

Crystalline modifications of N- α -(2,4,6-triisopropyl-phenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and/or salts thereof

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Description

The present invention relates to novel crystalline modifications of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and/or salts thereof, which can be used as pharmaceutical agents, and to pharmaceutical compositions and pharmaceutical uses comprising these novel crystalline modifications.

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The novel crystalline modifications of the present invention, which start from a compound which is known under the chemical name N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide, are efficacious inhibitors of serine protease urokinase and therefore particularly suitable for the treatment of urokinase-associated disorders, such as, for example, tumors and metastases, in particular for oral use. The free base form is designated as WX-671.

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The plasminogen activator of the urokinase type (uPA) plays a key role in tumor invasion and formation of metastases (Schmitt et al., J. Obst. Gyn. 21 (1995), 151-165). uPA is expressed in the most different types of tumor cells (Kwaan, Cancer Metastasis Rev. 11 (1992), 291-311) and binds to the tumor-associated uPA receptor (uPAR), where the activation of plasminogen to plasmin takes place. Plasmin is able to break down various components of the extracellular matrix (ECM), such as fibronectin, laminin and collagen type IV. It also activates some other ECM-degrading enzymes, in particular matrix metalloproteinases. High amounts of tumor-associated uPA correlate with a higher risk of

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metastasis for cancer patients (Harbeck et al., Cancer Research 62 (2002), 4617-4622). Inhibition of the proteolytic activity of uPA is therefore a good starting point for an antimetastatic therapy.

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A few active and selective urokinase inhibitors have already been described. Thus, uPA inhibitors of the benzamidine type are disclosed in EP 1 098 651, uPA inhibitors of the arylguanidine type in WO 01/96286 and
10 WO 02/14349. A common feature of these synthetic inhibitors is a basic residue consisting of an amidino or/and guanidino group.

International patent application WO 03/072559 discloses
15 WX-671 as an intermediate in the synthesis of the urokinase inhibitor N- α -(2,4,6-triisopropylphenyl-sulfonyl)-3-amidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide. WO 03/072559 also discloses a process for the preparation of the compound WX-671 as a
20 free base or in the form of its salts formed with acids. Special salts, in particular the hydrogen-sulfate and the sulfate form, are not mentioned. Process for crystallization are likewise not described. In the processes described, the free base WX-671 is
25 obtained as an amorphous product, which is thermally slightly unstable and hygroscopic and which has unsuitable filtration and drying properties. For this reason, it is not suitable for preparation on a large scale and must be especially protected from heat and
30 moisture.

WO 2004/011449 likewise discloses processes for the preparation of phenylalanine derivatives, which can also be present as salts, e.g. as salts of mineral
35 acids or as salts of organic acids. One of the compounds prepared is WX-671. Here too, no indication is given of the fact that the compounds disclosed can also be obtained in a stable crystalline form.

PCT/EP2004/005682 discloses hydroxyamine and hydroxyguanidine compounds as urokinase inhibitors. The medicaments disclosed comprise as active compound, inter alia, WX-671 and the active compounds can be
5 present as salts, e.g. as hydrochloride or hydrogensulfate or as salts of organic acids. A better bioavailability on oral administration was asserted for the medicaments claimed there. This publication too, however, does not disclose any sulfate salt compound of
10 said urokinase inhibitors.

The object of the present invention was the preparation of crystalline modifications of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenyl-
15 alanine 4-ethoxycarbonylpiperazide and/or salts thereof, which have advantageous properties compared to the compounds of the prior art.

The present invention provides crystalline
20 modifications of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and/or salts thereof.

According to the present invention, novel crystalline
25 modifications of the abovementioned urokinase inhibitor were found which have crucial advantages compared to the amorphous form of this compound. The crystalline modifications according to the invention have important advantages in handling, storage and formulation.

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Surprisingly, the compound N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and/or salts thereof can be crystallized, which was not possible hitherto, and is
35 therefore superior in its properties to the amorphous compounds from the prior art. Thus the crystalline modifications according to the invention are distinguished by very low hygroscopicity. They are moreover very resistant to decomposition and are

therefore also suitable for longer storage. In addition, the crystalline modifications according to the invention have improved filtration and drying properties. Moreover, the crystalline modifications of
5 N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxy-amidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and its salts are ideally suitable for formulation in pharmaceutical compositions.

