimethoxyphenyl)ethyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzamide utilising 2-(3,4-dimethoxyphenyl)ethylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.27 (s, 3H); 2.75 (t, 2H); 3.43 (m, 2H); 3.67 (s, 6H); 6.70 (dd, 1H); 6.77-6.83 (m, 2H); 7.30 (d,1H); 7.42 (d, 1H); 7.46-7.57 (m, 2H); 8.07 (d, 1H); 8.36-8.46 (m, 2H); 8.49 (d, 1H); 8.66 (dd, 1H); 9.05 (s, 1H); 9.22 (m, 1H).

Example 16: 2-[[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]amino]-4-thiazole-acetic acid ethyl ester utilising ethyl 2-amino-4-thiazoleacetate (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.16 (t, 3H); 2.32 (s, 3H); 3.70 (s, 2H); 4.06 (q, 2H); 7.01 (s, 1H); 7.36 (d,1H); 7.42-7.54 (m, 2H); 7.82 (d, 1H); 8.34-8.47 (m, 2H); 8.52 (d, 1H); 8.66 (m, 1H); 9.08 (s, 1H); 9.24 (m, 1H); 12.57 (br., 1H).

Example 17: *N*-[3-(1H-Imidazol-1-yl)propyl]-4-methyl-3-[[4-(3-pyridinyl)-2pyrimidinyl]amino]-benzamide utilising 1-(3-aminopropyl)imidazole (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.96 (qui, 2H); 2.30 (s, 3H); 3.24 (m, 2H); 4.01 (t, 2H); 6.91 (s,1H); 7.22 (m, 1H); 7.34 (d,1H); 7.45 (d, 1H); 7.51 (ddd, 1H); 7.59 (dd, 1H); 7.70 (s, 1H); 8.14 (d, 1H); 8.42 (m, 1H); 8.47 (t, 1H); 8.52 (d, 1H); 8.68 (dd, 1H); 9.10 (s, 1H); 9.25 (m, 1H).

Example 18: *N*-(Cyclopropylmethyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising cyclopropanemethylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 0.17-0.22 (m, 2H); 0.36-0.42 (m, 2H); 0.96-1.06 (m, 1H); 2.28 (s, 3H); 3.11 (m, 2H); 7.31 (d,1H); 7.43 (d, 1H); 7.50 (ddd, 1H); 7.58 (dd, 1H); 8.10 (d, 1H); 8.40 (m, 1H); 8.47 (t, 1H); 8.50 (d, 1H); 8.67 (dd, 1H); 9.07 (s, 1H); 9.23 (m, 1H).

Example 19: *N*-(2-methoxyethyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising 2-methoxyethylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.28 (s, 3H); 3.23 (s, 3H); 3.36-3.46 (m, 4H); 7.31 (d,1H); 7.43 (d, 1H); 7.51 (ddd, 1H); 7.57 (dd, 1H); 8.10 (d, 1H); 8.38-8.47 (m, 2H); 8.50 (d, 1H); 8.68 (dd, 1H); 9.07 (s, 1H); 9.23 (m, 1H).

Example 20: 4-Methyl-*N*-[3-(2-oxo-1-pyrrolidinyl)propyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzamide utilising 1-(3-aminopropyl)-2-pyrrolidinone (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.67 (m, 2H); 1.89 (m, 2H); 2.18 (t, 2H); 2.28 (s, 3H); 3.19 (m, 4H); 3.32 (m, 2H); 7.30 (d, 1H); 7.42 (d, 1H); 7.49 (ddd, 1H); 7.54 (dd, 1H); 8.09 (d, 1H); 8.31-8.42 (m, 2H); 8.49 (d, 1H); 8.66 (dd, 1H); 9.04 (s, 1H); 9.22 (m, 1H).

Example 21: *N*,4-Dimethyl-N-(phenylmethyl)-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising N-benzylmethylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.28 (s, 3H); 2.86 (s, 3H); 4.51-4.68 (m, 2H); 7.08-7.35 (m,7H); 7.43 (d, 1H); 7.48 (m, 1H); 7.71 (s, 1H); 8.35-8.54 (m, 2H); 8.67 (m, 1H); 8.97-9.09 (m, 1H); 9.24 (m, 1H).

Example 22: *N*-[4-(Acetylamino)phenyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 4-aminoacetanilide (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.01 (s, 3H); 2.32 (s, 3H); 7.38 (d,1H); 7.45 (d, 1H); 7.47-7.54 (m, 3H); 7.63-7.71 (m, 3H); 8.22 (m, 1H); 8.43 (m, 1H); 8.52 (d, 1H); 8.67 (dd, 1H); 9.13 (s, 1H); 9.25 (m, 1H); 9.90 (s, 1H); 10.11 (s, 1H).

Example 23: *N*-(4-Methoxy-2-methylphenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 4-methoxy-2-methylaniline (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.16 (s, 3H); 2.32 (s, 3H); 3.73 (s, 3H); 6.75 (dd, 1H); 6.82 (m, 1H); 7.16

(d, 1H); 7.37 (d,1H); 7.45 (d, 1H); 7.49 (ddd, 1H); 7.69 (dd, 1H); 8.25 (d, 1H); 8.41 (m, 1H); 8.52 (d, 1H); 8.67 (dd, 1H); 9.12 (s, 1H); 9.25 (m, 1H); 9.69 (s, 1H).

Example 24: 4-Methyl-*N*-[4-(methylsulfonyl)benzyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 4-methylsulfonylbenzylamine hydrochloride (Acros, Morris Plains, NJ). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.30 (s, 3H); 3.16 (s, 3H); 4.54 (d, 2H); 7.34 (d,1H); 7.44 (d, 1H); 7.49 (ddd, 1H); 7.55 (m, 2H); 7.63 (dd, 1H); 7.86 (m, 2H); 8.16 (d, 1H); 8.40 (m, 1H); 8.51 (d, 1H); 8.67 (dd, 1H); 9.10 (m, 2H); 9.24 (m, 1H).

Example 25: *N*-[[4-(Dimethylamino)phenyl]methyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzamide utilising 4-(dimethylamino)benzylamine dihydrochloride (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.28 (s, 3H); 2.82 (s, 6H); 4.32 (d, 2H); 6.64 (m, 2H); 7.11 (m, 2H); 7.31 (d, 1H); 7.43 (d, 1H); 7.48 (ddd, 1H); 7.59 (dd, 1H); 8.12 (d, 1H); 8.39 (m, 1H); 8.50 (d, 1H); 8.68 (dd, 1H); 8.81 (t, 1H); 9.07 (s, 1H); 9.24 (m, 1H).

Example 26: *N*-(2-Amino-2-oxoethyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising glycinamide hydrochloride (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.29 (s, 3H); 3.78 (d, 2H); 7.02 (s, 1H); 7.30-7.36 (m, 2H); 7.44 (d, 1H); 7.53 (ddd, 1H); 7.61 (dd, 1H); 8.11 (m, 1H); 8.41 (m, 1H); 8.50 (d, 1H); 8.57 (t, 1H); 8.67 (dd, 1H); 9.08 (s, 1H); 9.24 (m, 1H).

Example 27: *N*-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]glycine methyl ester utilising glycine methylester hydrochloride (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.29 (s, 3H); 3.63 (s, 3H); 3.98 (d, 2H); 7.34 (d,1H); 7.44 (d, 1H); 7.52 (ddd, 1H); 7.59 (dd, 1H); 8.11 (d, 1H); 8.41 (m, 1H); 8.50 (d, 1H); 8.67 (dd, 1H); 8.87 (t, 1H); 9.09 (s, 1H); 9.23 (m, 1H).

Example 28: *N*-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]beta-alanine methyl ester utilising beta-alanine methylester hydrochloride (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.27 (s, 3H); 2.57 (t, 2H); 3.46 (m, 2H); 3.57 (s, 3H); 7.31 (d, 1H); 7.43 (d, 1H); 7.50-7.55 (m, 2H); 8.07 (d, 1H); 8.40 (m, 1H); 8.47 (t, 1H); 8.50 (d, 1H); 8.67 (dd, 1H); 9.07 (s, 1H); 9.23 (m, 1H).

Example 29: *N*-[[4-(Aminosulfonyl)phenyl]methyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzamide utilising p-(aminomethyl)benzenesulfonamide hydrochloride (Sigma, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.29 (s, 3H); 4.51 (d, 2H); 7.30 (s, 2H); 7.34 (d, 1H); 7.43-7.50 (m, 4H); 7.62 (dd, 1H); 7.75 (m, 2H); 8.16 (d, 1H); 8.40 (m, 1H); 8.51 (d, 1H); 8.68 (dd, 1H); 9.06 (t, 1H); 9.09 (s, 1H); 9.24 (m, 1H).

Example 30: *N*-(3-Hydroxypropyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising 3-amino-1-propanol (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.65 (qui, 2H); 2.28 (s, 3H); 3.29 (m, 2H); 3.42 (m, 2H); 4.50 (m, 1H); 7.30 (d, 1H); 7.43 (d, 1H); 7.51 (ddd, 1H); 7.56 (dd, 1H); 8.09 (d, 1H); 8.36-8.43 (m, 2H); 8.50 (d, 1H); 8.67 (dd, 1H); 9.07 (s, 1H); 9.23 (m, 1H).

Example 31: *N,N*-Diethyl-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising diethylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.04 (m, 6H); 2.28 (s, 3H); 3.31 (m, 4H); 7.02 (dd, 1H); 7.27 (d, 1H); 7.44 (d, 1H); 7.51 (ddd, 1H); 7.61 (m, 1H); 8.39 (m, 1H); 8.51 (d, 1H); 8.68 (dd, 1H); 9.01 (s, 1H); 9.23 (m, 1H).

Example 32: *N*-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-(L)-phenylalanine 1,1-dimethylethyl ester utilising L-phenylalanine t-butylester hydrochloride (Novabiochem (Juro), Lucerne, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.32 (s, 9H); 2.28 (s, 3H); 3.07 (m, 2H); 4.53 (m, 1H); 7.13-7.29 (m, 5H); 7.32 (d, 1H); 7.44 (d, 1H); 7.50 (ddd, 1H); 7.55 (dd, 1H); 8.05 (m, 1H); 8.39 (m, 1H); 8.49 (d, 1H); 8.63 (d, 1H); 8.67 (dd, 1H); 9.08 (s, 1H); 9.23 (m, 1H).

Example 33: *N*-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-(D)-alanine 1,1-dimethylethyl ester utilising D-alanine t-butylester hydrochloride (Novabiochem (Juro), Lucerne, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.34 (d, 3H); 1.38 (s, 9H); 2.28 (s, 3H); 4.32 (m, 1H); 7.33 (d, 1H); 7.43 (d, 1H); 7.51 (ddd, 1H); 7.61 (dd, 1H); 8.14 (m, 1H); 8.40 (m, 1H); 8.50 (m, 1H); 8.58 (d, 1H); 8.67 (dd, 1H); 9.08 (s, 1H); 9.23 (m, 1H).

Example 34: *N*-[1-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-4-piperidinyl]-benzamide utilising N-4-piperidinyl-benzamide (Maybridge Chemical Co. Ltd). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.49 (m, 2H); 1.68-1.94 (m, 2H); 2.30 (s, 3H); 2.92 (m, 1H); 3.16 (m, 1H); 3.79 (m, 1H); 4.05 (m, 1H); 4.42 (m, 1H); 7.08 (dd, 1H); 7.31 (d, 1H); 7.41-7.54 (m, 5H); 7.63

(m, 1H); 7.79-7.84 (m, 2H); 8.28 (d, 1H); 8.40 (m, 1H); 8.51 (d, 1H); 8.66 (dd, 1H); 9.06 (s, 1H); 9.24 (m, 1H).

Example 35: 4-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-morpholine utilising morpholine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.29 (s, 3H); 3.47 (m, 8H); 7.10 (dd, 1H); 7.30 (d, 1H); 7.44 (m, 1H); 7.52 (ddd, 1H); 7.65 (m, 1H); 8.40 (m, 1H); 8.51 (d, 1H); 8.69 (dd, 1H); 9.05 (s, 1H); 9.23 (m, 1H).

Example 36: 1-(4-Methoxyphenyl)-4-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoyl]piperazine utilising 1-(4-methoxyphenyl)-piperazine (Emka Chemie, Neufahrn, Germany). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.30 (s, 3H); 2.87-3.08 (m, 4H); 3.50-3.75 (m, 4H); 3.67 (s, 3H); 6.78-6.88 (m, 4H); 7.12 (dd, 1H); 7.31 (d, 1H); 7.44 (m, 1H); 7.51 (ddd, 1H); 7.67 (m, 1H); 8.38 (m, 1H); 8.52 (m, 1H); 8.67 (dd, 1H); 9.06 (s, 1H); 9.23 (m, 1H).

Example 37: 1-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-4-(4-pyridinyl)-piperazine utilising 1-(4-pyridyl)-piperazine (Emka Chemie, Neufahrn, Germany). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.31 (s, 3H); 3.30 (m, 4H); 3.59 (m, 4H); 6.77 (m, 2H); 7.14 (dd, 1H); 7.32 (d, 1H); 7.45 (d, 1H); 7.52 (ddd, 1H); 7.70 (m, 1H); 8.16 (m, 2H); 8.41 (m, 1H); 8.53 (d, 1H); 8.67 (dd, 1H); 9.07 (s, 1H); 9.24 (m, 1H).

Example 38: 1-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-4-(pyrazinyl)-piperazine utilising 1-(2-pyrazinyl)-piperazine (Emka Chemie, Neufahrn, Germany). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.31 (s, 3H); 3.57 (m, 8H); 7.14 (dd, 1H); 7.32 (d, 1H); 7.45 (d, 1H); 7.51 (ddd, 1H); 7.72 (m, 1H); 7.85 (d, 1H); 8.08 (d, 1H); 8.29 (d, 1H); 8.40 (m, 1H); 8.53 (d, 1H); 8.65 (dd, 1H); 9.06 (s, 1H); 9.24 (m, 1H).

Example 39: 1-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-4-(phenylmethyl)-piperazine utilising 1-benzyl-piperazine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.21-2.42 (m, 4H); 2.28 (s, 3H); 3.34-3.63 (m, 6H); 7.07 (dd, 1H); 7.21-7.34 (m, 6H); 7.43-7.50 (m, 2H); 7.63 (m, 1H); 8.38 (m, 1H); 8.50 (d, 1H); 8.65 (dd, 1H); 9.03 (s, 1H); 9.22 (m, 1H).

Example 40: 1-Cyclopentyl-4-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-piperazine utilising 1-cyclopentyl-piperazine (Emka Chemie, Neufahrn, Germany). ^{1}H-NMR

(400 MHz, DMSO-d_6, δ): 1.20-1.31 (m, 2H); 1.39-1.62 (m, 4H); 1.65-1.75 (m, 2H); 2.18-2.47 (m, 8H); 3.27-3.62 (m, 4H); 7.08 (dd, 1H); 7.29 (d, 1H); 7.44 (d, 1H); 7.51 (ddd, 1H); 7.62 (m, 1H); 8.38 (m, 1H); 8.51 (d, 1H); 8.68 (dd, 1H); 9.04 (s, 1H); 9.22 (m, 1H).

Example 41: 4-{{4-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-1-piperazinyl}-acetyl}morpholine utilising 4-[2-(piperazin-1-yl)-acetyl]-morpholine (Emka Chemie, Neufahrn, Germany). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.29 (s, 3H); 2.31-2.49 (m, 4H); 3.16 (s, 2H); 3.37-3.60 (m, 12H); 7.07 (dd, 1H); 7.29 (d,1H); 7.45 (d, 1H); 7.52 (ddd, 1H); 7.65 (m, 1H); 8.39 (m, 1H); 8.51 (d, 1H); 8.68 (dd, 1H); 9.04 (s, 1H); 9.23 (m, 1H).

Example 42: 1-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-4-[2-oxo-2-(1-pyrrolidinyl)ethyl]piperazine utilising 1-[2-(piperazin-1-yl)-acetyl-pyrrolidine (Emka Chemie, Neufahrn, Germany). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.73 (m, 2H); 1.83 (m, 2H); 2.29 (s, 3H); 2.43 (m, 4H); 3.09 (s, 2H); 3.25 (m, 2H); 3.34-3.63 (m, 6H); 7.07 (dd, 1H); 7.29 (d, 1H); 7.45 (d, 1H); 7.52 (ddd, 1H); 7.64 (m, 1H); 8.39 (m, 1H); 8.51 (d, 1H); 8.68 (dd, 1H); 9.04 (s, 1H); 9.22 (m, 1H).

Example 43: 4-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-1-piperazine-carboxylic acid ethyl ester utilising ethyl 1-piperazinecarboxylate (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.16 (t, 3H); 2.29 (s, 3H); 3.19-3.63 (m, 8H); 4.02 (q, 2H); 7.10 (dd, 1H); 7.30 (d, 1H); 7.45 (d, 1H); 7.52 (ddd, 1H); 7.66 (m, 1H); 8.40 (m, 1H); 8.51 (d, 1H); 8.68 (dd, 1H); 9.06 (s, 1H); 9.23 (m, 1H).

Example 44: 2-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-1,2,3,4-tetrahydro-isoquinoline utilising 1,2,3,4-tetrahydroisoquinoline (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.31 (s, 3H); 2.79 (m, 2H); 3.57-3.90 (m, 2H); 4.58-4.79 (m, 2H); 7.08-7.23 (m, 5H); 7.32 (d, 1H); 7.42-7.50 (m, 2H); 7.70 (m, 1H); 8.39 (m, 1H); 8.51 (d, 1H); 8.67 (dd, 1H); 9.05 (s, 1H); 9.24 (m, 1H).

Example 45: *N,N*-bis(2-Methoxyethyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising bis(2-methoxyethyl)amine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.28 (s, 3H); 3.09 (br.s, 3H); 3.23 (br.s, 3H); 3.47 (m, 8H); 7.04 (dd, 1H); 7.27 (d, 1H); 7.44 (d, 1H); 7.51 (ddd, 1H); 7.62 (m, 1H); 8.39 (m, 1H); 8.51 (d, 1H); 8.68 (dd, 1H); 9.01 (s, 1H); 9.23 (m, 1H).

Example 46: 1'-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-1,4'-bipiperidine utilising 4-piperidinopiperidine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.21-1.50 (m, 8H); 1.51-1.83 (m, 2H); 2.29 (s, 3H); 2.39 (m, 4H); 2.68 (m, 1H); 2.95 (m, 1H); 3.71 (m, 1H); 4.42 (m, 1H); 7.07 (dd, 1H); 7.28 (d, 1H); 7.45 (d, 1H); 7.52 (ddd, 1H); 7.63 (m, 1H); 8.40 (m, 1H); 8.51 (d, 1H); 8.67 (dd, 1H); 9.03 (s, 1H); 9.23 (m, 1H).

Example 47: *N*-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-N-(phenylmethyl)-glycine ethyl ester utilising N-benzylglycine ethyl ester (Fluka, Buchs, Switzerland). ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 0.97-1.20 (m, 3H); 2.27 (s, 3H); 3.90-4.12 (m, 4H); 4.58-4.68 (m, 2H); 7.07 (m, 1H); 7.15-7.34 (m, 6H); 7.38-7.53 (m, 2H); 7.65-7.74 (m, 1H); 8.35-8.51 (m, 2H); 8.66 (dd, 1H); 8.96-9.04 (m, 1H); 9.22 (m, 1H).

Example 48: *N*-(3-Chlorophenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising 3-chlor-aniline (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.33 (s, 3H); 7.14 (m, 1H); 7.36 (m, 1H); 7.41 (d, 1H); 7.46 (d, 1H); 7.49 (ddd, 1H); 7.68-7.73 (m, 2H); 7.95 (m, 1H); 8.25 (m, 1H); 8.43 (m, 1H); 8.53 (d, 1H); 8.66 (dd, 1H); 9.15 (s, 1H); 9.26 (m, 1H); 10.33 (s, 1H).

Example 49: *N*-(2,2-Diphenylethyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 2,2-diphenylethylamine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.24 (s, 3H); 3.87 (m, 2H); 4.41 (m, 1H); 7.12-7.17 (m, 2H); 7.23-7.31 (m, 9H); 7.41-7.44 (m, 2H); 7.51 (ddd, 1H); 7.97 (m, 1H); 8.37-8.44 (m, 2H); 8.48 (d, 1H); 8.68 (dd, 1H); 9.05 (s, 1H); 9.23 (m, 1H).

Example 50: *N*-(2,3-Dihydro-1H-inden-1-yl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 1-Aminoindane (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.90-2.01 (m, 1H); 2.29 (s, 3H); 2.43 (m, 1H); 2.77-2.86 (m, 1H); 2.91-2.98 (m, 1H); 5,56 (m, 1H); 7.08-7.25 (m, 4H); 7.31 (d, 1H); 7.43 (d, 1H); 7.50 (ddd, 1H); 7.64 (dd, 1H); 8.20 (m, 1H); 8.40 (m, 1H); 8.50 (d, 1H); 8.68-8.72 (m, 2H); 9.08 (s, 1H); 9.24 (m, 1H).

Example 51: *N*-(Diphenylmethyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising alpha-aminodiphenylmethane (Fluka, Buchs, Switzerland) ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.29 (s, 3H); 6.41 (d, 1H); 7.20-7.36 (m, 11H); 7.43 (d, 1H); 7.46 (ddd, 1H);

7.67 (dd, 1H); 8.18 (m, 1H); 8.38 (m, 1H); 8.50 (d, 1H); 8.68 (dd, 1H); 9.10 (s, 1H); 9.20 (d, 1H); 9.24 (m, 1H).

Example 52: 4-Methyl-*N*-[2-(1-piperidinyl)ethyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 1-(2-aminoethyl)piperidine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.30-1.38 (m, 2H); 1.41-1.48 (m, 4H); 2.28 (s, 3H); 2.31-2.41 (m, 6H); 3.33 (m, 2H); 7.31 (d, 1H); 7.44 (d, 1H); 7.51 (ddd, 1H); 7.55 (dd, 1H); 8.08 (m, 1H); 8.28 (t, 1H); 8.40 (m, 1H); 8.51 (d, 1H); 8.67 (dd, 1H); 9.07 (s, 1H); 9.24 (m, 1H).

Example 53: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-N-(5,6,7,8-tetrahydro-1-naphthalenyl)benzamide utilising 5,6,7,8-tetrahydro-1-naphthylamine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.63-1.71 (m, 4H); 2.32 (s, 3H); 2.60 (m, 2H); 2.74 (m, 2H); 6.96 dd, 1H); 7.07-7.14 (m, 2H); 7.37 (d, 1H); 7.45 (d, 1H); 7.49 (ddd, 1H); 7.69 (dd, 1H); 8.25 (m, 1H); 8.41 (m, 1H); 8.52 (d, 1H); 8.67 (dd, 1H); 9.12 (s, 1H); 9.25 (m, 1H); 9.65 (br.s).

Example 54: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-N-[[4-(trifluoromethyl)phenyl]-methyl]benzamide utilising 4-(trifluoromethyl)benzylamine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.30 (s, 3H); 4.53 (d, 2H); 7.34 (d,1H); 7.44 (d, 1H); 7.46-7.53 (m, 3H); 7.62 (dd, 1H); 7.66 (m, 2H); 8.16 (m, 1H); 8.40 (m, 1H); 8.51 (d, 1H); 8.67 (dd, 1H); 9.08 (t, 1H); 9.10 (s, 1H); 9.24 (m, 1H).

Example 55: 4-Methyl-*N*-[(5-methylpyrazinyl)methyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 2-(aminomethyl)-5-methylpyrazine (TCI-JP, Distrib. Zürich, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.29 (s, 3H); 2.45 (s, 3H); 4.54 (d, 2H); 7.33 (d, 1H); 7.44 (d, 1H); 7.49 (ddd, 1H); 7.62 (dd, 1H); 8.14 (m, 1H); 8.40 (m, 1H); 8.45 (m, 2H); 8.50 (d, 1H); 8.66 (dd, 1H); 9.07 (t, 1H); 9.09 (s, 1H); 9.23 (m, 1H).

Example 56: *N*-(2-Ethoxyethyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising 2-ethoxyethylamine (TCI-JP, Distrib. Zurich, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ):1.07 (t, 3H); 2.28 (s, 3H); 3.30-3.49 (m, 6H); 7.31 (d, 1H); 7.43 (d, 1H); 7.51 (ddd, 1H); 7.57 (dd, 1H); 8.09 (m, 1H); 8.38-8.45 (m, 2H); 8.50 (d, 1H); 8.67 (dd, 1H); 9.07 (s, 1H); 9.24 (m, 1H).

Example 57: 4-Methyl-*N*-[2-(2-oxo-1-imidazolidinyl)ethyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzamide utilising 1-(2-aminoethyl)imidazolidin-2-one [Chem. Abstr. Reg. Number: 6281-42-1]. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.27 (s, 3H); 3.13-3.22 (m, 4H); 3.30-3.40 (m, 4H); 6.27 (br.s, 1H); 7.30 (d, 1H); 7.43 (d, 1H); 7.49-7.56 (m, 2H); 8.08 (d, 1H); 8.40 (m, 1H); 8.45 (t, 1H); 8.50 (d, 1H); 8.67 (dd, 1H); 9.06 (s, 1H); 9.23 (m, 1H).

Example 58: 4-Methyl-*N*-(5-methyl-2-pyridinyl)-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide utilising 2-amino-5-picoline (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.26 (s, 3H); 2.32 (s, 3H); 7.35 (d, 1H); 7.45 (d, 1H); 7.49 (ddd, 1H); 7.64 (dd, 1H); 7.77 (dd, 1H); 8.07 (d, 1H); 8.18 (m, 1H); 8.31 (d, 1H); 8.43 (m, 1H); 8.52 (d, 1H); 8.66 (dd, 1H); 9.08 (s, 1H); 9.25 (m, 1H); 10.58 (s, 1H).

Example 59: 1-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-4-phenyl-4-piperidinol utilising 4-hydroxy-4-phenylpiperidine (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.45-1.73 (m, 2H); 1.88 (m, 2H); 2.28 (s, 3H); 3.15 (m, 1H); 3.47 (m, 1H); 3.64 (m, 1H); 4.39 (m, 1H); 5.14 (s, 1H); 7.14 (dd, 1H); 7.19 (m, 1H); 7.26-7.31 (m, 3H); 7.43 (d, 1H); 7.45-7.51 (m, 3H); 7.69 (d, 1H); 8.40 (m, 1H); 8.48 (d, 1H); 8.67 (dd, 1H); 9.03 (s, 1H); 9.24 (m, 1H).

Example 60: *N*-(3-Benzoylphenyl)-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzamide utilising 3-aminobenzophenone (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.32 (s, 3H); 7.39 (d, 1H); 7.43-7.58 (m, 6H); 7.67 (m, 1H); 7.70-7.77 (m, 3H); 8.13 (m, 1H); 8.20 (m, 1H); 8.27 (m, 1H); 8.42 (m, 1H); 8.52 (d, 1H); 8.66 (dd, 1H); 9.14 (s, 1H); 9.25 (m, 1H); 10.41 (s, 1H).

Example 61: *N*-[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]-glycine 1,1-dimethylethyl ester utilising glycine t-butyl ester hydrochloride (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.40 (s, 9H); 2.29 (s, 3H); 3.86 (d, 2H); 7.33 (d, 1H); 7.43 (d, 1H); 7.51 (ddd, 1H); 7.58 (dd, 1H); 8.10 (d, 1H); 8.40 (m, 1H); 8.50 (d, 1H); 8.67 (dd, 1H); 8.75 (t, 1H); 9.08 (s, 1H); 9.23 (m, 1H).

Example 62: 4-[[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]amino]benzene-acetic acid ethyl ester utilising ethyl 4-aminophenylacetate (Maybridge Chemical Co. Ltd.). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.16 (t, 3H); 2.32 (s, 3H); 3.60 (s, 2H); 4.06 (q, 2H); 7.21

(m, 2H); 7.38 (d, 1H); 7.45 (d, 1H); 7.48 (ddd, 1H); 7.70 (m, 3H); 8.23 (m, 1H); 8.41 (m, 1H); 8.52 (d, 1H); 8.66 (dd, 1H); 9.13 (s, 1H); 9.25 (m, 1H); 10.16 (s, 1H).

Example 63: 4-Methyl-*N*-[3-(methylphenylamino)propyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzamide utilising N-(3-aminopropyl)-N-methylaniline (TCI-JP, Distrib. Zürich, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.73 (qui, 2H); 2.28 (s, 3H); 2.84 (s, 3H); 3.24-3.37 (m, 4H); 6.55 (m, 1H); 6.65 (m, 2H); 7.10 (m, 2H); 7.31 (d, 1H); 7.43 (d, 1H); 7.47 (ddd, 1H); 7.55 (dd, 1H); 8.10 (d, 1H); 8.37-8.44 (m, 2H); 8.50 (d, 1H); 8.65 (dd, 1H); 9.06 (s, 1H); 9.23 (m, 1H).

Example 64: 1-[[3-[[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]amino]phenyl]-methyl]-4-piperidinecarboxylic acid ethyl ester utilising ethyl 1-(3-aminobenzyl)piperidine-4-carboxylate (Maybridge Chemical Co. Ltd.). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.14 (t, 3H); 1.49-1.61 (m, 2H); 1.72-1.80 (m, 2H); 1.92-2.02 (m, 2H); 2.27 (m, 1H); 2.32 (s, 3H); 2.74 (m, 2H); 3.40 (s, 2H); 4.03 (q, 2H); 6.98 (d, 1H); 7.25 (m, 1H); 7.38 (d, 1H); 7.43-7.51 (m, 2H); 7.66-7.73 (m, 3H); 8.25 (s, 1H); 8.42 (m, 1H); 8.52 (d, 1H); 8.65 (dd, 1H); 9.12 (s, 1H); 9.25 (m, 1H); 10.14 (s, 1H).

Example 65: [[4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoyl]amino]propanedioic acid diethyl ester utilising diethyl aminomalonate hydrochloride (Aldrich, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.19 (t, 6H); 2.30 (s, 3H); 4.10-4.22 (m, 4H); 5.27 (d, 1H); 7.35 (d, 1H); 7.44 (d, 1H); 7.51 (ddd, 1H); 7.63 (dd, 1H); 8.15 (m, 1H); 8.40 (m, 1H); 8.50 (d, 1H); 8.67 (dd, 1H); 9.11 (s, 1H); 9.21-9.25 (m, 2H).

Example 66: *N*-[2-[bis(1-Methylethyl)amino]ethyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]-amino]benzamide utilising 2-diisopropylamino-ethylamine (Fluka, Buchs, Switzerland). ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 0.95 (m, 12H); 2.28 (s, 3H); 2.49 (m, 2H); 2.94 (m, 2H); 3.17 (m, 2H); 7.30 (d, 1H); 7.43 (d, 1H); 7.50 (ddd, 1H); 7.54 (dd, 1H); 8.09 (br.s, 1H); 8.27 (m, 1H); 8.40 (m, 1H); 8.50 (d, 1H); 8.67 (dd, 1H); 9.06 (s, 1H); 9.23 (m, 1H).

Example 67: *N*-[3-(Diethylamino)phenyl]-4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide

A solution containing ~50% of propylphosphonic anhydride in *N,N*-dimethylformamide (Fluka, Buchs, Switzerland; 674 µL, ~1.05 mmol) is added within 20 minutes to a stirred mixture of 4-

methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid (214.4 mg, 0.7 mmol), *N,N*-diethyl-1,3-benzenediamine (115 mg, 0.7 mmol) and triethylamine (776 µL, 5.6 mmol) in 2 mL *N,N*-dimethylformamide. After stirring for 24 hours at room temperature, the mixture is treated with a half-saturated aqueous solution of sodium hydrogen carbonate and extracted three times with ethyl acetate. The solvent is evaporated off under reduced pressure and the residue dried *in vacuo*. The crude product is purified by chromatography on silica gel, eluent 2% methanol in dichloromethane and crystallised from acetone to give the title compound as a crystalline solid. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 1.07 (t, 6H); 2.31 (s, 3H); 3.29 (m, 4H); 6.38 (m, 1H); 7.06 (m, 2H); 7.11 (m, 1H); 7.36 (d, 1H); 7.43-7.50 (m, 2H); 7.67 (m, 1H); 8.21 (m, 1H); 8.43 (m, 1H); 8.51 (d, 1H); 8.66 (dd, 1H); 9.12 (s, 1H); 9.24 (m, 1H); 9.90 (s, 1H).

Example 68: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[[3-[(1-hydroxy-1-methyl-ethyl)]-5-(1,1,1-trifluoromethyl)phenyl]methyl]benzamide

Diethylcyanophosphonate (Aldrich, Buchs, Switzerland; 0.33 mL, 2.0 mmol) is added to a stirred mixture of 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid (306 mg, 1.0 mmol), 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)benzeneamine (220 mg, 1.0 mmol) and triethylamine (560 µL, 4.0 mmol) in 5 mL *N,N*-dimethylformamide at 10°C. After stirring for 3 hours at 60°C, the mixture is treated with saturated aqueous solution of sodium hydrogen carbonate and extracted three times with ethyl acetate. The combined extracts are dried ($MgSO_4$), filtered and the solvent is evaporated off under reduced pressure to afford a crude product which is recrystallised from ethylacetate to give the title compound as a crystalline solid, m.p. 253-258°C.

Example 69: 3-[[4-(3-Pyridinyl)-2-pyrimidinyl]amino]-*N*-[(4-methyl-1-piperazinyl)methyl]-benzamide

Diethylcyanophosphonate (Aldrich, Buchs, Switzerland; 0.50 mL, 3.0 mmol) is added to a stirred mixture of 3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid (438 mg, 1.5 mmol), 4-[(4-methyl-1-piperazinyl)methyl]benzeneamine (308 mg, 1.5 mmol) and triethylamine (840 µL, 3.0 mmol) in 10 mL *N,N*-dimethylformamide at 10°C. After stirring for 12 hours at 60°C, the mixture is treated with an aqueous solution of sodium hydrogen carbonate and extracted three times with ethyl acetate. The combined extracts are washed with water, and the solvent is evaporated off under reduced pressure to give a residue. The residue is resuspended in water and filtered to afford the crude product which is recrystallised from tetrahydrofuran-

ethyl acetate to give *N*-[3-[[4-(3-Pyridinyl)-2-pyrimidinyl]amino]-*N*-[(4-methyl-1-piperazinyl)-methyl]benzamide as a crystalline solid, m.p. 220-224°C.

Example 69a: 3-[(Aminoiminomethyl)amino]-4-methylbenzoic acid methyl ester mononitrate
Utilising the procedure described in Example 1a, but with 3-aminobenzoic acid methyl ester (Fluka, Buchs, Switzerland) in lieu of 3-amino-4-methylbenzoic acid ethyl ester, afforded the title compound as a crystalline solid, m.p. 170-172°C.

Example 69b: 3-[[4-(3-Pyridinyl)-2-pyrimidinyl]amino]benzoic acid methyl ester
Utilising the procedure described in Example 1b, but with the intermediate of Example 69a in lieu of 4-methyl-3-[(aminoiminomethyl)amino]-4-methylbenzoic acid ethyl ester mononitrate, afforded the title compound as a crystalline solid, m.p. 195-200°C.

Example 69c: 3-[[4-(3-Pyridinyl)-2-pyrimidinyl]amino]benzoic acid
Utilising the procedure described in Example 1c, but with the intermediate of Example 69b in lieu of 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]benzoic acid ethyl ester, afforded the title compound as a crystalline solid, m.p. 285-293°C.

Example 70: 3-[[4-(3-Pyridinyl)-2-pyrimidinyl]amino]-*N*-[(3-(1-hydroxy-1-methylethyl)-5-(1,1,1-trifluoromethyl)phenyl]benzamide
Utilising the procedure described in Example 69, but with 3-(1-hydroxy-1-methylethyl)-5-(1,1,1-trifluoromethyl)benzeneamine in lieu of 4-[(4-methyl-1-piperazinyl)methyl]benzeneamine, afforded 3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[(3-(1-hydroxy-1-methylethyl)-5-(1,1,1-trifluoromethyl)phenyl]benzamide as a crystalline solid, m.p. 213-215°C.

Example 71: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-[3-(1H-imidazol-1-yl)propoxy]-phenyl]benzamide
Utilising the procedure described in Example 3, but employing 3-[3-(1H-imidazol-1-yl)propoxy]-benzenamine (Takao Nishi et al., JP 10182459) in lieu of 1-(2-pyridyl)piperazine, afforded the title compound as a solid. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.12-2.21 (m, 2H); 2.33 (s, 3H); 3.87 (t, 2H); 4.13 (t, 2H); 6.66 (dd, 1H); 6.87 (s, 1H); 7.15-7.26 (m, 2H); 7.32-7.42 (m, 2H); 7.44-7.52 (m, 3H); 7.61 (s, 1H); 7.70 (d, 1H); 8.24 (s, 1H); 8.43 (d, 1H); 8.53 (d, 1H); 8.67 (d, 1H); 9.13 (s, 1H); 9.26 (br. s, 1H); 10.13 (s, 1H).

Example 72: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-[2-(1H-imidazol-1-yl)ethoxy]phenyl]benzamide

Utilising the procedure described in Example 3, but employing 3-[2-(1H-imidazol-1-yl)ethoxy]-benzenamine (Rolf Paul et al., Journal of Medicinal Chemistry (1993), 36(19), 2716-25) in lieu of 1-(2-pyridyl)piperazine, afforded the title compound as a crystalline solid. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 2.34 (s, 3H); 4.22 (t, 2H); 4.37 (t, 2H); 6.68 (dd, 1H); 6.90 (s, 1H); 7.21-7.27 (m, 2H); 7.36-7.43 (m, 2H); 7.46-7.53 (m, 3H); 7.67-7.74 (m, 2H); 8.25 (br. s, 1H); 8.44 (dt, 1H); 8.54 (d, 1H); 8.68 (dd, 1H); 9.15 (s, 1H); 9.27 (br. d, 1H); 10.15 (s, 1H).

Example 73: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(ethylamino)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 69, but employing N-ethyl-2-(trifluoromethyl)-1,4-benzenediamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)-benzeneamine, afforded the title compound as a crystalline solid, m.p. 178–180°C.

The aniline is prepared as follows:

Example 73a: N-ethyl-2-(trifluoromethyl)-1,4-benzenediamine

A mixture of 2-bromo-5-nitrobenzotrifuoride (Lancaster Synthesis, GmbH; 5.4 g, 20 mmol) and a solution of ethylamine in ethanol (50 mL of 2M, 100 mmol) is heated at 80°C for 18 hours in a steel pressure vessel. The mixture is then cooled and the solvent is evaopated off under reduced pressure to yield the crude product which is purified by column chromatography (silica gel, eluent 20% ethyl acetate in hexane) to afford N-ethyl-4-nitro-6-(trifluoromethyl)-benzenamine as yellow oil. This product is dissolved in ethanol (180 mL) and hydrogenated at atmospheric pressure over Raney nickel (0.5 g) at 45°C. The calculated amount of hydrogen is taken up in 50 hours. The mixture is then filtered and the solvent is evaporated off under reduced pressure to yield the crude product which is purified by chromatography (silica gel; eluent 50% ethyl acetate in hexane) and recrystallised from ether – hexane to give the title compound as a beige crystalline solid. ^{1}H-NMR (400 MHz, DMSO-d_6): 1.11 (t, 3H), 3.05 (m, 2H), 4.18 (br t, 1H), 4.66 (br.s, 2H), 6.58 – 6.64 (m, 1H) and 6.68 – 6.75 (m, 2H).

Example 74: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(diethylamino)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 3, but employing N,N-diethyl-2-(trifluoromethyl)-1,4-benzenediamine (Toshio Niwa, DE 3524519) in lieu of 1-(2-pyridyl)piperazine, afforded the title compound as a crystalline solid, m.p. 128–131°C.

Example 75: (±)-4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-[(2-hydroxypropyl)amino]-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 3, but employing (±)-1-[[4-amino-2-(trifluoromethyl)phenyl]amino]-2-propanol (Tsutomu Mano, EP 299497) in lieu of 1-(2-pyridyl)piperazine, afforded the title compound as a crystalline solid, m.p. 184–186°C.

Example 76: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-[bis(2-methoxyethyl)amino]-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 3, but employing *N,N*-bis(2-methoxyethyl)-2-(trifluoromethyl)-1,4-benzenediamine (Toshio Niwa, DE 3524519) in lieu of 1-(2-pyridyl)piperazine, afforded the title compound as a crystalline solid, m.p. 156–157°C.

Example 77: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(4-methyl-1-piperazinyl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 3, but employing 4-(4-methyl-1-piperazinyl)-3-(trifluoromethyl)-benzenamine (Anthony David Baxter, WO 0119800) in lieu of 1-(2-pyridyl)piperazine, afforded the title compound as a crystalline solid, m.p. 214–217°C.

Example 78: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(1-piperidinyl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 3, but employing 4-(1-piperidinyl)-3-(trifluoromethyl)-benzenamine (Leping Li, WO 0151456) in lieu of 1-(2-pyridyl)piperazine, afforded the title compound as a crystalline solid, m.p. 201–202°C.

Example 79: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(1-pyrrolidinyl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 3, but employing 4-(1-pyrrolidinyl)-3-(trifluoromethyl)-benzenamine (Steven Lee Bender WO 0153274) in lieu of 1-(2-pyridyl)piperazine afforded the title compound as a crystalline solid, m.p. 129–130°C.

