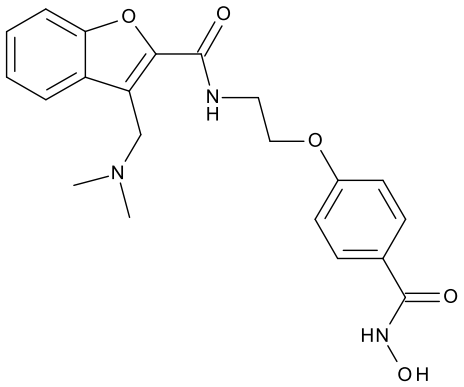
Novel abexinostat salt, associated crystalline form, preparation method thereof and the pharmaceutical compositions containing same

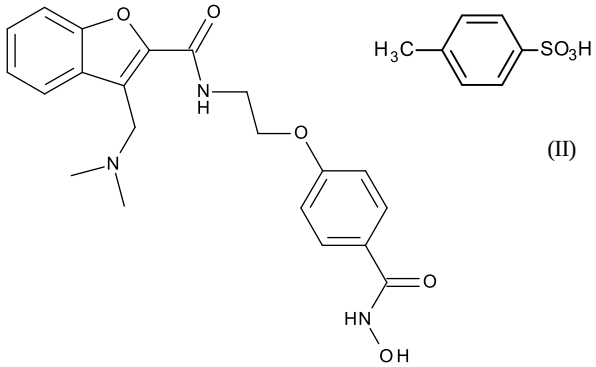
The present invention relates to *N*-hydroxy-4-{2-[3-(*N*,*N*-dimethylaminomethyl)-benzofuran-2-ylcarbonylamino]ethoxy}benzamide tosylate, or a solvate thereof.

Alternatively, the subject-matter of the invention relates to a tosylate salt of abexinostat of formula (I):



(I).

More particularly, the invention is directed to the salt of formula (II):



(II) (II)

The present invention relates also to the crystalline form I of *N*-hydroxy-4-{2-[3-(*N*,*N*-dimethylaminomethyl)benzofuran-2-ylcarbonylamino]ethoxy}benzamide tosylate, to the preparation method thereof and also to the pharmaceutical compositions containing same.

N-hydroxy-4-{2-[3-(*N*,*N*-dimethylaminomethyl)benzofuran-2-ylcarbonylamino]ethoxy}-benzamide, also known as abexinostat, is a histone deacetylase (HDAC) inhibitor described in patent application WO2004/092115. It allows inhibition of cell growth and induces

apoptosis in cultured tumour cells *in vitro*, and it inhibits tumour growth *in vivo* in xenograft models (Buggy *et al., Mol. Cancer Ther* **2006** 5(5) 1309). In view of its pharmacological profile, abexinostat is intended for use in the treatment of cancer.

From the industrial point of view it is imperative to be able to synthesize the compound with excellent purity, especially in a perfectly reproducible form, having valuable characteristics of dissolution, filtration, drying, ease of formulation and stability allowing its prolonged storage without particular requirements for temperature, light, humidity or oxygen levels.

Patent application WO2004/092115 describes two different routes for obtaining abexinostat. In both cases, 3-methyl-benzofuran-2-carboxylic acid is used as starting material, but functionalisation of this central nucleus by the dimethylaminomethyl group in the 3-position is carried out at different stages in the synthesis process, namely before or after coupling of the benzofuran-2-carboxylic acid derivative with methyl 4-(2-aminoethoxy)benzoate. Obtaining abexinostat hydrochloride is specifically described in the WO2004/092115 application. However, using this salt on an industrial scale is delicate because of its hygroscopic properties.

The present invention describes a process for obtaining abexinostat tosylate (abexinostat 4-methylbenzenesulfonate**)** in a well-defined, perfectly reproducible crystalline form, and presenting a very good stability compatible with the industrial constraints of preparation (especially drying) and storage of pharmaceutical compositions.

In a first aspect of the invention, there is provided *N*-hydroxy-4-{2-[3-(N,N-dimethylaminomethyl)benzofuran-2-ylcarbonylamino]-ethoxy}benzamide tosylate, or a solvate thereof.

