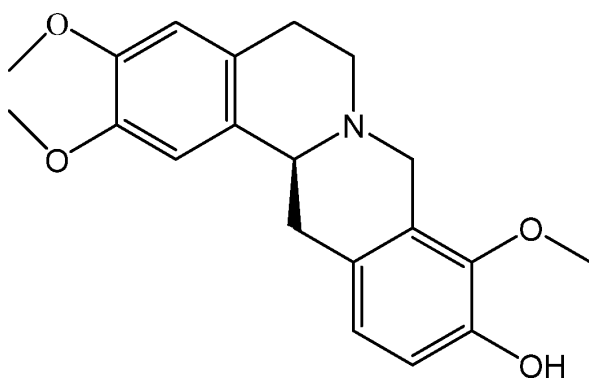


CRYSTALLINE SALTS OF CORYDALMINE

BACKGROUND

[001] 1. Field of the Discovery.

- 5 [002] The present disclosure relates to crystalline salt forms of the following Compound (1), also known as *l*-corydalmine or *l*-CDL, and methods for the preparation thereof, pharmaceutical compositions thereof, and their use in methods for the treatment of chronic pain, anxiety, insomnia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hepatic steatosis, and metabolic syndrome, et al.



Compound (1)

10 [003] 2. Background Information.

[004] Compound (1), also known as *l*-corydalmine or *l*-CDL, has CAS Number: 30413-84-

4. Compound (1) is a free base with an acid pKa of approximately 5.2. There is a need to produce Compound (1) in a form sufficient to enable formulations to meet exacting pharmaceutical requirements and specifications, while providing sufficient in-vivo exposure of the active drug. Further, the process by which Compound (1) is produced needs to be one which is amenable to large-scale production. Additionally, it is desirable that the product should be in a form that is easily processed, e.g. readily filterable and easily dried. Finally, it is desirable that the drug product of Compound (1) be stable for extended periods of time without the need for
15
20 3specialized storage conditions. Therefore, there is a need to identify crystalline pharmaceutically acceptable salts of Compound (1).

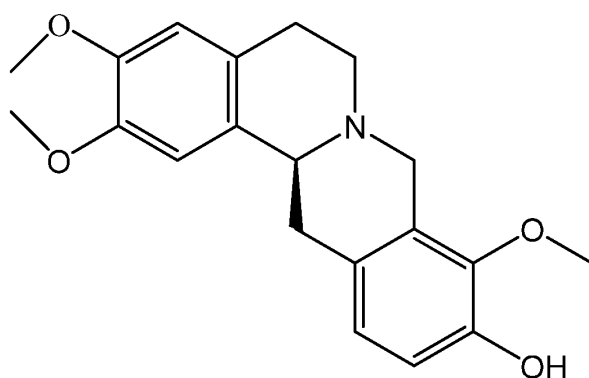
[005] In addition, many salt forms can exist in different crystal polymorphs, which exhibit different physical, chemical, and spectroscopic properties. For example, certain polymorphs of a salt may be more readily soluble in particular solvents, may flow more readily, or may compress

more easily than others. For a drug candidate, certain polymorphs may be more bioavailable than others, while others may be more stable under certain manufacturing, storage, and biological conditions. This is particularly important from a regulatory standpoint, since drugs are approved by agencies such as the U.S. Food and Drug Administration only if they meet
5 exacting purity and characterization standards. Indeed, the regulatory approval of one polymorph of a compound, which exhibits certain solubility and physico-chemical (including spectroscopic) properties, typically does not imply the ready approval of other polymorphs of that same compound. Therefore, there is a need to identify polymorphs of a salt that can provide a variety of advantages.

10

SUMMARY

[006] The present disclosure relates to crystalline salt forms of the following Compound (1), also known as *l*-corydalmine or *l*-CDL, and methods for the preparation thereof, pharmaceutical compositions thereof, and their use in methods for the treatment of chronic pain, anxiety,
15 insomnia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hepatic steatosis, and metabolic syndrome, et al.



Compound (1)

[007] In one aspect, the present disclosure relates to a crystalline salt form of Compound (1). In one specific aspect, the present disclosure relates to a crystalline hydrochloride, oxalate,
20 maleate, sulfate, or mesylate salt form of Compound (1).

[008] In one aspect, the present disclosure relates to a crystalline hydrochloride salt form of Compound (1). In one specific aspect, the crystalline hydrochloride salt form of Compound (1) is characterized by an X-ray powder diffraction ("XRPD") pattern comprising peaks selected from the group at approximately 9.7, 10.1, 14.3, 15.0, 17.2, 17.5, 18.9, 21.0, 21.4, 21.9, and 23.7

degrees 2θ and/or a differential scanning calorimetry (“DSC”) thermogram endotherm T_{onset} at approximately 252 °C.

[009] In one aspect, the present disclosure relates to a crystalline oxalate salt form of Compound (1). In one specific aspect, the crystalline oxalate salt form of Compound (1) is characterized by an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 11.7, 13.0, 15.4, 16.3, 19.0, 19.6, 20.4, 22.1, 24.5, and 25.2 degrees 2θ and/or a differential scanning calorimetry thermogram endotherm T_{onset} at approximately 205 °C.

[0010] In one aspect, the present disclosure relates to a crystalline maleate salt form of Compound (1). In one specific aspect, the crystalline maleate salt form of Compound (1) is characterized by an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 8.0, 10.4, 12.4, 14.0, 15.3, 16.4, 19.3, 20.8, 21.5, and 23.3 degrees 2θ and/or a differential scanning calorimetry thermogram endotherm T_{onset} at approximately 193 °C.

[0011] In one aspect, the present disclosure relates to a crystalline sulfate salt form of Compound (1). In one specific aspect, the crystalline sulfate salt form of Compound (1) is characterized by an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 8.4, 12.6, 13.7, 16.0, 16.7, 21.0, 21.8, 22.2, 23.4, and 27.5 degrees 2θ and/or a differential scanning calorimetry thermogram endotherm T_{onset} at approximately 229 °C.

[0012] In one aspect, the present disclosure relates to a crystalline mesylate salt form of Compound (1). In one specific aspect, the crystalline mesylate salt form of Compound (1) is characterized by an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 7.9, 11.3, 12.6, 12.9, 14.7, 16.1, 18.8, 20.0, 20.7, and 23.6 degrees 2θ and/or a differential scanning calorimetry thermogram endotherm T_{onset} at approximately 238 °C.

[0013] In one aspect, the present disclosure provides a pharmaceutical composition comprising any of the crystalline salt forms disclosed herein and a pharmaceutically acceptable carrier. In some specific aspects, the pharmaceutical composition is a single unit dosage form, and/or contains a substantially pure crystalline salt form disclosed herein.

[0014] In one aspect, the present disclosure provides a method of treating chronic pain, anxiety, insomnia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hepatic steatosis, and metabolic syndrome in a mammal comprising administering to the mammal a therapeutically-effective amount of any of the pharmaceutical compositions disclosed herein.

[0015] In another aspect, the present disclosure provides a method of treating chronic pain, anxiety, insomnia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hepatic steatosis, and metabolic syndrome in a mammal comprising administering to the mammal a pharmaceutical composition comprising a therapeutically-effective amount of any of the crystalline salt forms disclosed herein. The administration step can be carried out by oral, intravenous, intramuscular or subcutaneous in the amount of about 1 mg to about 1,000 mg, in the amount of about 5 mg to about 500 mg, in the amount of about 10 mg to about 200 mg, in the amount of about 10 mg to about 150 mg, in the amount of about 10 mg to about 120 mg, in the amount of about 10 mg, in the amount of about 20 mg, in the amount of about 40 mg, in the amount of about 80 mg, or in the amount of about 120 mg.

[0016] Further aspects, features, and advantages of the present disclosure will be apparent to those of ordinary skill in the art upon examining and reading the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present invention and, together with the description, serve to explain the principles of the invention. The drawings are only for the purpose of illustrating an embodiment of the invention and are not to be construed as limiting the invention. Further objects, features and advantages of the invention will become apparent from the following detailed description taken in conjunction with the accompanying figures showing illustrative embodiments of the invention, in which:

[0018] FIG. 1 illustrates a representative ¹HNMR spectrum of hydrochloride salt of Compound (1).

[0019] FIG. 2 illustrates a representative XRPD pattern of hydrochloride salt Form I of Compound (1).

[0020] FIG. 3 illustrates a representative polarized light microscopy ("PLM") image of hydrochloride salt Form I of Compound (1).

[0021] FIG. 4 illustrates representative differential scanning calorimetry ("DSC") and thermogravimetric analysis ("TGA") profiles of hydrochloride salt Form I of Compound (1).

[0022] FIG. 5 illustrates representative dynamic vapor sorption ("DVS") profiles of hydrochloride salt Form I of Compound (1).

- [0023] FIG. 6 illustrates a representative XRPD pattern of hydrochloride salt Form II of Compound (1).
- [0024] FIG. 7 illustrates representative DSC and TGA profiles of hydrochloride salt Form II of Compound (1).
- 5 [0025] FIG. 8 illustrates a representative XRPD pattern of hydrochloride salt Form III of Compound (1).
- [0026] FIG. 9 illustrates representative DSC and TGA profiles of hydrochloride salt Form III of Compound (1).
- [0027] FIG. 10 illustrates a representative XRPD pattern of hydrochloride salt Form IV of
10 Compound (1).
- [0028] FIG. 11 illustrates representative DSC and TGA profiles of hydrochloride salt Form IV of Compound (1).
- [0029] FIG. 12 illustrates a representative XRPD pattern of hydrochloride salt Form V of Compound (1).
- 15 [0030] FIG. 13 illustrates representative DSC and TGA profiles of hydrochloride salt Form V of Compound (1).
- [0031] FIG. 14 illustrates a representative XRPD pattern of hydrochloride salt Form VI of Compound (1).
- [0032] FIG. 15 illustrates representative DSC and TGA profiles of hydrochloride salt Form
20 VI of Compound (1).
- [0033] FIG. 16 illustrates a representative XRPD pattern of hydrochloride salt Form VII of Compound (1).
- [0034] FIG. 17 illustrates representative DSC and TGA profiles of hydrochloride salt Form VII of Compound (1).
- 25 [0035] FIG. 18 illustrates a representative XRPD patterns of hydrochloride salt Forms I to VII of Compound (1), from bottom to top, respectively.
- [0036] FIG. 19 illustrates a representative ¹HNMR spectrum of oxalate salt of Compound (1).
- [0037] FIG. 20 illustrates a representative XRPD pattern of oxalate salt Form I of Compound (1).
- 30 [0038] FIG. 21 illustrates a representative PLM image of oxalate salt Form I of Compound (1).