10 As used herein, WX-671 means the free base of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide. WX-671.1 designates the hydrogensulfate of said compound and WX-671.2 designates the sulfate of said compound.

15 As already mentioned at the outset, the modifications of WX-671 according to the invention and salts thereof are essentially crystalline. Hitherto, it was not possible to prepare hydroxyamidine compounds of this
20 type in crystalline form. Even the compounds already disclosed in the prior art were not crystallizable.

The crystalline modifications according to the invention of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-
25 hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and its salts preferably include the hydrogensulfate salt (WX-671.1) and the sulfate salt (WX-671.2), and the free base (WX-671).

30 The crystalline modifications according to the invention can include mixtures of the free base and of the salts or alternatively mixtures of the salts. Preferably, the crystalline modification according to the invention in each case includes either the free
35 base or the sulfate salt or the hydrogensulfate salt as a crystalline modification. Particularly preferably, the crystalline modification according to the invention includes single crystals of the respective crystalline modification.

The crystalline modifications according to the invention were investigated by X-ray diffractometry and preferably have the peaks shown in Table 3.1 (for the sulfate salt WX-671.2), Table 7.1 (for the hydrogen-sulfate salt WX-671.1) and Table 11.1 (for the free base). The crystalline modifications according to the invention essentially have the peaks shown in Figures 5 (for the sulfate salt WX-671.2), 13 (for the hydrogen-sulfate salt WX-671.1) and 19 (for the free base).

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As is described in the examples below, additionally to the X-ray diffractometry analyses, thermoanalytical investigations were also carried out (differential scanning calorimetry, DSC and thermogravimetry, TGA). It was seen here that the crystalline compound of the hydrogensulfate salt WX-671.1 decomposes at a temperature of approximately 175°C-195°C, more precisely in the range from approximately 185°C (at a heating rate of 2 Kmin⁻¹).

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The crystalline modification of WX-671.2 according to the invention preferably contains N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and sulfate anions in a molar ratio of approximately 2:1, where this ratio can vary in a range from 1.5 to 2.5:1. A ratio of approximately 1.25 to 2.25:1 is preferred, more highly preferably 1.1 to 2.1:1 and most highly preferably in the ratio of approximately 2:1.

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The crystalline modification of WX-671.2 preferably has units of in each case 2 molecules of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and a sulfate anion, where in each of these units the 2 molecules of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide can be present in the same conformation or they can preferably also be present in different conformations.

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In addition, the crystalline modification of WX-671.2 is preferably present as a hydrate, in particular as a trihydrate, i.e. per mole of salt approximately 3 mol of water are present. This ratio can, of course, also be subject to slight variations, i.e. per mole of salt on average between 2.5 and 3.5 mol of water can be present, preferably between 2.25 and 3.25, more highly preferably between 2.2 and 3.2, more highly preferably between 2.1 and 3.1.

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The sulfate salt of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide has, independently of the crystallinity, turned out to be the thermodynamically stable compound. Therefore the present invention also provides the novel compound of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide sulfate salt. The noncrystalline sulfate salt WX-671.2 is also suitable, as described below for the crystalline modifications, for the production of medicaments.

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In the crystalline modification of WX-671.1, N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and hydrogensulfate anions are preferably present in a molar ratio of 0.5 to 1.5:1, more highly preferably of 0.8 to 1.2:1, more highly preferably 0.9 to 1.1:1, more highly preferably approximately 1:1.

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A crystalline modification of WX-671.1 can also be present as a hydrate.

The crystalline modification of WX-671 (free base) is preferably not present as a hydrate. A hydrate form is, however, possible.

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The crystalline modifications according to the invention can optionally be used with suitable

pharmaceutical excipients and/or vehicles for the production of medicaments. Here, administration in combination with other active compounds, e.g. other urokinase inhibitors, such as, for example, antibodies
5 and/or peptides, but also with chemotherapeutics and cytostatics or/and other cytostatic and cytotoxic active compounds is possible.

The crystalline modifications according to the
10 invention can thus be prepared in a suitable pharmaceutical formulation, for example as tablets, coated tablets, capsules, pastilles, powder, syrup, suspension, solution or the like. In particular, a pharmaceutical preparation for oral administration is
15 preferred.

