

DESCRIPTION

Title of the Invention: CRYSTALS (2) OF PYRAZINO[2,1-
c][1,2,4]TRIAZINE COMPOUND

Technical Field

5 [0001]

The present invention relates to a crystal of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide (hereinafter to be referred to as compound 1).

10 Background Art

[0002]

Compound 1 described in patent document 1 is a compound that inhibits the TCP4/ β -Catenin transcription pathway by inhibiting CREB binding protein (CBP) in the Wnt signal
15 transduction pathway, and is expected to treat various carcinomass (e.g., lung cancer, breast cancer, stomach cancer, pancreatic cancer, liver cancer, uterine cancer, ovarian cancer, glioma, melanoma, rectal colon cancer, lymphoma, leukemia), restenosis relating to angioplasty, angiogenesis abnormality,
20 polycystic kidney, tuberous sclerosis, Alzheimer's disease, neurodegenerative diseases (e.g., glaucoma, macular degeneration, Parkinson's disease, Alzheimer's disease) and fibrosis (e.g., idiopathic pulmonary fibrosis).

[Document List]

25 Patent Documents

[0003]

patent document 1: WO 2009/148192

SUMMARY OF THE INVENTION

Problems to be Solved by the Invention

30 [0004]

In general, the property of a crystal of a compound utilizable as a pharmaceutical product exerts a marked influence on the bioavailability of a drug, purity of a drug substance, formulation of a preparation and the like.

35 [0005]

Therefore, the problem of the present invention is to provide a crystal of compound 1 expected to be utilized as a drug substance of a pharmaceutical product.

Means of Solving the Problems

5 [0006]

The present inventors have conducted intensive studies in an attempt to solve the aforementioned problem and found a crystal of compound 1, and completed the present invention.

[0007]

10 The present invention provides the following [1] - [16].

[1] A crystal of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide or hydrate thereof.

[2] A crystal of a hydrate of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide.

[3] A crystal of an anhydride of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide.

[4] The crystal of [2], showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 10.7° , 10.9° , 14.4° , 19.1° and 22.8° , in powder X-ray diffraction.

25 [4A] The crystal of [2], showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 10.7° , 10.9° , 14.4° , 16.8° , 19.1° , 20.3° , 22.2° , 22.8° , 23.8° and 27.4° , in powder X-ray diffraction.

[5] The crystal of [1], showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.2° , 8.9° , 13.2° , 16.4° and 22.4° , in powder X-ray diffraction.

[5A] The crystal of [1], showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.2° , 8.9° , 10.9° , 12.9° , 13.2° , 16.4° , 17.1° , 18.4° , 22.4° and 24.8° , in powder X-ray diffraction.

[6] The crystal of [3], showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 10.3° , 14.2° , 14.6° , 16.1° and 22.1° , in powder X-ray diffraction.

[6A] The crystal of [3], showing diffraction peaks at
5 diffraction angles ($2\theta \pm 0.2^\circ$) of 10.3° , 12.8° , 14.2° , 14.6° , 16.1° , 18.9° , 20.4° , 21.6° , 22.1° and 24.1° , in powder X-ray diffraction.

[7] The crystal of [1], showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.3° , 10.7° , 16.3° , 19.8° and
10 23.8° , in powder X-ray diffraction.

[7A] The crystal of [1], showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.3° , 9.9° , 10.7° , 12.2° , 15.4° , 16.3° , 17.7° , 19.8° , 23.1° , 23.8° and 25.0° , in powder X-ray diffraction.

15 [8] A pharmaceutical composition comprising the crystal of any of [1] - [7A] as an active ingredient.

[9] An antitumor agent comprising the crystal of any of [1] - [7A] as an active ingredient.

[10] A method of preventing or treating tumor, comprising
20 administering a pharmacologically effective amount of any of the crystals of [1] - [7A] to a patient.

[11] The crystal of any of [1] - [7A], which is used for the prophylaxis or treatment of tumor.

[12] Use of the crystal of any of [1] - [7A] in the production
25 of an antitumor agent.

Effect of the Invention

[0008]

The crystal of compound 1 provided by the present invention has good property as a drug substance of a
30 pharmaceutical product.

