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CA 3106484 C 2024/06/25

(11)(21) 3 106 484

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2019/07/19
(87) Date publication PCT/PCT Publication Date: 2020/01/23
(45) Date de délivrance/Issue Date: 2024/06/25
(85) Entrée phase nationale/National Entry: 2021/01/14
(86) N° demande PCT/PCT Application No.: CN 2019/096842
(87) N° publication PCT/PCT Publication No.: 2020/015745
(30) Priorité/Priority: 2018/07/20 (CN201810804068.3)

(51) Cl.Int./Int.Cl. *C07D 271/06* (2006.01), *A61K 31/13* (2006.01), *A61P 35/00* (2006.01)

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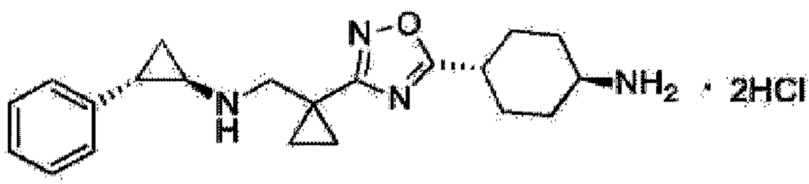
(54) Titre : SEL D'UN INHIBITEUR LSD1 ET FORME CRISTALLINE
(54) Title: A SALT OF AN LSD1 INHIBITOR AND ITS CRYSTAL FORM



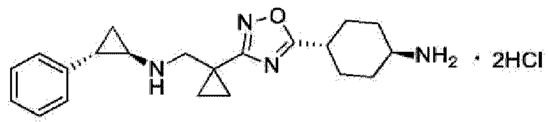
Compound III

(57) Abrégé/Abstract:
Provided are a compound III serving as an LSD1 inhibitor and a polymorph thereof, as well as an application of the compound and the polymorph thereof in the preparation of drugs for treating LSD1-associated diseases.

(see Compound III).

Abstract

Provided are a compound III serving as an LSD1 inhibitor and a crystal form thereof, as well as use of the compound and the crystal form thereof in preparation of a medicament for treating an LSD1 related disease.



Compound III

A SALT OF AN LSD1 INHIBITOR AND ITS CRYSTAL FORM

The present application claims the priority of CN201810804068.3 with an application date of July 20, 2018.

Technical field

The present disclosure relates to a compound III as an LSD1 inhibitor and its crystal form, and use of the compound and its crystal form in preparation of a medicament for treating an LSD1 related disease.

Background Art

Epigenetics regulates gene expression through different mechanisms, including covalent modifications to histones, such as methylation or demethylation; covalent modifications to DNA, such as methylation or hydroxymethylation; and reorganization of nuclear chromatin. Although these modifications do not change the basic sequence of DNA, such epigenetic change may persist throughout the cell life cycle or cell iteration process through cell division [Adrian Bird, Nature, 2007, 396-398]. Therefore, epigenetic dysfunction may cause and participate in pathological process of various diseases, such as various solid tumors, hematomas, viral infections, neurological abnormalities and other diseases. Therefore, epigenetics has now become a research hotspot in the field of drug development. Lysine-specific demethylase (LSD1, also called KDM1A) as the first demethylase discovered in 2004, belongs to the family of flavin adenine dinucleotide (FAD)-dependent amino oxidases. The structure of LSD1 consists of three major domains: an N-terminal SWIRM domain, a C-terminal amino oxidase domain (AOL) and a central protruding Tower domain. The C-terminal amino oxidase domain includes two active pockets, one is the site for binding to FDA, and the other is the site for recognizing and binding to a substrate. There is no clear conclusion about the function of the SWIRM domain. It does not directly participate in the binding of FAD or substrates, but mutation or removal of this region will reduce the activity of LSD1. Therefore, it is speculated that this region may affect active region by adjusting its conformation. The tower domain is the domain where LSD1 binds to other protein factors. LSD1 binds to different protein factors and acts on different substrates, thereby exerting different regulatory effects on histone and gene expression. For example, after combined with CoREST, LSD1 will preferentially act on histone H3K4, remove activation-related histone markers by demethylation, and thus inhibit gene transcription; and after combined with androgen receptor protein, the recombined LSD1 will preferentially act on H3K9, and activate androgen receptor-related gene transcription through demethylation. In addition, LSD1 has some non-histone receptors, such as p53, E2F1, DNMT1, MYPT1.

LSD1 is an FAD-dependent amino oxidase, in which proton transfer is

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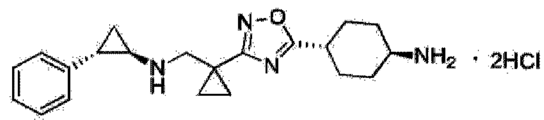
considered as the most likely oxidation mechanism. First, the N-CH_3 bond of the substrate is converted into an imine bond through proton transfer. This imine ion intermediate undergoes a hydrolysis reaction to generate a demethylated amine and a formaldehyde. During this catalytic cycle, FAD is reduced to $FADH_2$, which is then oxidized back to FAD by a molecule of oxygen, while generating a molecule of H_2O_2.

LSD1 is an indispensable regulator in epigenetics. It modifies histones through demethylation and is therefore called as the "eraser" enzyme in the organism. LSD1 can regulate gene expression, thereby regulating cell proliferation and differentiation.

Summary of the Invention

The present disclosure provides a compound III:



Compound III

The present disclosure also provides a crystal form A of the compound III, having an X-ray powder diffraction (XRPD) pattern with characteristic diffraction peaks at 2θ angles of: 4.72 ± 0.2°, 14.24 ± 0.2° and 21.78 ± 0.2°.

In some aspects of the present disclosure, the X -ray powder diffraction pattern of the above crystal form A has characteristic diffraction peaks at 2θ angles of: 4.72 ± 0.2°, 14.24 ± 0.2°, 16.28 ± 0.2°, 17.14 ± 0.2°, 20.72 ± 0.2°, 21.78 ± 0.2°, 23.98 ± 0.2° and 24.96 ± 0.2°.

In some aspects of the present disclosure, the X -ray powder diffraction pattern of the above crystal form A has characteristic diffraction peaks at 2θ angles of: 4.72 ± 0.2°, 14.24 ± 0.2°, 16.28 ± 0.2°, 17.14 ± 0.2°, 17.58 ± 0.2°, 18.70 ± 0.2°, 20.72 ± 0.2°, 21.78 ± 0.2°, 23.98 ± 0.2°, 24.96 ± 0.2° and 26.22 ± 0.2°.

