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Monohydrate and crystalline forms of 6-[(3S,4S)-4-methyl-1- (pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-3-tetrahydropyran-4-yl- 7H-imid azo [1,5- a] pyrazin-8-one

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(71) Applicant(s)
CARDURION PHARMACEUTICALS, INC.

(72) Inventor(s)
BUTTAR, Suzanne;PITAK, Mateusz;PATTERSON, Adam Ross;STRATFORD, Samuel Alexander;SOVAGO, Ioana;XU, Jun;ZHOU, Peng;WEI, Haojuan;DAI, Kuangchu

(74) Agent / Attorney
FB Rice Pty Ltd, L 33 477 Collins Street, Melbourne, VIC, 3000, AU

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(71) **Applicant: IMARA INC.** [US/US]; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US).

(72) **Inventors: BUTTAR, Suzanne**; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US). **PITAK, Mateusz**; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US). **PATTERSON, Adam Ross**; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US). **STRATFORD, Samuel Alexander**; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US). **SOVAGO, Ioana**; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US). **XU, Jun**; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US). **ZHOU, Peng**; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US). **WEI, Haojuan**; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US). **DAI, Kuangchu**; 700 Technology Square, 3rd Floor, Cambridge, Massachusetts 02139 (US).

(74) **Agent: JOHNSON, Lee Alan**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, California 94304-1050 (US).

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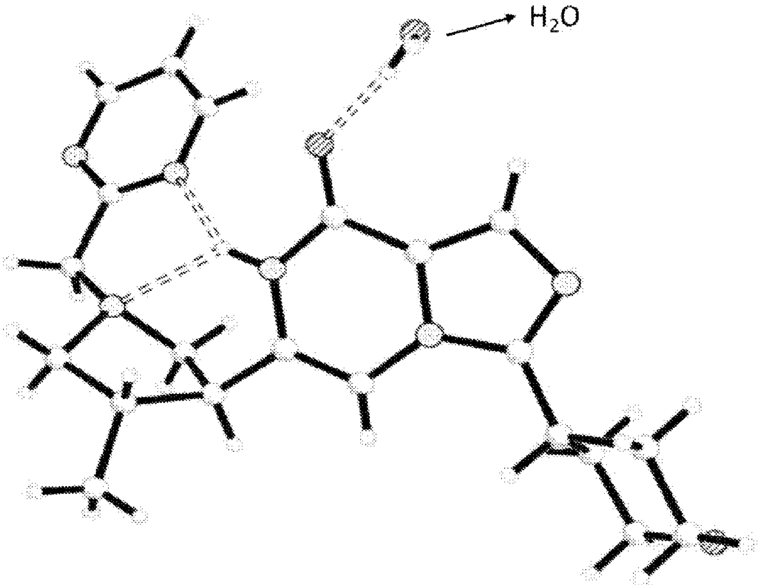
(54) **Title:** MONOHYDRATE AND CRYSTALLINE FORMS OF 6-[(3S,4S)-4-METHYL-1- (PYRIMIDIN-2-YLMETHYL)PYRROLIDIN-3-YL]-3-TETRAHYDROPYRAN-4-YL- 7H-IMID AZO [1,5- A] PYRAZIN-8-ONE

Fig. 1



(57) **Abstract:** The present disclosure relates to crystalline polymorph forms of 6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-3-tetrahydropyran-4-yl-7H-imidazo[1,5-a]pyrazin-8-one.

WO 2019/226944 A1

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MONOHYDRATE AND CRYSTALLINE FORMS OF 6-[(3S,4S)-4-METHYL-1-(PYRIMIDIN-2-YLMETHYL)PYRROLIDIN-3-YL]-3-TETRAHYDROPYRAN-4-YL-7H-IMIDAZO[1,5-A]PYRAZIN-8-ONE

REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit and priority to US Provisional Application No. 62/676,381 filed May 25, 2018, entitled MONOHYDRATE AND CRYSTALLINE FORMS OF 6-[(3S,4S)-4-METHYL-1-(PYRIMIDIN-2-YLMETHYL)PYRROLIDIN-3-YL]-3-TETRAHYDROPYRAN-4-YL-7H-IMIDAZO[1,5-A]PYRAZIN-8-ONE, and US Provisional Application No. 62/788,323 filed January 4, 2019, entitled MONOHYDRATE AND CRYSTALLINE FORMS OF 6-[(3S,4S)-4-METHYL-1-(PYRIMIDIN-2-YLMETHYL)PYRROLIDIN-3-YL]-3-TETRAHYDROPYRAN-4-YL-7H-IMIDAZO[1,5-A]PYRAZIN-8-ONE, the contents of each of which are incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

[0002] The present disclosure relates to polymorphic forms of a cyclic guanylate monophosphate (cGMP)-specific phosphodiesterase type 9 inhibitor (hereinafter referred to as PDE9 inhibitor).

BACKGROUND

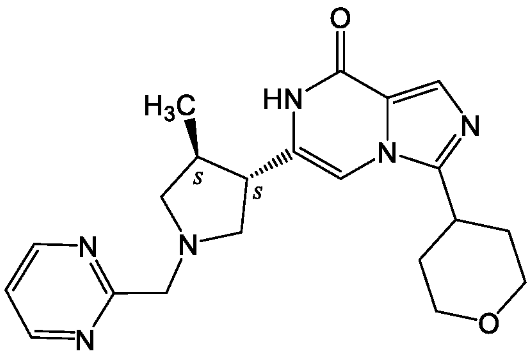
[0003] Solids exist in either amorphous or crystalline forms. Polymorphism relates to various crystalline forms of a chemical substance. These crystalline forms have different characteristics in structures and physical properties, such as XRPD spectrum, IR spectrum, and melting point. A particular polymorph form may have advantages over other forms and more suitable for the manufacture and use of the drug substance

[0004] 6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-3-tetrahydropyran-4-yl-7H-imidazo[1,5-a]pyrazin-8-one (Compound **1**) is a PDE9 inhibitor disclosed in WO 2017/005786 for treating various diseases such as sickle cell disease. Improved forms of Compound **1** are desired, particularly with regard to enhanced solubility, oral bioavailability, and/or physical stability.

SUMMARY OF THE DISCLOSURE

[0005] The present disclosure provides polymorph forms of a PDE9 inhibitor: 6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-3-tetrahydropyran-4-yl-7H-imidazo[1,5-a]pyrazin-8-one (Compound **1**), referred to herein as Form MH1 and Form MH2. The present disclosure also provides methods of making the polymorph forms, characterization of the polymorph forms, pharmaceutical compositions comprising the polymorph forms, and methods of using the polymorph forms and compositions.

[0006] One aspect of the disclosure provided herein comprises a monohydrate crystalline form of 6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-3-tetrahydropyran-4-yl-7H-imidazo[1,5-a]pyrazin-8-one



(Compound **1**). In some embodiments, the monohydrate crystalline form is MH1, having an XRPD pattern comprising peaks of 2θ angles at about 9.1, 11.5, 16.2, 16.7, 18.2, 18.9, 19.8, 22.6, and 26.4 degrees 2θ, each ±0.2 degrees 2θ. In some embodiments, the monohydrate crystalline form is MH1, having an XRPD pattern substantially as shown in FIG. 2A. In some embodiments, the monohydrate crystalline form is MH1, having a dehydration endothermic peak at about 40-100 °C and a melting endothermic peak at about 184.4 °C in a differential scanning calorimetry (DSC) thermogram. In some embodiments, the monohydrate crystalline form is MH1, having a DSC thermogram substantially in accordance with FIG. 5. In some embodiments, the monohydrate crystalline form is MH1, exhibiting dehydration between ambient and about 90 °C with a weight loss of about 4.4% in a thermogravimetric analysis (TGA). In some embodiments, the monohydrate crystalline form is MH1, having a TGA substantially in accordance with FIG. 5. In some embodiments, the monohydrate crystalline form is MH1, having characteristic absorptions at about 782cm^{-1}, 1123 cm^{-1}, 1562cm^{-1} and 1655cm^{-1} in an infrared (IR) spectrum. In some embodiments, the monohydrate crystalline form is MH1, having an infrared spectrum substantially in accordance with FIG. 3. In some embodiments, the monohydrate crystalline form is MH2, having an XRPD pattern comprising peaks of 2θ angles at about 9.0, 11.6, 15.0, 16.0, 18.6, 19.1, 20.4, or 20.6 degrees 2θ, each ±0.2 degrees 2θ. In some embodiments, the monohydrate crystalline form is MH2, having an XRPD pattern substantially as shown in FIG. 7. In some embodiments, the monohydrate crystalline form is MH2, having an endothermic peak at about 59.1°C (±5 °C) and at about 184.7 °C (±5 °C) in a differential scanning calorimetry (DSC) thermogram. In some embodiments, the monohydrate crystalline form is MH2, having a DSC thermogram substantially in accordance with FIG. 9. In some embodiments, the monohydrate crystalline form is MH2, exhibiting dehydration at about 25°C to about 100°C with a weight loss of about 4.4% in a thermogravimetric analysis (TGA). In some embodiments, the monohydrate crystalline form is MH2, having a TGA substantially in accordance with FIG. 9. In some embodiments, the monohydrate crystalline form is at least 95, 96, 97, 98, or 99% purified.

[0007] Another aspect described herein comprises a pharmaceutical composition comprising a therapeutically effective amount of the monohydrate crystalline form in any one of the embodiments described herein, and a pharmaceutically acceptable excipient. In some embodiments, the monohydrate crystalline form is present in an amount of at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% by weight. In some embodiments, the monohydrate crystalline form is present in an amount of at least about 91% by weight.

[0008] Another aspect described herein comprises a pharmaceutical composition consisting essentially of the monohydrate crystalline form of any one of the embodiments described herein.

[0009] Another aspect described herein comprises a pharmaceutical composition consisting essentially of the monohydrate crystalline form MH1 of any one of the embodiments described herein.

[0010] Another aspect described herein comprises a pharmaceutical composition consisting essentially of the monohydrate crystalline form MH2 of any one of the embodiments described herein. In some embodiments, the composition is in tablet or capsule form.

[0011] Another aspect described herein comprises a process for preparing a monohydrate crystalline form of Compound **1**, comprising precipitating the monohydrate crystalline form from a solution comprising Compound **1** and a solvent selected from the group consisting of n-propyl acetate, isopropyl acetate, anisole, methylisobutyl ketone, cumene, isopropanol, 2-methyl tetrahydrofuran, and combinations thereof. In some embodiments, the solvent is n-propyl acetate. In some embodiments, the process further comprises cooling the solution.

[0012] Another aspect described herein comprises a monohydrate crystalline form of Compound **1** prepared by the process of any one of the embodiments described herein.

[0013] Another aspect described herein comprises a method of inhibiting PDE9 activity in a patient, comprising administering to the patient the monohydrate crystalline form of any one of the embodiments described herein.

[0014] Another aspect described herein comprises a method of treating sickle cell disease in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of the monohydrate crystalline form of any one of the embodiments described herein.

[0015] Another aspect described herein comprises a process for preparing Monohydrate Form 1 (MH1) of Compound **1**, the steps comprising: (i) dissolving Compound **1** in a first solvent to get a solution; (ii) adding a second solvent to get a mixture; and (iii) filtering the mixture to get a solid, wherein the first and second solvent is each individually selected from selected from isopropyl acetate; ethanol; tetrahydrofuran; water; dichloromethane; acetonitrile; anisole; methylisobutyl ketone; nitromethane; 1,2-dimethoxyethane; methylethyl ketone; n-heptane; 1,4-dioxane; n-propyl acetate; 2-propanol; acetone; cumene; N,N-dimethylformamide; dimethyl sulfoxide; and combinations thereof. In some embodiments, the method further comprises heating the solution to a temperature above room temperature at about 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80 °C.

[0016] In some embodiments, the first solvent comprises 2-propanol. In some embodiments, the second solvent comprises n-heptane. In some embodiments, the first solvent in step (i) comprises water and 2-propanol. In some embodiments, the water content of the solution obtained in step (i) is about 0.5%, 1%, or 1.5%. In some embodiments, the water content of the solution obtained in step (i) is about 1%. In some embodiments, the solid obtained in step (ii) is optionally washed one or more times with n-heptane. In some embodiments, the process further comprises drying the solid after step (iii). In some embodiments, the solid is dried in a humidified.

[0017] Another aspect described herein comprises a process for preparing Monohydrate Form 2 (MH2) of Compound **1**, the steps comprising: a) treating Compound 1 or MH1 with a first solvent system to obtain a suspension; b)filtering the suspension to obtain the solid; c) washing the solid with heptane; and d) drying to remove the solvent to get MH2; wherein the solvent is a mixture of water and ethyl acetate (EtOAc) or methyl acetate (MeOAc), selected from 2% (v/v) EtOAc / water, 2.7% (v/v) EtOAc / water, and 7.5% (v/v) MeOAc / water.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] **FIG. 1** is the ball and stick diagram of MH1 molecular structure.

[0019] **FIG. 2A** is the experimental XRPD pattern of MH1 crystal at room temperature.

[0020] **FIG. 2B** is an overlay of the experimental XRPD pattern of MH1 at room temperature (top line) and the calculated XRPD pattern of MH1 at 293K (middle line) and 100 K (bottom line).

[0021] **FIG. 3** is the FTIR spectrum of MH1.

[0022] **FIG. 4** is the Raman spectrum of MH1.

[0023] **FIG. 5** is the TGA and DSC analysis of MH1.

[0024] **FIG. 6** is the ball and stick diagram of MH2 molecular structure.

[0025] **FIG. 7** is an overlay of the experimental XRPD pattern of MH2 at room temperature and the calculated XRPD pattern of MH2 at 100 K.

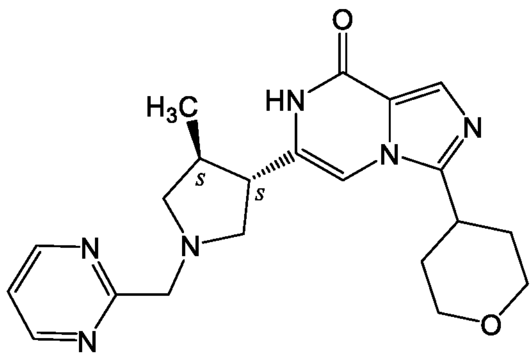
[0026] **FIG. 8** is an XRPD overlay of the scale up of MH2.

[0027] **FIG. 9** is the TGA and DSC analysis of MH2.

DETAILED DESCRIPTION

I. Polymorph Forms of Compound **1**

[0028] A racemate form of Compound **1** and an anhydrous form of Compound **1** have been described in WO 2013/053690 and WO 2017/005786. The anhydrous form of Compound **1** has the following structure:



6-[(3S,4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-3-tetrahydropyran-4-yl-7H-imidazo[1,5-a]pyrazin-8-one.

[0029] Two different monohydrate polymorph forms of Compound **1** have been discovered, Monohydrate Form 1 (MH1) and Monohydrate Form 2 (MH2). The two monohydrate forms and the anhydrate form (AH) differ in their crystal structure as determined by single crystal X-ray Powder Diffraction (XRPD). The main peaks of MH1 and MH2 below 30° 2θ were identified and their relative intensities are listed in Table 1. As will be understood by a person skilled in the art, the relative intensities of the peaks within Table 1 may vary due to various factors such as the purity of the material being analyzed, orientation effects of crystals in the X-ray beam, the degree of crystallinity of the sample, and so on. The peak positions may also shift for variations of sample height, but the peak positions will remain substantially as defined in Table 1. A person skilled in the art will also understand that measurements using a different wavelength will result in different shifts according to the Bragg equation ($n\lambda=2d \sin \theta$). Such further XRPD patterns generated by use of alternative wavelengths are alternative representations of the XRPD patterns of the crystalline materials.

Table 1. XRPD peak listing for MH1 and MH2.

MH1		MH2	
°2θ	**Intensity %**	**°2θ**	**Intensity %**
8.2	9.7	8.5	12.8
9.1	70.4	9.0	48.1
11.5	36.8	10.2	4.1
12.6	4.3	11.6	45.8
14.8	2.8	12.2	8.9
15.0	9.2	12.6	8.7
16.2	100	13.0	11.9
16.5	10	15.0	40.2
16.7	23.6	16.0	100
17.7	3.3	16.5	7.1
18.2	22.4	17.0	28.1
18.9	46.3	17.7	15.6
19.3	5.2	18.6	80.9
19.8	42.7	18.7	7.7
20.7	18.3	19.1	79.3
21.0	18.1	19.2	35.6
21.4	3.8	20.4	43.3
22.3	6.1	20.6	55
22.6	20.9	20.8	10.9
23.0	4.8	21.1	11.6
24.4	14	22.1	18.5
25.2	7.9	22.6	9.4
25.8	3.7	22.7	12.5
26.4	21.5	24.1	30.4
26.7	7.3	24.7	6.7
27.6	4	25.1	7.1
29.2	8.8	25.3	12.3
		25.9	8.4
		26.2	23.9
		26.7	32.6
		27.3	24.1
		27.9	20.7
		28.4	6.4
		28.5	5.7
		28.9	11.3
		29.6	8.9

i. Crystalline Form MH1

[0030] Form MH1 may be characterized by any of its peaks in Table 1. For example, MH1 may be characterized by any of the following peaks, among others: 9.1, 11.5, 16.2, 16.7, 18.2, 18.9, and 19.8 degrees 2θ, each ±0.2 degrees 2θ.