Brief Description of the Drawings

[0009]

Fig. 1 shows a powder X-ray diffraction pattern of crystal A of compound 1 obtained in Example 1, wherein the
35 horizontal axis shows diffraction angle (2θ), and the vertical

axis shows peak intensity.

Fig. 2 shows a powder X-ray diffraction pattern of crystal B of compound 1 obtained in Example 2, wherein the horizontal axis shows diffraction angle (2θ), and the vertical axis shows peak intensity.

Fig. 3 shows a powder X-ray diffraction pattern of crystal C of compound 1 obtained in Example 3, wherein the horizontal axis shows diffraction angle (2θ), and the vertical axis shows peak intensity.

Fig. 4 shows a powder X-ray diffraction pattern of crystal D of compound 1 obtained in Example 4, wherein the horizontal axis shows diffraction angle (2θ), and the vertical axis shows peak intensity.

Fig. 5 shows a powder X-ray diffraction pattern of an amorphous form of compound 1 obtained in Comparative Example 1, wherein the horizontal axis shows diffraction angle (2θ), and the vertical axis shows peak intensity.

Fig. 6 is a graph showing the hygroscopicity of crystal A of compound 1 obtained in Example 1, wherein the horizontal axis shows relative humidity (RH), and the vertical axis shows variation (%) in the mass.

Fig. 7 is a graph showing the hygroscopicity of crystal C of compound 1 obtained in Example 3, wherein the horizontal axis shows relative humidity (RH), and the vertical axis shows variation (%) in the mass.

Fig. 8 is a graph showing the hygroscopicity of compound 1 obtained in Comparative Example 1, wherein the horizontal axis shows relative humidity (RH), and the vertical axis shows variation (%) in the mass.

Description of Embodiments

[0010]

Preferable crystals in the present specification include a crystal of a hydrate of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-

carboxamide, showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 10.7° , 10.9° , 14.4° , 19.1° and 22.8° , in powder X-ray diffraction;

a crystal of a hydrate of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 10.7° , 10.9° , 14.4° , 16.8° , 19.1° , 20.3° , 22.2° , 22.8° , 23.8° and 27.4° , in powder X-ray diffraction;

10 a crystal of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.2° , 8.9° , 13.2° , 16.4° and 22.4° , in powder X-ray diffraction;

15 a crystal of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.2° , 8.9° , 10.9° , 12.9° , 13.2° , 16.4° , 17.1° , 18.4° , 22.4° and 24.8° , in
20 powder X-ray diffraction;

a crystal of an anhydride of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, showing diffraction peaks at diffraction angles
25 ($2\theta \pm 0.2^\circ$) of 10.3° , 14.2° , 14.6° , 16.1° and 22.1° , in powder X-ray diffraction;

a crystal of an anhydride of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, showing diffraction peaks at diffraction angles
30 ($2\theta \pm 0.2^\circ$) of 10.3° , 12.8° , 14.2° , 14.6° , 16.1° , 18.9° , 20.4° , 21.6° , 22.1° and 24.1° , in powder X-ray diffraction;

a crystal of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, showing
35

diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.3° , 10.7° , 16.3° , 19.8° and 23.8° , in powder X-ray diffraction;

a crystal of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide, showing
5 diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.3° , 9.9° , 10.7° , 12.2° , 15.4° , 16.3° , 17.7° , 19.8° , 23.1° , 23.8° and 25.0° , in powder X-ray diffraction, and the like.
[0011]

10 In general, the diffraction angle (2θ) in powder X-ray diffraction produces an error within the range of $\pm 0.2^\circ$. Therefore, the value of the above-mentioned diffraction angle needs to be understood to include numerical values within the range of about $\pm 0.2^\circ$. Accordingly, not only the crystals
15 showing peaks at completely identical diffraction angles in powder X-ray diffraction, but also crystals showing peaks at diffraction angles different only by about $\pm 0.2^\circ$ are also encompassed in the present invention.

[0012]

20 Therefore, in the present specification, for example, "showing a diffraction peak at a diffraction angle ($2\theta \pm 0.2^\circ$) of 10.5° " means that it "shows a diffraction peak at a diffraction angle (2θ) of $10.3^\circ - 10.7^\circ$ ", and the same applies to other diffraction angles.