- [0039] FIG. 22 illustrates representative DSC and TGA profiles of oxalate salt Form I of Compound (1).
- [0040] FIG. 23 illustrates representative DVS profiles of oxalate salt Form I of Compound (1).
- 5 [0041] FIG. 24 illustrates a representative XRPD pattern of oxalate salt Form II of Compound (1).
- [0042] FIG. 25 illustrates representative DSC and TGA profiles of oxalate salt Form II of Compound (1).
- [0043] FIG. 26 illustrates a representative ¹HNMR spectrum of maleate salt of Compound
10 (1).
- [0044] FIG. 27 illustrates a representative XRPD pattern of maleate salt of Compound (1).
- [0045] FIG. 28 illustrates a representative PLM image of maleate salt Compound (1).
- [0046] FIG. 29 illustrates representative DSC and TGA profiles of maleate salt of Compound (1).
- 15 [0047] FIG. 30 illustrates representative DVS profiles of maleate salt of Compound (1).
- [0048] FIG. 31 illustrates representative ¹HNMR spectrum of (a) Compound (1), (b) sulfate salt, (c) mesylate salt, and (d) benzene sulfonate salt of Compound (1).
- [0049] FIG. 32 illustrates representative XRPD patterns of sulfate salt of Compound (1).
- [0050] FIG. 33 illustrates representative XRPD patterns of mesylate salt of Compound (1).
- 20 [0051] FIG. 34 illustrates a representative PLM image of (a) sulfate and (b) mesylate salts of Compound (1).
- [0052] FIG. 35 illustrates representative DSC and TGA profiles of sulfate salt of Compound (1).
- [0053] FIG. 36 illustrates representative DSC and TGA profiles of mesylate salt of
25 Compound (1).
- [0054] FIGs. 37A and 37B show the plasma drug-time curves obtained from male rats administrated with TLZ-16 freebase (compound (1)) at 3 mg/kg IV and 10 mg/kg POA.
- [0055] FIG. 38 shows the plasma drug-time curve obtained from male rats administrated with TLZ-16 HCl at 10 mg/kg POB.
- 30 [0056] FIG. 39 shows the plasma drug-time curve obtained from male rats administrated with TLZ-16 maleate at 10 mg/kg POC.

[0057] FIG. 40 shows the plasma drug-time curves obtained from male rats administrated with TLZ-16 mesylate at 10 mg/kg POD.

[0058] FIG. 41 shows the plasma drug-time curves obtained from male rats administrated with TLZ-16 oxalate at 10 mg/kg POE.

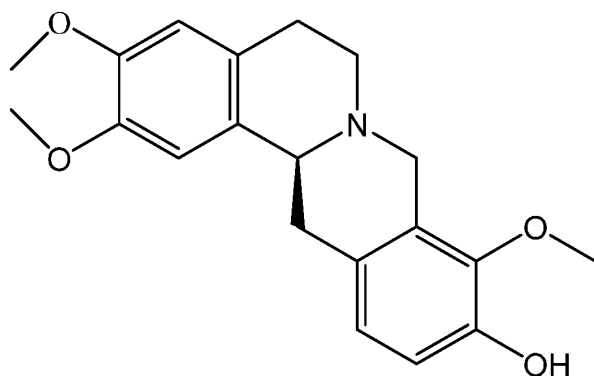
5 [0059] FIG. 42 shows the plasma drug-time curves obtained from male rats administrated with TLZ-16 sulfate at 10 mg/kg POF.

[0060] FIG. 43 shows the comparison of TLZ-16 plasma drug-time curves: male SD rats, 3 mg/kg IV, 10 mg/kg POA & POB & POC & POD & POE & POF (n=3).

10 DETAILED DESCRIPTION

[0061] The following is a detailed description provided to aid those skilled in the art in practicing the present disclosure. Those of ordinary skill in the art may make modifications and variations in the embodiments described herein without departing from the spirit or scope of the present disclosure. All publications, patent applications, patents, figures and other references
15 mentioned herein are expressly incorporated by reference in their entirety.

[0062] The present description provides crystalline salt forms of the following Compound (1), also known as *l*-corydalmine or *l*-CDL, and methods for the preparation thereof, pharmaceutical compositions thereof, and their use in methods for the treatment of chronic pain, anxiety, insomnia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hepatic steatosis,
20 and metabolic syndrome, et al.



Compound (1)

[0063] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure

belongs. The terminology used in the description is for describing particular embodiments only and is not intended to be limiting of the disclosure.

[0064] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise (such as in the case of a group containing a number of carbon atoms in which case each carbon atom number falling within the range is provided), between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the disclosure.

[0065] The following terms are used to describe the present disclosure. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The terminology used in the description is for describing particular embodiments only and is not intended to be limiting of the disclosure.

[0066] The articles “a” and “an” as used herein and in the appended claims are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article unless the context clearly indicates otherwise. By way of example, “an element” means one element or more than one element.

[0067] The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B,” when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0068] As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted
5 items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e., “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.”

10 [0069] In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent
15 Office Manual of Patent Examining Procedures, Section 2111.03.

[0070] As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from anyone or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and
20 not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in
25 one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

30 [0071] As used herein in the specification and in the claims, the term “pharmaceutically acceptable” with respect to a substance as used herein means that substance which is, within the

scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for the intended use when the substance is used in a pharmaceutical composition.

- 5 **[0072]** As used herein in the specification and in the claims, the terms “treat,” “treating” and “treatment” refer to the alleviation of a disease or disorder and/or at least one of its attendant symptoms.

- 10 **[0073]** As used herein in the specification and in the claims, the terms “polymorph” and “polymorphic form” refer to solid crystalline forms of a compound or a complex, such as a solvate or a hydrate. Different polymorphs of the same compound can exhibit different physical, chemical and/or spectroscopic properties. Different physical properties include, but are not limited to stability (e.g., to heat or light), compressibility and density (important in formulation and product manufacturing), and dissolution rates (which can affect bioavailability). Differences in stability can result from changes in chemical reactivity (e.g., differential oxidation, such that a dosage form discolors more rapidly when comprised of one polymorph than when comprised of another polymorph) or mechanical characteristics (e.g., tablets crumble on storage as a kinetically favored polymorph converts to thermodynamically more stable polymorph) or both (e.g., tablets of one polymorph are more susceptible to breakdown at high humidity). Different physical properties of polymorphs can affect their processing. For example, one polymorph might be more likely to form solvates or might be more difficult to filter or wash free of impurities than another due to, for example, the shape or size distribution of particles of it.

[0074] As used herein to refer to the spectra or data presented in graphical form (e.g., XRPD, DSC and NMR spectra), and unless otherwise indicated, the term “peak” refers to a peak or other special feature that one skilled in the art would recognize as not attributable to background noise.

- 25 **[0075] General Analytical Methods**

[0076] X-ray powder diffraction Analysis (XRPD)

The solid samples were determined by powder X-ray diffractometer (Bruker D8 advance) with LynxEye detector. The instrument parameters were listed below.

Scan: 3 degree 2θ to 40 degree 2θ

Increment: 0.02 degree 2θ

Scan speed: 0.3 sec/step
Voltage: 40 KV
Current: 40 mA
Rotation: On
Sample holder: Zero-background sample holder

It should be understood that relative intensities may vary depending on various factors, including sample preparation, instrumentation, and installation procedures and setting and for obtaining molecular spectrum. Therefore, the peak assignments listed herein are intended to include a variation of plus or minus 0.2 degrees 2 θ .

[0077] Thermogravimetric analysis (TGA)

Total weight loss was obtained on a TA Discovery TGA 55. The sample was heated in a tared aluminum pan, ramping 10 °C /min to the final temperature.

[0078] Differential scanning calorimetry analysis (DSC)

- 10 Thermal analysis was performed using a TA Discovery DSC 250. The sample was heated in an aluminum pin-hole hermetic pan, ramping 10 °C /min with 50 mL/min nitrogen purge to the final temperature. Note that the temperatures may vary depending on various factors, including sample preparation, instrumentation, and sample purity. Therefore, the temperatures reported herein are intended to include a variation of plus or minus 0.5 °C.

15 **[0079] Polarizing microscope (PLM)**

PLM analysis was performed on a Polarizing Microscope ECLIPSE LV100POL (Nikon, JPN).

[0080] ¹H NMR analysis

¹H NMR was performed using Bruker Advance 300 equipped with automated sampler (B-ACS 120).

20 **[0081] Dynamic vapor sorption (DVS)**

DVS was determined using DVS Intrinsic (SMS, UK). Transfer about 20 to 50 mg of the compound into a SMS dynamic vapor sorption model DVS and record the weight change with respect to a varying atmospheric humidity at 25 °C. Use the following parameters:

Equilibrium: 60 min
RH (%) measurement points:

Adsorption: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90

Desorption: 90, 80, 70, 60, 50, 40, 30, 20, 10, 0

[0082] Hydrochloride Salt of Compound (1)

[0083] Hydrochloride Salt of Compound (1) exhibits multiple crystalline polymorphs.

[0084] Form I

[0085] Hydrochloride ("HCl") salt Form I can be obtained from various solvents, including, but not limited to Acetone, isopropanol ("IPA"), or ethyl acetate ("EtOAc"). In some embodiment, Form I of crystalline hydrochloride salt was prepared by adding concentrated HCl aqueous solution (12 mol/L) to Compound (1) free base solutions of Acetone, IPA, or EtOAc, and Table 1 lists the details of the experimental conditions. In one specific embodiment, 1.07 g free base reacted with 333.3 μ L HCl in 25 mL Acetone by stirring overnight, the crystalline solids were filtered and dried at 40 °C, under vacuum. Form I was characterized by ¹HNMR, XRPD, PLM, DVS, TGA and DSC.

[0086] Table 1. Preparation of Form I of hydrochloride salt (1 eq. concentrated HCl)

Lot	Solvent	Volume (μ L)	Solid (mg)	Anti-solvent (μ L)	
32519-014A1	Acetone	700	30.5	N/A	N/A
32519-019A1		2200	100.5	N/A	N/A
32519-014B1	IPA	5000	29.7	Heptane	2500
32519-016A1	EtOAc	1800	30.9	N/A	N/A

[0087] FIG. 1 shows a representative ¹HNMR spectrum of Form I. The ¹HNMR spectrum shows that there is ~ 0.17 chemical shift occurred near 6.84 ppm, indicating that free base converts into salt with HCl.

[0088] FIG. 2 shows a representative XRPD pattern of Form I. The pattern is characterized by peaks, preferably three or more significant peaks, at approximately 9.7, 10.1, 14.3, 15.0, 17.2, 17.5, 18.9, 21.0, 21.4, 21.9, and 23.7 degrees 2 θ . XRPD pattern illustrates that Form I crystals has good crystallinity. Table 2 lists the XRPD peaks of Form I of hydrochloride salt.

[0089] Table 2. List of representative XRPD peaks of hydrochloride salt Form I

	Angle	Intensity %	d value
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	2-Theta degree	%	Angstrom
1	9.693	48.6	9.11751
2	10.118	100	8.73526
3	12.038	15.5	7.34598
4	12.28	18.5	7.20205
5	14.027	14.1	6.30851
6	14.28	68	6.19753
7	14.95	50.9	5.92118
8	15.901	10.8	5.56902
9	16.305	20.8	5.43208
10	17.243	35.8	5.13864
11	17.465	60.7	5.07365
12	18.637	15.1	4.75713
13	18.898	36.4	4.69202
14	19.616	16.3	4.52191
15	20.52	8.7	4.3248
16	20.971	33.3	4.23276
17	21.402	32.1	4.14846
18	21.914	31.6	4.0526
19	23.469	19.3	3.78751
20	23.748	97.3	3.74374
21	24.378	20.2	3.64837
22	25.138	22.5	3.53972
23	25.666	11.8	3.46807
24	26.181	6.6	3.40097
25	28.113	6.4	3.17156
26	28.721	10.5	3.10582
27	29.234	13.3	3.0524
28	29.884	11.1	2.98753
29	30.541	20.2	2.92475
30	31.086	11.6	2.8747
31	31.482	18.5	2.83937
32	32.34	7.2	2.76598
33	32.836	8.8	2.72533
34	33.506	10.7	2.67235
35	34.358	6.9	2.60802
36	35.209	7	2.54691
37	37.203	7.2	2.41488
38	38.146	6.9	2.35732
39	38.62	7.6	2.32943

[0090] Representative PLM image are shown in FIG. 3, which illustrates that Form I is rod-like crystalline solid.