[0031] In some embodiments, the monohydrate crystalline form of Compound **1** is MH1 and has an X-ray powder diffraction (XRPD) pattern comprising one or more peaks of any of 9.1, 11.5, 16.2, 16.7, 18.2, 18.9, and 19.8 degrees 2θ, each ±0.2 degrees 2θ.

[0032] In some embodiments, the monohydrate crystalline form of Compound **1** is MH1 and has an X-ray powder diffraction (XRPD) pattern comprising peaks of 9.1, 11.5, 16.2, 16.7, 18.2, 18.9, and 19.8 degrees 2θ, each ±0.2 degrees 2θ.

[0033] In some embodiments, MH1 analysed with infrared (IR) spectroscopy exhibits characteristic absorptions at about 782cm^{-1}, 1123 cm^{-1}, 1562cm^{-1} and 1655cm^{-1} (±0.5cm^{-1}) as shown in FIG. 3.

[0034] In some embodiments, MH1 analysed by differential scanning calorimetry (DSC) thermogram shows a dehydration endothermic peak at about 40-100°C (±10°C) and a melting endothermic peak at about 184.4 °C (±5°C) as shown in FIG. 5.

[0035] In some embodiments, MH1 analysed by thermogravimetric analysis (TGA) exhibits a dehydration at ambient to about 90°C with a weight loss of about 3.8% as shown in FIG. 5.

ii. Crystalline Form MH2

[0036] Form MH2 may be characterized by any of its peaks in Table 1. For example, MH2 may be characterized by any of the following peaks, among others: 9.0, 11.6, 15.0, 16.0, 18.6, 19.1, 20.4, and 20.6 degrees 2θ, each ±0.2 degrees 2θ.

[0037] In some embodiments, the monohydrate crystalline form of Compound **1** is MH2 and has an X-ray powder diffraction (XRPD) pattern comprising one or more peaks of any of 9.0, 11.6, 15.0, 16.0, 18.6, 19.1, 20.4, or 20.6 degrees 2θ, each ±0.2 degrees 2θ.

[0038] In some embodiments, the monohydrate crystalline form of Compound **1** is MH2 and has an X-ray powder diffraction (XRPD) pattern comprising peaks of 9.0, 11.6, 15.0, 16.0, 18.6, 19.1, 20.4, and 20.6 degrees 2θ, each ±0.2 degrees 2θ.

[0039] In some embodiments, MH2 analysed by differential scanning calorimetry (DSC) thermogram shows an endothermic peak at about 59.1°C (±5°C) and at about 184.7 °C (±5°C) as shown in FIG. 9.

[0040] In some embodiments, MH2 analysed by thermogravimetric analysis (TGA) exhibits a dehydration at about 25°C to about 100°C with a weight loss of about 4.4% as shown in FIG. 9.

[0041] In some embodiments, the crystalline form of Compound **1** (e.g. MH1 or MH2) is substantially pure. In some embodiments, the crystalline form MH1 or MH2 is at least 80%, 85%, 90%, or 95% pure. In some embodiments, the crystalline form MH1 or MH2 is at least 95%, 96%, 97%, 98%, or 99% pure. In some embodiments, the crystalline form MH1 or MH2 contains no more than 10%, 5%, 3%, or 1% impurity.

II. Pharmaceutical compositions

[0042] The present disclosure further provides a pharmaceutical composition comprising a therapeutically effective amount of any of the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) and a pharmaceutically acceptable excipient, carrier or diluent. In some embodiments, the pharmaceutical composition is for oral administration. In some embodiments, the pharmaceutical composition is in tablet form or capsule form.

[0043] The polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) may be administered alone or in combination with pharmaceutically acceptable carriers, diluents or excipients, in either single or multiple doses. The pharmaceutical compositions according to the disclosure may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in Remington: The Science and Practice of Pharmacy, 22nd Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 2013.

[0044] Pharmaceutical compositions for oral administration include solid dosage forms such as capsules, tablets, dragees, pills, lozenges, powders and granules. Where appropriate, the compositions may be prepared with coatings such as enteric coatings or they may be formulated so as to provide controlled release of the active ingredient such as sustained or prolonged release according to methods well known in the art. Liquid dosage forms for oral administration include solutions, emulsions, suspensions, syrups and elixirs.

[0045] Pharmaceutical compositions for parenteral administration include sterile aqueous and nonaqueous injectable solutions, dispersions, suspensions or emulsions as well as sterile powders to be reconstituted in sterile injectable solutions or dispersions prior to use. Other suitable administration forms include, but are not limited to, suppositories, sprays, ointments, creams, gels, inhalants, dermal patches and implants.

[0046] Typical oral dosages range from about 0.001 to about 100 mg/kg body weight per day. Typical oral dosages also range from about 0.01 to about 50 mg/kg body weight per day. Typical oral dosages further range from about 0.05 to about 10 mg/kg body weight per day. Oral dosages are usually administered in one or more dosages, typically, one to three dosages per day. The exact dosage will depend upon the frequency and mode of administration, the gender, age, weight and general health of the subject treated, the nature and severity of the condition treated and any concomitant diseases to be treated and other factors evident to those skilled in the art.

[0047] The formulations may also be presented in a unit dosage form by methods known to those skilled in the art. For illustrative purposes, a typical unit dosage form for oral administration may contain from about 0.01 to about 1000 mg, from about 0.05 to about 500 mg, or from about 0.5 mg to about 200 mg.

[0048] For parenteral routes such as intravenous, intrathecal, intramuscular and similar administration, typical doses are on the order of half the dose employed for oral administration.

[0049] The present disclosure also provides a process for making a pharmaceutical composition comprising admixing a therapeutically effective amount of any of the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) and at least one pharmaceutically acceptable carrier or diluent.

[0050] Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solutions and various organic solvents. Examples of solid carriers include lactose, terra alba, sucrose, cyclodextrin, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and lower alkyl ethers of cellulose. Examples of liquid carriers include, but are not limited to, syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene and water. Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax. The pharmaceutical compositions formed by combining the compounds of the present disclosure and a pharmaceutically acceptable carrier are then readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The formulations may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

[0051] Formulations of the present disclosure suitable for oral administration may be presented as discrete units such as capsules or tablets, each containing a predetermined amount of the active ingredient, and optionally a suitable excipient. Furthermore, the orally available formulations may be in the form of a powder or granules, a solution or suspension in an aqueous or non-aqueous liquid, or an oil-in-water or water-in-oil liquid emulsion.

[0052] If a solid carrier is used for oral administration, the preparation may be tabletted, placed in a hard gelatine capsule in powder or pellet form or it may be in the form of a troche or lozenge. The amount of solid carrier will vary widely but will range from about 25 mg to about 1 g per dosage unit. If a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, soft gelatine capsule or sterile injectable liquid such as an aqueous or non-aqueous liquid suspension or solution.

[0053] The pharmaceutical compositions of the disclosure may be prepared by conventional methods in the art. For example, tablets may be prepared by mixing the active ingredient with ordinary adjuvants and/or diluents and subsequently compressing the mixture in a conventional tabletting machine prepare tablets. Examples of adjuvants or diluents comprise: corn starch, potato starch, talcum, magnesium stearate, gelatin, lactose, gums, and the like. Any other adjuvants or additives usually used for such purposes such as colorings, flavorings, preservatives etc. may be used provided that they are compatible with the active ingredients.

[0054] In some embodiments, the pharmaceutical compositions comprise at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% by weight of a polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2). In some embodiments, the pharmaceutical compositions comprise at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% by weight of a polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2).

[0055] In one embodiment, the pharmaceutical composition comprising compounds of the present disclosure is used in combination with one or more additional active agents, such as hydroxyurea ("HU").

III. Methods of Making Polymorph Forms of Compound 1

[0056] Crystallization can be precipitated from a solution comprising a compound and a solvent. For example, crystallizations can be obtained by lowering the temperature of a clear solution. The solubility of most materials decreases with temperature, and hence, cooling can be used to cause supersaturation. Crystallization can also be obtained by fast evaporation.

[0057] In some embodiments, MH1 crystals are made in a crystallization process. In some embodiments, MH2 crystals are made in a crystallization process.

[0058] Crystals of Compound **1** can be prepared by dissolving Compound **1** in a solvent to obtain a saturated solution of Compound **1** and then cooling the saturated solution to precipitate the crystals.

[0059] Crystals of monohydrate form MH1 or MH2 can be prepared by precipitating the crystals from a solution comprising Compound **1** and a solvent. The solvent is selected from the group consisting of ethanol; tetrahydrofuran; water; dichloromethane; acetonitrile; nitromethane; 1,2-dimethoxyethane; methylethyl ketone; 1,4-dioxane; 2-propanol; acetone; cumene; N,N-dimethylformamide; dimethyl sulfoxide; n-propyl acetate; isopropyl acetate; anisole; methylisobutyl ketone; 2-methyl tetrahydrofuran; and combinations thereof. For example, Compound **1** can be suspended in a solvent, e.g. n-propyl acetate, to obtain a suspension, filtering the suspension to get a solution (i.e., mother liquor) and then cooling the solution (i.e., mother liquor) to precipitate the crystals.

[0060] In some embodiments, MH1 or MH2 crystals can be prepared by the steps comprising:

(i) suspending Compound **1** in a first solvent at room temperature (RT) to form a suspension;

(ii) heating the suspension obtained in step (i) to a temperature above RT, (e.g., from about 40°C to about 60°C);

(iii) adding a second solvent to the suspension obtained in step (ii) to form a mixture and heating the mixture to a temperature above RT, (e.g., from about 40°C to about 60°C);

(iv) optionally filtering the mixture obtained in step (iii) to obtain a solution (i.e., mother liquors); and

(v) cooling the mixture obtained in step (iii) or the solution obtained in step (iv) to a temperature below RT, (e.g., about 4 °C), to precipitate MH1 or MH2 crystals;

wherein the first and second solvent are each independently selected from the group consisting of n-heptane; ethanol; tetrahydrofuran; water; dichloromethane; acetonitrile; nitromethane; 1,2-dimethoxyethane; methylethyl ketone; 1,4-dioxane; 2-propanol; acetone; cumene; N,N-dimethylformamide; dimethyl sulfoxide; n-propyl acetate; isopropyl acetate; anisole; methylisobutyl ketone; 2-methyl tetrahydrofuran; and combinations thereof.

[0061] In some embodiments, the first and second solvents are each independently selected from methylethyl ketone; 1,4-dioxane; 2-propanol; acetone; n-propyl acetate; isopropyl acetate; anisole; methylisobutyl ketone; cumene; n-heptane; 2-methyl tetrahydrofuran; and combinations thereof. In some embodiments, the first and second solvent are each independently selected from n-propyl acetate or n-heptane.

[0062] In some embodiments, the first and second solvents are the same. In some embodiments, the first and second solvents are different.

[0063] In some embodiments of MH1, the first solvent is selected from n-prolyl acetate and the second solvent is selected from n-heptane.

[0064] In some embodiments, the volume of the first solvent used in step (i) is less than the volume of the second solvent used in step (iii). For example, the ratio of the volume of the first solvent used in step (i) to the volume of the second solvent used in step (iii) is around 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, or 1:5. In one example, the ratio of the volume of the first solvent used in step (i) to the volume of the second solvent used in step (ii) is around 1:2.

[0065] In some embodiments, the temperature above RT in steps (ii) and (iii) is from about 30 °C to about 100 °C. In some embodiments, the temperature above RT is from about 40 °C to about 60 °C. In some embodiments, the temperature above RT is about 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80 °C.

[0066] In some embodiments, MH1 crystals can be prepared by the steps comprising:

(i) dissolving Compound **1** in a first solvent to get a solution;

(ii) adding a second solvent to get a mixture; and

(iii) filtering the mixture to obtain the crystalline solid MH1.

[0067] In some embodiments, the first solvent is selected from methylethyl ketone; 2-propanol; cumene; n-propyl acetate; isopropyl acetate; anisole; methylisobutyl ketone; n-heptane; 2-methyl tetrahydrofuran; and combinations thereof. In some embodiments, the second solvent is selected from methylethyl ketone; 2-propanol; n-propyl acetate; isopropyl acetate; anisole; methylisobutyl ketone; cumene; n-heptane; 2-methyl tetrahydrofuran; and combinations thereof. In some embodiments, the first solvent is 2-propanol. In some embodiments, the second solvent is n-heptane.

[0068] In some embodiments, the first solvent of step (i) further comprises water. . In some embodiments, the first solvent of step (i) comprises 2-propanol and water. In some embodiments, the water content of the solution obtained in step (i) is about 1%, 2% or 3%. In some embodiments, the water content of the solution obtained in step (i) is about 1%. In some embodiments, the ratio (weight/weight) of water to 2-propanol is about 1:70.

[0069] In some embodiments, step (i) of the process is protected by nitrogen gas.

[0070] In some embodiments, step (i) is carried out at a temperature of about 25 °C to about 40 °C. In some embodiments, step (i) is carried out at a temperature of about 27 °C to about 35 °C.

[0071] In some embodiments, seed crystals are added after step (i) to induce the crystallization of MH1. In some embodiments, the temperature of the mixture is adjusted to from about 20 °C to about 30 °C, before the seed crystals are added. In some embodiments, the temperature of the mixture is adjusted to from about 22 °C to about 28 °C, before the seed crystals are added. The weight of the seed crystals is from about 0.05% to about 2%, , of the weight of Compound **1** added in step (i). In some embodiments, the weight of the seed crystals is about 0.05, 1%, or 2%, of Compound **1** in step (i).

[0072] In some embodiments, steps (ii) and (iii) are each independently carried out at a temperature from about 20 °C to about 30 °C., such as about 22 °C to about 28 °C. In some embodiments, steps (ii) and (iii) are each independently carried out at a temperature from about 22 °C to about 28 °C.

[0073] In some embodiments, the solid obtained in step (iii) is optionally washed one or more times with a solvent selected from methylethyl ketone; 1,4-dioxane; 2-propanol; acetone; cumene; n-propyl acetate; isopropyl acetate; anisole; methylisobutyl ketone; n-heptane; and 2-methyl tetrahydrofuran; and combinations thereof. In some embodiments, the solid obtained in step (iii) is optionally washed with n-heptane. In some embodiments, the solid obtained in step (iii) or after washing with n-heptane is pressed until dried. In some embodiments, the resulting dried solid is optionally subsequently dried under a flow of nitrogen gas. In some embodiments, the solid is further dried in a humidified environment, optionally under a flow of nitrogen gas. Different salt solutions, such as a saturated solution of sodium chloride, can provide a humidified environment with different relative humidity (RH). Commercially available equipment providing gas with adjustable RH and temperature may also be used.

[0074] In some embodiments, the anhydrous (AH) form of Compound **1** can be converted to MH1 when exposed to normal laboratory air (containing some moisture). In some embodiments, the anhydrous form of Compound **1** is left under ambient conditions (e.g. 25 °C) for at least about 12 hours, about 24 hours, about 36 hours, or about 48 hours.

[0075] In some embodiments, anhydrate (AH) or MH2 is made in a crystallization process. Drying and humidified drying of the anhydrate (AH) or MH2 then yields MH1 crystals.

[0076] Crystals of monohydrate form MH2 are prepared by the steps comprising:

(i) treating dry Compound **1** or MH1 with a first solvent system to get a suspension;

(ii) filtering the suspension to obtain the solid;

(iii) washing the solid one or more times with a second solvent; and

(iv) air drying the solid to obtain crystalline MH2,

wherein the first solvent system is selected from water, ethyl acetate (EtOAc), and methyl acetate (MeOAc); and combinations thereof; and wherein the second solvent is selected from acetone; n-heptane; and 2-methyl tetrahydrofuran.

[0077] In some embodiments, the first solvent system is a mixture of water and ethyl acetate (EtOAc) or methyl acetate (MeOAc). In some embodiments, the first solvent system selected from 2% (v/v) EtOAc / water; 2.7% (v/v) EtOAc / water; or 7.5% (v/v) MeOAc / water. In some embodiments, the suspension is kept for about 4 days at about 5 °C or about 25 °C.

[0078] In some embodiments, the second solvent is heptane.

[0079] In some embodiments, Compound **1** or MH1 of step (i) is dried in a vacuum oven. In some embodiments, Compound **1** or MH1 of step (i) is air dried.

[0080] In one embodiment, crystalline MH2 is prepared by treating Compound **1** or MH1 with a first solvent system comprising 7.5% (v/v) MeOAc / water at around 5°C, filtering the solid, and then air drying the solid.

[0081] In one embodiment, crystalline MH2 is prepared by:

(i) treating Compound 1 or MH1 with a first solvent system to obtain a suspension;

(ii) filtering the suspension to obtain the solid;

(iii) washing the solid with heptane; and

(iv) air drying to remove the solvent to get MH2;

wherein the solvent is a mixture of water and ethyl acetate (EtOAc) or methyl acetate (MeOAc), selected from 2% (v/v) EtOAc / water, 2.7% (v/v) EtOAc / water, and 7.5% (v/v) MeOAc / water.

