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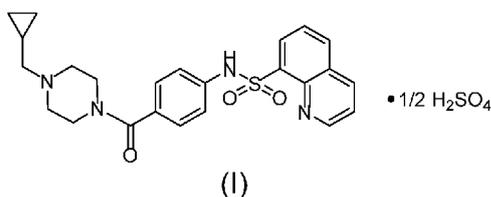
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(54) Title: CRYSTALLINE FORMS OF N-(4-(4-(CYCLOPROPYLMETHYL) PIPERAZINE-1-CARBONYL)PHENYL)QUINOLINE-8-SULFONAMIDE



(57) Abstract: Provided herein are amorphous and crystalline hemisulfate salt forms of the formula (I). Also provided are pharmaceutical compositions comprising the amorphous and crystalline hemisulfate salt forms, methods for their manufacture, and uses thereof for treating conditions associated with pyruvate kinase such as e.g., pyruvate kinase deficiency.



WO 2019/104134 A1

**CRYSTALLINE FORMS OF N-(4-(4-(CYCLOPROPYLMETHYL)PIPERAZINE-1-CARBONYL)PHENYL)QUINOLINE-8-SULFONAMIDE**

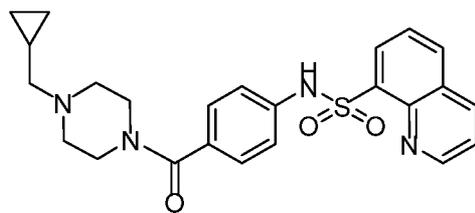
**RELATED APPLICATIONS**

**[0001]** This application claims priority to U.S. Provisional Application No. 62/589,822, filed November 22, 2017 and U.S. Provisional Application No. 62/691,709, filed June 29, 2018, each of which are incorporated herein in their entirety.

**BACKGROUND**

**[0002]** Pyruvate kinase deficiency (PKD) is a disease of the red blood cells caused by a deficiency of the pyruvate kinase R (PKR) enzyme due to recessive mutations of PKLR gene (Wijk et al. *Human Mutation*, **2008**, *30* (3) 446-453). PKR activators can be beneficial to treat PKD, thalassemia (*e.g.*, beta-thalassemia), abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, anemia (*e.g.*, congenital anemias (*e.g.*, enzymopathies), hemolytic anemia (*e.g.* hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, chronic hemolytic anemia caused by phosphoglycerate kinase deficiency, anemia of chronic diseases, non-spherocytic hemolytic anemia or hereditary spherocytosis). Treatment of PKD is supportive, including blood transfusions, splenectomy, chelation therapy to address iron overload, and/or interventions for other disease-related morbidity. Currently, however, there is no approved medicine that treats the underlying cause of PKD, and thus the etiology of life-long hemolytic anemia.

**[0003]** N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide, herein referred to as Compound **1**, is an allosteric activator of red cell isoform of pyruvate kinase (PKR). See *e.g.*, WO 2011/002817 and WO 2016/201227, the contents of which are incorporated herein by reference.

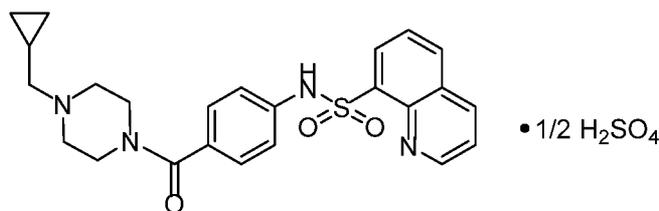


(Compound **1**)

**[0004]** Compound **1** was developed to treat PKD and is currently being investigated in phase 2 clinical trials. See *e.g.*, U.S. clinical trials identifier NCT02476916. Given its therapeutic benefits, there is a need to develop alternative forms of Compound **1** in an effort to facilitate isolation, manufacturing, and formulation development, as well as to enhance storage stability.

## SUMMARY

[0005] Provided herein are amorphous and crystalline hemisulfate salt forms of a compound having the formula.



[0006] Also provided herein are pharmaceutical compositions comprising the amorphous and crystalline hemisulfate salt forms, methods for their manufacture, and uses thereof for treating conditions associated with pyruvate kinase such as e.g., PKD.

## BRIEF DESCRIPTION OF THE FIGURES

[0007] FIG. 1 depicts an X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form A.

[0008] FIG. 2 depicts the combined thermogravimetric analysis (TGA) thermogram and differential scanning calorimetry (DSC) thermogram for crystalline hemisulfate salt Form A.

[0009] FIG. 3 depicts the differential scanning calorimetry (DSC) thermogram for crystalline hemisulfate salt Form A.

[0010] FIG. 4 depicts the dynamic vapor sorption (DVS) isotherm for crystalline hemisulfate salt Form A.

[0011] FIG. 5 (i) depicts X-ray powder diffraction pattern (XRPD) overlay for crystalline hemisulfate salt Form A and after exposure to 11%, 48%, and 75% relative humidity and 40 °C for two weeks. The XRPD remained unchanged after two-week test under these three humidity conditions. FIG. 5 (ii) depicts XRPD overlay for crystalline hemisulfate salt Form A and after exposure to phosphorus pentoxide ( $\text{P}_2\text{O}_5$ ) granules at room temperature and 50°C for one week, as well as ambient temperature in a vial for 24hrs. It was observed that there was no change in XRPD pattern at room temperature after one week, but some peaks were slightly shifted at 50°C. However, these peaks shifted back to original positions after exposure to ambient for 24 hrs, indicating these changes were reversible.

[0012] FIG. 6 depicts an X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form B obtained in MeOH:EtOH (3:7).

[0013] FIG. 7(i) depicts the combined thermogravimetric analysis (TGA) thermogram and differential scanning calorimetry (DSC) thermogram for crystalline hemisulfate salt Form

B. **FIG. 7(ii)** depicts the XRPD for crystalline hemisulfate salt Form B before and after dynamic vapor sorption (DVS). After DVS, Form B was converted to a different Form K.

**[0014]** **FIG. 8** depicts an X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form D.

**[0015]** **FIG. 9** depicts the thermogravimetric analysis (TGA) thermogram and differential scanning calorimetry (DSC) thermogram for crystalline hemisulfate salt Form D.

**[0016]** **FIG. 10** depicts the X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form D before and after dynamic vapor sorption (DVS).

**[0017]** **FIG. 11** depicts an X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form E.

**[0018]** **FIG. 12** depicts an X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form F.

**[0019]** **FIG. 13** depicts the thermogravimetric analysis (TGA) thermogram and differential scanning calorimetry (DSC) thermogram for crystalline hemisulfate salt Form F.

**[0020]** **FIG. 14** depicts an X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form G.

**[0021]** **FIG. 15** depicts the thermogravimetric analysis (TGA) thermogram and differential scanning calorimetry (DSC) thermogram for crystalline hemisulfate salt Form G.

**[0022]** **FIG. 16** depicts an X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form H.

**[0023]** **FIG. 17** depicts an X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form I.

**[0024]** **FIG. 18** depicts the thermogravimetric analysis (TGA) thermogram and differential scanning calorimetry (DSC) thermogram for crystalline hemisulfate salt Form I.

**[0025]** **FIG. 19** depicts an X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form J.

**[0026]** **FIG. 20** depicts the X-ray powder diffraction pattern (XRPD) for amorphous hemisulfate salt form of Compound 1.

**[0027]** **FIG. 21** depicts the thermogravimetric analysis (TGA) thermogram and differential scanning calorimetry (DSC) thermogram for amorphous hemisulfate salt of Compound 1 dried in vacuum oven at 50 °C for overnight.

**[0028]** **FIG. 22** depicts Mean plasma concentration-time profiles of crystalline Form A after a PO dose in different forms at 200 mg/kg in SD rats (N=6). The dose of crystalline Form A is calculated based on the equivalence to 200 mg/kg of compound 1.

[0029] FIG. 23 depicts the X-ray powder diffraction pattern (XRPD) for amorphous free base form of Compound 1.

[0030] FIG. 24 depicts the TGA and DSC thermograms for amorphous free base form of Compound 1.

[0031] FIG. 25 depicts the X-ray powder diffraction pattern (XRPD) for crystalline free base form of Compound 1.

## DETAILED DESCRIPTION

### Definitions

[0032] When used alone, the terms “Form A”, “Form B”, “Form C”, “Form D”, “Form E”, “Form F”, “Form G”, “Form H”, “Form I”, and “Form J” refer to the crystalline hemisulfate salt forms A, B, C, D, E, F, G, H, I, and J of Compound 1, respectively. The terms “Form A”, “crystalline Form A”, and “crystalline hemisulfate salt Form A of Compound 1” are used interchangeably. Similarly, “Form B”, “crystalline Form B”, and “crystalline hemisulfate salt Form B of Compound 1” are used interchangeably. Similarly, “Form C”, “crystalline Form C”, and “crystalline hemisulfate salt Form C of Compound 1” are used interchangeably. Similarly, “Form D”, “crystalline Form D”, and “crystalline hemisulfate salt Form D of Compound 1” are used interchangeably. Similarly, “Form E”, “crystalline Form E”, and “crystalline hemisulfate salt Form E of Compound 1” are used interchangeably. Similarly, “Form F”, “crystalline Form F”, and “crystalline hemisulfate salt Form F of Compound 1” are used interchangeably. Similarly, “Form G”, “crystalline Form G”, and “crystalline hemisulfate salt Form G of Compound 1” are used interchangeably. Similarly, “Form H”, “crystalline Form H”, and “crystalline hemisulfate salt Form H of Compound 1” are used interchangeably. Similarly, “Form I”, “crystalline Form I”, and “crystalline hemisulfate salt Form I of Compound 1” are used interchangeably. Similarly, “Form J”, and “crystalline Form J”, “crystalline hemisulfate salt Form J of Compound 1” are used interchangeably.

[0033] “Pattern A”, “Pattern B”, “Pattern C”, “Pattern D”, “Pattern E”, “Pattern F”, “Pattern G”, “Pattern H”, “Pattern I”, and “Pattern J” refer to the X-ray powder diffraction pattern (XRPD) for crystalline hemisulfate salt Form A, B, C, D, E, F, G, H, I and J respectively.

[0034] The term “crystalline free base,” “free-base crystalline form of Compound 1,” “crystalline free base form of Compound 1,” and “crystalline free base of Compound 1” are

used interchangeably and mean the free base or non-salt form of Compound **1**, which is present in a crystalline form.

**[0035]** As used herein, “anhydrous” means that the referenced crystalline form has substantially no water in the crystal lattice e.g., less than 0.1% by weight as determined by Karl Fisher analysis.

**[0036]** The term “amorphous” means a solid that is present in a non-crystalline state or form. Amorphous solids are disordered arrangements of molecules and therefore possess no distinguishable crystal lattice or unit cell and consequently have no definable long range ordering. Solid state ordering of solids may be determined by standard techniques known in the art, e.g., by X-ray powder diffraction (XRPD) or differential scanning calorimetry (DSC). Amorphous solids can also be differentiated from crystalline solids e.g., by birefringence using polarized light microscopy.

**[0037]** As used herein, chemical purity refers to extent by which the disclosed form is free from materials having different chemical structures. Chemical purity of the compound in the disclosed crystal forms means the weight of the compound divided by the sum of the weight of the compound plus materials/impurities having different chemical structures multiplied by 100%, i.e., percent by weight. In one embodiment, the compound in the disclosed crystalline forms has a chemical purity of at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% by weight.

**[0038]** As used herein, “crystalline” refers to a solid form of a compound wherein there exists long-range atomic order in the positions of the atoms. The crystalline nature of a solid can be confirmed, for example, by examination of the X-ray powder diffraction pattern. If the XRPD shows sharp intensity peaks in the XRPD then the compound is crystalline.

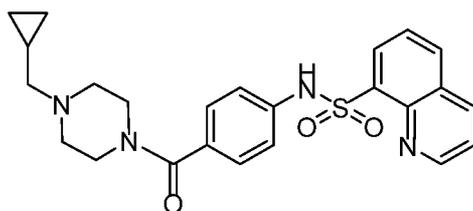
**[0039]** The term “solvate” refers to a crystalline compound wherein a stoichiometric or non-stoichiometric amount of solvent, or mixture of solvents, is incorporated into the crystal structure.

**[0040]** The term “hydrate” refers to a crystalline compound where a stoichiometric or non-stoichiometric amount of water is incorporated into the crystal structure. A hydrate is a solvate wherein the solvent incorporated into the crystal structure is water. The term “anhydrous” when used with respect to a compound means substantially no solvent incorporated into the crystal structure.

**[0041]** A single crystalline form of the disclosed crystalline hemisulfate salt means that N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide hemisulfate salt is present as a single crystal or a plurality of crystals in which each crystal

has the same crystal form (i.e., Form A, B, C, D, E, F, G, H, I, or J). When the crystal form is defined as a specified percentage of one particular single crystalline form of the compound, the remainder is made up of amorphous form and/or crystalline forms other than the one or more particular forms that are specified. In one embodiment, the crystalline form is at least 60% a single crystalline form, at least 70% a single crystalline form, at least 80% a single crystalline form, at least 90% a single crystalline form, at least 95% a single crystalline form, or at least 99% a single crystalline form by weight. Percent by weight of a particular crystal form is determined by the weight of the particular crystal form divided by the sum weight of the particular crystal, plus the weight of the other crystal forms present plus the weight of amorphous form present multiplied by 100%.

**[0042]** As used herein, “N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide” is used interchangeably with “Compound **1**”, “free base of Compound **1**” with the following structure::



**[0043]** The 2-theta values of the X-ray powder diffraction patterns for the crystalline forms described herein may vary slightly from one instrument to another and also depending on variations in sample preparation and batch to batch variation. Therefore, unless otherwise defined, the XRPD patterns / assignments recited herein are not to be construed as absolute and can vary  $\pm 0.2$  degrees. The 2-theta values provided herein were obtained using Cu K $\alpha$ 1 radiation.

**[0044]** Temperature values, e.g., for DSC peaks herein may vary slightly from one instrument to another and also depending on variations in sample preparation, batch to batch variation, and environmental factors. Therefore, unless otherwise defined, temperature values recited herein are not to be construed as absolute and can vary  $\pm 5$  degrees or  $\pm 2$  degrees.

**[0045]** “Substantially the same XRPD pattern” or “an X-ray powder diffraction pattern substantially similar to” a defined figure means that for comparison purposes, at least 90% of the peaks shown are present. It is to be further understood that for comparison purposes some variability in peak intensities from those shown are allowed, such as  $\pm 0.2$  degrees.

**[0046]** A “therapeutically effective amount” of a compound described herein is an amount sufficient to provide a therapeutic benefit in the treatment of a condition or to delay

or minimize one or more symptoms associated with the condition. The term “therapeutically effective amount” and “effective amount” are used interchangeably. In one aspect, a therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the condition. The term “therapeutically effective amount” can encompass an amount that improves overall therapy, reduces or avoids symptoms, signs, or causes of the condition, and/or enhances the therapeutic efficacy of another therapeutic agent. In certain embodiments, a therapeutically effective amount is an amount sufficient for eliciting measurable activation of wild-type or mutant PKR. In certain embodiments, a therapeutically effective amount is an amount sufficient for regulating 2,3-diphosphoglycerate levels in blood in need thereof or for treating pyruvate kinase deficiency (PKD), hemolytic anemia (e.g., chronic hemolytic anemia, hereditary non-spherocytic anemia), sickle cell disease, thalassemia (e.g., alfa thalassemia, beta-thalassemia or non-transfusion-dependent thalassemia), hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia (or Bassen-Kornzweig syndrome), paroxysmal nocturnal hemoglobinuria, acquired hemolytic anemia (e.g., congenital anemias (e.g., enzymopathies)), anemia of chronic diseases or treating diseases or conditions that are associated with increased 2,3-diphosphoglycerate levels (e.g., liver diseases). In certain embodiments, a therapeutically effective amount is an amount sufficient for eliciting measurable activation of wild-type or mutant PKR and for regulating 2,3-diphosphoglycerate levels in blood in need thereof or for treating pyruvate kinase deficiency (PKD), hemolytic anemia (e.g., chronic hemolytic anemia, hereditary non-spherocytic anemia), sickle cell disease, thalassemia (e.g., alfa thalassemia, beta-thalassemia or non-transfusion-dependent thalassemia), hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia (or Bassen-Kornzweig syndrome), paroxysmal nocturnal hemoglobinuria, acquired hemolytic anemia (e.g., congenital anemias (e.g., enzymopathies)), anemia of chronic diseases or treating diseases or conditions that are associated with increased 2,3-diphosphoglycerate levels (e.g., liver diseases). In one aspect, the therapeutically effective amount is the amount required to generate a subject’s hemoglobin response of  $\geq 1.0$  g/dL (such as  $\geq 1.5$  g/dL or  $\geq 2.0$  g/dL) increase in Hb concentration from baseline. In one aspect, the subject’s baseline Hb concentration is the average of all available Hb concentrations before treatment with a compound described herein. In certain aspects, the therapeutically effective amount is the amount required to reduce the patient’s transfusion burden. In one aspect, the therapeutically effective amount is between 0.01 - 100 mg/kg body weight/day of the provided compound, such as e.g., 0.1 - 100 mg/kg body weight/day.

**[0047]** As used herein, reduction in transfusion burden means at least 20% reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, the reduction in transfusion burden is  $\geq 33\%$  reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, reduction of transfusion burden is  $\geq 33\%$  reduction in the number of RBC units transfused within at least 10 weeks (e.g., at least 20 weeks or at least 24 weeks) of treatment.

**[0048]** As used herein, sickle cell disease (SCD), Hemoglobin SS disease, and sickle cell anemia are used interchangeably. Sickle cell disease (SCD) is an inherited blood disorder caused by the presence of sickle hemoglobin (HbS). In certain embodiments, subjects with SCD have abnormal hemoglobin, called hemoglobin S or sickle hemoglobin, in their red blood cells. In certain embodiments, people having SCD have at least one abnormal genes causing the body to make hemoglobin S. In certain embodiments, people having SCD have two hemoglobin S genes, Hemoglobin SS.

**[0049]** Thalassemia is an inherited blood disorder in which the normal ratio of  $\alpha$ - to  $\beta$ -globin production is disrupted due to a disease-causing variant in 1 or more of the globin genes. In certain embodiments, Alpha-globin aggregates (as found in  $\beta$ -thalassemia) readily precipitate, which disrupts the red blood cell (RBC) membrane and results in oxidative stress. In certain embodiments, Beta-globin tetramers (Hb H, found in  $\alpha$ -thalassemia) are generally more soluble, but are still unstable and can form precipitates. The imbalance of the globin chain synthesis can lead to a net reduction in Hb concentrations and has dramatic effects on the survival of RBC precursors, ultimately resulting in their premature destruction in the bone marrow and in extramedullary sites (Cappellini et al, 2014). In certain embodiments, the disorder results in large numbers of red blood cells being destroyed, which leads to anemia. In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia. In other embodiments, the thalassemia is non-transfusion-dependent thalassemia. In other embodiments, the thalassemia is beta thalassemia intermedia. In other embodiments, the thalassemia is Hb E beta thalassemia. In other embodiments, the thalassemia is beta thalassemia with mutations of 1 or more alfa genes.

**[0050]** The term “activating” as used herein means an agent that (measurably) increases the activity of wild type pyruvate kinase R (wt PKR) or causes wild type pyruvate kinase R (wt PKR) activity to increase to a level that is greater than wt PKR’s basal levels of activity or an agent that (measurably) increases the activity of a mutant pyruvate kinase R (mPKR) or causes mutant pyruvate kinase R (mPKR) activity to increase to a level that is greater than

that mutant PKR's basal levels of activity, for examples, to a level that is 20%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of the activity of wild type PKR.

**[0051]** The term "packed red blood cells" or PRBCs as used herein refer to red blood cells made from a unit of whole blood by centrifugation and removal of most of the plasma. In certain embodiments, a PRBC unit has a hematocrit of at least about 95%. In certain embodiments, a PRBC unit has a hematocrit of at least about 90%. In certain embodiments, a PRBC unit has a hematocrit of at least about 80%. In certain embodiments, a PRBC unit has a hematocrit of at least about 70%. In certain embodiments, a PRBC unit has a hematocrit of at least about 60%. In certain embodiments, a PRBC unit has a hematocrit of at least about 50%. In certain embodiments, a PRBC unit has a hematocrit of at least about 40%. In certain embodiments, a PRBC unit has a hematocrit of at least about 30%. In certain embodiments, a PRBC unit has a hematocrit of at least about 20%. In certain embodiments, a PRBC unit has a hematocrit of at least about 10%.

**[0052]** The terms "treatment," "treat," and "treating" refer to reversing, alleviating, reducing the likelihood of developing, or inhibiting the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed, *i.e.*, therapeutic treatment. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of genetic or other susceptibility factors), *i.e.*, prophylactic treatment. Treatment may also be continued after symptoms have resolved, for example to reduce the likelihood of or delay their recurrence.

**[0053]** As used herein the terms "subject" and "patient" may be used interchangeably, and means a mammal in need of treatment, *e.g.*, companion animals (*e.g.*, dogs, cats, and the like), farm animals (*e.g.*, cows, pigs, horses, sheep, goats and the like) and laboratory animals (*e.g.*, rats, mice, guinea pigs and the like). Typically, the subject is a human in need of treatment. In certain embodiments, the term "subject" refers to a human subject in need of treatment of a disease. In certain embodiments, the term "subject" refers to a human subject in need of treatment of PKD. In certain embodiments, the term "subject" refers to a human subject in need of treatment of thalassemia. In certain embodiments, the term "subject" refers to a human subject in need of treatment of sickle cell disease. In certain embodiments, the term "subject" refers to a human adult over 18 years old in need of treatment of a disease. In certain embodiments, the term "subject" refers to a human child no more than 18 years old in need of treatment of a disease. In certain embodiments, the subject is a patient in need of

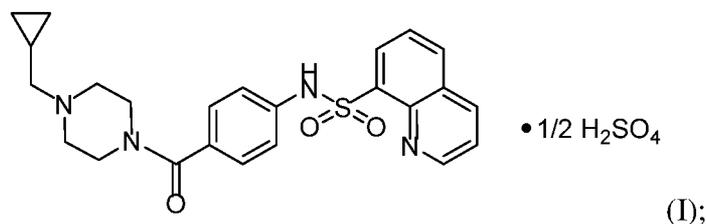
regular blood transfusion. As used here, the regular blood transfusion refers to at least 4 transfusion episodes in a 52-week period prior to the treatment. In certain embodiments, the regular blood transfusion refers to at least 5 transfusion episodes in a 52-week period prior to the treatment. In certain embodiments, the regular blood transfusion refers to at least 6 transfusion episodes in a 52-week period prior to the treatment. In certain embodiments, the regular blood transfusion refers to at least 7 transfusion episodes in a 52-week period prior to the treatment. In certain embodiments, the subject with a least one of the indications selected from the sickle cell disease, thalassemia, PKD under regular transfusion, and non-transfusion dependent PKD, has not been exposed to sotatercept (ACE-011), luspaterecept (ACE-536), ruxolitinib, or gene therapy. In certain embodiments, such subject is not taking inhibitors of cytochrome P450 (CYP)3A4, strong inducers of CYP3A4, strong inhibitors of P-glycoprotein (P-gp), or digoxin. In certain embodiments, such subject is not receiving chronic anticoagulant therapy, anabolic steroids, hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins), or allergic to sulfonamides.

**[0054]** The term “pharmaceutically acceptable carrier” refers to a non-toxic carrier, adjuvant, or vehicle that does not adversely affect the pharmacological activity of the compound with which it is formulated, and which is also safe for human use.

**[0055]** As used herein, the terms “about” and “approximately” when used in combination with a numeric value or range of values used to characterize a particular crystal form, amorphous form, or mixture thereof of a compound mean the value or range of values may deviate to an extent deemed reasonable to one of ordinary skill in the art while describing the particular crystal form, amorphous form, or mixture thereof.

### Compounds

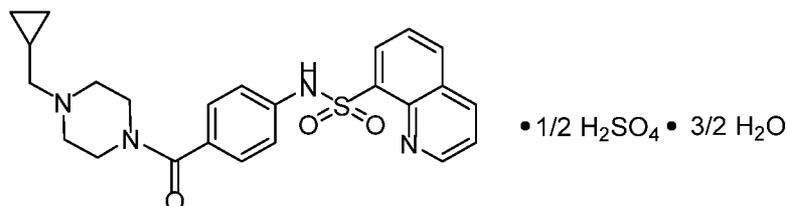
**[0056]** Provided herein is a crystalline Form A of a hemisulfate salt of a compound having the formula (I):



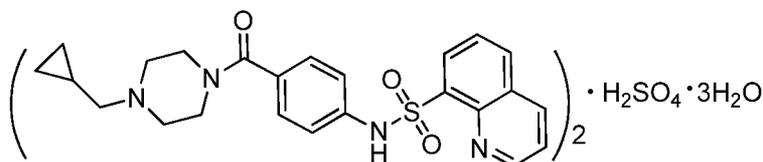
wherein the hemisulfate salt of the compound in crystalline Form A is a sesquihydrate.

**[0057]** As used herein, crystalline Form A is a hemisulfate sesquihydrate of compound 1, N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide. It is to

be understood that crystalline Form A can be named “1-(cyclopropylmethyl)-4-(4-(quinoline-8-sulfonamido)benzoyl)piperazin-1-ium hemisulfate sesquihydrate” having Formula A, or alternatively “1-(cyclopropylmethyl)-4-(4-(quinoline-8-sulfonamido)benzoyl)piperazin-1-ium sulfate trihydrate” having Formula B as shown below:



Formula A



Formula B.

**[0058]** It is to be understood that crystalline Form A can be referred to either Formula A or Formula B interchangeably.

**[0059]** As used herein, “hemisulfate” means the stoichiometric ratio of compound 1 to H<sub>2</sub>SO<sub>4</sub> is 2:1 in a crystalline form (i.e. a crystalline form contains two molecules of compound 1 per one molecule of H<sub>2</sub>SO<sub>4</sub>).

**[0060]** As used herein, “sesquihydrate” or “trihydrate” means the stoichiometric ratio of compound 1 to H<sub>2</sub>O is 2:3 in crystalline Form A (i.e. crystalline Form A contains two molecules of compound 1 per three molecules of water).

**[0061]** In one aspect, crystalline Form A is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 9.9°, 15.8°, and 22.6°. In certain embodiments, crystalline Form A is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 9.9°, 15.8°, and 22.6° and at least one additional x-ray powder diffraction peak at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 15.0°, 17.1°, 21.3°, and 21.9°. In certain embodiments, crystalline Form A is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 9.9°, 15.8°, and 22.6°; and at least two additional x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 15.0°, 17.1°, 21.3°, and 21.9°. In yet another alternative, crystalline Form A is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 9.9°, 15.8°, and 22.6°; and at least three additional x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 15.0°, 17.1°, 21.3°, and 21.9°. In certain embodiments, crystalline Form A is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 9.9°, 15.0°, 15.8°, 17.1°, 21.3°, 21.9°, and 22.6°. In certain embodiments, crystalline Form A is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 9.9°, 11.4°, 15.0°, 15.3°, 15.8°, 17.1°, 17.7°, 21.3°,

21.9°, 22.6°, and 23.5°. In certain embodiments, crystalline Form A is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) 4.9°, 9.9°, 11.0°, 11.4°, 11.7°, 12.3°, 12.8°, 13.6°, 13.9°, 14.2°, 15.0°, 15.3°, 15.8°, 17.1°, 17.4°, 17.7°, 18.8°, 19.1°, 19.8°, 21.3°, 21.9°, 22.6°, 23.0°, 23.2°, 23.5°, 23.8°, 24.1°, 24.5°, 25.3°, 25.6°, 26.1°, 27.1°, 28.1°, and 29.8°. In certain embodiments, crystalline Form A is characterized by an X-ray powder diffraction pattern substantially similar to **FIG. 1**. In yet another alternative, crystalline Form A is characterized by a differential scanning calorimetry (DSC) thermograph comprising endotherm peaks at about  $159\text{ }^\circ\text{C} \pm 5\text{ }^\circ\text{C}$  and  $199\text{ }^\circ\text{C} \pm 5\text{ }^\circ\text{C}$ . In yet another alternative, crystalline Form A is characterized by a differential scanning calorimetry (DSC) thermogram substantially similar to the one depicted in **FIG. 2**. In yet another alternative, crystalline Form A is characterized by a thermogravimetric analysis (TGA) thermogram comprising a weight loss of about  $4.5 \pm 0.5\%$  up to  $180\text{ }^\circ\text{C} \pm 2\text{ }^\circ\text{C}$ . In yet another alternative, crystalline Form A is characterized by a thermogravimetric analysis (TGA) thermogram substantially similar to the one depicted in **FIG 2**. In yet another alternative, crystalline Form A is characterized by a DSC substantially similar to the one depicted in **FIG 3**.

**[0062]** As discussed in greater detail in the Examples, crystalline Form A of a hemisulfate salt of a compound having the formula (I) was found to have a variety of favorable physicochemical properties, including high crystallinity, stability in multiple solvent systems (e.g. especially containing water), relatively small particle size (e.g. below  $20\text{ }\mu\text{m}$  under microscope so as to potentially avoid the subsequent micronization), and stability in humidity (e.g. at least 20% RH or at least a water activity of 0.2), and demonstrate favorable plasma concentration-time profiles and pharmacokinetic parameters.

**[0063]** Also provided herein is a crystalline Form B of a hemisulfate salt of a compound having the formula:

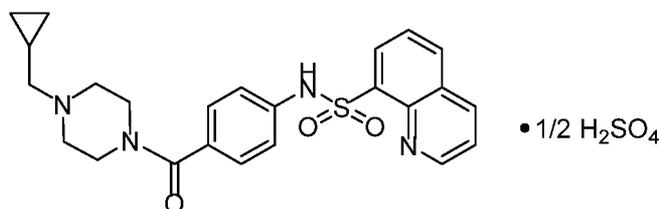


wherein the hemisulfate salt of the compound in crystalline Form B is an ethanol solvate.

**[0064]** In one aspect, crystalline Form B is characterized by at least three x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 9.9°, 10.6°, 12.7°, 15.7°, 16.9°, 22.0°, and 22.5°. Alternatively, crystalline Form B is characterized by at least four x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 9.9°, 10.6°, 12.7°, 15.7°, 16.9°, 22.0°,

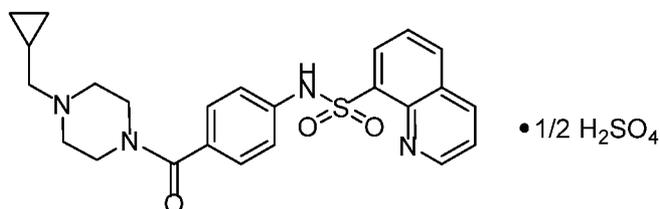
and 22.5°. In another alternative, crystalline Form B is characterized by at five three x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 9.9°, 10.6°, 12.7°, 15.7°, 16.9°, 22.0°, and 22.5°. In yet another alternative, crystalline Form B is characterized by at least six x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 9.9°, 10.6°, 12.7°, 15.7°, 16.9°, 22.0°, and 22.5°. In yet another alternative, crystalline Form B is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 9.9°, 10.6°, 12.7°, 15.7°, 16.9°, 22.0°, and 22.5°. In yet another alternative, crystalline Form B is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 9.9°, 10.6°, 12.7°, 13.9°, 14.6°, 15.7°, 16.9°, 22.0°, 22.5°, and 27.6°. In yet another alternative, crystalline Form B is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 7.0°, 7.8°, 9.9°, 10.6°, 11.7°, 12.7°, 13.1°, 13.5°, 13.9°, 14.6°, 14.9°, 15.3°, 15.7°, 16.1°, 16.9°, 17.6°, 19.3°, 19.7°, 20.7°, 21.2°, 22.0°, 22.5°, 23.3°, 24.0°, 24.7°, 25.1°, 25.7°, 26.1°, 27.2°, 27.6°, 28.4°, 29.3°, and 29.8°. In yet another alternative, crystalline Form B is characterized by an X-ray powder diffraction pattern substantially similar to **FIG 6**. In yet another alternative, crystalline Form B is characterized by a TGA or DSC pattern substantially similar to **FIG 7**. In yet another alternative, crystalline Form B is characterized by a differential scanning calorimetry (DSC) thermograph comprising endotherm peaks at about 154  $\pm$  5 °C. In yet another alternative, crystalline Form B is characterized by a TGA comprising a weight loss of about 4.3  $\pm$  0.5 % up to 200 °C  $\pm$  2 °C.

**[0065]** Also provided herein is a crystalline Form C of a hemisulfate salt of a compound having the formula:



**[0066]** In one aspect, crystalline Form C is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 6.9°, 10.4°, and 12.0°.

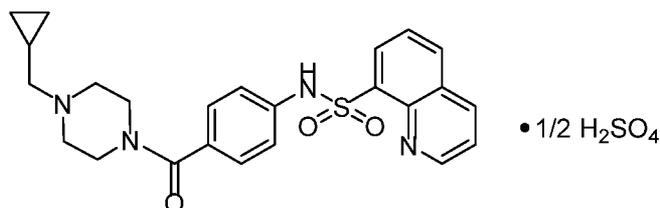
**[0067]** Also provided herein is a crystalline Form D of a hemisulfate salt of a compound having the formula:



wherein the hemisulfate salt of the compound in Form D is anhydrous.

