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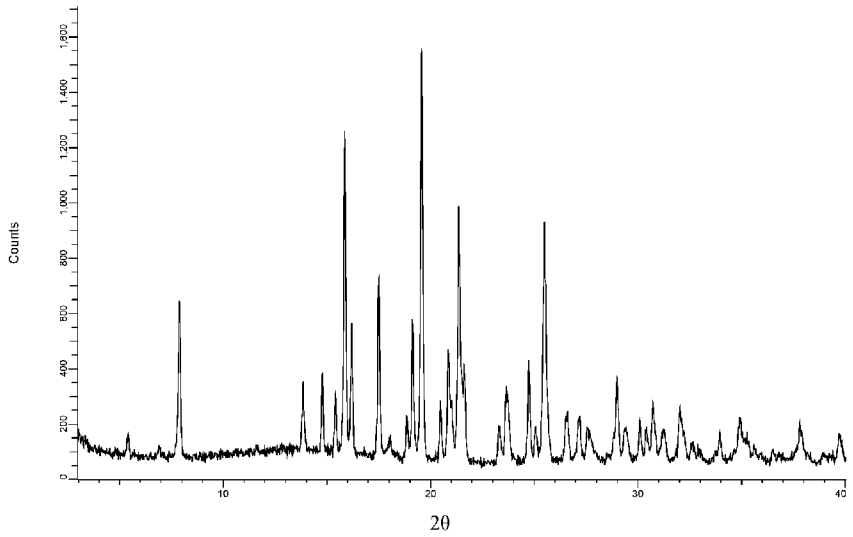

Figure 2

(57) Abstract: Provided herein are a fused heterocycle derivative compound, a crystal form and a hydrate and thereof, a pharmaceutical composition comprising these compounds and their use in the treatment of diseases associated with HBV infection.

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[Continued on next page]

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A crystal form of a fused heterocycle derivative compound

FIELD

The application relates to a crystal form and a hydrate of a fused heterocycle derivative compound, a process for preparing the same and their application in the treatment of diseases associated with HBV infection.

BACKGROUND

Chronic hepatitis B virus (HBV) infection is a significant global health problem, affecting over 5% of the world population (over 350 million people worldwide and 1.25 million individuals in the U.S.).

Despite the availability of a prophylactic HBV vaccine, the burden of chronic HBV infection continues to be a significant unmet worldwide medical problem, due to suboptimal treatment options and sustained rates of new infections in most parts of the developing world. Current treatments do not provide a cure and are limited to only two classes of agents (interferon alpha and nucleoside analogues/inhibitors of the viral polymerase); drug resistance, low efficacy, and tolerability issues limit their impact. The low cure rates of HBV are attributed at least in part to the fact that complete suppression of virus production is difficult to achieve with a single antiviral agent. However, persistent suppression of HBV DNA slows liver disease progression and helps to prevent hepatocellular carcinoma. Current therapy goals for HBV-infected patients are directed to reducing serum HBV DNA to low or undetectable levels, and to ultimately reducing or preventing the development of cirrhosis and hepatocellular carcinoma.

The HBV capsid protein plays essential functions during the viral life cycle. HBV capsid/core proteins form metastable viral particles or protein shells that protect the viral genome during intercellular passage, and also play a central role in viral replication processes, including genome encapsidation, genome replication, and virion morphogenesis and egress. Capsid structures also respond to environmental cues to allow un-coating after viral entry. Consistently, the appropriate timing of capsid assembly and dis-assembly, the appropriate capsid stability and the function of core protein have been found to be critical for viral infectivity.

The crucial function of HBV capsid proteins imposes stringent evolutionary constraints on the viral capsid protein sequence, leading to the observed low sequence variability and high conservation. Consistently, mutations in HBV capsid that disrupt its assembly are lethal, and mutations that perturb capsid stability severely attenuate viral replication. The high functional constraints on the multi-functional HBV core/capsid protein is consistent with a high sequence conservation, as many mutations are deleterious to function. Indeed, the core/capsid protein

sequences are >90% identical across HBV genotypes and show only a small number of polymorphic residues. Resistance selection to HBV core/capsid protein binding compounds may therefore be difficult to select without large impacts on virus replication fitness.

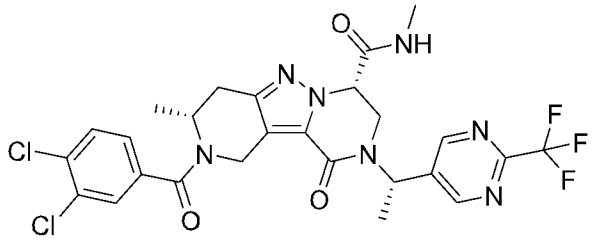
Reports describing compounds that bind viral capsids and inhibit replication of HIV, rhinovirus and HBV provide strong pharmacological proof of concept for viral capsid proteins as antiviral drug targets.

There is a need in the art for therapeutic agents and applicable forms thereof that can increase the suppression of virus production and that can treat, ameliorate, and/or prevent HBV infection.

SUMMARY

The present disclosure is directed to the general and preferred embodiments defined, respectively, by the independent and dependent claims appended hereto, which are incorporated by reference herein.

In an aspect, the present disclosure is directed to a crystal form of the compound of Formula (I)



(I).

In a further embodiment, the crystal form includes Crystal Form C.

In an embodiment, the Crystal Form C according to the present disclosure has an X-ray powder diffraction (XRPD) pattern comprising diffraction peaks at 7.9±0.2°, 14.8± 0.2°, 15.8±0.2°, 17.5± 0.2°, and 19.6± 0.2° (2θ).

In another aspect, the present disclosure is further directed to a hydrate of the compound of Formula (I).

The present disclosure is also directed to pharmaceutical compositions comprising the crystal form or hydrate according to the present disclosure or a combination thereof. The pharmaceutical compositions may further comprise one or more pharmaceutically acceptable excipients or one or more other agents or therapeutics.

The present disclosure is also directed to methods of using or uses of the crystal form or hydrate. In embodiments, the crystal form or hydrate are used to treat or ameliorate hepatitis B viral (HBV) infection, increase the suppression of HBV production, interfere with HBV capsid assembly or other HBV viral replication steps or products thereof. The methods comprise administering to a subject in need of such method an effective amount of the hydrate or the crystal form according to the present disclosure. Additional embodiments of methods of

treatment are set forth in the detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A and 1B show XRPD patterns of the amorphous forms of compound (I).

Figure 2 shows XRPD pattern of the crystal form C of an embodiment according to the present disclosure.

Figure 3 shows TGA pattern of the crystal form C of an embodiment according to the present disclosure.

Figure 4 shows XRPD pattern of the crystal form C of an embodiment according to the present disclosure.

Figure 5 shows DSC pattern of the crystal form C of an embodiment according to the present disclosure.

Figure 6 shows TGA pattern of the crystal form C of an embodiment according to the present disclosure.

Figure 7 shows XRPD pattern of the crystal form C of an embodiment according to the present disclosure.

Figure 8 shows DSC pattern of the crystal form C of an embodiment according to the present disclosure.

Figure 9 shows TGA pattern of the crystal form C of an embodiment according to the present disclosure.

Figure 10 shows results of the DVS test.

Figure 11 shows XRPD patterns in the DVS test.

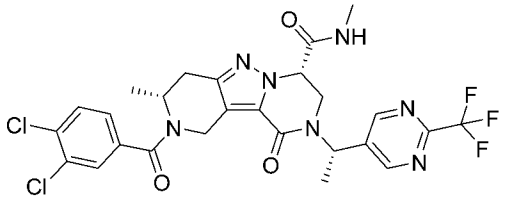
Figure 12 shows XRPD patterns in the VH test.

DETAILED DESCRIPTION

Additional embodiments, features, and advantages of the subject matter of the present disclosure will be apparent from the following detailed description of such disclosure and through its practice. For the sake of brevity, the publications, including patents, cited in this specification are herein incorporated by reference.

Crystal Form of the present disclosure

In another aspect, provided is a crystal form of compound (I):



(I)

In an embodiment, the crystal form of compound (I) according to the present disclosure comprises Crystal Form C.

The crystal form may be characterized by an X-ray powder diffraction (XRPD) pattern.

In an embodiment, the Crystal Form C according to the present disclosure has an X-ray powder diffraction (XRPD) pattern comprising diffraction peaks at 7.9±0.2°, 14.8± 0.2°, 15.8±0.2°, 17.5± 0.2°, and 19.6± 0.2° (2θ).

In a further embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern, which further comprises at least one diffraction peak of 5.4± 0.2°, 13.8± 0.2°, 15.4± 0.2°, 19.1± 0.2°, 20.5± 0.2°, and 24.7± 0.2° (2θ).

In yet another embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern, which further comprises two, three, four, five, or six diffraction peaks of 5.4± 0.2°, 13.8± 0.2°, 15.4± 0.2°, 19.1± 0.2°, 20.5± 0.2°, and 24.7± 0.2° (2θ); and may particularly further comprises diffraction peaks at 5.4± 0.2°, 13.8± 0.2°, 15.4± 0.2°, 19.1± 0.2°, 20.5± 0.2°, and 24.7± 0.2° (2θ).

In a preferable embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern, which further comprises at least one diffraction peak of 6.9± 0.2°, 16.2± 0.2°, 18.9± 0.2°, 21.4± 0.2°, 23.7± 0.2°, and 26.6± 0.2° (2θ).

In yet another embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern, which further comprises two, three, four, five, or six diffraction peaks of 6.9± 0.2°, 16.2± 0.2°, 18.9± 0.2°, 21.4± 0.2°, 23.7± 0.2°, and 26.6± 0.2° (2θ); and may particularly further comprises diffraction peaks at 6.9± 0.2°, 16.2± 0.2°, 18.9± 0.2°, 21.4± 0.2°, 23.7± 0.2°, and 26.6± 0.2° (2θ).

In a specific embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern comprises comprising diffraction peaks at 7.9±0.2°, 14.8± 0.2°, 15.8±0.2°, 17.5± 0.2°, and 19.6± 0.2° (2θ); and at least one diffraction peak of 5.4± 0.2°, 13.8± 0.2°, 15.4± 0.2°, 19.1± 0.2°, 20.5± 0.2°, and 24.7± 0.2° (2θ).

In another specific embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern comprises comprising diffraction peaks at 7.9±0.2°, 14.8± 0.2°, 15.8±0.2°, 17.5± 0.2°, and 19.6± 0.2° (2θ); and at least one diffraction peak of 6.9± 0.2°, 16.2± 0.2°, 18.9± 0.2°, 21.4± 0.2°, 23.7± 0.2°, and 26.6± 0.2° (2θ).

In a more specific embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern comprises comprising diffraction peaks at 7.9±0.2°, 14.8± 0.2°, 15.8±0.2°, 17.5± 0.2°, and 19.6± 0.2° (2θ); and at least one diffraction peak of 5.4± 0.2°, 13.8± 0.2°, 15.4± 0.2°, 19.1± 0.2°, 20.5± 0.2°, and 24.7± 0.2° (2θ); and at least one diffraction peak of 6.9± 0.2°, 16.2± 0.2°, 18.9± 0.2°, 21.4± 0.2°, 23.7± 0.2°, and 26.6± 0.2° (2θ).

In an alternative embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern, which may further comprise at least one diffraction peak of 20.8± 0.2°, 21.6± 0.2°, 25.5± 0.2°, 27.2± 0.2°, 29.0± 0.2°, 30.7± 0.2°, 32.1± 0.2°, and 34.9± 0.2° (2θ).

The Crystal Form C may be characterized by an X-ray powder diffraction (XRPD) pattern comprising those peaks identified in Table 1, wherein the relative intensity of the peaks is greater than about 2%, preferably greater than about 5%, more preferably greater than about 10%, more preferably greater than about 15%. Those skilled in the art will know that the relative intensity of the peaks may vary between different samples and different measurements on the same sample while it is more important to note the relative positions of peaks rather than their relative intensities.

Accordingly, in a specific embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern comprising at least one diffraction peak of those listed in Table 1, or comprising the diffraction peaks listed in Table 1.

Table 1 Peak listing and relative intensity for the XRPD of Crystal Form C

No.	Pos. (° 2θ)	Rel. Int. (%)	No.	Pos. (° 2θ)	Rel. Int. (%)
1	5.4	5.4	20	24.7	23.7
2	6.9	2.8	21	25.1	6.4
3	7.9	39.0	22	25.5	58.0
4	13.8	16.7	23	26.6	11.4
5	14.8	19.2	24	27.2	10.2
6	15.4	14.3	25	27.6	5.0
7	15.8	77.0	26	29.0	21.0
8	16.2	33.0	27	29.4	7.0
9	17.5	45.3	28	30.1	9.3
10	18.1	3.8	29	30.4	6.0
11	18.9	10.9	30	30.7	13.8
12	19.1	35.0	31	31.2	6.9
13	19.6	100.0	32	32.1	12.5
14	20.5	14.0	33	32.7	4.9
15	20.8	25.2	34	33.1	5.6
16	21.4	64.1	35	34.0	7.8
17	21.6	24.7	36	34.9	10.5
18	23.3	9.3	37	35.2	6.9
19	23.7	18.8	38	37.8	10.3

In a more preferable embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern as substantially shown in Figure 2. In another preferable embodiment, the Crystal Form C has an X-ray powder diffraction (XRPD) pattern as shown in Figure 2.

Those skilled in the art will know that the intensities of the peaks depicted in the Figure(s) and table(s) herein as well as those specified above are not a prerequisite but may vary.

The crystal form according to the present disclosure may also be characterized by Differential Scanning Calorimetric (DSC), Thermal Gravimetric Analysis (TGA) or other conventional technical means in the art. It should be noted that the specific DSC pattern and/or TGA pattern may vary with the batches or testing conditions and thus such patterns are provided for exemplary purpose.

In an embodiment, the TGA pattern shows about 2.1% weight loss at about 160°C±2°C, e.g., as shown in Figure 3.

In another exemplary embodiment, the DSC pattern of crystal form has a dehydration peak at T_{onset} of about 16.4°C, and a melting peak at T_{onset} of about 173.7°C, e.g., as shown in Figure 5. In another exemplary embodiment, the TGA pattern shows about 2.2% weight loss at about 150°C±2°C, e.g., as shown in Figure 6.

In still another exemplary embodiment, the DSC pattern of crystal form has a dehydration peak at T_{onset} of about 16.2°C, and a melting peak at T_{onset} of about 171.9°C, e.g., as shown in shown in Figure 8. In still another exemplary embodiment, the TGA pattern shows about 2.0% weight loss at about 150°C±2°C, e.g., as shown in shown in Figure 9.

In an embodiment, the crystal form C according to the present disclosure is hygroscopic with the water content varying with relative humidity. For example, during the preparation of the crystal form, when the obtained crystal form is obtained and/or dried under ambient condition, the obtained solid may comprise various water contents (such as 0.1-1.8, e.g., 0.7-1.7 molar equivalents) but the obtained product will still present in a stable crystal form with (substantially) consistent XRPD patterns.

Accordingly, in an embodiment, the crystal form C according to the present disclosure may comprise 0.1-1.8 molar equivalents of water (or the water content is 0.1 – 1.8 molar equivalents). For example 0.1-1.8, 0.7-1.7, 0.8-1.3, such as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8 molar equivalents of water, as well as any range constituted by any two values thereof, and particularly, 0.7, 0.8, 1.1, 1.2, 1.3, 1.6, 1.7 molar equivalents of water etc. The water content may be determined with Karl Fischer (KF) method or in DVS test as described herein.

Under different RH conditions, the crystal form C may have variable water content or water content range but it will still present in a stable crystal form with (substantially) consistent XRPD patterns. The crystal form C can dehydrate completely at 0% RH, that is it may exist in anhydrous form.

In a specific embodiment, the crystal form C according to the present disclosure has a water content of 0.8 to 1.3 equivalents of water at RH of 40% to 80%. The content may be determined at room temperature, e.g., 25°C. The water content may be determined with Karl Fischer (KF) method or in DVS test as described herein.

Accordingly, depending on the conditions, the crystal form C according to the present disclosure may comprise or may not comprise water. Alternatively, it may exist in anhydrous

form or hydrated form. The term "hydrate" as used herein is intended to describe a molecular complex comprising the compound (I) and solvent molecules as water. In general, the hydrated forms and anhydrate forms and are intended to be encompassed within the scope of the present invention.

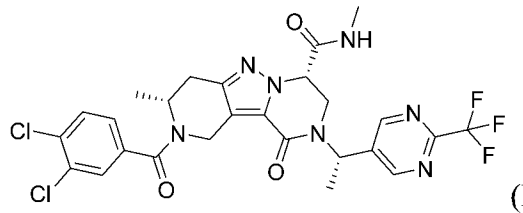
Although the hydrate phase typically contains a range of water, for example, as those listed above, it may also occur with lower or higher water content. Water uptake is reversible if humidity in the environment is raised again. The water content in the isolated product may depend on the drying conditions used during work up of the hydrate after crystallization or ambient condition.

In an embodiment, the water content in the crystal form according to the present disclosure comprises channel water (channel hydrate). In a channel hydrate, the water molecules may lie in lattice channels where they are next to other water molecules. When the water is relatively weakly bound, as in channel water, the water content may be dependent on humidity and drying conditions. In such cases, non-stoichiometry may be observed. Although the water content may be non-stoichiometry, the characteristic XRPD patterns remain substantially the same among various embodiments.

Accordingly, in an embodiment, the crystal form C according to the present disclosure may be a hydrate. In a further embodiment, the hydrate is a channel hydrate.

For example, the crystal form C may be hygroscopic with the water content varying with relative humidity and could dehydrate completely at 0% RH. Alternatively, the crystal form at equilibrium may contain 0.8 to 1.3 molar equivalents of water at RH of 40% to 80%. The content may be determined at room temperature, e.g., 25°C. The water content may be determined with Karl Fischer (KF) method or in DVS test as described herein.

In another aspect, provided is also a hydrate of compound (I):



(I).

In an embodiment, the hydrate comprises 0.1 – 1.8 molar equivalents of water. For example, 0.5-1.8, 0.7-1.7, 0.8-1.3, such as 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8 molar equivalents of water, as well as any range constituted by any two values thereof, and particularly, 0.7, 0.8, 1.1, 1.2, 1.3, 1.6, 1.7 molar equivalents of water etc. In an embodiment, the water content in hydrate comprises channel water (channel hydrate). In a further embodiment, the hydrate is a channel hydrate.