25 [0013]

The production method of the crystal of compound 1 is explained below.

[0014]

Unless otherwise specified, the water content in the
30 present specification is a numerical value measured based on the Karl Fischer method.

[0015]

production method of a crystal of compound 1 or hydrate thereof

A crystal of compound 1 can be obtained by a general
35 crystallization method and using compound 1 or a salt thereof.

Examples of the general crystallization method include a method of crystallizing a slurry of compound 1 added with a solvent, a method including dissolving compound 1 in a solvent and adding a poor solvent (solvent showing low solubility of compound 1) to performed crystallization, a method including dissolving compound 1 in a solvent with heating, and gradually cooling the mixture with stirring to perform crystallization, and a method including dissolving compound 1 in a solvent, concentrating and standing the mixture, or gradually concentrating with stirring to perform crystallization.

[0016]

Compound 1 or a salt thereof to be used for crystallization may have any form, may be a solvate or an anhydride, may be amorphous or crystalline (including one composed of plural polycrystallines), and may be a mixture of these. The solvate includes hydrate, methanolate and the like.

[0017]

The solvent used for crystallization includes, for example, alcohol solvents such as methanol, ethanol, isopropanol, 1-propanol and the like; acetonitrile; dimethyl sulfoxide (DMSO); amide solvents such as N,N-dimethylformamide (DMF) and the like; ester solvents such as methyl acetate, ethyl acetate, isopropyl acetate and the like; saturated hydrocarbon solvents such as hexane, heptane and the like; ketone solvents such as acetone, methylethyl ketone and the like; ether solvents such as t-butyl methyl ether (hereinafter to be referred to as MTBE), tetrahydrofuran (THF) and the like; dichloromethane and water. These solvents may be used alone or a mixture of two or more kinds thereof may be used. Preferred is a mixed solvent of alcohol solvent and water.

[0018]

The amount of the solvent to be used can be appropriately selected wherein an amount capable of dissolving compound 1 or a salt thereof by heating or an amount that enables stirring of a suspension obtained by adding a solvent to compound 1 or a

salt thereof is the lower limit, and an amount free of a marked decrease in the yield of crystal is the upper limit.

[0019]

In crystallization, a seed crystal (crystal of desired
5 compound 1 and the like) may or may not be added. While the temperature at which a seed crystal is added is not particularly limited, it is preferably 0°C - 60°C.

[0020]

As the temperature at which compound 1 or a salt thereof
10 is dissolved by heating, a temperature at which compound 1 is dissolved can be appropriately selected according to the solvent. Preferred is a temperature range from room temperature to a temperature at which a solvent used for crystallization starts refluxing, and more preferred is room
15 temperature - 100°C.

[0021]

Since cooling during crystallization may afford a crystal with different form (polymorphism) when the cooling is rapid. Therefore, cooling is desirably performed after appropriately
20 adjusting the cooling rate in consideration of the quality of crystal and influence on the particle size and the like. Cooling at 5°C - 40°C/h is preferable, and cooling at a rate of 5°C - 25°C/h is more preferable.

[0022]

25 While the final crystallization temperature can be appropriately selected according to the desired object such as yield, quality and the like of the crystal, it is preferably - 25°C - 30°C.

[0023]

30 The object crystal can be obtained by separating the crystal obtained by crystallization by a general filtration operation, washing the filtered crystal with a solvent as necessary, and drying same. As the solvent to be used for washing the crystal, those similar to the solvents used for
35 crystallization can be used. Preferred are, for example,

ethanol, acetone, ethyl acetate, isopropyl acetate, and MTBE.

[0024]

The crystal separated by the filtration operation can be dried by leaving in the air or a nitrogen stream as appropriate,
5 or by heating.

[0025]

The drying time can be selected as appropriate as the time necessary for the residual solvent to become less than a given amount, and according to the production amount, dryer,
10 drying temperature and the like. Drying can be performed under ventilation or under reduced pressure. The depressurization level can be appropriately selected according to the production amount, dryer, drying temperature and the like. The obtained crystal can also be left in the air as necessary after drying.

15 [0026]

The above-mentioned crystal can also be produced by, in the above-mentioned production method of compound 1, performing the production method of the crystal of compound 1 sequentially after the synthesis of compound 1.