IV. Methods of Using Polymorph Forms of Compound 1

[0082] PDE9 is expressed specifically in the human haematopoietic system including neutrophils, reticulocytes erythroid and erythroleukaemic cells. Furthermore, sickle cell disease (SCD) patients exhibit a marked and significant elevation of PDE9 expression in reticulocytes and neutrophils compared to healthy individuals (Almeida et al., Br J Haematol. 2008 Sep; 142(5):836-44). Evidence additionally demonstrates a link between PDE9 and cell adhesion since pharmacologic PDE9 inhibition ameliorates the increased adhesive properties of SCD neutrophils (Miguel et al., Inflamm Res. 2011 Jul; 60(7):633-42). The mechanism by which PDE9 inhibition decreases cell adhesion has been shown to be mediated by increased cGMP and decreased endothelial adhesion molecule expression. Importantly, in an animal model of SCD, the PDE9 inhibitor-mediated decrease in cell adhesion had the functional effect of increased cell survival. In addition to demonstrating decreased cell adhesion comparable to hydroxyurea (HU), PDE9 inhibition resulted in increased fetal non-sickled haemoglobin (HbF) production, which reduced the cellular concentration of abnormal haemoglobin (HbS) within red blood cells (RBCs) resulting in less polymerization of the abnormal haemoglobin and its associated sequelae. The importance of increasing HbF in treating SCD is evidenced by results of large studies like the Cooperative Study of Sickle Cell Disease, as well as studies in a variety of patient cohorts outside of the US, showing that HbF is among the most important modifiers of this disease (Alsultan et al., *Am J Hematol.*, 88(6):531-2 (2013)) as well as data showing that modifiers of HbF improve other hematological parameters (Akinsheye, *Blood*, 118(1):19-27 (2011)). Finally, Almeida and colleagues demonstrated that treatment with HU combined with PDE9 inhibition in a mouse model of SCD leads to an additional beneficial amplification of the cGMP elevating effects of HU (Almeida et al., Blood. 2012 Oct 4;120(14):2879-88). In conclusion, PDE9 inhibition can modulate both the expression of fetal haemoglobin production as well as decrease cell adhesion, both mechanisms key for the treatment of SCD.

[0083] One aspect of the present disclosure provides methods of using any of the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) and pharmaceutical compositions comprising any of the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2).

[0084] The polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) may be used to treat sickle cell disease or any disease and/or symptom related to sickle cell disease, such as anemia, sickle-hemoglobin C disease (SC), beta thalassemia (beta-plus thalassemia and beta-zero thalassemia), vaso-occlusive crisis, attacks of pain (sickle cell crisis), splenic sequestration crisis, acute chest syndrome, aplastic crisis, hemolytic crisis, long-term pain, bacterial infections, and stroke.

[0085] In one embodiment, the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) are used to treat beta thalassemia of a subject and/or to increase hemoglobin levels in the subject.

[0086] In another embodiment, the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) are used to increase cGMP levels in a cell or in the plasma of a subject, wherein the subject has sickle cell disease. The cell may be, but not limited to, red blood cells and/or white blood cells. The cGMP level may be increased by at least 50%, 100%, or 150%. In some embodiments, the cGMP level is increased at least 2 times, 3 times, 4 times, 5 times, 10 times, 15 times, 20 times, or 25 times.

[0087] In another embodiment, the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) are used to increase fetal hemoglobin (HbF) positive red blood cell number in a subject, wherein the subject has sickle cell disease. The HbF positive red blood cell number is increased by at least 50%, 100%, or 150%. In some embodiments, the HbF positive red blood cell number is increased at least 2 times, 3 times, 4 times, 5 times, 10 times, 15 times, 20 times, or 25 times.

[0088] In another embodiment, the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) are used to reduce sickle red blood cell percentage (% sickle RBC), stasis percentage (% stasis), total bilirubin, or total leucocyte count in a subject, wherein the subject has sickle cell disease. The % sickle RBC, % stasis, total bilirubin, total leucocyte count or spleen weight is decreased by at least 10%, 20%, 30%, 40%, 50%, 60% or 70%.

[0089] cGMP level may be measured with any suitable method in the art, such as enzyme immunoassay.

[0090] HbF positive cells, as used herein, means red blood cells with HbF. HbF positive cells may be measured from a blood sample with any suitable method in the art, such as electrophoresis and/or colorimetric methods.

[0091] Sickle red blood cells, sickled red blood cells, as used herein, means red blood cells with a crescent or sickle shape. % sickle red blood cell may be measured from a blood sample with any suitable method in the art.

[0092] Stasis or microvascular stasis, as used herein, is serious slowing, or complete cessation, of blood or lymph flow through vessels. % stasis is the number of static (no flow) venules divided by the number of flowing venules times 100. % stasis may be measured with any suitable method in the art.

[0093] Total bilirubin, as used herein, means both unconjugated and conjugated bilirubin. Total bilirubin levels may be measured from a blood sample with any suitable method in the art.

[0094] Total leucocyte count or total white blood cell count, as used herein, is a blood test that measures the number of white blood cells in the body. It may be measured from a blood sample with any suitable method in the art.

[0095] Another aspect of the present disclosure provides methods of using the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) in combination with at least one other active agent. They may be administered simultaneously or sequentially. They may be present as a mixture for simultaneous administration, or may each be present in separate containers for sequential administration.

[0096] The term "simultaneous administration", as used herein, is not specifically restricted and means that the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) and the at least one other active agent are substantially administered at the same time, e.g. as a mixture or in immediate subsequent sequence.

[0097] The term "sequential administration", as used herein, is not specifically restricted and means that the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) and the at least one other active agent are not administered at the same time but one after the other, or in groups, with a specific time interval between administrations. The time interval may be the same or different between the respective administrations of the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) and the at least one other active agent and may be selected, for example, from the range of 2 minutes to 96 hours, 1 to 7 days or one, two or three weeks. Generally, the time interval between the administrations may be in the range of a few minutes to hours, such as in the range of 2 minutes to 72 hours, 30 minutes to 24 hours, or 1 to 12 hours. Further examples include time intervals in the range of 24 to 96 hours, 12 to 36 hours, 8 to 24 hours, and 6 to 12 hours.

[0098] The molar ratio of the polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 or MH2) and the at least one other active agent is not particularly restricted. For example, when a polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2) and one other active agent are combined in a composition, the molar ratio of them may be in the range of 1:500 to 500:1, or of 1:100 to 100:1, or of 1:50 to 50:1, or of 1:20 to 20:1, or of 1:5 to 5:1, or 1:1. Similar molar ratios apply when a polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2) and two or more other active agent are combined in a composition. The polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2) may comprise a predetermined molar weight percentage from about 1% to 10%, or about 10% to about 20%, or about 20% to about 30%, or about 30% to 40%, or about 40% to 50%, or about 50% to 60%, or about 60% to 70%, or about 70% to 80%, or about 80% to 90%, or about 90% to 99% of the composition.

[0099] The other active agent may be a different PDE9 inhibitor of the present disclosure or HU. The other active agent may also be an antibiotic agent such as penicillin, a nonsteroidal anti-inflammatory drug (NSAIDS) such as diclofenac or naproxen, a pain relief medication such as opioid, or folic acid.

[00100] Yet another aspect of the present disclosure provides methods of using a polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2) in combination with at least one other therapy, such as but not limited to blood transfusion, bone marrow transplant, or gene therapy.

V. Kits and Devices

[00101] The disclosure provides a variety of kits and devices for conveniently and/or effectively carrying out methods of the present disclosure. Typically, kits will comprise sufficient amounts and/or numbers of components to allow a user to perform multiple treatments of a subject(s) and/or to perform multiple experiments.

[00102] In one embodiment, the present disclosure provides kits for treating sickle cell disease, comprising a polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2) or a combination of polymorph forms of Compound **1** (such as monohydrate crystalline form MH1 and MH2), optionally in combination with any other active agents, such as HU, an antibiotic agent such as penicillin, a nonsteroidal anti-inflammatory drug (NSAIDS) such as diclofenac or naproxen, a pain relief medication such as opioid, or folic acid.

[00103] The kit may further comprise packaging and instructions and/or a delivery agent to form a formulation composition. The delivery agent may comprise a saline, a buffered solution, or any delivery agent disclosed herein. The amount of each component may be varied to enable consistent, reproducible higher concentration saline or simple buffer formulations. The components may also be varied in order to increase the stability of PDE9 inhibitor compounds in the buffer solution over a period of time and/or under a variety of conditions.

[00104] The present disclosure provides for devices that may incorporate a polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2). These devices contain in a stable formulation available to be immediately delivered to a subject in need thereof, such as a human patient with sickle cell disease or beta thalassemia.

[00105] Non-limiting examples of the devices include a pump, a catheter, a needle, a transdermal patch, a pressurized olfactory delivery device, iontophoresis devices, multi-layered microfluidic devices. The devices may be employed to deliver a polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2) according to single, multi- or split-dosing regiments. The devices may be employed to deliver a polymorph form of Compound **1** (such as monohydrate crystalline form MH1 or MH2) across biological tissue, intradermal, subcutaneously, or intramuscularly. More examples of devices suitable for delivering a polymorph forms of compounds include but not limited to a medical device for intravesical drug delivery disclosed in International Publication WO

2014036555, a glass bottle made of type I glass disclosed in US Publication No. 20080108697, a drug-eluting device comprising a film made of a degradable polymer and an active agent as disclosed in US Publication No. 20140308336, an infusion device having an injection micropump, or a container containing a pharmaceutically stable preparation of an active agent as disclosed in US Patent No. 5716988, an implantable device comprising a reservoir and a channeled member in fluid communication with the reservoir as disclosed in International Publication WO 2015023557, a hollow-fibre-based biocompatible drug delivery device with one or more layers as disclosed in US Publication No. 20090220612, an implantable device for drug delivery including an elongated, flexible device having a housing defining a reservoir that contains a drug in solid or semi-solid form as disclosed in International Publication WO 2013170069, a bioresorbable implant device disclosed in US Patent No. 7326421, contents of each of which are incorporated herein by reference in their entirety.

VI. Definitions

[00106] The articles “a” and “an,” as used herein, should be understood to mean “at least one,” unless clearly indicated to the contrary.

[00107] The phrase “and/or,” as used herein, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified unless clearly indicated to the contrary. Thus, as a non-limiting example, a reference to “A and/or B,” when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A without B (optionally including elements other than B); in another embodiment, to B without A (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements).

[00108] As used herein, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements.

[00109] In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[00110] As used herein, the phrase "at least one" in reference to a list of one or more elements should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified.

[00111] Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[00112] As used herein, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," and the like are to be understood to be open-ended, i.e., to mean including but not limited to.

[00113] Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures.

[00114] As used herein, a "subject" or a "patient" refers to any mammal (e.g., a human), such as a mammal that may be susceptible to a disease or disorder, for example, tumorigenesis or cancer. Examples include a human, a non-human primate, a cow, a horse, a pig, a sheep, a goat, a dog, a cat, or a rodent such as a mouse, a rat, a hamster, or a guinea pig. In various embodiments, a subject refers to one that has been or will be the object of treatment, observation, or experiment. For example, a subject can be a subject diagnosed with cancer or otherwise known to have cancer or one selected for treatment, observation, or experiment on the basis of a known cancer in the subject.

[00115] As used herein, "process" and "method" can be used interchangeably.

[00116] As used herein, "treatment" or "treating" refers to amelioration of a disease or disorder, or at least one sign or symptom thereof. "Treatment" or "treating" can refer to reducing the progression of a disease or disorder, as determined by, e.g., stabilization of at least one sign or symptom or a reduction in the rate of progression as determined by a reduction in the rate of progression of at least one sign or symptom. In another embodiment, "treatment" or "treating" refers to delaying the onset of a disease or disorder.

[00117] As used herein, "prevention" or "preventing" refers to a reduction of the risk of acquiring or having a sign or symptom a given disease or disorder, i.e., prophylactic treatment.

[00118] The phrase "therapeutically effective amount" as used herein means that amount of a compound, material, or composition comprising a compound of the present teachings that is effective for producing a desired therapeutic effect. Accordingly, a therapeutically effective amount treats or prevents a disease or a disorder, e.g., ameliorates at least one sign or symptom of the disorder. In various embodiments, the disease or disorder is a cancer.

[00119] A dash ("–") that is not between two letters or symbols is used to indicate a point of attachment for a substituent. For example, $-CONH_2$ is attached through the carbon atom (C).

[00120] By "optional" or "optionally," it is meant that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" encompasses both "aryl" and "substituted aryl" as defined herein. It will be understood by those ordinarily skilled in the art, with respect to any group containing one or more substituents, that such groups are not intended to introduce any substitution or substitution patterns that are sterically impractical, synthetically non-feasible, and/or inherently unstable.

[00121] As used herein, the term "substantially" refers to the complete or nearly complete extent or degree of an action, characteristic, property, state, structure, item, or result. For example, an object that is "substantially the same" or "substantially in accordance with" a second object would mean that the object is either completely or nearly completely the same as the second object. The exact allowable degree of deviation from absolute completeness may in some cases depend on the specific context. However, generally speaking, the nearness of completion will be so as to have the same overall result as if absolute and total completion were obtained.

[00122] The use of "substantially" is equally applicable when used in a negative connotation to refer to the complete or near complete lack of an action, characteristic, property, state, structure, item, or result. For example, a composition that is "substantially free of" impurities would either completely lack impurities, or so nearly completely lack impurities that the effect would be the same as if it completely lacked impurities. In other words, a composition that is "substantially free of" an ingredient or element may still actually contain such item as long as there is no measurable effect thereof.

[00123] Unless otherwise specified, all numbers expressing quantities of ingredients, reaction conditions, and other properties or parameters used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless otherwise indicated, it should be understood that the numerical parameters set forth in the following specification and attached claims are approximations. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, numerical parameters should be read in light of the number of reported significant digits and the application of ordinary rounding techniques. For example, the term "about" can encompass variations of ±10%, ±5%, ±2%, ±1%, ±0.5%, or ±0.1% of the numerical value of the number, which the term "about" modifies. In various embodiments, the term "about" encompasses variations of ±5%, ±2%, ±1%, or ±0.5% of the numerical value of the number. In some embodiments, the term "about" encompasses variations of ±5%, ±2%, or ±1% of the numerical value of the number. In certain embodiments, the term "about" encompasses variations of ±5% of the numerical value of the number. In certain embodiments, the term "about" encompasses variations of ±2% of the numerical value of the number. In certain embodiments, the term "about" encompasses variations of ±1% of the numerical value of the number.

[00124] All numerical ranges herein include all numerical values and ranges of all numerical values within the recited range of numerical values. As a non-limiting example, (C_1-C_6) alkyls also include any one of C_1, C_2, C_3, C_4, C_5, C_6, (C_1-C_2), (C_1-C_3), (C_1-C_4), (C_1-C_5), (C_2-C_3), (C_2-C_4), (C_2-C_5), (C_2-C_6), (C_3-C_4), (C_3-C_5), (C_3-C_6), (C_4-C_5), (C_4-C_6), and (C_5-C_6) alkyls.

[00125] Further, while the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations as discussed above, the numerical values set forth in the Examples section are reported as precisely as possible. It should be understood, however, that such numerical values inherently contain certain errors resulting from the measurement equipment and/or measurement technique.

EXAMPLES

[00126] It will be appreciated that the following examples are intended to illustrate but not to limit the present disclosure. Various other examples and modifications of the foregoing description and examples will be apparent to a person skilled in the art after reading the disclosure without departing from the spirit and scope of the disclosure, and it is intended that all such examples or modifications be included within the scope of the appended claims. All publications and patents referenced herein are hereby incorporated by reference in their entirety.

List of abbreviations:

^{1}H-NMR	Proton Nuclear Magnetic Resonance
ATR	Attenuated Total Reflectance
ca.	Approximately
DMSO	Dimethylsulfoxide
DSC	Differential Scanning Calorimetry
DVS	Dynamic Vapour Sorption
EtOAc	Ethyl acetate
EtOH	Ethanol
FBRM	Focussed Beam Reflectance Measurement
GVS	Gravimetric Vapour Sorption
HPLC	High Performance Liquid Chromatography
HSM	Hot Stage Microscopy
ID	Identification
IPA	Propan-2-ol
IPrOAc	Iso-Propyl Acetate
KF	Karl Fischer
MeOH	Methanol
MeOAc	Methyl acetate
N/A	Not Applicable
PLM	Polarised Light Microscopy
RH	Relative Humidity
RT	Room Temperature
SCXRD	Single Crystal X-Ray Diffraction
TFA	Tri-Fluoro Acetic Acid
TGA	Thermal Gravimetric Analysis
THF	Tetrahydrofuran
Vol	Volume
VT-XRPD	Variable Temperature X-Ray Powder Diffraction
XRPD	X-Ray Powder Diffraction

Instrument and Methodology Details

X-Ray Powder Diffraction (XRPD)

[00127] XRPD diffractograms were collected on a Bruker D8 diffractometer using Cu Kα radiation (40 kV, 40 mA) and a θ-2θ goniometer fitted with a Ge monochromator. The incident beam passes through a 2.0 mm divergence slit followed by a 0.2 mm anti-scatter slit and knife edge. The diffracted beam passes through an 8.0 mm receiving slit with 2.5°

Soller slits followed by the Lynxeye Detector. The software used for data collection and analysis was Diffrac Plus XRD Commander and Diffrac Plus EVA respectively.