**[0068]** In one aspect, crystalline Form D is characterized by at least three x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $5.8^\circ$ ,  $10.0^\circ$ ,  $10.2^\circ$ ,  $19.3^\circ$ ,  $22.9^\circ$ ,  $23.3^\circ$ , and  $25.2^\circ$ . Alternatively, crystalline Form D is characterized by at least four x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $5.8^\circ$ ,  $10.0^\circ$ ,  $10.2^\circ$ ,  $19.3^\circ$ ,  $22.9^\circ$ ,  $23.3^\circ$ , and  $25.2^\circ$ . In another alternative, crystalline Form D is characterized by at least five x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $5.8^\circ$ ,  $10.0^\circ$ ,  $10.2^\circ$ ,  $19.3^\circ$ ,  $22.9^\circ$ ,  $23.3^\circ$ , and  $25.2^\circ$ . In yet another alternative, crystalline Form D is characterized by at least six x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $5.8^\circ$ ,  $10.0^\circ$ ,  $10.2^\circ$ ,  $19.3^\circ$ ,  $22.9^\circ$ ,  $23.3^\circ$ , and  $25.2^\circ$ . In yet another alternative, crystalline Form D is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $5.8^\circ$ ,  $10.0^\circ$ ,  $10.2^\circ$ ,  $19.3^\circ$ ,  $22.9^\circ$ ,  $23.3^\circ$ , and  $25.2^\circ$ . In yet another alternative, crystalline Form D is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $5.8^\circ$ ,  $10.0^\circ$ ,  $10.2^\circ$ ,  $12.2^\circ$ ,  $17.3^\circ$ ,  $17.6^\circ$ ,  $19.3^\circ$ ,  $22.9^\circ$ ,  $23.3^\circ$ ,  $23.6^\circ$ , and  $25.2^\circ$ . In yet another alternative, crystalline Form D is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $5.8^\circ$ ,  $10.0^\circ$ ,  $10.2^\circ$ ,  $11.3^\circ$ ,  $11.5^\circ$ ,  $12.2^\circ$ ,  $13.6^\circ$ ,  $14.1^\circ$ ,  $14.7^\circ$ ,  $15.4^\circ$ ,  $16.0^\circ$ ,  $17.3^\circ$ ,  $17.6^\circ$ ,  $19.3^\circ$ ,  $20.0^\circ$ ,  $20.8^\circ$ ,  $22.1^\circ$ ,  $22.9^\circ$ ,  $23.3^\circ$ ,  $23.6^\circ$ ,  $24.4^\circ$ ,  $25.2^\circ$ ,  $26.4^\circ$ ,  $27.4^\circ$ ,  $28.3^\circ$ , and  $29.6^\circ$ . In yet another alternative, crystalline Form D is characterized by an X-ray powder diffraction pattern substantially similar to **FIG. 8**. In yet another alternative, crystalline Form D is characterized by a differential scanning calorimetry (DSC) pattern having a peak at  $239.0^\circ\text{C} \pm 2^\circ\text{C}$ . In yet another alternative, crystalline Form D is characterized by a DSC substantially similar to **FIG. 9**. In yet another alternative, crystalline Form D, is characterized by a TGA comprising a weight loss of about  $0.62 \pm 0.5\%$  up to  $220^\circ\text{C} \pm 2^\circ\text{C}$ . In yet another alternative, crystalline Form D is characterized by a TGA substantially similar to **FIG. 9**.

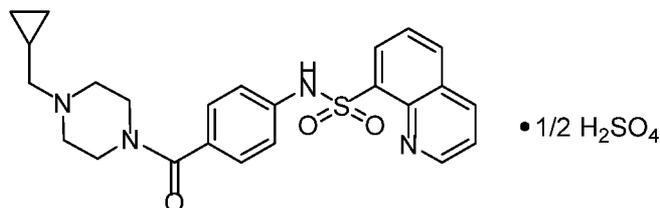
**[0069]** Also provided is a crystalline Form E of a hemisulfate salt of a compound having the formula:



**[0070]** In one aspect, crystalline Form E is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $4.6^\circ$ ,  $9.0^\circ$ ,  $13.5^\circ$ , and  $22.5^\circ$ . In another aspect, crystalline Form E is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )

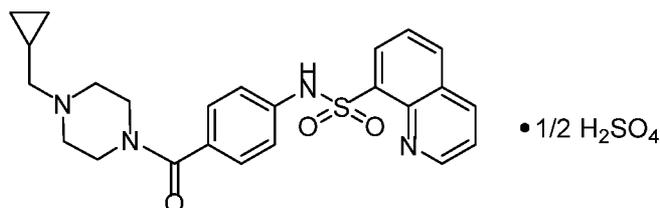
4.6°, 9.0°, 13.5°, 15.1°, 18.5°, 21.7°, and 22.5°. In another alternative, crystalline Form E is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) 4.6°, 9.0°, 9.9°, 11.0°, 13.5°, 15.1°, 15.8°, 18.5°, 19.8°, 20.4°, 21.7°, 22.5°, and 28.1°. In yet another alternative, crystalline Form E is characterized by an X-ray powder diffraction pattern substantially similar to **FIG. 11**.

**[0071]** Also provided herein is a crystalline Form F of a hemisulfate salt of a compound having the formula:



**[0072]** In one aspect, crystalline Form F is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 5.0°, 9.9°, and 14.7°. Alternatively, crystalline Form F is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 5.0°, 9.9°, 14.7°, 16.5°, 19.6°, 21.6°, and 24.4°. In another alternative, crystalline Form F is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) 5.0°, 9.9°, 11.1°, 14.7°, 16.5°, 19.6°, 21.6°, 22.8°, and 24.4°. In yet another alternative, crystalline Form F is characterized by an X-ray powder diffraction pattern substantially similar to **FIG. 12**. In yet another alternative, crystalline Form F, is characterized by a DSC pattern having a peak at  $101.0^\circ\text{C} \pm 2^\circ\text{C}$ . In yet another alternative, crystalline Form F, is characterized by a TGA comprising a weight loss of about  $8.8 \pm 0.5\%$  up to  $182^\circ\text{C} \pm 2^\circ\text{C}$ . In yet another alternative, crystalline Form F is characterized by a TGA or DSC substantially similar to **FIG. 13**.

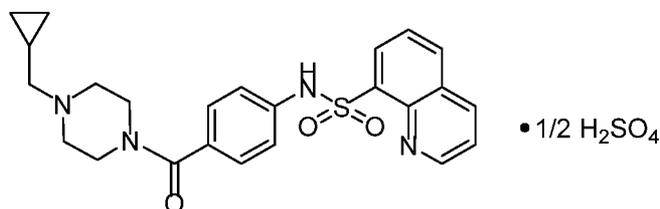
**[0073]** Also provided is crystalline Form G of a hemisulfate salt of a compound having the formula:



**[0074]** In one aspect, crystalline Form G is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 4.7°, 9.4°, and 14.1°. Alternatively, crystalline Form G is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 4.7°, 9.4°, 11.0°, 14.1°, 18.9°, 21.2°, and 23.8°. In another alternative, crystalline Form G is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) 4.7°, 9.4°, 11.0°,

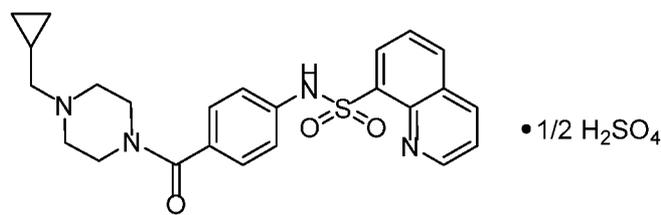
13.3°, 14.1°, 15.9°, 16.2°, 18.9°, 21.2°, 22.8°, 23.8°, 26.7°, and 28.5°. In yet another alternative, crystalline Form G is characterized by an X-ray powder diffraction pattern substantially similar to **FIG 14**. In yet another alternative, crystalline Form G, is characterized by a DSC pattern having a peak at 156.7 °C ± 2 °C. In yet another alternative, crystalline Form G, is characterized by a TGA comprising a weight loss of about 2.6 ± 0.5 % up to 176 °C ± 2 °C. In yet another alternative, crystalline Form G is characterized by a TGA or DSC substantially similar to **FIG. 15**.

**[0075]** Also provided is a crystalline Form H of a hemisulfate salt of a compound having the formula:



**[0076]** In one aspect, crystalline Form H is characterized by at least three x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 4.6°, 7.4°, 9.2°, 11.1°, 13.5°, 14.9°, and 22.3°. Alternatively, crystalline Form H is characterized by at least four x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 4.6°, 7.4°, 9.2°, 11.1°, 13.5°, 14.9°, and 22.3°. In another alternative, crystalline Form H is characterized by at least five x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 4.6°, 7.4°, 9.2°, 11.1°, 13.5°, 14.9°, and 22.3°. In yet another alternative, crystalline Form H is characterized by at least six x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) selected from 4.6°, 7.4°, 9.2°, 11.1°, 13.5°, 14.9°, and 22.3°. In yet another alternative, crystalline Form H is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 4.6°, 7.4°, 9.2°, 11.1°, 13.5°, 14.9°, and 22.3°. In yet another alternative, crystalline Form H is characterized by x-ray powder diffraction peaks at 2 $\Theta$  angles ( $\pm 0.2^\circ$ ) 4.6°, 5.4°, 7.4°, 9.2°, 10.3°, 11.1°, 13.5°, 13.8°, 14.9°, 16.9°, 17.6°, 18.4°, 19.5°, 20.7°, 22.3°, 22.9°, 23.4°, 24.1°, 24.8°, 26.5°, 27.2°, and 29.5°. In yet another alternative, crystalline Form H is characterized by an X-ray powder diffraction pattern substantially similar to **FIG. 16**.

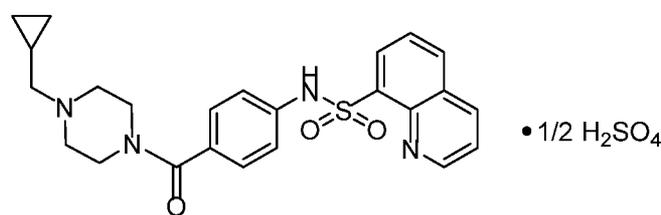
**[0077]** Also provided is a crystalline Form I of a hemisulfate salt of a compound having the formula:



wherein the hemisulfate salt of the compound in crystalline Form I is an ethanol solvate.

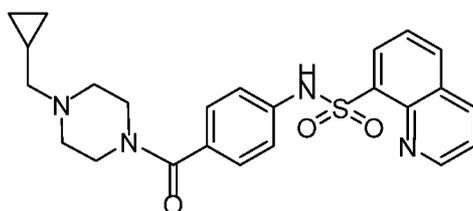
**[0078]** In one aspect, crystalline Form I is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $6.7^\circ$ ,  $9.5^\circ$ , and  $19.7^\circ$ . Alternatively, crystalline Form I is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $6.7^\circ$ ,  $9.5^\circ$ , and  $19.7^\circ$ ; and at least one additional x-ray powder diffraction peak at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $9.9^\circ$ ,  $12.6^\circ$ ,  $15.8^\circ$ ,  $21.9^\circ$ , and  $22.3^\circ$ . In another alternative, crystalline Form I is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $6.7^\circ$ ,  $9.5^\circ$ , and  $19.7^\circ$ ; and at least two additional x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $9.9^\circ$ ,  $12.6^\circ$ ,  $15.8^\circ$ ,  $21.9^\circ$ , and  $22.3^\circ$ . In yet another alternative, crystalline Form I is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $6.7^\circ$ ,  $9.5^\circ$ , and  $19.7^\circ$ ; and at least three additional x-ray powder diffraction peak at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $9.9^\circ$ ,  $12.6^\circ$ ,  $15.8^\circ$ ,  $21.9^\circ$ , and  $22.3^\circ$ . In yet another alternative, crystalline Form I is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $6.7^\circ$ ,  $7.7^\circ$ ,  $9.5^\circ$ ,  $9.9^\circ$ ,  $10.5^\circ$ ,  $11.6^\circ$ ,  $12.6^\circ$ ,  $13.4^\circ$ ,  $13.8^\circ$ ,  $14.3^\circ$ ,  $15.2^\circ$ ,  $15.8^\circ$ ,  $16.8^\circ$ ,  $17.2^\circ$ ,  $19.0^\circ$ ,  $19.7^\circ$ ,  $20.5^\circ$ ,  $20.9^\circ$ ,  $21.9^\circ$ ,  $22.3^\circ$ ,  $23.9^\circ$ ,  $24.6^\circ$ ,  $25.5^\circ$ ,  $26.0^\circ$ ,  $27.5^\circ$ ,  $28.3^\circ$ , and  $29.3^\circ$ . In yet another alternative, crystalline Form I is characterized by an X-ray powder diffraction pattern substantially similar to **FIG. 17**. In yet another alternative, crystalline Form I, is characterized by a DSC pattern having a peak at  $134.7^\circ\text{C} \pm 2^\circ\text{C}$ . In yet another alternative, crystalline Form I, is characterized by a TGA comprising a weight loss of about  $6.9 \pm 0.5\%$  up to  $180^\circ\text{C} \pm 2^\circ\text{C}$ . In yet another alternative, crystalline Form I is characterized by a TGA or DSC substantially similar to **FIG. 18**.

**[0079]** Also provided is a crystalline Form J of a hemisulfate salt of a compound having the formula:



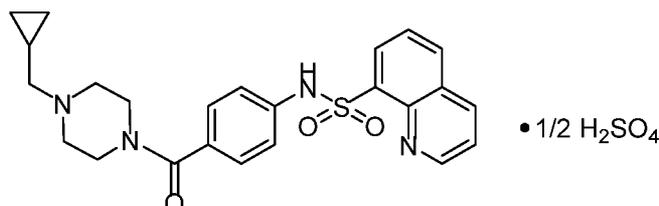
[0080] In one aspect, crystalline Form J is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $12.4^\circ$ ,  $13.2^\circ$ ,  $14.6^\circ$ ,  $20.4^\circ$ , and  $23.7^\circ$ . Alternatively, crystalline Form J is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $12.4^\circ$ ,  $13.2^\circ$ ,  $14.6^\circ$ ,  $15.7^\circ$ ,  $20.4^\circ$ ,  $23.3^\circ$ , and  $23.7^\circ$ . In another alternative, crystalline Form J is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $12.4^\circ$ ,  $13.2^\circ$ ,  $14.6^\circ$ ,  $15.7^\circ$ ,  $20.4^\circ$ ,  $22.0^\circ$ ,  $23.3^\circ$ ,  $23.7^\circ$ , and  $28.0^\circ$ . In another alternative, crystalline Form J is characterized by an X-ray powder diffraction pattern substantially similar to **FIG. 19**.

[0081] Also provided herein is a free-base crystalline form of Compound **1** having the formula:



[0082] In one aspect, the free-base crystalline form of Compound **1** is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $6.9^\circ$ ,  $13.5^\circ$ ,  $19.8^\circ$ , and  $20.3^\circ$ . Alternatively, the free-base crystalline form of Compound **1** is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $6.9^\circ$ ,  $13.5^\circ$ ,  $19.8^\circ$ ,  $20.3^\circ$ , and  $25.7^\circ$ . In another alternative the free-base crystalline form of Compound **1** is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $6.9^\circ$ ,  $13.5^\circ$ ,  $15.7^\circ$ ,  $15.9^\circ$ ,  $19.8^\circ$ ,  $20.3^\circ$ ,  $23.6^\circ$ , and  $25.7^\circ$ . In yet another alternative, the free-base crystalline form of Compound **1** is characterized by an X-ray powder diffraction pattern substantially similar to **FIG. 25**.

[0083] Also provided herein is an amorphous Form of a hemisulfate salt of a compound having the formula:



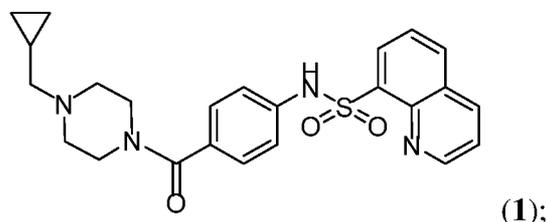
[0084] In one aspect, the compounds described herein are at least 60% a single crystalline form, at least 70% a single crystalline form, at least 80% a single crystalline form, at least 90% a single crystalline form, at least 95% a single crystalline form, or at least 99% a single crystalline form by weight.

**[0085]** In one aspect, the compounds described herein have a chemical purity of at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% by weight.

**[0086]** In one aspect, the compounds described herein are substantially free of amorphous forms, i.e., less than 10% of the amorphous form is present e.g., less than 5%, less than 3%, less than 2%, or less than 1% of the amorphous form is present.

**[0087]** Also provided are processes for making the disclosed crystalline and amorphous forms.

**[0088]** In one aspect, provided herein is a method of forming crystalline Form A, the method comprising reacting Compound **1**:



with  $\text{H}_2\text{SO}_4$  in an alcoholic solution. In one aspect, the molar ratio of the compound of Formula **1** to  $\text{H}_2\text{SO}_4$  is about 2:1. In another aspect, the alcoholic solution further comprises water. In one aspect, the method of forming crystalline Form A described above further comprises the step of, after the reaction with  $\text{H}_2\text{SO}_4$ , adding a sufficient amount of water to precipitate the crystalline form. In one aspect, the alcohol is methanol or ethanol. In another aspect, the solution further comprises an aromatic solvent. In another aspect, the aromatic solvent is toluene.

**[0089]** In one alternative, crystalline Form A is prepared by reacting Compound **1** with  $\text{H}_2\text{SO}_4$  in a solution comprising acetone and water. In one aspect, the solution is acetone:water (9:1/v:v).

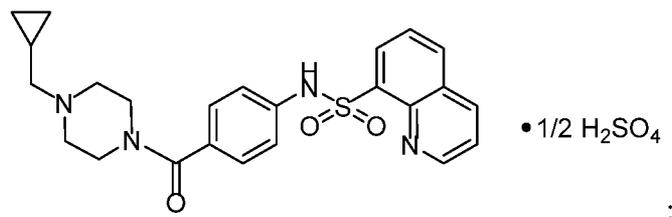
**[0090]** Also provided is a method of forming a hemisulfate salt of a compound having the formula:



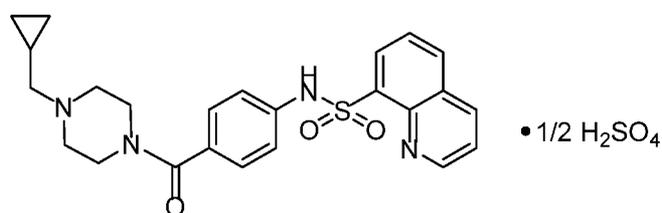
the method comprising reacting the non-crystalline freebase of compound 1 with a solution of sulfuric acid in EtOAc. In one aspect, the concentration of sulfuric acid in EtOAc is about 15 wt% to about 30 wt%. In certain embodiments, the concentration of sulfuric acid in EtOAc is about 24 wt%.

[0091] In one alternative, the hemisulfate salt is prepared by reacting Compound **1** with  $\text{H}_2\text{SO}_4$  in a solution comprising water and an alcohol such as MeOH or EtOH.

[0092] Also provided is a method of forming an amorphous form of a hemisulfate salt of a compound of the formula:



the method comprising crystallizing Form A of a hemisulfate salt of a compound having the formula:



via evaporation from MeOH. Alternatively, the amorphous form of the hemisulfate salt can be prepared by crystallizing the crystalline Form A of a hemisulfate salt from THF. In one aspect, the evaporative crystallization is performed in methanol or THF at about 50 °C.

### Compositions and Administration

[0093] Provided herein are pharmaceutical compositions comprising one or more of the disclosed crystalline forms (e.g. crystalline Form A), or the disclosed amorphous form, together with a pharmaceutically acceptable carrier. The amount of crystalline or amorphous form in a provided composition is such that is effective to measurably modulate PKR in a subject.

[0094] Pharmaceutical compositions described herein can be prepared by any method known in the art of pharmacology. In general, such preparatory methods include the steps of bringing one or more of the disclosed crystalline forms (e.g. crystalline Form A) into association with a carrier and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0095] Pharmaceutically acceptable carriers used in the manufacture of provided pharmaceutical compositions include inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Carriers such as cocoa butter and suppository

waxes, coloring agents, coating agents, sweetening, flavoring, and perfuming agents may also be present in the composition.

**[0096]** Exemplary diluents include calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, and mixtures thereof.

**[0097]** Exemplary granulating and/or dispersing agents include potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose, and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, and mixtures thereof.

**[0098]** Exemplary surface active agents and/or emulsifiers include natural emulsifiers (*e.g.* acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (*e.g.* bentonite (aluminum silicate) and Veegum (magnesium aluminum silicate)), long chain amino acid derivatives, high molecular weight alcohols (*e.g.* stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (*e.g.* carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (*e.g.* carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (*e.g.* polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan (Tween 60), polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60), sorbitan tristearate (Span 65), glyceryl monooleate, sorbitan monooleate (Span 80)), polyoxyethylene esters (*e.g.* polyoxyethylene monostearate (Myrj 45), polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and Solutol), sucrose fatty acid esters, polyethylene glycol fatty acid esters (*e.g.* Cremophor™), polyoxyethylene ethers, (*e.g.* polyoxyethylene lauryl ether (Brij 30)), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid,

ethyl laurate, sodium lauryl sulfate, Pluronic F-68, Poloxamer-188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, and/or mixtures thereof.

**[0099]** Exemplary binding agents include starch (*e.g.* cornstarch and starch paste), gelatin, sugars (*e.g.* sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol, *etc.*), natural and synthetic gums (*e.g.* acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (Veegum), and larch arabogalactan), alginates, polyethylene oxide, polyethylene glycol, inorganic calcium salts, silicic acid, polymethacrylates, waxes, water, alcohol, and/or mixtures thereof.

**[00100]** Exemplary preservatives include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives. In certain embodiments, the preservative is an antioxidant. In other embodiments, the preservative is a chelating agent.

**[00101]** Exemplary antioxidants include alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfite.

**[00102]** Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA) and salts and hydrates thereof (*e.g.*, sodium edetate, disodium edetate, trisodium edetate, calcium disodium edetate, dipotassium edetate, and the like), citric acid and salts and hydrates thereof (*e.g.*, citric acid monohydrate), fumaric acid and salts and hydrates thereof, malic acid and salts and hydrates thereof, phosphoric acid and salts and hydrates thereof, and tartaric acid and salts and hydrates thereof. Exemplary antimicrobial preservatives include benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and thimerosal.

**[00103]** Exemplary antifungal preservatives include butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and sorbic acid.

**[00104]** Exemplary alcohol preservatives include ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and phenylethyl alcohol.

**[00105]** Exemplary acidic preservatives include vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and phytic acid.

**[00106]** Other preservatives include tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, Glydant Plus, Phenonip, methylparaben, Germall 115, Germaben II, Neolone, Kathon, and Euxyl.

**[00107]** Exemplary buffering agents include citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium gluconate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and mixtures thereof.

**[00108]** Exemplary lubricating agents include magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, sodium stearyl fumarate, and mixtures thereof.

**[00109]** Exemplary natural oils include almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary synthetic oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric

triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and mixtures thereof.

**[00110]** Compositions described herein may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, transmucosally, or in an ophthalmic preparation. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. In one aspect, the pharmaceutical compositions provided herewith are orally administered in an orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

**[00111]** The amount of provided crystalline or amorphous form that may be combined with carrier materials to produce a composition in a single dosage form will vary depending upon the subject to be treated and the particular mode of administration. For example, a specific dosage and treatment regimen for any particular subject will depend upon a variety of factors, including age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, the judgment of the treating physician, and the severity of the particular disease being treated. The amount of a provided crystalline form in the composition will also depend upon the particular form (e.g., Form A, B, C, D, E, F, G, H, I, or J) in the composition. In one aspect, a provided composition may be formulated such that a dosage equivalent to about 0.001 to about 100 mg/kg body weight/day of compound 1 (e.g., about 0.5 to about 100 mg/kg of compound 1) can be administered to a subject receiving these compositions. Alternatively, dosages equivalent to 1 mg/kg and 1000 mg/kg of compound 1 every 4 to 120 hours is also acceptable. As used herein, the dose refers to the amount of compound 1 in the particular crystalline form. The amount of the particular crystalline form will be calculated based on the equivalence to the free-base form of compound 1.

**[00112]** In one aspect, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose of equivalent to about 2 mg to about 3000 mg of

compound 1. In certain embodiments, the dose is oral dose. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 2 mg to about 3000 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 5 mg to about 350 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 5 mg to about 200 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 5 mg to about 100 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 5 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 10 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 15 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 20 mg of compound 1. In certain 25 mg. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 30 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 40 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 45 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 50 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 60 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 70 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 80 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 90 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 100 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 110 mg of compound 1. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated equivalent to about 120 mg of compound 1.

[00113] In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 2 mg to about 3000 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 5 mg to about 500 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 5 mg to about 200 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 5 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 5 mg to about 10 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose of about 15 mg equivalent to compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 20 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 25 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 30 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 35 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 40 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 45 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 50 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 60 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 70 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 80 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form

A) or amorphous form is formulated for administration at a dose equivalent to about 90 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 100 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 110 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 120 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 130 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 140 mg of compound 1 per day. In certain embodiments, a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 150 mg of compound 1 per day. Dosing can be once, twice, or three times daily. In one aspect, e.g., a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 5 mg of compound 1 twice per day. In one aspect, e.g., a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 20 mg of compound 1 twice per day. In one aspect, e.g., a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 50 mg of compound 1 twice per day. In one aspect, e.g., a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 100 mg of compound 1 twice per day. In one aspect, e.g., a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 5 mg of compound 1 once every other day. In one aspect, e.g., a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 20 mg of compound 1 once every other day. In one aspect, e.g., a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 50 mg of compound 1 once every other day. In one aspect, e.g., a disclosed crystalline (e.g. crystalline Form A) or amorphous form is formulated for administration at a dose equivalent to about 100 mg of compound 1 once every other day.

**[00114]** In one aspect, a disclosed form (crystalline Form A, B, C, D, E, F, G, H, I, J, the crystalline free-base, or the amorphous form) is formulated as a tablet composition together with a pharmaceutically acceptable carrier. In one aspect, the carrier is selected from one or

more of microcrystalline cellulose, mannitol, Croscarmellose Sodium, and Sodium Stearyl Fumarate. In one aspect, the carrier is microcrystalline cellulose e.g., present in an amount of 50% w/w to 70% w/w ( $\pm 2\%$ ), 55% w/w to 65% w/w ( $\pm 2\%$ ), 58% w/w to 62% w/w ( $\pm 2\%$ ), 59% w/w ( $\pm 2\%$ ), 60% w/w ( $\pm 2\%$ ), 61% w/w ( $\pm 2\%$ ), 62% w/w ( $\pm 2\%$ ), 61% w/w, or 62% w/w. In another aspect, the carrier is mannitol e.g., present in an amount of 15% w/w ( $\pm 2\%$ ) to 35% w/w ( $\pm 2\%$ ), 20% w/w ( $\pm 2\%$ ) to 30% w/w ( $\pm 2\%$ ), 22% w/w ( $\pm 2\%$ ) to 26% w/w ( $\pm 2\%$ ), 22% w/w ( $\pm 2\%$ ), 23% w/w ( $\pm 2\%$ ), 24% w/w ( $\pm 2\%$ ), or 23% w/w. In another aspect, the carrier is croscarmellose sodium e.g., present in an amount of 1% w/w to 5% w/w ( $\pm 2\%$ ), 2% w/w to 4% w/w ( $\pm 2\%$ ), 2% w/w ( $\pm 2\%$ ), 3% w/w ( $\pm 2\%$ ), 4% w/w ( $\pm 2\%$ ) or 3% w/w. In another aspect, the carrier is stearyl fumarate e.g., present in an amount of 1% w/w to 5% w/w ( $\pm 2\%$ ), 2% w/w to 4% w/w ( $\pm 2\%$ ), 1% w/w ( $\pm 2\%$ ), 2% w/w ( $\pm 2\%$ ), 3% w/w ( $\pm 2\%$ ) or 2% w/w. In some embodiments, crystalline form A is present in the tablet composition in an amount equivalent to about 1 to about 200 mg of compound 1. In some embodiments, crystalline form A is present in the tablet composition in an amount equivalent to about 1 to about 150 mg of compound 1. In some embodiments, crystalline form A is present in the tablet composition in an amount equivalent to about 1 to about 100 mg of compound 1. In some embodiments, crystalline form A is present in the tablet composition in an amount equivalent to about 5 mg of compound 1. In some embodiments, crystalline form A is present in the tablet composition in an amount equivalent to about 20 mg of compound 1. In some embodiments, crystalline form A is present in the tablet composition in an amount equivalent to about 50 mg of compound 1. In some embodiments, crystalline form A is present in the tablet composition in an amount equivalent to about 75 mg of compound 1. In some embodiments, crystalline form A is present in a tablet composition in an amount equivalent to about 100 mg of compound 1.

**[00115]** As used herein, the dose amount of crystalline Form A, B, C, D, E, F, G, H, I, J, or the amorphous form is based on the equivalence to the free-base form of compound 1. For example, “crystalline form A present in the composition in an amount equivalent to about 1.0 mg of compound 1” means about 1.18 mg of crystalline Form A is present in the composition and is equivalent to about 1.0 mg of free base compound 1.

**[00116]** In one aspect, the tablet composition comprises 10% w/w ( $\pm 1\%$ ) of the crystalline free-base; 62% w/w ( $\pm 2\%$ ) microcrystalline cellulose; 23% w/w ( $\pm 2\%$ ) mannitol, 3% w/w ( $\pm 2\%$ ) croscarmellose sodium, and 2% w/w ( $\pm 2\%$ ) stearyl fumarate.

**[00117]** In one aspect, the tablet composition comprises 11.78% w/w ( $\pm 1\%$ ) of crystalline Form A; 62% w/w ( $\pm 2\%$ ) microcrystalline cellulose; 23% w/w ( $\pm 2\%$ ) mannitol; 3% w/w ( $\pm 2\%$ ) croscarmellose sodium; and 2% w/w ( $\pm 2\%$ ) stearyl fumarate.

**[00118]** In certain embodiments, provided is a pharmaceutical composition comprising crystalline Form A and a pharmaceutically acceptable carrier. In certain embodiments, provided is a pharmaceutical composition comprising the crystalline Form A is substantially free of Compound IM-1 and/or Compound IM-2 (see Exemplification). In certain embodiments, the pharmaceutical composition comprising Form A is substantially free of other crystal forms of the hemisulfate salt of compound 1. In certain embodiments, the one or more carriers are processed to generate particles of a consistent size. In certain embodiments, processing the powder (i.e., crystalline Form A) comprises milling the powder for an amount of time suitable to bring about a desired particle size (“milled powder”). In some embodiments, the particle size of the milled powder is less than about 400  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 300  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 200  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 100  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 90  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 80  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 70  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 60  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 50  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 40  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 30  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder is less than about 20  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder ranges from about 10  $\mu\text{m}$  to about 400  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder ranges from about 10  $\mu\text{m}$  to about 300  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder ranges from about 10  $\mu\text{m}$  to about 200  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder ranges from about 10  $\mu\text{m}$  to about 100  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder ranges from about 10  $\mu\text{m}$  to about 80  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder ranges from about 10  $\mu\text{m}$  to about 70  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder ranges from about 10  $\mu\text{m}$  to about 60  $\mu\text{m}$ . In some embodiments, the particle size of the milled powder ranges from about 20  $\mu\text{m}$  to about 60  $\mu\text{m}$ . The term “about,” as used herein with respect to particle size, means  $\pm 5 \mu\text{m}$ .

[00119] In some embodiments, at least 90% of a representative sample of the milled powder has a particle size of less than about 100, about 80, about 70, about 60, about 50, about 40, about 30, about 20, or about 10  $\mu\text{m}$ . In some embodiments, at least about 90% of a representative sample of the milled powder has a particle size of less than about 60  $\mu\text{m}$ .

**Methods of Treatment and Uses of Compounds and Compositions**

[00120] In one aspect, the crystalline and amorphous forms described herein and compositions thereof are allosteric activators of PKR, and are generally useful for treating the underlying condition of PKD.

[00121] Thus, provided herein are methods of treating Pyruvate Kinase Deficiency (PKD) in a subject in need thereof, comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I); or a pharmaceutical composition thereof for use in treating Pyruvate Kinase Deficiency (PKD) in a subject in need thereof. Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J, or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for treating Pyruvate Kinase Deficiency (PKD). Exemplified conditions related to PKD include, but are not limited to, anemias, cholecystolithiasis, gallstones, tachycardia, hemochromatosis, icteric sclera, splenomegaly, leg ulcers, jaundice, fatigue, and shortness of breath. As described herein, PKD is a deficiency of PKR. In certain embodiments, the deficiency of PKR is associated with a PKR mutation.

[00122] Pyruvate kinase deficiency (PKD) is a glycolytic enzymopathy that results in life-long hemolytic anemia. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene and at least one is a missense mutation. See Canu. et.al , Blood Cells, Molecules and Diseases **2016**, 57, pp. 100-109. In certain embodiments, a subject having PKD has an Hb concentration less than or equal to 10.0 g/dL. In certain embodiments, the subject having PKD is an adult not under regular transfusion (e.g. having had no more than 4 transfusion episodes in the 12-month period up to the treatment). In certain embodiments, the subject having PKD is an adult transfusion independent (e.g. having no more than 3 units of RBCs transfused in the 12-month period

prior to the treatment). In certain embodiments, the subject having PKD is an adult under regular transfusion (e.g. having had at least 4 transfusion episodes (e.g., at least 6 transfusion episodes) in the 12-month period prior to the treatment). In certain embodiments, the subject having PKD has a total number of at least 5 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 10 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 15 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 20 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 25 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 30 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 40 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 50 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 60 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 70 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD is not homozygous for the R479H mutation or does not have 2 non-missense mutations in the PKLR gene. In certain embodiments, the subject having PKD, under regular transfusion, has hemoglobin (Hb)  $\leq 12.0$  g/dL (if male) or  $\leq 11.0$  g/dL (if female), prior to the treatment. In certain embodiments, the subject having PKD, under regular transfusion, has transfusion occurring on average less than or equal to once every three weeks. In certain embodiments, the subject having PKD has received at least 0.8 mg (e.g. at least 1.0 mg) folic acid daily (e.g. for at least 21 days) prior to the treatment. In certain embodiments, the subject with PKD under regular transfusion achieves a reduction in transfusion burden (e.g. at least 33% reduction in the number of RBC units transfused) during the 5 weeks, 10 weeks, 15 weeks, 20 weeks, or 24 weeks, 28 weeks, or 32 weeks of treatment. In certain embodiments, the subject having PKD, not under regular transfusion (having had no more than 4 transfusion episodes in the 12-month period prior to the treatment and/or no transfusion in the 3 months prior to the treatment), has hemoglobin (Hb)  $\leq 10.0$  g/dL regardless of gender prior to the treatment. In certain embodiments, the subject having PKD has undergone splenectomy.

**[00123]** In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.0 g/dL increase in Hb concentration after the treatment compared to the baseline of

prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.5 g/dL increase in Hb concentration from baseline prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 2.0 g/dL increase in Hb concentration from baseline prior to the treatment.

**[00124]** In an embodiment, the mutant PKR is selected from the group consisting of A31V, A36G, G37Q, R40W, R40Q, L73P, S80P, P82H, R86P, I90N, T93I, G95R, M107T, G111R, A115P, S120F, H121Q, S130P, S130Y, V134D, R135D, A137T, G143S, I153T, A154T, L155P, G159V, R163C, R163L, T164N, G165V, L167M, G169G, E172Q, W201R, I219T, A221Y, D221N, G222A, I224T, G232C, N253D, G263R, G263W, E266K, V269F, L272V, L272P, G275R, G275R, E277K, V280G, D281N, F287V, F287L, V288L, D293N, D293V, A295I, A295V, I310N, I314T, E315K, N316K, V320L, V320M, S330R, D331N, D331G, D331E, G332S, V335M, A336S, R337W, R337P, R337Q, D339N, D339Q, G341A, G341D, I342F, K348N, A352D, I357T, G358R, G358E, R359C, R359H, C360Y, N361D, G364D, K365M, V368F, T371I, L374P, S376I, T384M, R385W, R385K, E387G, D390N, A392T, N393D, N393S, N393K, A394S, A394D, A394V, V395L, D397V, G398A, M403I, G406R, E407K, E407G, T408P, T408A, T408I, K410E, G411S, G411A, Q421K, A423A, A423A, R426W, R426Q, E427A, E427N, A431T, R449C, I457V, G458D, A459V, V460M, A468V, A468G, A470D, T477A, R479C, R479H, S485F, R486W, R486L, R488Q, R490W, I494T, A495T, A495V, R498C, R498H, A503V, R504L, Q505E, V506I, R510Q, G511R, G511E, R518S, R531C, R532W, R532Q, E538D, G540R, D550V, V552M, G557A, R559G, R559P, N566K, M568V, R569Q, R569L, Q58X, E174X, W201X, E241X, R270X, E440X, R486X, Q501X, L508X, R510X, E538X, R559X. These mutations are described in Canu et.al., Blood Cells, Molecules and Diseases **2016**, 57, pp. 100-109. In an embodiment, the mutant PKR is selected from G332S, G364D, T384M, K410E, R479H, R479K, R486W, R532W, R510Q, and R490W. In certain embodiments, the mutant PKR is selected from A468V, A495V, I90N, T408I, and Q421K, and R498H. In certain embodiments, the mutant PKR is R532W, K410E, or R510Q. In certain embodiments, the mutant PKR is R510Q, R486W, or R479H.