Example 80: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(4-morpholinyl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 69, but employing 4-(4-morpholinyl)-3-(trifluoromethyl)-benzenamine (Steven Lee Bender WO 0153274) in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)benzeneamine, afforded the title compound as a crystalline solid, m.p. 216–218°C.

Example 81: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-phenyl-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 3, but employing 4-(phenyl)-3-(trifluoromethyl)-benzenamine in lieu of 1-(2-pyridyl)piperazine afforded the title compound as a crystalline solid, m.p. 172–174°C.

The aniline is prepared as follows:

Example 81a: 4-(Phenyl)-3-(trifluoromethyl)benzenamine

Phenyl boronic acid (Aldrich, Buchs, Switzerland; 2.7 g, 22 mmol), Palladium II acetate (0.225 g, 1 mmol), tri-o-tolylphosphine (0.608 g, 2 mmol) and aqueous potassium carbonate solution (50 mL of 1 M) is added to a stirred solution of 2-bromo-5-nitrobenzotrifuoride (Lancaster Synthesis, GmbH; 5.4 g, 20 mmol) in dimethylformamide (200 mL) and heated at 120°C under an argon atmosphere for 1 h. The mixture is then evaporated to dryness under reduced pressure and the residue is treated with water (100 mL) and extracted with ethyl acetate (3 x 80 mL). The combined extracts are washed (brine), dried (MgSO4), filltered and the solvent is evorated off under reduced pressure to afford 4'-nitro-2'-(trifluoromethyl)-[1,1'-Biphenyl]. The biphenyl is dissolved in ethanol (200 mL) and hydrogenated at atmospheric pressure over Raney nickel (2 g) at 22°C. The calculated amount of hydrogen is taken up in 11 hours. The mixture is then filtered and the solvent is evaporated off under reduced pressure to yield the crude product which is purified by chromatograghy (silica gel; eluent ethyl acetate) to give the title compound as a brown oil. ^{1}H-NMR (400 MHz, DMSO-d_6): 5.62 (br.s, 2H), 6.80 (dd, 1H), 6.96 (d, 1H), 6.99 (d, 1H), 7.19 – 7.23 (m, 2H), and 7.29 – 7.39 (m, 3H).

Example 82: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-N-[3-[4-(3-pyridinyl)-3-(trifluoromethyl)phenyl]methyl]benzamide

Utilising the procedure described in Example 69, but employing 4-(3-pyridinyl)-3-(trifluoromethyl)-benzenamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)benzeneamine, afforded the title compound as a crystalline solid, m.p. 276–280°C.

The aniline is prepared as follows:

Example 82a: 4-(3-Pyridinyl)-3-(trifluoromethyl)benzenamine
A stirred solution of 2-bromo-5-nitrobenzotrifuoride (Lancaster Synthesis, GmbH; 3.37 g, 12.5 mmol) and 3-(tri-n-butylstannyl)pyridine (Maybridge Chemical Co. Ltd., England; 5.0 g, 13.6 mmol) in xylene (75 mL) was purged with argon for 10 minutes at 20°C. Tetrakis(triphenylphosphine)palladium (0) (1.4 g, 1.25 mmol) is then added and the resulting mixture is heated at 130°C for 24 hours under an argon atmosphere. The mixture is then cooled, treated with an aqueous solution of sodium hydroxide (150 mL of 0.1 M) and purged with air for 2 hours. The resulting mixture is then diluted with ethylacetate (200 mL) and filtered. The orgainic phase is then sequentially washed with water (2 x 80 mL) and saturated aqueous sodium chloride (1 x 80 mL), dried ($MgSO_4$), filtered and the solvent is evaporated off under reduced pressure to yield the crude product which is purified by column chromatography (silica gel, eluent 50% ethyl acetate in hexane) to afford 3-[(4-nitro-3-(trifluoromethyl)phenyl]pyridine. This product is dissolved in ethanol (200 mL) and hydrogenated at atmospheric pressure over Raney nickel (0.23 g) at 22°C. The calculated amount of hydrogen is taken up in 24 hours. The mixture is then filtered and the solvent is evaporated off under reduced pressure to yield the crude product which is purified by chromatography (silica gel; eluent 50% ethyl acetate in hexane) and recrystallised from ether – hexane to give the title compound as a colourless crystalline solid, m.p. 92–93°C.

Example 83: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide
Utilising the procedure described in Example 3, but employing 4-(1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine (Steven Lee Bender WO 0153274) in lieu of 1-(2-pyridyl)piperazine afforded the title compound as a crystalline solid, m.p. 226–229°C.

Example 84: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(2,4-dimethyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 69, but employing 4-(2,4-dimethyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)benzeneamine, afforded the title compound as an amorphous solid.

The aniline is prepared as follows:

Example 84a: 4-(2,4-dimethyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine
A mixture of 2-bromo-5-nitrobenzotrifuoride (Lancaster Synthesis, GmbH; 6.0 g, 22 mmol) and 2,4-dimethylimidazole (10.6, 110 mmol) is heated at 120°C for 36 hours under an argon atmosphere. The mixture is then cooled and the residue is treated with water (150 mL) and extracted with ethyl acetate (3 x 80 mL). The combined extracts are washed (brine), dried ($MgSO_4$), filtered and the solvent is evaporated off under reduced pressure to yield the crude product which is purified by column chromatography (silica gel, eluent ethyl acetate) to afford 1-[4-nitro-2-(trifluoromethyl)phenyl]-1H-imidazole as yellow crystalline solid. This product is dissolved in ethanol (290 mL) and hydrogenated at atmospheric pressure over Raney nickel (1.15 g) at 25°C. The calculated amount of hydrogen is taken up in 14 hours. The mixture is then filtered and the solvent is evaporated off under reduced pressure to yield the crude product which is purified by recrystallisation from ether – hexane to give the title compound as a crystalline solid, m.p. 163–164°C.

Example 85: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide
Utilising the procedure described in Example 69, but employing 4-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoro-methyl)benzeneamine, afforded the title compound as a crystalline solid, m.p. 154–163°C.

The aniline is prepared as follows:

Example 85a: 4-(4-Methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine
Utilising the procedure described in Example 84a, but employing 4(5)-methyl-1H-imidazole in lieu of 2,4-dimethylimidazole, afforded the title compound as a beige crystalline solid, m.p. 141–143°C.

Example 86: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[4-(2-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 69, but employing 4-(2-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)benzeneamine, afforded the title compound as a crystalline solid, m.p. 154–163°C.

The aniline is prepared as follows:

Example 86a: 4-(2-Methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine

Utilising the procedure described in Example 84a, but employing 2-methyl-1H-imidazole in lieu of 2,4-dimethylimidazole, afforded the title compound as a colourless crystalline solid, m.p. 117–119°C.

Example 87: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-(4-morpholinyl)-5-[(methylamino)carbonyl]phenyl]benzamide

Utilising the procedure described in Example 69, but employing 3-amino-5-(4-morpholinyl)-N-(methyl)-benzamide in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)-benzeneamine, afforded the title compound as a crystalline solid, m.p. 153–156°C.

The aniline is prepared as follows:

Example 87a: 3-Bromo-5-nitro-benzoic acid, 1,1-dimethylethyl ester

A solution of *n*-butyllithium in hexane (12.8 mL of 2.5 M, 32 mmol) is added with stirring to *t*-butanol (46 mL) at 25°C under an argon atmosphere. After 30 min the mixture is treated dropwise with a solution of 3-bromo-5-nitro-benzoyl chloride (J. Mindl, Collect. Czech. Chem. Commun. (1973), 38, 3496-505; 32 mmol) in dry THF (40 mL) and stirred for a further 17 h. The mixture is then treated with ether (250 mL) and washed with brine. The ether solution was dried ($MgSO_4$) and the solvent is evaporated off under reduced pressure to give the crude product which is purified by column chromatography (silica gel, eluent 20% ethyl acetate in hexane) and recrystallised from ether – hexane to afford the title compound as colourless crystalline solid, m.p. 77–78°C.

Example 87b: 3-(4-Morpholinyl)-5-nitro-benzoic acid, 1,1-dimethylethyl ester

A stirred mixture of 3-bromo-5-nitro-benzoic acid, 1,1-dimethylethyl ester (example 86a; 3.02 g, 10 mmol) and morpholine (1.22 mL, 14 mmol) in toluene (50 mL) is treated with sodium *t*-butylate (1.34 g, 14 mmol), tri-*t*-butylphosphine (3 mL, 1.5 mmol) and tris-(dibenzylidene-acetone)dipalladium[0] (0.45 g, 0.5 mmol) under an argon atmosphere, and then heated at 60°C for 18 h. The mixture is diluted with ethyl acetate (150 mL), filtered, washed with brine (2 x 50 mL), dried ($MgSO_4$) and the solvent is evaporated off under reduced pressure to give the crude product which is purified by column chromatography (silica gel, eluent 15% ethyl acetate in hexane) and recrystallised from ethyl acetate – hexane to afford the title compound as colourless crystalline solid, m.p. 116–118°C.

Example 87c: 3-(4-Morpholinyl)-5-nitro-benzoic acid, methyl ester

A mixture of 3-(4-morpholinyl)-5-nitro-benzoic acid, 1,1-dimethylethyl ester (Example 87b; 0.77 g, 2.5 mmol), 1,8-diazabicyclo[5,4,0]undec-7-ene (o.56 mL, 3.75 mL), and potassium bromide (1.09 g, 12.5 mmol) in methanol (25 mL) is stirred at 90 °C for 250 min. The cooled mixture is then added to hydrochloric acid (50 mL of 0.1 M) and extracted with ethyl acetate (3 x 100 mL). The combined extacts are washed with saturated aqueous sodium hydro-carbonate (2 x 25 mL), water (2 x 25 mL) and brine (2 x 50 mL), dried ($MgSO_4$) and the solvent is evaporated off under reduced pressure to give the crude product which is purified by recrystallisation from ethyl acetate – hexane to afford the title compound as yellow crystalline solid.

Example 87d: 3-(4-Morpholinyl)-5-nitro-N-(methyl)-benzamide

A stirred solution of 3-(4-morpholinyl)-5-nitro-benzoic acid, methyl ester (Example 86c; 0.53 g, 2 mmol) in toluene (5 mL) under an argon atmosphere, is treated with a mixture of methylamine hydrochloride (0.27 g, 4 mmol), trimethylaluminium (2 mL of a 2 M solution in toluene, 4 mmol) in toluene (5 mL) and heated at 60 °C for 18 h. The cooled mixture is then treated with hydrochloric acid (10 mL of 2 M), stirred for 5 min and then treated with aqueous sodium hydroxide (5 mL of 4 M). The mixture is then treated with water (100 mL) and extracted with ethyl acetate (3 x 100 mL). The combined extacts are washed with brine (2 x 50 mL), dried ($MgSO_4$) and the solvent is evaporated off under reduced pressure to give the crude product which is purified by recrystallisation from ethyl acetate to afford the title compound as yellow crystalline solid, m.p. 204–207°C.

Example 87e: 3-Amino-5-(4-Morpholinyl)-N-(methyl)-benzamide

A solution of 3-(4-morpholinyl)-5-nitro-N-(methyl)-benzamide (Example 86d; 300 mg, 1.12 mmol) in ethanol (20 mL) is hydrogenated at atmospheric pressure over Raney nickel (0.2 g) at 25°C. The calculated amount of hydrogen is taken up in 19 hours. The mixture is then filtered and the solvent is evaporated off under reduced pressure to yield the crude product which is purified by recrystallisation from ethyl acetate to give the title compound as a beige crystalline solid, m.p. 201–204°C.

Example 88: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-[(methylamino)carbonyl]-5-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 69, but employing 3-amino-5-(trifluoromethyl)-N-(methyl)-benzamide in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)-benzeneamine, afforded the title compound as a crystalline solid, m.p. 245–249°C.

Example 88a: 3-Amino-5-(trifluoromethyl)-N-(methyl)-benzamide

Utilising the procedure described in Example 86e, but employing α,α,α-trifluoro-N-methyl-5-nitro-m-toluamide (Dean E. Welch, J. Med. Chem. (1969), 12, 299-303) in lieu of 3-(4-morpholinyl)-5-nitro-N-(methyl)-benzamide, afforded the title compound as a beige crystalline solid, m.p. 113-115°C.

Example 89: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(3-pyridinyl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 69, but employing 5-(3-pyridinyl)-3-(trifluoro-methyl)-benzenamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)-benzeneamine, afforded the title compound as a crystalline solid, m.p. 275–279°C.

The aniline is prepared as follows:

Example 89a: 5-(3-Pyridinyl)-3-(trifluoromethyl)benzenamine

A stirred solution of 3-amino-5-bromo-benzotrifuoride (Apollo, England; 1.12 g, 5 mmol) and 3-(tri-n-butylstannyl)pyridine (Maybridge Chemical Co. Ltd., England; 2.0 g, 5.4 mmol) in xylene (30 mL) was purged with argon for 10 minutes at 20°C. Tetrakis(triphenylphosphine)-palladium (0) (1.16 g, 1.0 mmol) is then added and the resulting mixture is heated at 140°C for 36 hours under an argon atmosphere. The mixture is then cooled, treated with an aqueous solution of sodium hydroxide (100 mL of 0.1 M) and purged with air for 2 hours. The

resulting mixture is then diluted with ethylacetate (200 mL) and filtered. The orgainic phase is then sequentially washed with water (2 x 80 mL) and saturated aqueous sodium chloride (1 x 80 mL), dried ($MgSO_4$), filtered and the solvent is evaporated off under reduced pressure to yield the crude product which is purified by column chromatography (silica gel, eluent ethyl acetate) to afford the title compound as a brown oil. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 5.73 (br s, 2H), 6.83 (dd, 1H), 6.99 (d, 1H), 7.04 (d, 1H), 7.39 (dd, 1H), 7.64 (d, 1H), 8.42 (m, 1H) and 8.53 (dd, 1H).

Example 90: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(4-morpholinyl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 69, but employing 5-(4-morpholinyl)-3-(trifluoromethyl)-benzenamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoro-methyl)benzeneamine, afforded the title compound as a crystalline solid, m.p. 208–211°C.

The aniline is prepared as follows:

Example 90a: [3-Bromo-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester

A mixture of 3-amino-5-bromo-benzotrifuoride (Apollo, England; 12 g, 50 mmol), di-*t*-butyl-dicarbonate (12 g, 55 mmol) and 4-dimethylaminopyridine (0.61 g, 5 mmol) in acetonitrile (100 mL) is stirred at 60°C for 8 h. The solvent is then evaporated off under reduced pressure to yield the crude product which is purified by column chromatography (silica gel, eluent 10% ethyl acetate in hexane) and recrystallised from hexane to afford the title compound as a colourless crystalline solid, m.p. 113-115°C.

Example 90b: [3-(4-Morpholinyl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester

Utilising the procedure described in Example 86b but employing [3-bromo-5-(trifluoromethyl)-phenyl]-carbamic acid, 1,1-dimethylethyl ester (Example 90a) in lieu of 3-bromo-5-nitro-benzoic acid, 1,1-dimethylethyl ester, afforded the title compound as a crystalline solid, m.p. 146–148°C.

Example 90c: 5-(4-Morpholinyl)-3-(trifluoromethyl)-benzenamine

[3-(4-morpholinyl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester (Example 90b; 1.7 g, 5 mmol) is treated with a solution of hydrogen chloride in isopropanol (30 mL of 4

M) and heated at 60°C for 5 h. The solvent is evaporated off under reduced pressure and the residue is treated with aqueous sodium hydrogen carbonate solution (80 mL) and extracted with ethyl acetate (3 x 80 mL). The combined extacts are washed with brine (2 x 50 mL), dried ($MgSO_4$) and the solvent is evaporated off under reduced pressure to give the crude product which is purified by recrystallisation from ether - hexane to afford the title compound as yellow crystalline solid, m.p. 96-97°C.

Example 91: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(2-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 69, but employing 5-(2-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)benzeneamine, afforded the title compound as a crystalline solid, m.p. 242–247°C.

The was aniline is prepared as follows:

Example 91a: 3-(2-Methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzonitrile

A mixture of 3-fluoro-5-(trifluoromethyl)-benzonitrile (Lancaster Synthesis GmbH; 17 g, 89 mmol) and 2-methylimidazole (Fluka, Buchs, Switzerland; 22.2 g, 270 mmol) in *N,N*-dimethylacetamide (80 mL) is stirred at 145°C for 19 h. The solvent is evaporated off under reduced pressure and the residue is dissolved in ethyl acetate (200 mL). The solution is washed with brine (200 mL), dried (Na_2SO_4) and the solvent is evaporated off under reduced pressure to give the crude product which is purified by recrystallisation from ether - hexane to afford the title compound as yellow crystalline solid, m.p. 132-134°C.

Example 91b: 3-(2-Methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzoic acid

A solution of 3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzonitrile (Example 91a; 16.7 g, 66 mmol) in dioxane (300 mL) is added to an aqueous solution of sodium hydroxide (275 mL of 1 M) and the mixture is heated at 95°C for 18 h.The solvent is evaporated off under reduced pressure and the residue is neutralised with hydrochloric acid (1 M) and extracted with butanol (2 x 250 mL). The solvent is evaporated of under reduced pressure to give the title compound. ^{1}H-NMR (400 MHz, DMSO-d_6, δ): 7.17 (s, 1H); 8.03 (s, 1H); 8.12 (s, 1H); 8.35 (s, 1H); 8.41 (s, 1H); 8.53 (s, 1H); 13.90 (br., 1H).

Example 91c: [3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester

Triethylamine (5.23 mL, 37.5 mmol) is added to a stirred suspension of 3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzoic acid (Example 91b; 6.8 g, 25 mmol) in *t*-butanol (200 mL). Diphenylphosphorylazide (7.6 g, 27.5 mmol) is added to the resulting solution and the mixture is heated 80°C for 16 h. The solvent is evaporated off under reduced pressure and the residue is treated with water (100 mL) and extracted with ethyl acetate (2 x 100 mL). The combined extracts are washed with brine (100 mL), dried (Na_2SO_4) and the solvent is evaporated off under reduced pressure to give the crude product which is purified by column chromatography (silica gel, eluent 2% ethanol in ethyl acetate) and recrystallised from ether - hexane to afford the title compound as a colourless crystalline solid, m.p. 203-208°C.

Example 91d: 5-(2-Methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine

Utilising the procedure described in Example 90c but employing [3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester (Example 91c) in lieu of [3-(4-morpholinyl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester, afforded the title compound as a yellow crystalline solid, m.p. 130-133°C.

Example 92: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 69, but employing 5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)benzeneamine, afforded the title compound as a crystalline solid, m.p. 235–236°C.

The was aniline is prepared as follows:

Example 92a: 3-(4-Methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzonitrile

Utilising the procedure described in Example 91a, but employing 4-methyl-1H-imidazole in lieu of 2-methylimidazole, afforded the title compound as a crystalline solid, m.p. 127–128°C.

Example 92b: 3-(4-Methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzoic acid

Utilising the procedure described in Example 91b, but employing 3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzonitrile (Example 92a) in lieu of 3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzonitrile, afforded the title compound as a crystalline solid, m.p. > 300°C.

Example 92c: [3-(4-Methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester
Utilising the procedure described in Example 91c, but employing 3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzoic acid (Example 92b) in lieu of 3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzoic acid, afforded the title compound as a crystalline solid, m.p. 186-188°C.

Example 92d: 5-(2-Methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine
Utilising the procedure described in Example 91d, but employing [3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester (Example 92c) in lieu of [3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester, afforded the title compound as a colourless crystalline solid, m.p. 127-131°C.

Example 93: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(5-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide
Utilising the procedure described in Example 3, but employing 5-(5-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine in lieu of 1-(2-pyridyl)piperazine, afforded the title compound as a crystalline solid, m.p. 231-233°C.

The aniline is prepared as follows:

Example 93a: 3-(5-Methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzonitrile
Utilising the procedure described in Example 91a, but employing 4-methyl-1H-imidazole in lieu of 2-methylimidazole, afforded the title compound as a crystalline solid, m.p. 99–101°C.

Example 93b: 3-(5-Methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzoic acid
Utilising the procedure described in Example 91b, but employing 3-(5-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzonitrile (Example 93a) in lieu of 3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzonitrile, afforded the title compound as a colourless crystalline solid, m.p. 243-245°C.

Example 93c: [3-(5-Methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester

size of about 1 to 3 μm. 0.419 g portions of the mixture are then introduced into soft gelatin capsules using a capsule-filling machine.

Example 98: Pharmacokinetic data:

The compound of formula I to be tested is formulated for administration to female OF1 mice from IFACREDO, France, by first dissolving in NMP, and then by diluting with PEG300 to a final concentration of 10 % v/v NMP: 90 % v/v PEG300, producing a clear solution of the compound. The concentrations were adjusted to deliver a constant volume of 10 mL/kg body weight. The compound is prepared immediately before use. The formulated compound is administered perorally by gavage to provide dosages of 50 mg/kg. At the allotted time points mice (4 at each time) are anesthetized with 3 % isoflurane in medical oxygen and blood samples are obtained by heart puncture into heparinized tubes (ca. 30 IU/mL). The animals are subsequently killed without recovering from the anesthetic. Plasma is prepared from the blood by centrifugation (10,000 g, 5 min) and either analyzed immediately or stored frozen at – 70 °C.

The plasma samples (10 – 250 μL) are e.g. spiked with 5 μL of internal standard, mixed with 200 μL 0.1 M NaOH and 500 μL chloroform in a 1.5 mL Eppendorf tube and shaken vigorously for 10 minutes on an Eppendorf mixer. Thereafter, the mixture is centrifuged (3 min at 10'000xg), the organic phase transferred to a second Eppendorf tube and evaporated to dryness in a vacuum centrifuge (Speedvac 5301). The dry residue e.g. is dissolved in 250 μL of 10 % v/v Acetonitrile in water containing 0.1 % formic acid. The subsequent analysis is carried out e.g. by HPLC/MS-MS using an Agilent 1100 Series (Agilent, Palo Alto, CA, USA) HPLC system with vacuum degasser, binary pump, and thermostated column compartment combined with a cooled autosampler system (HTS PAL, CTC Analytics, Zwingen, Switzerland). The sample (5-15 μL) is injected e.g. onto an Ultra Phenyl column (particle size 3 μm, 50 x1 mm; Restek, Bellefonte, USA) with a guard column (4 x 2 mm) of the same material (Phenomenex, Torrance, USA). After equilibration e.g. with water and a latency period of 1 min the sample is eluted e.g. by a linear gradient of 0 – 100 % acetonitrile in water containing 0.2 % v/v formic acid over a period of 11 min at a flow rate of 60 μL/min. The column is prepared for the next sample e.g. by re-equilibrating for 3 min with 100 % water to the starting conditions. The separation is performed e.g. at a column temperature of 40 °C. The column effluent is introduced e.g. directly into the ion source of a triple stage quadropole mass spectrometer (Quattro Ultima™, Micromass, Manchester, UK) controlled by Masslynx™ 3.5 software (Micromass, Manchester, UK) using as ionization technique

Utilising the procedure described in Example 91c, but employing 3-(5-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzoic acid (Example 93b) in lieu of 3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)-benzoic acid, afforded the title compound as a crystalline solid, m.p. 169-171°C.

Example 93d: 5-(5-Methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine
Utilising the procedure described in Example 91d, but employing [3-(5-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester (Example 93c) in lieu of [3-(2-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester, afforded the title compound as a colourless crystalline solid, m.p. 131-133°C.

Example 94: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[3-(4-methyl-1-piperazinyl)-5-(trifluoromethyl)phenyl]benzamide
Utilising the procedure described in Example 69, but employing 3-(4-methyl-1-piperazinyl)-5-(trifluoromethyl)-benzenamine in lieu of 3-[(1-hydroxy-1-methylethyl)]-5-(1,1,1-trifluoromethyl)benzeneamine, afforded the title compound as a crystalline solid, m.p. 192–194°C.

The aniline is prepared as follows:

Example 94a: [3-(4-methyl-1-piperazinyl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester
Utilising the procedure described in Example 87b, but employing 1-methyl-1-piperazine in lieu of morpholine, afforded the title compound as a crystalline solid, m.p. 225°C.

Example 94b: 3-(4-methyl-1-piperazinyl)-5-(trifluoromethyl)-benzenamine
Utilising the procedure described in Example 90c, but employing [3-(4-methyl-1-piperazinyl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester (Example 94a) in lieu of [3-(4-morpholinyl)-5-(trifluoromethyl)phenyl]-carbamic acid, 1,1-dimethylethyl ester, afforded the title compound as oil. ^{1}H-NMR (400 MHz, DMSO-d_6): 2.20 (s, 3H), 2.42 (m, 4H), 3.07 (m, 4H), 3.32 (br s, 2H), 5.34 (s, 1H) and 6.31 (s, 2H).

Example 95: 4-Methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[2-(1-pyrrolidinyl)-5-(trifluoromethyl)phenyl]benzamide

Utilising the procedure described in Example 3, but employing 2-(1-pyrrolidinyl)-5-(trifluoromethyl)-benzenamine (Lancaster Synthesis Ltd.; Yasuhiro Ohtake et al., WO 9965874) in lieu of 1-(2-pyridyl)piperazine, afforded the title compound as a crystalline solid. ^{1}H-NMR (400 MHz, DMSO-d_6): 1.77-1.82 (m, 4H); 2.34 (s, 3H); 3.31-3.37 (m, 4H); 6.86 (d, 1H); 7.34-7.44 (m, 2H); 7.47 (d, 1H); 7.49-7.53 (m, 1H); 7.73 (dd, 1H); 8.27 (d, 1H); 8.43 (dt, 1H); 8.53 (d, 1H); 8.69 (dd, 1H); 9.13 (s, 1H); 9.27 (d, 1H); 9.96 (s, 1H).

Example 96: 3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-*N*-[5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)phenyl]benzamide
Utilising the procedure described in example 1, but employing 3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid in lieu of 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid and 5-(4-methyl-1H-imidazol-1-yl)-3-(trifluoromethyl)-benzenamine in lieu of furfurylamine, afforded the title compound as as pale-yellow crystalline solid, m.p. 264-266°C.

Example 96a: 3-[(Aminoiminomethyl)amino]-benzoic acid ethyl ester mononitrate
Utilising the procedure described in example 1a but employing 3-amino-benzoic acid ethyl ester (Fluka, Buchs, Switzerland) in lieu of 3-amino-4-methylbenzoic acid ethyl ester, afforded the title compound as a crystalline solid, m.p. 170-172°C.

Example 96b: 3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid ethyl ester
Utilising the procedure described in example 1b but employing 3-[(aminoiminomethyl)amino]-benzoic acid ethyl ester mononitrate in lieu of 3-[(aminoiminomethyl)amino]-4-methyl-benzoic acid ethyl ester mononitrate, afforded the title compound as a crystalline solid, m.p. 197-199°C.

Example 96c: 3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid
Utilising the procedure described in example 1c but employing 3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid ethyl ester in lieu of 4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzoic acid ethyl ester, afforded the title compound as a crystalline solid, m.p. 291-295°C.

Example 97: Soft Capsules
5000 soft gelatin capsules, each comprising as active ingredient 0.05 g of one of the compounds of formula I mentioned in the preceding Examples, are prepared as follows:
250 g pulverized active ingredient is suspended in 2L Lauroglykol® (propylene glycol laurate, Gattefossé S.A., Saint Priest, France) and ground in a wet pulverizer to produce a particle

electrospray ionization positive mode (ESI +). The compound is detected by MS/MS following fragmentation of the parent ions. The limit of quantitation is determined at e.g. 0.002 nmol/L. A calibration curve is constructed with known amounts of compound including a fixed amount of internal standard in plasma which is processed as described above. The concentration of unknown samples is calculated from a plot of the peak area ratio of the selected daughter ion of the analyte to the product of its internal standard (ordinate) against the nominal concentration (abscissa). Regression analysis is performed using Quanlynx ™, Masslynx™ software 3.5 (Micromass, Manchester, UK).

Example 99: In vitro inhibition data:

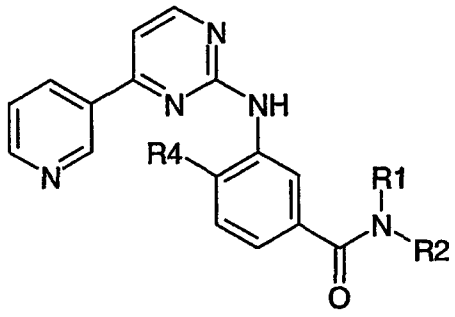
Enzymatic (c-Abl, KDR, Flt3) in vitro inhibition data are presented as % inhibition at 10 μM. The measurements are made as described above in the general description.

Example	Abl%@10μM	KDR%@10μM	Flt3%@10μM
1	51	57	
2	97	73	
3	66	71	
4	77	46	
5	71	60	
6	51	56	
7	72	45	
8	70	81	
9	44	39	
10	57	48	
11	53	41	
12	22	33	
13	78	48	
14	49	54	
15	60	23	
16	42	10	
17	54	62	
18	56	62	
19	41	33	
20	56	22	
21	30	93	
22	59	7	
23	90	67	
24	80	70	
25	44	73	
26	55	56	
27	54	51	
28	73	61	
29	78		
30	57	37	
31	68	83	
32	90	37	
33	97	51	
34	73	89	
35	27	47	
36	57	77	
37	28	82	
38	74	91	
39	64	74	
40	65	78	

Example	Abl%@10μM	KDR%@10μM	Flt3%@10μM
41	13	52	
42	32	56	
43	37	63	
44	75	97	
45	34	61	
46	1	43	
47	39	74	
48	90	50	
49	72	37	
50	87	83	
51	92	52	
52	78	37	
53	88	79	
54	69	74	
55	43	54	
56	40	44	
57	8	42	
58	40	26	
59	75	83	
60	79	36	
61	95	65	
62	59	44	
63	74	82	
64	56	59	
65	96	60	
66	67	23	
67	98	88	41
68	99	96	

WHAT IS CLAIMED IS:

1. A compound of formula



(I)

wherein

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or more identical or different radicals R_3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted; and

R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising zero, one, two or three ring nitrogen atoms and zero or one oxygen atom and zero or one sulfur atom, which groups in each case are unsubstituted or mono- or polysubstituted; or wherein

R_1 and R_2 together represent alkylene with four, five or six carbon atoms optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, oxo, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms; oxaalkylene with one oxygen and three or four carbon atoms; or azaalkylene with one nitrogen and three or four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, carboxy, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

2. A compound of formula I according to claim 1 wherein

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, acyloxy-lower alkyl, carboxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or two identical or different radicals R_3, cycloalkyl, benzcycloalkyl, heterocyclyl, an aryl group, or a mono- or bicyclic heteroaryl group comprising one, two or three nitrogen atoms or one sulfur atom, which aryl and heteroaryl groups in each case are unsubstituted or mono- or polysubstituted; and

R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, mono- or disubstituted amino, cycloalkyl, heterocyclyl, an aryl group, furanoyl, thienoyl, or a mono- or bicyclic heteroaryl group comprising one, two or three ring nitrogen atoms, zero or one ring oxygen atom and zero or one ring sulphur atom, which aryl and heteroaryl groups in each case are unsubstituted or mono- or polysubstituted; or wherein

R_1 and R_2 together represent alkylene with four or five carbon atoms, optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms in the alkylene group; oxaalkylene with one oxygen and three or four carbon atoms, or azaalkylene with one nitrogen and three or four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

3. A compound of formula I according to claim 1 wherein

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen, lower alkyl, optionally substituted by one or two identical or different radicals R_3, cyclopentyl, benzcyclopentyl, cylcohexyl, pyrrolidinyl, oxazolinyl, piperidinyl, N-substituted piperidinyl, morpholinyl, azepinyl, oxo-azepinyl, oxazepinyl, phenyl, naphthalinyl, tetrahydronaphthalinyl or a mono- or bicyclic heteroaryl group comprising one

or two nitrogen atoms, which phenyl, naphthalinyl and heteroaryl groups in each case are unsubstituted or mono- or polysubstituted, thienyl, or lower alkoxycarbonyl-lower alkylthienyl; and

R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, N-mono- or N,N-disubstituted carbamoyl, amino, lower alkylamino, di-lower alkylamino, phenylamino, N-lower alkyl-N-phenylamino, pyrrolidino, oxopyrrolidino, piperidino, morpholino, imidazolino, oxoimidazolino, cycloalkyl, heterocyclyl, furyl, phenyl, naphthalinyl, tetrahydronaphthalinyl, or a mono- or bicyclic heteroaryl group comprising one or two nitrogen atoms, which phenyl, naphthalinyl and heteroaryl group are unsubstituted or mono- or polysubstituted; or wherein

R_1 and R_2 together represent alkylene with four or five carbon atoms, optionally mono- or disubstituted by lower alkyl, cycloalkyl, heterocyclyl, phenyl, hydroxy, lower alkoxy, amino, mono- or disubstituted amino, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms in the alkylene group; oxaalkylene with one oxygen and four carbon atoms; or azaalkylene with one nitrogen and four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, N-mono- or N,N-disubstituted carbamoyl-lower alkyl, cycloalkyl, lower alkoxycarbonyl, phenyl, substituted phenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen, lower alkyl, or halogen;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

4. A compound of formula I according to claim 1 wherein

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, lower alkoxycarbonyl-lower alkyl, or phenyl-lower alkyl;

R_2 represents hydrogen; lower alkyl, optionally substituted by one radical R_3, by two phenyl groups, by two lower alkoycarbonyl groups, by phenyl and lower alkoxycarbonyl, or by hydroxyphenyl and lower alkoxycarbonyl; cyclopentyl; benzcyclopentyl; cylcohexyl; pyrrolidinyl; oxazolinyl; piperidinyl; N-lower alkylpiperidinyl; N-benzylpiperidinyl; N-pyrimidinylpiperidinyl; morpholinyl; azepinyl; oxo-azepinyl; oxazepinyl; phenyl, naphthalinyl, tetrahydronaphthalinyl or a mono- or bicyclic heteroaryl group comprising one or two nitrogen atoms, which phenyl, naphthalinyl and heteroaryl groups in each case are unsubstituted or substituted by one or two substituents selected from the group consisting of lower alkyl, trifluoro-lower alkyl, hydroxy-lower alkyl, lower alkoxy-lower alkyl, amino-

lower alkyl, lower alkylamino-lower alkyl, di-lower alkylamino-lower alkyl, N-cyclohexyl-N-lower alkylamino-lower alkyl, lower alkoxycarbonylpiperidino-lower alkyl, N-lower alkylpiperazino-lower alkyl, lower alkoxycarbonyl-lower alkyl, hydroxy, lower alkoxy, trifluoro-lower alkoxy, 1H-imidazolyl-lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, lower alkyl carbamoyl, amino, lower alkanoylamino, benzoylamino, amino mono- or disubstituted by lower alkyl, by hydroxy-lower alkyl or by lower alkoxy-lower alkyl, 1H-imidazolyl, mono- or di-lower alkyl-1H-imidazolyl, pyrrolidino, piperidino, piperazino, N-lower alkylpiperazino, morpholino, sulfamoyl, lower alkylsulfonyl, phenylsulfonyl, lower alkylsulfinyl, phenylsulfinyl, lower alkylthio, phenylthio, phenyl, pyridyl, halogenyl, or benzoyl; thienyl; or lower alkoxycarbonyl-lower alkylthienyl; and

R_3 represents hydroxy, lower alkoxy, acyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, carbamoyl mono-or disubstituted by lower alkyl, phenyl or lower alkylene, amino, lower alkylamino, di-lower alkylamino, phenylamino, N-lower alkyl-N-phenylamino, pyrrolidino, oxopyrrolidino, piperidino, morpholino, imidazolino, oxoimidazolino, cycloalkyl, heterocyclyl, furyl; phenyl, naphthalinyl, tetrahydronaphthalinyl, or a mono- or bicyclic heteroaryl group comprising one or two nitrogen atoms, which phenyl, naphthalinyl and heteroaryl group is unsubstituted or substituted by one or two substituents selected from the group consisting of lower alkyl, trifluoro-lower alkyl, lower alkoxycarbonyl-lower alkyl, hydroxy, lower alkoxy, trifluoro-lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, amino, lower alkanoylamino, benzoylamino, amino mono- or disubstituted by lower alkyl, by hydroxy-lower alkyl or by loweralkoxy-lower alkyl, pyrrolidino, piperidino, morpholino, piperazino, N-lower alkylpiperazino, N-lower alkoxycarbonylpiperazino, phenyl, pyridyl, 1H-imidazolyl, lower alkyl-1H-imidazolyl, sulfamoyl, lower alkylsulfonyl, phenylsulfonyl, lower alkylsulfinyl, phenylsulfinyl, lower alkylthio, phenylthio, halogenyl, or benzoyl; or wherein

R_1 and R_2 together represent alkylene with four or five carbon atoms, optionally mono- or disubstituted by lower alkyl, cycloalkyl, phenyl, hydroxy, lower alkoxy, amino, benzoylamino, piperidino, pyridyl, pyrazinyl or pyrimidinyl; benzalkylene with four or five carbon atoms in the alkylene group; oxaalkylene with one oxygen and four carbon atoms; or azaalkylene with one nitrogen and four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carboxy-lower alkyl, carbamoyl-lower alkyl, carbamoyl-lower alkyl N-mono- or N,N-disubstituted by lower alkyl, phenyl, lower alkylene or oxa-lower alkylene, cycloalkyl, lower alkoxycarbonyl,

phenyl, methoxyphenyl, trifluoromethylphenyl, trifluoromethoxyphenyl, pyridinyl, pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen or lower alkyl;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

5. A compound of formula I according to claim 1 wherein

R_1 represents hydrogen, lower alkyl, lower alkoxy-lower alkyl, or benzyl;

R_2 represents lower alkyl, optionally substituted by one radical R_3, by two phenyl groups, by two lower alkoycarbonyl groups, by phenyl and lower alkoxycarbonyl, or by hydroxyphenyl and lower alkoxycarbonyl; cyclopentyl; benzcyclopentyl; cylcohexyl; pyrrolidinyl; piperidinyl; N-lower alkylpiperidinyl; N-benzylpiperidinyl; N-pyrimidinylpiperidinyl; morpholinyl; azepinyl; oxoazepinyl; phenyl; naphthalinyl; tetrahydronaphthalinyl; pyridyl; lower alkyl-pyridyl; quinolinyl; thienyl; lower alkoxycarbonylmethylthienyl; or phenyl substituted by one or two substituents selected from the group consisting of lower alkyl, trifluoro-lower alkyl, hydroxy-lower alkyl, amino-lower alkyl, lower alkylamino-lower alkyl, di-lower alkylamino-lower alkyl, N-cyclohexyl-N-lower alkylamino-lower alkyl, lower alkoxycarbonylpiperidino-lower alkyl, N-lower alkylpiperazino-lower alkyl, lower alkoxycarbonyl-lower alkyl, hydroxy, lower alkoxy, trifluoro-lower alkoxy, 1H-imidazolyl-lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, lower alkylcarbamoyl, amino, lower alkanoylamino, benzoylamino, amino mono- or disubstituted by lower alkyl, by hydroxy-lower alkyl or by loweralkoxy-lower alkyl, 1H-imidazolyl, lower alkyl-1H-imidazolyl, pyrrolidino, piperidino, piperazino, N-lower alkylpiperazino, morpholino, sulfamoyl, lower alkylsulfonyl, phenyl, pyridyl, halogenyl, or benzoyl; and

R_3 represents hydroxy, lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, amino, lower alkylamino, di-lower alkylamino, phenylamino, N-lower alkyl-N-phenylamino, pyrrolidino, oxopyrrolidino, piperidino, morpholino, imidazolino, oxoimidazolino, cyclopropyl, cyclopentyl, cyclohexyl, tetrahydrofuranyl, phenyl, naphthalinyl, tetrahydronaphthalinyl, furyl, a mono- or bicyclic heteroaryl group comprising one or two nitrogen atoms, which heteroaryl group is unsubstituted or mono- or disubstituted by lower alkyl, hydroxy and lower alkoxy, or phenyl substituted by one or two substituents selected from the group consisting of lower alkyl, trifluoro-lower alkyl, lower alkoxycarbonyl-lower alkyl, hydroxy, lower alkoxy, trifluoro-lower alkoxy, lower alkanoyloxy, benzoyloxy, carboxy, lower alkoxycarbonyl, carbamoyl, amino, lower alkanoylamino, benzoylamino, amino mono- or disubstituted by lower alkyl, by hydroxy-lower alkyl or by

loweralkoxy-lower alkyl, pyrrolidino, piperidino, morpholino, piperazino, N-lower alkylpiperazino, N-lower alkoxycarbonylpiperazino, phenyl, pyridyl, 1H-imidazolyl, lower alkyl-1H-imidazolyl, sulfamoyl, lower alkylsulfonyl, halogenyl, or benzoyl; or wherein

R_1 and R_2 together represent alkylene with four or five carbon atoms, optionally mono- or disubstituted by phenyl, hydroxy, amino, benzoylamino, or piperidino; benzalkylene with four or five carbon atoms in the alkylene group; oxaalkylene with one oxygen and four carbon atoms; or azaalkylene with one nitrogen and four carbon atoms wherein nitrogen is unsubstituted or substituted by lower alkyl, phenyl-lower alkyl, lower alkoxycarbonyl-lower alkyl, carbamoyl-lower alkyl, pyrrolidinocarbonyl-lower alkyl, morpholinocarbonyl-lower alkyl, cyclopentyl, lower alkoxycarbonyl, phenyl, methoxyphenyl, trifluoromethylphenyl, pyridinyl; pyrimidinyl, or pyrazinyl;

R_4 represents hydrogen or methyl;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

6. A compound of formula I according to claim 1 wherein

R_1 represents hydrogen;

R_2 represents phenyl substituted by trifluoromethyl and optionally a further substituent selected from the group consisting of hydroxy-lower alkyl, lower alkylamino, hydroxy-lower alkylamino, di-lower alkylamino, 1H-imidazolyl, lower alkyl-1H-imidazolyl, carbamoyl, lower alkylcarbamoyl, pyrrolidino, piperidino, piperazino, lower alkylpiperazino, morpholino, lower alkoxy, trifluoro-lower alkoxy, phenyl, pyridyl, and halogenyl;

R_4 represents methyl;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

7. A compound of formula I according to claim 1 wherein

R_1 represents hydrogen;

R_2 represents phenyl substituted by 3-trifluoromethyl and optionally a further substituent selected from the group consisting of 1-hydroxy-1-methylethyl, methylamino, ethylamino, 2-hydroxy-1-propylamino, 2-hydroxy-2-propylamino, diethylamino, 1H-imidazolyl, 2- and 4-methyl-1H-imidazolyl, carbamoyl, methylcarbamoyl, pyrrolidino, piperidino, piperazino, 4-methylpiperazino, morpholino, methoxy, trifluoromethoxy, 2,2,2-trifluoroethoxy, phenyl, 2-, 3- and 4-pyridyl, chloro, and fluoro;

R_4 represents methyl;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

8. The compound of formula I according to claim 1 wherein

R_1 represents hydrogen;

R_2 represents 3-(1-hydroxy-1-methylethyl)-5-(trifluoromethyl)phenyl;

R_4 represents methyl;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

9. A compound according to any one of claim 1 wherein

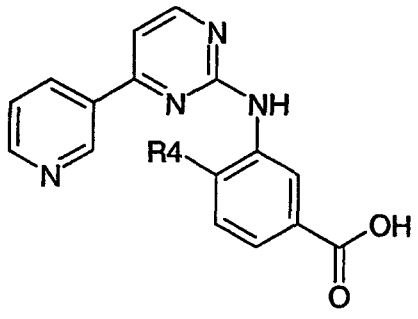
R_1 is hydrogen;

R_2 represents phenyl which is mono- or disubstituted by imidazol-lower alkoxy, lower alkyl amino, trifluoromethyl, hydroxy lower alkyl amino, bis-(lower alkoxy lower alkyl) amino, lower alkyl piperazinyl, piperidinyl, pyrrolidinyl, morpholinyl, phenyl, pyridyl, imidazolyl which is unsubstituted or mono- or disubstituted by lower alkyl or N-lower alkyl carbamoyl;

R_4 is lower alkyl;

and a N-oxide or a pharmaceutically acceptable salt of such a compound.