In a specific embodiment, the hydrate according to the present disclosure has a water content of 0.8 to 1.3 equivalents of water at RH of 40% to 80%. The content may be determined at room temperature like 25°C.

Process for preparing Crystal Form

Provided is also a process for preparing the crystal form C according to the present disclosure, comprising:

dissolving a compound of formula (I) in a crystallizing solvent with stirring,

wherein the crystallizing solvent comprises water, aqueous solution, organic solvent or a combination thereof;

allowing crystallization of the compound; and

recovering and drying the obtained product to give the crystal form C.

The compound of formula (I) used in the process may in any form. In an embodiment, the compound of formula (I) is amorphous form. In another embodiment, the compound (I) is a crystal form, for example a crystal form C according to the present disclosure.

In another embodiment of the process, a seed is added to promote the crystallization. The seed used may be a crystal form according to the present disclosure.

In an embodiment, the aqueous solution may comprise a solvent miscible with water. Examples comprise but not limited to C_{1-6} alcohol (such as C_{1-4} alcohol, e.g., ethanol), organic or inorganic acid or base (e.g., HCl, NaOH), dioxane (e.g., 1,4-dioxane), or a combination thereof.

In an embodiment, the organic solvent may be miscible or immiscible with water. In a specific embodiment, the organic solvent comprises C_{1-6} alcohol (e.g., ethanol), dioxane (e.g., 1,4-dioxane), C_{1-6} alkyl acetate (e.g., ethyl acetate), C_{5-8} alkane (e.g., heptane), or a combination thereof.

In the preparing process, the dissolution and/or stirring may be conducted at ambient condition, for example, being open to the atmospheric condition. The dissolution and/or stirring may be conducted under room temperature, e.g., about 25°C.

The stirring may be conducted with conventional means in the art, for example with a stirring bar. The stirring rate may be about 10-600 rpm, preferably about 100-500 rpm, more preferably about 300-400 rpm.

The stirring time may be hours to days, for example, about 1-24 hours e.g., about 4-20 hours, or about 10-14 hours, or about 1-14 days, e.g., about 1-8 days, or about 1-5 days.

The crystallization may occur during the dissolution/stirring process. For example, a suspension may be obtained when compound (I) is dissolved with stirring, which may then be collected for drying. The system may also optionally cooled or allowed to stand for crystallization. Alternatively, an antisolvent may be added for precipitation and the precipitated solids are then collected for drying.

The drying may be conducted under ambient condition. The drying time may be determined according to practical requirements and may be hours, for example about 1-24 hours, e.g., about 6-12 hours.

Pharmaceutical Compositions

Also disclosed herein are pharmaceutical compositions comprising the crystal form or hydrate, in any one of the embodiments defined above, and

at least one pharmaceutically acceptable excipient.

In embodiments, the pharmaceutical composition comprises at least one additional active or therapeutic agent. Additional active therapeutic agents may include, for example, an anti-HBV agent such as an HBV polymerase inhibitor, interferon, viral entry inhibitor, viral maturation inhibitor, capsid assembly modulator, reverse transcriptase inhibitor, immunomodulatory agent such as a TLR-agonist, or any other agents that affect the HBV life cycle and/or the consequences of HBV infection. The active agents of the present disclosure are used, alone or in combination with one or more additional active agents, to formulate pharmaceutical compositions of the present disclosure.

As used herein, the term "composition" or "pharmaceutical composition" refers to a mixture of at least one compound useful within the present disclosure with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a patient or subject. Multiple techniques of administering a compound exist in the art including, but not limited to, intravenous, oral, aerosol, parenteral, ophthalmic, pulmonary and topical administration.

As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the present disclosure within or to the patient such that it may perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, including the compound useful within the present disclosure, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound useful within the present disclosure and are physiologically acceptable to the patient. Supplementary active compounds may also be incorporated into the compositions. Other additional ingredients that may be included in the pharmaceutical compositions used in the practice of the present disclosure are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

A "pharmaceutically acceptable excipient" refers to a substance that is non-toxic, biologically tolerable, and otherwise biologically suitable for administration to a subject, such as an inert substance, added to a pharmacological composition or otherwise used as a vehicle, carrier, or diluent to facilitate administration of an agent and that is compatible therewith. Examples of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils, and polyethylene glycols.

Delivery forms of the pharmaceutical compositions containing one or more dosage units of the active agents may be prepared using suitable pharmaceutical excipients and compounding techniques known or that become available to those skilled in the art. The compositions may be administered in the inventive methods by a suitable route of delivery, e.g., oral, parenteral, rectal, topical, or ocular routes, or by inhalation.

The preparation may be in the form of tablets, capsules, sachets, dragees, powders, granules, lozenges, powders for reconstitution, liquid preparations, or suppositories. Preferably, the compositions are formulated for intravenous infusion, topical administration, or oral administration.

For oral administration, the compounds of the present disclosure can be provided in the form of tablets or capsules, or as a solution, emulsion, or suspension. To prepare the oral compositions, the compounds may be formulated to yield a dosage of, e.g., from about 0.05 to about 100 mg/kg daily, or from about 0.05 to about 35 mg/kg daily, or from about 0.1 to about 10 mg/kg daily. For example, a total daily dosage of about 5 mg to 5 g daily may be accomplished by dosing once, twice, three, or four times per day.

Oral tablets may include a compound according to the present disclosure mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservative agents. Suitable inert fillers include sodium and calcium carbonate, sodium and calcium phosphate, lactose, starch, sugar, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol, and the like. Exemplary liquid oral excipients include ethanol, glycerol, water, and the like. Starch, polyvinyl-pyrrolidone (PVP), sodium starch glycolate, microcrystalline cellulose, and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin. The lubricating agent, if present, may be magnesium stearate,

stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate to delay absorption in the gastrointestinal tract or may be coated with an enteric coating.

Capsules for oral administration include hard and soft gelatin capsules. To prepare hard gelatin capsules, compounds of the present disclosure may be mixed with a solid, semi-solid, or liquid diluent. Soft gelatin capsules may be prepared by mixing the compound of the present disclosure with water, an oil such as peanut oil or olive oil, liquid paraffin, a mixture of mono and di-glycerides of short chain fatty acids, polyethylene glycol 400, or propylene glycol.

Liquids for oral administration may be in the form of suspensions, solutions, emulsions or syrups or may be lyophilized or presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid compositions may optionally contain: pharmaceutically-acceptable excipients such as suspending agents (for example, sorbitol, methyl cellulose, sodium alginate, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminum stearate gel and the like); non-aqueous vehicles, e.g., oil (for example, almond oil or fractionated coconut oil), propylene glycol, ethyl alcohol, or water; preservatives (for example, methyl or propyl p-hydroxybenzoate or sorbic acid); wetting agents such as lecithin; and, if desired, flavoring or coloring agents.

The compound of this present disclosure may also be administered by non-oral routes. For example, the compositions may be formulated for rectal administration as a suppository. For parenteral use, including intravenous, intramuscular, intraperitoneal, or subcutaneous routes, the compounds of the present disclosure may be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity or in parenterally acceptable oil. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride. Such forms will be presented in unit-dose form such as ampules or disposable injection devices, in multi-dose forms such as vials from which the appropriate dose may be withdrawn, or in a solid form or pre-concentrate that can be used to prepare an injectable formulation. Illustrative infusion doses may range from about 1 to 1000 μg/kg/minute of compound, admixed with a pharmaceutical carrier over a period ranging from several minutes to several days.

For topical administration, the compounds may be mixed with a pharmaceutical carrier at a concentration of about 0.1% to about 10% of drug to vehicle. Another mode of administering the compounds of the present disclosure may utilize a patch formulation to affect transdermal delivery.

Compounds of the present disclosure may alternatively be administered in methods of this present disclosure by inhalation, via the nasal or oral routes, e.g., in a spray formulation also containing a suitable carrier.

Methods of Use

The disclosed compounds are useful in the prevention or treatment of an HBV infection

or of an HBV-induced disease in mammal in need thereof, more particularly in a human in need thereof.

In a non-limiting aspect, these compounds may (i) modulate or disrupt HBV assembly and other HBV core protein functions necessary for HBV replication or the generation of infectious particles, (ii) inhibit the production of infectious virus particles or infection, or (iii) interact with HBV capsid to effect defective viral particles with reduced infectivity or replication capacity acting as capsid assembly modulators. In particular, and without being bound to any particular mechanism of action, it is believed that the disclosed compounds are useful in HBV treatment by disrupting, accelerating, reducing, delaying and/or inhibiting normal viral capsid assembly and/or disassembly of immature or mature particles, thereby inducing aberrant capsid morphology leading to antiviral effects such as disruption of virion assembly and/or disassembly, virion maturation, virus egress and/or infection of target cells. The disclosed compounds may act as a disruptor of capsid assembly interacting with mature or immature viral capsid to perturb the stability of the capsid, thus affecting its assembly and/or disassembly. The disclosed compounds may perturb protein folding and/or salt bridges required for stability, function and/or normal morphology of the viral capsid, thereby disrupting and/or accelerating capsid assembly and/or disassembly. The disclosed compounds may bind capsid and alter metabolism of cellular polyproteins and precursors, leading to abnormal accumulation of protein monomers and/or oligomers and/or abnormal particles, which causes cellular toxicity and death of infected cells. The disclosed compounds may cause failure of the formation of capsids of optimal stability, affecting efficient uncoating and/or disassembly of viruses (e.g., during infectivity). The disclosed compounds may disrupt and/or accelerate capsid assembly and/or disassembly when the capsid protein is immature. The disclosed compounds may disrupt and/or accelerate capsid assembly and/or disassembly when the capsid protein is mature. The disclosed compounds may disrupt and/or accelerate capsid assembly and/or disassembly during viral infectivity which may further attenuate HBV viral infectivity and/or reduce viral load. The disruption, acceleration, inhibition, delay and/or reduction of capsid assembly and/or disassembly by the disclosed compounds may eradicate the virus from the host organism. Eradication of HBV from a subject by the disclosed compounds advantageously obviates the need for chronic long-term therapy and/or reduces the duration of long-term therapy.

An additional embodiment of the present disclosure is a method of treating a subject suffering from an HBV infection, comprising administering to a subject in need of such treatment an effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of reducing the viral load associated with an HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of reducing reoccurrence of an HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of a compound of Formula (I), or a pharmaceutically acceptable salt thereof.

Additionally, HBV acts as a helper virus to hepatitis delta virus (HDV), and it is estimated that more than 15 million people may be HBV/HDV co-infected worldwide, with an increased risk of rapid progression to cirrhosis and increased hepatic decompensation, than patients suffering from HBV alone (Hughes, S.A. et al. Lancet 2011, 378, 73-85). HDV, infects therefore subjects suffering from HBV infection. In a particular embodiment, the compounds of the present disclosure may be used in the treatment and/or prophylaxis of HBV/HDV co-infection, or diseases associated with HBV/HDV co infection. Therefore, in a particular embodiment, the HBV infection is in particular HBV/HDV co-infection, and the mammal, in particular the human, may be HBV/HDV co-infected, or be at risk of HBV/HDV co infection.

In another aspect, provided herein is a method of inhibiting or reducing the formation or presence of HBV DNA-containing particles or HBV RNA-containing particles in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of reducing an adverse physiological impact of an HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of inducing remission of hepatic injury from an HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of reducing the physiological impact of long-term antiviral therapy for HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of prophylactically treating an HBV infection in an individual in need thereof, wherein the individual is afflicted with a latent HBV infection, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In embodiments, the disclosed crystal form or hydrate are suitable for monotherapy. In embodiments, the disclosed compounds are effective against natural or native HBV strains. In embodiments, the disclosed crystal form or hydrate are effective against HBV strains resistant to currently known drugs.

In another embodiment, the crystal form or hydrate provided herein can be used in

methods of modulating (e.g., inhibiting or disrupting) the activity, stability, function, and viral replication properties of HBV cccDNA.

In yet another embodiment, the crystal form or hydrate provided herein can be used in methods of diminishing or preventing the formation of HBV cccDNA.

In another embodiment, the crystal form or hydrate provided herein can be used in methods of modulating (e.g., inhibiting or disrupting) the activity of HBV cccDNA.

In yet another embodiment, the crystal form or hydrate provided herein can be used in methods of diminishing the formation of HBV cccDNA.

In another embodiment, the crystal form or hydrate provided herein can be used in methods of modulating, inhibiting, or disrupting the generation or release of HBV RNA particles from within the infected cell.

In a further embodiment, the total burden (or concentration) of HBV RNA particles is modulated. In a preferred embodiment, the total burden of HBV RNA is diminished.

In another embodiment, the methods provided herein reduce the viral load in the individual to a greater extent or at a faster rate compared to the administering of a compound selected from the group consisting of an HBV polymerase inhibitor, interferon, viral entry inhibitor, viral maturation inhibitor, distinct capsid assembly modulator, antiviral compounds of distinct or unknown mechanism, and any combination thereof.

In another embodiment, the methods provided herein cause a lower incidence of viral mutation and/or viral resistance than the administering of a compound selected from the group consisting of an HBV polymerase inhibitor, interferon, viral entry inhibitor, viral maturation inhibitor, distinct capsid assembly modulator, antiviral compounds of distinct or unknown mechanism, and combination thereof.

In another embodiment, the methods provided herein further comprise administering to the individual at least one HBV vaccine, a nucleoside HBV inhibitor, an interferon or any combination thereof.

In an aspect, provided herein is a method of treating an HBV infection in an individual in need thereof, comprising reducing the HBV viral load by administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure, alone or in combination with a reverse transcriptase inhibitor; and further administering to the individual a therapeutically effective amount of HBV vaccine.

An additional embodiment of the present disclosure is a method of treating a subject suffering from an HBV infection, comprising administering to a subject in need of such treatment an effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of reducing the viral load associated with an HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of reducing reoccurrence of an HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of inhibiting or reducing the formation or presence of HBV DNA-containing particles or HBV RNA-containing particles in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of reducing an adverse physiological impact of an HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of inducing remission of hepatic injury from an HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of reducing the physiological impact of long-term antiviral therapy for HBV infection in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In another aspect, provided herein is a method of prophylactically treating an HBV infection in an individual in need thereof, wherein the individual is afflicted with a latent HBV infection, comprising administering to the individual a therapeutically effective amount of the crystal form or hydrate according to the present disclosure.

In an embodiment, the methods provided herein further comprise monitoring the HBV viral load of the subject, wherein the method is carried out for a period of time such that the HBV virus is undetectable.

Combinations

Provided herein are combinations of one or more of the disclosed crystal form or hydrate with at least one additional therapeutic agent. In embodiments, the methods provided herein can further comprise administering to the individual at least one additional therapeutic agent. In embodiments, the disclosed compounds are suitable for use in combination therapy. The crystal form or hydrate of the present disclosure may be useful in combination with one or more additional compounds useful for treating HBV infection. These additional compounds may comprise the crystal form or hydrate of the present disclosure or compounds known to treat, prevent, or reduce the symptoms or effects of HBV infection.

In an exemplary embodiment, additional active ingredients are those that are known or

discovered to be effective in the treatment of conditions or disorders involved in HBV infection, such as another HBV capsid assembly modulator or a compound active against another target associated with the particular condition or disorder involved in HBV infection, or the HBV infection itself. The combination may serve to increase efficacy (e.g., by including in the combination a compound potentiating the potency or effectiveness of an active agent according to the present disclosure), decrease one or more side effects, or decrease the required dose of the active agent according to the present disclosure. In a further embodiment, the methods provided herein allow for administering of the at least one additional therapeutic agent at a lower dose or frequency as compared to the administering of the at least one additional therapeutic agent alone that is required to achieve similar results in prophylactically treating an HBV infection in an individual in need thereof.

Such compounds include but are not limited to HBV combination drugs, HBV vaccines, HBV DNA polymerase inhibitors, immunomodulatory agents, toll-like receptor (TLR) modulators, interferon alpha receptor ligands, hyaluronidase inhibitors, hepatitis b surface antigen (HBsAg) inhibitors, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors, cyclophilin inhibitors, HBV viral entry inhibitors, antisense oligonucleotide targeting viral mRNA, short interfering RNAs (siRNA) and ddRNAi endonuclease modulators, ribonucleotide reductase inhibitors, HBV E antigen inhibitors, covalently closed circular DNA (cccDNA) inhibitors, famesoid X receptor agonists, HBV antibodies, CCR2 chemokine antagonists, thymosin agonists, cytokines, nucleoprotein modulators, retinoic acid-inducible gene 1 simulators, NOD2 stimulators, phosphatidylinositol 3-kinase (PI3K) inhibitors, indoleamine-2, 3-dioxygenase (IDO) pathway inhibitors, PD-1 inhibitors, PD-L1 inhibitors, recombinant thymosin alpha-1, bruton's tyrosine kinase (BTK) inhibitors, KDM inhibitors, HBV replication inhibitors, arginase inhibitors, and any other agent that affects the HBV life cycle and/or affect the consequences of HBV infection or combinations thereof.

In embodiments, the compounds of the present disclosure may be used in combination with an HBV polymerase inhibitor, immunomodulatory agents, interferon such as pegylated interferon, viral entry inhibitor, viral maturation inhibitor, capsid assembly modulator, reverse transcriptase inhibitor, a cyclophilin/TNF inhibitor, immunomodulatory agent such as a TLR-agonist, an HBV vaccine, and any other agent that affects the HBV life cycle and/or affect the consequences of HBV infection or combinations thereof. In particular, the compounds of the present disclosure may be used in combination with one or more agents (or a salt thereof) selected from the group consisting of

HBV reverse transcriptase inhibitors, and DNA and RNA polymerase inhibitors, including but not limited to: lamivudine (3TC, Zeffix, Heptovir, Epivir, and Epivir-HBV), entecavir (Baraclude, Entavir), adefovir dipivoxil (Hepsara, Preveon, bis-POM PMEA), tenofovir disoproxil fumarate (Viread, TDF or PMPA);

interferons, including but not limited to interferon alpha (IFN-α), interferon beta (IFN-

β), interferon lambda (IFN-λ), and interferon gamma (IFN-γ);

viral entry inhibitors;

viral maturation inhibitors;

literature-described capsid assembly modulators, such as, but not limited to BAY 41-4109;

reverse transcriptase inhibitor;

an immunomodulatory agent such as a TLR-agonist; and

agents of distinct or unknown mechanism, such as but not limited to AT-61 ((E)-N-(1-chloro-3-oxo-1-phenyl-3-(piperidin-1-yl)prop-1-en-2-yl)benzamide), AT-130 ((E)-N-(1-bromo-1-(2-methoxyphenyl)-3-oxo-3-(piperidin-1-yl)prop-1-en-2-yl)-4-nitrobenzamide), and similar analogs.