In a second aspect of the invention, there is provided a crystalline form I of abexinostat tosylate, wherein it has an X-ray powder diffraction pattern presenting the following diffraction lines (Bragg's angle 2 theta, expressed in degrees ±0.2°): 6.50; 9.94; 11.35; 12.33; 14.08; 18.95; 21.08; 27.05.

In a third aspect of the invention, there is provided a pharmaceutical composition containing as active ingredient abexinostat tosylate according to the first aspect of the invention in association with one or more pharmaceutically acceptable excipients.

In a fourth aspect of the invention, there is provided a pharmaceutical composition containing as active ingredient abexinostat tosylate according to the second aspect of the invention in association with one or more pharmaceutically acceptable excipients.

In a fifth aspect of the invention, there is provided use of the pharmaceutical composition according to the third or fourth aspects of the invention, in the manufacture of a medicament for the treatment of cancer.

In a sixth aspect of the invention, there is provided a preparation method of the crystalline form I of abexinostat tosylate according to the second aspect of the invention, wherein the abexinostat is crystallised in the presence of *para*-toluenesulphonic acid in a polar medium.

The crystalline form I of abexinostat tosylate is characterised by an powder diffraction pattern X having the following diffraction lines (Bragg's angle 2 theta, expressed in degrees ±0.2°): 6.50; 9.94; 11.35; 12.33; 14.08; 18.95; 21.08; 27.05. Even more particularly, the crystalline form I of abexinostat tosylate is characterised by the following diffraction lines: 6.50; 9.94; 11.35; 12.33; 14.08; 18.95; 19.61; 19.96; 21.08; 22.82; 23.61; 27.05.

More specifically, crystalline form I of abexinostat tosylate is characterised by the powder diffraction pattern X hereinbelow, measured using a PANalytical X'Pert Pro MPD diffractometer with an X'Celerator detector and expressed in terms of line position (Bragg's angle 2 theta, expressed in degrees ±0.2°) and interplanar distance d (expressed in Å):

Line no.	Angle 2-theta (degrees)	Interplanar distance (Å)
1	6.50	13.581
2	9.94	8.894
3	11.35	7.789
4	12.33	7.173
5	14.08	6.285
6	18.95	4.683
7	19.61	4.526
8	19.96	4.449
9	21.08	4.215
10	22.82	3.897
11	23.61	3.768
12	27.05	3.296

Furthermore, the crystalline form I of abexinostat tosylate has been characterised by Raman spectroscopy. Significant peaks were observed at the following positions: 940 cm^{-1}, 1088 cm^{-1}, 1132 cm^{-1}, 1242 cm^{-1}, 1360 cm^{-1}, 1608 cm^{-1}.

Alternatively, the crystalline form I of abexinostat tosylate may be characterised by the powder diffraction pattern X which includes the 12 significant lines presented previously and also by a Raman spectrum having a significant peak at the position 1608 cm^{-1}.

Finally, the crystalline form I of abexinostat tosylate has also been characterised by solid-state NMR spectroscopy. Significant peaks were observed at 121.2 ppm, 122.1 ppm, 123.5 ppm, 126.0 ppm, 126.8 ppm, 128.2 ppm, 128.9 ppm, 143.4 ppm, 144.6 ppm, 153.8 ppm, 159 ppm, 161.2 ppm and 162.1 ppm.

More specifically, the ^{13}C CP/MAS (Cross Polarization Magic Angle Spinning) spectra have the following peaks (expressed in ppm ± 0.2 ppm):

Peak no.	Chemical shift (ppm)	Peak no.	Chemical shift (ppm)
1	162.1	10	126.0
2	161.2	11	123.5
3	159.0	12	122.1
4	153.8	13	121.3
5	144.6	14	65.9
6	143.4	15	50.6
7	128.9	16	46.9
8	128.2	17	45.0
9	126.8	18	21.9

The invention relates also to a preparation method of crystalline form I of abexinostat tosylate, characterised in that abexinostat is crystallised from a polar medium in the presence of *para*-toluenesulphonic acid. Preferably, the polar medium is composed of one or more solvents selected from water, alcohols, ketones and esters, it being understood that:

- "alcohols" means the C_1-C_6 alcohols such as methanol, ethanol, propanol, isopropanol, butanol, isobutanol, pentanol, 2-pentanol, 3-pentanol, isopentanol, hexanol,
- "ketones" means the C_3-C_6 ketones such as acetone, methyl ethyl ketone, 2-pentanone, 3-pentanone, 3-methyl-2-butanone, 2-hexanone, 3-hexanone, ethyl isopropyl ketone, methyl isopropyl ketone, 2,2-dimethyl-3-butanone,
- "esters" means the C_3-C_8 esters such as ethyl formate, isopropyl formate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate, *tert*-butyl acetate, pentyl acetate, isopentyl acetate, hexyl acetate.

Preferred alcohols are ethanol and isopropanol. Among the preferred solvents preference will also be given to acetone and methyl ethyl ketone among the ketones and to ethyl acetate among the esters.

Alternatively, the polar medium is a binary mixture, one constituent of which is water. Even more preferably, the polar medium is a binary mixture selected among: acetone/water, ethanol/water, isopropanol/water, and methyl ethyl ketone/water.

In the crystallisation process according to the invention, abexinostat (free base) obtained by any process may be used.

The invention relates also to another preparation method of the crystalline form I of abexinostat tosylate, in which process the crystallisation is seeded using a very small amount of the crystalline form I of abexinostat tosylate.

In this second crystallisation process according to the invention, abexinostat (free base) obtained by any process may also be used.

Obtaining the crystalline form I of abexinostat tosylate has the advantage of making it possible to prepare pharmaceutical formulations having a consistent and reproducible composition, presenting good characteristics of dissolution and stability, which is especially advantageous when the formulations are intended for oral administration. More specifically, use of the crystalline form I of abexinostat tosylate is especially valuable in an industrial context in view of its low hygroscopicity.

The crystalline form I of abexinostat tosylate is intended for the treatment of cancer, more particularly for the treatment of a carcinoma, a tumour, a neoplasm, a lymphoma, a melanoma, a glioma, a sarcoma, or a blastoma.

The invention relates also to the pharmaceutical compositions comprising, as active ingredient, a tosylate salt of abexinostat, even more particularly the crystalline form I of abexinostat tosylate, together with one or more appropriate, non-toxic, inert excipients. Among the pharmaceutical compositions according to the invention those that are suitable for oral, parenteral (intravenous or subcutaneous) or nasal administration, tablets or dragées, granules, sublingual tablets, capsules, lozenges, suppositories, creams, ointments, dermal gels, injectable preparations, drinkable suspensions and chewing gums can be more particularly mentioned.

Preference is given to pharmaceutical compositions administered orally.

The useful dosage varies according to the sex, age and weight of the patient, the administration route, the nature of the cancer and any associated treatments; and the useful dosage ranges from 20 mg to 480 mg of *N*-hydroxy-4-{2-[3-(*N*,*N*-dimethylaminomethyl)-benzofuran-2-ylcarbonylamino]ethoxy}benzamide per day expressed in terms of the free base.

The Examples below illustrate the invention but do not limit it in any way.

Example 1: Process for obtaining the crystalline form I of abexinostat tosylate

1.66 kg of abexinostat (free base) are placed in 9.48 kg of a mixture of isopropanol/water (50/50 weight/weight) at ambient temperature. The *para*-toluenesulphonic acid monohydrate (0.83 kg) is added in 2.36 kg of water at ambient temperature. The mixture is then heated at 75°C for 30 minutes before being cooled to 0°C. When crystallisation is complete, the suspension is filtered at 20°C. After drying, the crystalline form I of abexinostat tosylate is obtained with a yield of about 85 % and a purity greater than 99 %. The solid was characterised by the powder diffraction pattern X, Raman spectrum and NMR spectrum as set out in the following Examples 3-6.

Example 2: Process for obtaining the crystalline form I of abexinostat tosylate (seeding)

33.9 kg of abexinostat (free base) are placed in 170 kg of a mixture of isopropanol/water (45.6/54.4 weight/weight) at ambient temperature. A solution composed of *para*-toluenesulphonic acid monohydrate (17.06 kg) in water (24.1 kg) is added. The medium is then heated at 70-75°C, cooled, and seeded with 1.935 kg of crystalline form I of abexinostat tosylate. The suspension is then filtered at 20°C. After drying, the crystalline form I of abexinostat tosylate is obtained with a yield of about 86 % and a purity greater than 99 %. The solid was characterised by the powder diffraction pattern X, Raman spectrum, and NMR spectrum as set out in the following Examples 3-6.

