FRESENIUS KABI

Date: February 07, 2014

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The Controller of Patents
The Patent Office
Boudhik Sampada Bhawan
Plot No. 32, Sector-14
New Delhi 110075

Subject: Representation U/S 25 (1) read with Rule 55 in respect of Indian Patent Application No. 1960/DELNP/2007 dated March 14, 2007
Representation filed by: Fresenius Kabi Oncology Ltd.

Dear Sir,

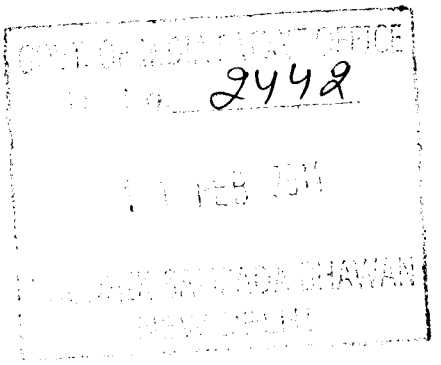
We submit herewith a representation U/S 25 (1) read with Rule 55 of the Patents Act, 1970 as amended by the Patents (Amendment) Act 2005. In reference to this we submit herewith following documents:

(i) Representation U/S 25 (1) in duplicate;
(ii) Annexure I and Exhibits 1 to 14.

The controller is requested to take the documents on record and proceed further in the matter and keep the opponent advised of each and every step taken in the matter.

We crave leave of the Controller to provide additional documents as evidence as called for by the Controller or if necessary to support any of the averments in the representation.

Lastly, we request the Controller to grant us an opportunity of being heard before the above representation is finally decided.



Thanking you,

Dr. Prachi Tiwari
(Of Fresenius Kabi Oncology Ltd.)

11 FEB 2014

BEFORE THE CONTROLLER OF PATENTS,

NEW DELHI

REPRESENTATION UDNER SECTION 25 (1) IN RESPECT OF

PATENT APPLICATION NO. 1960/DELNP/2007

FRESENIUS KABI ONCOLOGY LIMITED

...PETITIONER / OPPONENT

-VS-

BAYER HEALTHCARE LLC

...RESPONDENT/ APPLICANT

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Date: Feb 07, 2014

Dr. Prachi Tiwari
Of Fresenius Kabi Oncology Ltd.
(Opponent)

The Controller of Patents
The Patent Office, Delhi

BEFORE THE CONTROLLER OF PATENTS

PATENT OFFICE,

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, 2013

and

IN THE MATTER of Patent Application No. 1960/DELNP/2007 dated March 14, 2007 in the name of BAYER HEALTHCARE LLC; 100 Bayer Boulevard Whippany, New Jersey 07981, USA

..... Applicant

and

IN THE MATTER of opposition thereto by FRESENIUS KABI ONCOLOGY LIMITED, B-310, Som Datt Chambers-I Bhikaji Cama Place, New Delhi-110066, India

..... Opponent

REPRESENTATION UNDER SECTION 25(1)

We, FRESENIUS KABI ONCOLOGY LIMITED B-310,Som Datt Chambers-I Bhikaji Cama Place, New Delhi -110 066, India; (hereinafter called 'opponent') make the following representation under Section 25(1) of the Act in opposing the grant of patent on the application indicated in the cause title.

Opponent's business and activities

The opponent is a Company incorporated under the Companies Act, 1956, and having its principal office FRESENIUS KABI ONCOLOGY LIMITED, B-310,Som Datt Chambers-I Bhikaji Cama Place, New Delhi -110 066, India; carrying on business, inter alia, of manufacture, research and sale of anticancer agents.

Locus standi

Locus standi is not a condition precedent for an opposition under Section 25(1). In any event it is stated that the application under opposition relates to an alleged invention in the field of pharmaceutical products. The opponent being engaged in the research and development as well as in the manufacture of drugs / medicinal compositions for many years has interest in opposing the grant of patent to the application under opposition and is a person interested and therefore has locus standi to initiate the present proceedings.

GROUNDS OF OPPOSITION

1. The application is opposed on the following grounds:

a). Section 25(1) (b): Novelty / Anticipation

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: Provided that the ground specified in sub-clause (ii) shall not be available where such publication does not constitute an anticipation of the invention by virtue of sub-section (2) or subsection (3) of section 29;

b). Section 25(1) (d): Prior Knowledge / Prior 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.

***Explanation**.—For the purposes of this clause, an invention relating to a process for which a patent is claimed shall be deemed to have been publicly known or publicly used in India before the priority date of the claim if a product made by that process had already been imported into India before that date except where such importation has been for the purpose of reasonable trial or experiment only;*

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 as mentioned in clause (b) or having regard to what was used in India before the priority date of the applicant's claim;

d). Section 25(1) (f): Not Patentable Subject Matter

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;

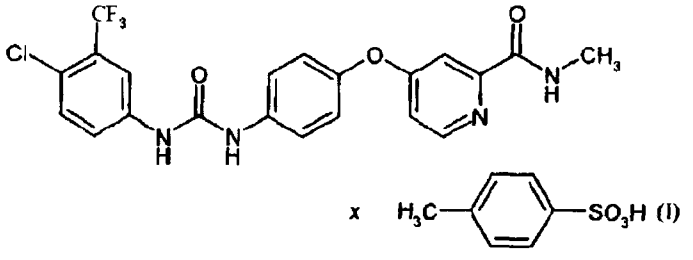
e) **Section 25(1) (g): Insufficient disclosure**

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

2. ANALYSIS OF THE IMPUGNED APPLICATION

2.1. Patent application 1960/DELNP/2007 (hereinafter referred as impugned application) entitled, "THERMODYNAMICALLY STABLE FORM OF A TOSYLATE SALT", dated March 14, 2007 was nationalized in India from International Application Number PCT/EP2005/010119 (International Publication Number WO 2006/034797) dated September 20, 2005. The impugned application claims priority from European Application 04/023130 filed on September 29, 2004. The impugned application was filed by "BAYER PHARMA AKTIENGESELLSCHAFT" (currently assigned to BAYER HEALTHCARE LLC). It was published U/S 11A in the Official Journal of Indian Patent Office on August 17, 2007.

2.2. The impugned application relates to a specific form of the known compound Sorafenib tosylate. The compound Sorafenib tosylate is represented hereinbelow as formula (I),



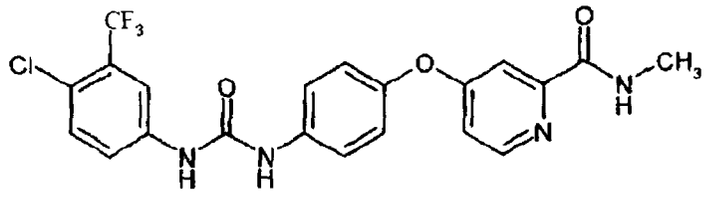
Formula (I)

In particular, the impugned application has been filed in respect of polymorph-I of Sorafenib tosylate, to processes for its preparation, to a pharmaceutical composition comprising it and to its use in the treatment of certain disorders.

a. ADMITTED PRIOR ART

Impugned application acknowledges the international application PCT/US00/00648, published as WO 00/42012 on July 20, 2000 which

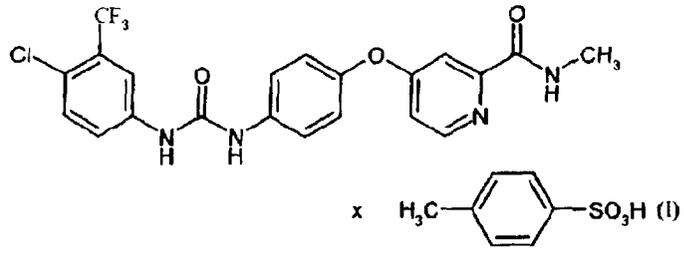
discloses the free base compound Sorafenib, represented hereinbelow as a compound of formula (II) and process for the preparation thereof,



Formula (II) i.e. Sorafenib

The compounds and their salts, disclosed in WO 00/42012, for example tosylate, are described there as inhibitors of the enzyme Raf kinase and may be used for the treatment of disorders, for example cancer (see page 01, fourth paragraph, lines 12-17).

Additionally, the impugned application acknowledges the international application PCT/US03/04103, published as WO 03/068228 on August 21, 2003 which discloses the compound of formula (I) **(i.e. Sorafenib tosylate),**



Formula (I)

WO 03/068228 (referred as Document D1 in this representation) relates, *inter alia,* to the use of Sorafenib tosylate for the treatment of disorders in which angiogenesis plays an important role, for example in tumor growth (see page 01, second paragraph, lines 05-09).

The applicant acknowledges the existence of Sorafenib tosylate in two additional forms, namely polymorph-II and polymorph-III (see Example 1 and Example 3 of the impugned application).

b. PROBLEM TO BE SOLVED

The impugned application alleges that polymorph-II and polymorph-III of Sorafenib tosylate have certain disadvantages. It mentions that polymorph-II is metastable. Accordingly, it is stated that safety and quality of preparations would be compromised unless a stable polymorph is employed in the pharmaceutical preparations. Hence, there was a need to provide a stable polymorph suitable for the preparations of Sorafenib tosylate for the treatment of diseases or conditions mediated by one or more cellular signal transduction pathways involving Raf, VEGFR, PDGFR, p38, and/or flt-3 kinases.

c. ALLEGED SOLUTION

Accordingly, the impugned application provides a solution to the existing problem of the prior art by providing polymorph-I of Sorafenib tosylate which ensures undesired conversion to another polymorph and associated properties of the Sorafenib tosylate e.g. solubility or bioavailability.

d. The impugned application contains a statement of 18 claims annexed hereto as "Annexure 1" (as downloaded from patent office website). A brief review of the Applicant's claims is provided hereinbelow for ready reference:

- Claim 1 corresponds to polymorph-I of Sorafenib tosylate;
- Claims 2, 3, 4, 13 and 14 relates to conversion of polymorph-II of Sorafenib tosylate to polymorph-I of Sorafenib tosylate;
- Subject matter of Claims 5 to 12 corresponds to a pharmaceutical composition of the compound of claim 1, use of compound of claim 1 or a pharmaceutical composition thereto for the treatment of certain disorders;
- Subject matter of claims 15 and 18 corresponds to a combination comprising compound of claim 1 and one or more other pharmaceutical agents and a pharmaceutical composition thereto.

2.3. The present representation U/S 25(1) is framed on claims set out above (enclosed as Annexure-1). 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.

2.4. 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.

3. DOCUMENTS RELIED ON IN THE PRESENT REPRESENTATION:

- WO 03/068228 (referred to hereinafter as D1) published on August 21, 2003; annexed hereto as Exhibit 1;

- WO 03/050111 (referred to hereinafter as D2) published on Jun 19, 2003; annexed hereto as Exhibit 2;

- A document downloaded from the EMA website (hereinafter referred to as D3) which is annexed hereto as Exhibit 3 (can be downloaded from the link http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000690/WC500027707.pdf);

- A document downloaded from the FDA website (hereinafter referred to as D4) which is annexed hereto as Exhibit 4 (can be downloaded from the link http://www.accessdata.fda.gov/drugsatfda_docs/appletter/2005/021923ltr.pdf);

- Chemistry & industry, 1989, pages 527-529 (hereinafter D5) which is annexed hereto as Exhibit 5;

- Pharmaceutical Research, vol. 12, No. 7, pages 1995, 945-954 (hereinafter D6) which is annexed hereto as Exhibit 6;

- X-Ray diffraction data of products obtained by repeating (four times) the example 1 of the impugned application are annexed hereto as Exhibit 7 to Exhibit 10:

    1. X-Ray Diffraction Data of Experiment No.: SOR/CRD0330/010/SOR-3 (Our Ref. No.) as Exhibit 7;

    2. X-Ray Diffraction Data of Experiment No.: SOR/CRD0330/012/SOR-3 (Our Ref. No.) as Exhibit 8;

    3. X-Ray Diffraction Data of Experiment No.: SOR/CRD0330/014/SOR-3 (Our Ref. No.) as Exhibit 9;

    4. X-Ray Diffraction Data of Experiment No.: SOR/CRD0317/004/SOR-3 (Our Ref. No.) as Exhibit 10;

- X-Ray diffraction data of product obtained by following the method of D2 is annexed hereto as Exhibit 11 [Experiment No.SOR/CRD0316/090/SOR-3/SP-1 (Our Ref. No.)];

- Affidavits of Ms. Sandeep Kaur and Mr. Nikunj Kachhadia, who repeated the experiment of example 1 of the impugned application are annexed hereto as Exhibit 12 and Exhibit 13;

- Affidavit of Mr. Varun Sharma who performed the method as given in D2 is annexed hereto as Exhibit 14.

Having regard to the aforesaid discussions, the opponent now proceeds to deal with the various grounds of opposition.

4. LACK OF NOVELTY / ANTICIPATION GROUNDS:

4.1. CLAIM 1 - LACK OF NOVELTY [U/S 25(1) (b)] AND [U/S 25(1) (e)]

Claim 1 of the impugned application relates to polymorph-I of Sorafenib tosylate (in general referred to as compound of claim 1).

The impugned application on page 02, first paragraph, lines 02- 04 states that "*The compound of the formula (1)* [i.e. Sorafenib tosylate] *is prepared according to a general standard method for the preparation of tosylate salts, as described in example 1 of the working examples. In this method, the compound of the formula (I) is obtained in one crystal polymorph which is referred to hereinbelow as polymorph II.*"

4.1.1 Therefore, according to the applicant's statement if the tosylate salt of Sorafenib is prepared according to general standard methods (i.e. prior art methods for the preparation of tosylate salts) it is yielded as polymorph-II. An example of such a prior art method is given in example 1 of the working examples of the impugned application.

The opponent being engaged in the research and development as well as in the manufacture of drugs / medicinal compositions repeated the experiment given in working example 1 of the impugned application for the preparation of the tosylate salt of Sorafenib.

However, contrary to the applicant's statement polymorph-I was obtained by following the procedure given in example 1 of the impugned application.

4.1.2. To ensure the reproducibility, the experiment of example 1 was repeated four times and X-Ray diffraction patterns of the obtained product from respective experiments are annexed hereto as Exhibit 7 to Exhibit 10.

Affidavits of Ms. Sandeep Kaur and Mr. Nikunj Kachhadia, who repeated the experiment of example 1 of the impugned application, are annexed hereto as Exhibit 12 and Exhibit 13.

4.1.3. Additionally, the opponent prepared the tosylate salt of Sorafenib by following an alternative method known in the art that is found in document D2 (see page 11, example 3).

Example 3 of D2 is reproduced verbatim hereinbelow for ready reference:
"A solution of p-toluensulfonic acid monohydrate (3.19 g) in acetone (5 ml) was added to a stirring solution of 5- [4- [2- (N-methyl-N- (2-pyridyl) amino) ethoxy] benzyl] thiazolidine-2,4-dione (6.0 g) in acetone (90 ml) at reflux. The reaction was heated at reflux for 25 minutes, then cooled to 21 C with stirring for 45 minutes. The white solid was collected by filtration, washed with acetone (25 ml), dried under vacuum for 15.5 hours at 21 C to give 5- [4- [2- (N-methyl-N- (2-pyridyl) amino) ethoxy] benzyl] thiazolidine- 2, 4-dione p-toluenesulfonate (8.6 g) as a white crystalline solid."

The above example was re-worked replacing the thiazolidine compound by Sorafenib, and again polymorph-I of Sorafenib tosylate was obtained.

The X-Ray diffraction analysis of the product obtained from the above described experiment is annexed hereto as Exhibit 11.

The affidavit of Mr. Varun Sharma, who performed the method as given in D2, is annexed hereto as Exhibit 14.

4.1.4. A table comparing the X-Ray diffraction pattern of

- the compound of claim 1 of the impugned application with,
- Sorafenib tosylate obtained by repeating the Example 1, and
- Sorafenib tosylate obtained by following the method given in prior art document D2

is presented hereinbelow in Table-1,

Table-I

Peak maxima of the ^{0}2Th. according to the impugned application	**Product obtained by repeating Example 1 of the Impugned Application**				**By following method of Example 3 of D2**
	Peak maxima of the ^{0}2Th. according to Exhibit 7	Peak maxima of the ^{0}2Th. according to Exhibit 8	Peak maxima of the ^{0}2Th. according to Exhibit 9	Peak maxima of the ^{0}2Th. according to Exhibit 10	Peak maxima of the ^{0}2Th. according to Exhibit 11
4.4	**4.4**	**4.3**	**4.4**	**4.4**	**4.4**
10.7	**10.7**	**10.6**	**10.7**	**10.7**	**10.7**
11.1	11.1	11.0	11.1	11.1	11.1
11.4				11.3	11.4
11.6	11.6	11.6	11.6	11.6	11.6
12.2	12.2	12.1	12.2	12.2	12.2
12.8	12.8	12.7	12.8	12.8	12.8
13.2	**13.2**	**13.1**	**13.2**	**13.2**	**13.2**
14.8	**14.7**	**14.7**	**14.7**	**14.8**	**14.8**
16.5				16.4	16.4
16.7	**16.6**	**16.6**	**16.6**	**16.6**	**16.7**
17.7	17.6			17.6	17.6
17.9	**17.9**	**17.8**	**17.8**	**17.8**	**17.9**
18.8	18.8	18.7	18.8	18.8	18.8
19.3	19.4	19.3	19.4	19.3	19.4
19.6				19.4	
20.1	20.0	20.0	20.0	20.0	20.1
20.5	**20.4**	**20.4**	**20.4**	**20.5**	**20.5**
20.8	**20.8**	**20.7**	**20.8**	**20.8**	**20.8**
21.5	**21.5**	**21.4**	**21.5**	**21.5**	**21.5**
21.7				21.7	

22.3	22.1		22.1	22.1	22.1
22.5			22.4	22.5	22.5
22.9	**22.9**	**22.8**	**22.9**	**22.8**	**22.9**
23.4	23.3	23.3	23.3	23.3	23.4
23.7	23.7	23.6	23.7	23.6	23.7
24.0			24.0	24.0	24.1
24.5	24.5	24.4	24.5	24.5	24.5
25.1	25.0	25.0	25.0	25.0	25.0
25.4	25.4	25.3	25.4	25.3	25.4
26.0	26.0	25.9	26.0	26.0	26.0
26.4					26.3
26.6	26.6	26.5	26.6	26.6	26.6
27.0	26.9	26.9	26.9	26.9	26.9
27.6	27.3	27.3	27.3	27.3	27.3
28.2	28.1	28.0	28.1	28.1	28.1
28.6	28.6	28.5	28.6	28.5	28.5
28.8				28.6	28.7
29.3			29.2	29.2	
29.6	29.5	29.5	29.5	29.5	29.5
29.9	29.8	29.8	29.8	29.8	29.8
30.8	30.8	30.7	30.8	30.8	30.8
31.2	31.1	31.1	31.1	31.1	31.1
31.6	31.5	31.5	31.5	31.5	31.6
31.8					
32.1	32.0	32.0	32.0	32.0	32.0
32.4					
32.7	32.6	32.6	32.6	32.6	32.6
33.1	33.1	33.0	33.1		33.1
33.8	33.8	33.8	33.8		33.8
34.2	34.2	34.1	34.2		34.2
34.6					
35.4	35.3	35.2	35.3		

35.7	35.7	35.6	35.7		35.7
37.1	37.3	37.0	37.0		

Note: A deviation of up to + / 2 degree 2-theta is to be regarded as normal. This may arise as rounding and measurement error and may result in differing measurement conditions and from the presence of impurities (e.g. residual solvents) in the samples.

From the above table it is evident that the Sorafenib tosylate manufactured by the opponent following the example 1 of the impugned application and by other known methods corresponds to the polymorph-I of the impugned application.

4.1.5. Hence, it is clear that polymorph-I of Sorafenib tosylate was obtainable by standard methods known in the art. Accordingly, the subject matter of claim 1 of the impugned application is not new. Hence, D1 disclosing Sorafenib tosylate inherently anticipates subject matter of claim 1.

4.1.6. Although, D1 does not refer to any particular parameters of the Sorafenib tosylate, however, it is a well settled rule that "*a prior art experiment which, when performed, reliably produced a particular result "more than 99 percent of the occasions on which it is conducted" would be regarded for the purposes of disclosure as "inevitably" leading to the result in question. It follows that a claim which defines an invention by reference to parameters, for example, of a process or a product, is anticipated by a disclosure, which when put into practice would necessarily fall within the scope of the claim,* ***even if the disclosure does not refer to these particular parameters"*** *(see section 3.4.7, pages 25 and 26, Manual Of Patent Practice And Procedure 2008).*

In other words, a compound can be anticipated if executing the directions in a prior specification would inevitably result in that compound, even if the description in the prior specifications does not refer to particular parameters of that compound.

Therefore, based on the above discussions and experimental data Sorafenib tosylate of D1 (which is an admitted prior art document) anticipates compound of claim 1 of the impugned application.

Hence, claim 1 of the impugned application lacks novelty, and ought to be rejected U/S 25(1) (b) alone.

4.2. CLAIM 1 - LACK OF NOVELTY [U/S 25(1) (d)]

Compound of Claim 1 i.e. polymorph-I of Sorafenib tosylate was well known and widely used before the priority date of the impugned application.

4.2.1. D3 and D4 are post published documents; however, it is a well set rule that publication is not necessary to establish prior knowledge or prior use. A matter may be publicly known even if unpublished, if for instance, it is publicly used. Hence, these documents can be used as proof of evidence to establish prior use or prior knowledge of polymorph-I of Sorafenib tosylate.

4.2.2. D3 describes the use of the compound of claim 1 before the priority date of the impugned application. D3 on page **3/49** under the heading, "***Active Substance***" describes that, *"The active substance exhibits polymorphism and it crystallizes in three different modifications (Mod I, Mod II and Mod III)."* Under the heading, "***Manufacture***", it states that, "*The active substance is visually tested for appearance and its identity is confirmed by NIR and HPLC, and the desired modification of Sorafenib tosylate (Mod I) is confirmed by Raman spectroscopy.* Also, it discloses that (on page 4/49), *"The potential for polymorphism was investigated by Raman spectroscopy and found to be unchanged."*

D3, on page 28/49 under the heading, ***"Main studies",*** it relates to the studies 100391 and 11213 performed during Phase II and Phase III trials. Also, it has been acknowledged on page 30/49 under the heading, "*Blinding (masking)*", that, "*The active and placebo tablets were identical in appearance*".

Hence, from the above, it is clear that polymorph-I of Sorafenib tosylate was used in phase II and phase III clinical studies.

4.2.3. From D4, it is evident that the clinical studies 100391 and 11213 for Sorafenib tosylate were started on September 25, 2002 and November 15, 2003 (Page 3, Point 5) respectively, i.e. before the priority date of the impugned application.

It is also known that the product which is ultimately approved is necessarily the one which has been clinically tested, this being a strict requirement of drug approving agencies such as the FDA or EMA.

Hence, it is concluded that, during phase-II and Phase-III studies polymorph-I of Sorafenib tosylate was handed out to the patients prior to the priority date of the impugned application.

In *Bilcare Limited v. Amartara (P) Ltd.* (IA Nos. 10848/2006, 13971/2006 and 11160/2006 in CSOS No.1847/2006), *"Prior public knowledge of the alleged invention which would disqualify the grant of patent can be by word of mouth or by publication through books or other media.* ***If the public once become possessed of an invention, says Hindmarch on Patents, by any means whatsoever,*** *no subsequent patent for it can be granted either to the true or first inventor himself or any other person, for the public cannot be deprived of the right to use the invention... the public already possessing everything that he could give" [Source www.judis.nic.in].*

As shown above, the subject matter of impugned application was in possession of the public before the priority date of the impugned application, hence lacks novelty **U/S 25(1) (d)**, and hence, ought to be rejected on this ground too.

4.3. CLAIMS 5 TO 12 – LACK OF NOVELTY [Section 25(1) (b)]

D1, *inter alia* discloses the subject matter of claims 5 to 18.

A comparison of claims 5 to 18 of the impugned application and D1 is represented hereinbelow in table 2 for ready reference:

Table 2

Claims 5 to 12 of the impugned application	**Disclosure in D1**
5. A compound of the formula (I) in the polymorph I for the treatment of disorders which feature abnormal angiogenesis or hyperpermeability processes, bone marrow diseases, carcinoma or carcinogenic cell growth. *6. A compound of the formula (I) in the polymorph I as claimed in claim 5 for the treatment of leukemia or for the treatment of carcinoma of the lung, of the pancreas, of the thyroid gland, of the kidney or of the intestine.* *7. A pharmaceutical composition comprising the compound of the formula (I) mainly in the polymorph I and no significant fractions of another form of the compound of the formula (I).* *8. The pharmaceutical composition as claimed in claim 7 containing more than 90 percent by weight of the compound of the formula (I) in the polymorph I related to the total*	D1 on page 72, claim 12 and 13; *"12. A method as in claim 1 wherein the disease is mediated by the VEGF- induced signal transduction pathway.* *13. A method as in claim 12 wherein disease mediated by the VEGF-induced signal transduction pathway that is treated is characterized by abnormal angiogenesis or hyperpermiability processes."* D1 on page 75, claim 22, Sorafenib tosylate is used; *Claim 22. "A method of treating diseases mediated by the VEGF-induced signal transduction pathway comprising administering the compound N- (4-chloro-3- (trifluoromethyl) phenyl)-N'- (4- (2- (N-methylcarbamoyl)-4-pyridyloxy) phenyl) urea tosylate."* D1, page 02, third paragraph, lines 15-32 and continuing paragraph on page 03, lines 1-5: *"Angiogenesis is regarded as an absolute prerequisite for growth of tumors beyond about 1-2 mm. Oxygen and nutrients may be supplied to cells in tumor smaller than*

amount of the compound of the formula (I) present in the composition. *9. The pharmaceutical composition as claimed in one of claims 7 or 8 for the treatment of disorders.* *10. The pharmaceutical composition as claimed in one of claims 7 to 9 for the treatment of disorders which feature abnormal angiogenesis or hyperpermeability processes, bone marrow diseases, carcinoma or carcinogenic cell growth.* *11. The pharmaceutical composition as claimed in one of claims 7 to 10 for the treatment of leukemia or for the treatment of carcinoma of the lung, of the pancreas, of the thyroid gland, of the kidney or of the intestine.* *12. The pharmaceutical composition as claimed in one of claims 7 to 11, comprising one or more inert, nontoxic, pharmaceutically suitable excipients.*	*this limit through diffusion. However, every tumor is dependent on angiogenesis for continued growth after it has reached a certain size. .....* *In situ hybridization studies have demonstrated VEGF mRNA is strongly upregulated in a wide variety of human tumors, including lung, thyroid,... breast,.... gastrointestional tract, kidney and bladder),... ovary,.. and cervical ... carcinomas, as well as angiosacroma,.... and several intracranial tumors..... "* **D1 on page 25, line 9; "***The compounds may be administered orally, topically, parenterally, by inhalation or spray or vaginally, sublingually, or rectally in dosage unit formulations..... Compositions intended for oral use may be prepared according to any suitable method known to the art for the manufacture of pharmaceutical compositions. Such compositions may contain one or more agents selected from the group consisting of diluents, sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide palatable preparations. Tablets contain the active*

	ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets."
15. A combination comprising the compound of the formula (I) in the polymorph I and one or more other pharmaceutical agents. *16. The combination as claimed in claim 19 wherein the one or more other pharmaceutical agents are cytotoxic agents, signal transduction inhibitors, anti-cancer agents, or antiemetics.* *17. The pharmaceutical composition as claimed in one of the claims 7 to 12 comprising one or more other pharmaceutical agents.* *18. The pharmaceutical composition as claimed in claim 17 wherein the one or more other pharmaceutical agents are cytotoxic agents, signal transduction inhibitors, anti-cancer agents, or antiemetics.*	**D1 on Page 16, first paragraph:** *"A compound according to the invention can be administered simultaneously with another angiogenesis inhibiting agent to a patient with such a disorder, in the same formulation or, more typically in separate formulations and, often, using different administration routes. Administration can also be sequentially, in any order."* **D1 on page 19, first paragraph:** *"This invention further relates to kits comprising separate doses of the two mentioned chemotherapeutic agents in separate containers. The combinations of angiogenesis inhibiting agents can also be formed in vivo, e. g., in a patient's body.* *These angiogenesis inhibiting agents can be administered in the conventional formulations and regimens in which they are known for use alone."*

Hence, from above it is evident that subject matter of claims 5 to 18 of the impugned application does not specify anything novel or new over D1. Hence, these claims ought to be rejected on this ground.

5. OBVIOUSNESS / LACK OF INVENTIVE STEP GROUNDS

5.1. CLAIM 1 - OBVIOUSNESS / LACK OF INVENTIVE STEP [Section 25(1) (e)]

Claim 1 of the impugned application relates to polymorph-I of Sorafenib tosylate.

Page 02, second paragraph of the impugned application states that "*The inventive polymorph I of the compound of the formula (I) is thermodynamically stable at room temperature and is storage-stable even after processing via suspensions and is therefore particularly suitable for use in pharmaceutical formulations, for example suspensions or creams, but also in other preparations which are prepared via suspended active ingredient, for example in aqueous granulation or wet grinding.*"

Page 15 and continuing paragraph at page 16 of the impugned application explain that "*Preference is given to preparing the compound of the formula (I) in the polymorph I by effecting the compound of the formula (I) in the polymorph II, obtained as described in example 1, in methanol, ethanol, a mixture of both solvents or a mixture of both solvents with water, preferably a 1:1 mixture with water, and shaking or stirring at a temperature of from 50°C up to the reflux temperature of the solvent,..*"

5.1.1. From the above, it is clear that polymorph-I of Sorafenib tosylate was identified by shaking or stirring a known compound of formula (I) in a solvent system at a temperature up to reflux.

In D1, which is an admitted prior art document, Sorafenib tosylate is disclosed: *"A method of treating diseases mediated by the VEGF-induced signal transduction pathway comprising administering the compound N- (4-chloro-3- (trifluoromethyl) phenyl)-N'- (4- (2- (N-methylcarbamoyl)-4-pyridyloxy) phenyl) urea tosylate*" (see D1, page 75, claim 22).

D5, which reflects the common general knowledge in the field of polymorphism, discloses that, *"The thermodynamically stable polymorph needs to be identified. If the compound is enantiotropic, there will be two or more stable polymorphs and transition temperatures as well. These can be identified by simple techniques, for example by stirring or shaking excess solid with solid at different temperatures"* (see D5, page 528, column 02).

Thus in view of D5, a thermodynamically stable polymorph of a known compound can be identified by mere stirring and shaking the said compound at different temperatures, which cannot be considered as inventive.

Also, it is a well set rule that, *"If an invention lies merely in verifying the previous predictions, without substantially adding anything for technical advancement or economic significance in the art, the inventive step is lacking"* (see revised Manual of Patent Office Practice and Procedure Version 01.11 as modified on March 22, 2011, page 79).

Therefore, compound of claim 1 cannot be considered as inventive over D1 in view of the teachings of D5. Hence, claim 1 of the impugned application ought to be rejected also on this ground.

5.2. CLAIMS 2, 3, 4, 13 AND 14 - OBVIOUSNESS AND LACK OF INVENTIVE STEP [Section 25(1) (e)]

The subject matter of Claims 2, 3, 4, 13 and 14 corresponds to the conversion of one polymorph to another thermodynamically stable polymorph. In particular, a process for the conversion of polymorph-II to polymorph-I is claimed.

The impugned application discloses the process for the conversion of polymorph-II to polymorph-I of Sorafenib tosylate; "*Preference is given to preparing the compound of the formula (I) in the polymorph I by effecting the compound of the formula (I) in the polymorph II, obtained as described in example 1, in methanol, ethanol, a mixture of both solvents or a mixture of both solvents with water, preferably a 1:1 mixture with water, and shaking or stirring at a temperature of from 50°C up to the reflux temperature of the solvent, preferably at from 60 to 80°C, in the absence of crystals of a solvate of the compound of the formula (I), for example in the absence of crystals of the methanol solvate or the ethanol solvate of the compound of formula (I), for up to one day. The crystals are cooled to from -30°C to room temperature, preferably from -25 to 10°C, isolated and dried. The compound of the formula (I) is thus obtained in the polymorph I. Most preferably isopropanol, ethylacetate or a mixture thereof is used as solvent.*

"Preference is likewise given to preparing the compound of the formula (I) in the polymorph I by effecting the compound of the formula (I) in the polymorph II, obtained as described in example 1, in methanol, ethanol, a mixture of both solvents or a mixture of both solvents with water, and shaking or stirring at a temperature of from 10°C up to the reflux temperature of the solvent, preferably at room temperature, for up to 1 day. The mixture is subsequently seeded with crystals of the compound of the formula (I) in the polymorph I and stirred or shaken, for example at room temperature, for from 1 hour to 14 days, preferably from 2 hours to 7 days. The crystals are isolated and dried. The compound of the formula (1) is thus obtained in the polymorph I. Most preferably isopropanol, ethylacetate or a mixture thereof is used as solvent" (see page 15 and continuing paragraph at page 16 of the impugned application).

D1 IN VIEW OF D5

5.2.1. From the above, it is clear that, according to the impugned application, polymorph-I of Sorafenib tosylate is obtained by heating the polymorph-II of

Sorafenib tosylate in an inert solvent. Subsequently, it mentions that seeding is preferred for obtaining the polymorph-I of Sorafenib tosylate.

The opponent states that such techniques or methods have been well known in the field of drug development for years. D5, which is a general document in the field of polymorphism in process development, states that *"The thermodynamically stable polymorph needs to be identified. If the compound is enantiotropic, there will be two or more stable polymorphs and transition temperatures as well. These can be identified by simple techniques, for example by stirring or shaking excess solid with solid at different temperatures."* (see on page 528, column 02)

On page 527, column 02 under section *"Crystals and Crystallisation", "The use of seed crystals can be helpful in obtaining a desired polymorph. Manufacturing processes seem to be worked out by trial and error aided by serendipity, and then adhered to rigidity."*

As evident from D5, stirring or shaking and seeding technology was already known in the art prior to the priority date of the impugned application to obtain a thermodynamically stable polymorph. It is submitted that stirring and shaking is just needed to accelerate the process which is also well known to a person skilled in the art in the pharmaceutical drug development.

5.2.2. Therefore, considering the applicant's statement that Sorafenib tosylate of D1 exist in polymorph-II (see page 02, first paragraph of the impugned application); starting from D1 conversion of a known compound to another thermodynamically stable polymorph cannot be considered as inventive in view of D5.

Thus, the process as claimed in the impugned application involves no inventive step of any sort and does not satisfy the criteria of non-obviousness. Therefore, claims 2, 3, 4, 13 and 14 lack inventive step and hence ought to be rejected on this ground alone.

D1 IN VIEW OF D5 AND D6

5.2.3. Claims 2, 3, 13 and 14 relate to the use of certain solvents in the process for the preparation of polymorph-I of Sorafenib tosylate. Example of such solvents are further provided on pages 18 and 19, examples 2.2, 2.3, 2.4, 2.5, 2.6; such solvents include ethanol / water, methanol, ethanol, ethanol / water, isopropanol, acetone, tetrahydrofuran, acetonitrile, ethyl acetate, toluene.

It is submitted that the use of such solvents does not add any inventive merit to the alleged claims. Still to provide an argument, the opponent wishes to draw the attention of Ld. Controller towards the salient features in the field of polymorph chemistry as documented in D6.

5.2.4. D6 provides a review of strategic approaches to remove much of uncertainty by presenting concepts and ideas in the form of flow charts to control the crystal form (polymorph) of drug substance.

It outlines investigations of the formation of polymorphs and the controls needed to ensure the integrity of the drug substance containing either a single or mixture of polymorphs. D6 on page 946, under the heading; *"Formation of Polymorphs – Have Polymorphs Been Discovered? "The first step in the polymorph decision tree is to crystallize the substance from a number of different solvents in order to answer the question: Are polymorphs possible? Solvents should include those used in the final crystallisastion steps and those used during formulation and processing and may also include water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, hexane and mixtures if appropriate. New crystal forms can often be obtained by cooling hot saturated solutions or partly evaporating clear saturated solutions......"*

Incidentally, D6 specifically mentions the use of solvents such as water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, hexane and mixtures, which are the same as employed in the examples of the impugned application.

Hence, in view of the above, a person skilled in the art setting out to convert one polymorph to another polymorph would find the teachings of D5 as highly relevant prior art. Further to this, D6 provides the list of important solvents useful in the formation of polymorphs.

Thus combining the teachings of D5 and D6, a person skilled in the art would reach the impugned process without ingenuity of thought.

5.2.5. The Draft Manual of Patent Practice and Procedure (2008) page 34, states that *"To judge the inventive step, the question to be answered is:"Would a person with ordinary skills in the art have thought of the alleged invention?" If the answer is 'No', then the invention is non- obvious. The question, "Is there an inventive step?" arises only if there is novelty in the invention. If the invention makes available to the person skilled in the art something that he would not reach by normal exercise of his skill; then the inventor has made a contribution to the art which justifies the grant of a patent. This does not mean that an invention has to be technically complex."*

Further under 3.10.7, on the same page it is explained that "*The term "obvious" means that the invention does not go beyond the normal progress of technology but merely follows plainly or logically from the prior art, i.e. something which does not involve the exercise of any skill or ability beyond that to be expected of the person skilled in the art. For this purpose a person skilled in the art is presumed to be an ordinary practitioner aware of what was general common knowledge in the relevant art at the relevant date. In some cases the person skilled in the art may be thought of as a group or team of persons rather than as a single person."*

The revised Manual of Patent Office Practice and procedure Version 01.11 as modified on March 22, 2011 states on page 79 - the general principle of inventive step:

"e) If an invention lies merely in verifying the previous predictions, without substantially adding anything for technical advancement or economic significance in the art, the inventive step is lacking."

"g) *If the invention is predictable based on the available prior art, merely requiring workshop improvement by a person skilled in the art, the inventive step is lacking."*

In view of the given teachings of prior art, it is evident that the impugned process does not involve the exercise of any skill or ability beyond which is expected of the person skilled in the art. Thus based on facts presented above, the subject matter of claims 2, 3, 4, 13 and 14 lacks inventiveness (under The Act) and hence ought to be rejected.

6. 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 [SECTION 25(1) (f)]

6.1. NOT AN INVENTION WITHIN THE MEANING OF SECTION 2(1)(ja)

Section 2 (1)(ja) of the Indian Patent Act defines "Inventive step" and states that "*a feature of an invention that involves technical advance as compared to the existing knowledge or having economic significance or both and that makes the invention not obvious to a person skilled in the art.*"

As according to the definition of inventive step, the invention should involve a technical advancement over the prior art or it should demonstrate economic significance or both and should not be obvious to a person skilled in the art. There is neither any technical advancement nor economic significance in the impugned invention. There is no data in the impugned application that establishes the advantageous effects of the claimed matter over the prior art. Therefore the impugned application is liable to be rejected on this ground too.

6.2. NOT PATENTABLE UNDER SECTION 3(d)

The subject matter of the impugned application 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). It 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 substance, unless they differ significantly in properties with regard to efficacy."

Assuming but not conceding that the polymorph of the impugned application differs from compound inherently disclosed in D1 (on page 75, claim 22), the subject matter of the impugned application is not patentable under Section 3(d) of the Patents Act, 1970. The clarification provided in Section 3(d) states that derivatives of known compounds are deemed to be the same substance unless they differ significantly in efficacy in comparison with the compounds known from the prior art.

6.2.1. The compound or molecular structure of the material or the drug in the impugned application is the same as disclosed in D1. It is only a different physical form of the same compound. Patent Law in India does not recognise polymorphs as new entities and therefore the claims should be rejected.

6.2.2. The Applicant has not shown any difference in efficacy between the known compounds (e.g. compound of D1) and compound of the impugned

application. It is submitted that physico-chemical properties such as solubility or bioavailability and efficacy are not proportional.

From the above, it is evident that the impugned application has been filed to retain monopoly over compounds that have already fallen into the public domain, which is strictly not allowed under the practices of the Indian Patent System.

6.3. NOT PATENTABLE UNDER SECTION 3(e)

Claims 7, 8 and 12 relate to a pharmaceutical composition comprising the compound of claim 1 along with one or more inert, nontoxic, pharmaceutically suitable excipients. Similarly, claims 15-18 relate to a composition comprising the compound of claim 1 in combination with one or more other pharmaceutical agents.

6.3.1. The subject matter of these claims is not an invention within the meaning of Section 3(e) of the Act. It states that, *"a substance obtained by a mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance;"* are not inventions.

The specification of the impugned application fails to provide any data to support the statement that these pharmaceutical compositions or combinations provide any synergistic effect. In the absence of such data, the compositions or combinations in these claims are a mere admixture of the known substances. Hence, such claims ought to be rejected solely on the basis of this ground.

6.4. NOT PATENTABLE UNDER SECTION 3(i)

Claims 5 to 6 and 9 to 11 relate to the use of the compound of claim 1 or a pharmaceutical composition thereof for the treatment of certain disorders or diseases.

6.4.1. The subject matter of these claims is not an invention within the meaning of Section 3(i) of the Act. It states that *"(i) any process for the medicinal, surgical, curative, prophylactic diagnostic, therapeutic 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 or that of their products;"* are not inventions.

Hence, such claims ought to be rejected solely on the basis of this ground.

7. THAT THE COMPLETE SPECIFICATION DOES NOT SUFFICIENTLY AND CLEARLY DESCRIBE THE INVENTION OR THE METHOD BY WHICH IT IS TO BE PERFORMED [SECTION 25(1) (g)]

At the onset, the Petitioner states that the whole objective of a patent grant is that a *quid pro quo* system is followed, whereby the Patent Office grants a patent to an inventor when he discloses the mode and method of performing an invention, along with details pertaining to the invention such as prior art, description etc. The very basis of granting a patent is to provide monopoly right to the inventor/applicant in lieu of the disclosure of the working of the invention to enable an unimaginative individual having sufficient skill in the art, to perform the invention in its best embodiment.

7.1. Claims 2, 3, 4, 13 and 14 relates to a process for the preparation of polymorph-I from polymorph-II. The impugned application provides working example 1 and states that polymorph-II is obtainable by following the experiment given in example 1 or by any general standard methods for the preparation of tosylate salts (see page 02, lines 02-04 of the impugned application).

However, reworking of example 1 does not lead to the formation of polymorph-II. This leads to an unsolvable problem to the skilled person in the field. In fact, general standard methods known in the art do not lead to the formation of polymorph-II of Sorafenib tosylate (as outlined in section 4.1 of this representation).

Thus, it is not possible to obtain the starting material, without which the object of claims 2, 3, 4, 13 and 14 cannot be achieved. Hence, such claims ought to be rejected U/25(1) (g) of the Act.

7.2. 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. Although the applicant claims a long list of diseases which can allegedly be cured by the compound of claim 1, the applicant fails to demonstrate the efficacy of the impugned compound in the treatment of any of these diseases. He does not provide any data demonstrating the in-vivo effect of the compound of claim 1.

7.3. In addition, the impugned application does not provide any comparative data provided demonstrating an improved efficacy or any unexpected advantage of the claimed compound, pharmaceutical composition or method of treatment over the prior art compound.

It is absolutely distressing that the impugned application does not adhere to the aforementioned criteria in addition to those of novelty and inventive merit.

Hence, the impugned application ought to be rejected on the ground of insufficiency in that it does not sufficiently disclose how the claimed invention may be put to practice.

8. RELIEF SOUGHT

The opponent states that he 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.

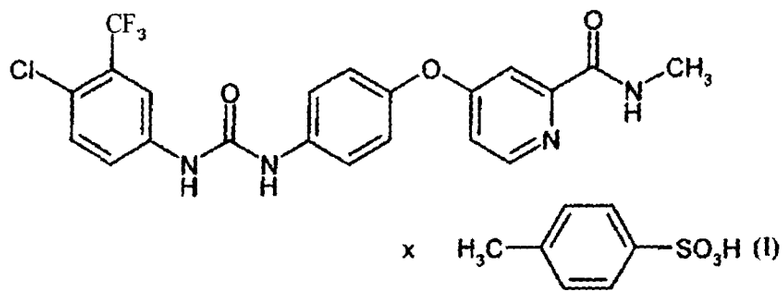
Dated this the 07th day of February 2014.

Dr. Prachi Tiwari (IN/PA-2045)
Of Fresenius Kabi Oncology Ltd.
(Opponent)

Annexure I

What is claimed is:

1. A compound of the formula (I)



in the polymorph I.

2. A preparation of the compound of the formula (I) in the polymorph I, which comprises effecting the compound of the formula (I) in the polymorph II in an inert solvent until quantitative conversion to the polymorph I.

3. The preparation of the compound of the formula (I) in the polymorph I of claim 2, wherein the compound of the formula (I) in the polymorph II is effected in an inert solvent and seeded with crystals of the compound of the formula (I) in the polymorph I.

4. The preparation of the compound of the formula (I) in the polymorph I, wherein the compound of the formula (I) in the polymorph II is heated to from 195 to 222°C at a heating rate of from 10 to 30°C per minute and subsequently cooled to from 10 to 30°C at a cooling rate of from 1 to 4°C per minute.

5. A compound of the formula (I) in the polymorph I for the treatment of disorders which feature abnormal angiogenesis or hyperpermeability processes, bone marrow diseases, carcinoma or carcinogenic cell growth.

6. A compound of the formula (I) in the polymorph I as claimed in claim 5 for the treatment of leukemia or for the treatment of carcinoma of the lung, of the pancreas, of the thyroid gland, of the kidney or of the intestine.

7. A pharmaceutical composition comprising the compound of the formula (I) mainly in the polymorph I and no significant fractions of another form of the compound of the formula (I).

8. The pharmaceutical composition as claimed in claim 7 containing more than 90 percent by weight of the compound of the formula (I) in the polymorph I related to the total amount of the compound of the formula (I) present in the composition.

9. The pharmaceutical composition as claimed in one of claims 7 or 8 for the treatment of disorders.

10 The pharmaceutical composition as claimed in one of claims 7 to 9 for the treatment of disorders which feature abnormal angiogenesis or hyperpermeability processes, bone marrow diseases, carcinoma or carcinogenic cell growth.

11. The pharmaceutical composition as claimed in one of claims 7 to 10 for the treatment of leukemia or for the treatment of carcinoma of the lung, of the pancreas, of the thyroid gland, of the kidney or of the intestine.

12. The pharmaceutical composition as claimed in one of claims 7 to 11, comprising one or more inert, nontoxic, pharmaceutically suitable excipients.

13 A compound of the formula (I) in the polymorph I, obtainable by dissolving or suspending the compound of the formula (I) in the polymorph II in an inert solvent and stirring or shaking it until quantitative conversion to the polymorph I.

14 A compound of the formula (I) as claimed in claim 13, obtainable by dissolving or suspending the compound of the formula (I) in the polymorph II in an inert solvent and seeding it with crystals of the compound of the formula (I) in the polymorph I.

15 A combination comprising the compound of the formula (I) in the polymorph I and one or more other pharmaceutical agents.

16 The combination as claimed in claim 19 wherein the one or more other pharmaceutical agents are cytotoxic agents, signal transduction inhibitors, anti-cancer agents, or antiemetics.

17. The pharmaceutical composition as claimed in one of the claims 7 to 12 comprising one or more other pharmaceutical agents.

18. The pharmaceutical composition as claimed in claim 17 wherein the one or more other pharmaceutical agents are cytotoxic agents, signal transduction inhibitors, anti-cancer agents, or antiemetics.

Dated this 14/3/2007

(DIPAK MUNDRA)
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS.

Exhibit 1

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(54) Title: ARYL UREAS WITH ANGIOGENESIS INHIBITING ACTIVITY

(57) Abstract: This invention relates to methods of using aryl ureas to treat diseases mediated by the VEGF induced signal transduction pathway characterized by abnormal angiogenesis or hyperpermeability processes.

ARYL UREAS WITH ANGIOGENESIS INHIBITING ACTIVITY

Field of the Invention

This invention relates to methods of treating diseases mediated by the VEGF induced signal transduction pathway characterized by abnormal angiogenesis or hyperpermeability processes.

Background of the Invention

Vasculogenesis involves the *de novo* formation of blood vessels from endothelial cell precursors or angioblasts. The first vascular structures in the embryo are formed by vasculogenesis. Angiogenesis involves the development of capillaries from existing blood vessels, and is the principle mechanism by which organs, such as the brain and the kidney are vascularized. While vasculogenesis is restricted to embryonic development, angiogenesis can occur in the adult, for example during pregnancy, the female cycle, or wound healing.

One major regulator of angiogenesis and vasculogenesis in both embryonic development and some angiogenic-dependent diseases is vascular endothelial growth factor (VEGF; also called vascular permeability factor, VPF). VEGF represents a family of isoforms of mitogens existing in homodimeric forms due to alternative RNA splicing. The VEGF isoforms are highly specific for vascular endothelial cells (for reviews, see: Farrara et al. *Endocr. Rev.* **1992**, *13*, 18; Neufield et al. *FASEB J.* **1999**, *13*, 9).

VEGF expression is induced by hypoxia (Shweiki et al. *Nature* **1992**, *359*, 843), as well as by a variety of cytokines and growth factors, such as interleukin-1, interleukin-6, epidermal growth factor and transforming growth factor-α and -β.

To date VEGF and the VEGF family members have been reported to bind to one or more of three transmembrane receptor tyrosine kinases (Mustonen et al. *J. Cell Biol.*, **1995**, *129*, 895), VEGF receptor-1 (also known as flt-1 (fms-like tyrosine kinase-1)), VEGFR-2 (also known as kinase insert domain containing receptor

(KDR); the murine analogue of KDR is known as fetal liver kinase-1 (flk-1)), and VEGFR-3 (also known as flt-4). KDR and flt-1 have been shown to have different signal transduction properties (Waltenberger et al. *J. Biol. Chem.* **1994**, *269*, 26988); Park et al. *Oncogene* **1995**, *10*, 135). Thus, KDR undergoes strong ligand-dependant tyrosine phosphorylation in intact cells, whereas flt-1 displays a weak response. Thus, binding to KDR is a critical requirement for induction of the full spectrum of VEGF-mediated biological responses.

In vivo, VEGF plays a central role in vasculogenesis, and induces angiogenesis and permeabilization of blood vessels. Deregulated VEGF expression contributes to the development of a number of diseases that are characterized by abnormal angiogenesis and/or hyperpermeability processes. Regulation of the VEGF-mediated signal transduction cascade will therefore provide a useful mode for control of abnormal angiogenesis and/or hyperpermeability processes.

Angiogenesis is regarded as an absolute prerequisite for growth of tumors beyond about 1-2 mm. Oxygen and nutrients may be supplied to cells in tumor smaller than this limit through diffusion. However, every tumor is dependent on angiogenesis for continued growth after it has reached a certain size. Tumorigenic cells within hypoxic regions of tumors respond by stimulation of VEGF production, which triggers activation of quiescent endothelial cells to stimulate new blood vessel formation. (Shweiki et al. *Proc. Nat'l. Acad. Sci.*, **1995**, *92*, 768). In addition, VEGF production in tumor regions where there is no angiogenesis may proceed through the ras signal transduction pathway (Grugel et al. *J. Biol. Chem.*, **1995**, *270*, 25915; Rak et al. *Cancer Res.* **1995**, *55*, 4575). In situ hybridization studies have demonstrated VEGF mRNA is strongly upregulated in a wide variety of human tumors, including lung (Mattern et al. *Br. J. Cancer* **1996**, *73*, 931), thyroid (Viglietto et al. *Oncogene* **1995**, *11*, 1569), breast (Brown et al. *Human Pathol.* **1995**, *26*, 86), gastrointestional tract (Brown et al. *Cancer Res.* **1993**, *53*, 4727; Suzuki et al. *Cancer Res.* **1996**, *56*, 3004), kidney and bladder (Brown et al. *Am. J. Pathol.* **1993**, *143I, 1255*), ovary (Olson et al. *Cancer Res.* **1994**, *54*, 1255), and cervical (Guidi et al. *J. Nat'l Cancer Inst.* **1995**, *87*, 12137) carcinomas, as well as angiosacroma (Hashimoto et al. *Lab.*

Invest. **1995**, *73*, 859) and several intracranial tumors (Plate et al. *Nature* **1992**, *359*, 845; Phillips et al. *Int. J. Oncol.* **1993**, *2*, 913; Berkman et al. *J. Clin. Invest.*, **1993**, *91*, 153). Neutralizing monoclonal antibodies to KDR have been shown to be efficacious in blocking tumor angiogenesis (Kim et al. *Nature* **1993**, *362*, 841; Rockwell et al. *Mol. Cell. Differ.* **1995**, *3*, 315).

Over expression of VEGF, for example under conditions of extreme hypoxia, can lead to intraocular angiogenesis, resulting in hyperproliferation of blood vessels, leading eventually to blindness. Such a cascade of events has been observed for a number of retinopathies, including diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity (Aiello et al. *New Engl. J. Med.* **1994**, *331*, 1480; Peer et al. *Lab. Invest.* **1995**, *72*, 638), and age-related macular degeneration (AMD; see, Lopez et al. *Invest. Opthalmol. Vis. Sci.* **1996**, *37*, 855).

In rheumatoid arthritis (RA), the in-growth of vascular pannus may be mediated by production of angiogenic factors. Levels of immunoreactive VEGF are high in the synovial fluid of RA patients, while VEGF levels were low in the synovial fluid of patients with other forms of arthritis of with degenerative joint disease (Koch et al. *J. Immunol.* **1994**, *152*, 4149). The angiogenesis inhibitor AGM-170 has been shown to prevent neovascularization of the joint in the rat collagen arthritis model (Peacock et al. *J. Exper. Med.* **1992**, *175*, 1135).

Increased VEGF expression has also been shown in psoriatic skin, as well as bullous disorders associated with subepidermal blister formation, such as bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis (Brown et al. *J. Invest. Dermatol.* **1995**, *104*, 744).

Because inhibition of KDR leads to inhibition of VEGF-mediated angiogenesis and permeabilization, KDR inhibitors will be useful in treatment of diseases characterized by abnormal angiogenesis and/or hyperpermeability processes, including the above listed diseases

Summary of the Invention

The present invention provides a method for treating diseases in humans or other mammals which are mediated by the VEGF induced signal transduction pathway, including those characterized by abnormal angiogenesis or hyperpermiability processes. These methods comprise administering a compound of formula I below or a salt, prodrug or stereoisomer thereof to a human or other mammal with a disease characterized by abnormal angiogenesis or hyperpermiability processes.

The compounds of formula I, which include all stereoisomeric forms (both isolated and in mixtures) salts thereof and prodrugs thereof are collectively referred to herein as the "compounds of the invention."

Formula I is as follows:

A-NH-C(O)-NH-B I

wherein A is selected from the group consisting of

(i) phenyl, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano, and nitro;

(ii) naphthyl, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano, and nitro;

(iii) 5 and 6 membered monocyclic heteroaryl groups, having 1-3 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano, and nitro; and

(iv) 8 to 10 membered bicyclic heteroaryl group in which the first ring is bonded to the NH of Figure I and contains 1-3 heteroatoms independently selected from the group consisting of O, N, and S, and the second ring is fused to the first ring using 3 to 4 carbon atoms. The bicyclic heteroaryl group is optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano, and nitro.

B is selected from the group consisting of

(i) phenyl, optionally substituted with 1-3 substituents independently selected from the group consisting of –L-M, C_1-C_5 linear or branched alkyl, C_1-C_5 linear or branched haloalkyl, C_1-C_3 alkoxy, hydroxy, amino, C_1-C_3 alkylamino, C_1-C_6 dialkylamino, halogen, cyano, and nitro;

(ii) naphthyl, optionally substituted with 1-3 substituents independently selected from the group consisting of –L-M, C_1-C_5 linear or branched alkyl, C_1-C_5 linear or branched haloalkyl, C_1-C_3 alkoxy, hydroxy, amino, C_1-C_3 alkylamino, C_1-C_6 dialkylamino, halogen, cyano, and nitro;

(iii) 5 and 6 membered monocyclic heteroaryl groups, having 1-3 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of –L-M, C_1-C_5 linear or branched alkyl, C_1-C_5 linear or branched haloalkyl, C_1-C_3 alkoxy, hydroxy, amino, C_1-C_3 alkylamino, C_1-C_6 dialkylamino, halogen, cyano, and nitro; and

(iv) 8 to 10 membered bicyclic heteroaryl groups having 1-6 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of –L-M, C_1-C_5 linear or branched alkyl, C_1-C_5 linear or branched haloalkyl, C_1-C_3 alkoxy, hydroxy, amino, C_1-C_3 alkylamino, C_1-C_6 dialkylamino, halogen, cyano, and nitro.

L is selected from the group consisting of :

(a) $-(CH_2)_m-O-(CH_2)_l-$,

(b) $-(CH_2)_m-(CH_2)_l-$,

(c) $-(CH_2)_m-C(O)-(CH_2)_l-$,

(d) $-(CH_2)_m-NR^3-(CH_2)_l-$,

(e) $-(CH_2)_m-NR^3C(O)-(CH_2)_l-$,

(f) $-(CH_2)_m-S-(CH_2)_l-$,

(g) $-(CH_2)_m-C(O)NR^3-(CH_2)_l-$,

(h) $-(CH_2)_m-CF_2-(CH_2)_l-$,

(i) $-(CH_2)_m-CCl_2-(CH_2)_l-$,

(j) $-(CH_2)_m-CHF-(CH_2)_l-$,

(k) $-(CH_2)_m-CH(OH)-(CH_2)_l-$;

(l) $-(CH_2)_m-C\equiv C-(CH_2)_l-$;

(m) $-(CH_2)_m-C=C-(CH_2)_l-$; and

(n) a single bond, where m and l are 0.;

(o) $-(CH_2)_m-CR^4R^5-(CH_2)_l-$;

The variables m and l are integers independently selected from 0-4.

M is selected from the group consisting of :

(i) phenyl, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro;

(ii) naphthyl, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro;

(iii) 5 and 6 membered monocyclic heteroaryl groups, having 1-3 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$,

$NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro and also oxides (e.g. =O, $-O^-$ or –OH); and

(iv) 8 to 10 membered bicyclic heteroaryl groups, having 1-6 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro and also oxides (e.g. =O, $-O^-$ or –OH).

(v) saturated and partially saturated C_3-C_6 monocyclic carbocyclic moiety optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and, nitro;

(vi) saturated and partially saturated C_8-C_{10} bicyclic carbocyclic moiety, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro;

(vii) saturated and partially saturated 5 and 6 membered monocyclic heterocyclic moiety, having 1-3 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro, and also oxides (e.g. =O, $-O^-$ or –OH); and

(viii) saturated and partially saturated 8 to 10 membered bicyclic heterocyclic moiety, having 1-6 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro, and also oxides (e.g. =O, $-O^-$ or –OH).

Each R^1- R^5 are independently selected from the group consisting of:

(a) hydrogen,

(b) C_1-C_6 alkyl, preferably, C_1-C_5 linear, branched, or cyclic alkyl, wherein said alkyl is optionally substituted with halogen up to per-halo;

(c) phenyl;

(d) 5-6 membered monocyclic heteroaryl having 1-4 heteroatoms selected from the group consisting of O, N and S or 8-10 membered bicyclic heteroaryl having 1-6 heteroatoms selected from the group consisting of O, N and S;

(e) C_1-C_3 alkyl-phenyl wherein said alkyl moiety is optionally substituted with halogen up to per-halo; and

(f) C_1-C_3 alkyl-heteroaryl having 1-4 heteroatoms selected from the group consisting of O, N and S, wherein said heteroaryl group is a 5-6 membered monocyclic heteroaryl or a 8-10 membered bicyclic heteroaryl, and wherein said alkyl moiety is optionally substituted with halogen up to per-halo.

Each R^1 - R^5, when not hydrogen is optionally substituted with 1-3 substituents independently selected from the group consisting of C_1-C_5 linear branched or cyclic alkyl, wherein said alkyl is optionally substituted with halogen up to per-halo, C_1-C_3 alkoxy, wherein said alkoxy is optionally substituted with halogen up to per-halo, hydroxy, amino, C_1-C_3 alkylamino, C_2-C_6 dialkylamino, halogen, cyano, and nitro;

Each variable q is independently selected from 0, 1, or 2.

Suitable substituted and unsubstituted heteroaryl groups for the compounds of this invention, such as those for A, B and M of formula I, include, but are not limited to the following monocyclic heteroaryl groups:

2- and 3-furyl, 2- and 3-thienyl, 2- and 4-triazinyl, 1-, 2- and 3-pyrrolyl, 1-, 2-, 4- and 5-imidazolyl, 1-, 3-, 4- and 5-pyrazolyl, 2-, 4- and 5-oxazolyl, 3-, 4- and 5-isoxazolyl, 2-, 4- and 5-thiazolyl, 3-, 4- and 5-isothiazolyl, 2-, 3- and 4-pyridyl, 2-, 4-, 5- and 6-pyrimidinyl, 1,2,3-triazol-1-, -4- and –5-yl, 1,2,4-triazol-1-, -3- and –5-yl, 1- and 5-tetrazolyl, 1,2,3-oxadiazol-4- and –5-yl, 1,2,4-oxadiazol-3- and –5-yl, 1,3,4-thiadiazol-2- and –5-yl, 1,2,4-oxadiazol-3- and –5-yl, 1,3,4-thiadiazol-2- and –5-yl,

1,3,4-thiadiazol-3- and –5-yl, 1,2,3-thiadiazol-4- and –5-yl, 2-, 3-, 4-, 5- and 6-2H-thiopyranyl, 2-, 3- and 4-4H-thiopyranyl, 3- and 4-pyridazinyl, 2-,3-pyrazinyl,

and bicyclic heteroaryl groups such as :

Benzofuryl, benzothienyl, indolyl, benzimidazolyl, benzopyrazolyl, benzoxazolyl, benzisoxazolyl, benzothiazolyl, benzisothiazolyl, benz-1,3-oxadiazolyl, quinolinyl, isoquinolinyl, quinazolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, dihydrobenzofuryl, pyrazolo[3,4-b]pyrimidinyl, purinyl, benzodiazine, pterindinyl, pyrrolo[2,3-b]pyridinyl, pyrazolo[3,4-b]pyridinyl, oxazo[4,5-b]pyridinyl, imidazo[4,5-b]pyridinyl, cyclopentenopyridine, cyclohexanopyridine, cyclopentanopyrimidine, cyclohexanopyrimidine, cyclcopentanopyrazine, cyclohexanopyrazine, cyclopentanopyridiazine, cyclohexanopyridazine, cyclopentanoimidazole, cyclohexanoimidazole, cyclopentanothiophene and cyclohexanothiophene.