20 [0027]

The crystal of compound 1 can be formulated according to a conventional method. Examples of the dosage form of formulation include oral preparation (tablet, granule, powder, capsule, syrup etc.), injection (for intravenous administration,
25 intramuscular administration, subcutaneous administration, intraperitoneal administration etc.), and external preparation (transdermal absorption preparation (ointment, adhesive preparation etc.), eye drop, nasal drop, suppository etc.).

[0028]

30 When an oral solid preparation (tablet, granule, powder, capsule etc.) is produced, additives such as excipient, binder, disintegrant, lubricant, colorant and the like are added as necessary to the crystal of compound 1, and an oral solid preparation is produced by a conventional method. When an oral
35 solid preparation is produced, coating may be applied as

necessary.

[0029]

Examples of the excipient include lactose, cornstarch, crystalline cellulose and the like, and examples of the binder
5 include hydroxypropylcellulose, hydroxypropylmethylcellulose and the like. Examples of the disintegrant include calcium carboxymethylcellulose, croscarmellose sodium and the like, and examples of the lubricant include magnesium stearate, calcium stearate and the like. Examples of the colorant include
10 titanium oxide and the like, and examples of the coating agent include hydroxypropylcellulose, hydroxypropylmethylcellulose, methylcellulose and the like. Usable additives are not limited to these.

[0030]

15 These oral solid preparations can generally contain 0.001 - 99.5 wt%, preferably 0.01 - 90 wt% and the like, of the crystal of compound 1.

[0031]

When an injection (for intravenous administration,
20 intramuscular administration, subcutaneous administration, intraperitoneal administration etc.) is produced, pH adjuster, buffering agent, suspending agent, solubilizing agent, antioxidant, preservative (antiseptic), isotonicity agent and the like are added as necessary to the crystal of compound 1,
25 and injection can be produced by a conventional method. The injection may be formed as a use-time dissolution type freeze-dry preparation by freeze-drying.

[0032]

As the pH adjuster and buffering agent, organic acid or
30 inorganic acid and/or a salt thereof and the like can be used. As the suspending agent, methylcellulose, polysorbate 80, sodium carboxymethylcellulose and the like can be used; as the solubilizing agent, polysorbate 80, polyoxyethylene sorbitan monolaurate and the like can be used. As the antioxidant, α -
35 tocopherol and the like can be used; as the preservative,

methyl p-hydroxybenzoate, ethyl parahydroxybenzoate and the like can be used; as the isotonicity agent, glucose, sodium chloride, mannitol and the like can be used. The pH adjuster, buffering agent, suspending agent, solubilizing agent,
5 antioxidant, preservative (antiseptic), and isotonicity agent are not limited to these.

[0033]

These injections can generally contain 0.000001 - 99.5 mass %, preferably 0.00001 - 90 mass % and the like of the
10 crystal of compound 1 relative to the total mass of the injection.

[0034]

When an external preparation is produced, a base starting material is added to the crystal of compound 1 and, for example,
15 preservative, stabilizer, pH adjuster, antioxidant, colorant and the like are added as necessary, and an external preparation can be produced by a conventional method.

[0035]

As the base starting material to be used, various
20 starting materials generally used for pharmaceutical products, quasi-drugs, cosmetics and the like can be used. Specifically, for example, starting materials such as animal and vegetable oils, mineral oil, ester oil, waxes, emulsifier, higher alcohols, fatty acids, silicon oil, surfactant, phospholipids,
25 alcohols, polyvalent alcohols, water-soluble polymers, clay minerals, purified water and the like can be mentioned.

[0036]

These external preparations can generally contain 0.000001 - 99.5 wt%, preferably 0.00001 - 90 wt% and the like
30 of the crystal of compound 1.

[0037]

While the dose of the crystal of compound 1 varies depending on the level of symptom, age, sex, body weight, administration form, kind of salt, specific kind of the disease
35 and the like, in the case of an adult, generally about 30 µg -

10 g, preferably 100 μ g - 5 g, further preferably 100 μ g - 1 g,
is administered per day by oral administration, and about 30 μ g
- 1 g, preferably 100 μ g - 500 mg, further preferably 100 μ g -
300 mg, is administered per day by injection administration,
5 each in one to several portions.