**[00125]** In other aspects, provided are methods of treating a disease selected from hemolytic anemia, sickle cell disease, thalassemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof, comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is

crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof for use in treating disease selected from hemolytic anemia, sickle cell disease, thalassemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject. Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for treating a disease selected from hemolytic anemia, sickle cell disease, thalassemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof. In one aspect, the disease to be treated is hemolytic anemia.

**[00126]** In other aspects, provided herein are methods for treating thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia) in a subject in need thereof, comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof for use in treating thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia). Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for treating thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia).

**[00127]** In other aspects, provided herein are methods for treating thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia) in a subject in need thereof, comprising administering to the subject an effective amount of crystalline Form A, or a pharmaceutical composition thereof. Also provided is crystalline Form A or a pharmaceutical composition thereof for use in treating thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia). Further provided is the use of crystalline Form A, or a pharmaceutical composition thereof, in the manufacture of a medicament for treating thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia).

**[00128]** In other aspects, provided herein are methods for treating thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia) in a subject in need thereof, comprising administering to the subject an effective amount of crystalline Form D, or a pharmaceutical composition thereof. Also provided is crystalline Form D, or a

pharmaceutical composition thereof for use in treating thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia). Further provided is the use of crystalline Form D, or a pharmaceutical composition thereof, in the manufacture of a medicament for treating thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia).

**[00129]** In certain embodiments, the subject is an adult subject with thalassemia. In certain embodiments, the subject has thalassemia such as  $\beta$ -thalassemia intermedia, Hb E  $\beta$ -thalassemia,  $\alpha$ -thalassemia (Hb H disease), or  $\beta$ -thalassemia with mutations of 1 or more  $\alpha$  genes. In certain embodiments, the subject has beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject is an adult male subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject is a female subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject is an adult female subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 6.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 7.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 8.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 9.0 g/dL. In certain aspects, the subject having non-transfusion-dependent thalassemia does not have a known history (e.g., has been diagnosed in the past) of Hb S or Hb C forms of thalassemia. In certain embodiments, the term “non-transfusion dependent” thalassemia refers to subjects with thalassemia having no more than 4 (e.g. five) units of RBCs transfused during a 24-week period up to the first day of administration of a crystalline or amorphous form described herein and/or no RBC transfusions in the 8 weeks prior to the first day of administration of a crystalline or amorphous form described herein.

**[00130]** In other aspects, provided herein are methods for increasing the lifetime of red blood cells (RBCs) in a subject in need thereof comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof for use in increasing the lifetime of red blood cells (RBCs) in a subject in need thereof. Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for increasing the lifetime of red blood cells (RBCs). In one aspect, crystalline Form A, B, C, D, E, F, G, H,

I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof is added directly to whole blood or packed red blood cells extracorporeally.

**[00131]** In other aspects, provided herein are methods for regulating 2,3-diphosphoglycerate levels in blood in a subject in need thereof comprising contacting blood with an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof for use in regulating 2,3-diphosphoglycerate levels in blood in a subject in need thereof. Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for regulating 2,3-diphosphoglycerate levels in blood.

**[00132]** In other aspects, provided herein are methods for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof for use in treating anemia in a subject in need thereof. Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for treating anemia. In one aspect, the anemia to be treated is dyserythropoietic anemia.

**[00133]** In certain embodiments, the anemia is a dyserythropoietic anemia such as congenital dyserythropoietic anemia type I, II, III, or IV. In certain embodiments, the anemia is hemolytic anemia. In certain embodiments, the hemolytic anemia is a congenital and/or hereditary form of hemolytic anemia such as PKD, sickle cell disease, thalassemias (e.g. alpha or beta or non-transfusion-dependent thalassemia), hereditary spherocytosis, hereditary elliptocytosis), paroxysmal nocturnal hemoglobinuria, abeta-lipoproteinemia (Bassen-Kornzweig syndrome). In certain embodiments, the hemolytic anemia is acquired hemolytic anemia such as autoimmune hemolytic anemia, drug-induced hemolytic anemia. In certain embodiments, the hemolytic anemia is anemia as part of a multi-system disease, such as the anemia of Congenital Erythropoietic Purpura, Fanconi, Diamond-Blackfan.

**[00134]** As used herein, the term “anemia” refers to a deficiency of red blood cells (RBCs) and/or hemoglobin. As used herein, anemia includes all types of clinical anemia, for example (but not limited to): microcytic anemia, iron deficiency anemia, hemoglobinopathies, heme synthesis defect, globin synthesis defect, sideroblastic defect, normocytic anemia, anemia of chronic disease, aplastic anemia, hemolytic anemia, macrocytic anemia, megaloblastic anemia, pernicious anemia, dimorphic anemia, anemia of prematurity, Fanconi anemia, hereditary spherocytosis, sickle cell disease, warm autoimmune hemolytic anemia, cold agglutinin hemolytic anemia, osteopetrosis, thalassemia, and myelodysplastic syndrome.

**[00135]** In certain embodiments, anemia can be diagnosed on a complete blood count. In certain embodiments, anemia can be diagnosed based on the measurement of one or more markers of hemolysis (e.g. RBC count, hemoglobin, reticulocytes, schistocytes, lactate Dehydrogenase (LDH), haptoglobin, bilirubin, and ferritin) and/or hemosiderinuria mean corpuscular volume (MCV) and/or red cell distribution width (RDW). In the context of the present invention, anemia is present if an individual has a hemoglobin (Hb) less than the desired level, for example, the Hb concentration of less than 14 g/dL, more preferably of less than 13 g/dL, more preferably of less than 12 g/dL, more preferably of less than 11 g/dL, or most preferably of less than 10 g/dL.

**[00136]** In certain embodiments, provided herein is a method of increasing the amount of hemoglobin in a subject by administering an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof as described herein. In certain embodiments, also provided herein is a method of increasing the amount of hemoglobin in a subject having thalassemia comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J, or the crystalline free base, or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Further provided is a method of increasing the amount of hemoglobin in subjects having non-transfusion-dependent thalassemia comprising administering an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J, or the crystalline free base, or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof as described herein to the subject. In certain embodiments, the provided methods increase hemoglobin concentration in the subject. In certain embodiments, the provided methods increase Hb concentration to a desired level, for example, above 10 g/dL, more preferably above 11 g/dL, more preferably above 12 g/dL, more preferably above 13 g/dL, or most preferably above 14 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 0.5 g/dL. In

certain embodiments, the provided methods increase Hb concentration by at least about 1.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 1.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 6.0 g/dL. In certain embodiments, the increase in Hb concentration is determined from baseline at one or more assessment between week 1 and week 20 (e.g., between week 2 and week 15, between week 3 and week 15, and between week 4 and week 12) of treatment with an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J, or the crystalline free base or amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof as described herein. In certain embodiments, the provided methods increase Hb concentration as described above in female subjects having thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 12 g/dL in female subjects having thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration as described above in male subjects having thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 13 g/dL in male subjects having thalassemia (e.g., beta-thalassemia or non-transfusion-dependent thalassemia).

**[00137]** In some aspects, provided herein are methods for treating hemolytic anemia in a subject in need thereof comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof for use in treating hemolytic anemia in a subject in need thereof. Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J or the

amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for treating hemolytic anemia. In one aspect, the hemolytic anemia to be treated is hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, or anemia as part of a multi-system disease.

**[00138]** In some aspects, provided herein are methods for treating sickle cell disease in a subject in need thereof comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof for use in treating sickle cell disease in a subject in need thereof. Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for treating sickle cell disease.

**[00139]** In some aspects, provided herein are methods for treating thalassemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, acquired hemolytic anemia, or anemia of chronic diseases in a subject in need thereof comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof for use in treating thalassemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, acquired hemolytic anemia, or anemia in a subject in need thereof. Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for treating thalassemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, acquired hemolytic anemia, or anemia.

**[00140]** In some aspects, provided herein are methods for activating wild-type or mutant PKR in red blood cells in a subject in need thereof comprising administering to the subject an effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof. Also provided is crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of

formula (I), or a pharmaceutical composition thereof for use in activating wild-type or mutant PKR in red blood cells in a subject in need thereof. Further provided is the use of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof in the manufacture of a medicament for activating wild-type or mutant PKR in red blood cells.

**[00141]** The provided crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), and pharmaceutical compositions described herein are activators of PKR mutants having lower activities compared to the wild type, thus are useful for methods of the present disclosure. Such mutations in PKR can affect enzyme activity (catalytic efficiency), regulatory properties (modulation by fructose bisphosphate (FBP)/ATP), and/or thermostability of the enzyme. Examples of such mutations are described in Valentini et al, JBC 2002. Some examples of the mutants that are activated by the compounds described herein include G332S, G364D, T384M, R479H, R479K, R486W, R532W, R510Q, and R490W. Without being bound by theory, in certain embodiments, the compounds described herein affect the activities of PKR mutants by activating FBP non-responsive PKR mutants, restoring thermostability to mutants with decreased stability, or restoring catalytic efficiency to impaired mutants. The activating activity of the present compounds against PKR mutants may be tested following a method described in the Examples. Compounds described herein are also activators of wild type PKR.

**[00142]** In certain embodiments, the provided crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), and pharmaceutical compositions described herein increase the affinity of PKR to phosphoenolpyruvate (PEP). In certain embodiments, the provided crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), and pharmaceutical compositions described herein restore the ability of RBCs to convert PEP and ADP to pyruvate and ATP.

**[00143]** In certain embodiments, provided herein are methods of reducing transfusion frequency of a subject with PKD comprising administering to the subject crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), and pharmaceutical compositions described herein. In certain embodiments, crystalline Form A is administered. In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 15 weeks. In certain embodiments,

the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks.

**[00144]** In some aspects, provided herein are methods of evaluating a subject, the method comprising: administering to the subject crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof; and acquiring a value for the level of the crystalline or amorphous form, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby evaluate the subject. In some aspects, the value for the level is acquired by analyzing the plasma concentration of crystalline or amorphous form. In some aspects, the level of 2,3-DPG is acquired by analyzing the blood concentration of 2,3-DPG. In some aspects, the level of ATP is acquired by analyzing the blood concentration of ATP. In some aspects, the activity of PKR is acquired by analyzing the blood concentration of a <sup>13</sup>C-label in the blood. In some aspects, the analysis is performed by sample analysis of bodily fluid. In some aspects, the bodily fluid is blood. In some aspects, the analysis is performed by mass spectroscopy. In some aspects, the analysis is performed by LC-MS.

**[00145]** In some aspects, provided herein are methods of evaluating a subject, the method comprising acquiring, the value for the level of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof, the level of 2,3-DPG, the level of ATP, or the activity of PKR in a subject that has been treated with crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof, to thereby evaluate the subject. In some aspects, acquiring comprises receiving a sample from the subject. In some aspects, acquiring comprises transmitting the value to another party. In some aspects, the other party is the party that administered crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof.

**[00146]** In some aspects, provided herein are methods of treating a subject, the method comprising: administering to the subject a therapeutically effective amount of crystalline Form A, B, C, D, E, F, G, H, I, or J or the amorphous Form of the compound of formula (I), or a pharmaceutical composition thereof; and acquiring a value for the level of the crystalline or amorphous form, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby treat the subject.

**[00147]** In another aspect, provided herein are methods of treating Pyruvate Kinase Deficiency (PKD) in a subject in need thereof, comprising administering to the subject an effective amount of crystalline free-base form of Compound 1 or a pharmaceutical composition thereof. In certain embodiments, the deficiency of PKR is associated with a PKR mutation. Also provided is crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, for use in treating Pyruvate Kinase Deficiency (PKD) in a subject in need thereof. Further provided is the use of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, in the manufacture of a medicament for treating Pyruvate Kinase Deficiency (PKD).

**[00148]** In other aspects, provided are methods of treating a disease selected from hemolytic anemia, sickle cell anemia, thalassemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof, comprising administering to the subject an effective amount of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof. In one aspect, the disease to be treated is hemolytic anemia.

**[00149]** In other aspects, provided herein are methods for treating hemolytic anemia, comprising administering to the subject an effective amount of crystalline free-base form of

Compound 1, or a pharmaceutical composition thereof. Also provided is crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, for use in treating hemolytic anemia in a subject in need thereof. Further provided is the use of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, in the manufacture of a medicament for treating hemolytic anemia.

**[00150]** In other aspects, provided herein are methods for treating sickle cell disease, comprising administering to the subject an effective amount of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof. Also provided is crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, for use in treating sickle cell disease in a subject in need thereof. Further provided is the use of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, in the manufacture of a medicament for treating sickle cell disease.

**[00151]** In other aspects, provided herein are methods for treating thalassemia (e.g., beta-thalassemia), comprising administering to the subject an effective amount of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof. Also provided is crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, for use in treating thalassemia (e.g., beta-thalassemia) in a subject in need thereof. Further provided is the use of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, in the manufacture of a medicament for treating thalassemia (e.g., beta-thalassemia).

**[00152]** In other aspects, provided herein are methods for increasing the lifetime of red blood cells (RBCs) in a subject in need thereof comprising administering to the subject an effective amount of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof. In one aspect, crystalline free-base form of Compound 1, or a pharmaceutical composition thereof is added directly to whole blood or packed red blood cells extracorporeally. Also provided is crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, for use in increasing the lifetime of red blood cells (RBCs) in a subject in need thereof. Further provided is the use of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, in the manufacture of a medicament for increasing the lifetime of red blood cells (RBCs).

**[00153]** In other aspects, provided herein are methods for regulating 2,3-diphosphoglycerate levels in blood in a subject in need thereof comprising contacting blood with an effective amount of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof. Also provided is crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, for use in regulating 2,3-diphosphoglycerate levels in

blood in a subject in need thereof. Further provided is the use of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, in the manufacture of a medicament for regulating 2,3-diphosphoglycerate levels in blood.

**[00154]** In other aspects, provided herein are methods for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof. In one aspect, the anemia to be treated is dyserythropoietic anemia. Also provided is crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, for use in treating anemia in blood in a subject in need thereof. Further provided is the use of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, in the manufacture of a medicament for treating anemia.

**[00155]** In certain embodiments, provided herein is a method of increasing the amount of hemoglobin in a subject by administering an effective amount of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof. Also provided is crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, for use in increasing the amount of hemoglobin in a subject in need thereof. Further provided is the use of crystalline free-base form of Compound 1, or a pharmaceutical composition thereof, in the manufacture of a medicament for increasing the amount of hemoglobin.

**[00156]** In some aspects, a therapeutically effective amount of a disclosed form (crystalline Form A, B, C, D, E, F, G, H, I, J, the crystalline free-base, or the amorphous form) can be administered to cells in culture, e.g. *in vitro* or *ex vivo*, or to a subject, e.g., *in vivo*, to treat, prevent, and/or diagnose a variety of disorders, including those described herein below.

**[00157]** In one aspect, the disclosed compositions, methods of treatment, and uses thereof, comprising a disclosed form (crystalline Form A, B, C, D, E, F, G, H, I, J, the crystalline free-base, or the amorphous form) further comprise the administration or use of folic acid. The administration or use of folic acid can be prior to, during, and/or following the administration or use of a crystalline or amorphous form described herein. In one aspect, however, the folic acid is administered or used prior to and/or concurrently with a disclosed form (crystalline Form A, B, C, D, E, F, G, H, I, J, the crystalline free-base, or the amorphous form). Thus, in one aspect, provided herein is a method for treating a condition described herein (e.g., PKD, anemia such as hemolytic anemia, acquired hemolytic anemia, and sickle cell anemia, thalassemia (e.g., beta-thalassemia, alpha-thalassemia, non-transfusion dependent thalassemia, etc.), sickle cell disease, hereditary spherocytosis, hereditary

elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria); increasing the lifetime of RBCs; regulating 2,3-diphosphoglycerate levels in blood; activating wild-type or mutant PKR in red blood cells; increasing the amount of hemoglobin; evaluating the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR; evaluating the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR; in a subject in need thereof, comprising administering to the subject an effective of a disclosed form (crystalline Form A, B, C, D, E, F, G, H, I, J, the crystalline free-base, or the amorphous form) and folic acid.

**[00158]** In aspects where folic acid is administered or used prior to a disclosed form (crystalline Form A, B, C, D, E, F, G, H, I, J, the crystalline free-base, or the amorphous form), the folic acid may be used at least 5 days, at least 10 days, at least 15 days, at least 20 days, or at least 25 days prior to the administration or use of disclosed form. In one aspect, the folic acid is administered or used at least 20, at least 21, at least 22, at least 23, at least 24, or at least 25 days prior to the administration or use of disclosed form. In another aspect, the folic acid is administered at least 21 days prior to the administration or use of disclosed form. In another aspect, the folic acid is administered or used from 1 to 30 days prior to the administration or use of disclosed form. In another aspect, the folic acid is administered or used from 5 to 25 days prior to the administration or use of disclosed form. In another aspect, the folic acid is administered or used from 10 to 30 days prior to the administration or use of disclosed form. In another aspect, the folic acid is administered or used from 10 to 25 days prior to the administration or use of disclosed form. In another aspect, the folic acid is administered or used from 15 to 25 days prior to the administration or use of disclosed form. In another aspect, the folic acid is administered or used from 20 to 25 days prior to the administration or use of disclosed form.

**[00159]** Specific amounts of folic acid to be administered or used with a disclosed form will vary depending upon the subject to be treated and the particular mode of administration. In certain aspects, the effective amount of folic acid is about 0.1 mg to about 10 mg daily. In certain aspects, the effective amount of folic acid is at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1.0 mg daily. In one aspect, the effective amount of folic acid is at least 0.8 mg daily or at least 1.0 mg daily.

**[00160]** The amount of folic acid is intended to be combined with any amount of a disclosed form described herein. Thus, in certain aspects, provided herein is a method for treating a condition described herein (e.g., PKD, anemia such as hemolytic anemia, acquired

hemolytic anemia, and sickle cell anemia, thalassemia (e.g., beta-thalassemia, alpha-thalassemia, non-transfusion dependent thalassemia, etc.), sickle cell disease, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria); increasing the lifetime of RBCs; regulating 2,3-diphosphoglycerate levels in blood; activating wild-type or mutant PKR in red blood cells; increasing the amount of hemoglobin; evaluating the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR; evaluating the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR; in a subject in need thereof, comprising administering to the subject an effective amount of a disclosed form described herein (crystalline Form A, B, C, D, E, F, G, H, I, J, the crystalline free-base, or the amorphous form) and folic acid, wherein the folic acid is administered prior to and/or concurrently with the disclosed form (e.g., at least 21 days prior), the disclosed form (e.g. Form A) is administered in an amount of 5, 20, or 50 mg BID and wherein the folic acid is administered in an amount of at least 0.8 mg/day .

### EXEMPLIFICATION

**[00161]** As depicted in the Examples below, crystalline and amorphous forms are prepared according to the following general procedures.

**[00162]** Typical abbreviations used are outlined below.

<b>Solvents</b>	
<b>Name</b>	<b>Abbreviation</b>
1-propanol	1-PA
2-propanol	IPA
Acetonitrile	ACN
Benzyl Alcohol	BA
Dichloromethane	DCM
Dimethyl Sulfoxide	DMSO
Ethanol	EtOH
Ethyl Acetate	EtOAc
Isopropyl Acetate	IPAc
Methanol	MeOH
Methyl Acetate	MeOAc
Methyl Butyl Ketone	MBK
Methyl Ethyl Ketone	MEK
Methyl Isobutyl Ketone	MIBK
N,N-Dimethylacetamide	DMAc
N,N-Dimethylformamide	DMF
N-Methyl Pyrrolidone	NMP
tert-Butyl Methyl Ether	MtBE

Tetrahydrofuran	THF
Trifluoroacetic Acid	TFA
Trifluoroethanol	TFE
Units	
Name	Abbreviation
Celsius	C
Degrees	°
Equivalents	eq.
Gram	g
Hour	hr
Kelvin	K
Liters	L
Milligrams	mg
Milliliters	mL
Minute	min
Second	sec
volume	vol.
Watt	W
weight	wt.

**[00163]** Powder X-ray diffraction was done using a Rigaku MiniFlex 600. Samples were prepared on Si zero-return wafers. A typical scan is from  $2\theta$  of 4 to 30 degrees, with step size 0.05 degrees over five minutes with 40 kV and 15 mA. A high-resolution scan is from  $2\theta$  of 4 to 40 degrees, with step size 0.05 degrees over thirty minutes with 40 kV and 15 mA. Typical parameters for XRPD are listed below.

Parameters for Reflection Mode	
X-ray wavelength	Cu K $\alpha$ 1, 1.540598 Å,
X-ray tube setting	40 kV, 15 mA
Slit condition	Variable + Fixed Slit System
Scan mode	Continuous
Scan range ( $^{\circ}2\theta$ )	4 - 30
Step size ( $^{\circ}2\theta$ )	0.05
Scan speed ( $^{\circ}/\text{min}$ )	5

**[00164]** Differential scanning calorimetry was done using a Mettler Toledo DSC<sup>3+</sup>. The desired amount of sample is weighed directly in a hermetic aluminum pan with pin-hole. A typical sample mass for is 3-5 mg. A typical temperature range is 30 °C to 300 °C at a heating rate of 10 °C per minute (total time of 27 minutes). Typical parameters for DSC are listed below.

Parameters	
Method	Ramp
Sample size	3-5 mg
Heating rate	10.0 °C/min
Temperature range	30 to 300 °C
Method gas	N <sub>2</sub> at 60.00 mL/min

**[00165]** Thermogravimetric analysis and differential scanning calorimetry was done using a Mettler Toledo TGA/DSC<sup>3+</sup>. The desired amount of sample is weighed directly in a hermetic aluminum pan with pin-hole. A typical sample mass for the measurement is 5-10 mg. A typical temperature range is 30 °C to 300 °C at a heating rate of 10 °C per minute (total time of 27 minutes). Protective and purge gasses are nitrogen (20 – 30 mL/min and 50 – 100 mL/min). Typical parameters for DSC/TGA are listed below.

Parameters	
Method	Ramp
Sample size	5-10 mg
Heating rate	10.0 °C/min
Temperature range	30 to 300 °C

**[00166]** Dynamic Vapor Sorption (DVS) was done using a DVS Intrinsic 1. The sample is loaded into a sample pan and suspended from a microbalance. A typical sample mass for DVS measurement is 25 mg. Nitrogen gas bubbled through distilled water provides the desired relative humidity. A typical measurement comprises the steps:

1. Equilibrate at 50% RH
2. 50% to 2%. (50%, 40%, 30%, 20%, 10% and 2%)
  - a. Hold minimum of 5 mins and maximum of 60 minutes at each humidity. The pass criteria is less than 0.002% change
3. 2% to 95% (2%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%)
  - b. Hold minimum of 5 mins and maximum of 60 minutes at each humidity. The pass criteria is less than 0.002% change
4. 95% to 2% (95%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 2%)
  - c. Hold minimum of 5 mins and maximum of 60 minutes at each humidity. The pass criteria is less than 0.002% change
5. 2% to 50% (2%, 10%, 20%, 30%, 40%, 50%)
  - d. Hold minimum of 5 mins and maximum of 60 minutes at each humidity. The pass criteria is less than 0.002% change

**[00167]** Proton NMR was done on a Bruker Avance 300 MHz spectrometer. Solids are dissolved in 0.75 mL deuterated solvent in a 4 mL vial and transferred to an NMR tube

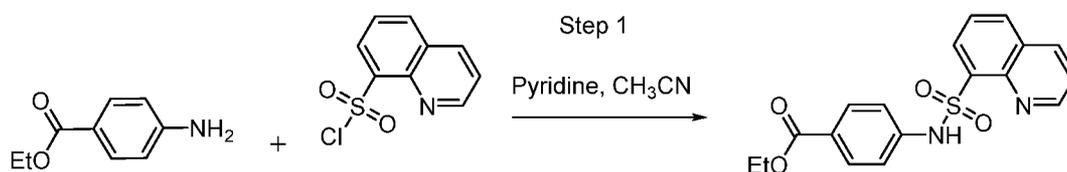
(Wilmad 5mm thin wall 8" 200MHz, 506-PP-8). A typical measurement is usually 16 scans. Typical parameters for NMR are listed below.

Parameters	
Instrument	Bruker Avance 300 MHz spectrometer Bruker Avance 500 MHz spectrometer
Temperature	300 K
Probe	5 mm PABBO BB-1H/DZ-GRD Z104275/0170
Number of scans	16
Relaxation delay	1.000 s
Pulse width	14.2500 $\mu$ s
Acquisition time	2.9999 s
Spectrometer frequency	300.15 Hz 500.13 Hz
Nucleus	$^1\text{H}$

**[00168]** Karl Fischer titration for water determination was done using a 785 DMP Titrino and 703 Ti Stand equipped with 6.0338.100 double platinum wire electrodes. Samples are dissolved in HPLC grade or anhydrous methanol and titrated with Hydranal-Composite 5. A typical sample mass for the measurement is 0.03 - 0.10 g. Hydranal 1 wt.% water standard is used for calibration.

**[00169]** Compound **1**, i.e., the non-crystalline free base, can be prepared following the procedures described below.

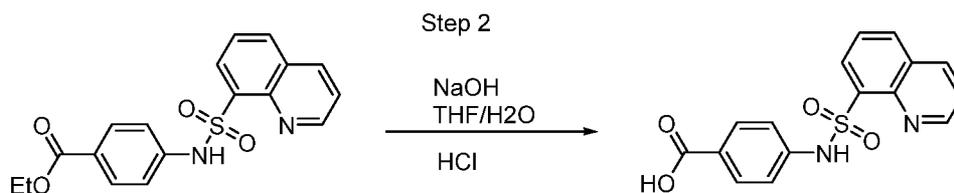
#### Preparation of ethyl -4-(quinoline-8-sulfonamido) benzoate



**[00170]** A solution containing ethyl-4-aminobenzoate (16.0g, 97mmol) and pyridine (14.0g, 177mmol) in acetonitrile (55mL) was added over 1.2 hours to a stirred suspension of quinoline-8-sulfonyl chloride (20.0g, 88mmol) in anhydrous acetonitrile (100 mL) at 65°C. The mixture was stirred for 3.5 hours at 65°C, cooled to 20°C over 1.5 hours and held until water (140 mL) was added over 1 hour. Solids were recovered by filtration, washed 2 times (100mL each) with acetonitrile/water (40/60 wt./wt.) and dried to constant weight in a vacuum oven at 85°C. Analyses of the white solid (30.8g, 87mmol) found (A) HPLC purity = 99.4% ethyl -4-(quinoline-8-sulfonamido) benzoate, (B) LC-MS consistent with structure, (M+1)= 357 (C18 column eluting 95-5, CH<sub>3</sub>CN/water, modified with formic acid, over 2 minutes), and (C)  $^1\text{H}$  NMR consistent with structure (400 MHz, DMSO-*d*<sub>6</sub>) =  $\delta$  10.71 (s, 1H),

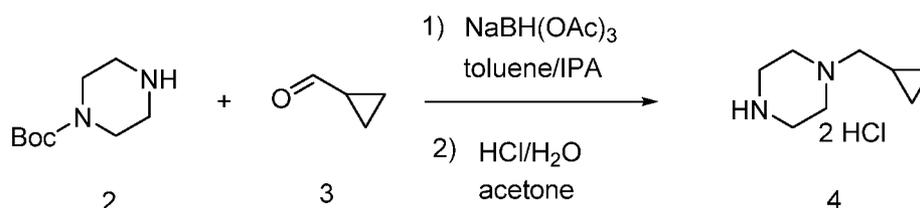
9.09 (dd,  $J = 4.3, 1.6$  Hz, 1H), 8.46 (ddt,  $J = 15.1, 7.3, 1.5$  Hz, 2H), 8.26 (dd,  $J = 8.3, 1.4$  Hz, 1H), 7.84 – 7.54 (m, 4H), 7.18 (dd,  $J = 8.6, 1.3$  Hz, 2H), 4.26 – 4.07 (m, 2H), 1.19 (td,  $J = 7.1, 1.2$  Hz, 3H).

#### Preparation of 4-(quinoline-8-sulfonamide) benzoic acid



**[00171]** A NaOH solution (16.2g, 122mmol) was added over 30 minutes to a stirred suspension of ethyl -4-(quinoline-8-sulfonamido) benzoate (20.0g, 56.2mmol) in water (125 mL) at 75°C. The mixture was stirred at 75°-80°C for 3 hours, cooled 20°C and held until THF (150 mL) was added. Hydrochloric acid (11% HCL, 81mL, 132mmol) was added over >1 hour to the pH of 3.0. The solids were recovered by filtration at 5°C, washed with water (2X 100mL) and dried to constant weight in a vacuum oven at 85°C. Analysis of the white solid (16.7g, 51 mmol) found (A) HPLC puurity = >99.9% 4-(quinoline-8-sulfonamide)benzoic acid, LC-MS consistent with structure ( $M+1 = 329$  (C18 column eluting 95-5 CH<sub>3</sub>CN/water, modified with formic acid, over 2 minutes.) and <sup>1</sup>H NMR consistent with structure (400 MHz, DMSO-*d*<sub>6</sub>) =  $\delta$  12.60 (s, 1H), 10.67 (s, 1H), 9.09 (dd,  $J = 4.2, 1.7$  Hz, 1H), 8.46 (ddt,  $J = 13.1, 7.3, 1.5$  Hz, 2H), 8.26 (dd,  $J = 8.2, 1.5$  Hz, 1H), 7.77 – 7.62 (m, 3H), 7.64 (d,  $J = 1.3$  Hz, 1H), 7.16 (dd,  $J = 8.7, 1.4$  Hz, 2H).

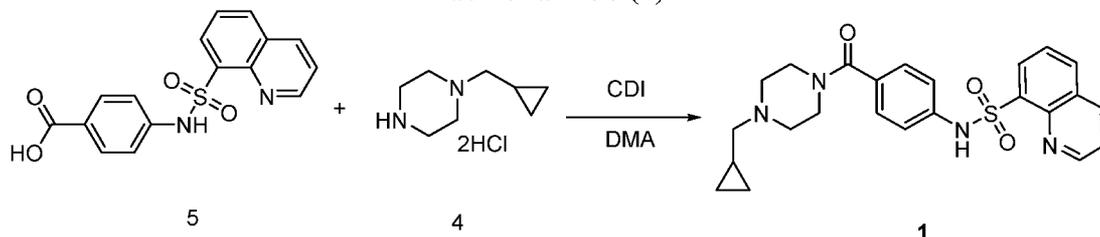
#### Preparation of 1-(cyclopropylmethyl)piperazine dihydrochloride (4)



**[00172]** To a 1 L reactor under N<sub>2</sub> was charged tert-butyl piperazine-1-carboxylate (2) (100.0 g, 536.9 mmol), cyclopropanecarbaldehyde (3) (41.4 g, 590.7 mmol), toluene (500.0 mL) and 2-propanol (50.0 mL). To the obtained solution was added NaBH(OAc)<sub>3</sub> (136.6 g, 644.5 mmol) in portions at 25-35 °C and the mixture was stirred at 25 °C for 2 h. Water (300.0 mL) was added followed by NaOH solution (30%, 225.0 mL) to the pH of 12. The layers were separated and the organic layer was washed with water (100.0 mLx2). To the organic layer was added hydrochloric acid (37%, 135.0 mL, 1.62 mol) and the mixture was stirred at 25 °C for 6 h. The layers were separated and the aqueous layer was added to acetone

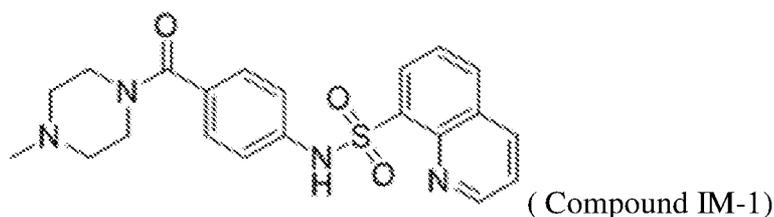
(2.0 L) at 25 °C in 1h. The resulted suspension was cooled to 0 °C. The solid was filtered at 0 °C, washed with acetone (100.0 mLx2) and dried to afford **4** (105.0 g) in 92% isolated yield. LC-MS (C18 column eluting 90-10 CH<sub>3</sub>CN/water over 2 minutes) found (M+1) =141. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 11.93 (br.s, 1H), 10.08 (br., 2H), 3.65 (br.s, 2H), 3.46 (br.s, 6H), 3.04 (d, *J* = 7.3 Hz, 2H), 1.14 – 1.04 (m, 1H), 0.65 – 0.54 (m, 2H), 0.45 – 0.34 (m, 2H) ppm.

**Preparation of N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide (1)**



**[00173]** To a 2 L reactor under N<sub>2</sub> was charged 4-(quinoline-8-sulfonamido) benzoic acid (**5**) (100.0 g, 304.5 mmol) and DMA (500.0 mL). To the resulted suspension was added CDI (74.0 g, 456.4 mmol) in portions at 25 °C and the mixture was stirred at 25 °C for 2 h. To the resulted suspension was added 1-(cyclopropylmethyl)piperazine dihydrochloride (**4**) (97.4 g, 457.0 mmol) in one portion at 25 °C and the mixture was stirred at 25 °C for 4 h. Water (1.0 L) was added in 2 h. The solid was filtered at 25 °C, washed with water and dried under vacuum at 65 °C to afford **1** (124.0 g) in 90 % isolated yield. LC-MS (C18 column eluting 90-10 CH<sub>3</sub>CN/water over 2 minutes) found (M+1) =451. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 10.40 (br.s, 1H), 9.11 (dd, *J* = 4.3, 1.6 Hz, 1H), 8.48 (dd, *J* = 8.4, 1.7 Hz, 1H), 8.40 (dt, *J* = 7.4, 1.1 Hz, 1H), 8.25 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.76 – 7.63 (m, 2H), 7.17 – 7.05 (m, 4H), 3.57 – 3.06 (m, 4H), 2.44 – 2.23 (m, 4H), 2.13 (d, *J* = 6.6 Hz, 2H), 0.79 – 0.72 (m, 1H), 0.45 – 0.34 (m, 2H), 0.07 – 0.01 (m, 2H) ppm.