In some aspects of the present disclosure, the X -ray powder diffraction pattern of the above crystal form A has characteristic diffraction peaks at 2θ angles of: 4.721°, 9.479°, 14.242°, 16.279°, 17.141°, 17.581°, 18.082°, 18.702°, 20.719°, 21.780°, 22.278°, 23.978°, 24.959°, 26.22°, 26.779°, 27.358°, 27.978°, 28.656°, 29.244°, 30.738°, 32.699°, 33.159°, 33.940°, 35.201° and 37.637°.

In some aspects of the present disclosure, the XRPD pattern of the above crystal form A is substantially as shown in FIG. 1.

In some aspects of the present disclosure, the XRPD pattern analysis data of the above crystal form A is shown in Table 1:

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Table 1: XRPD pattern analysis data of crystal form A

No.	2θ (°)	Interplanar spacing (Angstrom)	Background	Intensity	Relative Intensity (%)	area	area %	Half width
1	4.721	18.7027	77	776	82.6	11380	67.8	0.249
2	9.479	9.3229	62	46	4.9	418	2.5	0.155
3	14.242	6.2139	75	208	22.2	4345	25.9	0.355
4	16.279	5.4406	73	587	62.4	12072	72	0.35
5	17.141	5.1688	73	364	38.7	7120	42.4	0.333
6	17.581	5.0405	73	196	20.8	6533	38.9	0.567
7	18.082	4.9018	73	99	10.5	1030	6.1	0.177
8	18.702	4.7408	137	249	26.5	5177	30.9	0.353
9	20.719	4.2836	153	231	24.5	3782	22.5	0.279
10	21.78	4.0773	155	940	100	16777	100	0.304
11	22.278	3.9873	115	143	15.2	7131	42.5	0.846
12	23.978	3.7082	121	334	35.6	4100	24.4	0.209
13	24.959	3.5647	153	495	52.7	7533	44.9	0.259
14	26.22	3.3961	168	169	18	4286	25.5	0.432
15	26.779	3.3264	173	130	13.8	2327	13.9	0.305
16	27.358	3.2573	92	71	7.6	2553	15.2	0.61
17	27.978	3.1865	92	85	9.1	1421	8.5	0.284
18	28.656	3.1126	96	89	9.5	1567	9.3	0.299
19	29.244	3.0514	91	62	6.6	2223	13.3	0.613
20	30.738	2.9064	58	79	8.4	1769	10.5	0.381
21	32.699	2.7364	70	83	8.9	4417	26.3	0.902
22	33.159	2.6995	70	88	9.4	4082	24.3	0.786
23	33.94	2.6392	70	106	11.3	2576	15.4	0.412
24	35.201	2.5474	104	111	11.8	1810	10.8	0.278
25	37.637	2.388	68	35	3.7	1058	6.3	0.514

In some aspects of the present disclosure, the differential scanning calorimetry (DSC) curve of the above crystal form A has an onset of the exothermic peak at 194.66 ± 3°C.

In some aspects of the present disclosure, the DSC pattern of the above crystal form A is substantially as shown in FIG. 2.

In some aspects of the present disclosure, the thermogravimetric analysis (TGA) curve of the above crystal form A has a weight loss of 1.331% at 194.21 ± 3°C.

In some aspects of the present disclosure, the TGA pattern of the above crystal form A is substantially as shown in FIG. 3.

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The present disclosure also provides the use of the above compound III or the above crystal form A in preparation of a medicament for treating an LSD1 related disease.

The present disclosure also provides the use of the above compound III or the above crystal form A in preparation of a medicament for treating lung cancer, especially small cell lung cancer.

Technical effect

The compound III and its crystal form A of the present disclosure have good LSD1 inhibitory activity and superior in vivo effects; and as compared to the free base thereof and other salts, they have good stability and excellent solubility, and are less affected by light, heat and humidity, and thus have a promising prospect for becoming a medicine.

Definition and description

Unless otherwise stated, the following terms and phrases used herein are intended to have the following meanings. A specific phrase or term should not be considered uncertain or unclear without a special definition, but should be understood in its ordinary meaning. When a trade name appears herein, it is intended to refer to its corresponding commodity or its active ingredient.

The intermediates of the present disclosure can be prepared by a variety of synthetic methods well known to those skilled in the art, including the specific embodiments listed below, the embodiments formed by combining them with other chemical synthesis methods, and the equivalent alternative embodiments well-known by those skilled in the arts, preferred embodiments include but are not limited to the examples of the present disclosure.

The chemical reaction in the specific embodiment of the present disclosure is completed in a suitable solvent, and the solvent must be suitable for the chemical change of the present disclosure and the required reagents and materials. In order to obtain the compounds of the present disclosure, it is sometimes necessary for those skilled in the art to modify or select the synthesis steps or reaction schemes based on the existing embodiments.

The present disclosure will be specifically described below through examples, and these examples are not intended to limit the present disclosure in any way.

All solvents used in the present disclosure are commercially available and can be used without further purification.

The solvents used in the present disclosure are commercially available. The present disclosure employs the following abbreviations: DCM represents dichloromethane; DMF represents N,N-dimethylformamide; DMSO represents dimethyl sulfoxide; EtOH represents ethanol; MeOH represents methanol; TFA

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represents trifluoroacetic acid; TsOH represents p-toluenesulfonic acid; mp represents melting point; $EtSO_3H$ represents ethanesulfonic acid; $MeSO_3H$ represents methanesulfonic acid; ATP represents adenosine triphosphate; HEPES represents 4-hydroxyethylpiperazine ethanesulfonic acid; EGTA represents ethylene glycol bis(2-aminoethylether)tetraacetic acid; $MgCl_2$ represents magnesium dichloride; $MnCl_2$ represents manganese dichloride; DTT represents dithiothreitol; DCC represents dicyclohexylcarbodiimide; DMAP represents 4-dimethylaminopyridine.

X-ray powder diffraction (by X-ray powder diffractometer, XRPD) method of the present disclosure

Instrument model: DX-2700BH X -ray diffractometer
Test method: approximately 10 to 20 mg of sample is used for XRPD detection.
The detailed XRPD parameters are as follows:
Ray source: Cu, k-Alpha1 (λ=1.54184Å)
Light tube voltage: 40 kV, light tube current: 30 mA
Divergence Slit: 1 mm
The first Soller Slit: 28 mm, the second Soller Slit: 28 mm
Receiving Slit: 0.3 mm, anti-scatter slit: 1 mm
Measuring time: 0.5 s
Scanning angle range: 3-40 deg
Step width angle: 0.02 deg

The Differential Scanning Calorimeter (DSC) analytic method of the present disclosure

Instrument model: TA Q2000 Differential Scanning Calorimeter
Test method: a sample (about 1 mg) was taken and placed in a DSC aluminum pan for testing. The sample was heated from 30°C (room temperature) to 300°C (or 350°C) at a heating rate of 10°C/min under 50 mL/min of N_2.