In embodiments, the additional therapeutic agent is an interferon. The term "interferon" or "IFN" refers to any member the family of highly homologous species-specific proteins that inhibit viral replication and cellular proliferation and modulate immune response. Human interferons are grouped into three classes; Type I, which include interferon-alpha (IFN-α), interferon-beta (IFN-β), and interferon-omega (IFN-ω), Type II, which includes interferon-gamma (IFN-γ), and Type III, which includes interferon-lambda (IFN-λ). Recombinant forms of interferons that have been developed and are commercially available are encompassed by the term "interferon" as used herein. Subtypes of interferons, such as chemically modified or mutated interferons, are also encompassed by the term "interferon" as used herein. Chemically modified interferons include pegylated interferons and glycosylated interferons. Examples of interferons also include, but are not limited to, interferon-alpha-2a, interferon-alpha-2b, interferon-alpha-n1, interferon-beta-1a, interferon-beta-1b, interferon-lamda-1, interferon-lamda-2, and interferon-lamda-3. Examples of pegylated interferons include pegylated interferon-alpha-2a and pegylated interferon alpha-2b.

Accordingly, in one embodiment, the compounds of Formula I, can be administered in combination with an interferon selected from the group consisting of interferon alpha (IFN-α), interferon beta (IFN-β), interferon lambda (IFN-λ), and interferon gamma (IFN-γ). In one specific embodiment, the interferon is interferon-alpha-2a, interferon-alpha-2b, or interferon-alpha-n1. In another specific embodiment, the interferon-alpha-2a or interferon-alpha-2b is pegylated. In a preferred embodiment, the interferon-alpha-2a is pegylated interferon-alpha-2a (PEGASYS).

In another embodiment, the additional therapeutic agent is selected from immune modulator or immune stimulator therapies, which includes biological agents belonging to the interferon class.

Further, the additional therapeutic agent may be an agent that disrupts the function of other essential viral protein(s) or host proteins required for HBV replication or persistence.

In another embodiment, the additional therapeutic agent is an antiviral agent that blocks

viral entry or maturation or targets the HBV polymerase such as nucleoside or nucleotide or non-nucleos(t)ide polymerase inhibitors. In a further embodiment of the combination therapy, the reverse transcriptase inhibitor and/or DNA and/or RNA polymerase inhibitor is Zidovudine, Didanosine, Zalcitabine, ddA, Stavudine, Lamivudine, Abacavir, Emtricitabine, Entecavir, Apricitabine, Atevirapine, ribavirin, acyclovir, famciclovir, valacyclovir, ganciclovir, valganciclovir, Tenofovir, Adefovir, PMPA, cidofovir, Efavirenz, Nevirapine, Delavirdine, or Etravirine.

In an embodiment, the additional therapeutic agent is an immunomodulatory agent that induces a natural, limited immune response leading to induction of immune responses against unrelated viruses. In other words, the immunomodulatory agent can affect maturation of antigen presenting cells, proliferation of T-cells and cytokine release (e.g., IL-12, IL-18, IFN-alpha, -beta, and -gamma and TNF-alpha among others).

In a further embodiment, the additional therapeutic agent is a TLR modulator or a TLR agonist, such as a TLR-7 agonist or TLR-9 agonist. In further embodiment of the combination therapy, the TLR-7 agonist is selected from the group consisting of SM360320 (9-benzyl-8-hydroxy-2-(2-methoxy-ethoxy)adenine) and AZD 8848 (methyl [3-({[3-(6-amino-2-butoxy-8-oxo-7,8-dihydro-9H-purin-9-yl)propyl][3-(4-morpholinyl)propyl]amino}methyl)phenyl]acetate).

In any of the methods provided herein, the method may further comprise administering to the individual at least one HBV vaccine, a nucleoside HBV inhibitor, an interferon or any combination thereof. In an embodiment, the HBV vaccine is at least one of RECOMBIVAX HB, ENGERIX-B, ELOVAC B, GENEVAC-B, or SHANVAC B.

In another aspect, provided herein is method of treating an HBV infection in an individual in need thereof, comprising reducing the HBV viral load by administering to the individual a therapeutically effective amount of a compound of the present disclosure alone or in combination with a reverse transcriptase inhibitor; and further administering to the individual a therapeutically effective amount of HBV vaccine. The reverse transcriptase inhibitor may be one of Zidovudine, Didanosine, Zalcitabine, ddA, Stavudine, Lamivudine, Abacavir, Emtricitabine, Entecavir, Apricitabine, Atevirapine, ribavirin, acyclovir, famciclovir, valacyclovir, ganciclovir, valganciclovir, Tenofovir, Adefovir, PMPA, cidofovir, Efavirenz, Nevirapine, Delavirdine, or Etravirine.

For any combination therapy described herein, synergistic effect may be calculated, for example, using suitable methods such as the Sigmoid-E_{max} equation (Holford & Scheiner, 19981, Clin. Pharmacokinet. 6: 429-453), the equation of Loewe additivity (Loewe & Muischnek, 1926, Arch. Exp. Pathol Pharmacol. 114: 313-326) and the median-effect equation (Chou & Talalay, 1984, Adv. Enzyme Regul. 22: 27-55). Each equation referred to above may be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to

above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

Beneficial Effect

The present disclosure is directed to compounds capable of capsid assembly modulation. The compounds of the present disclosure may provide a beneficial balance of properties with respect to prior art compounds, e.g., they may display a different profile, display improved solubility, etc.

The present compounds have improved human liver microsome stability as well as reasonable anti-HBV activity. As compared to comparative compounds, the half-life (t1/2) of the present compounds are significantly increased, showing great improvement in human metabolic stability and the advantages in pharmaceutical applications.

Additionally, the crystal form and hydrate according to the present disclosure have advantageous characteristics, such as good stability for example against heat, moisture and/or light, good storage stability and/or coloring stability, and/or high purity, and thus are promising for manufacturing medicine. Particularly, the crystal form and hydrate according to the present disclosure show great stability against moisture and can keep stable under a wide range of humidity, especially under those (for example, 20%-95% RH, e.g., about 40%-80% RH) typically used for API storage and delivery, e.g., without the risk of transformation into another form which may not be desirable. Even undergoing conditions with overly high or low humidity, the crystal form and hydrate can revert to normal and stable state. Therefore, the crystal form and hydrate are in particular suitable for the preparation of medicaments and pharmaceutical compositions with improved stability.

Definitions

Listed below are definitions of various terms used to describe this present disclosure. These definitions apply to the terms as they are used throughout this specification and claims, unless otherwise limited in specific instances, either individually or as part of a larger group.

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the applicable art. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, and peptide chemistry are those well-known and commonly employed in the art.

As used herein, the articles “a” and “an” refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element. Furthermore, use of the term “including” as well as other forms, such as “include,” “includes,” and “included,” is not limiting.

As used in the specification and in the claims, the term “comprising” can include the

embodiments "consisting of" and "consisting essentially of." The terms "comprise(s)," "include(s)," "having," "has," "can," "contain(s)," and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that require the presence of the named ingredients/steps and permit the presence of other ingredients/steps. However, such description should be construed as also describing compositions or processes as "consisting of" and "consisting essentially of" the enumerated compounds, which allows the presence of only the named compounds, along with any pharmaceutically acceptable carriers, and excludes other compounds. All ranges disclosed herein are inclusive of the recited endpoint and independently combinable (for example, the range of "from 50 mg to 300 mg" is inclusive of the endpoints, 50 mg and 300 mg, and all the intermediate values). The endpoints of the ranges and any values disclosed herein are not limited to the precise range or value; they are sufficiently imprecise to include values approximating these ranges and/or values.

As used herein, approximating language can be applied to modify any quantitative representation that can vary without resulting in a change in the basic function to which it is related. Accordingly, a value modified by a term or terms, such as "substantially," cannot be limited to the precise value specified, in some cases. In at least some instances, the approximating language can correspond to the precision of an instrument for measuring the value.

As used herein, the term "at least one" or "one or more" refers to 1, 2, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more.

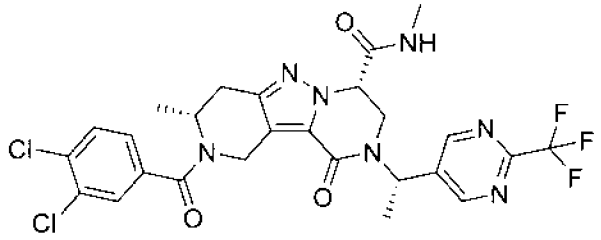
To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term "about". It is understood that, whether the term "about" is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value. Whenever a yield is given as a percentage, such yield refers to a mass of the entity for which the yield is given with respect to the maximum amount of the same entity that could be obtained under the particular stoichiometric conditions. Concentrations that are given as percentages refer to mass ratios, unless indicated differently.

The term "room temperature" (RT) refers to a temperature of from about 15 °C to about 30°C, in particular from about 20°C to about 30°C. Preferably, room temperature is a temperature of about 25°C.

The terms "buffered" solution or "buffer" solution are used herein interchangeably according to their standard meaning. Buffered solutions are used to control the pH of a medium, and their choice, use, and function is known to those of ordinary skill in the art. See, for example, G.D. Considine, ed., Van Nostrand's Encyclopedia of Chemistry, p. 261, 5th ed. (2005), describing, inter alia, buffer solutions and how the concentrations of the buffer constituents

relate to the pH of the buffer.

The term "compound (I)", "compound of formula (I)" or "compound of (I)" refers to a compound with the chemical name of (3R,7S)-2-(3,4-Dichlorobenzoyl)-N,3-dimethyl-10-oxo-9-((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxamide. The compound may also be shown as the following structural formula:



(I).

The term "amorphous or "amorphous form" used herein refers to a substance, compound or product which is not substantially crystalline as determined by X-ray (powder) diffraction. For example, it may describe a disordered solid form, i.e., a solid form lacking long range crystalline order. On the other hand, the term "crystalline", "crystalline form" or "crystal form" is defined as a form in which the position of the molecules relative to one another is organized according to a three-dimensional lattice structure.

Accordingly, as used herein and unless otherwise specified, the term "crystal form", "crystalline form", "crystalline" and relevant terms used herein, when used to describe a solid substance or product mean that the substance or product is substantially crystalline as determined for example, by X-ray powder diffractometry (XRPD).

Characterizing information for crystalline forms is provided herein. It should be understood that the determination of a particular form can be achieved using any portion of the characterizing information that one skilled in the art would recognize as sufficient for establishing the presence of a particular form. For example, even a single distinguishing peak can be sufficient for one skilled in the art to appreciate that a particular form is present.

In X-ray powder diffraction (XRPD) pattern, the diffraction pattern obtained from a crystalline compound is generally characteristic for a particular crystal form in which the relative intensities of the bands (especially at low angles) may vary with the dominant orientation effect due to the difference of crystallization conditions, particle diameters, and other measuring conditions. Therefore, it is more important to note the relative positions of peaks rather than their relative intensities when determining a crystal form. In addition, there may be slight errors in the positions of the peaks for any given crystal form, which is also well known in the art of crystallography.

When a crystalline form is identified using one or more XRPD peaks given as angles 2θ (two theta), each of the 2θ values is understood to mean the given value ± 0.2 degrees 2θ (two theta), unless otherwise expressed. Accordingly, when a particular crystal form of a chemical

entity is identified using one or more XRPD peaks given as 2θ, each of the 2θ values is understood to mean the given value ± 0.2° (degrees). If a crystal form or pattern is described as substantially as shown in a figure or substantially consistent or comparable with another one, the term "substantially" is also intended to encompass such differences in the diffraction peaks.

One skilled in the art will also recognize that diffraction patterns and peak positions are typically substantially independent of the diffractometer used and whether a specific calibration method is utilized. Typically, the peak positions may differ by about ± 0.2° 2θ (two theta), or less. The intensities (and relative intensities) of each specific diffraction peak may also vary as a function of various factors, including, but not limited to particle size, orientation, sample purity, etc.

As an example, the X-ray powder diffraction pattern may be determined on Bruker D8 Advance diffractometer with the exemplary parameters of Cu K-Alpha1 (λ=1.5406 Å) as radiation.

Other techniques for describing crystal forms and amorphous forms are well-known in the art and include, but are not limited to, thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), electron crystallography and quantitative analysis, particle size analysis (PSA), surface area analysis, solubility measurements, dissolution measurements, elemental analysis, Karl Fischer analysis or the like.

DSC (Differential Scanning Calorimetry) is a method for thermal analysis for the thermal properties of the substance. From a DSC curve, the information about the onset temperature, melting endothermic maximum and enthalpy of a compound can be obtained. It is known that the observed temperature may depend on rate of temperature change, the sample preparations techniques or the specific devices. Therefore, when a crystalline form of a chemical entity is identified using one or more temperatures from a DSC profile (e.g., onset of endothermic transition, melt, etc.), each of the temperature values is understood to mean the given value ±2°C.

As an example, DSC herein may be measured by the following method: apparatus: TA Discovery 2500; Temperature range: 0 to 250°C or before decomposition; Heating rate: 10°C/min.

Dynamic vapor sorption (DVS) is a gravimetric technique for measuring how quickly and how much of water is absorbed and desorbed by a sample in several relative humidity (RH). For example, a degree of water absorption is calculated by weight change in controlled humidity increased from 0% RH to 95% RH stepped 5% or 10%. Similarly, a degree of water desorption is calculated in humidity decreased from 95% RH to 0% RH. An absorption-desorption isotherm is obtained by plotting a value of weight change in each humidity. These results can provide the information of adhered water absorption and desorption. The results of absorption and desorption behavior of adhered water or crystal water may be affected by particle diameter, crystallinity and crystal habit.

The term "water activity (a_w)" is used herein as known in the art and means a measure of the energy status of water in a solvent system. It is defined as the vapor pressure of a liquid divided by that of pure water at the same temperature. Specifically, it is defined as $a_w = p/p_0$, where p is the vapor pressure of water in the liquid, and p_0 is the vapor pressure of pure water at the same temperature, or as $a_w = l_w \times x_w$, where l_w is the activity coefficient of water and x_w is the mole fraction of water in the aqueous fraction. For example, pure water has a water activity value of 1.0. Water activity values can typically be obtained by either a capacitance hygrometer or a dew point hygrometer. Various types of water activity measuring instruments are also commercially available. Alternatively, water activity values of mixtures of two or more solvents can be calculated based on the amounts of the solvents and the known water activity values of the solvents.

Unless indicated otherwise, the description or naming of a particular compound in the specification and claims is intended to include both individual enantiomers and mixtures, racemic or otherwise, thereof. The methods for the determination of stereochemistry and the separation of stereoisomers are well-known in the art.

The symbols  and are used as meaning the same spatial arrangement in chemical structures shown herein. Analogously, the symbols and are used as meaning the same spatial arrangement in chemical structures shown herein.

According to the foregoing interpretive considerations on assignments and nomenclature, it is understood that explicit reference herein to a set implies, where chemically meaningful and unless indicated otherwise, independent reference to embodiments of such set, and reference to each and every one of the possible embodiments of subsets of the set referred to explicitly.

The nomenclature "C_{i-j}" with j > i, when applied herein to a class of substituents, is meant to refer to embodiments of this present disclosure for which each and every one of the number of carbon members, from i to j including i and j, is independently realized. By way of example, the term C_{1-4} refers independently to embodiments that have one carbon member (C_1), embodiments that have two carbon members (C_2), embodiments that have three carbon members (C_3), and embodiments that have four carbon members (C_4).

The term "pharmaceutically acceptable" means approved or approvable by a regulatory agency of Federal or a state government or the corresponding agency in countries other than the United States, or that is listed in the U. S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly, in humans.

As used herein, the term "composition" or "pharmaceutical composition" refers to a mixture of at least one compound provided herein with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a patient or subject. Multiple techniques of administering a compound exist in the art including, but not limited to, intravenous, oral, aerosol, parenteral, ophthalmic, pulmonary and topical

administration.

As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound provided herein within or to the patient such that it can perform its intended function. Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, including the compound provided herein, and not injurious to the patient. Some examples of materials that can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound provided herein, and are physiologically acceptable to the patient. Supplementary active compounds can also be incorporated into the compositions. The "pharmaceutically acceptable carrier" can further include a pharmaceutically acceptable salt of the compound provided herein. Other additional ingredients that can be included in the pharmaceutical compositions provided herein are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

The term "stabilizer," as used herein, refers to polymers capable of chemically inhibiting or preventing degradation of a compound disclosed herein. Stabilizers are added to formulations of compounds to improve chemical and physical stability of the compound.

The term "tablet," as used herein, denotes an orally administrable, single-dose, solid dosage form that can be produced by compressing a drug substance or a pharmaceutically acceptable salt thereof, with suitable excipients (e.g., fillers, disintegrants, lubricants, glidants, and/or surfactants) by conventional tableting processes.

As used herein, the term "capsule" refers to a solid dosage form in which the drug is enclosed within either a hard or soft soluble container or "shell." The container or shell can be formed from gelatin, starch and/or other suitable substances.

As used herein, the terms "effective amount," "pharmaceutically effective amount," and "therapeutically effective amount" refer to a nontoxic but sufficient amount of an agent to provide the desired biological result. That result may be reduction or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. An appropriate therapeutic amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

The term "combination," "therapeutic combination," "pharmaceutical combination," or "combination product" as used herein refer to a non-fixed combination or a kit of parts for the combined administration where two or more therapeutic agents can be administered independently, at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, e.g., synergistic, effect.

The term "modulators" include both inhibitors and activators, where "inhibitors" refer to compounds that decrease, prevent, inactivate, desensitize, or down-regulate HBV assembly and other HBV core protein functions necessary for HBV replication or the generation of infectious particles.