[00128] Samples were run under ambient conditions as flat plate specimens using powder as received. The sample was prepared on a polished, zero-background (510) silicon wafer by gently pressing onto the flat surface or packed into a cut cavity. The sample was rotated in its own plane.

[00129] The details of the standard Pharmorphix data collection method are: Angular range: 2 to 42° 2θ; Step size: 0.05° 2θ; Collection time: 0.5 s/step (total collection time: 6.40 min).

PANalytical Empyrean

[00130] XRPD diffractograms were collected on a PANalytical Empyrean diffractometer using Cu Kα radiation (45 kV, 40 mA) in transmission geometry. A 0.5° slit, 4 mm mask and 0.04 rad Soller slits with a focusing mirror were used on the incident beam. A PI Xcel30 detector, placed on the diffracted beam, was fitted with a receiving slit and 0.04 rad Soller slits. The software used for data collect ion was X'Pert Data Collector using X'Pert Operator Interface. The data were analysed and presented using Diffrac Plus EVA or

HighScore Plus.

[00131] Samples were prepared and analysed in either a metal or Millipore 96 well-plate in transmission mode. X-ray transparent film was used between the metal sheets on the metal well-plate and powders (approximately 1 - 2 mg) were used as received. The Millipore plate was used to isolate and analyse solids from suspensions by adding a small amount of suspension directly to the plate before filtration under a light vacuum.

[00132] The scan mode for the metal plate used the gonio scan axis, whereas a 2θ scan was utilized for the Millipore plate.

[00133] The details of the standard screening data collection method are: Angular range: 2.5 to 32.0° 2θ; Step size: 0.0130° 2θ; Collection time: 12.75 s/step (total collection time of 2.07 min).

Non-Ambient Conditions

[00134] XRPD diffractograms were collected on a PANalytical Empyrean diffractometer using Cu Kα radiation (45 kV, 40 mA) in reflection geometry. The instrument is fitted with an Anton Paar CHC plus+ stage fitted with graphite/Kapton windows and equipped with air cooling and a low vacuum pump system using an Edwards RV3 pump. A programmable divergence slit (in automatic mode), with a 10 mm fixed incident beam mask, Ni filter and 0.04 rad Soller slits were used on the incident beam. A PIXcel^{3D} detector, placed on the diffracted beam, was fitted with a programmable anti-scatter slit (in automatic mode) and 0.04 rad Soller slits.

[00135] The software used for data collection was X'Pert Data Collector and the data analysed and presented using Diffrac Plus EVA or Highscore Plus.

[00136] For variable temperature (VT) experiments the samples were prepared and analysed in an Anton Paar chromed sample holder. A heating/cooling rate of 10 °C/min was used with a 2 min isothermal hold before the measurement started. The measurement parameters are as per the standard screening data collection method (detailed above). Measurements were taken at the following temperatures: 25, 50, 75, 100, 160, and 25 °C. The sample was then reanalyzed by XRPD after 1 h to check for complete rehydration.

[00137] For vacuum experiments the samples were prepared and analysed in an Anton Paar chromed sample holder. The measurement parameters are as per the standard screening data collection method (detailed above), at 25 °C with no vacuum (I2). A vacuum of around 50 mbar was then applied and the sample measured every 5 min until the anhydrate pattern was obtained for three consecutive measurements (to ensure complete dehydration, up to sample I8). The vacuum was then released and the sample analysed every 5 min for 6 measurements (until sample I22). The sample was then reanalyzed by XRPD after 1 h to check for complete rehydration.

^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR)

[00138] ^{1}H NMR spectra were collected on a Bruker 400 MHz instrument equipped with an auto-sampler and controlled by a DRX400 console. Samples were prepared in DMSO-*d6* solvent, unless otherwise stated. Automated experiments were acquired using ICON-NMR configuration within Topspin software, using standard Bruker-loaded experiments (^{1}H). Off-line analysis was performed using ACD Spectrus Processor.

Differential Scanning Calorimetry (DSC)

[00139] DSC data were collected on a TA Instruments Discovery DSC equipped with a 50 position auto-sampler. Typically, 0.5- 3 mg of each sample, in a pin-holed aluminum pan, was heated at 10 °C/min from 25 °C to 300 °C. A purge of dry nitrogen at 50 ml/min was maintained over the sample.

[00140] The instrument control software was TRIOS and the data were analysed using TRIOS or Universal Analysis.

Thermo-Gravimetric Analysis (TGA)

[00141] TGA data were collected on a TA Instruments Discovery TGA, equipped with a 25 position auto-sampler. Typically, 5 - 10 mg of each sample was loaded onto a pre-tared aluminum DSC pan and heated at 10 °C/min from ambient temperature to 350 °C. A nitrogen purge at 25 ml/min was maintained over the sample.

[00142] The instrument control software was TRIOS and the data were analysed using TRIOS or Universal Analysis.

Polarized Light Microscopy (PLM)

[00143] Samples were studied on a Nikon SMZ1500 polarized light microscope with a digital video camera connected to a DS Camera control unit DS-L2 for image capture. The sample was viewed with appropriate magnification and partially polarized light, coupled to a λ false-color filter.

Hot Stage Microscopy (HSM)

[00144] Hot Stage Microscopy was carried out using a Leica LM/DM polarized light microscope combined with a Mettler-Toledo FP82HT hot-stage and a digital video camera for image capture. A small amount of each sample was placed onto a glass slide with individual particles separated as well as possible. The sample was viewed with appropriate magnification and partially polarized light, coupled to a λ false-color filter, whilst being heated under ambient temperature, typically at 10 - 20 °C/ min. Data was collected using StudioCapture.

Gravimetric Vapour Sorption (GVS)

[00145] Sorption isotherms were obtained using a SMS DVS Intrinsic moisture sorption analyzer, controlled by DVS Intrinsic Control software. The sample temperature was maintained at 25 °C by the instrument controls. The humidity was controlled by mixing streams of dry and wet nitrogen, with a total flow rate of 200 ml/min. The relative humidity was measured by a calibrated Rotronic probe (dynamic range of 1.0 - 100 %RH), located near the sample. The weight change, (mass relaxation) of the sample as a function of %RH was constantly monitored by a microbalance (accuracy ±0.005 mg).

[00146] Typically, 5 - 30 mg of sample was placed in a tared mesh stainless steel basket under ambient conditions. The sample was loaded and unloaded at 40 %RH and 25 °C (typical room conditions). A moisture sorption isotherm was performed as outlined below

(2 scans per complete cycle). The standard isotherm was performed at 25 °C at 10 %RH intervals over a 0 - 90 %RH range. Typically, a double cycle (4 scans) was carried out. Data analysis was carried out within Microsoft Excel using the DVS Analysis Suite.

Table 2. Method for SMS DVS intrinsic experiments.

Parameter	Value
Adsorption - Scan 1	40-90
Desorption, Adsorption -Scan 2	90- 0, 0- 40
Intervals (%RH)	10
Number of Scans	4
Flow rate (ml/min)	200
Temperature (°C)	25
Stability (°C/min)	0.2
Sorption Time (hours)	6 hour time out
Number of cycles	2

[00147] The sample was recovered after completion of the isotherm and re-analyzed by XRPD.

Chemical Purity Determination by HPLC

[00148] Purity analysis was performed on an Agilent HP1100 series system equipped with a diode array detector and using ChemStation software. The full method details are provided below in Table 3.

Table 3. HPLC method for chemical purity determination.

Parameter	**Value**		
Type of method	Reverse phase with gradient elution		
Sample Preparation	0.5 mg/ml in acetonitrile : water 1:1		
Column	Supelco Ascentis Express C18, 100x4.6mm, 2.7μm		
Column Temperature (°C)	25		
Injection (μL)	5		
Wavelength Bandwidth (nm)	255, 90		
Flow Rate (ml/min)	2		
Phase A	0.1% TFA in water		
Phase B	0.085% TFA in acetonitrile		
Timetable	**Time (min)**	**% Phase A**	**% Phase B**
	0	95	5
	6	5	95
	6.2	95	5
	8	95	5

Water Determination by Karl Fischer Titration (KF)

[00149] The water content of each sample was measured on a Metrohm 874 Oven Sample Processor at 1.50 °C with 851 Titrano Coulometer using Hydranal Coulomat AG oven reagent and nitrogen purge. Weighed solid samples were introduced into a sealed sample vial. Approximately 10 mg of sample was used per titration and duplicate determinations were made. An average of these results is presented unless otherwise stated. Data collection and analysis were performed using Tiamo software.

Thermodynamic Aqueous Solubility

[00150] Aqueous solubility was determined by suspending sufficient compound in relevant media to give a maximum final concentration of ≥200 mg/ml of the parent freeform of the compound. The suspension was equilibrated at 25 °C, on a Heidolph plate shaker set to 750 rpm for 24 hours. The pH of the saturated solution was then measured and the suspension filtered through a glass fibre C filter (particle retention 1.2 µm) and diluted appropriately. Quantitation was by HPLC with reference to a standard solution of approximately 0.15 mg/ml in DMSO. Different volumes of the standard, diluted and undiluted sample solutions were injected.

[00151] The solubility was calculated using the peak areas determined by integration of the peak found at the same retention time as the principal peak in the standard injection.

Table 4. HPLC method for solubility measurements.

Parameter	**Value**		
Type of method	Reverse phase with gradient elution		
Column	Phenomenex Luna, C18 (2) 5 µm 50 x 4.6 mm		
Column Temperature (°C)	25		
Standard Injections (µl)	1, 2, 3 4, 5, 7		
Test Injections (µl)	1, 2, 3, 10, 15, 20		
Detection: Wavelength, Bandwidth (nm)	260, 90		
Flow Rate (ml/min)	2		
Phase A	0.1% TFA in water		
Phase B	0.085% TFA in acetonitrile		
Timetable	**Time (min)**	**% Phase A**	**% Phase B**
	0	95	5
	1	80	20
	2.3	5	95
	3.3	5	95
	3.5	95	5
	4.4	95	5

[00152] Analysis was performed on an Agilent HP1100 series system equipped with a diode array detector and using ChemStation software.

Raman Spectroscopy

[00153] Data were collected on a Renishaw inVia Qontor. Instrument control, data analysis and presentation software was WiRE.

[00154] Method: excitation source, λex = 633 nm or 785 nm laser, attenuated appropriately to avoid sample degradation. Raman shift range: 100 - 5000 cm^{-1}. Exposure time: 0.02 -10 s. Accumulations: 1 – 3. Alternatively, Raman shift range: 180 - 1700 cm^{-1}. Exposure time: 30 s. Accumulations: 3.

Crystal 16

[00155] A Crystal16 crystallization system (Technobis, NL) was used to determine the solubility and metastable zone of the material as a function of temperature.

[00156] Slurries of the API, in different overall concentrations/ were prepared by adding a known amount of solid to a known amount of chilled solvent (between 0.5 and 1.5 ml) and stirred at 400 rpm using a magnetic bar. The saturation temperature was measured through cycles of heating and cooling from -8 to 70 °C at 0.5 °C/min.

[00157] Upon increasing the temperature/ the solid completely dissolved and the suspension became a clear solution such that the light transmission reached its maximum value. This temperature is assigned as the clear point/ which was assumed to coincide with the saturation temperature. Then by cooling the solution at a rate of 0.5 °C/min, the temperature at which particles first formed was detected by a decrease in the light transmission. This is assigned as the cloud point. The points were fitted by a Van't Hoff equation and the difference between the cloud and the clear points defined the metastable zone width (MSZW) of the system. The instrument control software was Crystallization Systems and the data were analysed using Crystal Clear and Microsoft Excel.

Focused Beam Reflectance Measurement (FBRM)

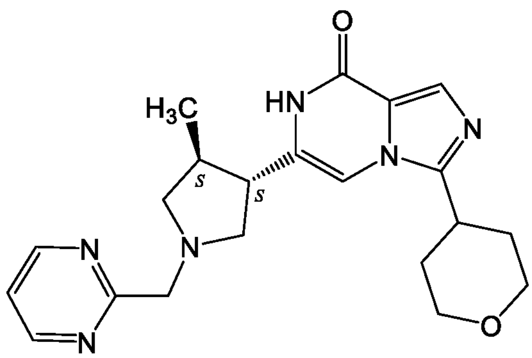
[00158] Particle size distribution was collected using an FBRM probe G400 by collecting data every 10 seconds. Data was processed with iC FBRM SP1 software.

Single Crystal X-Ray Diffraction (SCXRD)

[00159] Data were collected on a Rigaku Oxford Diffraction Supernova Dual Source, Cu at Zero, Atlas CCD diffractometer equipped with an Oxford Cryosystems Cobra cooling device. The data were collected using Cu Kα or Mo Kα radiation as stated in the experimental tables. Structures were solved and refined using the Bruker AXS SHELXTL suite or the OLEX2 crystallographic software. Full details can be found in the CIF. Unless otherwise stated, hydrogen atoms attached to carbon were placed geometrically and allowed to refine with a riding isotropic displacement parameter. Hydrogen atoms attached to a heteroatom were located in a difference Fourier synthesis and were allowed to refine freely with an isotropic displacement parameter. A reference diffractogram for the crystal structure was generated using Mercury.

Example 1. Synthesis of Compound **1.**

[00160] Compound **1** is an enantiomer of 6-[4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-3-tetrahydropyran-4-yl-7H-imidazo[1,5-a]pyrazin-8-one disclosed in WO 2013/053690. Compound **1** may be prepared from chiral-selective purification from 6-[4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-3-tetrahydropyran-4-yl-7H-imidazo[1,5-a]pyrazin-8-one prepared according to the method disclosed in WO 2013/053690, the contents of which are incorporated herein by reference in their entirety. Compound **1** may also be prepared with the method disclosed in WO 2017/005786, the contents of which are incorporated herein by reference in their entirety.



Example 2. Crystal Structure Determination and Characterization of MH1.

Single Crystal Growth Experiments

[00161] Crystallization can be obtained by lowering the temperature of a clear solution. The solubility of most materials decreases with temperature, and hence, cooling can be used to cause supersaturation. Solvents used in this study were Isopropyl Acetate; Ethanol; Tetrahydrofuran; Water; Dichloromethane; Acetonitrile; Anisole; Methylisobutyl Ketone; Nitromethane; 1,2-Dimethoxyethane; Methylethyl Ketone; 1,4-Dioxane; n-Propyl Acetate; 2-Propanol; Acetone; Cumene; N,N-Dimethylformamide; Dimethyl Sulfoxide; Methanol;

2-Methyl Tetrahydrofuran; MeCN/ 5% water; IPA/ 5% water; EtOH/ water 1:1; and THF/ 10% water.

[00162] 5.0 mg MH1 (off-white powder) was weighed into 24 HPLC vials and treated with various solvents (50 µl) at room temperature (RT). The sample was then placed at 50 °C for 5 minutes. Any solutions obtained at RT or 50 °C were placed in a refrigerator at 4 °C. A further aliquot of solvent (100 µl) was added to any suspensions and these were then placed at (50 °C) for 1 hour. Any remaining suspensions were then filtered by syringe and the mother liquors placed in the fridge at 4 °C. Any solutions obtained after the addition of solvent after 1 hour were also placed in a refrigerator.

[00163] Crystals suitable for analysis were only obtained initially on cooling from isopropyl acetate, anisole, methylisobutyl ketone, n-propyl acetate, cumene and 2-methyl THF. A crystal of sufficient size and quality for analysis by single crystal X-ray diffraction was isolated with approximate dimensions 0.40 x 0.15 x 0.08 mm obtained by cooling the mother liquor from a saturated solution of the compound in n-propyl acetate.

[00164] The crystal structure of MH1 was determined at 293 and 100 K. The crystals are orthorhombic, space group $P2_12_12_1$ with the final R1=[$I > 2\sigma(I)$] = 4.25 and 3.46 % respectively and the Flack parameter = 0.02(8) and -0.05(7) at 293 K and 100 K respectively. The absolute stereochemistry of the compound has been determined as (*S*, *S*). The compound was identified as depicted in FIG. 1. There is one molecule of Compound **1** and one molecule of water in the asymmetric unit, both fully ordered. XRPD patterns were calculated from the crystal structures and compared to the experimental diffractogram for the material as received at room temperature. An overlay of the experimental diffractogram at room temperature (FIG. 2B) and the simulated XRPD patterns for MH1 at 293 K and 100 K shows they are consistent. Any slight differences are attributable to lattice variations with temperature and preferred orientation.

[00165] A summary of the characterization data for MH1 is in Table 5.

Table 5. Characterization data for MH1.