Examples

[0038]

The present invention is explained in detail in the
following by referring to Reference Examples and Examples,
10 which are not to be construed as limitative. The "room
temperature" is a temperature within the range of 1 - 30°C.
[0039]

In the following, 4 kinds of crystals are explained as
crystals A, B, C and D. However, the crystal of compound 1 of
15 the present invention is not limited to them.

[0040]

Crystal A is a crystal of a hydrate of compound 1 and has
a melting point of 112°C. Crystal A can be obtained, for
example, by crystallizing compound 1 from a mixed solvent of an
20 alcohol solvent such as methanol, ethanol, isopropanol and the
like and water.

[0041]

While crystal A is not transited by heating at 50°C
overnight, it transits to an amorphous form by heating at 70°C
25 overnight.

[0042]

Crystal B is a crystal having a melting point of 140°C.
Crystal B can be obtained, for example, by crystallizing
compound 1 from methanol. It can also be obtained by adding
30 methanol to crystal A and leaving the mixture at room
temperature for 2 days.

[0043]

Crystal C is a crystal of an anhydride of compound 1 and
has a melting point of 187°C. Crystal C can be obtained, for
35 example, by crystallizing compound 1 from a mixed solvent of

ethanol, isopropanol or ethyl acetate, and heptane. It can also be obtained by dissolving compound 1 in isopropyl acetate, and crystallizing same by heating under reduced pressure at 40°C. While crystal C is transited to crystal D by adding a
5 mixed solvent of heptane and ethanol (volume ratio 16:3) which has a water content of not less than 0.5% and leaving the mixture overnight, it is not transited by adding a mixed solvent having a water content of less than 0.5% and leaving the mixture overnight.

10 [0044]

Crystal D is a crystal having a melting point of 105°C. It can be obtained, for example, by adding a mixed solvent of heptane and ethanol (volume ratio 16:3) which has a water content of not less than 0.5% to crystal C and leaving the
15 mixture overnight.

[0045]

A part of crystal D transits to an amorphous form by heating at 50°C overnight, and crystal D transits to an amorphous form by heating at 70°C overnight. In addition,
20 crystal D transits to crystal C by adding a mixed solvent of heptane and ethanol (volume ratio 16:3) which has a water content of less than 0.5% and leaving the mixture overnight.

[0046]

The chemical shift of ^1H -NMR (proton nuclear magnetic
25 resonance) spectrum was recorded in δ unit (ppm) to tetramethylsilane, and the coupling constant was recorded in hertz (Hz). The abbreviations of splitting patterns are as follows. s: singlet, d: doublet, t: triplet, q: quartette, m: multiplet, brs: broad singlet, brd: broad doublet.

30 [0047]

Comparative Example 1

According to the production method described in patent document 1, compound 1 was obtained as a solid. The powder X-ray diffraction of the obtained solid was measured, and the
35 results thereof are shown in Fig. 5. From Fig. 5, it was

confirm that compound 1 obtained in Comparative Example 1 was amorphous.

¹H-NMR (600 MHz, CHLOROFORM-d) δ (ppm): 1.27 (d, J=6 Hz, 3H), 2.59 (s, 3H), 3.01 (d, J=17 Hz, 1H), 3.24 (dd, J=14, 9 Hz, 1H),
5 3.35 (d, J=17 Hz, 1H), 3.43 (dd, J=14, 5 Hz, 1H), 4.03 (m, 1H), 4.32 (dd, J=15, 6 Hz, 1H), 4.51 (dd, J=15, 7 Hz, 1H), 5.35 (d, J=16 Hz, 1H), 5.50 (d, J=16 Hz, 1H), 5.56 (dd, J=9, 5 Hz, 1H), 5.83 (d, J=10 Hz, 1H), 6.16 (br. s, 1H), 6.63 (d, J=8 Hz, 2H), 6.90 (t, J=6 Hz, 1H), 7.07 (d, J=8 Hz, 2H), 7.26 (d, J=8 Hz,
10 2H), 7.30 (t, J=7 Hz, 1H), 7.36 (dd, J=8, 7 Hz, 2H), 7.41 (dd, J=8, 4 Hz, 1H), 7.52 (t, J=8 Hz, 1H), 7.61 (d, J=7 Hz, 1H), 7.75 (d, J=8 Hz, 1H), 8.16 (dd, J=8, 2 Hz, 1H), 8.88 (dd, J=4, 2 Hz, 1H).