As used herein, the term "capsid assembly modulator" refers to a compound that disrupts or accelerates or inhibits or hinders or delays or reduces or modifies normal capsid assembly (e.g., during maturation) or normal capsid disassembly (e.g., during infectivity) or perturbs capsid stability, thereby inducing aberrant capsid morphology and function. In one embodiment, a capsid assembly modulator accelerates capsid assembly or disassembly, thereby inducing aberrant capsid morphology. In another embodiment, a capsid assembly modulator interacts (e.g. binds at an active site, binds at an allosteric site, modifies and/or hinders folding and the like) with the major capsid assembly protein (CA), thereby disrupting capsid assembly or disassembly. In yet another embodiment, a capsid assembly modulator causes a perturbation in structure or function of CA (e.g., ability of CA to assemble, disassemble, bind to a substrate, fold into a suitable conformation, or the like), which attenuates viral infectivity and/or is lethal to the virus.

As used herein, the term "treatment" or "treating," is defined as the application or administration of a therapeutic agent, i.e., a compound of the present disclosure (alone or in combination with another pharmaceutical agent), to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient (e.g., for diagnosis or ex vivo applications), who has an HBV infection, a symptom of HBV infection or the potential to develop an HBV infection, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the HBV infection, the symptoms of HBV infection or the potential to develop an HBV infection. Such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

As used herein, the term "prevent" or "prevention" means no disorder or disease development if none had occurred, or no further disorder or disease development if there had

already been development of the disorder or disease. Also considered is the ability of one to prevent some or all of the symptoms associated with the disorder or disease.

As used herein, the term "patient," "individual" or "subject" refers to a human or a non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. Preferably, the patient, subject or individual is human.

In treatment methods according to the present disclosure, an effective amount of a pharmaceutical agent according to the present disclosure is administered to a subject suffering from or diagnosed as having such a disease, disorder, or condition. An "effective amount" means an amount or dose sufficient to generally bring about the desired therapeutic or prophylactic benefit in patients in need of such treatment for the designated disease, disorder, or condition. Effective amounts or doses of the compounds of the present disclosure may be ascertained by routine methods such as modeling, dose escalation studies or clinical trials, and by taking into consideration routine factors, e.g., the mode or route of administration or drug delivery, the pharmacokinetics of the compound, the severity and course of the disease, disorder, or condition, the subject's previous or ongoing therapy, the subject's health status and response to drugs, and the judgment of the treating physician. An example of a dose is in the range of from about 0.001 to about 200 mg of compound per kg of subject's body weight per day. An example of a dose of a compound is from about 1 mg to about 2,500 mg.

Once improvement of the patient's disease, disorder, or condition has occurred, the dose may be adjusted for preventative or maintenance treatment. For example, the dosage or the frequency of administration, or both, may be reduced as a function of the symptoms, to a level at which the desired therapeutic or prophylactic effect is maintained. Of course, if symptoms have been alleviated to an appropriate level, treatment may cease. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of symptoms.

HBV infections that may be treated according to the disclosed methods include HBV genotype A, B, C, and/or D infections. However, in an embodiment, the methods disclosed may treat any HBV genotype ("pan-genotypic treatment"). HBV genotyping may be performed using methods known in the art, for example, INNO-LIPA® HBV Genotyping, Innogenetics N.V., Ghent, Belgium).

Examples

Several methods for preparing the compounds of the present disclosure are illustrated in the following examples. Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification, or alternatively can be synthesized by a skilled person by using well-known methods.

Instruments and methods

X-ray powder diffraction (XRPD) analysis was carried out on a Bruker D8 Advance diffractometer. Samples were run on XRPD using the method below:

Radiation: Cu K-Alpha1 (λ=1.5406 Å)

Tube voltage/current: 40 kV/ 40 mA

Primary/secondary beam slit: 10.0 mm, 2.5°/5.2 mm, 2.5°

Geometry: Bragg-Brentano

Scan mode: Continuous Scan

Scan Range: 3-40° 2θ

Step size: 0.02° 2θ

Scan speed: 0.12 s/step

Rotation speed: 15 rpm

Detector: LYNXEYE_XE_T (1D mode)

^{1}H NMR spectra were recorded on 1) a Bruker DPX 400 MHz spectrometer or 2) a Bruker Avance 400 MHz spectrometer or c) Bruker Avance III 400 MHz spectrometer or d) Bruker Avance 600 MHz spectrometer or e) a Bruker Avance NEO 400 MHz spectrometer or f) Bruker model AVIII 400 spectrometer g) ZKNJ BIXI-1 300 MHz, Bruker Avance III 400 MHz or h) Bruker AVANCE Neo 400 MHz.

NMR spectra were recorded at ambient temperature unless otherwise stated. Data are reported as follow: chemical shift in parts per million (ppm) relative to TMS (δ = 0 ppm) on the scale, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sext = sextet, sept = septet, m = multiplet, b = broad, or a combination of these), coupling constant(s) *J* in Hertz (Hz).

Mass spectra were obtained on a Shimadzu LCMS-2020 MSD or Agilent 1200/G6110A MSD using electrospray ionization (ESI) in positive mode unless otherwise indicated.

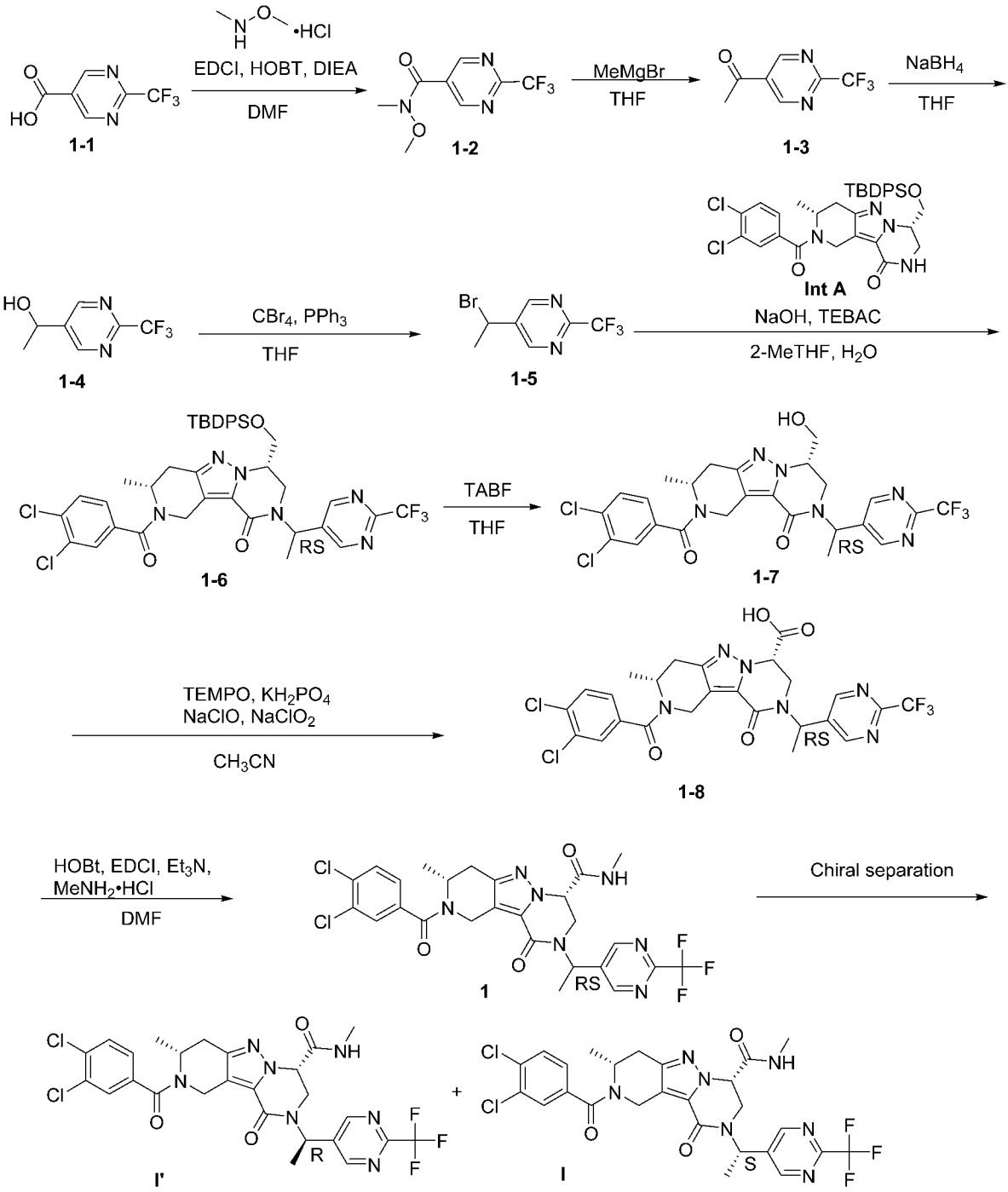
Differential Scanning Calorimetric (DSC) was carried out on TA Discovery 2500 with temperature range of 0 to 250°C and heating rate of 10°C/min.

Thermal Gravimetric Analysis (TGA) was carried out on TA Discovery 5500 with nitrogen flow of balance 10mL/min and sample chamber 25mL/min and heating rate of 10°C/min.

Karl Fischer (KF) was carried out on Mettler Toledo Coulometric KF Titrator C30 with coulometric method.

Preparing Example: Preparation of Compound (I)

Method A



Intermediate 1-2:

N-Methoxy-N-methyl-2-(trifluoromethyl)pyrimidine-5-carboxamide

To the solution of 2-(trifluoromethyl)pyrimidine-5-carboxylic acid **1-1** (5.0 g, 26.0 mmol) in N,N-dimethylformamide (55 mL) was added N,O-dimethylhydroxylamine hydrochloride (5.1g, 52.3 mmol), benzotriazol-1-ol (7.1 g, 52.5 mmol), 1-ethyl-(3-(3-dimethylamino)propyl)-carbodiimide hydrochloride (10.1 g, 52.7 mmol) and N-ethyl-N-isopropylpropan-2-amine (20.5 g, 158.6 mmol) at 0 °C. The mixture was stirred at room

temperature for 16 hours. The mixture was diluted with water (100 mL), acidized with 0.5 M hydrochloric acid aqueous solution to pH ~ 5 and extracted with ethyl acetate (100 mL) twice. The combined organic layers were washed with brine (100 mL), dried over $Na_2SO_{4(s)}$ and filtered. The filtrate was concentrated and purified by C18 column (acetonitrile: water = 30 % to 60 %) to give the title compound (5 g, 100 % purity from LCMS, 81.7 % yield) as yellow solids. LC-MS (ESI): R_T = 1.40 min, mass calcd. for $C_8H_8F_3N_3O_2$ 235.1, m/z found 236.1 $[M+H]^+$. ^1H NMR (400 M Hz, $CDCl_3$) δ 9.24 (s, 2H), 3.62 (s, 3H), 3.44 (s, 3H).

Intermediate 1-3:

1-(2-(Trifluoromethyl)pyrimidin-5-yl)ethanone

To a solution of N-Methoxy-N-methyl-2-(trifluoromethyl)pyrimidine-5-carboxamide **1-2** (5 g, 100 % purity, 21.3 mmol) in tetrahydrofuran (50 mL) was added 1 M methylmagnesium bromide in 2-methyltetrahydrofuran (33.3 mL, 33.3 mmol) dropwise at 0 °C. After being stirred at 0 °C for 2 hours, the reaction mixture was poured into saturated aqueous ammonium chloride solution (50 mL) and extracted with ethyl acetate (50 mL) for twice. The combined organic layers were washed with brine (50 mL), dried over $Na_2SO_{4(s)}$ and filtered. The filtrate was concentrated to give the title compound (4.5 g, 77 % purity from LCMS, 85.7 % yield) as a yellow oil. LC-MS (ESI): R_T = 1.36 min, mass calcd. for $C_7H_5F_3N_2O$ 190.0, m/z found 189.0 $[M-H]^-$.

Intermediate 1-4:

1-(2-(Trifluoromethyl)pyrimidin-5-yl)ethanol

To the solution of 1-(2-(trifluoromethyl)pyrimidin-5-yl)ethanone **1-3** (4.5 g, 77 % purity, 18.2 mmol) in tetrahydrofuran (50 mL) was added sodium borohydride (1.54 g, 40.7 mmol) at 0 °C. After being stirred at 0 °C for 1 hour, the mixture was quenched with saturated aqueous ammonium chloride solution (50 mL), which was diluted with ethyl acetate (50 mL), washed with water (50 mL) twice, dried over $Na_2SO_{4(s)}$ and concentrated under vacuum to give the residue, which was purified by C18 (acetonitrile : water = 40 % to 65 %) to give the desired product (2.3 g, 90 % purity, 59.1 % yield) as a yellow oil. ^1H NMR (400 M Hz, $CDCl_3$) δ 8.92 (s, 2H), 5.15 - 5.06 (m, 1H), 1.62 - 1.61 (m, 3H).

Intermediate 1-5:

5-(1-bromoethyl)-2-(trifluoromethyl)pyrimidine

To a solution of 1-(2-(trifluoromethyl)pyrimidin-5-yl)ethanol **1-4** (2 g, 90 % purity, 9.37 mmol) in tetrahydrofuran (20 mL) were added triphenylphosphine (5 g, 19.1 mmol) and perbromomethane (5 g, 15.1 mmol) at 0 °C. After being stirred at 25 °C for 0.5 hour, the mixture was filtered. The filtrate was concentrated under reduced pressure to give the residue, which was purified by silica gel column chromatography (petroleum ether: ethyl acetate = 100 : 1) to give the title compound (1 g, 90 % purity from ^1H NMR, 37.7 % yield) as a yellow oil. ^1H NMR (400 M Hz, $CDCl_3$) δ 8.96 (s, 2H), 5.18 - 5.17 (m, 1H), 2.13 - 2.11 (m, 3H).

Intermediate 1-6:

(3R,7S)-7-(((tert-Butyldiphenylsilyl)oxy)methyl)-2-(3,4-dichlorobenzoyl)-3-methyl-9-(1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,8,9-hexahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazin-10(7H)-one

To a solution of (3R,7S)-7-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,4-dichlorobenzoyl)-3-methyl-1,2,3,4,8,9-hexahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazin-10(7H)-one (1 g, 100 % purity, 1.54 mmol), N-benzyl-N,N-diethylethanaminium chloride (2 mg, 0.009 mmol) and 5-(1-bromoethyl)-2-(trifluoromethyl)pyrimidine **1-5** (1 g, 3.53 mmol) in 2-methyltetrahydrofuran (12 mL) was added 50 % wt. sodium hydroxide in water (12 mL) slowly at 30 °C. After being stirred at 30 °C for 2 hours, the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (15 mL) twice. The combined organic layers were washed with brine (15 mL) twice, dried over $Na_2SO_{4(s)}$ and filtered. The filtrate was concentrated under reduced pressure to give the title compound (1.2 g, 73 % purity, 69 % yield) as a yellow oil. LC-MS (ESI): R_T = 1.94 min, mass calcd. for $C_{41}H_{41}Cl_2F_3N_6O_3Si$ 820.2, m/z found 821.5 $[M+H]^+$.

Intermediate 1-7:

(3R,7S)-2-(3,4-Dichlorobenzoyl)-7-(hydroxymethyl)-3-methyl-9-(1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,8,9-hexahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazin-10(7H)-one

To the solution of (3R,7S)-7-(((tert-butyldiphenylsilyl)oxy)methyl)-2-(3,4-dichlorobenzoyl)-3-methyl-9-(1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,8,9-hexahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazin-10(7H)-one **1-6** (1.2 g, 73 % purity, 1.07 mmol) in tetrahydrofuran (12 mL) was added 1 M tetrabutylammonium fluoride solution (1.6 mL). After being stirred at room temperature for 1 hour, the mixture was diluted with water (20 mL), and extracted with ethyl acetate (20 mL) twice. The combined organic layers were washed with brine (20 mL), dried over $Na_2SO_{4(s)}$, and filtered. The filtrate was concentrated, and purified by C18 column (acetonitrile : water = 20 % to 40 %) to give the title compound (450 mg, 97 % purity, 70 % yield) as white solids. LC-MS (ESI): R_T = 1.59 min, mass calcd. for $C_{25}H_{23}Cl_2F_3N_6O_3$ 582.1, m/z found 583.4 $[M+H]^+$.

Intermediate 1-8:

(3R,7S)-2-(3,4-Dichlorobenzoyl)-3-methyl-10-oxo-9-(1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxylic acid

To a solution of (3R,7S)-2-(3,4-dichlorobenzoyl)-7-(hydroxymethyl)-3-methyl-9-(1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,8,9-hexahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazin-10(7H)-one **1-7** (390 mg, 97 % purity, 0.648 mmol) in acetonitrile (5 mL) was added saturated potassium dihydrogen phosphate (5 mL), 2,2,6,6-tetramethylpiperidinooxy (203 mg, 1.3 mmol), sodium chlorite (147 mg, 1.3mmol), dropwise 5.5 % sodium hypochlorite solution (1.5 mL, 1.39 mmol) at 0 °C. The reaction mixture was allowed to slowly return to room

temperature. After being stirred at room temperature overnight, the reaction mixture was quenched with saturated sodium thiosulfate aqueous solution (10 mL), acidized with 1 M hydrochloric acid solution to pH = 4 ~ 5, and extracted with ethyl acetate (10 mL) for three times. The combined organic layers were washed with brine (10 mL), dried over $Na_2SO_{4(s)}$, and filtered. The filtrate was concentrated and purified by C18 column (acetonitrile : water = 5 % to 35 %) to give the title compound (311 mg, 100 % purity, 80 % yield) as white solids. LC-MS (ESI): R_T = 1.28 min, mass calcd. for $C_{25}H_{21}Cl_2F_3N_6O_4$ 596.1, m/z found 595.1 $[M-H]^-$.