Technique	Characterization
XRPD	Crystalline MH1 (FIG. 2A)
1H-NMR	Consistent with structure
TGA	3.8% w/w mass loss ambient – 90 °C. 0.1% w/w loss at the melting point. (FIG. 5)
DSC	Broad endotherm from 40 – 100 °C (73 J/g)

	Large endotherm onset 184.4 °C (68 J/g) (FIG. 5)
Purity by HPLC	99.4%
KF	2.7%
GVS	Max uptake of 4.7%, no hysteresis, large step between 1020% RH
FTIR	Collected as reference (FIG. 3; Table 6)
Raman	Collected as reference (785 nm) (FIG. 4; Table 7)

Table 6. FTIR peak table for MH1.

cm^{-1}	%T	Intensity	cm^{-1}	%T	Intensity	cm^{-1}	%T	Intensity	cm^{-1}	%T	Intensity
668	92.3	M	996	94.0	W	1257	94.0	W	1476	93.9	W
712	93.6	W	1004	94.2	W	1265	94.0	W	1544	93.5	W
746	90.5	M	1014	93.6	W	1284	93.6	W	1562	87.8	S
782	88.7	S	1021	94.7	W	1292	94.4	W	1604	94.7	W
808	89.631	M	1046	95.0	W	1303	93.2	M	1655	82.8	VS
816	89.127	M	1055	94.1	W	1324	91.4	M	2807	94.8	W
836	94.18	W	1085	90.1	M	1333	92.4	M	2852	95.6	W
845	93.857	W	1110	92.8	M	1351	90.6	M	2870	95.9	W
870	94.546	W	1123	88.6	S	1361	90.2	M	2917	95.0	W
888	90.823	M	1136	93.8	W	1381	93.7	W	2955	94.3	W
913	95.345	W	1148	93.7	W	1390	94.2	W	2963	94.6	W
927	93.207	M	1161	92.2	M	1413	90.7	M	3078	95.2	W
943	93.438	W	1167	92.4	M	1433	90.7	M	3107	95.3	W
964	93.947	W	1199	95.0	W	1452	92.3	M	3431	96.4	W
987	90.082	M	1241	92.9	M	1465	94.6	W	3537	96.5	VW

Key: W-weak, M-medium, S-strong, VS-very strong

Table 7. Raman peak table for MH1.

Band centre/ cm^{-1}	Absolute intensity/ a.u	Band centre/ cm^{-1}	Absolute intensity/ a.u	Band centre/ cm^{-1}	Absolute intensity/ a.u	Band centre/ cm^{-1}	Absolute intensity/ a.u
217.3	12597	634.2	7887	1076.4	38792	1455.0	37645
233.9	8008	642.9	16466	1090.8	6061	1477.3	120271
256.0	41662	668.3	6531	1111.0	31567	1545.1	42516
279.6	17895	712.2	10035	1123.1	13450	1571.9	11241
313.9	18410	790.6	5200	1168.5	72312	-	-
339.5	8409	816.4	47312	1199.9	8600	-	-
369.4	8047	836.4	27888	1240.0	4881	-	-
398.1	9543	847.5	6629	1269.5	13599	-	-
457.7	11219	870.6	26632	1278.5	21228	-	-
476.0	7098	887.4	6800	1293.7	12122	-	-
534.7	10630	941.0	7904	1306.7	18711	-	-
554.2	3744	997.6	53279	1327.8	16145	-	-
573.5	11517	988.7	5529	1366.0	60204	-	-
605.1	5479	1021.0	19238	1383.4	10783	-	-
623.8	5852	1037.5	5146	1434.8	23876	-	-

Example 3. Crystal Structure Determination of MH2 of Compound **1**.

Single Crystal Growth Experiments

[00166] The supplied MH1 (1.6 g) was placed in a vacuum oven at 50 °C for 3 h. The sample was then treated with 7.5% Water/MeOAc (10 vol, 16 ml) at 5 °C. After 12 h at 5 °C, the suspension was filtered, washed with heptane and air dried.

[00167] Crystals suitable for analysis were obtained after washing the vial with n-heptane during the filtration procedure. These crystals were used to determine the single crystal structure at 100 K.

Crystallisation Method	Form
Fast evaporation (heptane wash)	MH2

Crystal Structure of MH2

[00168] Crystals of MH2 were obtained by fast evaporation when washing with heptane. A crystal of sufficient size and quality for analysis by single crystal X-ray diffraction was isolated with approximate dimensions 0.65 x 0.26 x 0.18 mm. The crystal structure of MH2 was determined at 100 K. The crystals are orthorhombic, space group $P2_12_12_1$ with the final $R_1 = [I > 2\sigma(I)] = 3.07$ %. The compound was identified as depicted in FIG. 6. The asymmetric unit contains one molecule of Compound 1 and one molecule of water, both fully ordered. The absolute configuration of MH2 has been determined with C7 and C9 in the (*S*,*S*) configuration, with the Flack parameter = -0.01(8).

[00169] An XRPD pattern was calculated from the crystal structure and compared to the experimental diffractogram of the MH2 material at room temperature (FIG. 7). The simulated diffractogram was generally consistent with the bulk material. Any slight differences are mostly likely attributable to lattice variations with temperature and preferred orientation.

Example 4. Transformations between Anhydrous (AH) and Hydrated Forms (MH1 and MH2).

Dehydration Investigations of MH1

[00170] MH1 was initially placed in a vacuum oven at 50 °C overnight. The sample was then returned to the vacuum oven and stored for 5 days at 50 °C and then for 4 hours at 90 °C. An aliquot was then removed from storage at 90 °C and held at ambient conditions for 2 hours. At each time point, analysis by TGA was undertaken.

[00171] A weight loss attributed to water was observed in all samples post drying. This is due to the sample either not losing the water completely at the temperatures investigated or reabsorbing water at ambient conditions. To determine the impact of vacuum and heating on the crystalline form further XRPD investigations were performed.

VT XRPD

[00172] MH1 was analysed at 25, 50, 75, 100, 160 and 25 °C (10 °C/min heating rate and a 2 min wait before the measurement started) on the Empyrean. The sample was then reanalysed by XRPD after 10 and 20 min to check for rehydration.

[00173] Initially the sample is MH1. As the temperature is increased, the sample converts to the anhydrate form and by 75 °C it is fully anhydrous by XRPD. Upon re-cooling to 25 °C, peaks corresponding to MH1 are present. Upon further standing at 25 °C these peaks become more intense as those corresponding to the anhydrate become less intense. This indicates that under ambient conditions, the anhydrate readily converts to MH1. TGA post VT XRPD gives 3.2% mass loss, confirming re-uptake of water.

XRPD Under Vacuum

[00174] MH1 was analysed at 25 °C with no vacuum. A vacuum of around 50 mbar was then applied and the sample measured every 5 min until the anhydrate pattern was obtained for three consecutive measurements (to ensure complete dehydration). The vacuum was then released and the sample analysed every 5 min for 6 measurements before the front of the sample stage was removed.

[00175] Initially, the sample is MH1, as the sample is stored under vacuum the sample converts to the anhydrate form (after 10 min it is fully anhydrous by XRPD). Upon releasing the vacuum, no change is initially seen for 30 min. The front of the sample stage is then taken off to allow the ambient air to penetrate the sample stage. Peaks corresponding to MH1 immediately appear and the sample is fully hydrated by XRPD after 15 min. This indicates that under ambient conditions, the anhydrate readily converts to MH1. TGA post vacuum XRPD gives 3.4% mass loss.

[00176] The results of the drying investigations confirm that although it is possible to obtain the anhydrous form, this material quickly converts back to the monohydrate, MH1. Compression studies on MH1 showed no conversion to the anhydrate by XRPD.

Water Activity Experiments for MH1

[00177] MH1 (30.0 mg) was weighed into HPLC vials and placed in a vacuum oven to dry over the weekend. All samples were then placed in a desiccator prior to use to ensure dryness. Solvent (300 µl) was added and slurries agitated at either 25 ^{0}C or 5 ^{0}C. Further MH1 was added to any solutions formed to return to a slurry. Ethyl acetate and methyl acetate were both dried prior to use. After four days, the samples were analysed by XRPD with minimum exposure to ambient conditions.

[00178] Selected samples were also characterised after standing at ambient for 2 days by XRPD, 1H NMR, TGA and DSC.

[00179] The results obtained from the water activity experiments show at water activity of $a_w \leq 0.4$ the monohydrate MH1 was obtained. At a_w= 0.5 and 0.6 at 25 °C the monohydrate MH1 was also obtained. However, a new form was observed at 5 °C. This form was then observed at both 5 and 25 °C at a_w=0.7-0.9. Re-analysis of selected samples of this form after two days at ambient conditions confirmed they remained the same form. The new form is also a monohydrate form and has been identified as MH2.

Table 8. Results of Water Activity Experiments.

Solvent	Water activity	Results for 5 °C	Results for 25 °C
EtOAc	Sieves	MH1	MH1
0.5% water / EtOAc	Aw = 0.3	MH1	MH1
0.8% water / MeOAc	Aw = 0.4	MH1	MH1
1% water / EtOAc	Aw = 0.5	New Form	MH1
1.5% water / EtOAc	Aw = 0.6	New Form	MH1
2% water / EtOAc	Aw = 0.7	New Form	New Form
2.7% water / EtOAc	Aw = 0.8	New Form	New Form
7.5% water / MeOAc	Aw = 0.9	New Form	New Form

Scale up of MH2

[00180] MH 1 (1.6 g) was placed in a vacuum oven at 50 °C for 3 h. The sample was then treated with 7.5% Water/MeOAc (10 Vol, 16 ml) at 5 °C. After 12 h at 5 °C, an aliquot of the sample was, analysed by XRPD. The suspension was then filtered, washed with heptane, air dried and analysed by XRPD and the appropriate techniques. Washing the vial with heptane caused crystals to form on the wall of the vial which were analysed by SCXRD. A summary of the characterization results is shown in Table 9.

[00181] ***Results and*** discussion*:* Attempts to prepare MH2 were successful. XRPD overlay of the scale up of MH2 is shown in Fig. 8. The TGA data revealed a 4.4% mass loss between 25 and 100 °C corresponding to 1 equivalent (eq) of water, indicating that the sample is a monohydrate. This was confirmed by the KF measurement with 4.5% water detected in the sample. The water loss was also noted in the DSC data with a broad endotherm at 59.1 °C followed by an endotherm indicative of melting at 184.7 °C. This endotherm corresponds to the melt of the AH and is very close in value with the melting point obtained when starting from MH1 (185.6 °C). The ^{1}H-NMR spectrum is consistent with the structure as well as the reference spectrum of MH1. GVS data showed a lack of uptake in the first sorption cycle from 40 to 90% RH. This was followed by 4.8% weight loss in the desorption cycle. An uptake of 4.5% was observed in the second sorption cycle. This suggested that MH2 transformed into AH during the desorption cycle followed by conversion to a monohydrate during the sorption cycle. The transformat ion was confirmed by the post-GVS XRPD analysis, which indicated that it had rehydrated as MH1. The Raman spectra of MH1 and MH2 contain similar features with the main differences at 1350 and 1650 cm-1. This is expected, as any differences in Raman spectra between polymorphs are often minor. The stereochemistry of MH2 was determined by SCXRD as the *S,S*-enantiomer (same as MH1). PLM shows that the morphology of the sample is crystalline plates mixed with irregular shapes. The thermodynamic solubility in water of MH2 at 25 °C is 36.5 mg/ml

Table 9. Characterization data for MH2.

XRPD	Crystalline form - Matches Monohydrate Form 2 (MH2)
Purity HPLC	99.3%
^{1}H-NMR	Consistent with reference spectrum
DSC	Broad endotherm at 59.1 °C followed by sharp endotherm corresponding to melt ing at 184.7 °C
TGA	4.4% w/w between 25-100 °C
KF	4.5% water detected
GVS	First sorption cycle from 40 to 90% RH, no uptake. Followed by 4.8% weight loss in the desorption cycle. Second sorption cycle, uptake of 4.5%.
XRPD post-GVS	Crystalline - Matches MH1
PLM	Crystalline plates and irregular shapes
Thermodynamic Solubility in water	36.5 mg/ml (25 °C)
Raman	Similar spectrum between MH1 and MH2 with main differences observed at 1350 and 1650 cm^{-1}
SCXRD	Monohydrate S,S-enantiomer (different to MH1)

Further Analysis of MH1 and MH2

Example 5. XRPD Investigation of MH2.

[00182] ***Procedure***: A second batch of MH2 material was prepared using the same procedure. This sample was then analysed by VTXRPD and by XRPD under vacuum.

[00183] ***Results and discussion***: MH2 converts to AH (anhydrate) upon heating. A mixture of MH2 and AH was formed at 50 °C followed by a complete conversion to AH at 75 °C. After cooling back to 25 °C the AH has completely transformed into MH1. The MH1 formation was confirmed by XRPD after 1 h storage at ambient conditions.

[00184] The MH2 sample dehydrated into AH after 5 minutes exposure to vacuum. After 15 minutes the vacuum was released. XRPD data were collected for 55 minutes and the final pattern collected was a mixture of AH and MH1. The sample was reanalyzed after 1 h and a complete rehydration to MH 1 was observed.

Example 6. Drying Investigation of the Two Hydrates (MH1 and MH2).

[00185] ***Procedure:*** MH1 and MH2 were placed in a vacuum oven at 50 °C and RT for 24 h. The samples were then analysed by TGA and XRPD immediately after removal and after 4 hours standing under ambient conditions.

[00186] ***Results and discussion*:** The samples were analyzed by XRPD and TGA immediately after they were removed from the oven (T=0) and then remeasured after 4 h (T=4 h). At T=0 both MH1 and MH2 (dried at RT and 50 °C) converted to a mixture of MH1 and AH by XRPD. A small amount of water loss was recorded by TGA for both samples. This indicates that both hydrates were converted to AH during vacuum oven drying. After 4 h at ambient conditions the XRPD data show a full conversion of both samples into MH1. However, the TGA data indicated that the rehydration is not fully complete with only ~1.8-2.7% water lost. Based on these results, it appears that MH2 converts into AH during drying, followed by a conversion to MH1 at ambient storage.

Table 10. Drying investigation of MH1 and MH2.

Compound	MH 1	MH2
XRPD (T=0, RT)	Matches MH1 and AH	Matches MH1 and AH
TGA (T=0, RT)	0.3 % w/w lost between 25 and 100 °C	0.2 % w/w lost between 25 and 100 °C
XRPD (T=0, 50 °C)	Matches MH1 and AH	Matches MH1 and AH
TGA (T=0, 50 °C)	0.3 % w/w lost between 25 and 100 °C	0.6 % w/w lost between 25 and 100 °C
XRPD (T=4 h, RT)	Matches MH1	Matches MH1
TGA (T=4 h, RT)	1.8 % w/w lost between 25 and 100 °C	1.9 % w/w lost between 25 and 100 °C

XRPD (T=4 h, 50 °C)	Matches MH1	Matches MH1
TGA (T=4 h, 50 °C)	2.7 % w/w lost between 25 and 100 °C	1.9 % w/w lost between 25 and 100 °C

Example 7. Stability Studies with Competitive Slurries of the Two Hydrates (MHl and MH2).

[00187] ***Procedure:*** Saturated solutions of the supplied MH 1 (J08343) were prepared in different solvents/systems (1 ml). The saturated solutions were then filtered and used for competitive slurry experiments.

[00188] MH1 and MH2 (ca. 15 mg each) were physically mixed before they were treated with the filtered saturated solutions (300 μL). The samples were stirred at 25 °C for 24 h then filtered, air dried and analysed by XRPD.

[00189] The same solvents were used as for samples in water activity experiments with additional IPA/ heptane mixtures as this is the current crystallisation solvent. This procedure was also followed for IPA/heptane at 5 and 50 °C (3 days slurrying).

[00190] ***Results and Discussion*:** MH2 was predominantly obtained during competitive slurries at 25 °C (Table 11). The solvents that yielded MH2 had a range of different values for water activity. Mixtures of MH1 and MH2 were obtained even though water was absent from the solvent systems (i.e. no conversion to AH was noted). MH2 is preferred in IPA and a mixture of IPA with heptane at 5 °C. However, mixtures of MH 1 and MH2 or pure MH1 were produced when a higher temperature was employed (50 °C) (Table 12).

[00191] Therefore, competitive slurries indicate that MH2 is preferred at a lower isolation temperature as well as at a higher water activity. Whereas, MH1 is preferred at a higher isolation temperature and a lower water activity.

[00192] Further experiments were designed to increase the diversity of solvents/systems studied while still investigating the effect of water activity on the form obtained. This was with a view to define a solvent list for Phase 3 (Solvent Selection). Further saturated solutions were prepared as described in the procedure section using MH1 as supplied.

[00193] Some samples dissolved during competitive slurries, this is likely because complete saturation had not occurred. MH2 is again favoured at lower temperature with a high water activity (Table 13). A mixture of MH1 and MH2 was obtainedfrom: heptane at 5 °C, IPA or a mixture of IPA: heptane at 50 °C. The IPrOAc: 0.5% water mixture (a_w = 0.35) favoured the formation of MH1 at 50 °C, but MH2 at 5 °C. Pure MH 1 was also obtained when THF solvent was used at 50 °C.