[0048]

15 Example 1

Preparation of crystal of compound 1 (crystal A)

Water (2 mL) was added to compound 1 (303 mg) obtained in Comparative Example 1, and isopropanol (0.52 mL) and ethanol (0.5 mL) were added with heat-stirring. As a result, it was
20 completely dissolved at about 93°C. The mixture was allowed to slowly cool to room temperature, and precipitation of a solid was confirmed. The precipitate was collected by filtration through a glass filter, washed with heptane, and air dried at 60°C to give the title crystal (221 mg) as a white solid.

25 [0049]

Example 2

Preparation of crystal of compound 1 (crystal B)

Compound 1 (100 mg) obtained in Example 1 was dissolved in methanol (400 mg), and the mixture was stirred at room
30 temperature for 5 - 10 hr. The precipitated solid was collected by filtration and dried to give the title crystal.

[0050]

Example 3

Preparation of crystal of compound 1 (crystal C)

35 Ethyl acetate (1 mL) was added to compound 1 (300 mg)

obtained in Comparative Example 1 and the mixture was heat-stirred. As a result, it was completely dissolved at about 68°C. When heating was stopped, a solid was precipitated at about 66°C, and the mixture was allowed to slowly cool to room
5 temperature. The precipitate was collected by filtration through a glass filter, washed with heptane, and air dried at 60°C to give the title crystal (228 mg) as a white solid.
[0051]

Example 4

10 Preparation of crystal of compound 1 (crystal D)

Water (14 mg) was added to ethanol (240 mg) and heptane (1100 mg), and the mixture was stirred. Compound 1 (100 mg) obtained in Example 3 was added to the obtained mixed solvent, and the mixture was stirred at 5 - 25°C for 15 hr. The
15 precipitated solid was collected by filtration and dried to give the title crystal.

[0052]

Experimental Example 1: powder X-ray diffraction measurement

For powder X-ray diffraction measurement, crystals A - D
20 were placed on a sample table of a powder X-ray diffraction apparatus, and measurement was performed under the following conditions.

(measurement conditions)

apparatus: Rigaku MiniFlex II Desktop X-ray Diffractometer

25 X-ray used: Cu K α ray

detector: scintillation counter

tube voltage: 30 kV

tube electric current: 15 mA

K β filter: nickel filter

30 divergence slit: 1.25°

scattering slit: 1.25°

receiving slit: 0.3 mm

soller slit: 5° (divergence angle)

scan rate: 5°/min

35 sampling interval: 0.02°

scan range: 3° - 36°

sample holder: aluminum holder

[0053]

As a result of the powder X-ray diffraction measurement
5 of crystals A, B, C and D, diffraction patterns shown in Figs.
1 - 4 were obtained.

[0054]

The powder X-ray diffraction of crystal A obtained in
Example 1 was measured, and the diffraction pattern shown in
10 Fig. 1 and Table 1 was obtained.

Table 1

2θ [°]	peak intensity [counts]
9.1	87
9.6	67
10.7	404
10.9	550
11.9	87
12.2	48
13.4	113
14.4	401
16.1	91
16.8	127
17.1	62
17.7	49
18.6	112
19.1	330
19.6	53
20.3	167
20.7	33
21.1	102
22.2	205
22.8	313
23.3	88
23.8	199
24.2	97
25.4	66
27.4	118
28.2	72
28.9	29
29.3	42
30.2	27
33.5	59
34.0	32

[0055]

The powder X-ray diffraction of crystal B obtained in Example 2 was measured, and the diffraction pattern shown in Fig. 2 and Table 2 was obtained.