Compound 1:
(3R,7S)-2-(3,4-Dichlorobenzoyl)-N,3-dimethyl-10-oxo-9-(1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxamide

To the solution of (3R,7S)-2-(3,4-dichlorobenzoyl)-3-methyl-10-oxo-9-(1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxylic acid **1-8** (270 mg, 100 % purity, 0.452 mmol) in N,N-dimethylformamide (9 mL) was added methanamine hydrochloride (76 mg, 1.13 mmol), 1-hydroxybenzotriazole (122 mg, 0.903 mmol), 1-ethyl-(3-(3-dimethylamino)propyl)-carbodiimide hydrochloride (173 mg, 0.902 mmol) and triethylamine (0.4 mL, 2.88 mmol) at 0 °C. The mixture was stirred at room temperature for 3 hours. The mixture was diluted with water (10 mL), acidized with 0.5 M hydrochloric acid aqueous solution to pH ~ 5 and extracted with ethyl acetate (10 mL) twice. The combined organic layers were washed with brine (10 mL), dried over $Na_2SO_{4(s)}$ and filtered. The filtrate was concentrated and purified by C18 column (acetonitrile : water = 30 % to 60 %) to give the title compound P1 (210 mg, 100 % purity from LCMS, 76 % yield) as yellow solids. LC-MS (ESI): R_T = 1.58 min, mass calcd. for $C_{26}H_{24}Cl_2F_3N_7O_3$ 609.1, m/z found 610.3 $[M+H]^+$.

Compounds (I') and (I):
(3R,7S)-2-(3,4-Dichlorobenzoyl)-N,3-dimethyl-10-oxo-9-((R)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxamide (I'), and
(3R,7S)-2-(3,4-dichlorobenzoyl)-N,3-dimethyl-10-oxo-9-((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxamide (I)

A racemic of (3R,7S)-2-(3,4-dichlorobenzoyl)-N,3-dimethyl-10-oxo-9-(1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxamide **compound 1** (280 mg, 100 % purity, 0.104 mmol) was separated by chiral Prep. HPLC separation condition: (Column: Chiralpak IC 5 μm 20*250mm; Mobile Phase: MeOH : EtOH= 50 : 50 at 30 mL/min; Temp: 30 °C; Wavelength: 254 nm) to give **compound (I')** (33.8 mg, 98.4 % purity, 17.6 % yield, 99.9 % stereopure) as white solids and **compound (I)** (51.3 mg, 99.3 % purity, 27 % yield,

100 % stereopure) as white solids.

Compound (I'):

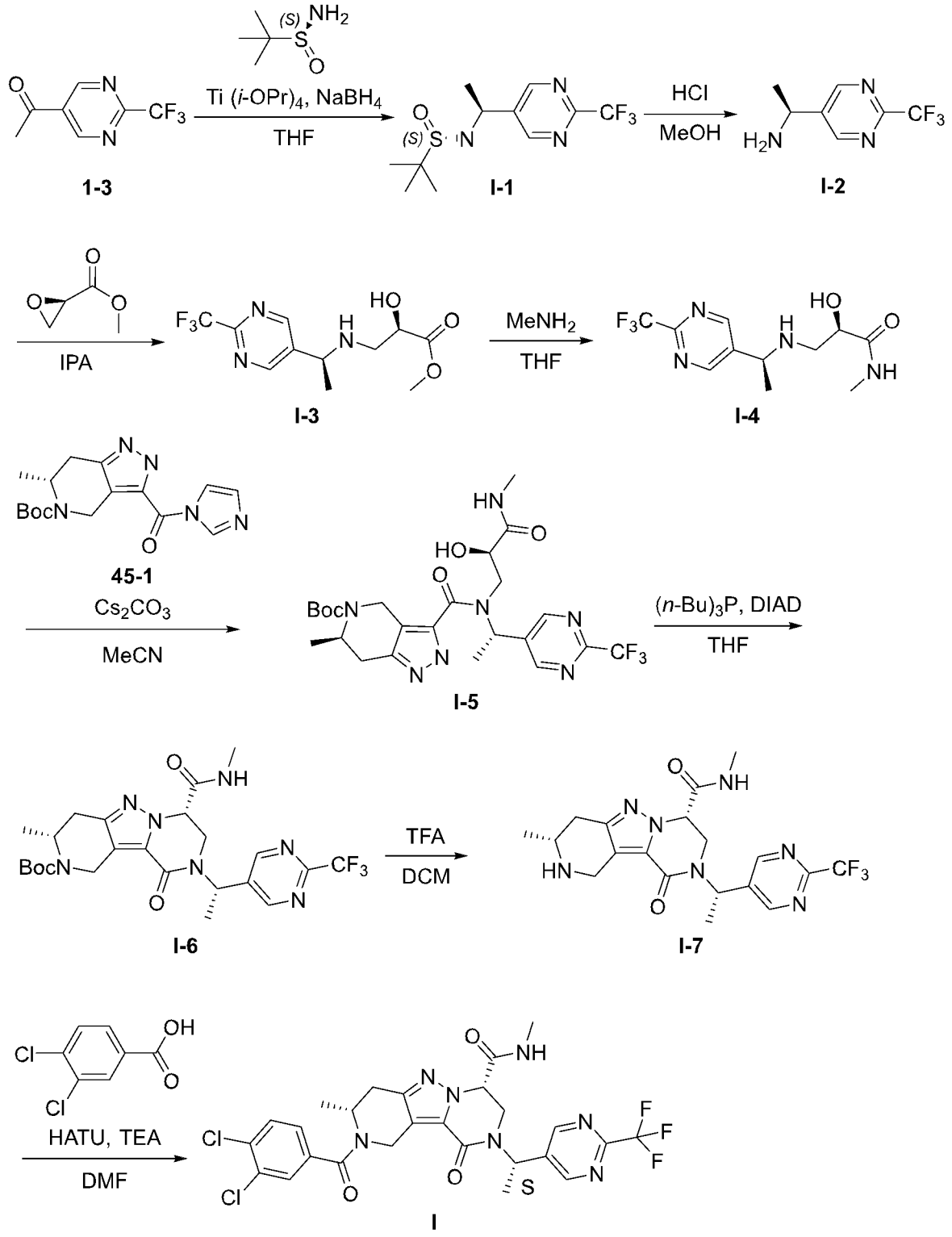
LC-MS (ESI): R_T = 3.233 min, mass calcd. for $C_{26}H_{24}Cl_2F_3N_7O_3$ 609.1, m/z found 610.2 $[M+H]^+$. Chiral analysis (Column: Chiralpak IE 5 μm 4.6 * 250 mm; Mobile Phase: MeOH : EtOH = 50 : 50 at 1 mL/min; Temp: 30 °C; Wavelength: 254 nm; R_T = 5.195 min). ^1H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 2H), 7.53 - 7.51 (m, 2H), 7.27 - 7. 4 (m, 1H), 5.99 - 5.44 (m, 3H), 4.89 - 4.46 (m, 3H), 4.13 - 4.09 (m, 1H), 3.94 - 3.90 (m, 1H), 3.05 (br s, 1H), 2.74 - 2.68 (m, 4H), 1.72 - 1.70 (m, 3H), 1.32 - 1.30 (m, 3H). ^{19}F NMR (376 MHz, $CDCl_3$) - 70.22.

Compound (I):

LC-MS (ESI): R_T = 2.978 min, mass calcd. for $C_{26}H_{24}Cl_2F_3N_7O_3$ 609.1, m/z found 610.2 $[M+H]^+$. Chiral analysis (Column: Chiralpak IE 5 μm 4.6 * 250 mm; Mobile Phase: MeOH : EtOH = 50 : 50 at 1 mL/min; Temp: 30 °C; Wavelength: 254 nm; R_T = 6.976 min). ^1H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 2H), 7.54 - 7.52 (m, 2H), 7.26 - 7.24 (m, 1H), 6.09 (br s, 1H), 5.85 - 5.53 (m, 2H), 4.93 - 4.30 (m, 4H), 3.64 - 3.59 (m, 1H), 3.07 (br s, 1H), 2.83 - 2.70 (m, 4H), 1.76 - 1.74 (m, 3H), 1.29 - 1.28 (m, 3H). ^{19}F NMR (376 MHz, $CDCl_3$) - 70.26.

The obtained product of compound (I) was subjected to X-ray Powder Diffractometer (XRPD) and was shown to be an amorphous form. See Figure 1A.

Method B



Intermediate I-1:

(S)-2-Methyl-N-((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)propane-2-sulfinamide

To a solution of 1-(2-(trifluoromethyl)pyrimidin-5-yl)ethanone **1-3** (19 g, 100 % purity, 99.9 mmol) and (S)-2-methylpropane-2-sulfinamide (20 g, 165 mmol) in tetrahydrofuran 450

mL) was added titanium (IV) tetraisopropanolate (60 mL, 205 mmol). After stirred at 70 °C for 16 hours, the reaction mixture was cooled to 0 °C, then sodium borohydride (2 g, 52.9 mmol) was added and stirred at room temperature for 1 hour. The reaction was quenched with methanol (30 mL), poured into water (60 mL), filtered with kieselguhr. The cake was washed with ethyl acetate (1 L). The filtrate was concentrated, purified by silica gel column chromatography (petroleum ether : ethyl acetate = 3 : 1) to give the title compound (19 g, 96 % purity, 62 % yield, 100 % stereopure) as yellow oil. LC-MS (ESI): R_T = 1.45 min, mass calcd. for $C_{11}H_{16}F_3N_3OS$ 295.3, m/z found 296.1 $[M+H]^+$. Chiral analysis (Column: Chiralpak IG 5 μm 4.6 * 250 mm; Mobile Phase: Hex : EtOH = 80 : 20 at 1 mL/min; Temp: 30 °C; Wavelength: 254 nm; R_T = 10.051 min).

Intermediate I-2:

(S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethan-1-amine

To the solution of (S)-2-methyl-N-((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)propane-2-sulfinamide **I-1** (14 g, 96 % purity, 45.5 mmol) in methanol (100 mL) was added 4 M hydrochloride in 1,4-dioxane (50 mL, 200 mmol). The reaction mixture was stirred at 0 °C for 2 hours. The mixture was concentrated and adjusted pH ~ 8 with saturated sodium bicarbonate aqueous solution, then extracted with ethyl acetate (60 mL) twice. The combined organic layers were washed with brine (120 mL) and dried over $Na_2SO_{4(s)}$ and filtered. The filtrate was concentrated to give the title compound (7.0 g, 100 % purity, 80 % yield, 99.7 % stereopure) as yellow oil. LC-MS (ESI): R_T = 1.18 min, mass calcd. for $C_7H_8F_3N_3$ 191.2, m/z found 192.0 $[M+H]^+$. Chiral analysis (Column: Chiralpak ID 5 μm 4.6 * 250 mm; Mobile Phase: Hex : EtOH : DEA = 95 : 5 : 0.2 at 1 mL/min; Temp: 30 °C; Wavelength: 254 nm; R_T = 10.854 min).

Intermediate I-3:

Methyl-(R)-2-hydroxy-3-(((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)amino)propanoate

(S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethan-1-amine **I-2** (7.0 g, 100 % purity, 26.5 mmol), (R)-methyl oxirane-2-carboxylate (5 g, 49.0 mmol) were mixed in propan-2-ol (60 mL) at 30 °C in a sealed tube. After stirred at 60 °C under nitrogen atmosphere for 16 hours, the mixture was concentrated and purified by silica gel column chromatography (petroleum ether : ethyl acetate = 1 : 1) to give the title compound (6.5 g, 79% yield, 95% purity, 99.7 % stereopure) as yellow oil. LC-MS (ESI): R_T = 1.31 min, mass calcd. for $C_{11}H_{14}F_3N_3O_3$ 293.2, m/z found 294.1 $[M+H]^+$. Chiral analysis (Column: Chiralpak IE 5 μm 4.6 * 250 mm; Mobile Phase: Hex : IPA = 90 : 10 at 1 mL/min; Temp: 30 °C; Wavelength: 254 nm; R_T = 12.408 min).

Intermediate I-4:

(R)-2-Hydroxy-N-methyl-3-(((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)amino) propanamide

A solution of methyl-(R)-2-hydroxy-3-(((S)-1-(2-(trifluoromethyl)pyrimidin-5-

yl)ethyl)amino)propanoate **I-3** (6.5 g, 95 % purity, 21.1 mmol) in 2 M methylamine in tetrahydrofuran (50 mL, 100 mmol) was stirred at 80 °C for 16 hours in a sealed tube (100 mL). The mixture was concentrated to give the residue and purified by C18 column (acetonitrile : water (+ 0.02 % ammonium acetate) = 5 % to 100 %) to give the title compound (6.2 g, 98 % purity, 99% yield) as while solids. LC-MS (ESI): R_T = 1.16 min, mass calcd. for $C_{11}H_{14}F_3N_3O_3$ 292.2, m/z found 293.3 $[M+H]^+$. Chiral analysis (Column: Chiralpak IH 5 μm 4.6 * 250 mm; Mobile Phase: Hex : EtOH : DEA = 90 : 10 : 0.2 at 1 mL/min; Temp: 30 °C; Wavelength: 254 nm; R_T = 7.772 min).

Intermediate I-5:

***tert*-Butyl (6R)-3-(((R)-2-hydroxy-3-(methylamino)-3-oxopropyl)((S)1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)carbamoyl)-6-methyl-2,4,6,7-tetrahydro-5H-pyrazolo[4,3-c]pyridine-5-carboxylate**

To a solution of *tert*-butyl (R)-3-(1H-imidazole-1-carbonyl)-6-methyl-2,4,6,7-tetrahydro-5H-pyrazolo[4,3-c]pyridine-5-carboxylate **45-1** (7.5 g, 100 % purity, 22.6 mmol), (R)-2-hydroxy-N-methyl-3-(((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl) amino)propanamide **I-4** (6.2 g, 98 % purity, 20.8 mmol) in acetonitrile (120 mL) was added cesium carbonate (13 g, 39.9 mmol) at 30 °C. After stirred at 45 °C for 3 hours, the mixture was added water (200 mL), then washed with ethyl acetate (200 mL) and brine (200 mL), dried over $Na_2SO_{4(s)}$ and filtered. The filtrate was concentrated and purified by silica gel column chromatography (dichloromethane : methanol = 10 : 1) to give the title compound (7.6 g, 100 % purity, 66 % yield) as light yellow oil. LC-MS (ESI): R_T = 2.29 min, mass calcd. for $C_{24}H_{32}F_3N_7O_5$ 555.6, m/z found 556.2 $[M+H]^+$. Chiral analysis (Column: Chiralpak IC 5 μm 4.6 * 250 mm; Mobile Phase: Hex : EtOH : DEA = 85 : 15 : 0.2 at 1 mL/min; Temp: 30 °C; Wavelength: 254 nm; R_T = 14.702 min).

Intermediate I-6:

***tert*-Butyl (3R,7S)-3-methyl-7-(methylcarbamoyl)-10-oxo-9-((S)-1-(2-(trifluoromethyl) pyrimidin-5-yl)ethyl)-3,4,7,8,9,10-hexahydropyrido[4',3':3,4] pyrazolo[1,5-a]pyrazine -2(1H)-carboxylate**

Tert-butyl (6R)-3-(((R)-2-hydroxy-3-(methylamino)-3-oxopropyl)((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)carbamoyl)-6-methyl-2,4,6,7-tetrahydro-5H-pyrazolo[4,3-c]pyridine-5-carboxylate **I-5** (7.6 g, 100 % purity, 13.7 mmol), tributylphosphane (4.2 g, 20.8 mmol) and (E)-diisopropyl diazene-1,2-dicarboxylate (4.2 g, 20.8 mmol) were mixed in tetrahydrofuran (120 mL) at 0 °C. After stirred at 0 °C for 2 hours, the reaction mixture was concentrated under reduced pressure to give a residue, which was purified by C18 column (acetonitrile : water (+ 0.02 % ammonium acetate) = 5 % to 100 %) to give the title compound (13 g, 50 % purity (including 50 % Bu_3PO MS: [219]), 88 % yield) as yellow oil. LC-MS (ESI): R_T = 2.64 min and 2.67 min, mass calcd. for $C_{24}H_{30}F_3N_7O_4$ 537.5, m/z found 555.2 $[M+18]^+$.

Intermediate I-7:

(3R,7S)-N,3-Dimethyl-10-oxo-9-((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxamide

To the solution of *tert*-butyl (3R,7S)-3-methyl-7-(methylcarbamoyl)-10-oxo-9-((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-3,4,7,8,9,10-hexahydropyrido[4',3':3,4]pyrazolo [1,5-a]pyrazine-2(1H)-carboxylate **I-6** ((9 g, 50 % purity, 8.37 mmol) in dichloromethane (60 mL) was added trifluoroacetic acid (15 mL) at 0 °C. After stirred at 0 °C for 2 hours, the mixture was concentrated and adjusted to pH ~ 8 with saturated sodium bicarbonate aqueous solution, then extracted with dichloromethane (100 mL) twice. The combined organic layers were washed with brine (150 mL) and dried over $Na_2SO_{4(s)}$ and filtered. The filtrate was concentrated to give the title compound (3 g, 75% yield, 91% purity) as yellow oil. LC-MS (ESI): R_T = 1.76 min and 1.88 min, mass calcd. for $C_{19}H_{22}F_3N_7O_2$ 437.1, m/z found 436.5 $[M-H]^-$.

Compound of formula (I):

(3R,7S)-2-(3,4-Dichlorobenzoyl)-N,3-dimethyl-10-oxo-9-((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxamide

(3R,7S)-N,3-Dimethyl-10-oxo-9-((S)-1-(2-(trifluoromethyl)pyrimidin-5-yl)ethyl)-1,2,3,4,7,8,9,10-octahydropyrido[4',3':3,4]pyrazolo[1,5-a]pyrazine-7-carboxamide **I-7** (3.0 g, 91 % purity, 6.2 mmol), 3,4-dichlorobenzoic acid (1.5 g, 7.85 mmol), N-ethyl-N-isopropylpropan-2-amine (3 mL, 16.2 mmol) and O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (3 g, 7.89 mmol) were mixed in N,N-dimethylformamide (45 mL) at 0 °C. After stirred at 30 °C for 1 hour, the mixture was concentrated and purified by C18 column (acetonitrile: water (+ 0.02 % ammonium acetate) = 5 % to 100 %) to give the title crude compound (3.2 g, 100 % purity, 84% yield) as white solids. LC-MS (ESI): R_T = 3.26 min, mass calcd. for $C_{26}H_{24}Cl_2F_3N_7O_3$ 609.1, m/z found 608.6 $[M-H]^-$. Then compound (I) ((3.2 g, 100 % purity, 5.24 mmol) was separated by chiral HPLC (separation condition: Column: Chiralpak IC 5 μm 30 * 250 mm; Mobile Phase: ACN = 100 at 20 mL/ min; Temp: 30 °C; Wavelength: 214 nm) to afford the title product (2.8 g, 99.8 % purity, recovery yield 87.3%, 100 % stereopure) as white solids. LC-MS (ESI): R_T = 8.337 min, mass calcd. for $C_{26}H_{24}Cl_2F_3N_7O_3$ 609.1, m/z found 610.0 $[M+H]^+$. Chiral analysis (Column: Chiralpak IC 5 μm 4.6 * 250 mm; Mobile Phase: ACN = 100 % at 1 mL/min; Temp: 30 °C; Wavelength: 214 nm; R_T = 10.028 min). ^1H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 2H), 7.54 - 7.51 (m, 2H), 7.26 - 7.24 (m, 1H), 6.08 - 5.44 (m, 3H), 4.93 - 4.30 (m, 4H), 3.65 - 3.60 (m, 1H), 3.14 - 2.96 (m, 1H), 2.83 - 2.70 (m, 4H), 1.75 (d, *J* = 7.2 Hz, 3H), 1.28 (d, *J* = 6.8 Hz, 3H). ^{19}F NMR (376 MHz, $CDCl_3$) δ - 70.28.