The crystalline modifications according to the invention are suitable for the control of diseases which are associated with a pathological overexpression
20 of uPA or/and urokinase plasminogen activator receptor (uPAR). It is, for example, able to inhibit the growth or/and the spread of malignant tumors and the metastasis of tumors highly efficiently. Examples of this are oncoses, e.g. breast cancer, lung cancer,
25 bladder cancer, stomach cancer, cervical cancer, ovarian cancer, kidney cancer, prostate cancer and soft tissue sarcomas, in particular tumors associated with a high metastasis rate.

30 The modifications according to the invention can be employed on their own or in combination with other physiologically active substances, e.g. with radiotherapeutics or with cytotoxic or/and cytostatic agents, e.g. chemotherapeutics, such as, for example,
35 cis-platin, doxorubicin, 5-fluorouracil, taxol derivatives, or/and other chemotherapeutic agents, for example selected from the group consisting of the alkylating agents, antimetabolites, antibiotics, epidophyllotoxins and vinca alkaloids. A combination

with radiation therapy or/and surgical interventions is likewise possible.

Furthermore, the crystalline modifications according to the invention are also efficacious for other uPA-associated diseases. Examples of diseases of this type are, for example, pulmonary high blood pressure and/or heart diseases (e.g. WO 02/00248), gastric and bowel diseases, such as, for example, inflammatory bowel diseases, premalignant colon adenomas, inflammatory diseases, such as, for example, septic arthritis, or other diseases, such as osteoporosis, cholesteatoma, skin and eye diseases, such as, for example, age-related macular degeneration (AMD), and viral or bacterial infections, reference being made expressly to the diseases mentioned in EP-A-0 691 350, EP-A-1 182 207 and US patent 5,712,291.

A further subject of the present invention is a medicament which includes a crystalline modification according to the invention as an active compound. Such a medicament can optionally additionally include pharmaceutically tolerable carriers and/or excipients. The medicaments can be administered to man or animals topically, orally, rectally or parenterally, e.g. intravenously, subcutaneously, intramuscularly, intraperitoneally, or alternatively sublingually, nasally and/or inhalatively. Suitable administration forms are, for example, tablets, coated tablets, capsules, pastilles, pellets, powder, suppositories, solutions, syrup, emulsions, suspensions, liposomes, inhalation sprays or transdermal systems, such as, for example, patches. A particularly preferred pharmaceutical composition is suitable for oral administration, e.g. also as a slow-release depot.

In addition, the present invention provides a use of the crystalline modifications according to the invention for the production of pharmaceutical

composition for the control of diseases which are associated with a pathological overexpression of urokinase and/or the urokinase receptor. In particular, such a medicament containing the active compound according to the invention is suitable for tumor treatment and/or prevention and in particular also for the treatment or prevention of formation of metastases, and for the treatment of primary tumors and secondary tumors.

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By means of the present invention, one possibility for urokinase inhibition in living beings, in particular man, is provided by administration of an efficacious amount of the modification according to the invention. The dose to be administered depends on the nature and severity of the diseases to be treated. For example, the daily dose is in the range from 0.01-100 mg/kg of active substance per body weight, more highly preferably 0.1-50 mg/kg, more highly preferably 0.5-40 mg/kg, more highly preferably 1-30 mg/kg, more highly preferably 5-25 mg/kg.

A further subject of the present invention is a process for the production of an essentially crystalline modification of an N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide salt, comprising the steps:

- (a) preparation of the compound N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide or of one of its salts,
- (b) dissolution or/and suspension of the compound or of the salt itself from step (a) in a solvent suitable for the formation of the crystalline modification,
- (c) separation of the crystalline modification.

It has surprisingly been shown that the crystalline modifications of WX-671, WX-671.1 and WX-671.2 can be prepared in crystalline form in a simple manner. Preferably, the starting material used is a salt of N-
5 α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide, particularly preferably the hydrogensulfate salt.

However, other salt compounds are also suitable as
10 starting compounds for the formation of crystalline modifications, for example the besylate salt, hydrochloride salt, mesylate salt, tartrate salt and others.

15 Solvents used for step (b) can preferably be various organic solvents. Those suitable are, for example, water and various alcohols, e.g. methanol, ethanol, propanol, butanol and their isoforms, such as, for example, isopropanol, isobutanol etc., and furthermore
20 glycols, ethers, glycol ethers, acetone and the like. Further suitable solvents are tetrahydrofuran (THF) and acetonitrile. Preferred solvents are in particular acetone and acetonitrile.

25 However, water can also be used as a solvent. In particular for the recrystallization (see step (d)), water is preferably used.