The Thermal Gravimetric Analyzer (TGA) analytic method of the present disclosure

Instrument model: TA Q5000 thermogravimetric analyzer
Test method: a sample (2 to 5 mg) was taken and placed in a TGA platinum pan for testing. The sample was heated from room temperature to 300°C or to a weight loss of 20% at a heating rate of 10°C/min under 25 mL/min of N_2.

The dynamic vapor adsorption (DVS) analytic method of the present disclosure

Instrument model: SMS DVS Advantage dynamic vapor adsorption instrument
Test conditions: a sample (10 ~ 15 mg) was taken and placed in the DVS sample pan

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for testing.

The detailed DVS parameters are as follows:

Temperature: 25°C

Balance: dm/dt = 0.01%/min (shortest: 10 min, longest: 180 min)

drying: drying at 0% RH for 120 min

RH (%) test step: 10%

RH (%) test step range: 0%-90%-0%

The classification of hygroscopicity evaluation is as follows:

Classification of hygroscopicity	**ΔW%**
Deliquescence	Absorb enough water to form a liquid
Very hygroscopic	$\Delta W\% \geq 15\%$
Hygroscopic	$15\% > \Delta W\% \geq 2\%$
Slightly hygroscopic	$2\% > \Delta W\% \geq 0.2\%$
No/little hygroscopicity	$\Delta W\% < 0.2\%$

Note: ΔW% represents the moisture gain of the test product at 25 ± 1°C and 80 ± 2% RH.

Description of the drawings

Figure 1 is the XRPD pattern by Cu-Kα radiation of crystal form A of compound III;

Figure 2 is the DSC pattern of crystal form A of compound III;

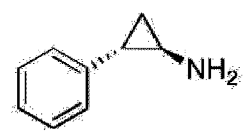
Figure 3 is the TGA pattern of crystal form A of compound III;

Figure 4 is the DVS isotherm of crystal form A of compound III.

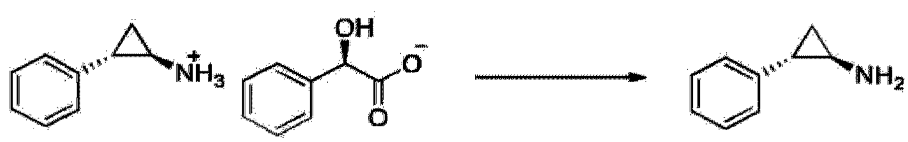
Detailed Embodiments

In order to better understand the content of the present disclosure, a further description is given below in combination with specific examples, but the specific embodiments are not intended to limit the content of the present disclosure.

Example 1 : Preparation of Compound I



Synthetic scheme:



A

I

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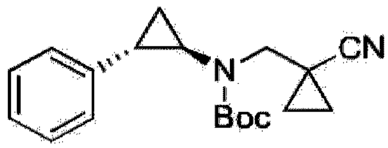
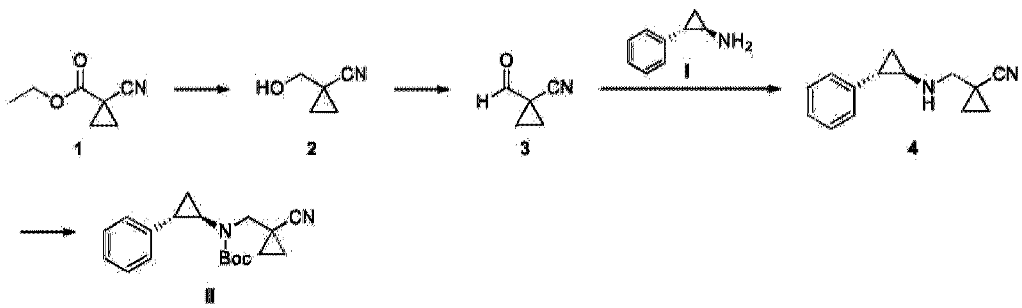
Sodium hydroxide (279 g, 6.99 mol) was dissolved in water (3.00 L), maintained at about 10°C, and added with the compound A (997 g, 3.49 mol) in batches. After the solid was completely dissolved, the mixture was extracted by ethyl acetate (2.00 L x 2). The combined organic phase was washed with water (1.50 L) and saturated brine (1.50 L) successively, dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated under reduced pressure to give the compound **I.** ^{1}H NMR (400MHz, $CDCl_3$) δ 7.18-7.14 (m, 2H), 7.07-7.05 (m, 1H), 6.94-6.92 (m, 2H), 2.47-2.44 (m, 1H), 1.78-1.76 (m, 1H), 0.97-0.94 (m, 1H), 0.92-0.89 (m, 1H).

Example 2 : Preparation of Compound II



Synthetic scheme:



Step I

The compound **1** (260 g, 1.87 mol) was dissolved in tetrahydrofuran (2.00 L) and methanol (200 mL), maintained at about 20°C, and added with sodium borohydride (70.8 g, 1.87 mol) in batches. The reaction mixture was stirred at 20°C for 18 hours, added dropwise with a saturated sodium bicarbonate solution (2.00 L) at 0°C to quench the reaction until no bubbles were generated. A small amount of solid was formed. The reaction mixture was filtered. The solid residue was washed with ethyl acetate (1.00 L x 2). The filtrate was added with solid sodium chloride to supersaturation and layered. The organic phase was washed with a saturated brine (1.00 L). The combined aqueous phase was extracted with a mixed solution of ethyl acetate and tetrahydrofuran (ethyl acetate : tetrahydrofuran = 10:1, 1.00 L x 3). The combined organic phase was washed with a saturated brine (1.00 L), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the compound **2**. ^{1}H NMR (400MHz, $CDCl_3$) δ 3.63 (s, 2H), 2.20 (s, 1H), 1.29 (dd, J_1=5.2 Hz, J_2=2.0 Hz, 2H), 0.99 (dd, J_1=5.2 Hz, J_2=2.0 Hz, 2H).