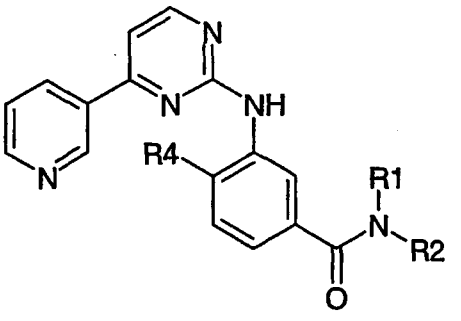
10. A compound of formula



(II)

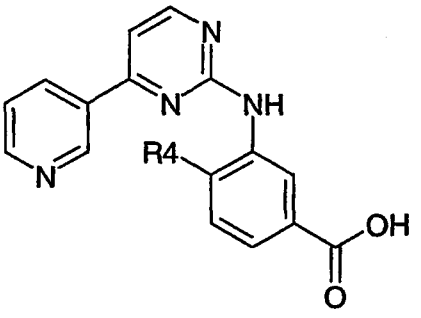
wherein R_4 is methyl or hydrogen.

11. A process for the synthesis of a compound of the formula



(I)

or an N-oxide or a salt thereof, wherein the symbols R_1, R_2 and R_4 are as defined in claim 1, characterized in that a compound of formula II



(II)

wherein R_4 is as defined for a compound of formula I, or a derivative thereof wherein the carboxy group –COOH is in activated form, is reacted with an amine of the formula III

$$R_1\text{-NH-}R_2 \qquad \text{(III)}$$

wherein R_1 and R_2 are as defined for a compound of the formula I, optionally in the presence of a dehydrating agent and an inert base and/or a suitable catalyst, and optionally in the presence of an inert solvent;

where the above starting compounds II and III may also be present with functional groups in protected form if necessary and/or in the form of salts, provided a salt-forming group is present and the reaction in salt form is possible;

any protecting groups in a protected derivative of a compound of the formula I are removed;

and, if so desired, an obtainable compound of formula I is converted into another compound of formula I or a N-oxide thereof, a free compound of formula I is converted into a salt, an obtainable salt of a compound of formula I is converted into the free compound or another

salt, and/or a mixture of isomeric compounds of formula I is separated into the individual isomers.

12. A pharmaceutical composition comprising as an active ingredient a compound of formula I according to any one of claims 1 to 10 or a N-oxide or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier.

13. A method for the treatment of a disease which responds to an inhibition of protein kinase activity, which comprises administering a compound of formula I according to any one of claims 1 to 10 or a N-oxide or a pharmaceutically acceptable salt thereof.

14. The use of a compound of formula I according to any one of claims 1 to 10 or a N-oxide or a possible tautomer thereof or of a pharmaceutically acceptable salt thereof for the preparation of a pharmaceutical composition for the treatment of a disease which responds to an inhibition of protein kinase activity.

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Exhibit A4

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REVIEW ARTICLE

Pharmaceutical Salts

STEPHEN M. BERGE *‡, LYLE D. BIGHLEY *, and DONALD C. MONKHOUSE *

Keyphrases □ Pharmaceutical salts—general pharmacy, physicochemical properties, bioavailability, pharmaceutical properties, toxicology, review □ Salts, pharmaceutical—general pharmacy, physicochemical properties, bioavailability, pharmaceutical properties, toxicology, review □ Physicochemical properties—dissolution, solubility, stability, and organoleptic properties of pharmaceutical salts, review □ Bioavailability—formulation effects, absorption alteration and pharmacokinetics of pharmaceutical salts, review □ Toxicology—pharmaceutical salts, review

CONTENTS

The chemical, biological, physical, and economic characteristics of medicinal agents can be manipulated and, hence, often optimized by conversion to a salt form. Choosing the appropriate salt, however, can be a very difficult task, since each salt imparts unique properties to the parent compound.

Salt-forming agents are often chosen empirically. Of the many salts synthesized, the preferred form is selected by pharmaceutical chemists primarily on a practical basis: cost of raw materials, ease of crystallization, and percent yield. Other basic considerations include stability, hygroscopicity, and flowability of the resulting bulk drug. Unfortunately, there is no reliable way of predicting the influence of a particular salt species on the behavior of the parent compound. Furthermore, even after many salts of the same basic agent have been prepared, no efficient screening techniques exist to facilitate selection of the salt most likely to exhibit the desired pharmacokinetic, solubility, and formulation profiles.

Some decision-making models have, however, been developed to help predict salt performance. For example, Walkling and Appino (1) described two techniques, "decision analysis" and "potential problem analysis," and applied them to the selection of the most suitable derivative of an organic acid for development as a tablet. The derivatives considered were the free acid and the potassium, sodium, and calcium salts. Both techniques are based on the chemical, physical, and biological properties of these specific derivatives and offer a promising avenue for developing optimal salt forms.

Information on salts is widely dispersed throughout the pharmaceutical literature, much of which addresses the use of salt formation to prolong the release of the active component, thereby eliminating various undesirable drug properties (2–6). This review surveys literature of the last 25 years, emphasizing comparisons between the properties of different salt forms of the same compound. Included also is a discussion of potentially useful salt forms. Our purpose is twofold: to present an overview of the many different salts from which new drug candidates can be chosen and

Table I—FDA-Approved Commercially Marketed Salts

Anion	Percent[a]	Anion	Percent[a]
Acetate	1.26	Iodide	2.02
Benzenesulfonate	0.25	Isethionate[c]	0.88
Benzoate	0.51	Lactate	0.76
Bicarbonate	0.13	Lactobionate	0.13
Bitartrate	0.63	Malate	0.13
Bromide	4.68	Maleate	3.03
Calcium edetate	0.25	Mandelate	0.38
Camsylate[b]	0.25	Mesylate	2.02
Carbonate	0.38	Methylbromide	0.76
Chloride	4.17	Methylnitrate	0.38
Citrate	3.03	Methylsulfate	0.88
Dihydrochloride	0.51	Mucate	0.13
Edetate	0.25	Napsylate	0.25
Edisylate[c]	0.38	Nitrate	0.64
Estolate[d]	0.13	Pamoate (Embonate)	1.01
Esylate[e]	0.13	Pantothenate	0.25
Fumarate	0.25	Phosphate/diphosphate	3.16
Gluceptate[f]	0.18	Polygalacturonate	0.13
Gluconate	0.51	Salicylate	0.88
Glutamate	0.25	Stearate	0.25
Glycollylarsanilate[g]	0.13	Subacetate	0.38
Hexylresorcinate	0.13	Succinate	0.38
Hydrabamine[h]	0.25	Sulfate	7.46
Hydrobromide	1.90	Tannate	0.88
Hydrochloride	42.98	Tartrate	3.54
Hydroxynaphthoate	0.25	Teoclate[i]	0.13
		Triethiodide	0.13

Cation	Percent[a]	Cation	Percent[a]
Organic:		Metallic:	
Benzathine[j]	0.66	Aluminum	0.66
Chloroprocaine	0.33	Calcium	10.49
Choline	0.33	Lithium	1.64
Diethanolamine	0.98	Magnesium	1.31
Ethylenediamine	0.66	Potassium	10.82
Meglumine[k]	2.29	Sodium	61.97
Procaine	0.66	Zinc	2.95

[a] Percent is based on total number of anionic or cationic salts in use through 1974. [b] Camphorsulfonate. [c] 1,2-Ethanedisulfonate. [d] Lauryl sulfate. [e] Ethanesulfonate. [f] Glucoheptonate. [g] *p*-Glycollamidophenylarsonate. [h] *N,N'*-Di(dehydroabietyl)ethylenediamine. [i] 2-Hydroxyethanesulfonate. [j] 8-Chlorotheophyllinate. [k] *N,N'*-Dibenzylethylenediamine. [l] *N*-Methylglucamine.

to assemble data that will provide, for the student and practitioner alike, a rational basis for selecting a suitable salt form.

POTENTIALLY USEFUL SALTS

Salt formation is an acid–base reaction involving either a proton-transfer or neutralization reaction and is therefore controlled by factors influencing such reactions. Theoretically, every compound that exhibits acid or base characteristics can participate in salt formation. Particularly important is the relative strength of the acid or base—the acidity and basicity constants of the chemical species involved. These factors determine whether or not formation occurs and are a measure of the stability of the resulting salt.

The number of salt forms available to a chemist is large; surveys of patent literature show numerous new salts being synthesized annually. Various salts of the same compound often behave quite differently because of the physical, chemical, and thermodynamic properties they impart to the parent compound. For example, a salt's hydrophobicity and high crystal lattice energy can affect dissolution rate and, hence, bioavailability. Ideally, it would be desirable if one could predict how a pharmaceutical agent's properties would be affected by salt formation.

Tables I and II list all salts that were commercially marketed through 1974. The list was compiled from all agents listed in "Martindale The Extra Pharmacopoeia," 26th ed. (7). Table I categorizes all salt forms approved by the Food and Drug Administration (FDA), while Table II lists those not approved by the FDA but in use in other countries. (Only salts of organic compounds are considered because most drugs are organic substances.) The relative frequency with which each salt type has been used is calculated as a percentage, based on the total number of anionic or cationic salts in use through 1974. Because of simple availability and physiological reasons, the monoprotic hydrochlorides have been by far the most frequent choice of the available anionic salt-forming radicals, outnumbering the sulfates nearly six to one. For similar reasons, sodium has been the most predominant cation.

Knowledge that one salt form imparts greater water solubility, is less toxic, or slows dissolution rate would greatly benefit chemists and formulators. In some cases, such generalizations can be made. Miller and Heller (8) discussed some properties associated with specific classes of salt forms. They stated that, in general, salt combinations with monocarboxylic acids are insoluble in water and lend themselves to repository preparations, while those of dicarboxylic acids confer water solubility if one carboxylic group is left free. Pamoic acid, an aromatic dicarboxylic acid, is an exception since it is used as a means of obtaining prolonged action by forming slightly soluble salts with certain basic drugs. Saias *et al.* (9) reviewed the use of this salt form in preparing sustained-release preparations. More recently, latentiation of dihydrostreptomycin (10)

Table II—Non-FDA-Approved Commercially Marketed Salts

Anion	Percent[a]
Adipate	0.13
Alginate	0.13
Aminosalicylate	0.25
Anhydromethylenecitrate	0.13
Arecoline	0.13
Aspartate	0.25
Bisulfate	0.25
Butylbromide	0.13
Camphorate	0.13
Digluconate	0.13
Dihydrobromide	0.13
Disuccinate	0.13
Glycerophosphate	0.88
Hemisulfate	0.13
Hydrofluoride	0.13
Hydroiodide	0.25
Methylenebis(salicylate)	0.13
Napadisylate[b]	0.13
Oxalate	0.25
Pectinate	0.13
Persulfate	0.13
Phenylethylbarbiturate	0.13
Picrate	0.13
Propionate	0.13
Thiocyanate	0.13
Tosylate	0.13
Undecanoate	0.13
Cation	**Percent[a]**
Organic:	
Benethamine[c]	0.33
Clemizole[d]	0.33
Diethylamine	0.33
Piperazine	0.98
Tromethamine[e]	0.33
Metallic:	
Barium	0.33
Bismuth	0.98

[a] Percent is based on total number of anionic and cationic salts in use through 1974. [b] 1,5-Naphthalenedisulfonate. [c] *N*-Benzylphenethylamine. [d] 1-*p*-Chlorobenzyl-2-pyrrolidin-1′-ylmethylbenzimidazole. [e] Tris(hydroxymethyl)aminomethane.

using p[illegible]noic acid resulted in the formation of a delayed-action [illegible]paration. Numerous studies using pamoate salts are di[illegible]ed throughout the literature (11–15).

Alg[illegible]e rel[illegible]id also has been used to prepare long-acting pharm[illegible]ration[illegible]cals. Streptomycin alginate was prepared (16) and s[illegible]review[illegible] be effective in sustained-release preparations. [illegible]ent sal[illegible] ing example of a long-acting alginate salt is that of[illegible]es.[illegible]; the [illegible]rpine. When dispersed in sterile water and dried to[illegible]pon [illegible]d gel, this compound was found useful in the prepara[illegible]f the preparations of the alginate and hydrochloride salt[illegible]e sa[illegible]sess similar miotic activity, studies showed that soli[illegible]t for[illegible] carpine alginate flakes constricted pupil size more effe[illegible]ely and increased the duration of miosis significantly [illegible]hen compared with the liquid preparations. Solid dose pilocarpine may be more uniformly available, because it diffuses more slowly through the gel matrix which holds the drug in reserve. In contrast, drops of the commonly employed solution dosage form release the dose immediately to the conjunctival fluid.

Málek *et al.* (18) devised a unique way of prolonging action through salt formation; they showed that the distribution of several antibiotics could be markedly altered by merely preparing macromolecular salts. Since macromolecules and colloidal particles have an affinity for the lymphatic system, streptomycin, neomycin, viomycin, and streptothrycin were combined with high molecular weight compounds such as polyacrylic acids, sulfonic or phosphorylated polysaccharides, and polyuronic derivatives. Parenteral administration of these compounds produced low blood levels of the antibiotic for long periods, while lymph levels were high. (In comparison, streptomycin sulfate gave high blood levels but low lymph levels.) This alteration in distribution caused the streptomycin to prolong its passage through the body, since lymphatic circulation is quite slow.

The appropriate choice of a salt form has been found to reduce toxicity. It can be rationalized that any compound associated with the normal metabolism of food and drink must be essentially nontoxic. The approach of choosing organic radicals that are readily excreted or metabolized opened up a new class of substances from which to select a salt form. For example, certain salts of the strong base choline have proven to be considerably less toxic than their parent compound. The preparation and properties of choline salts of a series of theophylline derivatives were reported (19), and it was shown that choline theophyllinate possessed a greater LD_{50} than theophylline or its other salts (20). It was postulated that this agent would be less irritating to the GI tract than aminophylline, because "its basic constituent, choline, is an almost completely nontoxic substance of actual importance to the physiologic economy." This evidence led to the preparation of choline salicylate (21) as an attempt to reduce the GI disturbances associated with salicylate administration. Clinical studies indicated that choline salicylate elicited a lower incidence of GI distress, was tolerated in higher doses, and was of greater benefit to the patient than was acetylsalicylic acid (aspirin).

Amino acids and acid vitamins also have been used as salt-forming agents. Based on the evidence that coadministration of amino acids with aminoglycoside antibiotics reduced their toxicity, a series of amino acid salts of dihydrostreptomycin was prepared (22). In all but one case, the acute toxicities of these salts were lower than the toxicity of the sulfate. The ascorbate and pantothenate also were synthesized and shown to be less toxic than the sulfate. Of the salts prepared, the ascorbate had the highest LD_{50}.

The vitamins most commonly used for forming salts exhibiting reduced toxicity are ascorbic and pantothenic acids. Keller *et al.* (23) were the first to use pantothenic acid as a means of "detoxifying" the basic streptomyces antibiotics. Parenteral administration of the pantothenates of streptomycin and dihydrostreptomycin had a significantly reduced incidence of acute neurotoxicity in cats as compared with the sulfates. Subsequent studies (24–28) supported this finding and showed that the pantothenates of neomycin and viomycin also are less toxic. The ascorbate of oleandomycin was synthesized and its pharmacological properties were reported (29). Upon intramuscular injection in rats, it produced less irritation than the phosphate.

p-Acetamidobenzoic acid, an innocuous metabolite of folic acid present in normal blood and urine, has been used in preparing salts. In particular, it yields stable salts with amines that otherwise tend to form hygroscopic products with conventional acid components (30).

Often the salt form is chosen by determining a salt

component that will pharmacologically antagonize an unfavorable property or properties exhibited by the basic agent. Salts of *N*-cyclohexylsulfamic acid are an example of the practical application of this approach. *N*-Cyclohexylsulfamic acid salts, better known as cyclamates, have a characteristic sweet, pleasing taste. Although presently under investigation by the FDA for potentially carcinogenic properties, salts incorporating this compound can render unpleasant or bitter-tasting drugs acceptable. For example, the cyclamates of dextromethorphan and chlorpheniramine exhibit greatly improved bitterness thresholds compared to commonly occurring salts (31). Furthermore, their stability in aqueous solution was described as good when maintained at a pH not greater than 4.

N-Cyclohexylsulfamic acid salts of thiamine hydrochloride and lincomycin also have been synthesized. Thiamine *N*-cyclohexylsulfamate hydrochloride was reported to have a more pleasant taste than other thiamine salts while having an equal or greater stability (32). Lincomycin cyclamate, shown to possess an enhanced thermal stability over its hydrochloride, was prepared (33) to test the hypothesis that reduced lincomycin absorption in the presence of small quantities of cyclamates was due to a simple metathetic reaction. However, this assumption was found not to be true. An extensive study of the preparation and characterization of cyclamic acid salts of several widely used classes of drugs including antihistamines, antibiotics, antitussives, myospasmolytics, and local anesthetics was reported (34, 35).

Various salts of penicillin and basic amine compounds have been formulated in an effort to produce a long-acting, nonallergenic form of penicillin. Since antihistamines appear to mitigate the symptomatology of penicillin reactions in some patients, coadministration of the two has been advocated. The preparation of the benzhydralamine salt of penicillin was an attempt to produce a repository form of penicillin with antiallergic properties (36). Blood levels achieved with this salt were comparable to those of penicillin G potassium; however, its antiallergic properties were not evaluated. In fact, the investigators noted that antihistamines can actually cause sensitization at times and stated that "despite their occasionally favorable influence on the symptoms of penicillin sensitivity, they contribute directly to the potential of drug sensitivity when co-administered with penicillin."

Silver salts of sulfanilamide, penicillin, and other antibiotics have been prepared and represent cases where the species (ions) are complementary. When aqueous solutions of the salts were applied topically to burned tissue, they yielded the combined benefits of the oligodynamic action of silver and the advantages of the antibacterial agents (37).

The use of 8-substituted xanthines, particularly the 8-substituted theophyllines, as salt-forming agents was first reported in the preparation of a series of antihistamine salts (38–41). Synthesis of these xanthine salts was an attempt to find a drug to counteract the drowsiness caused by the antihistamines with the stimulant properties of the xanthines. When an electronegative group is introduced into the xanthine molecule at the 8-position, the electron-drawing capacity of the substituent results in the creation of an acidic hydrogen at position 7. Thus, these moderately strong acidic compounds can undergo salt formation with various organic bases.

The 8-halotheophyllines were the first group of xanthines studied as potential salt-forming agents. Since the report on the preparation of the 8-chlorotheophylline salt of diphenhydramine (42), synthesis of the 8-halotheophyllinates of a number of organic bases has been attempted. The 8-chlorotheophylline salts of quinine, ephedrine, and strychnine were prepared and characterized (43). These salts were less water soluble than the corresponding free alkaloidal bases. In a similar report, the 8-chlorotheophyllinates of three synthetic narcotics, meperidine, levorphanol, and metopon, were prepared (44).

Pharmacological and clinical studies involving the 8-bromotheophylline pyrilamine salt revealed the unusual diuretic properties associated with the 8-halotheophylline portion of the compound (45, 46). This finding initiated an investigation into the preparation of a soluble 8-bromotheophylline salt of high diuretic activity. With readily available amines, over 30 salts were synthesized and screened for diuretic activity (47). When tested against theophylline salts of the same amines, the 8-bromotheophyllinates showed greater activity in every case.

With the successful formation of 8-halotheophyllinates of organic bases, Morozowich and Bope (48) proposed that, if the halogen moiety was replaced with a more electronegative substituent such as a nitro group, a more acidic compound would be formed. Presumably, more stable salts would result and precipitation of the free xanthine derivative in the stomach would be less likely to occur. On this premise, they successfully prepared pharmacologically effective 8-nitrotheophyllinates of several pharmaceutically useful bases.

Duesel *et al.* (19), in their study of choline theophyllinate, prepared the 8-chloro-, 8-bromo-, and 8-nitrotheophylline salts of choline. Oral toxicity studies in mice showed that the LD_{50} of the 8-nitrotheophyllinate was much greater than that of either 8-halotheophylline. In fact, it remained nonlethal at doses as high as [illegible] mg/kg.

Polygalacturonic acid, a derivative of pectic acid, has been used to prepare quinidine salts exhibiting reduced bitterness (49, 50). The compound possesses specific adherent properties and inhibits mucosal irritation. The rationale for use of this agent is to reduce the ionic shock to the mucosa resulting from the flood of irritating ions generated by rapid dissociation of the conventional inorganic quinidine salts. Studies have shown that it is [illegible] less toxic orally than the sulfate. This difference was attributed to the slower release of quinidine from the [illegible] galacturonate.

Other compounds reported to be potentially useful as pharmaceutical salt forms are listed in Table II.

PHYSICOCHEMICAL STUDIES

Biological activity of a drug molecule is influenced by two factors: its chemical structure and effect at a specific site and its ability to reach—and then be removed from—the site of action. Thus, a knowledge of the physicochemical properties of a compound that influence its absorption, distribution, metabolism, and excretion is essential for a complete understanding of the onset and duration of ac-

Table III—Potentially Useful Salt Forms of Pharmaceutical Agents

Salt-Forming Agent	Compound Modified	Modification	Reference
Acetylaminoacetic acid	Doxycycline	Solubility	51
N-Acetyl-L-asparagine	Erythromycin	Solubility, activity, stability	52
N-Acetylcystine	Doxycycline	Combined effect useful in pneumonia	53
Adamantoic acid	Alkylbiguanides	Prolonged action	54
Adipic acid	Piperazine	Stability, toxicity, organoleptic properties	55
N-Alkylsulfamates	Ampicillin	Absorption (oral)	56
	Lincomycin	Solubility	57
Anthraquinone-1,5-disulfonic acid	Cephalexin	Stability, absorption	58
Arabogalactan sulfate (arabino)	Various alkaloids	Prolonged action	59, 60
Arginine	Cephalosporins	Toxicity	61
	α-Sulfobenzylpenicillin	Stability, hygroscopicity, toxicity	62
Aspartate	Erythromycin	Solubility	63
Betaine	Tetracycline	Gastric absorption	64
Bis(2-carboxychromon-5-yloxy)alkanes	7-(Aminoalkyl)theophyllines	Activity, prolonged prophylactic effect	65
Carnitine	Metformin	Toxicity	66
4-Chloro-*m*-toluenesulfonic acid	Propoxyphene	Organoleptic properties	67
Decanoate	Heptaminol	Prolonged action	68
Diacetyl sulfate	Thiamine	Stability, hygroscopicity	69
Dibenzylethylenediamine	Ampicillin	Prolonged action	70, 71
Diethylamine	Cephalosporins	Reduced pain on injection	72
Diguaiacyl phosphate	Tetracycline	Activity	73
Dioctyl sulfosuccinate	Vincamine	Organoleptic properties	74
Embonic (pamoic) acid	Kanamycin	Toxicity	75
	2-Phenyl-3-methylmorpholine	Toxicity	76
Fructose 1,6-diphosphoric acid	Tetracycline	Solubility	77
	Erythromycin	Solubility	
Glucose 1-phosphoric acid, glucose 6-phosphoric acid	Tetracycline	Solubility	77
	Erythromycin	Solubility	
L-Glutamine	Erythromycin	Solubility, activity, stability	52
Hydroxynaphthoate	Bephenium	Toxicity	78
2-(4-Imidazolyl)ethylamine	Prostaglandin	Prolonged action	79
Isobutanolamine	Theophylline	Stability	80
Lauryl sulfate	Vincamine	Organoleptic properties	81
Lysine	α-Sulfobenzylpenicillin	Toxicity, stability, hygroscopicity	62
	Cephalosporins		61
Methanesulfonic acid	Pralidoxime (2-PAM)	Solubility	82
N-Methylglucamine	α-Sulfobenzylpenicillin	Toxicity, stability, hygroscopicity	62
	Cephalosporins	Reduced pain on injection	72
N-Methylpiperazine	Phenylbutazone	Toxicity, faster onset of action	83
Morpholine	Cephalosporins	Reduced pain on injection	72
2-Naphthalenesulfonic acid	Propoxyphene	Organoleptic properties	84
Octanoate	Heptaminol	Prolonged action	68
Probenecid	Pivampicillin	Organoleptic properties	85
Tannic acid	Various amines	Prolonged action	86, 87
Theobromine acetic acid	Propoxyphene	Activity	88
3,4,5-Trimethoxybenzoate	Tetracycline	Organoleptic properties	89
	Heptaminol	Prolonged action	68
Tromethamine	Aspirin	Absorption (oral)	90
	Dinoprost (prostaglandin $F_{2\alpha}$)	Physical state	91

tion, the relative toxicity, and the possible routes of administration (2).

In a review in 1960, Miller and Holland (92) stated that "different salts of the same drug rarely differ pharmacologically; the differences are usually based on the physical properties." In a subsequent review (93), Wagner expanded upon this statement, asserting that, although the nature of the biological responses elicited by a series of salts of the same parent compound may not differ appreciably, the intensities of response may differ markedly.

The salt form is known to influence a number of physicochemical properties of the parent compound including dissolution rate, solubility, stability, and hygroscopicity. These properties, in turn, affect the availability and formulation characteristics of the drug. Consequently, the pharmaceutical industry has systematically engaged in extensive preformulation studies of the physicochemical properties of each new drug entity to determine the most suitable form for drug formulation. Published information concerning such studies, however, is sparse. Preformulation studies have been outlined, and the influence of the salt form on the volatility and hygroscopicity of an agent under investigation was discussed (94).

In one such study, methylpyridinium-2-aldoxime (pralidoxime) salts were investigated (95). This study set out to prepare a salt with water solubility adequate to allow intramuscular injection of a low volume (2–3 ml) therapeutic dose. The original compound, the methiodide, had the disadvantages of limited aqueous solubility and high potential toxicity, since its high iodide content could result in iodism. On the basis of physiological compatibility, better water solubility, favorable stability, and relatively high percentage of oxime, the chloride salt of pralidoxime was selected for therapeutic administration; it was claimed that "the anion used to form the salt can confer physical properties of importance and significance for the formulation and administration of the compound" (95).

Some physicochemical properties of a series of mineral acid salts of lidocaine also were determined (96). While the hydrochloride and hydrobromide were more hygroscopic, they were more soluble in a number of solvents than the nitrate, perchlorate, phosphate, or sulfate salts.

Dissolution Rate—The dissolution rate of a pharmaceutical agent is of major importance to the formulator. In many cases, particularly with poorly soluble drugs, this characteristic best reflects the bioavailability of the com-

ound. As a rule, a pharmaceutical salt exhibits a higher issolution rate than the corresponding conjugate acid or e at an equal pH, even though they may have the same quilibrium solubility. The explanation for this result lies n the processes that control dissolution.

Dissolution can be described by a diffusion layer model[1] n terms of an equation developed by Nernst and Brunner 97):

$$\frac{dW}{dt} = \frac{DS}{h}(C_s - C) \qquad \text{(Eq. 1)}$$

where W is the mass of the solute dissolved at time t, dW/dt is the rate of mass transfer per unit time, D is the solute molecule diffusion coefficient, S is the surface area of the dissolving solid, h is the diffusion layer thickness, C is the concentration of the drug in the bulk solution at time t, and C_s is the saturation solubility of the solute in the diffusion layer.

The driving force for dissolution in Eq. 1 is the difference between the saturation solubility of the drug and the concentration of the drug in the bulk fluid. If the drug is not rapidly absorbed after it dissolves, then C, the concentration in the bulk solution, approaches C_s and the dissolution rate is retarded. When this occurs, absorption is "absorption rate" limited (or "membrane transport" limited). If the absorption rate is rapid (or if the absorption mass transfer coefficient is much larger than DS/h of Eq. 1), however, C becomes negligible compared to C_s and dissolution occurs under "sink" conditions. Absorption is then said to be dissolution rate limited, which is what occurs with most poorly soluble drugs. In either case, an increase in C_s, as in salt formation, increases dissolution.

Salts often speed dissolution by effectively acting as their own buffers to alter the pH of the diffusion layer, thus increasing the solubility of the parent compound, C_s, in that layer over its inherent solubility at the pH of the dissolution medium. Hence, dissolution is controlled by solubility in the diffusion layer which, in turn, is determined by the pH of that layer. The influence of K_{sp} on the solubility term, C_s, and dissolution rate, should an accumulation of ions be allowed to occur, will be treated later.

Nelson (98), in a study of theophylline salts, was the first to show the correlation between diffusion layer pH and dissolution rate. The major impact that this study had on the pharmaceutical sciences was its conclusion that, if other factors remained constant, the dissolution rate of a compound determined the rate of buildup of blood levels with time and the maximum levels obtained. Those salts of the acidic theophylline with high diffusion layer pH's had greater *in vitro* dissolution rates than those exhibiting a lower diffusion layer pH. And, indeed, the rank order of dissolution rates correlated well with clinically determined blood levels. Presumably, the higher pH in the diffusion layer retards hydrolysis of the salt, thereby maintaining the anionic charge of the theophyllinate ion. This report led to many additional studies which illustrate the influence of the salt form on dissolution and the beneficial effects of changing nonionized drugs into salts.

Juncher and Raaschou (99) demonstrated that the rank order of peak blood levels of penicillin V, obtained upon administration of three different salts and the free acid, was the same as the rank order of their rates of dissolution *in vitro*. While the investigators ascribed these differences to the solubility properties of the salts, their experiments actually compared dissolution rates, not solubilities. The relative order of dissolution rates and mean maximal blood levels was: potassium salt > calcium salt > free acid > benzathine salt.

Nelson (100) determined dissolution rates for several weak acids and their sodium salts in media whose pH's represented GI fluids. In all cases, the sodium salt dissolved more rapidly than the free acid. This finding resolved the misconception that absorption of drugs is related only to solubility in the appropriate medium; rather, solubility affects absorption only to the extent that it affects dissolution rate. Absorption of drugs is a dynamic process, and the ultimate solubility of a drug in fluid at absorption sites is of limited consequence since absorption prevents the attainment of saturated solutions. Therefore, dissolution rate, more than solubility, influences absorption since it is a preceding process.

In two subsequent studies, Nelson and coworkers further illustrated the effects of changing nonionized drugs into salts. A report concerning tolbutamide (101), a weak acid, showed that the initial dissolution rate of tolbutamide sodium was approximately 5000 times more rapid than the free acid in acidic media and 300 times more rapid in neutral media. This difference, measured *in vitro*, reflected the differences observed between the free acid and the salt when administered to human subjects. Oral administration of tolbutamide sodium produced an immediate drop in blood sugar comparable to that produced by intravenous injection of the salt, while the slowly dissolving tolbutamide produced a smooth, sustained fall in blood sugar (102).

Correlation of urinary excretion rates and dissolution rates of tetracycline and some of its acid salts also was demonstrated by Nelson (103). The salts that exhibited greater *in vitro* dissolution rates showed greater urinary excretion rates, indicating more rapid absorption.

Benet (104), in a discussion of the biopharmaceutical basis for drug design, referred to the influence of the salt form on dissolution. He compared the dissolution rates of tetracycline and tolbutamide and their salts, as reported in the studies previously cited, and explained why the rates differ at the pH's exhibited by physiological fluids.

Although salt formation usually increases the dissolution rate of a drug, studies with aluminum acetylsalicylate (105, 106), warfarin sodium (107), and benzphetamine pamoate (108) showed that administration of the salt *slowed* dissolution of the drug and subsequent absorption compared to the nonionized form. This decrease appeared to result from precipitation of an insoluble particle or film on the surface of the tablet. Such a phenomenon decreases the effective surface area and prevents deaggregation of the particles. Theoretical considerations of the processes controlling dissolution of an acid salt of a base (108) and the sodium salt of a weak acid (109, 110) in reactive media have been discussed.

Tablet processing and various formulation factors can decrease the dissolution rate of a salt in human gastric juice over its nonionized form (111). Granulation and tableting caused the dissolution rate of phenobarbital sodium to

[1] The authors recognize the existence of other models; this one was chosen simply for illustrative purposes.

decrease but had the opposite effect on phenobarbital. Therefore, as a tablet dosage form, the dissolution rate of the sodium salt was slower than that of the free acid. These results were attributed to differences in the disintegrating properties of the tablets; in some instances, rapid dissolution may in fact be a problem for very soluble drugs.

Others have illustrated a phenomenon that decreases the dissolution rate of a salt below that of its nonionized form. Lin *et al.* (112) studied the relationship between salts and biological activity by dissolving three salts and the free base of an experimental antihypertensive in water, 0.1 *N* HCl, and pH 7.2 phosphate buffer. The dissolution rate of the monohydrochloride salt was lower than that of the free base in 0.1 *N* HCl and higher than the free base in both water and phosphate buffer. These authors ascribed this variation to the common ion effect and substantiated it experimentally. Although the biological activity of the monohydrochloride was greater than that of the free base, the implications of altered absorption characteristics on the activity of any other hydrochloride salt in GI fluids must be considered. Similar results also were reported for hydrochloride salts of some tetracyclines (113).

Some consideration must be given to the influence of salt formation on oral toxicity, which often reflects the relationship between the *in vivo* dissolution rate and the appearance of drug in the circulation (114, 115). Morozowich *et al.* (114) showed that the relative toxicities of a series of salts of a drug reflect the rate of absorption, providing the salt-forming agents themselves are relatively nontoxic. They stated that "when absorption is rate-limited by dissolution of the salt in the gastrointestinal tract, as will be the case with slowly soluble salts, the toxicity of a slowly dissolving salt will most probably be lower than that of a more rapidly dissolving salt." The implications of salt formation on toxicology will be discussed under *Toxicological Considerations.*

Several reviews dealt with the influence of the dissolution rate on drug availability and, in particular, salt effects (116, 117). Other reports illustrating the influence of salts and salt form on dissolution rate are listed in Table IV.

Solubility—Knowledge of the solubility characteristics of a pharmaceutical agent is essential, because solubility is usually an important factor in the pharmacokinetic profile, the chemical stability, and, ultimately, the formulation of the drug. As discussed previously, it is certainly a primary factor in controlling dissolution rates. The solubility of a compound depends basically upon the physical and chemical properties of the solute; *e.g.*, a lower melting point for a compound within a series reflects a decreased lattice energy, which would suggest a higher solubility. Solubility depends as well upon such elements as temperature, pressure, solvent properties (such as resulting pH), and, to a lesser extent, the state of subdivision of the solute.

An important solvent property which is often overlooked involves the common ion effect; in particular, hydrochloride salts of drugs often exhibit less than desirable solubility in gastric juice because of the abundance of chloride ions. The equilibrium involved is shown in Scheme I.

$$DH^+Cl^-_{(solid)} \overset{K_{sp}}{\rightleftharpoons} (DH^+)_{aq} + (Cl^-)_{aq}$$

Scheme I

Salt formation is perhaps one of the first approaches considered as a means of increasing a compound's water solubility. As with dissolution rates, however, salt formation does not always confer greater solubility. Liberally dispersed throughout the pharmaceutical literature are studies that compare the solubilities of different salt forms of the same compound with those of its free acid or base (Table IV). Selection of the salt form exhibiting the desired solubility properties is critical, since these properties often dictate the formulation characteristics of the drug.

Table IV—Additional References on Salt Form and Dissolution Rate

Topic	Reference
Dissolution rate of mixtures of weak acids and tribasic sodium phosphate	118
Physiological availability and *in vitro* dissolution characteristics of some solid dosage formulations of aminosalicylic acid and its salts	119
Biopharmaceutics, rate of dissolution: chronological bibliography	120
Biopharmaceutics: rate of dissolution *in vitro* and *in vivo*	121
Dissolution tests and interpretation of anomalies observed in the dissolution process of sulfaquinoxaline based on salt formation	122
Influence of the dissolution rate of lithium tablets on side effects	123
Dissolution kinetics of drugs in human gastric juice	124
Comparison of dissolution and absorption rates of different commercial aspirin tablets	125
In vitro dissolution rates of aminorex dosage forms and their correlation with *in vitro* availability	126

Phase solubility techniques were used to study the formation of complex salts of triamterene (127). The results indicated that the organic acid salts of basic drugs, such as amines, were more soluble in water than the corresponding inorganic (halide) salts. This consideration is important in the synthesis and selection of a salt form that will exhibit enhanced bioavailability and desirable formulation characteristics.

The hydrogen-ion concentration can significantly affect the solubility of a salt. Anderson (128) discussed the influence of pH on the solubility of pharmaceuticals. Mathematical relationships between pH and solubility of therapeutically useful weak acids and bases and their salts were given along with some considerations in the formulation of solutions of these particular agents.

An extensive study on the solubility interrelationships of the hydrochloride and free base of two pharmaceutically useful amines was reported (129). Mathematical equations describing the total solubility at an arbitrary pH in terms of the independent solubilities of the hydrochloride and free base species and the dissociation constant of the salt were derived and fitted to experimental data with good results. This report elucidated the point that, while the solubility of the amine hydrochloride generally sets the maximum obtainable concentration for a given amine, the solubility of the free base and the pKa determine the maximum pH at which formulation as a solution is possible (assuming that the desired concentration exceeds the free base solubility). Shifting the pH–solubility profile to higher pH values for formulation purposes may require increasing the solubility of the free base. This increase might be accomplished by using an appropriate cosolvent. Since the dissociation characteristics of carboxylic acids and other acidic organic species are similar to those of organic hydrochlorides, it is expected that the pH–solubility profiles

of these organic acids, although reversed, can be characterized theoretically using the same treatment.

Several reports showed that the structure of an organic salt-forming radical influences the solubility of the resulting salt. The water solubilities of 16 salts (carboxylates, sulfates, sulfamates, and phosphates) of the weak base erythromycin were dependent on the size of the alkyl group of the acid (130). In a study with *N*-alkylsulfamates of lincomycin (66), a similar phenomenon was observed: solubility of these salts decreased as the size of the alkyl group attached to the acidic function increased.

Senior (131), in a study on the formulation and properties of the antibacterial chlorhexidine, determined the water solubilities of 35 salts and the free base. He found that inorganic salts had remarkably low solubilities while those of the lower aliphatic acids proved to be somewhat more soluble. Hydroxylation of the acid increased solubility, since salt formation with polyhydroxy acids, particularly the sugar acids, conferred extensive water solubility to the molecule.

Several investigators reported the influence of the solubility of a drug on its formulation and subsequent availability from the dosage form. In a discussion of the preparation and formulation of epinephrine salts in an aerosol system using liquefied gas propellants, Sciarra *et al.* (132) pointed out that the solubility characteristics of the agent are important in two respects. First, the solubility of the therapeutically active ingredient in the various propellants is an important consideration if the product is to be used for either local action in the lungs or systemic therapy. Second, the solubility of the drug in extracellular fluids plays an important role in selection of the compound. The bitartrate, malate, maleate, and fumarate salts of epinephrine were prepared and subjected to solubility and stability studies. While all salts had similar partition coefficients, the solubility of the maleate in several propellants and its stability in formulated aerosols made it the drug form of choice.