If the free base is used as a starting material for the
30 preparation of a crystalline modification of a salt of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide, suitable salts or acids are additionally added in step (b) in order to obtain the respective desired salt of
35 N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide.

The process according to the invention can also include a further step (d) of recrystallizing the crystalline

compounds from step (c). This applies in particular for the preparation of WX-671.2 from WX-671.1. Here, the crystalline modification resulting in step (c) is in turn preferably recrystallized in a suitable solvent or
5 a mixture of solvents. Here too, as solvents those mentioned beforehand can be employed. In particular, water is preferred, or recrystallization is preferably carried out with a content of water sufficient for the formation of the desired crystalline modification.
10 Water is preferred in particular if, as, for example, in the case of WX-671.2, a hydrate (in the case of WX-671.2 a trihydrate) is formed.

The invention will now be illustrated in more detail by
15 the following figures and the examples.

Description of the figures

Figure 1 shows an Ortep plot (50%) with symbol plot for molecule A (WX-671.2).

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Figure 2 shows an Ortep plot (50%) with symbol plot for molecule B (WX-671.2).

Figure 3 shows independent molecules within a unit cell of a unit of two molecules of WX-671.2 and sulfate anion.

Figure 4 shows a simulated X-ray diffractogram using single crystal data of WX-671.2.

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Figure 5 shows an experimental X-ray diffractogram of WX-671.2.

Figure 6a shows the superimposition of the simulated X-ray diffraction pattern and of the experimental X-ray diffraction pattern according to Figures 4 and 5 for WX-671.2.

Figure 6b shows the DSC and TGA thermograms of WX-671.2 after stirring in water.

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Figure 7 lists the crystal data and structure refinement for WX-671.2.

Figure 8 shows the bond lengths [$\text{\AA}$] and angles [$^\circ$] for WX-671.2.

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Figure 9 shows the torsion angles [$^\circ$] for WX-671.2.

Figure 10 shows the DSC and TGA thermograms of WX-671.1 (modification A).

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Figure 11 shows the DSC and TGA thermograms of a sample (mesophase B) of WX-671.1.

Figure 12 shows the DSC and TGA thermograms of
5 mesophase C of WX-671.1.

Figure 13 shows the X-ray diffractogram of modification A of WX-671.1.

10 **Figure 14** shows the X-ray diffractogram of mesophase B of WX-671.1.

Figure 15 shows the X-ray diffractogram of mesophase C of WX-671.1.
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Figure 16 shows the DSC and TGA thermograms of the amorphous starting substance of the free base WX-671.

Figure 17 shows the DSC and TGA thermograms of a sample
20 of the free base WX-671 crystallized from acetonitrile in the freezer.

Figure 18 shows a micrograph of the free base WX-671 after crystallization from acetonitrile in the freezer.
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Figure 19 shows the X-ray diffractogram of the free base WX-671 after crystallization from acetonitrile in the freezer.

30 **Figure 20** shows the sorption isotherm of water vapor on the free base WX-671 at 22°C.

Examples

Example 1

Preparation of various salts of N- α -(2,4,6-tri-isopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenyl-alanine 4-ethoxycarbonylpiperazide

The salts were prepared by dissolving 6.0 g of WX-671 in 50 ml of acetone. The acids used were added in a 25% excess without dilution and the mixture was stirred at room temperature for two hours.

Crystallization conditions

Table 1.1

Acid	Equivalent	Crystallization conditions	Drying
HCl	1.25 mol eq.	Clear solution	High vacuum
H ₂ SO ₄	1.25 mol eq.	Crystallized from acetone solution	High vacuum
MsOH	1.25 mol eq.	Crystallized from acetone solution	High vacuum
BsOH	1.25 mol eq.	Clear solution	High vacuum
Tartaric acid	1.25 mol eq.	Clear solution	High vacuum

MsOH: methanesulfonic acid; BsOH: benzenesulfonic acid

In a second step, the salts were suspended for 7 days in a suitable solvent, filtered and dried at room temperature.