Step II

The compound **2** (101 g, 1.04 mol) was dissolved in anhydrous dichloromethane (1.50

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L), maintained at 5°C to 10°C, and added with Dess-martin periodinane (486 g, 1.14 mol) in batches. The reaction mixture was stirred at 25°C for 12 hours, and then controlled below 15°C, and slowly added with a saturated aqueous sodium bicarbonate (4.00 L), followed by slow addition of a saturated sodium thiosulfate solution (4.00 L). After stirred for 30 minutes, the reaction mixture was stood to be layered. The aqueous phase was extracted with dichloromethane (1.00 L x 3), and the combined organic phase was washed with water (1.00 L x 1) and saturated brine (1.00 L x 1) in sequence, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to obtain the compound **3**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.31 (s, 1H), 1.71-1.68 (m, 4H).

Step III

The compound **I** (97.5 g, 732 mmol) and the compound **3** (83.5 g, 878 mmol) were dissolved in dry dichloromethane (1.50 L) and added with acetic acid (4.40 g, 73.2 mmol). The reaction mixture was stirred at 26°C for 4 hours, added with sodium triacetoxyborohydride (232 g, 1.10 mol), and stirred at 25°C for 12 hours. After slowly addition of a saturated sodium bicarbonate solution (3.50 L) until no bubbles were generated, the reaction mixture was stood to be layered. The aqueous phase was extracted with dichloromethane (1.00 L x 1), and the combined organic phase was concentrated under reduced pressure to remove the organic solvent. The residue was added with water (800 mL), adjusted pH to 3 with an aqueous hydrochloric acid (1 M) and extracted with tert-butyl methyl ether (800 mL x 2). The aqueous phase was adjusted pH with saturated sodium bicarbonate to 8 and extracted with dichloromethane (1.00 L x 2). The combined organic phase was dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give the compound **4**.

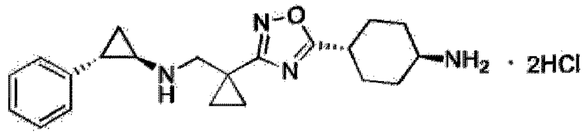
^{1}H NMR (400MHz, $CDCl_3$) δ 7.29-7.26 (m, 2H), 7.19-7.16 (m, 1H), 7.06-7.04 (m, 2H), 2.83 (s, 2H), 2.51-2.48 (m, 1H), 2.01-1.96 (m, 1H), 1.28-1.24 (m, 2H), 1.18-1.13 (m, 1H), 1.05-1.01 (m, 1H), 0.88-0.79 (m, 2H).

MS-ESI calculated: $[M+H]^+$ 213, found: 213.

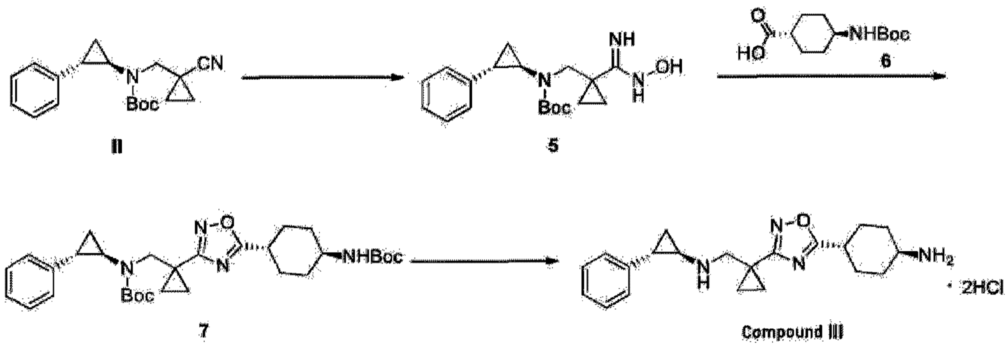
Step IV

The compound **4** (113 g, 534 mmol) was dissolved in tetrahydrofuran (1.20 L) and water (300 mL), added with di-tert-butyl dicarbonate (128 g, 588 mmol) and lithium hydroxide monohydrate (26.9 g, 641 mmol). The reaction mixture was stirred at 25°C for 12 hours, adjusted pH with aqueous hydrochloric acid (1 M) to 3, and extracted with ethyl acetate (800 mL x 2). The organic phase was washed with saturated brine (1.00 L x 1), dried with anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was added with n-heptane (1.00 L), stirred for 12 hours to generate a large amount of white solid, and filtered. The filter cake was dried under reduced pressure to obtain the compound **II**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.23-7.21 (m, 2H), 7.13-7.10 (m, 1H), 7.07-7.05 (m, 2H), 3.42-3.31 (m, 2H), 2.90-2.88 (m, 1H), 2.10-2.05 (m, 1H), 1.37 (s, 9H), 1.28-1.16 (m, 4H), 1.00-0.90 (m, 2H). MS-ESI calculated: $[M+H]^+$ 313, found: 313.

Example 3: Preparation of Compound Ⅲ and its crystal form A



Synthetic scheme:



Step I

The compound **II** (202 g, 647 mmol) was dissolved in absolute ethanol (500 mL) at room temperature, added with diisopropylethylamine (209 g, 1.62 mol) and hydroxylamine hydrochloride (90.0 g, 1.30 mol), heated to 80°C and stirred for 16 hours. The reaction solution was cooled to room temperature, and concentrated under reduced pressure to remove ethanol. The residue was dissolved in ethyl acetate (2.00 L). The organic phase was washed with water (500 mL x 3), dried over anhydrous sodium sulfate, and filtered. The filtrate was concentrated. The residue was dissolved in ethyl acetate (200 mL), added with n-heptane (2.00L) under stirring, and further stirred for 12 hours to precipitate a white solid. The resultant was filtered, and the filter cake was washed with n-heptane (200 mL), and dried at 45°C under vacuum for 12 hours to obtain the compound **5**. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.92 (s, 1H), 7.27-7.23 (m, 2H), 7.16-7.08 (m, 3H), 5.26 (s, 2H), 3.53-3.50 (m, 1H), 3.32-3.28 (m, 1H), 2.78-2.76 (m, 1H), 2.03-2.00 (m, 1H), 1.33 (s, 9H), 1.14-1.11 (m, 2H), 0.71-0.59 (m, 4H). MS-ESI calculated: $[M+H]^+$ 346, found: 346.