Suitable aryl groups which do not contain heteroatoms include, for example, phenyl and 1- and 2-naphthyl, tetrahydronaphthyl, indanyl, indenyl, benzocyclobutanyl, benzocycloheptanyl and benzocycloheptenyl.

Suitable linear alkyl groups and alkyl portions of groups, e.g., alkoxy, alkylphenyl and alkylheteroaryl etc. throughout include methyl, ethyl, propyl, butyl, pentyl, etc. Suitable branched alkyl groups include all branched isomers such as isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, etc.

Suitable halogen groups include F, Cl, Br, and/or I, from one to per-substitution (i.e. all H atoms on a group replaced by a halogen atom) being possible where an alkyl group is substituted by halogen, mixed substitution of halogen atom types also being possible on a given moiety. Preferred halogens are Cl, Br and F.

The term "up to perhalo substituted linear and branched alkyl," includes alkyl groups having one alkyl hydrogen replaced with halogen, alkyl groups wherein all hydrogens are replaced with halogen, alkyl groups wherein more than one but less than all hydrogens are replaced by halogen and alkyl groups having alkyl hydrogens replaced by halogen and other substituents.

The term "cycloalkyl", as used herein, refers to cyclic structures having 3-8 members in the ring such as cyclopropyl, cyclobutyl and cyclopentyl and cyclic structures having 3-8 members with alkyl substituents such that, for example, "C_3 cycloalkyl" includes methyl substituted cyclopropyl groups.

The term "saturated carbocyclic moieties" defines only the cyclic structure, i.e. cyclopentyl, cyclohexyl, etc. Any alkyl substitution on these cyclic structures is specifically identified.

Saturated monocyclic and bicyclic carbocyclic moieties include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and decahydronapthalene.

Partially saturated monocyclic and bicyclic carbocyclic moieties include cyclopentenyl, cyclohexenyl, cyclohexadienyl and tetrahydronaphthalene.

Saturated monocyclic and bicyclic heterocyclic moieties include tetrahydropyranyl, tetrahydrofuranyl, 1,3-dioxolane, 1,4-dioxanyl, morpholinyl, thiomorpholinyl, piperazinyl, piperidinyl, piperidinonyl, tetrahydropyrimidonyl, pentamethylene sulfide and tetramethylene sulfide.

Partially saturated monocyclic and bicyclic heterocyclic moieties include dihydropyranyl, dihydrofuranyl, dihydrothienyl, dihydropiperidinyl, and dihydropyrimidonyl.

A subclass of compounds of this invention is defined by formula I, wherein A B and M are selected from phenyl, naphthyl, furyl, isoindolinyl, oxadiazolyl, oxazolyl, isooxazolyl, pyrazolyl, pyridinyl, pyrimidinyl, pyrrolyl, quinolinyl, isoquinolinyl, tetrazolyl, thiadiazolyl, thiazolyl and thienyl and are optionally substituted as defined above.

Preferred substituents for B include methyl, trifluoromethyl, ethyl, n-propyl, n-butyl, n-pentyl, isopropyl, isobutyl, sec-butyl, tert-butyl, cyclopropyl, cyclobutyl, cyclopentyl, methoxy, ethoxy, propoxy, Cl, Br and F, cyano, nitro, hydroxy, amino, methylamino, dimethylamino, ethylamino and diethylamino as well as the structure -L-M.

Preferred substituents for A and M include methyl, trifluoromethyl, ethyl, n-propyl, n-butyl, n-pentyl, isopropyl, *tert*-butyl, sec-butyl, isobutyl, cyclopropyl, cyclobutyl, cyclopentyl, methoxy, ethoxy, propoxy, Cl, Br and F, cyano, nitro, hydroxy, amino, methylamino, dimethylamino, ethylamino and diethylamino and further include:

phenyl, pyridinyl, pyrimidinyl, chlorophenyl, dichlorophenyl, bromophenyl, dibromophenyl, chloropyridinyl, bromopyridinyl, dichloropyridinyl, dibromopyridinyl methylphenyl, methylpyridinyl quinolinyl, isoquinolinyl, isoindolinyl, pyrazinyl, pyridazinyl, pyrrolinyl, imidazolinyl, thienyl, furyl, isoxazolinyl, isothiazolinyl, benzopyridinyl, benzothiazolyl,

C_1-C_5 acyl;

$NH(C_1$-C_5 alkyl, phenyl or pyridinyl), such as aminophenyl;

$N(C_1$-C_5 alkyl)(C_1-C_5 alkyl, phenyl or pyridinyl), such as diethylamino and dimethyl amino;

$S(O)_q$ (C_1-C_5 alkyl); such as methanesulfonyl;

$S(O)_q$ H;

SO_2NH_2;

$SO_2NH(C_1$-C_5 alkyl);

$SO_2N(C_1$-C_5 alkyl)(C_1-C_5 alkyl);

$NHSO_2(C_1$-C_5 alkyl); $N(C_1$-C_3 alkyl) $SO_2(C_1$-C_5 alkyl);

$CO(C_1$-C_6 alkyl or phenyl);

C(O)H;

C(O)O(C_1-C_6 alkyl or phenyl), such as $C(O)OCH_3$, $-C(O)OCH_2CH_3$, $-C(O)OCH_2CH_2CH_3$;

C(O)OH;

$C(O)NH_2$ (carbamoyl);

C(O)NH(C_1-C_6 alkyl or phenyl), such as N-methylethyl carbamoyl, N-methyl carbamoyl, N-ethylcarbamoyl, or N-dimethylamino ethyl carbamoyl;

C(O)N(C_1-C_6 alkyl or phenyl)(C_1-C_6 alkyl, phenyl or pyridinyl), such as N-dimethyl carbamoyl;

C(N(C_1-C_5 alkyl)) (C_1-C_5 alkyl);

NHC(O)(C_1-C_6 alkyl or phenyl) and

N(C_1-C_5 alkyl,)C(O)(C_1-C_5 alkyl).

Each of the above substituents is optionally partially or fully halogenated, such as difluoromethyl sulfonyl.

An embodiment of this invention includes the administration of compounds of this invention wherein in formula I, A, B and M follow one of the following of combinations:

A= phenyl, B=phenyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= phenyl, B=pyridinyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=phenyl, B = naphthyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=pyridinyl, B= phenyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=pyridinyl, B= pyridinyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=pyridinyl, B= naphthyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=isoquinolinyl, B= phenyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= isoquinolinyl, B= pyridinyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= isoquinolinyl, B= naphthyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= quinolinyl, B= phenyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= quinolinyl, B= pyridinyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= quinolinyl, B= naphthyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present.

The structure L of formula I is preferably -O-, a single bond, -S-, -NH-, -$N(CH_3)$-, -$NHCH_2$-, - NC_2H_4-, -CH_2-, -C(O)-, -CH(OH)-, -$NHC(O)N(CH_3)CH_2$-, -$N(CH_3)C(O)N(CH_3)CH_2$-, -$CH_2C(O)N(CH_3)$-, -$C(O)N(CH_3)CH_2$-, -NHC(O)-, -$N(CH_3)C(O)$-, -$C(O)N(CH_3)$-, -C(O)NH-, -CH_2O-, -CH_2S-, -$CH_2N(CH_3)$-, -OCH_2-, -CHF-, -CF_2-,-CCl_2-, -S-CH_2- , and –$N(CH_3)CH_2$- .

One of ordinary skill in the art will recognize that some of the compounds of Formula (I) can exist in different geometrical isomeric forms. A number of the compounds of Formula I possess asymmetric carbons and can therefore exist in racemic and optically active forms as well as in the form of racemic or non-racemic mixtures thereof, and in the form of diastereomers and diastereomeric mixtures. All of these compounds, including *cis* isomers, *trans* isomers, diastereomic mixtures, racemates, non-racemic mixtures of enantiomers, substantially pure, and pure enantiomers, are considered to be within the scope of the present invention and are collectively referred to when reference is made to compounds of this invention.

Methods of separation of enantiomeric and diastereomeric mixtures are well known to one skilled in the art. The optical isomers can be obtained by resolution of the racemic mixtures according to conventional processes, for example, by the formation of diastereoisomeric salts using an optically active acid or base. Examples of appropriate acids are tartaric, diacetyltartaric, dibenzoyltartaric, ditoluoyltartaric and camphorsulfonic acid. Mixtures of diastereoisomers can be separated into their individual diastereomers on the basis of their physical chemical differences by methods known to those skilled in the art, for example, by chromatography or

fractional crystallization. The optically active bases or acids are liberated from the separated diastereomeric salts.

Another process for separation of optical isomers involves the use of a chiral chromatography column (e.g., chiral HPLC columns) optimally chosen to maximize the separation of the enantiomers. Suitable chiral HPLC columns are manufactured by Diacel, e.g., Chiracel OD and Chiracel OJ. The optically active compounds of Formula (I) can likewise be obtained by utilizing optically active starting materials.

The present invention encompasses any isolated racemic or optically active form of compounds described in Formula I which possess angiogenesis inhibitory activity. The term stereoisomer is understood to encompass diastereoisomers, enantiomers, geometric isomers, etc. Herein, substantially pure enantiomers is intended to mean that no more than 5% w/w of the corresponding opposite enantiomer is present.

Pharmaceutically acceptable salts of these compounds as well as commonly used prodrugs of these compounds are also within the scope of the invention.

Salts are especially the pharmaceutically acceptable salts of compounds of formula (I) or such as, for example, organic or inorganic acid addition salts of compounds of formula (I). Suitable inorganic acids include but are not limited to halogen acids (such as hydrochloric acid and hydrobromic acid), sulfuric acid, or phosphoric acid. Suitable organic acids include but are not limited to carboxylic, phosphonic, sulfonic, or sulfamic acids, with examples including acetic acid, propionic acid, octanoic acid, decanoic acid, trifluoroacetic acid, dodecanoic acid, glycolic acid, lactic acid, 2- or 3-hydroxybutyric acid, γ-aminobutyric acid (GABA), gluconic acid, glucosemonocarboxylic acid, benzoic acid, salicylic acid, phenylacetic acid and mandelic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azeiaic acid, malic acid, tartaric acid, citric acid, glucaric acid, galactaric acid, amino acids (such as glutamic acid, aspartic acid, N-methylglycine, acetytaminoacetic acid, N-acetylasparagine or N-acetylcysteine), pyruvic acid, acetoacetic acid,

methanesulfonic acid, tri-fluoromethane sulfonic acid, 4-toluene sulfonic acid, benzenesulfonic acid, 1-naphthalenesulfonic acid, 2-naphthalenesulfonic acid, phosphoserine, and 2- or 3-glycerophosphoric acid.

In addition, pharmaceutically acceptable salts include acid salts of inorganic bases, such as salts containing alkaline cations (e.g., Li^+ Na^+ or K^+), alkaline earth cations (e.g., Mg^{+2}, Ca^{+2} or Ba^{+2}), the ammonium cation, as well as acid salts of organic bases, including aliphatic and aromatic substituted ammonium, and quaternary ammonium cations, such as those arising from protonation or peralkylation of triethylamine, *N,N*-diethylamine, *N,N*-dicyclohexylamine, lysine, pyridine, *N,N*-dimethylaminopyridine (DMAP), 1,4-diazabiclo[2.2.2]octane (DABCO), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

The formation of prodrugs is well known in the art in order to enhance the properties of the parent compound; such properties include solubility, absorption, biostability and release time (see *"Pharmaceutical Dosage Form and Drug Delivery Systems"* (Sixth Edition), edited by Ansel et al., published by Williams & Wilkins, pages 27-29, (1995) which is hereby incorporated by reference). Commonly used prodrugs of the disclosed oxazolyl-phenyl-2,4-diamino-pyrimidine compounds are designed to take advantage of the major drug biotransformation reactions and are also to be considered within the scope of the invention. Major drug biotransformation reactions include N-dealkylation, O-dealkylation, aliphatic hydroxylation, aromatic hydroxylation, N-oxidation, S-oxidation, deamination, hydrolysis reactions, glucuronidation, sulfation and acetylation (see *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Ninth Edition), editor Molinoff et al., pub. by McGraw-Hill, pages 11-13, (1996), which is hereby incorporated by reference).

The invention also relates to methods for treating and preventing diseases, for example, angiogenesis disorders in mammals by administering a compound of this invention or a pharmaceutical composition comprising one or more compounds of this invention.

A compound according to the invention can be administered simultaneously with another angiogenesis inhibiting agent to a patient with such a disorder, in the same formulation or, more typically in separate formulations and, often, using different administration routes. Administration can also be sequentially, in any order.

A compound according to the invention can be administered in tandem with another angiogenesis inhibiting agent, wherein a compound according to the invention can be administered to a patient once or more per day for up to 28 consecutive days with the concurrent or intermittent administration of another angiogenesis inhibiting agent over the same total time period.

A compound according to the invention can be administered to a patient at an oral, intravenous, intramuscular, subcutaneous, or parenteral dosage which can range from about 0.1 to about 200 mg/kg of total body weight and the additional angiogenesis inhibiting agent can be administered to a patient at an intravenous, intramuscular, subcutaneous, or parenteral dosage which can range from about 0.1 mg to 200 mg/kg of patient body weight.

An embodiment of the present invention is a method for treating diseases in humans and/or other mammals which are mediated by the VEGF induced signal transduction pathway which comprises administering a compound of this invention to a human or other mammal.

Another embodiment of this invention is a method for treating diseases in humans and/or other mammals which are characterized by abnormal angiogenesis or hyperpermiability processes with a compound of this invention to a human or other mammal.

Another embodiment of this invention is a method for treating diseases in humans and/or other mammals which are characterized by abnormal angiogenesis or hyperpermiability processes, which are not raf-mediated, which comprises administering a compound of this invention to a human or other mammal.

Another embodiment of this invention is a method for treating diseases in humans and/or other mammals which are characterized by abnormal angiogenesis or hyperpermiability processes, which are not raf mediated or p38-mediated, which comprises administering a compound of this invention to a human or other mammal.

Another embodiment of this invention is a method for treating diseases in humans and/or other mammals which are characterized by abnormal angiogenesis or hyperpermiability processes, which are raf-mediated and/or p38 mediated, which comprises administering a compound of this invention to a human or other mammal.

Another embodiment of this invention is a method for treating one or more of the following conditions in humans and/or other mammals: tumor growth, retinopathy, including diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity and age related macular degeneration; rheumatoid arthritis, psoriasis, or bullous disorder associated with subepidermal blister formation, including bullous pemphigoid, erythema multiforme, or dermatitis herpetiformis, which comprises administering a compound of this invention to a human or other mammal with one or more of these conditions.

Another embodiment of this invention is a method for treating one or more of the following conditions in humans and/or other mammals: tumor growth, retinopathy, diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, bullous disorder associated with subepidermal blister formation, bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis in combination with another condition selected from the group consisting of:

rheumatic fever, bone resorption, postmenopausal osteoporosis, sepsis, gram negative sepsis, septic shock, endotoxic shock, toxic shock syndrome, systemic inflammatory response syndrome, inflammatory bowel disease (Crohn's disease and ulcerative colitis), Jarisch-Herxheimer reaction, asthma, adult respiratory distress syndrome, acute pulmonary fibrotic disease, pulmonary sarcoidosis, allergic respiratory disease, silicosis, coal worker's pneumoconiosis, alveolar injury, hepatic failure, liver disease during acute inflammation, severe alcoholic hepatitis, malaria (Plasmodium falciparum malaria and cerebral malaria), non-insulin-dependent

diabetes mellitus (NIDDM), congestive heart failure, damage following heart disease, atherosclerosis, Alzheimer's disease, acute encephalitis, brain injury, multiple sclerosis (demyelation and oligiodendrocyte loss in multiple sclerosis), advanced cancer, lymphoid malignancy, pancreatitis, impaired wound healing in infection, inflammation and cancer, myelodysplastic syndromes, systemic lupus erythematosus, biliary cirrhosis, bowel necrosis, radiation injury/ toxicity following administration of monoclonal antibodies, host-versus-graft reaction (ischemia reperfusion injury and allograft rejections of kidney, liver, heart, and skin), lung allograft rejection (obliterative bronchitis) or complications due to total hip replacement. This method comprises administering a compound of this invention to a human or other mammal with one of the above combinations of conditions.

Another embodiment of this invention is a method for treating one or more of the following conditions in humans and/or other mammals:

tumor growth, retinopathy, diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, bullous disorder associated with subepidermal blister formation, bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis,

in combination with an infectious disease selected from the group consisting of:

tuberculosis, Helicobacter pylori infection during peptic ulcer disease, Chaga's disease resulting from Trypanosoma cruzi infection, effects of Shiga-like toxin resulting from E. coli infection, effects of enterotoxin A resulting from Staphylococcus infection, meningococcal infection, and infections from Borrelia burgdorferi, Treponema pallidum, cytomegalovirus, influenza virus, Theiler's encephalomyelitis virus, and the human immunodeficiency virus (HIV). These methods comprise administering a compound of this invention to a human or other mammal with a combination of one of the above infectious diseases and one of the above diseases characterized by abnormal angiogenesis or hyperpermiability processes.

This invention further relates to kits comprising separate doses of the two mentioned chemotherapeutic agents in separate containers. The combinations of angiogenesis inhibiting agents can also be formed in vivo, e.g., in a patient's body.

These angiogenesis inhibiting agents can be administered in the conventional formulations and regimens in which they are known for use alone.

Conditions within a human or other mammal which can be treated by administering a compound of this invention are those characterized by abnormal angiogenesis or hyperpermiability processes. Conditions to be treated include tumor growth, retinopathy, including diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity and age related macular degeneration; rheumatoid arthritis, psoriasis, or a bullous disorder associated with subepidermal blister formation, including bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis.

Methods of interest include the treatment of combinations of the conditions above (tumor growth, retinopathy, diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, bullous disorder associated with subepidermal blister formation, bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis) and another condition selected from the group consisting of:

rheumatic fever, bone resorption, postmenopausal osteoperosis, sepsis, gram negative sepsis, septic shock, endotoxic shock, toxic shock syndrome, systemic inflammatory response syndrome, inflammatory bowel disease (Crohn's disease and ulcerative colitis), Jarisch-Herxheimer reaction, asthma, adult respiratory distress syndrome, acute pulmonary fibrotic disease, pulmonary sarcoidosis, allergic respiratory disease, silicosis, coal worker's pneumoconiosis, alveolar injury, hepatic failure, liver disease during acute inflammation, severe alcoholic hepatitis, malaria (Plasmodium falciparum malaria and cerebral malaria), non-insulin-dependent diabetes mellitus (NIDDM), congestive heart failure, damage following heart disease, atherosclerosis, Alzheimer's disease, acute encephalitis, brain injury, multiple sclerosis (demyelation and oligiodendrocyte loss in multiple sclerosis), advanced

cancer, lymphoid malignancy, pancreatitis, impaired wound healing in infection, inflammation and cancer, myelodysplastic syndromes, systemic lupus erythematosus, biliary cirrhosis, bowel necrosis, radiation injury/ toxicity following administration of monoclonal antibodies, host-versus-graft reaction (ischemia reperfusion injury and allograft rejections of kidney, liver, heart, and skin), lung allograft rejection (obliterative bronchitis) or complications due to total hip replacement.

Also provided is a method for treating combinations of the conditions above (tumor growth, retinopathy, diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, bullous disorder associated with subepidermal blister formation, bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis) and an infectious disease selected from the group consisting of:

tuberculosis, Helicobacter pylori infection during peptic ulcer disease, Chaga's disease resulting from Trypanosoma cruzi infection, effects of Shiga-like toxin resulting from E. coli infection, effects of enterotoxin A resulting from Staphylococcus infection, meningococcal infection, and infections from Borrelia burgdorferi, Treponema pallidum, cytomegalovirus, influenza virus, Theiler's encephalomyelitis virus, and the human immunodeficiency virus (HIV).

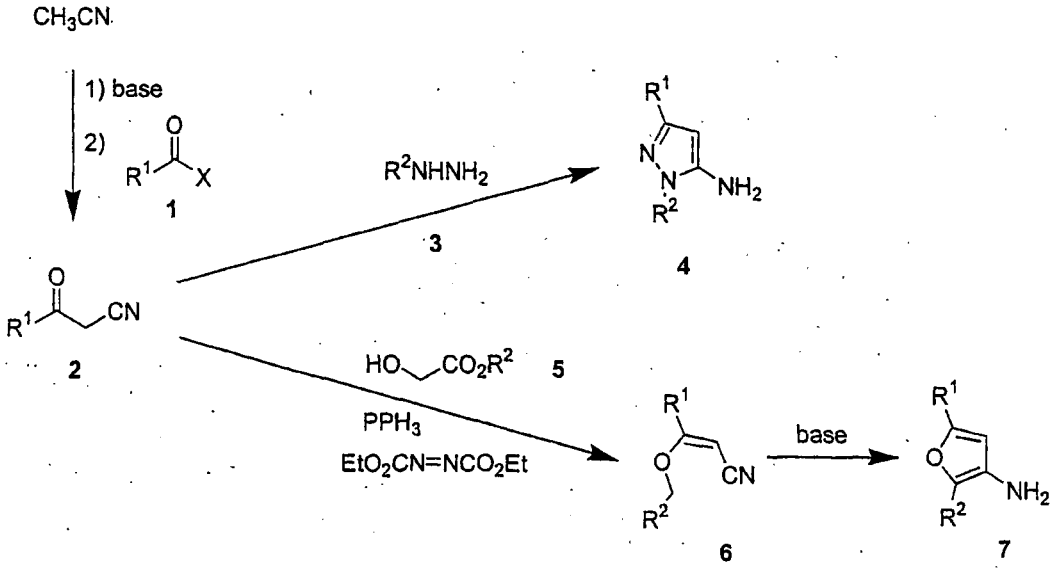
The compounds of this invention can be made according to conventional chemical methods, and/or as disclosed below, from starting materials which are either commercially available or producible according to routine, conventional chemical methods. General methods for the preparation of the compounds are given below, and the preparation of a suitable compound is specifically illustrated in the Examples.

Ureas of formula (I) can be prepared by a variety of simple methods known in the art. General approaches for the formation of those compounds can be found in *"Advanced Organic Chemistry"*, by J. March, *John Wiley and Sons,* 1985 and in *"Comprehensive Organic Transformations"*, by R. C. Larock, *VCH Publishers,* 1989), which are hereby incorporated by reference. Nevertheless, the following

general preparative methods are presented to aid one of skill in the art in synthesizing these compounds, with more detailed examples being presented in the experimental section describing the working examples.

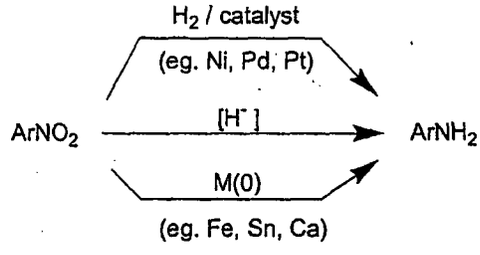
General Preparative Methods

Heterocyclic amines may be synthesized utilizing known methodology (Katritzky, et al. *Comprehensive Heterocyclic Chemistry*; Permagon Press: Oxford, UK (1984). March. *Advanced Organic Chemistry*, 3rd Ed.; John Wiley: New York (1985)). For example, as shown in Scheme I, 5-aminopyrazoles substituted at the *N*-1 position with either aryl or heteroaryl moieties may be synthesized by the reaction of an α-cyanoketone (**2**) with the appropriate aryl- or heteroaryl hydrazine (**3**, R^2=aryl or heteroaryl). Cyanoketone **2**, in turn, is available from the reaction of acetamidate ion with an appropriate acyl derivative, such as an ester, an acid halide, or an acid anhydride. In cases where the R^2 moiety offers suitable anion stabilization, 2-aryl- and 2-heteroarylfurans may be synthesized from a Mitsunobu reaction of cyanoketone **2** with alcohol **5**, followed by base catalyzed cyclization of enol ether **6** to give furylamine **7**.



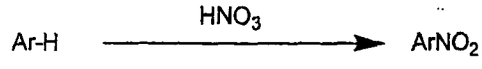
Scheme I. Selected General Methods for Heterocyclic Amine Synthesis

Substituted anilines may be generated using standard methods (March. *Advanced Organic Chemistry*, 3rd Ed.; John Wiley: New York (1985). Larock. *Comprehensive Organic Transformations*; VCH Publishers: New York (1989)). As shown in Scheme II, aryl amines are commonly synthesized by reduction of nitroaryls using a metal catalyst, such as Ni, Pd, or Pt, and H_2 or a hydride transfer agent, such as formate, cyclohexadiene, or a borohydride (Rylander. *Hydrogenation Methods*; Academic Press: London, UK (1985)). Nitroaryls may also be directly reduced using a strong hydride source, such as $LiAlH_4$ (Seyden-Penne. *Reductions by the Alumino- and Borohydrides in Organic Synthesis*; VCH Publishers: New York (1991)), or using a zero valent metal, such as Fe, Sn or Ca, often in acidic media. Many methods exist for the synthesis of nitroaryls (March. *Advanced Organic Chemistry*, 3rd Ed.; John Wiley: New York (1985). Larock. *Comprehensive Organic Transformations*; VCH Publishers: New York (1989)).

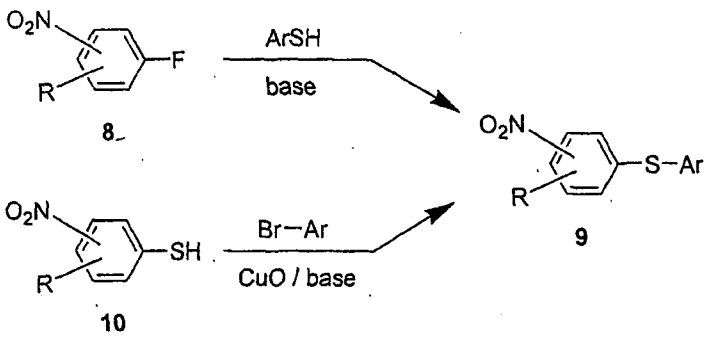


Scheme II Reduction of Nitroaryls to Aryl Amines

Nitroaryls are commonly formed by electrophilic aromatic nitration using HNO_3, or an alternative NO_2^+ source. Nitro aryls may be further elaborated prior to reduction. Thus, nitroaryls substituted with

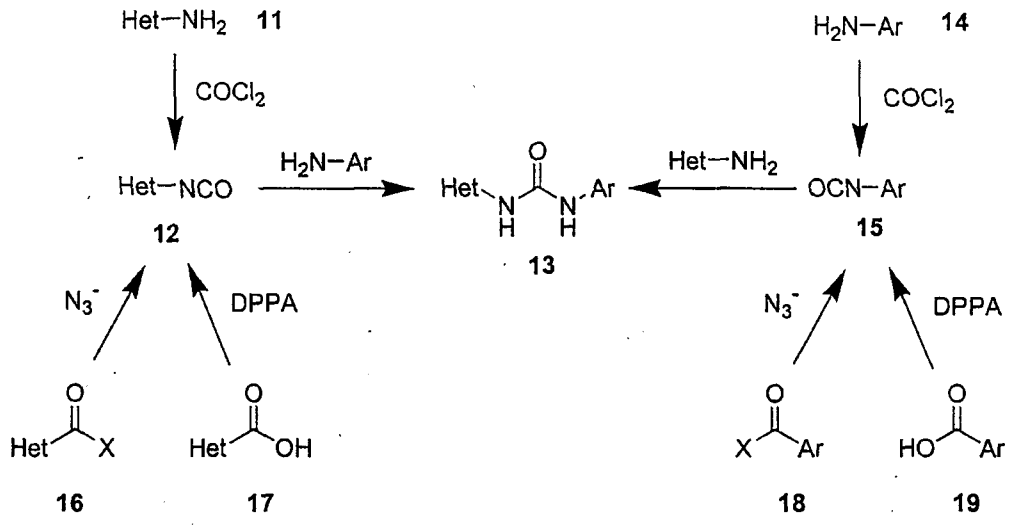


potential leaving groups (eg. F, Cl, Br, etc.) may undergo substitution reactions on treatment with nucleophiles, such as thiolate (exemplified in Scheme III) or phenoxide. Nitroaryls may also undergo Ullman-type coupling reactions (Scheme III).



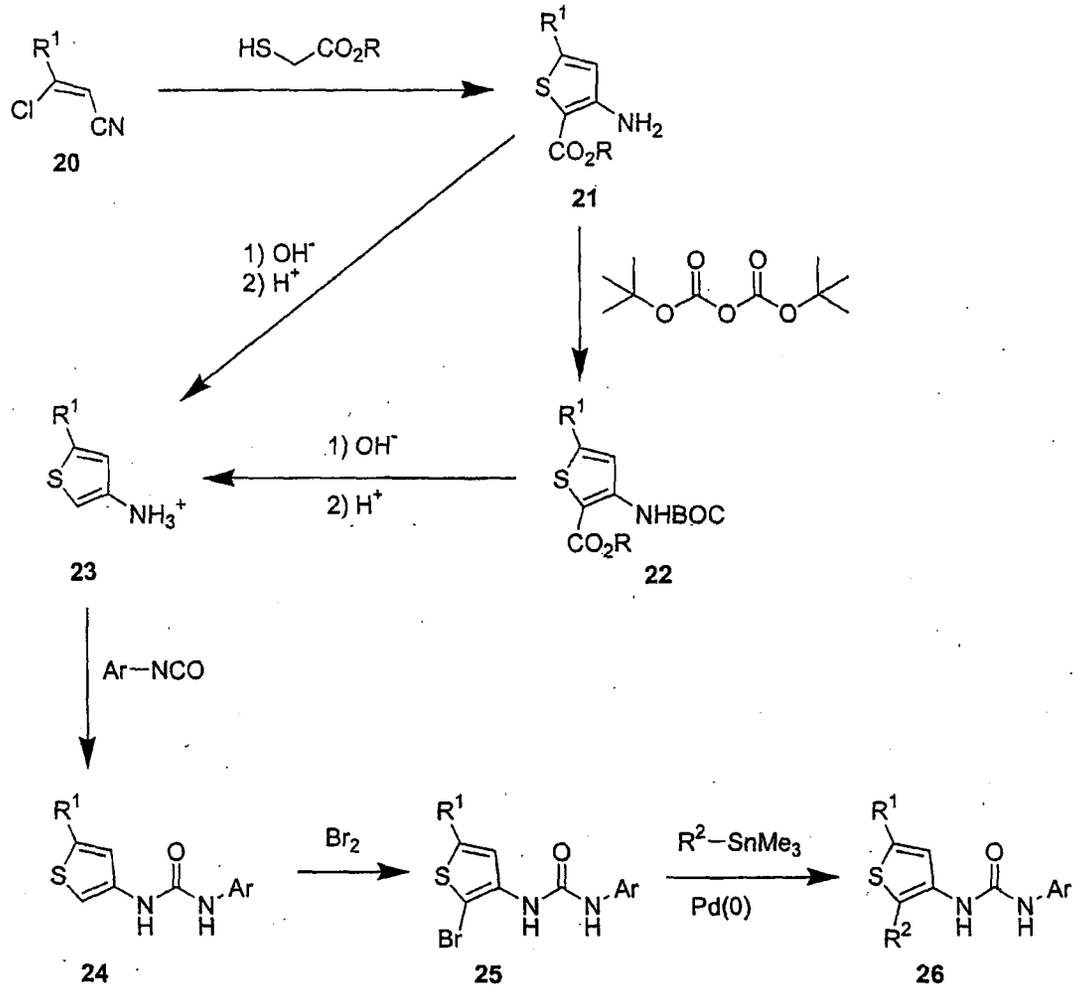
Scheme III Selected Nucleophilic Aromatic Substitution using Nitroaryls

As shown in Scheme IV, urea formation may involve reaction of a heteroaryl isocyanate (**12**) with an aryl amine (**11**). The heteroaryl isocyanate may be synthesized from a heteroaryl amine by treatment with phosgene or a phosgene equivalent, such as trichloromethyl chloroformate (diphosgene), bis(trichloromethyl) carbonate (triphosgene), or *N,N'*-carbonyldiimidazole (CDI). The isocyanate may also be derived from a heterocyclic carboxylic acid derivative, such as an ester, an acid halide or an anhydride by a Curtius-type rearrangement. Thus, reaction of acid derivative **16** with an azide source, followed by rearrangement affords the isocyanate. The corresponding carboxylic acid (**17**) may also be subjected to Curtius-type rearrangements using diphenylphosphoryl azide (DPPA) or a similar reagent. A urea may also be generated from the reaction of an aryl isocyanate (**15**) with a heterocyclic amine.



Scheme IV Selected Methods of Urea Formation (Het = heterocycle)

Finally, ureas may be further manipulated using methods familiar to those skilled in the art. For example, 2-aryl and 2-heteroarylthienyl ureas are available from the corresponding 2-halothienyl urea through transition metal mediated cross coupling reactions (exemplified with 2-bromothiophene **25**, Scheme V). Thus, reaction of nitrile **20** with an α-thioacetate ester gives 5-substituted-3-amino-2-thiophenecarboxylate **21** (Ishizaki et al. JP 6025221). Decarboxylation of ester **21** may be achieved by protection of the amine, for example as the *tert*-butoxy (BOC) carbamate (**22**), followed by saponification and treatment with acid. When BOC protection is used, decarboxylation may be accompanied by deprotection giving the substituted 3-thiopheneammonium salt **23**. Alternatively, ammonium salt **23** may be directly generated through saponification of ester **21** followed by treatment with acid. Following urea formation as described above, bromination affords penultimate halothiophene **25**. Palladium mediated cross coupling of thiophene **25** with an appropriate tributyl- or trimethyltin (R^2= aryl or heteroaryl) then affords the desired 2-aryl- or 2-heteroarylthienyl urea.



Scheme V Synthesis and Interconversion of Ureas

Finally, ureas may be further manipulated using methods familiar to those skilled in the art.

The compounds may be administered orally, topically, parenterally, by inhalation or spray or vaginally, sublingually, or rectally in dosage unit formulations.

The term 'administration by injection' includes intravenous, intramuscular, subcutaneous and parenteral injections, as well as use of infusion techniques. Dermal administration may include topical application or transdermal administration. One or

more compounds may be present in association with one or more non-toxic pharmaceutically acceptable carriers and if desired other active ingredients.

Compositions intended for oral use may be prepared according to any suitable method known to the art for the manufacture of pharmaceutical compositions. Such compositions may contain one or more agents selected from the group consisting of diluents, sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, microcrystalline cellulose, carboxymethyl cellulose, hydroxypropylmethylcellulose or alginic acid; and binding agents, for example magnesium stearate, stearic acid or talc and lubricants/surfactants such as sodium lauryl sulfate. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. These compounds may also be prepared in solid, rapidly released form.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally occurring phosphatide, for

example, lecithin, or condensation products or an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl *p*-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, sweetening, flavoring and coloring agents, may also be present.

The compounds may also be in the form of non-aqueous liquid formulations, e.g., oily suspensions which may be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or peanut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide palatable oral preparations. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and

esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents.

The compounds may also be administered in the form of suppositories for rectal or vaginal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal or vaginal temperature and will therefore melt in the rectum or vagina to release the drug. Such materials include cocoa butter and polyethylene glycols.

Compounds of the invention may also be administrated transdermally using methods known to those skilled in the art (see, for example: Chien; "Transdermal Controlled Systemic Medications"; Marcel Dekker, Inc.; 1987. Lipp et al. WO94/04157 3Mar94). For example, a solution or suspension of a compound of Formula I in a suitable volatile solvent optionally containing penetration enhancing agents can be combined with additional additives known to those skilled in the art, such as matrix materials and bacteriocides. After sterilization, the resulting mixture can be formulated following known procedures into dosage forms. In addition, on treatment with emulsifying agents and water, a solution or suspension of a compound of Formula I may be formulated into a lotion or salve.

Suitable solvents for processing transdermal delivery systems are known to those skilled in the art, and include lower alcohols such as ethanol or isopropyl alcohol, lower ketones such as acetone, lower carboxylic acid esters such as ethyl acetate, polar ethers such as tetrahydrofuran, lower hydrocarbons such as hexane, cyclohexane or benzene, or halogenated hydrocarbons such as dichloromethane,

chloroform, trichlorotrifluoroethane, or trichlorofluoroethane. Suitable solvents may also include mixtures of one or more materials selected from lower alcohols, lower ketones, lower carboxylic acid esters, polar ethers, lower hydrocarbons, halogenated hydrocarbons.

Suitable penetration enhancing materials for transdermal delivery system are known to those skilled in the art, and include, for example, monohydroxy or polyhydroxy alcohols such as ethanol, propylene glycol or benzyl alcohol, saturated or unsaturated C_8–C_{18} fatty alcohols such as lauryl alcohol or cetyl alcohol, saturated or unsaturated C_8–C_{18} fatty acids such as stearic acid, saturated or unsaturated fatty esters with up to 24 carbons such as methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl isobutyl tertbutyl or monoglycerin esters of acetic acid, capronic acid, lauric acid, myristinic acid, stearic acid, or palmitic acid, or diesters of saturated or unsaturated dicarboxylic acids with a total of up to 24 carbons such as diisopropyl adipate, diisobutyl adipate, diisopropyl sebacate, diisopropyl maleate, or diisopropyl fumarate. Additional penetration enhancing materials include phosphatidyl derivatives such as lecithin or cephalin, terpenes, amides, ketones, ureas and their derivatives, and ethers such as dimethyl isosorbid and diethyleneglycol monoethyl ether. Suitable penetration enhancing formulations may also include mixtures of one or more materials selected from monohydroxy or polyhydroxy alcohols, saturated or unsaturated C_8–C_{18} fatty alcohols, saturated or unsaturated C_8–C_{18} fatty acids, saturated or unsaturated fatty esters with up to 24 carbons, diesters of saturated or unsaturated discarboxylic acids with a total of up to 24 carbons, phosphatidyl derivatives, terpenes, amides, ketones, ureas and their derivatives, and ethers.

Suitable binding materials for transdermal delivery systems are known to those skilled in the art and include polyacrylates, silicones, polyurethanes, block polymers, styrenebutadiene coploymers, and natural and synthetic rubbers. Cellulose ethers, derivatized polyethylenes, and silicates may also be used as matrix components. Additional additives, such as viscous resins or oils may be added to increase the viscosity of the matrix.

For all regimens of use disclosed herein for compounds of Formula I, the daily oral dosage regimen will preferably be from 0.01 to 200 mg/Kg of total body weight. The daily dosage for administration by injection, including intravenous, intramuscular, subcutaneous and parenteral injections, and use of infusion techniques will preferably be from 0.01 to 200 mg/Kg of total body weight. The daily vaginal dosage regimen will preferably be from 0.01 to 200 mg/Kg of total body weight. The daily rectal dosage regime will preferably be from 0.01 to 200 mg/Kg of total body weight. The daily topical dosage regime will preferably be from 0.1 to 200 mg administered between one to four times daily. The transdermal concentration will preferably be that required to maintain a daily dose of from 0.01 to 200 mg/Kg. The daily inhalation dosage regime will preferably be from 0.01 to 10 mg/Kg of total body weight. These dosages regimes can be achieved with multiple dosages within a single day or extended dosages, such as those given on a weekly or monthly basis.

It will be appreciated by those skilled in the art that the particular method of administration will depend on a variety of factors, all of which are considered routinely when administering therapeutics. It will also be appreciated by one skilled in the art that the specific dose level for any given patient will depend upon a variety of factors, including, the activity of the specific compound employed, the age of the patient, the body weight of the patient, the general health of the patient, the gender of the patient, the diet of the patient, time of administration, route of administration, rate of excretion, drug combinations, and the severity of the condition undergoing therapy.

It will be further appreciated by one skilled in the art that the optimal course of treatment, i.e., the mode of treatment and the daily number of doses of a compound of this invention given for a defined number of days, can be ascertained by those skilled in the art using conventional treatment tests.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of

administration, route of administration, and rate of excretion, drug combination and the severity of the condition undergoing therapy.

Specific preparations of the compounds of this invention are already described in the patent literature, and can be adapted to the compounds of the present invention. For example, Miller S. et al, "Inhibition of p38 Kinase using Symmetrical and Unsymmetrical Diphenyl Ureas" *PCT Int. Appl.* WO 99 32463, Miller, S et al. "Inhibition of raf Kinase using Symmetrical and Unsymmetrical Substituted Diphenyl Ureas" *PCT Int. Appl.*, WO 99 32436, Dumas, J. et al., "Inhibition of p38 Kinase Activity using Substituted Heterocyclic Ureas" *PCT Int. Appl.*, WO 99 32111, Dumas, J. et al., "Inhibition of RAF Kinase Activity using Substituted Heterocyclic Ureas" *PCT Int. Appl.*, WO 99 32106, Dumas, J. et al., "Method for the Treatment of Neoplasm by Inhibition of raf Kinase using N-Heteroaryl-N'-(hetero)arylureas" *PCT Int. Appl.*, WO 99 32106, Dumas, J. et al., "Inhibition of p38 Kinase Activity using Aryl- and Heteroaryl- Substituted Heterocyclic Ureas" *PCT Int. Appl.*, WO 99 32110, Dumas, J., et al., "Inhibition of raf Kinase using Aryl- and Heteroaryl-Substituted Heterocyclic Ureas" *PCT Int. Appl.*, WO 99 32455, Riedl, B., et al., "O-Carboxy Aryl Substituted Diphenyl Ureas as raf Kinase Inhibitors" *PCT Int. Appl.*, WO 00 42012, Riedl, B., et al., "O-Carboxy Aryl Substituted Diphenyl Ureas as p38 Kinase Inhibitors" *PCT Int. Appl.*, WO 00 41698.

Methods for preparaing the compounds of this invention are also described in the following U.S. applications, some of which correspond to the PCT applications listed above.

08/863,022, filed May 23, 1997;

08/996,344, filed December 22, 1997;

08/996,343, filed December 22, 1997;

08/996,181, filed December 22, 1997;

08/995,749, filed December 22, 1997;

08/995,750, filed December 22, 1997;

08/995,751, filed December 22, 1997;

09/083,399, filed May 22, 1998;

09/425,228, filed October 22, 1999;

09/777,920, filed February 7, 2001.

09/722,418 filed November 28, 2000

09/838,285, filed April 20, 2001;

09/838,286, filed April 20, 2001;

09/458,548, filed January 12, 2001;

09/948,915, filed September 10, 2001, and

Serial Number (attorney docket number: Bayer 34 V1), filed December 3, 2001.

The entire disclosure of all applications, patents and publications cited above and below are hereby incorporated by reference.

The compounds of this invention are producible from known compounds (or from starting materials which, in turn, are producible from known compounds), e.g., through the general preparative methods shown below. The activity of a given compound to inhibit angiogenesis activity can be routinely assayed, e.g., according to procedures disclosed below.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are, therefore, to be construed as merely illustrative and not limitative of the remainder of the disclosure in any way whatsoever. The following examples are for illustrative purposes only and are not intended, nor should they be construed to limit the invention in any way.

EXAMPLES

All reactions were performed in flame-dried or oven-dried glassware under a positive pressure of dry argon or dry nitrogen, and were stirred magnetically unless otherwise indicated. Sensitive liquids and solutions were transferred via syringe or cannula, and introduced into reaction vessels through rubber septa. Unless otherwise stated, the term 'concentration under reduced pressure' refers to use of a Buchi rotary evaporator at approximately 15 mmHg.

All temperatures are reported uncorrected in degrees Celsius (°C). Unless otherwise indicated, all parts and percentages are by weight.

Commercial grade reagents and solvents were used without further purification.

Thin-layer chromatography (TLC) was performed on Whatman® pre-coated glass-backed silica gel 60A F-254 250 μm plates. Visualization of plates was effected by one or more of the following techniques: (a) ultraviolet illumination, (b) exposure to iodine vapor, (c) immersion of the plate in a 10% solution of phosphomolybdic acid in ethanol followed by heating, (d) immersion of the plate in a cerium sulfate solution followed by heating, and/or (e) immersion of the plate in an acidic ethanol solution of 2,4-dinitrophenylhydrazine followed by heating. Column chromatography (flash chromatography) was performed using 230-400 mesh EM Science® silica gel.

Melting points (mp) were determined using a Thomas-Hoover melting point apparatus or a Mettler FP66 automated melting point apparatus and are uncorrected. Proton (^{1}H) nuclear magnetic resonance (NMR) spectra were measured with a General Electric GN-Omega 300 (300 MHz) spectrometer with either Me_4Si (δ 0.00) or residual protonated solvent ($CHCl_3$ δ 7.26; MeOH δ 3.30; DMSO δ 2.49) as standard. Carbon (^{13}C) NMR spectra were measured with a General Electric GN-Omega 300 (75 MHz) spectrometer with solvent ($CDCl_3$ δ 77.0; MeOD-d_3; δ 49.0; DMSO-d_6 δ 39.5) as standard. Low resolution mass spectra (MS) and high resolution mass spectra (HRMS) were either obtained as electron impact (EI) mass spectra or as fast atom bombardment (FAB) mass spectra. Electron impact mass spectra (EI-MS) were obtained with a Hewlett Packard 5989A mass spectrometer equipped with a Vacumetrics Desorption Chemical Ionization Probe for sample introduction. The ion source was maintained at 250 °C. Electron impact ionization was performed with electron energy of 70 eV and a trap current of 300 μA. Liquid-cesium secondary ion mass spectra (FAB-MS), an updated version of fast atom bombardment were obtained using a Kratos Concept 1-H spectrometer.

Chemical ionization mass spectra (CI-MS) were obtained using a Hewlett Packard MS-Engine (5989A) with methane as the reagent gas ($1x10^{-4}$ torr to $2.5x10^{-4}$ torr). The direct insertion desorption chemical ionization (DCI) probe (Vaccumetrics, Inc.) was ramped from 0-1.5 amps in 10 sec and held at 10 amps until all traces of the sample disappeared (~1-2 min). Spectra were scanned from 50-800 amu at 2 sec per scan. HPLC - electrospray mass spectra (HPLC ES-MS) were obtained using a Hewlett-Packard 1100 HPLC equipped with a quaternary pump, a variable wavelength detector, a C-18 column, and a Finnigan LCQ ion trap mass spectrometer with electrospray ionization. Spectra were scanned from 120-800 amu using a variable ion time according to the number of ions in the source.

Gas chromatography - ion selective mass spectra (GC-MS) were obtained with a Hewlett Packard 5890 gas chromatograph equipped with an HP-1 methyl silicone column (0.33 mM coating; 25 m x 0.2 mm) and a Hewlett Packard 5971 Mass Selective Detector (ionization energy 70 eV).

Elemental analyses were conducted by Robertson Microlit Labs, Madison NJ. All compounds displayed NMR spectra, LRMS and either elemental analysis or HRMS consistent with assigned structures.

List of Abbreviations and Acronyms:

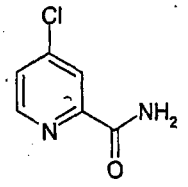
AcOH	acetic acid
anh	anhydrous
BOC	*tert*-butoxycarbonyl
conc	concentrated
dec	decomposition
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DPPA	diphenylphosphoryl azide
EtOAc	ethyl acetate
EtOH	ethanol (100%)

Et_2O	diethyl ether
Et_3N	triethylamine
m-CPBA	3-chloroperoxybenzoic acid
MeOH	methanol
pet. ether	petroleum ether (boiling range 30-60 °C)
THF	tetrahydrofuran
TFA	trifluoroacetic acid
Tf	trifluoromethanesulfonyl

EXAMPLE A

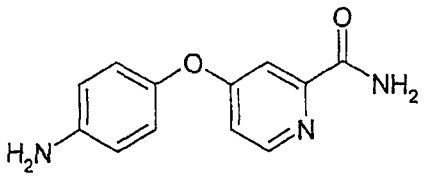
N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-{4-[2-carbamoyl-(4-yridyloxy)]phenyl} urea.

Step 1: Preparation of 4-chloro-2-pyridinecarboxamide



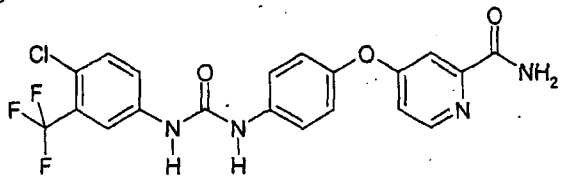
To a stirred mixture of methyl 4-chloro-2-pyridinecarboxylate hydrochloride (1.0 g, 4.81 mmol) dissolved in conc. aqueous ammonia (32 mL) was added ammonium chloride (96.2 mg, 1.8 mmol, 0.37 equiv.), and the heterogeneous reaction mixture was stirred at ambient temperature for 16h. The reaction mixture was poured into EtOAc (500 mL) and water (300 mL). The organic layer was washed with water (2 x 300 mL) and a saturated NaCl solution (1 x 300 mL), dried ($MgSO_4$), concentrated in *vacuo* to give 4-chloro-2-pyridinecarboxamide as a beige solid (604.3 mg, 80.3%): TLC (50% EtOAc / hexane) R_f 0.20; ^{1}H-NMR (DMSO-d_6) δ 8.61 (d, J = 5.4 Hz, 1H), 8.20 (broad s, 1H), 8.02 (d, J = 1.8 Hz, 1H), 7.81 (broad s, 1H), 7.76 to 7.73 (m, 1H).

Step 2: Preparation of 4-(4-aminophenoxy)-2-pyridinecarboxamide



To 4-aminophenol (418 mg, 3.83 mmol) in anh DMF(7.7 mL) was added potassium *tert*-butoxide (447 mg, 3.98 mmol, 1.04 equiv.) in one portion. The reaction mixture was stirred at room temperature for 2 h, and a solution of 4-chloro-2-pyridinecarboxamide (600 mg, 3.83 mmol, 1.0 equiv.) in anh DMF (4 mL) was then added. The reaction mixture was stirred at 80 °C for 3 days and poured into a mixture of EtOAc and a saturated NaCl solution. The organic layer was sequentially washed with a saturated NH_4Cl solution then a saturated NaCl solution, dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was purified using MPLC chromatography (Biotage®; gradient from 100% EtOAc to followed by 10% MeOH / 50% EtOAc / 40% hexane) to give the 4-chloro-5-trifluoromethylaniline as a brown solid (510 mg, 58%). ^{1}H-NMR (DMSO-d_6) δ 8.43 (d, J = 5.7 Hz, 1H), 8.07 (br s, 1H), 7.66 (br s, 1H), 7.31 (d, J = 2.7 Hz, 1H), 7.07 (dd, J = 5.7 Hz, 2.7 Hz, 1H), 6.85 (d, J = 9.0 Hz, 2 H), 6.62 (d, J = 8.7 Hz, 2H), 5.17 (broad s, 2H); HPLC EI-MS *m/z* 230 ($(M+H)^+$.

Step 3: Preparation of N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-{4-[2-carbamoyl-(4-pyridyloxy)]phenyl} urea

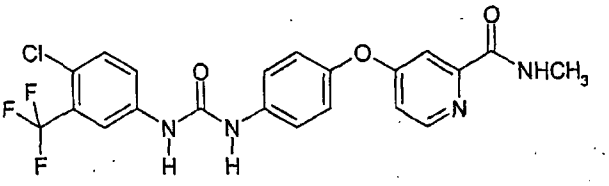


A mixture of 4-chloro-5-trifluoromethylaniline (451 mg, 2.31 mmol, 1.1 equiv.) and 1,1'-carbonyl diimidazole (419 mg, 2.54 mmol, 1.2 equiv.) in anh dichloroethane (5.5 mL) was stirred under argon at 65 °C for 16 h. Once cooled to room temperature, a solution of 4-(4-aminophenoxy)-2-pyridinecarboxamide (480 mg, 2.09 mmol) in anh THF (4.0 mL) was added, and the reaction mixture was stirred at 60°C for 4 h. The reaction mixture was poured into EtOAc, and the organic layer was washed with water (2x) and a saturated NaCl solution (1x), dried ($MgSO_4$), filtered, and evaporated in *vacuo*. Purification using MPLC chromatography (Biotage®; gradient from 100% EtOAc to 2% MeOH / EtOAc) gave N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-{4-[2-carbamoyl-(4-pyridyloxy)]phenyl} urea as a white solid (770 mg, 82%): TLC (EtOAc) R_f 0.11, 100% ethyl acetate ^{1}H-NMR (DMSO-d_6)

δ 9.21 (s, 1H), 8.99 (s, 1H), 8.50 (d, J = 5.6 Hz, 1H), 8.11 (s, 1H), 8.10 (s, 1H), 7.69 (broad s, 1H), 7.64 (dd, J = 8.2 Hz, 2.1 Hz, 1H), 7.61 (s, 1H), 7.59 (d, J = 8.8 Hz, 2H), 7.39 (d, J = 2.5 Hz, 1H), 7.15 (d, J = 8.9 Hz, 2H), 7.14 (m, 1H); MS LC-MS (MH^+ = 451). Anal. calcd for $C_{20}H_{14}ClF_3N_4O_3$: C 53.29% H 3.13% N 12.43%. Found: C 53.33% H 3.21% N 12.60%;.

Example B

N-[4-chloro-3-(trifluoromethyl)phenyl]-N'-{4-[2-N-methylcarbamoyl-4-pyridyloxy]phenyl} urea



Step 1: 4-Chloro-*N*-methyl-2-pyridinecarboxamide is first synthesized from 4-chloropyridine-2-carbonyl chloride by adding 4-chloropyridine-2-carbonyl chloride HCl salt (7.0 g, 32.95 mmol) in portions to a mixture of a 2.0 M methylamine solution in THF (100 mL) and MeOH (20 mL) at 0 °C. The resulting mixture is stored at 3 °C for 4 h, then concentrated under reduced pressure. The resulting nearly dry solids are suspended in EtOAc (100 mL) and filtered. The filtrate is washed with a saturated NaCl solution (2 x 100 mL), dried (Na_2SO_4) and concentrated under reduced pressure to provide 4-chloro-*N*-methyl-2-pyridinecarboxamide as a yellow, crystalline solid.

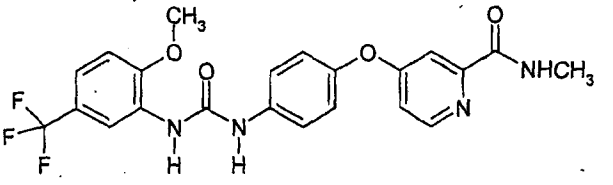
Step 2: A solution of 4-aminophenol (9.60 g, 88.0 mmol) in anh. DMF (150 mL) is treated with potassium *tert*-butoxide (10.29 g, 91.7 mmol), and the reddish-brown mixture is stirred at room temp. for 2 h. The contents are treated with 4-chloro-*N*-methyl-2-pyridinecarboxamide (15.0 g, 87.9 mmol) from Step 1 and K_2CO_3 (6.50 g, 47.0 mmol) and then heated at 80 °C for 8 h. The mixture is cooled to room temp. and separated between EtOAc (500 mL) and a saturated NaCl solution (500 mL). The aqueous phase is back-extracted with EtOAc (300 mL). The combined organic layers are washed with a saturated NaCl solution (4 x 1000 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The resulting solids are dried under reduced

pressure at 35 °C for 3 h to afford 4-(2-(*N*-methylcarbamoyl)-4-pyridyloxy)aniline as a light-brown solid. ^{1}H-NMR (DMSO-d_6) δ 2.77 (d, *J*=4.8 Hz, 3H), 5.17 (br s, 2H), 6.64, 6.86 (AA'BB' quartet, *J*=8.4 Hz, 4H), 7.06 (dd, *J*=5.5, 2.5 Hz, 1H), 7.33 (d, *J*=2.5 Hz, 1H), 8.44 (d, *J*=5.5 Hz, 1H), 8.73 (br d, 1H); HPLC ES-MS *m/z* 244 ($(M+H)^+$).

Step 3: A solution of 4-chloro-3-(trifluoromethyl)phenyl isocyanate (14.60 g, 65.90 mmol) in CH_2Cl_2 (35 mL) is added dropwise to a suspension of 4-(2-(*N*-methylcarbamoyl)-4-pyridyloxy)aniline from Step 2; (16.0 g, 65.77 mmol) in CH_2Cl_2 (35 mL) at 0 °C. The resulting mixture is stirred at room temp. for 22 h. The resulting yellow solids are removed by filtration, then washed with CH_2Cl_2 (2 x 30 mL) and dried under reduced pressure (approximately 1 mmHg) to afford *N*-(4-chloro-3-(trifluoromethyl)phenyl)-*N'*-(4-(2-(*N*-methylcarbamoyl)-4-pyridyloxy)phenyl) urea as an off-white solid: mp 207-209 °C; ^{1}H-NMR (DMSO-d_6) δ 2.77 (d, *J*=4.8 Hz, 3H), 7.16 (m, 3H), 7.37 (d, *J*=2.5 Hz, 1H), 7.62 (m, 4H), 8.11 (d, *J*=2.5 Hz, 1H), 8.49 (d, *J*=5.5 Hz, 1H), 8.77 (br d, 1H), 8.99 (s, 1H), 9.21 (s, 1H); HPLC ES-MS *m/z* 465 ($(M+H)^+$).

Example C

N-[2-methoxy-5-(trifluoromethyl)phenyl]-N'-{4-[2-N-methylcarbamoyl-4-pyridyloxy]phenyl} urea



Step 1: 4-Chloro-*N*-methyl-2-pyridinecarboxamide is first synthesized from 4-chloropyridine-2-carbonyl chloride by adding 4-chloropyridine-2-carbonyl chloride HCl salt (7.0 g, 32.95 mmol) in portions to a mixture of a 2.0 M methylamine solution in THF (100 mL) and MeOH (20 mL) at 0 °C. The resulting mixture is stored at 3 °C for 4 h, then concentrated under reduced pressure. The resulting nearly dry solids are

suspended in EtOAc (100 mL) and filtered. The filtrate is washed with a saturated NaCl solution (2 x 100 mL), dried (Na_2SO_4) and concentrated under reduced pressure to provide 4-chloro-*N*-methyl-2-pyridinecarboxamide as a yellow, crystalline solid.

Step 2: A solution of 4-aminophenol (9.60 g, 88.0 mmol) in anh. DMF (150 mL) is treated with potassium *tert*-butoxide (10.29 g, 91.7 mmol), and the reddish-brown mixture is stirred at room temp. for 2 h. The contents are treated with 4-chloro-*N*-methyl-2-pyridinecarboxamide (15.0 g, 87.9 mmol) from Step 1 and K_2CO_3 (6.50 g, 47.0 mmol) and then heated at 80 °C for 8 h. The mixture is cooled to room temp. and separated between EtOAc (500 mL) and a saturated NaCl solution (500 mL). The aqueous phase is back-extracted with EtOAc (300 mL). The combined organic layers are washed with a saturated NaCl solution (4 x 1000 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The resulting solids are dried under reduced pressure at 35 °C for 3 h to afford 4-(2-(*N*-methylcarbamoyl)-4-pyridyloxy)aniline as a light-brown solid. ^{1}H-NMR (DMSO-d_6) δ 2.77 (d, *J*=4.8 Hz, 3H), 5.17 (br s, 2H), 6.64, 6.86 (AA'BB' quartet, *J*=8.4 Hz, 4H), 7.06 (dd, *J*=5.5, 2.5 Hz, 1H), 7.33 (d, *J*=2.5 Hz, 1H), 8.44 (d, *J*=5.5 Hz, 1H), 8.73 (br d, 1H); HPLC ES-MS *m/z* 244 ($(M+H)^+$).

Step 3: To a solution of 2-methoxy-5-(trifluoromethyl)aniline (0.15 g) in anh CH_2Cl_2 (15 mL) at 0 °C is added CDI (0.13 g). The resulting solution is allowed to warm to room temp. over 1 h, is stirred at room temp. for 16 h, then is treated with 4-(2-(*N*-methylcarbamoyl)-4-pyridyloxy)aniline (0.18 g) from Step 2. The resulting yellow solution is stirred at room temp. for 72 h, then is treated with H_2O (125 mL). The resulting aqueous mixture is extracted with EtOAc (2 x 150 mL). The combined organics are washed with a saturated NaCl solution (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue is triturated (90% EtOAc/10% hexane). The resulting white solids are collected by filtration and washed with EtOAc. The filtrate is concentrated under reduced pressure and the residual oil purified by column chromatography (gradient from 33% EtOAc/67% hexane to 50% EtOAc/50% hexane to 100% EtOAc) to give *N*-(2-methoxy-5-(trifluoromethyl)phenyl)-*N'*-(4-(2-(*N*-methylcarbamoyl)-4-pyridyloxy)phenyl) urea as

a light tan solid: TLC (100% EtOAc) R_f 0.62; ^{1}H NMR (DMSO-d_6) δ 2.76 (d, *J*=4.8 Hz, 3H), 3.96 (s, 3H), 7.1-7.6 and 8.4-8.6 (m, 11H), 8.75 (d, *J*=4.8 Hz, 1H), 9.55 (s, 1 H); FAB-MS *m/z* 461 ($(M+H)^+$).

BIOLOGICAL EXAMPLES

KDR (VEGFR2) Assay:

The cytosolic kinase domain of KDR kinase was expressed as a 6His fusion protein in Sf9 insect cells. The KDR kinase domain fusion protein was purified over a Ni++ chelating column. Ninety-six well ELISA plates were coated with 5 µl HEPES buffer (20 mM poly(Glu4;Tyrl) (Sigma Chemical Co., St. Louis, MO) in 100 µk HEPES buffer (20 mM HEPES, pH 7.5, 150 mM Na Cl, 0.02% Thimerosal) at 4° overnight. Before use, the plate was washed with HEPES, NaCl buffer and the plates were blocked with 1% BSA, 0.1% Tween 20 in HEPES, NaCl buffer.