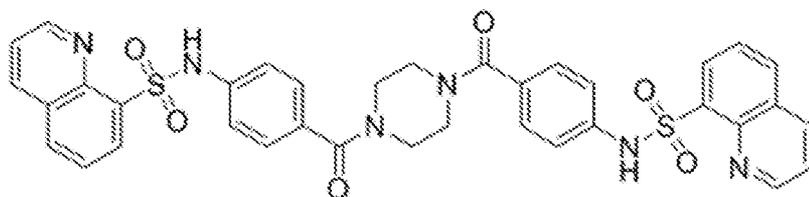
**[00174]** Two impurities are also identified from this step of synthesis. The first impurity is Compound IM-1 (about 0.11% area percent based on representative HPLC) with the following structure:



Compound IM-1 was generated due to the presence of N-methyl piperazine, an impurity in compound **2**, and was carried along to react with compound **5**. LC-MS found (M+1) =411.2;

(M-1)= 409.2.  $^1\text{H}$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  10.43 (brs, 1H) 9.13-9.12 (m, 1H), 8.52-8.50 (m, 1H), 8.43-8.41 (m, 1H), 8.26 (d, *J*=4.0 Hz, 1 H), 7.73-7.70 (m, 2H), 7.15-7.097.69 (m, 4H), 3.60-3.25 (brs, 4H), 2.21 (brs, 4H), 2.13 (s, 3H).

**[00175]** The second impurity is Compound IM-2 (about 0.07% area percent based on the representative HPLC) with the following structure:



(Compound IM-2)

Compound IM-2 was due to the presence of piperazine, an impurity generated by deprotection of compound 2. The piperazine residue was carried along to react with two molecules of compound 5 to give Compound IM-2. LC-MS found (M+1) = 707.  $^1\text{H}$  NMR (400 MHz, CF<sub>3</sub>COOD)  $\delta$  9.30-9.23 (m, 4H), 8.51 (s, 4H), 8.20-8.00 (m, 4H), 7.38-7.28 (m, 8H), 4.02-3.54 (m, 8H).

### *Solubility Experiments*

**[00176]** Solubility measurements were done by gravimetric method in 20 different solvents at two temperatures (23 °C and 50 °C). About 20-30 mg of Form A, the synthesis of which is described below, was weighed and 0.75 mL solvent was added to form a slurry. The slurry was then stirred for two days at the specified temperature. The vial was centrifuged and the supernatant was collected for solubility measurement through gravimetric method. The saturated supernatant was transferred into pre-weighed 2 mL HPLC vials and weighed again (vial + liquid). The uncapped vial was then left on a 50 °C hot plate to slowly evaporate the solvent overnight. The vials were then left in the oven at 50 °C and under vacuum to remove the residual solvent so that only the dissolved solid remained. The vial was then weighed (vial + solid). From these three weights; vial, vial+liquid and vial+solid; the weight of dissolved solid and the solvent were calculated. Then using solvent density the solubility was calculated as mg solid/mL of solvent. Solubility data are summarized in **Table 1**.

Table 1

Solvent (ratios are volumetric)	Solubility, mg solid/mL solvent	
	23 °C	50 °C
MeOH	393	>765
EtOH	0	1
IPA	0	2
Acetone	1	6
MtBE	1	3
EtOAc	0	2
Acetonitrile	4	6
Cyclohexane	1	1
MEK:water (20:1)	0	2
Toluene:EtOH:water (10:5:1)	immiscible	immiscible
Toluene:EtOH:water (10:5:0.5)	1	4
Toluene	0	2
THF	2	26
IPAc	0	1
acetone:water (8:2)	5	16
THF:water (8:2)	14	77
IPA:DMSO (8:2)	11	28
IPA:DMSO:water (80:18:2)	1	6
IPA:DMSO:water (70:25:5)	2	9
IPA:DMSO:water (70:20:10)	1	8
DCM	2	N/A

[00177] Since the solubility in methanol was significantly high, the measurement was extended to more solvent systems mixed with methanol. Results are shown in **Table 2**. 20-30 mg of Form A was weighed into 2 mL vial and 500  $\mu$ L MeOH solvent system as outlined in the table was added. If dissolved, more solids were added to form the slurry. The slurries were then stirred for two days. The vials were centrifuged and the supernatant was collected for solubility measurement through gravimetric method by evaporation at 50 °C. XRPD analysis was conducted on the solid after filtration of the slurries.

[00178] Water had a significant impact on solubility of Form A. For example in MeOH:water (1:1) system, the room temperature solubility were 208 mg/mL, 118, 39 and 5 for 0 vol%, 1%, 2.5% and 5%, respectively. Therefore, just adding 5 vol% water decreased the solubility 42 fold. Three solvent compositions were used as MeOH:Water (99:1 vol), (98:2) and (95:5). About 25mg of Form A was weighed into 2 mL vial and 6 volume solvent was added, then slowly heated to dissolve. For experiment with 5% water, an additional test was conducted with 8 vol solvents. See **Table 3**.

Table 2

Solvent (ratios are volumetric)	Solubility, mg solid/mL solvent	
	23 °C	50 °C
MeOH:Water (1:1)	7	27
MeOH:water (8:2)	10	59
EtOAc:MeOH (1:1)	21	240
EtOAc:MeOH (9:1)	0	9
EtOAc:MeOH (1:9)	359	531
EtOH:MeOH (1:1)	2	11
EtOAc:MeOH (1:1) and 5 vol% water	3	12
MeOAc:MeOH (1:1) and 5 vol% water	5	26
MeOAc:MeOH (1:1)	208	340
MeOAc:MeOH (1:1) and 1_vol% water	118	236
MeOAc:MeOH (1:1) and 2.5_vol% water	39	143

Table 3

Solvent	Solvent vol	Temperature when dissolution, °C	Solubility, mg solid/mL solvent	Extra water	XRPD Pattern
MeOH:Water (99:1)	6 vol	23	>167	N/A	B + extra
MeOH:Water (98:2)	6 vol	35	167	N/A	B
MeOH:Water (95:5)	6 vol	60	167	4 vol	A
MeOH:Water (95:5)	8 vol	50	125	4 vol	A

[00179] For the sake of comparison, the solubility of Compound 1, i.e., the non-crystalline freebase, was also measured in two solvent systems to see the effect of water. 2.5 vol% water did not have any impact on the solubility of Compound 1. See Table 4.

Table 4

Solvent	Solubility, mg solid/mL solvent	
	23 °C	50 °C
MeOAc:MeOH (1:1)	15	34
MeOAc:MeOH (1:1) and 2.5_vol% water	15	36

### Screening Experiments

#### 1. Short-Term Slurries

[00180] Solids from the slurry of solubility measurement experiments were collected for XRPD analysis, shown in Table 5. Pattern A remained unchanged in most solvent systems

over the course of two days slurry. In some cases, it lost its crystallinity indicating that stirring longer would possibly change the form. Three new patterns were observed: Pattern B in EtOH at RT and 50 °C; Pattern A + C in IPA at RT; Pattern D in IPA and acetonitrile at 50 °C. Pattern D + crystalline free-base in IPA:DMSO (8:2) at 50 °C.

[00181] In addition, 2 days slurry was performed in methanol solvent mixtures and the results are presented in **Table 6**. Where at least 2.5 vol% water was present, Pattern A remained unchanged. When only 1% water was present, Pattern A lost its crystallinity at 50 °C while remaining unchanged at room temperature over two days slurry. In anhydrous mixtures where there was substantial amount of methanol, Pattern A was not stable.

**Table 5**

Solvent	XRPD pattern after 2 days slurry	
	23 °C	50 °C
MeOH	A	Low crystalline A
EtOH	B	B
IPA	A + C	D
Acetone	A	A (Extra peak at ~5.8)
MtBE	A	A
EtOAc	A	A
Acetonitrile	A	D
Cyclohexane	A	A
MEK:water (20:1)	A	A
Toluene:EtOH:water (10:5:0.5)	A	A
Toluene	A	A
THF	A	A
IPAc	A	A
acetone:water (8:2)	A	A
THF:water (8:2)	A	A
IPA:DMSO (8:2)	A	D + crystalline free-base
IPA:DMSO:water (80:18:12)	A	A
IPA:DMSO:water (70:25:5)	A	A
IPA:DMSO:water (70:20:10)	A	A
DCM	A	N/A

**Table 6**

Solvent	XPRD pattern after slurry	
	RT	50 °C
MeOH:Water (1:1)	A	A
MeOH:water (8:2)	A	A
EtOAc:MeOH (1:1)	B	D
EtOAc:MeOH (9:1)	A	A

EtOAc:MeOH (1:9)	A	low crystalline A
EtOH:MeOH (1:1)	B	B
EtOAc:MeOH (1:1) and 5 vol% water	A	A
MeOAc:MeOH (1:1) and 5 vol% water	A	A
MeOAc:MeOH (1:1)	A	Lower crystalline A
MeOAc:MeOH (1:1) and 1 vol% water	A	Lower crystalline A
MeOAc:MeOH (1:1) and 2.5 vol% water	A	A

## 2. Crystalline Freebase Slurry Experiments

[00182] Compound **1**, i.e., the crystalline freebase, was also slurried in a few solvent systems in which the hemisulfate salt showed a different XRPD pattern for the sake of comparison. The freebase remained unchanged after two days slurry. Results are shown in **Table 7**.

**Table 7**

Solvent	XRPD pattern after slurry	
	23 °C	50 °C
MeOAc:MeOH (1:1)	FB-A	FB-A
MeOAc:MeOH (1:1) and 2.5_vol% water	FB-A	FB-A
EtOH	Not performed	FB-A
ACN	Not performed	FB-A
IPA:water(8:2)	Not performed	FB-A

FB-A refers to the crystalline free base form of Compound **1**

## 3. Amorphous Generation Experiments

[00183] Lyophilization in ACN:water (2:1 vol) was performed to generate amorphous material. About 250 mg of Form A was weighed into 20 mL vial and 6 mL solvent mixture was added, then the mixture was heated to 40 °C and held for 0.5 hr, then put into the freezer (-20 °C). Part of the frozen mixture (about half) was scooped on a filter before melting to analyze. Another half was melted, then 1 mL ACN:water (2:1 vol) and 1 mL water were added, and put it into the freezer again. Finally this mixture was placed in the freeze dryer overnight. XRPD analysis was conducted on the resulting solids. See **Table 8**.

**Table 8**

<b>Solvent</b>	<b>XRPD pattern</b>	<b>Method</b>
6 mL ACN:water (2:1)	Form E (wet, filter and XRD immediately, 57-1-wet) Form F (dry vacuum oven, 57-1-dry) Form E converted to Form A when the wet cake of Form E was left in the vial overnight	filter
1 mL ACN:water (2:1) + 2mL water	Form A	Freeze dry

**[00184]** Based on evaporative crystallization result, amorphous material was observed for the sample in THF. This experiment was repeated, but Pattern D was observed instead. See **Table 9**.

**Table 9**

<b>Solvent</b>	<b>Solvent vol, <math>\mu</math>L</b>	<b>XRPD Pattern</b>
THF	13380	Form D

**[00185]** The evaporative crystallization in MeOH solvent generated amorphous material. This experiment was repeated at 55 °C by adding 1.5 vol methanol to dissolve Form A. Then the solution was put in vacuum oven at 50 °C overnight. XPRD showed the resulting solid was amorphous. TGA/DSC also showed a small endothermic event accompanied by 2.7% gradual weight loss. See **Table 10**.

**Table 10**

<b>Solvent</b>	<b>Solvent vol, <math>\mu</math>L</b>	<b>XRPD Pattern</b>
MeOH	70	amorphous

**[00186]** A method to form the free base amorphous form of Compound **1** was found and described as follows.

**[00187]** About 4 mL of acetonitrile was combined with 2 mL of water. In a 20 mL scintillation vial, excess solid of Compound **1** was suspended in the solvent mixture such that the solids were not completely dissolved. The scintillation vial was capped and the system was placed in room temperature sonication bath for 10 – 15 minutes. The suspension was filtered through 0.45  $\mu$ m syringe filter into a 100 mL round bottom flask to obtain a clear solution. The solution was frozen using a dry ice/acetone bath and the frozen solution was placed in a lyophilizer for at least 18 h to obtain the amorphous form of compound **1**. The

XRPD of the obtained amorphous form is shown in **FIG. 23**. The TGA and DSC thermograms for the amorphous free base of Compound 1 are shown in **FIG. 24**.

#### 4. Evaporative Crystallization

**[00188]** About 0.3 mL to 0.5 mL of saturated solution of Form A in various solvent systems were evaporated to dryness and XRPD analysis was performed where applicable. The evaporation was done at 50 °C and under atmospheric pressure (**Table 11**). The solid was very little for many cases due to low solubility. XRPD pattern remained Pattern A for the samples in MeOH at RT, acetone:water (8:2) at RT and 50 °C; low crystalline freebase was observed in ACN at RT and 50 °C; amorphous was generated in MeOH at 50 °C and in THF at 50 °C.

**Table 11**

Solvent	XRPD pattern by evaporative crystallization	
	Slurried at 23 °C	Slurried at 50 °C
MeOH	Form A	amorphous
EtOH	little solid	little solid
IPA	little solid	little solid
Acetone	little solid	little solid
MeBE	little solid	little solid
EtOAc	little solid	little solid
ACN	Low crystalline freebase	Low crystalline freebase
Cyclohexane	little solid	little solid
MEK:water(20:1)	little solid	little solid
Toluene:EtOH:water(10:5:1)	little solid	little solid
Toluene	little solid	little solid
THF	little solid	amorphous
IPAc	little solid	little solid
Acetone:water(8:2)	Form A	Form A
THF:water(8:2)	little solid	Low crystalline Form A
IPA:DMSO(8:2)	little solid	little solid
IPA:DMSO:water(80:18:12)	little solid	little solid
IPA:DMSO:water(70:25:5)	little solid	little solid
IPA:DMSO:water(70:20:10)	little solid	little solid
DCM	little solid	N/A

**[00189]** Evaporative crystallization was performed in MeOH solvent system after solubility measurement. The results are shown in **Table 12**. The solid was very little for some cases due to low solubility. XRPD pattern remained Pattern A for the samples in

MeOH:Water (1:1) at 50 °C, MeOH:water (8:2) at RT and 50 °C, and EtOAc:MeOH:water (1:1:0.05) at 50 °C; Pattern B was found in EtOH:MeOH (1:1) at 50 °C; Pattern F was observed in MeOAc:MeOH (1:1:0.05) at 50 °C; Amorphous was generated in EtOAc:MeOH (1:1) at 50 °C and MeOAc:MeOH(1:9) at RT and 50 °C.

Table 12

Solvent	XRPD pattern by evaporative crystallization	
	Slurried at 23 °C	Slurried at 50 °C
MeOH:Water (1:1)	Little solid	Form A
MeOH:water (8:2)	Form A	Form A
EtOAc:MeOH (1:1)	Little solid	Amorphous
EtOAc:MeOH (9:1)	Little solid	Little solid
EtOAc:MeOH (1:9)	Amorphous	Amorphous
EtOH:MeOH (1:1)	Little solid	Form B
EtOAc:MeOH (1:1) and 5vol% water	Little solid	Form A
MeOAc:MeOH (1:1) and 5vol% water	Little solid	Form F

### 5. Slow and Fast Cooling Crystallization

**[00190]** Slow and fast cooling experiments were conducted to study the polymorphic behavior of Form A. In the slow cooling experiments, about 20-35 mg Form A was dissolved at 55 °C in various solvent systems and then cooled to RT (23 °C) over 5 hours, and if no precipitation was observed, samples were cooled to 5 °C over 1 hour. In the fast cooling experiments, Form A solutions at 55 °C were cooled quickly to 0 °C by placing the vial in an ice water bath and if no precipitation, samples were placed in -20 °C freezer without mixing to cool down further.

**[00191]** In both fast and slow cooling experiments, no precipitation was observed in IPA:DMSO (7:3) and in IPA:DMSO:water (65:30:5). Form A did not dissolve in 77 volume MeOH:EtOH (3:7). The slurry in MeOH:EtOH (3:7) was filtered and analyzed, which showed conversion to Pattern B. In the fast cooling experiment, two new patterns were obtained: Pattern G in Acetone:water (8:2) and Pattern H in MeOH:EtOAc (1:1). In the slow cooling experiments, resulting solid pattern was Pattern A in Acetone:water (8:2), and Pattern B in MeOH:EtOAc (1:1). See **Table 13**.

Table 13

Solvent	Solvent vol	XRPD pattern by cooling crystallization	
		Fast	Slow
Acetone:Water (8:2)	72	Form G	Form A
IPA:DMSO (7:3)	14	Not precipitated	Not precipitated
MeOH:EtOAc (1:1)	17	Form H	Form B
IPA:DMSO:water (65:30:5)	196	Not precipitated	Not precipitated
MeOH:EtOH (3:7)	77	undissolved	Undissolved (slurry was Pattern B)

## 6. Anti-solvent Crystallization

[00192] Anti-solvent experiments were conducted to further study the polymorphic behavior of the Form A. In direct anti-solvent experiments, the Anti-solvent was added to a solution of 20-25 mg Form A dissolved in 3 volume MeOH with 25  $\mu$ L increments every time. In reverse addition experiments, the solution was added to 6 volume anti-solvent at once. The experiments were performed at RT. The results are shown in **Table 14**. Only in MeOH/EtOH system solids were generated during the course of experiments.

Table 14

Solvent (3vol)	Anti-solvent (6 vol)	Anti-solvent crystallization		XRPD pattern	
		Direct	Reverse	Direct	Reverse
MeOH	EtOAc	Precipitated and dissolved quick (no solid)	Precipitated and dissolved quick (no solid)	N/A	N/A
MeOH	IPAc	Precipitated and thin slurry, little solid after filtration	Precipitated and gummed	N/A	N/A
MeOH	Acetone	Not precipitated	Not precipitated	N/A	N/A
MeOH	Ethanol	Precipitated	Gummed	Form B	low crystallinity Form B
MeOH	Water	Not precipitated	Not precipitated	N/A	N/A

## 7. Solvent Drop Milling Experiment of Salt Pattern A

[00193] Solvent drop milling experiments were performed to evaluate the polymorphic behavior of Form A. About 25 mg Form A was weighed in the ball mill capsule, then 25  $\mu$ L solvent was added. The solid was milled three times, 30s each time. The solid was scraped

off the capsule wall each time to prevent caking. Besides, dry milling of Form A without solvent was designed as reference. XRPD showed that resulting solids remained unchanged after solvent drop milling process except the dry milling sample, which led to a low crystallinity of Form A, almost amorphous. See **Table 15**.

**Table 15**

Solvent	Solvent vol, $\mu\text{L}$	XRPD pattern
MeOH:water (95:5)	25	Form A
Actone:water (8:2)	25	Form A
MeOH:MeOAc (1:1)	25	Form A
IPAc	25	Low crystalline Form A
Anisole	25	Form A
dry solid	no solvent	Almost amorphous

## 8. Hemisulfate Salt Formation

**[00194]** Salt formation was conducted by slurrying Compound **1**, i.e., the non-crystalline free base in various solvents followed by adding sulfuric acid solution in EtOAc. First, a sulfuric solution in EtOAc was prepared (concentration approximately 24 wt.%). Then 25 to 30 mg of non-crystalline free base of Compound **1** was weighed in a 2 mL vial, followed by adding 15 vol solvent. The desired number of equivalents of sulfuric acid were then added. The slurries were heated to 45 °C and held for 1 hour and then cooled down to RT over 2 hours, then held overnight. The slurry was filtered and then analyzed by XRPD. The results was shown in **Table 16**.

**Table 16**

Solvent	observation		XRPD pattern	
	0.5 eq. Sulfuric acid	1.0 eq. sulfuric acid	0.5 eq. Sulfuric acid	1.0 eq. sulfuric acid
IPA	thin slurry and slight gum	thin slurry and slight gum	low crystallinity FB-A	low crystallinity FB-A
Acetonitrile	thin slurry and slight gum	Solution to slurry	Low crystallinity Form A	Form D
MeOAc:MeOH (1:1)	Solution*	Solution*	Amorphous	Amorphous
EtOAc:MeOH (1:1)	Solution*	Solution*	Amorphous	Amorphous

MeOAc:MeOH (1:1); 5% water	Solution*	Solution*	Low crystallinity Form A	Amorphous
EtOAc:MeOH (1:1); 5% water	solution to slurry	Solution*	Form A	Amorphous
MeOAc:MeOH (1:1); 10% water	Solution*	Solution*	Form A	Amorphous
Acetone	Slurry	thin slurry and slight gum	low crystallinity FB-A	Form D
THF	Slurry	Slurry	low crystallinity FB-A	low crystallinity FB-A
Ethanol	Slurry	solution to slurry	Form I	Form D
IPAc	thin slurry and slight gum	thin slurry and slight gum	FB-A	low crystallinity FB-A
MeOAc:MeOH (1:1); 1% water	Solution*	Solution*	low crystallinity FB-A	Amorphous

\* No precipitation after 3-4 hrs of stirring, then the solvent was evaporated. FB-A refers to the crystalline free-base form of Compound 1

[00195] The hemisulfate was also formed in MeOAc:MeOH (1:1) mixture with and without water. A sulfuric acid solution in water of 43 wt% (density: 1.3072 g/mL) was prepared and used for hemisalt formation. See **Table 17**.

**Table 17- Salt formation in MeOAc:MeOH solvent system with 0.52eq sulfuric acid solution in water (40 to 50wt%)**

sulfuric acid, eq	sulfuric acid solution in water, $\mu\text{L}$	Solvent, $\mu\text{L}$	Experiment description	XPRD Pattern-wet solid
0.52	19.4	578.4	Added 6 volume MeOAc:MeOH (1:1) at RT; Added 0.52 eq. acid; solution achieved with some specs of solid Added Pattern A as seed. Added Pattern B as seed Held 1hr. became thick. Sampled for XRPD. Heated to 50 °C	Form J
0.52	18.6	555	Added 6 volume MeOAc:MeOH (1:1) at RT Added 0.52 eq. acid; solution achieved with some specs of solid	Form A

			Added 0.4 vol water dropwise Hold 2hrs. became thick. Filtered for XRPD	
0.52	19.8	591	Added 6 volume MeOAc:MeOH (1:1) at RT Heated to 45 C Added 0.52 eq. acid; solution achieved with some specs of solid Added 0.4 vol water dropwise Held for 30 mins and cool to RT over 1 hr Held 1hr. became thick. Filtered for XRPD	Form A
0.52	18.6	555.6	Added 6 volume MeOAc:MeOH (1:1) at RT Added 0.52 eq. acid; solution achieved with some specs of solid Added 1 vol water over 2 hrs Held 1hr. became thick. Filtered for XRPD	Form A

**[00196]** Hemisulfate salt formation was also conducted by slurring Compound **1** in methanol followed by adding sulfuric acid solution in water (43 wt% aq.). About 100 mg Compound **1**, the non-crystalline free base, was weighed in a 4 mL vial, followed by adding 6 vol solvent. 0.52 equivalents of sulfuric acid solution in water (concentration approximately 43wt.%) was then added which resulted in complete dissolution at room temperature. For experiment 1, one volume water was added dropwise first, tiny amount of Pattern A was added as seeds, then 5 volume water was added over 1 hour; for experiment 2, no water was added, both Patterns A and B were added as seeds; for experiment 3, six volume water was added over 2 hours, no seed. All vials were stirred overnight (O/N). The slurry was filtered and then analyzed by XRPD. Yield was about 80% for the experiment with adding water and about 60% for the experiment without adding water, refer to **Table 18**.

**Table 18**

Solvent	Solvent vol, $\mu\text{L}$	Sulfuric acid in Water $\mu\text{L}$	Water Added $\mu\text{L}$	Final composition	Seeds added	XRPD pattern	Yield
MeOH	572	19.2	595	MeOH:water (50:50)	Form A	Form A	84%
MeOH	552	18.4	no water	MeOH:water (98.5:1.5)	Form A+B	Form B	63%
MeOH	577	19.4	600	MeOH:water (50:50)	no seed	Form A	82%

[00197] Hemisulfate salt formation was also performed in methanol:water (95:5) by using freebase and sulfuric acid. About 150 mg of Compound **1** was weighed into 4 mL vial and 8 volume solvent was added. The mixture was stirred at 500 rpm and then 0.52 eq. sulfuric acid solution in water (concentration approximately 43 wt.%) was added. This mixture was heated to 45 °C which resulted in complete dissolution followed by adding 0.5 volume water, then cooled to RT and held O/N. This resulted in a flowable slurry. 3.5 vol water was then added and filtered. XRPD analysis showed Pattern A. Yield was about 84%. Refer to **Table 19**.

**Table 19**

Solvent	Solvent $\mu\text{L}$	Sulfuric acid in Water $\mu\text{L}$	Temperature (heated to), °C	Final composition	XRPD pattern	yield
MeOH:water (95:5)	1200	30.2	45	MeOH:water (63:37)	Form A	84%

[00198] A scale-up experiment for Form A proceeded as follows.

[00199] About 1.0253 g of Compound **1** was added to a 100 mL flask. 8 vol MeOH:water (95:5 vol) solvent (8.2 mL) was then added and the mixture was stirred at 300 rpm using a 4-pitch blade turbine (4-PBT) impeller with diameter of 3.5 cm. 0.52 eq. sulfuric acid in water (~43 wt.%) (206  $\mu\text{L}$ ) was manually added and the mixture was heated from 23 °C to 45 °C over 10 min, which resulted in complete dissolution. 0.5 vol water (0.512 mL) was added and the solution was held for 10 mins, cooled to 23 °C over 1 hr and held while stirring overnight. No precipitation was observed. 4 vol water was then over 1 hr and the solution remained clear throughout water addition at room temperature. Precipitation started shortly (15-30 mins) after completion of water. A sample was analyzed via XRPD which showed Pattern A. The mixture was then cooled down to 15 °C over 1 hour, held for 3 hrs while stirring, and then filtered and washed by 2 vol MeOH:water(1:1). XRPD of the wet cake showed Pattern A. The product was dried overnight in a vacuum oven at 50 °C. Yield 1.1143 g salt Pattern A by XRPD. Residual methanol was 0.18 wt% by NMR. pH of the filtrate was measured using pH meter and showed a value of 2.63.

## 9. Hemisulfate salt Scale-up

[00200] Hemisulfate salt was produced at 2 gram scale as detailed below. The resulting solid was hemisulfate salt Form A. The yield was about 88%.

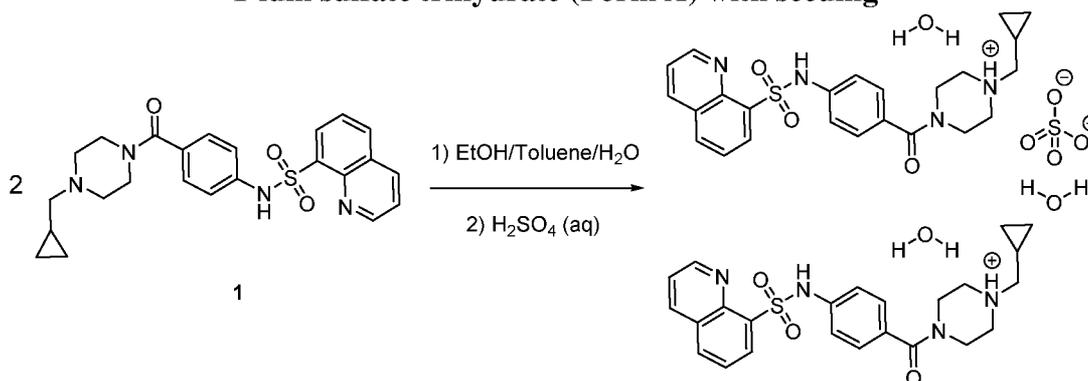
[00201] About 1.9581 g Compound **1** was added to a 100 mL flask. 8 vol MeOH:water (95:5 vol) solvent (15.9 mL) was added and the mixture was stirred at 300 rpm using a 4-PBT

impeller with diameter of 3.5 cm. 0.52 eq. of sulfuric acid in water (~43 wt.%) (399  $\mu$ L) was manually added. The mixture was heated from 23 °C to 45 °C over 10 min which resulted in complete dissolution. 0.5 vol water (0.993 mL) was added, and the solution was held for 10 mins and cooled to 23 °C over 1 hr. 4 vol water was added over 1 hour and nucleation was observed after about 45 mins of hold while stirring. The mixture was cooled down to 15 °C over 1 hour, held for about 30 mins while stirring, and filtered and washed by 2 vol MeOH:water (1:1). XRPD of the wet cake showed Pattern A. The product was dried overnight in the vacuum oven at 50 °C. This Yielded in 2.04 g (88%) salt Pattern A by XRPD. The residual methanol was 0.16 wt% by NMR.

### 10. Optimized Crystalline Form A Hemisulfate Salt Scale-up Procedure

[00202] An optimized preparation of Form A as a hemisulfate sesquihydrate salt with and without seeding is provided below.

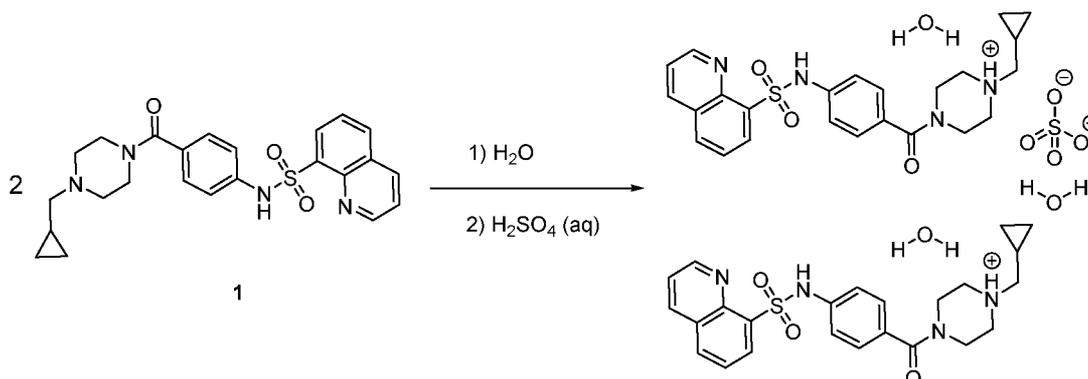
#### Preparation of 1-(cyclopropylmethyl)-4-(4-(quinoline-8-sulfonamido)benzoyl)piperazin-1-ium sulfate trihydrate (Form A) with seeding



[00203] To a 2 L reactor under N<sub>2</sub> was charged N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide (5) (111.0 g, 246.4 mmol), and a pre-mixed process solvent of ethanol (638.6 g), toluene (266.1 g) and water (159.6 g). The suspension was stirred and heated above 60°C to dissolve the solids, and then the resulting solution was cooled to 50°C. To the solution was added an aqueous solution of H<sub>2</sub>SO<sub>4</sub> (2.4 M, 14.1 mL, 33.8 mmol), followed by 1-(cyclopropylmethyl)-4-(4-(quinoline-8-sulfonamido)benzoyl)piperazin-1-ium sulfate trihydrate (6) (1.1 g, 2.1 mmol). After 1 h stirring, to the suspension was added an aqueous solution of H<sub>2</sub>SO<sub>4</sub> (2.4 M, 42.3 mL, 101.5 mmol) over 5 h. The suspension was cooled to 22°C and stirred for 8 h. The solids were filtered at 22°C, washed with fresh process solvent (2 x 175 g) and dried to give the product (121.6 g) in 94% isolated yield. LC-MS (C18 column eluting 90-10 CH<sub>3</sub>CN/water over 2 minutes) found (M+1) = 451. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  10.45 (s, 1H), 9.11 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.50 (dd, *J* = 8.4, 1.7 Hz, 1H), 8.41 (dd, *J* = 7.3, 1.5 Hz, 1H), 8.27 (dd, *J* =

8.2, 1.5 Hz, 1H), 7.79 – 7.60 (m, 2H), 7.17 (d,  $J = 8.4$  Hz, 2H), 7.11 (d,  $J = 8.4$  Hz, 2H), 3.44 (d,  $J = 8.9$  Hz, 5H), 3.03 – 2.50 (m, 6H), 0.88 (p,  $J = 6.3$  Hz, 1H), 0.50 (d,  $J = 7.6$  Hz, 2H), 0.17 (d,  $J = 4.9$  Hz, 2H).

**Preparation of 1-(cyclopropylmethyl)-4-(4-(quinoline-8-sulfonamido)benzoyl)piperazine-1-ium sulfate trihydrate (Form A) without seeding**



**[00204]** To a 50 L reactor was charged N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide (5) (1.20 kg, 2.66 mol) and water (23.23 L) at 28°C. While stirring the suspension, an aqueous solution of H<sub>2</sub>SO<sub>4</sub> (1.0 M, 261 g) was added dropwise over 2 h. The reaction was stirred at 25 - 30°C for 24 h. The solids were filtered and dried under vacuum below 30°C for 96 h to give the product (1.26 kg) in 90% isolated yield.

## 11. Reproduction and Preparation of Various Patterns

**[00205]** The patterns observed during the previous experiments were reproduced for characterization. Patterns B, D, E, F were reproducible. Pattern G was reproduced at lower crystallinity. Pattern I was reproduced, although, it was missing a few peaks. Refer to **Table 20**.

**Table 20**

Target Pattern	Actual Pattern	Solvent	Method	Description
Form B	Form B	EtOH	slurry at RT	Holds methanol or ethanol. Upon drying loses crystallinity
Form C+A	Form A	IPA	slurry at RT	Pattern C was not reproducible as it might be so unstable and converts to Pattern A
Form D	Form A+D	ACN	slurry at 50 °C	Pattern D is Anhydrous
Form B	Form B	EtOH:MeOH (3:7)	Slurry at 55 °C	Pattern B obtained

Form E	Form E	ACN:water (2:1)	-20 °C cooling crystallization	Very low yield (5%) Unstable upon drying and converts to F
Form F	Form F	N/A	Obtained by drying Pattern E at 50 °C and vacuum	About 8 wt% loss in TGA which should be water based on NMR
Form G	Low crystalline Form G	ACN:water (8:2)	Fast cooling crystallization & -20 °C	About 5 wt% loss in TGA which should be water based on NMR
Form H	Almost amorphous solid. Most peaks are missing	MeOH:EtOAc (1:1)	Fast cooling crystallization & -20 °C	Possible solvate very low crystalline
Form B	Form B	MeOH:water (99:1)	Dissolved at RT and seed with pattern A and B	Pattern B obtained.
Form I	Form I – missing a few peaks	EtOH	Salt formation with freebase and sulfuric in EtOAc	Contained 8.5 wt% EtOH. Possible solvate
Form D	Form D	ACN	Salt formation with freebase and sulfuric acid in EtOAc	Pattern D is Anhydrous

### Form B

[00206] Form B can be prepared by slurring Form A in about 10 volumes ethanol at room temperature for a number of days until Form B is obtained. Form B is typically dried at 50 °C under vacuum. Other methods of forming Form B are described above, e.g., in Tables 5, 6, 12-14, 18, and 20.