Ephedrine hydrochloride was more rapidly released than the free base from theobroma oil suppositories containing different surfactants (133). This enhanced rate of release (dialysis) was ascribed primarily to the greater aqueous solubility of the hydrochloride, which solubilized faster from the oil-in-water emulsion, whereas the ephedrine alkaloid base tended to remain behind in the oil phase.

The solubility of the active ingredient in ointment bases can dramatically influence its diffusion properties. A study of salicylic acid and its sodium salt showed that the diffusion of both was very low from hydrophobic bases, whereas the solubility of the drug significantly affected the diffusion from hydrophilic bases. The more soluble sodium salicylate diffused much faster from these latter bases than did salicylic acid (134).

Additional references on the relationship of salt form and solubility are listed in Table V.

Organoleptic Properties—Modern medicine requires that a pharmaceutical formulation be efficacious, safe, stable, and acceptable to the patient. Of primary importance in the development of oral dosage forms is taste acceptability. This factor presents no major problems when the drug is to be administered as a tablet or a capsule and swallowed as a unit but is clearly a prominent factor in patient acceptability when it is to be administered as a liquid, chewable tablet, or lozenge.

Table V—Additional References on Salt Form and Solubility

Topic	Reference
Influence of solubility on the rate of GI absorption of aspirin	135
Effect of dosage form upon the GI absorption rate of salicylates	136
Physical–chemical properties of polyene macrolide esters and their water-soluble salts	137
Isolation and reaction products of orotic acid and amines and their solubility in water	138
Solubility and stability of erythromycin salts	139
Studies on pharmaceutical preparations of orotic acid: water-soluble properties of orotic acid salts	140
Solubility of antibiotics in 24 solvents	141, 142
Solubility of antibiotics in 26 solvents	143

Since taste is a chemical sense, a substance must be dissolved if it is to elicit a taste sensation—either by taking it as a solution or by its dissolving in the saliva. Therefore, one method used to minimize undesirable organoleptic properties of pharmaceuticals involves the preparation of a poorly soluble salt form of the drug such that the saturation concentration is less than the taste threshold.

Erythromycin estolate (lauryl sulfate) has approximately one-twelfth the solubility of the free base, is tasteless, and is useful in the formulation of oral suspensions (144). A study on erythromycin salts showed that the bitterness level was dependent on two properties: (*a*) the water solubility of the salt, which is dependent on the size of the alkyl group attached to the acid function; and (*b*) the strength of the acid used to form the salt, *i.e.*, the stability of the salt (130). The stearyl sulfamate salt possessed the most desirable organoleptic properties.

Many problems concerned with formulation and stability of topical and oral pharmaceuticals containing bacitracin have been overcome by incorporating bacitracin into the formulation as its zinc salt. One distinct advantage over the parent compound is its lack of taste, caused by its relative insolubility. Thus, it is the preferred drug form for preparations where taste is a factor (145). Taste panel evaluations of the comparative bitterness of bacitracin zinc and bacitracin indicate that the taste of the zinc salt is more easily masked and that the presence of a bitterness-masking adjuvant, such as sucrose, increases the bitterness threshold ratio differences between the two compounds even further (146).

Propoxyphene napsylate, nearly water insoluble, is only slightly bitter to the taste as compared to the highly water-soluble hydrochloride (147) and can be readily formulated into a flavored aqueous suspension. The taste of these suspensions can be improved significantly by the addition of a common ion (sodium or calcium napsylate) to depress solubility further.

A newer approach to the improvement of drug palatability has been to form insoluble salts with ion-exchange resins. Several investigators described and tested the practical application of this method (148–150). Spross *et al.* (149) outlined the conditions necessary for improving the palatability of a drug by adsorbing it onto an ion-exchange resin without appreciably modifying its pharmacological effects. They found that: (*a*) the degree of drug release from the ion-exchange adsorbate depends on the

equivalent quotient between the electrolytes in the surrounding fluid and the ionic drugs, (*b*) the amount of ions is far less in the saliva than in the gastric juice (the temporary electrolyte contents can be estimated at 0.05 mEq in the saliva and at 10 mEq in the gastric juice), and (*c*) the exchange rates should allow the equilibria to be attained within a fairly short period. Insoluble drug resinates formed between dextran gel[2] cation exchangers and several basic drugs were in many cases much more pleasant tasting than their parent compounds. Furthermore, release of the drug from the ion-exchange adsorbate was quite rapid and complete under conditions prevailing in the GI tract.

Similar findings were reported using a polymethacrylic acid ion-exchange resin (150). In addition, coating the adsorbate particles with a 4:1 ethylcellulose–hydroxypropyl methylcellulose mixture further reduced bitterness. While *in vitro* release from the uncoated resinate was rapid and complete, release from coated adsorbates varied with the extent of coating.

Another approach to improving the taste properties of pharmaceutical agents is to prepare a pleasant-tasting soluble salt of a poor-tasting parent drug. This approach often can be very difficult, however, since solubilization of the parent compound usually imparts its unpleasant taste to the preparation. Nevertheless, some success has been reported using the artificial sweeteners cyclamate sodium and saccharin.

As described earlier, formation of *N*-cyclohexylsulfamate salts of several drug substances has produced better tasting derivatives with enhanced solubility properties (31, 32). The physicochemical and toxicological properties of benzalkonium saccharinate and a series of saccharinates of other quaternary ammonium compounds were reported (151). While conventional quaternary ammonium compounds have a very bitter taste, their saccharin analogs are sweet.

Potassium salts frequently possess an unpleasant taste and a metallic aftertaste. The palatability of some potassium salts in flavored vehicles was reported (152); while the salts had similar taste thresholds at effective therapeutic levels, all potassium salts exhibited inferior palatability.

Table III includes several samples of other salts that exhibit an improved taste relative to their free acid or base forms.

Stability—The chemical and physical stability of a pharmaceutical must be known, because it can influence the choice of dosage form, the manufacturing and packaging, and the therapeutic efficacy of the final preparation. Systematic determination of the thermal stability, solution stability (at various pH's), and light sensitivity of a drug and its derivatives, both alone and in the presence of common additives, provides essential input toward selecting the most suitable derivative and dosage form.

Depending on the route of degradation, different salt forms impart different stability characteristics to the parent drug by various mechanisms. Most commonly used are sparingly soluble salts which, when used in the formulation of suspensions, reduce the amount of drug in solution and, hence, its degradation. Differences in hygroscopicity of several salts influence the stability of the drug in the dry state. In some cases, the salt-forming radical itself enhances the stability of the parent agent.

The stability of penicillin G and its salts has been widely studied due to the drug's therapeutic importance and its characteristic instability. Schwartz and Buckwalter (153) described some of the stability characteristics of this antibiotic, stating that, with present techniques, a solution of penicillin cannot be made stable for more than 2 weeks, even at refrigerator temperatures. They also discussed the use of suspensions of sparingly soluble amine salts in aqueous vehicles as a means of "allowing marketing of a 'ready-made' penicillin product." Procaine, benzathine, and hydrabamine salts are marketed, and their acceptable stability as aqueous suspensions is based mainly on their insolubility and the minimization of degradation in solution.

A theoretical treatment of the solubility of these salts was presented in which equations were derived for calculating the solubility as a function of pH and the pH of minimum solubility (154, 155). These equations are based on the mass action law and its relationship to the ionization constants of the amine and the penicillin and the solubility of the salt in water. Since the salt in solution is partially dissociated, further suppression of the solubility may be achieved by the common ion effect. Swintosky *et al.* (156) demonstrated this effect with penicillin G procaine by adding procaine hydrochloride to the preparation and further enhancing its stability. The 8-chlorotheophylline salt (or complex) of penicillin G was reported to be water soluble, yet stable in solution (157). Since 8-chlorotheophylline is acidic, it has been postulated that a buffer effect could account for the stabilization of this "salt."

While penicillin G procaine is more stable in aqueous vehicles, it is less thermally stable than the sodium or potassium salts, decomposing if heated much above 60°. The sodium and potassium salts are known to withstand heating up to 100° for 4 days with little loss in potency (158). This behavior might well be due to differences in melting points—*viz.*, 106° for penicillin G procaine and ~215° dec. for the potassium salt.

Since hydrolysis of penicillin is dependent on moisture content, preparations in which moisture is rigorously excluded are quite stable in the dry state. A study on the effect of moisture on penicillin salts found the calcium salt to be less hygroscopic than the sodium salt and, hence, more stable in moist atmospheres (159). Similarly, penicillin G potassium is also much less hygroscopic than penicillin G sodium and has become the preferred form for marketing in the dry state (160).

Several studies reported the relative stabilities of thiamine salts, particularly the hydrochloride and the mononitrate (161–163). The mononitrate is observed to be less hygroscopic and is accordingly much less water soluble than the hydrochloride. Investigations of various preparations including compressed tablets, multivitamin capsules, and dry-filled vitamin B complex capsules at various temperatures showed that the mononitrate was more stable than the hydrochloride (164, 165). The stabilities of numerous thiamine salts were studied in aqueous solution and in dry powder preparations with various excipients (166, 167). In aqueous solution, the resulting pH was the chief factor controlling hydrolysis and oxidative decomposition of thiamine salts; their stability as powder

[2] Sephadex.

preparations was related to their aqueous solubility, with the sparingly soluble salts being more stable (and presumably less hygroscopic).

An orally administered drug must be stable in acidic solution because it generally must pass intact through the acidic environment of the stomach if it is to exhibit therapeutic blood levels. The advantage of erythromycin estolate over the free base lies in its low solubility in gastric juice, which enables it to be administered with food without any decrease in attained blood levels. The salt is more stable in the stomach because it remains undissolved. Therefore, it retains its potency even when exposed to acidic environments for extended periods (144).

In a study on the preparation and characterization of lincomycin cyclamate (33), it was noted that the cyclamate salt had an enhanced thermal stability over the hydrochloride. In a subsequent report (168), differential thermal analysis and thermogravimetric analysis showed that while the hydrochloride easily undergoes thermal degradation, the cyclamate anion confers a considerably greater thermal stability on the lincomycin moiety.

Mullins and Macek (169) showed that the physical and chemical stability of the calcium salt of novobiocin makes it the form of choice for the formulation of a liquid preparation of the antibiotic. The amorphous calcium novobiocin salt proved to be tasteless, yet fully biologically active and perfectly stable in aqueous suspension. Neither the sodium salt nor the free acid is suitable; the sodium salt cannot be formulated in a liquid due to its chemical instability, while the crystalline free acid is not absorbed from the GI tract. Amorphous novobiocin is absorbed but is metastable in solution and slowly converts to the unabsorbed crystalline form.

Other reports of alterations in stability characteristics due to salt formation are listed in Table VI.

Miscellaneous Properties—The salt form has been reported to influence other physicochemical properties of a drug substance. Studies illustrating the effect of the salt-forming radical on surface tension, deaggregation behavior, and ion-pair extraction have appeared.

The influence of the anion on the absorption processes of two charged species, dextromethorphan and tetracycline, was studied in the rat stomach (186, 187). A linear relationship existed between the rate of absorption from buffer solutions of the anions under investigation and their surface tensions. Thus, the absorption process was related to the surface activity of the various salts and not to their lipid solubilities. This change of surface activity with the buffer (or salt) species is similar to the results reported in a study of the surface activity of various phenothiazine salts (188).

The antibacterial chlorhexidine possesses surface activity. A study of the colloidal properties of some chlorhexidine salts showed that the counterion can affect the critical micelle concentration of a surface-active agent, and this effect was usually associated with a change in micellar size (189).

The deaggregation behavior of a relatively insoluble acid and its sodium salt was studied, and deaggregation was postulated to be a possible rate-limiting step in the absorption of a drug from a dosage form (190). While no direct comparisons of the two forms were made, inspection of the data shows that the deaggregation rate of the salt was considerably more rapid than that of the free acid in equivalent dosage forms. Therefore, if absorption is dependent on the dissolution rate, which in turn is dependent on the deaggregation rate, the salt should produce the highest and earliest blood levels. On the other hand, it is possible that hygroscopic (and deliquescent) salts can absorb atmospheric moisture, cause a sticky surface, and inhibit deaggregation.

Table VI—Additional References on Salt Form and Stability

Topic	Reference
Stability of chlorhexidine solutions	170
Stability of chlorhexidine when autoclaved	171
Anhydrotetracycline and 4-epianhydrotetracycline in marketed tetracycline and aged tetracycline products	172
Solid-state stability of some crystalline vitamin A compounds	173
Physicochemical studies on the stability of penicillin salts	174
Light sensitivity of tetracyclines	175
Hygroscopic properties, thermostability, and solubility of oleandomycin salts	176
Stability of orotic acid and its amine salts in aqueous solution	177
Some factors influencing the stability of tablets (aspirin)	178
Stability of aqueous solutions of sodium aminosalicylate	179
Hygroscopic properties of various preparations of erythromycin	180
Physicochemical studies on the decomposition of aminosalicylic acid and its salts	181
Stabilities of aqueous solutions of 2-diethylaminoethyl-3-methyl-2-phenylvalerate hydrochloride and its methobromide	182
Investigation of some properties of penicillin G salts	183
Stability of ferrous iron tablets on storage	184
Stability of aspirin aluminum compounded with antacids	185

Higuchi and coworkers presented an extensive study on the physicochemical basis of the ion-pair extraction of pharmaceutical amines. Distribution ratios of dextromethorphan (191) and chlorpheniramine (192) between an organic layer and water were highly dependent on the concentration and nature of the anion present. Less hydrophilic anions yielded more readily extractable ion-pairs. A study of the thermodynamic properties, enthalpy, free energy, and entropy, involved in the extraction equilibria of dextromethorphan ion-pairs indicated that the entropy change associated with transfer of the different anions between phases is the main controlling factor in the extraction process (193).

BIOAVAILABILITY

Most drugs prescribed in the United States are administered in solid and polyphasic dosage forms. Consequently, dissolution of the drug must precede the absorption process. The simplest model that adequately describes this process is shown in Scheme II.

$$\text{solid drug} \xrightarrow[1]{\text{dissolution}} \text{dissolved drug} \xrightarrow[2]{\text{absorption}} \text{drug in circulation}$$

Scheme II

Since the dissolution rate is generally slow for drugs with poor solubility, Step 1 is frequently rate limiting in the overall absorption process. As a result, the onset, intensity, duration of pharmacological activity, and, hence, bioavailability are affected by changes in dissolution rate. As discussed previously, administering a salt of the parent drug often proves to be an effective means of altering dissolution rate and absorption.

Table VII—Additional References on Bioavailability and Formulation Effects

Topic	Reference
Effects of various substances on the absorption of tetracycline in rats	197
Effects of dosage form upon the GI absorption rate of salicylates	136
Determination of *in vivo* and *in vitro* release of theophylline aminoisobutanol in a prolonged-action system	198
Ion-exchange resin salts for oral therapy: carbinoxamine	199
Latentiation of dihydrostreptomycin by pamoate formation	10
Solid-state ophthalmic dosage systems in effecting prolonged release of pilocarpine in the cul-de-sac	17
Absorption of erythromycin: various pharmaceutical forms	200
Comparative study of the absorption of drugs from old and new rectal preparations	201

Formulation Effects—Choice of the salt form of a drug may have a pronounced effect on the formulation of the parent compound. For example, Fenton and Warren (194) found there was no release of medicament from proflavine cream BPC, a water-in-oil emulsion containing 0.1% proflavine as the hemisulfate salt. They also investigated the release of various salts of proflavine with aliphatic carboxylic acids from water-in-oil cream emulsions. Salts formed with the water-soluble, oil-insoluble "lower" acids, such as formic and acetic acids, showed very poor release from a water-in-oil cream. The "higher" acid salts (*e.g.*, *n*-valeric, caproic, cyclohexanecarboxylic, and caprylic) all showed increased diffusion from similar emulsions since these salts are soluble in both water and oil. Their release was even greater from oil-in-water emulsions, however, in agreement with their preferential oil solubility. The *n*-valerate salt provided the most effective water-in-oil cream. The primary factor responsible for diffusion of proflavine from a water-in-oil cream is the low hydrophilic–lipophilic balance conveyed to the salt by the acid. This finding illustrates the desirability of carefully selecting the salt anion of a cationic drug in lieu of the nature of the dosage form.

Studies of the effect of formulation on the bioavailability of warfarin sodium relative to warfarin yielded interesting results (107, 195). Absorption of warfarin upon administration of the sodium salt as a lactose-base tablet was no better than that from a similar formulation of the free acid. In fact, absorption was further depressed when the salt was formulated with starch instead of lactose. Later results indicated that the *in vitro* water dissolution rate of a warfarin sodium tablet was 350 times that of a slowly dissolving warfarin tablet formulation, yet the latter exhibited rapid and complete absorption *in vivo*. The virtual insolubility of warfarin in acidic gastric fluids precluded its absorption from the stomach. However, the strongly acidic medium was necessary for tablet disintegration, which, in turn, was critical for absorption. Following initial exposure to 0.1 *N* HCl, *in vitro* dissolution of the warfarin tablet in pH 7.4 buffer was 14 times faster than that of the sodium salt, a result that explained the otherwise contradictory *in vivo* blood level data. Therefore, absorption was ultimately dependent upon gastric emptying rate and gastric pH, as long as the formulation disintegrated properly in the stomach.

The rectal absorption of aspirin, aspirin aluminum, and calcium carbaspirin from several suppository bases was studied in dogs (196). The absorption of aspirin aluminum from either cocoa butter or a polyethylene glycol base was poor. While the maximum salicylate levels produced by aspirin and calcium carbaspirin from the cocoa butter base occurred at a later time than from the other bases studied, minimal plasma levels were exhibited by a polysorbate 61 base formulation. The highest peak and largest area under the blood level curve were seen with calcium carbaspirin in a vegetable fatty acid glyceride base. The poor absorption of aspirin aluminum from suppositories was not unexpected since it is poorly soluble. Furthermore, as pointed out for aspirin aluminum tablets (105, 106), an insoluble aluminum compound may form on the surface of the dissolving drug, further impeding its dissolution rate and bioavailability.

Additional references on bioavailability and formulation effects are given in Table VII.

Absorption Alteration—Several years ago, clinicians claimed that certain salts of theophylline were therapeutically preferable to other salts or to the free acid (202–204). For example, Schluger *et al.* (204) found higher blood theophylline levels after administering uncoated tablets of theophylline ethylenediamine than were observed with enteric-coated tablets of choline theophyllinate. These results were at variance with the *in vivo* work of Gagliani *et al.* (202), who found that the oral ingestion of choline theophyllinate produced significantly higher blood levels than the ethylenediamine salt. This apparent discrepancy could be explained by the formulation effects of tablet coating, *etc.*, which was not discussed in the work of Gagliani *et al.* In another study, a slightly more rapid rise in blood concentration and a greater area under the curve were observed with theophylline isopropanolamine than with theophylline ethylenediamine (203). It was suggested that the difference was a result of the greater water solubility of the isopropanolamine salt.

The *in vitro* dissolution rates of the choline and isopropanolamine salts of theophylline have been observed to be three to five times greater than the ethylenediamine salt, depending on the dissolution medium (98). It has been suggested that these differences in dissolution rate are consistent with, and offer an explanation for, the clinical results.

In a comparative study of the absorption of ampicillin trihydrate and ampicillin potassium following oral administration (205), the potassium salt yielded 37% higher peak levels and a larger area under the curve. Only 36% of the administered ampicillin trihydrate was absorbed while 53% of the potassium salt was absorbed. Determining the percentage of each drug eliminated in the urine showed that 39% was eliminated following administration of the trihydrate and 52% from the potassium salt. The entire difference between the two drug forms was accounted for in the initial 4 hr postadministration.

Several studies compared blood levels obtained with erythromycin and its salts and esters. Erythromycin estolate produced blood levels that were severalfold higher than those obtained with erythromycin base or erythromycin stearate (206–208). These differences were found to persist in the fasting as well as nonfasting subjects (207), indicating that food did not appreciably alter the absorption of erythromycin estolate when given under single or

multiple dosing (207). This finding is explained by the fact that this salt is acid stable and alkaline dissociable, permitting its passage through the acid of the stomach both in fasting and nonfasting subjects (209). Accordingly, the antibacterial activity remained essentially the same when this form of erythromycin was given, regardless of the state of fasting of the subject (209).

On first inspection, the higher serum levels attained with erythromycin estolate suggest that the salt form is more readily absorbed. Also critical to efficacy, however, is the volume of distribution of the drug, since extensive binding to plasma proteins can render a drug unavailable for activity at the biophase. Therefore, the significance of blood level data greatly depends on the measure of the free or unbound fraction of total antibiotics in the blood, which more directly indicates probable therapeutic benefit.

Wiegand and Chun (210) showed that, despite the higher blood levels attained with erythromycin estolate (after correction for half-life differences), the stearate salt produced seven times more free drug in serum than did the estolate salt. This finding explained the higher total tissue levels observed on administration of the stearate. They attributed the difference to a greater serum protein binding of the intact erythromycin estolate, proving that its higher serum levels did not necessarily reflect more efficient absorption.

Marked differences have been observed following oral administration of various salts of penicillin. Penicillin G potassium has been compared with penicillin G benzathine (benzethacil) (211, 212), penicillin G hydrabamine (213), penicillin V (214), penicillin G procaine (212), and penicillin G ammonium (215). As anticipated, penicillin G potassium produced higher and earlier blood levels than penicillin G benzathine (211, 212). Furthermore, tablets of penicillin G potassium buffered with sodium citrate yielded higher peak levels than unbuffered penicillin G potassium. While the absorption of buffered tablets was apparently not significantly affected by food intake, the unbuffered tablets yielded lower average levels and irregular absorption under similar conditions.

When penicillin G potassium was compared with penicillin G hydrabamine (213), a less soluble salt, blood levels similar to those produced by penicillin G benzathine were observed. When equivalent doses were administered, the penicillemia that occurred with penicillin G hydrabamine was only one-third or one-fourth as great and was of shorter duration than that produced by penicillin G potassium.

Budolfsen *et al.* (212) found that peak concentrations of penicillin G potassium were three to four times those obtained with penicillin G procaine and five to six times those of penicillin G benzathine, indicating a more rapid rate of absorption. The therapeutic action was related to the maximum concentration attained, but it also depended on the persistence of penicillemia, which was greater with penicillin G potassium than with the other two compounds. The authors suggested that the lower blood levels attained with the relatively insoluble penicillin G benzathine were caused by its destruction in the GI tract prior to absorption. This suggestion seems unlikely, however, since the drug should not degrade very rapidly as an undissolved suspension.

Because of its superior stability in gastric juice, penicillin V produces higher blood penicillin levels than corresponding doses of penicillin G. As a result, extensive investigations have been conducted with various salts of penicillin V (99, 214–220). For instance, tablets, capsules, and oral suspensions of penicillin V acid produced significantly higher blood concentrations than comparable penicillin G preparations (214). In the same study, the average serum levels produced by the benzathine salt of penicillin V were significantly higher than comparable doses of penicillin G benzathine for the first 4 hr but lower thereafter, again illustrating the value of using an insoluble salt to prolong blood levels of an acid-unstable compound. In another study (215), penicillin V acid was shown to produce higher and more prolonged plasma concentrations than either penicillin G potassium or ammonium, whose properties were comparable.

Plasma levels were correlated with dissolution rates of various forms of penicillin V (99). As solid dosage forms, the readily soluble potassium and calcium salts produced earlier and higher blood levels in fasting subjects than either the free acid or its benzathine salt. On the other hand, when the potassium and benzathine salts were administered orally as solutions, absorption was the same, implying that the poor solubility of the benzathine salt was responsible for the inferior blood levels obtained from its solid dosage forms. Other studies found that, while the potassium salt is 40% better absorbed than the free acid in fasting subjects, both forms produce therapeutic levels when administered with food (218).

Experiments with fistulated dogs indicated that penicillin V is absorbed primarily from the stomach. Therefore, it is not surprising that the potassium salt should show higher blood levels on oral administration since it is the most soluble salt in gastric pH (216). In accordance with this observation, it was also reported that the benzathine salt exhibited higher serum levels in patients with gastric achlorhydria (pernicious anemia) than in patients with normal gastric function (219). Differences in gastric emptying time may also explain this result.

Several studies compared the absorption of tetracycline and its salts (103, 221, 222). For example, serum concentrations in dogs and humans showed that a phosphate complex salt of tetracycline was absorbed more rapidly and gave higher blood levels during the first 6–8 hr than did tetracycline hydrochloride (221). The total amount of drug absorbed was about twice as great with the former compound.

Another study (222) suggested that tetracycline base produced higher blood levels than tetracycline hydrochloride. However, a subsequent investigation (223) showed that, in the absence of adjuvants or fillers, tetracycline hydrochloride and tetracycline base were absorbed equally well. Results obtained in this same study indicated that tetracycline hydrochloride encapsulated with citric acid produced higher serum concentrations than tetracycline hydrochloride mixed with hexametaphosphate or the phosphate complex salt of tetracycline.

In a study comparing urinary excretion rates and *in vitro* dissolution, the absorption of tetracycline and tetracycline phenolsulfonaphthaleinate was rate limited by their dissolution rates, whereas tetracycline hydrochloride absorption was rate limited by the absorption process itself (103).

Several lincomycin salts were studied for their comparative availability (224). In particular, the blood levels obtained with the relatively water-insoluble hexadecylsulfamate salt were compared to those of the hydrochloride following oral administration. Higher and extended whole blood and serum concentrations were obtained in mice, rats, and dogs with the hexadecylsulfamate. However, subcutaneously administered lincomycin did not produce significantly different fractions absorbed, regardless of which salt was administered. It is not known whether the greater area under the curve with oral administration of lincomycin hexadecylsulfamate is due to greater absorption from the GI tract, slower renal clearance, or greater enterohepatic circulations.

Salts of streptomycin, neomycin, viomycin, and streptothrycin have been formed with: (*a*) polyacrylic acids, (*b*) sulfonic or phosphorylated polysaccharides, and (*c*) natural polycarboxyl acids from a series of polyuronic substances and polysaccharide derivatives containing carboxyl groups (18). The report indicated that these salts were absorbed from the injection locus primarily by the lymph system. Blood levels from the salts were generally lower but were maintained for a longer time than the equivalent amount of antibiotic alone, and higher concentrations of longer duration may actually be produced in the lymphatic drainage.

The influence of salt formation on the onset and duration of pharmacological activity also was illustrated with tolbutamide and several of its salts (101). Following oral administration, the sodium salt produced a rapid decrease in blood sugar level followed by a rapid recovery. By contrast, the free acid of tolbutamide caused a slow and prolonged drop in blood sugar level, a preferred effect since the chance of hypoglycemic shock would be lessened. This finding also illustrates the often overriding influence of the actual disease state on the choice of drug form.

Additional references on the implications of salt formation on absorption are listed in Table VIII.

Pharmacokinetics—Because of the various new properties that are usually imposed on a compound by salt formation, the pharmacokinetics of the drug necessarily change as a function of these properties.

For example, a pharmacokinetic evaluation comparing ampicillin sodium and potassium with ampicillin trihydrate was performed after oral administration to beagle dogs (243). The absorption rate constants of the sodium and potassium salts, which were similar, proved significantly greater than the rate constant of ampicillin trihydrate, resulting in earlier, higher peak concentrations with two to three times higher serum concentrations during the 1st hr. Yet, any differences between the fraction absorbed for the three products were not statistically significant. Apparently, although dissolution of the ampicillin trihydrate was the rate-limiting step in its absorption, the overall extent of bioavailability remained unaffected.

An interesting study of the biliary excretion of erythromycin base and erythromycin estolate was reported (244). The biliary excretion of erythromycin base was high, while that of erythromycin estolate was much lower; preferential secretion of erythromycin in the bile could partially account for the lower serum levels exhibited by the base. However, the proportion of the ingested dose secreted in the bile was small, and the total amounts involved were not sufficient to account entirely for the differences in serum concentrations attained. Undoubtedly, the protein binding studies of Wiegand and Chun (210) (discussed under *Absorption Alteration*) more satisfactorily explain the difference in serum concentrations.

Table VIII—Additional References on Bioavailability and Absorption Alteration

Topic	Reference
Blood levels produced by three theophylline-containing elixirs	225
Naproxen oral absorption characteristics	226
Effect of food on absorption of a new form of erythromycin propionate	227
Effect of the anion on the absorption of tetracycline from the rat stomach	186
Blood levels following oral administration of different preparations of novobiocin	228
Absorption of iopanoic acid and its sodium salt	229
Oral absorption of secobarbital (quinalbarbitone) and its sodium salt	230
Absorption rate of barbiturates in humans	231
Morphine and atropine mucate	232
Excretion of bephenium salts in urine of human volunteers	233
Polymethylene bis(isothiuronium) salts: antituberculosis activity	234
Prolonged antitussive action of a resin-bound noscapine preparation	235
Pharmacology of sulfapyridine and sulfathiazole	236
Evaluation of plasma concentrations of propoxyphene utilizing a hybrid principal component analysis of variance technique: equimolar doses	237
Antrycide, a new trypanocidal drug	238
Pralidoxime methanesulfonate: plasma levels and pharmacokinetics after oral administration to humans	239
Intestinal absorption of pralidoxime and other aldoximes	240
Blood plasma levels and elimination of salts of pralidoxime (2-PAM) in humans after oral administration	241
Enhancement of GI absorption of a quaternary ammonium compound by trichloroacetate	242

Often, salt formation can be used to modify drug absorption and dose tolerance favorably. For example, aminosalicylic acid exhibits a short half-life and, therefore, requires large and frequent doses which may cause gastric irritation. Consequently, different chemical forms such as salts have been prepared (119, 245–247) to reduce the incidence of gastric irritation, increase absorption, and prolong blood levels.

Aminosalicylic acid is an interesting example in other ways; considerable confusion about this drug exists because many fail to recognize its nonlinear pharmacokinetics. Several definitive studies were reported regarding the absorption of the acid and its sodium, potassium, and calcium salts from solution, suspension, and tablet formulations (245, 246). Comparison of the relative bioavailabilities of aminosalicylic acid suspended in water and its salts dissolved in water showed that, while differences in rate of absorption were found to exist, absorption of both the acid and its salts was essentially complete. Absorption of the free acid from tablets reached only 77% of the dose, whereas that of the tableted salts was rapid and complete.

Regardless of formulation, the area under the plasma concentration–time curve of unmetabolized drug from free acid administration was less than that for the salts. This result was attributed to concentration-dependent metabolism during absorption: when the rate of absorption is high, the metabolic processes become saturated and more unmetabolized drug remains in the blood; conversely,

Table IX—Additional References on Bioavailability and Pharmacokinetics

Topic	Reference
Pharmacodynamics of fosfomycin (phosphonomycin) after intravenous administration to humans	248
Pharmacodynamics of phosphonomycin after oral administration to humans	249
Comparative studies on distribution, excretion, and metabolism of ^{3}H-hydroxyzine and its ^{14}C-methiodide in rats	250
Pharmacokinetics of ampicillin trihydrate, ampicillin sodium, and dicloxacillin sodium following intramuscular injection	251
Physiological disposition of fenoprofen in humans: pharmacokinetic comparison of calcium and sodium salts administered orally	252

when the absorption rate is low, as for the free acid, a higher percentage of drug is metabolized.

Additional references regarding bioavailability and pharmacokinetics are presented in Table IX.

GENERAL PHARMACY

Pharmacological Effect—Chlorpromazine hydrochloride and quaternary chlorpromazine chloride were investigated with respect to their effects on the central nervous system (CNS) (253). The quaternized compound was less potent and more toxic in rodents than the parent tertiary compound.

Naloxone, an effective opiate antagonist, is generally used as the hydrochloride salt; however, the drug has a very short duration of action. The mucate salt was prepared to extend its duration of action, since mucic acid is only slightly soluble in water (254). In a test on the blocking of morphine activity in mice, however, the mucate salt did not differ in duration from the hydrochloride. These investigators assumed the same receptor site for naloxone as for morphine and, since Heron's (232) work suggested that the receptor had a greater affinity for morphine mucate than for the free base, it also should have a greater affinity for naloxone mucate. The results disproved this hypothesis. Furthermore, this theory implies that intact salt reaches the receptor, which is highly unlikely, regardless of whether the drug is administered as a solution or as a suspension.

A series of salts of 9-aminoacridine and its derivatives was prepared and screened for antifungal and antibacterial activity (255–257). By using salts of fatty acids, the antifungal action was found to parallel the length of the carbon chain of the anion, with maximal activity occurring with acridine caproate, undecylate, and undecylenate (where undecylenic acid also exhibits some intrinsic antifungal activity) (255). This result appears reasonable, because these salts would be more lipid soluble and could be expected to pass through the cell wall of the infecting organism more readily, probably as an ion-pair.

The efficacy of bases or salts as topical anesthetics for relieving cutaneous itch, burning, and pain in unbroken skin has also been examined (258). In these experiments, itching and pricking were induced by an alternating current of low amperage and voltage applied to the skin or by exposure of the skin to UV light. Interestingly, aqueous solutions of salts of the local anesthetics did not alleviate itching or burning in any of the subjects, although saturated solutions of their bases in a mixture of water, 40% alcohol, and 10% glycerol were claimed to be effective. Such transport phenomena across the stratum corneum are often dependent on the polarity of the drug and vehicle and on the binding of the drug to keratin.

Table X—Additional References on General Pharmacy and Pharmacological Effect

Topic	Reference
Differential excretion of bromide and chloride ions and its role in bromide retention	259
Pharmacological study of calcium methionate	260
Synthesis and *in vitro* fungistatic evaluation of some *N*-substituted amides and amine salts of sorbic acid	261
Antiamebic studies on clamoxyquin [5-chloro-7-[[(3-diethylaminopropyl)amino]methyl]-8-quinolinol] *in vitro* and in experimentally infected animals	262
Adjunctive value of oral prophylaxis with the oximes pralidoxime (2-PAM) lactate and pralidoxime methanesulfonate to therapeutic administration of atropine in dogs poisoned by inhaled sarin vapor	263
Pralidoxime (2-hydroxyiminomethyl-*N*-methylpyridinium) methanesulfonate and atropine in the treatment of severe organophosphate poisoning	264
Efficacy and limitations of oxime–atropine treatment of organophosphorus anticholinesterase poisoning	265
Antitussive activity of enoxolone (glycyrrhetinic acid) and its derivatives	266
Pharmacological properties of glycyrrhetinic acid hydrogen succinate (disodium salt)	267
Ganglionic blocking activity of diastereomeric dimethylaminobornyl acetates and their methiodides	268
A new potent nonnarcotic antitussive, 1-methyl-3-[bis(2-thienyl)methylene]piperidine: pharmacology and clinical efficacy	269

Additional references on pharmacological effects can be found in Table X.

Dialysis—Dialysis through a cellophane membrane of the hydrochloride or sodium salts has been studied with several drugs (270). In many cases, it appeared that the ionic form of the drug was bound to the membrane whereas the nonionized form was not. Ephedrine hydrochloride presented an interesting example, however, since it dialyzed considerably faster than its corresponding base. It was theorized that the chloride ion dialyzed rapidly, enhancing the rate of dialysis of the ephedrine ion. Accordingly, when chloride ion was present on both sides of the membrane, the observed rate of dialysis for the ephedrine ion was comparable to the ephedrine base.

The diffusion of sodium chloride through a lipoprotein interface was very slow, especially if calcium chloride was present on both sides of the interface (271). In the presence of low concentrations of choline chloride or carbamylcholine chloride, the diffusion of sodium chloride is more rapid. Apparently, choline salts are able to increase the permeability of the lipoprotein to salts, which may relate to the physiological action of choline salts.

Miscellaneous—Release rates were determined for aminophylline, ephedrine alkaloid, and ephedrine hydrochloride from theobroma oil suppositories containing nonionic surfactants (133). While surfactants with hydrophilic–lipophilic balance (HLB) values less than 11 only minimally affected release rate, rates increased with surfactants of HLB values greater than 11. Under optimal conditions, aminophylline was faster than ephedrine hydrochloride which, in turn, was superior to the ephedrine base.

Willis and Banker (272) reported on the formation of polymer–drug salts as an approach to the physicochemical design of dosage forms. Poly(methyl vinyl ether/maleic

anhydride) salts of methapyrilene were prepared and tested with the free base for *in vitro* dissolution and dialysis. Their dissolution and dialysis rates were not appreciably different from the free drug or its hydrochloride salt. [redacted]ous poly(methyl vinyl ether/maleic anhydride) hemiester salts of methapyrilene exhibited substantially slower release than the polymer ether salts, hydrochloride salt, or free base forms. Polymer–drug salts thus appear to have promise.

A series of metallic salts of edetic (ethylenediaminetetraacetic) acid were tested *in vitro* to determine their effect on blood coagulation (273). The results showed that only the dipotassium and disodium salts had any effect on coagulation. It was theorized that the lack of anticoagulant activity resulted from an almost complete suppression of ionization of the heavy metal salts.

Interesting research regarding the angina-preventive effect of some chromone-2-carboxylate salts showed a direct correlation between biological activity and pKa of the salt-forming amines (274).

Lin *et al.* (112) investigated the relationship between salt form and biological activity of a given antihypertensive. While the intrinsic dissolution rates of the dihydrochloride and disulfate salts were many fold greater than the monohydrochloride, the hypotensive potencies of the salts did not differ significantly from one another in an anesthetized dog study. Yet, when administered to renal hypertensive dogs, the dihydrochloride and disulfate salts produced greater hypotensive effects than did the monohydrochloride.

TOXICOLOGICAL CONSIDERATIONS

Toxicity of Salt Ion—Any discussion regarding the toxicity of salts of a drug must consider the pharmacological properties of the cation or anion used to form the salt as well as those of the free drug, since any of these may produce toxic effects. The toxicology of several ions that are commonly used to form salts and that are relevant to this review were discussed in depth (275).

Toxicity from ingestion of calcium salts of drugs is rare. If hypercalcemia occurs, however, calcium deposits in the kidney can bring on a reduction of renal function. The principal toxic effects of lithium also involve the kidneys. When small amounts of lithium are taken, no apparent damage occurs; yet large amounts of the metal can lead to irreversible damage. An apparent correlation was observed between lithium dosage and sodium intake (276). When lithium dosage was low or sodium intake was high, rats were able to excrete all lithium given and sustained a reversible polyuria. Conversely, if large amounts of lithium were administered to the rats or if their sodium intake was lowered, they incurred irreversible kidney damage. Ammonium ion, although it serves a major role in maintenance of the acid–base balance of the body, can be toxic in high concentrations and initiate CNS derangements.

Sulfate ions given orally tend to be minimally absorbed and may act as a laxative. The nitrate ion is irritating to the GI tract, causing nausea and gastric distress. Also, intestinal bacteria may convert the nitrate ion to nitrite which oxidizes hemoglobin to methemoglobin. The citrate ion, an intermediary in carbohydrate metabolism, can form a soluble complex with calcium which is poorly dissociable and rarely causes any toxic reactions. While tartrate ions are usually absorbed minimally from the GI tract, high concentrations reaching the circulation can cause renal damage.

Acetate and lactate ions are normal metabolites and appear to be well tolerated in relatively large amounts. Iodide and bromide ions can produce conditions known as iodism and bromidism, respectively. Bromide intoxication occurs quite frequently, since bromides are used as ingredients in some nonprescription preparations (277–280). Bromide is slowly excreted by the kidney (its half-life is 12 days) and tends to accumulate when taken for prolonged periods or when used by patients with decreased renal function (277).

Toxicity of Salt Form—Provided the salt-forming agents are nontoxic, the relative toxicities of a series of salts of a compound are often observed to reflect directly their absorption rates. For example, the toxicities of dibromide, dichloride, diiodide, and dimethylsulfate salts of quinapyramine[1], a trypanocidal drug, were determined (238). The sparingly soluble halogen salts were much less toxic subcutaneously or intramuscularly than the freely soluble dimethylsulfate, yet all salts showed about equal toxicity upon intravenous administration. The difference in toxicities obviously resulted from rapid absorption of the methylsulfate compared to the slowly absorbed, poorly soluble halogen salts. Similar reasoning has been used to explain the acute oral toxicity of propoxyphene hydrochloride in rodents, which is twice that of equimolar doses of the napsylate salt (281).

Several salts of benzphetamine and etryptamine were prepared as potential sustained-release formulations (114). The water solubility, *in vitro* dissolution rates at pH 1.0 and 7.2, and the median lethal times (LT_{50}) were determined for each salt. Both the LT_{50} and LD_{50} (determined on only a few salts) increased as the *in vitro* dissolution rate at pH 7.2 decreased. While dissolution at pH 1.0 did not correlate well with toxicity, the LT_{50}'s were inversely related to the square root of the dissolution rates at pH 7.2.

Toxicity studies comparing iopanoic acid, a cholecystographic contrast medium, with its sodium salt (115) showed that the salt form has 10-fold greater toxicity. The LD_{50}'s of the free acid and the salt were 22 and 2.32 g/kg, respectively. It was postulated that the free acid precipitated from the sodium salt upon its reaction with gastric hydrochloric acid. The fine, amorphous particles of precipitated acid had a greatly increased surface area and, therefore, dissolved more rapidly than even fine crystals of the free acid. The faster and more complete drug absorption then resulted in increased toxicity.

Salts exhibiting greater water solubility than their parent compounds or less soluble salts are not always more toxic. For example, various inorganic and organic ions were used to prepare salts of methyl pyridinium-2-aldoxime that would have greater water solubility and would eliminate undesirable side effects due to the iodide ion (95). Even though the aqueous solubility of the majority of these salts was many times greater than the iodide, their toxicity on a molar basis was not significantly different, with the exception of the dihydrogen phosphate salt which was 15%

[1] Aritryride.