[0091] Representative thermal characteristics of Form I are shown in FIG. 4. TGA data indicates that there is ~0.3% weight loss between RT and 150 °C, and the residual solvent is low. DSC data illustrates that Form I has one endothermic peak with the onset and peak temperatures of 252 and 259 °C respectively, and the enthalpy of the endothermic peak is about 120 J/g.

[0092] Representative DVS data are plotted in FIG. 5, which suggest that Form I of hydrochloride salt is slightly hygroscopic, and absorb about 0.57% at 80% RH. The solid form does not change after DVS test.

[0093] Solubility studies of Form I hydrochloride salt in water at 2 hours were higher than that of the free base, which were improved from free base solubility of less than 1 mg/mL to the Form I hydrochloride salt solubility of approximately 19.1 mg/mL.

[0094] Form II

[0095] Hydrochloride salt Form II can be obtained from methanol. In one embodiment, Form II can be obtained by stirring a Hydrochloride salt Form I slurry in methanol at RT for 3 days, and/or stirring the slurry at 50 °C for one day. In another embodiment, Form II can be obtained by evaporate a saturated hydrochloride salt methanol solution. In yet another embodiment, Form II can be obtained by adding an anti-solvent, e.g. methyl t-butyl ether ("MTBE"), Toluene, EtOAc, or IPA, into a methanol solution.

[0096] FIG. 6 shows a representative XRPD pattern of Form II. Representative thermal characteristics of Form II are shown in FIG. 7. The TGA and DSC data further indicate that Form II is a methanol solvate.

[0097] Form III

[0098] Hydrochloride salt Form III can be obtained from ethanol. In one embodiment, Form III can be obtained by stirring a Hydrochloride salt Form I slurry in ethanol at RT for 3 days, and/or stirring the slurry at 50 °C for one day.

[0099] FIG. 8 shows a representative XRPD pattern of Form III. Representative thermal characteristics of Form III are shown in FIG. 9. The TGA and DSC data further indicate that Form III is an ethanol solvate.

[0100] Form IV

[00101] Hydrochloride salt Form IV can be obtained from water. In one embodiment, Form IV can be obtained by stirring a Hydrochloride salt Form I slurry in water at RT for 3 days.

[00102] FIG. 10 shows a representative XRPD pattern of Form IV. Representative thermal characteristics of Form IV are shown in FIG. 11. The TGA and DSC data further indicate that Form IV is a hydrate.

[00103] Form V

[00104] Hydrochloride salt Form V can be obtained from water. In one embodiment, Form V can be obtained by stirring a Hydrochloride salt Form I slurry in water at RT 50 °C for one day.

[00105] FIG. 12 shows a representative XRPD pattern of Form V. Representative thermal characteristics of Form V are shown in FIG. 13. The TGA and DSC data further indicate that Form V is a hydrate.

[00106] Form VI

[00107] Hydrochloride salt Form VI can be obtained from acetonitrile ("ACN"). In one embodiment, Form VI can be obtained by stirring a hydrochloride salt Form I slurry in acetonitrile at RT 50 °C for one day.

[00108] FIG. 14 shows a representative XRPD pattern of Form VI. Representative thermal characteristics of Form VI are shown in FIG. 15. The TGA and DSC data further indicate that Form VI is an acetonitrile solvate.

[00109] Form VII

[00110] Hydrochloride salt Form VII can be obtained from ethanol and/or water. In one embodiment, Form VII can be obtained by evaporating a saturated hydrochloride salt ethanol solution or water solution.

[00111] FIG. 16 shows a representative XRPD pattern of Form VII. Representative thermal characteristics of Form VI are shown in FIG. 17. The TGA and DSC data further indicate that Form VII is an ethanol solvate or a hydrate. The XRPD patterns of the ethanol solvate and the hydrate are nearly the same, indicating the ethanol solvate and the hydrate of Form VII have similar crystal lattice.

[00112] FIG. 18 illustrates a comparison of XRPD patterns of Forms I – VII. Table 3 summarizes the DSC and TGA data of Form II – VII of hydrochloride salt.

[00113] **Table 3.** DSC and TGA of Form II – VII of Hydrochloride salt.

Lot	XRPD	DSC	TGA	Comment
32519-054B1	Form II	36/61 °C; 12 J/g 182/194 °C; 44 J/g 242/248 °C; 9 J/g	RT-56 °C: 1.1% 56-200 °C: 7.1%	MeOH solvate
32519-059A1	Form III	129/144 °C; 49 J/g 167/172 °C; 3 J/g 254/261 °C; 94 J/g	RT-180 °C: 11.2%	EtOH solvate
32519-059B1	Form IV	54/77 °C; 38 J/g 102/122 °C; 43 J/g exo: 195/200 °C; 31 J/g 250/258 °C; 101 J/g	RT-150 °C: 2.8%	Hydrate
32519-059C1	Form V	58/78 °C; 60 J/g 98/118 °C; 23 J/g exo: 168/176 °C; 6.2 J/g 253/262 °C; 107 J/g	RT-61 °C: 2.1% 61-150 °C: 1.4%	Hydrate
32519-059D1	Form VI	113/119 °C; 62 J/g 167/179 °C; 12 J/g exo: 191/194 °C; 21 J/g exo: 200/206 °C; 3.6 J/g 247/257 °C; 94 J/g	RT-150 °C: 7.3%	ACN solvate
32519-045B2	Form VII	82/95 °C; 11 J/g exo: 133/150 °C; 14 J/g 221/234 °C; 60 J/g	RT-200 °C: 3.4%	EtOH solvate

[00114] Oxalate Salt of Compound (1)

[00115] Oxalate Salt of Compound (1) exhibits two crystalline polymorphs.

[00116] Form I

- 5 **[00117]** Oxalate salt Form I can be obtained from various solvents, including, but not limited to EtOAc. In one embodiment, 1.07 g free base was reacted with 420 mg oxalic acid in 72 mL EtOAc by stirring overnight, the crystalline solids were filtered and dried at 40 °C, under vacuum. Oxalate salt Form I was characterized by ¹HNMR, XRPD, PLM, DVS, TGA and DSC.

- [00118]** FIG. 19 shows a representative ¹HNMR spectrum of oxalate salt Form I. The
10 ¹HNMR spectrum shows that there is ~ 0.17 chemical shift occurred near 6.84 ppm, indicating that free base converts into oxalate salt.

[00119] FIG. 20 shows a representative XRPD pattern of oxalate salt Form I. The pattern is characterized by peaks, preferably three or more significant peaks, at approximately 11.7, 13.0, 15.4, 16.3, 19.0, 19.6, 20.4, 22.1, 24.5, and 25.2 degrees 2 θ . XRPD pattern illustrates that oxalate salt Form I crystals have good crystallinity. Table 4 lists the XRPD peaks of Form I of oxalate salt.

[00120] **Table 4.** List of representative XRPD peaks of oxalate salt Form I

	Angle	Intensity %	d value
	2-Theta degree	%	Angstrom
1	10.886	19.9	8.12063
2	11.715	100	7.54772
3	12.311	15.5	7.1837
4	12.522	47	7.063
5	13.008	26.9	6.8002
6	15.129	19.3	5.85166
7	15.377	29.4	5.75774
8	15.786	12.7	5.60954
9	16.336	61.3	5.42178
10	17.469	12.8	5.07268
11	18.969	21.4	4.67461
12	19.568	83.3	4.53297
13	20.385	29.8	4.3531
14	20.788	14.7	4.26951
15	21.709	11.3	4.09055
16	22.107	48.2	4.0178
17	22.921	17.5	3.87688
18	23.549	10.8	3.7749
19	23.888	18.7	3.72209
20	24.518	58.7	3.62775
21	24.954	20	3.56538
22	25.196	31.9	3.53176
23	25.798	14.7	3.45065
24	26.419	17.6	3.37092
25	26.853	11.2	3.31737
26	27.795	7.6	3.20709
27	28.381	16	3.14222
28	29.767	14.5	2.99893
29	30.116	11.2	2.96499
30	30.758	9.3	2.90456
31	31.233	9.1	2.86148

32	31.981	9.5	2.79624
33	35.162	8	2.55022
34	35.657	11.8	2.51596
35	37.703	6.7	2.38395
36	38.1	6.7	2.36003

[00121] Representative PLM image are shown in FIG. 21.

[00122] Representative thermal characteristics of oxalate salt Form I are shown in FIG. 22. TGA data indicates that there is ~0.56% weight loss between RT and 150 °C, and the residual solvent is low. DSC data illustrates that Form I has one endothermic peak with the onset and peak temperatures of 205 and 206 °C respectively, and the enthalpy of the endothermic peak is about 100 J/g.

[00123] Representative DVS data are plotted in FIG. 23, which suggest that Form I of oxalate salt is slightly hygroscopic, and absorb about 0.25 wt% at 80% RH. The solid form does not change after DVS test.

[00124] Solubility studies of Form I oxalate salt in water (in 2 hours) were higher than that of the free base, which were improved from free base solubility of less than 1 mg/mL to the Form I oxalate salt solubility of approximately 19.2 mg/mL.

[00125] Form II

[00126] Oxalate salt Form II can be obtained from methanol. In one embodiment, Form II can be obtained by stirring a oxalate salt Form I slurry in methanol at RT for 3 days, and/or stirring the slurry at 50 °C for one day. In another embodiment, oxalate salt Form II can be obtained by adding an anti-solvent, e.g. MTBE, Toluene, EtOAc, or IPA, into a methanol solution.

[00127] FIG. 24 shows a representative XRPD pattern of Form II. Representative thermal characteristics of Form II are shown in FIG. 25. The TGA and DSC data further indicate that oxalate salt Form II is a methanol solvate.

[00128] **Maleate Salt of Compound (1)**

[00129] Maleate Salt

[00130] Maleate salt can be obtained from various solvents, including, but not limited to EtOAc and MeOH. In one embodiment, 1.03 g free base was reacted with 356 mg maleic acid in

72 mL EtOAc by stirring overnight, the crystalline solids were filtered and dried at 40 °C, under vacuum. Maleate salt was characterized by ¹HNMR, XRPD, PLM, DVS, TGA and DSC.

[00131] FIG. 26 shows a representative ¹HNMR spectrum of maleate salt. The ¹HNMR spectrum shows that that maleic acid formed maleate with free base in a molar ratio of 1:1.

- 5 [00132] FIG. 27 shows a representative XRPD pattern of maleate salt. The pattern is characterized by peaks, preferably three or more significant peaks, at approximately 8.0, 10.4, 12.4, 14.0, 15.3, 16.4, 19.3, 20.8, 21.5, and 23.3 degrees 2θ. XRPD pattern illustrates that maleate salt crystals have good crystallinity. Table 5 lists the XRPD peaks of maleate salt.