Example 3: The crystalline form I of abexinostat tosylate (powder diffraction pattern X)

Recording of the data was carried out using a PANalytical X'Pert Pro MPD diffractometer with an X'Celerator detector under the following conditions:

- Voltage 45 kV, current 40 mA,
- Mounting: theta/theta,
- Anode: copper,
- K alpha-1 wavelength: 1.54060 Å,
- K alpha-2 wavelength: 1.54443 Å,
- K alpha-2/K alpha-1 ratio: 0.5,
- Measurement mode: continuous from 3° to 55° (Bragg's angle 2 theta) in increments of 0.017°,
- Measurement time per step: 35.53 s.

The powder diffraction pattern X of the form I of abexinostat tosylate obtained according to the process of Example 1 or 2 is expressed in terms of line position (Bragg's angle 2 theta, expressed in degrees ±0.2°), interplanar distance (expressed in Å) and relative intensity (expressed as a percentage relative to the most intense line). The significant lines have been collated in the following table:

Line no.	Angle 2-theta (degrees)	Interplanar distance (Å)	Relative intensity (%)
1	6.50	13.581	75.6
2	9.94	8.894	58.4
3	11.35	7.789	19.1
4	12.33	7.173	23.7
5	14.08	6.285	33.1
6	18.95	4.683	100
7	19.61	4.526	53.9
8	19.96	4.449	50.9
9	21.08	4.215	93.5
10	22.82	3.897	28.5
11	23.61	3.768	32.6
12	27.05	3.296	16.0

Example 4: Crystalline form I of abexinostat tosylate (crystal unit cell)

A saturated solution of abexinostat tosylate in 2,2,2-trifluoroethanol is prepared by stirring a suspension for 24 hours at ambient temperature, followed by filtration. 1 mL of the resulting solution is then poured into a 1.8-mL HPLC vial, to which 0.25 mL of water is added. The solution is maintained at ambient temperature for 75 minutes. After centrifuging and then drying, the solid is isolated for analysis. From among the crystals obtained a crystal of sufficient quality is taken for single-crystal X-ray diffraction analysis.

The crystalline structure of the above single crystal was determined using a Bruker Kappa CCD diffractometer equipped with an FR590 generator having a molybdenum anticathode ($\lambda MoK\alpha 1$ = 0.7093 Å) with an angular range from 2° to 27.5° in terms of θ. The following parameters were established:

- crystal unit cell: triclinic
- unit cell parameters: a = 10.467 Å, b = 14.631 Å, c = 20.159 Å, α = 73.971°, β = 79.040°, γ = 72.683°
- space group: P -1
- number of molecules in the unit cell: 4
- volume of the unit cell: $V_{unit\ cell}$ = 2813.0 $Å^3$
- density: d = 1.345 g/cm^3.

Example 5: Crystalline form I of abexinostat tosylate (Raman spectrum)

The form I of abexinostat tosylate was characterised by Raman spectroscopy. The spectra were recorded in diffuse reflectance mode (Raman Station 400, PerkinElmer) using a 785 nm laser. The signal was recorded by a CCD detector. The wavelength shift depends on the material and is characteristic of that material, which allows analysis of the chemical composition and of the molecular arrangement of the sample studied. The spectra were acquired with maximum power (100 % laser capacity), a spot size of 100 µm, twenty exposures of 2 seconds and a spectral resolution of 2 cm^{-1}. The spectral range explored ranges from 0 to 3278 cm^{-1}.

Significant peaks were observed at the following positions: 940 cm^{-1}, 1088 cm^{-1}, 1132 cm^{-1}, 1242 cm^{-1}, 1360 cm^{-1}, 1608 cm^{-1}.