5 Table 2

2 θ [°]	peak intensity [counts]
7.2	266
8.1	54
8.9	268
10.9	139
11.5	27
12.9	149
13.2	271
16.4	592
17.1	189
18.4	121
19.6	95
20.2	109
21.3	59
22.4	297
23.4	87
24.8	203
26.2	113
26.8	74
27.4	32
30.1	44
32.9	29
34.6	28

[0056]

The powder X-ray diffraction of crystal C obtained in Example 3 was measured, and the diffraction pattern shown in
10 Fig. 3 and Table 3 was obtained.

Table 3

2 θ [°]	peak intensity [counts]
9.0	41
10.3	745
11.0	186
11.6	130
12.8	211
14.2	305
14.6	265
16.1	300
17.5	85
18.6	135
18.9	263
19.4	120
19.8	96
20.4	248
20.9	48
21.6	228
22.1	348
23.6	55
24.1	218
25.2	78
26.2	41
27.0	81
27.5	95
28.1	76
29.0	26
29.6	43
31.0	24
33.4	36
34.8	32
35.2	30

[0057]

The powder X-ray diffraction of crystal D obtained in
 5 Example 4 was measured, and the diffraction pattern shown in
 Fig. 4 and Table 4 was obtained.

Table 4

2 θ [°]	peak intensity [counts]
3.8	29
7.3	396
9.2	96
9.9	112
10.7	267
11.5	139
12.2	174
13.2	147
14.7	99
15.4	160
16.3	249
17.7	164
19.0	80
19.8	284
21.2	151
21.7	113
22.3	135
23.1	212
23.8	283
24.6	68
25.0	213
26.4	95
26.9	58
28.1	97
29.8	51
33.1	74
34.4	35

[0058]

Experimental Example 2: hygroscopicity evaluation

5 The hygroscopicity of Examples 1, 3 and Comparative
Example 1 was evaluated by a dynamic water adsorption measuring
apparatus. A sample mounting part of the apparatus was
maintained at 25°C, and the relative humidity (RH) was set
stepwisely within the range of 5 - 95%RH. The humidity was
10 controlled by changing the relative flow of 0%RH dry nitrogen
and 100%RH humidified nitrogen. The sample weight was
confirmed at 2 min intervals by a micro balance, and the
humidity was sequentially changed at the time point when the
weight shift range for 5 min was below 0.01%. The results
15 thereof are shown in Figs. 6 - 8.

[0059]

In Comparative Example 1, the weight increased along with an increase in the humidity, as shown in Fig. 8. In contrast, the weight did not increase in Examples 1 and 3 even when the humidity increased, as shown in Fig. 6 and Fig. 7, respectively. Therefore, it was clarified that the hygroscopicity of crystals A and C is low.

[0060]

Experimental Example 3: stability evaluation of crystal A

Crystal A was added in excess to the solvents described in Table 1 to give a slurry, which was stood at room temperature for 2 days, and the crystal form thereof was measured using powder X-ray diffraction and differential scanning calorimeter (DSC). The results thereof are shown in Table 5.

[0061]

Table 5

	solvent	crystal form
1	ethanol	crystal C
2	ethyl acetate	mixture of crystal A and crystal C
3	isopropanol	crystal C
4	isopropyl acetate	crystal A
5	MTBE	crystal A
6	methanol	crystal B
7	water	crystal A
8	tetrahydrofuran	amorphous
9	acetonitrile	crystal A
10	methylethyl ketone	crystal A

[0062]

When isopropyl acetate, MTBE, acetonitrile, methylethyl ketone or water was added to crystal A and the mixture was stood at room temperature for 2 days, transition did not occur. When methanol was added to crystal A and the mixture was stood at room temperature for 2 days, transition to crystal B occurred. When ethanol, isopropanol or ethyl acetate was added and the mixture was stood at room temperature for 2 days, transition to crystal C occurred gradually. When

tetrahydrofuran was added to crystal A and the mixture was stood at room temperature for 2 days, transition to an amorphous form occurred.

[0063]

- 5 Experimental Example 4: relationship between water content of solvent and crystal form

To a saturated solution of crystal C in a mixed solvent of heptane and ethanol (volume ratio 16:3) were added crystal C (100 mg) and crystal D (100 mg) at a temperature shown in Table 2, water in the amount shown in Table 6 was further added, and the mixture was stood overnight. The water content of the solution after the standing was measured by the Karl Fischer method, and the crystal form of compound 1 was measured by powder X-ray diffraction. The results thereof are shown in Table 6. The amount of the water added is shown in mass % relative to the mass of the whole solvent.