[00133] Table 5. List of representative XRPD peaks of maleate salt

10

	Angle	Intensity %	d value
	2-Theta degree	%	Angstrom
1	8.029	46.3	11.0025
2	10.392	33.9	8.50589
3	11.223	10.6	7.87745
4	12.362	46.3	7.1544
5	13.372	8.3	6.61605
6	14.032	70.6	6.30655
7	15.347	65.9	5.76892
8	16.168	13.9	5.47786
9	16.367	46.7	5.41155
10	16.823	15.4	5.2658
11	18.266	6.6	4.85293
12	18.794	10.2	4.71788
13	19.293	100	4.59699
14	20.557	27	4.31692
15	20.832	34.3	4.2607
16	21.471	69.5	4.13526
17	22.509	9.7	3.94683
18	23.32	54.8	3.81148
19	24.35	7.9	3.6525
20	24.934	15.6	3.56819
21	25.381	7.4	3.50642
22	26.159	15.5	3.40378
23	26.574	26.2	3.35157
24	27.037	27.5	3.29528
25	27.606	7.9	3.22861
26	28.627	16	3.11574

27	29.065	6.7	3.06977
28	29.636	6.1	3.01191
29	31.017	14.7	2.88089
30	31.638	7.2	2.82579
31	32.607	6.7	2.74392
32	33.121	6.2	2.70256
33	33.745	4.9	2.65397
34	34.323	5.3	2.61061
35	35.065	4.2	2.55706
36	35.242	5.2	2.54461
37	35.819	4.9	2.50489
38	37.778	5.7	2.37939
39	38.867	4.6	2.31521

[00134] Representative PLM image is shown in FIG. 28.

[00135] Representative thermal characteristics of maleate salt are shown in FIG. 29. TGA data indicates that there is ~0.92% weight loss between RT and 150 °C, which is due to the loss of the residue solvent. DSC data illustrates that has one endothermic peak with the onset and peak temperatures of 193 and 197 °C respectively, and the enthalpy of the endothermic peak is about 104 J/g.

[00136] Representative DVS data are plotted in FIG. 30, which suggest that of maleate salt is slightly hygroscopic, and absorb about 0.085 wt% at 80% RH. The solid form does not change after DVS test.

[00137] Solubility studies of maleate salt in water (in 2 hours) is higher than that of the free base, which is improved from free base solubility of less than 1 mg/mL to the maleate salt solubility of approximately 11.0 mg/mL.

[00138] **Sulfate, Mesylate, and Benzene sulfonate Salts of Compound (1)**

[00139] Sulfate Salt

[00140] Sulfate salt can be obtained from various solvents, including, but not limited to EtOAc and Acetone. In one embodiment, 1 mL EtOAc solution of free base (15 mg/mL) was added into 500 µL EtOAc solution of H₂SO₄ (0.07 mol/L), crystalline solids were obtained by stirring overnight, the crystalline solids were filtered and dried at 40 °C, under vacuum. Sulfate salt was characterized by ¹HNMR, XRPD, PLM, TGA and DSC.

[00141] Mesylate Salt

[00142] Mesylate salt can be obtained from various solvents, including, but not limited to EtOAc and Acetone. In one embodiment, 2.6 μ L (1 eq) methanesulfonic acid was added into 1 mL EtOAc solution of free base (15 mg/mL), crystalline solids were obtained by stirring overnight, the crystalline solids were filtered and dried at 40 °C, under vacuum. Mesylate salt was characterized by ¹HNMR, XRPD, PLM, TGA and DSC.

[00143] Benzene Sulfonate Salt

[00144] Benzene sulfonate salt can be obtained from various solvents, including, but not limited to EtOAc and Acetone. In one embodiment, 7.6 mg (1 eq) Benzenesulfonic acid was added into 1 mL EtOAc solution of free base (15 mg/mL), crystalline solids were obtained by stirring overnight, the crystalline solids were filtered and dried at 40 °C, under vacuum. Benzene sulfonate salt was characterized by ¹HNMR, XRPD, PLM, TGA and DSC. XRPD pattern illustrates that sulfate and mesylate crystals have good crystallinity, and benzene sulfonate salt has poor crystallinity.

[00145] FIG. 31 shows a representative ¹HNMR spectrum of sulfate, mesylate, and benzene sulfonate salts of Compound (1). The ¹HNMR spectrum shows that that Compound (1) forms salt with sulfuric acid, methanesulfonic acid, and benzenesulfonic acid.

[00146] FIG. 32 shows a representative XRPD pattern of sulfate salt. The XRPD pattern of sulfate salt is characterized by peaks, preferably three or more significant peaks, at approximately 8.4, 12.6, 13.7, 16.0, 16.7, 21.0, 21.8, 22.2, 23.4, and 27.5 degrees 2 θ . FIG. 33 shows a representative XRPD pattern of mesylate salt. The XRPD pattern of The XRPD pattern of mesylate salt is characterized by peaks, preferably three or more significant peaks, at approximately 7.9, 11.3, 12.6, 12.9, 14.7, 16.1, 18.8, 20.0, 20.7, and 23.6 degrees 2 θ . Tables 6 and 7 list the XRPD peaks of sulfate salt and mesylate salt, respectively.

[00147] Table 6. List of representative XRPD peaks of sulfate salt

	Angle	Intensity %	d value
	2-Theta degree	%	Angstrom
1	8.433	100	10.47628
2	11.259	17.6	7.85273
3	12.601	39.5	7.019
4	13.716	45.9	6.45111
5	14.737	14.5	6.00615
6	15.633	25.9	5.6641
7	16.034	30.4	5.52328

8	16.658	34.9	5.31764
9	17.048	20.5	5.1968
10	18.191	5.7	4.87295
11	18.818	8.1	4.71183
12	19.367	17	4.57955
13	19.62	23.7	4.5211
14	21.011	82.2	4.22479
15	21.784	61.7	4.07652
16	22.181	36.3	4.00454
17	22.649	8.1	3.92287
18	22.961	13.3	3.87017
19	23.356	36.3	3.8056
20	23.771	22.2	3.74003
21	24.042	11.1	3.69862
22	25.498	12.7	3.49051
23	26.236	7.7	3.39404
24	26.718	12.8	3.33393
25	27.466	30.4	3.24479
26	28.034	11.9	3.18035
27	29.242	4.4	3.05159
28	29.657	4.9	3.00983
29	30.522	6.3	2.92648
30	31.352	4.2	2.85091
31	32.056	8	2.78983
32	32.983	5.5	2.71353
33	33.485	5	2.67396
34	35.071	5.3	2.55661
35	37.409	4.9	2.40202
36	39.314	3.5	2.2899

[00148] Table 7. List of representative XRPD peaks of mesylate salt

	Angle	Intensity %	d value
	2-Theta degree	%	Angstrom
1	6.814	22.9	12.96112
2	7.858	53.5	11.24213
3	11.285	34.3	7.83485
4	12.576	51.5	7.03303
5	12.877	39.7	6.86939
6	13.877	10.7	6.37637
7	14.708	39.5	6.01816
8	15.147	15	5.84465

9	15.835	28.3	5.59217
10	16.132	43.1	5.48993
11	17.554	9.4	5.04811
12	18.248	22.8	4.8577
13	18.791	35.9	4.71852
14	19.255	12.6	4.60591
15	19.682	17.4	4.50693
16	20.03	100	4.42932
17	20.737	64.1	4.27995
18	21.295	10.1	4.16914
19	22.147	13.6	4.01062
20	22.583	28.6	3.93402
21	23.053	16.5	3.85497
22	23.383	22.9	3.80128
23	23.592	34.5	3.76808
24	24.135	29.7	3.68452
25	25.047	20.3	3.55244
26	25.516	13.7	3.48817
27	25.991	35	3.4255
28	26.504	21.9	3.36029
29	28.399	7.1	3.14023
30	29.874	13.2	2.98846
31	30.479	10.9	2.93049
32	30.912	10.6	2.89045
33	31.496	10.8	2.83821
34	32.603	7.8	2.7443
35	38.306	8.9	2.3478

[00149] Representative PLM images of sulfate salt and mesylate salt are shown in FIG. 34.

[00150] Representative thermal characteristics of sulfate salt are shown in FIG. 35. TGA data indicates that there is ~0.89% weight loss between RT and 150 °C, which is due to the loss of the residue solvent. DSC data illustrates that sulfate salt has one endothermic peak with the onset and peak temperatures of 229 and 234 °C respectively, and the enthalpy of the endothermic peak is about 101 J/g. Representative thermal characteristics of mesylate salt are shown in FIG. 36. TGA data indicates that there is ~0.73% weight loss between RT and 150 °C, which is due to the loss of the residue solvent. DSC data illustrates that mesylate salt has one endothermic peak with the onset and peak temperatures of 238 and 240 °C respectively, and the enthalpy of the endothermic peak is about 83 J/g.

[00151] Solubility studies of sulfate and mesylate salt in water (in 2 hours) is higher than that of the free base, which is improved from free base solubility of less than 1 mg/mL to the salt solubility of approximately 20 mg/mL.

[00152] Methods of Use and Pharmaceutical Compositions

5 [00153] Crystalline salt forms of the invention exhibit physical characteristics that are beneficial for drug manufacture, storage and/or use.

[00154] This invention encompasses methods of treating and preventing a wide variety of diseases and conditions using crystalline salt forms of the invention. In each of the methods, a therapeutically or prophylactically effective amount of the crystalline salt or polymorph is administered to a patient in need of such treatment or prevention. Examples of such disease and conditions include, but are not limited to, chronic pain, anxiety, insomnia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hepatic steatosis, and metabolic syndrome. Examples of these and other diseases and disorders that can be treated or prevented using compositions of the invention are described in Pat. Appl. No. US20180071269A1, 10 WO2009002873A1, EP1911456A1, CN101327214A, and CN106176740A. The entirety of each of the patents and patent applications cited herein is incorporated herein by reference.

[00155] The therapeutically-effective amount of any of the crystalline salt forms disclosed herein is not particular limited, and the administration step can be carried out by oral, intravenous, intramuscular or subcutaneous in the amount of about 1 mg to about 1,000 mg, in 20 the amount of about 5 mg to about 500 mg, in the amount of about 10 mg to about 200 mg, in the amount of about 10 mg to about 150 mg, in the amount of about 10 mg to about 120 mg, in the amount of about 10 mg, in the amount of about 20 mg, in the amount of about 40 mg, in the amount of about 80 mg, or in the amount of about 120 mg.

[00156] Depending on the disease to be treated and the subject's condition, crystalline salt 25 forms of the invention can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous, ICV, intracisternal injection or infusion, subcutaneous injection, or implantation), inhalation spray, nasal, vaginal, rectal, sublingual, or topical routes of administration and may be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles 30 appropriate for each route of administration. Because individual crystalline salt forms have different dissolution, stability, and other properties, the optimal polymorph used in methods of

treatment may depend on the route of administration. For example, crystalline salt forms that are readily soluble in aqueous solutions are preferably used to provide liquid dosage forms, whereas crystalline salt forms that exhibit great thermal stability may be preferred in the manufacture of solid dosage forms (e.g., tablets and capsules).

- 5 [00157] Although the physical characteristics of crystalline salt forms can, in some cases, affect their bioavailability, amounts of the polymorphs that are therapeutically or prophylactically effective in the treatment of various disease and conditions can be readily determined by those of ordinary skill in the pharmacy or medical arts.

[00158] The invention encompasses pharmaceutical compositions and single unit dosage
10 forms that can be used in methods of treatment and prevention, which comprise one or more crystalline salt forms of Compound (1) and optionally one or more excipients or diluents. Specific compositions and dosage forms are disclosed in the various patents and patent applications incorporated herein by reference.