Example 6: Crystalline form I of abexinostat tosylate (solid NMR spectrum)

The form I of abexinostat tosylate was also characterised by solid-state NMR spectroscopy. The ^{13}C NMR spectra were recorded at ambient temperature using a Bruker SB Avance spectrometer with a 4-mm CP/MAS SB VTN type probe under the following conditions:

- Frequency: 125.76 MHz,
- Spectral width: 40 kHz,
- Magic angle spinning rate of sample: 10 kHz,
- Pulse sequence: CP (Cross Polarization) with SPINAL64 decoupling (decoupling power: 80 kHz),
- Repetition delay: 10 s,
- Acquisition time: 35 ms,
- Contact time: 4 ms,
- Number of scans: 4096.

An apodisation function ("5 Hz line broadening") is applied to the collected signal before the Fourier transform. The spectra thereby obtained were referenced relative to a sample of adamantane (the highest-frequency peak of adamantane has a chemical shift of 38.48 ppm). The peaks observed have been collated in the following table (expressed in ppm ± 0.2 ppm):

Peak no.	Chemical shift (ppm)	Peak no.	Chemical shift (ppm)
1	162.1	10	126.0
2	161.2	11	123.5
3	159.0	12	122.1
4	153.8	13	121.3
5	144.6	14	65.9
6	143.4	15	50.6
7	128.9	16	46.9
8	128.2	17	45.0
9	126.8	18	21.9

Example 7: Pharmaceutical composition

Formula for the preparation of 1000 tablets each containing 100 mg of abexinostat (expressed in terms of the base equivalent):

Abexinostat tosylate ..... 143.4 g
Lactose monohydrate..... 213.1 g
Magnesium stearate ..... 2.5 g
Maize starch..... 75 g
Maltodextrin ..... 50 g
Anhydrous colloidal silica ..... 1 g
Sodium carboxymethylcellulose..... 15 g

Example 8: Hygroscopicity

Hygroscopicity of the form I of abexinostat tosylate was assessed using gravimetric water vapor adsorption (DVS – Dynamic Vapor Sorption). A sample of 5 to 10 mg of the drug substance, accurately weighed, was placed into a DVS sample pan working at 25°C under controlled humidity. The mass variation was recorded whilst drying under 0 per cent RH (relative humidity) and during two subsequent cycles of increasing and decreasing linear variations of relative humidity in the range 0-90 per cent RH at a rate of 10 per cent per hour. The relative humidity was maintained constant when it reached either 0 or 90 per cent RH

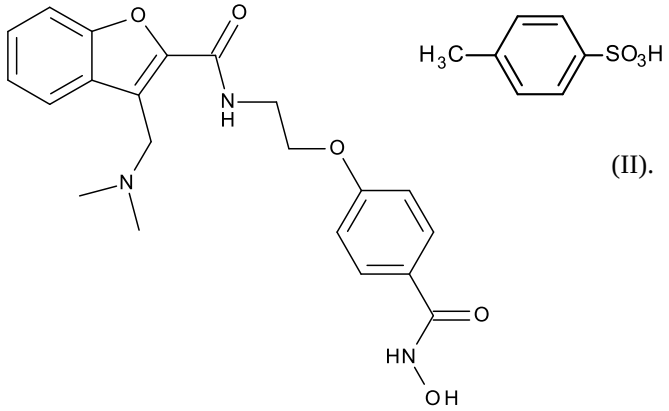
until the mass variation was less than 0.002 per cent per minute within a limit of time of 15 h.

An increase in weight lower than 0.5% was detected by DVS analysis when a sample was exposed to relative humidities included between 0% to 90% at 25°C.

CLAIMS

1. *N*-hydroxy-4-{2-[3-(*N*,*N*-dimethylaminomethyl)benzofuran-2-ylcarbonylamino]-ethoxy}benzamide tosylate, or a solvate thereof.

2. Salt according to claim 1 of formula (II):



(II).

3. A crystalline form I of abexinostat tosylate, wherein it has an X-ray powder diffraction pattern presenting the following diffraction lines (Bragg's angle 2 theta, expressed in degrees ±0.2°): 6.50; 9.94; 11.35; 12.33; 14.08; 18.95; 21.08; 27.05.