[00194] Although MH2 was preferred at low temperature and high a_w, MH1 was selected for further development as MH2 converts to MH1 (via AH) and it can be obtained using higher temperatures and lower water activities.

Table 11. Competitive slurry of the two hydrates at RT (MH1 and MH2).

Sample ID	Solvent	Water activity	Observations after solvent addition	Observation after 24 h at 25 °C	XRPD
32-01	EtOAc	-	Suspension	Suspension	MH1+MH2
32-02	0.5% water/EtOAc	$a_w = 0.3$	Suspension	Suspension	MH2
32-03	0.8% water/MeOAc	$a_w = 0.4$	Suspension	Suspension	MH2
32-04	1% water/EtOAc	$a_w = 0.5$	Suspension	Suspension	MH2
32-05	1.5% water/EtOAc	$a_w = 0.6$	Suspension	Suspension	MH2
32-06	2% water/EtOAc	$a_w = 0.7$	Suspension	Suspension	MH2
32-07	2.7% water/EtOAc	$a_w = 0.8$	Suspension	Suspension	MH2
32-08	7.5% water/MeOAc	$a_w = 0.9$	Suspension	Suspension	MH2
32-09	IPA	-	Suspension	Suspension	MH1+MH2
34-02	IPA:Heptane 1:3	-	Suspension	Suspension	MH1+MH2
34-03	IPA:Heptane 1:2	-	Suspension	Suspension	MH1+MH2

Table 12. Competitive slurry of the two hydrates 5 and 50 °C (MH1 and MH2).

Sample ID	Solvent	Temperature (°C)	Observations after solvent addition	Observations after 24 h	XRPD
34-04	IPA:Heptane 1:3	5	Suspension	Suspension	MH2
34-05	IPA:Heptane 1:2	5	Suspension	Suspension	MH2
34-08	IPA	5	Suspension	Suspension	MH2
34-06	IPA:Heptane 1:3	50	Suspension	Suspension	MH1
34-07	IPA:Heptane 1:2	50	Suspension	Suspension	MH1+MH2
34-09	IPA	50	Suspension	Suspension	MH1+MH2

Table 13. Additional competitive slurry of the two hydrates at 5 and 50 °C (MH1 and MH2).

Sample ID	Solvent	Water activity	Temp. (°C)	Observations after solvent addition	Observations after 3 days 24 h	XRPD
35-01	Water	$a_w = 1$	5	Suspension	Solution	-
35-02	THF:10%water	$a_w = 0.9$	5	Suspension	Solution	-
35-03	IPA:10%water	$a_w = 0.75$	5	Suspension	Solution	-
35-04	Ethanol:7%water	$a_w = 0.5$	5	Suspension	Solution	-
35-05	IPrOAc:0.5%water	$a_w = 0.35$	5	Suspension	Suspension	MH2
35-06	MeOH:5%water	$a_w = 0.2$	5	Suspension	Solution	-
35-07	IPA	-	5	Suspension	Solution	-
35-08	IPA:Heptane (1:2)	-	5	Suspension	Suspension	MH2
35-09	THF	-	5	Suspension	Suspension	-
35-10	Heptane	-	5	Suspension	Suspension	MH1+MH2
35-11	Water	$a_w = 1$	50	Suspension	Suspension	MH2
35-12	THF:10%water	$a_w = 0.9$	50	Suspension	Solution	-
35-13	IPA:10%water	$a_w = 0.75$	50	Suspension	Suspension	MH2
35-14	Ethanol:7%water	$a_w = 0.5$	50	Suspension	Solution	-
35-15	IPrOAc:0.5%water	$a_w = 0.35$	50	Suspension	Suspension	MH1
35-16	MeOH:5%water	$a_w = 0.2$	50	Suspension	Solution	-
35-17	IPA	-	50	Suspension	Suspension	MH1+MH2
35-18	IPA:Heptane (1:2)	-	50	Suspension	Suspension	MH1+MH2
35-19	THF	-	50	Suspension	Suspension	MH1
35-20	Heptane	-	50	Suspension	Solution	-

Structure Comparison of MH1 and MH2

[00195] The unit cell and the asymmetric units of MH1 and MH2 were compared (Table 14, measured at 100 K). The two hydrated structures have the same space group and have similar size unit cells. However, the asymmetric units significantly differ.

[00196] In MH1 the water and the API (Compound **1**) in the asymmetric unit participate in one O-H---O intermolecular hydrogen bond. In addition, there is an intramolecular, bifurcated, asymmetrical hydrogen bond between the nitrogen atom of the imidazopyrazine ring and the nitrogen atoms of pyrimidine and pyrrolidine rings.

[00197] In MH2 the water molecule is located between the imidazopyrazine and pyrimidine rings, hence the intramolecular hydrogen bonding present in MH1 is replaced by the intermolecular interaction between water and the API.

Table 14. Single crystal structure comparison of MH1 and MH2.

MH1		MH2	
Crystal system		Crystal system	
Orthorhombic		Orthorhombic	
Space group	$P2_12_12_1$	Space group	$P2_12_12_1$
Unite cell dimensions		Unite cell dimensions	
a = 8.97778(18) Å	α = 90°	a = 9.20995(16) Å	α = 90°
b = 10.84333(16) Å	β = 90°	b = 11.01308(16) Å	β = 90°
c = 21.2411(4) Å	γ = 90°	c = 20.7812(3) Å	γ = 90°
Volumne = 2067.80(6) $Å^3$		Volumne = 2107.84(6) $Å^3$	
R factor = 3.46%		R factor = 3.07%	
Z' = 4		Z' = 4	

[00198] ***Results and discussion*:** The experiments performed in this study showed that MH1 is the most stable form at ambient conditions. Solvent mixtures with water (high water activities) and at lower temperature (5 °C) produced MH2 and hence should be avoided. Although MH1 was solely produced at the end of the crystallization process, care should be taken to avoid AH or MH2 formation.

<u>Example 8. Solubility Assessment of MH1.</u>

[00199] ***Procedure:*** Four solvents were chosen based on the competitive slurry experiments that favored MH1 as well as for diversity. Combinations of those solvents with water (a_w = 0.35) were also utilized to verify which hydrate is favored. Mixtures of the solvents with heptane (1:1) were used to check for crystal form with a good antisolvent.

[00200] MH1 (22 x 104 mg) was suspended in various solvents (11 x 0.5 ml) and stirred at either 5 or 50 °C for 24 hours at 750 rpm. The solids were isolated by filtration and centrifugation and the liquors were analysed by HPLC to determine their solubility (relative to a standard made up). The solids were also investigated by XRPD. This procedure was also followed for an additional five solvent systems comprising different ratios of THF:heptane and IPA:heptane at either 5 or 50 °C. The isolated solids were investigated by XRPD, HPLC and ^{1}HNMR.

[00201] ***Results and discussion*****:** Samples that formed clear solutions are considered to have solubility > 200 mg/ml (Table 15). This was the case for six of the samples at 50 °C such as: THF, ethanol, IPA, as well as several of those solvents combined with water or heptane. At 5 °C, solubility > 200 mg/ml was achieved in ethanol and the ethanol mixture with water. A large solubility was also measured using ethanol:heptane (1:1) (170 mg/ml) at 5 °C.

[00202] The samples produced a variation of MH1 and MH2, as well as a mixture of MH1 with AH at both 5 and 50 °C. As IPA and THF gave high solubility at 50 °C (>200 mg/ml) and THF:heptane (1:1) gave MH1 at both temperatures, further solubility assessments were carried out using mixtures of IPA:heptane (1:1. 1:2, 1:3) and THF:heptane (2:1, 1:2) (Table 16).

[00203] Based on the solubility observations, it was decided to proceed with the IPA/Heptane (1:3) system for the antisolvent crystallisation, as it gives a low solubility at 5 °C and no MH2 was observed. This solvent/system was selected for solubility and MSZW experiments, to explore the temperature dependence on the solubility of MH1.

Table 15. Solubility assessment of MH1.

Sample ID	Analysis Temperature (°C)	Media	Weight (mg)	Appearance	Solubility (mg/ml)	XRPD
40-01	5	THF	104.46	Suspension	79	MH2 + New peaks
40-02		THF:0.8%water	104.02	Suspension	76	MH2
40-03		THF:Heptane 1:1	103.93	Suspension	6.3	MH1
40-04		IPrOAc	103.90	Suspension	11	MH1
40-05		IPrOAc:0.5%water	103.89	Suspension	3.8	MH2
40-06		IPrOAc:Heptane 1:1	103.86	Suspension	1.9	MH1
40-07		Ethanol	103.73	Clear Solution	>200	-
40-08		Ethanol:4.2%water	103.93	Clear Solution	>200	-

40-09		Ethanol:Heptane 1:1	103.75	Suspension	170	MH1+AH
40-10		IPA	103.89	Suspension	130	MH1+AH
40-11		Heptane	103.80	Suspension	N/A*	MH1
40-12	50	THF	103.88	Clear Solution	>200	-
40-13		THF:0.8%water	103.85	Clear Solution	>200	-
40-14		THF:Heptane 1:1	106.30	Suspension & Solid on bottom	11	MH1
40-15		IPrOAc	103.84	Suspension & Solid on bottom	6.3	MH1+AH
40-16		IPrOAc:0.5%water	103.82	Suspension & Solid on bottom	5.6	MH1
40-17		IPrOAc:Heptane 1:1	103.91	Suspension & Solid on bottom	2.1	MH1
40-18		Ethanol	103.91	Clear Solution	>200	-
40-19		Ethanol:4.2%water	103.76	Clear Solution	>200	-
40-20		Ethanol:Heptane 1:1	103.75	Clear Solution	>200	-
40-21		IPA	103.85	Clear Solution	>200	-
40-22		Heptane	103.94	Suspension & Solid on bottom	N/A	MH1+AH

Table 16. Additional solubility assessment for MH1.

Sample ID	Analysis Temperature (°C)	Media	Weight (mg)	Appearance	Solubility (mg/ml)	XRPD	PLM
44-01	5	THF:Heptane 2:1	103.94	Suspension & Solid on bottom	17	MH2 (small MH1)	Small crystals, some agglomerates
44-02		THF:Heptane 1:2	104.01	Suspension & Solid on bottom/sides	0.33	MH1	Small crystals, some agglomerates
44-03		IPA:Heptane 1:1	104.16	Suspension & Solid on bottom	40	MH1	Small crystals, some agglomerates
44-04		IPA:Heptane 1:3	104.12	Suspension & Solid on bottom	8	MH1	Small crystals, some agglomerates
44-05		IPA:Heptane 1:2	104.02	Suspension & Solid on bottom/sides	14	MH1	Small crystals, some agglomerates
44-06	50	THF:Heptane 2:1	103.93	Suspension & Solid on bottom/sides	21	MH1	Large crystals
44-07		THF:Heptane 1:2	104.01	Suspension & Solid on bottom/sides	0.62	MH1	Small crystals
44-08		IPA:Heptane 1:1	104.04	Clear Yellow Solution	>200	-	-
44-09		IPA:Heptane 1:3	103.95	Suspension & Solid on sides	10	MH1	Small crystals, some agglomerates

44-10		IPA:Heptane 1:2	103.99	Suspension & Solid on bottom/sides	19	MH1	Small crystals, some agglomerates

Example 9. Solubility Studies on MH1 and MH2.

[00204] ***Procedure*****:** pH profiling solubility experiments were performed on MH1 and MH2. Solubility was determined in singlicate by suspending sufficient compound in relevant media (1.00 ml) to give a maximum final concentration as shown in the Table 17.

Table 17. Maximum Final Sample Concentrations.

Sample	pH	Max. Concentration (mg/ml)
A	1.2	1100
B	4.0	140
C	6.5	20
D	7.5	20

[00205] The suspensions were equilibrated at 25 °C, on a Heidolph plate shaker set to 750 rpm for 24 hours. Samples were pH adjusted as required using 0.5M/1M HCl and 0.2M NaOH to within 0.1 of the desired pH unit (where possible). The pHs of the saturated solutions were measured (where applicable) and an appearance was recorded. The suspensions were filtered through a glass fibre C filter (particle retention 1.2 µm) and diluted appropriately. Quantitation was by HPLC with reference to a standard solution of approximately 0.15 mg/ml in DMSO. Different volumes of the standard, diluted and undiluted sample solutions were injected. The solubility was calculated using the peak areas determined by integration of the peak found at the same retention time as the principal peak in the standard injection.

[00206] ***Results and discussion*****:** Upon the addition of media to MH1-A and MH2-A, a thick paste was observed indicating the media had been absorbed, therefore additional media was added to the sample vial and a suspension was observed.

[00207] After *ca.* 1 hour MH1-B, C and D and MH2-C and D were clear solutions, therefore additional material was added to the sample vials.

[00208] During the first pH adjustment of MH1-A, little shift in pH was observed when using 0.5M HCl, it was then decided that 1M HCl was to be used as the adjusting solution. The volume capacity of the vial was reached with the pH of the sample was 5.02. The volume in the vial was also reached for MH1-A with a measured pH of 6.63.

Table 18. Results from solubility experiments.

Sample ID	Weight (mg)	Media	Appearance	Final pH	Solubility (mg/ml)
MH1-A	1101.4	pH 1.2	Clear Solution	5.02	> 275
MH1-B	194.1	pH 4.0	Clear Solution	4.02	> 106
MH1-C	193.7	pH 6.5	Clear Solution	6.53	> 164
MH1-D	191.9	pH 7.5	Solid on bottom	7.51	62
MH2-A	1109.1	pH 1.2	Clear Solution	6.63	> 275
MH2-A	140.9	pH 4.0	Clear Solution	3.76	> 99
MH2-A	191.7	pH 6.5	Clear Solution	6.47	> 147
MH2-A	193.3	pH 7.5	Solid on bottom	7.52	58

[00209] The solubility for the A samples (initially in pH 1.2 media) was determined as >275 mg/ml, however, it is worth noting that the pH was not able to be maintained at pH 1.2 as the volume capacity of the vial was reached. Therefore, the solubility value obtained is for the final pH. These experiments were repeated using a reverse addition approach.

Solubility Repeat

[00210] ***Procedure:*** pH 1.2 buffered media (0.4 ml) was added to two separate 7 ml vials. For each compound, material was added in portions to the pH 1.2 media. After each addition of compound, the vial was vortex mixed and the pH and appearance was recorded. The samples were adjusted to pH 1.2 with 1M HCl to within 0.05 of the desired pH unit. This was repeated until no further material was available (~400 mg for each form, which would give a maximum concentration of around 1000 mg/ml if no adjustment was required).

[00211] ***Results and discussion:*** Clear solutions were observed for both samples due to the volume of adjusting solution required. However, the pH of both solutions was maintained at pH 1.2 throughout.

Table 19. Solubility of MH1 and MH2 at pH 1.2

Sample	Solubility (mg/ml)
MH1	>153
MH2	>161

<u>Example 10. Cystallizaton Process for the Preparation of MH 1.</u>

[00212] Dry (KF≤ 0.1%) 2-propanol (67 kg) was charged into the reactor under N_2 followed by crude solid Compound **1** (20.4 kg). Purified water (1 kg) and dry 2-propanol (3 kg) were subsequently added and the reactor temperature control was adjusted to 27-

35°C. The resulting reaction mixture was stirred under N_2 protection until all solid material had dissolved. Optionally, an in-process control (IPC) sample (KF) was performed to determine the water contents of the reaction mixture and enough dry 2-propanol was added to bring the water content to 1.0% (as verified by KF).

[00213] The temperature of the reaction mixture was adjusted to 22-28°C and seed crystals (0.24 kg) were added. The resulting reaction mixture was stirred for 0.5-2.0 h at 22-28°C. n-Heptane (246 kg) was charged slowly into the reactor at 22-28°C using a pump, such as a diaphragm pump, and the resulting reaction mixture was stirred at 22-28°C. In order to achieve complete desupersaturation and maximize yield, the resulting reaction mixture may be stirred for 8-12 hr. Optionally, at this point an IPC sample was taken to determine moisture, residual Compound **1** in the supernatant, purity and crystallinity of the precipitated solid.

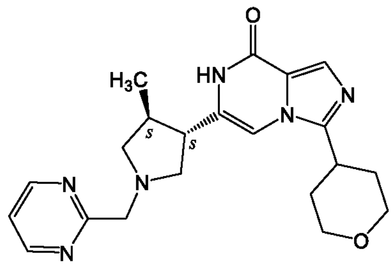
[00214] The reaction mixture was filtered at 22-28°C and the resulting solid was washed with n-heptane (27.8 kg). The filter cake was pressed until dried and subsequently dried under a flow of N_2 for 1-2 hrs. Optionally, at this point an IPC sample confirmed purity and crystallinity of the isolated solid.

[00215] The solid was dried over a saturated solution of sodium chloride in the drying chamber at 20-27°C under a constant flow of nitrogen for 10-18 hr. The solid was removed from the dryer, sieved and packed into a drum lined with LDPE bags. A release sample confirmed moisture (KF = 4.2%) and crystallinity (XRPD: MH 1) along with other purity related release methods. Yield: 17.46 kg MH1.