15 EXAMPLES

[00159] The embodiments described above in addition to other embodiments can be further understood with reference to the following examples:

[00160] Methods Identifying Different Polymorphs

[00161] Slurry Study at room temperature

- 20 [00162] Certain amount of solid was added into specific solvent, and the mixture slurry was stirred at RT for 3 days. The slurry were filtered and characterized by XRPD, and new patterns obtained were further analyzed by DSC and TGA.

[00163] Slurry Study at 50 °C

- [00164] The slurry study at room temperature samples were heated to 50 °C, and stirred for 1
25 day. The slurry was filtered and characterized by XRPD, and new patterns obtained were further analyzed by DSC and TGA.

[00165] Evaporation crystallization

- [00166] Certain amount of solvent was added into the slurry study at room temperature
30 samples. The slurry samples were filtered by 0.22 μm membrane, and then the saturated solutions were evaporated under ambient condition. New patterns obtained were further analyzed by DSC and TGA.

[00167] Anti-solvent crystallization

[00168] Certain amount of crystalline salt was added MeOH and/or H₂O to form a solution, followed by adding anti-solvent to precipitate out the solids. The solids were characterized by XRPD and new patterns obtained were further analyzed by DSC and TGA.

5 [00169] Preparing the hydrochloride salt Form I

[00170] 1.07 g Compound (1) was dissolved in 25 mL acetone, followed by reacting with 333.3 μ L concentrated HCl. The reaction mixture was stirred overnight, and solids were precipitated out from the solution. Solids were collected by filtration and dried at 40 °C under vacuum for 5 hours. The solids were characterized as crystalline materials by PLM and XRPD,
10 with a DSC melting onset temperature (“T_{onset}”) of about 252 °C.

[00171] Preparing the oxalate salt Form I

[00172] 1.07 g Compound (1) was dissolved in 72 mL EtOAc, followed by reacting with 420 mg oxalic acid. The reaction mixture was stirred overnight, and solids were precipitated out from the solution. Solids were collected by filtration and dried at 40 °C under vacuum for 5
15 hours. The solids were characterized as crystalline materials by PLM and XRPD, with a DSC melting T_{onset} of about 205 °C.

[00173] Preparing the maleate salt

[00174] 1.03 g Compound (1) was dissolved in 72 mL EtOAc, followed by reacting with 356.2 mg maleic acid. The reaction mixture was stirred overnight, and solids were precipitated
20 out from the solution. Solids were collected by filtration and dried at 40 °C under vacuum for 5 hours. The solids were characterized as crystalline materials by PLM and XRPD, with a DSC melting T_{onset} of about 193 °C.

[00175] Preparing the sulfate salt

[00176] 15 mg Compound (1) was dissolved in 1 mL EtOAc, followed by reacting with
25 500 μ L H₂SO₄ (0.07 mol/L) EtOAc solution. The reaction mixture was stirred overnight, and solids were precipitated out from the solution. Solids were collected by filtration and dried at 40 °C under vacuum for 5 hours. The solids were characterized as crystalline materials by PLM and XRPD, with a DSC melting T_{onset} of about 229 °C.

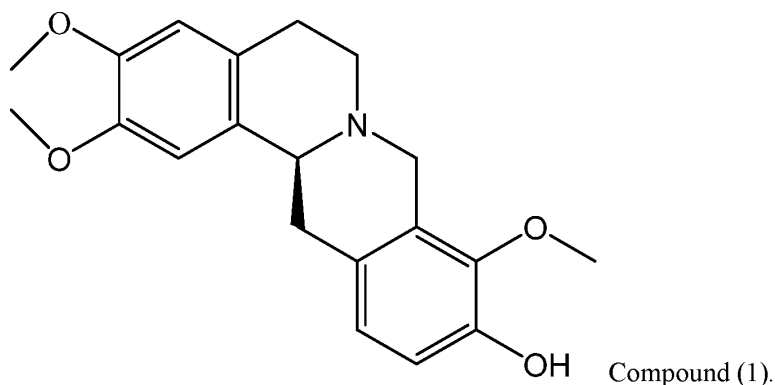
[00177] Preparing the mesylate salt

30 [00178] 15 mg Compound (1) was dissolved in 1 mL EtOAc, followed by reacting with 2.6 μ L methanesulfonic acid. The reaction mixture was stirred overnight, and solids were

precipitated out from the solution. Solids were collected by filtration and dried at 40 °C under vacuum for 5 hours. The solids were characterized as crystalline materials by PLM and XRPD, with a DSC melting T_{onset} of about 238 °C.

[00179] EXEMPLARY EMBODIMENTS

- 5 **[00180]** A crystalline salt form of Compound (1)



[00181] A crystalline salt form of Compound (1), wherein the crystalline salt form is a hydrochloride, oxalate, maleate, sulfate, or mesylate salt.

- 10 **[00182]** A crystalline salt form of Compound (1), wherein the crystalline salt form is a hydrochloride salt having an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 9.7, 10.1, 14.3, 15.0, 17.5, 18.9, 21.9, and 23.7 degrees 2θ .

- [00183]** A crystalline salt form of Compound (1), wherein the crystalline salt form is a hydrochloride salt having a differential scanning calorimetry thermogram endotherm T_{onset} at approximately 252 °C and an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 9.7, 10.1, 14.3, 15.0, 17.5, 18.9, 21.9, and 23.7 degrees 2θ .

- 15 **[00184]** A crystalline salt form of Compound (1), wherein the crystalline salt form is an oxalate salt having an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 11.7, 13.0, 15.4, 16.3, 19.6, 22.1, and 24.5 degrees 2θ .

- 20 **[00185]** A crystalline salt form of Compound (1), wherein the crystalline salt form is an oxalate salt having a differential scanning calorimetry thermogram endotherm T_{onset} at approximately 205 °C and an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 11.7, 13.0, 15.4, 16.3, 19.6, 22.1, and 24.5 degrees 2θ .

[00186] A crystalline salt form of Compound (1), wherein the crystalline salt form is a maleate salt having an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 8.0, 10.4, 12.4, 14.0, 15.3, 16.4, 19.3, 21.5, and 23.3 degrees 2 θ .

[00187] A crystalline salt form of Compound (1), wherein the crystalline salt form is a maleate salt having a differential scanning calorimetry thermogram endotherm T_{onset} at approximately 193 °C and an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 8.0, 10.4, 12.4, 14.0, 15.3, 16.4, 19.3, 21.5, and 23.3 degrees 2 θ .

[00188] A crystalline salt form of Compound (1), wherein the crystalline salt form is a sulfate salt having an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 8.4, 12.6, 13.7, 16.7, 21.0, 21.8, 22.2, and 23.4 degrees 2 θ .

[00189] A crystalline salt form of Compound (1), wherein the crystalline salt form is a sulfate salt having a differential scanning calorimetry thermogram endotherm T_{onset} at approximately 229 °C and an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 8.4, 12.6, 13.7, 16.7, 21.0, 21.8, 22.2, and 23.4 degrees 2 θ .

[00190] A crystalline salt form of Compound (1), wherein the crystalline salt form is a mesylate salt having an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 7.9, 11.3, 12.6, 12.9, 14.7, 16.1, 20.0, and 20.7 degrees 2 θ .

[00191] A crystalline salt form of Compound (1), wherein the crystalline salt form is a mesylate salt having a differential scanning calorimetry thermogram endotherm T_{onset} at approximately 238 °C and an X-ray powder diffraction pattern comprising peaks selected from the group at approximately 7.9, 11.3, 12.6, 12.9, 14.7, 16.1, 20.0, and 20.7 degrees 2 θ .

[00192] A pharmaceutical composition comprising any of the crystalline salt forms disclosed herein and a pharmaceutically acceptable carrier.

[00193] A pharmaceutical composition comprising any of the crystalline salt forms disclosed herein, wherein the pharmaceutical composition is a single unit dosage form.

[00194] A pharmaceutical composition comprising any of the crystalline salt forms disclosed herein, wherein the crystalline salt form is in substantially pure form.

[00195] A method of treating chronic pain, anxiety, insomnia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hepatic steatosis, and metabolic syndrome in a mammal comprising administering to the mammal a therapeutically-effective amount of any of the pharmaceutical compositions disclosed herein.

[00196] A method of treating chronic pain, anxiety, insomnia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hepatic steatosis, and metabolic syndrome in a mammal comprising administering to the mammal a pharmaceutical composition comprising a therapeutically-effective amount of any of the crystalline salt forms disclosed herein.

5

EXAMPLE 1. PHARMACOKINETIC AND BRAIN PENETRATION STUDY

[00197] Purpose

[00198] To determine pharmacokinetic profile and brain penetration of TLZ-16 freebase (Compound (1)) and its five salts

10 [00199] Study Design

[00200] The study groups are shown in the following table.

Group	Treatment	Dose Level (mg/kg) (Equal to freebase)	Dose Volume (mL/kg)	Conc. (mg/mL)	Administration Route	No. of Animals
1	TLZ-16 freebase	3	5	0.6	IV	3 MALE
2		10	10	1	PO-A	3 MALE
3	TLZ-16 freebase	10	10	1	PO-A	6 MALE
4	TLZ-16 HCl	10	10	1	PO-B	3 MALE
5		10	10	1	PO-B	6 MALE
6	TLZ-16 maleate	10	10	1	PO-C	3 MALE
7		10	10	1	PO-C	6 MALE
8	TLZ-16 mesylate	10	10	1	PO-D	3 MALE
9		10	10	1	PO-D	6 MALE
10	TLZ-16 oxalate	10	10	1	PO-E	3 MALE
11		10	10	1	PO-E	6 MALE

12	TLZ-16 sulfate	10	10	1	PO-F	3 MALE
13		10	10	1	PO-F	6 MALE

Note: the dose level of salt forms should be corrected by the salt factor

[00201] Experimental procedure

A. Pharmacokinetics (PK) Schedule:

Group	PK time points
1	Plasma: 0, 0.083h, 0.25h, 0.5h, 1h, 2h, 4h, 8h, 24h post dose
2, 4, 6, 8, 10, 12	Plasma: 0, 0.25h, 0.5h, 1h, 2h, 4h, 8h, 24h post dose Brain: 24h
3, 5, 7, 9, 11, 13	Plasma and Brain: 1h, 2h, 4h (N=2/time point) post dose

5

Acceptable Time Ranges for Blood Collection

Time Points	Acceptable Time Ranges
5-30 min	Within ± 0.25 minute
1- 2 hr	Within ± 2 minutes
4-24 hr	Within ± 10 minutes

B. Blood Sample Collection and Processing

Collection Site:	Jugular vein
Volume Collected:	~ 0.2 mL per time point
Anticoagulant:	EDTA-K2
Blood Samples Processing and Storage:	1) Approximately 0.2 mL blood will be collected at each time point. Blood of each sample will be transferred into plastic micro centrifuge tubes containing anticoagulant. 2) All blood samples will be centrifuged at 4,000 g for 5 minutes at 4°C to obtain plasma. The samples will be stored in a freezer at $-75\pm 15^{\circ}\text{C}$ prior to analysis.

Brain Samples Processing and Storage:	1) The animal will be fully exsanguinated prior to brain collection. Procedure: open chest cavity, cut ventricle and perform a gentle iv saline flush (saline flush volume ~ 20 ml) with the animal placed head down at a 45 degree angle to facilitate blood removal. 2) Brain samples will be collected at adopted time point, quick frozen in ice box. 3) All brain samples will be weighted and homogenated with water by brain weight (g) to water volume (mL) at ratio 1:3 before analysis. The actual concentration is the detected value multiplied by the dilution factor.
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C. Evaluations during the In-life Phase:

Procedure	Frequency
Cageside Observations:	Twice daily
Detailed Clinical Observations:	Once prior to dosing on Day 1
Body Weight:	Once prior to each dosing

Note: Unscheduled clinical observations (cageside or detailed) will also be performed, as needed.