Step II

The compound **6** (126 g, 532 mmol) was dissolved in anhydrous N,N-dimethylformamide (1.40 L), added with carbonyldiimidazole (88.8 g, 557 mmol) at 30°C under nitrogen protection and stirred for 3 hours. The reaction mixture was added with the compound **5** (175 g, 506 mmol), heated to 110°C and stirred for 12 hours. The reaction solution was cooled to room temperature, slowly added to water (14 L) under stirring to precipitate a lot of white solid, and filtered. The filter cake was washed with water (3 L x 3) and dried at 30°C under vacuum to give the

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compound **7**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.23 (m, 2H), 7.17-7.14 (m, 1H), 7.06-7.05 (m, 2H), 4.43-4.41 (m, 1H), 3.88-3.70 (m, 2H), 3.48-3.47 (m, 1H), 2.76-2.73 (m, 2H), 2.14-2.07 (m, 5H), 1.64-1.61 (m, 2H), 1.46 (s, 9H), 1.41 (s, 9H), 1.24-1.00 (m, 7H). MS-ESI calculated: $[M+Na]^+$ 575, found: 575.

Step III

The compound **7** (240 g, 434 mmol) was dissolved in ethyl acetate (240 mL), added with a solution of hydrochloric acid in ethyl acetate (4M, 820 mL) under stirring at 0°C, stirred at 0°C to 25°C for 3 hours to precipitate a lot of white solid, and filtered. The filter cake was washed with ethyl acetate (500 mL x 5), and dried at 40°C under vacuum to give the compound **III**. The compound **III** was added to absolute ethanol (1.20 L), heated to reflux under stirring until all the solids were dissolved, and filtered hot to remove mechanical impurities. A small amount of solids were precipitated in the filtrate. The refluxing was continued so that all the solids were dissolved and the stirring was stopped. The filtrate was cooled at a rate of 10°C to 20°C per 1 to 2 hours. After cooled to 45°C, the filtrate was maintained for 12 hours to precipitate a lot of white solid. Then the filtrate was cooled at a rate of 10°C to 20°C per 1 to 2 hours again until the temperature was 25°C, stirred evenly and filtered. The filter cake was washed with isopropanol (260 mL x 3), and dried under vacuum at 45°C. After detected by XRPD, the **crystal form A** of the compound **III** was obtained. ^{1}H NMR (400MHz, CD_3OD) δ 7.32-7.29 (m, 2H), 7.25-7.21 (m, 1H), 7.17-7.14 (m, 2H), 3.70-3.62 (m, 2H), 3.21-3.14 (m, 1H), 3.09-3.05 (m, 1H), 3.01-2.95 (m, 1H), 2.57-2.52 (m, 1H), 2.26-2.22 (m, 2H), 2.18-2.15 (m, 2H), 1.75-1.64 (m, 2H), 1.61-1.54 (m, 3H), 1.44-1.41 (m, 2H), 1.39-1.36 (m, 1H), 1.34-1.32 (m, 2H). MS-ESI calculated: $[M+H]^+$ 353, found: 353.

Example 4: Test of chloride ion content in the crystal form A of the compound III

Experimental instrument: Ion chromatography ICS5000

Chromatographic parameters:

Guard column: Dionex™ IonPac™ AG11-HC，4 x 50 mm Guard column

Chromatographic column: Dionex™ IonPac™ AS11-HC，4 x 250 mm Guard column

Column temperature: 30°C

Detection mode: conductivity detection

Flow rate: 1.0 mL/min

ASRS-4mm suppressor 18 mA

Injection volume: 25 µL

Analysis time: 20 min

Mobile phase: 7 mM KOH

Sample preparation:

Three samples of 50 mg crystal form A of Compound III were weighed accurately and

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labeled as Sample 1, Sample 2 and Sample 3. They were dissolved with deionized water and brought to volume to prepare three solutions of 0.2 mg/mL.

Experimental results:

Table 2: Test result of chloride ion content in the crystal form A of the compound III

Content of Chloride ion (%)		
Calculated Value	Measured Value (averages value of 3 measurement)	error
16.7	17.0	0.3

Experimental conclusions:

The measured value of chloride ion content in the crystal form A of the compound III is consistent with the theoretical value thereof with an error of less than 0.3%, and this product is a dihydrochloride.

Example 5: Study of hygroscopicity of the crystal form A of the compound III

Experimental Materials: SMS DVS Advantage dynamic vapor adsorption instrument

Experimental Methods

10 to 15 mg of the crystal form A of the compound **III** was taken and placed in a DVS sample pan for testing.

Experimental results:

The DVS spectrum of the crystal form A of the compound **III** is shown in Figure 4 with $\Delta W = 1.14\%$.

Experimental conclusion:

The crystal form A of the compound **III** has a moisture weight gain of 1.14% at 25°C and 80% RH, exhibiting slightly hygroscopic.

Example 6: Test of solid stability of the crystal form A of the compound III

According to the "Guiding Principles for Testing of Stability of API and Preparations" (Chinese Pharmacopoeia 2015, Edition Four, General Principles 9001), the stability of the crystal form A of the compound **III** was investigated under conditions of high temperature (60°C, open), high humidity (room temperature/relative humidity 92.5%, open) and light (total illuminance = 1.2×10^6 Lux • hr/near-UV = 200W • hr/m^2, open).

10 mg of the crystal form A of the compound **III** was weighed, placed in the bottom of a glass vial, and spread into a thin layer. For the samples placed under the conditions of high temperature (60°C) and high humidity (relative humidity of 92.5%

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RH), the bottle opening was sealed with an aluminum foil, and some small holes were pierced in the aluminum foil to ensure that the sample can be sufficiently in contact with ambient air, and the samples were placed in the corresponding constant temperature and humidity ovens. The sample exposed to light (open, not covered with aluminum foil) and the control sample (the entire sample bottle was covered with aluminum foil) were placed in a light box. At each time point, 2 samples were weighed as formal test samples. Another 50 mg of the crystal form A of the compound **III** was weighed for XRPD testing. The sample bottles were wrapped with an aluminum foil, and the aluminum foil was pierced with small holes. They were also placed in the corresponding constant temperature and humidity ovens. The sample was taken and detected (by XRPD) at the 5th day and 10th day, and the detection results were compared with the initial detection result at 0 day. The test results are as shown in the following table 3:

Table 3: Test result of solid stability of the crystal form A of the compound III

Test conditions	**Time point**	**Crystal form**
-	0 day	Crystal Form A
High temperature (60°C, open)	5^{th} day	Crystal Form A
	10^{th} day	Crystal Form A
High humidity (relative humidity 92.5%, open)	5^{th} day	Crystal Form A
	10^{th} day	Crystal Form A
Light (total illuminance $=1.2\times10^6$ Lux•hr/ near ultraviolet =200 w•hr/m^2, open)	5^{th} day	Crystal Form A
	10^{th} day	Crystal Form A

Conclusion: The crystal form A of the compound **III** has good stability under the conditions of high temperature, high humidity and strong light.