Investigation of the crystallinity

Processes: X-ray diffractometry (XRD); microscopy

Table 1.2

Species	XRD	Microscopy
Free base	mainly crystalline	small particles
Besylate	mainly amorphous	agglomerates
Hydrochloride	completely amorphous	small particles
Mesylate	liquid crystalline	agglomerates
Hydrogensulfate	liquid crystalline	agglomerates
Tartrate	completely amorphous	glass

Investigation of the hygroscopicity

Process: storage for 1 week/85% relative humidity;
thermogravimetric analysis (TGA)

5 **Table 1.3**

Species	TGA
Free base	1.5% (semihydrate)
Besylate	10.1% (adsorptive)
Hydrochloride	7.5% (adsorptive)
10 Mesylate	7.8% (adsorptive)
Hydrogensulfate	0.8% (adsorptive)
Tartrate	10.5% (adsorptive)

Example 2

15 **Crystallization and single crystal X-ray structural
analysis of WX-671.2 (sulfate salt)**

The novel form of N- α -(2,4,6-triisopropylphenyl-
sulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxy-
20 carbonylpiperazide sulfate is obtained by suspending
the hydrogensulfate salt (WX-671.1) of the compound WX-
671 in water and by separating off the crystalline
compound formed.

25 Crystalline WX-671.2 was obtained in two different
ways:

a) About 50 mg of WX-671.1 were suspended in about
0.5 ml of water. The suspension was allowed to stand at
30 room temperature. After 6 days, the suspension was
filtered and the residue was dried in air at room
temperature.

b) About 0.2 g of WX-671.1 was suspended in about 2 ml
35 of water. The suspension was shaken at 25°C. After
3 days, the residue was filtered off and the crystals
of WX-671.2 were dried in air at room temperature.

The X-ray structure of the crystalline material (WX-671.2) prepared here was determined. Single crystals were also obtained here.

5 The crystal structure determination was carried out using a Bruker-Nonius diffractometer equipped with a Proteum CCD surface detector, an FR591 rotating anode with CuK α radiation, Montel mirror as a monochromator and a Kryoflex low temperature apparatus (T $\approx$ 90K). Full
10 sphere data determination omega and phi scans. Programs used: Proteum data collection V.1.37 (Bruker-Nonius 2002), data reduction Saint+ version 6.22 (Bruker-Nonius 2001) and absorption correction SADABS V.2.03 (2002). Crystal structure resolution was achieved by
15 means of direct methods as implemented in SHELXTL version 6.10 (Sheldrick, University of Göttingen) and visualized by means of XP program.

Missing atoms were located by means of differential
20 Fourier synthesis and added to the atom list. "Least squares refinement" on F² over all measured intensities were performed using the program SHELXTL version 6.10 (Sheldrick, University of Göttingen, 2000). All non-hydrogen atoms were "refined" with the inclusion of the
25 "anisotropic displacement parameters".

Table 2.1

Chirality Check*	Correct structure	Inverted structure
Flack parameter (standard deviation)	0.0298 (0.0282)	0.9694 (0.0307)
Twin Basf (standard deviation)	0.03 (3)	0.97 (3)
wR2 value (with Flack parameter)	0.2016	0.2219
Chirality	S (C9)	R (C9)

*

H. D. Flack, *Acta Cryst.*, **1983**, A39, 876-881

30 H. D. Flack, G. Bernardinelli, *Acta Cryst.*, **1999**, A55, 908-915

H. D. Flack, G. Bernardinelli, *J. Appl. Cryst.*, **2000**,
33, 1143-1148

The results of the X-ray structural analysis are shown
5 in Figures 1 to 3 and 7 to 9.

Example 3

X-ray diffractometry of WX-671.2 (sulfate salt)

10 X-ray diffractograms were obtained using a STOE STADI-P
Debye-Scherrer diffractometer, equipped with a
position-sensitive detector (PSD, 5°), a germanium
[1 1 1] primary monochromator and a CuK α 1.6 kW ceramic
X-ray tube (1.5406 Å). Program used: Stoe WinXpow,
15 version 2.03 (2003).

Table 3.1

X-ray diffractometry peak list for WX-671.2

WX 671.2	
Reflections 2 theta	Reflections 2 theta
3.7	26.8
4.2	27.2
7.1	27.5
7.4	27.8
10.3	28.1
10.8	29.1
11.1	29.7
11.6	30.2
12.1	30.6
12.6	31.1
13.3	31.4
13.8	31.9
14.2	32.1
14.8	33.2
15.1	33.8
15.7	34.2
16.4	36.3
16.8	37.3
17.3	
17.8	
18.0	
18.6	
19.2	
19.8	

20.1	
20.4	
20.8	
21.0	
21.2	
21.8	
22.2	
22.9	
23.4	
23.9	
24.6	
25.0	
25.5	
26.2	
26.3	
26.5	

Example 4

Recrystallizations of WX-671.1 (hydrogensulfate salt)

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WX-671.1 was dissolved in solvents (isopropanol, ethanol, methanol) of different polarity. The solutions were filtered, divided into four, and the solvents were removed at different rates.