5

D. Test System

Species/Strain/Sex:	Male SD Rat
Source:	Si Bei Fu Laboratory Animal Technology Co. Ltd
Age at Day of Dosing:	Approximately 6-8 weeks
Weight at Day of Dosing:	Approximately 200-300 g

[00202] Test Article and Dose Formulations

A. Test Article Information

Test Article Identification	Lot No.	Retest Date	MW	FW	Storage Condition
TLZ-16 freebase			341.41	341.41	RT
TLZ-16 HCl			341.41	377.87	RT
TLZ-16 maleate			341.41	457.48	RT
TLZ-16 mesylate			341.41	437.51	RT
TLZ-16 oxalate			341.41	431.44	RT
TLZ-16 sulfate			341.41	439.48	RT

B. Dose Formulation

Formulation Frequency:	Freshly prepared on of the day of dosing
Vehicle Composition:	IV/POA: 20%HP- β -CD in water
	POB/POC/POD/POE/POF: Water
Storage Condition:	Dose formulation for dosing: Room temperature
Preparation Procedures:	TBD

[00203] PK Samples Analyses

5 **[00204]** Concentrations of Test Article in the plasma samples will be analyzed using a LC-MS/MS method.

[00205] WinNonlin (PhoenixTM, version 6.1) or other similar software will be used for pharmacokinetic calculations. The following pharmacokinetic parameters will be calculated, whenever possible from the plasma concentration versus time data:

10 **[00206]** IV administration: $T_{1/2}$, C_0 , AUC_{last} , AUC_{inf} , MRT_{inf} , Cl , V_{ss} , Number of Points for Regression.

[00207] PO administration: $T_{1/2}$, C_{max} , T_{max} , MRT_{inf} , AUC_{inf} , AUC_{last} , $F\%$, Number of Points for Regression.

15 **[00208]** The pharmacokinetic data will be described using descriptive statistics such as mean, standard deviation.

[00209] Additional pharmacokinetic or statistical analysis may be performed at the discretion of the contributing scientist, and will be documented in the data summary.

[00210]

[00211] Results

20 **[00212] (1) TLZ-16 freebase**

TLZ-16 freebase concentration in plasma (IV, 3 mg/kg)

Time (h)	Conc. (ng/mL)			Mean	RSD
	Rat 1	Rat 2	Rat 3	(ng/mL)	(ng/mL)
0	BLOQ	BLOQ	BLOQ	NA	NA
0.083	1420	1410	1610	1480	113
0.25	974	960	995	976	17.6

0.5	401	496	536	478	69.3
1	192	174	206	191	16.0
2	63.3	81.2	60.6	68.4	11.2
4	28.6	43.0	20.2	30.6	11.5
8	5.24	11.7	2.20	6.38	4.85
24	BLOQ	BLOQ	0.500	NA	NA

Note: BLOQ = below limit of quantitation (0.2 ng/mL)

TLZ-16 freebase concentration in plasma (POA, 10 mg/kg)

Time (h)	Conc. (ng/mL)			Mean	RSD
	Rat 4	Rat 5	Rat 6	(ng/mL)	(ng/mL)
0	BLOQ	BLOQ	BLOQ	NA	NA
0.25	127	358	86.7	191	146
0.5	139	344	109	197	128
1	109	167	98.9	125	36.8
2	75.1	53.1	71.9	66.7	11.9
4	66.3	47.1	40.1	51.2	13.6
8	68.7	35.7	45.3	49.9	17.0
24	BLOQ	0.278	0.869	0.574	NA

TLZ-16 freebase Plasma PK parameters (IV, 3 mg/kg)

PK parameters	Unit	R1	R2	R3	Mean	SD	CV (%)
Cl _{obs}	mL/min/kg	52.6	46.5	48.6	49.2	3.1	6.30
T _{1/2}	h	1.66	2.14	4.37	2.73	1.44	53.0
C ₀	ng/mL	1713	1707	2045	1822	194	10.6
AUC _{last}	h*ng/mL	937	1038	1027	1001	55	5.51
AUC _{Inf}	h*ng/mL	950	1074	1030	1018	63	6.19
AUC _{%Extrap_obs}	%	1.32	3.37	0.306	1.67	1.56	93.6
MRT _{Inf_obs}	h	1.09	1.52	1.08	1.23	0.25	20.6
AUC _{last/D}	h*mg/mL	312	346	342	334	18	5.51
V _{ss_obs}	L/kg	3.43	4.26	3.16	3.61	0.57	15.8

TLZ-16 freebase plasma PK parameters (POA, 10 mg/kg)

PK parameters	Unit	R4	R5	R6	Mean	SD	CV (%)
T _{1/2}	h	8.80	2.57	3.34	4.90	3.39	69.3
T _{max}	h	0.5	0.25	0.5	0.417	0.144	34.6
C _{max}	ng/mL	139	358	109	202	136	67.3
AUC _{last}	h*ng/mL	615	924	825	788	158	20.1
AUC _{Inf}	h*ng/mL	1486	925	829	1080	355	32.9
AUC_%Extrap_obs	%	58.7	0.111	0.505	19.8	33.7	171
MRT _{Inf_obs}	h	13.6	4.19	5.68	7.84	5.08	64.8
AUC _{last/D}	h*mg/mL	61.5	92.4	82.5	78.8	15.8	20.1
F	%	18.4	27.3	24.4	23.4	4.5	19.3

[00213] (2) TLZ-16 HCl

TLZ-16 HCl concentration in plasma (POB, 10 mg/kg)

Time (h)	Conc. (ng/mL)			Mean	RSD
	R13	R14	R15	(ng/mL)	(ng/mL)
0	BLOQ	BLOQ	BLOQ	NA	NA
0.25	236	239	385	287	85.2
0.5	225	217	356	266	78.0
1	130	149	173	151	21.5
2	135	129	115	126	10.3
4	109	60.3	79.2	82.8	24.6
8	44.3	15.6	14.1	24.7	17.0
24	0.356	BLOQ	BLOQ	NA	NA

Note: BLOQ = below limit of quantitation (0.2 ng/mL)

TLZ-16 HCl Plasma PK parameters (POB, 10 mg/kg)

PK parameters	Unit	R13	R14	R15	Mean	SD	CV(%)
T _{1/2}	h	2.39	1.98	1.92	2.09	0.25	12.1
T _{max}	h	0.25	0.25	0.25	0.250	0.000	0.000
C _{max}	ng/mL	236	239	385	287	85	29.7

AUC _{last}	h*ng/mL	1216	658	798	891	290	32.6
AUC _{Inf}	h*ng/mL	1217	703	837	919	267	29.0
AUC_%Extrap_obs	%	0.101	6.34	4.66	3.70	3.23	87.2
MRT _{Inf_obs}	h	4.53	2.86	2.58	3.32	1.05	31.7
AUC _{last} /D	h*mg/mL	122	65.8	79.8	89	29	32.6
F	%	35.9	20.7	24.7	27.1	7.9	29.0

[00214] (3) TLZ-16 maleate**TLZ-16 maleate concentration in plasma (POC, 10 mg/kg)**

Time (h)	Conc. (ng/mL)			Mean	RSD
	R22	R23	R24	(ng/mL)	(ng/mL)
0	BLOQ	BLOQ	BLOQ	NA	NA
0.25	138	257	438	278	151
0.5	132	290	287	236	90.4
1	118	206	176	167	44.7
2	106	175	55.8	112	59.8
4	104	116	69.3	96.4	24.3
8	40.8	34.5	34.1	36.5	3.76
24	BLOQ	BLOQ	BLOQ	NA	NA

Note: BLOQ = below limit of quantitation (0.2 ng/mL)

TLZ-16 maleate Plasma PK parameters (POC, 10 mg/kg)

PK parameters	Unit	R22	R23	R24	Mean	SD	CV(%)
T _{1/2}	h	4.08	2.52	3.83	3.48	0.84	24.2
T _{max}	h	0.25	0.5	0.25	0.333	0.144	43.3
C _{max}	ng/mL	138	290	438	289	150	52.0
AUC _{last}	h*ng/mL	725	1007	709	814	168	20.6
AUC _{Inf}	h*ng/mL	965	1132	898	998	121	12.1
AUC_%Extrap_obs	%	24.9	11.1	21.0	19.0	7.1	37.5
MRT _{Inf_obs}	h	5.88	3.67	4.80	4.78	1.11	23.2
AUC _{last} /D	h*mg/mL	72.5	101	70.9	81	17	20.6

F	%	21.7	33.4	21.3	25.5	6.9	26.9
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[00215] (4) TLZ-16 mesylate**TLZ-16 mesylate concentration in plasma (POD, 10 mg/kg)**

Time (h)	Conc. (ng/mL)			Mean	RSD
	R31	R32	R33	(ng/mL)	(ng/mL)
0	BLOQ	BLOQ	BLOQ	NA	NA
0.25	227	253	256	245	15.9
0.5	192	233	338	254	75.3
1	162	197	208	189	24.0
2	211	180	271	221	46.3
4	208	155	122	162	43.4
8	70.3	40.7	35.2	48.7	18.9
24	BLOQ	0.454	BLOQ	NA	NA

Note: BLOQ = below limit of quantitation (0.2 ng/mL)

TLZ-16 mesylate plasma PK parameters (POD, 10 mg/kg)

PK parameters	Unit	R31	R32	R33	Mean	SD	CV(%)
T _{1/2}	h	3.54	2.40	2.06	2.67	0.77	29.0
T _{max}	h	0.25	0.25	0.5	0.333	0.144	43.3
C _{max}	ng/mL	227	253	338	273	58	21.3
AUC _{last}	h*ng/mL	1331	1444	1190	1322	127	9.64
AUC _{Inf}	h*ng/mL	1690	1446	1294	1477	200	13.5
AUC_%Extrap_obs	%	21.2	0.109	8.09	9.82	10.7	109
MRT _{Inf_obs}	h	5.40	4.15	3.26	4.27	1.07	25.2
AUC _{last} /D	h*mg/mL	133	144	119	132	13	9.64
F	%	39.9	42.6	38.1	40.2	2.2	5.58

5 [00216] (5) TLZ-16 oxalate**TLZ-16 oxalate concentration in plasma (POE, 10 mg/kg)**

Time (h)	Conc. (ng/mL)	Mean	RSD
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	R40	R41	R42	(ng/mL)	(ng/mL)
0	BLOQ	BLOQ	BLOQ	NA	NA
0.25	302	368	131	267	122
0.5	270	309	148	242	84.0
1	143	200	121	155	40.8
2	70.8	86.0	156	104	45.4
4	63.1	120	95.0	92.7	28.5
8	10.1	56.8	29.1	32.0	23.5
24	BLOQ	BLOQ	BLOQ	NA	NA

Note: BLOQ = below limit of quantitation (0.2 ng/mL)

TLZ-16 oxalate Plasma PK parameters (POE, 10 mg/kg)