Example 7: Test of solvent stability of the crystal form A of the compound III

Appropriate amounts of the compound III were weighed and added to different glass vials, and added respectively with appropriate amount of a solvent or a solvent mixture to prepare a suspension. After added with a magnet rotor and placed at room temperature, the above samples were placed on a constant temperature mixer (40°C) to be shaken for 2 days (avoid from light). In order to ensure that the sample was as suspended as possible, the amount of the compound and the solvent would be adjusted according to the test phenomenon and even the container used in the experiment may be changed during the experiment. (The dissolved sample was evaporated naturally

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to dryness). The test results were shown in the following Table 4:

Table 4: Test result of solvent stability of the crystal form A of the compound III

No.	Solvent	Weight (mg)	Solvent volume (μL)	Status	Crystal form
1	Acetonitrile	32	300+200	Suspending	Crystal form A
2	Tetrahydrofuran	31	200+200	Suspending	Crystal form A
3	Isopropyl acetate	30	200+400	Suspending	Crystal form A
4	Isopropanol	31	200+200	Suspending	Crystal form A
5	Methanol	20	100	Dissolved, and then Precipitated	Crystal form A

Conclusion: The crystal form A of the compound **III** has good stability in the solvent.

Experimental Example 1: Study of LSD1 Inhibition of the crystal form A of the compound III

1.1 Experimental purpose:

The experimental purpose was to evaluate IC_{50} of the crystal form A of the compound **III** at 10 concentrations against LSD1. The experiment was conducted in duplicate with an initial concentration of 10 μM diluted in a gradient of 3 times, and it was repeated twice at different dates.

1.2 Test conditions:

LSD1 buffer composition: 50 mM Tris-HCl, pH 7.5, 0.05% CHAPS, 1% DMSO.

Reaction time: reacted at room temperature for 1 hour

Reaction process:

1.2.1 Adding enzyme to a freshly prepared buffer

1.2.2 Adding a DMSO solution of the compound to the enzyme mixture using Acoustic Technology (Echo 550, LabCyte Inc. Sunnyvale, CA) at nL level, and incubating at room temperature for 30 minutes

1.2.3 Adding the substrate to a freshly prepared buffer

1.2.4 Incubating at room temperature for 1 hour

1.2.5 Preparing to test the mixture

1.2.6 Using Perkin Elmer Envision to read data

1.2.7 Using Excel and GraphPad Prism software to analyze data

1.3 Test results:

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Table 5. LSD1 Inhibition of the crystal form A of the compound III

Compound	IC_{50} (nM) Mean ± standard deviation
Crystal form A of Compound III	8.0±0.6

Conclusion: This experiment evaluated LSD1 inhibition of the crystal form A of the compound III with the enzyme fluorescent conjugated method. The results showed that the crystal form A of compound III has a significant inhibitory effect against LSD1 with IC_{50}=8 nM.

Experimental Example 2: In vivo efficacy study of the crystal form A of the compound III on human small cell lung cancer NCI-H1417 cell subcutaneous xenograft tumor in CB-17 SCID mouse model

2.1 Experimental purpose:

The experimental purpose is to evaluate the in vivo efficacy of the crystal form A of the compound III of the present disclosure on human small cell lung cancer NCI-H1417 cell subcutaneous xenograft in a CB-17 SCID mouse model.

2.2 Experimental animals:

Species: Mouse

Strain: CB-17 SCID mouse

Week age and weight: 6-8 weeks old, weight: 16-21 grams

Sex: female

Supplier: Shanghai Lingchang Biological Technology Co., Ltd.

2.3 Experimental method and procedures

2.3.1 Cell culture

Human small cell lung cancer NCI-H1417 cells were cultured in a monolayer in vitro with RPMI-1640 medium plus 10% fetal bovine serum, at 37°C and 5% CO_2 to culture and passage. When the cell saturation was 80%-90%, the cells were collected by trypsin-EDTA digestion, counted, adjusted to 10×10^7 cells/mL and resuspended in PBS.

2.3.2 Tumor cell inoculation

0.2 mL (10×10^6 cells) of NCI-H1417 cells (with Matrigel™, volume ratio 1:1) were subcutaneously inoculated on the right back of each mouse. When the average tumor volume reached about 100-150 mm^3, the mice were grouped randomly and started to be administered.

2.3.3 Preparation of test substance

The experimental vehicle was a 0.5% methyl cellulose solution. 5 g of methyl cellulose was weighed, dissolved in 800 mL of ultrapure water, stirred evenly and brought to the volume of 1000 mL with ultrapure water. The test substance was

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dissolved in the vehicle, prepared into a uniform solution of a certain concentration, and stored at 4°C.

2.3.4 Tumor measurement and experimental indicator

The experimental indicator was to investigate whether the tumor growth was inhibited, delayed or cured. The tumor diameter was measured with a vernier caliper twice a week. The tumor volume is calculated by the formula of: $V = 0.5a \times b^2$, where a and b represent the long diameter and short diameter of the tumor, respectively.

TGI (%) is used for the anti-tumor efficacy of the compound. TGI (%) reflects the tumor growth inhibition rate. TGI(%)=[(1-(Average tumor volume at the end of the administration of a certain treatment group - average tumor volume at the beginning of the administration of the treatment group))/(Average tumor volume at the end of treatment in the vehicle control group - Average tumor volume at the beginning of the treatment in the vehicle control group)]×100%. Vehicle control group: Vehicle (0.5% methylcellulose solution).

Table 6: Evaluation of anti-tumor efficacy of the crystal form A of the compound III on human small cell lung cancer NCI-H1417 xenograft tumor model (Calculated based on tumor volume on day 35 after administration)

Group	Tumor volume (mm^3) (Day 35)	TGI (%) (Day 35)
Vehicle	443±41	--
Crystal form A of Compound III (1 mg/kg, PO, QD)	178±17	81.9
Crystal form A of Compound III (1.5 mg/kg, PO, QD)	121±15	99.2
Crystal form A of Compound III (3 mg/kg, PO, QD)	68±13	115.6

Conclusion: The crystal form A of the compound III of the present disclosure has an excellent anti-tumor effect on the human small cell lung cancer NCI-H1417 xenograft tumor model.

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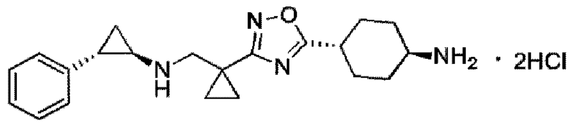
Claims

1. A crystal form A of compound III, which has an X-ray powder diffraction (XRPD) pattern with characteristic diffraction peaks at 2θ angles of: 4.72 ± 0.2°, 14.24 ± 0.2° and 21.78 ± 0.2°,



Compound III.