10

WX-671.1 exists in its crystalline form (modification A) and two mesophases B and C. It decomposes from about 185°C (modification A and mesophase B) or 156°C (mesophase C). For the demonstration of the crystallinity of modification A, an X-ray structure determination is carried out. Modification A is the thermodynamically stable form at room temperature.

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Example 5

Differential scanning calorimetry (DSC) and thermogravimetry (TGA) of WX-671.1 (hydrogensulfate salt)

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In this example, thermograms were plotted by differential scanning calorimetry (DSC) and thermogravimetry (TGA). Figure 10 shows the DSC and TGA thermograms of the WX-671.1 (modification A) stirred in isopropanol at 25°C for one week. It decomposes from

25

about 180°C (exothermic peak in the DSC thermogram, mass loss in the TGA thermogram in the corresponding temperature range). The decomposition temperatures are strongly heating rate-dependent and were determined in
5 the DSC calorimeter at a heating rate of 2 Kmin⁻¹. The DSC table measurements shown were recorded at a heating rate of 2 Kmin⁻¹. The decomposition is correspondingly recorded at higher temperatures.

10 At 150°C, no significant mass loss is recorded. The weakly pronounced endothermic effect before the decomposition peak could be caused by partial melting or partial conversion.

15 Figure 11 shows the DSC thermogram of a sample of WX-671.1 (mesophase B) employed for this screening. Modification A and mesophase B are thermoanalytically identical. Mesophase A was obtained from isopropanol (room temperature/refrigerator) and ethanol (room
20 temperature).

Figure 12 shows the DSC and TGA thermograms of the active compound after a crystallization attempt from methanol at room temperature (mesophase C). It
25 decomposes from about 156°C (heating rate 2 Kmin⁻¹). Up to 175°C, a mass loss of 4.5% is recorded. In the DSC thermogram, the endothermic effect before the decomposition peak is missing. This form is present in a less ordered state than modification A and mesophase
30 B. It is formed after crystallization attempts from methanol (room temperature, refrigerator) and ethanol (refrigerator). Mass losses of between 4.5 and 4.8% by weight were recorded in the samples. The stoichiometric value for the mass loss of 2 molecules of water per
35 active compound molecule is 4.7%. This form, however, is not a hydrate.

Example 6

Thermomicroscopy of WX-671.1 (hydrogensulfate salt)

Thermomicroscopic recordings of a sample of WX-671.1
5 were made (not shown). Agglomerates were observed which
show no specific habit. The active compound decomposes
with bubble formation from about 197°C. Differences to
the decomposition temperature observed in the DSC
10 calorimeter come about due to the different heating
rates. WX-671.1 is obtained in nonspecific form from
solvents. The active compound shows double diffraction
in some cases, as is characteristic of crystalline and
mesomorphic substances.

15 **Example 7**

X-ray diffractometry of WX-671.1 (hydrogensulfate salt)

The X-ray diffractogram of modification A (Figure 13)
shows the pattern of numerous sharp peaks at higher 2-
20 theta angles characteristic of crystalline phases. For
the confirmation of the existence of a crystalline
phase, further investigations, e.g. an X-ray structural
analysis, are carried out.

25 In the X-ray diffractogram of mesophase B (Figure 14),
a sharp peak was observed at a 2-theta angle of about
5° and further reflections of low intensity between
about 8° and about 25°. The position and number of the
peaks is similar to those of modification A. The peak
30 present at low theta angle indicates the presence of a
remote arrangement of the molecules; the peaks of low
intensity verify the presence of a close arrangement.
It can be concluded from this that mesophase B is
present neither in crystalline nor in amorphous form,
35 but presumably as a mesophase.

The X-ray diffractogram of mesophase C (Figure 15)
likewise shows a pattern characteristic of mesomorphic
compounds: an intensive peak at low 2-theta angle. The

X-ray diffractogram verifies that mesophase C is not a crystalline phase and because of the remote arrangement present cannot be considered part of the amorphous phase. Comparison with the X-ray diffractogram of mesophase B shows that mesophase C presumably forms a phase having a low degree of organization. An indication of this is the strongly pronounced and more intensive reflections in mesophase B between the 2-theta angles of about 8° and about 25°.