The obtained product of compound (I) was subjected to X-ray Powder Diffractometer (XRPD) and was shown to be an amorphous form. See Figure 1B.

Example 1. Preparation of Crystal Form C of compound (I)

About 20 mg of amorphous form of compound (I) was weighed and dissolved in 0.25 mL of water at 25°C with a stirring bar on a magnetic stirring plate at a rate of 300-400 rpm for 13 days. The obtained suspensions were filtered through a 0.45 μm nylon membrane filter by centrifugation at 14,000 rpm.

The obtained solid parts were dried at ambient condition and the resulting solids were investigated by XRPD. The obtained product was identified as crystal form (designated as Crystal Form C). The XRPD pattern was shown in Figure 2.

TGA test was also performed, and the result was shown in Figure 3, showing about 2.1% weight loss at about 160°C.

Example 2. Preparation of Crystal Form C of compound (I)

Example 2.1

300 mg of amorphous form of compound (I) was weighed into an 8 mL glass vial. 4.75 mL of water was added into the vial under stirring at 25°C.

About 1 mg seeds of crystal form C were added to the vial. After stirring at the magnetic stirring plate for 1 day, 3 mL of water was added to the vial. After further stirring at the magnetic stirring plate for 8 days, the suspension was transferred to a 20 mL glass vial. 4 mL of water was added into the vial under stirring at 25°C. Then the mixture was stirred at 25°C for 1 day. Solids were collected by centrifugation through a 0.45 μm nylon membrane filter by centrifugation at 14,000 rpm and then dried at ambient condition for about 12 hours.

About 260 mg of product was obtained as an off-white solid in 88% yield. The resulting solids were investigated by XRPD. The XRPD pattern was shown in Figure 4 (top pattern), which was substantially consistent to that obtained for Example 1 (bottom pattern, Figure 4), confirming that crystal form C was obtained.

The obtained crystal form C was determined to have water content and the water content was determined to be around 4.8% by weight, corresponding to 1.7 equivalents by molar ratio (according to Karl Fisher method).

DSC showed a dehydration peak at T_{onset} of about 16.4°C with an enthalpy of about 37J/g, and a melting peak at T_{onset} of about 173.7°C°C with an enthalpy of about 53J/g (Figure 5). TGA showed about 2.2% weight loss at about 150°C (Figure 6).

Example 2.2

2.3 g of amorphous free form of compound (I) was weighed into a 150 mL crystallizer. 37 mL of water was added into the crystallizer under stirring at 25°C.

About 1 mg seeds of crystal form C were added to the stirrer. Then the mixture was stirred at 25°C for 3 days. Solids were collected by centrifugation through a 0.45 μm nylon

membrane filter by centrifugation at 14,000 rpm and then dried at ambient condition for about 12 hours.

About 2.1 g of product was obtained as an off-white solid in 91% yield. The resulting solids were investigated by XRPD. The XRPD pattern was shown in Figure 7 (top pattern), which was substantially consistent to that obtained for Example 1 (bottom pattern, Figure 7), confirming that crystal form C was obtained.

The obtained crystal form C was determined to have water content and the water content was determined to be around 2.1% by weight, corresponding to 0.7 equivalents by molar ratio (according to Karl Fisher method).

DSC showed a dehydration peak at T_{onset} of about 16.2°C with an enthalpy of about 9J/g, and a melting peak at T_{onset} of about 171.9°C with an enthalpy of about 53J/g (Figure 8). TGA showed about 2.0% weight loss at about 150°C (Figure 9).

Example 3

Example 3.1 Water sorption and desorption

Water sorption and desorption behavior of crystal form C (the initial sample was the crystal form of Example 2.1) was investigated by DVS.

The moisture sorption analysis (DVS) was performed using Surface Measurement Systems' Advantage model dynamic vapor sorption apparatus. The moisture profile was evaluated by monitoring vapor adsorption and desorption over the range of 0 to 95% RH at 25°C. The sample weight equilibrium criteria were set at ≤0.002% weight change per minute with minimum and maximum time of acclimation at 60 min and 360 min, respectively. The moisture profile consisted of 2 cycles of vapor adsorption and desorption.

The DVS isothermal change in mass plot of the crystal form showed that the crystal form was hygroscopic with the water content varying with relative humidity and could dehydrate completely at 0% RH. In the humidity range of 40-80% RH, the crystalline form adsorbed and desorbed moisture slowly and reversibly up to 1% by mass on average. Based on DVS, the crystal form C at equilibrium can contain around 0.8 to 1.3 molar equivalents of water (2 to 3.6% total moisture mass) at common ambient RH of 40% to 80%. It was also shown that the crystal form can contain up to around 1.6 molar equivalents of water at 95% RH. The results of DVS test were shown in Figure 10. The results showed that the crystal form with water content was a channel hydrate.

The XRPD pattern of the fraction obtained after the DVS test (top pattern, Figure 11) was substantially consistent to that obtained for Example 1 (bottom pattern, Figure 11). No indication of a solid-state form change was observed. The test indicated a good stability, e.g., in respect of hygroscopicity, particularly under humidity condition.

Example 3.2 Solid Form testing under variable humidity (VH)

In this example, the crystal form C was subjected to solid form testing under variable humidity conditions. Before testing, the crystal was treated with Ethyl Acetate (EA)/Heptane system, which was briefly summarized as the following steps: 2 g of crystal form C was dissolved in 14 ml of EA (contain 0.5% water) at 50°C; 19.6 ml of n-heptane was added at 50°C; 2% of crystal form C was further charged and bred for 2 h at 50°C; 29.4 ml of n-heptane were added and the system was maintained for 10 h at 50°C; the system was cooled down to 25°C over 2 h and aged for 10 h at 25°C; the obtained solids were filtered, washed by 28 ml of n-heptane and vacuum dried at 40°C and were used as the initial samples, which were placed at ambient condition (28°C 36%RH) for further use.

XRPD pattern of the initial sample of crystal form C was obtained and as shown in Figure 12, which was substantially consistent to that obtained for the crystal form of Example 1.

The VH test was performed with the following procedures:

Samples	Chamber RH/Temp.
initial	Ambient (28°C 36%RH)
initial-0RH	0% RH at 25°C
initial-0-20RH	20% RH at 25°C
initial-0-20-40RH	40% RH at 25°C
initial-0-20-40-60RH	60% RH at 25°C
initial-0-20-40-60-80RH	80% RH at 25°C
initial-0-20-40-60-80-95RH	95% RH at 25°C
initial-0-20-40-60-80-95-40RH	40% RH at 25°C
Sample used amount: ~ 15mg XRPD Testing time: ~20mins VH procedure: Initial-0%-20%-40%-60%-80%-95%-40%RH (each step equilibration for 3h)	

XRPD patterns were investigated for each crystal form sample under different relative humidity (RH) conditions and the results were shown in Figure 12, where the patterns from bottom to top, were shown according to the VH procedures.

As mentioned previously, the crystal form was hygroscopic with the water content varying with relative humidity. Although a few peak shifts were observed at 0% and 20% RH, which were consistent with shrinking of crystal lattice in conjunction with reduction in water content, the contraction and expansion of crystal lattice with water content/environment RH were reversible as evident by the fact that the crystal form returned to its initial form at ambient after RH reaches 40% (as proved by the substantially consistent XRPD patterns). No further lattice expansion was observed above 40% RH, showing the same XRPD patterns as the initial form, where the XRPD patterns all remained substantially consistent.

The VH testing results showed superior stability of the crystal form C under a wide

range of humidity conditions. Although small changes of the solid form may be observed under very dry conditions like 0% and 20% RH, which were slight but not significant, the crystal form was still stable and will revert to its normal state under ambient condition.

Example 3.3 Bulk Stability (BS) Testing

In this example, the crystal form was subjected to Bulk Stability Testing.

Briefly, the product was placed at 25°C/92% RH in an open container, at 40°C/75% RH in an open container and at 60°C in a closed container for 10 days and the solid form (by XRPD), purity (by HPLC) and appearance like color (by visualization) were examined.

Example 3.4 Water Activity Test

In this example, the crystal form was subjected to Water Activity (WA) Test. Such a test can be used to determine the behavior like stability under water system. Briefly, the water activity experiments were conducted at 25°C in EtOH/water system to determine critical water activity. The product was added to 0.5 - 1 mL saturated solutions of the EtOH/water systems in the following table.

Exp. ID	Solvents
AW1	EtOH/water (96.5:3.5, v:v) (a.w.= 0.1)*
AW2	EtOH/water (88:12, v:v) (a.w.= 0.3)*
AW3	EtOH/water (76:24, v:v) (a.w.= 0.5)*
AW4	EtOH/water (58:42,v:v) (a.w.= 0.7)*
AW5	EtOH/water (25:75, v:v) (a.w.= 0.9)*

*: water activity is calculated by UNIFAC method.

Efficacy Example

Anti-HBV activity of Compound of formula (I)

Procedure

The anti HBV activity was measured using the HepG2.117 cell line, a stable, inducibly HBV producing cell line, which replicates HBV in the absence of doxycycline (Tet-off system). The HepG2 cell line is available from ATCCR under number HB-8065. Transfection of the HepG2 cell line can be as described in Sun and Nassal 2006 Journal of Hepatology 45 (2006) 636-645 "*Stable HepG2- and Huh7-based human hepatoma cell lines for efficient regulated expression of infectious hepatitis B virus*".

For the antiviral assay, HBV replication was induced, followed by a treatment with serially diluted compound in 96-well plates. After 3 days of treatment, the antiviral activity was determined by quantification of intracellular HBV DNA using real-time PCR and an HBV specific primer set and probe.

Forward Primer	GTGTCTGCGGCGTTTTATCA	SEQ ID. No: 1
Reverse Primer	GACAAACGGGCAACATACCTT	SEQ ID. No: 2
Taqman Probe	CCTCTKCATCCTGCTGCTATGCCTCATC_FAM-BHQ1	SEQ ID. No: 3

Cytotoxicity of the compounds was tested using HepG2 or HepG2.117 cells, incubated for 3 or 4 days in the presence of compounds. The viability of the cells was assessed using the PERKIN ELMER ATPlite Luminescence Assay System.

Human liver microsome stability t1/2 (min) of Compound (I)

Procedure

Pooled human liver microsomes (0.5 mg/mL) was pre-incubated with 1 μM test compounds or control compounds (verapamil, warfarin and cerivastatin) in 0.1 M phosphate buffer pH 7.4 containing 1 mM $MgCl_2$ at 37 °C. The final concentration of DMSO and acetonitrile are 0.05% and 0.2%, respectively. All incubations are performed singularly for each compound. NADPH was added to initiate the reaction with the final concentration of 1 mM and the final incubation volume of 400μL.

At 6 time points (0, 5, 10, 20, 40 and 60 min) reactions were stopped by the removal of 50μL of the incubation mixture into plates containing acetonitrile at a of 1:3 (v:v). The plates were centrifuged at 3000rpm for 10min at 4 °C to precipitate the protein. Following protein precipitation, the sample supernatants were combined in cassettes of up to 8 compounds. The internal standard was added (1:1 supernatant to internal standard solution) and samples were analyzed using standard LC-MS conditions.

From a plot of ln peak area ratio (compound peak area/internal standard peak area) against time, the gradient of the line is determined. Subsequently, half-life is calculated using the equations below:

Elimination rate constant (k) = (- gradient)

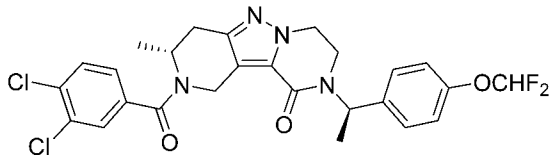
Half-life ($t_{1/2}$) (min) = 0.693/k

Results

N.A. = not available

CC50 values: 3-days incubation unless marked with * (* = 4-days incubation)

Compound Number	EC50 (μM, mean value)	CC50 (μM, mean value)	HLM Metabolism $t_{1/2}$ (min)
Compound (I)	0.16	>50.0	>180
Ref 1	0.069	>50	15.4
Ref 2	0.026	19.84	7.05
Ref 3	0.072	>50	6.6
Ref 4	0.076	>50	13.5
Ref 5	0.022	>50	7.9



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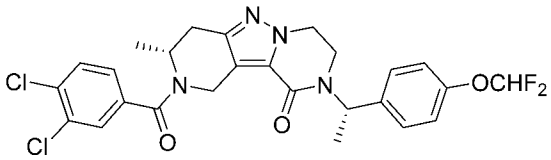

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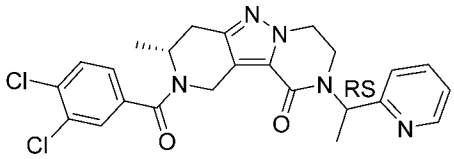

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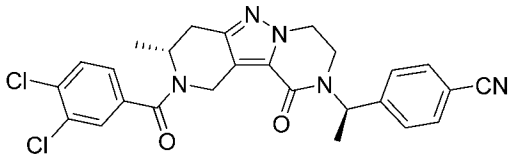

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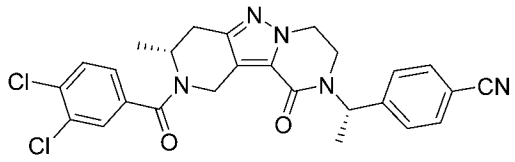


Ref 5

Ref 1, Ref 2, Ref 3, Ref 4 and Ref 5 can be prepared according to the procedures of WO2020/243135.

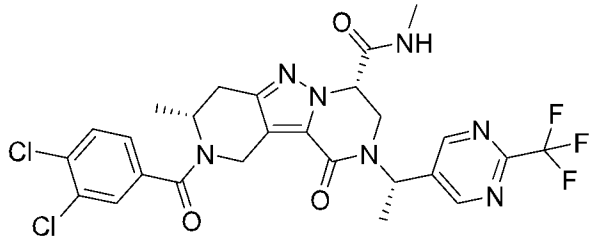
According to the experimental results, Compound of formula (I) showed improved human liver microsome stability as well as reasonable anti-HBV activity. As compared to comparative compounds (e.g., reference compounds 1-5), the half-life ($t_{1/2}$) of the present compounds are significantly increased, showing great improvement in human metabolic stability.

The disclosed subject matter is not to be limited in scope by the specific embodiments and examples described herein. Indeed, various modifications of the disclosure in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the

appended claims. All references (e.g., publications or patents or patent applications) cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual reference (e.g., publication or patent or patent application) was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. Other embodiments are within the following claims.

CLAIMS

1. A Crystal Form C of a compound of Formula (I), having an X-ray powder diffraction (XRPD) pattern comprising diffraction peaks at 7.9±0.2°, 14.8± 0.2°, 15.8±0.2°, 17.5± 0.2°, and 19.6± 0.2° (2θ)



(I).

2. The Crystal Form C according to claim 1, wherein the X-ray powder diffraction (XRPD) pattern further comprises at least one diffraction peak of 5.4± 0.2°, 13.8± 0.2°, 15.4± 0.2°, 19.1± 0.2°, 20.5± 0.2°, and 24.7± 0.2° (2θ).

3. The Crystal Form C according to claim 1 or 2, wherein the X-ray powder diffraction (XRPD) pattern further comprises at least one diffraction peak of 6.9± 0.2°, 16.2± 0.2°, 18.9± 0.2°, 21.4± 0.2°, 23.7± 0.2°, and 26.6± 0.2° (2θ).

4. The Crystal Form C according to any one of claims 1-3, having an X-ray powder diffraction (XRPD) pattern as substantially shown in Figure 2.

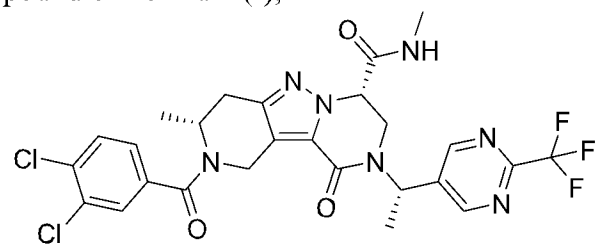
5. The Crystal Form C according to any one of claims 1-4, which is a hydrate.

6. The Crystal Form C according to claim 5, which is a channel hydrate.

7. The Crystal Form C according to any one of claims 1-6, comprising 0.1-1.8 molar equivalent of water, particularly 0.7-1.7 molar equivalent of water.

8. The Crystal Form C according to any one of claims 1-7, comprising 0.8 to 1.3 molar equivalent of water at RH of 40% to 80%.

9. A hydrate of a compound of Formula (I),



(I).

10. The hydrate according to claim 9, wherein the hydrate comprises 0.1-1.8 molar equivalent of water, particularly 0.7-1.7 molar equivalent of water.

11. The hydrate according to claim 9 or 10, wherein the hydrate comprises 0.8 to 1.3 molar equivalent of water at RH of 40% to 80%.

12. The hydrate according to any one of claims 9-11, which is a channel hydrate.

13. A pharmaceutical composition, which comprises the Crystal Form C according to any one of claims 1-8 or the hydrate according to any one of claims 9-12, and which further comprises at least one pharmaceutically acceptable excipient.

14. The Crystal Form C according to any one of claims 1-8 or the hydrate according to any one of claims 9-12, or the pharmaceutical composition of claim 13, for use as a medicament.