### Form C

[00207] Form C is formed as a mixture with Form A after slurring Form A in IPA for 2 days at about 23 °C. See e.g., Table 5. XRPD peaks for Form C are obtained from subtracting the Form A peaks.

### Form D

[00208] Form D is formed by adding 15 vol acetonitrile and then 0.52 eq. sulfuric acid to compound **1** and heating the mixture to 50 °C. The mixture is then held at 50 °C for 30 mins and cooled to RT. The resulting product, Form D, is then Filtered and dried. In an alternative, Form A can be slurried in IPA or acetonitrile at more than 50 °C for at least 2 days to convert to Form D. The latter method was not as robust as the first procedure in which freebase is used. Additional methods are described above, e.g., in Tables **5, 6, 9, 16, and 20**.

### Form E

[00209] Form E can be prepared by adding 24 volume acetonitrile:water (2:1) to Form A and heating the mixture to 50 °C to dissolve the material. The solution is then cooled to -20 °C and left overnight. The resulting product, Form E, is then filtered. See e.g., Table **8 and 20**.

### Form F

[00210] Form F can be prepared by drying Form E at 50 °C under vacuum. See e.g., Table **20**. Additional methods are described above, e.g., in Tables **8 and 12**.

### Form G

[00211] Form G can be prepared by adding 72 volume acetonitrile:water (8:2) to Form A and then heating the mixture to 50 °C to dissolve the solids. The solution is then rapidly cooled to -20 °C and left overnight to produce Form G. See e.g., Table **20**. Additional methods are described above, e.g., in Table **13**.

### Form H

[00212] Form H can be prepared by dissolved Form A in 40 volume MeOH:EtOAc (1:1) at 50 °C, and then placing the solution in ice to crash cool the solution and then cooling the solution to -20 °C. The resulting precipitate, Form H, is then filtered and analyzed as wet cake. See e.g., Table **20**. Reproducing Pattern H resulted in an amorphous like solid which does not have peaks of Form H. Additional methods are described above, e.g., in Tables **13**.

### Form I

[00213] Form I is a possible solvate that can be prepared by adding 15 volumes of ethanol to compound **1** and heating the mixture to 45 °C to obtain a slurry. 0.52 eq. sulfuric acid in EtOAc is then added and the mixture is held for 1 hr then cooled to room temperature over 2 hours, held for 1 hr and filtered. The product, Form I, is then dried at 50 °C and vacuum. See e.g., Tables **16 and 20**.



16.4 X H<sub>2</sub>O was added dropwise at 70 °C and the reaction was stirred for 2h at 70 °C. The reaction was adjusted to 30 °C over 5.5h and stirred for 2h at 30 °C. The reaction was centrifuged and 21.75 kg wet cake was obtained. The material was dried under vacuum at 70°C for 25h. 16.55 kg of the crystalline free base form of compound **1** was obtained. Purity of 99.6%.

## 12. Solubility in Water and Simulated Fluid

**[00217]** Solubility of Patterns A, B and D were measured in water. The solids were slurried in the fluids for two days and at 37 °C. The supernatant was syringe filtered and used for HPLC analysis. For Patterns A and D the solubility measurement was repeated and the sample was taken after 1 hr slurry. A calibration curve was developed using Pattern A. This was not assay corrected and therefore the solubility values are based on the hemi-sulfate, sesqui-hydrate salt.

**[00218]** Both Patterns B and D converted to Pattern A after two days slurry. Pattern D also converted to Pattern A within 1 hour slurry in Fasted state simulated gastric fluid (FaSSGF) or water. Only in Fasted State Simulated Intestinal Fluid (FaSSIF), Patterns A and B disproportionated to crystalline free base after two days slurry and showed lower solubility. Pattern D, however, did not disproportionate in FaSSIF and instead converted to Pattern D. The solubility in water and the simulated fluid of the three patterns were not significantly different. This could be because of the conversion of Patterns B and D to Pattern A. The solubility data and the resulting XRPD Pattern after the measurement are reported in **Table 21**.

**Table 21- Solubility in simulated fluids at 37 °C**

	Pattern	Solubility mg Solid /mL solvent	Pattern	Slurry time
Water	A	2.69	A	2 days
Water	B	3.02	A	2 days
Water	D	2.71	A	2 days
FaSSIF	A	0.24	FB	2 days
FaSSIF	B	0.20	FB	2 days
FaSSIF	D	3.49	A	2 days
FaSSGF	A	6.80	Not enough solid to do XRPD	2 days
FaSSGF	B	6.53	A	2 days
FaSSGF	D	5.94	A	2 days
Water	A	3.05	A	1 hr
FaSSGF	A	7.07	A	1 hr

Water	D	2.62	A	1 hr
FaSSGF	D	5.43	A	1 hr

FB means crystalline free base of compound 1.

### 13. Competitive Slurry

[00219] Four different solvents were used for competitive slurry experiments. Four solvent systems were selected for competitive slurry at two different temperatures. The solvents were acetone, acetone:water (9:1), IPA and MeOH:water (95:5) and the temperatures were 23 °C and 50 °C. All the solvents were initially saturated by slurrying Pattern A at the target temperatures for about 2 hours then the stir bars were removed and kept the vial at the target temperature allowing the solid to settle. Then the saturated supernatant was transferred into new empty vials which were already heated to the target temperatures on hot plate. Then 5-10 mg each of Patterns A, B and D was added to these saturated solutions. The solid mixture was slurried using stir bar and the first sample was taken after 2 days of slurry. The second sample was taken after 1 week. Pattern A was stable in the solvents that contained water. Pattern D was stable in anhydrous systems especially at higher temperatures. It appears that Pattern D which is anhydrous solid is the most stable solid when anhydrous organic solvents are used. See **Table 22**.

**Table 22**

Solvent	Initial Pattern	XPRD pattern - 2 days		XPRD pattern - 1 week	
		RT	50 °C	RT	50 °C
Acetone	A + B + D	A+D	D	A+D	D
Acetone:water (9:1)	A + B + D	A	A	A	A
IPA	A + B + D	D	D	D	D
MeOH:water (95:5)	A + B + D	A	Not enough sample for XRPD	A	---

### 14. Stability Study

No degradation was observed in the stability study of Form A in the following three conditions: one month under 40±2°C/75±5% RH, three months under 25±2°C /60±5% RH, three months at 30±2°C/65±5% RH, three months under 40±2°C/75% RH, twelve months under 25±2°C/60±5% RH.

## 15. Exemplified tablet composition

Form A has been formulated into a tablet either through direct compression of the direct blend formulation or via a dry granulation or wet granulation process. Other excipients can be added, such as binders, and/or surfactants, and coating films to aid tablet integrity, taste masking and aesthetics. An exemplified tablet composition is as follows:

Component	% w/w of Component
Form A	11.70%
Microcrystalline Cellulose NF	60.18%
Mannitol	23.12%
Croscarmellose Sodium	3.00%
Sodium Stearyl Fumarate	2.00%
% Total	100.00%

## 15. Characterization Summary

### *i). Form A*

[00220] The XRPD for Form A is shown by **FIG. 1** and the peak listings are shown in **Table 23**.

**Table 23**

Angle, 2- $\theta$	d spacing (Å)	Height (counts)	Rel. Int.
4.9	17.8305	40	0.28%
9.9	8.9545	9363	66.02%
11.0	8.02613	2418	17.05%
11.4	7.7313	4177	29.45%
11.7	7.56421	3209	22.63%
12.3	7.16163	635	4.48%
12.8	6.93632	2500	17.63%
13.6	6.51539	2286	16.12%
13.9	6.38771	1644	11.59%
14.2	6.23136	3520	24.82%
15.0	5.88897	5689	40.11%
15.3	5.78752	4085	28.80%
15.8	5.60806	12313	86.82%
17.1	5.17018	7400	52.18%

17.4	5.07818	2144	15.12%
17.7	4.99463	4146	29.23%
18.8	4.71113	2437	17.18%
19.1	4.63884	1177	8.30%
19.8	4.49007	3657	25.79%
21.3	4.17694	6577	46.38%
21.9	4.04794	6503	45.85%
22.6	3.92853	14182	100.00%
23.0	3.86945	1915	13.50%
23.2	3.82274	2764	19.49%
23.5	3.78695	4288	30.24%
23.8	3.73813	2177	15.35%
24.1	3.68699	1723	12.15%
24.5	3.63374	2387	16.83%
25.3	3.51767	3957	27.90%
25.6	3.47637	2258	15.92%
26.1	3.41137	1463	10.32%
27.1	3.28258	3975	28.03%
28.1	3.17265	2581	18.20%
29.8	3.00049	2969	20.93%

[00221] TGA of Form A showed about 4.5% weight loss up to 180 °C. See FIG. 2. The water content by Karl Fisher was about 5.3%. Two thermal events were observed in DSC thermogram with the first peak at 159.9 °C and the second peak at 199.1 °C. See FIG. 3. The first peak also showed a shoulder. Dynamic vapor sorption of salt Form A at 25 °C showed that the solid picks up about 1.3% moisture from 2% to 95% relative humidity. See FIG. 4. The DVS showed a rapid weight loss at humidity of less than 10% which was reversible upon sorption cycle. A DVS isotherm taken at 40 °C was essentially the same as the one at 25°C. The XRPD remained unchanged after DVS at both temperatures. Humidity test was conducted on Form A with exposure to 11%, 48%, and 75% relative humidity at 40°C for 2 weeks followed by XRPD analysis. The XRPD remained unchanged after two week test. See FIG. 5. Crystallographic data for Form A is as follows:

Empirical formula: C<sub>48</sub>H<sub>6</sub> N<sub>8</sub>O<sub>13</sub> S<sub>3</sub>

Formula weight: 1053.22

Temperature: 173(2) K

Wavelength: 1.54178 Å

Crystal system, space group: Monoclinic, C 2/c

Unit cell dimensions:

$$a = 3.0748(5) \text{ \AA}$$

$$b = 10.2638(4) \text{ \AA}$$

$$c = 36.1371(12) \text{ \AA}$$

$$\alpha = 90 \text{ deg.}$$

$$\beta = 97.340(3) \text{ deg.}$$

$$\gamma = 90 \text{ deg.}$$

Volume: 4809.8(3)  $\text{\AA}^3$

Z = 4

Calculated density: 1.454 Mg/m<sup>3</sup>

Absorption coefficient: 2.046 mm<sup>-1</sup>

F(000): 2224

Crystal size: 0.171 x 0.156 x 0.061 mm<sup>3</sup>

Theta range for data collection: 2.47 to 72.03°. deg.

Limiting indices:

$$-15 \leq h \leq 16$$

$$-12 \leq k \leq 12$$

$$-43 \leq l \leq 44$$

Reflections collected / unique: 16676 / 4532 [R(int) = 0.0767] Completeness: 95.6%

Refinement method: Full-matrix least-squares on F<sup>2</sup>

Data / restraints / parameters: 4532 / 0 / 336

Goodness-of-fit on F<sup>2</sup>: 1.096

ii). **Form B**

[00222] The XRPD for Form B is shown by **FIG. 6** and the peak listings are shown in **Table 24**.

**Table 24**

Angle, 2- $\theta$	d spacing (Å)	Height (counts)	Rel. Int.
7.0	12.57535	1083	8.57%
7.8	11.27583	1748	13.83%
9.9	8.89921	10912	86.36%
10.6	8.33283	3619	28.64%
11.7	7.55341	2313	18.31%
12.7	6.95632	7739	61.25%
13.1	6.7694	1609	12.73%
13.5	6.53423	2490	19.71%

13.9	6.38636	3216	25.45%
14.6	6.06413	3446	27.27%
14.9	5.94981	2751	21.77%
15.3	5.79313	2471	19.56%
15.7	5.62485	4393	34.77%
16.1	5.51086	1536	12.16%
16.9	5.2332	7462	59.06%
17.6	5.02985	2722	21.54%
19.3	4.60381	2787	22.06%
19.7	4.50087	2503	19.81%
20.7	4.29561	2454	19.42%
21.2	4.18049	1540	12.19%
22.0	4.03572	8193	64.84%
22.5	3.95619	12635	100.00%
23.3	3.81776	1530	12.11%
24.0	3.70488	985	7.80%
24.7	3.60111	2053	16.25%
25.1	3.54205	2015	15.95%
25.7	3.46451	521	4.12%
26.1	3.41725	1020	8.07%
27.2	3.27757	1417	11.21%
27.6	3.23088	3271	25.89%
28.4	3.1406	1887	14.93%
29.3	3.04578	1720	13.61%
29.8	2.99111	750	5.94%

[00223] Form B tends to hold substantial amount of solvent. However, the TGA data did not correspond to the NMR data for organic solvent, which could be due to hygroscopic nature of Form B. See e.g., FIG. 7. The DVS of Form B showed the solid picks up about 14% moisture between 2% and 95% relative humidity.

iv). *Form D*

[00224] The XRPD for Form D is shown by FIG. 9 and the peak listings are shown in Table 26.

Table 26

Angle, 2- $\theta$	d spacing (Å)	Height (counts)	Rel. Int.
5.8	15.28058	4971	70.74%
10.0	8.87509	4389	62.46%
10.2	8.65751	6043	86.00%

11.3	7.82263	2566	36.52%
11.5	7.66971	749	10.66%
12.2	7.27684	3233	46.01%
13.6	6.51388	2448	34.84%
14.1	6.28225	1365	19.43%
14.7	6.01924	2881	41.00%
15.4	5.74733	250	3.56%
16.0	5.52579	1652	23.51%
17.3	5.13037	3250	46.25%
17.6	5.03792	3136	44.63%
19.3	4.59918	5083	72.34%
20.0	4.42807	1495	21.28%
20.8	4.26377	1928	27.44%
22.1	4.01598	1363	19.40%
22.9	3.88059	4961	70.60%
23.3	3.81849	7027	100.00%
23.6	3.76707	3176	45.20%
24.4	3.6518	1755	24.98%
25.2	3.52644	4136	58.86%
26.4	3.37296	1305	18.57%
27.4	3.25211	610	8.68%
28.3	3.15078	553	7.87%
29.6	3.01482	615	8.75%

[00225] Form D was determined to be anhydrous with a melting peak at 239 °C. See e.g., FIG. 9. DVS of Pattern D was performed and solid picked up about 2% moisture from 2% to 95% relative humidity. See FIG. 10. The XRPD pattern remained unchanged after DVS and one week exposure to 75% relative humidity at 40 °C also did not change the form.

v). **Form E**

[00226] The XRPD for Form E is shown by FIG. 11 and the peak listings are shown in Table 27.

**Table 27**

Angle, 2- $\theta$	d spacing (Å)	Height (counts)	Rel. Int.
4.6	19.33569	1738.68	42.25%
9.0	9.8034	4115.26	100.00%
9.9	8.90634	170.17	4.14%
11.0	8.05091	182.67	4.44%
13.5	6.56847	2221.93	53.99%

15.1	5.86939	909.83	22.11%
15.8	5.59154	280.55	6.82%
18.5	4.78855	309.99	7.53%
19.8	4.48316	260.12	6.32%
20.4	4.34963	461.71	11.22%
21.7	4.09107	862.85	20.97%
22.5	3.94718	1259.97	30.62%
28.1	3.1714	143.14	3.48%

vi). *Form F*

[00227] The XRPD for Form F is shown by **FIG. 12** and the peak listings are shown in **Table 28**. A combined TGA and DSC is shown by **FIG. 13**.

**Table 28**

Angle, 2- $\theta$	d spacing (Å)	Height (counts)	Rel. Int.
5.0	17.63127	3501.15	68.88%
9.9	8.953	5083.17	100.00%
11.1	7.95783	175.54	3.45%
14.7	6.02129	1144.71	22.52%
16.5	5.3774	319.64	6.29%
19.6	4.52559	557.84	10.97%
21.6	4.11341	625.78	12.31%
22.8	3.89996	383.12	7.54%
24.4	3.64257	313.08	6.16%

vii). *Form G*

[00228] The XRPD for Form G is shown by **FIG. 14** and the peak listings are shown in **Table 29**. A combined TGA and DSC is shown by **FIG. 15**.

**Table 29**

Angle, 2- $\theta$	d spacing (Å)	Height (counts)	Rel. Int.
4.7	18.70556	1242.62	59.33%
9.4	9.36302	2094.33	100.00%
11.0	8.02324	393.11	18.77%
13.3	6.67148	95.37	4.55%
14.1	6.2664	1061.57	50.69%
15.9	5.58681	287.25	13.72%
16.2	5.46454	135.26	6.46%
18.9	4.69155	302.27	14.43%
21.1	4.20095	210.62	10.06%

21.2	4.17959	623.95	29.79%
22.8	3.89083	287.75	13.74%
23.8	3.74261	339.41	16.21%
26.7	3.3332	36.96	1.76%
28.5	3.12882	200.93	9.59%

viii). *Form H*

[00229] The XRPD for Form H is shown by **FIG. 16** and the peak listings are shown in **Table 30**.

**Table 30**

Angle, 2- $\theta$	d spacing (Å)	Height (counts)	Rel. Int.
4.6	19.00577	2892.58	100.00%
5.4	16.21955	656.39	22.69%
7.4	11.92606	1637.2	56.60%
9.2	9.58199	2031.32	70.23%
10.3	8.60629	244.93	8.47%
11.1	7.96834	1041.86	36.02%
13.5	6.53326	1064.3	36.79%
13.8	6.3908	495.71	17.14%
14.9	5.92137	1640.91	56.73%
16.9	5.24557	238.8	8.26%
17.6	5.03079	330.16	11.41%
18.4	4.82818	764.47	26.43%
19.5	4.55426	201.62	6.97%
20.7	4.28199	421.57	14.57%
22.3	3.98173	914.58	31.62%
22.9	3.88793	641.59	22.18%
23.4	3.80422	445.25	15.39%
24.1	3.68517	219.37	7.58%
24.8	3.58024	766.52	26.50%
26.5	3.36223	292.29	10.10%
27.2	3.27175	129.95	4.49%
29.5	3.0237	272.48	9.42%

ix). *Form I*

[00230] The XRPD for Form I is shown by **FIG. 17** and the peak listings are shown in **Table 31**. A combined TGA and DSC is shown by **FIG. 18**. NMR analysis revealed the present of about 4.6% EtOH, indicating a possible solvate.

**Table 31**

Angle, 2- $\theta$	d spacing ( $\text{\AA}$ )	Height (counts)	Rel. Int.
6.7	13.16055	3581.49	96.93%
7.7	11.44901	740.16	20.03%
9.5	9.319	1710.55	46.29%
9.9	8.97128	3519.19	95.24%
10.5	8.45555	1052.24	28.48%
11.6	7.61244	855.25	23.15%
12.6	7.01279	1799.14	48.69%
13.4	6.60237	1385.62	37.50%
13.8	6.43395	850.03	23.00%
14.3	6.19667	516.23	13.97%
15.2	5.8277	722.04	19.54%
15.8	5.59406	1926.11	52.13%
16.8	5.26642	1316.92	35.64%
17.2	5.1627	745.55	20.18%
19.0	4.65907	716.28	19.39%
19.7	4.50851	3694.98	100.00%
20.5	4.32796	526.56	14.25%
20.9	4.25526	540.88	14.64%
21.9	4.05301	2830.08	76.59%
22.3	3.97817	2205.69	59.69%
23.9	3.71963	1226.09	33.18%
24.6	3.61051	976.08	26.42%
25.5	3.48946	531.17	14.38%
26.0	3.42073	440.36	11.92%
27.5	3.24167	511.68	13.85%
28.3	3.15203	747.77	20.24%
29.3	3.0427	455.27	12.32%

x). *Form J*

[00231] The XRPD for Form J is shown by **FIG. 19** and the peak listings are shown in **Table 32**.

**Table 32**

Angle, 2- $\theta$	d spacing ( $\text{\AA}$ )	Height (counts)	Rel. Int.
12.4	7.14209	722.37	100.00%
13.2	6.69075	254.54	35.24%
14.6	6.06522	348.12	48.19%
15.7	5.64077	168.89	23.38%

20.4	4.34313	213.59	29.57%
22.0	4.0429	79.01	10.94%
23.3	3.8076	179.4	24.83%
23.7	3.74601	213.97	29.62%
28.0	3.18475	87.25	12.08%

*xi). Crystalline Free Base of Compound 1*

[00232] The XRPD for the crystalline free base form of Compound 1 is shown by **FIG. 25** and the peak listings are shown in **Table 33**.

**Table 33**

Angle, 2- $\theta$	d spacing ( $\text{\AA}$ )	Rel. Intensity (%)
6.9	12.8	46.1
10.6	8.4	1.9
12.1	7.3	0.8
13.5	6.5	100
14.3	6.2	2.2
15.7	5.6	10.3
15.9	5.6	10.1
17.3	5.1	5.9
17.9	4.9	2.9
19.8	4.5	32.6
20.3	4.4	44.0
21.0	4.2	3.0
21.7	4.1	2.8
22.2	4.0	1.0
23.6	3.8	13.5
24.0	3.7	7.8
24.8	3.6	10.6
25.7	3.5	23.1
26.0	3.4	2.2
26.7	3.3	1.7
27.1	3.3	2.9
27.6	3.2	0.6
28.4	3.1	4.0
29.7	3.0	9.7
30.6	2.9	3.3
31.0	2.9	0.7
31.5	2.8	1.4
32.1	2.8	5.8
33.9	2.6	2.2
35.6	2.5	0.4
36.3	2.5	0.7

37.1	2.4	0.8
39.0	2.3	0.6
39.5	2.3	2.9

### **Pharmacokinetic Evaluation**

**[00233] Methods:**

**[00234]** Single dose in suspension using Crystalline Form A micronized and unmiconized, and crystalline free base of Compound **1** were administered to male Sprague-Dawley rat at 200 mg/kg. Blood samples were collected in the presence of K<sub>2</sub>EDTA, and were centrifuged to obtain plasma. The plasma was then analyzed for these compounds by LC/MS.

**[00235] Preparation of test article for suspension dosing in rats:**

**[00236]** All animals involved in the dosing schedule were weighted and assigned numbers. All suspension formulations were freshly made prior use. Each solution was prepared at 40 mg/mL in 0.5% methyl-cellulose solution in water. The tubes were vortex for 2 min and sonicate for 30 min to achieve a white homogenous suspension.

**[00237]** All animals (n=6/ group) were dosed orally in a volume of 5 mL/kg. Following dosing, each rat was bled at each of the designated time points. Blood samples were collected from the tail vein. Blood aliquots (150 µl) were collected in tubes coated with K<sub>2</sub>EDTA, mixed gently then kept on ice and centrifuged at 2,000 g for 5 minutes at 4 °C, within 15 min of collection. The plasma layer was collected and maintained frozen at -70 °C until further processing.

**[00238] Bioanalytical Methods**

**[00239]** Bioanalytical quantitation using HPLC/triple quadruple mass spectrometry (HPLC-MS) was performed. Plasma concentrations and T<sub>1/2</sub> were calculated and reported.

**[00240] *Plasma Assay:***

**[00241]** A 1 mg/mL standard solution in DMSO: methanol (20:80, v/v) was diluted in 50 fold and subsequently serial diluted in 50% methanol water. Aliquots (10 µl) of the serial dilutions were mixed with 190 ul of control plasma for use as a standard curve. Plasma samples (50 µl) and standard samples (non-diluted) were diluted 10x with ice cold acetonitrile containing 40 ng/ml dexamethasone as internal standard. Acetonitrile precipitated samples and standards were vortexed at 5 g for 2 min (IKA vortex), then centrifuged at 5000 g for 10 min.

[00242] For 10-fold dilution standard samples: an aliquot of 5  $\mu$ L sample was added with 45  $\mu$ L control plasma to obtain the diluted samples. If a 50-fold dilution standard sample was required: an aliquot of 10  $\mu$ L 10-fold diluted sample was added with 40  $\mu$ L blank plasma to obtain the final diluted samples. Then, the extraction procedure for diluted samples was same as those for non-diluted samples.

[00243] Samples and standards (10  $\mu$ L) were injected into the LC-MS system, as described below. Concentrations of dose solutions were reported in mg/mL.

[00244] *LC-MS analysis:*

[00245] LC: 7.5  $\mu$ L of each sample and standard were injected onto a Waters BEH C18 (2.1x50 mm, 1.7  $\mu$ m, maintained at 60  $^{\circ}$ C) column at 0.6 mL/min by an UPLC. The column was equilibrated at 10% acetonitrile. Compounds were eluted with a gradient to 95% acetonitrile. All mobile phase contained 0.025% (v/v) formic acid with 1 mM ammonium acetate.

**Table 34.** Chromatographic elution conditions.

Time (min)	Mobile Phase B (%)
Initial	10
0.20	10
0.60	95
1.10	95
1.15	10
1.50	10

[00246] MS: Column eluent was analyzed by electrospray ionization into a triple quadrupole mass spectrometer system. Eluent composition was analyzed for ion pair specifics to the internal standard and the analyte respectively.

[00247] **Pharmacokinetic Analyses**

[00248] Experimental samples were compared with standard curve samples to determine compound concentrations. Average compound concentrations (in ng/mL  $\pm$  standard deviation) were reported for each time-point. Limit of detection (LLOQ) was reported as the lowest standard curve sample demonstrating a deviation of less than 20% of nominal concentration. PK analysis was performed in Phoenix Winnonlin;  $C_{max}$  were determined as

the maximum average concentration observed at a given point, the area under the curve (AUC) was reported for  $t_0$  to  $t_{last}$  hours.

**[00249]** As shown in **FIG. 22** and **Table 35**, crystalline Form A (micronized) and crystalline Form A (unmicronized) were shown to be more soluble in phosphate buffer pH7.4 compared to crystalline free-base of Compound **1**. When the plasma concentration-time profiles and pharmacokinetic parameters of crystalline Form A (micronized and unmicronized) were compared to the crystalline free-base of Compound **1** following oral dosing as suspension to fasted male Sprague-Dawley rats, AUC and Cmax were significantly different. The micronized and unmicronized of crystalline Form A showed AUCs 3.8 and 2.7 fold higher compared to crystalline free-base of Compound **1** respectively. Similarly, the micronized and unmicronized of crystalline Form A showed Cmax 2 and 1.6 fold higher compared to crystalline free-base of Compound **1** respectively. In contrast, no significant differences could be observed between the micronized and unmicronized forms of crystalline Form A.

**Table 35:** Characterization summary of compounds used in Pharmacokinetic Study

Materials	PSD
Crystalline Form A (micronized)	D90: 10 / D50= 4.6 / D10 = 1
Crystalline Form A (unmicronized)	D90= 71 / D50= 32.6 / D10 =11.55
Crystalline free-base of Compound <b>1</b>	D90= 521 / D50=206/ D10 =32

**Table 36:** Pharmacokinetic Parameters after PO administration to Sprague-Dawley Rats

Compound Form	Crystalline Form A (micronized)	Crystalline Form A (unmicronized)	Crystalline free-base of Compound <b>1</b>
Oral Dose (mg/kg)*	200	200	200
Cmax (ng/mL)	36700 ± 6800	29800 ± 5300	17900 ± 3600
AUC 0-last (ng/h/mL)	478000 ± 87900	355900 ± 82900	124800 ± 85800

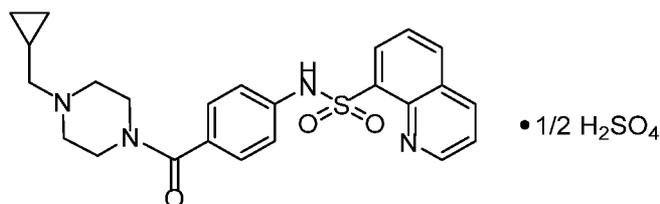
\*Dose shows the equivalence to the free base form of compound 1.

**[00250]** While a number of embodiments have been described, the scope of this disclosure is to be defined by the appended claims, and not by the specific embodiments that have been represented by way of example. The contents of all references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated herein in their entireties

by reference. Unless otherwise defined, all technical and scientific terms used herein are accorded the meaning commonly known to one with ordinary skill in the art.

Listing of Claims:

1. A crystalline form of a compound having the formula:



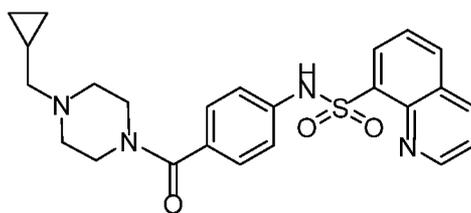
2. The crystalline form of Claim 1, wherein the compound is a solvate.
3. The crystalline form of Claim 2, wherein the compound is a hydrate.
4. The crystalline form of Claim 3, wherein the compound is a sesquihydrate.
5. The crystalline form of Claim 1, wherein the compound is anhydrous.
6. The crystalline form of Claim 2, wherein the compound is an ethanol solvate.
7. The crystalline form of Claim 4, wherein the crystalline form is crystalline Form A characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $9.9^\circ$ ,  $15.8^\circ$ , and  $22.6^\circ$ .
8. The crystalline Form A of Claim 7, wherein the crystalline form is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $9.9^\circ$ ,  $15.8^\circ$ , and  $22.6^\circ$ ; and at least one, at least two, or at least three additional x-ray powder diffraction peak at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $15.0^\circ$ ,  $17.1^\circ$ ,  $21.3^\circ$ , and  $21.9^\circ$ .
9. The crystalline Form A of Claim 8, wherein the crystalline form is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $9.9^\circ$ ,  $11.4^\circ$ ,  $15.0^\circ$ ,  $15.3^\circ$ ,  $15.8^\circ$ ,  $17.1^\circ$ ,  $17.7^\circ$ ,  $21.3^\circ$ ,  $21.9^\circ$ ,  $22.6^\circ$ , and  $23.5^\circ$ .
10. The crystalline Form A of Claim 9, wherein the crystalline form is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $4.9^\circ$ ,  $9.9^\circ$ ,  $11.0^\circ$ ,  $11.4^\circ$ ,  $11.7^\circ$ ,  $12.3^\circ$ ,  $12.8^\circ$ ,  $13.6^\circ$ ,  $13.9^\circ$ ,  $14.2^\circ$ ,  $15.0^\circ$ ,  $15.3^\circ$ ,  $15.8^\circ$ ,  $17.1^\circ$ ,  $17.4^\circ$ ,  $17.7^\circ$ ,  $18.8^\circ$ ,  $19.1^\circ$ ,  $19.8^\circ$ ,  $21.3^\circ$ ,  $21.9^\circ$ ,  $22.6^\circ$ ,  $23.0^\circ$ ,  $23.2^\circ$ ,  $23.5^\circ$ ,  $23.8^\circ$ ,  $24.1^\circ$ ,  $24.5^\circ$ ,  $25.3^\circ$ ,  $25.6^\circ$ ,  $26.1^\circ$ ,  $27.1^\circ$ ,  $28.1^\circ$ , and  $29.8^\circ$ .

11. The crystalline form of Claim 6, wherein the crystalline form is crystalline Form B characterized by at least three, at least four, at least five, or at least six x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $9.9^\circ$ ,  $10.6^\circ$ ,  $12.7^\circ$ ,  $15.7^\circ$ ,  $16.9^\circ$ ,  $22.0^\circ$ , and  $22.5^\circ$ .
12. The crystalline form of Claim 1, wherein the crystalline form is crystalline Form C characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $6.9^\circ$ ,  $10.4^\circ$ , and  $12.0^\circ$ .
13. The crystalline form of Claim 5, wherein the crystalline form is Crystalline Form D characterized by at least three, at least four, at least five, or at least six x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $5.8^\circ$ ,  $10.0^\circ$ ,  $10.2^\circ$ ,  $19.3^\circ$ ,  $22.9^\circ$ ,  $23.3^\circ$ , and  $25.2^\circ$ .
14. The crystalline form of Claim 1, wherein the crystalline form is Crystalline Form E characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $4.6^\circ$ ,  $9.0^\circ$ ,  $13.5^\circ$ , and  $22.5^\circ$ .
15. The crystalline form of Claim 1, wherein the crystalline form is Crystalline Form F characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $5.0^\circ$ ,  $9.9^\circ$ , and  $14.7^\circ$ .
16. The crystalline form of Claim 1, wherein the crystalline form is Crystalline Form G characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $4.7^\circ$ ,  $9.4^\circ$ , and  $14.1^\circ$ .
17. The crystalline form of Claim 1, wherein the crystalline form is Crystalline Form H is characterized by at least three, at least four, at least five, or at least six x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $4.6^\circ$ ,  $7.4^\circ$ ,  $9.2^\circ$ ,  $11.1^\circ$ ,  $13.5^\circ$ ,  $14.9^\circ$ , and  $22.3^\circ$ .
18. The crystalline form of Claim 6, wherein the crystalline form is Crystalline Form I characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $6.7^\circ$ ,  $9.5^\circ$ , and  $19.7^\circ$ .

19. The crystalline Form I of Claim 17, wherein the crystalline form is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ )  $6.7^\circ$ ,  $9.5^\circ$ , and  $19.7^\circ$ ; and at least one, at least two, or at least three additional x-ray powder diffraction peak at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $9.9^\circ$ ,  $12.6^\circ$ ,  $15.8^\circ$ ,  $21.9^\circ$ , and  $22.3^\circ$ .

20. The crystalline form of Claim 1, wherein the crystalline form is Crystalline Form J characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $12.4^\circ$ ,  $13.2^\circ$ ,  $14.6^\circ$ ,  $20.4^\circ$ , and  $23.7^\circ$ .

21. A crystalline free base of a compound having the formula:

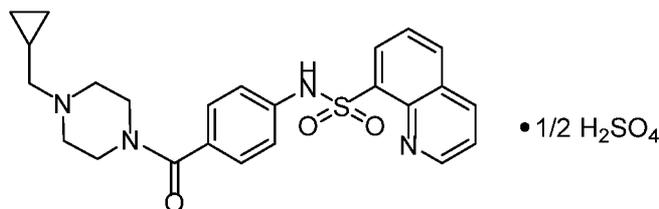


22. The crystalline free base of Claim 21, wherein the crystalline form is characterized by x-ray powder diffraction peaks at  $2\Theta$  angles ( $\pm 0.2^\circ$ ) selected from  $6.9^\circ$ ,  $13.5^\circ$ ,  $19.8^\circ$ , and  $20.3^\circ$ .

23. The crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 22, wherein the compound is at least 60% a single crystalline form, at least 70% a single crystalline form, at least 80% a single crystalline form, at least 90% a single crystalline form, at least 95% a single crystalline form, or at least 99% a single crystalline form by weight.

24. The crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 23, wherein the compound form is substantially free of amorphous forms of Formula (I).