Test compounds were serially diluted in 100% DMSO form 4 mM to 0.12 µM in half-log dilutions. These dilutions were further diluted twenty fold in H_2O to obtain compound solutions in 5% DMSO. Following loading of the assay plate with 85 µl of assay buffer (20 mM HEPES, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 3 mM $MnCl_2$, 0.05% glycerol, 0.005% Triton X-100, 1 mM -mercaptoethanol, with or without 3.3 µM ATP), 5 µl of the diluted compounds were added to a final assay volume of 100 µl. Final concentrations were between 10 µM, and 0.3 mM in 0.25% DMSO. The assay was initiated by the addition of 10µl (30 ng) of KDR kinase domain.

The assay was incubated with test compound or vehicle alone with gentle agitation at room temperature for 60 minutes. The wells were washed and phosphotyrosines (PY) were probed with an anti-phosphotyrosine (PY), mAb clone 4G10 (Upstate Biotechnology, Lake Placid, NY). PY/anti-PY complexes were detected with an anti-mouse IgG/HRP conjugate Iamersham International plc, Buckinghamshire, England). Phosphotyrosine was quantitated by incubating with 100 µl 3, 3', 5, 5' tetramethylbenzidine solution (Kirkegaard and Perry, TMB Microwell 1 Component peroxidase substrate). Color development was arrested by the addition of

100 µl 1% HCl-based stop solution (Kirkegaard and Perry, TMB 1 Component Stop Solution).

Optical densities were determined spectrophotometrically at 450 nm in a 96-well plate reader, SpectraMax 250 (Molecular Devices). Background (no ATP in assay) OD values were subtracted from all Ods and the percent inhibition was calculated according to the equation:

$$\% \text{ Inhibition} = \frac{(OD(\text{vehicle control}) - OD(\text{with compound}) \times 100}{OD(\text{vehicle control}) - OD(\text{no ATP added})}$$

The IC_{50} values were determined with a least squares analysis program using compound concentration versus percent inhibition.

The following compounds were tested in the assay described above and were found to have either an IC_{50} of less than 10 micromolar or showed greater than 30% inhibition at 1 micromolar. Compound names were generated using Nomenclator™ v 3.0 and may differ from those in the patent applications.

From WO 1999/32463:

Entry No	Name
73	N-[5-(tert-butyl)-2-(3-thienyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
96	{[4-(4-methoxyphenoxy)phenyl]amino}-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
99	N-[2-methoxy-5-(trifluoromethyl)phenyl][(3-(4-pyridylthio)phenyl)amino]carboxamide
100	N-[2-methoxy-5-(trifluoromethyl)phenyl][(3-(4-pyridylthio)phenyl)amino]carboxamide
101	N-[2-methoxy-5-(trifluoromethyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide

From WO 1999/32436:

Entry No	Name
11	N-[5-(tert-butyl)-2-methoxyphenyl][(3-(4-pyridylthio)phenyl)amino]carboxamide
12	N-[5-(tert-butyl)-2-(3-thienyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
17	N-[3-(tert-butyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
23	{[3-(tert-butyl)phenyl]amino}-N-(4-(3-pyridyl)phenyl)carboxamide
33	{[4-(4-methoxyphenoxy)phenyl]amino}-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
36	N-[2-methoxy-5-(trifluoromethyl)phenyl][(3-(4-pyridylthio)phenyl)amino]carboxamide
37	N-[2-methoxy-5-(trifluoromethyl)phenyl][(3-(4-pyridylthio)phenyl)amino]carboxamide
38	N-[2-methoxy-5-(trifluoromethyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
56	N-[2-methoxy-5-(trifluoromethyl)phenyl]{[3-(2-methyl(4-pyridyloxy))phenyl]amino}carboxamide
70	[(3-chloro-4-(6-quinolyloxy)phenyl)amino]-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
81	[(4-(4-pyridyloxy)phenyl)amino]-N-[3-(trifluoromethyl)phenyl]carboxamide
82	N-[2-chloro-5-(trifluoromethyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
83	N-[2-fluoro-5-(trifluoromethyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
91	{[4-chloro-3-(trifluoromethyl)phenyl]amino}-N-(2-methyl-4-(3-pyridyloxy)phenyl)carboxamide
102	N-[4-chloro-3-(trifluoromethyl)phenyl][(4-(4-

	pyridyloxy)phenyl)amino]carboxamide
103	N-[4-chloro-3-(trifluoromethyl)phenyl]{[4-(4-methoxyphenoxy)phenyl]amino}carboxamide
105	{[4-chloro-3-(trifluoromethyl)phenyl]amino}-N-[3-(4-pyridylcarbonyl)phenyl]carboxamide
106	{[4-chloro-3-(trifluoromethyl)phenyl]amino}-N-[3-(2-methyl(4-pyridyloxy))phenyl]carboxamide
119	N-[4-fluoro-3-(trifluoromethyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
132	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
133	N-[5-methoxy-3-(trifluoromethyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
135	[(3-bromo-4-chlorophenyl)amino]-N-(4-(4-pyridyloxy)phenyl)carboxamide
136	[(4-(4-pyridyloxy)phenyl)amino]-N-[3-(trifluoromethoxy)phenyl]carboxamide
141	N-[3,5-bis(trifluoromethyl)phenyl][(4-(4-pyridyloxy)phenyl)amino]carboxamide

From WO 1999/32111:

Entry No	**Name**
18	N-[5-(tert-butyl)isoxazol-3-yl][(4-(4-pyridylthio)phenyl)amino]carboxamide
32	{[5-(tert-butyl)isoxazol-3-yl]amino}-N-(3-(4-pyridylthio)phenyl)carboxamide
53	N-[5-(tert-butyl)isoxazol-3-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
59	N-[5-(tert-butyl)isoxazol-3-yl][(4-(3-pyridyloxy)phenyl)amino]carboxamide

67	{3-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy]phenyl}-N-methylcarboxamide
85	{[5-(tert-butyl)isoxazol-3-yl]amino}-N-[3-(2-methyl(4-pyridyloxy))phenyl]carboxamide
86	N-[5-(tert-butyl)isoxazol-3-yl]{[4-(2-methyl(4-pyridyloxy))phenyl]amino}carboxamide
103	4-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy]pyridine-2-carboxamide
104	{[5-(tert-butyl)isoxazol-3-yl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
105	4-[3-({N-[5-(tert-butyl)isoxazol-3-yl]carbamoyl}amino)phenoxy]pyridine-2-carboxamide
106	3-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy]benzamide
143	N-[3-(methylethyl)isoxazol-5-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
146	N-(3-cyclobutylisoxazol-5-yl)[(4-(4-pyridyloxy)phenyl)amino]carboxamide
147	N-(3-cyclobutylisoxazol-5-yl)[(4-(6-quinolyloxy)phenyl)amino]carboxamide
162	{[3-(tert-butyl)isoxazol-5-yl]amino}-N-(3-(4-pyridylthio)phenyl)carboxamide
163	N-[3-(tert-butyl)isoxazol-5-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
164	N-[3-(tert-butyl)isoxazol-5-yl]{[4-(4-methoxyphenoxy)phenyl]amino}carboxamide
188	N-[3-(1,1-dimethylpropyl)isoxazol-5-yl][(3-(4-pyridylthio)phenyl)amino]carboxamide
195	N-[3-(1,1-dimethylpropyl)isoxazol-5-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide

220	{[3-(tert-butyl)pyrazol-5-yl]amino}-N-(3-(4-pyridylthio)phenyl)carboxamide
221	N-[3-(tert-butyl)pyrazol-5-yl][(4-(4-pyridylthio)phenyl)amino]carboxamide
222	{3-[4-({[3-(tert-butyl)pyrazol-5-yl]amino}carbonylamino)phenoxy]phenyl}-N-methylcarboxamide, 2,2,2-trifluoroacetic acid
225	N-[3-(tert-butyl)-1-methylpyrazol-5-yl][(3-(4-pyridylthio)phenyl)amino]carboxamide
251	N-[3-(tert-butyl)-1-methylpyrazol-5-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
261	N-[3-(tert-butyl)-1-methylpyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
266	N-[3-(tert-butyl)-1-methylpyrazol-5-yl]{[4-(4-pyridylmethoxy)phenyl]amino}carboxamide
277	tert-butyl 3-(tert-butyl)-5-[({4-[3-(N-methylcarbamoyl)phenoxy]phenyl}amino)carbonylamino]pyrazolecarboxylate
280	N-[5-(tert-butyl)(1,3,4-thiadiazol-2-yl)][(4-(4-pyridyloxy)phenyl)amino]carboxamide
281	{[5-(tert-butyl)(1,3,4-thiadiazol-2-yl)]amino}-N-(3-(4-pyridylthio)phenyl)carboxamide
284	N-[5-(tert-butyl)(3-thienyl)][(4-(3-pyridyloxy)phenyl)amino]carboxamide
293	N-[5-(tert-butyl)(3-thienyl)][(4-(4-pyridyloxy)phenyl)amino]carboxamide
299	N-(6-chloro(1H-indazol-3-yl))[(4-(4-pyridyloxy)phenyl)amino]carboxamide
302	({4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-(1-methyl-3-phenylpyrazol-5-yl)carboxamide

From WO 1999/

Entry No	Name
21	N-[5-(tert-butyl)isoxazol-3-yl][(4-(4-pyridylthio)phenyl)amino]carboxamide
42	{[5-(tert-butyl)isoxazol-3-yl]amino}-N-(3-(4-pyridylthio)phenyl)carboxamide
59	N-[5-(tert-butyl)isoxazol-3-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
64	N-[5-(tert-butyl)isoxazol-3-yl][(4-(3-pyridyloxy)phenyl)amino]carboxamide
69	{3-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy]phenyl}-N-methylcarboxamide
81	{[5-(tert-butyl)isoxazol-3-yl]amino}-N-[3-(2-methyl(4-pyridyloxy))phenyl]carboxamide
82	N-[5-(tert-butyl)isoxazol-3-yl]{[4-(2-methyl(4-pyridyloxy))phenyl]amino}carboxamide
101	{4-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-methylcarboxamide
103	4-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy]pyridine-2-carboxamide
104	4-[3-({N-[5-(tert-butyl)isoxazol-3-yl]carbamoyl}amino)phenoxy]pyridine-2-carboxamide
105	{[5-(tert-butyl)isoxazol-3-yl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
106	3-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy]benzamide
118	{4-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)-3-chlorophenoxy](2-pyridyl)}-N-methylcarboxamide

124	{3-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy]phenyl}-N-(2-morpholin-4-ylethyl)carboxamide
125	{4-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-ethylcarboxamide
126	{4-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)-2-chlorophenoxy](2-pyridyl)}-N-methylcarboxamide
127	{[5-(tert-butyl)isoxazol-3-yl]amino}-N-{2-methyl-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
128	{[5-(tert-butyl)isoxazol-3-yl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}carboxamide
130	{3-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenoxy]phenyl}-N-(3-pyridyl)carboxamide
140	{4-[4-({[5-(tert-butyl)isoxazol-3-yl]amino}carbonylamino)phenylthio](2-pyridyl)}-N-methylcarboxamide
182	N-methyl{4-[4-({[3-(methylethyl)isoxazol-5-yl]amino}carbonylamino)phenoxy](2-pyridyl)}carboxamide
186	N-methyl{4-[3-({[3-(methylethyl)isoxazol-5-yl]amino}carbonylamino)phenoxy](2-pyridyl)}carboxamide
187	N-(3-cyclobutylisoxazol-5-yl)[(4-(6-quinolyloxy)phenyl)amino]carboxamide
188	N-(3-cyclobutylisoxazol-5-yl)[(4-(4-pyridyloxy)phenyl)amino]carboxamide
194	N-[3-(tert-butyl)isoxazol-5-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
195	N-[3-(tert-butyl)isoxazol-5-yl]{[4-(4-methoxyphenoxy)phenyl]amino}carboxamide
206	{[3-(tert-butyl)isoxazol-5-yl]amino}-N-(3-(4-

	pyridylthio)phenyl)carboxamide
212	N-[3-(tert-butyl)isoxazol-5-yl]{[4-(1,3-dioxoisoindolin-5-yloxy)phenyl]amino}carboxamide
213	{[3-(tert-butyl)isoxazol-5-yl]amino}-N-[4-(1-oxoisoindolin-5-yloxy)phenyl]carboxamide
214	{4-[4-({[3-(tert-butyl)isoxazol-5-yl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-ethylcarboxamide
215	{4-[4-({[3-(tert-butyl)isoxazol-5-yl]amino}carbonylamino)-2-chlorophenoxy](2-pyridyl)}-N-methylcarboxamide
216	{[3-(tert-butyl)isoxazol-5-yl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}carboxamide
217	{4-[4-({[3-(tert-butyl)isoxazol-5-yl]amino}carbonylamino)phenylthio](2-pyridyl)}-N-methylcarboxamide
218	{4-[4-({[3-(tert-butyl)isoxazol-5-yl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-methylcarboxamide
228	{[3-(tert-butyl)isoxazol-5-yl]amino}-N-[3-(6-methyl(3-pyridyloxy))phenyl]carboxamide
240	N-[3-(tert-butyl)isoxazol-5-yl][(6-(4-pyridylthio)(3-pyridyl))amino]carboxamide
247	{[3-(tert-butyl)isoxazol-5-yl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
253	N-[3-(1,1-dimethylpropyl)isoxazol-5-yl][(3-(4-pyridylthio)phenyl)amino]carboxamide
255	N-[3-(1,1-dimethylpropyl)isoxazol-5-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
261	N-[3-(1,1-dimethylpropyl)isoxazol-5-yl]({4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
263	N-[3-(1,1-dimethylpropyl)isoxazol-5-yl]{[4-(2-methyl(4-

	pyridylthio))phenyl]amino}carboxamide
292	N-[3-(tert-butyl)pyrazol-5-yl]{[4-(6-methyl(3-pyridyloxy))phenyl]amino}carboxamide
298	{[3-(tert-butyl)pyrazol-5-yl]amino}-N-(3-(4-pyridylthio)phenyl)carboxamide
299	N-[3-(tert-butyl)pyrazol-5-yl][(4-(4-pyridylthio)phenyl)amino]carboxamide
300	{3-[4-({[3-(tert-butyl)pyrazol-5-yl]amino}carbonylamino)phenoxy]phenyl}-N-methylcarboxamide, 2,2,2-trifluoroacetic acid
304	N-[3-(tert-butyl)-1-methylpyrazol-5-yl]{[4-(4-pyridylmethoxy)phenyl]amino}carboxamide
305	{5-[4-({[3-(tert-butyl)-1-methylpyrazol-5-yl]amino}carbonylamino)phenoxy]-2-methoxyphenyl}-N-methylcarboxamide
309	N-[3-(tert-butyl)-1-methylpyrazol-5-yl][(3-(4-pyridylthio)phenyl)amino]carboxamide
321	N-[3-(tert-butyl)-1-methylpyrazol-5-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
326	N-[3-(tert-butyl)-1-methylpyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
339	tert-butyl 3-(tert-butyl)-5-[({4-[3-(N-methylcarbamoyl)phenoxy]phenyl}amino)carbonylamino]pyrazolecarboxylate
341	N-[5-(tert-butyl)(1,3,4-thiadiazol-2-yl)][(4-(4-pyridyloxy)phenyl)amino]carboxamide
342	{[5-(tert-butyl)(1,3,4-thiadiazol-2-yl)]amino}-N-(3-(4-pyridylthio)phenyl)carboxamide
356	N-[5-(tert-butyl)(1,3,4-thiadiazol-2-yl)]{[6-(6-methyl(3-pyridyloxy))(3-pyridyl)]amino}carboxamide
366	N-[5-(1,1-dimethylpropyl)(1,3,4-thiadiazol-2-yl)][(4-(4-

	pyridyloxy)phenyl)amino]carboxamide
367	N-[5-(1,1-dimethylpropyl)(1,3,4-thiadiazol-2-yl)][(3-(4-pyridylthio)phenyl)amino]carboxamide
376	N-[5-(tert-butyl)(3-thienyl)][(4-(3-pyridyloxy)phenyl)amino]carboxamide
388	{3-[4-({[5-(tert-butyl)(1,3,4-oxadiazol-2-yl)]amino}carbonylamino)phenoxy]phenyl}-N-ethylcarboxamide
389	{3-[4-({[5-(tert-butyl)(1,3,4-oxadiazol-2-yl)]amino}carbonylamino)phenoxy]phenyl}-N-(methylethyl)carboxamide
390	{3-[4-({[5-(tert-butyl)(1,3,4-oxadiazol-2-yl)]amino}carbonylamino)phenoxy]phenyl}-N-methylcarboxamide
391	N-[5-(tert-butyl)(1,3,4-oxadiazol-2-yl)][(4-(4-pyridyloxy)phenyl)amino]carboxamide
392	N-(3-cyclopropyl-1-methylpyrazol-5-yl)[(4-(6-quinolyloxy)phenyl)amino]carboxamide
393	({4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-(1-methyl-3-phenylpyrazol-5-yl)carboxamide
395	N-[2-(tert-butyl)(1,3-thiazol-5-yl)]{[4-(6-methyl(3-pyridyloxy))phenyl]amino}carboxamide

From WO 1999/32110

Entry No	**Name**
1	[(2,3-dichlorophenyl)amino]-N-[3-(tert-butyl)-1-phenylpyrazol-5-yl]carboxamide
2	N-[1-(4-aminophenyl)-3-(tert-butyl)pyrazol-5-yl][(2,3-dichlorophenyl)amino]carboxamide
11	N-[1-(3-aminophenyl)-3-(tert-butyl)pyrazol-5-yl][(2,3-

	dichlorophenyl)amino]carboxamide
18	N-[3-(3-(tert-butyl)-5-{[(4-phenoxyphenyl) amino]carbonylamino}pyrazolyl)phenyl]acetamide
23	N-[1-(2,6-dichlorophenyl)-3-(tert-butyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
24	N-[3-(tert-butyl)-1-(4-fluorophenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
25	N-[3-(tert-butyl)-1-(2-methylphenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
26	N-[3-(tert-butyl)-1-(3-fluorophenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
27	N-{3-(tert-butyl)-1-[4-(methylsulfonyl)phenyl]pyrazol-5-yl}{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
28	N-[3-(tert-butyl)-1-(4-nitrophenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
29	N-[3-(tert-butyl)-1-(3-methoxyphenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
30	N-[1-(3-aminophenyl)-3-(tert-butyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
32	N-[1-(3-aminophenyl)-3-(tert-butyl)pyrazol-5-yl][(4-(4-pyridylthio)phenyl)amino]carboxamide
34	N-[3-(tert-butyl)-1-(3-fluorophenyl)pyrazol-5-yl][(3-(4-pyridylthio)phenyl)amino]carboxamide
35	N-[3-(tert-butyl)-1-(4-fluorophenyl)pyrazol-5-yl][(3-(4-pyridylthio)phenyl)amino]carboxamide
36	N-[3-(tert-butyl)-1-(3-fluorophenyl)pyrazol-5-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide
37	N-[3-(tert-butyl)-1-(4-fluorophenyl)pyrazol-5-yl][(4-(4-pyridyloxy)phenyl)amino]carboxamide

From WO 1999/32455

Entry No	Name
1	[(2,3-dichlorophenyl)amino]-N-[3-(tert-butyl)-1-phenylpyrazol-5-yl]carboxamide
2	N-[1-(4-aminophenyl)-3-(tert-butyl)pyrazol-5-yl][(2,3-dichlorophenyl)amino]carboxamide
14	N-[1-(3-aminophenyl)-3-(tert-butyl)pyrazol-5-yl][(2,3-dichlorophenyl)amino]carboxamide
22	N-[3-(3-(tert-butyl)-5-{[(4-phenoxyphenyl) amino]carbonylamino}pyrazolyl)phenyl]acetamide
27	N-[1-(2,6-dichlorophenyl)-3-(tert-butyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
28	N-[3-(tert-butyl)-1-(4-fluorophenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
29	N-[3-(tert-butyl)-1-(2-methylphenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
30	N-[3-(tert-butyl)-1-(3-fluorophenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
31	N-{3-(tert-butyl)-1-[4-(methylsulfonyl)phenyl]pyrazol-5-yl}{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
32	N-[3-(tert-butyl)-1-(4-nitrophenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
33	N-[3-(tert-butyl)-1-(3-methoxyphenyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
34	N-[1-(3-aminophenyl)-3-(tert-butyl)pyrazol-5-yl]{[4-(4-pyridylmethyl)phenyl]amino}carboxamide
36	N-[1-(3-aminophenyl)-3-(tert-butyl)pyrazol-5-yl][(4-(4-pyridylthio)phenyl)amino]carboxamide

From WO 2000/41698

Entry No	Name
1	{3-[4-({[3-(tert-butyl)phenyl]amino}carbonylamino)phenoxy]phenyl}-N-methylcarboxamide
11	N-[2-methoxy-5-(trifluoromethyl)phenyl]({3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
12	4-[3-({N-[2-methoxy-5-(trifluoromethyl)phenyl]carbamoyl}amino)phenoxy]pyridine-2-carboxamide
13	N-[2-methoxy-5-(trifluoromethyl)phenyl]({4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
14	4-[4-({N-[2-methoxy-5-(trifluoromethyl)phenyl]carbamoyl}amino)phenoxy]pyridine-2-carboxamide
16	{4-[4-({N-[2-methoxy-5-(trifluoromethyl)phenyl]carbamoyl}amino)-3-methylphenoxy](2-pyridyl)}-N-methylcarboxamide
17	({2-chloro-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
19	({4-[2-(N-ethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
20	({3-chloro-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
22	3-[4-({N-[2-methoxy-5-(trifluoromethyl)phenyl]carbamoyl}amino)phenoxy]benzamide
24	({4-[2-(N,N-dimethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
27	N-[2-methoxy-5-(trifluoromethyl)phenyl]({4-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}amino)carboxamide
29	N-[2-methoxy-5-(trifluoromethyl)phenyl]({3-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}amino)carboxamide
31	N-[2-methoxy-5-(trifluoromethyl)phenyl][(4-{5-[N-(2-morpholin-4-ylethyl)carbamoyl](3-pyridyloxy)}phenyl)amino]carboxamide
32	N-[2-methoxy-5-(trifluoromethyl)phenyl]({4-[5-(N-

	methylcarbamoyl)(3-pyridyloxy)]phenyl}amino)carboxamide
34	N-[2-methoxy-5-(trifluoromethyl)phenyl]({4-[3-(N-(3-pyridyl)carbamoyl)phenoxy]phenyl}amino)carboxamide
42	{4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-methylcarboxamide
43	4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy]pyridine-2-carboxamide
44	4-[3-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy]pyridine-2-carboxamide
45	{[4-chloro-3-(trifluoromethyl)phenyl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
47	{[4-chloro-3-(trifluoromethyl)phenyl]amino}-N-{2-methyl-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
49	{4-[3-chloro-4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-methylcarboxamide
51	N-[4-chloro-3-(trifluoromethyl)phenyl]({4-[2-(N-ethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
61	{3-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy]phenyl}-N-(2-morpholin-4-ylethyl)carboxamide
62	{3-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy]phenyl}-N-(2-piperidylethyl)carboxamide
65	{4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenylthio](2-pyridyl)}-N-methylcarboxamide
69	{[4-chloro-3-(trifluoromethyl)phenyl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}carboxamide
70	{4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-(2-morpholin-4-ylethyl)carboxamide
72	{5-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](3-

	pyridyl)}-N-methylcarboxamide
75	N-[4-chloro-3-(trifluoromethyl)phenyl]({4-[3-(N-(3-pyridyl)carbamoyl)phenoxy]phenyl}amino)carboxamide
84	{4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-(2-hydroxyethyl)carboxamide
87	{4-[4-({[4-bromo-3-(trifluoromethyl)phenyl]amino}carbonylamino)-2-chlorophenoxy](2-pyridyl)}-N-methylcarboxamide
88	N-[4-bromo-3-(trifluoromethyl)phenyl]({4-[2-(N-ethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
89	{[4-bromo-3-(trifluoromethyl)phenyl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
90	{[4-bromo-3-(trifluoromethyl)phenyl]amino}-N-{4-methyl-3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
93	{[4-bromo-3-(trifluoromethyl)phenyl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}carboxamide
94	{4-[4-({[4-bromo-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-(2-morpholin-4-ylethyl)carboxamide
95	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
96	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({2-chloro-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
97	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({3-chloro-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
98	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
99	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({4-[2-(N-ethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide

From WO 2000/42012

Entry No	**Name**
1	{3-[4-({[3-(tert-

	butyl)phenyl]amino}carbonylamino)phenoxy]phenyl}-N-methylcarboxamide
11	N-[2-methoxy-5-(trifluoromethyl)phenyl]({3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
12	4-[3-({N-[2-methoxy-5-(trifluoromethyl)phenyl]carbamoyl}amino)phenoxy]pyridine-2-carboxamide
13	N-[2-methoxy-5-(trifluoromethyl)phenyl]({4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
14	4-[4-({N-[2-methoxy-5-(trifluoromethyl)phenyl]carbamoyl}amino)phenoxy]pyridine-2-carboxamide
16	{4-[4-({N-[2-methoxy-5-(trifluoromethyl)phenyl]carbamoyl}amino)-3-methylphenoxy](2-pyridyl)}-N-methylcarboxamide
17	({2-chloro-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
19	({4-[2-(N-ethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
20	({3-chloro-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
22	3-[4-({N-[2-methoxy-5-(trifluoromethyl)phenyl]carbamoyl}amino)phenoxy]benzamide
24	({4-[2-(N,N-dimethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)-N-[2-methoxy-5-(trifluoromethyl)phenyl]carboxamide
27	N-[2-methoxy-5-(trifluoromethyl)phenyl]({4-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}amino)carboxamide
29	N-[2-methoxy-5-(trifluoromethyl)phenyl]({3-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}amino)carboxamide
31	N-[2-methoxy-5-(trifluoromethyl)phenyl][(4-{5-[N-(2-morpholin-4-ylethyl)carbamoyl](3-pyridyloxy)}phenyl)amino]carboxamide
32	N-[2-methoxy-5-(trifluoromethyl)phenyl]({4-[5-(N-methylcarbamoyl)(3-pyridyloxy)]phenyl}amino)carboxamide
34	N-[2-methoxy-5-(trifluoromethyl)phenyl]({4-[3-(N-(3-

	pyridyl)carbamoyl)phenoxy]phenyl}amino)carboxamide
42	{4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-methylcarboxamide
43	4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy]pyridine-2-carboxamide
44	4-[3-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy]pyridine-2-carboxamide
45	{[4-chloro-3-(trifluoromethyl)phenyl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
47	{[4-chloro-3-(trifluoromethyl)phenyl]amino}-N-{2-methyl-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
49	{4-[3-chloro-4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-methylcarboxamide
51	N-[4-chloro-3-(trifluoromethyl)phenyl]({4-[2-(N-ethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
61	{3-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy]phenyl}-N-(2-morpholin-4-ylethyl)carboxamide
62	{3-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy]phenyl}-N-(2-piperidylethyl)carboxamide
65	{4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenylthio](2-pyridyl)}-N-methylcarboxamide
69	{[4-chloro-3-(trifluoromethyl)phenyl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}carboxamide
70	{4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-(2-morpholin-4-ylethyl)carboxamide
72	{5-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](3-pyridyl)}-N-methylcarboxamide
75	N-[4-chloro-3-(trifluoromethyl)phenyl]({4-[3-(N-(3-

	pyridyl)carbamoyl)phenoxy]phenyl}amino)carboxamide
84	{4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-(2-hydroxyethyl)carboxamide
87	{4-[4-({[4-bromo-3-(trifluoromethyl)phenyl]amino}carbonylamino)-2-chlorophenoxy](2-pyridyl)}-N-methylcarboxamide
88	N-[4-bromo-3-(trifluoromethyl)phenyl]({4-[2-(N-ethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
89	{[4-bromo-3-(trifluoromethyl)phenyl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
90	{[4-bromo-3-(trifluoromethyl)phenyl]amino}-N-{4-methyl-3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}carboxamide
93	{[4-bromo-3-(trifluoromethyl)phenyl]amino}-N-{3-[2-(N-methylcarbamoyl)(4-pyridylthio)]phenyl}carboxamide
94	{4-[4-({[4-bromo-3-(trifluoromethyl)phenyl]amino}carbonylamino)phenoxy](2-pyridyl)}-N-(2-morpholin-4-ylethyl)carboxamide
95	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
96	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({2-chloro-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
97	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({3-chloro-4-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
98	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({3-[2-(N-methylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide
99	N-[4-chloro-2-methoxy-5-(trifluoromethyl)phenyl]({4-[2-(N-ethylcarbamoyl)(4-pyridyloxy)]phenyl}amino)carboxamide

From WO 2002/85859

Entry No	**Name**
16	[(4-fluorophenyl)amino]-N-(3-isoquinolyl)carboxamide
25	N-(2-methoxy(3-quinolyl))[(4-(4-

	pyridyloxy)phenyl)amino]carboxamide
27	N-(2-methoxy(3-quinolyl))[(3-(4-pyridylthio)phenyl)amino]carboxamide
28	N-[1-(4-methylpiperazinyl)(3-isoquinolyl)][(4-(4-pyridyloxy)phenyl)amino]carboxamide

From WO 2002/85857

Entry No	Name
25	N-(2-methoxy(3-quinolyl))[(4-(4-pyridyloxy)phenyl)amino]carboxamide
27	N-(2-methoxy(3-quinolyl))[(3-(4-pyridylthio)phenyl)amino]carboxamide
28	N-[1-(4-methylpiperazinyl)(3-isoquinolyl)][(4-(4-pyridyloxy)phenyl)amino]carboxamide

Cell mechanistic assay-Inhibition of 3T3 KDR phosphorylation:

NIH3T3 cells expressing the full length KDR receptor are grown in DMEM (Life Technologies, Inc., Grand Island, NY) supplemented with 10% newborn calf serum, low glucose, 25 mM/L sodium pyruvate, pyridoxine hydrochloride and 0.2 mg/ml of G418 (Life Technologies Inc., Grand Island, NY). The cells are maintained in collagen I-coated T75 flasks (Becton Dickinson Labware, Bedford, MA) in a humidified 5% CO2 atmosphere at 37°C.

Fifteen thousand cells are plated into each well of a collagen I-coated 96-well plate in the DMEM growth medium. Six hours later, the cells are washed and the medium is replaced with DMEM without serum. After overnight culture to quiesce the cells,t he medium is replaced by Dulbecco's phosphate-buffered saline (Life Technologies Inc., Grand Island, NY) with 0.1% bovine albumin (Sigma Chemical Co., St. Louis, MO). After adding various concentrations (0-300 nM) of test compounds to the cells in 1% final concentration of DMSO, the cells are incubated at room temperature for 30 minutes. Following VEGF stimulation, the buffer is

removed and the cells are lysed by addtion of 150 µl of extraction buffer (50 mM Tris, pH 7.8, supplemented with 10% glycerol, 50 mM BGP, 2 mM EDTA, 10 mM NaF, 0.5 mM NaVO4, and 0.3% TX-100) at 4°C for 30 minutes.

To assess receptor phosphorylation, 100 microliters of each cell lysate are added to the wells of an ELISA plate precoated with 300 ng of antibody C20 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Following a 60-minute incubation, the plate is washed and bound KDR is probed for phosphotyrosine using an anti-phosphotyrosine mAb clone 4G10 (Upstate Biotechnology, Lake Placid, NY). The plate is washed and wells are incubated with anti-mouse IgG/HRP conjugate (Amersham International plc, Buckinghamshire, England) for 60 minutes. Wells are washed and phosphotyrosine is quantitated by addition of 100 µl per well of 3,3',5,5' tetramethylbenzidine (Kirkegaard and Perry, TMB 1 Component Stop Solution).

Optical densities (OD) are determined spectrophotometrically at 450 mm in a 96-well plate reader (SpectraMax 250, Molecular Devices). Background (no VEGF added) OD values are subtracted from all Ods and percent inhibition is calculated according to the equation:

$$\% \text{ Inhibition} = \frac{(OD(\text{VEGF control}) - OD(\text{with test compound}) \times 100}{OD(\text{VEGF control}) - OD(\text{no VEGF added})}$$

IC_{50s} are determined on some of the exemplary materials with at least squares analysis program using compound concentration versus percent inhibition.

Matrigel® Angiogenesis Model:

Preparation of Martigel Plugs and *in vivo* Phase: Matrigel® (Collaborative Biomedical Products, Bedord, MA) is a basement membrane extract from a murine tumor composed primarily of laminin, collagen IV and heparan sulfate proteoglycan. It is provided as a sterile liquid at 4°C, but rapidly forms a solid gel at 37°C.

Liquid Matrigel at 4°C is mixed with SK-MEL2 human tumor cells that are transfected with a plasmid containing the murine VEGF gene with a selectable marker. Tumor cells are grown *in vitro* under selection and cells are mixed with cold liquid Matrigel at a ratio of 2 X 10^6 per 0.5 ml. One half milliliter is implanted subcutaneously near the abdominal midline using a 25 gauge needle. Test compounds are dosed as solutions in Ethanol/Ceremaphor EL/saline (12.5%:12.5%:75%) at 30, 100, and 300 mg/kg po once daily starting on the day of implantation. Mice are euthanized 12 days post-implantation and the Matrigel pellets are harvested for analysis of hemoglobin content.

Hemoglobin Assay: The Matrigel pellets are placed in 4 volumes (w/v) of 4°C Lysis Buffer (20mM Tris pH 7.5, 1mM EGTA, 1mM EDTA, 1% Triton X-100 [EM Science, Gibbstown, N.J.], and complete EDTA-free protease inhibitor cocktail [Mannheim, Germany]), and homogenized at 4°C. homogenates are incubated on ice for 30 minutes with shaking and centrifuged at 14K x g for 30 minutes at 4°C. Supernatants are transferred to chilled microfuge tubes and stored at 4°C for hemoglobin assay.

Mouse hemoglobin (Sigma Chemical Co., St. Louis, MO) is suspended in autoclaved water (BioWhittaker, Inc, Walkersville, MD.) at 5 mg/ml. A standard curve is generated from 500 micrograms/ml to 30 micrograms/ml in Lysis Buffer (see above). Standard curve and lysate samples are added at 5 microliters/well in duplicate to a polystyrene 96-well plate. Using the Sigma Plasma Hemoglobin Kit (Sigma Chemical Co., St. Louis, MO), TMB substrate is reconstituted in 50 mls room temperature acetic acid solution. One hundred microliters of substrate is added to each well, followed by 100 microliters/well of Hydrogen Peroxide Solution at room temperature. The plate is incubated at room temperature for 10 minutes.

Optical densities are determined spectrophotometrically at 600 nm in a 96-well plate reader, SpectraMax 250 Microplate Spectrophotometer System (Molecular Devices, Sunnyvale, CA). Background Lysis Buffer readings are subtracted from all wells.

Total sample hemoglobin content is calculated according to the following equation:

Total Hemoglobin = (Sample Lysate Volume) x (Hemoglobin Concentration)

The average Total Hemoglobin of Matrigel samples without cells is subtracted from each Total Hemoglobin Matrigel sample with cells. Percent inhibition is calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{\text{(Average Total Hemoglobin Drug-Treated Tumor Lysates) X 100}}{\text{(Average Total Hemoglobin Non-Treated Tumore Lysates).}}$$

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various conditions and usages.

WHAT IS CLAIMED IS:

1. A method for treating or preventing a disease in a human or other mammal regulated by tyrosine kinase,(associated with an aberration in the tyrosine kinase signal transduction pathway) comprising administering to a human or other mammal in need thereof a compound of Formula I, a salt form of a compound of Formula I, an isomer of a compound of Formula I or a prodrug of a compound of Formula I

A-NH-C(O)-NH-B I

wherein A is selected from the group consisting of

(i) phenyl, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano, and nitro;

(ii) naphthyl, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano, and nitro;

(iii) 5 and 6 membered monocyclic heteroaryl groups, having 1-3 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano, and nitro; and

(iv) 8 to 10 membered bicyclic heteroaryl group in which the first ring is bonded to the NH of Figure I and contains 1-3 heteroatoms independently selected from the group consisting of O, N, and S, and the second ring is fused to the first ring

using 3 to 4 carbon atoms. The bicyclic heteroaryl group is optionally substituted with 1-3 substituients independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano, and nitro,

B is selected from the group consisting of

(i) phenyl, optionally substituted with 1-3 substituents independently selected from the group consisting of –L-M, C_1-C_5 linear or branched alkyl, C_1-C_5 linear or branched haloalkyl, C_1-C_3 alkoxy, hydroxy, amino, C_1-C_3 alkylamino, C_1-C_6 dialkylamino, halogen, cyano, and nitro;

(ii) naphthyl, optionally substituted with 1-3 substituents independently selected from the group consisting of –L-M, C_1-C_5 linear or branched alkyl, C_1-C_5 linear or branched haloalkyl, C_1-C_3 alkoxy, hydroxy, amino, C_1-C_3 alkylamino, C_1-C_6 dialkylamino, halogen, cyano, and nitro;

(iii) 5 and 6 membered monocyclic heteroaryl groups, having 1-3 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of –L-M, C_1-C_5 linear or branched alkyl, C_1-C_5 linear or branched haloalkyl, C_1-C_3 alkoxy, hydroxy, amino, C_1-C_3 alkylamino, C_1-C_6 dialkylamino, halogen, cyano, and nitro; and

(iv) 8 to 10 membered bicyclic heteroaryl groups having 1-6 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of –L-M, C_1-C_5 linear or branched alkyl, C_1-C_5 linear or branched haloalkyl, C_1-C_3 alkoxy, hydroxy, amino, C_1-C_3 alkylamino, C_1-C_6 dialkylamino, halogen, cyano, and nitro.

L is selected from the group consisting of :

(a) $-(CH_2)_m-O-(CH_2)_l-$,

(b) $-(CH_2)_m-(CH_2)_l-$,

(c) $-(CH_2)_m-C(O)-(CH_2)_l-$,

(d) $-(CH_2)_m-NR^3-(CH_2)_l-$,

(e) $-(CH_2)_m- NR^3C(O)-(CH_2)_l-$,

(f) $-(CH_2)_m-S-(CH_2)_l-$,

(g) $-(CH_2)_m-C(O)NR^3-(CH_2)_l-$,

(h) $-(CH_2)_m-CF_2-(CH_2)_l-$,

(i) $-(CH_2)_m-CCl_2-(CH_2)_l-$,

(j) $-(CH_2)_m-CHF-(CH_2)_l-$,

(k) $-(CH_2)_m-CH(OH)-(CH_2)_l-$;

(l) $-(CH_2)_m-C\equiv C-(CH_2)_l-$;

(m) $-(CH_2)_m-C=C-(CH_2)_l-$; and

(n) a single bond, where m and l are 0.;

(o) $-(CH_2)_m-CR^4R^5-(CH_2)_l-$;

wherein the variables m and l are integers independently selected from 0-4,

M is selected from the group consisting of :

(i) phenyl, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro;

(ii) naphthyl, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro;

(iii) 5 and 6 membered monocyclic heteroaryl groups, having 1-3 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro and also oxides (e.g. =O, $-O^-$ or –OH); and

(iv) 8 to 10 membered bicyclic heteroaryl groups, having 1-6 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro and also oxides (e.g. =O, $-O^-$ or –OH).

(v) saturated and partially saturated C_3-C_6 monocyclic carbocyclic moiety optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and, nitro;

(vi) saturated and partially saturated C_8-C_{10} bicyclic carbocyclic moiety, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro;

(vii) saturated and partially saturated 5 and 6 membered monocyclic heterocyclic moiety, having 1-3 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro, and also oxides (e.g. =O, $-O^-$ or –OH); and

(viii) saturated and partially saturated 8 to 10 membered bicyclic heterocyclic moiety, having 1-6 heteroatoms independently selected from the group consisting of O, N and S, optionally substituted with 1-3 substituents independently selected from the group consisting of R^1, OR^1, NR^1R^2, $S(O)_qR^1$, $SO_2NR^1R^2$, $NR^1SO_2R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$, $NR^1C(O)R^2$, $NR^1C(O)OR^2$, halogen, cyano and nitro, and also oxides (e.g. =O, $-O^-$ or –OH);

wherein each R^1 - R^5 is independently selected from the group consisting of:

(a) hydrogen,

(b) C_1-C_6 alkyl, preferably, C_1-C_5 linear, branched, or cyclic alkyl, wherein said alkyl is optionally substituted with halogen up to per-halo;

(c) phenyl;

(d) 5-6 membered monocyclic heteroaryl having 1-4 heteroatoms selected from the group consisting of O, N and S or 8-10 membered bicyclic heteroaryl having 1-6 heteroatoms selected from the group consisting of O, N and S;

(e) C_1-C_3 alkyl-phenyl wherein said alkyl moiety is optionally substituted with halogen up to per-halo; and

(f) C_1-C_3 alkyl-heteroaryl having 1-4 heteroatoms selected from the group consisting of O, N and S, wherein said heteroaryl group is a 5-6 membered monocyclic heteroaryl or a 8-10 membered bicyclic heteroaryl, and wherein said alkyl moiety is optionally substituted with halogen up to per-halo,

wherein each R^1 - R^5, when not hydrogen is optionally substituted with 1-3 substituents independently selected from the group consisting of C_1-C_5 linear branched or cyclic alkyl, wherein said alkyl is optionally substituted with halogen up to per-halo, C_1-C_3 alkoxy, wherein said alkoxy is optionally substituted with halogen up to per-halo, hydroxy, amino, C_1-C_3 alkylamino, C_2-C_6 dialkylamino, halogen, cyano, and nitro; and

each variable q is independently selected from 0, 1, or 2.

2. A method of claim 1 wherein A, B, and M are each, independently,

(i) a substituted or unsubstituted monocyclic heteroaryl group selected from the group consisting of :

2- and 3-furyl, 2- and 3-thienyl, 2- and 4-triazinyl, 1-, 2- and 3-pyrrolyl, 1-, 2-, 4- and 5-imidazolyl, 1-, 3-, 4- and 5-pyrazolyl, 2-, 4- and 5-oxazolyl, 3-, 4- and 5-isoxazolyl, 2-, 4- and 5-thiazolyl, 3-, 4- and 5-isothiazolyl, 2-, 3- and 4-pyridyl, 2-, 4-, 5- and 6-pyrimidinyl, 1,2,3-triazol-1-, -4- and –5-yl, 1,2,4-triazol-1-, -3- and –5-yl, 1- and 5-tetrazolyl, 1,2,3-oxadiazol-4- and –5-yl, 1,2,4-oxadiazol-3- and –5-yl, 1,3,4-thiadiazol-2- and –5-yl, 1,2,4-oxadiazol-3- and –5-yl, 1,3,4-thiadiazol-2- and –5-yl, 1,3,4-thiadiazol-3- and –5-yl, 1,2,3-thiadiazol-4- and –5-yl, 2-, 3-, 4-, 5- and 6-2H-thiopyranyl, 2-, 3- and 4-4H-thiopyranyl, 3- and 4-pyridazinyl, 2-,3-pyrazinyl,

(ii) a substituted or unsubstituted bicyclic heteroaryl groups selected from the group consisting of :

benzofuryl, benzothienyl, indolyl, benzimidazolyl, benzopyrazolyl, benzoxazolyl, benzisoxazolyl, benzothiazolyl, benzisothiazolyl, benz-1,3-oxadiazolyl, quinolinyl, isoquinolinyl, quinazolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, dihydrobenzofuryl, pyrazolo[3,4-b]pyrimidinyl, purinyl, benzodiazine, pterindinyl, pyrrolo[2,3-b]pyridinyl, pyrazolo[3,4-b]pyridinyl, oxazo[4,5-b]pyridinyl, imidazo[4,5-b]pyridinyl, cyclopentenopyridine, cyclohexanopyridine, cyclopentanopyrimidine, cyclohexanopyrimidine, cyclcopentanopyrazine, cyclohexanopyrazine, cyclopentanopyridiazine, cyclohexanopyridazine, cyclopentanoimidazole, cyclohexanoimidazole, cyclopentanothiophene and cyclohexanothiophene, or

(iii) a substituted or unsubstituted aryl group without heteroatoms selected from the group consisting of:

phenyl, 1-naphthyl, 2-naphthyl, tetrahydronaphthyl, indanyl, indenyl, benzocyclobutanyl, benzocycloheptanyl and benzocycloheptenyl.

3. A method as in claim 2 wherein the substituents on the groups for A, B, and M are selected from the group consisting of: methyl, ethyl, propyl, butyl, pentyl, isopropyl, isobutyl, *sec*-butyl, and *tert*-butyl, F, Cl, Br, and I.

4. A method of claim 1 wherein

the saturated monocyclic and bicyclic carbocyclic moieties are selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and decahydronapthalene,

the partially saturated monocyclic and bicyclic carbocyclic moieties are selected from the group consisting of cyclopentenyl, cyclohexenyl, cyclohexadienyl and tetrahydronaphthalene,

the saturated monocyclic and bicyclic heterocyclic moieties are selected from the group consisting of include tetrahydropyranyl, tetrahydrofuranyl, 1,3-dioxolane, 1,4-dioxanyl, morpholinyl, thiomorpholinyl, piperazinyl, piperidinyl, piperidinonyl, tetrahydropyrimidonyl, pentamethylene sulfide and tetramethylene sulfide and

the partially saturated monocyclic and bicyclic heterocyclic moieties are selected from the group consisting of dihydropyranyl, dihydrofuranyl, dihydrothienyl, dihydropiperidinyl, and dihydropyrimidonyl.

5. A method of claim 1 wherein the structures of A, B and M are each independently selected from the group consisting of optionally substituted phenyl, naphthyl, furyl, isoindolinyl, oxadiazolyl, oxazolyl, isooxazolyl, pyrazolyl, pyridinyl, pyrimidinyl, pyrrolyl, quinolinyl, isoquinolinyl, tetrazolyl, thiadiazolyl, thiazolyl and thienyl.

6. A method of claim 5 wherein the substituents of the substituted structures of B are each, independently, selected from the group consisting of methyl, trifluoromethyl, ethyl, n-propyl, n-butyl, n-pentyl, isopropyl, isobutyl, sec-butyl, tert-butyl, cyclopropyl, cyclobutyl, cyclopentyl, methoxy, ethoxy, propoxy, Cl, Br and F, cyano, nitro, hydroxy, amino, methylamino, dimethylamino, ethylamino, diethylamino and the structure -L-M.

7. A method of claim 5 wherein the substituents of the substituted structures of A and M are each, independently, selected from the group consisting of

methyl, trifluoromethyl, ethyl, n-propyl, n-butyl, n-pentyl, isopropyl, *tert*-butyl, sec-butyl, isobutyl, cyclopropyl, cyclobutyl, cyclopentyl, methoxy, ethoxy, propoxy, Cl, Br and F, cyano, nitro, hydroxy, amino, methylamino, dimethylamino, ethylamino and diethylamino and further include:

phenyl, pyridinyl, pyrimidinyl, chlorophenyl, dichlorophenyl, bromophenyl, dibromophenyl, chloropyridinyl, bromopyridinyl, dichloropyridinyl, dibromopyridinyl methylphenyl, methylpyridinyl quinolinyl, isoquinolinyl, isoindolinyl, pyrazinyl, pyridazinyl, pyrrolinyl, imidazolinyl, thienyl, furyl, isoxazolinyl, isothiazolinyl, benzopyridinyl, benzothiazolyl,

C_1-C_5 acyl;

NH(C_1-C_5 alkyl, phenyl or pyridinyl), such as aminophenyl;

N(C_1-C_5 alkyl)(C_1-C_5 alkyl, phenyl or pyridinyl), such as diethylamino and dimethyl amino;

$S(O)_q$ (C_1-C_5 alkyl); such as methanesulfonyl;

$S(O)_q$ H;

SO_2NH_2;

$SO_2NH(C_1$-C_5 alkyl);

$SO_2N(C_1$-C_5 alkyl)(C_1-C_5 alkyl);

$NHSO_2(C_1$-C_5 alkyl); $N(C_1$-C_3 alkyl) $SO_2(C_1$-C_5 alkyl);

$CO(C_1$-C_6 alkyl or phenyl);

C(O)H;

$C(O)O(C_1$-C_6 alkyl or phenyl), such as $C(O)OCH_3$, $-C(O)OCH_2CH_3$, $-C(O)OCH_2CH_2CH_3$;

C(O)OH;

$C(O)NH_2$ (carbamoyl);

C(O)NH(C_1-C_6 alkyl or phenyl), such as N-methylethyl carbamoyl, N-methyl carbamoyl, N-ethylcarbamoyl, or N-dimethylamino ethyl carbamoyl;

C(O)N(C_1-C_6 alkyl or phenyl)(C_1-C_6 alkyl, phenyl or pyridinyl), such as N-dimethyl carbamoyl;

C(N(C_1-C_5 alkyl)) (C_1-C_5 alkyl);

NHC(O)(C_1-C_6 alkyl or phenyl) and

N(C_1-C_5 alkyl,)C(O)(C_1-C_5 alkyl).

wherein each of the above substituents is optionally partially or fully halogenated.

8. A method as in claim 1 wherein A, B or M of formula I or a combination thereof are independently selected from the group consisting of substituted or unsubstituted phenyl, pyridinyl, naphthyl, quinolinyl and isoquinolinyl.

9. A method as in claim 1 wherein A, B or M of formula I or a combination thereof are independently a phenyl or pyridinyl group, optionally substituted by halogen up to per halo and 0 to 3 times by one or more substituents selected from the group consisting of -CN, C_1-C_5 alkyl, C_1-C_5 alkoxy, -OH, phenyl, up to per halo

substituted C_1-C_5 alkyl, up to per halo substituted C_1-C_5 alkoxy and up to per halo substituted phenyl.

10. A method as in claim 1 wherein A, B and M of formula I follow one of the following combinations:

A= phenyl, B=phenyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= phenyl, B=pyridinyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=phenyl, B = naphthyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=pyridinyl, B= phenyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=pyridinyl, B= pyridinyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=pyridinyl, B= naphthyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A=isoquinolinyl, B= phenyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= isoquinolinyl, B= pyridinyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= isoquinolinyl, B= naphthyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= quinolinyl, B= phenyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= quinolinyl, B= pyridinyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present,

A= quinolinyl, B= naphthyl and M is phenyl, pyridinyl, quinolinyl, isoquinolinyl or not present.

11. A method as in claim 10 wherein L of formula I is -O-, a single bond, -S-, -NH-, -N(CH_3)-, -NHCH_2-, - NC_2H_4-, -CH_2-, -C(O)-, -CH(OH)-, -NHC(O)N(CH_3)CH_2-, -N(CH_3)C(O)N(CH_3)CH_2-, -CH_2C(O)N(CH_3)-, -C(O)N(CH_3)CH_2-, -NHC(O)-, -N(CH_3)C(O)-, -C(O)N(CH_3)-, -C(O)NH-, -CH_2O-, -CH_2S-, -CH_2N(CH_3)-, -OCH_2-, -CHF-, -CF_2-,-CCl_2-, -S-CH_2- , and –N(CH_3)CH_2- .

12. A method as in claim 1 wherein the disease is mediated by the VEGF-induced signal transduction pathway .

13. A method as in claim 12 wherein disease mediated by the VEGF-induced signal transduction pathway that is treated is characterized by abnormal angiogenesis or hyperpermiability processes.

14. A method as in claim 13 wherein a compound of Formula I, a salt form of a compound of Formula I, an isolated stereo-isomer of a compound of Formula I or a prodrug of a compound of Formula I is administered simultaneously with another angiogenesis inhibiting agent to a patient with such a disorder in the same formulation or in separate formulations.

15. A method as in claim 1 wherein the disease that is treated is one or more of the following conditions in humans and/or other mammals: tumor growth, retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, a bolos disorder associated with subepidermal blister formation, including bullous pemphigoid, erythema multiforme, or dermatitis herpetiformis.

16. A method as in claim 1 wherein the disease that is treated is one or more of the following conditions in humans and/or other mammals: tumor growth,

retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, a bullous disorder associated with subepidermal blister formation, including bullous pemphigoid, erythema multiforme, or dermatitis herpetiformis in combination with another condition selected from the group consisting of:

rheumatic fever, bone resorption, postmenopausal osteoporosis, sepsis, gram negative sepsis, septic shock, endotoxic shock, toxic shock syndrome, systemic inflammatory response syndrome, inflammatory bowel disease (Crohn's disease and ulcerative colitis), Jarisch-Herxheimer reaction, asthma, adult respiratory distress syndrome, acute pulmonary fibrotic disease, pulmonary sarcoidosis, allergic respiratory disease, silicosis, coal worker's pneumoconiosis, alveolar injury, hepatic failure, liver disease during acute inflammation, severe alcoholic hepatitis, malaria (Plasmodium falciparum malaria and cerebral malaria), non-insulin-dependent diabetes mellitus (NIDDM), congestive heart failure, damage following heart disease, atherosclerosis, Alzheimer's disease, acute encephalitis, brain injury, multiple sclerosis (demyelation and oligiodendrocyte loss in multiple sclerosis), advanced cancer, lymphoid malignancy, pancreatitis, impaired wound healing in infection, inflammation and cancer, myelodysplastic syndromes, systemic lupus erythematosus, biliary cirrhosis, bowel necrosis, radiation injury/ toxicity following administration of monoclonal antibodies, host-versus-graft reaction (ischemia reperfusion injury and allograft rejections of kidney, liver, heart, and skin), lung allograft rejection (obliterative bronchitis) and complications due to total hip replacement.

17. A method as in claim 1 wherein the disease that is treated is one or more of the following conditions in humans and/or other mammals:

tumor growth, retinopathy, diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, bullous disorder associated with subepidermal blister formation, bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis,

in combination with an infectious disease selected from the group consisting of:

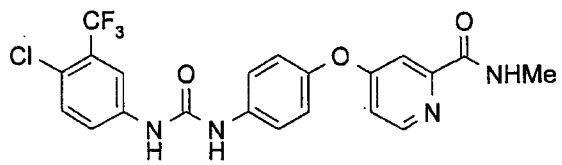
tuberculosis, Helicobacter pylori infection during peptic ulcer disease, Chaga's disease resulting from Trypanosoma cruzi infection, effects of Shiga-like toxin resulting from E. coli infection, effects of enterotoxin A resulting from Staphylococcus infection, meningococcal infection, and infections from Borrelia burgdorferi, Treponema pallidum, cytomegalovirus, influenza virus, Theiler's encephalomyelitis virus, and the human immunodeficiency virus (HIV).

18. A method as in claim 1, wherein M is substituted by at least one substituent selected from the group consisting of $S(O)_qR^1$, $SO_2NR^1R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$,
wherein q, R^1 and R^2 are independently as defined in claim 1.

19. A method as in claim 1, wherein M is substituted by at least one substituent selected from the group consisting of $-C(O)R^1$, $C(O)OR^1$, and $C(O)NR^1R^2$, wherein R^1 and R^2 are independently as defined in claim 1.

20. A method of claim 1 wherein M is substituted by $-C(O)\ NR^1R^2$, wherein R^1 and R^2 are independently as defined in claim 1.

21. A method of treating diseases mediated by the VEGF-induced signal transduction pathway comprising administering the compound N-(4-chloro-3-(trifluoromethyl)phenyl)-N'-(4-(2-(N-methylcarbamoyl)-4-pyridyloxy)phenyl) urea of the formula below or a pharmaceutically acceptable salt thereof



22. A method of treating diseases mediated by the VEGF-induced signal transduction pathway comprising administering the compound N-(4-chloro-3-(trifluoromethyl)phenyl)-N'-(4-(2-(N-methylcarbamoyl)-4-pyridyloxy)phenyl) urea tosylate.

23. A method of claim 1 wherein the structures of A, B and M are each, independently selected from the group consisting of phenyl, substituted phenyl, pyridinyl, substituted pyridinyl, pyrimidinyl, substituted pyrimidinyl, naphthyl, substituted naphthyl, isoquinolinyl, substituted isoquinolinyl, quinolinyl and substituted quinolinyl.

24. A method as in claim 23, wherein M is substituted by at least one substituent selected from the group consisting of $S(O)_qR^1$, $SO_2NR^1R^2$, $C(O)R^1$, $C(O)OR^1$, $C(O)NR^1R^2$,
wherein q, R^1 and R^2 are independently as defined in claim 1.

25. A method of claim 24 wherein M is additionally substituted 1 to 3 times by one or more substituents selected from the group consisting of C_1-C_{10} alkyl, up to per halo substituted C_1-C_{10} alkyl, -CN, -OH, halogen, C_1-C_{10} alkoxy and up to per halo substituted C_1-C_{10} alkoxy.

26. A method as in claim 1 wherein L of formula I is -O-, a single bond, -S-, -NH-, $-N(CH_3)-$, $-NHCH_2-$, $-NC_2H_4-$, $-CH_2-$, -C(O)-, -CH(OH)-, $-NHC(O)N(CH_3)CH_2-$, $-NCH_3C(O)N(CH_3)CH_2-$, $-CH_2C(O)N(CH_3)-$, $C(O)N(CH_3)CH_2-$, -NHC(O)-, $-N(CH_3)C(O)-$, $-C(O)N(CH_3)-$, -C(O)NH-, $-CH_2O-$, $-CH_2S-$, $-CH_2N(CH_3)-$, $-OCH_2-$, -CHF-, $-CF_2-$, $-CCl_2-$, $-S-CH_2-$ or $-N(CH_3)CH_2-$.

27. A method of claim 1 wherein L of formula I is selected from the group consisting of -O-, -S-, $-N(R^{35})-$, $-(CH_2)_m-$, -C(O)-, -CH(OH)-, $-(CH_2)_mO$, where m= 1-3 and R^{35} is hydrogen, C_1-C_{10} alkyl, up to per halo substituted C_1-C_{10} alkyl, -CN, -OH, halogen, C_1-C_{10} alkoxy or up to per halo substituted C_1-C_{10} alkoxy.

28. A method of claim 1 wherein M is substituted by $-C(O)NR^1R^2$ and R^1 and R^2 are as defined in claim 1.

29. A method of claim 1 wherein M is

a saturated C_3-C_6 monocyclic carbocyclic moiety selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentanyl, and cyclohexanyl;

a saturated C_8-C_{10} bicyclic carbocyclic moiety selected from the group consisting of bicyclopentanyl and bicyclohexanyl;

a partially saturated C_3-C_6 monocyclic carbocyclic moiety selected from the group consisting of cyclopentenyl, cyclohexenyl and cyclohexadienyl;

the partially saturated C_8-C_{10} bicyclic carbocyclic moiety bicyclohexenyl;

a substituted naphthyl group selected from benzocyclobutanyl, indanyl, indenyl, dihydronaphthyl and tetrahydronaphthyl; or

an 8 to 10 membered bicyclic heteroaryl group selected from cyclopentenopyridine, cyclohexanopyridine, cyclopentanopyrimidine, cyclohexanopyrimidine, cyclcopentanopyrazine, cyclohexanopyrazine, cyclopentanopyridiazine, cyclohexanopyridazine, cyclopentanothiophene and cyclohexanothiophene.

30. A method as in claim 12 wherein the disease that is treated or prevented is a KDR-mediated disorder.

31. A method as in claim 12 wherein the disease that is treated or prevented is a Flk-1 mediated disorder.

32. A method of regulating ***tyrosine kinase*** signal transduction comprising administering to a human or other mammal one or more compounds of claim 1.

Exhibit 2

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(54) Title: TOLUENESULFONATE SALTS OF A THIAZOLIDINEDIONE DERIVATIVE

(57) Abstract: A 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione Toluenesulphonate salt, a process for preparing such a salt, a pharmaceutical composition containing such as salt and the use of such a salt in medicine.

TOLUENESULFONATE SALTS OF A THIAZOLIDINEDIONE DERIVATIVE

This invention relates to a novel pharmaceutical, to a process for the preparation of the pharmaceutical and to the use of the pharmaceutical in medicine.

EP-A-0 306 228 relates to certain thiazolidinedione derivatives disclosed as having hypoglycaemic and hypolipidaemic activity. The compound of Example 30 of EP-A-0 306 228 is 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione (hereinafter referred to as "Compound (I)").

WO 94/05659 discloses certain salts of the compounds of EP-A-0 306 228. The preferred salt of WO 94/05659 is the maleic acid salt.

There remains a need for alternative salt forms which have properties suitable for pharmaceutical processing on a commercial scale.

We have now prepared and characterised a para-toluenesulfonate salt of Compound (I) (hereinafter also referred to as the "Toluenesulfonate") and discovered that the toluenesulfonate salt is particularly stable and hence is suitable for bulk preparation and handling. The novel Toluenesulfonate possesses good bulk flow properties and is a high melting crystalline material and hence is amenable to large scale pharmaceutical processing, especially in manufacturing processes which require or generate heat, for example milling, fluid bed drying, spray drying, hot melt processing and sterilisation by autoclaving.

The novel salt can be prepared by an efficient and economic process particularly suited to large-scale preparation.

The novel toluenesulfonate also has useful pharmaceutical properties and in particular it is indicated to be useful for the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof.

Accordingly, the present invention provides a 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate salt as a novel compound.

The Toluenesulfonate has been isolated with a low (e.g. 0.2% by wt.) water content, which is consistent with regarding the isolated salt as an anhydrate (1 molar equivalent of H_2O = 3.3% by wt.)

Accordingly, the present invention also provides 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate anhydrate as a novel compound.

In one suitable embodiment, there is provided a Toluenesulfonate characterised by

(i) an infrared spectrum containing peaks at about 1755, 1706, 1301, 1224, 1170 cm^{-1}; and/or

(ii) a Raman spectrum containing peaks at about 3054, 2927, 1302, 1260, and 602 cm^{-1}; and/or

(iii) an X-ray powder diffraction (XRPD) pattern containing peaks at about 4.5, 4.7, 5.8, 9.2, 17.6 and 24.2 degrees 2θ; and/or

(iv) a Solid State ^{13}C NMR spectrum containing peaks at about 50.8, 53.5, 65.0, 155.5 ppm

In one favoured aspect, the Toluenesulfonate provides an infrared spectrum substantially in accordance with Figure 1.

In one favoured aspect, the Toluenesulfonate provides a Raman spectrum substantially in accordance with Figure 2.

In one favoured aspect, the Toluenesulfonate provides an X-Ray powder diffraction pattern (XRPD) substantially in accordance with Table 1 or Figure 3.

In one favoured aspect, the Toluenesulfonate provides a Solid State ^{13}C NMR spectrum substantially in accordance with Figure 4.