15. The Crystal Form C according to any one of claims 1-8 or the hydrate according to any one of claims 9-12, or the pharmaceutical composition of claim 13, for use in the prevention or treatment of an HBV infection or of an HBV-induced disease in a subject in need thereof.

16. The Crystal Form C according to any one of claims 1-8 or the hydrate according to any one of claims 9-12, or the pharmaceutical composition of claim 13, for use in the prevention or treatment of chronic hepatitis B.

17. A method of treating an HBV infection or an HBV-induced disease in an individual in need thereof, comprising administering to the individual a therapeutically effective amount of Crystal Form C according to any one of claims 1-8 or the hydrate according to any one of claims 9-12, or the pharmaceutical composition of claim 13.

18. A product comprising a first compound and a second compound as a combined preparation for simultaneous, separate or sequential use in the prevention or treatment of an HBV infection or of an HBV-induced disease in a subject in need thereof, wherein said first compound is different from said second compound, wherein said first compound is the Crystal Form C according to any one of claims 1-8 or the hydrate according to any one of claims 9-12, or the pharmaceutical composition of claim 13, and wherein said second compound is another HBV inhibitor.

19. The product of claim 18, wherein said second compound is another HBV inhibitor which is

selected from the group consisting of: therapeutic agents selected from HBV combination drugs, HBV vaccines, HBV DNA polymerase inhibitors, immunomodulatory agents, toll-like receptor (TLR) modulators, interferon alpha receptor ligands, hyaluronidase inhibitors, hepatitis b surface antigen (HBsAg) inhibitors, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors, cyclophilin inhibitors, HBV viral entry inhibitors, antisense oligonucleotide targeting viral mRNA, short interfering RNAs (siRNA) and ddRNAi endonuclease modulators, ribonucleotide reductase inhibitors, HBV E antigen inhibitors, covalently closed circular DNA (cccDNA) inhibitors, famesoid X receptor agonists, HBV antibodies, CCR2 chemokine antagonists, thymosin agonists, cytokines, nucleoprotein modulators, retinoic acid-inducible gene 1 simulators, NOD2 stimulators, phosphatidylinositol 3-kinase (PI3K) inhibitors, indoleamine-2, 3-dioxygenase (IDO) pathway inhibitors, PD-1 inhibitors, PD-L1 inhibitors, recombinant thymosin alpha-1, bruton's tyrosine kinase (BTK) inhibitors, KDM inhibitors, HBV replication inhibitors, arginase inhibitors, and other HBV drugs.

20. The Crystal Form C according to any one of claims 1-8 or the hydrate according to any one of claims 9-12, or the pharmaceutical composition of claim 13 for use in the prevention or treatment of an HBV infection or an HBV-induced disease in a subject, wherein the compound or pharmaceutical composition is administered to the subject in combination with another HBV inhibitor.

21. A process for preparing the Crystal Form C according to any one of claims 1-8, comprising:

dissolving a compound of formula (I) in a crystallizing solvent with stirring,

wherein the crystallizing solvent comprises water, aqueous solution, organic solvent or a combination thereof;

allowing crystallization of the compound; and

recovering and drying the product to give the Crystal Form C.