PK parameters	Unit	R40	R41	R42	Mean	SD	CV(%)
T _{1/2}	h	2.02	4.98	2.46	3.15	1.60	50.8
T _{max}	h	0.25	0.25	2	0.833	1.010	121
C _{max}	ng/mL	302	368	156	275	108	39.4
AUC _{last}	h*ng/mL	600	960	756	772	181	23.4
AUC _{Inf}	h*ng/mL	629	1369	859	952	378	39.7
AUC_%Extrap_obs	%	4.67	29.8	12.0	15.5	12.9	83.5
MRT _{Inf_obs}	h	2.59	6.60	3.95	4.38	2.04	46.6
AUC _{last} /D	h*mg/mL	60.0	96.0	75.6	77.2	18.1	23.4
F	%	18.5	28.8	25.3	24.2	5.2	21.5

[00217] (6) TLZ-16 sulfate

TLZ-16 sulfate concentration in plasma (POF, 10 mg/kg)

Time (h)	Conc. (ng/mL)			Mean	RSD
	R49	R50	R51	(ng/mL)	(ng/mL)
0	BLOQ	BLOQ	BLOQ	NA	NA
0.25	231	226	349	269	69.6
0.5	229	223	401	284	101
1	117	161	240	173	62.3
2	119	152	189	153	35.0

4	103	110	203	139	55.8
8	22.4	39.9	87.8	50.0	33.9
24	BLOQ	BLOQ	BLOQ	NA	NA

Note: BLOQ = below limit of quantitation (0.2 ng/mL)

TLZ-16 sulfate plasma PK parameters (POF, 10 mg/kg)

PK parameters	Unit	R49	R50	R51	Mean	SD	CV (%)
T _{1/2}	h	2.37	3.05	4.97	3.46	1.35	39.0
T _{max}	h	0.25	0.25	0.5	0.333	0.144	43.3
C _{max}	ng/mL	231	226	401	286	100	34.8
AUC _{last}	h*ng/mL	764	899	1486	1049	384	36.6
AUC _{Inf}	h*ng/mL	840	1074	2115	1343	679	50.5
AUC _{%Extrap_obs}	%	9.10	16.3	29.8	18.4	10.5	57.0
MRT _{Inf_obs}	h	3.53	4.44	6.73	4.90	1.65	33.6
AUC _{last/D}	h*mg/mL	76.4	89.9	149	105	38	36.6
F	%	24.8	31.7	44.5	33.7	10.0	29.8

[00218] The experimental results are further summarized as below:

5 [00219] 1. Solubility comparison of TLZ-16 free base and various salts

[00220] Solubility studies of Form I hydrochloride salt in water (in 2 hours) were higher than that of the free base, which were improved from free base solubility of less than 1 mg/mL to the Form I hydrochloride salt solubility of approximately 19.1 mg/mL.

10 [00221] Solubility studies of Form I oxalate salt in water (in 2 hours) were higher than that of the free base, which were improved from free base solubility of less than 1 mg/mL to the Form I oxalate salt solubility of approximately 19.2 mg/mL.

[00222] Solubility studies of maleate salt in water (in 2 hours) is higher than that of the free base, which is improved from free base solubility of less than 1 mg/mL to the maleate salt solubility of approximately 11.0 mg/mL.

15 [00223] Solubility studies of sulfate and mesylate salt in water (in 2 hours) is higher than that of the free base, which is improved from free base solubility of less than 1 mg/mL to the salt solubility of approximately 20 mg/mL.

[00224] 2. Concentrations of TLZ-16 freebase and salts in rat plasma samples

Table 8. Concentrations of TLZ-16 freebase and its salts in individual plasma samples (animal number n=3)

5

Time (h)	Freebase 3mg/kg, iv (ng/mL)	Freebase 10mg/kg, p.o. (ng/mL)	Hydrochloride salt 10mg/kg, p.o. (ng/mL)	Maleate salt 10mg/kg, p.o. (ng/mL)	Mesylate salt 10mg/kg, p.o. (ng/mL)	Oxalate salt 10mg/kg, p.o. (ng/mL)	Sulfate salt 10mg/kg, p.o. (ng/mL)
0	NA	NA	NA	NA	NA	NA	NA
0.083	1480±113						
0.25	976±17.6	223±161	287±85.2	278±151	245±15.9	267±122	269±69.6
0.5	478±69	221±122	266±78.0	236±90.4	254±75.3	242±84.0	284±101
1	191±16	125±47	151±21.5	167±44.7	189±24.0	155±40.8	173±62.3
2	68.4±11.2	118±23	126±10.3	112±59.8	221±46.3	104±45.4	153±35.0
4	30.6±11.5	63.5±18	82.8±24.6	96.4±24.3	162±43.4	92.7±28.5	139±55.8
8	6.38±4.9	11.9±6.6	24.7±17.0	36.5±3.76	48.7±18.9	32.0±23.5	50.0±33.9
24	NA	NA	NA	NA	NA	NA	NA

Data are represented as mean ± SD. IV: intravenous administration; P.O.: orally administration.

NA: Not Available

[00225] 3. PK parameters of TLZ-16 free base

- 10 **[00226]** The PK parameters ($T_{1/2}$, AUC_{0-t} , AUC_{inf} , $AUC_{\%Extrap}$, Mean Residence Time (MRT), Clearance (CL), C_{max} , F (bioavailability) etc.) for TLZ-16 freebase in rat plasma are summarized in Table 9.

Table 9. The PK parameters of TLZ-16 freebase in rat plasma

IV 3 mg/kg		
PK parameters	Unit	Mean ± SD
Cl _{obs}	mL/min/kg	49.2±3.1
$T_{1/2}$	h	2.73±1.4
C_0	ng/mL	1822±194
AUC_{last}	h*ng/mL	1001±55
AUC_{inf}	h*ng/mL	1018±63
$AUC_{\%Extrap_obs}$	%	1.67±1.6
MRT_{inf_obs}	h	1.23±0.3

AUC _{last} /D	h*mg/mL	334±18
V _{ss_obs}	L/kg	3.61±0.6

[00227] Comparison of the PK parameters of TLZ-16 free base and its various salts

[00228] The PK parameters (T_{1/2}, AUC_{0-t}, AUC_{inf}, AUC%_{Extrap}, Mean Residence Time (MRT), Clearance (CL), C_{max}, F (bioavailability)) for TLZ-16 free base and salts in rat plasma are shown

5 in Table 10.

Table 10. The PK parameters of TLZ-16 freebase in rat plasma (n=3, mean ± SD)

Items	Unit	Free base, 10 mg/kg, p.o.	HCl salt, 10 mg/kg, p.o.	Maleate salt, 10 mg/kg, p.o.	Mesylate salt, 10 mg/kg, p.o.	Oxalate salt, 10 mg/kg, p.o.	Sulfate salt, 10 mg/kg, p.o.
T _{1/2}	h	1.8±0.5	2.1±0.25	3.5±0.8	2.7±0.8	3.2 ±1.6	3.5± 1.4
T _{max}	h	0.9±0.9	0.25±0.0	0.3±0.1	0.3±0.1	0.8±1.0	0.3±0.1
C _{max}	ng/mL	245±130	286.7±85.2	289±150	272.7±58.1	275.3±108.5	286.0±99.6
AUC _{last}	h*ng/mL	623±48	890.8±290.2	814±168	1321.7±127.5	772.1±180.9	1049.4±383.9
AUC _{Inf}	h*ng/mL	656±48	919.1±267	998±121	1476.8±199.9	952.4±378.5	1343.2±678.8
AUC _{ap_obs} % _{Extr}	%	5.3±3.8	3.7±3.2	19.0±7.1	9.8 ±10.7	15.5±12.9	18.4±10.5
MRT _{Inf_obs}	h	2.8±0.9	3.3±1.1	4.8±1.1	4.3±1.1	4.4 ±2.0	4.9±1.6
AUC _{last} /D	h*mg/mL	62.3±7.0	89.0±29.0	81±17	132.2±12.7	77.2±18.1	104.9±38.4
F	%	19.3±1.4	27.1±7.9	25.5±6.9	40.2±2.2	24.2 ±5.2	33.7±10.0

[00229] As shown in the above, the TLZ-16 free base has a bioavailability (F value) of 19.3 ± 1.4, which is significantly lower than the F values of the salts.

10 [00230] Comparing with the TLZ-16 free base, various salts show a great improvement in their solubility. Also, the above pharmacokinetic data show that the inventive salts have better bioavailability and bio-distribution.

EXAMPLE 2. Effects of TLZ-16-CL (TLZ-16 HCl salt) in a rat Complete Freund's adjuvant (CFA) Model of Inflammatory Pain

15

[00231] Study Objective: To evaluate the effects of TLZ-16-CL in the rat Complete Freund's adjuvant (CFA) Model of Inflammatory Pain

[00232] This study is a non-GLP study. All the procedures related to animal handling, care, and the treatment in this study were performed according to guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Pharmaron following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

5 [00233] **Study Design**

[00234] **Animals**

Species & Strain: SD rat, from Beijing Vital River Laboratory Animal Technology Co., Ltd.

Age: 6-8 weeks

10 Body weight: 203-234 g

Sex: Male

[00235] Housing: General procedures for animal and housing were in accordance with the standard, Commission on Life Sciences, National Research Council, Standard operating procedures (SOPs) of Pharmaron, Inc. The animals were kept in laminar flow rooms at constant
15 temperature and humidity with 3 animals in each cage. Animals were housed in polycarbonate cage and in an environmentally monitored, well-ventilated room maintained at a temperature of (22±3°C) and a relative humidity of 40%-80%. Fluorescent lighting was provided illumination approximately 12 hours per day. The bedding material was soft wood, which were changed once per week.

20 [00236] Animal ID: Each animal were assigned an identification number; the following identification method were applied. Each cage card were labeled with such information as study number, group, sex, dose, animal number, initiation date, study director and telephone number.

[00237] Diet: Animals had free access to irradiation sterilized dry granule food (Beijing Keaoxieli Feed Co., Ltd., Beijing, China) during the entire study period except for time periods
25 specified by the protocol.

[00238] Water: Sterile drinking water in a bottle was available to all animals ad libitum during the quarantine and study periods. The bottle and the stopper with attached sipper tube were autoclaved prior to use. Samples of water from the animal facility were analyzed and results of water analysis were retained in the facility records and were reviewed by the veterinarian, or
30 designee, to assure that no known contaminants were present that could interfere with or affect the outcome of the studies.

[00239] **Groups and Treatments**

[00240] Based on the body weight and baseline of Von Frey on Day 3, animals were randomly assigned to respective groups using a computer-generated randomization procedure. The study groups and treatment per group are shown in the following table.

Table. Groups and treatments

Group	Treatment	Dose (mg/kg)	Route	CFA	N	Von Frey
1	Vehicle	-	PO	Yes	8	
2	Naproxen	20	PO	Yes	8	
3	TLZ-16-CL	0.6	PO	Yes	8	# (1) Prior CFA
4	TLZ-16-CL	1.8	PO	Yes	8	(2) Prior to dose
5	TLZ-16-CL	5.4	PO	Yes	8	(3) 1, 2 and 4 hours post dose.
6	TLZ-16-CL	10.8	PO	Yes	8	

5 # Note: 10 animals were randomly selected to tested baseline of Von Frey prior CFA injection

[00241] Induction Reagent: CFA, purchased from Chondrex Inc.

[00242] Vehicle: Physiological saline, supplied by Shandong Hualu Pharmaceutical Co., Inc.