2. The crystal form A according to claim 1, wherein the X-ray powder diffraction pattern has characteristic diffraction peaks at 2θ angles of: 4.72 ± 0.2°, 14.24 ± 0.2°, 16.28 ± 0.2°, 17.14 ± 0.2°, 20.72 ± 0.2°, 21.78 ± 0.2°, 23.98 ± 0.2° and 24.96 ± 0.2°.

3. The crystal form A according to claim 2, wherein the X-ray powder diffraction pattern has characteristic diffraction peaks at 2θ angles of: 4.72 ± 0.2°, 14.24 ± 0.2°, 16.28 ± 0.2°, 17.14 ± 0.2°, 17.58 ± 0.2°, 18.70 ± 0.2°, 20.72 ± 0.2°, 21.78 ± 0.2°, 23.98 ± 0.2°, 24.96 ± 0.2° and 26.22 ± 0.2°.

4. The crystal form A according to claim 3, wherein the X-ray powder diffraction pattern has characteristic diffraction peaks at 2θ angles of: 4.721°, 9.479°, 14.242°, 16.279°, 17.141°, 17.581°, 18.082°, 18.702°, 20.719°, 21.780°, 22.278°, 23.978°, 24.959°, 26.22°, 26.779°, 27.358°, 27.978°, 28.656°, 29.244°, 30.738°, 32.699°, 33.159°, 33.940°, 35.201° and 37.637°.

5. The crystal form A according to claim 4, wherein the XRPD pattern is substantially as shown in FIG. 1.

6. The crystal form A according to any one of claims 1 to 5, which has a differential scanning calorimetry (DSC) curve with an onset of the exothermic peak at 194.66 ± 3°C.

7. The crystal form A according to claim 6, wherein the DSC pattern is substantially as shown in FIG. 2.

8. The crystal form A according to any one of claims 1 to 5, which has a thermogravimetric analysis (TGA) curve with a weight loss of 1.331% at 194.21 ± 3°C.

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9. The crystal form A according to claim 8, wherein the TGA pattern is substantially as shown in FIG. 3.

10. Use of the crystal form A as defined in any one of claims 1 to 9 in preparation of a medicament for treating a lysine-specific demethylase 1 related disease.

11. Use of the crystal form A as defined in any one of claims 1 to 9 in preparation of a medicament for treating a lung cancer.

12. Use according to claim 11, wherein the lung cancer is small cell lung cancer.

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Fig. 1

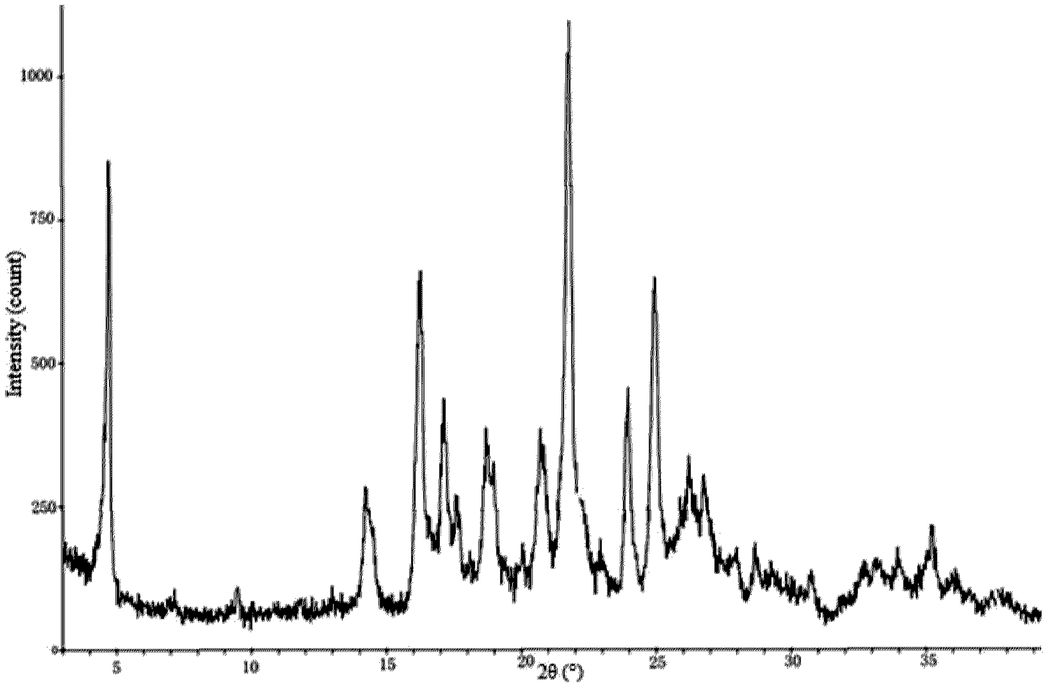

Intensity (count)
1000
750
500
250
0
5
10
15
20
25
30
35
2θ (°)