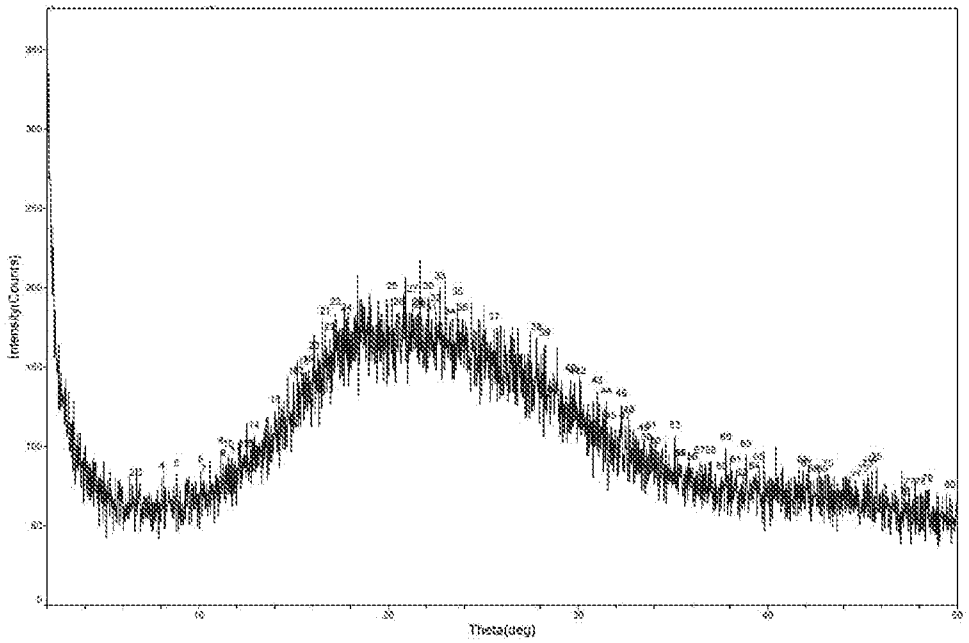


Figure 1A

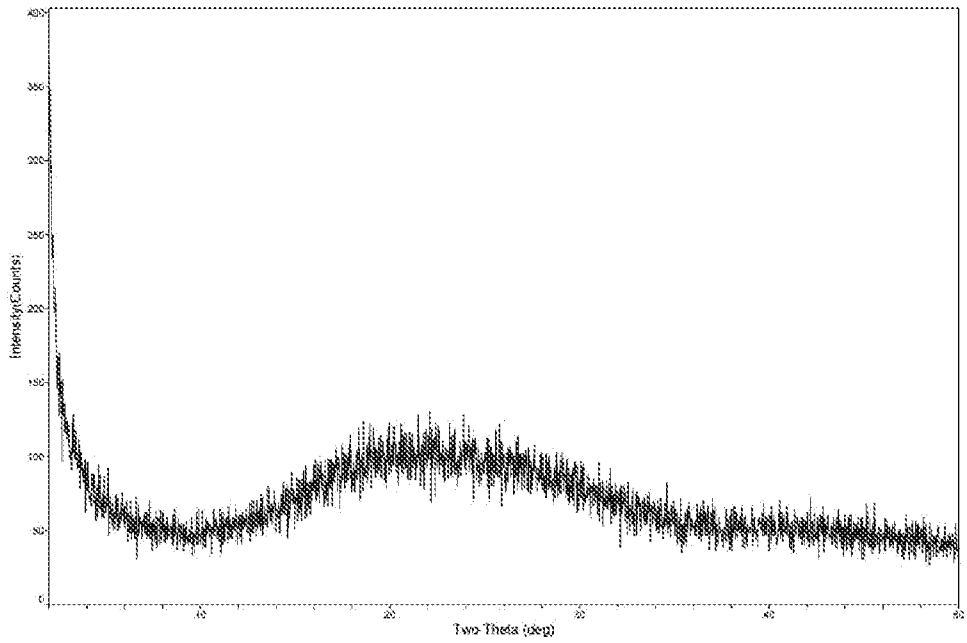


Figure 1B

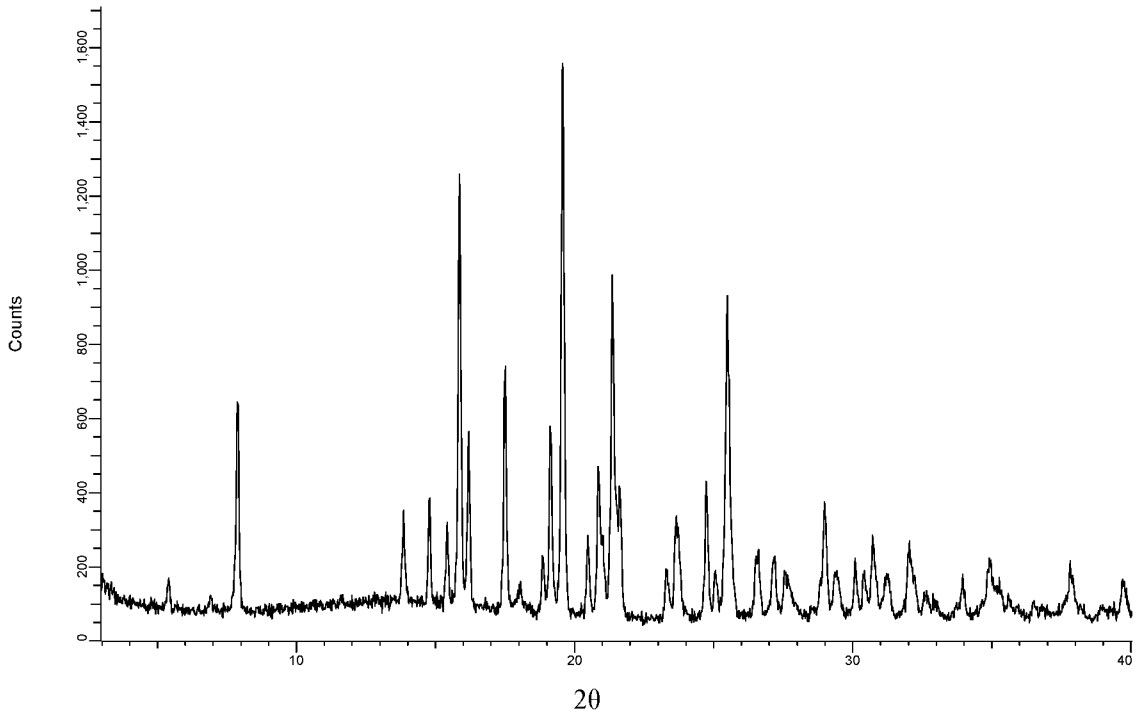


Figure 2

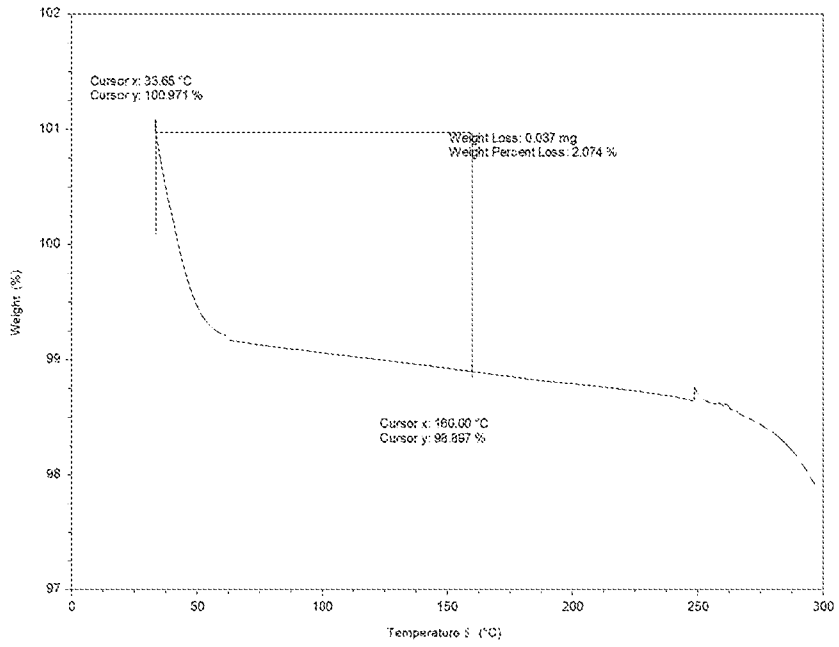


Figure 3

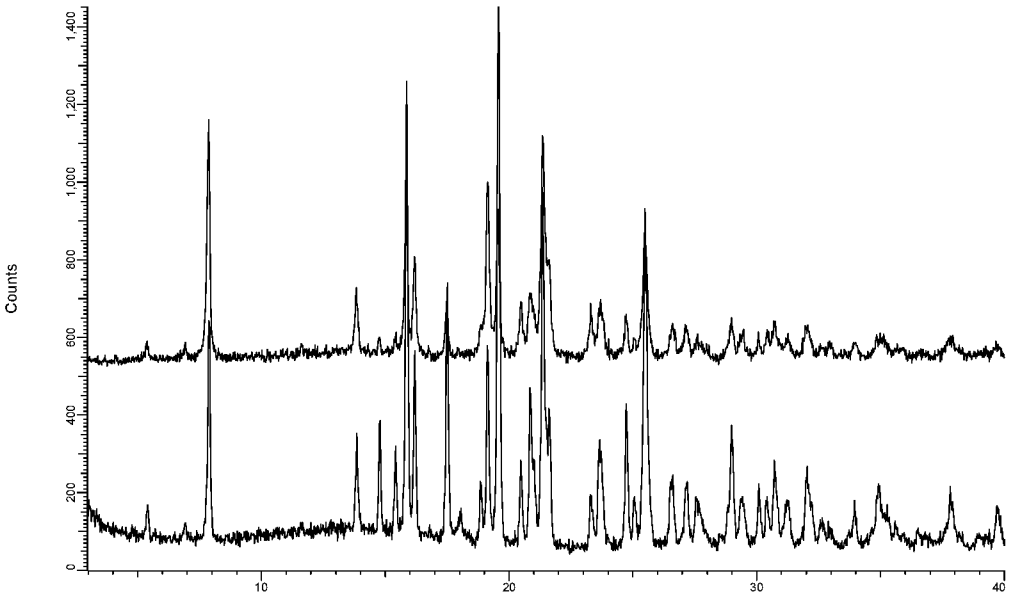


Figure 4

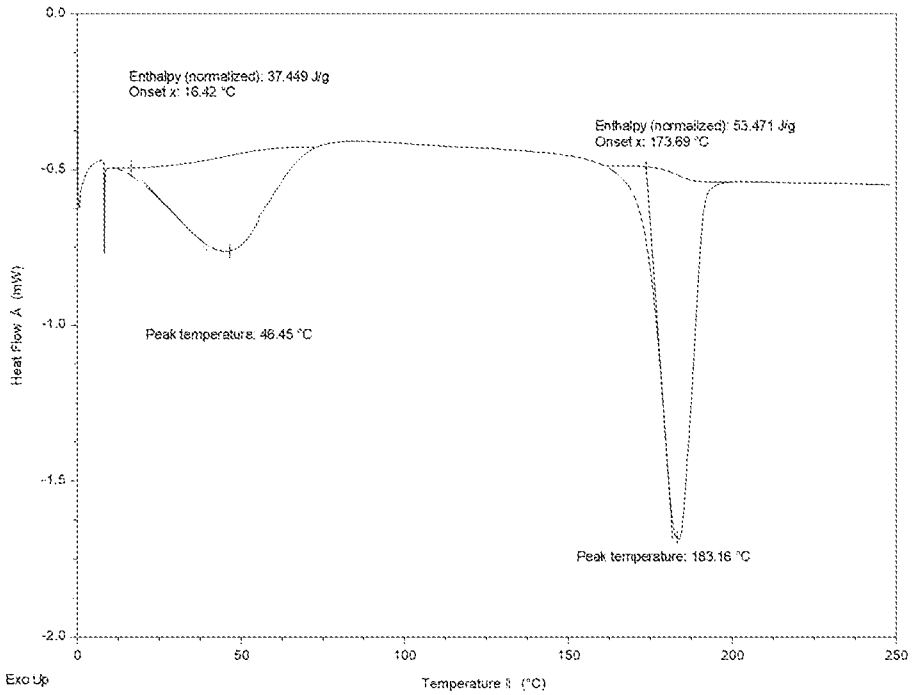


Figure 5

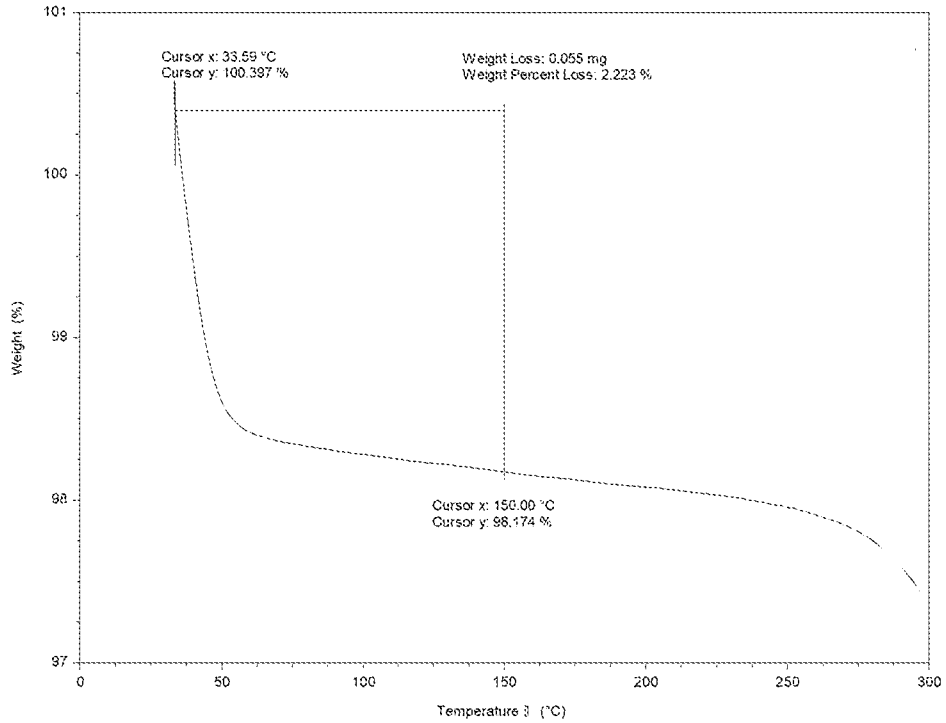


Figure 6

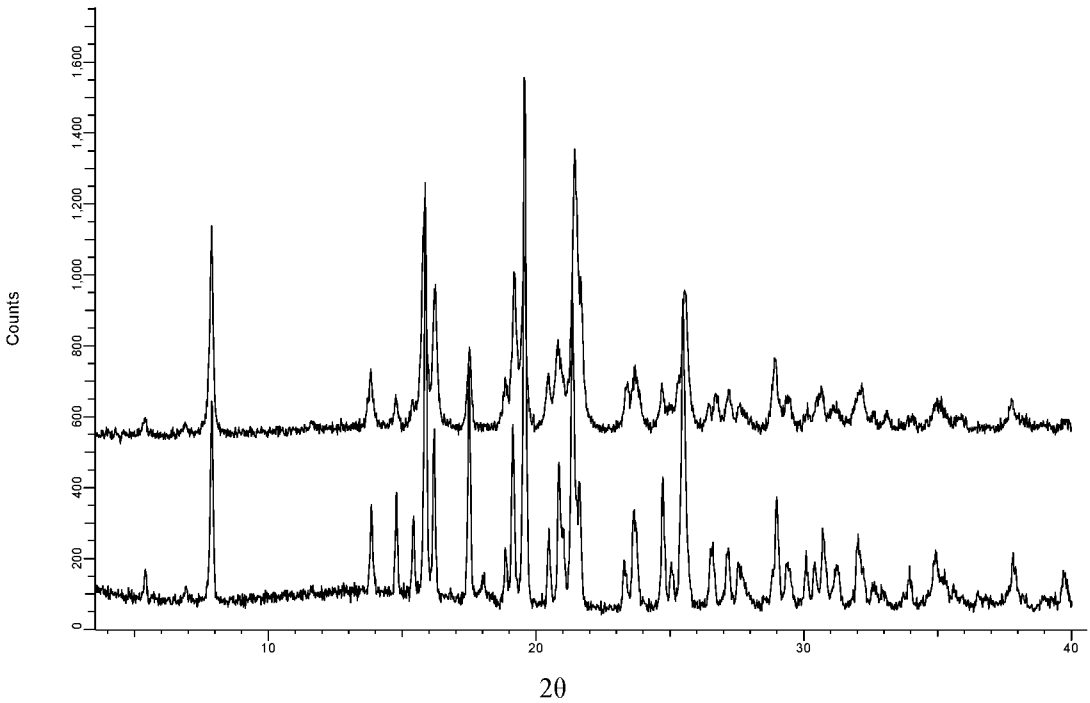


Figure 7

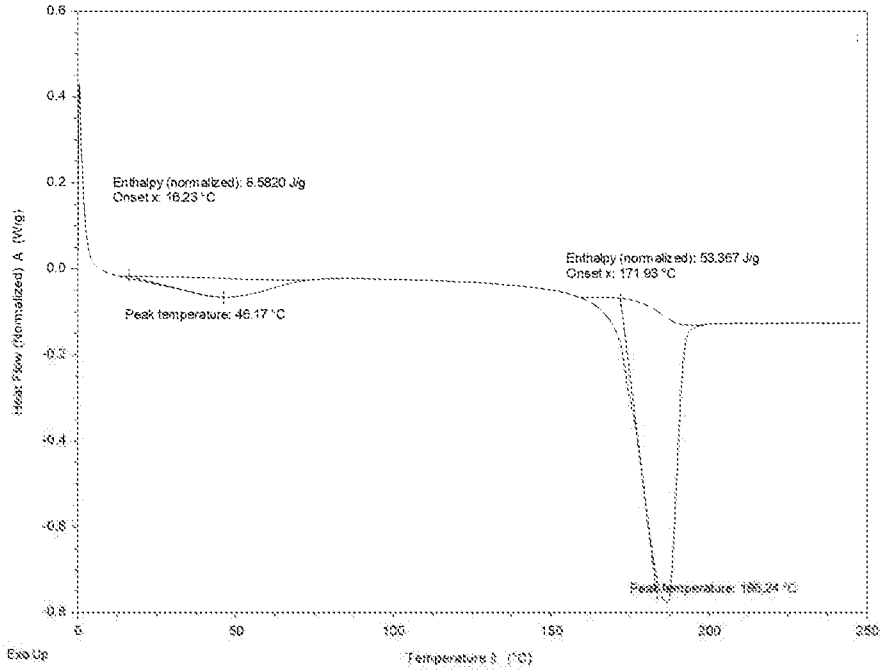


Figure 8

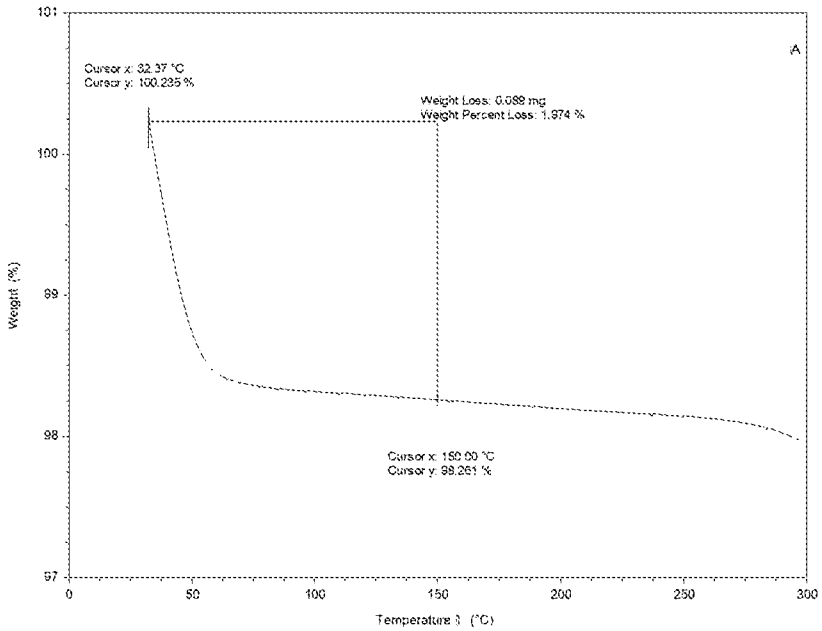
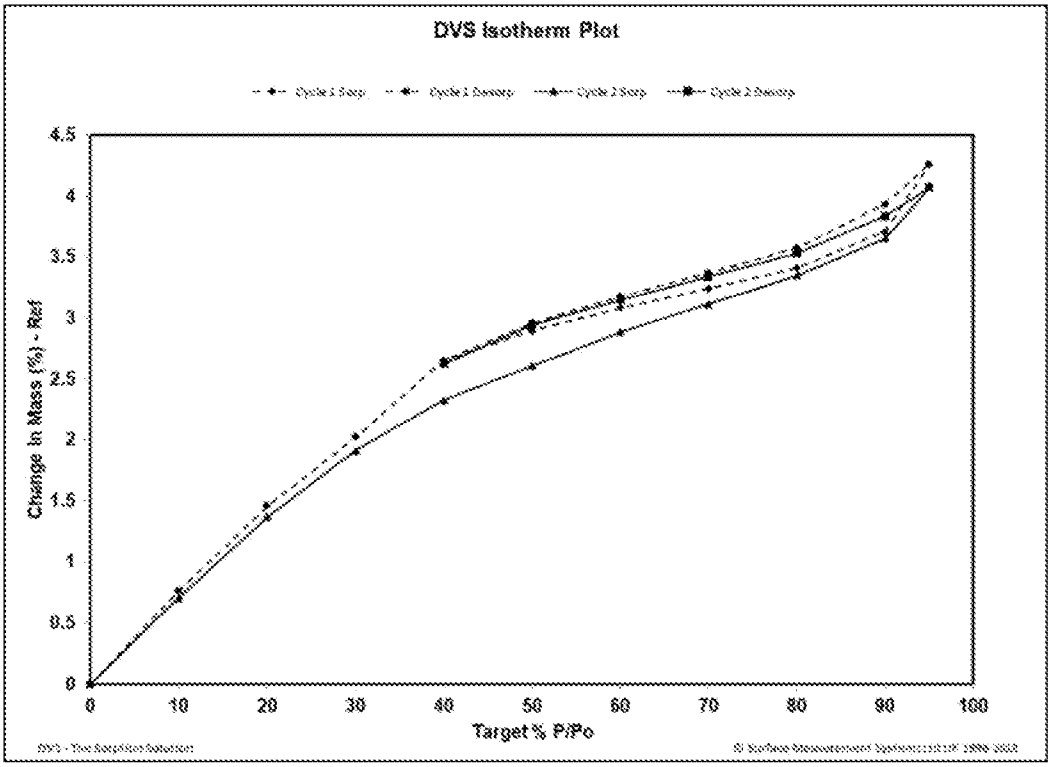


Figure 9


DVS Isotherm Plot
Change In Mass (%) - Ref
Target % P/Po
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
0
10
20
30
40
50
60
70
80
90
100

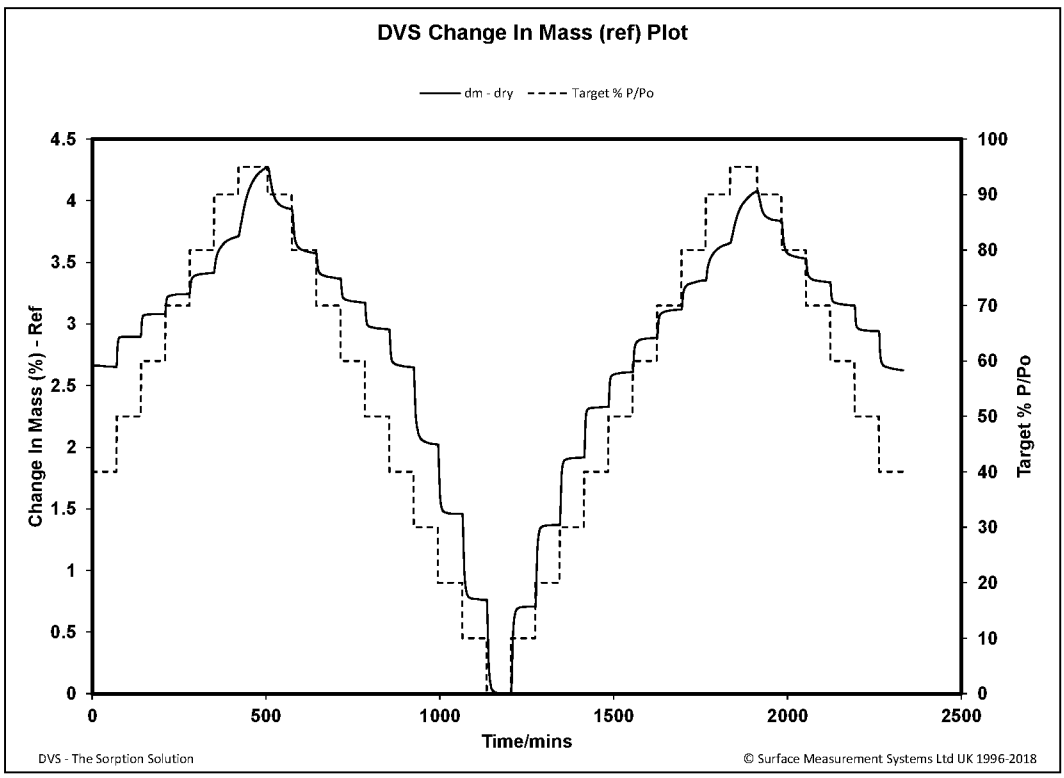

DVS Change In Mass (ref) Plot
dm - dry
Target % P/Po
Change In Mass (%) - Ref
Target % P/Po
Time/mins
0
500
1000
1500
2000
2500
DVS - The Sorption Solution
© Surface Measurement Systems Ltd UK 1996-2018

Figure 10

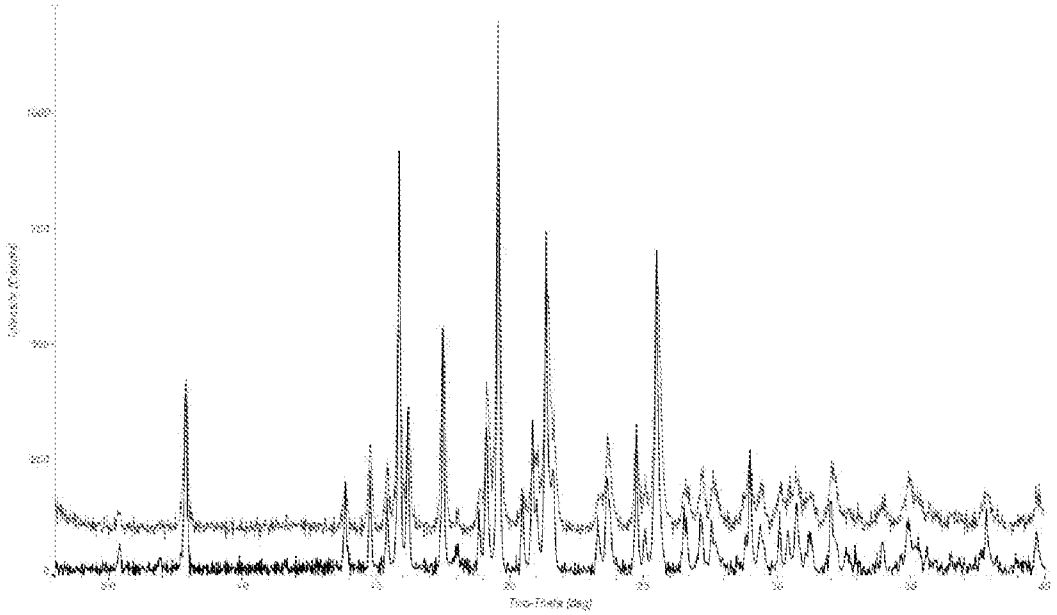


Figure 11

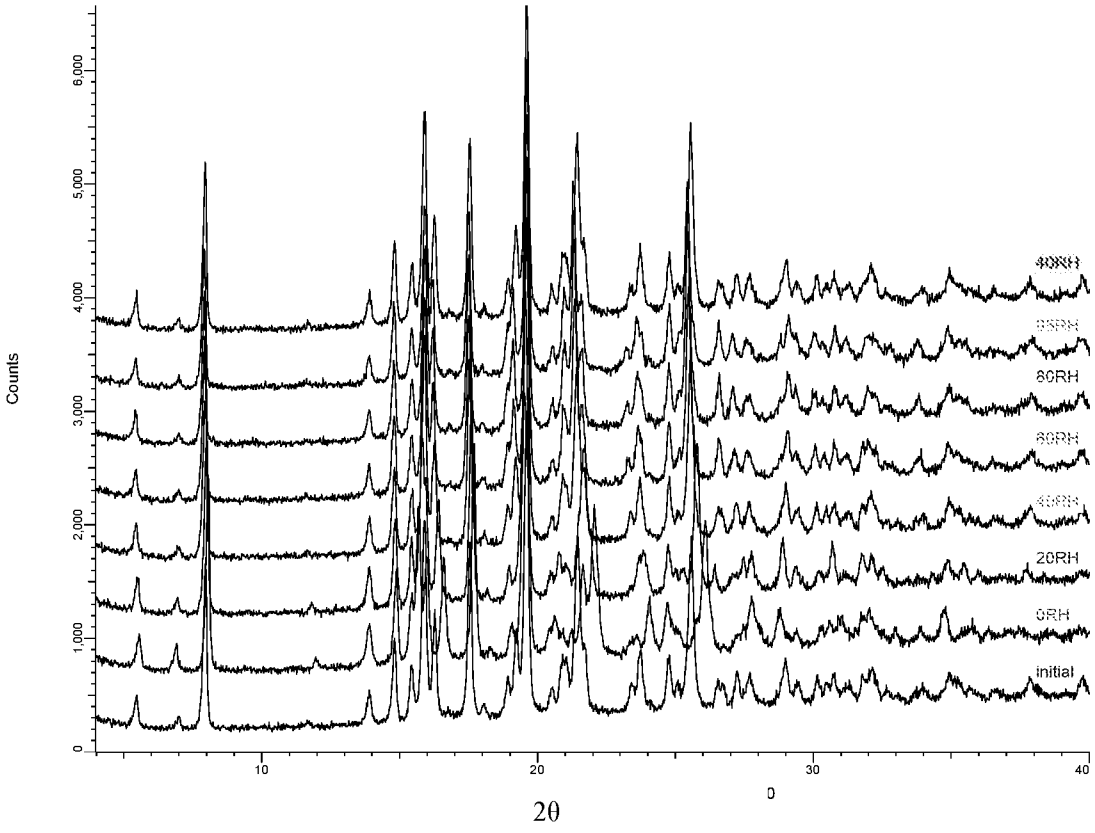


Figure 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/135272

A. CLASSIFICATION OF SUBJECT MATTER

C07D471/14(2006.01)i; A61K31/4985(2006.01)i; A61P31/20(2006.01)i

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)

IPC: C07D, A61K, A61P

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)

CNABS;EPTXT;USTXT;WOTXT;JPTXT;CNTXT;CNKI, Registry (STN), Marpat (STN): hydrate, crystal+, HBV, hepatitis, pyrazolo, pyrazine, pyrimidin?, structure search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	WO 2022253255 A1 (JANSSEN SCIENCES IRELAND UNLIMITED COMPANY et al.) 08 December 2022 (2022-12-08) claims 1-26	1-21
X	WO 2022116997 A1 (JANSSEN SCIENCES IRELAND UNLIMITED COMPANY et al.) 09 June 2022 (2022-06-09) claims 1, 13-21, description, pages 13, 25-26, 29, 73	1-21
A	US 2022348592 A1 (JANSSEN SCIENCES IRELAND UNLIMITED COMPANY) 03 November 2022 (2022-11-03) description, pages 1-3, claim 29	1-21
A	CN 113906028 A (JANSSEN SCIENCES IRELAND UNLIMITED COMPANY) 07 January 2022 (2022-01-07) claims 1-28	1-21
A	CN 109641896 A (NOVIRA THERAPEUTICS,INC.) 16 April 2019 (2019-04-16) claims 1-32	1-21

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 February 2024	**07 February 2024**

Name and mailing address of the ISA/CN	Authorized officer
CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China**	**FENG,Yuan** Telephone No. **(+86) 010-53962319**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/135272

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 113939512 A (JANSSEN SCIENCES IRELAND UNLIMITED COMPANY) 14 January 2022 (2022-01-14) claims 1-26	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/135272

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.: **17**
because they relate to subject matter not required to be searched by this Authority, namely:

The subject matter of claim 17 relates to a method for treatment of human body by therapy as defined in PCT Rule 39.1(iv). This search has been carried out and based on the use of the claimed compounds for manufacturing medicaments for treating the alleged diseases.

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).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2023/135272

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
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		EP 3976616 A1	06 April 2022
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		KR 20220012321 A	03 February 2022

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2023/135272

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
		AU 2020285719 A1	03 February 2022