25. An amorphous Form of a hemisulfate salt of a compound having the formula:



26. The amorphous Form of Claim 25, wherein the compound is substantially free of crystalline forms of Formula (I).
27. A pharmaceutical composition comprising the crystalline Form A, B, C, D, E, F, G, H, I, J, or crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26; and a pharmaceutically acceptable carrier.
28. A tablet composition comprising the crystalline Form A, B, C, D, E, F, G, H, I, J, or crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26; and a pharmaceutically acceptable carrier.
29. The composition of Claim 27 or tablet composition of Claim 28, wherein the carrier is selected from one or more of microcrystalline cellulose, mannitol, Croscarmellose Sodium, and Sodium Stearyl Fumarate.
30. The tablet composition of Claim 28 or Claim 29, wherein the composition comprises about 5.7 to about 5.9 mg, about 23.4 to about 23.6 mg, or about 58.7 to about 58.9 mg crystalline Form A; 62% w/w ( $\pm 2\%$ ) microcrystalline cellulose; 23% w/w ( $\pm 2\%$ ) mannitol, 3% w/w ( $\pm 2\%$ ) croscarmellose sodium, and 2% w/w ( $\pm 2\%$ ) stearyl fumarate.
31. The composition of any one of Claims 28 to 30, wherein the crystalline Form is Form A.
32. A method of treating Pyruvate Kinase Deficiency (PKD) in a subject in need thereof, comprising administering to the subject an effective amount of the crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26, or the pharmaceutical composition of any one of Claims 27 to 31.

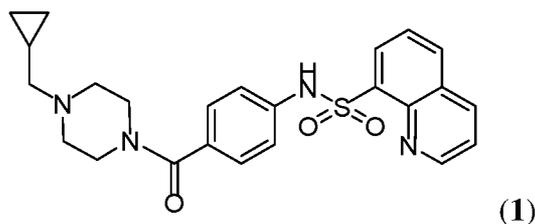
33. A method of treating sickle cell disease (SCD) in a subject in need thereof, comprising administering to the subject an effective amount of the crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26, or the pharmaceutical composition of any one of Claims 27 to 31.
34. A method of treating thalassemia (such as beta-thalassemia, non-transfusion-dependent thalassemia, and transfusion-dependent thalassemia) in a subject in need thereof, comprising administering to the subject an effective amount of the crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26, or the pharmaceutical composition of any one of Claims 27 to 31.
35. A method of treating hemolytic anemia in a subject in need thereof, comprising administering to the subject an effective amount of the crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26, or the pharmaceutical composition of any one of Claims 27 to 31.
36. A method of treating a disease selected from hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof, comprising administering to the subject an effective amount of the crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26, or the pharmaceutical composition of any one of Claims 27 to 31.
37. A method of regulating 2,3-diphosphoglycerate levels in blood in a subject in need thereof, comprising administering to the subject an effective amount of the crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26, or the pharmaceutical composition of any one of Claims 27 to 31.
38. A method of activating wild-type or mutant PKR in red blood cells in a subject in need thereof, comprising administering to the subject an effective amount of the crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26, or the pharmaceutical composition of any one of Claims 27 to 31.

39. A method of increasing the amount of hemoglobin in a subject in need thereof, comprising administering to the subject an effective amount of the crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26, or the pharmaceutical composition of any one of Claims 27 to 31.

40. A method of evaluating the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in a subject in need thereof, comprising administering to the subject an effective amount of the crystalline Form A, B, C, D, E, F, G, H, I, J, or the crystalline free base of any one of Claims 1 to 24, or the amorphous Form of Claim 25 or 26, or the pharmaceutical composition of any one of Claims 27 to 31.

41. The method of any one of claims 32 to 40, wherein the crystalline form is Form A.

42. A method of forming crystalline Form A of any one of Claims 7-10, 23, and 24, the method comprising reacting a compound of Formula 1:



with H<sub>2</sub>SO<sub>4</sub> in an alcoholic solution.

FIG. 1

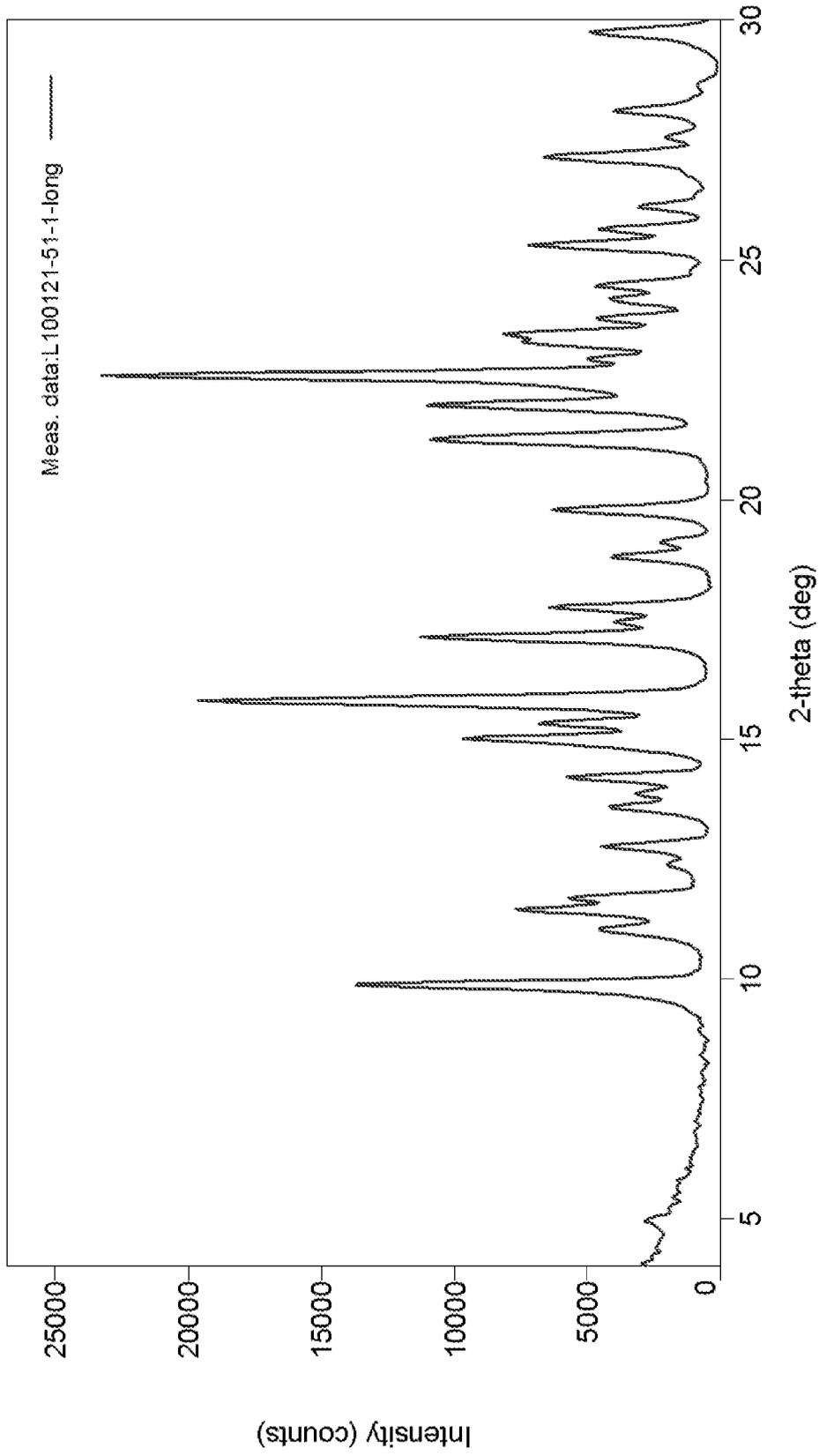


FIG. 2

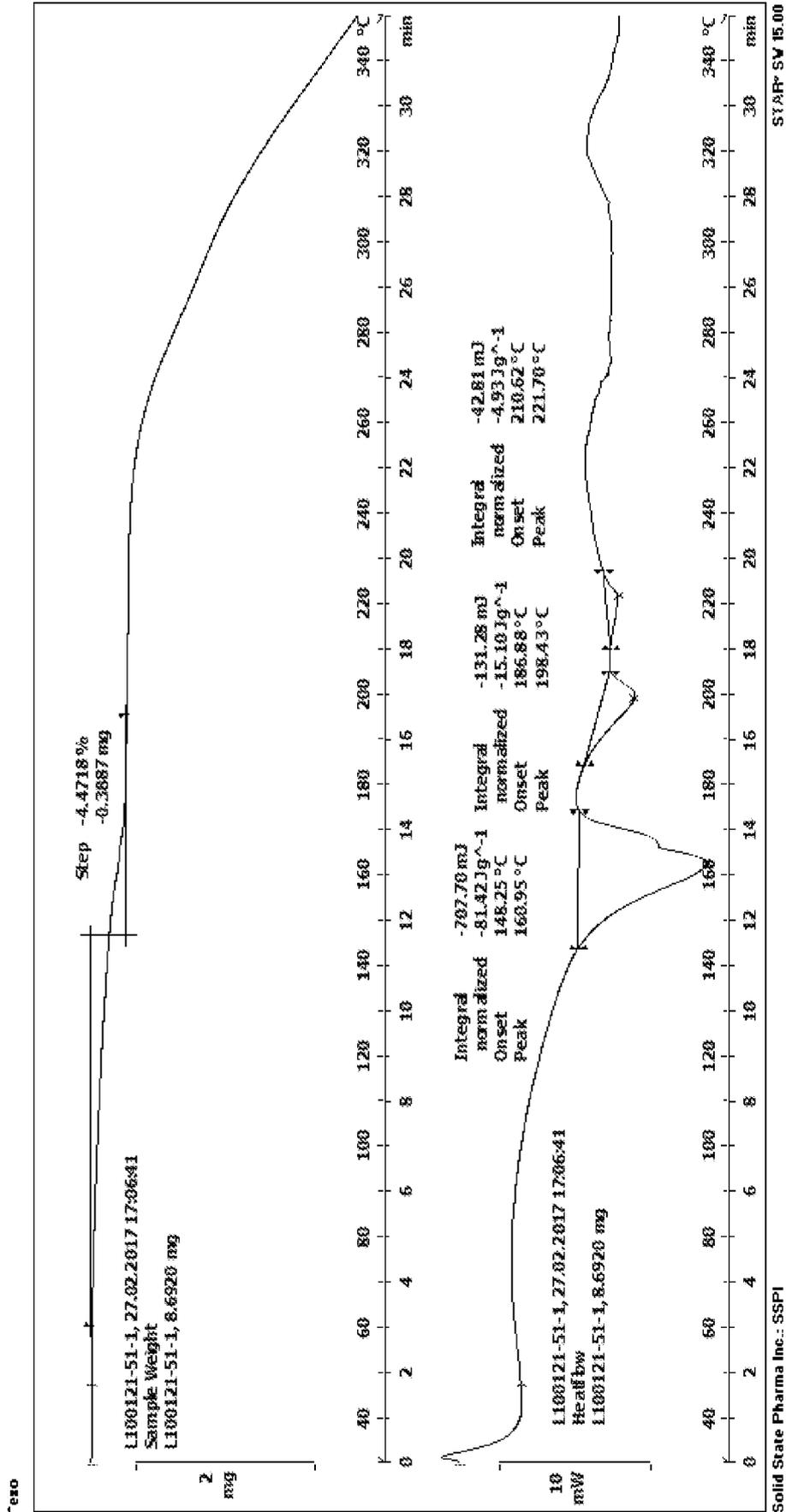


FIG. 3

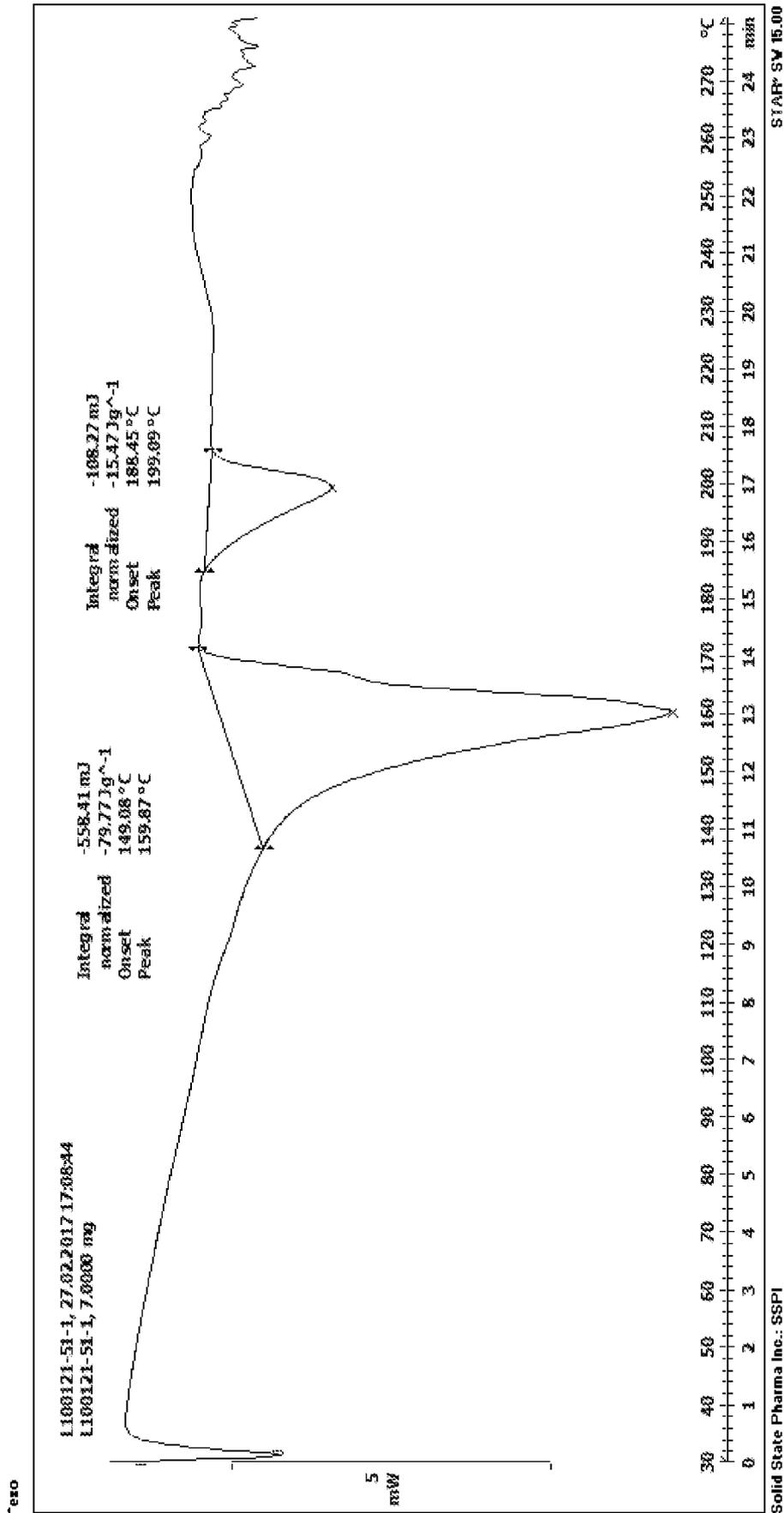


FIG. 4

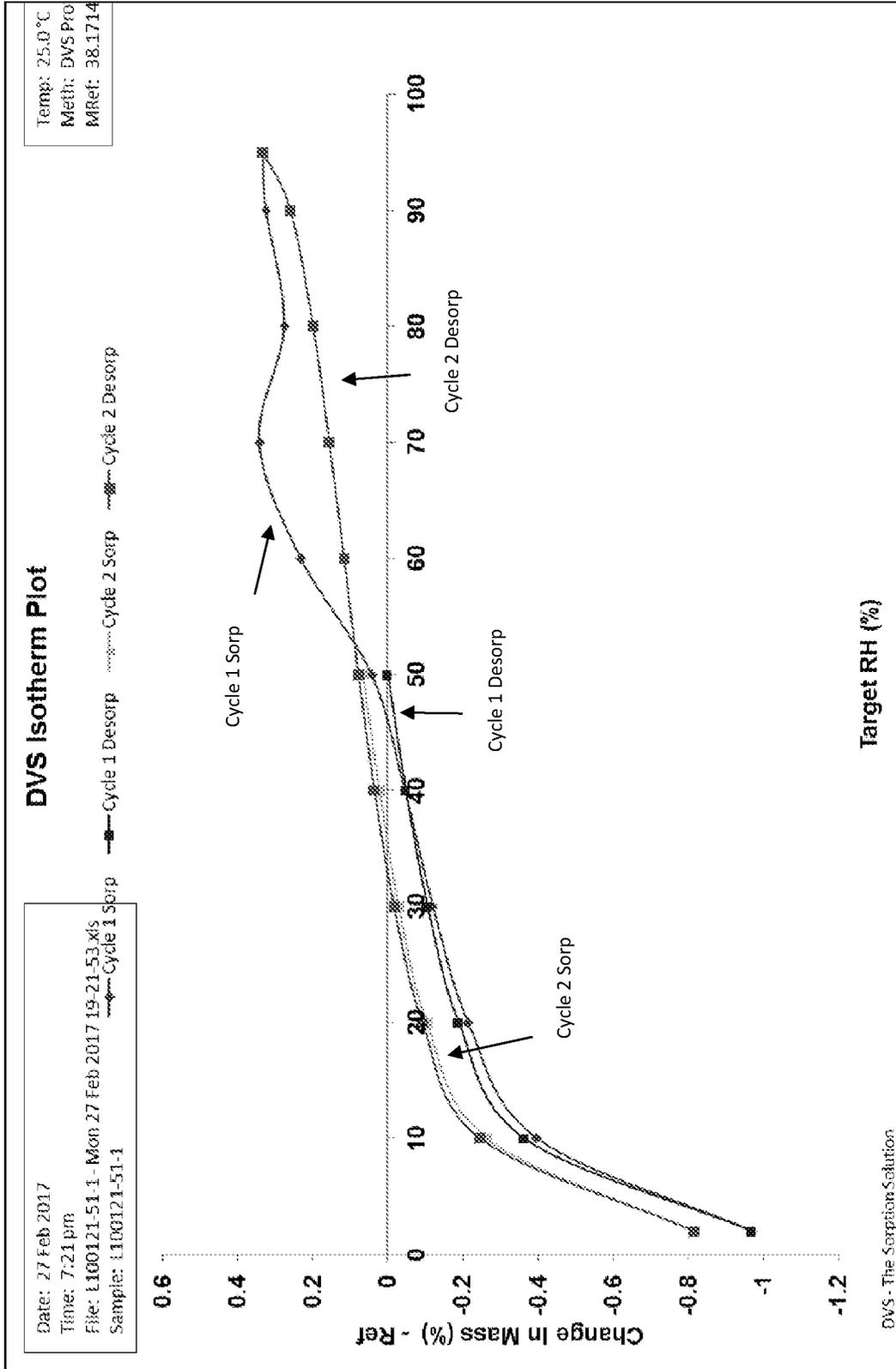


FIG. 5(i)

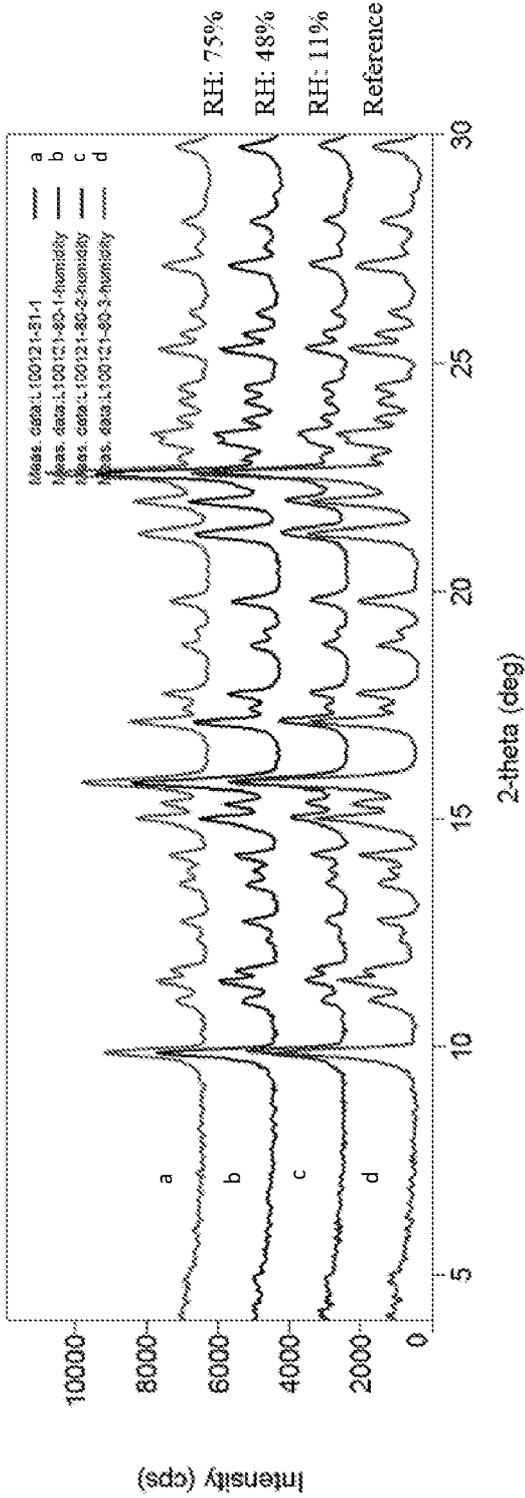


FIG. 5(ii)