In a further favoured aspect, the Toluenesulfonate provides a melting point in the range of from 160 to 175 °C, such as 163 to 170°C, for example 166.7 °C .

In a preferred aspect, the invention provides 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione toluenesulfonate, characterised in that it provides:

(i) an infrared spectrum substantially in accordance with Figure 1; and

(ii) a Raman spectrum substantially in accordance with Figure 2; and

(iii) an X-Ray powder diffraction pattern (XRPD) substantially in accordance with Table 1 or Figure 3; and

(iv) a Solid State ^{13}C NMR spectrum substantially in accordance with Figure 4.

The present invention encompasses the Toluenesulfonate isolated in pure form or when admixed with other materials.

Thus in one aspect there is provided the Toluenesulfonate in isolated form.

In a further aspect there is provided the Toluenesulfonate in substantially pure form.

In yet a further aspect there is provided the Toluenesulfonate in crystalline form.

Also, the invention provides the Toluenesulfonate or solvate thereof in a solid pharmaceutically acceptable form, such as a solid dosage form, especially when adapted for oral administration.

Moreover, the invention also provides the Toluenesulfonate or solvate thereof in a pharmaceutically acceptable form, especially in bulk form, such form being particularly capable of pharmaceutical processing, especially in manufacturing processes which require or generate heat, for example milling; for example heat-drying especially fluid-bed drying

or a spray drying; for example hot melt processing; for example heat-sterilisation such as autoclaving.

Examples of manufacturing processes which require or generate heat include milling, heat-drying especially fluid-bed drying, spray drying or hot melt processing and heat-sterilisation such as autoclaving. Particular examples of manufacturing processes which require or generate heat include milling, heat-drying especially fluid-bed drying, spray drying and heat-sterilisation such as autoclaving.

Furthermore, the invention provides the Toluenesulfonate, or a solvate thereof, in a pharmaceutically acceptable form, especially in a bulk formand especially in a form having been processed in a manufacturing process requiring or generating heat, for example in a milled form; for example in a heat-dried form, especially a fluid-bed dried form or a spray dried form; for example in a form having being hot melt processed; for example in a form having being heat-sterilised by such as autoclaving.

Furthermore, the invention provides the Toluenesulfonate or solvate thereof in a pharmaceutically acceptable form, especially in bulk form, such form having good flow properties, especially good bulk flow properties.

Suitable texts decribing the manufacturing processes referred to herein include "The Theory and Practice of Industrial Pharmacy" edited by Leon Lachman, Herbert A. Lieberman and Joseph L. Kanig, published by Lea & Febiger and for spray drying and fluid bed drying Advanced Drying Technologies by Kudra, Tadeusz.; Mujumdar, A. S, New York Marcel Dekker, Inc., 2001.

The invention also provides a process for preparing the Toluenesulfonate, characterised in that 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione (Compound (I)) or a salt thereof, preferably dispersed or dissolved in a suitable solvent, is reacted with a suitable source of p-toluenesulfonate ion; and optionally thereafter as required:

(i) forming a solvate thereof;

(ii) recovering the Toluenesulfonate or solvate thereof ; or

(iii) further processing the Toluenesulfonate or solvate therof in a manufacturing process requiring or generating heat.

A suitable reaction solvent is an ether, for example tetrahydrofuran, or an alkanol such as propan-2-ol, a hydrocarbon, such as toluene, a ketone, such as acetone, an ester, such as ethyl acetate, a nitrile such as acetonitrile, or a halogenated hydrocarbon such as dichloromethane or an organic acid such as acetic acid; or a mixture thereof.

Conveniently, the source of p-toluenesulfonate ion is p-toluenesulfonic acid. The p-toluenesulfonic acid may be added as a solid or in solution, for example in ether, ketone, nitrile or a lower alcohol such as methanol, ethanol, or propan-2-ol, or a mixture of solvents.

For example, a solution of p-toluenesulfonic acid, in particular p-toluenesulfonic acid monohydrate, in tetrahydrofuran may be added to a solution of Compound (I) in the same solvent.

Typically the salt is formed under generally anhydrous conditions e.g. preferably < 1% water.

An alternative source of p-toluenesulfonate ion may be provided by a base salt of p-toluenesulfonic acid for example ammonium p-toluenesulfonate, or the p-toluenesulfonic acid salt of an amine, for example ethylamine or diethylamine.

The concentration of Compound (I) is preferably in the range 3 to 50% weight/volume, more preferably in the range 5 to 20%. The concentration of p-toluenesulfonic acid solutions are preferably in the range of 5 to 75% weight/volume.

The reaction is usually carried out at ambient temperature or at an elevated temperature, for example at about 60°C, although any convenient temperature that provides the required product may be employed.

As indicated above the Toluenesulfonate can exist as a solvate. Suitable solvates are pharmaceutically acceptable solvates, such as non-aqueous solvates

Solvates of the Toluenesulfonate may be prepared according to conventional procedures, for example by crystallising or recrystallising from a solvent which provides or contains the solvate moiety, or by exposing the Toluenesulfonate to the solvate moiety as a vapour. When the solvate is formed by crystallization methods the nature of the solvate is typically dictated by the solvent from which the Toluenesulfonate is crystallized. A Toluenesulfonate solvate, especially a non-aqeous solvate, is a favoured aspect of the invention.

Solvates of the Toluenesulfonate are prepared according to conventional procedures.

Recovery of the required compound generally comprises crystallisation from an appropriate solvent, conveniently the reaction solvent, usually assisted by cooling. For example, the Toluenesulfonate may be crystallised from an ether such as tetrahydrofuran. An improved yield of the salt can be obtained by evaporation of some or all of the solvent or by crystallisation at elevated temperature followed by controlled cooling. Careful control of precipitation temperature and seeding may be used to improve the reproducibility of the product form.

Suitable manufacturing processes requiring or generating heat include milling, heat-drying, especially a fluid-bed drying, hot melt processing or heat-sterilisation, such as autoclaving.

Compound (I) is prepared according to known procedures, such as those disclosed in EP-A-0 306 228 and WO 94/05659. The disclosures of EP-A-0 306 228 and WO 94/05659 are incorporated herein by reference.

Para-Toluenesulfonic acid is a commercially available compound.

When used herein the term "T_{onset}" is generally determined by Differential Scanning Calorimetry and has a meaning generally understood in the art, as for example expressed in "Pharmaceutical Thermal Analysis, Techniques and Applications", Ford and Timmins, 1989 as "The temperature corresponding to the intersection of the pre-transition baseline with the extrapolated leading edge of the transition".

When used herein in respect of certain compounds the term "good flow properties" is suitably characterised by the said compound having a Hausner ratio of less than or equal to 1.5, especially of less than or equal to 1.25. "Hausner ratio" is an art accepted term.

When used herein the term 'prophylaxis of conditions associated with diabetes mellitus' includes the treatment of conditions such as insulin resistance, impaired glucose tolerance, hyperinsulinaemia and gestational diabetes.

Diabetes mellitus preferably means Type II diabetes mellitus.

Conditions associated with diabetes include hyperglycaemia and insulin resistance and obesity. Further conditions associated with diabetes include hypertension, cardiovascular disease, especially atherosclerosis, certain eating disorders, in particular the regulation of appetite and food intake in subjects suffering from disorders associated with under-eating, such as anorexia nervosa, and disorders associated with over-eating, such as obesity and anorexia bulimia. Additional conditions associated with diabetes include polycystic ovarian syndrome and steroid induced insulin resistance.

The complications of conditions associated with diabetes mellitus encompassed herein includes renal disease, especially renal disease associated with the development of Type II diabetes including diabetic nephropathy, glomerulonephritis, glomerular sclerosis, nephrotic syndrome, hypertensive nephrosclerosis and end stage renal disease.

As mentioned above the compound of the invention has useful therapeutic properties. The present invention accordingly provides the Toluenesulfonate for use as an active therapeutic substance.

More particularly, the present invention provides the Toluenesulfonate for use in the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof.

The Toluenesulfonate may be administered *per se* or, preferably, as a pharmaceutical composition also comprising a pharmaceutically acceptable carrier. Suitable methods for formulating the Toluenesulfonate are generally those disclosed for Compound (I) in the above mentioned publications.

Accordingly, the present invention also provides a pharmaceutical composition comprising the Toluenesulfonate and a pharmaceutically acceptable carrier therefor.

The Toluenesulfonate is normally administered in unit dosage form.

The active compound may be administered by any suitable route but usually by the oral or parenteral routes. For such use, the compound will normally be employed in the form of a pharmaceutical composition in association with a pharmaceutical carrier, diluent and/or excipient, although the exact form of the composition will naturally depend on the mode of administration.

Compositions are prepared by admixture and are suitably adapted for oral, parenteral or topical administration, and as such may be in the form of tablets, capsules, oral liquid preparations, powders, granules, lozenges, pastilles, reconstitutable powders, injectable and infusable solutions or suspensions, suppositories and transdermal devices. Orally administrable compositions are preferred, in particular shaped oral compositions, since they are more convenient for general use.

Tablets and capsules for oral administration are usually presented in a unit dose, and contain conventional excipients such as binding agents, fillers, diluents, tabletting agents, lubricants, disintegrants, colourants, flavourings, and wetting agents. The tablets may be coated according to well known methods in the art.

Suitable fillers for use include cellulose, mannitol, lactose and other similar agents. Suitable disintegrants include starch, polyvinylpyrrolidone and starch derivatives such as sodium starch glycollate. Suitable lubricants include, for example, magnesium stearate. Suitable pharmaceutically acceptable wetting agents include sodium lauryl toluenesulfonate.

Solid oral compositions may be prepared by conventional methods of blending, filling, tabletting or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers. Such operations are, of course, conventional in the art.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example, almond oil, fractionated coconut oil, oily esters such as esters of glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

For parenteral administration, fluid unit dose forms are prepared containing a compound of the present invention and a sterile vehicle. The compound, depending on the vehicle and the concentration, can be either suspended or dissolved. Parenteral solutions are normally prepared by dissolving the active compound in a vehicle and filter sterilising

before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anaesthetic, preservatives and buffering agents are also dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum.

Parenteral suspensions are prepared in substantially the same manner except that the active compound is suspended in the vehicle instead of being dissolved and sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the active compound.

As is common practice, the compositions will usually be accompanied by written or printed directions for use in the medical treatment concerned.

The present invention further provides a method for the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof, in a human or non-human mammal which comprises administering an effective, non-toxic, amount of Toluenesulfonate or a pharmaceutically acceptable solvate thereof to a human or non-human mammal in need thereof.

Conveniently, the active ingredient may be administered as a pharmaceutical composition hereinbefore defined, and this forms a particular aspect of the present invention.

In a further aspect the present invention provides the use of Toluenesulfonate or a pharmaceutically acceptable solvate thereof for the manufacture of a medicament for the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof.

In the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof the Toluenesulfonate or a pharmaceutically acceptable solvate thereof may be taken in amounts so as to provide Compound (I) in suitable doses, such as those disclosed in EP 0,306,228, WO94/05659 or WO98/55122.

The unit dose compositions of the invention comprise the Toluenesulfonate or a pharmaceutically acceptable solvate thereof in an amount providing up to 12mg, including 1-12mg such as 2-12mg of Compound (I), especially 2-4mg, 4-8mg or 8-12mg of Compound (I), for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12mg of Compound (I). Thus in particular there is provided a pharmaceutical composition comprising the Toluenesulfonate or a pharmaceutically acceptable solvate thereof and a pharmaceutically acceptable carrier therefor, wherein the Toluenesulfonate or a pharmaceutically acceptable solvate thereof is present in an amount providing 1, 2, 4, 8, 12, 4 to 8 or 8 to 12mg of Compound (I); such as 1mg of Compound (I); such as 2mg of Compound (I); such as 4mg of Compound (I); such as 8mg of Compound (I); such as 12mg of Compound (I).

The invention also provides a pharmaceutical composition comprising the Toluenesulfonate or a pharmaceutically acceptable solvate thereof in combination with one or more other anti-diabetic agents and optionally a pharmaceutically acceptable carrier thereof.

The invention also provides a method for the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof, in a human or non-human mammal which comprises administering an effective, non-toxic, amount of the Toluenesulfonate or a pharmaceutically acceptable solvate thereof in combination with one or more other anti-diabetic agents.

In a further aspect the present invention provides the use of the Toluenesulfonate or a pharmaceutically acceptable solvate thereof in combination with one or more other anti-diabetic agents, for the manufacture of a medicament for the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof.

In the above mentioned treatments the administration of the Toluenesulfonate or a pharmaceutically acceptable solvate thereof and the other anti-diabetic agent or agents includes co-administration or sequential administration of the active agents.

Suitably in the above mentioned compositions, including unit doses, or treatments the Toluenesulfonate or a pharmaceutically acceptable solvate thereof is present in an amount providing up to 12mg, including 1-12mg, such as 2-12mg of Compound (I), especially 2-4mg, 4-8mg or 8-12mg of Compound (I), for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12mg of Compound (I) or 4 to 8 or 8 to 12 mg of Compound (I). Thus for example in the above mentioned compositions, including unit doses, or treatments the Toluenesulfonate or a pharmaceutically acceptable solvate thereof is present in an amount providing 1mg of Compound (I); the Toluenesulfonate or a pharmaceutically acceptable solvate thereof is present in an amount providing 2mg of Compound (I); the Toluenesulfonate or a pharmaceutically acceptable solvate thereof is present in an amount providing 3mg of Compound (I); the Toluenesulfonate or a pharmaceutically acceptable solvate thereof is present in an amount providing 4mg of Compound (I); or the Toluenesulfonate or a pharmaceutically acceptable solvate thereof is present in an amount providing 8mg of Compound (I).

The other antidiabetic agents are suitably selected from biguanides, sulphonylureas and alpha glucosidase inhibitors. The other antidiabetic agent is suitably a biguanide. The other antidiabetic agent is suitably a sulphonylurea. The other antidiabetic agent is suitably a alpha glucosidase inhibitor. Suitable antidiabetic agents are those disclosed in WO98/57649, WO98/57634, WO98/57635, WO98/57636, WO99/03477, WO99/03476. The contents of the above mentioned publications are incorporated herein by reference as if set out in full herein.

No adverse toxicological effects are indicated in the above mentioned treatments for the compounds of the invention.

The following examples illustrate the invention but do not limit it in any way.

Reference Preparation 1 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl] thiazolidine-2,4-dione *p*-toluenesulfonate monohydrate

A mixture of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione (2.6 g) and acetone (55 ml) was stirred and heated to reflux until a clear solution was observed. A solution of p-toluenesulfonic acid monohydrate (1.4 g) in acetone (10 ml) was added and the resulting mixture heated to reflux for 10 minutes and then cooled to 22°C. After standing at 22°C in an unsealed vessel for 17 days the product was collected to give a 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione *p*-toluensulfonate hydrate (4.2 g) as a crystalline solid.

^{1}H-NMR (d^6-DMSO): consistent with 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione *p*-toluenesulfonate hydrate.

Example 1 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl] thiazolidine-2,4-dione *p*-toluenesulfonate

A solution of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl] thiazolidine-2,4-dione (4.0 g) in tetrahydrofuran (50 ml) was heated to 60°C and *p*-toluenesulfonic acid monohydrate (2.15 g) in tetrahydrofuran (6 ml) was added. The reaction mixture was stirred at 60°C for 40 minutes and then cooled to 50°C. The mixture was seeded with the product of Reference Preparation 1 and stirred for a further 20 minutes before cooling to 21°C. The product was collected by filtration and dried under vacuum over phosphorus pentoxide to give 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl] thiazolidine-2,4-dione *p*-toluenesulfonate (5.4 g) as a white crystalline solid.

^{1}H-NMR (d^6-DMSO): consistent with 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione *p*-toluenesulfonate.

Water content (Karl Fisher): 0.2% wt/wt

Example 2 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl] thiazolidine-2,4-dione *p*-toluenesulfonate

A solution of *p*-toluensulfonic acid monohydrate (5.4 g) in tetrahydrofuran (18 ml) was added to a stirred solution of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]

thiazolidine-2,4-dione (10.0 g) in tetrahydrofuran (125 ml) at 60°C. The reaction mixture was stirred for 40 minutes at 60°C, cooled to 30°C with stirring for 1 hour and then cooled to 21°C. The white solid was collected by filtration, washed with tetrahydrofuran (50 ml) and dried under vacuum at 21°C for 30 minutes to give 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione *p*-toluenesulfonate (15.5 g) as a white crystalline solid.

Example 3 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl] thiazolidine-2,4-dione *p*-toluenesulfonate

A solution of *p*-toluensulfonic acid monohydrate (3.19 g) in acetone (5 ml) was added to a stirring solution of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl] thiazolidine-2,4-dione(6.0 g) in acetone (90 ml) at reflux. The reaction was heated at reflux for 25 minutes, then cooled to 21°C with stirring for 45 minutes. The white solid was collected by filtration, washed with acetone (25 ml), dried under vacuum for 15.5 hours at 21°C to give 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione *p*-toluenesulfonate (8.6 g) as a white crystalline solid.

Characterising data recorded for the product of Example 1

The infrared absorption spectrum of a mineral oil dispersion of the product was obtained using a Nicolet 710 FT-IR spectrometer at 2 cm^{-1} resolution (**Figure 1**). Data were digitised at 1 cm^{-1} intervals. Bands were observed at: 3113, 2925, 2853, 1755, 1706, 1643, 1619, 1546, 1511, 1465, 1418, 1377, 1330, 1301, 1224, 1196, 1181, 1170, 1121, 1052, 1032, 1009, 817, 772, 721, 711, 682, 570, 565 cm^{-1}.

The infrared spectrum of the solid product was recorded using Perkin-Elmer Spectrum One FT-IR spectrometer fitted with a universal ATR accessory. Bands were observed at: 3116, 3036, 2774, 1755, 1703, 1643, 1618, 1547, 1511, 1467, 1418, 1366, 1329, 1301, 1224, 1195, 1168, 1121, 1053, 1032, 1009, 997, 932, 905, 816, 770, 738, 709, 681 cm^{-1}.

The Raman spectrum of the product (**Figure 2**) was recorded with the sample in an NMR tube using a Nicolet 960 E.S.P. FT-Raman spectrometer, at 4 cm^{-1} resolution with excitation from a Nd:V04 laser (1064 nm) with a power output of 400mW. Bands were observed at 3109, 3077, 3054, 2927, 2907, 1754, 1609, 1547, 1467, 1441, 1419, 1387, 1332, 1302, 1283, 1260, 1239, 1211, 1180, 1125, 1036, 1011, 984, 931, 914, 839, 820, 801, 775, 741, 711, 683, 653, 637, 619, 602, 565, 536, 470, 395, 345, 296, 233, 83 cm^{-1}.

The X-Ray Powder Diffractogram pattern of the product (**Figure 3**) was recorded using the following acquisition conditions: Tube anode: Cu, Generator tension: 40 kV, Generator current: 40 mA, Start angle: 2.0 °2θ, End angle: 35.0 °2θ, Step size: 0.02 °2θ, Time per step: 2.5 seconds.Characteristic XRPD angles and relative intensities are recorded in Table 1.

Table 1

Angle	Rel. Intensity
2-Theta °	%
2.3	4.4
4.5	28.5
4.7	16.6
5.8	21.6
7.2	6.6
9.0	12.8
9.2	16.2
9.7	21.0
11.7	100.0
13.5	17.2
14.1	13.6
14.4	20.4
15.2	28.5
15.9	21.6
16.6	14.2
17.6	56.5
18.1	20.8
18.6	66.7
19.4	23.4
20.7	34.1
21.0	63.5
21.6	57.5
22.1	53.7
23.5	31.9
24.2	62.7
24.4	41.7
25.4	27.5
25.7	42.9
26.5	70.1
27.1	36.9
28.4	26.9

29.5	35.1
30.0	30.1
33.5	19.2

The solid-state NMR spectrum of the product (**Figure 4**) was recorded on a Bruker AMX360 instrument operating at 90.55 MHz: The solid was packed into a 4 mm zirconia MAS rotor fitted with a Kel-F cap and rotor spun at ca.10 kHz. The ^{13}C MAS spectrum was acquired by cross-polarisation from Hartmann-Hahn matched protons (CP contact time 3 ms, repetition time 15 s) and protons were decoupled during acquisition using a two-pulse phase modulated (TPPM) composite sequence. Chemical shifts were externally referenced to the carboxylate signal of glycine at 176.4 ppm relative to TMS and were observed at: 21.7, 23.0, 37.2, 39.7, 40.7, 50.8, 53.5, 65.0, 111.0, 113.0, 114.7, 117.8, 126.1, 130.5, 135.3, 141.6, 143.8, 146.5, 149.7, 151.9, 155.5, 169.4, 175.0 ppm.

Properties of the *p*-Toluenesulfonate, recorded for the product of Example 2

Solid State Stability of the *p*-Toluenesulfonate

The solid state stability of the salt was determined by storing approximately 1.0 g of the material in a glass bottle at a) 40°C / 75% Relative Humidity (RH), open exposure, for 1 month and b) at 50°C, closed, for 1 month. The material was assayed by HPLC for final content and degradation products in both cases.

a) 40°C / 75% RH: No significant degradation observed (HPLC assay 98% initial).

b) 50°C: No significant degradation observed (HPLC assay 98% initial).

Solubility of the *p*-Toluenesulfonate

The solubility of the salt was determined by adding water in aliquots from 1 to 1000 ml to approximately 100 mg of the salt until the powder had dissolved. The visual solubility was confirmed by an HPLC assay of a saturated solution.

Solubility: 0.8 mg/ml.

Flow Properties of the *p*-Toluenesulfonate

The ratio between the bulk density and the tapped bulk density (Hausner Ratio) of the salt was determined using standard methods ("Pharmaceutics - The Science of Dosage Form Design", editor M. Aulton, 1988, published by:Churchill Livingstone).

Hausner Ratio: 1.1

Melting Point of the *p*-Toluenesulfonate

The melting point of the salt the was determined according to the method described in the U.S. Pharmacopoeia, USP 23, 1995, <741> "Melting range or temperature, Procedure for Class Ia", using a Buchi 545 melting point instrument.

Melting Point: 166.7°C

T_{onset} of the *p*-Toluenesulfonate

The T_{onset} of the salt was determined by Differential Scanning Calorimetry using a Perkin-Elmer DSC7 apparatus.

T_{onset} (10°C/minute, closed pan): 161.7 °C

CLAIMS

1. A 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate salt (the Toluensulphonate), or a non-aqueous solvate thereof.

2. A salt according to claim 1, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate anhydrate.

3. A salt according to claim 1 or claim 2, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate, characterised by an infrared spectrum containing peaks at about 1755, 1706, 1301, 1224, 1170 cm^{-1}.

4. A salt according to any one of claims 1 to 3, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate, characterised by a Raman spectrum containing peaks at about 3054, 2927, 1302, 1260, and 602 cm^{-1}.

5. A salt according to any one of claims 1 to 4, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate, characterised by an X-ray powder diffraction (XRPD) pattern containing peaks at about 4.5, 4.7, 5.8, 9.2, 17.6 and 24.2 degrees 2θ.

6. A salt according to any one of claims 1 to 5, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate, characterised by a Solid State ^{13}C NMR spectrum containing peaks at about 50.8, 53.5, 65.0, 155.5 ppm

7. A salt according to any one of claims 1 to 6, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate, characterised by an infrared spectrum substantially in accordance with Figure 1.

8. A salt according to any one of claims 1 to 6, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate, characterised by a Raman spectrum substantially in accordance with Figure 2.

9. A salt according to any one of claims 1 to 8, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate, characterised by an X-Ray powder diffraction pattern (XRPD) substantially in accordance with Table 1 or Figure 3.

10. A salt according to any one of claims 1 to 9, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate, characterised by a Solid State ^{13}C NMR spectrum substantially in accordance with Figure 4.

11. A salt according to any one of claims 1 to 10, being 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate, characterised by:
(i) an infrared spectrum substantially in accordance with Figure 1; and
(ii) a Raman spectrum substantially in accordance with Figure 2; and
(iii) an X-Ray powder diffraction pattern (XRPD) substantially in accordance with Table 1 or Figure 3; and
(iv) a Solid State ^{13}C NMR spectrum substantially in accordance with Figure 4.

12. A compound according to any one of claims 1 to 11, in isolated form.

13. A compound according to any one of claims 1 to 11, in substantially pure form.

14. A compound according to any one of claims 1 to 11, in crystalline form.

15. A compound according to any one of claims 1 to 11, in a form having been processed in a manufacturing process requiring or generating heat.

16. A compound according to claim 15, or a solvate thereof, in a bulk form.

17. A compound according to claim 15 or 16, or a solvate thereof, wherein the processed form is selected from: a milled form, a heat-dried form, a hot melt processed form and a heat-sterilised form.

18. A compound according to any one of claims 15 to 16, or a solvate thereof, in a milled form.

19. A process for preparing a compound according to any one of claims 1 to 18, characterised in that 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione (Compound (I))or a salt thereof, preferably dispersed or dissolved in a suitable solvent, is reacted with a source of p-toluenesulfonate ion; and optionally thereafter as required:
(i) forming a solvate thereof;
(ii) recovering the Toluenesulfonate or solvate thereof ; or

(iii) further processing the Toluenesulfonate or solvate therof in a manufacturing process requiring or generating heat.

20. A pharmaceutical composition comprising 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate salt (the Toluensulphonate), or a non-aqueous solvate thereof, according to claim 1, and a pharmaceutically acceptable carrier therefor.

21. A pharmaceutical composition comprising the 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate salt (the Toluensulphonate), or a non-aqueous solvate thereof , according to claim 1, in combination with one or more other anti-diabetic agents and optionally a pharmaceutically acceptable carrier therefor.

22. A pharmaceutical composition according to claim 20 or claim 21, wherein the 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate salt (the Toluensulphonate), or a non-aqueous solvate thereof, is present in an amount providing 1, 2, 4, 8, 12, 4 to 8 or 8 to 12 mg of 5-[4-[2-(N-methyl-N-(2-pyridyl)amino) ethoxy]benzyl]thiazolidine-2,4-dione (Compound (I)).

23. A compound5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate salt (the Toluensulphonate), or a non-aqueous solvate thereof, according to claim 1, for use as an active therapeutic substance.

24. A compound 5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate salt (the Toluensulphonate), or a non-aqueous solvate thereof, for use in the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof.

25. A use of5-[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate salt (the Toluensulphonate), or a non-aqueous solvate thereof, according to claim 1, for the manufacture of a medicament for the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof.

26. A method for the treatment and/or prophylaxis of diabetes mellitus, conditions associated with diabetes mellitus and certain complications thereof, in a human or non-human mammal which comprises administering an effective, non-toxic, amount of 5-

[4-[2-(N-methyl-N-(2-pyridyl)amino)ethoxy]benzyl]thiazolidine-2,4-dione p-toluenesulfonate salt (the Toluensulphonate), or a non-aqueous solvate thereof, according to claim 1, to a human or non-human mammal in need thereof.

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Figure 1 Infrared spectrum of the *p*-Toluenesulfonate

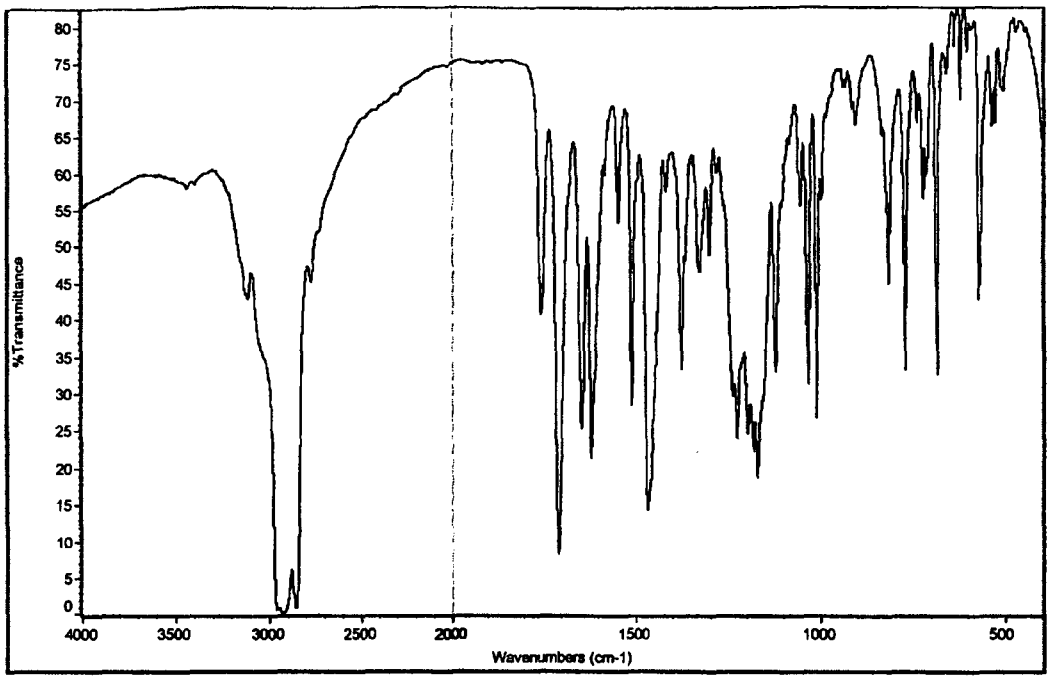

Figure 2 Raman Spectrum of the *p*-Toluenesulfonate

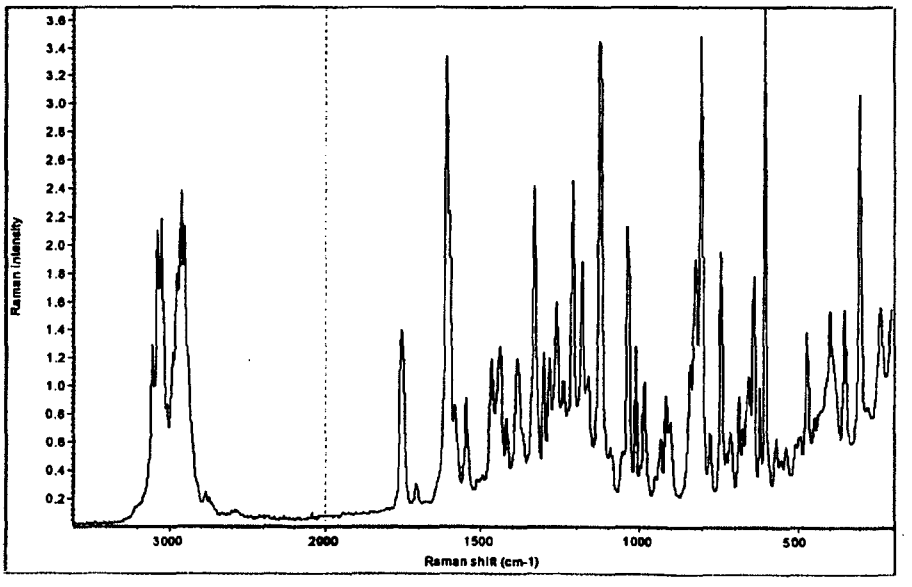

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Figure 3 X-Ray Powder Diffractogram of the *p*-Toluensulfonate

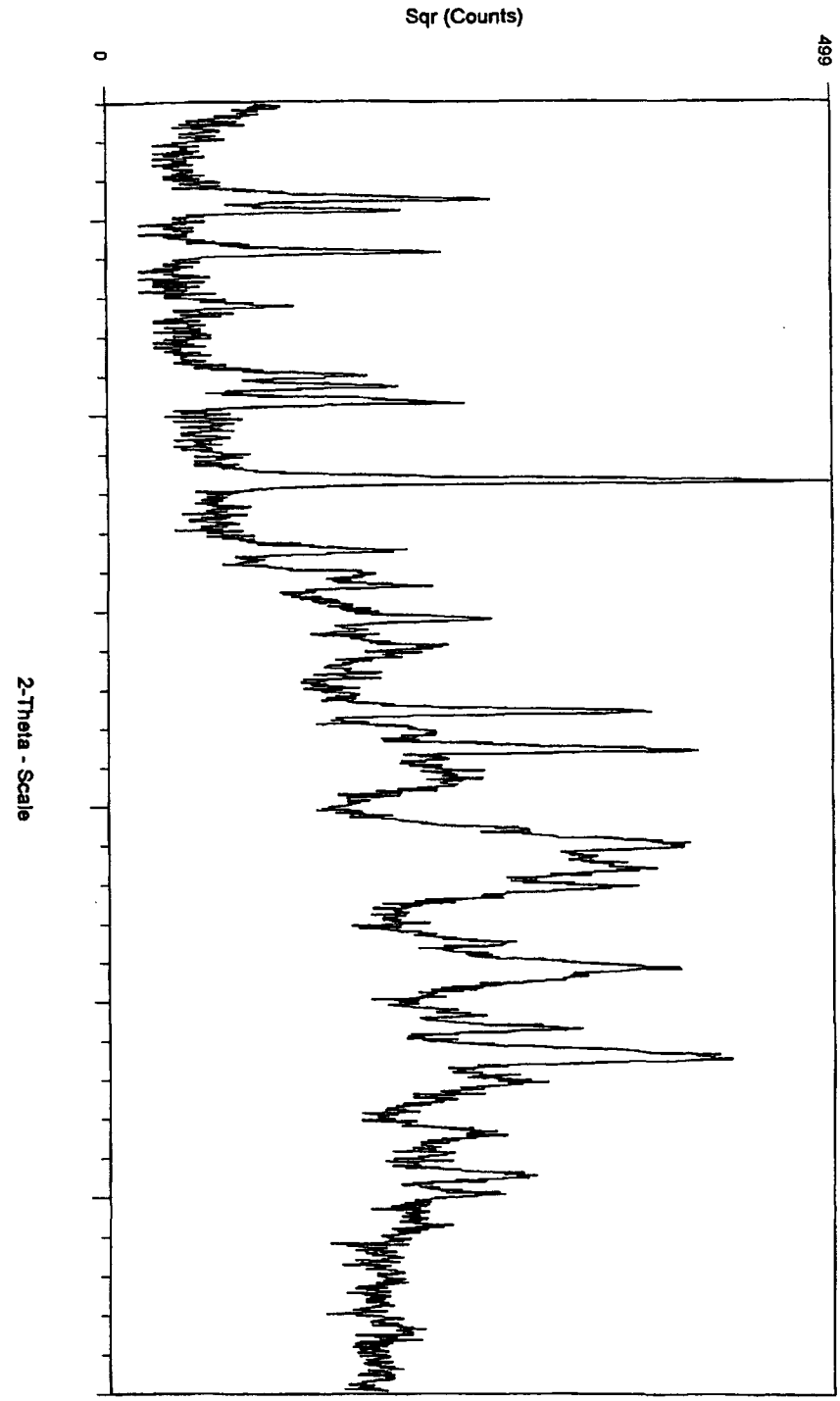

Figure 4 Solid State ^{13}C NMR spectrum of the *p*-Toluensulfonate

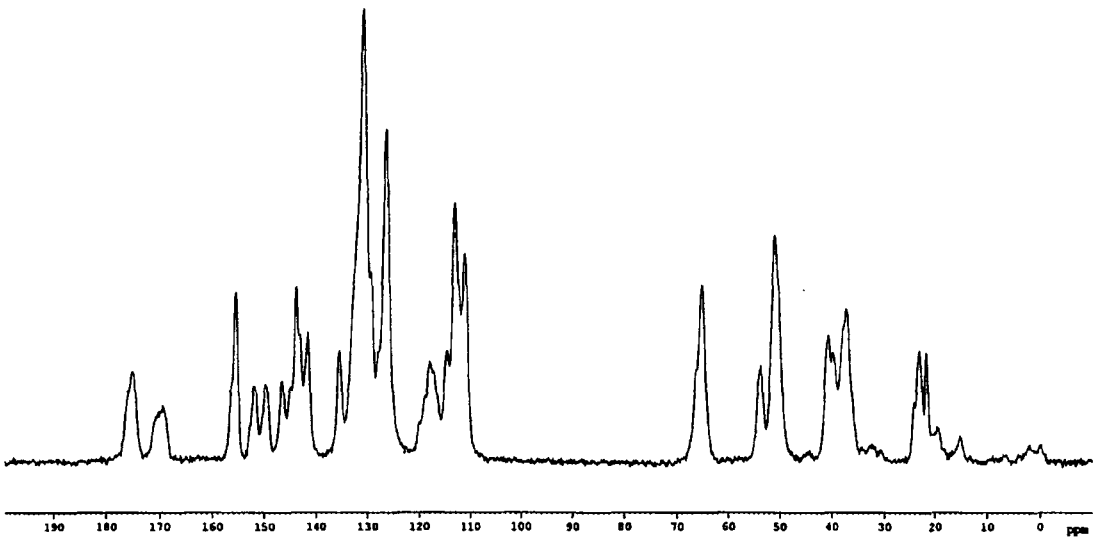

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Exhibit 3

1 SCIENTIFIC DISCUSSION

1.1 Introduction

Renal cell carcinoma

Renal cell carcinoma (RCC), also- called renal adenocarcinoma, is a form of kidney cancer that arises from the cells of the renal tubule. RCC accounts for 90-95% of tumours arising from the kidney, and represents approximately 2% of all adult malignant tumours [1]. Over the past five years decades, the number of individuals diagnosed with RCC has dramatically[1] increased: it is estimated that the prevalence in the US has risen by 126% in this period [2]. Several factors may explain the rise including significant improvements in diagnosing due CT and MRI, but also additional factors like smoking, hypertension, obesity and diet have contributed to the rising incidence of RCC [3]. The male to female ratio is 1.5:1 [4].
RCC is the sixth leading cause of cancer death [5] and is responsible for an estimated 95,000 deaths worldwide [6]. In the past 30 years, prognosis for RCC appears to have improved. The American Society of Cancer Surveillance Research showed an increase in the 5-year survival rates from kidney cancer in the period 1992–1999 (63%) compared with 1974–1976 (52%) and 1983–1985 (56%) [7]. However there is a marked difference in outcome between early and advanced RCC. Advanced RCC is defined as metastatic, locally advanced and/or unresectable RCC. A median survival time of 6-12 months and a 2-year survival rate of 10-2-% have been estimated for patients with metastatic RCC [8]. Approximately 25% of patients have metastatic disease when RCC is first diagnosed and this number rises to 33% when those who develop metastatic spread throughout the course of the disease are also considered [8].
The main histological subtypes of RCC are clear cell, chromophilic, chromophobic, oncocytic, collecting duct carcinomas and unclassified. Clear cell Carcinoma is the most common form of renal tumour, and accounts for 70-80% of all cases of RCC [9].

Treatment of advanced RCC

The management of a patient with RCC is determined by the stage of the disease. Due to the proposed indication only the treatment of patients with advanced real cell carcinoma is described. In the last 25 years, the overall prognosis for patients with metastatic RCC (mRCC) has not appreciably improved. RCC is highly resistant to systemic therapies, including chemotherapy and radiation therapy. Although RCC tumours express receptors for estrogen, progesterone and testosterone, hormone therapy is not considered sufficiently effective [10]. A large meta-analysis (over 50 trials) revealed on average an overall objective response rate of 5.5% for chemotherapy in mRCC patients [11].
Cytokine therapy is considered a modestly effective systemic treatment of RCC that may be limited by drug-related adverse events. An overview from multiple clinical trials with IFN revealed a response rate of 12-15% for IFN [12]. A metaanalysis of 4 randomized trials provided evidence for a reduced one-year mortality (hazard ratio 0.56) with IFN treatment [12]. However, in patients with intermediate risk, this has been challenged by recent data indicating no difference in progression-free survival (PFS) and overall survival (OS) between IFN or IL-2 treatment compared to a control with medroxyprogesterone acetate (Negrier et al, 2005).
Interleukin-2 (IL-2) has been extensively studied in RCC patients, showing widely varied response rates, with relatively few complete or durable responses [13]. The toxicity profile of high-dose IL-2 includes a 4% drug-related death rate due to a capillary leak syndrome associated with severe hypotension, pulmonary edema and renal dysfunction [14]. Low-dose subcutaneous IL-2 appears to be less effective as high-dose bolus IL-2 but better tolerable [15].

A recent randomized Phase III study evaluating IFN *versus* low-dose IL-2 *versus* IFN+IL-2 *versus* medroxyprogesterone acetate in RCC patients did not show a difference in PFS or OS for any treatment arm but a less favorable quality of life and safety profile for the cytokines [16]. The combination of immunotherapy with chemotherapy, in particular 5-FU, could not demonstrate unequivocal improvement of the outcome [15].

In summary advanced RCC remains incurable and is a serious, life-threatening condition with high unmet medical need.

Because current treatment options for patients with advanced RCC are limited, various new treatment modalities are being investigated with the aim of improving outcomes. The pathophysiology points out that the tumour growth is dependent on multiple factors, including tumour cell proliferation and the process of tumour neovascularization. Therefore the understanding of these factors is essential to develop a new drug.

Tumour cell proliferation is stimulated by signaling molecules that activate receptor tyrosine kinases (RTKs) [17, 18] including those for the epidermal growth factor (EGFR), platelet-derived growth factors (PDGFs), c-KIT and FLT3. The binding of a growth factor to its receptor activates the receptor's tyrosine kinase activity. RAS subsequently activates the RAF/MEK/ERK pathway [19]. Activated ERK translocates from the cytoplasm into the nucleus and modulates gene expression *via* the phosphorylation of transcription factors. Thus, activation of the RAS signaling pathway initiates a sequence of events that stimulate cellular growth. Constitutive activation of RAS signaling pathways occurs through overexpression or mutation of RTKs, mutational activation of *k-ras*, or mutational activation of downstream effectors of RAS such as *b-raf* [20]. EGFR is overexpressed in a subset of human lung cancer. PDGFs support the development of glioblastomas and play a role in chronic myeloproliferative cancers. Flt3 is mutationally activated in acute myelogenous leukaemia and c-Kit is activated in gastrointestinal stromal tumours. RAS mutations are present in at least 45% of all colon cancer and in greater than 90% of tumours of the exocrine pancreas [21]. Mutated BRAF (V600E) is present in a high percentage of melanomas [20].
Neovascularization is also a highly regulated process in which the proliferation of vascular endothelial cells, lymphangiogenic endothelial cells and smooth muscle cells that support new blood vessels is controlled by multiple growth factors that bind to RTKs. The growth factors involved in neovascularization include vascular endothelial growth factors (VEGFs), basic fibroblast growth factor (bFGF), and platelet-derived growth factors (PDGFs). In addition, PDGFs also support vessel stabilization through the recruitment and maturation of pericytes [22]. Moreover, bFGF and VEGF differentially activate RAF, resulting in endothelial cell protection from apoptosis [23, 24] in addition to stimulation of proliferation [25, 26], providing conditions conducive to neovascularization.
Therefore, activation of RTK signaling pathways is an important mechanism by which the majority of human tumours are stimulated to proliferate, and by which tumour associated neovascularization is initiated and stabilized.

About the product

Nexavar contains sorafenib, an antineoplastic agent that acts as protein kinase inhibitor (ATC code: L01XE05). Sorafenib inhibits tumour cell proliferation and the tumour vascularisation through activating the receptor tyrosine kinases (RTKs) signalling RAS/RAF/MEK/ERK cascade pathway.

The acronyms used during the development of the medicinal product were sorafenib (INN, USAN, JAN of the free base) coded BAY 43-9006, sorafenib tosylate (INN, USAN for the tosylate salt) coded BAY 54-9085, sorafenib tosilate (JAN for the tosylate salt).

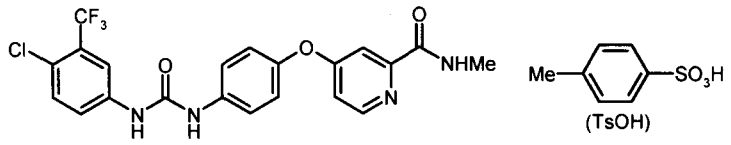
1.2 Quality aspects

Introduction

Nexavar is presented as film coated tablets containing 274 mg of sorafenib tosylate, which corresponds to 200 mg of sorafenib, as active substance. Other ingredients are microcrystalline cellulose, croscarmellose, hypromellose, sodium laurilsulfate, magnesium stearate, water, titanium dioxide and red ferric oxide (as colorants).
The capsules are packed in polypropylene (PP) blister and sealed with aluminium foil.

Active Substance

Sorafenib tosylate is a white to yellowish or brownish solid substance practically insoluble in water, slightly soluble in alcohols and soluble in DMSO and DMF. Sorafenib tosylate chemical name is 4-{4-[3-(4-Chloro-3-trifluoromethyl-phenyl)ureido]phenoxy}pyridine-2-carboxylic acid methylamide 4-methylbenzenesulfonate. The active substance exhibits polymorphism and it crystallizes in three different modifications (Mod I, Mod II and Mod III). It is a non-chiral molecule with the following structure:



The route of synthesis and data from structural analysis (IR, Raman, UV-VIS, ^{1}H-NMR, ^{13}C-NMR, MS, elementary analysis, X-ray structural analysis data) all support the above structure of sorafenib tosylate.

- Manufacture

Sorafenib tosylate is synthesised in six steps. The manufacture involves the synthesis of a key isolated intermediate, which is synthesized *via* three reaction steps, from the starting material. The next two steps involve the formation of sorafenib followed by the salt formation and crystallisation, resulting in sorafenib tosylate. The final step involves micronization in an air-jet mill.
The active substance is visually tested for appearance and its identity is confirmed by NIR and HPLC, and the desired modification of sorafenib tosylate (Mod I) is confirmed by Raman spectroscopy.
Batch analysis data on sorafenib tosylate micronized produced from both routes of synthesis showed that the active substance can be manufactured reproducibly.

- Specification

The specifications for sorafenib tosylate micronized and sorafenib tosylate N micronized include appearance (visual test), identity of sorafenib tosylate (HPLC and NIR), identity of modification I (Raman spectroscopy), water content (Karl Fisher), particle size distribution (laser diffraction method), heavy metals, assay on dried active substance (HPLC), related substances (HPLC and GC) and microbial purity.
Sorafenib tosylate is controlled by well-described analytical methods, which have been validated according to the ICH guidelines. The inorganic impurities are controlled by limits for sulphated ash and heavy metal. The residual solvents used in the last synthesis and purification steps are controlled in the final active substance and the specifications are within the ICH guideline on residual solvents.

- Stability

Sorafenib tosylate is a stable substance and no sign of degradation is observed after 24 months of storage under ICH long term or accelerated conditions (12 months). The active substance was found to be resistant to heat, oxidation and hydrolysis. ICH light stability studies have been performed on sorafenib tosylate in the solid state and it was concluded to be stable. When dissolved in methanol it was shown to be slightly sensitive to light. Stability data was provided on three pilot scale batches of sorafenib tosylate micronized packaged in polyamide/polyethylene (PA/PE) bags, whereas sorafenib tosylate N micronized stability data was provided on batches packaged in polypropylene (PP) bags.

The stability results justified the proposed re-test period for both sorafenib tosylate and sorafenib tosylate N.

Medicinal Product

- Pharmaceutical Development

Sorafenib tosylate tablets represent an immediate-release formulation for oral use packaged in transparent polypropylene/aluminium blisters.

Due to the very low solubility of sorafenib in aqueous media, the tosylate salt was used in the drug product. To enhance dissolution the active substance is micronized and the particle size is tightly controlled. The permeability of sorafenib tosylate using the Caco-2 cell model indicates that it is a 'high permeability' compound.
The excipients used in the manufacture of Nexavar are all commonly used in this type of formulation. For the tablet core, microcrystalline cellulose is used as filler, croscarmellose as disintegrant, hypromellose as binder, sodium lauryl sulfate as wetting agent and magnesium stearate is utilised as lubricant. The film-coat is composed of hypromellose as the film-forming agent and macrogol as a plasticizer. Water is used as suspending liquid, and titanium dioxide and red ferric oxide as colorants. All excipients comply with PhEur monographs, except ferric oxide, which complies with Directive 94/45 EC regarding colorants in food. No materials of human or animal origin are used in the synthesis of the drug substance or in the manufacture of the drug product.
The objective of the pharmaceutical development has been to obtain a small immediate release tablet with a high amount of active substance. Sorafenib tosylate exhibited good compression characteristics and therefore a high content of drug could be used in the formulation. A wet granulation process with water as granulating liquid was found to be suitable. The content of magnesium stearate in the debossed tablet was adjusted to avoid sticking to the tabletting tools. The tablets are film coated to facilitate swallowing and to add colour.

- Manufacture of the Product

The manufacture of the finished product involves conventional processes including (1) mixing, (2) wet high-shear granulation, (3) wet sizing, (4) drying of granulate, (5) tablet compression and (6) film-coating. During process development and scale-up, the impact of manufacturing conditions on target properties of the final dosage form, such as tablet hardness, disintegration, dissolution and stability were investigated. The dissolution rate was determined to be a critical property of the product and to be affected by several manufacturing conditions. The potential for polymorphism was investigated by Raman spectroscopy and found to be unchanged.
The in-process controls were found to be adequate to the manufacture of the film-coated tablets.
The manufacturing process has been validated using three batches of the intended standard batch size. Evaluation of the batches was based on manufacturing process parameters and in-process control data. All validation batches complied with the release specifications. Batch analysis data provided shows that the film-coated tablets can be manufactured with suitable quality according to the finished product specifications.

- Product Specification

The product specifications include tests for appearance (visual method), identity (HPLC and TLC or NIR), dissolution (PhEur), uniformity of dosage units (PhEur), unspecified degradation products (HPLC), assay (HPLC) and microbial purity (PhEur).
No specified impurities are part of the finished product specification. This is acceptable since these are controlled in the drug substance and they have been found not to increase upon storage. The finished product shelf-life specifications are the same as the release specifications, exception being the water content. The specifications are considered acceptable and justified.

- Stability of the Product

Stability studies were carried out according to the ICH guideline on stability. Long-term stability data was generated on three pilot scale batches at storage conditions of 25oC/60%RH and 30oC/70%RH for 30 months. Data demonstrates that the product is stable under climate zone I/II conditions. Long-term and accelerated conditions stability data was also provided for commercial scale batches. Stress testing data was provided covering thermal and hydrolytic stress, which confirmed that high humidity can influence the properties of the finished product. Bulk stability data was also provided for one batch at 25oC/60%RH and was found to be satisfactory.
Photostability studies performed according to the ICH guideline showed that the finished product is not light sensitive.
Based on the available stability data, the proposed shelf life and storage conditions as stated in the SPC are acceptable.

Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the drug substance and drug product have been presented in a satisfactory manner. The results of test carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

1.3 Non-clinical aspects

The pharmacology of sorafenib was investigated *in vitro* and *in vivo*. All pivotal toxicology studies were conducted according to the GLP standards, as claimed by the applicant.

Pharmacology

- Primary pharmacodynamics

The biological activity (IC50 values) of sorafenib was tested in biochemical assays against a selected panel of purified serine/threonine kinases and receptor tyrosine kinases. Sorafenib was active in biochemical assays against all kinases listed in Table X. Sorafenib was also tested in cellular assays (using 0.1% BSA) measuring the inhibition of the RAF/MEK/ERK pathway in human breast cancer cell line MDA-MB-231, the VEGFR-2 receptor autophosphorylation in NIH 3T3 cells expressing human VEGFR-2 receptor, and PDGF-dependent proliferation of human aortic smooth muscle cells (HAoSMC). The IC50 data is summarized in Table 1.

Table 1 - Summary of the *in vitro* profile of sorafenib

Biochemical Assay [a]	**IC50 (nM)**
CRAF [b]	6
BRAF wild-type	22
BRAF V600E mutant	38
VEGFR2	90
mVEGFR2	15
mVEGFR3	20
mPDGFR-β	57
Flt3	58
c-KIT	68
FGFR1	580
Cellular Mechanism [c]	**IC50 (nM)**
MDA MB 231 MEK phosphorylation (Human Breast)	40
MDA MB 231 ERK phosphorylation (Human Breast)	90
BxPC-3 ERK phosphorylation (Human Pancreatic)	1200
LOX ERK phosphorylation (Human Melanoma)	880
VEGFR-2 receptor phosphorylation (Human, 3T3 cells)[d]	30
VEGF-stimulated ERK phosphorylation (HUVECs)e	60
BFGF-stimulated ERK phosphorylation (HUVECs)	620
MVEGFR-3 receptor phosphorylation (Mouse, 293 cells)	100
PDGFR-β phosphorylation (HAoSMC)[f]	80
Flt-3 receptor phosphorylation (Human ITD, 293 cells)	20
Cellular Proliferation	**IC50 (nM)**
MDA MB 231 (10% FCS) [g]	2600
PDGFR-β-stimulated HAoSMC f (0.1% BSA) [h]	220

A: Recombinant enzyme assay; b: Raf kinase activated with Lck (full length CRAF) ; c: Mechanistic cellular assays all performed in 0.1% BSA ; d: Western blot assay format with Phospho-VEGFR-2 antibody; e: Human umbilical vein endothelial cells ; f: Human aortic smooth muscle cells; g: Fetal calf serum; h: Bovine serum albumin.

Sorafenib was a potent inhibitor of CRAF and wild-type and mutant (V600E) BRAF with inhibitory concentration (IC50s) of 6 nM, 22 nM and 38 nM, respectively. Sorafenib was also a potent inhibitor of several RTKs linked to tumour progression, including Flt-3, c-Kit, VEGFR2, VEGFR3, and PDGFR-ß. Sorafenib did not inhibit MEK-1, ERK-1, EGFR, HER2/NEU, c-MET, PKA, PKB, IGFR-1, Cdk-1/cyclin B, PIM-1, GSK 3-b, CK-2, PKC-α, PKC-β, or PKC-γ at concentrations up to 10 μM.

The *in vivo* anti-tumour efficacy of sorafenib, administered as a single daily oral treatment, has been studied against non-renal tumour xenograft models using athymic mice. In each model, sorafenib was administered p.o. once a day for 9 days starting when all animals in an experiment had established tumours (tumour weight of approximately 100 mg). All doses are expressed as free base equivalents irrespective of the form of compound administered (free base or the tosylate salt). The tumour cells were implanted sc into the flank region of female athymic mice (NCr-nu/nu). Sorafenib demonstrates anti-tumour efficacy as a single agent against a broad range of human tumour xenografts including the HCT-116 (64%TGI at 30mg/kg/dose free base), DLD-1 (66%TGI at 30mg/kg/dose free base), Colo-205 (66% TGI at 30mg/kg/dose free base), and HT-29 colon tumours (21) (71% TGI at 30mg/kg/dose free base), the NCI-H460 (56% TGI at 30mg/kg/dose free base), and A549b (60% TGI at 30mg/kg/dose free base) NSCLC, the MDA-MB-231 (92% TGI at 30mg/kg/dose free base) and MX-1 (51% TGI at 30mg/kg/dose free base) mammary tumours, the MiaPaCa-2 pancreatic tumour (66% TGI at 30mg/kg/dose free base), the MV4; 11 AML (84% TGI at 3mg/kg/dose free base), LOX IMVI melanoma (52% TGI at 30mg/kg/dose free base), and the SK-OV-3 (64% TGI at 30mg/kg/dose free base) ovarian tumour. The percent tumour growth inhibition (TGI) calculated as (1-T/C)*100, where T and C represent the mean size of the treated (T) and control (C) tumours on the day after the last dose of sorafenib in each experiment.

Sorafenib exhibited anti-tumour efficacy against tumour models that express either mutated k-ras (DLD-1, Mia-PaCa-2) or BRAF (Colo-205, HT-29). Sorafenib showed efficacy against the MDA-MB-231 model that exhibits activating mutations of both genes. Sorafenib was also effective against the SK-OV-3 human ovarian tumour line that contains a wild-type ras and BRAF but overexpresses both the EGF and Her2 growth factor receptors. These receptors also signal through the RAS/RAF/MEK pathway.

The anti-tumour efficacy of sorafenib against renal tumour has been studied using female athymic mice (NCr-nu/nu). The studied xenograft models were the A498 human renal cell cancer model, the CAKI-1 human renal tumour model and the RENCA murine renal cell cancer model. In the RENCA model growth inhibition ranging from 30% to 84% was observed following oral administration of doses from 7.5 mg/kg/day to 90 mg/kg/day. Preliminary anti-tumour efficacy studies were also conducted against the A498 and CAKI-1 human renal tumour xenograft models in athymic mice. Growth of CAKI-1 tumours was not significantly ($p=0.8074$) inhibited by sorafenib at dose levels up to 60 mg/kg.

The mechanism of action of sorafenib was investigated *ex vivo* in multiple tumour models including HT-29, DLD-1, HCT-116, and Colo-205 colon tumour models, the MDA-MB-231 breast tumour model, and the Mia-PaCa-2 pancreatic tumour model (21). In each study, animals bearing approximately 200 mg size tumours were treated with sorafenib (30-60 mg/kg/dose) for 5 days. Tumours were then collected 3 hours after the final dose and evaluated for modulation of ERK phosphorylation by Western staining and/or by immunohistochemistry (IHC) to assess the effect of sorafenib on signaling through the RAF/MREK/ERK pathway. Tumour samples in some models were also stained with a goat polyclonal antibody against CD31 and the microvessel density was assessed by image analysis to measure the inhibition of neovascularization. An inhibition of the RAF/MEK/ERK pathway in HT-29, DLD-1, HCT-116, and MDA-MB-231 tumour models but not in Colo-205 or Mia-PaCa-2 tumours was demonstrated. Analysis of the phosphoprotein values in the MDA-MB-231 model showed that RAF/MEK/ERK phosphorylation was reduced along with six other proteins (BTK, CDK1, Ekt/BMX, MAPK1, p38, src and Zap70 (RMC-01274). Among these the CDK1 also had lower levels of phosphorylation in LOX cells *in vitro* (MRC-01275). In addition, the phosphorylation state of one protein, GSK3 , was significantly upregulated. Anti-angiogenetic effects

were evaluated *in vivo* in human xenograft models (RMC-01264). Mice with 150-250 mg HT-29, Colo-205 or MDA-MB-231 tumours were treated for 5 days with either vehicle or oral sorafenib at 30 or 60 mg/kg. Tumours were collected, sectioned, and stained with anti-CD31 antibodies. The results demonstrated that sorafenib produced 50% to 80% reduction of microvessel area (MVA) and microvessel density (MVD) in drug-treated relative to vehicle- treated HT-29 and Colo-205 tumours despite a lack of RAF/MEK/ERK inhibition in the Colo-205 tumour samples.

Three metabolites (M-2, M-4, and M-5) were synthesized and characterized for their biochemical activities against wild-type CRAF and BRAF, mPDGFR-â, mVEGFR-2, and human FLT-3. The metabolites M-2, M-4 and M-5 of sorafenib potently inhibited several receptor tyrosine kinases including VEGFR-2 (IC 50 between 7-10nM), PDGFR-β (IC 50 between 14-42nM), and Flt-3 (IC 50 between 87-170nM). In cellular assays, these metabolites inhibited the RAF/MEK/ERK pathway in tumour cells and inhibit VEGFR-2 and PDGFR signalling. Two of the metabolites, M-2, and M-5 , with IC 50 of 22nM and 46nM respectively, were more potent than Sorafenib (IC 50285nM) at inhibiting PDGF-dependent human aortic smooth muscle cell proliferation. However, Sorafenib (IC 50: 6nM) was more potent than either M-2 (IC 50: 21nM) or M-5 (IC 50: 18nM) against CRAF. For both wild-type and mutant BRAF the IC 50 was higher for M2 and M5 than for Sorafenib. Sorafenib (IC 5058nM) was also significantly more potent against Flt-3 than M-5 (IC 50: 170nM). The three metabolites (M2, M4, and M5) had similar *in vitro* pharmacological activity as sorafenib but the metabolites were present at much lower plasma levels. The most predominant human metabolite, M-2, was also evaluated *in vivo* for anti-tumour efficacy against the MDA-MB-231 xenograft model. The studies indicated that M2 was efficacious when administered orally at a dose level of 120 mg/kg on a daily schedule against s.c. MDA-MB-231 human mammary tumour xenografts in athymic mice. Lower dose levels (30 or 60 mg/kg) of M2 were not efficacious. M2 was both less potent and less active than sorafenib. A dose level of 120 mg/kg of M2 produced less growth inhibition (63%-68% TGI) than administration of 30mg/kg sorafenib by the same route and schedule of administration (80%-91% TGI). However, the degree to which M-2 contributes to the anti-tumour efficacy of sorafenib was not determined as M-2 is partially converted to sorafenib in vivo.

- Secondary pharmacodynamics

No studies were conducted to assess the secondary pharmacodynamics of sorafenib.

- Safety pharmacology programme

The safety pharmacology studies were conducted in accordance with ICH S7A and S7B [27].The only central nervous system (CNS) related effects observed in male rats were transient tremor in 2 and 3 out of 6 animals treated with 100 and 300 mg/kg. No effects on psychomotoric activity, body temperature, anti-/or pro-convulsive effect, nociceptive responsiveness or hexobarbiton induced sleeping time were observed.
For the safety of the cardiovascular and respiratory system transfected CHO cells and Rabbit cardiac Purkinje fibers were used *in vitro*. Sorafenib had no statistically significant effect on hERG current at concentrations up to 3 μM even if a tendency for inhibition was seen. At 10 μM, hERG current inhibition could not be evaluated due to unspecific effects subsequent to substance precipitation. Measurements of the action potential duration indicated a small prolongation of the APD90 , which became significant at a concentration of 10μM sorafenib. There was no toxicologically relevant effect on pulmonary function, hemodynamics, cardiac contractility and ECG in anesthetized dogs after single intraduodenal sorafenib tosylate doses of up to 60 mg/kg (corresponding sorafenib plasma Cmax approximately 3 mg/l). A transient decrease in heart rate (mean maximum of 15%) was noted at the 60 mg/kg dose but no effect on blood pressure, blood flow, P-wave amplitude, P-Q interval, QRS interval and duration of QT interval were observed.
Potential effects on diuresis, blood pharmacological parameters, blood glucose, and GI-tract were investigated in Wistar-rats after single oral sorafenib tosylate doses of 30, 100, or 300 mg/kg (corresponding sorafenib plasma Cmax approximately 3.5, 12, and 35 mg/L). There was a dose-dependent anti-diuretic effect (up to -56%) with parallel decrease in blood erythrocyte counts, hematocrit and total hemoglobin concentration, and effects gaining statistical significance at 220 mg/kg) at a systemic exposure of about 35 mg/l. No effect on blood coagulation was observed. Blood glucose levels were statistically significant decreased (maximum approximately -20% in top dose

animals) at all dose levels (fed rats) and at 73 mg/kg and 220 mg/kg in fasted animals. Sorafenib had no effect on intestinal contractions or relaxation on guinea pig ileum *in vitro*.