10

By stirring in isopropanol at room temperature for one week, mesophase B changes into modification A. It does not change due to stirring in water:ethanol (1:1). It likewise does not alter due to mechanical stress (grinding in a mortar, compressing at 9 kbar).

15

Table 7.1

X-ray diffractometry peak list for crystalline modification of WX-671.1 (hydrogensulfate salt)

WX 671.1
Reflections
2 theta
4.3
8.6
10.1
10.3
10.6
11.0
11.2
11.3
11.6
12.0
12.3
13.8
14.8
15.9
17.5
17.7
19.3
19.7
20.2
21.0
21.4
21.7

21.9
22.6
23.3
24.0
25.8
26.9
27.4
28.0
29.7
30.8
31.3
32.2
33.3
35.5
37.1

Example 8

Recrystallizations of WX-671 (free base)

5 The active compound WX-671 is investigated for
polymorphism thermoanalytically (DSC, TGA), by X-ray
diffractometry and by crystallizations from organic
solvents. WX-671 crystallizes in one modification
(modification A). The active compound shows a very low
10 tendency for crystallization.

Modification A is the thermodynamically stable form at
room temperature. A final assessment of the
polymorphism and pseudopolymorphism of WX-671 is only
15 possible after carrying out a polymorphism study.

WX-671 was dissolved in solvents (tetrahydrofuran,
acetonitrile, methanol) of different polarity. The
solutions were filtered, divided into four and the
20 active compound was crystallized at different rates.
After drying at room temperature, the thermograms (DSC,
TGA) and the X-ray diffractograms were recorded.

The starting substance employed for the polymorphism
25 screening could be prepared in mainly crystalline form.
The crystallization was possible from acetonitrile in
the freezer at about -18°C.

Example 9

Differential scanning calorimetry (DSC) and thermogravimetry (TGA) of WX-671 (free base)

5 Figure 16 shows the DSC and TGA thermograms of the amorphous starting substance. Just like the DSC/TGA thermograms of the greatest part of the other samples investigated, it shows a thermal effect in the range between 45°C and 85°C. It was not possible to assign
10 this thermal effect in the course of the polymorphism screening. It could be a glass transition. Conclusions as to a crystal form having a melting point of about 190°C could follow from this. Depending on the heating rate, the substance decomposes from about 155°C. For
15 this reason, the melting point of the crystalline sample (GBA 190903-8c) cannot be determined. The thermograms of the amorphous and crystalline substance are therefore largely identical (Figures 16 and 17).

20 **Example 10**

Microscopy of WX-671 (free base)

WX-671 crystallizes from acetonitrile in the freezer in the form of prisms (Figure 18). The active compound
25 does not crystallize or only crystallizes to a very small amount from other solvents; it does not crystallize from the melt.

Example 11

30 **X-ray diffractometry of WX-671 (free base)**

Figure 19 shows the X-ray diffractogram of the active compound after crystallization from acetonitrile in the freezer (modification A).

Table 11.1

X-ray diffractometry peak list for WX-671 (free base)

The sample was crystallized from acetonitrile in the
5 freezer.

WX 671
Reflections 2 theta
3.2
5.5
6.4
8.5
9.7
10.2
10.7
11.2
11.5
11.7
12.1
13.4
13.8
14.1
14.6
14.8
15.5
16.5
18.1
19.1
19.7
20.5
20.7
21.3
22.4
22.7
23.6
26.5

Example 12

Moist sorption of WX-671 (free base)

10

Figure 20 shows the sorption isotherm of water vapor on
WX-671 at 22°C. The amorphous active compound takes up
water continuously from 0% r.h. to 95% r.h. On drying,
this water is given off again. At most points of the
15 isotherm, no equilibrium state was achieved within the

respective stopping time. The sample weight increased or fell further. There was no indication of hydrate formation in this experiment.

5 **Example 13**

Stability of WX-671 (free base)

By stirring in diisopropyl ether and ethanol/water (1:1) at room temperature for one week, the active
10 compound does not crystallize or crystallizes to a very small amount in modification A. It is not converted to any other polymorphic form. It is likewise not converted by mechanical stress (grinding in a mortar, compressing at 9 kbar); the crystallinity only
15 decreases further.