Table XI—Additional References on Toxicological Considerations

Topic	Reference
Toxicity and absorption of 2-sulfanilimidopyridine and its soluble sodium salt	285
Sorbic acid as a fungistatic agent for foods: harmlessness of sorbic acid as a dietary component	286
Toxicity and distribution of erythromycin	287
Further toxicological studies with erythromycin	288
Pharmacology and toxicology of erythromycin estolate	289
Erythromycin propionate (propionylerythromycin): a review of 20,525 case reports for side-effect data	290
New class of antibiotic salts of reduced toxicity	22
GI intolerance to oral iron preparations	291
Comparative toxicology of iron compounds	292
Influence of the dissolution rate of lithium tablets on side effect	123
Toxicity and tissue distribution studies on the hydrochloride, bismuth iodide complex, and a resinate of emetine	293
Bacitracin zinc in pharmaceutical preparations	145
New approach to quaternary ammonium compounds	151
Pharmacology of choline theophyllinate	294

more toxic. Further research with oximes revealed that other salts are also toxic (282).

GI bleeding is a common toxic effect of aspirin for a large percentage of the population. Consequently, a search was initiated for an aspirin derivative that would not induce GI blood loss (283). All compounds prepared, however, including the sodium and calcium salts, caused GI hemorrhage with a severity similar to aspirin.

Polyene antibiotics are potent antifungal agents but bear considerable toxicity. Even though the methyl ester hydrochlorides of these compounds are more soluble, they retain almost all of their antifungal activity and, more significantly, show a uniform decrease in toxicity compared to their parent compounds (284).

Additional references on toxicological considerations of salt formation are given in Table XI.

CONCLUSIONS

Salt formation is a means of altering the physical, chemical, and biological characteristics of a drug without modifying its chemical structure. Clearly, the salt form can have a dramatic influence on the overall properties of the parent compound. At present, selecting a salt form that exhibits the desired combination of properties is a difficult semiempirical choice. Pharmaceutical scientists now recognize these facts and are beginning to study the effects of different salt forms on the physicochemical properties, bioavailability, and toxicity of drug substances.

Although now only a few generalizations are available to predict the effect of particular salt forms on the characteristics of a drug, perhaps in time it will be possible to evolve increasingly more powerful generalizations regarding the effect of a salt on the properties of its parent compound. In addition, we predict that polymer–drug salts will have a revolutionary effect on future trends in drug therapy, particularly in the areas of reducing drug toxicity and in controlling the release profile of novel drug delivery systems.

REFERENCES

(1) D. Walkling and J. Appino, *Drug Cosmet. Ind.*, **112** (3), 39 (1973).

(2) N. J. Harper, *J. Med. Pharm. Chem.*, **1**, 467 (1959).

(3) N. J. Harper, *Progr. Drug Res.*, **4**, 221 (1962).

(4) K. Munzel, *ibid.*, **10**, 255 (1966).

(5) V. J. Stella, *Aust. J. Pharm. Sci.*, **NS2** (2), 57 (1973).

(6) A. A. Sinkula and S. H. Yalkowsky, *J. Pharm. Sci.*, **64**, 181 (1975).

(7) "Martindale The Extra Pharmacopoeia," 26th ed., Pharmaceutical Press, London, England, 1973.

(8) L. C. Miller and W. H. Heller, in "1974–75 Drugs of Choice," W. C. Modell, Ed., Mosby, St. Louis, Mo., p. 26.

(9) E. Saias, A. Jondet, and J. Phillipe, *Ann. Pharm. Fr.*, **27**, 557 (1969); through *Chem. Abstr.*, **72**, 125018e (1970).

(10) H. C. Caldwell, A. B. Rednick, G. C. Scott, G. J. Yakatan, and D. Ziv, *J. Pharm. Sci.*, **59**, 1689 (1970).

(11) G. R. Coatney, P. G. Contacos, and J. S. Lunn, *Am. J. Trop. Med. Hyg.*, **13**, 383 (1964).

(12) P. E. Thompson, B. J. Olszewski, E. F. Elsager, and D. F. Worth, *ibid.*, **12**, 481 (1963).

(13) W. C. Miller, Jr., D. B. Marcotte, and L. McCurdy, *Curr. Ther. Res., Clin. Exp.*, **15**, 700 (1973).

(14) H. L. Goldberg and L. Nathan, *Psychosomatics*, **13**, 131 (1972).

(15) E. F. Elsager, *Ann. Rep. Med. Chem.*, **1965**, 137.

(16) H. A. M. El Shibini, M. Abdel Nasser, and M. M. Motawi, *Pharmazie*, **26**, 630 (1971).

(17) S. P. Loucas and H. M. Haddad, *J. Pharm. Sci.*, **61**, 985 (1972).

(18) P. Málek, J. Kolc, M. Herold, and J. Hoffman, in "Antibiotics Annual, 1957–1958," Medical Encyclopedia, New York, N.Y., 1958, p. 546.

(19) B. F. Duesel, H. Berman, and R. J. Schacter, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 619 (1954).

(20) B. F. Duesel and T. I. Fand, *Int. Rec. Med. Gen. Pract. Clin.*, **167**, 245 (1954).

(21) R. H. Broh-Kahn, *ibid.*, **173**, 217 (1960).

(22) F. A. Alves, M. F. C. A. N. Graca, and H. L. Baptista, *Nature*, **181**, 182 (1958).

(23) H. Keller, W. Krüpe, H. Sous, and H. Mückter, *Arzneim.-Forsch.*, **5**, 170 (1955).

(24) *Ibid.*, **6**, 61 (1956).

(25) H. Keller, W. Krüpe, H. Sous, and H. Mückter, in "Antibiotics Annual, 1955–1956," Medical Encyclopedia, New York, N.Y., 1956, p. 35.

(26) A. C. Osterberg, J. J. Oleson, N. N. Yuda, C. E. Rauh, H. G. Parr, and L. W. Will, in "Antibiotics Annual, 1956–1957," Medical Encyclopedia, New York, N.Y., 1957, p. 564.

(27) R. Ducrot, O. Leau, and C. Coser, *Antibiot. Chemother.*, **6**, 404 (1956).

(28) R. S. Brigham and J. K. Nielsen, *ibid.*, **8**, 122 (1958).

(29) M. Khristov, *Khim.-Farmatsert. Zh.*, **11**, 19 (1972).

(30) A. Lasslo, C. Pfeiffer, and P. D. Waller, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 345 (1959).

(31) J. A. Campbell and J. G. Slater, *J. Pharm. Sci.*, **51**, 931 (1962).

(32) J. A. Campbell, *ibid.*, **51**, 270 (1962).

(33) G. A. Neville and J. C. Ethier, *ibid.*, **60**, 497 (1971).

(34) T. Sciortino, *Boll. Chim. Farm.*, **105**, 223 (1966); through *Chem. Abstr.*, **65**, 622h (1966).

(35) *Ibid.*, **104**, 292 (1965); through *Chem. Abstr.*, **63**, 6783g (1965).

(36) W. P. Boger, S. C. Strickland, and J. M. Gylfe, *Antibiot. Med. Clin. Ther.*, **1**, 372 (1955).

(37) C. L. Fox, Jr., South African pat. 68 03, 461 (Oct. 31, 1968); through *Chem. Abstr.*, **71**, 33401a (1969).

(38) J. W. Cusic, U.S. pat. 2,499,058 (Feb. 28, 1950); through *Chem. Abstr.*, **44**, 4926g (1950).

(39) J. W. Cusic, U.S. pats. 2,534,235–2,534,247 (Dec. 19, 1950); through *Chem. Abstr.*, **46**, 527b–i (1952).

(40) J. W. Cusic, British pat. 677,798 (Aug. 20, 1952); through *Chem. Abstr.*, **48**, 4010a (1954).

(41) G. D. Searle Co., British pats. 683,645 (Dec. 3, 1952) and 683,236 (Nov. 26, 1952); through *Chem. Abstr.*, **48**, 2769b (1954).

(42) J. W. Cusic, *Science*, **109**, 574 (1949).

(43) M. L. Robinette and F. W. Bope, *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 32 (1955).

(44) D. J. Lamb and F. W. Bope, *ibid.*, **45**, 178 (1956).

(45) W. Bickers and M. Woods, *Tex. Rep. Biol. Med.*, **9**, 406 (1951).

(46) W. Bickers and M. Woods, *N. Engl. J. Med.*, **245** 453 (1951).
(47) J. M. Holbert, I. W. Grote, and H. Smith, *J. Am. Pharm. Assoc., Sci. Ed.*, **44**, 355 (1955).
(48) W. Morozowich and F. W. Bope, *ibid.*, **47**, 173 (1958).
(49) A. Halpern, N. Shaftel, and G. Schwartz, *Antibiot. Chemother.*, (1959).
(50) A. Halpern, N. Shaftel, and A. J. Monte Bovi, *Am. J. Pharm.*, **130**, 190 (1958).
(51) P. A. Fernander, German Offen. 2,259,151 (June 7, 1973); through *Chem. Abstr.*, **79**, 53102x (1973).
(52) A. Reiner, German Offen. 2,330,380 (Jan. 31, 1974); through *Chem. Abstr.*, **80**, 100206b (1974).
(53) J. R. Blanco, F. J. U. Fernandez, and L. S. Vinals, German Offen. 2,144,679 (Dec. 21, 1972); through *Chem. Abstr.*, **78**, 71781b (1973).
(54) VEB Berlin-Chemie, German Offen. 2,316,721 (Dec. 20, 1973).
(55) M. T. Davies, J. Forrest, F. Hartley, and V. Petrow, *J. Pharm. Pharmacol.*, **6**, 707 (1954).
(56) Laboratorios Hosbon S. A., French Demande 2,138,498 (Feb. 9, 1973); through *Chem. Abstr.*, **79**, 83485s (1973).
(57) B. J. Magerlein, *J. Pharm. Sci.*, **54**, 1065 (1965).
(58) E. H. Massey, German Offen. 2,138,049 (Feb. 3, 1972); through *Chem. Abstr.*, **76**, 117507f (1972).
(59) R. Tixier, German Offen. 1,936,723 (May 14, 1970); through *Chem. Abstr.*, **73**, 28895b (1970).
(60) P. Wirth, German Offen. 2,117,902 (Nov. 4, 1971); through *Chem. Abstr.*, **76**, 46462z (1972).
(61) Takeda Chem. Ind. Ltd., German Offen. 2,332,878 (Jan. 17, 1974); through *Chem. Abstr.*, **80**, 96362v (1974).
(62) Takeda Chem. Ind. Ltd., German Offen. 2,332,840 (Jan. 17, 1974); through *Chem. Abstr.*, **80**, 87531x (1974).
(63) G. Fabrizio, U.S. pat. 3,764,595 (Oct. 9, 1973); through *Chem. Abstr.*, **80**, 6945k (1974) or *Chem. Abstr.*, **73**, 133984e (1970).
(64) Koniklijke Nederlandsche Gist en Spiritusfabriek, French pat. 2,126,443 (Oct. 6, 1972).
(65) R. Aries, French pat. 2,190,410 (Mar. 8, 1974).
(66) Otsuka Pharmaceutical Co., Ltd., Japanese pat. 71 03,600 (Jan. 28, 1971); through *Chem. Abstr.*, **75**, 40421t (1971).
(67) V. C. Stephens, U.S. pat. 3,728,379 (Apr. 17, 1973).
(68) Investigations Scientifiques Pharmaceutiques S. A., French Demande 2,097,064 (Apr. 7, 1972); through *Chem. Abstr.*, **77**, 168654e (1972).
(69) C. Jarowski, *Trans. N.Y. Acad. Sci., Ser. II*, **21**, 290 (1959).
(70) Andreu SA, West German pat. 2,237,267 (Feb. 1, 1973).
(71) E. N. Lazareva, L. A. Kovaleva, V. K. Vasil'evg, and P. S. Braginskaya, *Antibiotiki (Moscow)*, **14**, 813 (1969); through *Chem. Abstr.*, **71**, 111183x (1969).
(72) O. B. Ferno, T. O. E. Linderat, and B. Hansen, South African pat. 68 01,104 (July 12, 1968); through *Chem. Abstr.*, **70**, 60832c (1969).
(73) A. Allais and M. Paturet, French M. 5,309 (Sept. 25, 1967); through *Chem. Abstr.*, **71**, 64078e (1969).
(74) Cent. Ind. Pharmaceutique, French pat. 2,193,586 (Mar. 29, 1974).
(75) G. Guadagnini and F. Fabi, German Offen. 2,040,143 (Feb. 25, 1971); through *Chem. Abstr.*, **74**, 126005b (1971).
(76) Ciba-Geigy A.G., Canadian pat. 916,713 (Dec. 12, 1972).
(77) R. Ferrari, S. Magnaghi, and G. Ghielmetti, British pat. 1,205,441 (Sept. 16, 1970); through *Chem. Abstr.*, **73**, 133979e (1970).
(78) L. G. Goodwin, L. G. Jayewardene, and O. D. Standen, *Br. Med. J.*, **2**, 1572 (1958).
(79) A. Zaffaroni, U.S. pat. 3,708,492 (Jan. 2, 1973).
(80) F. K. Hansel, *Ann. Allergy*, **1**, 199 (1943).
(81) Cent. Ind. Pharmaceutique, French pat. 2,193,587 (Mar. 29, 1974).
(82) N. H. Creasey and A. L. Green, *J. Pharm. Pharmacol.*, **11**, 485 (1959).
(83) J. M. De Muylder, German Offen. 2,310,447 (Sept. 13, 1973); through *Chem. Abstr.*, **80**, 6982v (1974).
(84) V. C. Stephens, U.S. pat. 3,065,261 (1962).
(85) Merck & Co., Inc., West German pat. 2,256,538 (May 24, 1973).
(86) C. J. Cavallito and R. Jewell, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 165 (1958).
(87) R. L. Kile, *Antibiot. Med. Clin. Ther.*, **5**, 578 (1958).
(88) Whitefin Holding S. A., Belgian pat. 793,548 (Apr. 16, 1973).
(89) Officina Therapeutica Italiana SRL, French pat. 2,099,449 (Mar. 17, 1972).
(90) J. Klosa, West German pat. 2,134,672 (Jan. 25, 1973); through *Chem. Abstr.*, **78**, 97334h (1973).
(91) T. J. Roseman and S. H. Yalkowsky, *J. Pharm. Sci.*, **62**, 1680 (1973).
(92) L. C. Miller and A. H. Holland, *Mod. Med.*, **28**, 312 (1960).
(93) J. G. Wagner, *J. Pharm. Sci.*, **50**, 359 (1961).
(94) T. H. Simons, abstracted from a paper presented at the APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, Apr. 1967.
(95) A. A. Kondritzer, R. I. Ellin, and L. J. Edberg, *J. Pharm. Sci.*, **50**, 109 (1961).
(96) H. M. Koehler and J. J. Hefferren, *ibid.*, **53**, 1126 (1964).
(97) W. Nernst and E. Brunner, *Z. Phys. Chem.*, **47**, 52, 56 (1904).
(98) E. Nelson, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 607 (1957).
(99) H. Juncher and F. Rasschou, *Antibiot. Med. Clin. Ther.*, **4**, 497 (1957).
(100) E. Nelson, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 297 (1958).
(101) E. Nelson, E. L. Knoechel, W. E. Hamlin, and J. G. Wagner, *J. Pharm. Sci.*, **51**, 509 (1962).
(102) J. G. Wagner, *ibid.*, **50**, 375 (1961).
(103) E. Nelson, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 96 (1959).
(104) L. Benet, in "Drug Design," vol. 4, E. Ariens, Ed., Academic, New York, N.Y., 1973, p. 1.
(105) G. Levy and B. A. Sahli, *J. Pharm. Sci.*, **51**, 58 (1962).
(106) G. Levy and J. A. Procknal, *ibid.*, **51**, 294 (1962).
(107) R. A. O'Reilly, E. Nelson, and G. Levy, *ibid.*, **55**, 435 (1966).
(108) W. I. Higuchi and W. E. Hamlin, *ibid.*, **52**, 575 (1963).
(109) W. I. Higuchi, N. A. Mir, A. P. Parker, and W. E. Hamlin, *ibid.*, **54**, 8 (1965).
(110) W. I. Higuchi, E. Nelson, and J. G. Wagner, *ibid.*, **53**, 333 (1964).
(111) S. Solvang and P. Finholt, *ibid.*, **59**, 49 (1970).
(112) S.-L. Lin, L. Lachman, C. J. Swartz, and C. F. Huebner, *ibid.*, **61**, 1418 (1972).
(113) S. Miyazaki, M. Nakano, and T. Arita, *Chem. Pharm. Bull.*, **23**, 1197 (1975).
(114) W. Morozowich, T. Chulski, W. E. Hamlin, P. M. Jones, J. I. Northam, A. Purmalis, and J. G. Wagner, *J. Pharm. Sci.*, **51**, 993 (1962).
(115) R. Peterhoff, *Acta Radiol.*, **46**, 719 (1956).
(116) G. Levy, *J. Mond. Pharm.*, **3**, 237 (1967).
(117) S. S. Davis, *Br. Med. J.*, **1**, 102 (1972).
(118) E. Nelson, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 300 (1958).
(119) E. J. Middleton, H. S. Chang, and D. Cook, *Can. J. Pharm. Sci.*, **3**, 97 (1968).
(120) J. G. Wagner, *Drug Intell. Clin. Pharm.*, **4**, 17 (1970).
(121) *Ibid.*, **4**, 232 (1970).
(122) T. Paal and P. Regos, *Gyogyszereszet*, **17**, 59 (1973); through *Chem. Abstr.*, **78**, 128377f (1973).
(123) K. O. Borg, J. Jeppsson, and J. Sjögren, *Acta Pharm. Suec.*, **11**, 133 (1974).
(124) P. Finholt and S. Solvang, *J. Pharm. Sci.*, **57**, 1322 (1968).
(125) G. Levy, *ibid.*, **50**, 388 (1961).
(126) W. A. Cressman, C. A. Janicki, P. C. Johnson, J. T. Dolusio, and G. A. Braun, *ibid.*, **58**, 1516 (1969).
(127) L. W. Dittert, T. Higuchi, and D. R. Reese, *ibid.*, **53**, 1325 (1964).
(128) R. Anderson, *Aust. J. Pharm.*, **42**, 919 (1961).
(129) S. F. Kramer[4] and G. L. Flynn, *J. Pharm. Sci.*, **61**, 1896 (1972).
(130) P. H. Jones, E. K. Rowley, A. L. Weiss, D. L. Bishop, and A. H. C. Chun, *ibid.*, **58**, 337 (1969).
(131) N. Senior, *J. Soc. Cosmet. Chem.*, **24**, 259 (1973).
(132) J. J. Sciarra, J. M. Patel, and A. L. Kapoor, *J. Pharm. Sci.*, **61**, 219 (1972).
(133) J. M. Plaxco, Jr., C. B. Free, Jr., and C. R. Rowland, *ibid.*, **56**, 809 (1967).
(134) J. Kucera and V. Veber, *Cesk. Dermatol.*, **41**, 229 (1966); through *Chem. Abstr.*, **65**, 18431e (1966).
(135) J. R. Leonards, *Clin. Pharmacol. Ther.*, **4**, 476 (1963).
(136) G. Levy, R. H. Gumtow, and J. M. Rutowski, *Can. Med. Assoc. J.*, **85**, 414 (1961).
(137) C. P. Schaffner and W. Mechlinski, *J. Antibiot. (Tokyo)*, **25**, 259 (1972).
(138) H. Nakatani, *Yakugaku Zasshi*, **83**, 6 (1963); through *Chem. Abstr.*, **58**, 11170e (1963).
(139) H.-C. Wang and P.-Y. Wang, *Yao Hsueh Hsueh Pao*, **13**, 63

(1966); through *Chem. Abstr.*, **65**, 5307b (1966).
(140) H. Nakatani, *Yakugaku Zasshi*, **84**, 1057 (1964).
(141) P. J. Weiss, M. L. Andrew, and W. W. Wright, *Antibiot. Chemother.*, **7**, 374 (1957).
(142) P. J. Weiss and M. L. Andrew, *ibid.*, **9**, 277 (1959).
(143) J. Marsh and P. J. Weiss, *J. Assoc. Offic. Anal. Chem.*, **50**, 457 (1967).
(144) V. C. Stephens, J. W. Conine, and H. W. Murphy, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 620 (1959).
(145) H. M. Gross, *Drug Cosmet. Ind.*, **75**, 612 (1954).
(146) H. M. Gross, W. A. Johnson, and G. J. Lafferty, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 447 (1956).
(147) C. M. Gruber, Jr., V. C. Stephens, and P. M. Terrill, *Toxicol. Appl. Pharmacol.*, **19**, 423 (1971).
(148) N. Brudney, *Can. J. Pharm.*, **92**, 245 (1959).
(149) B. Spross, M. Ryde, and B. Nyström, *Acta Pharm. Suec.*, **2**, 1 (1965).
(150) S. BoRodkin and D. P. Sundberg, *J. Pharm. Sci.*, **60**, 1523 (1971).
(151) W. J. Shibe, Jr., and D. H. Hanson, *Soap Chem. Spec.*, **40**, 83 (1964).
(152) G. E. Schumacher and W. J. Crowell, *Am. J. Hosp. Pharm.*, **21**, 226 (1964).
(153) M. A. Schwartz and F. H. Buckwalter, *J. Pharm. Sci.*, **51**, 1119 (1962).
(154) R. Brunner, *Monatsh. Chem.*, **86**, 767 (1955).
(155) R. Brunner and H. Margreiter, *ibid.*, **86**, 958 (1955).
(156) J. V. Swintosky, E. Rosen, M. J. Robinson, R. E. Chamberlain, and J. R. Guarini, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 34 (1956).
(157) W. Storbeck, German pat. 971,830 (Apr. 2, 1959); through *Chem. Abstr.*, **55**, 4893d (1961).
(158) F. H. Buckwalter, *J. Am. Pharm. Assoc., Pract. Ed.*, **15**, 694 (1954).
(159) W. A. Woodward, *Q. J. Pharm. Pharmacol.*, **20**, 197 (1947).
(160) C. A. Johnson, in "Advances in Pharmaceutical Sciences," vol. 2, H. S. Bean, A. H. Beckett, and J. E. Carless, Eds., Academic, New York, N.Y., 1967, p. 227.
(161) A. Bojarski, D. Blitek, and B. Borkowski, *Diss. Pharm. Pharmacol.*, **19**, 297 (1967); through *Chem. Abstr.*, **67**, 102734t (1967).
(162) A. Bojarski, D. Blitek, and B. Borkowski, *Diss. Pharm.*, **17**, 345 (1965); through *Chem. Abstr.*, **64**, 4874c (1966).
(163) J. C. Bird and R. S. Shelton, *J. Am. Pharm. Assoc., Sci. Ed.*, **39**, 500 (1950).
(164) T. J. Macek, B. A. Feller, and E. J. Hanus, *ibid.*, **39**, 365 (1950).
(165) A. Taub, I. Katz, and M. Katz, *ibid.*, **38**, 119 (1949).
(166) R. Yamamoto, I. Takahashi, and M. Harada, *J. Pharm. Soc. Jpn.*, **76**, 853 (1956).
(167) R. Yamamoto, I. Takahashi, and K. Inazu, *ibid.*, **77**, 82 (1957).
(168) G. A. Nevill, J. C. Ethier, N. F. H. Bright, and R. H. Lake, *J. Assoc. Offic. Anal. Chem.*, **54**, 1200 (1971).
(169) J. D. Mullins and T. J. Macek, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 245 (1960).
(170) R. R. Goodall, J. Goldman, and J. Woods, *Pharm. J.*, **200**, 33 (1968).
(171) J. Dolby, B. Gunnarsson, L. Kronberg, and H. Wikner, *Pharm. Acta Helv.*, **47**, 615 (1972).
(172) V. C. Walton, M. R. Howlett, and G. B. Selzer, *J. Pharm. Sci.*, **59**, 1160 (1970).
(173) J. K. Guillory and T. Higuchi, *ibid.*, **51**, 100 (1962).
(174) N. Narasimhachari and G. R. Rao, *Hind. Antibiot. Bull.*, **4**, 163 (1962); through *Chem. Abstr.*, **57**, 16758b (1962).
(175) D. Kodrnja and K. Weber, *Sci. Pharm.*, **39**, 34 (1971); through *Chem. Abstr.*, **75**, 40339x (1971).
(176) A. M. Nagle, E. I. Rodionovskaya, D. M. Trakhtenberg, and G. I. Kleiner, *Antibiotiki*, **12**, 420 (1967); through *Chem. Abstr.*, **67**, 76253p (1967).
(177) H. Nakatani, *Yakuzaiguku*, **23**, 75 (1963); through *Chem. Abstr.*, **59**, 12596h (1963).
(178) L. Kiss, B. Rozsondai, and T. Scholz, *Gyogyszereszet*, **8**, 341 (1964); through *Chem. Abstr.*, **62**, 405d (1965).
(179) D. Stefanescu, N. Tuchel, and V. Antonescu, *Farmacia (Bucharest)*, **12**, 465 (1964); through *Chem. Abstr.*, **62**, 1520b (1965).
(180) M. G. Kostareva, *Antibiotiki (Moscow)*, **16**, 312 (1972); through *Chem. Abstr.*, **75**, 40334s (1971).
(181) N. Tanaka and S. Takino, *Yakugaku Zasshi*, **82**, 329 (1962).
(182) H. Nogami, K. Kigasawa, N. Ikari, H. Ohtani, and M. Takayama *ibid.*, **90**, 967 (1970).
(183) I. I. Inozemtseva, D. M. Trakhtenberg, and E. S. Zinatullina *Antibiotiki*, **19**, 448 (1974); through *Chem. Abstr.*, **81**, 158634s (1974).
(184) H. Jalil and A. W. H. Daoud, *J. Fac. Med., Baghdad*, **9**, 175 (1967); through *Chem. Abstr.*, **69**, 54272h (1968).
(185) F. Kubo, K. Imaoka, and A. Kaneko, *Kyoritsu Yakka Daigaku Kenkyu Nempo*, **6/7**, 5 (1961/2); through *Chem. Abstr.*, **60**, 375d (1964).
(186) G. Fiese and J. Perrin, *J. Pharm. Sci.*, **58**, 599 (1969).
(187) J. Perrin and J. Vallner, *J. Pharm. Pharmacol.*, **22**, 758 (1970).
(188) G. Zografi and I. Zarenda, *Biochem. Pharmacol.*, **15**, 591 (1966).
(189) D. D. Heard and R. W. Ashworth, *J. Pharm. Pharmacol.*, **20**, 505 (1968).
(190) A. J. Aguiar, J. E. Zelmer, and A. W. Kinkel, *J. Pharm. Sci.*, **56**, 1243 (1967).
(191) T. Higuchi, A. Michaelis, T. Tan, and A. Hurwitz, *Anal. Chem.*, **39**, 979 (1967).
(192) T. Higuchi and K. Kato, *J. Pharm. Sci.*, **55**, 1080 (1966).
(193) A. F. Michaelis and T. Higuchi, *ibid.*, **58**, 201 (1969).
(194) A. H. Fenton and M. Warren, *Pharm. J.*, **188**, 5 (1962).
(195) R. A. O'Reilly, P. M. Aggeler, and L. S. Leong, *Thromb. Diath. Haemorrhag.*, **11**, 1 (1964).
(196) W. Lowenthal, J. F. Borzelleca, and C. D. Corder, Jr., *J. Pharm. Sci.*, **59**, 1353 (1970).
(197) E. H. Dearborn, J. T. Litchfield, Jr., H. J. Eisner, J. J. Corbett, and C. W. Dunnett, *Antibiot. Med. Clin. Ther.*, **4**, 627 (1957).
(198) L. L. Kaplan, *J. Pharm. Sci.*, **54**, 457 (1965).
(199) D. A. Schlichting, *ibid.*, **51**, 134 (1962).
(200) L. E. Josselyn, C. Endicott, and J. C. Sylvester, in "Antibiotic Annual, 1954–1955," Medical Encyclopedia, New York, N.Y., 1955, pp 279–282.
(201) F. Neuwald and P. Ackad, *Am. J. Hosp. Pharm.*, **23**, 347 (1966).
(202) J. Gagliani, A. C. DeGraff, and H. S. Kupperman, *Int. Rec. Med. Gen. Pract. Clin.*, **167**, 251 (1954).
(203) A. E. Vivino, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 234 (1954).
(204) J. Schluger, J. T. McGinn, and D. J. Hennessy, *Am. J. Med. Sci.*, **233**, 296 (1957).
(205) G. Hitzenberger and I. Jaschek, *Int. J. Clin. Pharmacol., Ther. Toxicol.*, **9**, 114 (1974).
(206) R. S. Griffith and H. R. Black, *Antibiot. Chemother.*, **12**, 398 (1962).
(207) R. S. Griffith and H. R. Black, *Am. J. Med. Sci.*, **247**, 69 (1964).
(208) S. M. Bell, *Med. J. Aust.*, **2**, 1280 (1971).
(209) R. S. Griffith, *Antibiot. Med. Clin. Ther.*, **7**, 320 (1960).
(210) R. G. Wiegand and A. H. C. Chun, *J. Pharm. Sci.*, **61**, 425 (1972).
(211) S. S. Wright, E. M. Purcell, E. H. Cass, and M. Finland, *J. Lab. Clin. Med.*, **42**, 417 (1953).
(212) S. E. Budolfsen, S. E. J. Hansen, and E. Rud, *Acta Pharmacol. Toxicol.*, **11**, 49 (1955).
(213) S. C. Strickland, J. M. Gylfe, and W. P. Boger, *Antibiot. Med. Clin. Ther.*, **1**, 388 (1955).
(214) L. E. Putnam, W. W. Wright, A. DeNunzio, and H. Welch, in "Antibiotics Annual, 1955–1956," Medical Encyclopedia, New York, N.Y., 1956, p. 483.
(215) W. P. Boger and S. C. Strickland, *Antibiot. Med. Clin. Ther.*, **4**, 452 (1957).
(216) C. C. Lee, R. O. Froman, R. C. Anderson, and K. K. Chen, *Antibiot. Chemother.*, **8**, 354 (1958).
(217) W. J. Kaipainen and P. Härkonen, *Scand. J. Clin. Lab. Invest.* **8**, 18 (1956).
(218) F. B. Peck, Jr., and R. S. Griffith, in "Antibiotics Annual 1957–1958," Medical Encyclopedia, New York, N.Y., 1958, pp. 1004–1011.
(219) J. Colquhoun, E. C. Scorer, G. Sandler, and G. M. Wilson, *Br. Med. J.*, **1**, 1451 (1957).
(220) W. W. Wright and H. Welch, *Antibiot. Med. Clin. Ther.*, **5**, 13 (1958).
(221) M. A. Kaplan, H. L. Dickison, K. A. Hubel, and F. H. Buckwalter *ibid.*, **4**, 99 (1957).
(222) H. Welch, W. W. Wright, and A. Kirshbaum, *ibid.*, **4**, 29 (1957).

(223) W. M. Sweeney, S. M. Hardy, A. C. Dornbush, and J. M. Ruegsegger, *ibid.*, **4**, 642 (1957).
(224) C. Lewis, K. F. Stern, and J. E. Grady, *Antimicrob. Ag. Chemother.*, **1964**, 13.
(225) J. T. McGinn, *Curr. Ther. Res. Clin. Exp.*, **7**, 110 (1965).
(226) R. A. Runkel, K. S. Kraft, G. Boost, H. Sevelius, E. Forchielli, [illegible]ill, R. Magoun, J. B. Szakacs, and E. Segre, *Chem. Pharm. Bull.*, **20**, 1457 (1972).
(227) H. A. Hirsch, C. V. Pryles, and M. Finland, *Am. J. Med. Sci.*, **239**, 198 (1960).
(228) S. Fürész, *Antibiot. Chemother.*, **8**, 446 (1958).
(229) K. H. Holmdahl and H. Lodin, *Acta Radiol.*, **51**, 247 (1959).
(230) K. W. Anderson, *Arch. Int. Pharmacodyn. Ther.*, **147**, 171 (1964).
(231) J. Sjögren, L. Sölvell, and I. Karlsson, *Acta Med. Scand.*, **178**, 553 (1965).
(232) J. S. Heron, *Can. Med. Assoc. J.*, **72**, 302 (1955).
(233) E. W. Rogers, *Br. Med. J.*, **2**, 1576 (1958).
(234) A. C. Glasser and R. M. Doughty, *J. Pharm. Sci.*, **54**, 1055 (1965).
(235) O. Wulff, *ibid.*, **54**, 1058 (1965).
(236) O. W. Barlow and D. R. Climenko, *J. Am. Med. Assoc.*, **116**, 282 (1941).
(237) B. E. Rodda, N. E. Scholz, C. M. Gruber, Jr., and R. L. Wolen, *Toxicol. Appl. Pharmacol.*, **19**, 554 (1971).
(238) F. H. S. Curd and D. G. Davey, *Br. J. Pharmacol.*, **5**, 25 (1950).
(239) F. R. Sidell, W. A. Groff, and A. Kaminskis, *J. Pharm. Sci.*, **61**, 1136 (1972).
(240) R. R. Levine and G. M. Steinberg, *Nature*, **209**, 269 (1966).
(241) A. A. Kondritzer, P. Zvirblis, A. Goodman, and S. H. Paplanus, *J. Pharm. Sci.*, **57**, 1142 (1968).
(242) G. M. Irwin, H. B. Kostenbauder, L. W. Dittert, R. Staples, A. Misher, and J. V. Swintosky, *ibid.*, **58**, 313 (1969).
(243) B. E. Cabana, L. E. Willhite, and M. E. Bierwagen, *Antimicrob. Ag. Chemother.*, **1969**, 35.
(244) J. B. Hammond and R. S. Griffith, *Clin. Pharmacol. Ther.*, **2**, 308 (1961).
(245) S. H. Wan, P. J. Pentikainen, and D. L. Azarnoff, *J. Pharm. Sci.*, **63**, 708 (1974).
(246) S. H. Wan, P. Pentikainen, and D. L. Azarnoff, *J. Pharmacokinet. Biopharm.*, **2**, 1 (1974).
(247) R. V. Cohen, L. Molthan, and C. J. D. Zarafonetis, *Dis. Chest*, **30**, 418 (1956).
(248) E. L. Foltz and H. Wallick, *Antimicrob. Ag. Chemother.*, **1969**, 316.
(249) E. L. Foltz, H. Wallick, and C. Rosenblum, *ibid.*, **1969**, 322.
(250) S. F. Pong and C. L. Huang, *J. Pharm. Sci.*, **63**, 1527 (1974).
(251) J. T. Doluisio, J. C. LaPiana, and L. W. Dittert, *ibid.*, **60**, 715 (1971).
(252) A. Rubin, B. E. Rodda, P. Warrick, A. Ridolfo, and C. M. Gruber, *ibid.*, **60**, 1797 (1971).
(253) N. Watzman, A. A. Manian, H. Barry, III, and J. P. Buckley, *ibid.*, **57**, 2089 (1968).
(254) M. Jain, E. Bakutis, and J. C. Krantz, Jr., *Am. J. Pharm.*, **145**, 174 (1973).
(255) S. M. Viscia and D. C. Brodie, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 52 (1954).
(256) G. R. Goetchius and C. A. Lawrence, *J. Lab. Clin. Med.*, **29**, 134 (1944).
(257) *Ibid.*, **30**, 145 (1945).
(258) H. Dalili and J. Adriani, *Clin. Pharmacol. Ther.*, **12**, 913 (1971).
(259) O. Bodansky and W. Modell, *J. Pharmacol. Exp. Ther.*, **73**, 51 (1941).
(260) G. V. Rossi, T. S. Miya, and L. D. Edwards, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 47 (1956).
(261) L. W. Morgan, D. H. Cronk, and R. P. Knott, *J. Pharm. Sci.*, **58**, 942 (1969).
(262) P. E. Thompson, A. Bayles, P. McClay, and J. E. Meisenhelder, *J. Parasitol.*, **51**, 817 (1965).
(263) J. W. Crook, A. I. Goodman, J. L. Colbourn, P. Zvirblis, F. W. Oberst, and J. H. Wills, *J. Pharmacol. Exp. Ther.*, **136**, 397 (1962).
(264) D. R. Davies, A. L. Green, and G. L. Willey, *Br. J. Pharmacol.*, **14**, 5 (1959).
(265) J. F. O'Leary, A. M. Kunkel, and A. H. Jones, *J. Pharmacol. Exp. Ther.*, **132**, 50 (1961).
(266) D. M. Anderson and W. G. Smith, *J. Pharm. Pharmacol.*, **13**, 396 (1961).
(267) R. S. H. Finney and A. L. Tárnoky, *ibid.*, **12**, 49 (1960).
(268) G. H. Copper, D. M. Green, R. L. Rickard, and P. B. Thompson, *ibid.*, **23**, 662 (1971).
(269) Y. Kasé, T. Yukono, T. Yamasaki, T. Yamada, S. Io, M. Tamiya, and I. Kondo, *Chem. Pharm. Bull.*, **7**, 372 (1959).
(270) A. Agren, *Acta Pharm. Suec.*, **5**, 37 (1968).
(271) L. Saunders, *J. Pharm. Pharmacol.*, **15**, 348 (1963).
(272) C. R. Willis, Jr., and G. S. Banker, *J. Pharm. Sci.*, **57**, 1598 (1968).
(273) R. Brendel, V. Swayne, R. Preston, J. M. Beiler, and G. J. Martin, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 123 (1953).
(274) J. Couquelet, P. Bastide, J. B. Le Polles, and A. Paturet, *C. R. Soc. Biol.*, **164**, 329 (1970); through *Chem. Abstr.*, **74**, 21748t (1971).
(275) "The Pharmacological Basis of Therapeutics," 3rd ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1965, p. 741 and chaps. 37 and 38.
(276) M. Schou, *Acta Pharmacol. Toxicol.*, **15**, 70 (1958).
(277) G. Torosian, K. F. Finger, and R. B. Stewart, *Am. J. Hosp. Pharm.*, **30**, 716 (1973).
(278) M. W. P. Carney, *Lancet*, **2**, 523 (1971).
(279) J. A. Ewing and W. J. Grant, *South. Med. J.*, **58**, 148 (1965).
(280) R. B. Stewart, *Am. J. Hosp. Pharm.*, **30**, 85 (1973).
(281) J. L. Emerson, W. R. Gibson, and R. C. Anderson, *Toxicol. Appl. Pharmacol.*, **19**, 445 (1971).
(282) R. I. Ellin and J. H. Wills, *J. Pharm. Sci.*, **53**, 1143 (1964).
(283) P. H. N. Wood, E. A. Harvey-Smith, and A. St. J. Dixon, *Br. Med. J.*, **1**, 669 (1962).
(284) D. P. Bonner, W. Mechlinski, and C. P. Schaffner, *J. Antibiot. (Tokyo)*, **25**, 261 (1972).
(285) E. K. Marshall, Jr., A. C. Bratton, and J. T. Litchfield, Jr., *Science*, **88**, 597 (1938).
(286) H. J. Deuel, Jr., R. Alfin-Slater, C. S. Weil, and H. F. Smyth, Jr., *Food Res.*, **19**, 1 (1954).
(287) R. C. Anderson, P. N. Harris, and K. K. Chen, *J. Am. Pharm. Assoc., Sci. Ed.*, **41**, 555 (1952).
(288) *Ibid.*, **44**, 199 (1955).
(289) R. C. Anderson, C. C. Lee, H. M. Worth, and P. N. Harris, *ibid.*, **48**, 623 (1959).
(290) H. V. Kuder, *Clin. Pharmacol. Ther.*, **1**, 604 (1960).
(291) D. N. S. Kerr and S. Davidson, *Lancet*, **2**, 489 (1958).
(292) L. C. Weaver, R. W. Gardier, V. B. Robinson, and C. A. Bunde, *Am. J. Med. Sci.*, **241**, 296 (1961).
(293) K. J. Child, B. Davis, M. G. Dodds, and E. G. Tomich, *J. Pharm. Pharmacol.*, **16**, 65 (1964).
(294) R. J. Schachter, E. T. Kimura, G. M. Nowarra, and J. Mestern, *Int. Rec. Med. Gen. Pract. Clin.*, **167**, 248 (1954).

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Exhibit A5

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CHARACTERISATION OF SALTS OF DRUG SUBSTANCES

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Abstract

The properties of the solid-state of drug substances are critical factors that determine the choice of an appropriate salt form for the development of the pharmaceutical formulation. The most relevant properties may affect the therapeutic efficacy, toxicity, bioavailability, pharmaceutical processing and stability. The salt form must fulfil the needs of the targeted formulation, be suitable for full-scale production and its solid-state properties maintained batchwise as well as over time.

Comparison of the solid-state properties of different salt candidates may be quite complicated if each salt candidate exist as different solid phases: polymorphs, solvates or amorphous forms. Thermal analysis, microcalorimetry and combined techniques, X-ray diffraction, solubility, intrinsic dissolution, sorption-desorption and stability studies are basic techniques for the characterisation of the salt candidates. Some examples show the role of the salt form as well as the polymorphic form in the characteristics of the solid-state. Thermal analysis and combined techniques are efficient for the detection of unexpected phase transitions and for the comparison of the suitability of the salt candidates prepared for salt selection.