[0064]

Table 6

	temperature (°C)	amount of water added (mass%)	water content (%)	crystal form
1	5	0	0.32	crystal C
2	room temperature	0	0.33	crystal C
3	40	0	0.33	crystal C
4	5	0.5	0.93	crystal D
5	room temperature	0.5	0.93	crystal D
6	40	0.5	0.94	crystal D
7	5	1.0	1.56	crystal D
8	room temperature	1.0	1.57	crystal D
9	40	1.0	1.55	crystal D

20 [0065]

When the water content was less than 0.5%, crystal C was obtained, whereas when the water content was not less than 0.5%, crystal D was obtained. When the amount of water to be further added exceeded 2 mass %, crystal A was obtained. When crystal D was added to a mixed solvent of heptane and ethanol (volume ratio 16:3) which has a water content of less than 0.5% and the

mixture was stood, transition to crystal C occurred. When crystal C was added to a mixed solvent of heptane and ethanol (volume ratio 16:3) which has a water content of less than 0.5% and the mixture was stood, transition of crystal form did not
5 occur.

[0066]

Experimental Example 5: thermal stability of crystals A and D

Crystals A and D were heated in an oven at 50°C or 70°C overnight, and transition of each crystal was confirmed by
10 powder X-ray diffraction.

[0067]

[Table 7]

	temperature	crystals used	
		crystal A	crystal D
1	50°C	crystal A	mixture of crystal D and amorphous form
2	70°C	amorphous	amorphous

[0068]

15 As shown in Table 7, crystal A did not show transition under heating conditions at 50°C, whereas a part of crystal D transited to an amorphous form. Therefore, crystal A was more stable to heat than crystal D.

[0069]

20 Experimental Example 6: stability evaluation of crystals A, B and C

To a supersaturated solution of compound 1 using the solvents shown in Table 8 was added a mixture of equal amounts of crystals A, B and C at room temperature or 40°C, and the
25 form of the crystal obtained one day later was observed using powder X-ray diffraction. The results thereof are shown in Table 8.

[0070]

[Table 8]

	solvent	temperature	
		room temperature	40°C
1	isopropanol	crystal C	crystal C
2	isopropyl acetate	crystal C	crystal C
3	MTBE	crystal C	crystal C
4	ethyl acetate	crystal C	crystal C
5	water	crystal A	crystal A

[0071]

5 As shown in Table 8, when water was used as the solvent, crystal A was obtained; when isopropanol, isopropyl acetate, MTBE or ethyl acetate was used as the solvent, crystal C was obtained. Therefore, of crystals A, B and C, crystal A was most stable in water, and crystal C was most stable in other
10 nonaqueous solvents.

Claims

1. A crystal of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide or hydrate thereof.
5
2. A crystal of a hydrate of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide.
10
3. A crystal of an anhydride of (6S,9S)-N-benzyl-6-(4-hydroxybenzyl)-2,9-dimethyl-4,7-dioxo-8-(quinolin-8-ylmethyl)octahydro-1H-pyrazino[2,1-c][1,2,4]triazine-1-carboxamide.
15
4. The crystal according to claim 2, showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 10.7° , 10.9° , 14.4° , 19.1° and 22.8° , in powder X-ray diffraction.
20
5. The crystal according to claim 1, showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.2° , 8.9° , 13.2° , 16.4° and 22.4° , in powder X-ray diffraction.
- 25 6. The crystal according to claim 3, showing diffraction peaks at diffraction angles ($2\theta \pm 0.2^\circ$) of 10.3° , 14.2° , 14.6° , 16.1° and 22.1° , in powder X-ray diffraction.
7. The crystal according to claim 1, showing diffraction peaks
30 at diffraction angles ($2\theta \pm 0.2^\circ$) of 7.3° , 10.7° , 16.3° , 19.8° and 23.8° , in powder X-ray diffraction.
8. A pharmaceutical composition comprising the crystal according to any one of claims 1 to 7 as an active ingredient.

9. An antitumor agent comprising the crystal according to any one of claims 1 to 7 as an active ingredient.