- Pharmacodynamic drug interactions

The ability of sorafenib (60 or 30 mg/kg/dose, p.o.) to be combined with paclitaxel, irinotecan, gemcitabine, doxorubicin, gefitinib or cisplatin was evaluated in non-clinical human tumour xenograft models.
Combinations with paclitaxel were evaluated in two mice model systems. In the NCI-H460 model, a 14-day course of treatment with sorafenib initiated concurrently with a 5-day course of treatment with paclitaxel was tolerated and had no adverse effect on the anti-tumour efficacy of paclitaxel. 10 mg/kg/dose of paclitaxel or lower was well tolerated in combination with sorafenib regardless of the sequence of administration. An evaluation of the efficacy of the combination of sorafenib administered on the q1d x 14 schedule with paclitaxel administered on either a q.d.x1 or q15d x 2 schedule was conducted in the MX-1 model. A tumour growth delay (TGD) significantly ($p<0.005$) greater in the combination than those produced by paclitaxel or sorafenib alone was observed.
Combination chemotherapy involving irinotecan (40 or 26.8 mg/kg/dose, i.p) was evaluated in the DLD-1 human colon tumour xenograft model. A multiple tumour regression of these agents was observed with concurrent administration in two separate experiments, but not with either single agent therapy.
Combination chemotherapy with gemcitabine (120 and 80 mg/kg/dose, i.p) was investigated using the MiaPaCa-2 human pancreatic tumour model. All combination therapies were as well tolerated as either single agent therapy with no lethality.
Combination chemotherapy with cisplatin (5.4 and 3.6 mg/kg/inj, i.p. on a q4d x 3 schedule) was investigated using the NCI-H23 human NSCLC tumour model. TGD produced by the combination of 60 mg/kg sorafenib and 5.4 mg/kg cisplatin (27.5 days) was significantly greater than that produced by cisplatin alone (21.4 days) at the same dose level ($p<0.04$). The TGD of the 5.4mg/kg dose level of cisplatin with the lower dose of 30 mg/kg sorafenib was not significantly different from that of cisplatin alone.
Combination chemotherapy with doxorubicin (6 and 4 mg/kg/inj, i.v.) was investigated using the MX-1 mammary tumour model. This combination of was not well tolerated, producing significantly greater dose dependent weight loss and lethality (4/10 treatment-related deaths in mice treated with the combination of the highest dose of each agent). TGD for this combination was 86%, therefore not as high in response as it was to doxorubicin treatment alone (129%) at the higher tolerated dose level of 6 mg/kg.
Combination chemotherapy with gefitinib (150 and 75 mg/kg/dose, p.o., on a q.d. x 10 schedule) was investigated using the A549 human NSCLC tumour model.
No increased toxicity or decreased efficacy of either agent was observed with this combination.

Pharmacokinetics

Pharmacokinetics of sorafenib administered as tosylate salt was investigated *in vivo* in CD-1 mice, Wistar rats and in Beagle dogs. Additionally, *in vitro* studies were performed to investigate plasma protein binding, blood cell/plasma partitioning, and drug metabolism in rodents, dogs, monkeys and human.

- Absorption-Bioavailability

The absorption and the basic pharmacokinetics following a single dose of sorafenib tosylate were evaluated in female CD-1 mice, male Wistar rats, and female Beagle dogs.
For the determination of the absorption of sorafenib in rats, bile duct-cannulated rats (n=5/group) were used. Twenty-four hours after surgery [14C] sorafenib tosylate was administered orally or intravenously to the rats at a dose of 5 mg/kg sorafenib. The absorption of sorafenib was almost complete in female CD-1 mice (78.6%) and male Wistar rats (79.2%). In Beagle dogs the absorption (67.6 %, calculated from AUCnorm values after intravenous and oral administration) and the absolute bioavailability (59.9 %) were lower than in rodents.
Maximum plasma concentrations of radioactivity between 1.5 h and 2 h after oral administration were observed in all species. After intravenous administration of [14C] sorafenib tosylate to mice, rats, and

dogs the elimination of the radioactivity from plasma occurred with similar terminal half-lives of 6.8, 8.8, and 7.3 hours, respectively. The terminal half-lives of radioactivity after oral administration were 6.1 hours in mice and 5.8 hours in dogs. In rats, terminal half-live after oral administration was longer (11.2 h) than after intravenous administration. In rats, the elimination of the unchanged compound was slower (t1/2: 9.3 h) than in the mice (t1/2: 6.5 h) and dogs (t1/2:4.3 h). The total plasma clearance in rats was 0.044 l/(h·kg) corresponding to a blood clearance of 0.049 l/(h·kg). In mice and dogs the total plasma clearance was 0.13 and 0.15 l/(h·kg) respectively. The volume of distribution at steady state ranged from 0.65 l/kg to 0.74 l/kg, depending on the species.

- Distribution

The binding of sorafenib to plasma proteins of mice (male), rats (male and female), rabbits (female), dogs (female) and humans (male and female) was investigated *in vitro* by the distribution of sorafenib between diluted plasma and between solid-supported lipid membranes according to the method of SCHUHMACHER et al [28] . The protein binding was investigated in the concentration range from 0.1 mg/l to about 10 mg/l for undiluted rat, dog and mouse plasma and in the range from 0.1 mg/l to about 5 mg/l in undiluted plasma of human and rabbits. The protein binding was about 99.5% in mice, rats and humans, 99.1% in dogs and 98% in rabbits. Human serum albumin, α-, -globulin and the low-density lipoprotein (LDL) were the main binding proteins (fractions unbound from 1.02 to 3.55 %). In the blood of rats, dogs, and humans sorafenib was mainly equally distributed between plasma and blood cells. The plasma/blood concentration ratio was 1.12 for rats, 1.02 for dogs and 1.33 for humans. The binding of sorafenib was dependent on pH in plasma. The fraction unbound decreased to 0.165 % at pH 7.99 and increased to 1.80 % at the acidic pH 6.78.
Qualitative and quantitative distribution patterns were determined in male and female Wistar rats and male pigmented Long Evans rats by means of whole-body autoradiography. For the evaluation of the qualitative distribution patterns [14C]sorafinib was administered at a single oral dose of 10 mg/kg to male and female Wistar rats (one rat per time-point). For the evaluation of the quantitative distribution patterns [14C]sorafinib was administered at a single oral dose of 10 mg/kg to male albino Wistar rats (3 rats per time-point) and pigmented Long Evans rat (1 rat per time-point). [14C]sorafenib and/or its labeled metabolites were homogeneously distributed throughout the body, with the exception of the brain (maximum concentration of radioactivity (CEQ max) 0.391), seminal vesicles (0.363) and the compact bone (0.155). Maximum radioactivity concentration was mainly reached in most organs and tissues up to 4 hours post dosing. Blood/brain penetration was low as indicated by brain uptake less than 10 % of blood or plasma exposure. The highest exposure in terms of AUC was found in the liver (492mg-eq h/l). Similar exposure was observed in adrenal cortex (369mg-eq h/l), Harderian gland (306mg-eq h/l) and kidney outer medulla (285mg-eq h/l) and cortex (263mg-eq h/l). The rank order of the ratio organ/blood (heart) values were the following; liver (5.5) > adrenal cortex (4.3), adrenal gland (3.8), Harderian gland (2.9), pancreas (2.9), kidney outer medulla (2.7) and kidney cortex (2.6). Terminal elimination of radioactivity occurred rapidly for all organs and tissues, with half-lives mainly between 20 and 36 hours as derived from concentrations up to 7 days. Only the half-life in the skin was longer (72.8 hours).
Whole-body autoradiography was performed in pregnant Wistar rats at the 19th Day of gestation. [14C] sorafenib and/or its radiolabeled metabolites penetrated the placental barrier to a moderate extent (CEQ max ratio organ/blood: 0.988). The radioactivity was homogeneously distributed to most fetal organs and tissues, except fetal brain (CEQ max ratio organ/blood: 0.194) which contained less radioactivity. The average exposure in the fetuses reached 52 % of the exposure in maternal blood. After 24 hours, 54.4 % of the dose was present in pregnant rats, mainly in the dam, only 1.8 % of the dose could be attributed to the fetuses. Enrichments of radioactivity in the mammary glands were observed (CEQ max ratio organ/blood: 1.50).

- Metabolism

The biotransformation of sorafenib has been studied in rats, dogs, and humans *in vivo*. The biotransformation of sorafenib *in vitro* was investigated using liver microsomes, microsomes containing heterologously expressed human cytochromes or glucuronosyl transferases and rat and human hepatocytes. In vitro incubations of [14C] sorafenib tosylate with liver microsomes (protein concentration 0.5 mg/ml, 90 min) revealed two phase I prominent reactions: hydroxylation of the N-methyl group yielding metabolite M-3 and N-oxidation at the pyridine moiety yielding metabolite M-

2. Combination of both pathways led to metabolite M-1 and demethylation of sorafenib led to M-4. The percentage of radioactivity of M-2 in incubation of 16 µM [14C] sorafenib tosylate with liver microsomes was 36.4% in human, 29.4% in rhesus monkeys, 9.6% in rats and 3.8% in dogs. To identify the CYP isoforms involved in the *in vitro* phase I metabolism of sorafenib, incubations with human liver microsomes with/without CYP isoform-selective inhibitors or antibodies. CYP3A4 was found to be the responsible enzyme for phase I (oxidative metabolism) reactions of sorafenib. Sorafenib glucuronide (M-7) was identified as a minor metabolite in human plasma and was excreted into human urine (14.8 % of the dose).
From a panel of recombinant UGT enzymes UDP-glucuronosyltransferase 1A9 (UGT1A9) was identified as the main UGT isoform catalyzing conjugation of sorafenib with glucuronic acid to M-7. Kinetic parameters were determined for UGT1A9 catalyzed glucuronidation using recombinant enzyme, human kidney microsomes, and cultured human hepatocytes. High affinity to UGT1A9 was demonstrated by Km values of 5.8 µM, 8.1 µM, and 3 - 7 µM, in the respective *in vitro* model. Following incubated with cultured human hepatocytes formation of M-7 (glucuronidation) predominated at lower substrate concentrations, whereas preferentially M-2 (N-oxidation) was formed at higher concentrations of sorafenib. Intrinsic clearance (CLint = Vmax/Km) for N-oxidation was approximately 2-fold higher than for glucuronidation. Kidney tissue was also capable of forming glucuronide M-7.
In vivo, the biotransformation of sorafenib has been studied in mice, rats, dogs and humans.
Following a single oral administration, exposure to sorafenib was 71 - 73 % of the AUC of total radioactivity in the respective time interval. In contrast to man, in rat and dog plasma M-3 represented 12.1 % and 15.6 % of the AUC of total radioactivity in plasma. M-4 was found in plasma of all 3 species. Metabolite M-2 was a main metabolite in human plasma (16.7 % of AUC), but was found in small amounts in rat plasma (0.9 % of AUC) and was absent in dog plasma. The glucuronide of sorafenib (M-7) was a minor metabolite in human plasma and could not be detected in rat and dog plasma. In mice, dosed daily for 3 months, M-2, M-3, M-4 and M-5 exposures after multiple dosing were approximately 7%, 4%, 11% and 3%, respectively, of the total exposure (sum of parent and measured metabolites).

- Excretion

For the determination of absorption, excretion and mass balance of the radioactivity ([14C] sorafenib and radiolabeled metabolites) in rats and dogs the compound was administered as an intravenous bolus injection or as a solution *via* gavage to fasted intact male Wistar rats (5 rats/group) or bile duct-cannulated rats (5 rats/group) and as an intravenous bolus injection (5.77 mg/kg equivalent to 4.21 mg/kg of sorafenib) or as a solution in a capsule (5.65 mg/kg equivalent to 4.12 mg/kg sorafenib) to 6 fasted female Beagle dogs (3 dogs/group). After intravenous and oral administration the radioactivity was mainly excreted *via* the biliary/fecal route (90%), urinary excretion was low (<1%). In man, renal excretion of radioactivity was more pronounced than in rat and dog due to species-dependent formation of sorafenib glucuronide, which was excreted into human urine.

- Pharmacokinetic drug interactions

Warfarin, propranolol, nifedipine, furosemide, digitoxin, taxotere, taxol, iressa and cisplatin did not influence the binding extent of sorafinib in any concentration tested (3-15mg/l, 1-5mg/l, 1-5mg/l, 0.5-2.5mg/l, 0.1-0.5mg/l, 3-15mg/l, 2.5-12.5mg/l, 0.5-2.5mg/l, 10-5-mg/l, respectively. Salicylic acid and ibuprofen (200 and 50 mg/l) did not alter fraction unbound (control value 0.275 %) whereas at 1000 and 250 mg/l the fraction unbound increased to 1.50 and 2.70 %, respectively.
The potential of sorafenib to induce human CYP1A2 and 3A4 was investigated in cultured human hepatocytes. Cells were exposed with 0.01 to 50 µg/ml sorafenib for five days in comparison to the prototypic inducers omeprazole (OME, CYP1A2), rifampicin (RIF, CYP3A4), and phenobarbital (PB, CYP3A4). The study revealed no inductive effect of sorafenib on human CYP1A2 and CYP3A4 after repeated exposure up to a concentration of at least 3 µg/ml, whereas OME (100 µM), RIF (50 µM), and PB (2 mM) showed their inducer-specific changes of the CYP isoform activities.
The inhibitory potency of sorafenib towards human cytochrome P-450 isoforms was investigated. Only small inhibitory effects on CYP2C19, 2D6, and 3A4 were observed (Ki = 17 µM, 22 µM, and 29 µM, respectively). Sorafenib inhibited CYP2B6, 2C8, and 2C9 activities (Ki = 5 - 6 µM, 1 - 2 µM, and 7 - 8 µM, respectively). The potency of sorafenib to inhibit single CYP isoforms was according to

the following order: CYP2C8 > CYP2B6, CYP2C9 > CYP2C19, CYP2D6, CYP3A4. CYP inhibition caused by metabolite M-2 was in the same range as for sorafenib itself.
The inhibitory potency of sorafenib towards five human UDP-glucuronosyltransferases and towards acetaminophen glucuronidation, catalyzed by multiple UGTs, was investigated. Propofol was selected as suitable selective substrate for UGT1A9 and its glucuronidation was significantly inhibited by sorafenib (Ki = 2.2 μM). Sorafenib was also a substrate of UGT1A9 (Km = 3 – 8 μM). 3-Glucuronidation of estradiol as well as SN-38 glucuronidation were strongly inhibited (Ki =1.0 μM and 2.7 μM, respectively).
Sorafenib (100 and 200 μM) did not affect biotransformation of [14C] 5-fluorouracil *in vitro*, whereas uracil (100 μM, positive control) as a substrate of dihydropyrimidine dehydrogenase inhibited 5-fluorouracil metabolism to one third of control activity.

- Other pharmacokinetic studies

The metabolic pattern in plasma of male Wistar rats was investigated following oral administration of 5 mg/kg [14C] M2, the major human circulating metabolite identified. There was a time-dependent decrease of [14C] M2 from 96 % (after 30 minutes) to 6 % of the radioactivity present in plasma, 48 hours following administration. After 7 hours M2 was still the major component. In terms of AUC, M2 represented the majority of the radioactivity present in plasma (57.8 %). M2 moderately inhibited CYP2B6, 2C9, 2C19, 2D6, and 3A4 and exhibited a more pronounced inhibitory effect on CYP2C8.

Toxicology

- Single dose toxicity

The toxicity of single doses of sorafenib has been investigated *via* the oral route in mice, rats and dogs. The highest single oral sorafenib dose of 1460 mg/kg was tolerated in mice and rats without any sign of toxicity. In Beagle dogs, vomiting was observed after a single oral dose of 120 mg/kg solution, and 1000 mg/kg powder (1370 mg/kg of the tosylate salt) After administration of two times 24 hours i.p doses of 125-500 mg/kg of sorafenib in mice, apathy, roughened fur, hard abdomen, spasm, periodically stretching of body and difficulty in breathing were observed but mortality was not induced. At 500 mg/kgone male died. At the 1000 mg/kg dose, all males and one female died.

- Repeat dose toxicity (with toxicokinetics)

The long term toxicity of sorafenib was tested in mice (3 months), rats (up to 6-month), Beagle dogs (up to 12 months).
A group of 10 male and 10 female Wistar rats were treated orally for 4 weeks at 0 -125 mg/kg dose of sorafenib. Mortalities prior to the end of 4 weeks treatment included 3 males and 9 females at 25 mg/kg, 3 males and 1 female at 125 mg/kg. Dose-dependent clinical signs of toxicity were noted at 5 mg/kg and above. In the clinico-chemical investigations, increases were seen in AST, ALT (beginning at 1 mg/kg), GLDH and LDH (at 25 and 125 mg/kg), cholesterol and bilirubin (beginning at 5 mg/kg). An increase was seen in alkaline phosphatase (ALP) at 5 mg/kg with decreases at 25 and 125 mg/kg. In urine, increases were seen in protein, urine creatinine (in females only), NAG, and LDH, particularly at 25 and 125 mg/kg. Histopathological evaluation revealed treatment-related findings in the majority of animals treated at 25 or 125 mg/kg. Overall, the changes were classified as degenerative in the adrenal glands, liver, stomach, duodenum, pancreas, kidneys, heart, and ovaries. Regenerative changes were observed in the liver (bile duct proliferation), pancreas, duodenum and kidneys. Necrosis was observed in the spleen, lymph nodes, and thymus. Effects in male reproductive organs included retardation in testes, epididymides, prostate and seminal vesicles. Most of the findings could be shown to be reversible. However, bile duct proliferation, liver fibrosis, effects on lymphoreticular system were still visible at the end of the 1-month recovery period. A NOAEL was not established.
In a 6 month toxicity study,20 male and 20 female Wistar rats were treated daily orally by gavage at 0 -2.5 mg/kg dose of sorafenib. The lowest dose causing significant toxicity (LOAEL) was 1 mg/kg/day. A NOAEL was not established for females.

In a 4-week study in dogs with initial twice-daily administration of 10, 30, or 60 mg/kg, both 30 mg/kg bid and 60 mg/kg bid of sorafenib induced emesis, bloody diarrhoea and reduced body weight gain > Dosing was therefore reduced to once daily after one week of treatment.
After the administration of 30 mg/kg of sorafenib, once-daily for 3 or 12 months in dogs, effects on the GI tract (bloody diarrhoea) and on the skin (hair loss, inflammation) were observed. At histopathology, degenerative and regenerative processes were seen dose-dependently in multiple tissues including kidneys, lymphoreticular / hematopoetic system, GI tract, adrenals, teeth and bone. Marked morphological changes were observed in the liver and skin. The morphological no-effect-level after 3 or 12 months treatment was below 10 mg/kg/day.

- Genotoxicity

The genotoxicity of sorafenib tosylate was studied with respect to gene mutations in bacteria, chromosomal aberrations *in vitro* in Chinese hamster V79 cells, and *in vivo* in the mouse micronucleus test in bone marrow. Sorafinib was investigated using the Salmonella/microsome plate incorporation test for point mutagenic effects in doses of up to 5000 µg per plate on five Salmonella *typhimurium* LT2 mutants. Doses up to 8µg per plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed. At higher doses the substance had a strong, strain-specific bacteriotoxic effect. Evidence of mutagenic activity of sorafenib was not seen. No biologically relevant increase in the mutant count was observed. The cytogenetics *in vitro* test for clastogenicity using Chinese hamster V79 cells was negative without metabolic activation, but was positive for clastogenicity in the cytotoxic range of doses (cytotoxicity starting at 40 µg/ml) following addition of a liver microsome preparation (S9-mix). The Micronucleus test for clastogenicity in NMRI mice with intraperitoneal sorafenib tosylate doses of 0, 125, 250, or 500 mg/kg (sorafenib dose up to 365 mg/kg) was negative.

- Carcinogenicity

Carcinogenicity studies were not conducted with sorafenib.

- Reproductive and developmental toxicity

Developmental toxicity studies were performed in rats and rabbits. Twenty-two inseminated female Wistar rats were treated orally by gavage with sorafenib tosylate. The rats were treated from day 6 to 17 *post conceptionem* with sorafenib doses of 0 to 2.5 mg/kg/day. Treatment-related effects were observed at 2.5 mg/kg and included impaired gestation rate (one total late resorption), increased post-implantation loss (late resorptions) and consequently decreased mean litter size, increased incidence of necrotic placental borders and pale placentas, decreased placental and fetal weights, retarded fetal skeletal ossification in relation to reduced fetal weights and increased incidence of external and visceral deviations (pale appearance, missing innominate artery) and skeletal variations (supernumerary 14th ribs). Incidence of generally common fetal malformations of different types was as well increased at 2.5 mg/kg. The NOAELs determined for systemic maternal toxicity and intrauterine development were 1 mg/kg/day and 0.2 mg/kg/day, respectively.
Twenty female Himalayan rabbits were treated daily by oral administration (gavage) with sorafenib tosylate from days 6 to 20 *post conceptionem* with 0 to 3 mg/kg dose of sorafenib. Sorafenib induced teratogenic effects at 3 mg/kg/day ($AUC_{0\text{-}24h}$ 12 mg.h/l; 0.1 times the expected clinical exposure). Increased incidence of fetal malformations mainly malformations of kidneys, vertebrae, and ribs was seen at 3 mg/kg/day. A treatment-related effect on external and visceral deviations was not evident at doses up to and including the highest tested dose (3 mg/kg/day). Skeletal development (retardations/variations) revealed an increased incidence of fused sternebrae and retarded ossification of cervical vertebral bodies and frontal bones at 3 mg/kg/day. Fetal sex distribution was shifted to 40% males at 3 mg/kg/day. At 3 mg/kg/day, decreased maternal weights, decreased gestation rate due to the abortion of one female, and by three females, which showed total resorptions, incidences of placental findings (partly necrotic placentas), increased postimplantation loss in females with viable fetuses, mainly caused by late resorptions and decreased mean number of fetuses were observed. Increased

incidence of females with cold ears (transient occurrence) increasing with dose (NOAEL was < 0.3 mg/kg/day in maternal and NOAEL was 1 mg/kg in pups).
In the 4-,13- and 26- week study in rats, treatment-related effects at a dose of 25 mg/kg/day of sorafenib were observed (in male reproductive organs included retardation in testes, epididymides, prostate and seminal vesicles; in females there were retarded ovaries and central necrosis of corpora lutea).
Dogs showed tubular degeneration in a 12-month study at 30 mg/kg/day, and oligospermia in the epididymides was found at 60 mg/kg/day.

- Local tolerance

In rabbits, sorafenib was not irritant to the skin or to the eyes and there were no systemic intolerance reactions ([29, 30])

- Other toxicity studies

Immunotoxicity
No specific studies to investigate immunotoxicity were performed.

Metabolites
M-2 has been toxicologically characterised with respect to genotoxicity (*in vitro* bacterial mutation) and repeat dose toxicity (1-month oral rat study). M-2 did not induce genotoxic effects *in vitro* in the Ames test. In the repeat dose toxicity study survival as well as the appearance or behavior of the rats were not affected by treatment with M2. At 5 and 25 mg/kg, the animals exhibited slight body weight retardation. Results from urinalysis were unremarkable. Hematology revealed slightly elevated numbers of erythrocytes, hemoglobin and hematocrit at 5 and 25 mg/kg, in addition slightly reduced platelet counts in 25 mg/kg rats. Blood clinical chemistry revealed a tendency to increased activities of AST and ALT in rats receiving 5 or 25 mg/kg, plasma concentrations of triglycerides were slightly reduced after 25 mg/kg. At histopathology, the only remarkable finding was a treatment-related dentin alteration and increased numbers of adipocytes in the bone marrow of 5 and 25 mg/kg animals. There was no morphological correlate for the elevated serum markers for liver toxicity and decreased liver weights observed at necropsy. The NOAEL was 1 mg/kg/day.

Studies on impurities
Two impurities have been toxicologically characterised in genotoxicity trial (*in vitro* bacterial mutation).
An Ames test and a repeat-dose (4-week) toxicity study in rats were performed with PAPE-urea. There was no indication for a genotoxic potential of PAPE-urea. In the 4-week rat study with experimental conditions the highest dose tested, 1000 mg/kg/day, was tolerated without any signs of systemic toxicity.
For the second impurity (PAPE) an Ames test was positive after metabolic activation (S9-mix), with a lowest effect-dose of 624 µg/plate, but negative without metabolic activation. One batch, which contained 0.34% PAPE was tested in the Ames test, the chromosomal aberrations test and in the *in vivo* micronucleus test, no genotoxic effect was seen in either the Ames test or the micronucleus test. However, genotoxic effects were induced in the chromosomal aberration test.

Ecotoxicity/environmental risk assessment

The calculated $PEC_{SURFACEWATER}$ was below the action limit of 0.01µg/l, taking individual market penetration factors into consideration.

Discussion on the non-clinical aspects

Pharmacology

Sorafenib was highly efficacious *in vivo* in renal tumour xenograft models (RENCA model). Anti-tumour efficacy was also demonstrated *in vivo* in a number of different non-renal tumour xenograft models by affecting the RAS/RAF/MEK pathway. This included xenograft models of human colon, lung, breast, melanoma, leukaemia, pancreatic, and ovarian cancer. The efficacy of sorafenib against this tumour models suggested that a RAF kinase inhibitor may have utility not only in human tumours containing ras and/or b-raf mutations, but also in tumours that overexpress other growth factor receptors that signal through the same pathway. Furthermore, sorafenib was very effective against the MV4-11 AML model that expresses an activating Flt3 mutation. In cellular assays *in vitro*, sorafenib inhibited the RAF/MEK/ERK pathway in breast, pancreatic, melanoma, and colon tumour lines as evidenced by reduction of phospho-ERK levels including cell lines expressing either wild-type or mutant k-RAS or BRAF. However, inhibition of the RAF/MEK/ERK pathway was not observed in the non-small cell lung cancer (NSCLC) lines A549 and H460, at concentrations up to 20 µM sorafenib. The mechanism underlying the lack of inhibition of ERK phosphorylation in these cell lines has not been elucidated.
No direct comparison was made non-clinically between a tumour model that expresses a wild type VHL and a VHL mutant subline of the same model. Sorafenib was active against the 786-0 human renal tumour model that has a VHL deletion and was less active against the CAKI-1 human renal tumour model that expresses wild type VHL. However, since these two tumour lines were derived independently from separate patients, it could not be concluded that the difference in VHL status was solely responsible for the different sensitivity to sorafenib. Although this data could help to predict the sensitivity of a human tumour to sorafenib therapy, limited data did not support patient selection for sorafenib treatment based on this biomarker (see clinical pharmacology section).

Pharmacodynamic drug interactions were studied in several non-clinical tumour xenograft models. The results suggested that sorafenib can be combined with paclitaxel, irinotecan, gemcitabine, gefitinib and cisplatin with no significant increase in the toxicity and without diminishing their anti-tumour efficacy. However, the combination of sorafenib and doxorubicin required reduction of the dose level of both agents to attain acceptable tolerance and efficacy (see clinical pharmacokinetc section).

The metabolites M-2, M-4 and M-5 inhibited several receptor tyrosine kinases including VEGFR-2, PDGFR-β, and Flt-3, showing a similar *in vitro* pharmacological activity as sorafenib, but they were present at much lower plasma levels. Thus, the metabolites did not much contribute to the overall activity. The most predominant human metabolite, M-2, was also evaluated *in vivo* for anti-tumour efficacy against the MDA-MB-231 xenograft model and was less active than the parent compound. However, the degree to which M-2 contributed to the anti-tumour efficacy of sorafenib could not be determined as M-2 was partially converted to sorafenib *in vivo*.

Pharmakokinetics

Sorafenib was almost completely absorbed in mice and rats and moderately absorbed in dogs. Sorafenib and/or its metabolites were widely distributed, crossed the blood-brain barrier and penetrated the placental barrier. In animals, sorafenib and/or its metabolites were excreted into milk. It is not known whether sorafenib is excreted in human milk. Because sorafenib could harm infant growth and development, breast-feeding is contraindicated during sorafenib treatment (see SPC sections 4.3, 4.6 and 5.3).
In human, sorafenib was subject to two important biotransformation pathways. Formation of M-2 was catalyzed by CYP3A4 and formation of the drug glucuronide M-7 was mediated by UGT1A9. Additionally, human kidney was also capable of forming glucuronide M-7. *In vitro* incubations of

[^{14}C]sorafenib tosylate with liver microsomes of human and animal species revealed two phase I reactions to be prominent: hydroxylation of the N-methyl group yielding metabolite M-3 and N-oxidation at the pyridine moiety yielding metabolite M-2. Based on comparison of metabolite profiles, mouse and rat was considered roughly similar to human. M-2, however, was only formed in smaller amounts in dogs and rabbits. Rhesus monkey was regarded as most similar to human.
Sorafenib did not induce human CYP1A2 and CYP3A4 in cultured human hepatocytes. Sorafenib was capable of inhibiting CYP2C8, CYP2B6 and CYP2C9 activities in human liver microsomes whereas only small inhibitory effects on CYP2C19, CYP2D6, and CYP3A4 were observed. Glucuronidation of propofol (UGT1A9) was significantly inhibited. 3-Glucuronidation of estradiol (UGT1A1) as well as SN-38 glucuronidation (UGT1A1) were strongly inhibited. Sorafenib did not inhibit dihydropyrimidine dehydrogenase *in vitro*. Hence, clinical drug interactions might be expected due to inhibition specifically of the CYP2C8, CYP2B6, CYP2C9, UGT1A9 and UGT1A1 enzymes (see SPC, section 4.4 and 4.5, see also clinical pharmacokinetic section).

Toxicology

The toxicology of sorafenib was investigated in accordance with the European guidelines [31],[32]. Sorafenib was of low acute toxicity. Single-dose studies with intravenous administration were not performed due to the very low solubility of sorafenib in suitable vehicles. When comparing non-lethal doses, the multiple to human maximum dose (13 mg/kg) was approximately 100 in the mouse and rat, and 75 in the dog. In repeat-dose toxicity studies, the MTD was 100 mg/kg/day in mice (3-months), 5 mg/kg/day in rats (3-months) and >60 mg/kg/day in dogs (12-months). The main target organs of toxicity with possible impact on the human risk assessment were: liver, kidneys, gastrointestinal-tract, cardio-vascular system, lymphoreticular/hematopoietic system, adrenals, reproductive organs, teeth, bone, and skin. Some of the morphological lesions were fully reversible or showed at least a tendency towards recovery. However, bile duct proliferation, liver fibrosis, effects on lymphoreticular system were still visible at the end of the 1-month recovery period. Effects on the liver were observed in mice, rats and dogs after repeated oral administration. Increased levels of ALT, AST, GLDH and ALP in serum were accompanied by histopathological changes and also mostly by decreased liver weights. The estimated exposure multiple towards the estimated clinical exposure was 1-3, 0.7 and <0.2 in mice, rats and dogs, respectively. An anti-diuretic effect and histopathologically nephropathy including tubular dilation, hyaline casts and basophilic tubules were observed in different animal studies. The exposure multiple towards the estimated clinical exposure was 2-4, 0.1 and <0.2 in mice, rats and dogs, respectively.
Effects on peripheral white and red blood cell serum parameters or on blood coagulation parameters were observed at relatively low dose levels in the repeat dose studies. The observations were often accompanied by changes in the blood-forming elements (bone marrow, spleen) and immune organs (thymus, spleen) but then mostly at a slightly higher dose to where haematological changes were noted. The exposure multiple towards the estimated clinical exposure was 0.5-3, <0.7 and <0.2 in mice, rats and dogs, respectively. In young and growing dogs after repeated dosing, irregular thickening of the femoral growth plate, hypocellularity of the bone marrow next to the altered growth plate, and alterations of the dentin composition in the teeth were observed. In aged adult dogs, no treatment related morphological changes in the femoral bone or in the teeth were observed. In rats and mice after repeated dosing, marked effects on the teeth including altered dentin composition were observed. The extrapolation of these findings to the human is difficult as in contrast to humans, the teeth of the rodent species used (in particular the incisors) are continuously growing through the life of the animal. The LOAEL for bone was, 5 mg/kg/day (rat, 6-month) and 10 mg/kg/day (dog). The exposure multiple towards the estimated clinical exposure was 0.7 and <0.2 in rats and dogs, respectively. The LOAEL for teeth was 100 mg/kg/day (mice), 0.1 mg/kg/day (female rat, 6-month) and 30 mg/kg/day (dog). The exposure multiple towards the estimated clinical exposure was 1-3, 0.01 and <0.5 in mice, rats and dogs, respectively. Overall, a potential risk of toxicity on bone and teeth of children or adolescents cannot be excluded (see SPC section 5.3).
The standard program of genotoxicity studies was conducted and positive results were obtained as an increase in structural chromosomal aberrations in an *in vitro* mammalian cell assay (Chinese hamster ovary) for clastogenicity in the presence of metabolic activation was seen. Sorafenib was not genotoxic in the Ames test or in the *in vivo* mouse micronucleus assay. One intermediate in the manufacturing process, which is also present in the final drug substance (< 0.15%), was positive for

mutagenesis in the Ames test. Furthermore, the sorafenib batch tested in the standard genotoxicity program included 0.34% PAPE. Studies to evaluate the carcinogenic potential of sorafenib have not been performed. The omission of carcinogenicity studies is acceptable for this type of compound [5].
No specific studies have been conducted in animals to evaluate the effect of sorafenib on fertility. Repeat-dose studies in animals have shown changes in male and female reproductive organs at exposures below the anticipated clinical exposure (based on AUC). Sorafenib has been shown to be embryotoxic and teratogenic when administered to rats and rabbits at exposures below the clinical exposure. Therefore, it is recommended that sorafenib is not used during pregnancy unless clearly necessary, after careful considerations of the needs of the mother and the risk to the foetus. Women of childbearing potential must use effective contraception during treatment (see SPC sections 4.6 and 5.3).
Sorafenib and its metabolite M-2 had a similar toxicological profile. The specification limit of 0.15% for two impurities was considered toxicologically qualified and justified in view of its use in patients with advanced renal cancer. A third impurity was considered toxicologically qualified up to 0.74%. Sorafenib was considered unlikely to pose a risk to the environment [33] .

1.4 Clinical aspects

Introduction

The clinical programme of sorafenib comprised seven dose-finding and PK phase I studies involving patients with advanced solid tumours. Patients from these studies were allowed to continue treatment with sorafenib in an extension phase II protocol (study 10922). The phase II program explored signals for efficacy in several tumour types (study 100391 in advanced, refractory colorectal cancer and renal cell carcinoma, and study 10874 in hepatocellular carcinoma). In addition, four studies explored the combination of sorafenib with other antineoplastic agents [oxaliplatin (study 10954), doxorubicin (study 10916), gemcitabine (study 100374), irinotecan (study 10981)]. A phase III pivotal study (study 11213) compared sorafenib 400 mg twice daily with placebo in patients with advanced RCC. The clinical trials were performed in accordance with GCP as claimed by the applicant. The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Sorafenib treatment must be supervised by a physician experienced in the use of anticancer therapies. The recommended dose in adults is 400 mg (two tablets of 200 mg) twice daily (equivalent to a total daily dose of 800 mg). It is recommended that sorafenib is administered without food or with a low or moderate fat meal. If the patient intends to have a high-fat meal, sorafenib tablets should be taken at least 1 hour before or 2 hours after the meal. The tablets should be swallowed with a glass of water. The treatment should continue as long as clinical benefit is observed or until unacceptable toxicity occurs. Management of suspected adverse drug reactions may require temporary interruption and/or dose reduction of sorafenib therapy. When dose reduction is necessary, the sorafenib dose should be reduced to two tablets of 200 mg once daily (see section 4.2 and 4.4).

Pharmacokinetics

Pharmacokinetic (PK) plasma samples from clinical studies were analysed for sorafenib using validated HPLC assays with mass-spectrometric detection. The lower limit of quantitation in plasma was 1-10 µg/l for sorafenib (varied by study), 25 µg/l for M1 and 10 µg/l for metabolites M2, M3, M4 and M5. The lower limit of quantitation in urine was approximately 10 µg/l for sorafenib and metabolite M2. Metabolites M7 and M8, glucuronides of parent drug and metabolite M2, respectively, were measured indirectly as sorafenib and M2 after hydrolyzing the corresponding glucuronide. The lower limit of quantitation for both analytes was approximately 14 µg/l based on the conversion factor. Upper calibration range was approximately 10,000 µg/l for sorafenib and metabolite M2 and approximately 14,000 for metabolites M7 and M8. Precision for all analytes for all methods was within 15%, and accuracy was within 85 - 115%. Stability of all analytes was determined under sample handling conditions, freeze-thaw cycles and for the duration the samples from clinical studies were stored prior to analysis. All analytes were stable under those conditions. Cross-validation for

different analytical sites was performed. Pharmacokinetic parameters were calculated using non-compartmental methods.

- Absorption

In healthy volunteers, sorafenib taken with a moderate-fat meal was rapidly absorbed with a median T_{max} ranging from 4 to 8 hours. In patients, the median T_{max} was approximately 3 hours (range 0-24 hours). Secondary absorption peaks were observed at 8-12 and 24 hours postdose, indicating enterohepatic re-circulation.
Permeability evaluations in Caco-2 cells indicated that sorafenib is a highly permeable compound based on comparison with 22 reference compounds. The efflux ratio of sorafenib for transport from basolateral to apical side to transport from the apical to basolateral side of Caco-2 cells was 4.7 and 2.5 at 0.1 and 1 µM sorafenib, respectively, indicating significant but saturable efflux. However, the efflux ratios for known substrates of efflux pumps such as vinblastine (29.6) and sulphasalzine (42.3) were higher, indicating that sorafenib is a weak substrate for an efflux pump. Sorafenib efflux was also evaluated in monolayers of P-gp-expressing L-MDR1 cells in the presence and absence of ivermectin, a potent P-gp inhibitor. In this study the efflux ratio was 6.78 and 4.25 at 0.1 and 1 µM sorafenib, respectively. The sorafenib efflux was inhibited by ivermectin. The efflux ratios for sorafenib in this experiment were in the same range as those reported in the literature for prazosin (4.6) and vincristine (6.3). Efflux ratio reported for paclitaxel in P-gp over-expressing cells is >108. The efflux observed for sorafenib was low to moderate.

Bioavailability

The absolute bioavailability of sorafenib has not been determined as no intravenous formulation has been developed. The pharmacokinetic parameters of sorafenib following the administration of a single oral doses of sorafenib given as liquid formulation (polysorbate 80-based oral solution) or as 50 mg tablets to patients are provided in the table below.

Table 2: PK parameters of sorafenib following single oral dose given as liquid formulation or as 50 mg tablets [geometric means / (%CV)] (n=3 / cohort)

Parameter	Unit	Liquid formulation		50 mg tablet	
Dose		**100 mg**	**200 mg**	**100 mg**	**400 mg**
AUC	mg*h/l	64.6 / 63%	59.7 / 51%	24.5 / 52%	58.3 / 22%[b]
C_{max}	mg /l	2.10 / 22%	2.10 / 21%	0.75 / 34%	3.53 / 31%
t_{max}[a]	h	2.5 (1.0-6.0)	3.0 (2.5-3.0)	8.0 (8.0-12.0)	3.1(3.0-3.1)
$t_{½}$	h	34.9 / 51%	32.1 / 26%	38.0 / 10%	

[a] median (range); [b] AUC_{0-tn}

Bioequivalence

In one study (n=26-34), similar exposures were obtained with a single 400 mg dose given either as 50 mg tablets or as 200 mg tablets.

Influence of food

The pharmacokinetics of sorafenib after a single 400 mg (given as 200 mg tablets) dose were evaluated in 15 healthy volunteers (study 100484) following administration of a high-fat breakfast, a moderate-fat breakfast and administration in the fasted state. Sorafenib bioavailability from tablets following administration of a moderate-fat breakfast was almost similar to that when administered in the fasted state, although there was a trend towards increased AUC (14%). However, when given with a high-fat breakfast, sorafenib absorption was reduced by approximately 29% compared to administration in the fasted state. Study 100545 confirmed that concomitant administration of a moderate-fat meal with 400 mg sorafenib (given as 50 mg tablets) in 29 healthy volunteers had no influence on AUC of sorafenib compared to fasted dosing.
The solubility of sorafenib tosylate ranges from 0.034 mg/100ml at pH 1.0, to 0.013 mg/100ml at pH 4.5. The influence of intra-gastric pH on sorafenib pharmacokinetics is described in the PK interaction studies (antiacids) section (see also discussion on clinical PK).

- Distribution

As there was no study with intravenous administration of sorafenib, the volume of distribution was determined. Apparent volume (V/F) was not reported.
Sorafenib and its main metabolite in plasma (M2) were to 99.5% bound to plasma proteins. Protein binding was linear across concentrations. Sorafenib and M2 were primarily bound to serum albumin and to a lesser extent to α-globulins, β-globulins and LDL but not to γ-globulins and α_1-acid glycoprotein. Sorafenib was distributed between red blood cells and plasma with a plasma to blood ratio of 1.33. In man, total plasma radioactivity and sorafenib were subject to enterohepatic circulation as indicated by several secondary maxima of the plasma concentration time curves and was explained by two different processes: i) glucuronidation of the drug, biliary excretion of the glucuronidated metabolite M7 into the gut, cleavage of the glucuronide and reabsorption of sorafenib, ii) oxidation of the drug to metabolite M2, biliary excretion of M2 into the gut, reduction by colonic bacteria to sorafenib and reabsorption.

- Metabolism

The biotransformation of sorafenib *in vitro* was investigated using liver microsomes of several animal species and humans, microsomes containing heterologously expressed human cytochromes or glucuronosyl transferases and rat and human hepatocytes (see non-clinical section). Table 3 provides a summary of the identified metabolites formed in human, *in vivo* and/or *in vitro,* and their occurrence in plasma, faeces and urine over 192 hours sampling after a single dose in the mass-balance study.

Table 3: The human metabolites of sorafenib and their occurrence after a single dose

Substance		**Plasma (% of total radioactivity)**	**Faeces (% of dose)**	**Urine (% of dose)**
Sorafenib		73%	50.7%	nd
M1	Combination of N-oxidation and N-methylhydroxylation	nd	nd	nd
M2	N-oxidation	16.7%	nd	nd
M3	N-methylhydroxylation	trace amounts	0.4%	nd
M4	demethylation	1%	1.2%	nd
M5	oxidative metabolite	nd	nd	nd
M6	carboxylic acid	trace amounts	19.1%	nd
M7	glucuronide of sorafenib	0.5%	nd	14.8%
M8	glucuronide of M2	nd	nd	2.7%

nd = not detected

At 400 mg b.i.d. multiple dosing, metabolites M2 and M4 accumulate to a similar extent as sorafenib. At steady state, metabolite M2 exposure was16% (sum of parent and measured metabolites) and M4 exposure was 8% of the total exposure. Metabolites M2 and M4 reached a steady state after 7 days of dosing. M5 was 6% of the total exposure at steady state. M5 reached a steady state after 14 days of dosing. The major metabolite M2 was eliminated *via* further metabolism to M1, glucuronidation to M8 and possibly biliary excretion.

- Elimination

The mean terminal half-life determined for sorafenib across studies varied between 25-48 hours.
The disposition and excretion of sorafenib and its metabolites was evaluated in a clinical mass-balance study with a single oral administration of 100 mg ^{14}C-sorafenib as oral solution to four healthy male volunteers. A total of 96% of the dose was recovered in excreta within 14 days, and 77% of the radioactivity was recovered in faeces. Unchanged sorafenib was the major constituent in faecal extracts (51% of the dose). About 10% of the dose (5-8% of the dose as unchanged drug) was recovered in the first faecal sample (0-48hr), while the largest fractions of the dose (25-38% of the dose as total radioactivity or 19-25% as unchanged drug) was found in the second (48-72 hr) or third (72-96%) samples. The major metabolite in faeces was the carboxylic acid M6 (9.1 % of the dose).

M3 and M4 were minor metabolites (0.4% and 1.2% of the dose, respectively). M2 was not found in faeces, likely due to its instability towards intestinal bacteria.
Approximately 19% of the dose was recovered in urine as glucuronidated metabolites. No unchanged sorafenib was found in urine, but two metabolites, M7 and M8 could be detected and identified as glucuronide of sorafenib and glucuronide of the predominant plasma metabolite, M2, respectively. M7 accounted for 78.6 % and M8 for 14.1 % of the radioactivity in urine, respectively (14.8 % and 2.7 % of the dose, respectively).

- Dose proportionality and time dependencies

With the oral solution, the exposure did not increase linearly at doses of 100 mg and higher in a multiple-dose study in patients. However, with b.i.d. dosing, there was a dose-dependent increase in AUC with increasing dose up to 400 mg b.i.d. administered as tablets, although the variability was high within and between studies. At higher doses, exposure increased less than proportionally with dose: Mean $AUC_{(0-12),ss}$ values at the 600 mg b.i.d. dose level were slightly greater than those at 400 mg b.i.d. and mean $AUC_{(0-12),ss}$ at the 800 mg dose level was not greater than that at 600 mg b.i.d (see table 4).

Sorafenib exposures reach steady state after 7 days of dosing. With 400 mg b.i.d. dosing, there was on average a 4-fold and 4.7-fold accumulation at steady state in Cmax,ss and AUC(0-12),ss values, respectively.

Table 4: Mean plasma pharmacokinetic parameters following 28 days of dosing in patients (study 100277)

Dose		**AUC_{0-24} -(mg*h/l)**	**AUC_{0-12} (mg*h/l)**	**C_{max} (mg/l)**		**$t_{1/2}$ (h)**
		Day 1	**Day 28**	**Day1**	**Day 28**	**Day 28**
100 mg	N	3	4	4	4	2
once daily	*mean (CV%)*	8.75 (35.1)	15.06 (64.6)	0.81 (37.6)	0.85 (62.0)	23.8 (1.7)
100 mg	N	2	3	3	3	3
bid	*mean (CV%)*	6.13 (74.0)	45.98 (36.7)	0.81 (61.3)	5.4838.9)	35.5 (74.6)
200 mg	N	4	5	6	5	4
bid	*mean (CV%)*	10.88 (38.4)	34.72 (43.8)	1.34 (33.8)	3.95 (52.3)	31.8 (10.1)
400 mg	N	4	3	4	3	3
bid	*mean (CV%)*	**21.81** (58.8)	**47.78** (24.0)	**2.87** (68.4)	**5.37** (41.0)	**27.4** (24.1)
600 mg	N	3	5	7	5	4
bid	*mean (CV%)*	10.06 (96.7)	38.09 (36.8)	2.00 (71.5)	4.71 (28.5)	26.3 (27.1)

Sorafenib exhibits relatively high inter- and intra-patient pharmacokinetic variability in exposure with coefficients of variation around 61%-65% and 44%-47%, respectively.

- Special populations

Impaired renal function: A retrospective evaluation across studies was made to evaluate the relationship between calculated creatinine clearance and steady-state exposure to sorafenib. Pharmacokinetic data were available from 4 patients with a calculated creatinine clearance in the range of 30-50 ml/min, 24 patients in the range of 50-80 ml/min and 71 patients in the range of >80 ml/min. Data were only presented for patients receiving the 400 mg dose b.i.d (phase I studies 10164, 100277, 100283, 100342), which did not include patients with creatinine clearance < 60 ml/min. Steady state exposure to sorafenib was similar in patients with mild or moderate renal impairment compared to the exposures in patients with normal renal function.

Impaired hepatic function: Pharmacokinetic data was obtained in a phase II study (10874) in patients with hepatocellular carcinoma [Child-Pugh A (n=14) or B (n=8)] receiving sorafenib 400 mg bid. Patients with Child Pugh B, compared with patients with Child Pugh A, had numerically higher AUC_{0-8hr} [geometric mean (%CV) = 30.3 (82.1) *vs* 25.4 (38.4)] and C_{max} [geometric mean (%CV) = 5.97 (73.8) *vs* 4.92 (38.7)] values for sorafenib (differences not statistically significant). Exposure to

metabolite M5 was slightly lower in Child-Pugh B patients, while there were no differences between the two groups for M2 and M4.

Gender: A retrospective evaluation across studies (10164, 100277, 100283 and 100342) did not indicate a relationship between gender and steady state plasma sorafenib $AUC_{(0-12),ss}$ values following administration of 400 mg bid of sorafenib. No evaluation of the relationship between CL/F and gender was presented.

Race: Steady state plasma sorafenib AUC(0-12),ss and Cmax,ss values in Oriental (Japanese) patients (studies 10658 and 11497) were compared with those in Caucasian patients (studies 10164, 100277, 100283 and 100342) across studies. While the mean sorafenib $C_{max,ss}$ [geometric mean (%CV) = 4.91 (76) *vs* 8.3 (57.4)] and $AUC_{(0-12),ss}$ [geometric mean (%CV) = 36.7 (73) *vs* 67.3 (56.8)] values were lower in Japanese patients (n=6) compared to Caucasian patients (n=27) receiving sorafenib 400mg b.i.d. and for other dosages (data not shown), there was a significant overlap in the range of exposures observed in these two groups. Sorafenib had high inter-patient pharmacokinetic variability in both patient groups.

Weight: A retrospective evaluation across studies (10164, 100277, 100283 and 100342) did not indicate a relationship between body weight and steady state sorafenib $AUC_{(0-12),ss}$ values following administration of 400 mg bid of sorafenib.

Elderly: A retrospective evaluation across studies (10164, 100277, 100283 and 100342) did not indicate a relationship between age and steady-state exposure to sorafenib. No evaluation of the relationship between CL/F and age was made. There were only 3 patients above 65 years in the presented evaluation of the 400 mg dose.

Children: There were no PK data available in children.

- Pharmacokinetic interaction studies

Inhibition of cytochrome P450

The potential of sorafenib to inhibit cytochrome P450-mediated metabolism *in vitro* was investigated using recombinant enzymes as well as human liver microsomes. Table 5 summarises the results of these studies.

Table 5: Inhibitory effects of sorafenib on formation of metabolites from standard probes mediated by CYP isoforms

CYP isoform	Substrate	Enzyme source	Ki [µM]
1A2	Phenacetin	Recombinant enzyme	232
	Phenacetin	Human liver microsomes	n.i.
2A6	Coumarin	Recombinant enzyme	n.i.
2B6	7-Ethoxytrifluoromethylcoumarin	Recombinant enzyme	6.2
	Bupropion	Human liver microsomes	5.1
2C8	Taxol	Recombinant enzyme	2.4
	Amodiaquine	Recombinant enzyme	0.7
2C9	Diclofenac	Recombinant enzyme	7.3
	Tolbutamide	Recombinant enzyme	7.7
2C19	S-Mephenytoin	Recombinant enzyme	17
2D6	Bufuralol	Recombinant enzyme	4.0
	Dextromethorphan	Human liver microsomes	22.2
2E1	Chloroxazone	Recombinant enzyme	n.i.
3A4	Testosterone	Human liver microsomes	26.3
	Midazolam	Human liver microsomes	28.9

n.i = no inhibition

In an *in vitro* study with pre-incubation with sorafenib in the presence and absence of NADPH, the inhibition of CYP2C9, CYP2D6 and CYP3A5 was neither time- nor NADPH dependent. The inhibitory potency of M2, towards 8 human CYP isoforms was investigated. M2 moderately inhibited CYP2B6, 2C9, 2C19, 2D6, and 3A4 and exhibited a more pronounced inhibitory effect on CYP2C8.

Inhibition of UDP-glucuronosyltransferases

The inhibitory potency of sorafenib towards five human UDP-glucuronosyltransferases (UGTs) and towards acetaminophen glucuronidation, was investigated. The glucuronidation of propofol, a substrate for UGT1A9, was inhibited by sorafenib (Ki = 2.2 μM). Sorafenib also inhibited UGT1A1 as shown by an inhibition of the glucuronidation of estradiol as well as SN-38 (active metabolite of irinotecan) with Ki =1.0 μM and 2.7 μM, respectively. Following repeated administration of 400 mg b.i.d. of sorafenib to patients, plasma levels (C_{max}) were above Ki values towards UGT1A1 and 1A9. Sorafenib did not significantly inhibit UGT1A4, UGT1A6 and UGT2B7 as indicated by IC50 values of 61 μM (UGT1A4) or > 100 μM sorafenib. Acetaminophen glucuronidation was not significantly inhibited. Genetic polymorphisms of UGT1A9 have been identified. Two alleles UGT1A9*3 [34-37] and UGT1A9*5 [38] have been associated with decreased glucuronidation activity. However, functional consequences of the UGT1A9 polymorphism are not yet well understood.

Inhbition of dihydropyrimidine dehydrogenase

Hepatic dihydropyrimidine dehydrogenase has been recognised as the relevant enzyme determining plasma and tissue concentrations of 5-fluorouracil in man. The inhibitory potency of sorafenib towards this enzyme was investigated in human liver cytosolic fractions. Sorafenib (100 and 200 μM) did not affect biotransformation of [^{14}C] 5-fluorouracil *in vitro*.

Inhibition of P-gp

In L-MDR1 cells, sorafenib concentration-dependently inhibited the active efflux of loperamide and dipyridamole. The IC_{50} for the inhibition of loperamide and dipyridamole efflux amounted to 0.84 μM and 1.24 μM, respectively. These IC_{50} values were significantly lower than the plasma concentrations of sorafenib observed during clinical studies.

Induction of cytochrome P450

The potential of sorafenib (0.01 to 50 μg/ml) to induce human CYP1A2 and 3A4 was investigated in cultured human hepatocytes of two different donors. Omeprazole, rifampicin and phenobarbital were used as positive controls. No inductive effect of sorafenib on human CYP1A2 and CYP3A4 after repeated exposure up to a concentration of 3 μg/ml was observed.

Ketokonazol

An interaction study was performed with 50 mg sorafenib and 400 mg ketokonazole (CYP3A4 inhibitor) in a one-way cross-over study in 16 healthy male volunteers. Ketoconazole caused no increase in sorafenib AUC [ratios of LS means: 0.89, 90% CI (0.69, 1.14)] and Cmax [ratios of LS means: 0.74, 90% CI (0.56, 0.97)] values and no change in its half-life [ratios of LS means: 1.01, 90% CI (0.80, 1.29)] in the absence and presence of ketoconazole administration. These data indicate that CYP3A4 may not be the primary metabolic/elimination pathway for sorafenib. M2 was measurable in the absence of ketoconazole, but when sorafenib was co-administered with ketoconazole, M2 concentrations could not be measured, indicating that M2 formation *via* CYP3A4 was inhibited.

Warfarin

The effect of sorafenib on warfarin metabolism was assessed indirectly by measuring prothrombin time international normalized ratio (PT-INR) for patients treated with warfarin (pivotal phase III study; 11 patients in the sorafenib group, 10 patients in the placebo groups). The incidence rate of a 50% increase in PT-INR from baseline and a 100% increase in PT-INR from baseline, in patients on warfarin, were similar in the sorafenib vs. placebo groups. Mean percent maximum change in PT-INR ratios was lower in the sorafenib group vs. placebo group.

Omeprazol, dextromethorphan and midazolam

Substrates of CYP2C19, CYP2D6 and CYP3A4 were administered before and on the last day of a 28-day cycle with continuous administration of sorafenib 400 mg b.i.d to patients. There were no effects of sorafenib on omeprazol, dextromethorphan or midazolam plasma pharmacokinetics in this study.

Irinotecan

Following single intravenous infusions of 125mg/m^2 irinotecan together with 400 mg b.i.d. sorafenib to patients the exposure to the active metabolite of irinotecan (SN-38), increased approximately by a factor of 2, and there was a tendency to increased irinotecan exposure. Concomitant administration of 100 mg or 200 mg sorafenib b.i.d. did not result in significant changes in either irinotecan or SN-38. In a study involving 6 patients, irinotecan had no significant effect on sorafenib at the lower doses of sorafenib, but at 400 mg b.i.d. sorafenib + irinotecan 125 mg/m^2, sorafenib exposure increased by 68%

(90% CI 1.27-2.49). This was in a cohort of only 6 patients, and the Applicant suggests that the clinical relevance might be limited, given the relatively high variability of sorafenib.

Doxorubicin
In one group of patients (n=5) receiving 400 mg b.i.d. sorafenib, doxorubicin C_{max} and AUC increased by 103% (90% CI 1.27 - 3.26) and 47% (90% CI 1.18 - 1.81), respectively. In a larger cohort of patients (n=12) receiving 400 mg b.i.d. sorafenib the increase in doxorubicin AUC was 21% (90% CI 0.95 - 1.54). Mean AUC of sorafenib increased 22-36% at co-administration of doxorubicin with 100 or 200 mg b.i.d. sorafenib (n=5 per dose). There was no appreciable change in sorafenib AUC at 400 mg b.i.d. sorafenib.

Gemcitabine
Gemcitabine is rapidly converted by cytidine deaminase to deoxyfluorouridine (dFdU). Gemcitabine levels were too low to be adequately measured in the study evaluating concomitant treatment with gemcitabine and sorafenib. However, there were no effects of sorafenib on the metabolite dFdU, and no apparent effects of gemcitabine on sorafenib pharmacokinetics, but the variability in sorafenib concentrations was high.

Oxaliplatin
Oxaliplatin undergoes rapid non-enzymatic biotransformation. In small cohorts of patients receiving different doses of sorafenib together with oxaliplatin, there were no consistent changes in either sorafenib or oxaliplatin pharmacokinetics.

Discussion on clinical pharmacokinetics

Sorafenib was a low-solubility, high permeability compound. This was consistent with the finding that one single batch of 50 mg tablets exhibiting a slower *in vitro* dissolution rate compared to other batches of tablets had a lower bioavailability. *In vitro* studies indicated that sorafenib is a weak to moderate substrate of the efflux transporter P-gp. In cancer patients, across studies, the median T_{max} was approximately 3 hours (range 0-24 hours). Secondary absorption peaks were observed at 8-12 and 24 hours postdose, indicating enterohepatic re-circulation.

The absolute bioavailability is unknown. The bioavailability of the tablet was about 38%-49% of that of an oral solution. Doses below 100 mg (solution) and 400 mg (tablet), and b.i.d. dosing increased the bioavailability for tablets. The bioavailability was mainly limited by solubility. Solubility of sorafenib decreases at increased pH. The effect of anti-acidic medicinal products, such as antacids, H_2-antagonists or proton-pump inhibitors, on sorafenib bioavailability has not been studied. Decreased plasma concentrations of sorafenib cannot be excluded and, if possible, chronic treatment with anti-acidic drugs should be avoided during treatment with sorafenib (see SPC section 4.4 and 4.5). The applicant agreed to conduct, post-authorisation, a single dose, cross-over PK study in healthy volunteers to evaluate the effect of increased gastric pH (anti-acidic medication) on the bioavailability of sorafenib (400 mg, single dose).

Sorafenib bioavailability decreased with a high-fat meal, while there was no impact of a moderate-fat meal (approximately 30% of calories from fat) on sorafenib bioavailability compared to the fasted state. It is therefore recommended that sorafenib is administered without food or with a low or moderate fat meal. If the patient intends to have a high-fat meal, sorafenib tablets should be taken at least 1 hour before the meal or at least 2 hours after the meal, and should be swallowed with a glass of water (see SPC section 4.2).

Sorafenib is metabolised primarily in the liver and undergoes oxidative metabolism, mediated by CYP 3A4, as well as glucuronidation mediated by UGT1A9. Sorafenib accounts for approximately 70-85% of the circulating analytes in plasma at steady state. Eight metabolites of sorafenib have been identified, of which five have been detected in plasma. The main circulating metabolite of sorafenib in plasma, the pyridine N-oxide M 2, shows *in vitro* potency similar to that of sorafenib. This metabolite comprises approximately 9-16% of circulating analytes at steady state. The other active metabolites, M4 and M5, accounted for 8% and 6%, respectively, of the total exposure at steady state.

Most of the dose was absorbed and subsequently excreted, either as sorafenib or as metabolites. Unchanged sorafenib, accounting for 51% of the dose, was found in faeces but not in urine, indicating

that biliary excretion of unchanged drug might contribute to the elimination of sorafenib (see section 5.2 of the SPC). Metabolites were mainly recovered in feaces and only in small amounts in urine. Metabolism and biliary excretion of unchanged drug was therefore the major elimination pathways for sorafenib. As some of the unchanged drug found in faeces could have been re-formed from excreted metabolites, the relative contribution of the two pathways could not be determined.

Across studies, there was no apparent relationship between creatinine clearance and sorafenib exposure, but no pharmacokinetic data for patients with moderate or severe renal impairment was presented (CLcrea<60 ml/min). However, since there is no renal excretion of unchanged sorafenib or M2, a significant effect of renal function on the exposure is not expected. A specific study in patients with renal impairment was not considered necessary, and no dose adjustment is required in patients with mild to moderate renal impairment (creatinine clearance >30ml/min). Appropriate information is included in the SPC (section 4.2 and 5.2). The SPC also states that no data is available in patients with severe renal impairment (creatinine clearance <30 ml/min) or in patients requiring dialysis.

Sorafenib pharmacokinetics in patients with mild to moderate hepatic impairment (Child-Pugh A and B) was investigated in a phase II study in patients with hepatocellular carcinoma. Exposure (calculated for 8 hours) was increased in Child-Pugh B compared with Child-Pugh A patients, but the difference was not statistically significant. There was no comparison with patients with normal hepatic function in this study, but exposure in both groups was within the range observed in other studies (e.g. AUC_{0-12} = 48 mg*h/ml in study 100277). The degree of metabolic impairment vs. degree of cholestatis was not discussed and the results cannot be extrapolated to cirrhosis patients. However, no dose adjustment is required in patients with mild to moderate hepatic impairment. Given the hepatic elimination of sorafenib, a further increase in exposure in patients with severe hepatic impairment would be expected (see SPC section 4.2, 4.4 and 5.2).