Keywords: characterisation of salts, intrinsic dissolution, microcalorimetry, pharmaceutical salt form, phase transitions, polymorphism affected properties, polymorphism in salt selection, salt candidates, salt selection, sorption-desorption, thermal analysis, thermal analysis combined techniques

Introduction

The properties of the solid-state of drug candidates are critical factors for the development of the pharmaceutical formulation. The most relevant properties may affect the therapeutic efficacy, toxicity, bioavailability, pharmaceutical processing and stability. An estimated half of all the drugs molecules used in medicinal therapy are administrated as salts [1], therefore the choice of an appropriate salt form is an important task of the development [1–6]. The salt form must be suitable for full-scale production and its solid-state properties maintained batchwise as well as over time.

The different salts are different entities with different behavior in solid-state and also in liquid and vapor state. Comparison of the solid-state properties of different salt candidates may be quite complicated if each salt form exists as different solid phases: poly-

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morphs, solvates or amorphous forms. Polymorphs have different crystalline arrangements but the same liquid and vapor phases. Solvates are new crystalline compounds formed with the solvent. In the amorphous phase there is no ordered structure of the compound. All physico-chemical characteristics of the solid-state are involved in the polymorphism and pseudo-polymorphism (solvate or hydrate formation). The main properties affected are melting and sublimation temperatures, heat capacity, conductivity, volume, density, viscosity, crystal hardness, crystal shape, color, refractive index, solubility, dissolution rate, stability, hygroscopicity, processability and solid-state reactions [7–13]. The consequences of polymorphism and pseudo-polymorphism are found in all steps of manufacture and storage of drug substances and drug products. This impact is so high that the International Conference on Harmonization (ICH) requires proper investigations and analytical methods for drug substance and drug products following a decision tree [14].

The parameters affected by the processing of the drug products: solvent, excipients, temperature, pressure and humidity are relevant in the choice of the salt form considering the different phases which may exist for each salt form.

Therefore the solid-state properties such as solubility, dissolution, melting, density, morphology, hygroscopicity, processability, stability, compatibility have to be studied taking into account thermodynamic and kinetic factors. For this task automated thermal analysis techniques, microcalorimetry coupled or combined with other techniques play an important role [15, 16] in addition to solubility, dissolution and stability studies [17–19].

Instrumentation

For the examples given in this paper, automated DSC-7 of Perkin Elmer, automated TGA-850 of Mettler, the TG-MS of Mettler, TGA7 of Perkin Elmer, the X-ray diffraction with heating cell or moisture control of Scintag, type XDS 2000 and the FT-IR with heating cell of Brucker IFS 55 have been used. The intrinsic dissolution experiments have been carried out according to USP 24 with the Vankel instrumentation. The hygroscopicity has been measured by DVS instrument of Surface Measurement Systems Ltd, or by a previous internal developed instrument. Scanning electron microscopy has been carried out with the instrument Jeol JSM 6300.

Properties in the solid-state

Melting point

The choice of the counter-ion allows modifying the characteristics of the drug molecule. A drug candidate with a low melting point is not suitable for purification, handling, processing. Table 1 gives examples of the influence of the salt form on the melting point measured by DSC. The influence of the counter ion on the thermal behavior is based on the thermodynamic phase diagram between the counter-ion and the drug substance. Figure 1 exemplifies binary mixtures of a stable compound with a congruent melting and a compound which dissociates on melting with incongruent

behavior. The phase diagrams allow understanding why some eutectics between the salt form and the molecule may be encountered as well as different salts in crystallization. These types of phase diagrams are also valid for the solvates.

Table 1 Influence of the salt form on the melting point

Salt form	Substance 1	Substance 2
	Melting point/°C	
Base	40	98
Hydrogen fumarate	156	196
Hydrogen maleate	139	161
Hydrogen malonate	115	72
Hydrogen tartrate	–	122
Hydrochloride	210	251
Oxalate	197	–
Pamoate	154	–

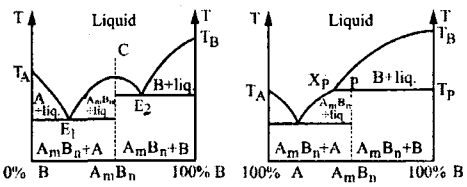

Fig. 1 Phase diagrams of binary mixtures of temperature *vs.* composition (e.g. mole fraction) of two chemical compounds, A and B, showing the following behaviour: left – formation of a compound with a congruent melting point at C, right – formation of a compound with an incongruent melting point at P

Morphology

Different salts may exhibit different morphology as exemplified in Fig. 2. Morphology depends also on the polymorph. The solvent of crystallisation and additives may be used for the modification of the morphology.

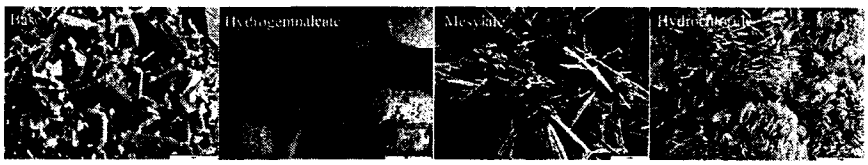

Fig. 2 SEM pictures showing different morphologies of several salt forms of a compound

Solubility/dissolution rate

The next property affected by the salt form is the solubility. As emphasised by Bastin *et al.* [6], the majority of the salts are developed to enhance the aqueous solubility of drug substances. In special cases, a retarding effect is suitable and insoluble counter-ion like pamoic acid or hydrophobic fatty acids are chosen. For albuterol, Jashani *et al.* [20] compared different salts, base, sulfate, adipate and stearate. All were crystalline. The densities were 1.15, 1.34, 1.22 and 1.07 g cm^{-3}. The solubilities were 15.7, 250, 353 and 0.6 mg mL^{-1}. Only the sulfate was found hygroscopic at relative humidity RH >93%. For inhaler performance in a high humidity environment, the hydrophobic stearate was found the best.

Metastable forms may have higher solubility giving wrong expectations for a salt form when the thermodynamic stable form is obtained with a lower solubility.

Figure 3 shows the effect of the equilibration time in order to obtain the equilibrium solubility of a drug molecule [21]. Therefore the solubility should be measured at different time and the insoluble analysed by DSC or X-ray diffraction. This point as well as the solubility profile *vs.* pH is discussed in several chapters of the reference [1]. Automated instrument has been developed for the determination of the pH solubility profile [19].

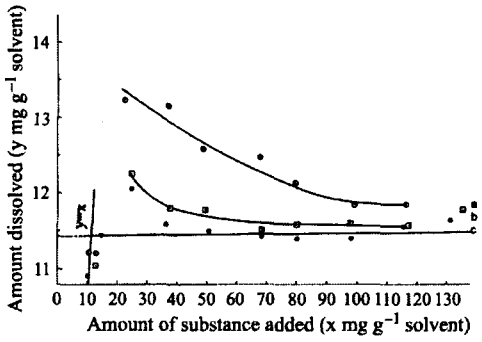

Fig. 3 Phase solubility analysis: transformation into a less soluble form depending on the time of equilibration a: 20 h, b: 63 h, c: 115 h [21]

In early development the measurement of the intrinsic dissolution is very useful since the dissolution rate is measured independently of the particle size. The influence of the salt form on the intrinsic dissolution rate measured at 50 rpm with the vankel instrument is demonstrated in Tables 2 and 3. Polymorphism has also its relevance as demonstrated in Table 4. If different salt forms with the same counter ion are possible, their IDR is also very relevant as demonstrated in Table 5.

The influence of the pH on the intrinsic dissolution rate (IDR) of a drug candidate is given in Fig. 4. When measuring salts there are differences among the salts as resulting from the behavior of the salts in solutions (Fig. 5).

Table 2 Influence of the salt form on the intrinsic dissolution rate of a poor soluble drug

Salt form	IDR/mg min^{-1} cm^{-2}	
	IDR in mg $min^{-1}cm^{-2}$ Buffer pH 5	IDR in mg $min^{-1}cm^{-2}$ Buffer pH 8
Base	0.014	0.0004
Hydrochloride	0.056	0.0016
Oxalate	1.359	0.0096
Tosylate	0.011	0.0032

Table 3 Influence of the salt form on the intrinsic dissolution rate of a soluble drug

Salt form	IDR/ mg min^{-1} cm^{-2}	
	IDR in mg $min^{-1}cm^{-2}$ HCl 0.1 N	IDR in mg $min^{-1}cm^{-2}$ Water
Base	2.61	–
Hydrochloride	1.09	4.04
Tartrate	1.78	–
Lactate	3.48	4.44
Succinate	3.38	–
Benzoate	10.85	–

Table 4 Influence of polymorphism on the intrinsic dissolution rate

Drug substance as base		Drug substance neutral	
Polymorph	IDR in water with 0.2% LDAO	Polymorph	IDR in water/ mg min^{-1} cm^{-2}
Amorphous form	0.048	Amorphous form	0.269
Form B	0.035	Form A	0.117
Form D	0.011	Form B	0.085

Table 5 Influence of the salt form and of the hydrate formation for a sodium salt of a drug substance

Salt form	IDR/ mg min^{-1} cm^{-2}	
	Water	Buffer pH 6.8
Monosalt Na	43.6	22.6
Monosalt monohydrate	17.6	16.5
Hemisalt	0.40	0.35

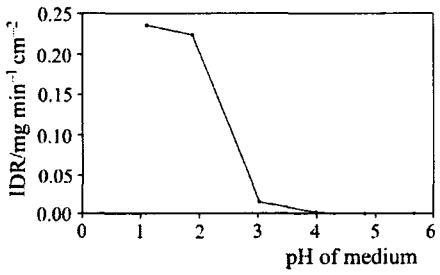

Fig. 4 Influence of the pH on the intrinsic dissolution rate with the same counter ion (HCl). pKa of the base is 6.9

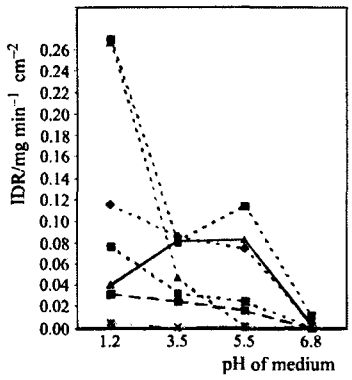

Fig. 5 Influence of the salt forms on the intrinsic dissolution rate in different buffers. -▲- – HCl, -◆- – Malonate, -■- – Maleate, -■- – Malate, –■– – Oxalate, -×- – Pamoate and -▲- – Base

Hygroscopicity/Interaction with water vapor

Water vapor is an omnipresent component of the atmosphere. The most excipients contain water. For solid dosage forms granulation in humid conditions are generally used. Therefore the study of the behaviour of substances in water vapor atmospheres is a prerequisite in the studies for the choice of the salt form. Some salts are deliquescent and cannot survive high humidities. At a given temperature, the ratio actual water vapor pressure/saturated vapor pressure at that temperature is called the relative humidity RH given as percentage of the saturation. The environmental humidity depends on the climatic zones. Sorption-desorption isotherms are measured as the mass change observed during the change of the relative humidity. Generally a hysteresis in the desorption is an indication of hydrate formation. But reversible desorption may also be observed for hydrates. X-ray diffraction during such studies is very fruitful [22]. Figure 6 shows the complexity of sorption-desorption of several salts of a drug candidate. The base and the hydrogen maleate salt were not hygroscopic while the

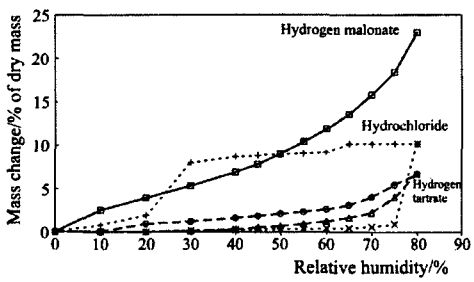

Fig. 6 Water vapour sorption isotherms of several salts of a basic investigational compound: – hydrogen malonate; (□ – sorption and desorption, water uptake 22%); – hydrochloride (X sorption+desorption, formation of hydrate occurs, corresponding to the plateau); – hydrogen tartrate (△ – sorption; ○ – desorption). The base and the hydrogen maleate are not hygroscopic, the water uptake is 0% until 90% relative humidity (RH)

hydrochloride transformed into a hydrate and the hydrogen malonate took until 22% water. The hydrogen tartrate was slightly hygroscopic.

In such studies the polymorphic form as well as the amorphous content of the samples used for the comparison of the salts is very important as exemplified in Figs 7 and 8. Figure 7 shows the different behaviours of two polymorphs of a hydrochloride [10] with an enantiotropic relationship. The high melting form, metastable at ambient temperature takes up water at lower RH value; the hydrate form loses water at RH values below 20%. A choice of the hydrated form was discussed. In fact a second polymorph of the hydrated form was also obtained in crystallisation studies in aqueous media.

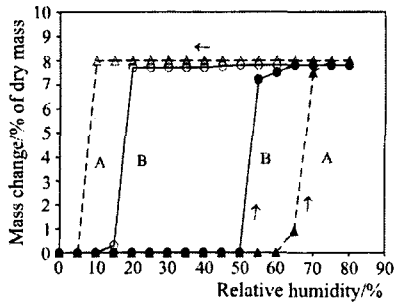

Fig. 7 Examples of water sorption-desorption isotherms of two polymorphs. The two polymorphs, A and B, transform into the same hydrated form. The metastable form B takes up water a lower RH than the stable form A. The hydrate form loses water at RH values below 20%

In Fig. 8, the salt form is not hygroscopic, but as resulting of milling the sample is partially amorphous and takes up moisture until a high level of humidity. The recrystallization is observed with lost of adsorbed water.

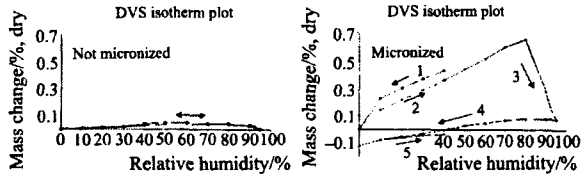

Fig. 8 Comparison of the behaviour of two samples in sorption-desorption experiment. Before milling, no change is observed. After milling the sample takes up water as resulting of the partial amorphisation during milling. Then an abrupt loss of water occurs resulting of the expulsion of water since the amorphous part crystallizes

Stability

The chemical stability behavior depends on the solid-state of the salt form [13] and also on the polymorphic form [23, 24]. The amorphous state is particularly critical for its chemical reactivity [25]. Microcalorimetry has been proposed for stability screening [26, 27]. An example of discriminative behavior between salt candidates is given in Fig. 9. Figure 10 exemplifies the influence of crystallinity. Table 6 gives 3 examples with the stability results of different polymorphs.

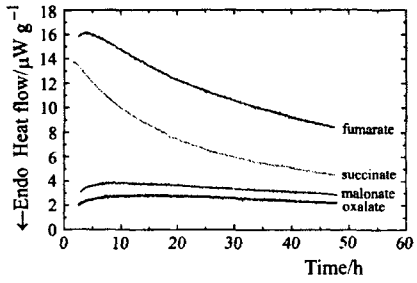

Fig. 9 Use of microcalorimetry for the study of the stability behaviour of 4 salts candidates of a drug

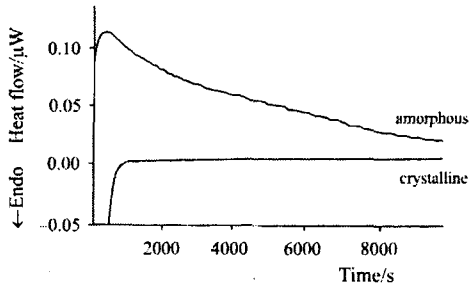

Fig. 10 Discriminative stability behaviour between amorphous and crystalline forms of a drug candidate

Table 6 Influence of polymorphism on the stability behaviour in solid-state

Examples	Degradation (HPLC)	
Example 1	1 month at 80°C (oxygen/water)	
Crystalline form A	No degradation	
Crystalline form B	0.5–1.5% degradation	
Amorphous form	2–3.5% degradation	
Example 2	2 weeks at 50°C	Exposition 1200 kluxh
Monohydrate A	No degradation	10%
Monohydrate B	12%	23%
Example 3	1 week at 70°C	Exposition 300 kluxh
Crystalline form	10%	2%
Amorphous form	80%	38%

Phase transitions

Polymorphic phase transitions

The process of transformation of one polymorph into another is a phase transition, which may occur on storage or during processing. If the phase transition is reversible, the two polymorphs are enantiotrops. If the phase transition is irreversible, the two polymorphs are monotrops and only one form is stable whatever the temperature. In case of solvates, the phase transitions are more complex since several compounds are involved. If a physical property of a crystalline substance is plotted *vs.* temperature, a sharp discontinuity occurs at the melting point. For amorphous substances, there is no melting point, and a change of slope occurs at the so-called glass transition temperature T_g. Below this temperature, the amorphous phase has certain properties of a crystalline solid (e.g. plastic deformation) and is termed 'glassy'. Above this temperature, the substance retains some of the properties of a liquid, e.g., molecular mobility, and is termed 'rubbery'. Above this temperature, the increase in molecular mobility facilitates spontaneous crystallization into the crystalline form with an exothermic enthalpy change after the glass transition. The amorphous state is thermodynamically unstable. The glass transition temperature, T_g, is lowered by water or other additives, facilitating crystallization.

All thermodynamically 'unstable' forms may behave like stable forms outside the phase diagrams for kinetic reasons. They are therefore called 'metastable' forms and may behave like stable forms. Therefore a limited polymorphic study is part of the salt selection. In the example of Fig. 10, the hydrochloride and the base were amorphous. The crystalline form of the base was obtained during the polymorphic study of the salt selection: After equilibration of a suspension the crystalline form

was obtained in the solid phase. The base which could be obtained crystalline and as result was chemically more stable was chosen for future development. The impurities may inhibit the transformation and the metastable form may not transform, giving rise to wrong selection. As the purification increases with the first batch the thermodynamic stable form appears. We had such an example for a drug candidate. The salt form had a better morphology for micronization. It was never possible to manufacture again this metastable form and the thermodynamic stable form had a needle shape habit and was difficult to be milled. In another case, the hydrochloride was rejected because of its hygroscopicity, the besylate was chosen. During development several forms including hydrates were identified for the besylate and a stable anhydrous hydrochloride could be obtained. In the last case from 12 salts showing hygro-

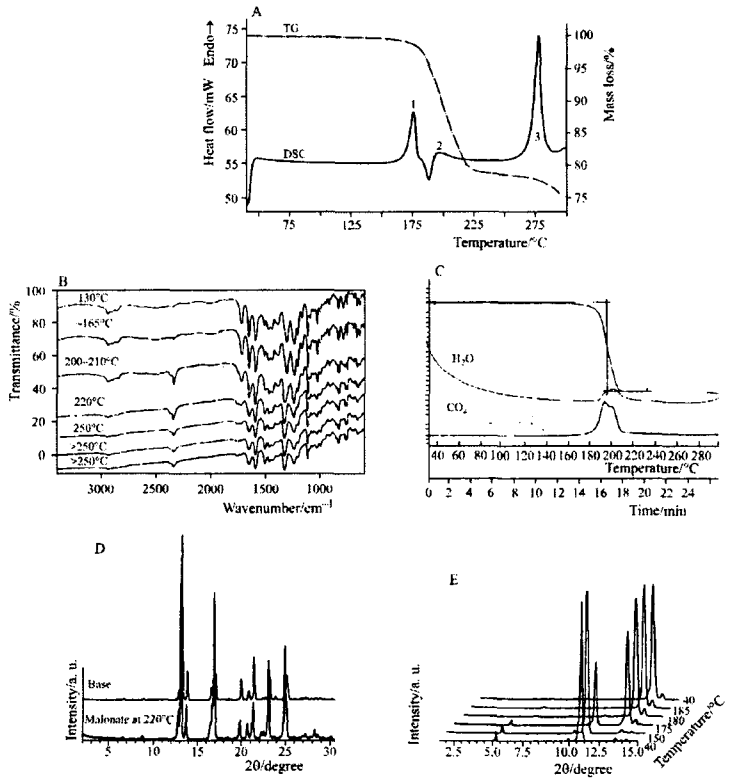

Fig. 11 Use of thermal analysis and combined techniques for the study of the thermal behaviour of a malonate salt. A: DSC and TG curves. B: FT-IR in the heating cell, bands of the base and of CO_2 appear. C: TG-MS experiment demonstrating the formation of water and CO_2 by decomposition of malonic acid. D: Comparison of the X-ray diffractograms of the base and of the compound obtained until 220°C. E: Temperature resolved X-ray diffraction scans following the decomposition

scopicity and polymorphic behavior, the base was chosen. Deeper polymorphic study revealed 3 forms of the base.

Another consequence of polymorphic transition is the chemical result of the stress testing if during this stress a polymorphic change occurs giving erroneous results (e.g. hydrate formation with high moisture, polymorphic change for testing at high temperatures).

Phase transitions observed by thermal analysis and combined techniques

The use of combined techniques is very efficient to understand results of pre-screening due to the very low amounts which can be studied with a lot of information. Figures 11 and 12 exemplify the use of TG-MS and combined techniques with X-ray diffraction or FT-IR for proper interpretation of the DSC curves. Figure 11 deals with a malonate salt [16]. The DSC scan with a dual melting would be wrongly attributed to a polymorphic behaviour if the TG curve had done parallel. The high amount of lost found by TG showed a decomposition which was easily attributed to the lost of malonic acid by TG-MS, FT-IR and X-ray diffraction with heating cell. The base was

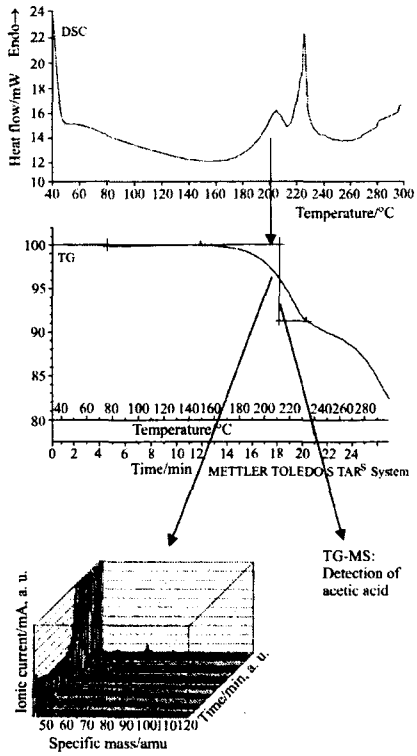

Fig. 12 Use of TG-MS for the interpretation of the DSC scan of an acetate which loses acetic acid by melting (pKa of the base 7.2)

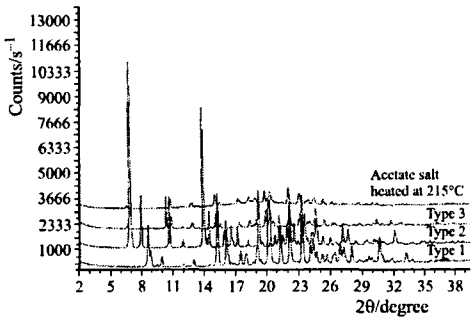

Fig. 13 Comparison of the base obtained in DSC (Fig. 12) with the 3 different polymorphs of the base

the product formed after recrystallization. The last DSC peak is the melting peak of the base and is not the melting peak of a polymorph of the malonate.

In Fig. 12, the acetate converts to the base as demonstrated by TG-MS and X-ray diffraction of the product compared to the 3 different forms of the base (Fig. 13). TG-MS is generally very helpful when salt forms contain high amount of entrapped residual solvents which play a role in the solubility results or which are bound as solvate or hydrated forms.

Change of salt form

Dissociation of the salt forms is often observed by analysing the undissolved residue as demonstrated in Figs 14 and 15. Figure 14 deals with a bi-methanesulfonate dihydrate, which dissolved, in a parenteral formulation. Upon storage a precipitate was observed. The monomethanesulfonate anhydrous was less soluble and was more stable in the formulation. In the case of Fig. 15, the salt was a hydrochloride and the

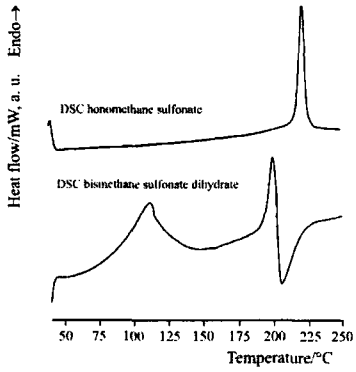

Fig. 14 Transformation of a soluble bi-methanesulfonate in the less soluble monosalt in a liquid formulation

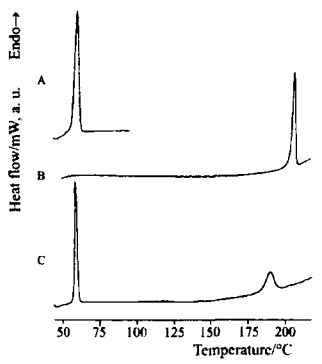

Fig. 15 Observation of the dissociation of a hydrochloride in the base in solubiliity experiments in water; A – DSC of the base, B – DSC of the hydrochloride and C – DSC of the insoluble crystals after vibration of an excess of hydrochloride with water

base was undissolved. In solubility experiments the amount of substance in solution increased with the amount of solid added resulting in a strong decrease of the pH, but the remaining base was undissolved and the solubility results completely erroneous.

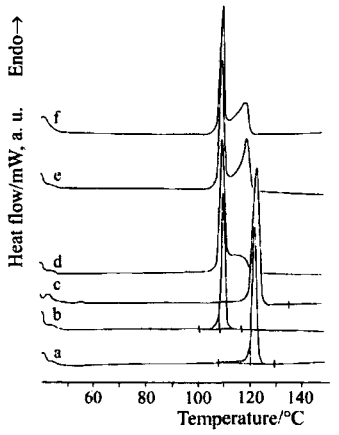

Fig. 16 DSC curves of mixtures of two salt forms of the base of an investigational drug substance with fumaric acid. a – fumarate salt (base:acid=1:1); b – fumarate salt (base:acid=3:2); c – mixture (1:1) of a and b, equilibrated in ethanol or 2-propanol; d – mixture (1:1) of a and b, ground; e – mixture (1:1) of a and b, placed directly in the sample pan; f – mixture (1:1) of a and b, equilibrated in ethyl acetate

Figure 16 shows the advantage of DSC for the study of the relative stability of two salts of fumaric acid with the drug substance in alcoholic solutions [28].

Figures 17 and 18 are example of the observation, which can be done when a phase transition occurs during the intrinsic dissolution experiment. The IDR curve of the hydro-

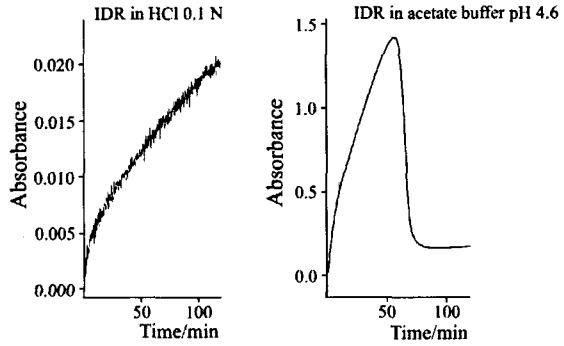

Fig. 17 UV absorbance measured curves during IDR experiment of a hydrochloride salt in HCl 0.1 N and acetate buffer. During the experiment in acetate buffer the hydrochloride transforms into a less soluble compound demosntrated to be the acetate (Fig. 18)

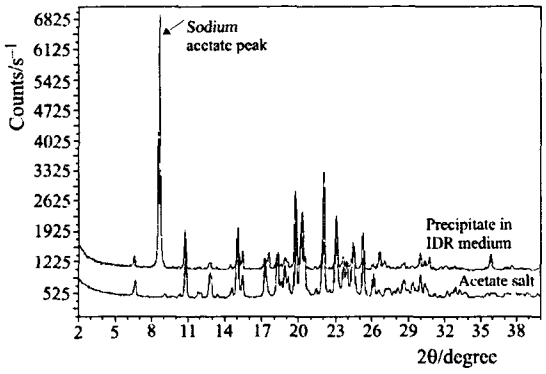

Fig. 18 Comparison of X-ray diffractograms of the acetate salt and the precipitate obtained in acetate buffer (Fig. 17)

chloride salt in HCl 0.1 N is a current behaviour. In acetate buffer the IDR is high and with the time an abrupt decrease of the curve is observed as result of a phase transition in a new entity. The X-ray diffraction (Fig. 18) confirms the formation of the acetate salt form. This salting out effect of buffers should be investigated in the salt selection.

The last example deals with a hydrochloride, which partially transforms into the base (pKa=4.9) in a gelatine capsule [28]. Figure 19 shows the mixture of drug substance with lactose after storage at 40°C/75% RH in accelerated conditions as required by ICH [29]. Needles sticking to the gelatine wall were growing in these storage conditions. The DSC curves of some needles are identical with the DSC of the base (Fig. 20). FT-IR microscopy could confirm the findings.

Fig. 19 SEM of a powder formulation in a gelatin capsule. After storage in accelerate conditions, needles appear due to the partial dissociation of the hydrochloride salt into the base

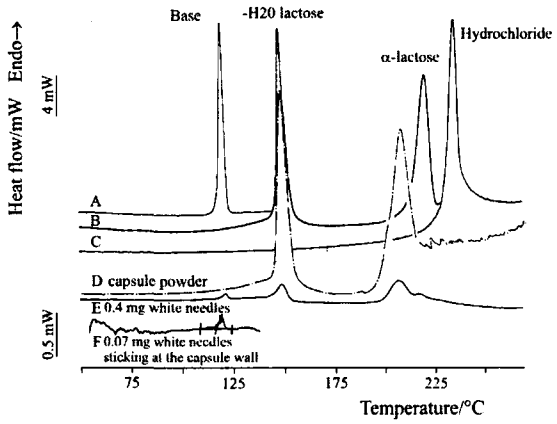

Fig. 20 DSC study of the mixture lactose, hydrochloride salt. Needles are pure base

Conclusions

The selection of the salt candidate requires a proper characterisation of the solid-state including chemical analysis, polymorphic behaviour and feasibility in different solvents as well as targeted studies for the dosage form. The decision takes into account several criteria. An example of a selection is given in Table 7. The 6 candidates were feasible and of good crystallinity. Three salt candidates showed a polymorphic behavior in the selected study. Two were hygroscopic. The selected hydrogen maleate had a solubility of 0.8%, was not hygroscopic, was monomorphic and was acceptable for stability and compatibility with excipients.

Thermal analysis, microcalorimetry and combined techniques play an important role in such studies for helping the manufacture of samples, for discriminating hydrates, solvates and amorphous samples from stable forms and for the characterisation of suitable salt forms.

Table 7 Results of screening of salts of a drug

Item	Base	hfu	hml	ch	hta	hmo
Melting/°C	98	196	161	251	121.5	71.7
DSC purity	99%	99%	>99.9%	–	–	–
Hygroscopicity	no	no	no	yes	yes	no
X-ray	cryst.	cryst.	cryst.	cryst.	cryst.	cryst.
Polymorphism behaviour	mono	mono	mono	poly	poly	poly
Feasibility	good	good	good	good	good	good
Solubility						
water	<0.01%	0.5%	0.8%	0.8%	>3%	>3%
HCl 0.1 N	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
pH 1% water	8.3	3.6	4.6	5.9	3.9	4.2
Stability bulk						
96 h Xenon	0%	0%	0%	2–5%	0%	0%
1 week 70°C	0%	0%	0%	<2%	0%	10–20%
1 w.70°C/95 RH	0%	0%	0%	<2%	>20%	10–20%
Methanol	0%	0%	0%	10–20%	<2%	0%
water	>20%	>90%	>20%	>20%	>90%	>20%
Compatibility						
mixture 1	0%	>20%	0%	<2%	0%	0%
mixture 1/95 RH	>90%	>20%	>20%	>20%	>90%	10–20%
mixture 2	2–5%	<2%	<2%	0%	10–20%	2–5%
mixture 2/95 RH	5–10%	>20%	5–10%	10–20%	>20%	10–20%
Particle size 99%/μm	115	50	17	–	–	–

* * *

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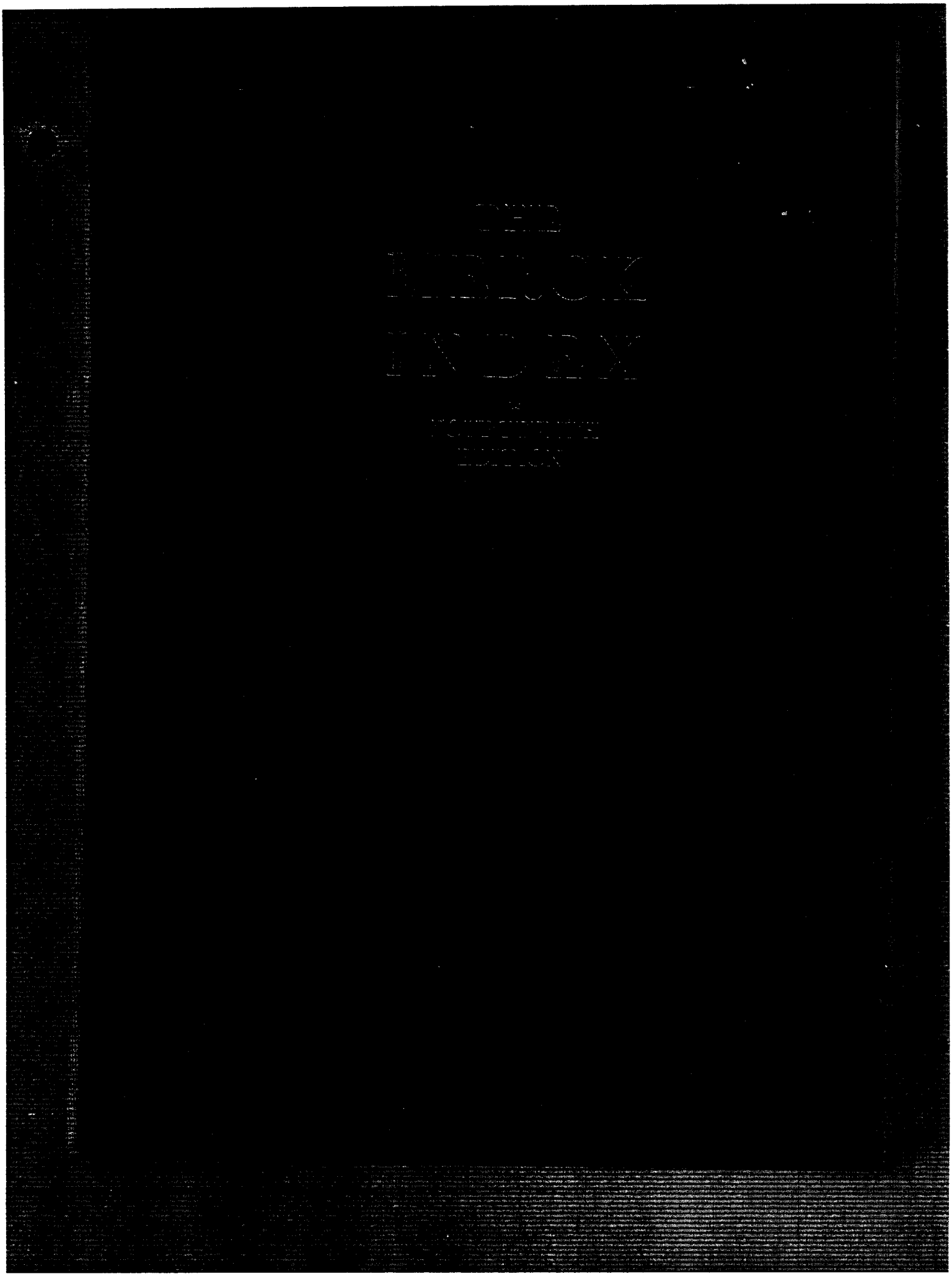
References

1 Handbook of Pharmaceutical Salts, Properties, Selection and Use, P. H. Stahl and C. G. Wermuth (Eds), IUPAC, Wiley-VCH, Verlag Helvetica Chimica Acta, Zürich 2002.
2 S. M. Berge, L. D. Bighley and D. C. Monckhouse, J. Pharm. Sci., 66 (1977) 1.
3 L. D. Bighley, S. M. Berge and D. C. Monkhouse, Salt forms of drugs and absorption. Encyclopaedia of pharmaceutical Technology, (Eds: J. Swarbrick, J. C. Boylan) Vol. 13, Marcel Dekker, New York 1996, p. 453.
4 P. L. Gould, Int. J. Pharm., 33 (1986) 201.

5 J. I. Wells, Pharmaceutical Preformulation, Ellis Horwood Series in Pharmaceutical Technology, Chichester 1988.
6 R. J. Bastin, M. J. Bowker and B. J. Slater, Organic Proc. Res. & Dev., 4 (2000) 427.
7 J. Haleblian, J. Pharm. Sci., 64 (1975) 1269.
8 D. Giron, Labo-Pharma, Probl. Techn., 307 (1981) 151.
9 S. R. Byrn, Solid State Chemistry of Drugs, Academic Press 1982.
10 D. Giron, Thermochim. Acta, 248 (1995) 1.
11 H. G. Brittain, 'Polymorphism in Pharmaceutical Solids', Ed., Marcel Dekker 1999.
12 D. Giron, S. T. P. Pharma, 4 (1988) 330.
13 W. D. Walkling, W. R. Sisco, M. P. Newton, B. Y. Fegely, H. N. Plampin and F. A. Chrzanowski, Acta Pharm. Technol., 32 (1986) 10.
14 International Conference on Harmonization (ICH), Guideline Specification Q6A 1999.
15 D. Giron, J. Therm. Anal. Cal., 64 (2001) 37.
16 D. Giron, J. Therm. Anal. Cal., 68 (2002) 335.
17 D. Giron and D. J. W. Grant, in Handbook of Pharmaceutical Salts, Properties, Selection and Use, Ch. 3 p. 42.
18 D. J. W. Grant and H. Brittain, Physical Characterization of Pharmaceutical Solids, (Ed.) H. G. Brittain, Marcel Dekker 1995, p. 321.
19 A. Avdeef, Pharm. Pharmacol. Commun., 4 (1998) 165.
20 R. N. Jashani and P. R. Byron, Int. J. Pharm., 130 (1996) 13.
21 D. Giron and C. Goldbronn, Analysis, 7 (1979) 109.
22 D. Giron, C. Goldbronn, M. Mutz, S. Pfeffer, P. Piechon and P. Schwab, J. Therm. Anal. Cal., 68 (2002) 453.
23 W. D. Walkling, B. E. Reynolds, B. Y. Fegely and C. Janicki, Drug Dev. Ind. Pharm., 9 (1983) 809.
24 D. Giron, Mol. Cryst. Liq. Cryst., 161 (1988) 77.
25 B. C. Hancock and G. Zografi, J. Pharm. Sci., 86 (1987) 1.
26 M. Anberg, C. Nyström and S. Castensson, Int. J. Pharm., 81 (1992) 153.
27 M. Koenigbauer, J. Pharm. Res., 11 (1994) 777.
28 D. Giron, PSTT, 1 (1998) 239, 262.
29 International Confederation of Harmonisation (ICH) Guideline 'Stability Testing of New Drug Substances and Products', ICHQ1A.

Exhibit A6



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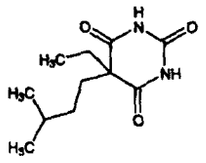
tabolism: Frey, Magnussen, *Arzneimittel-Forsch.* **16**, 612 (1966). Toxicity data: K. Irrgang, *ibid.* **15**, 688 (1965). Comprehensive description: N. A. A. Mian *et al., Anal. Profiles Drug Subs.* **19**, 27-58 (1990).



Slightly bitter crystals, mp 156-158°. One gram dissolves in 1300 ml water, in 5 ml alc, in 17 ml chloroform, in 6 ml ether. Freely sol in benzene; sol in alkaline solns. Insol in petr ether, aliphatic hydrocarbons. A said aq soln is acid to litmus paper. Dissolves in aq solns of alkali hydroxides and carbonates. pH of a saturated soln in water about 5.6. pKa (25°C) 8.0. LD_{50} in mice (mg/kg): 212 s.c. (Irrgang).

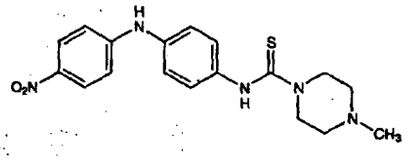
Sodium salt. [64-43-7] Sodium Amytal. $C_{11}H_{17}N_2NaO_3$; mol wt 248.25. Hygroscopic, friable granules of powder. Slightly bitter taste. Very soluble in water; sol in alcohol (1:1). Practically insol in ether.

Caution: May be habit forming: **21 CFR, 329.1** and is a controlled substance (depressant): **21 CFR, 1308.12** and **1308.13.**

THERAP CAT: Sedative, hypnotic.

THERAP CAT (VET): Sedative, hypnotic.