2019275075 29 Oct 2024

CLAIMS

1. A monohydrate crystalline form of Compound 1:



(Compound 1);

wherein the monohydrate crystalline form is Monohydrate Form 1 (MH1), having an XRPD pattern comprising peaks of 2θ angles at 9.1, 11.5, 16.2, 16.7, 18.2, 18.9, 19.8, 22.6, and 26.4 degrees 2θ, each ±0.2 degrees 2θ.

2. The monohydrate crystalline form of claim 1, having an XRPD pattern substantially the same as shown in FIG. 2A.

3. The monohydrate crystalline form of claim 1 or claim 2, having a dehydration endothermic peak at 40-100 °C and a melting endothermic peak at about 184.4 °C in a differential scanning calorimetry (DSC) thermogram.

4. The monohydrate crystalline form of any one of claims 1 to 3, having a DSC thermogram substantially in accordance with FIG. 5.

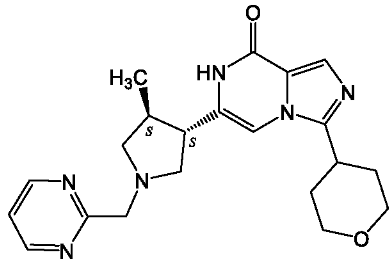
5. The monohydrate crystalline form of any one of claims 1 to 4, exhibiting dehydration between ambient and about 90 °C with a weight loss of about 3.8% in a thermogravimetric analysis (TGA).

6. The monohydrate crystalline form of any one of claims 1 to 5, having a TGA substantially in accordance with FIG. 5.

7. The monohydrate crystalline form of any one of claims 1 to 6, having characteristic absorptions at about 782 cm^{-1}, 1123 cm^{-1}, 1562 cm^{-1}, and 1655 cm^{-1} in an infrared (IR) spectrum.

8. The monohydrate crystalline form of any one of claims 1 to 7, having an infrared spectrum substantially in accordance with FIG. 3.

9. A monohydrate crystalline form of Compound 1:



(Compound 1);

wherein the monohydrate crystalline form is Monohydrate Form 2 (MH2), having an XRPD pattern comprising peaks of 2θ angles at 9.0, 11.6, 15.0, 16.0, 18.6, 19.1, 20.4, and 20.6 degrees 2θ, each ±0.2 degrees 2θ.

10. The monohydrate crystalline form of claim 9, having an XRPD pattern substantially the same as shown in FIG. 7A.

11. The monohydrate crystalline form of claim 9 or claim 10, having an endothermic peak at 59.1 °C (±5 °C) and at 184.7 °C (±5 °C) in a differential scanning calorimetry (DSC) thermogram.

12. The monohydrate crystalline form of any one of claims 9 to 11, having a DSC thermogram substantially in accordance with FIG. 9.

13. The monohydrate crystalline form of any one of claims 9 to 12, exhibiting dehydration at about 25 °C to about 100 °C with a weight loss of about 4.4% in a thermogravimetric analysis (TGA).

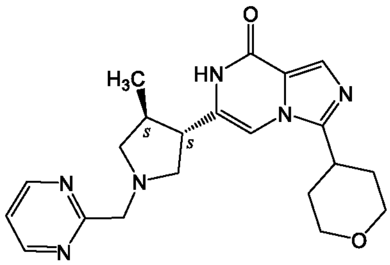
14. The monohydrate crystalline form of any one of claims 9 to 13, having a TGA substantially in accordance with FIG. 9.

15. The monohydrate crystalline form of any one of claims 1 to 14, which is at least 95%, 96%, 97%, 98%, or 99% purified.

2019275075 29 Oct 2024

16. A pharmaceutical composition comprising the monohydrate crystalline form (MH1) of any one of claims 1 to 8, or the monohydrate crystalline form (MH2) of any one of claims 9 to 15, and a pharmaceutically acceptable excipient.

17. A process for preparing a monohydrate crystalline form of Compound 1:



(Compound 1);

comprising precipitating the monohydrate crystalline form from a solution comprising Compound 1 and a solvent selected from the group comprising of n-propyl acetate, isopropyl acetate, anisole, methylisobutyl ketone, cumene, isopropanol, 2-methyl tetrahydrofuran, and combinations thereof.

18. A process for preparing Monohydrate Form 1 (MH1) of Compound 1, the steps comprising:

(i) dissolving Compound 1 in a first solvent to get a solution;

(ii) adding a second solvent to get a mixture; and

(iii) filtering the mixture to get a solid,

wherein the first and second solvent is each individually selected from isopropyl acetate; ethanol; tetrahydrofuran; water; dichloromethane; acetonitrile; anisole; methylisobutyl ketone; nitromethane; 1,2-dimethoxyethane; methylethyl ketone; n-heptane; 1,4-dioxane; n-propyl acetate; 2-propanol; acetone; cumene; N,N-dimethylformamide; dimethyl sulfoxide; and combinations thereof.

19. A process for preparing Monohydrate Form 2 (MH2) of Compound 1, the steps comprising:

(i) treating Compound 1 or MH1 with a first solvent system to obtain a suspension;

(ii) filtering the suspension to obtain the solid;

(iii) washing the solid with heptane; and

(iv) drying to remove the solvent to get MH2;

wherein the solvent is a mixture of water and ethyl acetate (EtOAc) or methyl acetate (MeOAc), selected from 2% (v/v) EtOAc / water, 2.7% (v/v) EtOAc / water, and 7.5% (v/v) MeOAc /

2019275075 29 Oct 2024

water.

20. A monohydrate crystalline form of Compound 1, prepared by the process of any one of claims 17 to 19.

21. A method of inhibiting PDE9 activity in a patient, comprising administering to the patient the monohydrate crystalline form of Compound 1 of any one of claims 1 to 15 or the pharmaceutical composition of claim 16.

Fig. 1

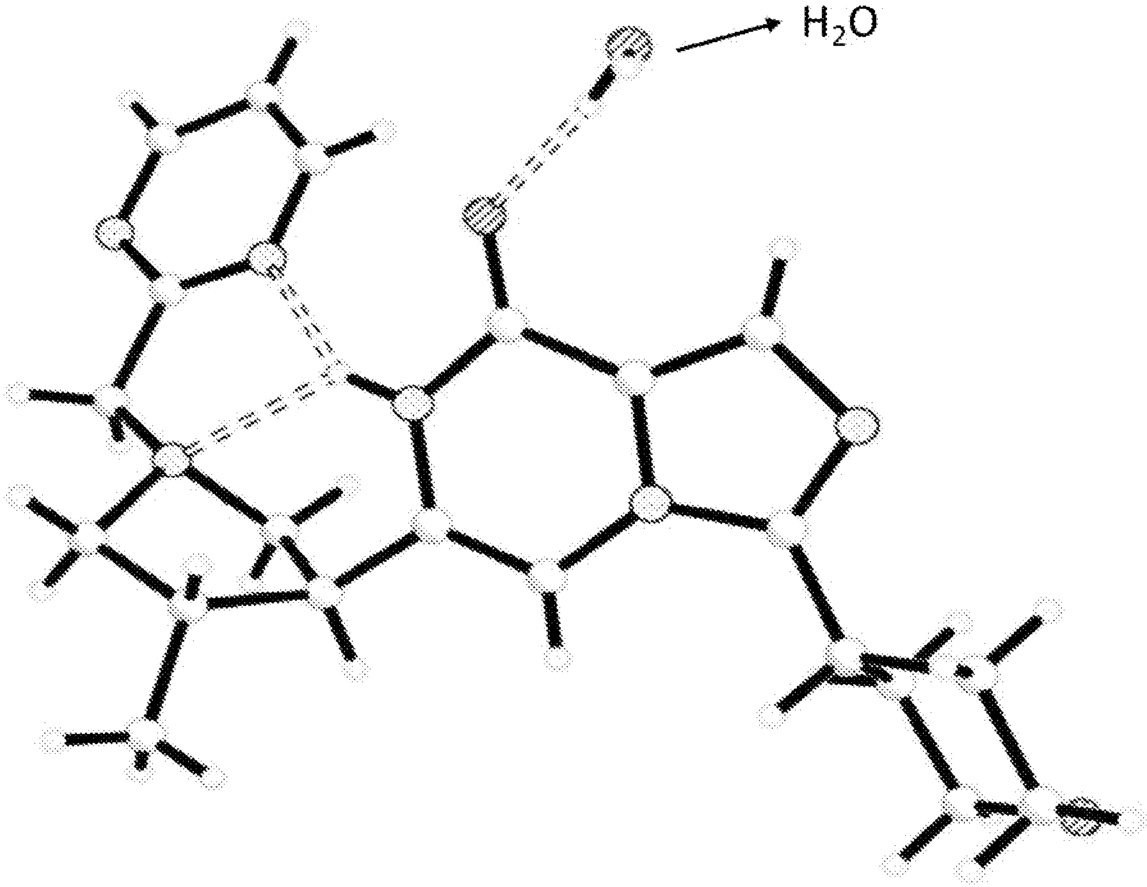
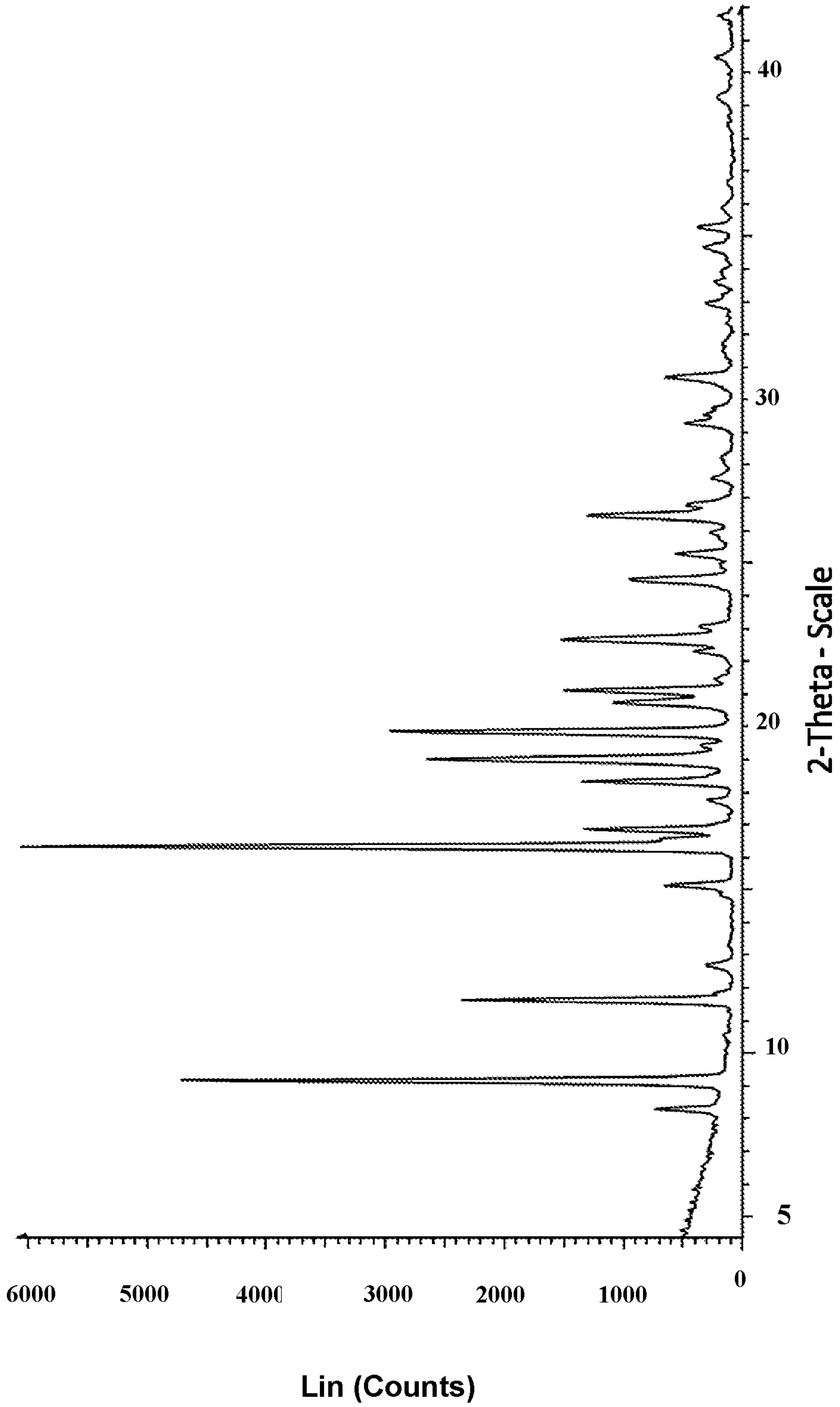