Fig. 2

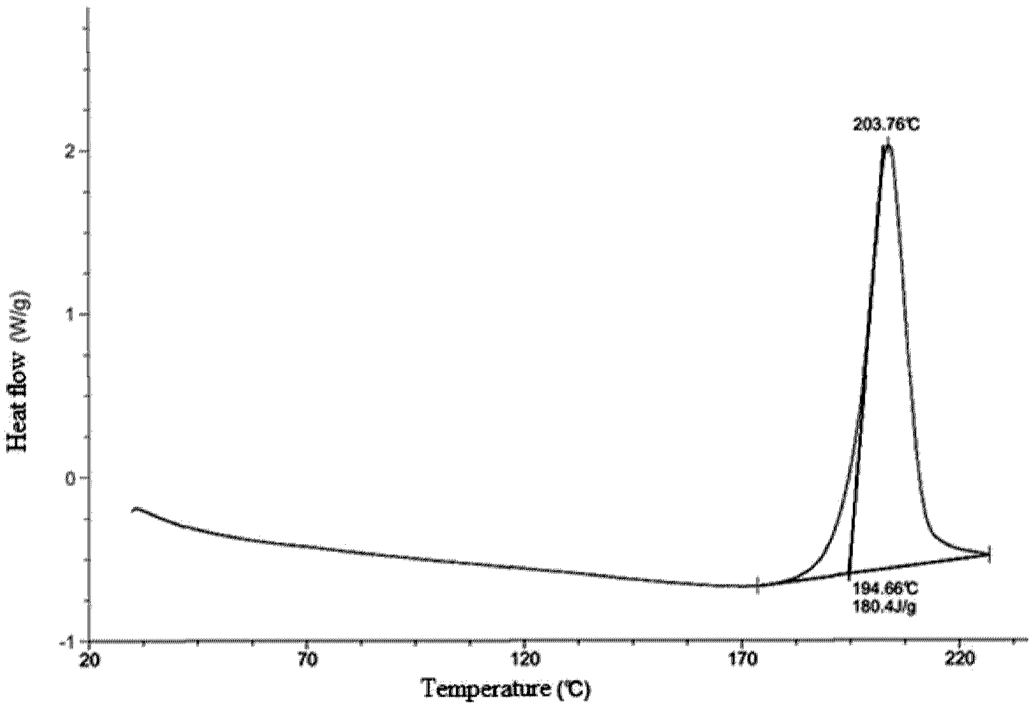

Heat flow (W/g)
2
1
0
-1
203.76℃
194.66℃
180.4J/g
20
70
120
170
220
Temperature (℃)

Fig. 3

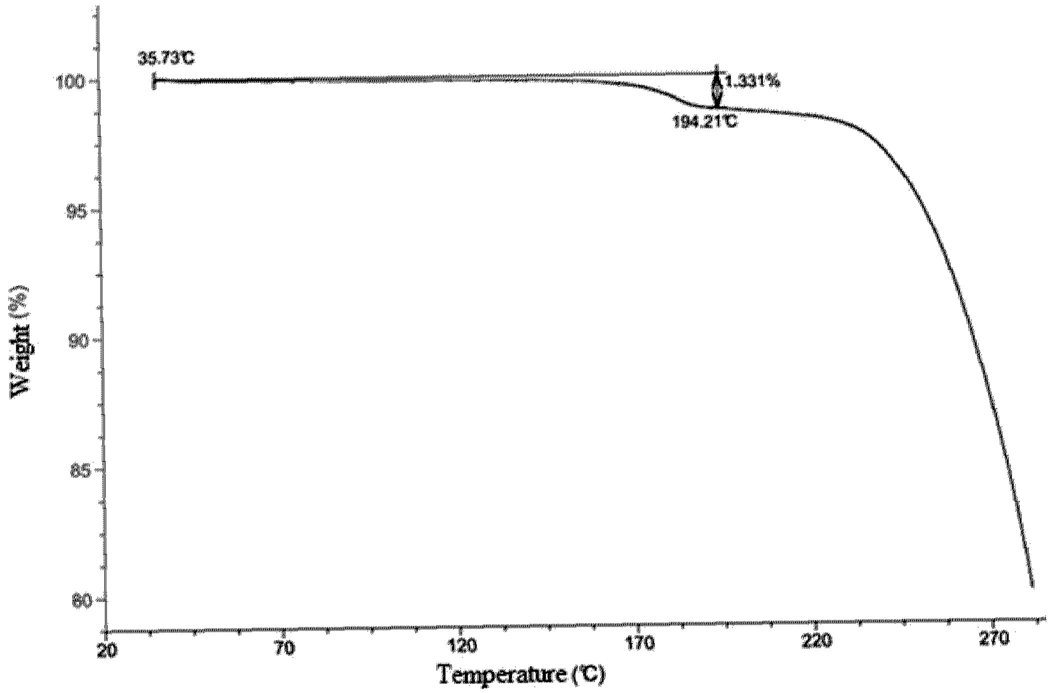
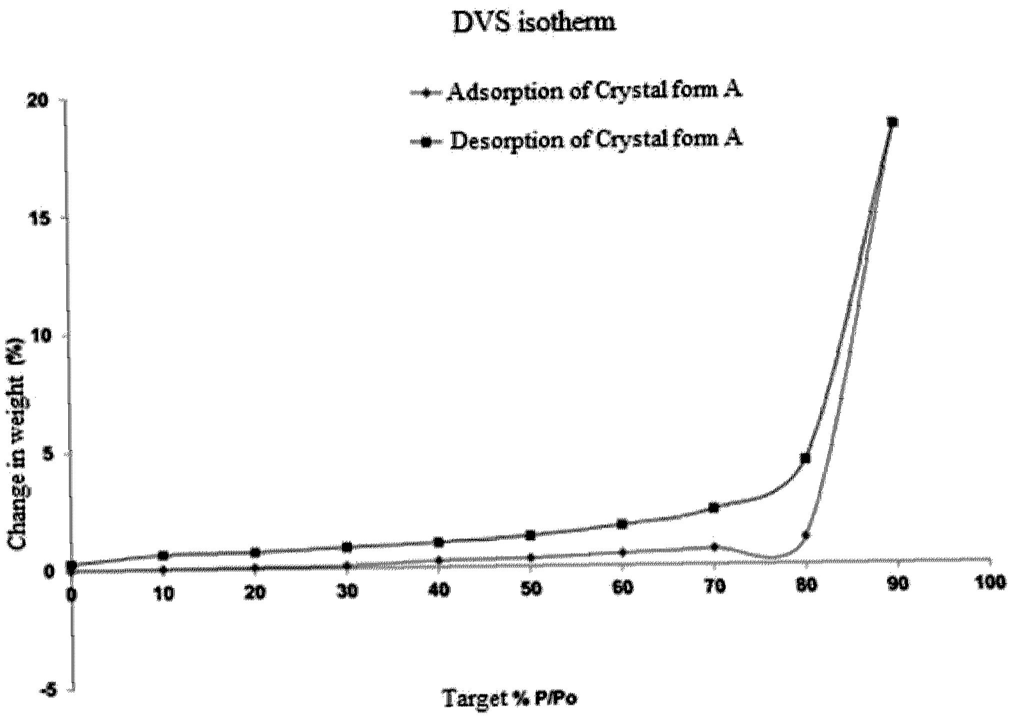
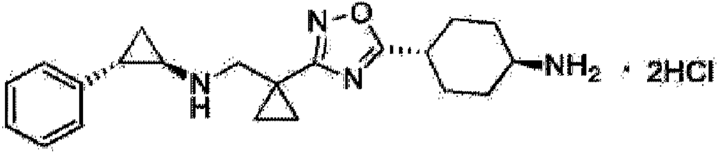

35.73℃
1.331%
194.21℃
Weight (%)
100
95
90
85
80
20
70
120
170
220
270
Temperature (℃)

Fig. 4


DVS isotherm
Adsorption of Crystal form A
Desorption of Crystal form A
Change in weight (%)
20
15
10
5
0
-5
0
10
20
30
40
50
60
70
80
90
100
Target % P/Po



Compound III