Claims

1. A crystalline modification of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide sulfate or hydrogensulfate.
2. The crystalline modification as claimed in claim 1, characterized in that it comprises N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide sulfate as a single crystal.
3. The crystalline modification of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide sulfate as claimed in one of claims 1 to 2, characterized in that it essentially contains the following 2-Theta-peaks according to an X-ray diffractometry analysis:

3.7°
10.3°
12.1°
13.8°
16.4°
19.2°
22.2°
4. The crystalline modification of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide sulfate as claimed in one of the preceding claims, characterized in that

it essentially has the peaks measured as in the X-ray diffractometry analysis according to Figure 5.

5. The crystalline modification as claimed in claim 1, characterized in that it comprises N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide hydrogensulfate as a single crystal.

6. The crystalline modification of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide hydrogensulfate as claimed in one of claims 1 and 5, characterized in that

it essentially contains the following 2-Theta-peaks according to an X-ray diffractometry analysis:

4.3°
10.1°
20.2°
21.4°
24.0°

7. The crystalline modification of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide hydrogensulfate as claimed in one of claims 1 and 5 to 6, characterized in that

it essentially has the peaks measured as in the X-ray diffractometry analysis according to Figure 13.

8. The crystalline modification of N- α -(2,4,6-tri-

isopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide hydrogensulfate as claimed in one of claims 1 and 5 to 7, characterized in that
it shows a decomposition from a temperature of 175°C-195°C (DSC, heating rate 2 Kmin⁻¹).

9. The crystalline modification as claimed in claims 1-4, characterized in that
it contains N-α-(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and sulfate anions in a molar ratio of 1.5-2.5:1.
10. The crystalline modification as claimed in claim 9, characterized in that
it contains N-α-(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and sulfate anions in a molar ratio of 2:1.
11. The crystalline modification as claimed in claims 1 and 5-8, characterized in that
it contains N-α-(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and hydrogensulfate anions in a molar ratio of 0.5 to 1.5:1.
12. The crystalline modification as claimed in claim 11, characterized in that
it contains N-α-(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and hydrogensulfate anions in a molar ratio of 1:1.

13. The crystalline modification as claimed in claims 1-4, characterised in that it comprises units of in each case approximately two molecules of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and a sulphate anion.
14. The crystalline modification as claimed in claim 13, characterized in that the two molecules of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide are present in one unit in various conformations.
15. The crystalline modification as claimed in one of the preceding claims, characterized in that it contains approximately three moles of water per mole of salt.
16. A process for the production of an essentially crystalline modification of N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide and/or salt thereof, comprising the steps:
- (a) preparation of the compound N- α -(2,4,6-triisopropylphenylsulfonyl)-3-hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide or of one of its salts,
 - (b) dissolution and/or suspension of the compound or of the salt itself from step (a) in a solvent suitable for the formation of the crystalline modification,
 - (c) separation of the crystalline modification.

17. The process as claimed in claim 16, comprising the additional step
(d) recrystallization of the crystalline modification from step (c) from a suitable solvent or a mixture of solvents.
18. The process as claimed in claim 16 or 17, in step (a) N- α -(2,4,6-triisopropylphenylsulfonyl)-3hydroxyamidino-(L)-phenylalanine 4-ethoxycarbonylpiperazide hydrogensulfate being prepared.
19. A medicament comprising as active compound a crystalline modification as claimed in one of claims 1 to 15, optionally together with a pharmaceutically tolerable carrier or/and excipient.
20. The medicament as claimed in claim 19, characterized in that
it is an orally, nasally, inhalatively, rectally or/and parenterally administrable agent.
21. The use of a crystalline modification as claimed in one of claims 1-15 for the production of a pharmaceutical composition for the control of diseases which are associated with a pathological overexpression of urokinase and/or the urokinase receptor.
22. The use as claimed in claim 21 for tumor treatment and/or prevention.
23. The use as claimed in claim 22 for the treatment or prevention of formation of metastases.
24. The use as claimed in claim 22 for the treatment of primary tumors.

25. The use as claimed in one of claims 21 to 24, an orally administrable composition being produced.
26. The use as claimed in claim 25, the composition being produced in the form of tablets, coated tablets, capsules, pellets, powder, suppositories, solutions, syrup, emulsions, liposomes or/and suspensions.