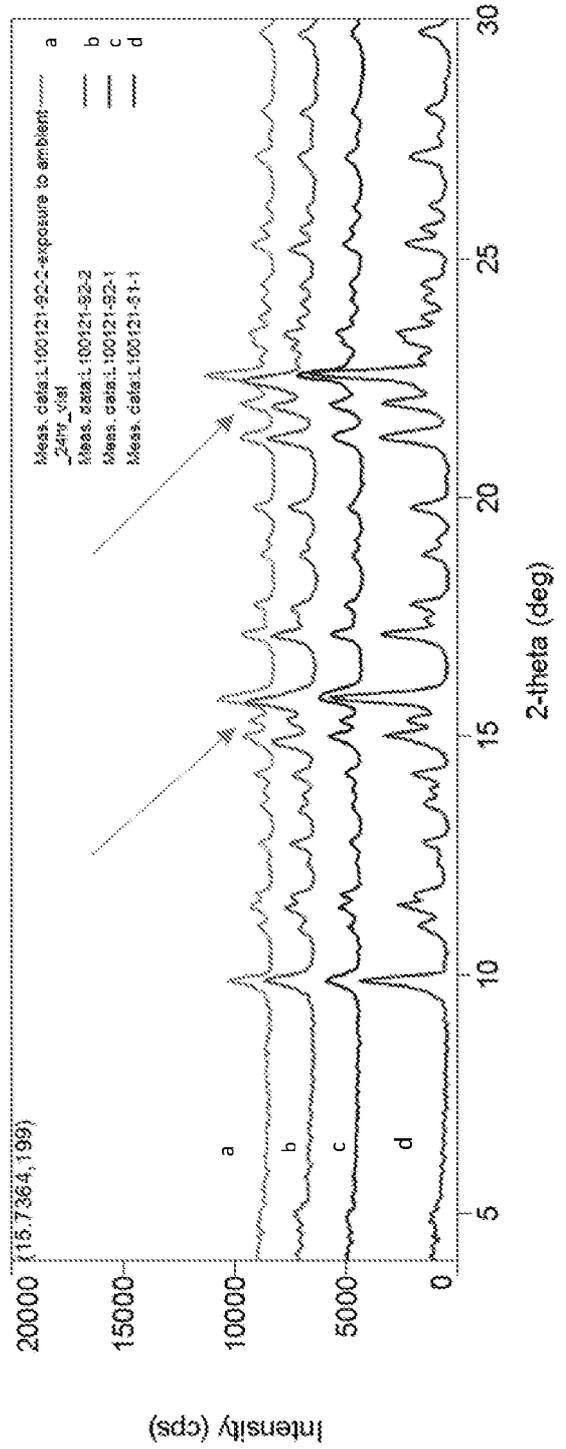


FIG. 6

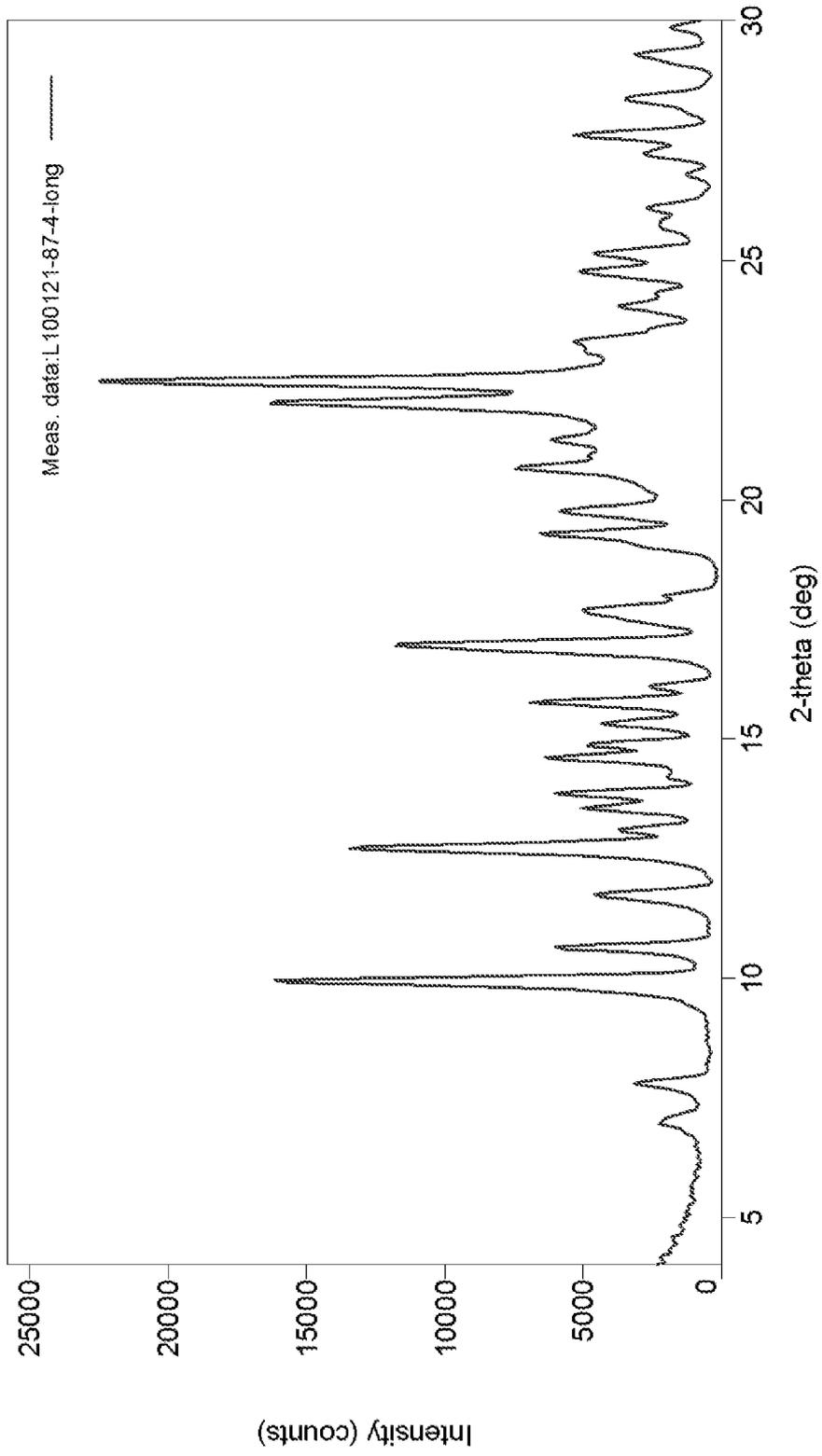


FIG. 7i

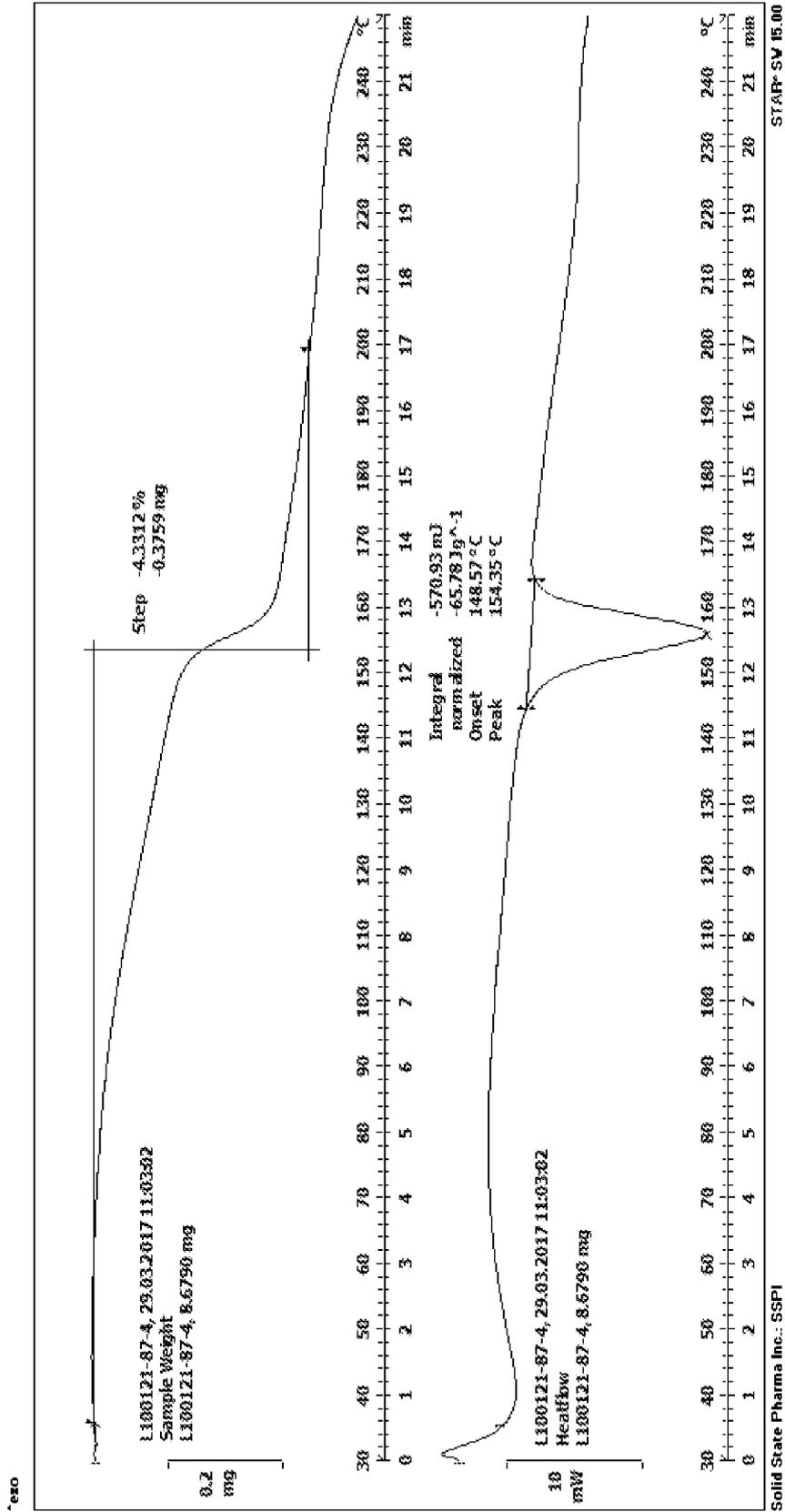


FIG. 7ii

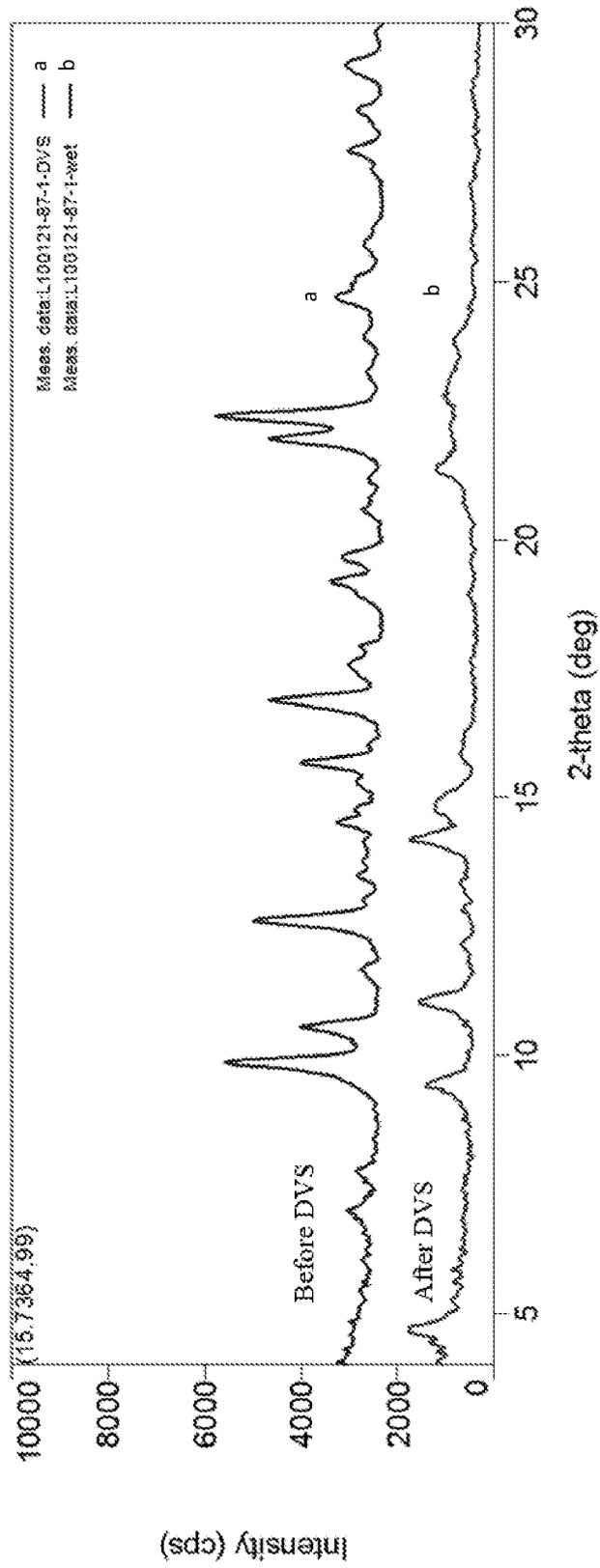


FIG. 8

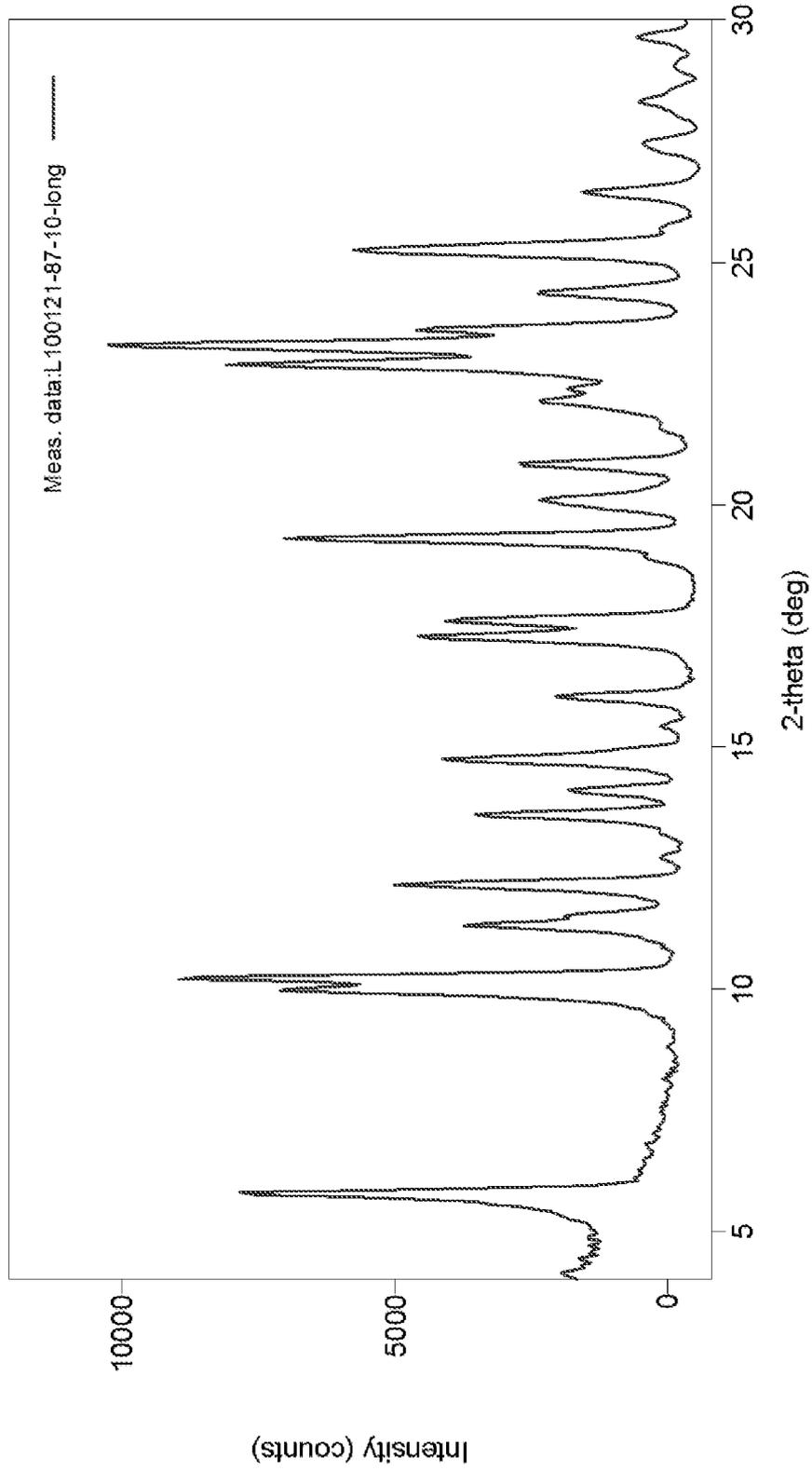


FIG. 9

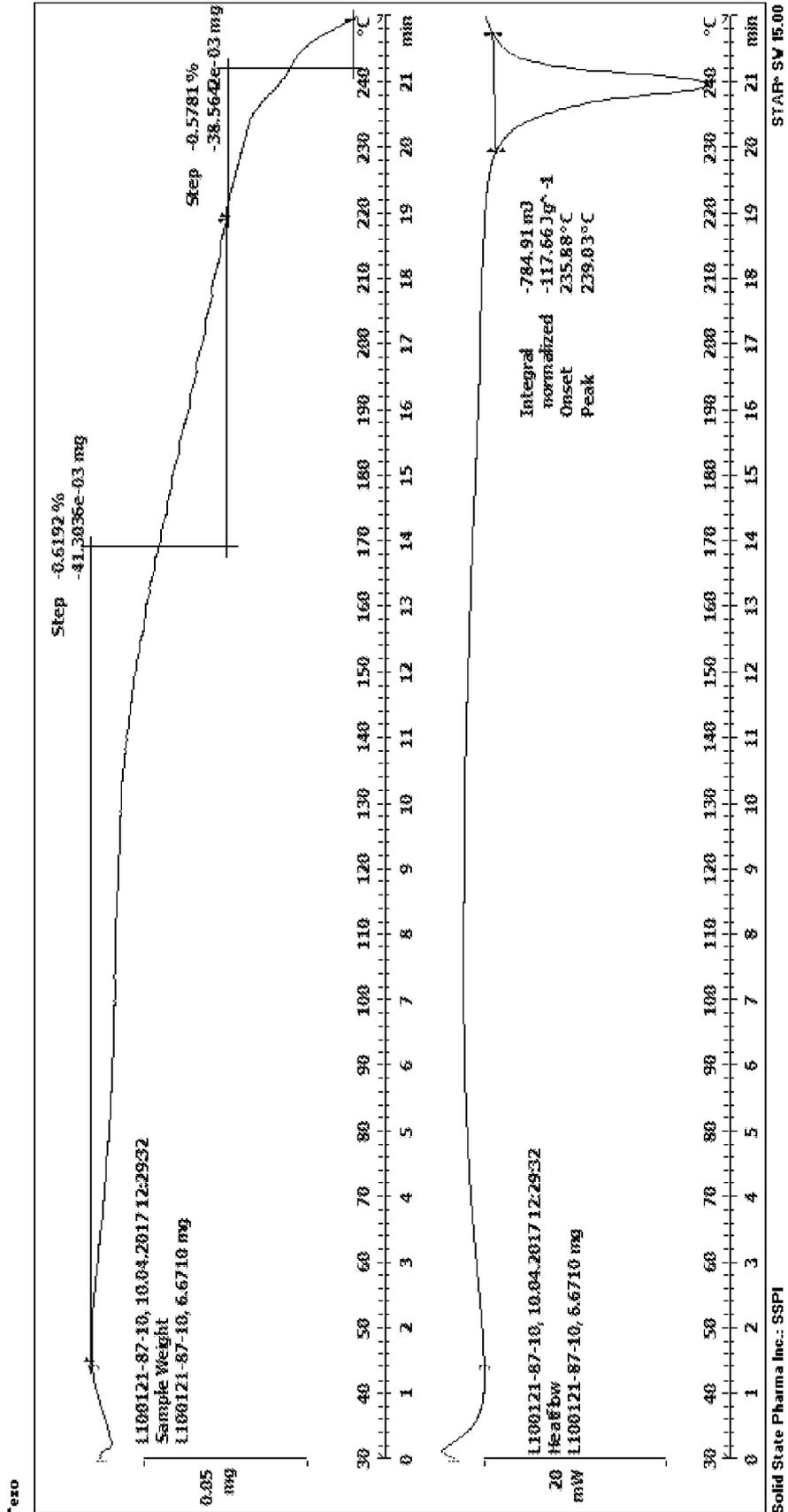


FIG. 10

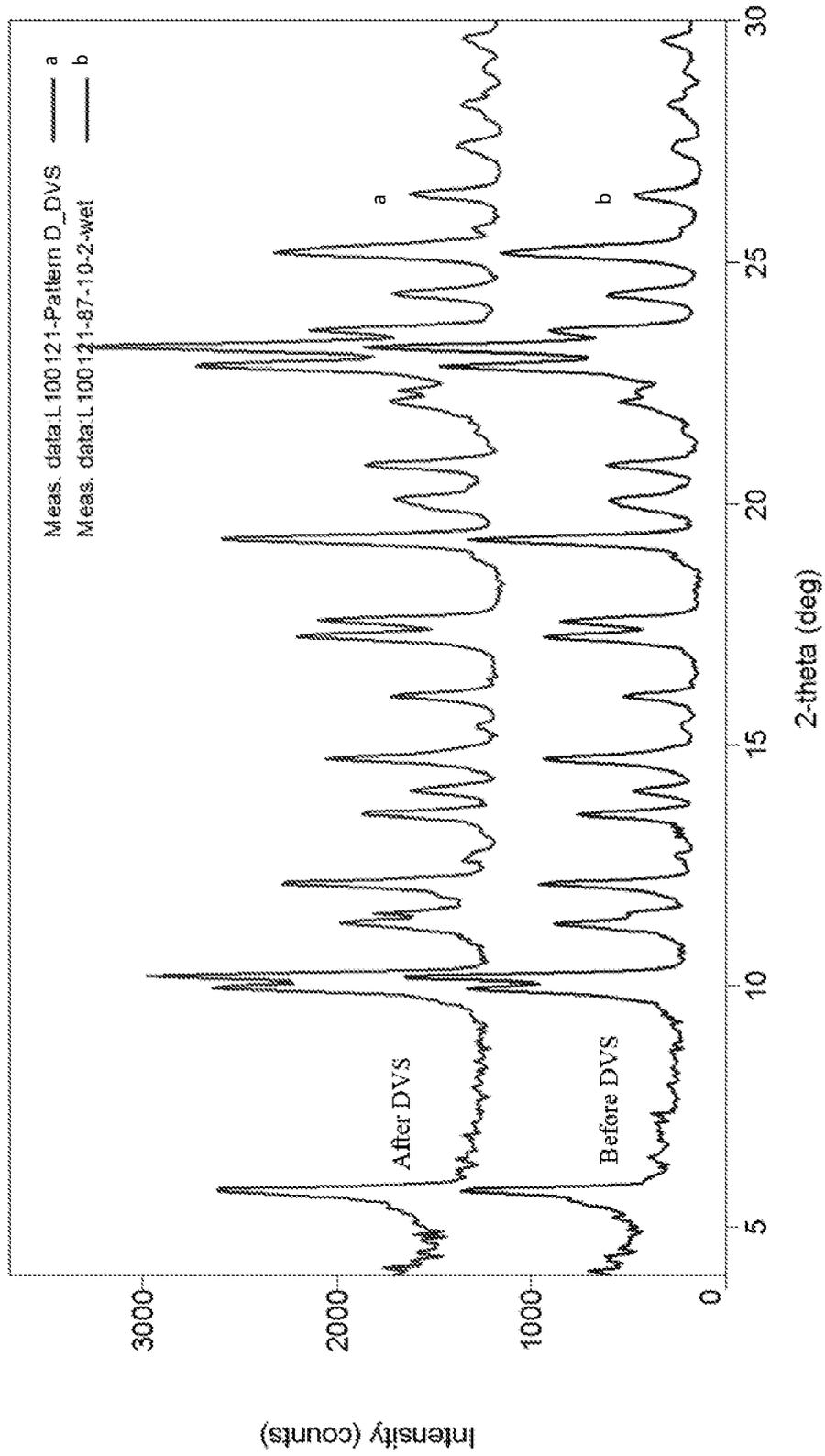


FIG. 11

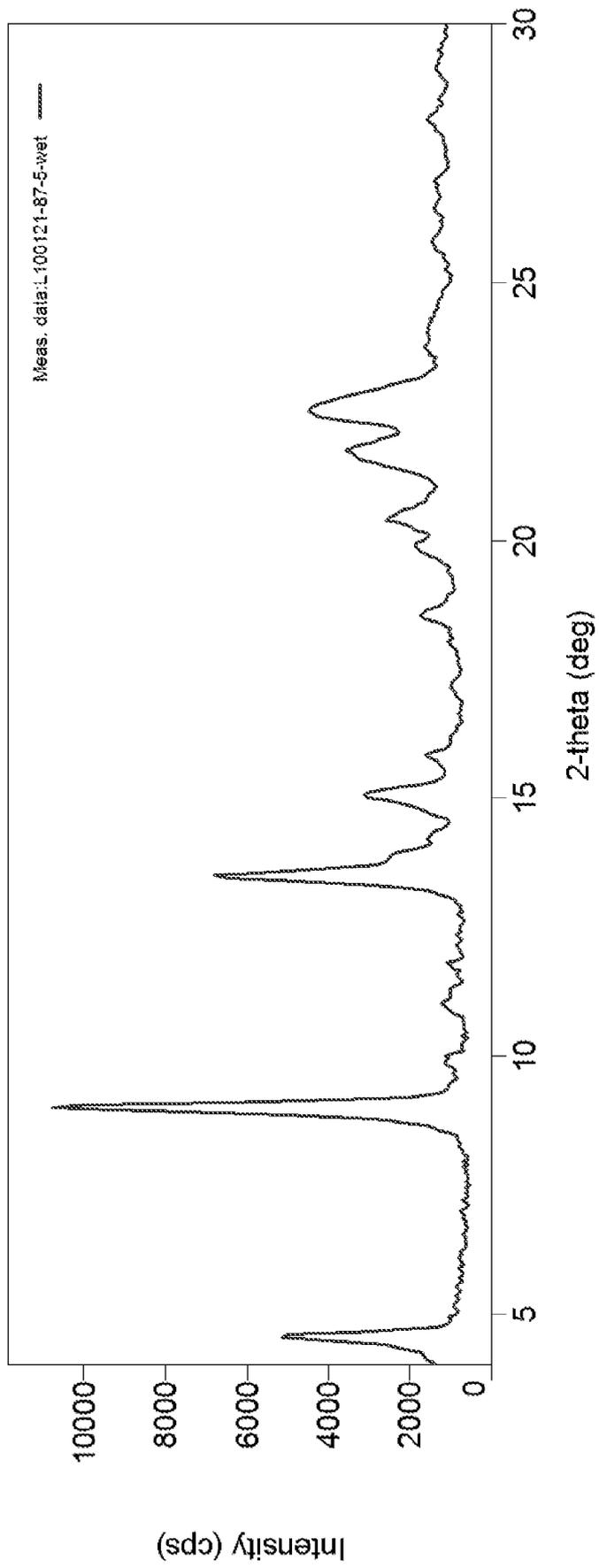


FIG. 12

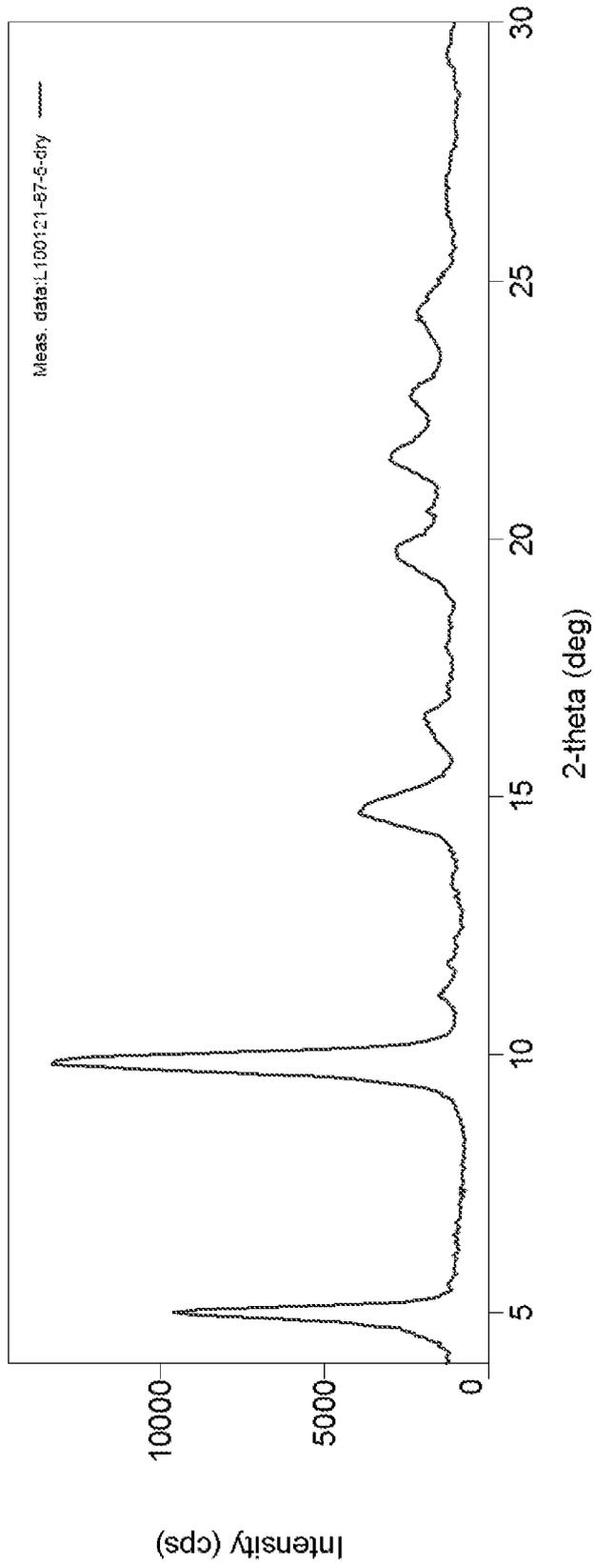


FIG. 13

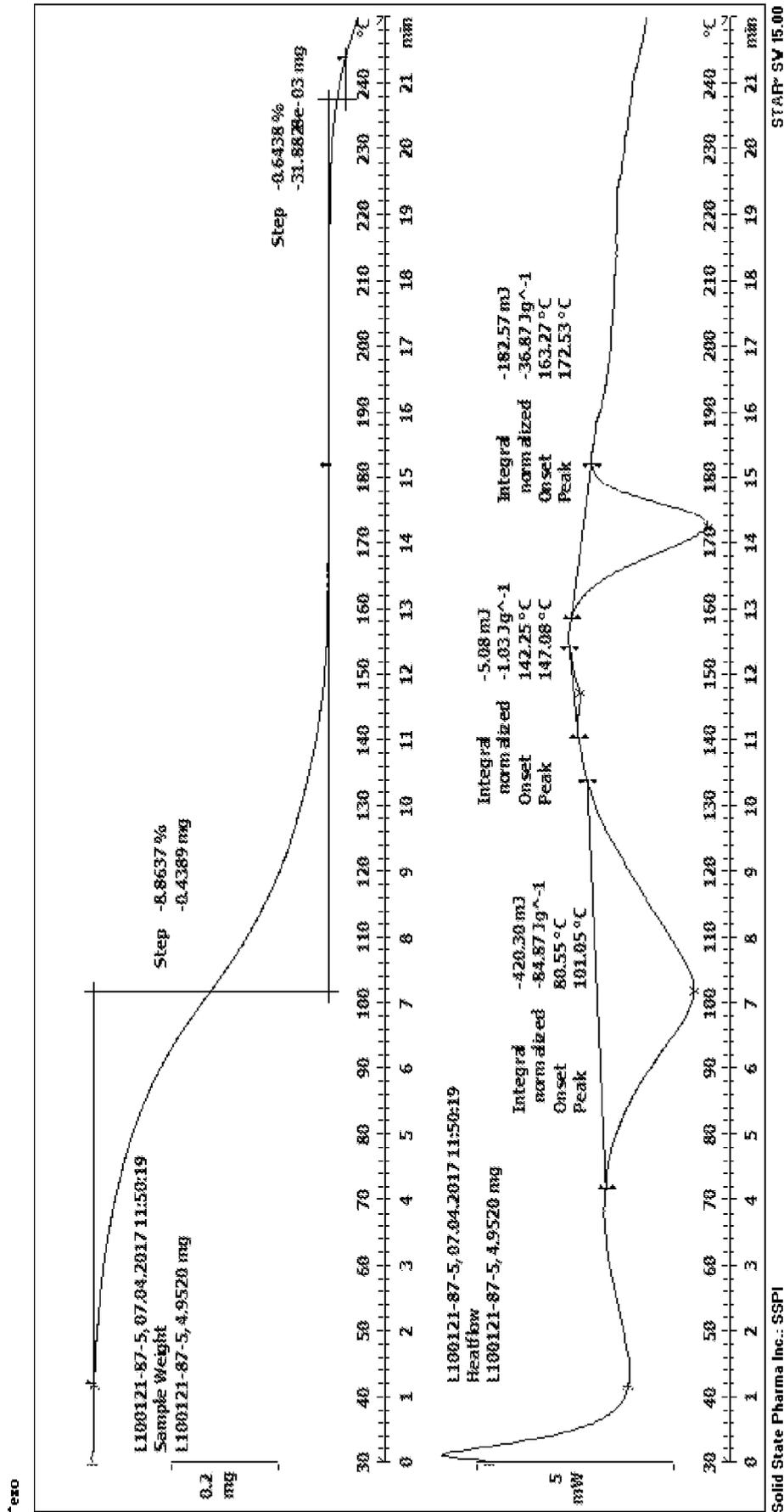


FIG. 14

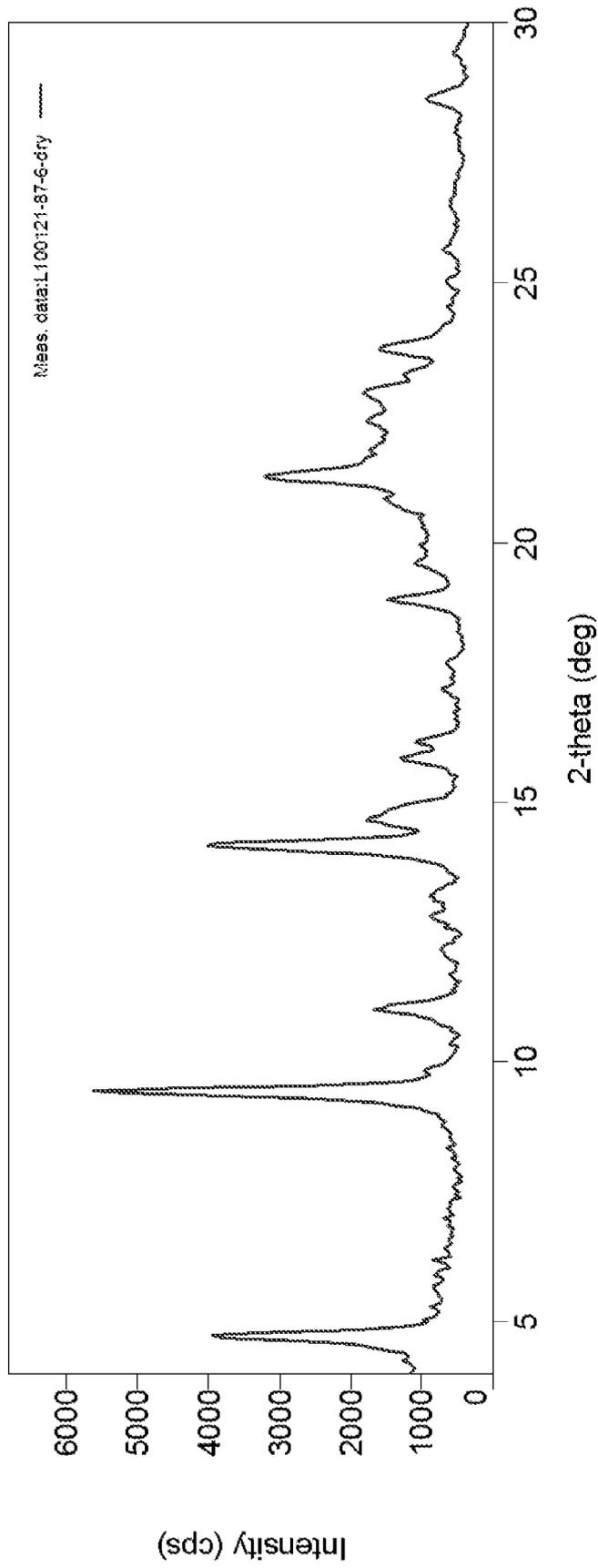


FIG. 15

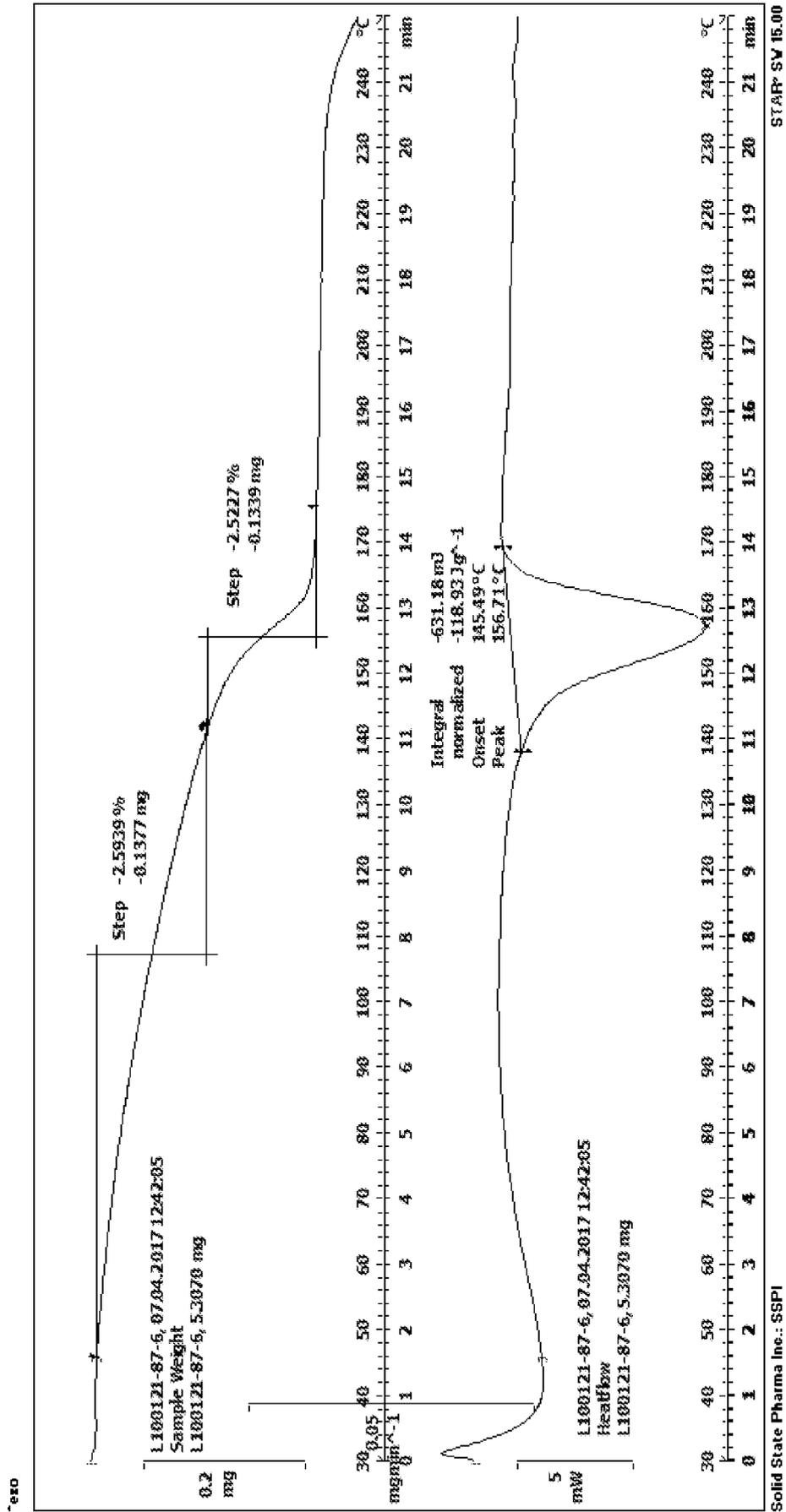


FIG. 16

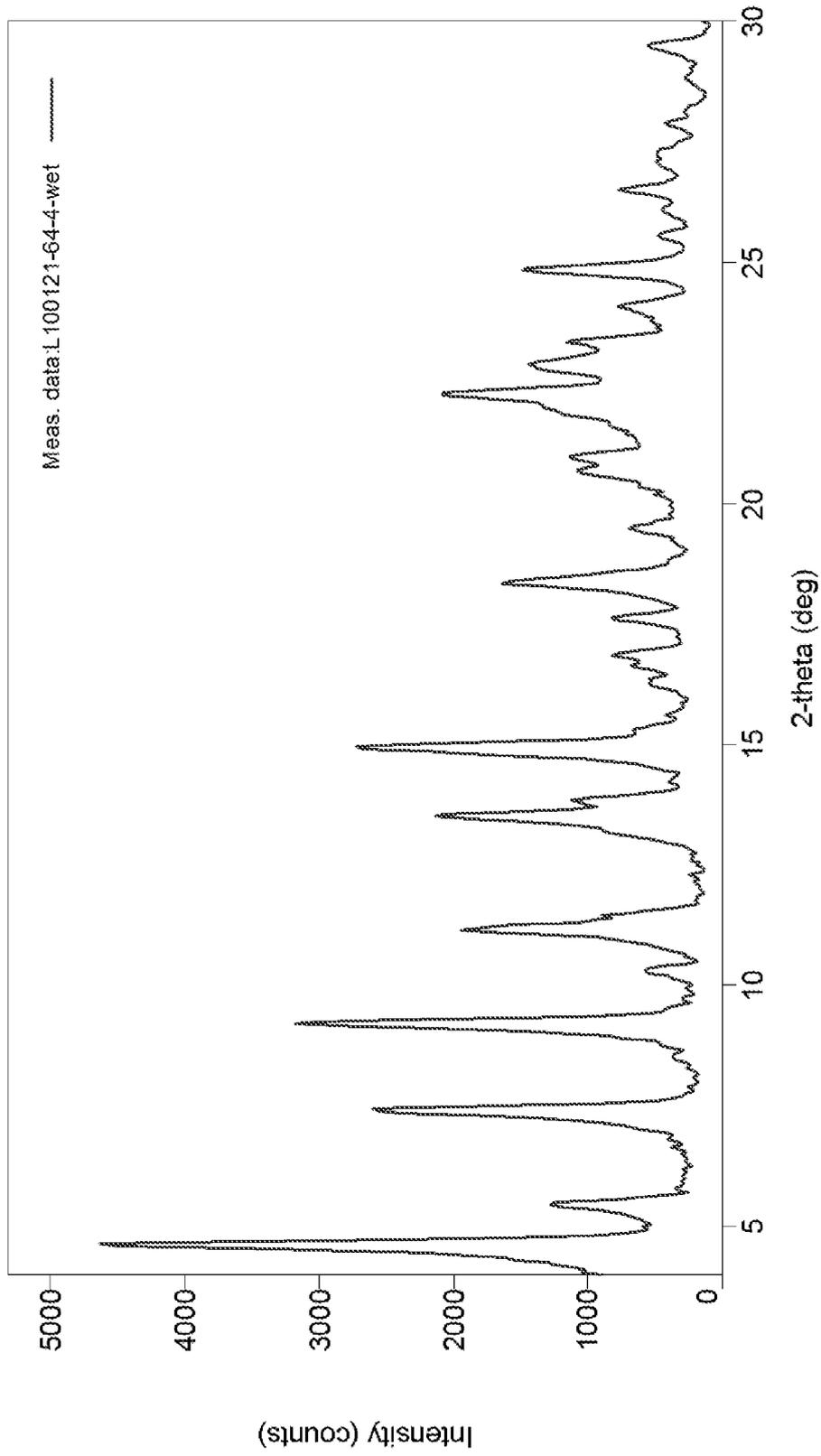


FIG. 17

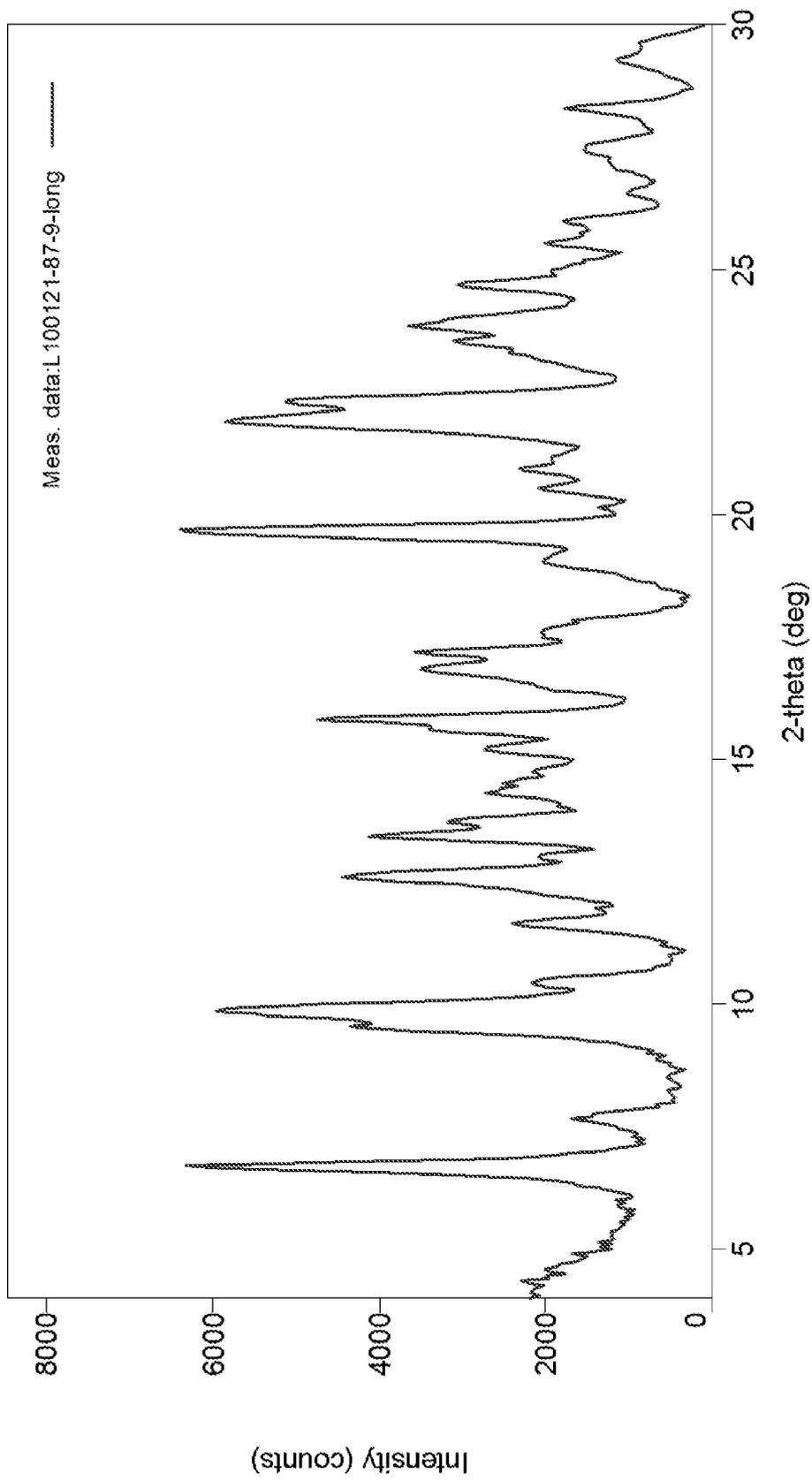


FIG. 18

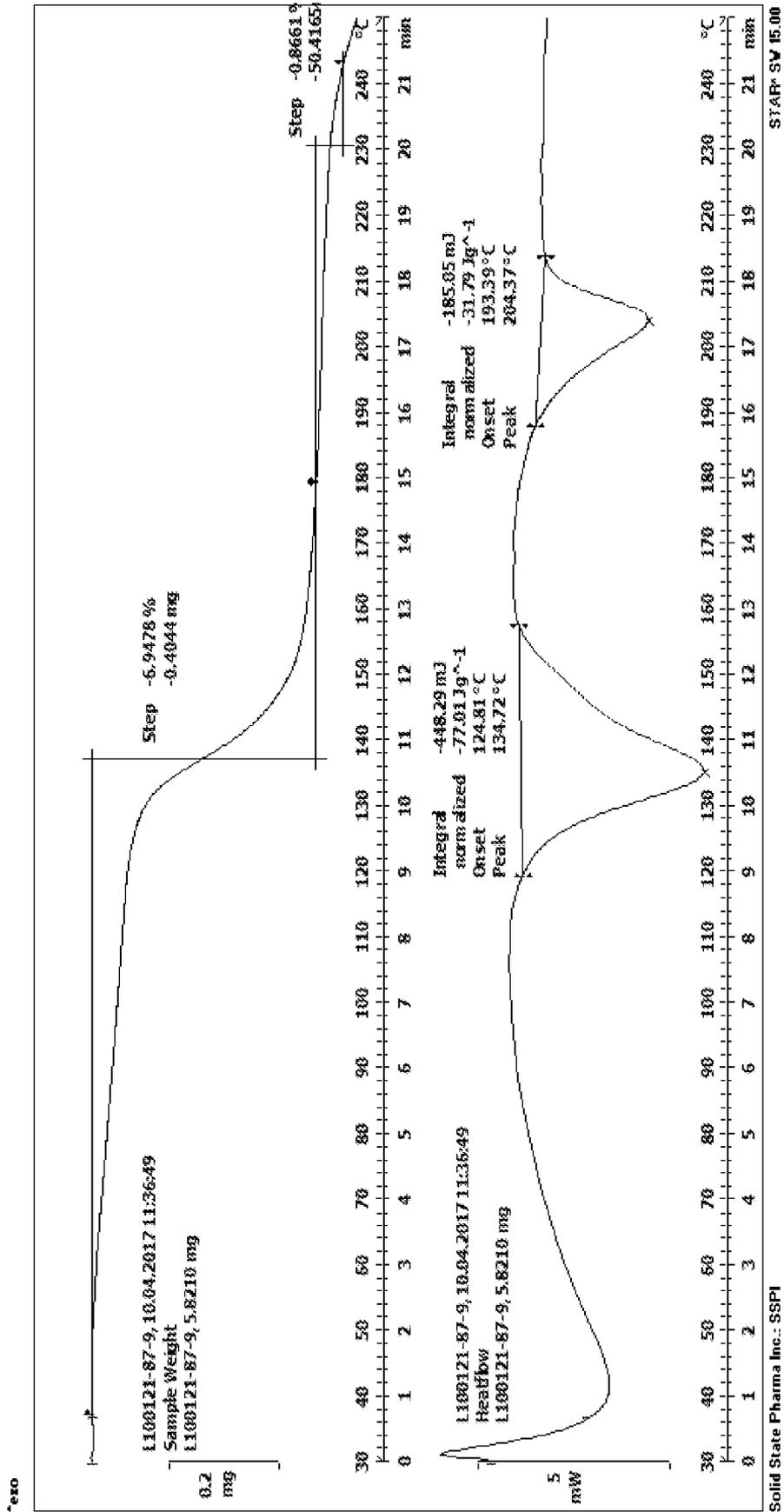


FIG. 19

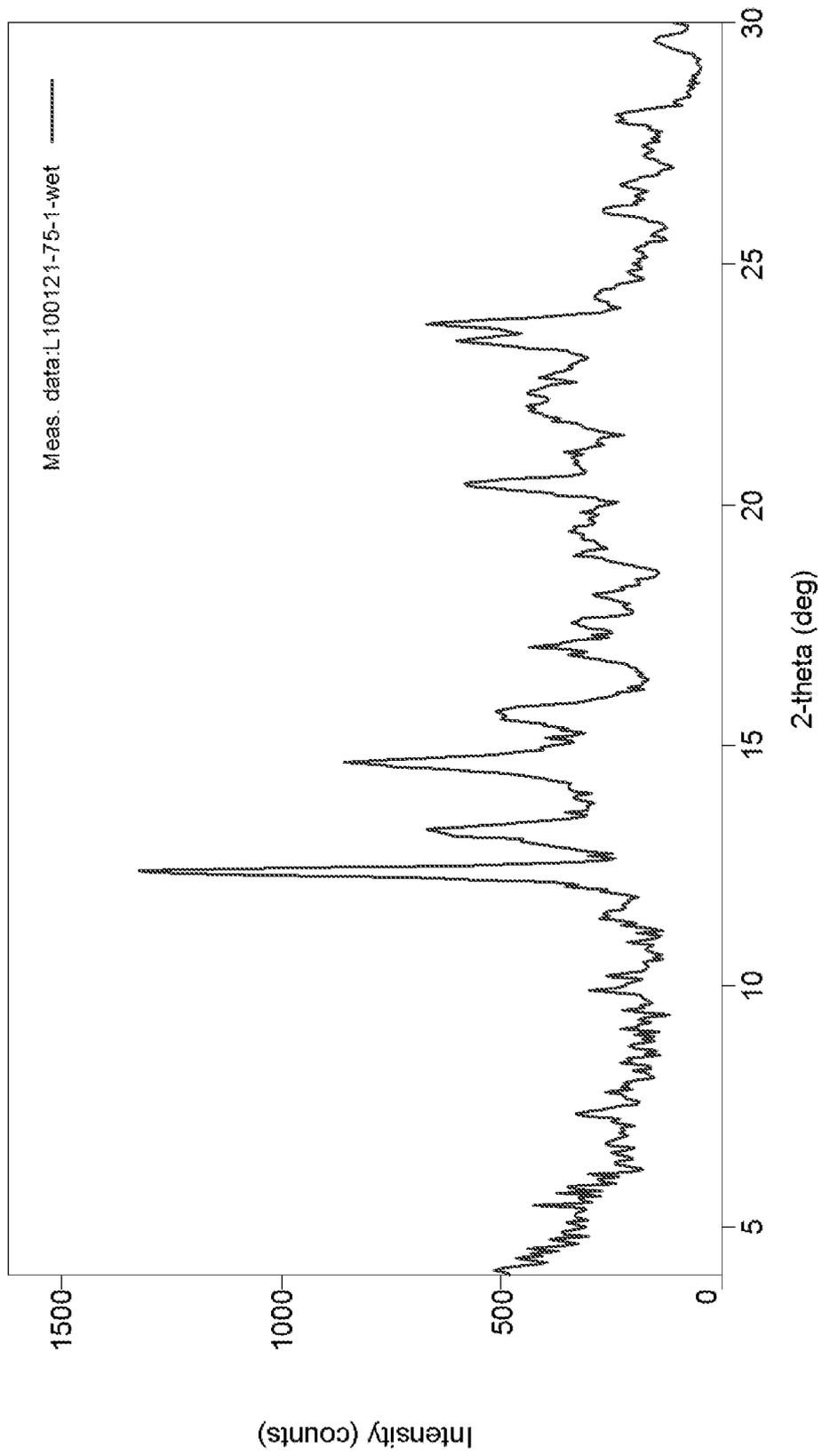


FIG. 20

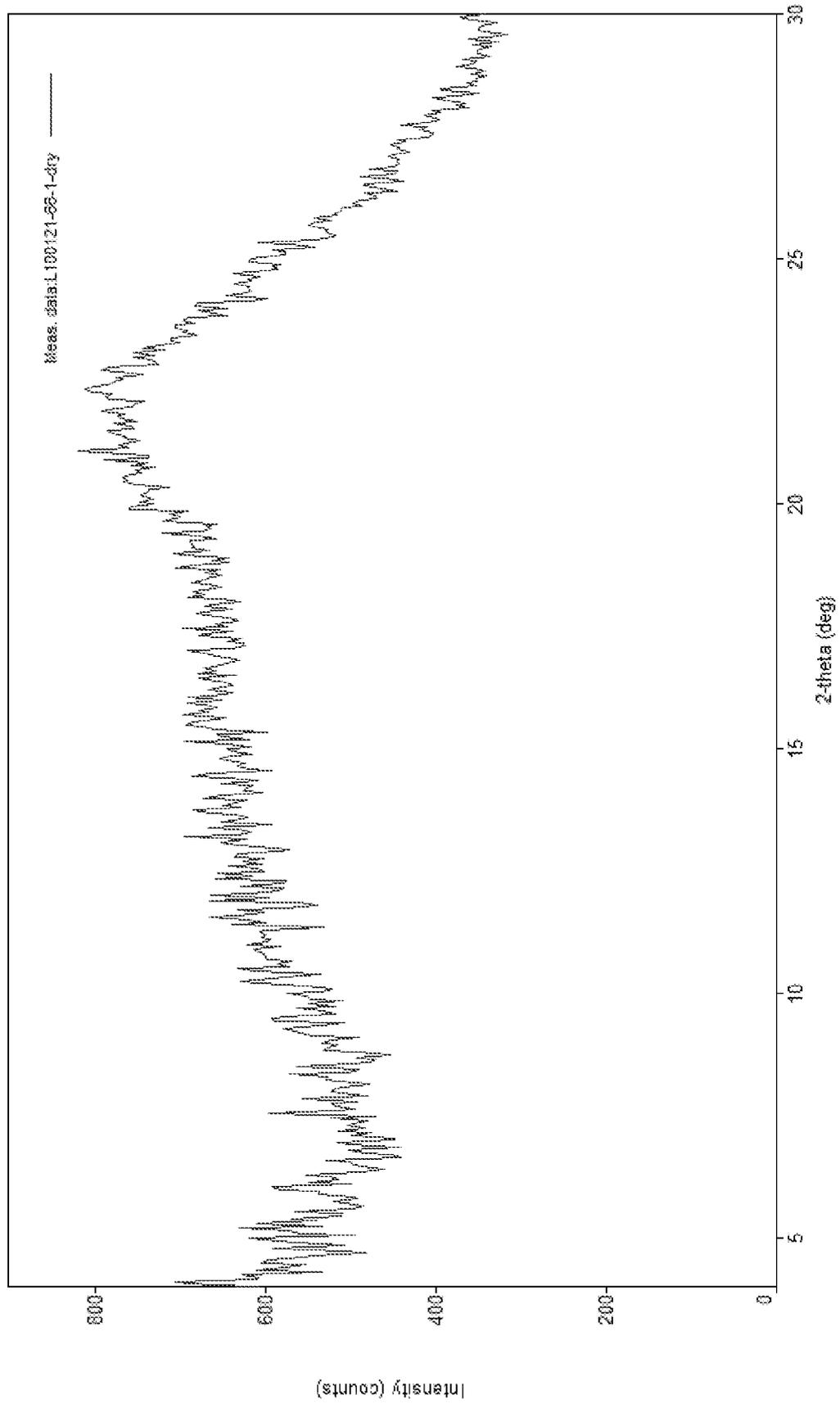


FIG. 21

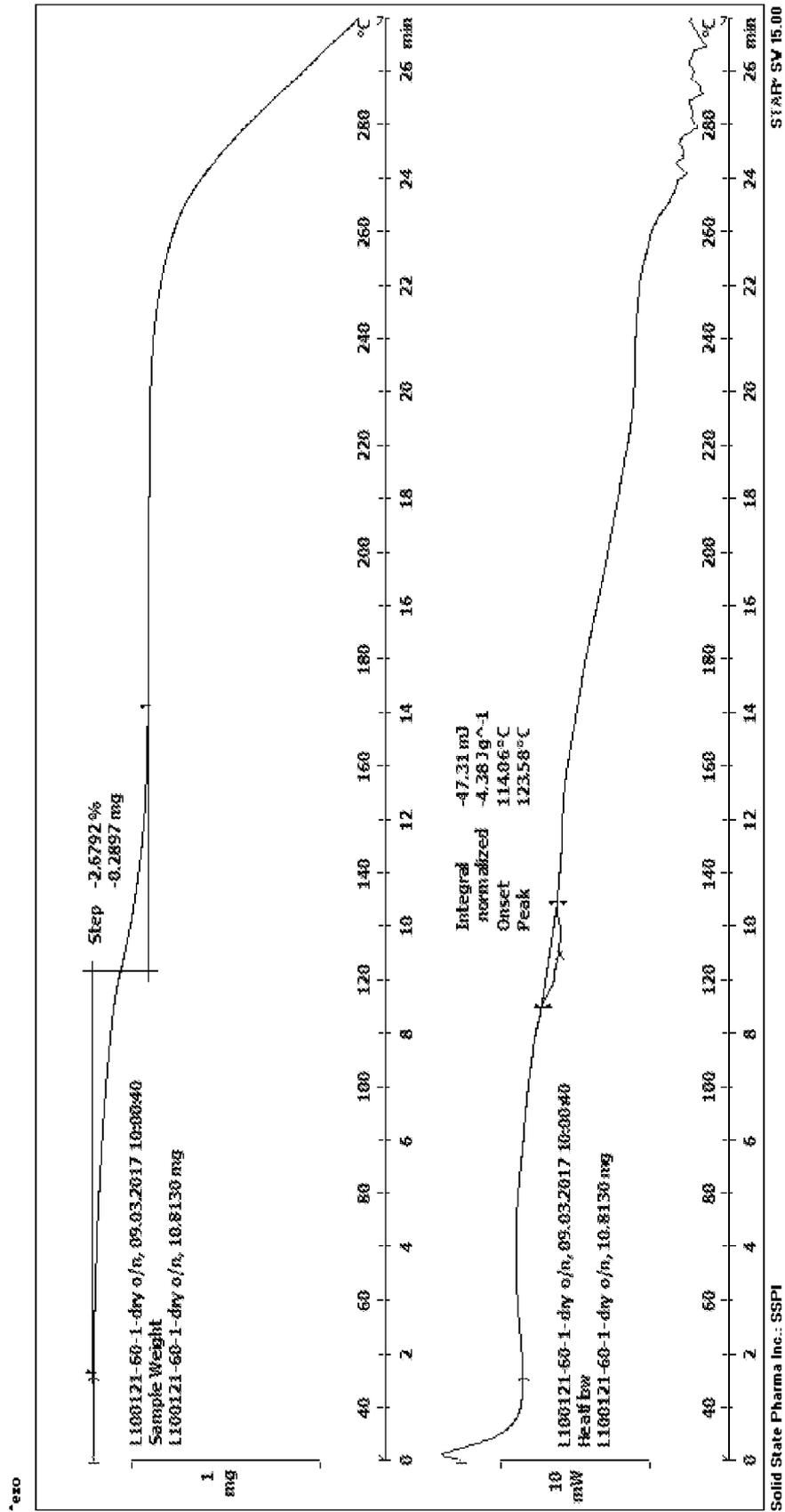


FIG. 22

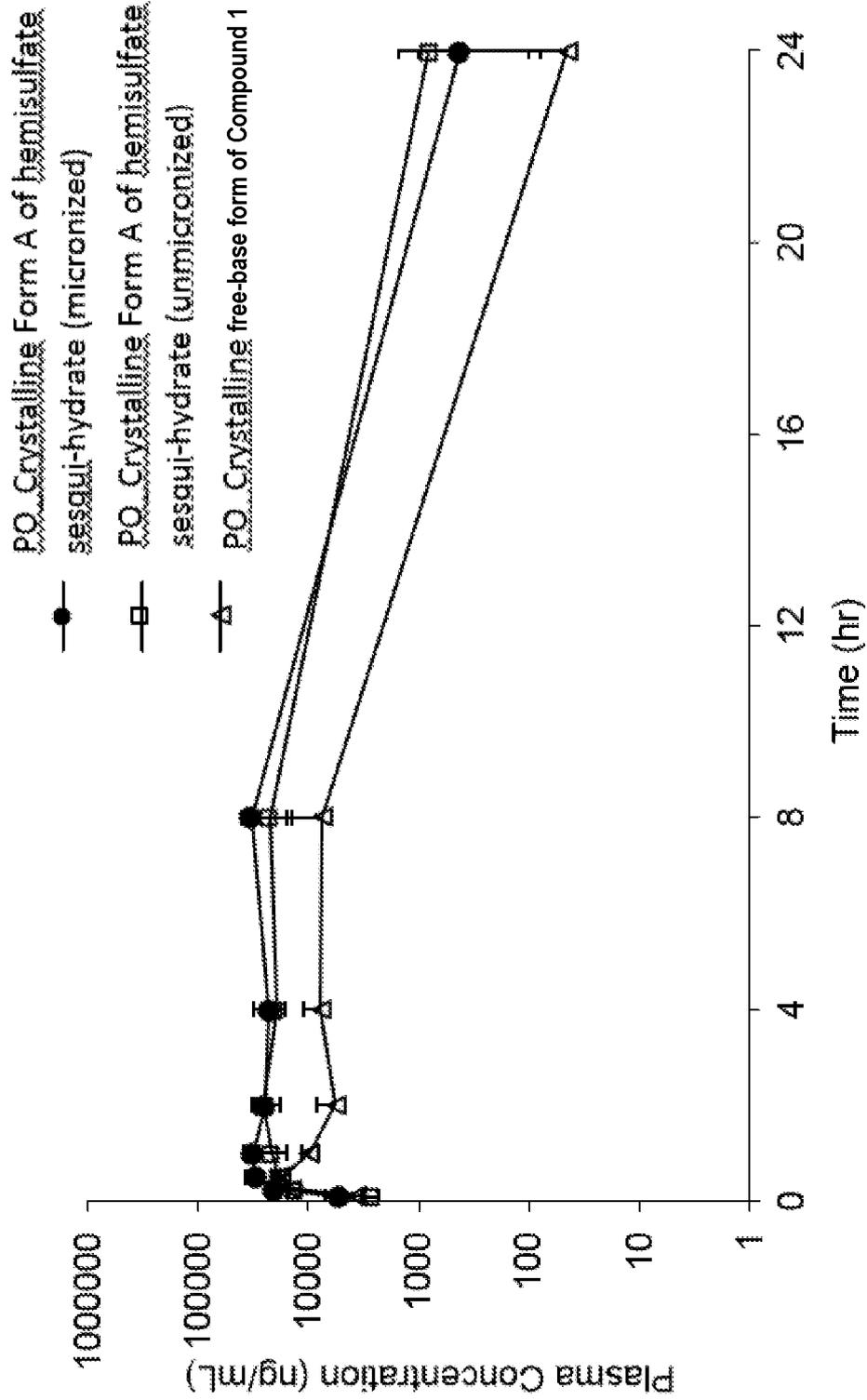


FIG. 23

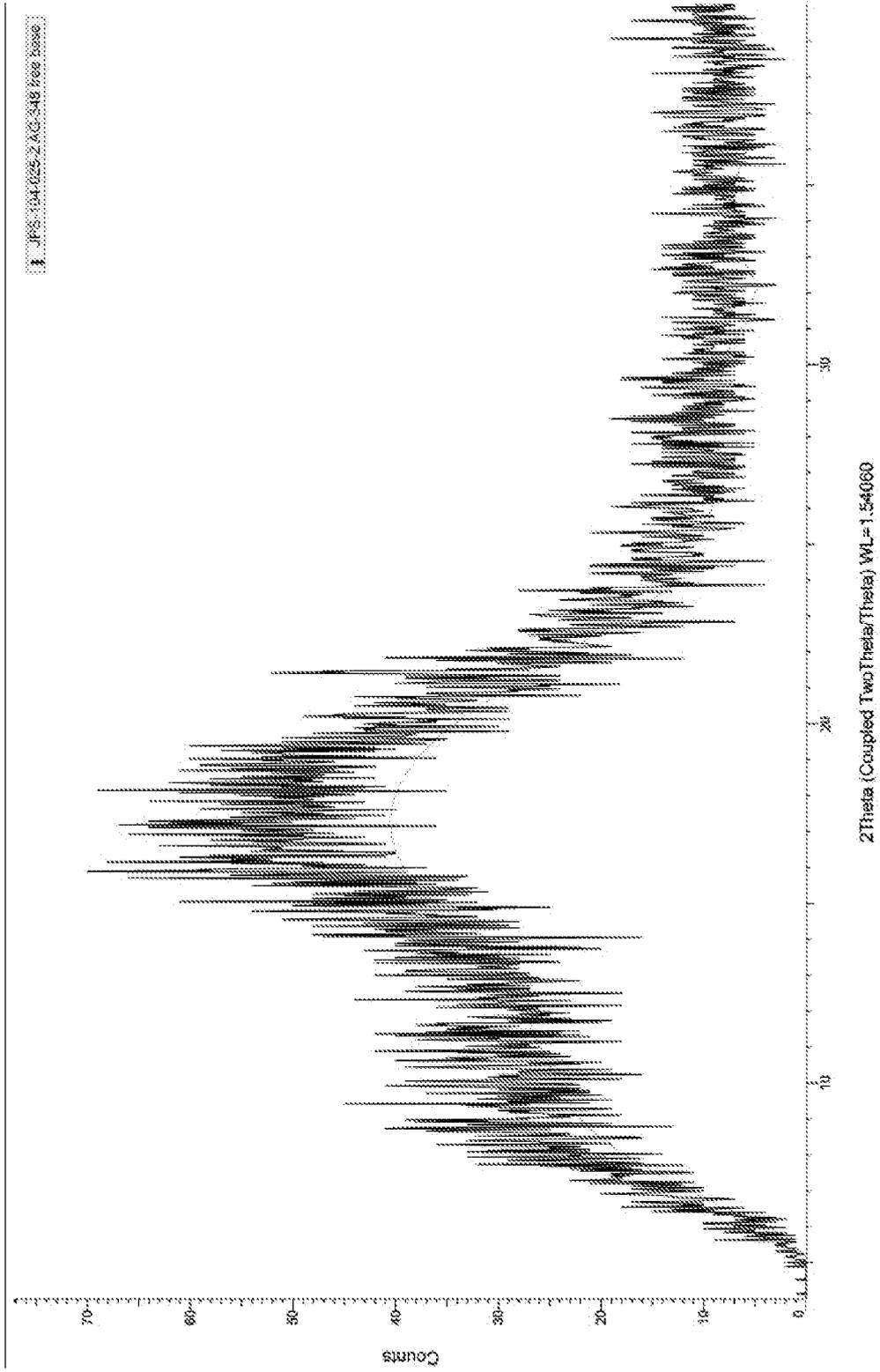


FIG. 24

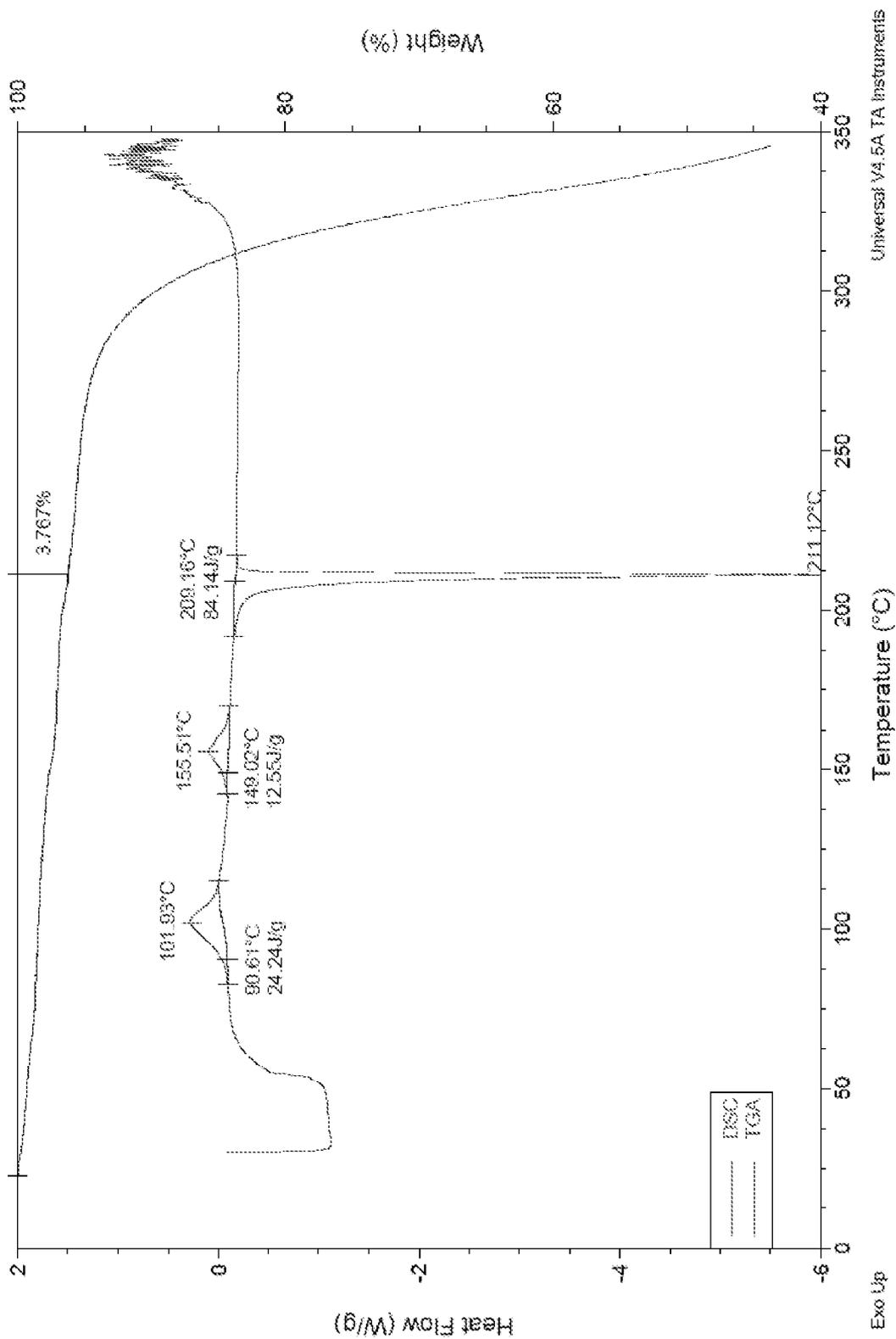
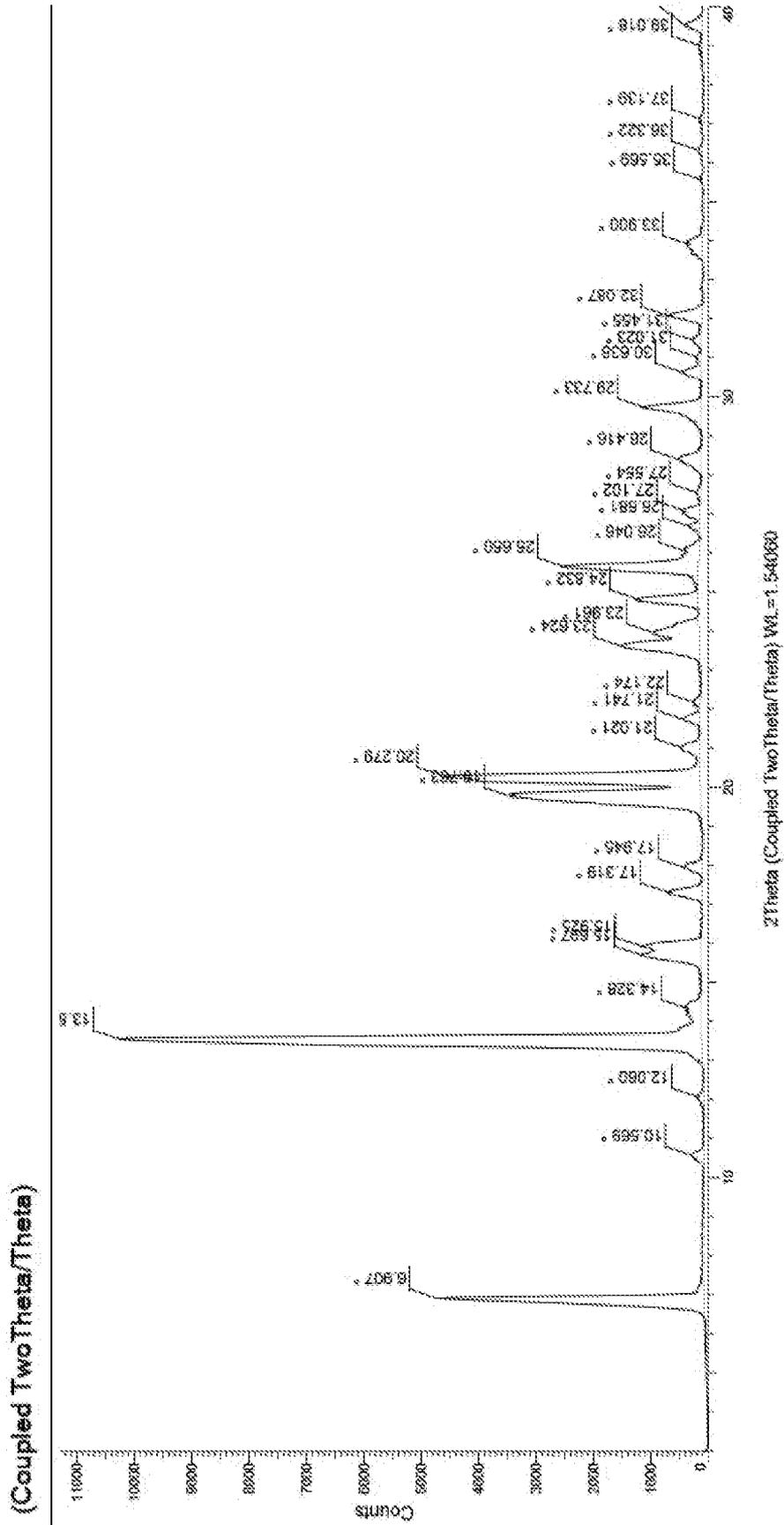


FIG. 25



**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/US2018/062197

A. CLASSIFICATION OF SUBJECT MATTER  
 INV. C07D215/36 A61P7/06 A61K31/4709  
 ADD.  
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED  
 Minimum documentation searched (classification system followed by classification symbols)  
 C07D A61P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 EPO-Internal, CHEM ABS Data, WPI Data, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/201227 A1 (AGIOS PHARMACEUTICALS INC [US]) 15 December 2016 (2016-12-15) cited in the application Claims 1, 17, 22, 38; page 35, example 1 with reference to US 8,785,450. -----	1-42
X	US 8 785 450 B2 (AGIOS PHARMACEUTICALS, INC.) 22 July 2014 (2014-07-22) Claims 1, 24, 30, 51; column 172, lines 55-67 and column 175, lines 6-25: compound VIII-8 = present compound 1. ----- -/--	1-42

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>
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Date of the actual completion of the international search  29 January 2019	Date of mailing of the international search report  20/02/2019
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Weisbrod, Thomas
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## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2018/062197

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; 5 December 2017 (2017-12-05), XP002788333, Database accession no. 2151847-10-6 abstract	1-42
T	----- DATABASE IMSRESEARCH [Online] IQVIA, LONDON, UK; 13 December 2018 (2018-12-13), XP002788334, Database accession no. 2014:8 abstract	1-42
L	----- BERGE ET AL.: "PHARMACEUTICALS SALTS", JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 66, no. 1, 1 January 1977 (1977-01-01), pages 1-19, XP000562636, ISSN: 0022-3549, DOI: 10.1002/JPS.2600660104 Page 1, paragraph bridging left and right columns; page 2, table 1.	1-20, 23-42