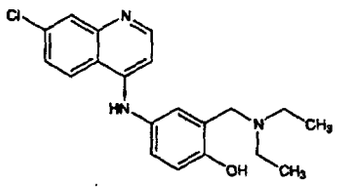
575. Amocarzine. [36590-19-9] 4-Methyl-*N*-[4-[(4-nitrophenyl)amino]phenyl]-1-piperazinecarbothioamide; 4-nitro-4'-[(*N*-methylpiperazinyl)thiocarbonylamino]diphenylamine; CGP-6140. $C_{18}H_{21}N_5O_2S$; mol wt 371.46. C 58.20%, H 5.70%, N 18.85%, O 8.61%, S 8.63%. Orally active macrofilaricide; derivative of amoscanate, *q.v.* Prepn: **BE 772053** (1971 to Agripat); R. Spaun *et al.*, **US 3781290** (1973 to Ciba-Geigy). Mechanism of action study: K. P. Davies *et al., Exp. Parasitol.* **68**, 382 (1989). HPLC determn in biological fluids: S. C. Bhatia *et al., J. Chromatog.* **434**, 288 (1988). Pharmacokinetics: J. B. Lecaillon *et al., Brit. J. Clin. Pharmacol.* **30**, 625, 629 (1990). Clinical trial in onchocerciasis: A. A. Poltera *et al., Lancet* **337**, 583 (1991).



Mp 191-196°. Sol in acetonitrile.

THERAP CAT: Anthelmintic (Nematodes).

576. Amodiaquin. [86-42-0] 4-[(7-Chloro-4-quinolinyl)amino]-2-[(diethylamino)methyl]phenol; 4-[(7-chloro-4-quinolyl)amino]-α-(diethylamino)-*o*-cresol; 7-chloro-4-(3-diethylaminomethyl-4-hydroxyanilino)quinoline; 7-chloro-4-(3-diethylaminomethyl-4-hydroxyphenylamino)quinoline; 4-(3'-diethylaminomethyl-4'-hydroxyanilino)-7-chloroquinoline; SN-10751. $C_{20}H_{22}ClN_3O$; mol wt 355.87. C 67.50%, H 6.23%, Cl 9.96%, N 11.81%, O 4.50%. Prepd from 4,7-dichloroquinoline and 4-acetamido-α-diethylamino-*o*-cresol: Burckhalter *et al., J. Am. Chem. Soc.* **70**, 1363 (1948); **US 2474819**; **US 2474821** (1949 to Parke, Davis). Alternate synthesis from 2-aminomethyl-*p*-aminophenol and 4,7-dichloroquinoline: Natarajan, Lan, *Arzneimittel-Forsch.* **22**, 1230 (1972). Spectral study: J. Kracmar *et al., Pharmazie* **29**, 773 (1974). HPLC determn in plasma, blood and urine: E. Pussard *et al., J. Chromatog.* **374**, 111 (1986). Comprehensive description: I. Ahmad *et al., Anal. Profiles Drug Subs.* **21**, 43-73 (1992).



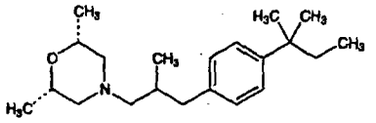
Crystals from abs. ethanol, mp 208° (dec).

Dihydrochloride dihydrate. [6398-98-7] CAM-AQ1; Camoquin; Flavoquine. $C_{20}H_{22}ClN_3O.2HCl.2H_2O$; mol wt 464.82. Yellow, bitter crystals, dec 150-160°. uv max (methanol): 342 nm ($E^{1\%}_{1cm}$ 349); (water): 341.5 nm ($E^{1\%}_{1cm}$ 389); (0.1*N* HCl): 342 nm ($E^{1\%}_{1cm}$ 396). Sol in water; sparingly sol in alc; very slightly sol in benzene, chloroform, ether. pH of 1% aq soln 4.0 to 4.8.

Dihydrochloride hemihydrate. Yellow crystals from methanol, mp 243°. Slightly sol in water, alc.

THERAP CAT: Antimalarial.

577. Amorolfine. [78613-35-1] *cis*-4-[3-[4-(1,1-Dimethylpropyl)phenyl]-2-methylpropyl]-2,6-dimethylmorpholine; *cis*-4-[3-(4-*tert*-amylphenyl)-2-methylpropyl]-2,6-dimethylmorpholine; (±)-*cis*-2,6-dimethyl-4-[2-methyl-3-(*p*-*tert*-pentylphenyl)propyl]morpholine; Ro-14-4767/000. $C_{21}H_{35}NO$; mol wt 317.51. C 79.44%, H 11.11%, N 4.41%, O 5.04%. Antimycotic morpholine derivative; inhibits fungal ergosterol biosynthesis. Prepn (unspec stereochem): A. Pfiffner, K. Bohnen, **DE 2752096**; A. Pfiffner, **US 4202894** (1978, 1980 both to Hoffmann-La Roche); of *cis*-form: **NL 8004537** (1980 to Hoffmann-La Roche). *In vitro* comparative antifungal spectrum: S. Shadomy *et al., Sabouraudia* **22**, 7 (1984). Mechanism of action: A. Polak-Wyss *et al., ibid.* **23**, 433 (1985); A. Polak, *Ann. N.Y. Acad. Sci.* **544**, 221 (1988). LC determn in pharmaceutical formulations: M. A. Czech *et al., J. Pharm. Biomed. Anal.* **9**, 1019 (1991). Series of articles on mode of action and clinical trials: *Clin. Exp. Dermatol.* **17**, Suppl. 1, 1-70 (1992). Review of pharmacology and clinical efficacy: M. Haria, H. M. Bryson, *Drugs* **49**, 103-120 (1995).

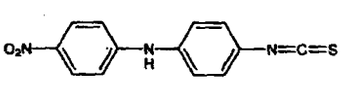


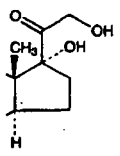
$bp_{0.1}$ 120°.

Hydrochloride. [78613-38-4] Ro-14-4767/002; Loceryl. $C_{21}H_{35}NO.HCl$; mol wt 353.98.

THERAP CAT: Antifungal (topical).

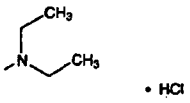
578. Amoscanate. [26328-53-0] 4-Isothiocyanato-*N*-(4-nitrophenyl)benzeneamine; isothiocyanic acid *p*-(*p*-nitroanilino)phenyl ester; 4-isothiocyanato-4'-nitrodiphenylamine; nithiocyamine; C-9333-Go; CGP-4540. $C_{13}H_9N_3O_2S$; mol wt 271.30. C 57.55%, H 3.34%, N 15.49%, O 11.79%, S 11.82%. Analog of nitroscanate, *q.v.* Prepn: K. Antos *et al.*, **DE 1932690** (1970 to Cesk. Akad. Ved), *C.A.* **72**, 100265 (1970); S. Rajappa *et al., J. Chem. Soc. Perkins Trans I* **1979**, 2001; N. Viswanathan, R. C. Desai, *Indian J. Chem.* **20B**, 308 (1981). Anthelmintic activity: H. P. Striebel, *Experientia* **32**, 457 (1976); K. R. Middleton *et al., ibid.* **35**, 243 (1979); H. G. Sen, B. N. Deb, *Am. J. Trop. Med. Hyg.* **30**, 992 (1981). HPLC determn in human plasma: W. M. Kofi-Tsekpo, C. W. Karekezi, *Drugs Exp. Clin. Res.* **14**, 31 (1988). Clinical pharmacology: A. B. Vaidya *et al., Brit. J. Clin. Pharmacol.* **4**, 463 (1977). Mutagenicity study: B. S. Reddy *et al., Antimicrob. Ag. Chemother.* **22**, 707 (1982). Brief review: J. I. Bruce, *Int. J. Parasitol.* **17**, 131-140 (1987).





ilan. $C_{23}H_{27}ClO_6$; mol wt
exane, mp 217-219°. uv max
$]_D$ +144° (chloroform).
pical).

ochloride. [3858-89-7] 4-
thylamino)ethyl ester mono-
nzoic acid diethylaminoeth-
. $C_{13}H_{20}Cl_2N_2O_2$; mol wt
3.08%, N 9.12%, O 10.42%.
tralh. 94, 384 (1955).



• HCl

ge). Bitter taste. Slowly sol
out one gram in 22 ml. Aq
rn yellow on standing. Soly
100 ml.

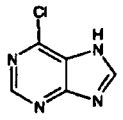
cid. [107-94-8] 3-Chloro-
wt 108.52. C 33.20%, H
$_2$ClCH$_2$COOH. Prepd by the
n with hydrochloric acid; by
aldehyde or of trimethylene
reu, Chaux, *Org. Syn.* **8**, 54
eck, *Ber.* **64**, 2142 (1931).
t hygroscopic. mp 41°. bp_{765}
8°. pK (25°) 4.00. Freely sol
less in ether.
om β-chloropropionic acid
rylic acid chloride and meth-

. [542-76-7] 3-Chloropro-
. C_3H_4ClN; mol wt 89.53. C
N 15.65%. ClCH$_2$CH$_2$CN.
drogen chloride or bromide:
France [4] **27**, 905 (1920);
oc. **69**, 714 (1947); Shirley,
ates (Wiley, New York, 1951)

dor. *Poisonous!* mp −51°.
p 132° (dec); bp_{50} 95.2°; bp_5
; 1.4341. Begins to dec when
Absorbs strongly in the in-
20 nm. Soly in water at 25°:
chloropropionitrile at 25°: 2.2
ether, acetone, benzene, car-
nice, rats: 9, 100 mg/kg, Fas-
xicology vol. 2, F. A. Patty,
ed., 1962) pp 2025-2026.
te should be avoided. Some-
itrile because of lower vapor
to produce systemic cyanide

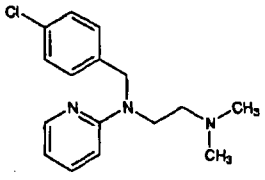
olymer synthesis. Combines
alkyl halide. Because of the
more reactive than in ordinary

2180. 6-Chloropurine. [87-42-3] $C_5H_3ClN_4$; mol wt 154.56. C 38.86%, H 1.96%, Cl 22.94%, N 36.25%. Prepd by the action of phosphorus oxychloride on hypoxanthine in *N,N*-dimethylaniline: Bendich *et al.*, *J. Am. Chem. Soc.* **76**, 6073 (1954). Antineoplastic activity *in vivo:* A. C. Sartorelli, B. A. Booth, *Experientia* **21**, 457 (1965).



Blunt needles from water, dec 175-177° (hot stage preheated to 170°). Also reported as not melted at 290°. uv max (pH 5.2): 265 nm (ε 9120); (pH 13): 274 nm (ε 8790). Soly in water: 0.5% at 20°, also reported as 1 g/182 ml at 24°. Sol in ether, dimethylformamide.

2181. Chloropyramine. [59-32-5] *N*-[(4-Chlorophenyl)methyl]-*N'*,*N'*-dimethyl-*N*-2-pyridinyl-1,2-ethanediamine; 2-[(*p*-chlorobenzyl)[2-(dimethylamino)ethyl]amino]pyridine; *N*-dimethylaminoethyl-*N*-*p*-chlorobenzyl-α-aminopyridine; *N,N*-dimethyl-*N'*-(*p*-chlorobenzyl)-*N'*-(2-pyridyl)ethylenediamine; halopyramine; Chloropyribenzamine. $C_{16}H_{20}ClN_3$; mol wt 289.81. C 66.31%, H 6.96%, Cl 12.23%, N 14.50%. Prepn: Phillips, Cates, US **2607778** (1952 to Merck & Co.); Howard, US **2569314** (1951 to Am. Cyanamid); **CH 264754**; **CH 266234**; **CH 266235** (1950); **GB 651596** (1951) (all to Geigy).

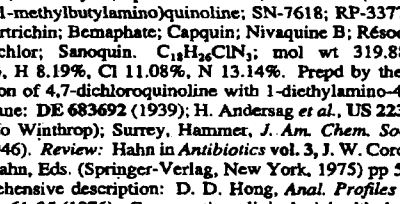


Light yellow, viscous, oily liquid. Pungent odor. $bp_{0.2}$ 154-155°.

Hydrochloride. [6170-42-9] Alegan S; Synopen; Synpen. $C_{16}H_{20}ClN_3$.HCl; mol wt 326.27. Crystals from acetone, mp 172-174°.

THERAP CAT: Antihistaminic.

2182. Chloroquine. [54-05-7] N^4-(7-Chloro-4-quinolinyl)-N^1,N^1-diethyl-1,4-pentanediamine; 7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline; SN-7618; RP-3377; Aralen; Artrichin; Bemaphate; Capquin; Nivaquine B; Résoquine; Reumachlor; Sanoquin. $C_{18}H_{26}ClN_3$; mol wt 319.88. C 67.59%, H 8.19%, Cl 11.08%, N 13.14%. Prepd by the condensation of 4,7-dichloroquinoline with 1-diethylamino-4-aminopentane: **DE 683692** (1939); H. Andersag *et al.*, **US 2233970** (1941 to Winthrop); Surrey, Hammer, *J. Am. Chem. Soc.* **68**, 113 (1946). *Review:* Hahn in *Antibiotics* vol. 3, J. W. Corcoran, F. E. Hahn, Eds. (Springer-Verlag, New York, 1975) pp 58-78. Comprehensive description: D. D. Hong, *Anal. Profiles Drug Subs.* **5**, 61-85 (1976). Comparative clinical trial with dapsone in rheumatoid arthritis: P. D. Fowler *et al.*, *Ann. Rheum. Dis.* **43**, 200 (1984); with penicillamine: T. Gibson *et al.*, *Brit. J. Rheumatol.* **26**, 279 (1987).



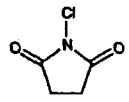
mp 87°.

Diphosphate. [50-63-5] Arechin; Avloclor; Imagon; Malaquin; Resochin; Tresochin. $C_{18}H_{26}ClN_3.2H_3PO_4$; mol wt 515.87. Bitter, colorless crystals. Dimorphic. One modification, mp 193-195°; the other, mp 215-218°. Freely sol in water; pH of 1% soln about 4.5; less sol at neutral and alkaline pH. Stable to heat in solns of pH 4.0 to 6.5. Practically insol in alcohol, benzene, chloroform, ether.

Sulfate. Nivaquine. $C_{18}H_{26}ClN_3.H_2SO_4$; mol wt 417.96.

THERAP CAT: Antimalarial; antiamebic; antirheumatic. Lupus erythematosus suppressant.

2183. *N*-Chlorosuccinimide. [128-09-6] 1-Chloro-2,5-pyrrolidinedione; succinchlorimide. $C_4H_4ClNO_2$; mol wt 133.53. C 35.98%, H 3.02%, Cl 26.55%, N 10.49%, O 23.96%. Prepn from succinimide: Hirst, Macbeth, *J. Chem. Soc.* **121**, 2169 (1922); Zimmer, Audrieth, *J. Am. Chem. Soc.* **76**, 3856 (1954). Crystal structure: Brown, *Acta Cryst.* **9**, 193 (1956); **14**, 711 (1961). Toxicity data: Stohlman, Smith, *U.S. Publ. Health Repts.* **59**, 541 (1944).



Orthorhombic crystals, mp 150-151°. Odor of chlorine. Acid to litmus (1:50 aq soln). One gram dissolves in about 70 ml water, 150 ml alcohol, 50 ml benzene. Sparingly sol in ether, chloroform, carbon tetrachloride. Liberates iodine from potassium iodide solns, and bromine from sodium bromide solns. MLD orally in rats: 2.7 g/kg (Stohlman, Smith).

USE: Chlorinating agent.

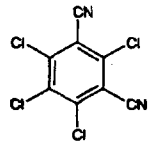
2184. Chlorosulfonic Acid. [7790-94-5] Sulfuric chlorohydrin; chlorosulfuric acid. $ClHO_3S$; mol wt 116.52. Cl 30.43%, H 0.87%, O 41.19%, S 27.52%. $SO_2(OH)Cl$. Prepd by the reaction of HCl gas with SO_3: Simon, Kratsch, *Z. Anorg. Allgem. Chem.* **242**, 369 (1939); Briggs, **US 1442335** (1922 to General Chem.). Purification: Kaplar, Shechter, *Inorg. Syn.* **4**, 52 (1953). *Review:* H. O. Burrus in *Kirk-Othmer Encyclopedia of Chemical Technology* vol. 5 (Wiley-Interscience, New York, 3rd ed., 1979) pp 873-880.

Colorless or slightly yellow, very corrosive liquid, causes severe burns; fumes in air; pungent odor. d_{20}^{20} 1.76-1.77; d_4^0 1.784; d_4^{20} 1.753. mp −80°. bp_{755} 151-152°; bp_{19} 74-75°; $bp_{2.4}$ 60-64°. n_D^{14} 1.437. On dropping into water dec with explosive violence. *Keep tightly closed.* When used for the prepn of sulfate esters, the common solvent is pyridine. Other solvents are liquid sulfur dioxide and dichloroethane.

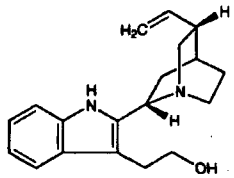
Caution: Highly irritating and corrosive to eyes, skin, mucous membranes.

USE: Manuf sulfone compds, saccharin. As chlorosulfonating and condensing agent in organic syntheses.

2185. Chlorothalonil. [1897-45-6] 2,4,5,6-Tetrachloro-1,3-benzenedicarbonitrile; tetrachloroisophthalonitrile; *m*-tetrachlorophthalodinitrile; 2,4,5,6-tetrachloro-1,3-dicyanobenzene; 1,3-dicyano-2,4,5,6-tetrachlorobenzene; chlorthalonil; DAC-2787; Daconil 2787; Bravo. $C_8Cl_4N_2$; mol wt 265.91. C 36.14%, Cl 53.33%, N 10.54%. Fungicidal properties first described by N. J. Turner *et al.*, *Contrib. Boyce Thompson Inst.* **22**, 303 (1964). Prepn: R. D. Battershell, H. Bluestone, US **3290353** (1966 to Diamond Alkali); R. M. Bimber, US **3652637** (1972 to Diamond Shamrock). Toxicity studies in mice: H. Yoshikawa, K. Kawai, *Ind. Health* **4**, 11 (1966). Review of carcinogenic risk: *IARC Monographs* **30**, 319-328 (1983).



synthetic studies: Battersby, Parry, *Chem. Commun.* **1971**, 31. Conversion of chinchona alkaloids of the quinoline series to those of the indole series: Ochiai *et al.*, *C.A.* **59**, 14040h (1963).

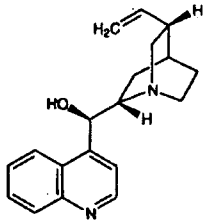


Triboluminescent, orthorhombic prisms from methanol, mp 186°, also reported as mp 194°. $[\alpha]_D^{20}$ +123° (c = 0.66 in ethanol). pK in 80% methyl Cellosolve: 8.28. uv max (methanol): 223, 292 nm (log ε 4.60, 3.88). One gram dissolves in about 35 ml alcohol, 105 ml ether. Sol in benzene, chloroform, petr ether, CS_2; practically insol in water.

Hydrochloride monohydrate. $C_{19}H_{24}N_2O.HCl.H_2O$. Cubic crystals. One gram dissolves in 200 ml water. Sol in alc.

2307. Cinchonidine. [485-71-2] (8α,9*R*)-Cinchonan-9-ol; cinchovatine; α-quinidine. $C_{19}H_{22}N_2O$; mol wt 294.39. C 77.52%, H 7.53%, N 9.52%, O 5.43%. Occurs in most varieties of cinchona bark, especially in bark of *Cinchona pubescens* Vahl. (*C. succirubra* Pav.) and *C. pitayensis* Wedd., *Rubiaceae*. Isoln: Leers, *Ann.* **82**, 147 (1852). Structure: Rabe, *ibid.* **365**, 359 (1909). Stereoisomeric with cinchonine: Koenigs, *ibid.* **347**, 182 (1906). Configuration: Prelog, Zalán, *Helv. Chim. Acta* **27**, 535 (1944); Prelog, Häfliger, *ibid.* **33**, 2021 (1950); Roth, *Pharmazie* **16**, 257 (1961); Lyle, Keefer, *Tetrahedron* **23**, 3253 (1967). Biosynthetic studies: Battersby, Parry, *Chem. Commun.* **1971**, 31. Toxicity studies: C. C. Johnson, C. F. Poe, *Acta Pharmacol.* **4**, 265 (1948); E. W. Schafer, Jr. *et al.*, *Ecotoxicol. Environ. Safety* **6**, 149 (1982).

Orthorhombic plates, prisms from alcohol, mp 210°. $[\alpha]_D^{20}$ −109.2° (alc). uv absorption data: Kamath *et al.*, *Indian J. Chem.* **6**, 510 (1968). Sol in alcohol and chloroform; moderately sol in ether. Practically insol in water. pK_1 5.80, pK_2 10.03. *Protect from light.* LD_{50} i.p. in rats: 206 mg/kg (Johnson, Poe). LD_{50} orally in quail: >316 mg/kg (Schafer).



Dihydrochloride. $C_{19}H_{22}N_2O.2HCl$. White or slightly yellow crystals or powder. Freely sol in water or alcohol. *Protect from light.*

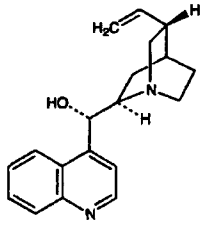
Hydrochloride dihydrate. $C_{19}H_{22}N_2O.HCl.2H_2O$. Cryst powder; loses all of its H_2O at 120°. $[\alpha]_D^{20}$ −117.5° (water): Sol in 25 parts cold water, more sol in boiling water; sol in alcohol, chloroform, slightly in ether. The aq soln is practically neutral. *Protect from light.*

Sulfate trihydrate. $(C_{19}H_{22}N_2O)_2.H_2SO_4.3H_2O$. Silky, acicular crystals; efflorescent on exposure to air and darkens in light. mp when anhydr ~240° with decompn. One gram dissolves in 70 ml water, 20 ml hot water, 90 ml alcohol, 40 ml hot alc, 620 ml chloroform. Practically insol in ether. The aq soln is practically neutral. *Protect from light.*

Epicinchonidine. [550-54-9] (8α,9*S*)-Cinchonan-9-ol. mp 104°, $[\alpha]_D^{20}$ +63° (c = 0.804 in alc): Rabe *et al.*, *Ann.* **492**, 253 (1932).

THERAP CAT: Antimalarial.

2308. Cinchonine. [118-10-5] (9*S*)-Cinchonan-9-ol. $C_{19}H_{22}N_2O$; mol wt 294.39. C 77.52%, H 7.53%, N 9.52%, O 5.43%. Occurs in most varieties of cinchona bark, especially in bark of *Cinchona micrantha* R. & P., *Rubiaceae*. Isoln and structure: Rabe, *Ber.* **41**, 63 (1908). Stereoisomeric with cinchonidine: Koenigs, *Ann.* **347**, 182 (1906). Configuration: Prelog, Zalán, *Helv. Chim. Acta* **27**, 535 (1944); Prelog, Häfliger, *ibid.* **33**, 2021 (1950); Roth, *Pharmazie* **16**, 257 (1961); Lyle, Keefer, *Tetrahedron* **23**, 3253 (1967). Crystal and molecular structure: B. Oleksyn *et al.*, *Acta Crystallogr.* **B35**, 440 (1979). Biosynthetic studies: Battersby, Parry, *Chem. Commun.* **1971**, 31. Toxicity study: C. C. Johnson, C. F. Poe, *Acta Pharmacol. Toxicol.* **4**, 265 (1949).



Prisms, needles from alcohol or ether. mp about 265°; begins to sublime at 220°. $[\alpha]_D$ +229° (alcohol). uv absorption data: Kamath *et al.*, *Indian J. Chem.* **6**, 510 (1968). One gram dissolves in 60 ml alcohol, 25 ml boiling alcohol, 110 ml chloroform, 500 ml ether. Practically insol in water. pK_1 5.85, pK_2 9.92. *Protect from light.* LD_{50} i.p. in rats: 152 mg/kg (Johnson, Poe).

Dihydrochloride. $C_{19}H_{22}N_2O.2HCl$. White or faintly yellow crystals or crystalline powder. Freely sol in water or alcohol. *Protect from light.*

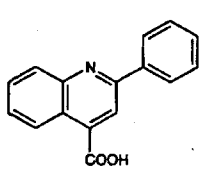
Hydrochloride dihydrate. $C_{19}H_{22}N_2O.HCl.2H_2O$. Fine crystals. mp when anhydr about 215° with dec. One gram dissolves in 20 ml water, 3.5 ml boiling water, 1.5 ml alcohol, 20 ml chloroform; slightly sol in ether. The aq soln is practically neutral. *Protect from light.*

Sulfate dihydrate. $(C_{19}H_{22}N_2O)_2.H_2SO_4.2H_2O$. Lustrous, very bitter crystals. mp when anhydr about 198°. One gram dissolves in 65 ml water, 30 ml hot water, 12.5 ml alc, 7 ml hot alc, 47 ml chloroform; slightly sol in ether. The aq soln is practically neutral.

Epicinchonine. [485-70-1] (9*R*)-Cinchonan-9-ol. mp 83°, $[\alpha]_D^{22}$ +120.3° (c = 0.806 in alc), Rabe *et al.*, *Ann.* **492**, 253 (1932).

THERAP CAT: Antimalarial.

2309. Cinchophen. [132-60-5] 2-Phenyl-4-quinolinecarboxylic acid; 2-phenylcinchoninic acid; Atophan; Quinophan; Phenoquin; Agotan; Artam; Alutyl; Cinconal; Vantyl; Viophan; Atocin; Mylofanol; Rhematan; Rheumin; Tophol; Polyphlogin; Quinofen; Tophosan. $C_{16}H_{11}NO_2$; mol wt 249.26. C 77.10%, H 4.45%, N 5.62%, O 12.84%. Quinoline derivative formerly used in treatment of chronic gout: A. B. Gutman, *Arthritis Rheum.* **16**, 431 (1973). Prepd by heating pyruvic acid with aniline and benzaldehyde or with benzylidene aniline in abs alcohol: Doebner, Gieseke, *Ann.* **242**, 290 (1887). Also from acetophenone and isatinic acid in alcoholic KOH: Pfitzinger, *J. Prakt. Chem.* **38**, 582 (1882); **56**, 293 (1897). Cinchophen as ulcerogenic agent: T. P. Churchill, F. H. van Wagoner, *Arch. Path.* **13**, 850 (1932); N. Umeda *et al.*, *Toxicol. Appl. Pharmacol.* **18**, 102 (1971); T. H. Stewart *et al.*, *J. Pathol.* **131**, 363 (1980). Mitochondrial toxicity of cinchophen and derivatives: H. Vainio *et al.*, *Biochem. Pharmacol.* **20**, 1589 (1971).

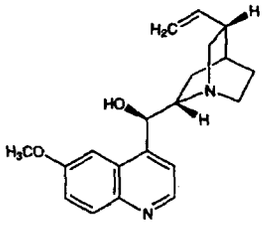


der, mp 180° (dec). Anhydr product is insol in methanol, ethanol, chloroform, ether, acetone, dioxane. Soly in hot 40% methanol or ethanol: 12%; in water at 25°: ~2%. LD_{50} in rats, mice (mg/kg): 3200 ± 350, 2680 ± 210 orally (Halpern, 1959).

THERAP CAT: Antiarrhythmic (class IA); antimalarial.

THERAP CAT (VET): Antiarrhythmic.

8151. Quinine. [130-95-0] (8α,9*R*)-6'-Methoxycinchonan-9-ol. $C_{20}H_{24}N_2O_2$; mol wt 324.42. C 74.04%, H 7.46%, N 8.63%, O 9.86%. Primary alkaloid of various species of *Cinchona* (Rubiaceae), *see* Cinchona. Representative samples of dried bark contain ~0.8 to 4% quinine. Optical isomer of quinidine, *q.v.* Isoln: Pelletier, Caventau, *Ann. Chem. Phys.* [2], **15**, 291 (1820). Extraction procedure: Jucker, Stoll, in *Ullmann's Enzyklopädie der technischen Chemie* **3**, 213-218 (1953). Configuration: Prelog, Zalán, *Helv. Chim. Acta* **27**, 535 (1944); Prelog, Häfliger, *ibid.* **33**, 2021 (1950); Roth, *Pharmazie* **16**, 257 (1961). Synthesis: Woodward, Doering, *J. Am. Chem. Soc.* **66**, 849 (1944); **67**, 860 (1945); Taylor, Martin, *ibid.* **94**, 6218 (1972); Gutzwiller, Uskokovic, *ibid.* **100**, 576 (1978); G. Grethe *et al., ibid.* 589; T. Imanishi *et al., Chem. Pharm. Bull.* **30**, 1925 (1982). Review of structural elucidation and early synthetic studies: R. B. Turner, R. B. Woodward in *The Alkaloids*, **vol. 3**, 1-63 (1953); of bioactivity: F. E. Hahn, Ed. in *Antibiotics* **vol. 5** (pt. 2) (Springer-Verlag, New York, 1979) pp 353-362. Comprehensive description of the hydrochloride: F. J. Muhtadi *et al., Anal. Profiles Drug Subs.* **12**, 547-621 (1983). LC determn in soft drinks: L. P. Valenti, *J. Assoc. Off. Anal. Chem.* **68**, 782 (1985). HPLC determn in blood: V. K. Dua *et al. J. Chromatog.* **614**, 87 (1993). Clinical evaluation to relieve nocturnal leg cramps: P. S. Connolly *et al., Arch. Intern Med.* **152**, 1877 (1992). Clinical efficacy in malaria: P. G. Kremsner *et al., J. Infect. Dis.* **169**, 467-470 (1994).



Triboluminescent, orthorhombic needles from abs alcohol, mp 177° (some decompn). Sublimes in high vacuum at 170-180°. $[\alpha]_D^{15}$ −169° (c = 2 in 97% alcohol), $[\alpha]_D^{17}$ −117° (c = 1.5 in chloroform), $[\alpha]_D^{15}$ −285° (c = 0.4*M* in 0.1*N* H_2SO_4). pK_1 (18°) 5.07; pK_2 9.7. pH of satd aq soln 8.8. Absorption spectra: Dobbie, Lauder, *J. Chem. Soc.* **99**, 1260 (1911); Dobbie, Fox, *ibid.* **101**, 78 (1912). Fluorescence: Rabe, Marschall, *Ann.* **382**, 362 (1911). The blue fluorescence is especially strong in dil H_2SO_4. One gram dissolves in 1900 ml water, 760 ml boiling water, 0.8 ml alcohol, 80 ml benzene (in 18 ml benzene at 50°), in 1.2 ml chloroform; 250 ml dry ether, 20 ml glycerol, 1900 ml of 10% ammonia water. Almost insol in petr ether.

Trihydrate. Microcrystalline powder, mp 57°, efflorescent, loses one H_2O in air, two H_2O over H_2SO_4, anhydr at 125°.

Bisulfate heptahydrate. [6183-68-2] (heptahydrate); [549-56-4] (anhydrous). Biquinate; Dentojel; Quinbisan. $C_{20}H_{24}N_2O_2.H_2SO_4.7H_2O$; mol wt 548.61. Very bitter crystals or cryst powder; efflorescent on exposure to air and darkens on exposure to light. One gram dissolves in 9 ml water, 0.7 ml boiling water, 23 ml alcohol, 0.7 ml alcohol at 60°, 625 ml chloroform, 2500 ml ether, 15 ml glycerol. pH: 3.5.

Dihydrochloride. [60-93-5] Quinine dichloride; quinine bimuriate; acid quinine hydrochloride. $C_{20}H_{24}N_2O_2.2HCl$. Very bitter powder or crystals. One gram dissolves in about 0.6 ml water, in about 12 ml alcohol. Slightly sol in chloroform, very slightly sol in ether. Aq solns are strongly acid to litmus paper (pH about 2.6).

Hydrochloride dihydrate. [6119-47-7] (dihydrate); [130-89-2] (anhydrous). $C_{20}H_{24}N_2O_2.HCl.2H_2O$. Bitter, silky needles. Effloresces on exposure to warm air. Does not lose all its water below 120°. One gram dissolves in 16 ml water, in 0.5 ml boiling water, in 1.0 ml alcohol, in about 7.0 ml glycerol, in about 1 ml chloroform, in about 350 ml ether. pH (1% aq soln): 6.0-7.0. Bitterness threshold 1:30000. *Protect from light.*

Sulfate dihydrate. [6119-70-6] (dihydrate); [804-63-7] (anhydrous). Quinamm; Quine; Quinate; Quinsan. $(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$; mol wt 782.96. Dull needles or rods, making a light and readily compressible mass. Becomes brownish on exposure to light. Loses its water of crystn at about 100°. $[\alpha]_D^{15}$ −220° (5% soln in about 0.5*N* HCl). One gram dissolves in 810 ml water, 32 ml boiling water, 120 ml alcohol, 10 ml alcohol at 78°. Slightly sol in chloroform, ether, but freely sol in a mixture of 2 vols chloroform and 1 vol abs alcohol. Aq solns are neutral to litmus, pH of satd soln 6.2.

USE: Flavor in carbonated beverages.

THERAP CAT: Antimalarial; muscle relaxant (skeletal).

THERAP CAT (VET): Antiprotozoal for fish.

8152. Quinine Iodosulfate. [7631-46-1] Herapathite; iodoquinine sulfate. $C_{80}H_{104}I_6N_8O_{20}S_3$; mol wt 2355.37. C 40.80%, H 4.45%, I 32.33%, N 4.76%, O 13.59%, S 4.08%. $4C_{20}H_{24}N_2O_2.3H_2SO_4.2HI.I_4$. Named after its discoverer, Herapath, an English physician.

Hexahydrate. Plate-like crystals of pale olive-green color by transmitted light and of a brilliant green to reddish green by reflected light. The crystals polarize light 5 times as much as tourmaline. Loses its water at 100° and becomes red. Almost insol in water. Sol in about 1000 parts boiling water; in 800 parts cold, 50 parts boiling alcohol, in 60 parts hot glacial acetic acid.

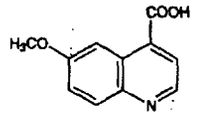
USE: In the manuf of polarizing glasses and plastics.

8153. Quinine Oleate. [10486-11-0] Usually with an excess of oleic acid. Contains about 21% anhydr quinine.

Brown, thick liquid. Insol in water. Sol in alcohol, ether, oils, petr ether.

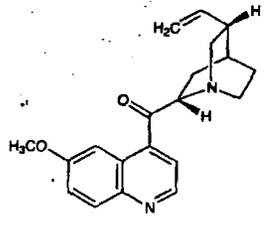
USE: Has been credited with opacity to ultraviolet rays and suggested as prophylactic against sunburn, applied as a 5-10% ointment. A soln of the oleate without excess of oleic acid in petr naphtha has been recommended for mothproofing of fabrics.

8154. Quininic Acid. [86-68-0] 6-Methoxy-4-quinolinecarboxylic acid; 6-methoxycinchoninic acid. $C_{11}H_9NO_3$; mol wt 203.19. C 65.02%, H 4.46%, N 6.89%, O 23.62%.



Pale yellow crystals, mp about 280° with dec. Slightly sol in water, cold alcohol or ether; sol in about 80 parts boiling abs alcohol; sol in aq alkalies.

8155. Quininone. [84-31-1] (8α)-6'-Methoxycinchonan-9-one. $C_{20}H_{22}N_2O_2$; mol wt 322.40. C 74.51%, H 6.88%, N 8.69%, O 9.93%. By careful oxidation of quinine or quinidine: Rabe, Kuliga, *Ann.* **364**, 346, 349 (1909); Woodward *et al., J. Am. Chem. Soc.* **67**, 1425 (1945). Alternate procedure: Rabe, Kindler, *Ber.* **51**, 466 (1918). Prepn of amorphous epimeric mixture of quininone and quinidinone from quinotoxine: Gutzwiller, Uskokovic, *Helv. Chim. Acta* **56**, 1494 (1973).



Crystals from et
otation, final $[\alpha]_D^{20}$
to litmus. Freely
Almost insol in wa
Hydrochloride.
mp 212°. Final $[\alpha]$

8156. Quinizi
cenedione; 1,4-dih
mol wt 240.21. C
p-chlorophenol and
Am. Chem. Soc. **48**
Org. Syn. **coll. vol.**
droquinone: Gatte
Chemikers (de Gru
prepd from diazotiz
GB 373999, *C.A.* **2**
with ammonium pe
Chem. [2] **54**, 90 (1
purin: *Org. Syn.* (*lo*
ed., 1971) p 4515.

Orange crystals fr
Orange plates from e
zene, toluene, xylene.
sorption spectrum:
(1916); Meek, *ibid.* **1**
sol in alcohol with re
yellow fluorescence.
ammonia. Black prec
about 13 g of boiling
Dimethyl ether.

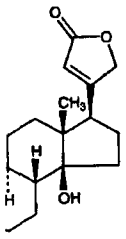
8157. Quinizari
thylphenyl)amino]-9,1
thraquinone; D & C
61565. $C_{28}H_{22}N_2O_2$;
6.69%, O 7.65%. Disc
Index **vol. 4** (3rd ed.,

Dark violet needles, m
a blue-green ppt on dilu
USE: For sutures: *Fe*
for use in externally a
14138 (1982).

8158. Quinmerac.
quinolinecarboxylic acid
mol wt 221.64. C 59.61
14.44%. Auxin-type he
3233089; *eidem*, US 47
ELISA determn in cerea

lepilatory.

28-4] (3β,5β)-3,5,14-Trihy-
; apocynamarin; convallatoxi-
notoxin; corchorgenin; corch-
50. C 68.29%, H 7.97%, O
drolysis of glycosides present
. *Apocynaceae.* Isoln: Jacobs,
253 (1922). Structure: Kon,
)56 (1934); Jacobs, Elderfield,
with corchorin, corchorgenin
v. Chim. Acta **40,** 588 (1957).
state, *q.v.*: E. Yoshii *et al., J.*
icity study: Graebner, Geisel,
1972). *Reviews:* Elderfield,
iesche, *Ergeb. Physiol.* **38,** 31
osides (Pharmaceutical Press,
ic Chemistry, **vol. II,** Gilman,
Fieser, Fieser, *Steroids* (Rein-
30, 736-750.



parts methanol and 10 parts wa-
½ mol H_2O which is given up at
ap 171-175° with effervescence.
ystals are found which melt at
in methanol). Sol in alcohol,
and glacial acetic acid. Practi-
ether. Color reaction with
erol. LD_{100} i.v. in cats: 0.337

$_{30}H_{36}O_7$. mp 230°. $[\alpha]_D^{26}$ +47.8°

nthidin. $C_{30}H_{33}BrO_7$. mp 222-
in acetone).
$_{23}H_{34}O_6$. mp 100-103°, resolidi-
°. $[\alpha]_D^{23}$ +34.9° (methanol).
Strophadogenin. $C_{23}H_{32}O_7$.
: 238-241°. $[\alpha]_D^{23}$ +52.9° (c =
) nm (log ε 4.3).

.005-63-3]. K-Strophanthin; K-
glycoside or a mixture of gly-
uthus kombé Oliv., *Apocynaceae.*
hoside contains α-glucose 19%,
%, strophanthidin 47%. K-Stro-
ains β-glucose, cymarose, and
Schweiz. Med. Wochenschr. **91,**
nd acute toxicity: H. Mehnert,
937).
: contg as much as 10% water,
without decompn. *Very poison-*
by light. Soluble in water and in
hol. Nearly insol in chloroform,
neutral to litmus. When bioas-
ess a potency per mg equivalent
Reference Standard. A deviation
cats, rats (mg/kg): 0.11, 9.4 i.v.

in

n used as a cardiotonic.

8935. Strophanthus. Dried ripe seeds of *Strophanthus kombé* Oliv., or of *S. hispidus* DC., *Apocynaceae,* deprived of the awns. *Habit.* East and Central Africa. *Constit.* 2-5% Strophanthin, kombic acid, choline, trigonelline, ~30% oil. *Poisonous!*

USE: As arrow poison by African natives.

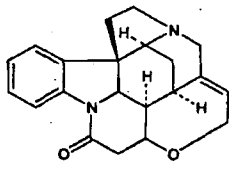
THERAP CAT: Cardiotonic.

THERAP CAT (VET): Has been used as a cardiotonic.

8936. Struvite. [15490-91-2] Magnesium ammonium phosphate hexahydrate; guanite. $H_4MgNO_5P.6H_2O$; mol wt 263.42. H 6.89%, Mg 9.23%, N 5.32%, O 66.81%, P 11.76%. $(NH_4)MgPO_4.6H_2O$. Phosphate mineral named in honor of H. C. G. von Sturve, a Russian naturalist, in 1845 by Ulex, a Swedish geologist. A component of urinary calculi and bat guano also found as a contaminant in canned foods, in waste management systems. Production by urease-splitting bacteria: H. Robinson, *Proc. Cambridge Phil. Soc.* **6,** 360 (1889); J. Beavon, N. G. Heatley, *J. Gen. Microbiol.* **31,** 167 (1962); M. T. Gonzalez-Munoz *et al., Chemosphere* **26,** 1881 (1993). Identification in tinned seafood: M. T. Gilles, Seafood Processing in *Food Processing Review* **22,** (Noyes Data Corp., Park Ridge, NJ, 1971) pp 31-58; in home-canned beef: J. G. Sebranek *et al., J. Muscle Foods* **4,** 81 (1993). Precipitation in sludge digesters: C. Maqueda *et al., Water Res.* **28,** 411 (1994). Crystal growth: R. J. C. McLean *et al., Urol. Res.* **18,** 39 (1990). Thermodynamics of formation: J. R. Buchanan *et al., Trans. Am. Soc. Agric. Engin.* **37,** 617 (1994). Chemical control in waste systems: J. R. Buchanan *et al., ibid.* 1301. Review of struvite urolithiasis: formation, detection and dissolution: C.A. Osborne *et al., Adv. Vet. Sci. Compar. Med.* **29,** 1-101 (1985). Review of struvite calculi: M. J. Gleeson, D. P. Griffith, *Brit. J. Urol.* **71,** 503-511 (1993).