Analyses of demographic data suggested that there is no relationship between pharmacokinetics and age (up to 65 years) gender or body weight. Data in elderly were supported by safety data. No specific dosing recommendations were considered necessary based on these demographic criteria. The mean sorafenib exposure was lower in Japanese patients than in Caucasian patients, but the exposure was highly variable. The clinical relevance of this observation is unknown (see SPC section 5.2).
Substances that are inducers of enzyme activity (e.g. rifampicin, *Hypericum perforatum* also known as St. John's wort, phenytoin, carbamazepine, phenobarbital, and dexamethasone) may increase metabolism of sorafenib *via* CYP3A4 and UGT1A9 and thus decrease sorafenib concentrations. These drugs should only be administered concomitantly after careful benefit-risk evaluation (see SPC section 4.4 and 4.5). The results of a drug-drug interaction study with rifampicin will be provided post-authorisation. Ketoconazole, a potent inhibitor of CYP3A4, administered once daily for 7 days to healthy male volunteers did not alter the mean AUC of a single 50 mg dose of sorafenib. These data suggest that clinical pharmacokinetic interactions of sorafenib with CYP3A4 inhibitors are unlikely. Sorafenib inhibited CYP2C9 *in vitro*. It cannot be excluded that sorafenib may increase the concentrations of concomitantly administered substrates of CYP2C9. The concomitant treatment with sorafenib and warfarin, a CYP2C9 substrate, did not result in changes in mean PT-INR compared to placebo. However, patients taking warfarin or phenprocoumon should have their INR checked regularly. Sorafenib inhibited CYP2B6 and CYP2C8 *in vitro*, but the clinical relevance of this inhibition has not been evaluated. It cannot be excluded that sorafenib may increase the concentrations of concomitantly administered substrates of CYP2B6 (e.g. bupropion, cyclophosphamide, efavirenz, ifosfamide, methadone) and CYP2C8 (e.g. paclitaxel, amodiaquine, repaglinide). *In vitro*, sorafenib inhibited glucuronidation *via* UGT1A1 and UGT1A9. The clinical relevance of this finding is unknown (see SPC section 4.5). Caution is recommended when administering sorafenib with compounds that are metabolised/eliminated predominantly by UGT1A1 (e.g. irinotecan) or UGT1A9 pathway (see SPC section 4.4). Concomitant administration of sorafenib and midazolam, dextromethorphan or omeprazole, which are substrates for cytochromes CYP3A4, CYP2D6 and CYP2C19, respectively, did not alter the exposure of these agents. This indicates that sorafenib is neither an inhibitor nor an inducer of these cytochrome P450 isoenzymes. Therefore, clinical pharmacokinetic interactions of sorafenib with substrates of these enzymes were considered unlikely. CYP1A2 and CYP3A4 activities were not increased after treatment of cultured human hepatocytes with sorafenib, indicating that sorafenib is unlikely to be an inducer of CYP1A2 and CYP3A4. *In vitro*, sorafenib has been shown to inhibit the transport protein p-glycoprotein (P-gp). Increased

plasma concentrations of P-gp substrates such as digoxin cannot be excluded at concomitant treatment with sorafenib (see SPC section 4.4 and 5.4). Sorafenib had no effect on the pharmacokinetics of gemcitabine or oxaliplatin. Concomitant treatment with sorafenib resulted in a 21% increase in the AUC of doxorubicin. When administered with irinotecan, whose active metabolite SN-38 is further metabolised by the UGT1A1 pathway, there was a 67 - 120% increase in the AUC of SN-38 and a 26 - 42% increase in the AUC of irinotecan. The clinical significance of these findings is unknown (see clinical efficacy section, and SPC section 4.4).

Pharmacodynamics

- Mechanism of action

Sorafenib was developed as an inhibitor of C-Raf (IC_{50} = 2 nM), but also inhibited wild type and mutant B-Raf (IC_{50} = 25 and 38 nM) *in vitro*. In addition, sorafenib inhibited the receptor tyrosine kinases for VEGFR-2, VEGFR-3, PDGFβ, c-KIT and FLT3. Based on in these properties, dual patterns of activity are possible: growth inhibition and anti-angiogenesis.

- Primary and secondary pharmacology

Primary pharmacology

There were no specific studies conducted in patients with RCC. Exploratory data were submitted from a single arm study conducted in patients with hepatocellular cancer (HCC). A number of biomarkers were explored, including phosphorylated ERK in tumour tissue, plasma HER-2/neu, plasma proteomics and blood cell RNA expression pattern. Higher intensity (immune histochemistry) of tumour pERK at baseline was correlated with longer time to tumour progression (p=0.00034, n=33). No correlation between baseline plasma HER-2/neu and response to sorafenib was seen. Twenty five proteomics components (mass spectroscopy) were found to differentiate between responders (PR, SD) and non-responders (PD). Based on data from 30 patients a panel of 18 genes was identified (Affymetrix GeneChip microarray) as differentiating between responders (PR, SD) and non-responders (PD).

To investigate the relationships between tumour levels of pERK and sorafenib associated patients outcome in RCC patients (study 11213), immunohistochemistry (IHC) was used to semi-quantitatively examine pERK levels in pre-treatment tumour biopsies. The maximum tumour staining intensity was graded on a standard IHC scale (neg = no staining, 1+ = weak staining, 2+ = moderate staining, 3+ = strong staining, 4+ = intense staining). The percent nuclei stained was assigned on a quartile basis (<5% = 0-5% nuclei stained, 1Q = 6-25% nuclei stained, 2Q = 26-50% nuclei stained, 3Q = 51-75% nuclei stained, 4Q = 76-100% nuclei stained). Paraffin-embedded tumour samples from 146 patients were subjected to pERK staining followed by pathologist's interpretation. Of these, appropriately prepared samples with sufficient material, pERK results, associated patient data, and antitumour activity data were available for only 125 patients (of the 903 subjects randomized in this trial). Patients were grouped based on percent nuclei expressing pERK (1Q *vs.* 2Q to 4Q), and also based on tumour staining intensity (Neg. to 3+ *vs.* 4+). These groupings separate those with high level staining from those with low level staining, and the ability to perform statistical analyses based on the numbers of samples available within each group. An analysis of PFS (using investigator assessed PFS) based on pERK staining is shown in table 6. Results related to survival were similar (data not shown).

Table 6 - Analysis of PFS based on pre-treatment tumour pERK staining

	N	# Events	# Censored	Median PFS (Days) Placebo	Sorafenib	Hazard Ratio (sorafenib/placebo) Estimate	95% CI
Tumour % Expressing							
pERK % expressing unknown	776	506	270	84	168	0.53	(0.44, 0.63)
pERK % expressing 1Q	76	57	19	96	184	0.49	(0.29, 0.84)
pERK % expressing 2Q, 3Q, 4Q	49	41	8	84	126	0.66	(0.34, 1.25)
Tumour Staining Intensity							
pERK staining intensity unknown	776	506	270	84	168	0.53	(0.44, 0.63)
pERK staining intensity Neg, 1+, 2+, 3+	29	25	4	88	200	0.52	(0.23, 1.18)
pERK staining intensity 4+	96	73	23	86	166	0.59	(0.37, 0.94)

VEGF and sVEGFR-2 levels were analyzed in plasma samples from study 11213 using immunoassays. The results are presented in table 7.

Table 7: Analysis of PFS based on baseline plasma VEGF levels in patients from study 11213

Baseline VEGF Status	N	Median PFS (Days) Placebo	Sorafenib	Hazard Ratio (sorafenib/placebo) Estimate	95% CI	p-value*
VEGF ≤ 131 pg/mL	356	100	168	0.64	(0.49, 0.83)	0.096
VEGF > 131 pg/mL	356	83	167	0.48	(0.38, 0.62)	

* comparison of the hazard ratio of sorafenib to placebo between low-level and high-level baseline VEGF groups

Inactivating somatic mutations of the VHL gene are frequently found in RCC. The resulting inactivation of the VHL protein leads to an increase in tumour VEGF activity through upregulation of hypoxia-inducible factor-1α (HIF-1α). This increase in VEGF, mediated by VHL inactivation, leads to activation of VEGFR-2, which contributes to tumour angiogenesis. Given this relationship between VHL and angiogenesis, the relationship between VHL mutational status and sorafenib antitumour activity was investigated. DNA was isolated from formalin-fixed paraffin-embedded tumour samples, subjected to the polymerase chain reaction (PCR) in order to amplify each of the three exons encoding the VHL gene, and the amplified DNA was sequenced. A total of 141 samples from 134 patients had sequence data for at least 1 exon that could be utilized for analysis. Among the 48 subjects from whom all 3 exons were sequenced successfully, 15 (31.3%) had VHL mutations. Analysis of VHL mutational status and sorafenib antitumour activity is provided in the table below. The correlative analyses of mutational status and PFS or OS were performed assuming that all nucleotide changes in the VHL gene are equivalent.

Table 8: Analysis of PFS based on VHL mutation status in a subset of patients in Study 11213

	N	# Events	# Censored	Median PFS (Days)		Hazard Ratio (sorafenib/placebo)	
				Placebo	Sorafenib	Estimate	95% CI
Exon 1							
No Mutation	90	72	18	79	166	0.55	(0.34, 0.89)
Mutation	26	20	6	45	165	0.39	(0.15, 1.04)
Missing	785	512	273	84	169	0.53	(0.45, 0.63)
Exon 2							
No Mutation	72	57	15	75	126	0.45	(0.26, 0.77)
Mutation	11	10	1	178	143	0.89	(0.24, 3.34)
Missing	818	537	281	84	169	0.54	(0.45, 0.64)
Exon 3							
No Mutation	88	68	20	84	126	0.62	(0.38, 1.00)
Mutation	3	3	0	141	402		
Missing	810	533	277	84	169	0.52	(0.44, 0.62)
Exon 1, 2 or 3*							
No Mutation	33	24	9	79	167	0.52	(0.23, 1.19)
Mutation	35	28	7	97	165	0.49	(0.22, 1.08)
Missing	833	552	281	84	168	0.54	(0.45, 0.63)

*patients that contained a mutation in any one exon were considered to have mutant VHL, even if one or more exons from that patient had not been sequenced. However, patients were only considered to have a wild-type VHL gene if all 3 exons had been successfully sequenced and all 3 exons contained no mutation.

Secondary pharmacology

Dermatologic events, including rash and hand-foot skin reaction, were the most common adverse events attributed to sorafenib in clinical studies (see clinical safety sections). Investigation of the association between clinical efficacy and dermatologic toxicity was based on PFS (according to investigator assessment in study 11213) analysis by presence or absence of rash and hand foot syndrome. The number of placebo patients with rash was 16% *versus* 40% in the sorafenib group. Median PFS in placebo patients with rash was 125 days *versus* 83 days in placebo patients without rash. PFS was analyzed for patients with or without a first report of rash in cycle 1 or 2. PFS was similar in patients receiving placebo who had a rash in cycle 1 or 2 (85 days) and those who did not have rash in cycle 1 or 2 (83 days), in patients receiving sorafenib PFS was 219 days in patients with a rash in cycle 1 or 2 vs. 164 days for patients with no rash in cycle 1 or 2. Analyses were also performed with PFS and hand-foot skin reaction. Hand-foot skin reaction was reported in 134 (29.7%) patients in the sorafenib group and 30 (6.7%) patients in the placebo group. Median PFS in sorafenib treated patients was found to be 181 days in patients with reaction *versus* 167 in those without.

Trough plasma sorafenib concentrations, defined as concentrations between 9 and 15 hours after administration of the previous dose, were evaluated as function of hypertension. Trough concentration data were evaluable in 67 patients of the 451 treated with sorafenib. Hand-foot skin reaction was reported in 10 (28.6%) patients with sorafenib concentrations < 3.2 mg/l and 12 (37.5%) patients with sorafenib concentrations ≥ 3.2 mg/l. Rash was reported in 9 (25.7%) patients with sorafenib concentrations < 3.2 mg/l and 9 (28.1%) patients with sorafenib concentrations ≥ 3.2 mg/l.
In a pooled analysis of 179 patients from 4 dose escalation studies, hand-foot skin reaction was reported in 45 (25%) patients and rash was reported in 32 (18%) patients. The frequency of dermatologic events correlated with dose and dermatologic events were most common in doses ≥ 400 mg bid.

In study 11213, hypertension was reported as an adverse event in 76 (16.9%) patients receiving sorafenib and 8 (1.8%) patients receiving placebo. Even in patients in whom hypertension was not reported as an adverse event, blood pressure tended to increase within the first 3 weeks of sorafenib therapy. Mean change in systolic blood pressure at day 21 of cycle 1 was 8.0 mmHg in sorafenib patients and 0 mmHg in placebo patients. The relationship between hypertension and efficacy was

investigated based on investigator-assessed PFS data from study 11213 analyzed by blood pressure data (see also clinical safety section). Post-baseline hypertension [defined as systolic blood pressure (SBP) ≥ 160 mmHg] was reported in 170 sorafenib-treated patients and 73 patients receiving placebo (see table 9).

Table 9 - Progression-free survival analyzed by systolic blood pressure (SBP ≥ 160 mmHg)

Treatment Group	SBP ≥160 mmHg	N	Number Failed	Number Censored	Median PFS (Days)
Sorafenib	Yes	170	89	81	224
	No	270	176	94	139
Placebo	Yes	73	43	30	172
	No	356	273	83	78

Progression-Free Survival in sorafenib-treated patients was analyzed by presence or absence of hypertension in cycle 1. Trough concentration data were evaluable in only 67 patients of the 451 treated with sorafenib. Pharmacokinetic exposure and hypertension adverse events reported from in study 11213 were presented (data not shown). Measurements of circulating concentration of VEGF, catecholamines, epinephrine, norepinephrine, endothelin I, urotensin I, urotensin II, rennin, and aldosterone were performed at baseline and after 3 weeks of therapy. There were no significant changes in the levels of these vasoactive, renal, and angiogenic factors and there was no correlation of levels of these factors with blood pressure.

No PK/PD modelling data from the pivotal RCC study were provided. No pharmacodynamic interactions studies with other medicinal products or substances were conducted.

Discussion on clinical pharmacodynamics

The observation from the phase III study 11213 that baseline tumour pERK levels are not predictive of sorafenib antitumour activity in RCC patients complemented the results from an uncontrolled phase II study of sorafenib in RCC patients in which a lack of correlation was observed between pERK staining and time to progression (TTP) (n=64). Without control arm, it was not possible in a phase II trial to determine whether an observed correlation was predictive of sorafenib treatment efficacy or simply indicative of a prognostic baseline biomarker. Taken together, these limited phase II and phase III data suggested that baseline tumour pERK levels may not be prognostic in RCC patients nor predictive of sorafenib antitumour activity in RCC. In contrast, in a limited number of samples from an uncontrolled phase II study of sorafenib in hepatocellular carcinoma (HCC) patients, pERK staining intensity showed a significant correlation with TTP (n=33). Most patients assayed from study 11213 had similar pERK levels as described by the semi-quantitative 5-point scales utilized (i.e. >70% of patients had a maximum staining intensity of 4+, and almost 70% of patients had <50% of tumour cell nuclei stained). This lack of differentiation may represent a true lack of diversity among the RCC tumour population tested. Alternatively, the lack of differentiation may suggest that a finer scale of IHC quantitation is necessary in order to effectively differentiate sub-populations of RCC tumours.

Patients with either high baseline VEGF or low baseline VEGF benefited from sorafenib treatment. However, the data suggested poorer prognosis for patients with high baseline VEGF and a trend towards greater improvement in PFS for high VEGF subjects upon sorafenib treatment. Baseline levels of plasma sVEGFR-2 did not show a significant relationship to sorafenib treatment effect as measured by progression-free survival (PFS) or overall survival (OS). Mean plasma VEGF levels increased significantly from baseline to treatment cycle 1 day 21 and from baseline to treatment cycle 3 day 1 in the sorafenib-treated group (31.7% and 47.0% increase, respectively). Concurrently, mean sVEGFR-2 levels decreased significantly (17.8%, and 23.9%, respectively). No significant changes were observed for VEGF and sVEGFR-2 levels in the placebo group. Altogether, baseline VEGF did not appear as an important predictor of sorafenib activity. The applicant agreed to submit further data on biomarkers (including proteomics, metabolomics) post-approval.
Limited data suggest that DNA level tumour VHL mutational status is not predictive of sorafenib antitumour activity. These limited data did not support patient selection for sorafenib treatment based on this biomarker. The relationship between mutation and loss of VHL protein function has not yet been investigated. However VHL mutations have been reported as typifying for Clear Cell RCC, therefore "silent" mutations are unlikely to be common. Further analyses are ongoing to determine

which of these nucleotide changes result in changes to the VHL protein, and, furthermore, which protein level changes may result in loss of VHL protein function. Amplification of receptors activating the MAPK pathway and mutations in MAPK pathway components associated with constitutive activation were not investigated.

For a multitargeted agent, a combination of signs and biomarkers, such as VEGF levels prior to and on therapy, might be needed to optimise the predictive value. Inhibition of angiogenesis has been associated with hypertension, particularly in patients treated with inhibitors of the vascular endothelial growth factor (VEGF) pathway [39].
The relationship between sorafenib exposure (dose, AUC, C_{max}) and response (inhibition of cellular proliferation, clinical toxicity, anti-tumour activity) were evaluated in phase I studies at doses of 100, 200, 300, 400, 600 and 800 mg bid. Increasing the dose from 400 to 600 mg bid did not increase the mean systemic exposure to sorafenib (13% in terms of $AUC_{(0-12),ss}$) yet significantly increased clinical toxicities. There was a trend towards increasing adverse events with dose, while there was no apparent relationship between steady state sorafenib $AUC_{(0-12),ss}$ or C_{max} values and the grade of drug-related adverse events at the 400 mg bid dose-level.
A relationship between dose and dermatologic events was observed. However, at the recommended dose (400 mg bid), the relationship between trough concentrations and skin reactions was weak. This observation was supported by plots of individual patient trough level data *versus* skin reactions (data not shown). The validity of the assessment of relationship between concentration and activity relies on the assumption that concentration data derive from samples taken prior to any dose reductions/interruptions. Rash (data from the two first treatment cycles), hypertension and higher trough levels in sorafenib treated patients was moderately predictive of prolonged PFS. This was not the case for hand-foot reactions. These observations were based on limited PK data.
There were no significant changes in the levels of vasoactive, renal, and angiogenic factors and there was no correlation of levels of these factors with blood pressure. A direct effect on the vasculature, possibly through endothelial cell function, was considered more likely than a humoral or renovascular mechanism for sorafenib-associated hypertension. Overall, considering the small sample size and the large number of patients who did not have PK samples collected, definitive conclusions on the predictive value of biomarkers could not be drawn. However, despite the large inter-individual variability in pharmacokinetics, no apparent relationship between exposure and dermatologic events, hypertension, or PFS was observed. Available systemic exposure data, although very limited, did not suggest any implications for the use of sorafenib in patients with renal cell carcinoma.

Clinical efficacy

- Dose response studies

Seven multiple dosing regimens/schedules studies were conducted. In these studies, 197 patients were exposed to sorafenib. The following schedules were investigated: 1 week on / 3 weeks off, 3 weeks on / 1 week off, 4 weeks on / 1 week off, once weekly to continuous dosing. Tolerability was used as activity marker aiming at defining a tolerated dose. Based on these data, 400 mg bid continuous dosing was proposed for the assessment of efficacy in the pivotal studies.

- Main studies

Two study reports of controlled clinical studies pertinent to the claimed indication were submitted.
Study 11213 was a phase III randomized, placebo-controlled trial to evaluate sorafenib in patients with advanced RCC who had received one prior systemic anticancer treatment [40].
Study 10039 was a phase II randomized, placebo-controlled discontinuation trial in patients with advanced, refractory solid tumours.

Study 11213

METHODS

Study Participants

This was a randomised, placebo-controlled, multicentre clinical trial. The main inclusion criteria were adult patients with life expectancy ≥ 12 weeks; histologically or cytologically documented unresectable and/or metastatic measurable RCC (excluding rare subtypes of RCC, e.g. pure papillary cell tumour, mixed tumour containing predominantly sarcomatoid cells, Bellini carcinoma, medullary carcinoma or chromophobe oncocytic tumours); no more than one systemic therapy for advanced disease, during or after which the patient experienced disease progression (prior treatment must have been completed at least 30 days before but not more that 8 months prior to randomisation); at least 1 unidimensional measurable lesion by computed tomography (CT) scan or magnetic resonance imaging (MRI) according to Response Evaluation Criteria in Solid Tumour (RECIST); risk rated "low" or "intermediate" according to Motzer score; performance status 0 or 1 according to Eastern Cooperative Oncology Group (ECOG) scale; total bilirubine < 4.5 x upper limit of normal (ULN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) < 2.5 x ULN, amylase and lipase < 1.5 x ULN, serum creatinine < 2.0 x ULN, prothrombin time (PT) and partial thromboplastin time < 2.5 x ULN.

Treatments

Patients were administered either sorafenib 400mg + best supportive care (BSC) or placebo + BSC twice daily on a continuous basis, without food or with a moderate fat meal. For the purpose of data recording, the treatment period was divided into 6-week cycles for the first 24 weeks; thereafter a cycle was defined as 8 weeks. Treatment continued until patients reached one of the criteria or withdrawal. Crossover from placebo at time of progression was not accepted.

Objectives

The primary objective was to evaluate the efficacy (survival) of sorafenib as compared with placebo. The secondary objectives were to evaluate the efficacy by progression-free survival (PFS), response rate (CR or PR), changes in health-related quality of life (HRQOL) and symptom response. Other objectives were duration of response, predictive value of pERK, HER-2, VEGF, plasma proteomics, gene expression profiling in blood cells and tumour biopsies.

Outcomes/endpoints

The primary outcome, overall survival, was defined as the time elapsed from randomization to death (from any cause). All randomized (ITT population) was included in the analyasis of the primary endpoint. Patients still alive at the time of the analysis were censored at their last date of follow-up.

The secondary outcome measures included PFS defined as the time from randomization to disease progression (radiological or clinical, whichever was earlier) or death (if death occurred before progression). Patients without tumour progression or death at the time of analysis were censored at their last date of tumour evaluation. PFS was primarily analysed by independent radiological review.

Estimate of the objective tumour response rates (confirmed CR and PR according to RESICT criteria) Confirmation scan for tumour responses assessed as CR or PR was to be conducted on day 1 of the next cycle, as long as it was at least 4 weeks after the first scan. The objective response rates were to be compared between treatment groups using Cochran-Mantel-Haenzel test adjusting for prognostic group (intermediate or low) and country.

Sample size

Sample size was based on the primary endpoint of overall survival. A clinically meaningful improvement was defined as a 33.3% increase in overall survival. Assuming a 2-sided α of 0.04, a total of 540 events are required to achieve 90% power if one interim and one final analysis are performed during this study.
The final analysis was to be performed (mature overall survival) when 540 events have been observed, if the stopping rule has not been met at the interim analysis.
The expected study duration was estimated at 29 months assuming patients enroll at a rate of 50 patients per month, an exponentially distributed event time, a 12 month median time for the control group and a 17 month long enrollment for a total of 856 subjects in the 2 treatment groups combined (428 subjects in each arm). Assuming a 3% rate for patients lost to follow-up, approximately 884 patients were to be randomized.

About 270 deaths (number of events required for the first interim analysis on overall survival) were expected in about 17 months. The planned final analysis of the secondary PFS endpoint was to be performed when approximately 363 progressions were observed. This analysis, presented in this report, would have power of 90% to detect a 50% increase in PFS at the 1% significant level (two-sided). Assuming a median PFS for the control arm of 4.5 months, the 363 progressions were projected to occur in approximately 13 to 14 months from the initiation of the trial.

Randomisation

Randomisation (central) was stratified by country (19 countries, including 9 EU countries) and prognostic risk category according to Motzer criteria [poor ECOG (PS≥2), high serum lactate dehydrogenase (≥1.5xUNL), low serum hemoglobin, high corrected serum calcium (≥10 mg/dl), absence of prior nephrectomy].

Blinding (masking)

The active and placebo tablets were identical in appearance. Medication containers were labeled with unique bottle numbers, which were to be assigned to patients using the IVRS. Copies of an unblinded randomization code, in the form of individual sealed envelopes for each patient, were provided to each investigator. Envelopes could be opened by the investigator (preferably in consultation with a sponsor representative) if knowledge of a patient's treatment became necessary for the clinical management of that patient. Unblinding after disease progression for entry into the open-label phase of the study proceeded *via* the IVRS rather than with the blinded code envelopes.

Statistical methods

In the primary analysis, the two treatment groups was compared using a two-sided log-rank test with an overall α of 0.04 stratified by country and Motzer risk factor (see randomisation section). Kaplan-Meier survival curves were also to be displayed. An α spending function was to be used to account for interim analysis(es) to ensure that the overall false positive rate, α, is less that or equal to 0.04.
The planned final analysis on the secondary PFS endpoint was to be performed when approximately 363 progressions or deaths are observed.
The best overall response rates will be compared between treatment groups using the two-sided Cochran-Mantel-Haenszel test adjusting for country and Motzer risk category at the 0.05 α level.
Patient-reported outcomes (PRO) data were collected at day 1 of each cycle, and at the end of treatment visit, prior to seeing the physician. HRQOL assessment was based on two PRO instruments: the Functional Assessment of Cancer Therapy-Kidney Symptom Index (FKSI) to assess kidney cancer-related symptoms, and the Functional Assessment of Cancer Therapy-General (FACT–G). The primary endpoint was the longitudinal evolution of mean score over the first 5 treatment cycles. Treatment differences were evaluated by random coefficient. Treatment and Motzer score were factors, and relative day and baseline measure of the response variable were covariates in these models. Pattern mixture models were used to evaluate the robustness of the results from the random coefficient model.

RESULTS

Participant flow

At the time of the data cutoff for the PFS analysis, 976 patients were enrolled, 769 were randomized and 768 received at least 1 dose of study medication. All 769 patients were included in the ITT population, all but 1 patient were included in the safety population. There were 207 patients enrolled as of 28 January 2005 data cutoff date who were not randomized: 164 of these patients were screening failures (more than one prior therapy, prior therapy completed outside of the protocol window, brain metastases). The remaining 43 patients were still in screening as of the data cutoff date. Of the 768 patients treated, 384 (50%) were randomized to placebo and 384 (50%) to sorafenib. By the data cutoff date for the PFS analysis, 226 (58.9%) patients receiving placebo and 144 (37.5%) patients receiving sorafenib had discontinued treatment. The most common reason for discontinuing treatment are provided in table 10.

Table 10: Reasons for discontinuation of double blind therapy as per the investigator (randomised patients) – Study 11213

	sorafenib (n=384)	**placebo (n=385)**

Discontinued double blind therapy	**144 (37.5%)**	**226 (58.9%)**
Adverse events	13	14
Non-compliant with medication	0	1
Progression by clinical judgement	14	22
Radiological progression	103	174
Consent withdrawn	3	7
Lost to follow-up	1	4
Death	7	3
Missing	3	1

Conduct of the study

Six amendments of the protocol were made during the study. The first dealt with modifications to PFS analysis, survival analysis and biological modifiers, before patients were enrolled. The second included clarifications of the inclusion and exclusion criteria. Amendment 3 involved the monitoring of congenital nevi and histopathological effects of sorafenib on the skin. Amendment 4 involved functional imaging of tumour vascularisation. Amendment 5 was approved when 632 patients had been enrolled, and included the following revisions: clarification of timing and requirements for pre-study tumour tissue samples, correction of number of events required for analysis, clarification on reporting of adverse events and serious adverse events, and correction to exclusion criteria clarifying that patients may not have had previous therapy for RCC within the past 30 days but no longer than 8 months prior to randomisation. Amendment 6 was approved after all patients had been enrolled and specified that patients were to be unblinded and placebo patients would be given the opportunity to cross over to sorafenib.

Baseline data

Prior therapy for RCC Baseline, demographic and diseases characteristics are shown in table 11 and 12. About 80% of the study population received systemic therapy with palliative intent, about 20% adjuvant therapy. More than 70% of patients were less than 65 year old with good performance status and relatively good prognosis.

Table 11 - Baseline demographic and diseases characteristics in study 11213 (ITT population)

Characteristics	sorafenib N = 384		placebo N = 385	
	n	(%)	n	(%)
Male	267	(69.5)	287	(74.5)
Female	116	(30.2)	98	(25.5)
Age Group, n (%)				
<65	255	(66.4)	280	(72.7)
≥65	127	(33.1)	103	(26.8)
ECOG Performance Status, n (%)				
0	184	(47.9)	180	(46.8)
1	191	(49.7)	201	(52.2)
2	6	(1.6)	1	(0.3)
Missing	3	(0.8)	3	(0.8)
Motzer Risk Factors, n (%)				
Low	200	(52.1)	194	(50.4)
Intermediate	184	(47.9)	191	(49.6)
RCC Subtype, n (%)				
Clear Cell	377	(98.2)	380	(98.7)
Papillary subtype	1	(0.3)	3	(0.8)
Other Variant	1	(0.3)	1	(0.3)
Missing	5	(1.3)	1	(0.3)
Duration of Disease (years)				
Mean (Range)	2.8	(0.1-19.4)	3.3	(0.1-19.9)
Median	1.6		1.9	
Duration of Metastatic Disease (years)				
Mean (Range)	1.3	(0.1-11.4)	1.3	(0-10.2)
Median	0.9		0.9	

Table 12 - Prior therapy for RCC in study 11213 (ITT population)

Characteristic	sorafenib N = 384		placebo N = 385	
	n	(%)	n	(%)
Type of Therapy				
Nephrectomy	356	(92.7)	362	(94.0)
Systemic Anticancer Therapy	381	(99.2)	382	(99.2)
Radiation Therapy	108	(28.1)	90	(23.4)
Type of Systemic Anticancer Therapy				
Interferon	260	(67.7)	264	(68.6)
Interleukin (IL-2)	168	(43.8)	170	(44.2)
Pyrimidine analogues	60	(15.6)	72	(18.7)
Vinca alkaloids	44	(11.5)	49	(12.7)
Progesterone agents	25	(6.5)	25	(6.5)
Investigational drugs	12	(3.1)	23	(6.0)
Intent of Systemic Anticancer Therapy				
Palliative therapy	315	(82.0)	304	(79.0)
Adjuvant therapy	65	(16.9)	80	(20.7)
Neoadjuvant therapy	2	(0.5)	5	(1.3)
Intent not reported	11	(2.9)	9	(2.3)
No palliative therapy	60	(15.6)	73	(19.0)
Intent of Cytokine Therapy				
IL-2 and/or Interferon, any Intent	319	(83.1)	313	(81.3)
Palliative IL-2 and/or Interferon	257	(67.2)	247	(64.2)
Adjuvant IL-2 and/or Interferon	58	(15.1)	66	(17.1)
Neoadjuvant IL-2 and/or Interferon	2	(0.5)	3	(0.8)
Intent not reported	8	(2.1)	8	(2.1)

Outcomes and estimation

Progression free survival

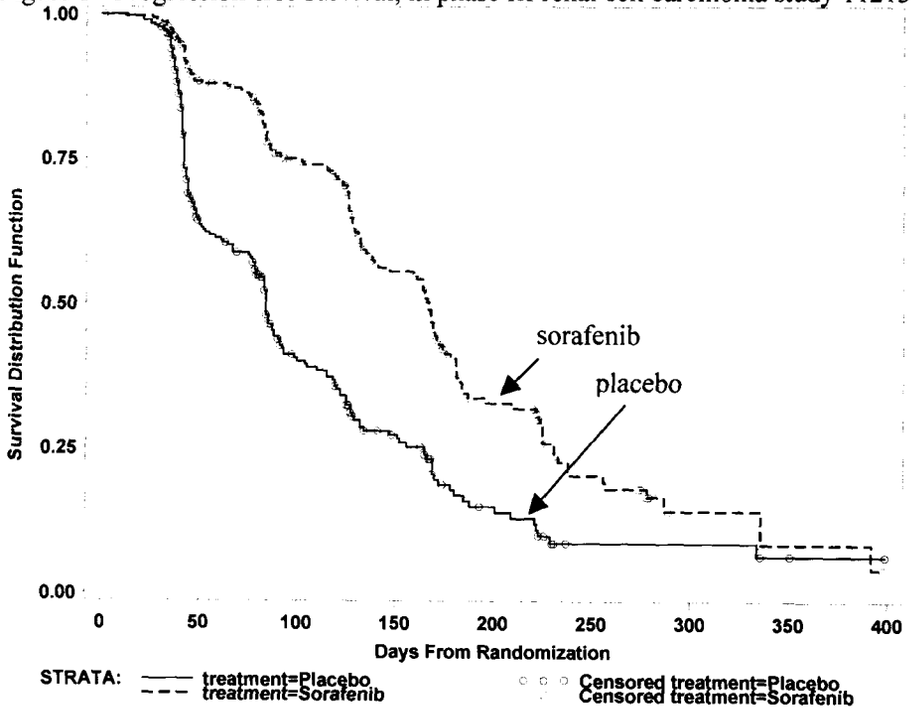
There were no meaningful differences in time from randomization to tumour evaluations (or scans) between the two treatment groups. The median time to first scan was 41 days for placebo and 42 days for sorafenib. The median time to the second scan was 84 days for both groups.

Median PFS was 84 days for patients randomised to placebo and 167 days for patients randomised to sorafenib. The stratified log-rank test had a two-sided p-value < 0.000001 (see table 13). The Kaplan-Meier curves for PFS by treatment group are shown in figure 1.

Table 13 - Progression-free survival, in phase III renal cell carcinoma study 11213

	sorafenib (N = 384)	**placebo (N = 385)**
Total failed	147 (38.3%)	195 (50.6%)
Total censored	237 (61.7%)	190 (49.4%)
Median PFS (days)	167	84
95% confidence interval for median	(139, 174)	(78, 91)
Hazard ratio (sorafenib/placebo)	0.44 (p<0.000001)	
95% confidence interval for hazard ratio	(0.35, 0.55)	

Figure 1 - Progression-free survival, in phase III renal cell carcinoma study 11213



Survival

First interim analysis (31 May 2005)

A total of 903 patients (451 in the sorafenib group and 452 in the placebo group) were enrolled in the study at the time of the survival analysis. The distribution of baseline demographics and patients characteristics was similar to that seen at the time of the PFS analysis conducted when 769 patients were enrolled. At the time of analysis there were a total of 220 deaths reported (123 in the placebo group and 97 in patients treated with sorafenib). The hazard ratio was 0.72 (p=0.018, two-sided log-rank, stratified by country and Motzer category; 95% CI: 0.55, 0.95). The median survival was 14.7 months in the placebo group. The median OS had not been reached for the sorafenib group.

Response rate

As defined in the protocol, the first post-baseline tumour evaluation was performed at the end of cycle 1 (6 weeks post randomization). There were 97 patients (49 in the sorafenib group and 48 in the placebo group) randomized within 6 weeks of the data cut-off and consequently did not have the opportunity to undergo a post-baseline tumour evaluation. The status of patients with regard to analysis of tumour response is detailed in table 14.

Table 14 - Overall best confirmed tumour response in phase III renal cell carcinoma study 11213

Best Response	**sorafenib N = 335 n (%)**	**placebo N = 337 n (%)**
Complete response (CR)	0 (0)	0 (0)
Parcial response (PR)	7 (2.1)	0 (0)
Stable disease (SD)	261 (77.9)	186 (55.2)
Progressive disease (PD)	29 (8.7)	102 (30.3)
Not Evaluated	38 (11.3)	49 (14.5)

For the 7 patients treated with sorafenib with confirmed PR, the time to response ranged from 42 to 129 days with a median of 84 days. Four of the 7 responders had eventual disease progression with time to progression ranging from 84 to 252 days; the other 3 responders did not have progression at the time of the data cut off for this analysis.

- **Ancillary analyses**

Subgroup analyses – study 11213

Analysis of PFS based on independent radiological review by demographic, baseline and prognostic characteristics are shown in table15.

Table 15– Subgroup analysis of progression free survival (ITT population) – study 11213

					Hazard Ratio (sorafenib/placebo)		**Median PFS (days)**	
Variable	**Subgroup**	**N**	**Number of events**	**Number censored**	**Estimate**	**95% CI**	**placebo**	**sorafenib**
Sex	Male	554	255	299	0.45	(0.35, 0.58)	84	166
	Female	214	87	127	0.45	(0.29, 0.69)	83	169
Age	<65 years	535	245	290	0.49	(0.38, 0.63)	84	165
	≥65 years	230	97	133	0.34	(0.22, 0.52)	83	181
Motzer score	**Low**	**394**	**157**	**237**	**0.53**	(0.39, 0.73)	**91**	171
	Intermediate	**375**	**185**	**190**	**0.39**	(0.29, 0.53)	**61**	147
Nephrectomy	No	51	15	36	0.2	(0.06, 0.68)	83	225
	Yes	718	327	391	0.48	(0.38, 0.60)	84	166
Baseline ECOG	0	364	160	204	0.48	(0.35, 0.66)	88	172
	1	392	176	216	0.44	(0.32, 0.59)	67	147
Stage at study entry	**Stage III**	**26**	**12**	**14**	**0.91**	(0.27, 3.07)	83	126
	Stage IV	740	330	410	0.45	(0.36, 0.56)	84	168
Prior IL2/Interferon	No	137	52	85	0.35	(0.19, 0.63)	85	172
	Yes	632	290	342	0.47	(0.37, 0.60)	84	164
Prior palliative therapy	No	133	62	71	0.56	(0.33,0.93)	78	132
	Yes	636	280	356	0.43	(0.34,0.55)	84	169
Time since initial diagnosis >1.5 years	No	333	156	177	0.41	(0.30, 0.57)	76	147
	Yes	419	181	238	0.47	(0.35, 0.63)	92	170
Time since initial diagnosis to metastatic disease >0.5 year	**No**	**285**	**127**	**158**	**0.38**	(0.27, 0.55)	**79**	164
	Yes	**374**	**168**	**206**	**0.45**	(0.33, 0.61)	**84**	169

Time since metastatic disease >1 year	No	411	193	218	0.42	(0.32, 0.57)	77	147
	Yes	339	143	196	0.46	(0.33, 0.65)	87	175
Top enrolling countries	France	186	114	72	0.47	(0.32, 0.68)	84	171
	US	**146**	**64**	**82**	**0.39**	(0.24, 0.65)	**76**	141
	Poland	**117**	**49**	**68**	**0.59**	(0.33, 1.05)	**122**	178

More favourable effects in terms of hazard ratios were observed in patients with more aggressive disease as indicated by early progression in the placebo group or by generally acknowledged risk factors (Motzer score "low" *vs.* "intermediate": median PFS for placebo group 91 *vs.* 61 days, HR 0.53 *vs.* 0.39; Time since initial diagnosis > 1.5 years, "yes" *vs.* "no": median PFS for placebo group 92 *vs.* 76 days, HR 0.47 *vs.* 0.41; Poland *vs.* United States: median PFS for placebo group 122 *vs.* 76 days, HR 0.59 *vs.* 0.39).

Survival second interim analysis (30 November 2005) - study 11213

At the time of analysis there were a total of 367 deaths reported and approximately 200 patients had crossed-over to sorafenib. The nominal alpha value for this analysis was 0.0094 (two-sided). The hazard ratio was 0.77 (p = 0.015, two-sided log-rank, stratified by country and Motzer category; 95% CI: 0.63, 0.95). The median survival was 19.3 months in the sorafenib group *vs.* 15.9 month in the placebo group). In the post-hoc subgroup analysis, the hazard ratio was 0.74 (95% CI: 0.57, 0.95) for Motzer score "intermediate" and 0.82 (95% CI, 0.57, 1.17) for Motzer score "low".
A pre-specified secondary analysis was performed in order to understand the potential effect of crossover on overall survival in placebo patients. In this analysis, survival data for placebo patients were censored at 30 Jun 2005, and data for sorafenib patients were those used in the primary analysis (30 Nov 2005). The latest survival data for sorafenib could be compared to the non-crossover placebo data. The hazard ratio was 0.74, p = 0.010.

Progression-free survival analysis (as off 31 May 2005) - study 11213

After the completion of the final PFS analysis in study 11213, independent reviews of radiologic studies were discontinued. The updated analysis was a descriptive analysis, which includes PFS data for all randomized patients as of 31 May 2005 (see table 16 and 17).

Table 16 - **Progression-free survival based on investigator assessment of radiological scans**

	sorafenib N=451	**placebo N=452**
Total Failed (n, %)	274 (60.9%)	330 (73.2%)
Total Censored (n, %)	176 (39.1%)	121 (26.8%)
Median PFS Days	168	84
Hazard ratio (sorafenib/placebo) (95% CI for hazard ratio)	0.51 (0.43, 0.60)	

Table 17- Analysis of investigator-assessed PFS as of 31 May 2005 by Motzer prognostic criteria

Prognostic Category	**HR (95% CI)**		**Median PFS (days)**	
			sorafenib	**placebo**
Low (n=460)	0.58	(0.46, 0.74)	181	97
Intermediate (n=441)	0.46	(0.36, 0.57)	166	64

- Analysis performed across trials (pooled analyses and meta-analysis)

There were no analysis performed across trials.

- Clinical studies in special populations

No studies were conducted to assess the efficacy of sorafenib in special population. No data were available in patients with impaired liver or kidney function.

- Supportive study(ies)

Study 100391

This was a multicenter, placebo-controlled, randomised, discontinuation phase II study evaluating sorafenib in patients with advanced refractory cancer. The primary objective was to determine, in patients with advanced solid tumours and stable disease after 12 weeks of treatment with sorafenib, the percentage of patients who were progression-free after continued sorafenib treatment *versus* placebo. Patients should have refractory solid cancer for which other treatments were considered ineffective or intolerant, at least one measurable tumour lesion (modified WHO tumour response criteria), ECOG performance status 0 or 1, life expectancy of 12 weeks. Originally, the study focused on patients with colorectal carcinoma, but as tumour responses were observed in patients with RCC and without treatment options, this group of patients was expanded and 202 patients with RCC were included.
Treatment: Sorafenib 2 x 200 mg tablets bid. Dose modification due to toxicity allowed 200 mg bid and 200 mg QD. All patients were treated with sorafenib 400 bid for 12 weeks. Patients with stable disease at 12 weeks were to be randomised to placebo or continued sorafenib while responders continued on active therapy and patients with progressive disease went off study. The population for efficacy analysis was the ITT population, i.e. all patients who received at least 1 dose of study medication. The primary efficacy endpoint was progression-free rate at 12 weeks after randomisation. The two groups were compared using Cochran-Mantel-Haenszel test stratified by baseline ECOG score. Secondary endpoints included response rate (confirmed PR and CR).
A total of 187 patients with RCC completed 12 weeks of sorafenib therapy and 65 underwent randomised withdrawal while 79 continued open label sorafenib. Progression-free survival after randomisation in the placebo group was 41 days *vs.* 163 days in the sorafenib continuation group (p=0.0001). At 12 weeks post randomisation, 16/32 of the patients randomised to sorafenib were progression free *vs.* 6/33 in the placebo group (p = 0.008). Independent review of tumour response was undertaken in 152 patients and the partial response rate was 4% (8/202). Only 17 patients with CRC were included in the randomised withdrawal phase. Two out of 8 patients in the sorafenib group were progression-free 12 weeks after randomisation *vs.* 1/9 in the placebo group (p=0.14).

- Discussion on clinical efficacy

Based on the results of the dose finding studies, 400 mg bid continuous dosing was selected as the optimal schedule for the treatment with sorafenib. For a compound exhibiting predominantly features of a cytostatic, continuous dosing was considered appropriate [32, 41]. Biomarkers were not used to identify signs of anti-tumour activity in relation to dose or exposure. Skin reactions and diarrhoea were not dose limiting toxicities. After discussion and consultation of the oncology scientific advisory group, this was considered acceptable, due to the multi-targeting nature of sorafenib and the absence of biomarkers identified which may be used for dose adjustment (see discussion on clinical pharmacology). Further biomarker data derived from the pivotal study will be provided post-authorisation.

The application was based on a phase III, randomised, placebo-controlled trial including patients with advanced clear cell renal cell carcinoma, receiving sorafenib as a second line therapy, with good performance status and without impaired organ function. The primary endpoints of the study were overall survival and progression free survival (PFS), and according to the statistical analysis plan, a PFS analysis was undertaken after 363 events of progression or death.
While the conduct of interim analyses for overall survival (OS) could be accepted, the conduct of analysis of PFS prior to the final OS analysis is not recommended for studies designed to show a survival benefit.
Among randomised patients, 27% receiving sorafenib discontinued double blind therapy due to progression (independently reviewed and excluding clinical progression) *versus* 45% receiving placebo (95% CI for difference 12; 25%).
PFS results were statistically robust and median time to progression or death was prolonged in the sorafenib group (167 vs. 84 days). The pre-specified α for this analysis was 0.01, and the stratified log-rank test had a two-sided p-value < 0.000001. The estimated hazard ratio (risk of progression with sorafenib *versus* placebo) was 0.44 (95% CI 0.35, 0.55), representing a 56% reduction in hazard over placebo. Only considering independent imaging review data (and deaths), in the event analysis (and

not timing of event), the difference between treatment groups was still convincing (95% CI for difference 11; 24%). Therefore no further sensitivity analyses were considered necessary.
The median difference in PFS in absolute terms was less than 3 months and after prolonged follow up the difference between treatment groups became small. This might illustrate that the activity expressed in terms of change in median is a poor measure of overall activity. More favourable effects were observed in terms of hazard ratios in patients with more aggressive disease as indicated by early progression in the placebo group. However, these differences were small and the interpretation of these data were limited by the small number of patients reported as having stage III disease and the small number of events among these patients. Due to the magnitude of the overall effect observed and the pattern of observed events, no major changes could be expected if late events had been recorded. No meaningful differences related to age and gender were observed.

A total of 903 patients (451 in the sorafenib group and 452 in the placebo group) were enrolled in the study at the time of the first survival analysis. A total of 220 deaths were reported (123 in the placebo group and 97 in patients treated with sorafenib). The hazard ratio was 0.72 (p=0.018, two-sided log-rank, stratified by country and Motzer category; 95% CI: 0.55, 0.95). The formal alpha level for this analysis was 0.0005. The survival analysis was thus non-significant. Survival data as of November 2005 were still considered immature (367 deaths in 903 patients). Results remained stable comparing data from the first cut-off date. Formally the results were still statistically non-significant in relation to the stopping criteria for interim analyses. However, based on PFS data, it was concluded that a favourable and clinically meaningful effect has been demonstrated.

A final analysis of OS will be conducted when approximately 540 deaths are observed. However, it is anticipated that the crossover of placebo patients form placebo to sorafenib arm after May 2005, may limit interpretation of the results.

It was considered unfortunate that the blind was broken and that patients were crossed-over based on PFS data, as survival results were expected to be either immature or highly confounded by cross-over to active therapy. It was anticipated that a good estimate of the survival benefit would be hard to obtain. However, after consultation of the oncology scientific advisory group, the CHMP considered that PFS *per se* could be considered as a measure of the clinical benefit of sorafenib. Based on PFS data, it was concluded that a favourable and clinically meaningful effect has been demonstrated.

The analysis of an enrichment study design (all patients were treated with sorafenib 400mg bid; patients with stable disease at 12 weeks were randomised to placebo or sorafenib; responders continued on active open label therapy; patients with progressive disease went off study) confirmed the activity of sorafenib in patients with advanced RCC and that the activity is mainly cytostatic, i.e. growth inhibiting. The methodology for assessing quality of life was considered adequate and the results (data not shown) suggested a possible advantage for the patients treated with sorafenib, to be weighed against an increased incidence of side effects.

Sorafenib is not recommended for use in children and adolescents due to a lack of data on safety and efficacy (see SPC sections 4.2 and 5.3).

Clinical safety

- Patient exposure

Safety data for sorafenib have been derived from six completed phase I single-agent studies (studies 10658, 10164, 100277, 100283, 100313, and 100342), one ongoing extension study (10922), two phase II studies (100391 and 10874) and one phase III study (11213). Adverse events were reported by the investigators as an investigator term, and as a term in the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) coding dictionary. An integrated safety analysis of data from phase I and II clinical studies was provided, with safety data presented in MedDRA, based on the investigator text terms provided.
A total of 1872 patients with cancer and 85 healthy volunteers were exposed to sorafenib as of 31 Dec 2004 in sponsor-initiated trials, thereof 1376 patients were exposed to single agent sorafenib. There were 1219 patients exposed to sorafenib in completed single agent studies with 253 patients being exposed for at least 6 months and 42 patients being exposed for at least 1 year (data cut-off

January 2005). The patient exposure to sorafenib in the pivotal trial are provided in table 18 and 19. There was no specific treatment for sorafenib overdose.

Table 18 - Exposure to sorafenib in completed single agent studies

	any exposure	≥ 6 months	≥ 12 months
Phase I	197[a]	33	13
Phase II[c]	638[b]	135	23
Phase III[d]	384[b]	85	6
Total	1219	253	42

a: 41 patients received sorafenib 400 mg bid; b: All patients received sorafenib 400 mg bid; c: At the time of the analysis of the two studies in the phase II pool, 86 patients were still receiving sorafenib; d: At the time of the PFS analysis in study 11213, 158 patients were still receiving sorafenib.

Table 19 - Duration of exposure in study 11213

Duration of Treatment	**As of 28 Jan 2005**		**As of 31 May 2005**	
	sorafenib N=384	**placebo N=385**	**sorafenib N=451**	**placebo N=452**
N	329	326	447	443
Range (weeks)	0.7-57.0	0.0-57.1	0.7-72.3	0.4-76.1
Mean (weeks)	19.4	13.4	25.4	15.5
SD (weeks)	13.0	10.6	16.1	12.6
Median (weeks)	18.0	9.2	23.3	12.0

n = number of patients with duration of exposure data available; N = total number of patients; SD, standard deviation

- Adverse events

Study 11213 (data cut-off of 31 May 2005)

A total of 902 patients, including 451 receiving sorafenib were involved in the safety analyses. Study medication was discontinued during double blind therapy due to adverse events in 46 patients in the sorafenib arm and 37 patients in the placebo arm. A total of 121 patients continued to receive open label sorafenib after disease progression. At the time of the safety analysis, 12 patients had crossed-over to sorafenib. An overview of safety events in study 11213 is provided below.

Table 20- Overview of safety events in study 11213

Event	**As of 28 Jan 2005**				**As of 31 May 2005**			
	sorafenib[a] N=384		**placebo[a] N=384**		**sorafenib[b] N=451**		**placebo[b] N=451**	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Treatment-emergent adverse event	325	(84.6)	283	(73.7)	428	(94.9)	387	(85.8)
Drug-related adverse event	282	(73.4)	171	(44.5)	375	(83.1)	229	(50.8)
Serious adverse event	91	(23.7)	68	(17.7)	153	(33.9)	110	(24.4)
Drug-related serious adverse event	25	(6.5)	10	(2.6)	35	(7.8)	12	(2.7)
Adverse event leading to permanent discontinuation	24	(6.3)	28	(7.3)	46	(10.2)	37	(8.2)
Deaths within 30 days of receiving study medication	23	(6.5)	18	(4.7)	48	(10.6)	28	(6.2)

a: The median treatment duration was 9 weeks for the placebo group and 18 weeks for the sorafenib group; b: The median treatment duration was 12.0 weeks for the placebo group and 23.1 weeks for the sorafenib group; n = number of patients with event; N = total number of patients in the group.

The most common drug reactions are reported in the table below. Cumulative event rates by 6-week cycles indicate that most of adverse reactions were reported already in cycle 1. An increase over time was seen for alopecia 9% cycle 1, > 20% cycle 3+, neuropathy 6% and 10%, and hypertension 6% and 10%.

Table 21: Adverse drug reaction reported in at least 5% of patients in any treatment group

MedDRA terms		sorfenib (N=451)			placebo (N=451)		
System organ class	**Preferred Term**	**all grades**	**grade 3**	**grade 4**	**all grades**	**grade 3**	**grade 4**
Metabolism and Nutrition Disorders	anorexia	9%	<1%	0%	5%	<1%	0%
Nervous System Disorders	headache	6%	0%	0%	3%	0%	0%
Vascular Disorders	hypertension	12%	2%	<1%	1%	<1%	0%
	flushing	6%	0%	0%	2%	0%	0%
Gastrointestinal Disorders	diarrhoea	38%	2%	0%	9%	<1%	0%
	nausea	16%	<1%	0%	12%	<1%	0%
	vomiting	10%	<1%	0%	6%	<1%	0%
	constipation	6%	0%	0%	3%	0%	0%
Skin and Subcutaneous Tissue Disorders	rash	28%	<1%	0%	9%	<1%	0%
	alopecia	25%	<1%	0%	3%	0%	0%
	hand foot syndrome	19%	4%	0%	3%	0%	0%
	pruritus	17%	<1%	0%	4%	0%	0%
	erythema	15%	0%	0%	4%	0%	0%
	dry skin	11%	0%	0%	2%	0%	0%
	skin exfoliation	7%	<1%	0%	2%	0%	0%
Musculoskeletal, Connective Tissue and Bone Disorders	arthralgia	6%	<1%	0%	3%	0%	0%
	pain in extremity	6%	<1%	0%	2%	0%	0%
General Disorders and Administration Site conditions	fatigue	15%	2%	0%	11%	<1%	0%
	asthenia	9%	<1%	0%	4%	<1%	0%

Haemorrhage: Bleeding events were more commonly reported in sorafenib treated patients (15% *versus* 8%), thereof events grade 3 and more in 2.5% vs. 1.7%.

Wound healing: there was no indication that wound-healing was affected by sorafenib monotherapy, but only 70 patients on sorafenib underwent surgical procedures, mainly minor. No formal studies of the effect of sorafenib on wound healing have been conducted.

Thromboembolism: The overall incidence of treatment related cardiac ischemia/infarction events was higher in the sorafenib group (2.9%) than in the placebo group (0.4%).

Neuropathy: Sensory neuropathy was more commonly reported in sorafenib treated patients (13.1%) than in the placebo group (6.4%).

Exploratory studies

The sorafenib dose received by patients involved in phase I trials (n=197) ranged from 100 bid to 800 bid. The rate of drug-related adverse reactions increased with higher doses of sorafenib. In particular, the incidence of diarrhoea, nausea, and stomatitis were higher in patients receiving 800 mg bid than in patients receiving 400 mg bid. Similarly, drug-related hand-foot skin reactions were reported in no patients at 100 mg bid, 11.8% at 200 mg bid, 12.2% of patients at 400 mg bid, 27.5% at 600 mg bid and 30.8% at 800 mg bid.
In the phase II trials, 91.8% of the adverse events were considered related to sorafenib. The most common drug-related adverse events were rash (38.4%), diarrhoea (37.5%) and hand-foot syndrome (35.0%). Drug-related hypertension had an incidence of 16.8%.

- Serious adverse events and deaths

Study 11213 (data cut-off of 31 May 2005)

Table 22: Grade 3 and 4 adverse drug reaction reported in at least 2% of patients

NCI-CTCAE Category/Term	sorafenib (N=451)				placebo (N=451)			
	Grade 3		Grade 4		Grade 3		Grade 4	
	n	(%)	n	(%)	n	(%)	n	(%)
Any event	139	(30.8)	32	(7.1)	97	(21.5)	27	(6.0)
Blood/bone marrow								
Decreased hemoglobin	9	(2.0)	3	(0.7)	16	(3.5)	4	(0.9)
Cardiovascular, general								
Hypertension	**15**	**(3.3)**	**1**	**(0.2)**	**2**	**(0.4)**	**0**	**(0.0)**
Constitutional symptoms								
Fatigue	21	(4.7)	1	(0.2)	14	(3.1)	2	(0.4)
Dermatology/skin								
Hand -foot skin reaction	**25**	**(5.5)**	**0**	**(0.0)**	**0**	**(0.0)**	**0**	**(0.0)**
Gastrointestinal								
Diarrhoea	11	(2.4)	0	(0.0)	3	(0.7)	0	(0.0)
Pain								
Pain, tumour pain	**13**	**(2.9)**	**0**	**(0.0)**	**7**	**(1.6)**	**1**	**(0.2)**
Pain, bone pain	2	(0.4)	1	(0.2)	14	(3.1)	1	(0.2)
Pulmonary								
Dyspnea	12	(2.7)	4	(0.9)	10	(2.2)	1	(0.2)

N: number of patients with event; N: total number of patients in the group; CTCAE: Common terminology criteria for AE

The incidence of deaths within 30 days of study drug was higher in the sorafenib group (48 patients, 10.6%) than in the placebo group (28 patients, 6.2%). Patients who were treated with sorafenib had the option of continuing sorafenib therapy after progression of disease, while cross-over was not permitted in patients progressing on placebo. Most deaths in both treatment groups were due to progressive disease.

Exploratory trials

In phase II trials, 40.1% of adverse events were grade 3 or 4 events. Grade 3 hypertension was reported in 95 (14.9%) patients and assessed as drug-related in 75 (11.8%). There was one Grade 4 hypertension, which was reported as hypertensive crisis and related to sorafenib. In addition to hypertension, Grade 3 events that occurred in at least 5% of patients included diarrhoea (5%), dyspnoea (5.3%) and hand-foot syndrome (7.5%), with an overall incidence of Grade 3 events of 51.6%. The most common Grade 4 events in the phase II database included dyspnoea (1.1%), increased bilirubin (0.9%), asthenia (0.8%), increased GGT (0.8%), anaemia (0.6%) and abdominal pain (0.6%), with an overall incidence of Grade 4 events of 12.1%. One patient involved in phase II trial had a drug-related adverse event (cerebrovascular ischemia) leading to death.

- Laboratory findings

Study 11213 (data cut-off of 31 May 2005)

A central laboratory was used to evaluate hematological and biochemical parameters. The Grade 3 and 4 laboratory abnormalities occurring at a higher rate (≥ 2%) in sorafenib vs. placebo patients were lymphopenia, neutropenia, elevated lipase, and hypophosphatemia (see Table 23). Sorafenib modestly suppresses bone marrow function.

Table 23: Grade 3 or 4 laboratory abnormalities observed in ≥ 2% of patients

CTCAE Category/Term	sorafenib (N = 451)				placebo (N = 451)			
	Grade 3		Grade 4		Grade 3		Grade 4	
	n/N[a]	(%)	n/N[a]	(%)	n/N[a]	(%)	n/N[a]	(%)
Blood/bone marrow								
Low Leukocytes	11/434	(2.5)	0/434	(0.0)	4/425	(0.9)	0/425	(0.0)
Lymphopenia	52/434	(12.0)	3/434	(0.7)	29/424	(6.8)	2/424	(0.5)
Low Neutrophils	12/434	(2.8)	11/434	(2.5)	6/424	(1.4)	4/424	(0.9)
Low Hemoglobin	8/434	(1.8)	1/434	(0.2)	11/425	(2.6)	4/425	(0.9)

CTCAE Category/Term	sorafenib (N = 451)				placebo (N = 451)			
	Grade 3		Grade 4		Grade 3		Grade 4	
	n/N[a]	(%)	n/N[a]	(%)	n/N[a]	(%)	n/N[a]	(%)
Low Platelets	2/433	(0.5)	1/433	(0.2)	0/425	(0.0)	0/425	(0.0)
Coagulation								
Prolonged INR	23/435	(5.3)	0/435	(0.0)	28/425	(6.6)	0/425	(0.0)
Metabolic/laboratory								
Hypophosphatemia	58/436	(13.3)	0/436	(0.0)	11/427	(2.6)	0/427	(0.0)
Elevated lipase	50/436	(11.5)	4/436	(0.9)	22/427	(5.2)	8/427	(1.9)
Hyponatremia	25/436	(5.7)	3/436	(0.7)	20/427	(4.7)	0/427	(0.0)
Hyperglycemia	14/436	(3.2)	1/436	(0.2)	22/427	(5.2)	1/427	(0.2)
Hyperkalemia	15/436	(3.4)	7/436	(1.6)	11/427	(2.6)	3/427	(0.7)
Elevated amylase	6/436	(1.4)	0/436	(0.0)	9/427	(2.1)	3/427	(0.7)
Hypercalcemia	1/436	(0.2)	3/436	(0.7)	6/427	(1.4)	8/427	(1.9)
Hypocalcemia	7/436	(1.6)	4/436	(0.9)	0/427	(0.0)	2/427	(0.5)

n = number of patients with the laboratory abnormality, N = total number of patients with the laboratory measurement reported; INR – international normalized ratio.

Increased lipase and amylase were very commonly reported and grade 3 or 4 lipase elevations occurred in 11% of patients in the sorafenib group compared with 7% of patients in the placebo group. Grade 3 or 4 amylase elevations were reported in 3% of patients in the sorafenib group compared to 1% of patients in the placebo group. Three cases of pancreatitis were reported in the sorafenib group *versus* one in the placebo group.

Hypophosphatemia, was observed in 45% of sorafenib patients and 12% of placebo patients had hypophosphatemia. There were no cases of Grade 4 hypophosphatemia, defined as below 1.0 mg/dl. Grade 3 hypophosphataemia (1 - 2 mg/dl) occurred in 13% on sorafenib treated patients and 3% of patients in the placebo group. The aetiology of hypophosphataemia associated with sorafenib could not be defined.

- Safety in special populations

As of 28 January 2005, 703 men and 266 women (approximately reflecting the prevalence of the disease) were treated with sorafenib. Hypertension, rash, hand-foot syndrome and alopecia tended to be reported in a higher frequency in women.

The majority of patients included in the clinical trials were between 45 and 64 years of age. There were no signs of an increased incidence of peripheral neuropathy in the elderly. Hypertension was reported in about 40% (53/128) of patients in the age group 45-64 and 25% (14/56) in those over 65 years. Nine of the 202 RCC patients in study 100391 (4.4%) and 32 of the 902 patients in study 11213 (3.5%) were $\geq$ 75 years of age. Although no renal toxic effects of sorafenib have been identified, there were 3 (16.7%) patients $\geq$75 years in the sorafenib group and none in the placebo group who had renal failure reported as an adverse event. Overall, renal failure was reported as an adverse event in 10 (2.2%) sorafenib patients and 4 (0.9%) placebo patients in study 11213.

Too few non-Caucasians were included in clinical trials to draw conclusion on safety in different ethnic groups. No safety data in patients with hepatic and renal impairment were submitted. The safety of sorafenib in paediatric patients has not been studied.