Fig. 2A


2-Theta - Scale
5
10
20
30
40
Lin (Counts)
0
1000
2000
3000
4000
5000
6000

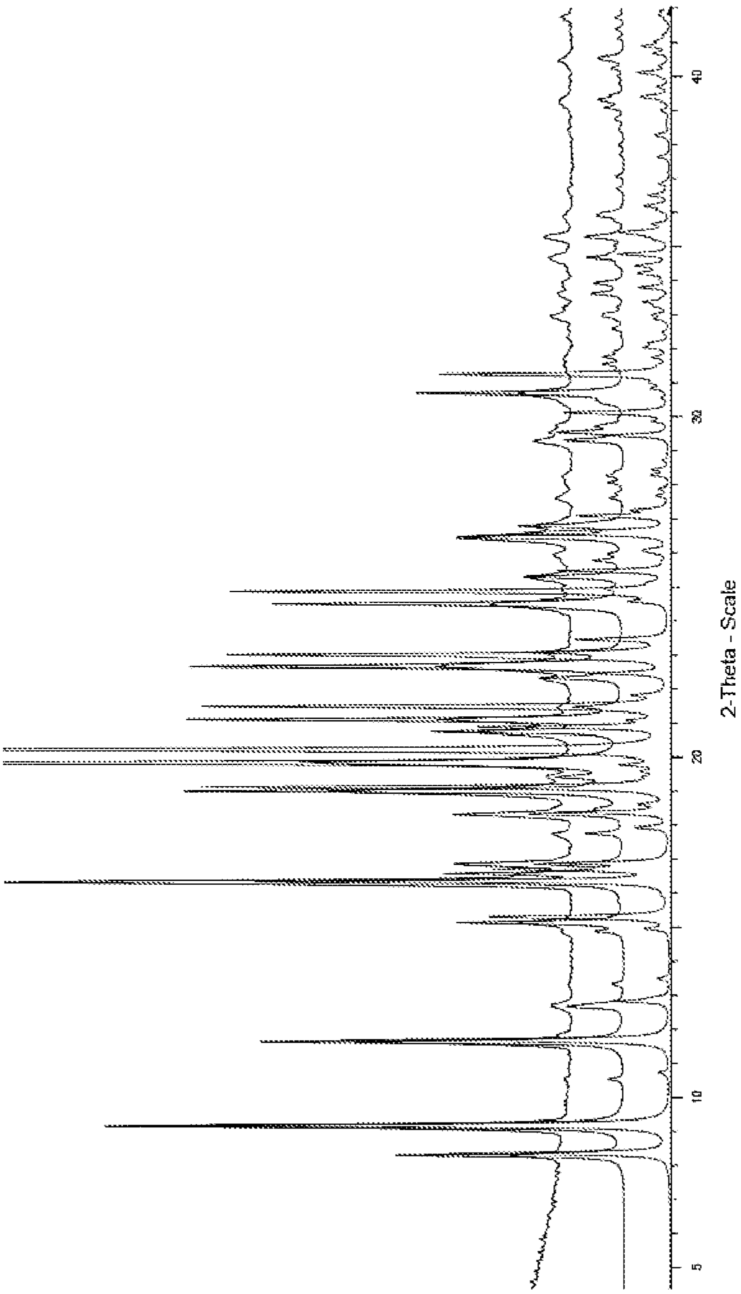


Fig. 2B

Fig. 3

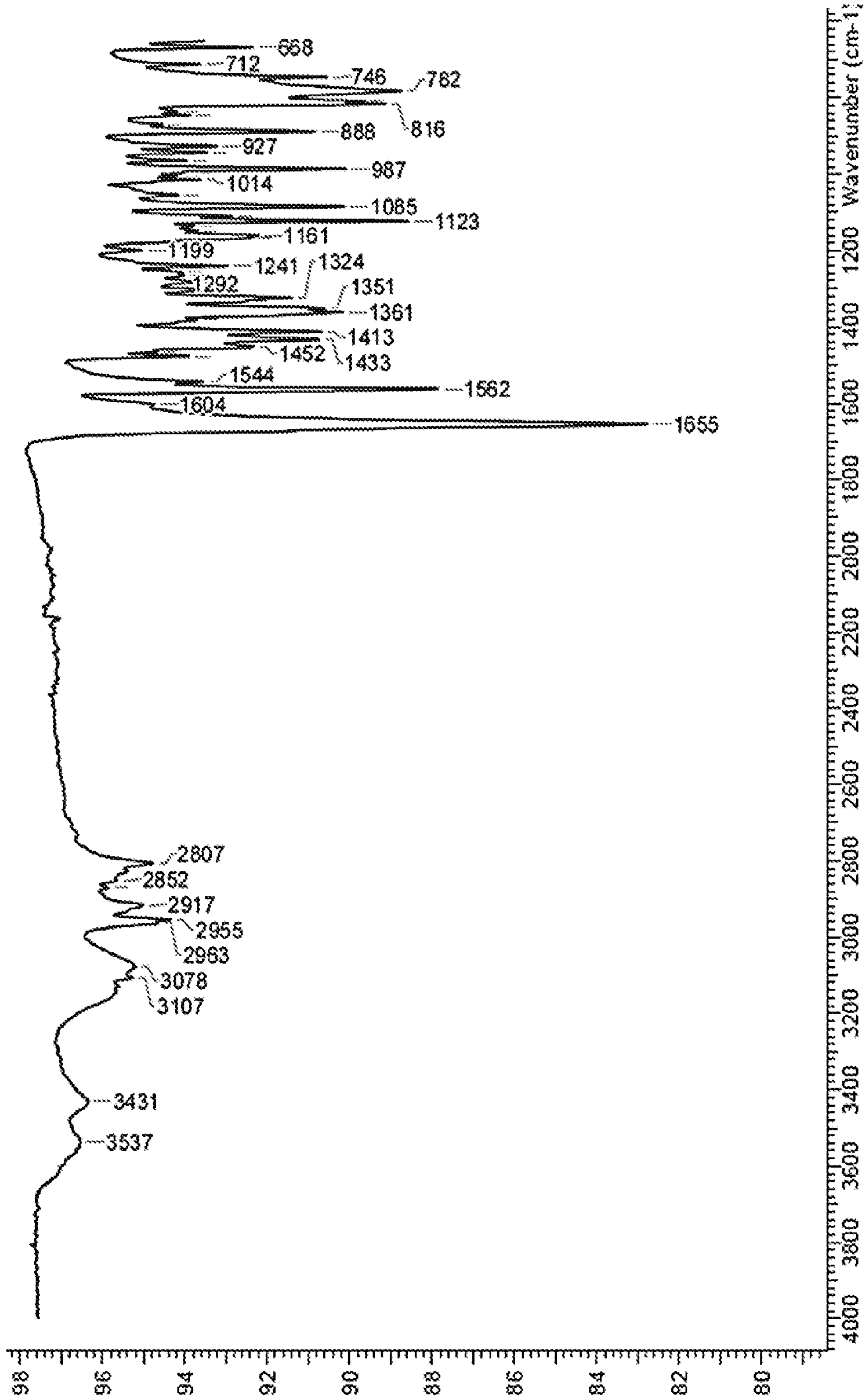

668
712
746
782
816
888
927
987
1014
1085
1123
1161
1199
1241
1324
1292
1351
1361
1413
1452
1433
1544
1562
1604
1655
2807
2852
2917
2955
2963
3078
3107
3431
3537
Wavenumber (cm-1)
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
4000
98
96
94
92
90
88
86
84
82
80

Fig. 4

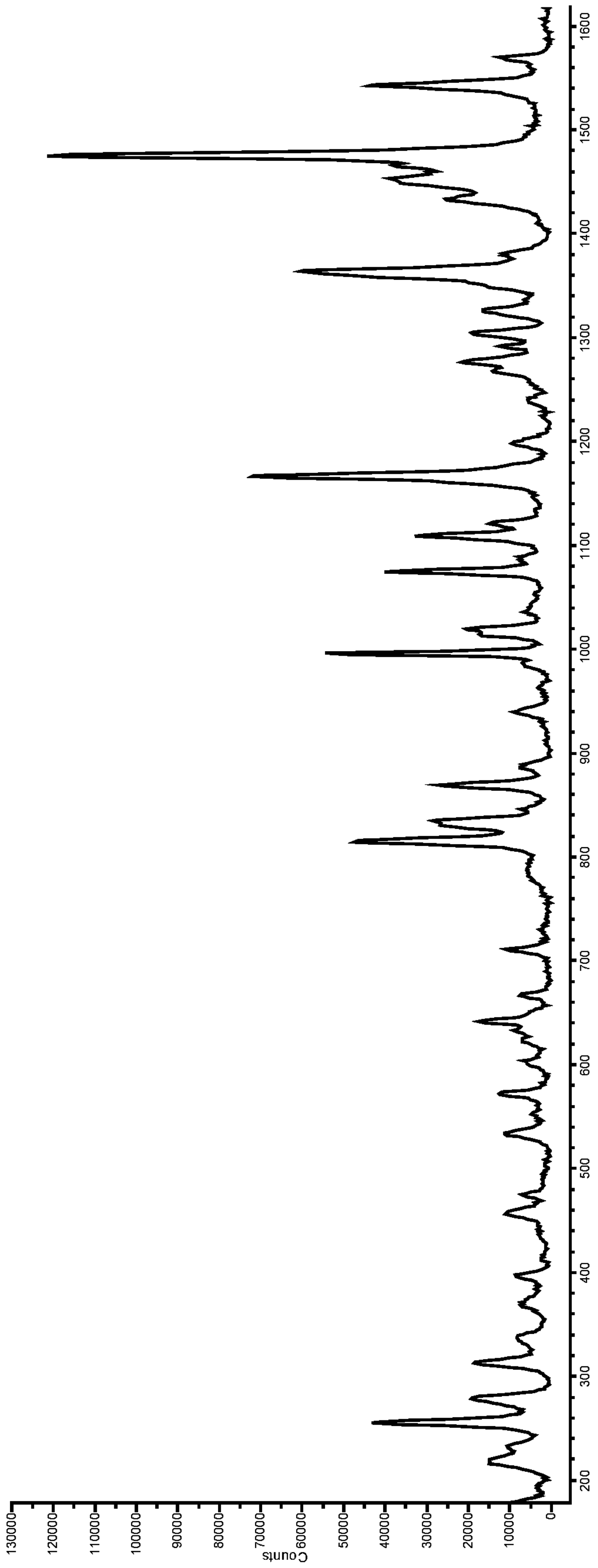

Raman shift / cm-1
Counts
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
0
10000
20000
30000
40000
50000
60000
70000
80000
90000
100000
110000
120000
130000

Fig. 5

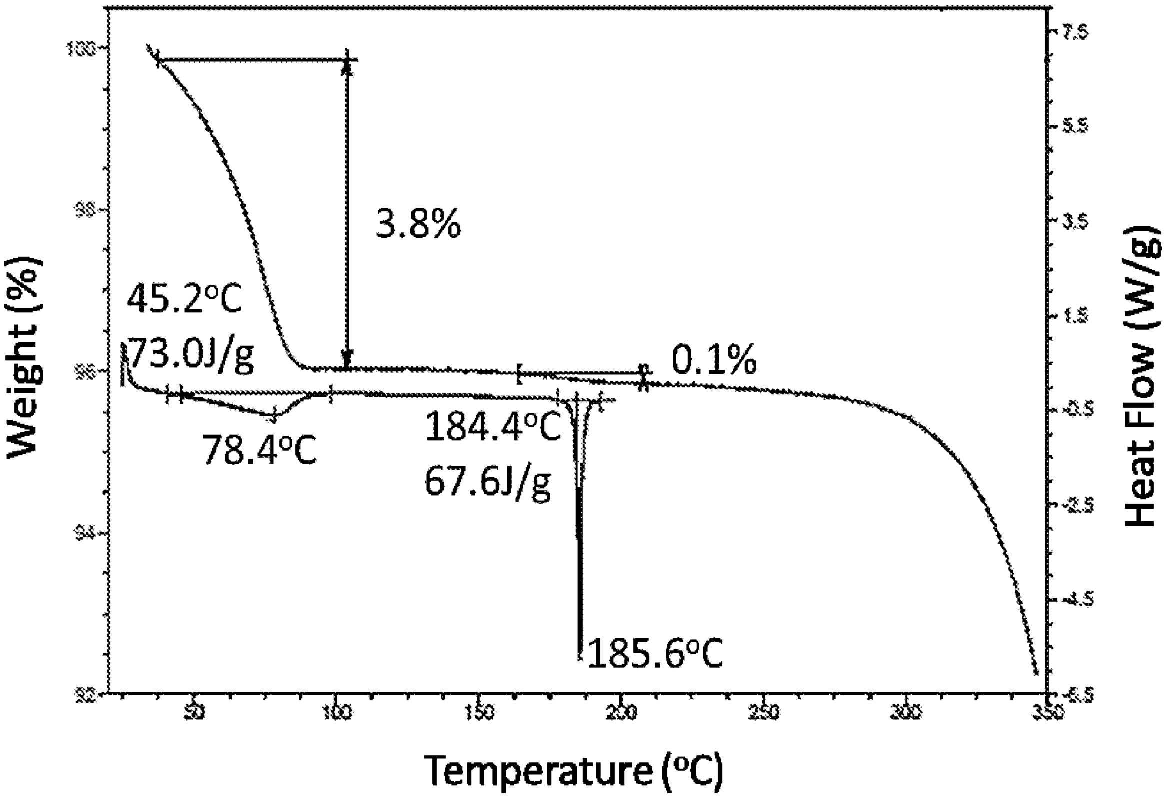

3.8%
45.2°C
73.0J/g
0.1%
78.4°C
184.4°C
67.6J/g
185.6°C
Weight (%)
Heat Flow (W/g)
Temperature (°C)

Fig. 6

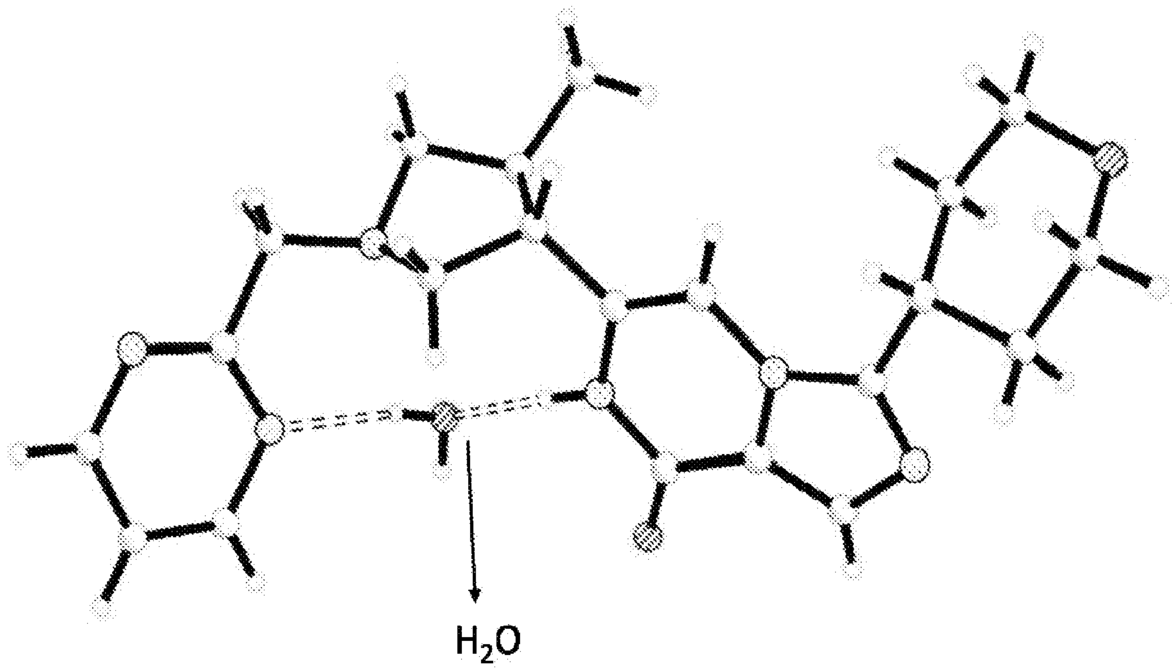


Fig. 7

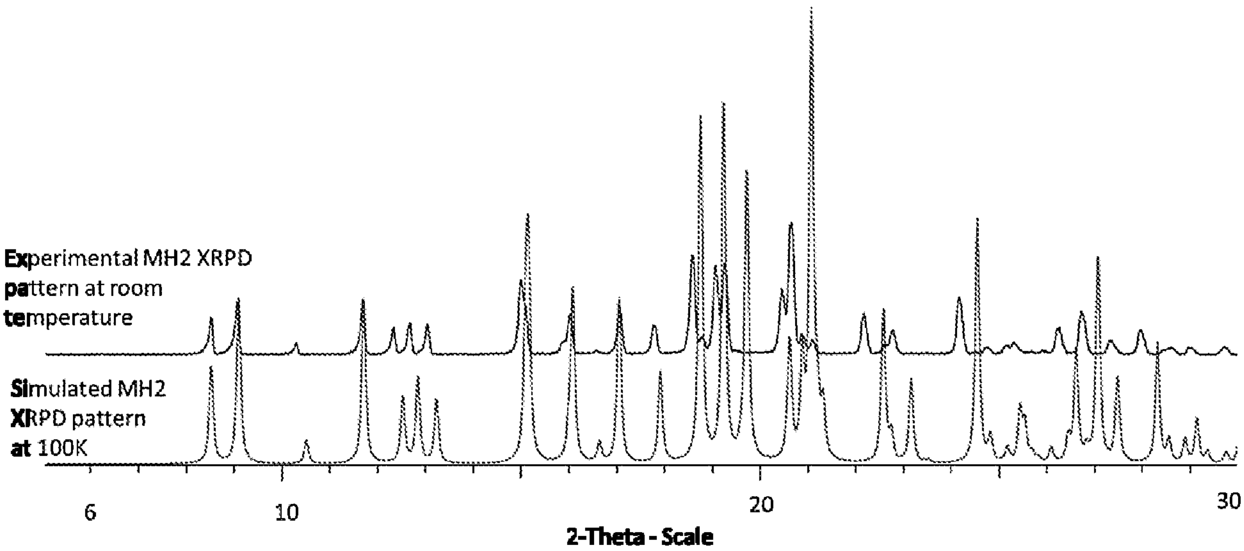

Experimental MH2 XRPD pattern at room temperature
Simulated MH2 XRPD pattern at 100K
6
10
20
30
2-Theta - Scale

Fig. 8

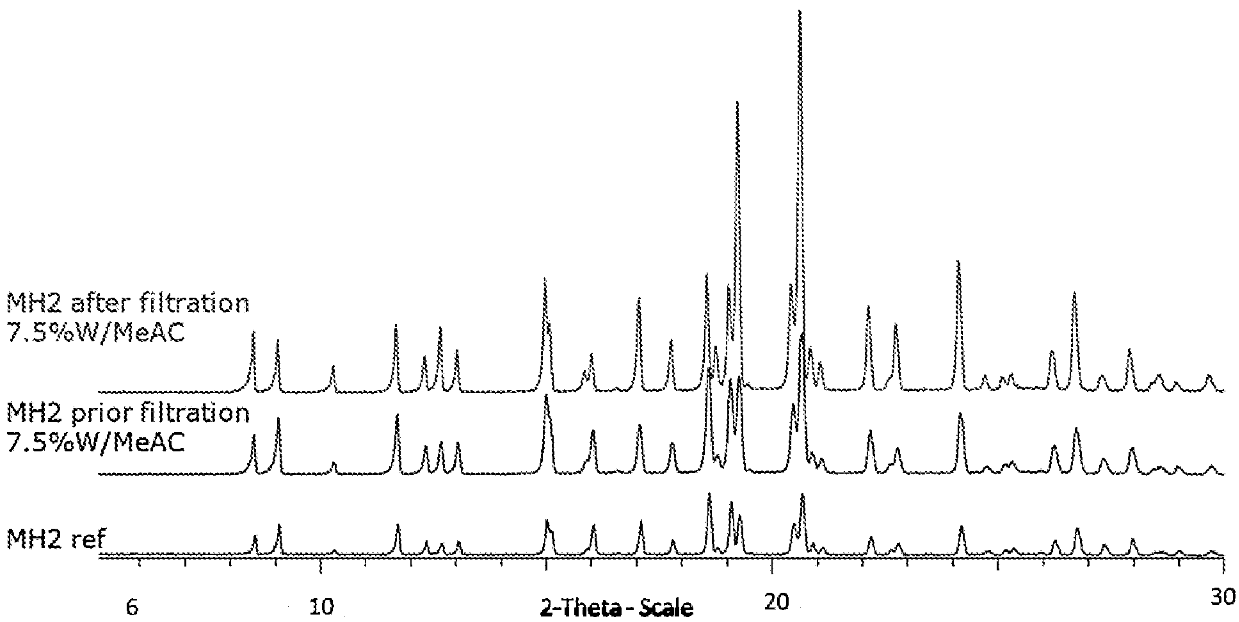

MH2 after filtration 7.5%W/MeAC
MH2 prior filtration 7.5%W/MeAC
MH2 ref
6
10
2-Theta - Scale
20
30

Fig. 9