L	----- STAHL ET AL.: "Pharmaceutical Salts: Properties, Selections, and Use, passages", 1 January 2002 (2002-01-01), HANDBOOK OF PHARMACEUTICAL SALTS : PROPERTIES, SELECTION, AND USE, ZÜRICH : VERL. HELVETICA CHIMICA ACTA ; WEINHEIM [U.A.] : WILEY-VCH, DE, PAGE(S) 212-214,254,305, XP002550486, ISBN: 978-3-906390-26-0 pages 212-214, 254, 305-306, Page 212, section 8.1.2; page 306: sulfuric acid. Cited as common general knowledge.	1-20, 23-42
L	----- BYRN ET AL.: "Pharmaceutical Solids: A Strategic Approach to Regulatory Considerations", PHARMACEUTICAL RESEARCH, vol. 12, no. 7, 1 January 1995 (1995-01-01), pages 945-954, XP055531015, DOI: 10.1023/A:1016241927429 Abstract; page 945, lwft column paragraph 1 to right column, paragraph 1; page 946, figure 1 and right column, paragraphs 2-3; page 949, figure 6 and left column, paragraph 2; page 952, figure 11 and left column, paragraph 2. Cited as common general knowledge.	1-26
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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2018/062197

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
L	CAIRA: "Crystalline Polymorphism of Organic Compounds", TOPICS IN CURRENT CHEMISTRY, vol. 198, 1 January 1998 (1998-01-01), pages 163-208, XP008166276, ISSN: 0340-1022 Paragraph bridging pages 165 and 166. Cited as common general knowledge. -----	1-26
L	HARWOOD; MOODY: "Experimental organic chemistry - Principles and practice", 1 January 1989 (1989-01-01), Blackwell Science, XP003025361, ISBN: 978-0-632-02016-4 pages 127-132, Pages 127-132, chapter: "Crystallization". Cited as common general knowledge. -----	1-24,42
L	"Chapter 11, Tools for Purifying the Product: Column Chromatography, Crystallization and Reslurrying" In: Anderson: "Practical Process Research & Development", 1 January 2000 (2000-01-01), Academic Press, San Diego, XP002565895, ISBN: 978-0-12-059475-7 pages 223-224, Paragraph bridging pages 223-224. Cited as common general knowledge. -----	1-24,42
L	HANCOCK; ZOGRAFI: "Characteristics and Significance of the Amorphous State in Pharmaceutical Systems", JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 86, no. 1, 1 January 1997 (1997-01-01), pages 1-12, XP055274556, DOI: 10.1021/js9601896 Page 1, abstract; page 1, left column and figure 1. Cited as common general knowledge. -----	25-40

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2018/062197

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-42

relating to the subject-matter of claims 1-42

1.1. claims: 1-20, 25, 26, 41, 42(completely); 23, 24, 27-40(partially)

relating to a crystalline form or amorphous form of  
N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide x 1/2 H<sub>2</sub>SO<sub>4</sub> (= compound 1 x 1/2 H<sub>2</sub>SO<sub>4</sub>)  
as shown in claims 1 and 25.

1.2. claims: 21, 22(completely); 23, 24, 27-40(partially)

relating to a crystalline free base of compound 1 as shown in claim 21.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2018/062197

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