- Safety related to drug-drug interactions and other interactions

No specific studies were conducted to assess the safety related to drug-drug interactions. Infrequent bleeding events or elevations in the International Normalized Ratio (INR) have been reported in some patients taking warfarin while on sorafenib therapy.

- Discontinuation due to adverse events

As of 31 May 2005, 97 patients treated with sorafenib had at least one dose interruption due to adverse events. Most common reasons for temporary discontinuation of sorafenib were hand foot skin reaction (22 patients) and diarrhoea (15 patients). In total, dose reductions due to adverse events were reported

in 58 sorafenib patients. The most common events leading to dose reductions were hand foot skin reaction (24 patients) and diarrhoea (10 patients). Hypertension led to dose interruption in 8 patients and dose reduction in 5 patients treated with sorafenib. Adverse events led to dose interruption in 27 patients receiving placebo and dose reduction in 14 patients receiving placebo. The most common reasons for dose reductions and interruptions in placebo patients were pain and diarrhoea.

- Post marketing experience

No post-marketing experience was available at the time of submission.

- Readability testing

The package leaflet was submitted to a pilot testing (involving four subjects) as well as two separate full rounds of user testing involving 10 subjects each. The package leaflet used in these testing rounds did not significantly differ from the version revised due to the scientific assessment.

- Discussion on clinical safety

Code terms in the NCI-CTC (National Cancer Institute Common Toxicity Criteria) and MedDRA dictionaries were used. Investigators provided both an investigator term and a CTC coded term for each event. The potential assignment of different CTC and MedDRA codes to the same event was acknowledged. However, NCI-CTC are commonly used in oncology trials. Terms were translated into MedDRA and the approach was considered acceptable by the CHMP.
The safety evaluation of sorafenib was based on 1286 patients who received sorafenib in single agent clinical studies. The most common drug-related adverse events were diarrhoea, rash, alopecia and hand-foot skin reaction. Events coded as drug related were reported in 80% of sorafenib treated patients *versus* 50% in the placebo group. Rash and hand-foot skin reaction were usually CTC Grade 1 and 2 and generally appeared during the first six weeks of treatment with sorafenib. Management of dermatologic toxicities may include topical therapies for symptomatic relief, temporary treatment interruption and/or dose modification of sorafenib, or in severe or persistent cases, permanent discontinuation of sorafenib (see section 4.4 and 4.8 of the SPC).
The events most commonly associated with permanent discontinuation of study drug were pulmonary events (9 patients in sorafenib group, 4 patients in placebo group), gastrointestinal events (8 patients in sorafenib group, 4 patients in placebo group), and constitutional symptoms (7 patients in sorafenib group, 3 patients in placebo group). An increased risk of bleeding may occur following sorafenib administration. If any bleeding event necessitates medical intervention, it is recommended that permanent discontinuation of sorafenib should be considered (see section 4.4 and 4.8 of the SPC).

Altogether 97 sorafenib patients had at least one dose interruption, thereof 22 due to hand-foot skin reactions and 10 due to diarrhoea. Most of these patients had a dose reduction upon resumption of therapy. Hypertension led to dose interruption in 8 patients and dose reduction in 5 patients. Hypertension was usually mild to moderate, occurred early in the course of treatment, and was amenable to management with standard antihypertensive therapy. Blood pressure should be monitored regularly and treated, if required, in accordance with standard medical practice. In cases of severe or persistent hypertension, or hypertensive crisis despite institution of antihypertensive therapy, permanent discontinuation of sorafenib should be considered (see section 4.4 and 4.8 of the SPC).
Treatment related cardiac ischaemia/infarction adverse events was higher in the sorafenib group (2.9%) compared with the placebo group (0.4%). Patients with unstable coronary artery disease or recent myocardial infarction were excluded from the pivotal study. Temporary or permanent discontinuation of sorafenib is recommended in patients who develop cardiac ischemia and/or infarction (see section 4.4 and 4.8 of the SPC). Patients taking concomitant warfarin should be monitored regularly for changes in prothrombin time, International Normalized Ratio (INR) or clinical bleeding episodes (see section 4.4 and 4.8 of the SPC).

In the pivotal trial, more adverse events were reported as of 31 May 2005, mainly due to prolonged follow-up. There were about 10% (absolute) more serious adverse events in the sorafenib group and 4% more deaths within 30 days of receiving study medication. The median duration of therapy in sorafenib patients increased from 18.0 weeks as of 28 Jan 2005 to 23.3 weeks as of 31 May 2005. The overall exposure was longer in the sorafenib group (218.3 patient-years) than in the placebo group

(132.0 patient-years). The increase in the rate of adverse events in the sorafenib arm was also explained by the continuation of sorafenib therapy in the setting of tumour progression, as per the protocol. Patients on placebo were to discontinue therapy upon progression (until the crossover was instituted in April 2005). Patients with progressive disease were expected to have a higher rate of adverse events.

In phase I and phase II trials, common and drug related adverse reactions were dose-dependent and the overall pattern of adverse events was similar to that in the phase III trial.
No data was available on patients with Child Pugh C (severe) hepatic impairment. Since sorafenib is mainly eliminated *via* the hepatic route, a special warning has been included in section 4.4 of the SPC as exposure might be increased in patients with severe hepatic impairment. Temporary interruption of sorafenib therapy is recommended for precautionary reasons in patients undergoing major surgical procedures. Cases of renal failure have been reported in elderly patients. Monitoring of renal function is recommended in these patients (see section 4.4 od the SPC). There is limited clinical experience regarding the timing of reinitiation of therapy following major surgical intervention. The decision to resume sorafenib therapy following a major surgical intervention should be based on clinical judgement of adequate wound healing (see SPC section 4.4). High risk patients, according to MSKCC (Memorial Sloan Kettering Cancer Center) prognostic group, were not included in the pivotal study and the benefit-risk balance in these patients has not been evaluated.

The highest dose of sorafenib studied clinically was 800 mg twice daily. The adverse events observed at this dose were primarily diarrhoea and dermatological events. In the event of suspected overdose, it is recommended that sorafenib is withheld and supportive care instituted, where necessary (see SPC section 4.9). No studies on the effects on the ability to drive and use machines have been performed. There is no evidence that sorafenib affects the ability to drive or to operate machinery (see SPC section 4.7). Sorafenib is contra-indicated in patients with hypersensitivity to the active substance or to any excipients (see SPC sections 4.3).
Hypophosphatemia was a commonly observed phenomenon in patients treated with sorafenib and has been reported also for other tyrosine kinase inhibitors (e.g. imatinib, erlotinib). The mechanisms behind this adverse reaction will be investigated post-authorisation.

1.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan.

Table 24: Summary of the risk management plan

Potential Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
Dermatological toxicities	-Routine pharmacovigilance activities -In cases of Steven-Johnson or Lyell syndrome, a targeted questionnaire to direct data collection on SAE's	-Warning in section 4.4 of the SPC that dermatological side effects occur generally during the first 6 months of treatment with Nexavar -Management of symptoms by topical therapy -Temporary treatment interruption or dose reduction -In severe cases permanent discontinuation of sorafenib is recommended -Listed as ADR's in Section 4.8 of the SPC
Hypertension	-Routine Pharmacovigilance activities -Additional clinical AE data collection from ongoing clinical studies. -A targeted questionnaire to direct data collection on SAEs reported as hypertensive crisis will be used.	-Warning in section 4.4 of the SPC that Regular blood pressure should be monitored and treated by standard medical practice, required. In cases of severe or persistent hypertension, or hypertensive crisis despite institution of antihypertensive therapy, permanent discontinuation of Nexavar should be considered. -Listed as ADR in section 4.8 of the SPC
Increases in lipase, amylase and symptomatic pancreatitis.	-Routine Pharmacovigilance activities -Additional clinical AE data collection from ongoing clinical studies. -Key Bayer sponsored clinical studies will continue to collect lipase and amylase data -SAE questionnaires will be used to collect data for ---SAE reports of significant lipase and amylase increases and clinical	-Listed as ADR's in section 4.8 of the SPC and data described in section 4.8 on laboratory test abnormalities

Potential Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
	pancreatitis. -A review will be performed to determine the rates of lipase and amylase increases, the incidence of pancreatitis in cancer patients and to assess the sensitivity and specificity of the relationship between biochemical and clinical sequelae	
Hypophosphataemia	-Routine Pharmacovigilance activities: -Additional clinical AE data collection from ongoing clinical studies. -A study will be conducted to understand the mechanism of hypophosphataemia. -Additionally, attempts will be made to evaluate mutations in the tyrosine kinase domain of the FGF Receptor 3 to perform correlative analysis with hypophosphataemia. -Results expected to be available in Q 2/2008	-Listed as ADR in section 4.8 of the SPC
Haemorrhage	-Routine Pharmacovigilance activities -Additional clinical AE data collection from ongoing clinical studies. -In the planned Phase 3 non-small cell lung cancer (NSCLC) trial case record forms will collect histological subtype of the tumour (squamous vs. adeno carcinomas) -Specific SAE follow up, using SAE questionnaires for: - Cerebral hemorrhage. - Significant lung bleeding -Epidemiological assessment - To determine in the advanced or metastatic RCC population the rates of cerebral bleeding, outcome of cerebral bleeding, prevalence of cerebral metastasis and cerebral bleeding from these metastases. - To determine the risk factors for and the incidence of haemoptysis and significant lung bleeding in the NSCLC population. - To assess bleeding rates across tumour types to highlight particular disease settings at higher risk of bleeding	-Warning in section 4.4 of the SPC that an increased risk of bleeding may occur following Nexavar treatment. If any bleeding event necessitates medical intervention, it is recommended that permanent discontinuation of Nexavar should be considered -Listed as ADR in section 4.8 of the SPC
Arterial thrombosis / Cardiac ischaemia and/or infarction	-Routine Pharmacovigilance activities -Additional clinical AE data collection from ongoing clinical studies. -Further assessment of the available epidemiological resources will be made to determine the rates of cardiovascular and cerebrovascular diseases in relevant cancer patient populations. -Specific SAE questionnaires on Myocardial infarction and cerebrovascular ischemic events will be used.	-Warning in section 4.4 of the SPC that in a randomised, placebo-controlled, double-blind study the incidence of treatment-emergent cardiac ischaemia/infarction events was higher in the Nexavar group (2.9%) compared with the placebo group (0.4%). Temporary or permanent discontinuation of Nexavar should be considered in patients who develop cardiac ischemia and/or infarction -Cardiac ischemia and/or infarction are listed as ADR in section 4.8 of the SPC
Wound healing complications	-Routine Pharmacovigilance activities -Additional clinical AE data collection from ongoing clinical studies. -SAE reports of surgical interventions will be followed up to query whether the investigator/ prescriber consider there to have been any adverse effects on wound healing. -When company sponsored adjuvant or neo adjuvant clinical trials are planned, appropriate data collection techniques will be defined to capture any effects on wound healing and scar formation.	-Warning of section 4.4 of the SPC that no formal studies of the effect of sorafenib on wound healing have been conducted and limited clinical experience is available regarding the timing of reinitiation of therapy following major surgical intervention. -Temporary interruption of Nexavar therapy is recommended for precautionary reasons in patients undergoing major surgical procedures. The decision to resume Nexavar therapy following a major surgical intervention should be based on clinical judgement of adequate wound healing.
Patients on Coumadin Anticoagulants / Warfarin co-administration	-Routine Pharmacovigilance activities.	-Warning in section 4.4 of the SPC that infrequent bleeding events or elevations in the International Normalized Ratio (INR) have been reported in some patients taking warfarin while on Nexavar therapy. Patients taking concomitant Warfarin or phenprocoumon should be monitored regularly for changes in Prothrombin time, INR or clinical bleeding. -Statement in section 4.5 (interaction with other medical products) that INR should be checked regularly if Sorafenib is administered concomitantly with warfarin. -Abnormal prothrombin, INR listed in section 4.8 as ADRs
Hepatic impairment	-Routine Pharmacovigilance activities	-Warning in section 4.4 of the SPC that since Sorafenib is *mainly eliminated via the hepatic route*, exposure might be increased in patients with severe hepatic impairment No data are available for patients with Child Pugh C (severe) hepatic impairment. -4.2 Posology and method of administration also indicates that no data are available in Child Pugh C patients.
Elderly	-Routine Pharmacovigilance activities	-Warning in section 4.4 of the SPC that the experience with the use of Nexavar in elderly patients is limited and

Potential Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
		cases of renal failure have been reported. -Monitoring of renal function should be considered.
Safety in children	-A paediatric study to be run by the CTEP Children's Oncology Group in children with refractory solid tumours or refractory leukaemias is planned to start in second quarter 2006.	-Warning in section 4.2 of the SPC that Nexavar is not recommended for use in children and adolescents due to a lack of data on safety and efficacy. After repeated dosing to young and growing dogs, effects on bone and teeth were observed at exposures below the clinical exposure.
Pregnancy and lactation	-All reports of pregnancy occurring on Sorafenib reported to Bayer will be followed up with targeted questionnaires at appropriate intervals to record the outcome of the pregnancy and any adverse outcomes. Any congenital abnormalities will be recorded as SAEs and reported accordingly	-Warning in section 4.6 of the SPC (pregnancy and lactation) that Women of childbearing potential must use effective contraception during treatment. -Product labelling clearly states the harmful potential effects to a pregnancy if it occurs during Sorafenib dosing and the potential adverse effects Sorafenib may have on an ongoing pregnancy. -Contraindicated for breast-feeding women: Breast-feeding is contra-indicated during sorafenib treatment
Non-Caucasians	-Database of experience of Sorafenib in non-caucasian patients will be further expanded by additional data from non-Caucasian population studies conducted in Japan and Asia.	-Statement in section 5.2 of the SPC: *Race*: The mean sorafenib exposure was lower in Japanese patients than in Caucasian patients, but the exposure was highly variable. The clinical relevance of this observation is unknown.

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

1.6 Overall conclusions, risk/benefit assessment and recommendation

Quality, Non-clinical pharmacology and toxicology

The quality of this product was considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

There were no issues concerning the quality, the non-clinical pharmacology or the toxicology of sorafenib that negatively affected the overall benefit-risk assessment. Some issues required further clarification, to be provided post-authorisation.

Efficacy

In a phase III, randomised, placebo-controlled trial, including patients with advanced clear cell renal cell carcinoma, with good performance status and without impaired organ function (half of the patients had an ECOG performance status of 0, and half of the patients were in the low risk MSKCC prognostic group), receiving sorafenib 400mg+best supportive care (BSC) or placebo+BSC twice daily as a second line therapy, the median time to progression or death was prolonged in the sorafenib group (167 vs. 84 days; $\alpha = 0.01$, two-sided p-value < 0.000001). These results corresponded to a 56% reduction in hazard over placebo.

A total of 903 patients were enrolled at the time of the survival analysis. Among them, 220 deaths were reported (123 in the placebo group, 97 in the sorafenib group). The hazard ratio was 0.72 (p=0.018). The survival analysis was non-significant. Survival data as of November 2005 were still considered immature (367 deaths in 903 patients). Formally the results were still statistically non-significant in relation to the stopping criteria for interim analyses. However, based on PFS data, it was concluded that a favourable and clinically meaningful effect had been demonstrated.

The analysis of an enrichment study design confirmed the activity of sorafenib in patients with advanced RCC and that the activity is mainly cytostatic.

Safety

During clinical trials, sorafenib was considered a well tolerated anti-cancer drug. Dermatological side effects and diarrhoea, and hypertension likely related to VEGF inhibition, were frequently observed and led to dose reductions and treatment withdrawal in some individuals. Neuropathy constitutes no major concern, and may be cumulative. There was a signal as regards pancreatitis. Further investigations will be conducted post-authorisation to understand the mechanism of hypophosphataemia.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

Risk-benefit assessment

The antitumour activity of sorafenib was demonstrated in treatment-naïve and previously treated patients, irrespective of age, gender and Motzer prognostic criteria (good or intermediate risk). In terms of PFS, the results were statistically robust and median time to progression or death was prolonged with slightly less than 3 months. Two interim survival analyses were conducted (data cut-off May and November 2005). The hazard ratios in both analyses were about 0.75 (corresponding p-values of 0.015, i.e. nominally non-significant). The estimated median survival benefit was about 3+ months, i.e. a treatment effect considered clinically relevant. A final analysis of OS will be conducted when approximately 540 deaths are observed, but it is anticipated that the crossover of placebo patients from placebo to sorafenib arm after May 2005, limit interpretation of the results. However, after consultation of the oncology scientific advisory group (SAG), the CHMP considered that PFS *per se* could be considered as a measure of the clinical benefit of sorafenib. Based on PFS data, it was concluded that a favourable and clinically meaningful effect has been demonstrated.

Positive results are available (OS and PFS) from randomised trials using cytokines-based therapies in advanced RCC. Although several major questions remain about their application, cytokines-based therapies are widely used in Europe, and in the absence of comparative data relative to available treatment options, the oncology SAG did not recommend the use of sorafenib in first line, as initially proposed by the applicant. An ongoing, first-line, interferon alpha comparative study aiming at showing superiority in terms of PFS is ongoing.
Moreover, the applicant was asked to justify the proposed first line indication taking into account the study population of the pivotal study i.e., "second-line patients", low-moderate Motzer risk and clear cell carcinoma (mutation of the VHL gene). In retrospect and as no relationship between VHL mutation status and clinical outcome was identified, the indication was not restricted to clear cell RCC. In addition, acknowledging that patients with, e.g. an expected survival of less than 3 months are frequently excluded from clinical trials without restrictions of the indication, the CHMP did not consider it appropriate to restrict the indications to patients with poor prognosis according to Motzer score. The baseline disease characteristics of the population included in the pivotal study are mentioned in section 5.1 of the SPC. In line with the oncology SAG recommendations, the final wording adopted by the CHMP for section 4.1 of the SPC was: "*treatment of patients with advanced renal cell carcinoma who have failed prior interferon-alpha or interleukin-2 based therapy or are considered unsuitable for such therapy*". *The* CHMP required a post-authorisation investigation of the activity of sorafenib in patients with papillary tumours through the conduct of an exploratory study utilising, e.g. effects on angiogenesis as outcome measure. Further post-authorisation exploratory biomarker studies were also required.
Based on available data, the benefit-risk balance of sorafenib was considered favourable, considering the relatively positive safety profile of this medicinal product. The benefit-risk assessment was strengthened by immature survival data (HR 0.7, p=0.02). However, it was acknowledged that the assessment of the full potential of sorafenib in terms of survival benefit in the treatment of advanced RCC would not be possible due to the early unblinding of study results and subsequent cross-over. Due to the availability of active authorised treatment for the first-line treatment of advanced renal cell carcinoma RCC, the indication was restricted to use in second-line.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns. No additional risk minimisation activities were required beyond those included in the product information.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Nexavar in the treatment of patients with advanced renal cell carcinoma who have failed prior interferon-alpha or interleukin-2 based therapy or are considered unsuitable for such therapy was favourable and therefore recommended the granting of the marketing authorisation

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Exhibit 4

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Rockville, MD 20857

NDA 21-923

Bayer Pharmaceuticals Corporation
Attention: Aileen Ryan, M.Sc.
Director, Global Regulatory Affairs Therapeutic Area Oncology
400 Morgan Lane
West Haven, CT 06516

Dear Ms. Ryan:

Please refer to your new drug application (NDA) dated July 6, 2005, received July 8, 2005, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Nexavar (sorafenib) 200 mg tablets.

We acknowledge receipt of your submissions dated April 28, June 1 and 17, August 8, 19, 23, and 25, September 13, 16, and 23, October 7, 13, 18, 24, and 31, November 4 (2 submissions) and 8, and December 2, 5, 6, 15 and 19, 2005.

This new drug application provides for the use of Nexavar (sorafenib) tablets 200 mg for the treatment of patients with advanced renal cell carcinoma.

We have completed our review of this application, as amended. It is approved, effective on the date of this letter, for use as recommended in the agreed-upon labeling text.

The final printed labeling (FPL) must be identical to the enclosed labeling (text for the package insert, text for the patient package insert, immediate container and carton labels). Marketing the product with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

Please submit an electronic version of the FPL according to the guidance for industry titled *Providing Regulatory Submissions in Electronic Format - NDA*. Alternatively, you may submit 20 paper copies of the FPL as soon as it is available but no more than 30 days after it is printed. Individually mount 15 of the copies on heavy-weight paper or similar material. For administrative purposes, designate this submission "**FPL for approved NDA 21-923**." Approval of this submission by FDA is not required before the labeling is used.

We remind you of your post-marketing study commitments as agreed upon on December 19, 2005. These commitments, along with any completion dates agreed upon, are listed below.

Clinical

Regarding Study 11213, "A phase 3 randomized study of BAY43-9006 in patients with unresectable and/or metastatic renal cell cancer":

1. Provide the results of the second interim analysis of overall survival (cutoff date of November 30, 2005).

   Protocol Submission: October 16, 2003 (IND 60,453, serial number 317)
   Study start: November 15, 2003
   Report submission on second interim analysis: February 2006

2. Provide the complete study report and datasets with the definitive statistical analysis of overall survival (after approximately 540 events).

   Protocol Submission: October 16, 2003 (IND 60,453, serial number 317)
   Study start: November 15, 2003
   Final report submission: March 2007

Clinical Pharmacology and Biopharmaceutics

3. Provide a full report for the dose-ranging Phase 1 study in Japan (Study 11497) and additional data in Asian patients from ongoing studies. The evaluation and submission of data from this Phase 1 study and other ongoing studies will be completed by December 2006. If the FDA concludes that further trials are warranted, you will conduct modeling and simulation analyses to devise a dosing regimen designed to achieve similar exposures between Asians and Caucasians. After agreement with the FDA on the proposed dosing regimen and study design, you will perform a clinical pharmacokinetic study to confirm that the proposed regimen achieves similar exposures between these two populations.

   The modeling and simulation analysis to determine a dosing regimen will be completed in March 2007. If a PK study to evaluate an alternative dosing regimen in Asian patients is warranted, the study will be reported by June 2008.

   Protocol submission: March 2007
   Study start: July 2007
   Final report submission: June 2008

4. Complete the ongoing study of the effect of sorafenib on paclitaxel (a CYP 2C8 substrate) pharmacokinetics: Study 100375.

   Protocol submission: November 29, 2001 (IND 60,453, serial number 038)
   Study start: July 15, 2002
   Final report submission: June 2006

5. Complete the ongoing investigation of biomarkers to identify patients who respond to sorafenib. This request will be fulfilled based on data from studies 100391 and 11213.

   Study 100391
   Protocol Submission: April 12, 2002 (IND 60,453, serial number 67)
   Study start: September 25, 2002
   Final report submission: September 2006

   Study 11213
   Protocol Submission: October 16, 2003 (IND 60,453, serial number 317)
   Study start: November 15, 2003
   Final report submission: September 2006

6. Complete the ongoing study examining rifampin's effects on sorafenib pharmacokinetics.

   Protocol submission: October 3, 2005 (IND 60,453, serial number 1109)
   Study start: October 27, 2005
   Final report submission: June 2006

7. Complete the ongoing study examining sorafenib pharmacokinetics in patients with renal impairment.

   Protocol submission: April 4, 2005 (IND 60,453, serial number 798)
   Study start: June 3, 2005
   Final report submission: September 2006

The FDA acknowledges your commitment to make appropriate changes as recommended by the USAN Council if the Council does not accept your proposed nonproprietary name.

In addition, although not considered post-marketing commitments, we have the following suggestions and comments.

1. Hemorrhage has been reported in association with sorafenib. Consider performing a study of platelet function [redacted] or similar assay) in patients before and during sorafenib therapy,

2. Hypophosphatemia occurs commonly during treatment with sorafenib and is an unusual adverse event of anti-neoplastic therapy. Consider further study to elucidate the mechanism of sorafenib-associated hypophosphatemia.

3. Thyroid changes and hypothyroidism were observed in nonclinical sorafenib studies. Although only two sorafenib-treated patients were diagnosed with clinical hypothyroidism in the phase 3 study, this adverse event was not prospectively assessed. We recommend close monitoring of post-marketing adverse event reports for hypothyroidism. Further study to assess changes in thyroid function may be warranted based on post-marketing reports.

4. Sorafenib may act as a VEGF-R inhibitor. Another approved VEGF inhibitor has been associated with thrombosis, hemorrhage, and delayed surgical wound healing. We recommend careful post-marketing monitoring of these adverse events.

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are waiving the pediatric study requirement for this application.

In addition, submit three copies of the introductory promotional materials that you propose to use for this product. Submit all proposed materials in draft or mock-up form, not final print. Send one copy to the Division of Drug Oncology Products and two copies of both the promotional materials and the package insert directly to:

Division of Drug Marketing, Advertising, and Communications
Food and Drug Administration
5901-B Ammendale Road
Beltsville, MD 20705-1266

We have not completed validation of the regulatory methods. However, we expect your continued cooperation to resolve any problems that may be identified.

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

If you have any questions, call Patty Garvey, Regulatory Project Manager, at (301) 796-1356.

Sincerely,

{See appended electronic signature page}

Richard Pazdur, M.D.
Director
Office of Oncology Drug Products
Center for Drug Evaluation and Research

Enclosure

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Richard Pazdur
12/20/2005 12:14:48 PM

Exhibit 5

PROCESS DEVELOPMENT

Polymorphism in Process Development

MIKE BAVIN

Unexpected crystal structure transformations can lead to critical changes in chemical behaviour. Mike Bavin proposes that the occurrence of crystal polymorphs should be greeted as a commercial opportunity in the pharmaceutical industry, and draws on his experience at Smith Kline & French to demonstrate that results can be achieved quickly with some collaboration.

Crystalline products are generally the easiest to isolate, purify, dry and, in a batch process, handle and formulate. Despite these advantages, the process of crystallisation is taken for granted by most chemists, and it takes a reaction vessel clogged with an unstirrable mass to provoke serious thought. Such an occurrence is likely to be due to the appearance of a previously unknown polymorph or solvate.

The shapes of crystals are called habits: needles, cubes, plates. These can be altered by inhibiting growth on particular faces and forcing it onto others. Changes of this sort are unlikely to be caused by alteration of the impurity profile of an established product, and sudden changes of crystal habit are much more likely to be due to the appearance of a polymorph or solvate.

Polymorphs have crystal lattices which differ in the ways in which the same molecule is bound in the unit cell. The differences may reflect different ways of packing molecules in the cell, or conformational changes, which can be large. Hydrogen-bonding will be involved for most molecules of interest to the pharmaceutical industry. It is self-evident that polymorphs will have different lattice energies, melting points, heats of fusion, solubilities and rates of solution. Differences can also be expected in density, hardness, etc. There are obvious implications for the pharmaceutical industry.

Two types of polymorphs are distinguished, defined in textbooks by the use of pressure-temperature (PT) diagrams. For monotropic polymorphs, only one form is thermodynamically stable at all temperatures at which the solid exists. The other forms are metastable and may transform into the stable one, particularly in the presence of a solvent. Despite the art of pharmacists, it seems sensible and prudent to use the stable polymorph for preparing suspensions. The presence of water in the process of granulation for tabletting can lead to polymorphic transitions.

Enantiotropic compounds have two or more polymorphs, each of which is thermodynamically stable over a limited temperature range. The limits of these ranges are marked by transition temperatures, and by the melting point at the upper limit. In this case it is simplest to prepare suspensions using the polymorph which is stable over the ambient temperature range. Obviously a transition temperature in the range 15-25°C can cause difficulties.

Crystals and crystallisation

The process of crystallisation and the factors governing the appearance of individual polymorphs are still poorly understood. Crystals are in equilibrium only with a saturated solution but crystallisation requires supersaturation, so that the Phase Rule is not applicable. Which of several possible polymorphs is obtained seems to depend upon various factors: the temperature at which crystallisation occurs, the nature of the solvent (hydrophilic, hydrophobic) and the degree of supersaturation when crystallisation commenced. The use of seed crystals can be helpful in obtaining a desired polymorph. Manufacturing processes seem to be worked out by trial and error aided by serendipity, and then adhered to rigidly. Even so, unwanted polymorphs are likely to be obtained occasionally and have to be reworked.

All decisions are based upon analytical data so that means are required for identifying polymorphs. They exist only in the solid state and infrared spectroscopy and X-ray diffraction are the most generally useful techniques. Nuclear magnetic resonance (NMR) spectroscopy of solid samples is less readily available but can be valuable despite complications arising from having molecules with different conformations in the unit cell. Quantitative analyses are time-consuming by X-ray powder diffraction. They are carried out much more quickly by infrared spectroscopy particularly now that spectrometers have laser sources and dedicated computers with powerful software. A major requirement for quantitative analysis is to have samples of the pure polymorphs.

It is essential to keep in mind that analyses are not only as good as the samples. Grinding is an energetic process and can bring about polymorphic changes. It may need to be limited to, say, 20 seconds, for reproducible results to be obtained. The self-orientation of crystal fragments can influence X-ray powder diffractograms. Whether or not a sample is truly representative and how it is prepared require careful attention.

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Polymorphic transitions can occur during granulation for tabletting.

Market opportunities

Few compounds reach development and fewer still are ever marketed. In giving each development candidate the best chance of progressing, it seems better to search for polymorphs rather than to leave their appearance to time and chance with the consequent disruption. The methods used in attempting to obtain polymorphs include crystallisation from a range of solvents (polar and non-polar, hydrophilic and hydrophobic) at different temperatures, by chilling solutions rapidly, by adding a second solvent in which the solute is sparingly soluble, by vigorously stirring excess solid with solvent, by heating excess solid with a high boiling solvent, by sublimation, and by very rapidly changing the pH of a solution to precipitate acidic or basic substances. Samples can then be prepared for evaluation and for use for reference. The identity and purity of such samples should always be checked, for example by solution NMR spectroscopy and high performance liquid chromatography.

The discovery, whether accidental or intended, of polymorphs is unlikely to be greeted with enthusiasm by senior management, and the situation is better treated as an opportunity rather than as a problem. Opportunities are likely to exist for increasing patent cover, for retaining a competitive edge through unpublished knowledge and in formulating pharmaceutical products. A metastable polymorph can be used in capsules or for tabletting, and the thermodynamically stable one for suspensions.

The bringing together of chemists, pharmacists, toxicologists, biochemists, patent attorneys and colleagues from marketing at an early stage in development can only be beneficial. The essential questions can then be posed so that the research is limited rather than open-ended. The following outlines ways and means of proceeding.

The thermodynamically stable polymorph needs to be identified. If the compound is enantiotropic, there will be two or more stable polymorphs and transition temperatures as well. These can be identified by simple techniques, for example by stirring or shaking excess solid with solid at different temperatures. The polymorphic form of the residual solid must be determined. These experiments can be scaled down to use a few crystals in a small dish of solvent on the stage of a hot stage microscope. If two or more polymorphs are available in gram quantities, then attempts should be made to determine their solubilities. This can be done using a shake-flask technique and a good thermostatted bath. Alternatively a filtered solution from a stirred suspension can be passed through the sample cell of an ultraviolet spectrometer. The plateau value of the absorbance is a measure of the solubility.

Whichever method is used, it is essential that the solid residue consists of only one polymorph, otherwise the system cannot have reached equilibrium. This follows from application of the Phase Rule. For this reason solubility measurements must be made at least in duplicate. Perseverance may be necessary due to polymorphic transitions but with luck as well, it should be possible to obtain solubility-temperature curves for the polymorphs. At any given temperature, the thermodynamically stable polymorph will have the lowest solubility.* Transition temperatures will be marked by the intersections of the solubility curves.

The differences in the physical properties of the polymorphs

* This makes the reasonable assumption that the heat of solution is negative.

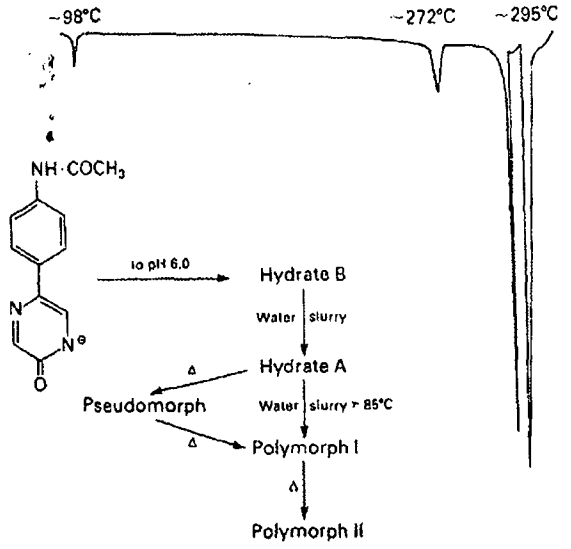


Figure Known relation of SK&F 94120 hydrates and polymorphs and DSC trace for a sample of SK&F 94120

of organic compounds will probably not be large except for bulk density. Melting points may differ by just a few degrees and data from differential scanning calorimetry (DSC), differential thermal analysis (DTA) and hot stage microscopy are likely to be difficult to interpret. This is because the polymorphs will have been crystallised from different solvents and may have different impurity profiles. But the combined technique of DSC-thermogravimetric analysis (TGA) (DTA-TGA) is essential for studying solvates, which are as likely as polymorphs to appear during development. The identity of the occluded solvent can be established by solution NMR spectroscopy and by gas liquid chromatography (with mass spectrometry if available).

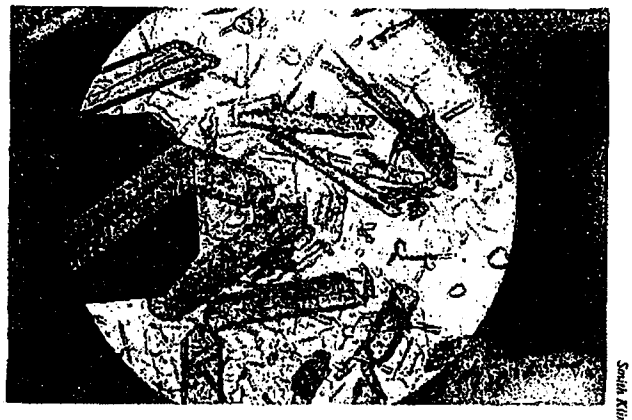
Hydrates and polymorphs of SK&F 94120

The following is an example of what can be achieved quickly by collaboration.

SK&F 94120 is weakly basic and this was made use of in its purification: a solution in alkali was filtered to remove impurities and the filtrate adjusted to pH 6. The precipitate (hydrate B) was finely divided and very difficult to collect and wash, but was prepared as a suspension for toxicological studies. In a short time, samples of the suspension recrystallised as a solid mass of long yellow needles, useless for the purpose for which they had been prepared. The new crystals were also a hydrate, A. Both hydrates A and B were characterised by DSC-TGA and infrared spectroscopy and X-ray powder diffraction** but water was lost too readily for their stoichiometry to be determined. We then showed that hydrate A could be obtained by slurrying B, simplifying the isolation of SK&F 94120 (see Figure).

Hydrates B and A could be dried in an oven at 110-120°C to give anhydrous SK&F 94120 (polymorph I). DSC-TGA showed that after hydrate A had lost its water, a small exotherm occurred at 98°C. This was interpreted as rearrangement of the pseudomorph† of hydrate A to polymorph I. The behaviour of SK&F 94120 close to its melting point is complex. Three endotherms are usually observed in the range 272-295°C (Figure), suggesting polymorphic transitions prior to melting. Sublimation complicates the situation and reduces the usefulness of hot stage microscopy. However, we were able to obtain a second polymorph (II) by heating excess SK&F 94120 with boiling ethyl benzoate. The samples always contained approximately 2 per cent of chemical impurities.

Finally, efforts to determine the transition temperature hydrate A–polymorph I showed that polymorph I was obtained from aqueous suspensions above ~85°C, facilitating drying. Large quantities of hydrate A and polymorph I were prepared before the project was terminated. The other polymorphs indicated by DSC were not investigated because they did not contribute to our objectives.



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Polymorph I shows melting and crystal change at 252°C (above); polymorph II at 250°C (below).

** Paul Brush was able to study the transformation of hydrate B to hydrate A in the X-ray powder diffractometer, using an aqueous suspension sealed in the sample holder with cling-film. A heated cell holder was very useful for infrared spectroscopic studies.

† The pseudomorph is equally-well described as an unstable polymorph.

Acknowledgement. The directors of SK&F Ltd are thanked for permission to quote unpublished data. I am particularly indebted to my former colleagues for their cooperation and suggestions, and to W A M Duncan for his trust.

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Exhibit 6

Pharmaceutical Research, Vol. 12, No. 7, 1995

Review

Pharmaceutical Solids: A Strategic Approach to Regulatory Considerations

Stephen Byrn,[1,4] Ralph Pfeiffer,[1] Michael Ganey,[2,3] Charles Hoiberg,[2] and Guirag Poochikian[2]

Purpose. This review describes a conceptual approach to the characterization of pharmaceutical solids. ***Methods.*** Four flow charts are presented: (1) polymorphs, (2) hydrates, (3) desolvated solvates, and (4) amorphous forms. ***Results.*** These flow charts (decision trees) are suggested as tools to develop information on pharmaceutical solids for both scientific and regulatory purposes. ***Conclusions.*** It is hoped that this review will lead to a more direct approach to the characterization of pharmaceutical solids and ultimately to faster approval of regulatory documents containing information on pharmaceutical solids.

KEY WORDS: polymorph; hydrate; amorphous form; desolvated solvate.

Interest in the subject of pharmaceutical solids stems in part from the Food and Drug Administration's (FDA's) drug substance guideline that states "appropriate" analytical procedures should be used to detect polymorphic, hydrated, or amorphous forms of the drug substance. These guidelines suggest the importance of controlling the crystal form of the drug substance. The guideline also states that it is the applicant's responsibility to control the crystal form of the drug substance and, if bioavailability is affected, to demonstrate the suitability of the control methods.

Thus, while it is clear that the New Drug Application (NDA) should contain information on solid state properties, particularly when bioavailability is an issue, the applicant may be unsure about how to scientifically approach the gathering of information and perhaps what kind of information is needed. This review is intended to provide a strategic approach to remove much of this uncertainty by presenting concepts and ideas in the form of flow charts rather than a set of guidelines or regulations. This is especially important because each individual compound has its own peculiarities which require flexibility in approach. The studies proposed herein are part of the Investigational New Drug (IND) process.

Solid drug substances display a wide and largely unpredictable variety of solid state properties. Nevertheless, application of basic physicochemical principles combined with appropriate analytical methodology can provide a strategy for scientific and regulatory decisions related to solid state behavior in the majority of cases. By addressing *fundamental* questions about solid state behavior at an early stage of drug development, both the applicant and the FDA are in a better position to assess the possible effects of any variations in the solid state properties of the drug substance. The resulting early interaction of the parties with regard to this area would not only tend to ensure uniformity of the materials used throughout the clinical trials but also fully resolve solid state issues before the critical stages of drug development. A further benefit of these scientific studies is the development of a meaningful set of solid state specifications which critically describe the solid form of the drug substance. These specifications would thus also facilitate the approval of a change in supplier or chemical process.

Our approach in this review is to suggest a sequence for collecting data on a drug substance that will efficiently answer specific questions about solid state behavior in a logical order. In "difficult" cases, perhaps where mixtures of forms must be dealt with, or other unusual properties are encountered, the suggested sequences would still have to be followed as a first stage in this investigation.

We have chosen to present this approach in the form of a series of decision trees, or flow charts (algorithms), one for each of the most common solid state forms. The charts are accompanied by examples from the literature representing the kind of data that would be useful in supporting the various decisions.

Decision trees provide conceptual frameworks for understanding how the justification for different crystal forms might be presented in the drug application. Industry may wish to use these decision trees as a strategic tool to organize the gathering of information early in the drug development process. Put another way, these decision trees provide a thought process that will lead to development of the most

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appropriate analytical controls. One should also note that it is the responsibility of the industry to select the appropriate test or tests to identify the phase of the solid and determine its relevant pharmaceutical properties. This approach is superior to simply performing a broad range of tests without regard to their relevance.

We should point out that, from a regulatory standpoint, if a company can establish a specification/test to ensure production of a well defined solid form of the drug substance, then it is not necessary to do all of the physical/chemical testing outlined in the decision trees. From a scientific standpoint, however, such an approach is risky since new forms may appear unpredictably during various stages of the development process. The appearance of these new forms usually slows the drug approval process and makes planning difficult.

Four decision trees are described in the sections that follow: Polymorphs; Hydrates (Solvates); Desolvated Solvates; and Amorphous Forms. Polymorphs exist when the drug substance crystallizes in different crystal packing arrangements all of which have the same elemental composition (Note that hydrates can exist in polymorphs). Hydrates exist when the drug substance incorporates water in the crystal lattice in either stoichiometric or non-stoichiometric amounts. Desolvated solvates are produced when a solvate is desolvated (either knowingly or unknowingly) and the crystal retains the structure of the solvate. Amorphous forms exist when a solid with no long range order and thus no crystallinity is produced. It is apparent that the appropriate flow chart can only be determined after the solid has been characterized using some of the tests described in the first decision point of the decision trees/flow charts (i.e. X-ray powder diffraction, elemental analysis, etc.). If there is no interest in marketing or producing an amorphous form or desolvated solvate at any stage in the process, then the corresponding flow charts do not need to be addressed. As already mentioned, it is advisable to investigate the drug substance for the existence of polymorphs and hydrates since these may be encountered at any stage of the drug manufacturing process or upon storage of the drug substance or dosage form.

All of the flow charts end (see for example Figure 1) with an indication of the types of controls which will be required based on whether a single morphic form or a mixture will be produced as the drug substance. Although this ending provides a simplistic view of a very complicated process of selecting appropriate controls, it is included to illustrate the consequence of the decisions made with regard to the drug substance. The reader should realize that the actual selection of the appropriate control could be the subject of another review which might contain another set of flow charts or decision trees.

POLYMORPHS

The flow chart/decision tree for polymorphs is shown in Figure 1. It outlines investigations of the formation of polymorphs, the analytical tests available for identifying polymorphs, studies of the physical properties of polymorphs and the controls needed to ensure the integrity of drug substance containing either a single morphic form or a mixture.

A. Formation of Polymorphs—Have Polymorphs Been Discovered?

The first step in the polymorphs decision tree is to crystallize the substance from a number of different solvents in order to attempt to answer the question: Are polymorphs possible? Solvents should include those used in the final crystallization steps and those used during formulation and processing and may also include water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, hexane and mixtures if appropriate. New crystal forms can often be obtained by cooling hot saturated solutions or partly evaporating clear saturated solutions. The solids produced are analyzed using X-ray diffraction and at least one of the other methods. In these analyses, care must be taken to show that the method of sample preparation (i.e. drying, grinding) has not affected the solid form. If the analyses show that the solids obtained are identical (e.g. have the same X-ray diffraction patterns and IR spectra) then the answer to the question "Are polymorphs possible?" is "No",

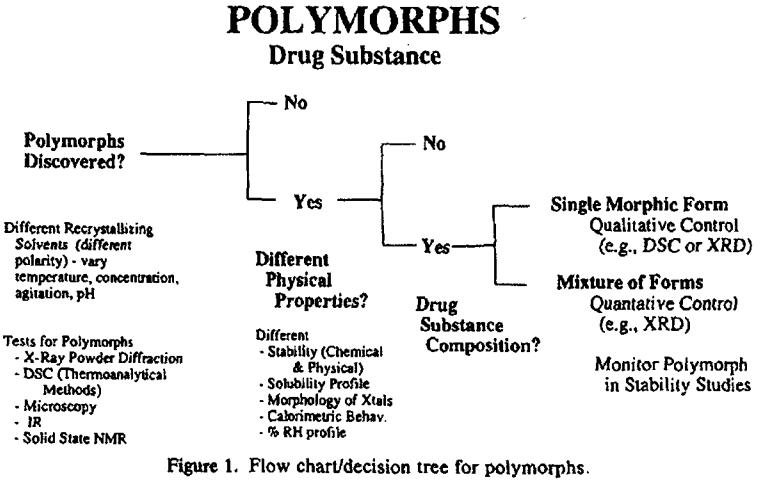

Figure 1. Flow chart/decision tree for polymorphs.

and further research is not needed. The work of Miyamae *et al.* serves as a good example of solid state studies of a drug substance which exists as polymorphs (1). Powder diffraction showed that there were two crystal forms (see Figure 2).

These workers also carried out single crystal analysis of the two crystal forms of the compound. The structures are shown in Figure 3. While such studies are not required, and indeed sometimes not possible, they provide an unequivocal confirmation of the existence of polymorphs. Moreover, once the single crystal structure of a phase has been determined, it is possible to calculate the corresponding X-ray powder pattern. This provides an irrefutable standard for identifying the phase by that method.

The DSC thermal curves of the two forms are slightly different, as shown in Figure 4 and thus may not be the preferred way of differentiating these polymorphs.

The IR spectra of the two polymorphs are quite similar(1), and IR does not appear to be a powerful method for differentiating the crystal forms in this case. Thus, for 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine, powder diffraction appears to be the best method for differentiating the two forms.

Solid-state NMR is another powerful technique for analyzing different crystal forms (2,3). Figure 5 shows the solid-state C-13 NMR spectra of Forms I and II of prednisolone. Differences in the positions of the two resonances in the 120 ppm range clearly differentiate the two forms. In principle, solid state NMR is an absolute technique in which the signal intensity is proportional to the number of nuclei provided appropriate conditions are met. In addition, solid state NMR is a bulk technique which is not very sensitive to surface changes. This method appears to be very sensitive and will undoubtedly be used more often in the future as a tool to detect different crystal forms. However, with present technology, errors in solid-state quantitative studies may be rather large.

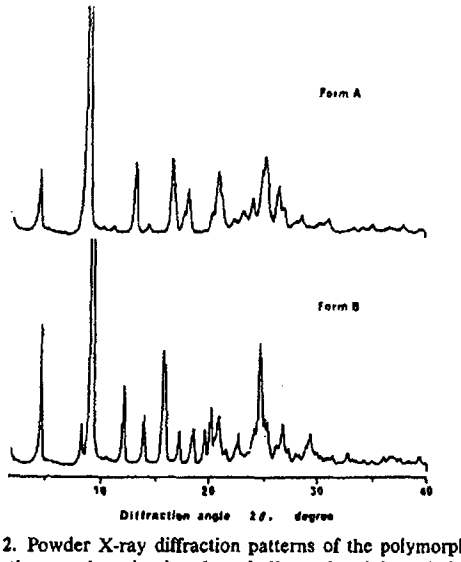

Figure 2. Powder X-ray diffraction patterns of the polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine (1).

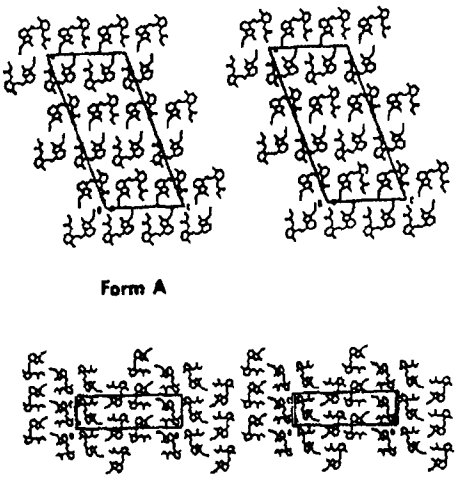

Figure 3. Stereoscopic drawings of the crystal packing of both polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine viewed along the shortest axis (Form A, b-axis; Form B, a-axis) (1).

B. Do the Polymorphs Have Different Physical Properties?

If polymorphs exist then it is necessary to examine the physical properties of the different polymorphs that can affect dosage form performance (bioavailability and stability) or manufacturing reproducibility. The properties of interest are solubility profile (intrinsic dissolution rate, equilibrium solubility), stability (chemical and physical), and crystal morphology (including both shape and particle size), calorimetric behavior, and %RH profile. If there are no discernible differences between these physico-chemical properties, then the answer to the second question in the decision tree, "Different physical properties?" is "No."

The variable physical properties of several drugs with different polymorphs are reported in the literature. For example, the dissolution profiles of the polymorphs of chloramphenicol are significantly different (4). In addition, van't Hoff solubility analysis has been used to elucidate the dif-

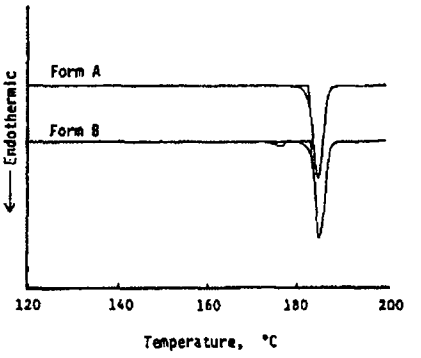

Figure 4. DSC thermal curves of the polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo{1,2-a}pyridine (1). These curves show that Form A melts whereas Form B undergoes a small endothermic transition and then melts at the same temperature as Form A.

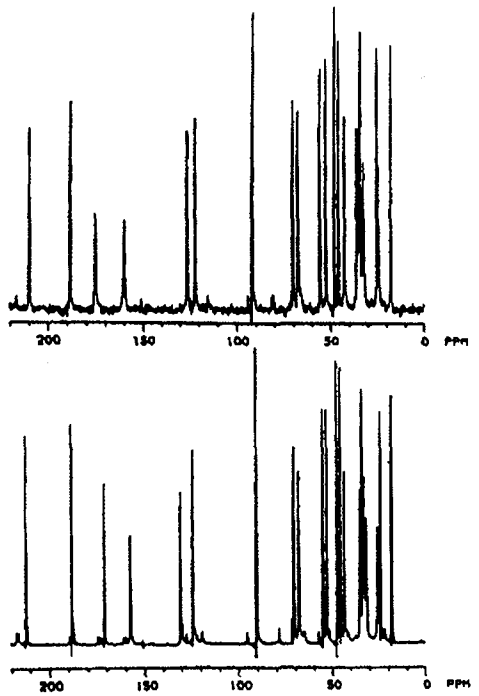

Figure 5. Solid state NMR of the two Crystal Forms of Prednisolone (2).

ferent solubilities of two polymorphs of methyl prednisolone(5). This method involves determining the equilibrium solubility of each polymorph at various temperatures. The log of the equilibrium solubility is then plotted vs 1/T. This should give straight lines for each polymorph and the temperature at which the curves intersect is the transition temperature. This technique does not work if the polymorphs interconvert.

For balance, it is important to point out that there are also cases where polymorphs exist but they have virtually identical dissolution properties(6).

C. Drug Substance Control

The important question lies in the properties that differ among polymorphs and whether those properties affect the dosage form performance (i.e., quality or bioavailability). If they do then from a regulatory standpoint it is appropriate to establish a specification/test (e.g. powder X-ray diffraction or IR) to ensure the proper form is produced. From a production standpoint, it is important to develop a process that reproducibly produces the desired polymorph.

If mixtures of forms cannot be avoided, then quantitative control is needed to ensure that a fixed proportion of forms is obtained. Furthermore, the method of analyzing for the proportion of forms would have to be validated. Also, the proportion of forms would have to remain within stated limits through the retest date of the drug substance and potentially throughout the shelf life of the product; a difficult requirement if the forms interconvert. Thus, the way to avoid a substantial amount of work in this area is to select a single solid form for production. Usually, this would be the most physically stable form when their bioavailabilities are not significantly different. Selection of the most stable from would, of course, insure that it there would be no conversion into other forms.

Powder diffraction is often a useful method to determine the percentages of polymorphs in a mixture; however, the detection limit is variable from case to case and can be as high as 15%. Matsuda (7) carried out a mixture analysis of phenylbutazone polymorphs. Diffraction lines disappear and appear as the ratio of the crystal forms change. Some of these calibration curves developed from this analysis are almost horizontal, meaning that any given mixture gives the same line intensity in this mixture range. However, other calibration curves are sloped and would appear to allow a reasonable analysis. It is fair (although Matsuda did not carry out an estimate) to estimate the errors in this analysis as ±15%.

Tanninen and Ylirussi (8) used computer curve fitting to carry out a mixture analysis of prazosin. In this particular case, they reported a highly accurate analysis, and, in fact, showed a calibration curve that could detect 0.5% of one form in another. This is obviously a highly accurate mixture analysis by powder diffraction and shows the power of this method for some applications. However, this analysis required extreme care in sample preparation and may be more difficult to carry out in a production setting where particle size may not be controlled. Similar comments apply to the analysis of mixtures by IR, where the accuracy and precision may also vary considerably from case to case. Given the analytical problems in dealing with mixtures of forms, it may generally be simpler to develop a method to prepare only one crystal form.

In summary, it is important to determine whether polymorphs are present and to solve any problems before pivotal clinical studies are initiated.

D. Determination of the Polymorph Present in the Drug Product

In cases where stability or bioavailability issues exist, the solid form present in the drug product should be investigated, if possible.

For bulk drug substances, X-ray powder diffraction and other techniques can identify the polymorph; however, solid state NMR appears to be the best method for the study of the drug substance in the dosage form (2, 3). Solid-state NMR study of three commercial products containing prednisolone showed that the products A and B contain Form I, whereas product C contains Form II.. This analysis was possible even though these tablets contain approximately 95 mg of excipients and 5 mg of drug. There are numerous cases, often involving complex mixtures or low dose products, where solid state NMR (and, in fact, any technique) will not be sensitive enough to identify the polymorph present in the drug product. However, the safety and efficacy is, of course, controlled by the potency assays and by the physical tests (e.g., dissolution).

HYDRATES (SOLVATES)

The flow chart/decision tree for hydrates (solvates) is shown in Figure 6. It outlines investigations of the formation

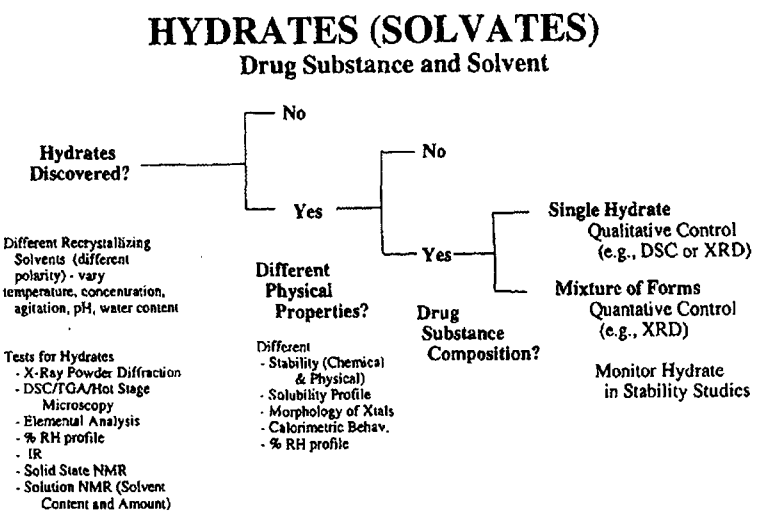

Figure 6. Flow chart for solvates or hydrates.

of hydrates (solvates), the analytical tests available for hydrates (solvates), studies of the physical properties of hydrates (solvates) and the controls needed to ensure the integrity of drug substance containing either a single morphic form or a mixture.

A. Have Hydrates (Solvates) Been Discovered?

The flow chart for hydrates (solvates) (Figure 7) is applied after the preliminary crystallizations have been completed. These are essentially the same as in the polymorph decision tree but, in addition, should include solvent-water mixtures in order to maximize the chance for hydrate formation. These experiments can be guided by the moisture uptake (% RH) studies. Any solids that indicate a significant change in water content as indicated by the % RH-moisture profile should also be examined. The resulting solid phases are preferably characterized by a combination of methods—two for phase identity and two to reveal composition and stoichiometry.

With a very few exceptions, the structural solvent contained in marketed crystalline drug products is water. It is nevertheless often desirable to characterize other solvated crystalline forms of a drug for several reasons: they may be the penultimate form used to crystallize the final product and thus require controlled characterization; they may form if the final crystallization from solvents, especially mixed solvents, is not well controlled; they may be the actual crystallized form of a final product that is desolvated during a final drying step; they may be the form used in recovery for subsequent rework. The relevance of these points will vary from case to case, but for the present discussion we shall treat the subject of solvates in its broadest form.

Examples taken from the literature serve to illustrate the kind of data that proves useful in characterizing solvated crystal forms. For example, a recent report from our laboratory showed that IR and solid state NMR was useful for the identification of the different crystal forms of dirithromycin(9). TGA is another powerful method for the analysis of solvates. For example, one early study showed that TGA could differentiate three different hydrated salts of fenoprofen(10). Combined with IR or other methods, TGA is an unequivocal method for the verification of the existence of solvates. In addition, TGA is a good method for looking at mixtures of solvated and unsolvated crystal forms, and probably can be developed into an analytical method for determining the ratios of solvated and unsolvated forms.

DSC is also a good method for detecting solvates since there is usually heat change involved in desolvation, especially for hydrates(11). Specifically, DSC by itself does not prove the existence of a solvate, but once other analytical

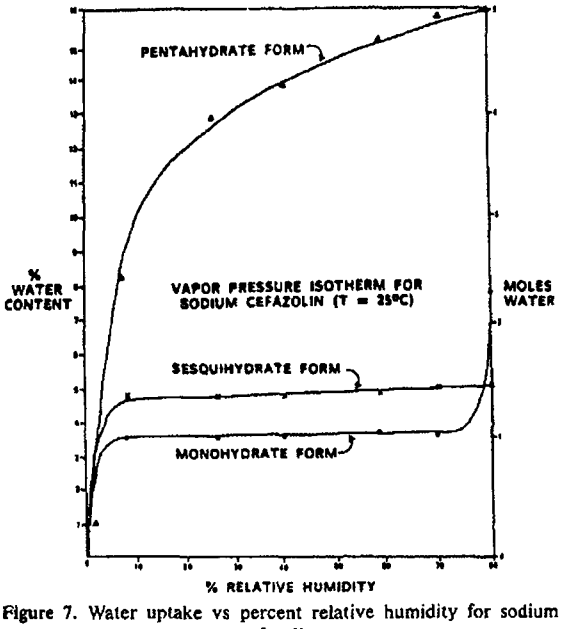

Figure 7. Water uptake vs percent relative humidity for sodium cefazolin.

data from TGA, NMR, etc. are available, DSC becomes a good method for analyzing solvates and determining a percentage of solvates present.

The three solvates of ethynylestradiol (0.5 acetonitrile, 1.0 methanol, 0.5 water) provides another interesting example (12). These solvates have different cell parameters and are crystallographically completely distinct materials. The hemihydrate was obtained from an organic solvent which is not completely miscible with water but was saturated with water. In fact, it is known that crystallization from water-immiscible solvents containing small but slightly different proportions of water can produce different hydrates of a substance.

The DSC/TGA of the three ethynylestradiol solvates(12) are unique and in this case it may be possible to develop DSC/TGA into an analytical procedure for determining the proportions of each solvate. The DSC in some of these traces appears to show a melt and recrystallization corresponding to the loss of solvent of crystallization. However, the exact interpretation of this is not possible without either a DSC microscope or interrupting the tracing to analyze the sample at various temperatures. The methanolate appears to lose solvent in two equal steps, indicating that there may also be a hemimethanolate of this compound. Again, confirmation of this would require interrupting the heating and analyzing the substance after the first solvent loss has occurred. In addition, the DSC/TGA traces suggest that all of the forms are converted to an anhydrous form which then melts at a higher temperature. Thus, interrupting any one of these thermal curves just prior to the final melt could reveal a new form that gives the powder pattern for the anhydrate. Unfortunately, no data of this type is provided in the case cited.

DSC analysis of solvates should be carried out using either an open pan or a pan with a pin-prick; otherwise, unusual and variable results will be obtained because the solvent is not provided a way of escape from the pan. One advantage of using an open pan for DSC is that it reproduces the conditions under which the TGA is performed.

Comparison of the ethinylestradiol powder diffraction patterns clearly establishes that these solvates are different crystal forms as would be expected from the single crystal data(12). In summary, DSC, TGA, and powder diffraction are all good methods for analysis of the different crystal forms of ethinylestradiol.

Figure 7 shows a percent relative humidity versus water uptake study of the type recommended by the USP committee on water(13) In this case, there are two hydrates which are relatively well behaved insofar as they are completely hydrated at about 10% relative humidity and remain uniformly hydrated throughout a wide humidity range. On the other hand, the so-called pentahydrate, which really is only a pentahydrate at very high humidity, changes water content considerably as the relative humidity is changed. The USP committee on moisture specifications recommended that moisture uptake vs relative humidity studies should be routinely performed on all drug substances and excipients (13).

B. Do the Hydrates (Solvates) Have Different Physical Properties?

The physical properties of hydrates are often quite different from the anhydrate form. Figure 8 shows the dissolution profile of theophylline hydrate and anhydrate. This figure shows that the anhydrate reaches a much higher solubility in water, and on extended exposure recrystallizes to the less soluble hydrate. Such differences must be further examined for possible effects on bioavailability.

In our laboratory we have described the different crystal forms of hydrocortisone-21-tertiary butylacetate(14). Our studies showed that the nonstoichiometric ethanolate is oxygen-sensitive and, of course, would show different physical properties from the stoichiometric ethanolate and the other solvates. Prednisolone tertiary-butylacetate also exists as a nonstoichiometric hydrate which is oxygen sensitive(15). Thus, these are cases where different crystal forms have different chemical stability, although there may be no significant differences in solubility.

C. Mixtures of Polymorphs and Hydrates

Other drug substances exist as both polymorphs and solvates. For example, furosemide exists in two polymorphs, two solvates, and an amorphous form (16, 17). The polymorphs are enantiotropically related, which means that at one temperature one polymorph is more stable, but at a different temperature the other polymorph is physically more stable. That is, plots of solubility versus temperature cross for the two polymorphs. In addition, the different crystal forms have different photostability (chemical stability in light) and moreover have different dissolution rates. Thus, there are significant differences in both chemical and physical properties.

The five different forms, or modifications of furosemide, give clearly different powder patterns. Thus, powder diffraction is a good method for analysis of these different forms. There are similarities between the IR spectra of the five different forms but there are also some significant differences, and expansion and careful analysis could lead to an FT/IR method for analysis of these different forms. IR would probably be a useful method for analysis at least for pairs of these compounds. However, it is not clear whether IR could be used to determine the percentages of several different forms in a more complex mixture. The DSC and TGA of the different forms are significantly different. As expected, the solvates show weight loss in the TGA.