4. The crystalline form I of abexinostat tosylate according to claim 3, wherein it has an X-ray powder diffraction pattern having the following diffraction lines (Bragg's angle 2 theta, expressed in degrees ±0.2°): 6.50; 9.94; 11.35; 12.33; 14.08; 18.95; 19.61; 19.96; 21.08; 22.82; 23.61; 27.05.

5. The crystalline form I of abexinostat tosylate according to claim 3 or claim 4, wherein it has the following X-ray powder diffraction pattern, measured using a PANalytical X'Pert Pro MPD diffractometer with an X'Celerator detector and expressed in terms of line position (Bragg's angle 2 theta, expressed in degrees ±0.2°) and interplanar distance d (expressed in Å):

Line no.	Angle 2-theta (degrees)	Interplanar distance (Å)
1	6.50	13.581
2	9.94	8.894
3	11.35	7.789
4	12.33	7.173
5	14.08	6.285
6	18.95	4.683
7	19.61	4.526
8	19.96	4.449
9	21.08	4.215
10	22.82	3.897
11	23.61	3.768
12	27.05	3.296

6. The crystalline form I of abexinostat tosylate according to any one of claims 3 to 5, wherein it has a Raman spectrum having a significant peak at the position 1608 cm^{-1}.

7. The crystalline form I of abexinostat tosylate according to any one of claims 3 to 6, wherein it has a Raman spectrum having significant peaks at the positions 940 cm^{-1}, 1088 cm^{-1}, 1132 cm^{-1}, 1242 cm^{-1}, 1360 cm^{-1}, and 1608 cm^{-1}.

8. The crystalline form I of abexinostat tosylate according to claim 1 or claim 2, wherein it has a solid-state ^{13}C CP/MAS NMR spectrum having the following peaks (expressed in ppm ± 0.2 ppm): 121.3 ppm, 122.1 ppm, 123.5 ppm, 126.0 ppm, 126.8 ppm, 128.2 ppm, 128.9 ppm, 143.4 ppm, 144.6 ppm, 153.8 ppm, 159 ppm, 161.2 ppm, and 162.1 ppm.

9. The crystalline form I of abexinostat tosylate according to claim 8, wherein it has a solid-state ^{13}C CP/MAS NMR spectrum having the following peaks (expressed in ppm ± 0.2 ppm):

Peak no.	Chemical shift (ppm)	Peak no.	Chemical shift (ppm)
1	162.1	10	126.0
2	161.2	11	123.5
3	159.0	12	122.1
4	153.8	13	121.3
5	144.6	14	65.9
6	143.4	15	50.6
7	128.9	16	46.9
8	128.2	17	45.0
9	126.8	18	21.9

10. A pharmaceutical composition containing as active ingredient abexinostat tosylate according to claim 1 or claim 2 in association with one or more pharmaceutically acceptable excipients.

11. A pharmaceutical composition containing as active ingredient the crystalline form I of abexinostat tosylate according to any one of claims 3 to 9 in association with one or more pharmaceutically acceptable excipients.

12. Use of the pharmaceutical composition according to claim 10 or claim 11, in the manufacture of a medicament for the treatment of cancer.

13. The use of claim 12, wherein the cancer is a carcinoma, a tumour, a neoplasm, a lymphoma, a melanoma, a glioma, a sarcoma or a blastoma.

14. A preparation method of the crystalline form I of abexinostat tosylate according to any one of claims 3 to 9, wherein the abexinostat is crystallised in the presence of *para*-toluenesulphonic acid in a polar medium.

15. The preparation method of the crystalline form I of abexinostat tosylate according to claim 14, wherein the polar medium is composed of one or more solvents selected from water, alcohols, ketones, and esters.

16. The preparation method of the crystalline form I of abexinostat tosylate according to claim 15, wherein the polar medium is a binary mixture, one constituent of which is water.

17. The preparation method of the crystalline form I of abexinostat tosylate according to claim 16, wherein the polar medium is a binary mixture selected from: acetone/water, ethanol/water, isopropanol/water, and methyl ethyl ketone/water.

18. The preparation method of the crystalline form I of abexinostat tosylate according to any one of claims 14 to 17, wherein the crystallisation is seeded using a very small amount of the crystalline form I of abexinostat tosylate.

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