Glass like crystals.

8937. Strychnine. [57-24-9] Strychnidin-10-one. $C_{21}H_{22}N_2O_2$; mol wt 334.41. C 75.42%, H 6.63%, N 8.38%, O 9.57%. Occurs most abundantly in seeds of *Strychnos nux-vomica* L., *Loganiaceae* and beans of *S. ignatti,* Berg. One of the first alkaloids isolated in pure form. Isoln: Pelletier, Caventou, *Ann. Chim. Phys.* **8,** 323 (1818); *ibid.* **10,** 142 (1819). Extraction procedure: C. Srinivasulu *et al., Res. Ind.* **23,** 224 (1978). Structure elucidation: L. H. Briggs *et al., J. Chem. Soc.* **1946,** 903; H. T. Openshaw, R. Robinson, *Nature* **157,** 438 (1946); R. B. Woodward *et al., J. Am. Chem. Soc.* **69,** 2250 (1947). Total synthesis: R. B. Woodward *et al., ibid.* **76,** 4749 (1954); *eidem, Tetrahedron* **19,** 247 (1963). Abs config: A. F. Peerdeman, *Acta Cryst.* **9,** 824 (1956); K. Nagarajan *et al., Helv. Chim. Acta* **46,** 1212 (1963). Stereoselective total synthesis: S. D. Knight *et al., J. Am. Chem. Soc.* **115,** 9293 (1993). ^{1}H- and ^{13}C-NMR analysis: E. Wenkert *et al., J. Org. Chem.* **43,** 1099 (1978). Pharmacology and pharmacokinetics: S. Weiss, R. A. Hatcher, *J. Pharmacol. Exp. Ther.* **19,** 419 (1921). Acute toxicity: I. Setnikar, M. J. Magistretti, *Arzneimittel-Forsch.* **14,** 996 (1964). HPLC determn in urine and tissues: T. Egloff *et al., J. Clin. Chem. Clin. Biochem.* **20,** 203 (1982). *Review:* J. B. Hendrickson in *The Alkaloids* **vol. VI,** R. H. F. Manske, Ed. (Academic Press, New York, 1960) pp 179-195; G. F. Smith, *ibid.* **vol. VIII** (1965) pp 591-671. Comprehensive description: F. J. Muhtadi, M. S. Hifnawy, *Anal. Profiles Drug Subs.* **15,** 563-646 (1986). Brief review of synthesis: U. Beifuss, *Angew. Chem. Int. Ed.* **33,** 1144-1149 (1994).



Brilliant, colorless cubes from chloroform-ether, mp 275-285°. d^{18} 1.359. $[\alpha]_D^{18}$ −104.3° (c = 0.254 in alc); $[\alpha]_D^{25}$ −139° (c = 0.4 in chloroform). pKa (25°) 8.26: A. J. Everett *et al., J. Chem. Soc.* **1957,** 1120. uv max (95% ethanol): 255, 280, 290 nm ($E_{1cm}^{1\%}$ 377, 130, 101): A. I. Biggs, *J. Pharm. Pharmacol.* **13,** 547 (1952). One gram dissolves in 182 ml ethanol, 6.5 ml chloroform, 150 ml benzene, 250 ml methanol, 83 ml pyridine; very slightly soluble in ether, water. Bitter taste in soln contg 1 part in 700,000 of water. LD_{50} i.v. (slow infusion) in rats: 0.96 mg/kg (Setnikar, Magistretti).

Acetate. $C_{23}H_{26}N_2O_4$. Crystals, sol in 20 parts water, sol in alcohol.

Gluconate pentahydrate. $C_{27}H_{34}N_2O_9.5H_2O$. Crystals, darken above 80°, sol in 2 parts water, ~40 parts alcohol; the aq soln is neutral.

Glycerophosphate hexahydrate. $C_{45}H_{53}N_4O_{10}P.6H_2O$. One gram dissolves in ~350 ml water, ~310 ml alcohol. Slightly sol in chloroform; very slightly in ether.

Hydrochloride dihydrate. $C_{21}H_{23}ClN_2O_2.2H_2O$. Efflorescent, trimetric prisms. One gram dissolves in ~40 ml water, ~80 ml alc. Insol in ether. pH of 0.01*M* soln 5.4.

Nitrate, *see* separate entry

Phosphate, *see* separate entry

Salicylate. $C_{28}H_{28}N_2O_5$. Leaflets, sol in ~30 parts water; sparingly sol in alcohol.

Sulfate, *see* separate entry

Caution: Extremely poisonous, *see:* G. D. Osweiler, *Curr. Vet. Ther.* **6,** 115 (1977). Potential symptoms of overexposure to strychnine are stiff neck and facial muscles; restlessness, apprehension and increased acuity of perception; increased reflex excitability; cyanosis; tetanic convulsions with opisthotonos. *See NIOSH Pocket Guide to Chemical Hazards* (DHHS/NIOSH 97-140, 1997) p 286.

USE: Chiefly in poison baits for rodents.

THERAP CAT (VET): Has been used as a tonic and central stimulant.

8938. Strychnine Nitrate. [66-32-0] $C_{21}H_{23}N_3O_5$; mol wt 397.42. C 63.47%, H 5.83%, N 10.57%, O 20.13%.

Colorless, odorless needles or white, cryst powder. *Violent poison!* One gram dissolves in 42 ml water, 10 ml boiling water, 150 ml alcohol, 80 ml alcohol at 60°, 105 ml chloroform, 50 ml glycerol; insol in ether. pH ~5.7. *Protect from light.*

THERAP CAT (VET): Has been used as tonic, stimulant.

8939. Strychnine N^6-Oxide. [7248-28-4] Genostrychnine; Movellan (tabl.). $C_{21}H_{22}N_2O_3$; mol wt 350.41. C 71.98%, H 6.33%, N 7.99%, O 13.70%. Prepn: Pictet, Mattison, *Ber.* **38,** 2782 (1905); Bailey, Robinson, *J. Chem. Soc.* **1948,** 703; Zellner, US 2758113 (1956 to Donau-Pharmazie).

Monoclinic prisms from water, dec 207°. pK 5.17. Freely sol in alc, glacial acetic acid, chloroform; fairly sol in water; sparingly sol in benzene. Practically insol in ether, petr ether.

Hydriodide. Light yellow plates from hot water, dec 253°.

Nitrate. Small transparent prisms from water, dec 250°.

Strychnic acid sodium salt. Movellan (amps.).

8940. Strychnine Phosphate. [509-42-2] $C_{21}H_{23}N_2O_6P$; mol wt 432.41. C 58.33%, H 5.83%, N 6.48%, O 22.20%, P 7.16%. Usually as $C_{21}H_{22}N_2O_2.H_3PO_4.2H_2O$.

Dihydrate. Colorless or white crystals or white powder. *Violent poison!* One gram dissolves slowly in ~30 ml water; more sol in hot water; slightly sol in alcohol. The aq soln is acid.

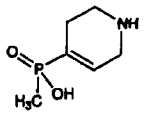
THERAP CAT (VET): Has been used as tonic, stimulant.

8941. Strychnine Sulfate. [60-41-3] $C_{42}H_{46}N_4O_8S$; mol wt 766.92. C 65.78%, H 6.05%, N 7.31%, O 16.69%, S 4.18%. Usually crystallizes as $2C_{21}H_{22}N_2O_2.H_2SO_4.5H_2O$. Also crystallizes with $6H_2O$ (12.36% water).

Incompatibility of all strychnine salts: alkalies, alkali carbonates and bicarbonates, benzoates, dichromates, bromides, iodides, tannic and picric acids, salicylates, borax, gold chloride and other alkaloid precipitants, piperazine, potassium-mercuric iodide (not if acacia is present).

Pentahydrate. Colorless, odorless, very bitter crystals, or white, cryst powder. *Violent poison!* Effloresces in dry air; loses all its water of crystn at 100°. mp when anhydr ~200° with decompn. One gram dissolves in 35 ml water, 7 ml boiling water, 81 ml alcohol, 26 ml alcohol at 60°, 220 ml chloroform, 6 ml glycerol; insol in ether. pH 5.5 in 1:100 soln. *Protect from*

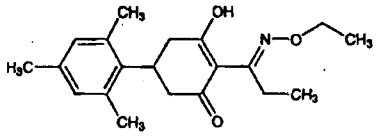
antagonist for $GABA_c$ receptors. Prepn: Y. Murata *et al., Bioorg. Med. Chem. Letters* **6**, 2073 (1996); R. Miledi *et al.*, US **5627169** (1997 to Univ. California). Design and *in vitro* pharmacology: D. Ragozzino *et al., Mol. Pharmacol.* **50**, 1024 (1996). Specificity vs human receptors and alternate synthesis: M. Chebib *et al., Eur. J. Pharmacol.* **357**, 227 (1998). Use as antagonist: A. Rozzo *et al., Neuroscience* **90**, 1085 (1999).



Off-white solid from ethanol, mp 252-254°. Soly in water 16 mg/ml. Insol in DMSO.

USE: Neurochemical tool.

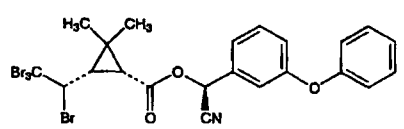
9640. Tralkoxydim. [87820-88-0] 2-[1-(Ethoxyimino)-propyl]-3-hydroxy-5-(2,4,6-trimethylphenyl)-2-cyclohexen-1-one; 2-[1-(ethoxyimino)propyl]-3-hydroxy-5-mesitylcyclohex-2-en-1-one; PP-604; Achieve; Grasp. $C_{20}H_{27}NO_3$; mol wt 329.43. C 72.92%, H 8.26%, N 4.25%, O 14.57%. Cereal selective post-emergent herbicide. Prepn: R. B. Warner *et al.*, EP **80301**; *eidem*, US **4717418** (1983, 1988 both to ICI). Physical properties and herbicidal activity: R. B. Warner *et al., Proc. Brit. Crop Prot. Conf. - Weeds* **1987**, 19. Field trials on grass weeds: J. Rola, *ibid.* 363; P. B. Sutton *et al., ibid.* 389. Mechanism of action study: J. Secor, C. Cseke, *Plant Physiol.* **86**, 10 (1988).



White crystalline solid, mp 106°. Vapor pressure at 20°: 4×10^{-10} kPa. Soly at 20° (mg/l): water 6 at pH 6.5, 5 at pH 5.0; at 24° (g/l): hexane 18; toluene 213; dichloromethane >500; methanol 25; acetone 89; ethyl acetate 110. LD_{50} orally in male and female rats, male and female mice, male rabbit (mg/kg): 1324, 934, 1231, 1100, 519; dermally in male and female rats: >2000, >2000 mg/kg (Warner 1987).

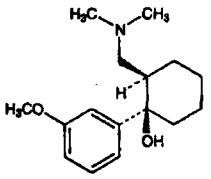
USE: Post-emergent herbicide.

9641. Tralomethrin. [66841-25-6] 2,2-Dimethyl-3-(1,-2,2,2-tetrabromoethyl)cyclopropanecarboxylic acid cyano(3-phenoxyphenyl)methyl ester; (*S*)-α-cyano-3-phenoxybenzyl (1*R*)-*cis*-2,2-dimethyl-3-[(*RS*)-1,2,2,2-tetrabromoethyl]cyclopropanecarboxylate; RU-25474; OMS-3048; Saga; Scout; Tracker; Tralate; Tralox. $C_{22}H_{19}Br_4NO_3$; mol wt 665.01. C 39.74%, H 2.88%, Br 48.06%, N 2.11%, O 7.22%. Synthetic pyrethroid consisting of two active diastereomers whose absolute configurations differ at the monobrominated carbon atom. Prepn: BE **873201**; J. Martel *et al.*, US **4279835** (1979, 1981 both to Roussel-Uclaf). Insecticidal activity: M. Benoit *et al., Pest. Biochem. Physiol.* **26**, 284 (1986). Mechanism of action study: M. Roche *et al., ibid.* **24**, 306 (1985). HPLC determn in water, sediment and fish tissue: J. Mao *et al., J. Agric. Food Chem.* **41**, 596 (1993). Field trials: D. D. Amalraj *et al., Indian J. Malariol.* **28**, 141 (1991); W. R. Halliday *et al., J. Agric. Entomol.* **9**, 145 (1992).



USE: Insecticide.

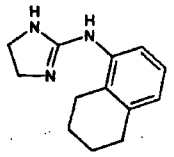
9642. Tramadol. [27203-92-5] (1*R*,2*R*)-*rel*-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol; E-265; CG-315E; U-26225A. $C_{16}H_{25}NO_2$; mol wt 263.37. C 72.97%, H 9.57%, N 5.32%, O 12.15%. Prepn: GB **997399**; K. Flick, E. Frankus, US **3652589** (1965, 1972 both to Grünenthal); K. Flick *et al., Arzneimittel-Forsch.* **28**, 107 (1978). Series of articles on pharmacology and clinical studies: *ibid.* 114-219. Toxicology: F. Lagler *et al., ibid.* 164. Mechanism of action study: R. B. Raffa *et al., J. Pharmacol. Exp. Ther.* **267**, 331 (1993). HPLC determn in urine: B. Elsing, G. Blaschke, *J. Chromatog.* **612**, 223 (1993). Symposium on pharmacology and clinical experience: *Drugs* **47**, Suppl. 1, 1-46 (1994).



Hydrochloride. [22204-88-2] Contramal; Crispin; Tramal; Ultram; Zydol. $C_{16}H_{25}NO_2.HCl$; mol wt 299.84. White crystals, mp 180-181°. Sol in water. LD_{50} in mice, rats (mg/kg): 350, 228 orally; 200, 286 s.c. (Lagler).

THERAP CAT: Analgesic.

9643. Tramazoline. [1082-57-1] 4,5-Dihydro-*N*-(5,6,7,-8-tetrahydro-1-naphthalenyl)-1*H*-imidazol-2-amine; 2-[(5,6,7,-8-tetrahydro-1-naphthyl)amino]-2-imidazoline. $C_{13}H_{17}N_3$; mol wt 215.29. C 72.53%, H 7.96%, N 19.52%. α-Adrenergic agonist. Prepn: Berg, **DE 1191381**; **DE 1195323** (both 1965 to Thomae). *C.A.* **63**, 8373c; 13274d (1965). Pharmacology and toxicity data: R. Engelhorn, H. Klupp, *Arzneimittel-Forsch.* **12**, 971 (1962). Activity studies: Sachsenröder *et al., ibid.* **22**, 392 (1972).

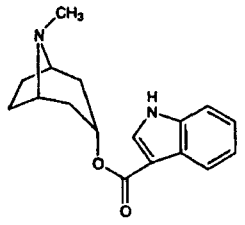


Crystals from isopropanol, mp 142-143°.

Hydrochloride monohydrate. [3715-90-0] KB-227; Bicitron; Ellatun; Rhinaspray; Rhinogutt; Rhinospray; Rinogutt; Towk. $C_{13}H_{17}N_3.HCl.H_2O$; mol wt 269.78. Crystals from alc + ether or acetone + ether, mp 172-174°. Sol in water. LD_{50} orally in mice: 195 mg/kg (Engelhorn, Klupp).

THERAP CAT: Decongestant.

9644. Trandolapril. [87679-37-6] (2*S*,3a*R*,7a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic acid; (3a*R*,7a*S*)-1-[*N*-[1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanyl]octahydroindole-2(*S*)-carboxylic acid; (2*S*,3a*R*,7a*S*)-1-[(*S*)-*N*-[(*S*)-1-carboxy-3-phenylpropyl]alanyl]hexahydro-2-indolinecarboxylic acid 1-ethyl ester; RU-44570; Mavik; Odrik; Gopten. $C_{24}H_{34}N_2O_5$; mol wt 430.54. C 66.95%, H 7.96%, N 6.51%, O 18.58%. Angiotensin converting enzyme (ACE) inhibitor. Prepn: H. Urbach *et al.*, EP **84164**; *eidem*, US **4933361** (1983, 1990 both to Hoechst). Enzyme inhibition and pharmacology: N. L. Brown *et al., Eur. J. Pharmacol.* **148**, 79 (1988). Clinical pharmacology: F. De Ponti *et al., Eur. J. Clin. Pharmacol.* **40**, 149 (1991). Clinical trial in prevention of death after myocardial infarction: L. Kober *et al., N. Engl. J. Med.* **333**, 1670 (1995). Series of articles on pharmacology and clinical trials: *Am. J. Hypertension* **8**, 63S-74S (1995). Clinical trial in diabetic neuropathy: R. A Malik *et al., Lancet* **352**, 1978 (1998).



mp 201-202° (from methylene chloride/ethyl acetate).

Monohydrochloride. [105826-92-4] Navoban; Novaban. $C_{17}H_{20}N_2O_2.HCl$; mol wt 320.82. mp 283-285° (dec).

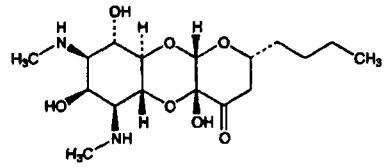
THERAP CAT: Antiemetic.

9854. Tropomyosins. Fibrous proteins involved in the regulation of muscle relaxation. They are present in all forms of striated and smooth muscles and probably in nonmuscle cells as well. Native tropomyosin consisting of two proteins, tropomyosin and ***troponin***, is the Ca^{2+}-sensitive regulatory protein that controls the interaction between actin and myosin necessary for the production of force in muscle. In all skeletal tissues, there are two forms of tropomyosin chains, designated α and β. Their ratio depends on the muscle source. Troponin consists of three subunits, troponin T (the tropomyosin binding subunit), troponin I (the actomyosin ATPase inhibitory subunit) and troponin C (the Ca^{2+}-binding subunit). All three subunits, in addition to tropomyosin, are responsible for the native tropomyosin activity. Isoln of tropomyosin from skeletal muscle and cardiac muscle: K. Bailey, *Nature* **157**, 368 (1946); *idem, Biochem. J.* **43**, 271 (1948). Structure studies: R. S. Hodges, L. B. Smillie, *Biochem. Biophys. Res. Commun.* **41**, 987 (1970). Amino acid sequence studies: J. Sodek *et al., Proc. Nat. Acad. Sci. USA* **69**, 3800 (1972). X-ray crystal structure of troponin C: M. Sundaralingam *et al., Science* **227**, 945 (1985). *Reviews:* C. E. Bodwell, K. Laki, in *Contractile Proteins and Muscle*, K. Laki, Ed. (Dekker, New York, 1971); W. F. Harrington in *The Proteins*, **vol. 4**, H. Neurath, R. L. Hill, Eds. (Academic Press, New York, 1979) pp 317-327. Comprehensive review of isolation, preparation, identification and role in the contractile process: *Methods Enzymol.* **vol. 85**, Part B, entitled "Structural and Contractile Proteins", D. W. Frederiksen, L. W. Cunningham, Eds. (Academic Press, New York, 1982) 774 pp.

9855. Tropylium Bromide. [5376-03-4] Cycloheptatrienylium bromide; cycloheptatrienocarbonium bromide. C_7H_7Br; mol wt 171.04. C 49.16%, H 4.13%, Br 46.72%. Prepd by bromination of 1,3,5-cycloheptatriene in carbon tetrachloride, followed by removal of the CCl_4 and heating the residue *in vacuo* for several days: von Doering, Knox, *J. Am. Chem. Soc.* **79**, 352 (1957); King, Stone, *Inorg. Syn.* **7**, 99 (1963).

Yellow prisms from ethanol, mp 203°. Freely sol in water. Practically insol in ether.

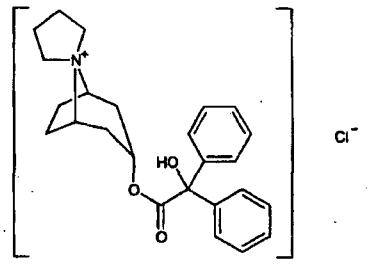
9856. Trospectomycin. [88669-04-9] (2*R*,4a*R*,5a*R*,6*S*,7*S*,-8*R*,9*S*,9a*R*,10a*S*)-2-Butyldecahydro-4a,7,9-trihydroxy-6,8-bis-(methylamino)-4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one; 6′-*n*-propylspectinomycin. $C_{17}H_{36}N_2O_7$; mol wt 374.43. C 54.53%, H 8.08%, N 7.48%, O 29.91%. Semi-synthetic analog of spectinomycin, *q.v.* Prepn: D. R. White, **DE 3308196**; *idem*, **US 4532336** (1983, 1985 both to Upjohn); D. R. White *et al., J. Antibiotics* **36**, 339 (1983); P. M. Herrinton *et al., J. Org. Chem.* **58**, 678 (1993). *In vitro* activity: G. E. Zurenko *et al., Antimicrob. Ag. Chemother.* **32**, 216 (1988). Mode of action: E. M. Veringa *et al., Int. J. Med. Microbiol.* **271**, 311 (1989). Pharmacokinetic and tolerance study: G. R. Peters *et al., Int. J. Clin. Pharmacol. Ther. Toxicol.* **28**, 361 (1990). HPLC determn in plasma and serum: R. J. Simmonds *et al., J. Liq. Chromatog.* **13**, 1125 (1990). Clinical study in gonorrhea: T. F. Mroczkowski *et al., Drugs Exptl. Clin. Res.* **19**, 41 (1993).



Sulfate pentahydrate. [88851-61-0] U-63366F; Spexil. $C_{17}H_{30}N_2O_7.H_2SO_4.5H_2O$; mol wt 562.59. Crystals from acetone/water.

THERAP CAT: Antibacterial.

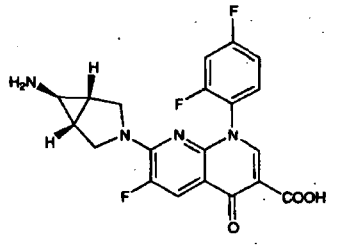
9857. Trospium Chloride. [10405-02-4] (1α,3β,5α)-3-[(Hydroxydiphenylacetyl)oxy]spiro[8-azoniabicyclo-[3.2.1]octane-8,1′-pyrrolidinium] chloride; 3α-hydroxyspiro-[1α*H*,5α*H*-nortropane-8,1′-pyrrolidinium] chloride benzilate; azoniaspiro(3α-benziloyloxynortropane-8,1′-pyrrolidine) chloride; azoniaspiro(3α-diphenylglycoloyloxynortropan-8,1′-pyrrolidine) chloride; 3α-benziloyloxyspiro(nortropane-8,1′-pyrrolidinium) chloride; Regurin; Relaspium; Spasmex. $C_{25}H_{30}ClNO_3$; mol wt 427.97. C 70.16%, H 7.07%, Cl 8.28%, N 3.27%, O 11.22%. Tropine derivative with anticholinergic activity. Prepn: **NL 6402155**; R. Pfleger *et al.*, **US 3480626** (1964, 1969 both to Pfleger); H. Bertholdt *et al., Arzneimittel-Forsch.* **17**, 719 (1967). Pharmacology and toxicology in animals: H. Antweiler *et al., ibid.* **16**, 1581 (1966). Inhibition of gastric motility and acid secretion in humans: G. Lux, P. Frühmorgen, *Fortschr. Med.* **96**, 2113 (1978). Fluorimetric determn in plasma and urine: G. Schladitz-Keil *et al., J. Chromatog.* **345**, 99 (1985). Bioavailability: *eidem, Arzneimittel-Forsch.* **36**, 984 (1986). Clinical trial in bladder hyper-reflexia: H. Madersbacher *et al., Brit. J. Urol.* **75**, 452 (1995).



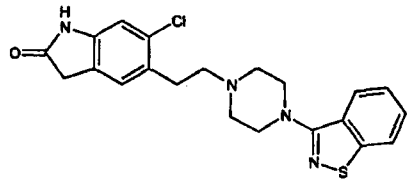
Crystals from ethanol-ether, mp 255-257° (dec). LD_{50} in mice (mg/kg): 12.3 i.v. (Antweiler).

THERAP CAT: Antispasmodic; in treatment of urinary incontinence.

9858. Trovafloxacin. [147059-72-1] (1α,5α,6α)-7-(6-Amino-3-azabicyclo[3.1.0]hex-3-yl)-1-(2,4-difluorophenyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid; CP-99219. $C_{20}H_{15}F_3N_4O_3$; mol wt 416.35. C 57.70%, H 3.63%,



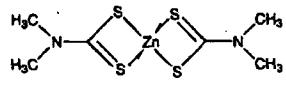
10224. Ziprasidone. [146939-27-7] 5-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2*H*-indol-2-one; 5-(2-(4-(1,2-benzisothiazol-3-yl)piperazinyl)ethyl)-6-chlorooxindole; CP-88059. $C_{21}H_{21}ClN_4OS$; mol wt 412.94. C 61.08%, H 5.13%, Cl 8.59%, N 13.57%, O 3.87%, S 7.77%. Dopamine D_2/serotonin 5-HT_2 antagonist. Prepn: J. A. Lowe III, A. A. Nagel, **EP 281309**; *eidem*, **US 4831031** (1988, 1989 both to Pfizer). Clinical pharmacology: C. J. Bench *et al.*, *Psychopharmacology* **112**, 308 (1993). HPLC determn in serum: J. S. Janiszewski *et al.*, *J. Chromatog. B* **668**, 133 (1995). Brief review of comparative pharmacology: J. M. Goldstein, *Exp. Opin. Invest. Drugs* **4**, 291-298 (1995).



Hydrochloride monohydrate. [138982-67-9] CP-88059-1. $C_{21}H_{21}ClN_4OS.HCl.H_2O$; mol wt 467.42. Also occurs as the hemihydrate, mp >300°.

THERAP CAT: Antipsychotic.

10225. Ziram. [137-30-4] (*T*-4)-Bis(dimethylcarbamodithioato-*S*,*S*')zinc; bis(dimethyldithiocarbamato)zinc; zinc dimethyldithiocarbamate; dimethyldithiocarbamic acid zinc salt; zinc bis(dimethylthiocarbamoyl) disulfide; methyl cymate; Crittam; Mezene; Pomarsol Z; Thionic; Triscabol. $C_6H_{12}N_2S_4Zn$; mol wt 305.87. C 23.56%, H 3.96%, N 9.16%, S 41.94%, Zn 21.38%. Prepd from zinc oxide, dimethylamine, and carbon disulfide: Olin, Deger, **US 2492314** (1949 to Sharples Chemicals). Crystal structure: Klug, *Acta Cryst.* **21**, 536 (1966). Toxicity study: Hodge *et al.*, *J. Pharmacol. Exp. Ther.* **118**, 174 (1956). Field analysis of residues in air: J. E. Woodrow *et al.*, *J. Agric. Food Chem.* **43**, 1524 (1995). Review and evaluation of toxicity studies: *IARC Monographs* **53**, 423-438 (1991).



Crystals from hot chloroform + alcohol, mp 250°. *Can form a flammable dust.* d_4^{25} 1.66. Practically insol in water. Soly per 100 ml of solvent at 25°: <0.2 g, alcohol; <0.5 g, acetone; <0.5 g, benzene; <0.2 g, carbon tetrachloride, more sol in chloroform; <0.2 g, ether; 0.5 g, naphtha. Sol in dil caustic solns. LD_{50} orally in rats: 1.4 g/kg (Hodge).

Caution: May be irritating to skin and mucous membranes. *See: Clinical Toxicology of Commercial Products*, R. E. Gosselin *et al.*, Eds. (Williams & Wilkins, Baltimore, 5th ed., 1984) Section II, p. 314.

USE: Rubber vulcanization accelerator; agricultural fungicide.

10226. Zirconium. [7440-67-7] Zr; at. wt 91.224; at. no. 40; valence 4; also 3. Group IVB(4). Five naturally occurring isotopes: 90 (51.46%); 91 (11.23%); 92 (17.11%); 94 (17.40%); 96 (2.80%); artificial radioactive isotopes: 81-89, 93, 95, 97-99. Occurrence in earth's crust: 0.023%. Occurs in the minerals zircon, malacon, baddeleyite, zirkelite, eudialyte; frequently found in the rare-earth minerals; in monazite sand. Discovered by Klaproth in 1789; prepd by Berzelius in 1824. Prepn: Fast, *Z. Anorg. Chem.* **239**, 145 (1938); purification of zirconium by ion exchange columns: Ayres, *J. Am. Chem. Soc.* **69**, 1879 (1947). Sepn of zirconium and hafnium: Fischer *et al.*, *Angew. Chem. Int. Ed.* **5**, 15 (1966). Reviews of zirconium and its compds: W. B. Blumenthal, *The Chemical Behavior of Zirconium* (Van Nostrand, Princeton, 1958); *Gmelin's Handb. Anorg. Chem., Zirconium* (8th ed.) **42**, (1958) 448 pp; Larsen, "Zirconium and Hafnium Chemistry" in *Advan. Inorg. Chem. Radiochem.* **13**, 1-333 (1970); Bradley, Thornton, "Zirconium and Hafnium" in *Comprehensive Inorganic Chemistry*, **vol. 3**, J. C. Bailar, Jr. *et al.*, Eds. (Pergamon Press, Oxford, 1973) pp 419-490.

Bluish-black, amorphous powder or grayish-white lustrous metal (platelets or flakes) of hexagonal lattice below 865°, body-centered cubic above 865°, mp 1857°; bp 3577°. d 6.5. Brinnell hardness: 85. Can absorb up to 10 atoms per cent of oxygen or nitrogen. Reacts with hydrofluoric acid, aqua regia, hot phosphoric acid. Not attacked by cold, very slightly attacked by hot, concd sulfuric or hydrochloric acid; not attacked by nitric acid. Attacked by fused potassium hydroxide or nitrate. On prolonged heating the compact form combines with oxygen, nitrogen, carbon, and the halogens. The powder form has a very low ignition temp and is very explosive when mixed with oxidizing agents.

Caution: Zirconium and its salts generally have low systemic toxicity. A granulomatous disease of the skin, particularly in the axilla, has been reported in users of a deodorant contg sodium zirconium lactate: *see* E. Browning, *Toxicity of Industrial Metals* (Appleton-Century-Crofts, New York, 2nd ed., 1969) pp 356-360. Consult latest Government regulations on use in aerosol antiperspirants.

USE: Pure zirconium (hafnium-free) is a valuable structural material for atomic reactors because of its low nuclear cross-section and high corrosion and heat resistance. Because of hafnium's high neutron absorption characteristics, it must be removed from zirconium which is to be used in nuclear reactors; removal unnecessary for other commercial purposes. As an ingredient of priming or explosive mixtures; flashlight powders; as deoxidizer in metallurgy; as "getter" in vacuum tubes; in constructing rayon spinnerets in lamp filaments, flash bulbs.

10227. Zirconium Chloride. [10026-11-6] Zirconium tetrachloride. Cl_4Zr; mol wt 233.04. Cl 60.85%, Zr 39.15%. $ZrCl_4$. In large-scale prepns zirconium oxide is converted to the carbide, which is chlorinated to yield the tetrachloride: Kroll *et al.*, *J. Electrochem. Soc.* **94**, 1 (1948). Lab prepn based on the equation $ZrO_2 + 2CCl_4 \rightarrow ZrCl_4 + 2COCl_2$: Hummers *et al.*, *Inorg. Syn.* **4**, 121 (1953). Toxicology study: N. A. Zhilova, A. A. Kasparov *Hygiene Sanit.* **31**, 328 (1966). *Review:* Blumenthal, *J. Chem. Ed.* **39**, 604-610 (1962).

Lustrous monoclinic crystals: Krebs, *Angew. Chem. Int. Ed.* **8**, 146 (1969); *idem*, *Z. Anorg. Allgem. Chem.* **378**, 263 (1970). Tetrahedral symmetry in gas phase with the Zr-Cl distance of 2.33 Å. Lewis acid. Extremely hygroscopic, forms HCl vapor and gives off fumes in moist air. d 2.803. Sublimes at 331°, mp 437° under its own pressure which is about 25 atm at this temp. Decomposed by water to form $ZrOCl_2$ and HCl; sol in alcohol, ether. LD_{50} in mice, rats (mg/kg): 665, 1688 orally (Zhilova, Kasparov).

USE: Friedel-Crafts catalyst. Component of Ziegler-type catalysts in the condensation of ethylene. Starting material in the synthesis of a number of organic derivs of zirconium, such as alkoxides and zircocene. The alkoxides have been shown to be of value in the curing of silicone plastic films. The alkoxyzirconium carboxylates are said to be useful in the water-repellent treatment of textiles and other fibrous materials.

10228. Zirconium Fluoride. [7783-64-4] Zirconium tetrafluoride. F_4Zr; mol wt 167.22. F 45.44%, Zr 54.55%. ZrF_4. Prepd by thermal decompn of $(NH_4)_2ZrF_6$: v. Hevesy, Dullenkamp, *Z. Anorg. Allgem. Chem.* **221**, 161 (1934); according to the equation $ZrCl_4 + 4HF \rightarrow ZrF_4 + 4HCl$: Wolter, *Chemiker-Ztg.* **51**, 607 (1908); from zirconium oxide and fluorine: Haendler *et al.*, *J. Am. Chem. Soc.* **76**, 2177 (1954).

Strongly refractive, crystalline mass (monoclinic system): d^{16} 4.6. Sublimes above 600°. Solubility in water (20°): 1.32 g/100 ml. Does not react with water; forms stable trihydrate. Freely soluble in hydrofluoric acid.

10229. Zirconium Hydride. [7704-99-6] Ideal composition: ZrH_2. Prepd by the reduction of zirconium oxide with

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Exhibit A7

9322 /DELNP/2007

FORM 3
THE PATENTS ACT, 1970
(39 of 1970)
&
The Patents Rules, 2003
STATEMENT AND UNDERTAKING UNDER SECTION 8
(See section 8, rule 12)

13 DEC 2007

I/We, Novartis AG., a corporation organized and existing under the laws of Switzerland, of Lichtstrasse 35, CH-4056 Basel Switzerland.

Hereby declare:

(i) That I/We have not made any application for the same/substantially the same invention outside India.

(ii) That I/We who have made this application No. **/DELNP/2007** dated 03rd December **2007** alone/jointly with made for the same/ substantially same invention, application(s) for patent in the other countries, the particulars of which are given below:

Country	Application No	Date of application	Status of the application	Date of publication	Date of grant
USA	60/701,406	July 20, 2005	Pending	-	-
USA	60/716,213	September 12, 2005	Pending	-	-
PCT	PCT/US2006/027878	July 18, 2006	-	-	-

(iii) That the rights in the application(s) has/have been assigned to.

I/We undertake that upto the date of grant of the patent, by the Controller, I/We would keep him informed in writing the details regarding corresponding applications for patents filed outside India within three months from the date of filing of such application.

Dated this 03rd day of December 2007

Snigdha Rani Dey
Of Anand And Anand Advocates
Attorney for the Applicant

ORIGINAL

To
The Controller of Patents,
The Patent Office, Delhi

The examination is being carried out on the **following application documents**:

Description, Pages

1-24 as published

Claims, Numbers

1-9 received on 12.02.2009 with letter of 06.02.2009

Drawings, Sheets

1-8 as published

1. Amendments (Article 123(2) EPC)

The amended set of claims are allowable with respect to Article 123(2) EPC.

2. Inventive Step (Article 56 EPC)

The present application does not meet the requirements of Article 52(1) EPC because the subject-matter of claims 2-4,6,8 does not involve an inventive step within the meaning of Article 56 EPC.

The problem to be solved by the applicant was the provision of an improved compound for use in the treatment of a disease which responds to an inhibition of protein kinase activity. This problem was clearly solved for the hydrochloride salt of compound A. For the phosphate salts this effect was not shown. The problem underlying the subject-matter of these salts was the provision of an alternative form of compound A. Starting from D1, a skilled person would come to the solution of the present application as he would try to make salts like phosphate salts of the compound disclosed in example 85.

Phosphoric acid was mentioned in D1 as a possible acid to make salts. Claims 2-4,6,8 are therefore not considered to involve an inventive step.

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Tel: +31 70 340 - 1097

Application No.	Ref.	Date
06 787 733.2 - 1211	34385-EP-EPT	09.09.2009
Applicant: NOVARTIS AG, et al		

Communication pursuant to Article 94(3) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(2) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to correct the indicated deficiencies within a period

of 4 months

from the notification of this communication, this period being computed in accordance with Rules 126(2) and 131(2) and (4) EPC. One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (R. 50(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

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Diederen, Jeroen
Primary Examiner
For the Examining Division

Enclosure(s): 2 page/s reasons (Form 2906)

The examination is being carried out on the **following application documents**:

Description, Pages

1-24 as published

Claims, Numbers

1-9 as annexed to the Int. Prel. Examination Report

Drawings, Sheets

1-8 as published

1. Inventive step (Article 56 EPC)

The present application does not meet the requirements of Article 52(1) EPC because the subject-matter of claims 1-9 does not involve an inventive step within the meaning of Article 56 EPC.

Reference is made to the following document; the numbering will be adhered to in the rest of the procedure:

D1: WO 2004/005281 A1 (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; BREITENSTEIN WERNER [CH];) 15 January 2004 (2004-01-15)

Document D1 discloses compounds of Formula I with the same use as the compounds of the present application. In claim 1 of the said document, pharmaceutically acceptable salts are claimed. On page 8 of the description, it is made clear which pharmaceutically acceptable salts are meant. Mentioned on the said page are amongst others, hydrochloric acid and phosphoric acid.

In example 85, 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-yl-amino)-benzamide is disclosed.

The problem to be solved by the applicant was to provide alternative compounds as tyrosine kinase inhibitors. Starting from D1, a skilled person would try a pharmaceutically acceptable salt like a hydrochloride acid or phosphate salt of the compound of example 85 and come to the solution of claims 1-3 without any inventive skill.

It is therefore considered, that the monohydrochloride monohydrate salt, the monophosphate and the diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-yl-amino)-benzamide are not inventive over the prior art with respect to Article 56 EPC. A method for the preparation, pharmaceutical compositions and methods of treatments comprising these compounds are not considered inventive over the prior art either.

Claims 1-9 are therefore not considered to involve an inventive step.

2. Method of treatment (Article 53(c) EPC)

The subject matter of claims 8,9 concerns a method of treatment which is expressly excluded from patentability (see Article 53(c) EPC). The claims as presently formulated are thus not allowable, and should therefore be either suppressed or reformulated in a correct manner (see Guidelines C-IV, 4.8).

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Application No.	Ref.	Date
06 787 733.2 - 1211	34385-EP-EPT	29.07.2008
Applicant: Novartis AG, et al		

Communication pursuant to Article 94(3) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(2) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to correct the indicated deficiencies within a period

of 4 months

from the notification of this communication, this period being computed in accordance with Rules 126(2) and 131(2) and (4) EPC. One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (R. 50(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

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Diederen, Jeroen
Primary Examiner
For the Examining Division

Enclosure(s): 2 page/s reasons (Form 2906)

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Date of receipt: 13 November 2007 (13.11.2007) PCT/US2006/027878

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 34385-WO-PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/US2006/027878	International filing date *(day/month/year)* 18.07.2006	Priority date *(day/month/year)* 20.07.2005
International Patent Classification (IPC) or national classification and IPC INV. C07D401/14		
Applicant NOVARTIS AG et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the International application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
2007-05-16	06.11.2007
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Diederen, Jeroen Telephone No. +31 70 340-1097

Date of receipt: 13 November 2007 (13.11.2007) PCT/US2006/027878

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2006/027878

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-24	as originally filed
Claims, Numbers	
1-9	filed with telefax on 16.05.2007
Drawings, Sheets	
1-8	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Date of receipt: 13 November 2007 (13.11.2007) PCT/US2006/027878

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No. PCT/US2006/027878

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 8,9

because:

☒ the said international application, or the said claims Nos. 8,9 (with respect to industrial application) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*.

☐ no international search report has been established for the said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Date of receipt: 13 November 2007 (13.11.2007) PCT/US2006/027878

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US2006/027878

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Date of receipt: 13 November 2007 (13.11.2007) PCT/US2006/027878

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No. PCT/US2006/027878

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 8,9 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Inventive Step (Article 33(2) PCT)

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1,4-9 does not involve an inventive step in the sense of Article 33(3) PCT.

Reference is made to the following document:

D1: WO 2004/005281 A1 (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; BREITENSTEIN WERNER [CH];) 15 January 2004 (2004-01-15) cited in the application

Document D1 discloses compounds of Formula I with the same use as the compounds of the present application. In claim 1 of the said document, pharmaceutically acceptable salts are claimed. On page 8 of the description, it is made clear which pharmaceutically acceptable salts are meant. Mentioned on the said page are amongst others, hydrochloric acid and phosphoric acid.

In example 85, 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-yl-amino)-benzamide is disclosed.

The problem to be solved by the applicant was to provide alternative compounds as tyrosine kinase inhibitors. Starting from D1, a skilled person would try a

Date of receipt: 13 November 2007 (13.11.2007) PCT/US2006/027878

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.
PCT/US2006/027878

pharmaceutically acceptable salt like a hydrochloride acid or phosphate salt of the compound of example 85 and come to the solution of claims 1-3 without any inventive skill. It is therefore considered, that the monohydrochloride monohydrate salt, the monophosphate and the diphosphate salt of 4-methyl-N-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-phenyl]-3-(4-pyridin-3-yl-pyrimidin-2-yl-amino)-benzamide are not inventive over the prior art with respect to Article 33(3) PCT. A method for the preparation, pharmaceutical compositions and methods of treatments comprising these compounds are not considered inventive over the prior art either.