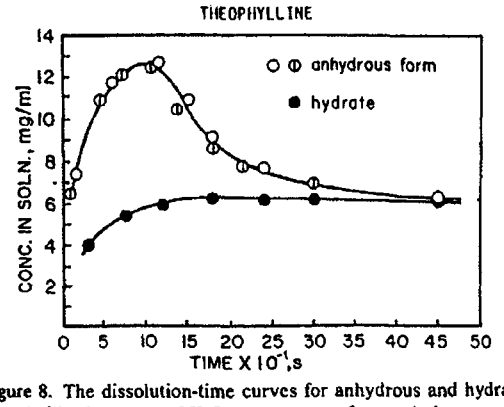

Figure 8. The dissolution-time curves for anhydrous and hydrated theophylline in water at 25°. The two types of open circles represent successive experiments (18).

The interconversion of the different forms of furosemide have been analyzed and a diagram constructed. Such a diagram can be experimentally difficult when so many pairs of crystal forms must be studied for possible interconversions and under different conditions. It is clear from this diagram that many of the forms of furosemide can be converted to form I. This study is one of the most complete reports of solvates and polymorphs available in the literature and serves as a model for studies of such systems for regulatory submissions.

D. Determination of the Hydrate Present in the Drug Product

Another important area is the analysis of the material which is produced after wet granulation of a substance which can form hydrates. We are aware of cases where the bulk drug substance is manufactured and stored as the anhydrate. However, upon wet granulation, there is a conversion (either partial or complete) to a hydrate. Subsequent drying is sometimes not adequate to convert the substance back to the anhydrate, and a hydrate or a mixture of hydrate and anhydrate remain. The formation of a hydrate and its subsequent drying can result in a change in particle size of the drug substance (19). It may also be possible to cause transformations during other processing steps. It is thus recommended that if wet granulation or processing that subjects the drug to even brief changes in temperature or pressure (e.g. milling or compression) is contemplated, then extensive studies of the ability to convert the drug substance to a new crystal form be carried out by mimicking the processing step in the laboratory.

DESOLVATED SOLVATES

The term "desolvated solvates" refers to compounds that are crystallized as solvates but undergo desolvation prior to analysis. Often these "desolvated solvates" retain the structure of the solvate with relatively small changes in the lattice parameters and atomic coordinates, but no longer contain the solvent. In addition, desolvated solvates are apt to be less ordered that their crystalline counterparts. These forms are particularly difficult to characterize properly since analytical studies indicate that they are unsolvated materials (anhydrous crystal forms) when, in fact, they have the structure of the solvated crystal form from which they were derived. Several observations may give clues that one is dealing with a desolvated form: (1) The form can be obtained from only one solvent; (2) On heating, the form converts to a structure known to be unsolvated; and (3) The form has a particularly low density compared to other forms of the same substance. Experiments that help to clarify whether an apparently solvent free modification is a desolvated form or a true anhydrate include: (1) Single crystal X-ray structure determination in the presence of mother liquor from the crystallization; (2) comparison of the X-ray powder diffraction patterns and solid state NMR spectra of the solvated and desolvated crystal forms; and (3) determination of the vapor pressure isotherm by varying the vapor pressure of the specific solvent involved. A desolvated form will often take up stoichiometric amounts of the relevant solvent. In addition, crystals of the form directly isolated from the crystallizing medium will show a plateau in their isotherm as the vapor pressure of the solvent is reduced.

Figure 9 shows the flow chart used to address regulatory issues involving desolvated solvates. It is similar to the polymorphs flow chart except that the first question involves determining whether a solvate was formed initially and then desolvated, perhaps by "air drying." The remaining questions are identical to the polymorphs flow chart.

Figure 10 shows the behavior of three different crystal forms of the same antibiotic. One crystal form takes up about two waters of hydration and further analysis indicated that it was the dimethanolate which had been desolvated by drying. The second crystal form takes up approximately one water and was originally the monomethanolate which had been desolvated by drying. The third crystal form also takes up

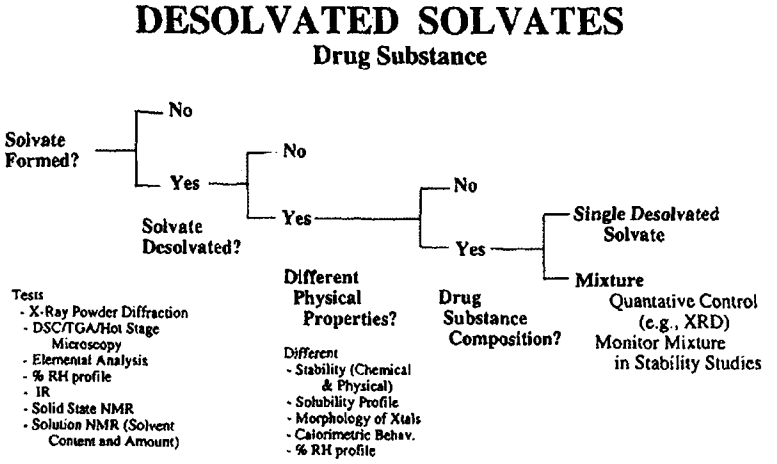

Figure 9. Flow chart for desolvated solvates.

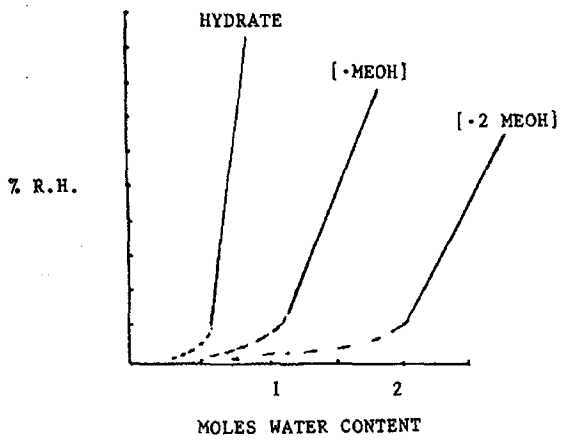

Figure 10. Water sorption by three crystal forms of cephaloridine. The brackets indicate the crystal form produced by desolvating the designated methanolate.

about one molecule of water and is the 0.75 hydrate typically obtained from water solution.

AMORPHOUS FORMS

Amorphous forms are of substantial interest because they usually are much more soluble than their crystalline counterparts. Indeed, there are cases where the amorphous forms is the only solid form that has adequate bioavailability. The initial question with this flow chart (Figure 11) is similar to the previous ones: "Are amorphous forms possible?" Amorphous forms can be prepared in different ways, for example, by spray drying or by freeze drying. One can test whether an amorphous form has been produced by using one of the methods listed. X-ray powder diffraction and microscopy are the two primary methods for determining whether an amorphous form has been produced. Powder diffraction is an excellent method for determining the existence of an amorphous form since they usually exhibit a broad hump between 2 and 20° 2θ. An amorphous form is expected to have no peaks in the powder diffraction pattern. The USP test for the presence of an amorphous form involves determining, by microscopy, whether the material lacks birefringence. IR and solid-state NMR may be useful for detecting amorphous forms since the amorphous nature of the solid sometimes results in broad lines, or in NMR, altered relaxation times. The next question on the flow chart is: "Do the amorphous forms have different physical properties?" The answer to this question will almost certainly be "Yes." Three differences from crystalline forms may generally be expected: 1) Amorphous forms would have greater solubility, 2) Amorphous forms take up water more extensively, and 3) Amorphous forms are sometimes less chemically stable. Another key question for an amorphous form is: "Does it crystallize, and how and when?" This question is very important since inadvertent crystallization can greatly affect the solubility and dissolution rate, and lead to other failures in formulation. Attempts to purposely cause amorphous forms to crystallize can provide information on the parameters involved in crystallization of amorphous forms. Specific questions include: (1) "Does the amorphous form crystallize upon exposure to heat and/or humidity?," and (2) "What other factors (e.g. mechanical pressure and seeding) can lead to the crystallization of the amorphous forms?"

The amorphous form of any substance can be partly characterized by the glass transition temperature, T_g (11). When heated to a temperature above T_g, the solid transforms from a glassy state to a more fluid-like rubbery state. The corresponding increased molecular mobility greatly raises the likelihood of two adverse events: (1) Crystallization and subsequent decreased solubility; and (2) Reduced chemical stability in the more reactive amorphous solid. Amorphous solids are also often prone to absorb moisture and this water sorption reduces the glass transition temperature further. The weight of water required to reduce the glass transition

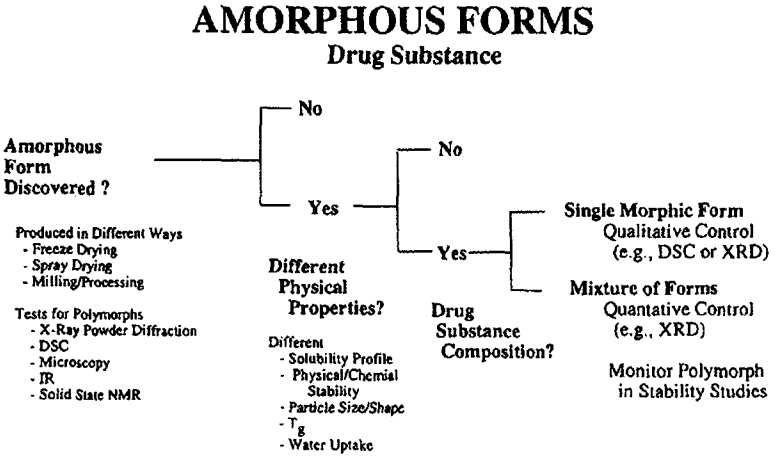

Figure 11. Flow chart for amorphous solids.

temperature to room temperature is of obvious interest and is termed W_g. Table I shows a series of interesting studies on amorphous forms of some common pharmaceuticals.

The table compares the glass transition temperatures (T_g) of a number of pharmaceutical solids with the melting temperatures (T_m). It is interesting that the average ratio of the glass transition temperature to the melting temperature is about 0.70. This table provides a simple rule of thumb which allows the prediction of the glass transition temperature of pharmaceuticals from the known melting point. Crystallization and other solid-state phenomena, such as degradation reactions, as we have said, would be more likely to occur at temperatures above the glass transition temperature. For stability, one might, therefore, wish to prepare amorphous forms only for drugs which have a T_g well above room temperature.

Amorphous indomethacin crystallizes upon standing at room temperature(Figure 12). Obviously, formulations containing amorphous indomethacin are at significant risk to crystallize and thus become less soluble. This has to some extent hampered preparing more bioavailable indomethacin dosage forms.

Quantitative analysis of mixtures of amorphous and crystalline forms provides some challenges. Cefixamine trihydrate is the subject of some early research in this area. This antibiotic, upon grinding, became a mixture of crystalline and amorphous forms. A calibration curve based upon analyzing the height of a selected powder X-ray peak was constructed and used to determine the crystallinity versus grinding time for this system. It is clear that powder diffraction provides a way to estimate the amount of amorphous cefiximine. These studies show that milling and other similar processing steps can create amorphous material and that this process may be detectable. As with wet granulation where transitions to hydrated forms can occur, processing of the drug substance can promote the formation of amorphous drug.

Pikal has compared the analysis of mixtures of crystalline and amorphous forms of several antibiotics by powder diffraction and calorimetry (20). His studies indicate that calorimetry can be a more accurate method for analysis of percent crystallinity but are complicated by water sorption. Zografi and co-workers (unpublished results) have developed a powerful method for the determination of low percentages of amorphous material based on the general propensity of amorphous materials to sorb moisture.

Table I. Pharmaceuticals Forming Glasses Above Room Temperature (21)

Pharmaceutical	T_g (K)	T_m (K)	T_g/T_m
Cholecalciferol	296	352	0.84
Sulfisoxazole	306	460	0.67
Stilbestrol	308	439	0.70
Phenobarbital	321	443	0.72
Quinidine	326	445	0.73
Salicin	333	466	0.71
Sulfathiazole	334	471	0.71
Sulfadimethoxine	339	465	0.73
Dehydrocholic acid	348	502	0.69
17β-Estradiol	351	445	0.80

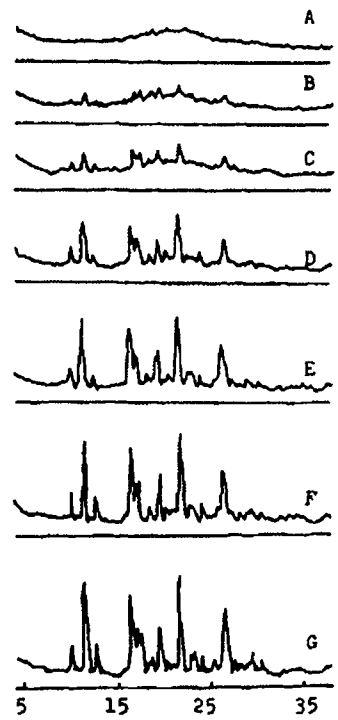

Figure 12. Behavior of amorphous indomethacin upon standing: A, at start; B 24 h; C, 48 h; D, 7d; E, 14d; F, 30d; G, 67d (22).

SUMMARY

Four flow charts which describe approaches to regulatory issues involving pharmaceutical solids have been developed. These flow charts are for the different types of solids generally encountered (polymorphs, solvates, desolvated solvates, and amorphous forms). It is hoped that these flow charts will guide the solid-state research needed to prepare a comprehensive regulatory submission on the physicochemical properties of a pharmaceutical. It is also hoped that this review has provided enough information to allow the generation of results and information necessary to prepare a drug substance submission that will be quickly approved.

ACKNOWLEDGMENTS

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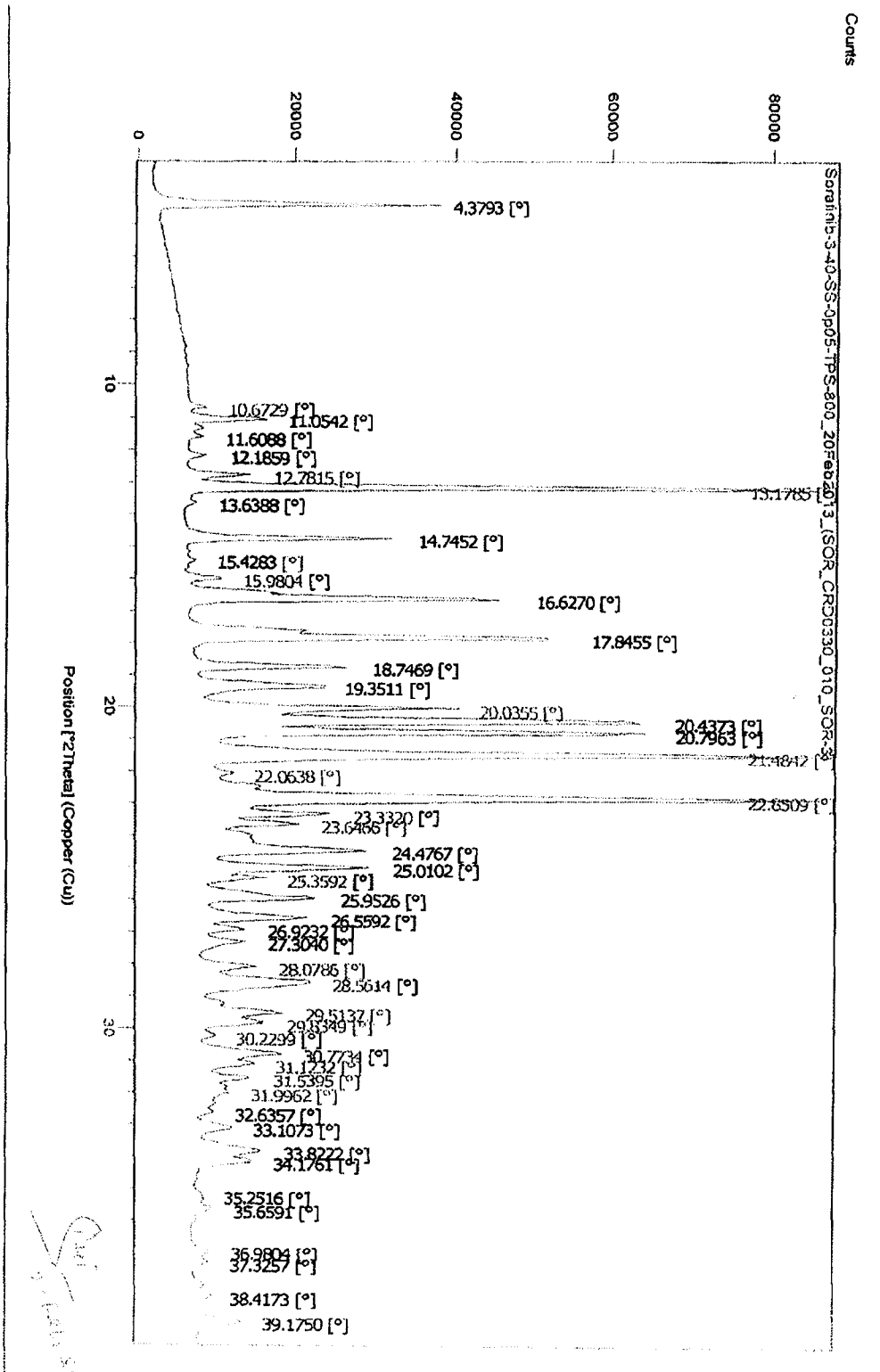
Exhibit 7



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Spinning	Yes

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10.6729	2556.13	0.1043	8.28927	3.05	263.00
11.0542	10101.75	0.1565	8.00420	12.05	1559.06
11.6088	2231.09	0.1043	7.62303	2.66	229.56
12.1859	2785.56	0.1565	7.26326	3.32	429.91
12.7815	8443.20	0.1043	6.92614	10.07	868.73
13.1785	83859.78	0.1565	6.71834	100.00	12942.58
13.6388	1699.84	0.1565	6.49262	2.03	262.35
14.7452	27112.19	0.1565	6.00785	32.33	4184.39
15.4283	1425.54	0.1565	5.74335	1.70	220.01
15.9804	4788.02	0.1565	5.54615	5.71	738.96
16.6270	42191.34	0.1565	5.33190	50.31	6511.64
17.8455	48886.13	0.1565	4.97050	58.30	7544.89
18.7469	19795.23	0.1565	4.73349	23.61	3055.12
19.3511	15548.68	0.2086	4.58704	18.54	3199.63
20.0355	31976.96	0.1565	4.43187	38.13	4935.20
20.4373	56945.57	0.1565	4.34563	67.91	8788.75
20.7963	56815.38	0.1565	4.27142	67.75	8768.66
21.4842	79379.27	0.1565	4.13618	94.66	12251.08
22.0638	2254.85	0.1043	4.02882	2.69	232.00
22.8509	77091.97	0.2086	3.89179	91.93	15864.09
23.3320	14106.29	0.1043	3.81263	16.82	1451.41
23.6466	10112.08	0.1043	3.76261	12.06	1040.44
24.4767	19654.04	0.1565	3.63686	23.44	3033.33
25.0102	20246.04	0.1565	3.56047	24.14	3124.69
25.3592	7099.05	0.1043	3.51226	8.47	730.43
25.9526	14183.16	0.1565	3.43329	16.91	2188.97
26.5592	13189.72	0.1565	3.35623	15.73	2035.65
26.9232	5561.80	0.1043	3.31167	6.63	572.26
27.3040	5819.99	0.1565	3.26635	6.94	898.23
28.0786	7353.79	0.1565	3.17798	8.77	1134.96
28.5614	13911.70	0.2608	3.12534	16.59	3578.46
29.5137	10452.28	0.1565	3.02664	12.46	1613.16
29.8349	8161.30	0.1043	2.99477	9.73	839.72
30.2299	1647.08	0.1043	2.95654	1.96	169.47
30.7734	10222.48	0.1565	2.90556	12.19	1577.70
31.1232	6707.40	0.1043	2.87369	8.00	690.13
31.5395	6445.19	0.1043	2.83670	7.69	663.15
31.9962	3753.86	0.2086	2.79725	4.48	772.47
32.6357	2059.58	0.1043	2.74388	2.46	211.91
33.1073	4571.35	0.2086	2.70587	5.45	940.70
33.8222	8529.99	0.1565	2.65029	10.17	1316.48
34.1761	7259.17	0.1565	2.62365	8.66	1120.35
35.2516	1323.78	0.2608	2.54604	1.58	340.51
35.6591	2634.49	0.1565	2.51787	3.14	406.60
36.9804	2400.35	0.1565	2.43089	2.86	370.46
37.3257	2270.65	0.1565	2.40918	2.71	350.44
38.4173	2192.58	0.2086	2.34321	2.61	451.19

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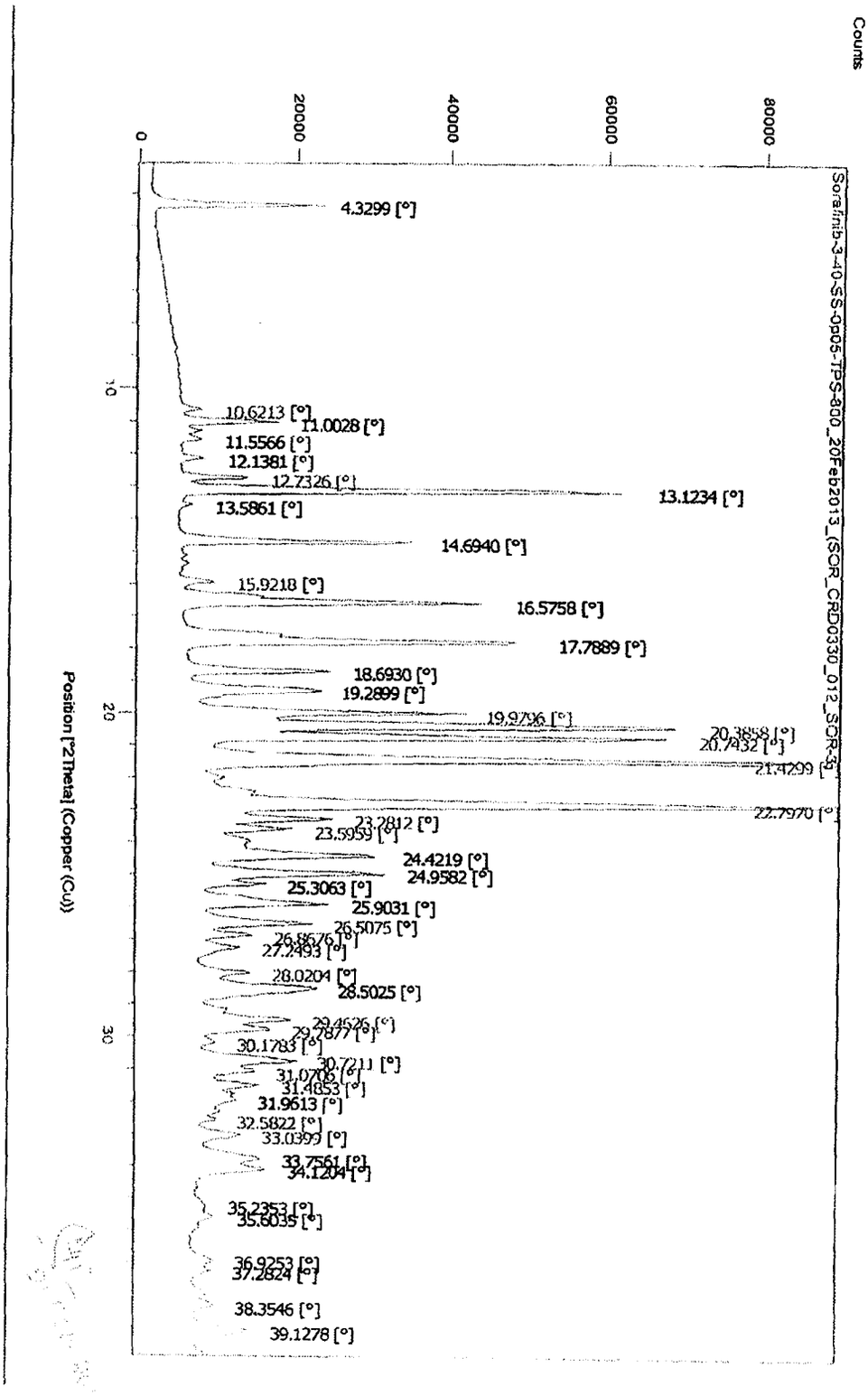
39.1750	6127.13	0.2608	2.29962	7.31	1576.06

Exhibit 8



File: Sorafinib-3-40-SS-0p05-TPS-800_20Feb2013 (SOR_CRD0330_012_SOR-3) User: PANalytical Service

Page: 1 of 1

Measurement Conditions:

Dataset Name	Sorafinib-3-40-SS-0p05-TPS-800_20Feb2013_(SOR_CRD0330_012_SOR-3)
File name	C:\XRD Data\X'Pert Data\2013\Sorafinib\Sorafinib-3-40-SS-0p05-TPS-800_20Feb2013_(SOR_CRD0330_012_SOR-3).xrdml
Sample Identification	(SOR_CRD0330_012_SOR-3)
Comment	Configuration=Reflection-transmission spinner, Owner=\\GGNXRD\PANalytical, Creation date=12/16/2010 2:41:23 PM Goniometer=Theta/Theta; Minimum step size 2Theta:0.0001; Minimum step size Omega:0.0001 Sample stage=Reflection-transmission spinner; Minimum step size Phi:0.1 Diffractometer system=EMPYREAN Measurement program=Routine-API-3-40-SS-0p05-TPS-800, Owner=\\GGNXRD\PANalytical, Creation date=8/11/2012 3:45:33 PM
Measurement Date / Time	2/20/2013 3:30:37 PM
Operator	\\GGNXRD\PANalytical
Raw Data Origin	XRD measurement (*.XRDML)
Scan Axis	Gonio
Start Position [°2Th.]	3.0263
End Position [°2Th.]	39.9143
Step Size [°2Th.]	0.0530
Scan Step Time [s]	798.7000
Scan Type	Continuous
PSD Mode	Scanning
PSD Length [°2Th.]	2.30
Offset [°2Th.]	0.0000
Divergence Slit Type	Automatic
Irradiated Length [mm]	10.00
Specimen Length [mm]	10.00
Measurement Temperature [°C]	25.00
Anode Material	Cu
K-Alpha1 [Å]	1.54060
K-Alpha2 [Å]	1.54443
K-Beta [Å]	1.39225
K-A2 / K-A1 Ratio	0.50000
Generator Settings	40 mA, 45 kV
Diffractometer Type	0000000011088667
Diffractometer Number	0
Goniometer Radius [mm]	240.00
Dist. Focus-Diverg. Slit [mm]	100.00
Incident Beam Monochromator	No
Spinning	Yes

Peak List:

21 Feb 2013

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Area [cts*°2Th.]
4.3299	21320.96	0.1565	20.40784	26.75	3290.59
10.6213	2901.48	0.1043	8.32942	3.64	298.53
11.0028	12574.28	0.1565	8.04149	15.78	1940.66
11.5566	2766.15	0.1043	7.65735	3.47	284.61
12.1381	3154.48	0.1043	7.29180	3.96	324.57
12.7326	8886.71	0.1043	6.95263	11.15	914.36
13.1234	59382.84	0.1565	6.74643	74.52	9164.91
13.5861	1850.63	0.1565	6.51772	2.32	285.62
14.6940	31466.02	0.1565	6.02867	39.49	4856.34
15.9218	4318.51	0.1565	5.56643	5.42	666.50
16.5758	40894.03	0.1565	5.34825	51.32	6311.42
17.7889	46009.11	0.1565	4.98618	57.73	7100.86
18.6930	18434.25	0.1565	4.74702	23.13	2845.07
19.2899	16208.37	0.2086	4.60145	20.34	3335.38
19.9796	34539.23	0.1565	4.44414	43.34	5330.65
20.3858	63196.00	0.1565	4.35650	79.30	9753.42
20.7432	61549.19	0.1565	4.28224	77.24	9499.26
21.4299	79594.76	0.1565	4.14653	99.88	12284.34
22.7970	79690.16	0.2086	3.90089	100.00	16398.75
23.2812	15039.74	0.1043	3.82083	18.87	1547.45
23.5959	9613.97	0.1043	3.77059	12.06	989.19
24.4219	22119.55	0.1565	3.64489	27.76	3413.84
24.9582	23280.49	0.1565	3.56777	29.21	3593.02
25.3063	7646.08	0.1043	3.51948	9.59	786.71
25.9031	16552.41	0.1565	3.43973	20.77	2554.63
26.5075	14340.92	0.1565	3.36266	18.00	2213.32
26.8676	6543.10	0.1043	3.31840	8.21	673.22
27.2493	5189.86	0.1565	3.27278	6.51	800.98
28.0204	6462.65	0.1565	3.18445	8.11	997.42
28.5025	15030.82	0.2608	3.13167	18.86	3866.33
29.4626	11463.98	0.1565	3.03177	14.39	1769.31
29.7877	8763.04	0.1043	2.99941	11.00	901.64
30.1783	1670.65	0.1565	2.96148	2.10	257.84
30.7211	11987.01	0.1565	2.91038	15.04	1850.03
31.0706	6542.07	0.1043	2.87844	8.21	673.12
31.4853	7248.06	0.1043	2.84146	9.10	745.76
31.9613	4253.17	0.2086	2.80023	5.34	875.22
32.5822	1923.62	0.1043	2.74827	2.41	197.92
33.0399	5371.54	0.2086	2.71124	6.74	1105.36
33.7561	8030.37	0.1565	2.65533	10.08	1239.37
34.1204	8961.58	0.1565	2.62781	11.25	1383.09
35.2353	1558.68	0.2086	2.54717	1.96	320.75
35.6035	2935.27	0.1565	2.52167	3.68	453.02
36.9253	2814.81	0.1565	2.43438	3.53	434.43
37.2824	2478.11	0.1565	2.41188	3.11	382.46
38.3546	2764.45	0.1565	2.34689	3.47	426.65
39.1278	7153.51	0.2608	2.30228	8.98	1840.07

PHI
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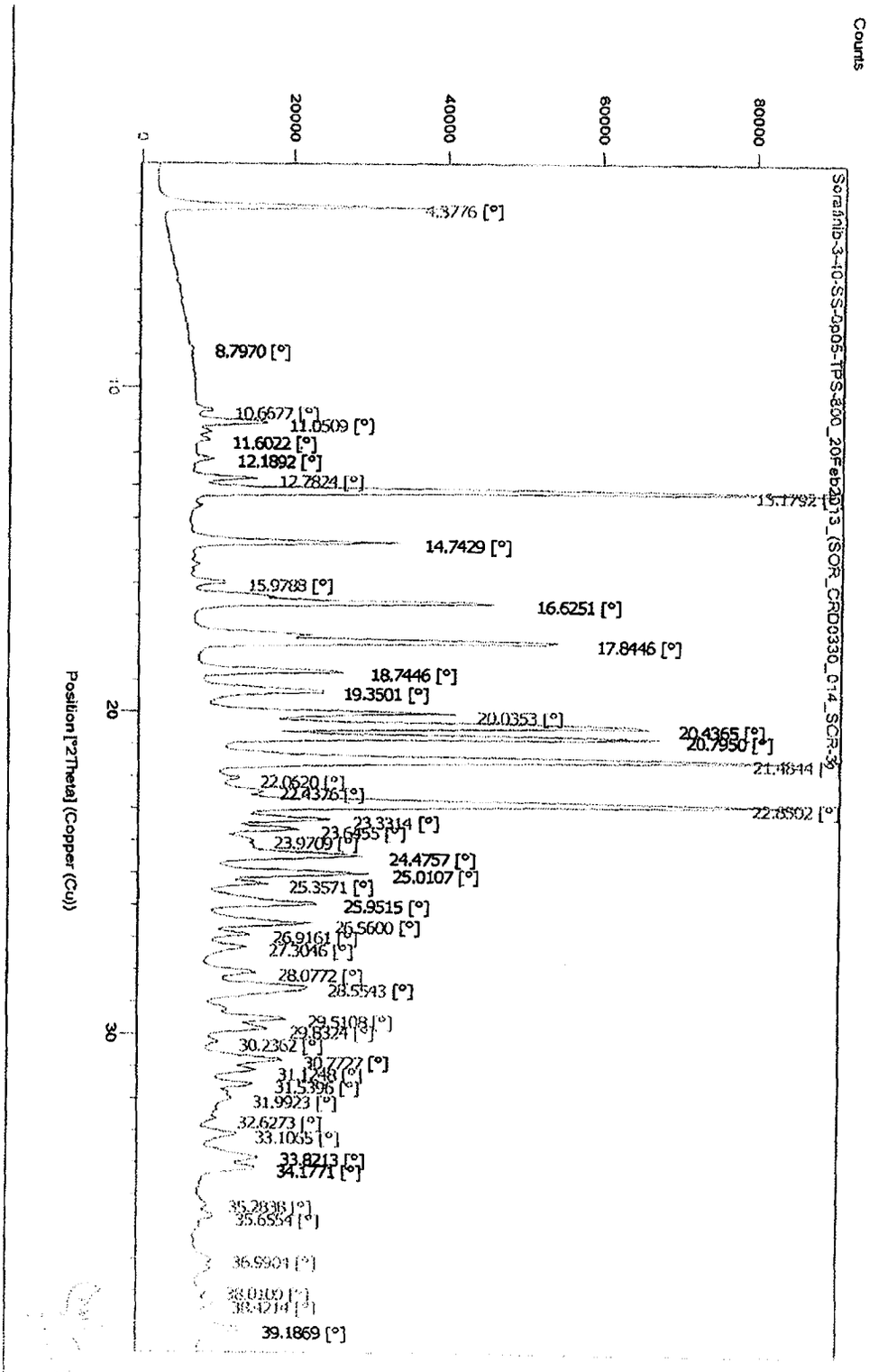
Exhibit 9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31.9923 [°]
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33.1055 [°]
33.8213 [°]
34.1771 [°]
35.2838 [°]
35.6554 [°]
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20
30
Position [°2Theta] (Copper (Cu))
File: Sorafinib-3-40-SS-0p05-TPS-800_20Feb2013_(SOR_CRD0330_014_SOR-3)
User: PANalytical Service
Page: 1 of 1

Measurement Conditions:

Dataset Name	Sorafinib-3-40-SS-0p05-TPS-800_20Feb2013_(SOR_CRD0330_014_SOR-3)
File name	C:\XRD Data\X'Pert Data\2013\Sorafinib\Sorafinib-3-40-SS-0p05-TPS-800_20Feb2013_(SOR_CRD0330_014_SOR-3).xrdml
Sample Identification	(SOR_CRD0330_014_SOR-3)
Comment	Configuration=Reflection-transmission spinner, Owner=\\GGNXRD\PANalytical, Creation date=12/16/2010 2:41:23 PM Goniometer=Theta/Theta; Minimum step size 2Theta:0.0001; Minimum step size Omega:0.0001 Sample stage=Reflection-transmission spinner; Minimum step size Phi:0.1 Diffractometer system=EMPYREAN Measurement program=Routine-API-3-40-SS-0p05-TPS-800, Owner=\\GGNXRD\PANalytical, Creation date=8/11/2012 3:45:33 PM
Measurement Date / Time	2/20/2013 4:28:28 PM
Operator	\\GGNXRD\PANalytical
Raw Data Origin	XRD measurement (*.XRDML)
Scan Axis	Gonio
Start Position [°2Th.]	3.0263
End Position [°2Th.]	39.9143
Step Size [°2Th.]	0.0530
Scan Step Time [s]	798.7000
Scan Type	Continuous
PSD Mode	Scanning
PSD Length [°2Th.]	2.30
Offset [°2Th.]	0.0000
Divergence Slit Type	Automatic
Irradiated Length [mm]	10.00
Specimen Length [mm]	10.00
Measurement Temperature [°C]	25.00
Anode Material	Cu
K-Alpha1 [Å]	1.54060
K-Alpha2 [Å]	1.54443
K-Beta [Å]	1.39225
K-A2 / K-A1 Ratio	0.50000
Generator Settings	40 mA, 45 kV
Diffractometer Type	00000000011088667
Diffractometer Number	0
Goniometer Radius [mm]	240.00
Dist. Focus-Diverg. Slit [mm]	100.00
Incident Beam Monochromator	No
Spinning	Yes

Peak List:

PHT
21 Feb 2013

Oncolo

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Area [cts*°2Th.]
4.3776	32229.99	0.1565	20.18556	36.63	4974.25
8.7970	505.92	0.3129	10.05224	0.57	156.16
10.6677	2526.09	0.1043	8.29330	2.87	259.91
11.0509	9917.68	0.1565	8.00656	11.27	1530.66
11.6022	2173.94	0.1043	7.62734	2.47	223.68
12.1892	2962.41	0.1565	7.26133	3.37	457.21
12.7824	8719.46	0.1043	6.92562	9.91	897.15
13.1792	87999.53	0.1565	6.71801	100.00	13581.50
14.7429	28446.03	0.1565	6.00881	32.33	4390.25
15.9788	4722.67	0.1565	5.54671	5.37	728.88
16.6251	42691.87	0.1565	5.33251	48.51	6588.89
17.8446	50345.88	0.1565	4.97074	57.21	7770.18
18.7446	19873.80	0.1565	4.73405	22.58	3067.24
19.3501	15546.24	0.2086	4.58727	17.67	3199.13
20.0353	32335.13	0.1565	4.43191	36.74	4990.47
20.4365	58719.69	0.1565	4.34581	66.73	9062.56
20.7950	59606.48	0.1565	4.27168	67.74	9199.43
21.4844	80679.25	0.1565	4.13615	91.68	12451.71
22.0620	2575.16	0.1043	4.02914	2.93	264.96
22.4376	4845.46	0.1043	3.96253	5.51	498.55
22.8502	77020.74	0.2086	3.89191	87.52	15849.43
23.3314	14390.81	0.1043	3.81273	16.35	1480.68
23.6455	10173.98	0.1043	3.76278	11.56	1046.81
23.9709	4034.54	0.1565	3.71244	4.58	622.67
24.4757	19747.71	0.1565	3.63701	22.44	3047.78
25.0107	20772.49	0.1565	3.56041	23.61	3205.94
25.3571	7171.29	0.1043	3.51254	8.15	737.86
25.9515	14806.33	0.1565	3.43342	16.83	2285.15
26.5600	13942.17	0.1565	3.35613	15.84	2151.78
26.9161	5929.07	0.1043	3.31253	6.74	610.05
27.3046	5659.77	0.1565	3.26627	6.43	873.51
28.0772	7096.50	0.1565	3.17814	8.06	1095.25
28.5543	13691.30	0.3129	3.12610	15.56	4226.12
29.5108	10885.97	0.1565	3.02693	12.37	1680.10
29.8324	8503.40	0.1043	2.99502	9.66	874.92
30.2362	1668.36	0.1565	2.95594	1.90	257.49
30.7727	10420.47	0.1565	2.90561	11.84	1608.25
31.1248	6815.20	0.1043	2.87355	7.74	701.22
31.5396	6551.54	0.1043	2.83669	7.44	674.09
31.9923	3713.57	0.2086	2.79758	4.22	764.18
32.6273	1989.16	0.1565	2.74457	2.26	307.00
33.1065	4594.89	0.1565	2.70593	5.22	709.16
33.8213	8056.47	0.1565	2.65036	9.16	1243.40
34.1771	7696.65	0.1565	2.62358	8.75	1187.87
35.2838	1564.73	0.2086	2.54379	1.78	321.99
35.6554	2622.90	0.1565	2.51812	2.98	404.81
36.9904	2424.36	0.1565	2.43025	2.75	374.17
38.0100	1473.50	0.2086	2.36738	1.67	303.22

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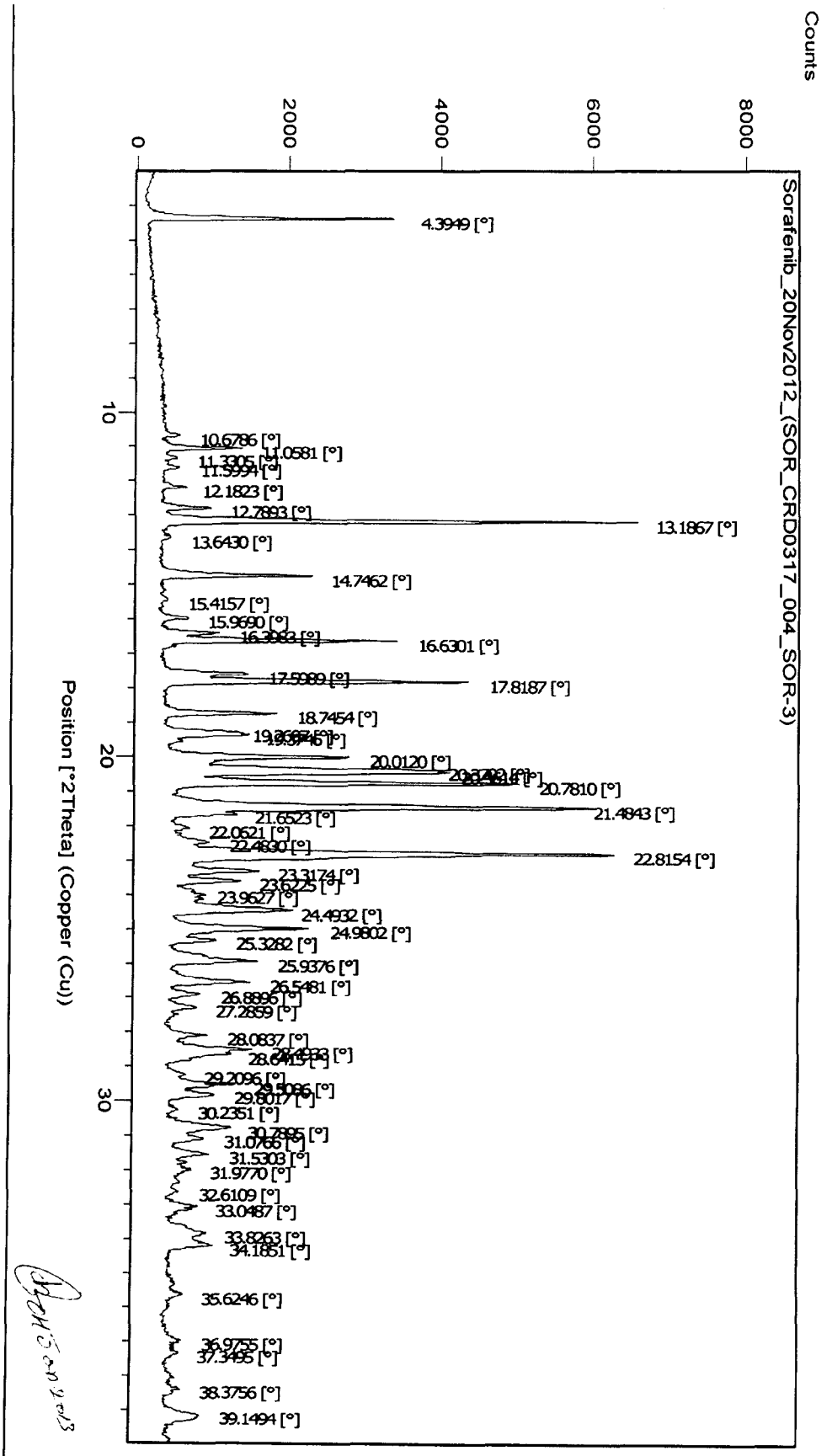
38.4214	2376.60	0.2086	2.34297	2.70	489.06
39.1869	6271.71	0.2608	2.29894	7.13	1613.25

Exhibit 10



File: Sorafenib_20Nov2012_(SOR_CRD0317_004_SOR-3)

User: PANalytical Service

Page: 1 of 1

Measurement Conditions:

Dataset Name	Sorafenib_20Nov2012_(SOR_CRD0317_004_SOR-3)
File name	C:\XRD Data\X'Pert Data\YEAR-2012\1st-Q-05-Jan2012-05Mar2012\Sorafenib Tosylate\Sorafenib_20Nov2012_(SOR_CRD0317_004_SOR-3).xrdml
Sample Identification	(SOR_CRD0317_004_SOR-3)
Comment	Configuration=Reflection-transmission spinner, Owner=\\GGNXRD\PANalytical, Creation date=12/16/2010 2:41:23 PM Goniometer=Theta/Theta; Minimum step size 2Theta:0.0001; Minimum step size Omega:0.0001 Sample stage=Reflection-transmission spinner; Minimum step size Phi:0.1 Diffractometer system=EMPYREAN Measurement program=Routine Scan Reflection, Owner=\\GGNXRD\PANalytical, Creation date=8/11/2012 2:34:48 PM
Measurement Date / Time	11/20/2012 9:15:46 AM
Operator	\\GGNXRD\PANalytical
Raw Data Origin	XRD measurement (*.XRDML)
Scan Axis	Gonio
Start Position [°2Th.]	3.0066
End Position [°2Th.]	39.9916
Step Size [°2Th.]	0.0130
Scan Step Time [s]	39.2700
Scan Type	Continuous
PSD Mode	Scanning
PSD Length [°2Th.]	3.35
Offset [°2Th.]	0.0000
Divergence Slit Type	Automatic
Irradiated Length [mm]	10.00
Specimen Length [mm]	10.00
Measurement Temperature [°C]	25.00
Anode Material	Cu
K-Alpha1 [Å]	1.54060
K-Alpha2 [Å]	1.54443
K-Beta [Å]	1.39225
K-A2 / K-A1 Ratio	0.50000
Generator Settings	40 mA, 45 kV
Diffractometer Type	0000000011088667
Diffractometer Number	0
Goniometer Radius [mm]	240.00
Dist. Focus-Diverg. Slit [mm]	100.00
Incident Beam Monochromator	No
Spinning	Yes

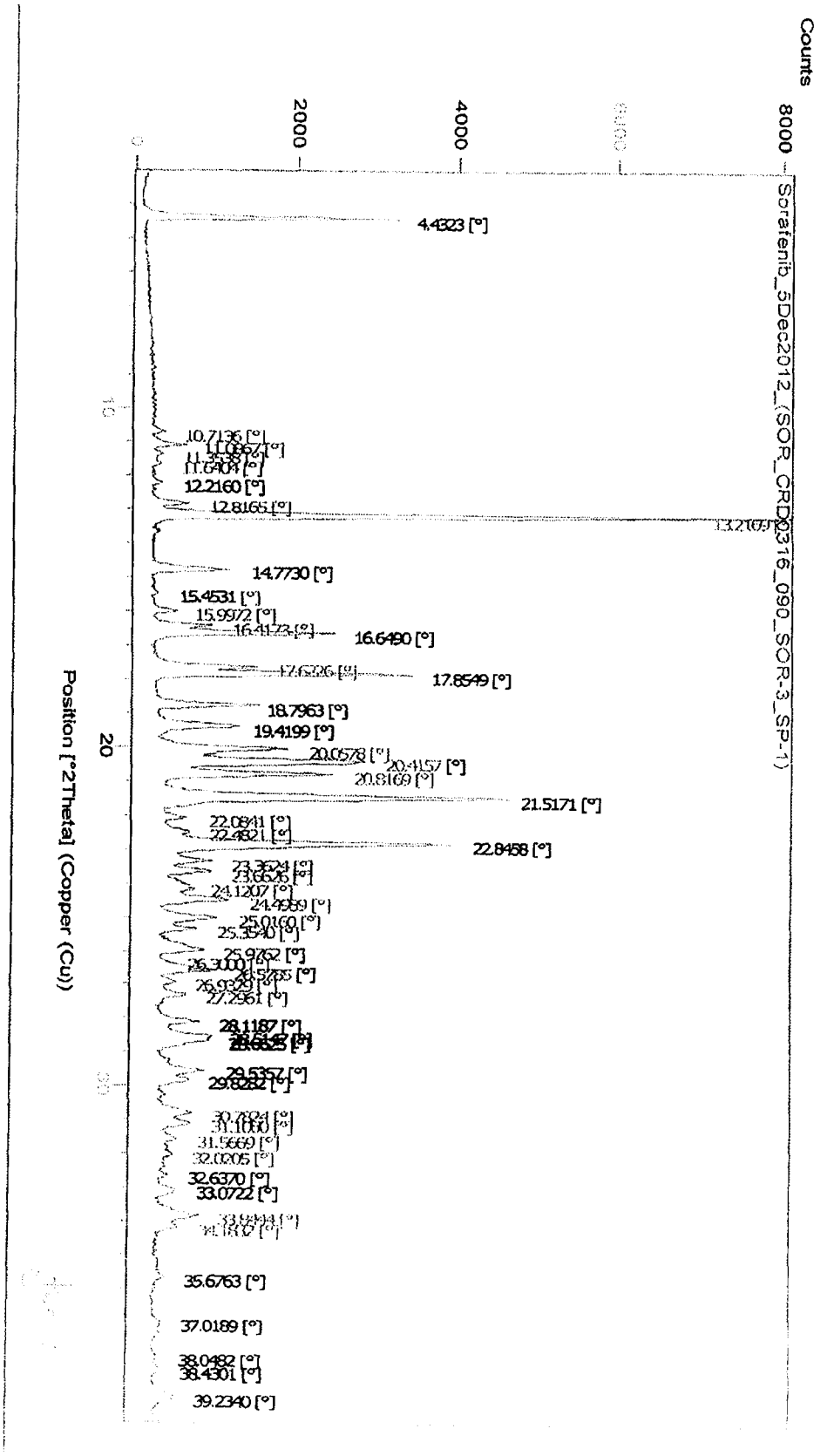
Peak List:

Pos. [°2Th.]	Height [cts]	FWHM Left	d-spacing [Å]	Rel. Int. [%]	Area

		[°2Th.]			[cts*°2Th.]
4.3949	3323.02	0.0384	20.10628	53.26	125.80
10.6786	226.78	0.0768	8.28486	3.63	17.17
11.0581	1040.42	0.0640	8.00135	16.67	65.64
11.3305	193.93	0.0640	7.80963	3.11	12.24
11.5994	239.70	0.0768	7.62917	3.84	18.15
12.1823	263.38	0.0512	7.26541	4.22	13.29
12.7893	641.52	0.0768	6.92193	10.28	48.57
13.1867	6239.57	0.0768	6.71418	100.00	472.41
13.6430	121.83	0.0768	6.49066	1.95	9.22
14.7462	1968.69	0.0768	6.00746	31.55	149.05
15.4157	90.27	0.1023	5.74802	1.45	9.11
15.9690	351.54	0.0895	5.55009	5.63	31.05
16.3983	769.07	0.0640	5.40574	12.33	48.52
16.6301	3126.71	0.0895	5.33091	50.11	276.18
17.5989	1144.08	0.0640	5.03959	18.34	72.18
17.8187	4055.27	0.0895	4.97790	64.99	358.20
18.7454	1528.93	0.0768	4.73385	24.50	115.76
19.2697	948.56	0.0768	4.60622	15.20	71.82
19.3746	1108.64	0.0640	4.58152	17.77	69.95
20.0120	2502.74	0.0768	4.43701	40.11	189.49
20.3702	3537.22	0.0780	4.35619	56.69	367.87
20.4614	3706.40	0.0640	4.34056	59.40	233.85
20.7810	4727.11	0.1023	4.27454	75.76	477.20
21.4843	5440.86	0.1151	4.13616	87.20	617.91
21.6523	974.28	0.0512	4.10444	15.61	49.18
22.0621	385.60	0.1023	4.02913	6.18	38.93
22.4830	647.72	0.1023	3.95464	10.38	65.39
22.8154	5972.63	0.0768	3.89777	95.72	452.20
23.3174	1298.88	0.0895	3.81498	20.82	114.73
23.6225	1044.90	0.0895	3.76640	16.75	92.30
23.9627	491.90	0.1535	3.71369	7.88	74.49
24.4932	1595.44	0.1151	3.63445	25.57	181.19
24.9802	1981.07	0.0768	3.56468	31.75	149.99
25.3282	745.83	0.0640	3.51649	11.95	47.06
25.9376	1290.00	0.0768	3.43523	20.67	97.67
26.5481	1196.48	0.0768	3.35760	19.18	90.59
26.8896	554.05	0.0384	3.31574	8.88	20.97
27.2859	481.02	0.0768	3.26848	7.71	36.42
28.0837	643.11	0.0512	3.17742	10.31	32.46
28.4933	1223.10	0.0768	3.13266	19.60	92.60
28.6415	906.87	0.0384	3.11679	14.53	34.33
29.2096	336.77	0.0768	3.05745	5.40	25.50
29.5086	988.85	0.0512	3.02715	15.85	49.91
29.8017	728.75	0.1151	2.99803	11.68	82.76
30.2351	256.07	0.0768	2.95604	4.10	19.39
30.7895	906.09	0.0895	2.90407	14.52	80.04
31.0766	608.99	0.0895	2.87789	9.76	53.79
31.5303	672.75	0.0640	2.83751	10.78	42.45
31.9770	430.23	0.0768	2.79888	6.90	32.57

32.6109	275.84	0.1023	2.74592	4.42	27.85

Exhibit 11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35.6763 [°]
37.0189 [°]
38.0482 [°]
38.4301 [°]
39.2340 [°]
10
20
30
Position [°2Theta] (Copper (Cu))
Page: 1 of 1

Dataset Name	Sorafenib 5Dec2012_(SOR_CRD0316_090_SOR-3_SP-1)
File name	C:\XRD Data\X'Pert Data\YEAR-2012\1st-Q-05-Jan2012-05Mar2012\Sorafenib Tosylate\Sorafenib_5Dec2012_(SOR_CRD0316_090_SOR-3_SP-1).xrdml
Sample Identification	(SOR_CRD0316_090_SOR-3_SP-1)
Comment	Configuration=Reflection-transmission spinner, Owner=\\GGNXRD\PANalytical, Creation date=12/16/2010 2:41:23 PM Goniometer=Theta/Theta; Minimum step size 2Theta:0.0001; Minimum step size Omega:0.0001 Sample stage=Reflection-transmission spinner; Minimum step size Phi:0.1 Diffractometer system=EMPYREAN Measurement program=Routine Scan Reflection, Owner=\\GGNXRD\PANalytical, Creation date=8/11/2012 2:34:48 PM
Measurement Date / Time	12/5/2012 10:26:52 AM
Operator	\\GGNXRD\PANalytical
Raw Data Origin	XRD measurement (*.XRDML)
Scan Axis	Gonio
Start Position [°2Th.]	3.0066
End Position [°2Th.]	39.9916
Step Size [°2Th.]	0.0130
Scan Step Time [s]	39.2700
Scan Type	Continuous
PSD Mode	Scanning
PSD Length [°2Th.]	3.35
Offset [°2Th.]	0.0000
Divergence Slit Type	Automatic
Irradiated Length [mm]	10.00
Specimen Length [mm]	10.00
Measurement Temperature [°C]	25.00
Anode Material	Cu
K-Alpha1 [Å]	1.54060
K-Alpha2 [Å]	1.54443
K-Beta [Å]	1.39225
K-A2 / K-A1 Ratio	0.50000
Generator Settings	40 mA, 45 kV
Diffractometer Type	0000000011088667
Diffractometer Number	0
Goniometer Radius [mm]	240.00
Dist. Focus-Diverg. Slit [mm]	100.00
Incident Beam Monochromator	No
Spinning	Yes

Peak List:

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Area [cts*°2Th.]

4.4323	2149.47	0.3753	19.33604		238.93
10.7135	148.24	0.1023	8.25722		14.96
11.0367	368.51	0.0768	7.98081		28.42
11.3538	137.45	0.1023	7.79264		13.88
11.6404	112.45	0.1023	7.60239		11.35
12.2160	139.10	0.0895	7.24546		12.29
12.8165	470.49	0.0895	6.90728		41.56
13.2169	7761.91	0.1023	6.69893	100.00	783.56
14.7730	1002.01	0.1023	5.99663	12.91	101.15
15.4531	94.50	0.0768	5.73421	1.22	7.15
15.9972	288.51	0.0895	5.54036	3.72	25.48
16.4173	765.66	0.0768	5.39955	9.86	57.97
16.6490	2290.09	0.0895	5.32492	29.50	202.28
17.6226	1308.24	0.0768	5.03287	16.85	99.05
17.8549	3268.27	0.0895	4.96791	42.11	288.69
18.7963	1191.60	0.1023	4.72116	15.35	120.29
19.4199	1015.76	0.1279	4.57093	13.09	128.17
20.0578	1722.24	0.0895	4.42699	22.19	152.13
20.4157	2691.96	0.1092	4.34658	34.68	391.95
20.4977	2380.76	0.0780	4.34014	30.67	247.60
20.8169	2290.98	0.1560	4.26371	29.52	476.52
21.5171	4438.26	0.1248	4.12652	57.18	738.53
22.0841	483.26	0.0936	4.02184	6.23	60.31
22.4821	497.13	0.1560	3.95153	6.40	103.40
22.8458	3814.18	0.1248	3.88944	49.14	634.68
23.3624	766.02	0.1248	3.80459	9.87	127.47
23.6626	761.25	0.1716	3.75700	9.81	174.18
24.1207	510.28	0.2496	3.68667	6.57	169.82
24.4989	993.69	0.1248	3.63060	12.80	165.35
25.0160	869.03	0.1248	3.55672	11.20	144.61
25.3540	600.72	0.1092	3.51007	7.74	87.47
25.9762	670.62	0.0780	3.42738	8.64	69.74
26.3000	229.30	0.4992	3.38592	2.95	152.62
26.5785	818.63	0.0780	3.35107	10.55	85.14
26.9329	335.12	0.1560	3.30776	4.32	69.70
27.2961	462.04	0.1560	3.26457	5.95	96.10
28.1187	626.53	0.0624	3.17091	8.07	52.13
28.5147	769.88	0.0936	3.12777	9.92	96.08
28.6625	751.06	0.0936	3.11197	9.68	93.73
29.5357	716.57	0.0780	3.02193	9.23	74.52
29.8282	497.94	0.1872	2.99295	6.42	124.29
30.7824	544.63	0.2496	2.90232	7.02	181.25
31.1080	544.59	0.1872	2.87268	7.02	135.93
31.5669	369.88	0.1560	2.83196	4.77	76.94
32.0205	308.09	0.1872	2.79287	3.97	76.90
32.6370	255.52	0.1248	2.74150	3.29	42.52
33.0722	363.12	0.2808	2.70641	4.68	135.95
33.8444	629.82	0.1872	2.64641	8.11	157.20
34.1832	381.15	0.1560	2.62096	4.91	79.28
35.6763	226.15	0.1560	2.51461	2.91	47.04

Exhibit 12

AFFIDAVIT

Declaration of Ms. Sandeep Kaur (Research Scientist)

I, Sandeep Kaur (Research Scientist), citizen of India reside at H.No-262/A, Model Colony, Yamuna Nagar, Haryana (India), hereby declare as follows:

I am employed as Research Scientist at Fresenius Kabi Oncology Ltd. based in Echelon Institutional Area, Plot No.-11, Sector-32, Gurgaon-122001, Haryana, India. I have been employed by Fresenius Kabi Oncology Ltd. since October 31, 2010.

I have been asked to repeat the Example -1 of IN 1960/DELNP/2007. I have conducted the experiment (**Experiment No.-SOR/CRD0317/004/SOR-3)** with the procedure described for the synthesis of Sorafenib tosylate as given in Example-1 of IN 1960/DELNP/2007.

I confirm that the repetition of this yielded Sorafenib Tosylate similar to polymorph I as shown by XRPD analysis as reported in IN 1960/DELNP/2007.

Respectfully submitted,

Date : January 06, 2014

Place : Fresenius Kabi Oncology Ltd.
Echelon Institutional Area, Plot No.-11, Sector-32, Gurgaon-122001, Haryana, India

Signature : Sandeep Kaur
06 Jan. 2014

Witnessed by : Bhuwan Bhaskar Mishra

Signed : 06 Jan 2014

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Exhibit 13

AFFIDAVIT

Declaration of Mr. Nikunj Kachhadia (Senior Research Scientist-II)

I, Nikunj Kachhadia (Senior Research Scientist-II), citizen of India reside at H.No-A/14, Kendriya Vihar, Sector-56, Gurgaon, Haryana (India), hereby declare as follows:

I am employed as Senior Research Scientist-II at Fresenius Kabi Oncology Ltd. based in Echelon Institutional Area, Plot No.-11, Sector-32, Gurgaon-122001, Haryana, India. I have been employed by Fresenius Kabi Oncology Ltd. since August 01, 2008.

I have been asked to repeat the Example -1 of IN 1960/DELNP/2007 three times. I have conducted the experiments (**Experiment No.-SOR/CRD0330/010/SOR-3, SOR/CRD0330/012/SOR-3 and SOR/CRD0330/014/SOR-3)** with the procedure described for the synthesis of Sorafenib tosylate as given in Example-1 of IN 1960/DELNP/2007.

I confirm that the repetition of these experiments yielded Sorafenib Tosylate similar to polymorph I as shown by XRPD analysis as reported in IN 1960/DELNP/2007.

Respectfully submitted,

Date : January 06, 2014

Place : Fresenius Kabi Oncology Ltd.
Echelon Institutional Area, Plot No.-11, Sector-32, Gurgaon-122001, Haryana, India

Signature : Kachhadia
06 Jan. 2014

Witnessed by : Bhuwan Bhaskar Mishra

Signed: 06 Jan 2014

Exhibit 14

AFFIDAVIT

Declaration of Mr. Varun Sharma (Research Scientist)

I, Varun Sharma (Research Scientist), citizen of India reside at H.No-356, Sector-39, Gurgaon, Haryana (India), hereby declare as follows:

I am employed as Research Scientist at Fresenius Kabi Oncology Ltd. based in Echelon Institutional Area, Plot No.-11, Sector-32, Gurgaon-122001, Haryana, India. I have been employed by Fresenius Kabi Oncology Ltd. since November 28, 2011.

I have been asked to prepare the Sorafenib tosylate as per process reported in WO 03/50111 **(Experiment No.-SOR/CRD0316/090/SOR-3/SP-1).**

I confirm that the repetition of this yielded Sorafenib Tosylate similar to polymorph I as shown by XRPD analysis as reported in IN 1960/DELNP/2007.

Respectfully submitted,

Date : January 06, 2014

Place : Fresenius Kabi Oncology Ltd.
Echelon Institutional Area, Plot No.-11, Sector-32, Gurgaon-122001, Haryana, India

Signature : Varun
06 Jan. 2014

Witnessed by : Bhuwan Bhaskar Mishra

Signed : 06 Jan 2014