[00243] Test compounds: TLZ-16-CL (TLZ-16 HCl salt), supplied by Beijing Taizhou Lize
10 Pharmaceutical Technology co., LTD

[00244] Control: Naproxen Sodium, supplied by National Institutes for Food and Drug
Control

[00245] Induction Procedure

15 Induction reagent: CFA
Route: Left paw sub-plantar injection
Dosing volume: 50 μ L/rat (M. Tuberculosis at 4 mg/mL)
Frequency: Single dose on Day 0

[00246] Dosing Procedure and Formulation

20 Route: P.O.
Volume: 5 mL/kg
Frequency: Single Dose
Dosing Time: 72 hours after CFA injection.

25 [00247] Formulation:

Preparation of test articles and vehicle are shown in the following table.

Table. Formulation

Treatment	Dose (mg/kg)	Preparation	Concentration	Storage
Naproxen	20	50.5 mg of Naproxen Sodium (Purity: 100%) was added to 11.525 mL Vehicle, vortex until a solution was achieved.	4 mg/mL	Prepared freshly
TLZ-16-CL	10.8	26.1 mg of TLZ-16-CL (Purity: 99.3%) were added to 11.999 mL Vehicle, vortex until a solution was achieved.	2.16 mg/mL	Prepared freshly
TLZ-16-CL	5.4	22.7 mg of TLZ-16-CL (Purity: 99.3%) were added to 20.871 mL Vehicle, vortex until a solution was achieved.	1.08 mg/mL	Prepared freshly
TLZ-16-CL	1.8	Take out 6.000 mL liquid of TLZ-16-CL (1.08 mg/mL) into 12.000 mL Vehicle by vortex until a solution was achieved.	0.36 mg/mL	Prepared freshly
TLZ-16-CL	0.6	Take out 5.000 mL liquid of TLZ-16-CL (0.36 mg/mL) into 10.000 mL Vehicle by vortex until a solution was achieved.	0.12 mg/mL	Prepared freshly

[00248] Experimental Method and Measurement Parameters

[00249] Von Frey Test

[00250] Mechanical allodynia of the left hind paw were measured during the course of study by determining withdrawal thresholds to an electronic Von Frey filament (Bioseb, France). The filament was applied perpendicularly to the plantar surface of the paw with increasing force. The force required to induce a reflex paw withdrawal were calculated by taking the average of 2-3 repeated stimuli (in g), which induced a reflex paw withdrawal.

[00251] The Von Frey test was performed 5 times during the course of the study including baseline of prior CFA, 72 hours post-CFA and 1, 2 and 4 hours post dose.

[00252] Blood Collection

[00253] The blood samples were collected into a tube. Blood samples were centrifuged at 4°C for 10 min at 8000 g to collect the serum samples, total 48 serum samples were stored at -80°C.

[00254] Statistical Analysis

[00255] One-way analysis of variance (ANOVA) following multiple comparisons testing was applied among the groups with software SPSS 16.0, and $P < 0.05$ was accepted as significant.

[00256] Results

[00257] Test compound TLZ-16-CL administered orally single dose at 10.8 mg/kg and 5.4 mg/kg showed a significantly antagonistic effect base on Von Frey test at 1, 2 and 4 hours post dosing and it's in a dose dependent fashion. As respected, the positive compound Naproxen after a single dose at 20 mg/kg showed a significant antagonistic effect base on Von Frey test at 1, 2 and 4 hours post dosing.

Table 11. Von Frey test results (Mean $\pm$ SD) of study groups (n=10)

Group	Von Frey (g)			
	Baseline	1h	2h	4h
CFA+ Vehicle	6.2 $\pm$ 0.4	6.2 $\pm$ 0.3	6.4 $\pm$ 0.3	6.2 $\pm$ 0.3
CFA+ Naproxen (20 mg/kg)	6.2 $\pm$ 0.3	8.3 $\pm$ 0.4**	9.5 $\pm$ 0.4**	9.9 $\pm$ 0.4**
CFA+TLZ-16-CL (0.6 mg/kg)	6.2 $\pm$ 0.5	6.4 $\pm$ 0.3	6.6 $\pm$ 0.3	6.4 $\pm$ 0.3
CFA+TLZ-16-CL (1.8 mg/kg)	6.1 $\pm$ 0.3	6.5 $\pm$ 0.4	6.4 $\pm$ 0.1	6.5 $\pm$ 0.3
CFA+TLZ-16-CL (5.4 mg/kg)	6.2 $\pm$ 0.4	6.8 $\pm$ 0.5**	8.0 $\pm$ 0.6**	8.4 $\pm$ 0.2**
CFA+TLZ-16-CL (10.8 mg/kg)	6.1 $\pm$ 0.4	7.8 $\pm$ 0.2**	9.1 $\pm$ 0.9**	8.7 $\pm$ 0.4**

Note: Compared with CFA + Vehicle1, ** P<0.01. The baseline of Von Frey prior CFA on day 0 was: 12.7 $\pm$ 0.3.

10 Example 3. Analgesic effect of TLZ-16-CL in a rat Model of Neuropathic Pain Induced by SNI

[00258] **Study Objective:** To evaluate the effect of TLZ-16-CL in rat's model of neuropathic pain induced by SNI.

[00259] This study is a non-GLP study. All the procedures related to animal handling, care, and the treatment in this study were performed according to guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Pharmaron following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

[00260] STUDY DESIGN

[00261] Animals

20 Species & Strain: SD rat, purchased from Beijing Vital River Co., LTD

Age: 6-8 weeks

Body weight: 192-233 g

Sex: male

[00262] Housing: General procedures for animal care and housing were in accordance with the standard, Commission on Life Sciences, National Research Council, Standard operating procedures (SOPs) of Pharmaron, Inc. The animals were kept in laminar flow rooms at constant temperature and humidity with 3 animals in each cage. Animals were housed in polycarbonate cage and in an environmentally monitored, well-ventilated room maintained at a temperature of (22±3°C) and a relative humidity of 40% 80%. During the entire study, all the animals were kept in an environment with a regular cycle of 12 h light (from 8:00 AM to 8:00 PM) and 12 h dark (from 8:00 PM to 8:00 AM). The bedding material was soft wood, which was changed once per week.

[00263] Animal ID: Each animal was assigned an identification number; the following identification method was applied. Each cage card was labeled with such information as study number, group, sex, dose, animal number, initiation date, study director and telephone number.

[00264] Diet: Animals had free access to irradiation sterilized dry granule food (Beijing Keaoxili Feed Co., Ltd., Beijing, China) during the entire study period except for time periods specified by the protocol.

[00265] Water: Sterile drinking water in a bottle was available to all animals ad libitum during the quarantine and study periods. The bottle and the stopper with attached sipper tube were autoclaved prior to use. Samples of water from the animal facility were analyzed and results of water analysis were retained in the facility records and were reviewed by the veterinarian or designee, to assure that no known contaminants were present that could interfere with or affect the outcome of studies.

[00266] Groups and Treatments

[00267] Based on the animal's body weight and baselines of Von Frey and Weight Bearing on Day 10, animals were randomly assigned to respective groups using a computer-generated randomization procedure. The study groups and treatments per group are shown in the following table.

Table. Groups and Treatments

Group	Compound	Dose (mg/kg)	Route	SNI	N	Dosing Volume (ml/kg)	Von Frey/Weight Bearing
1	Vehicle 1	-	PO	Yes	10	5	# (1)Baseline prior SNI on Day 0 (2)Baseline prior to
2	Pregabalin	30	PO	Yes	10	5	

3	Morphine Hydrochloride	3.2	IP	Yes	10	5	dose on Day10 (3)1, 2 and 4 hours post dose on Day10
4	TLZ-16-CL	2.7	PO	Yes	10	5	
5	TLZ-16-CL	5.4	PO	Yes	10	5	
6	TLZ-16-CL	10.8	PO	Yes	10	5	

Note: 10 animals were randomly selected to test and record baseline of Von Frey and weight bearing prior SNI surgery.

[00268] Test Compound: TLZ-16-CL, supplied by Beijing Taizhou Lize Pharmaceutical Technology co., LTD

[00269] Control Compounds: Pregabalin, supplied by Jiuding chemical technology co. LTD; and Morphine Hydrochloride, supplied by Beijing Taizhou Lize Pharmaceutical Technology co., LTD

[00270] Dosing Procedure and Formulation

10 Route: P.O. (G1.2.4.5.6), IP (G3)

Volume: 5 mL/kg

Frequency: Single dose on Day 10 post SNI surgery

Dosing Time: Day 10.

[00271] Formulation

15 [00272] Test compound was freshly prepared before dosing. The preparation of vehicle and compounds are listed in the following table.

Table. Formulation

Treatment	Dose (mg/kg)	Preparation	Concentration (mg/mL)	Storage
Vehicle 2	-	Dissolve 5.0000 g of MC in 1000 mL of water, stir was applied to obtain a clear solution of 0.5%MC (w/v).	-	2-8°C
Pregabalin	30	127.7 mg of Pregabalin (99%) was dissolved in 21.071 mL of 0.5%MC. vortex until a solution was achieved. The final concentration was 6 mg/mL	6	Prepared freshly
Morphine Hydrochloride	3.2	12.7 mg of Morphine Hydrochloride (100%) was dissolved in 19.844 mL of physiological saline. vortex until a solution was achieved. The final concentration was 0.64 mg/mL.	0.64	2-8°C
TLZ-16-CL	10.8	80.5 mg of TLZ-16-CL (99.3%) was added to 37.008 mL physiological saline, vortex until a solution was achieved. The final concentration was 2.16 mg/mL.	2.16	Prepared freshly

TLZ-16-CL	5.4	Take out 17.000 mL liquid of TLZ-16-CL (2.16 mg/mL) into 17.000 mL physiological saline by vortex until a solution was achieved. The final concentration was 1.08 mg/mL.	1.08	Prepared freshly
TLZ-16-CL	2.7	Take out 14.000 mL liquid of TLZ-16-CL (1.08 mg/mL) into 14.000 mL physiological saline by vortex until a solution was achieved. The final concentration was 0.54 mg/mL.	0.54	Prepared freshly

[00273] EXPERIMENTAL METHOD AND MEASUREMENT PARAMETERS

[00274] Body Weight

[00275] Body weights of all animals were recorded weekly and prior surgery and dosing during the course of the study.

[00276] Spared Nerve Injury (SNI)

[00277] The skin on the lateral surface of the thigh of the animals under anesthesia was incised and a section made directly through the biceps femoris muscle exposing the sciatic nerve and its three terminal branches: the sural, common peroneal and tibial nerves.

10 [00278] The SNI procedure comprised an axotomy and ligation of the tibial and common peroneal nerves leaving the sural nerve intact. The common peroneal and the tibial nerves were tight-ligated with 6.0 silk and sectioned distal to the ligation, removing 2±4 mm of the distal nerve stump. Great care was taken to avoid any contact with or stretching of the intact sural nerve. Muscle and skin was closed in two layers. All animals had the surgery on their left-lateral leg on Day 0.

[00279] Von Frey Test

[00280] Mechanical allodynia of the left hind paw was measured during the course of study by determining withdrawal thresholds to an electronic Von Frey filament (Bioseb, France). The filament was applied perpendicularly to the plantar surface of the paw with increasing force. The force required to induce a reflex paw withdrawal was calculated by taking the average of 2 repeated stimuli (in g), which induced a reflex paw withdrawal.

[00281] The Von Frey of animals from Groups 1~6 were measured five times including baseline prior surgery, baseline prior dosing, and 1, 2 and 4 hours post dosing on Day 10.

[00282] Difference of Weight Bearing Test

25 [00283] Rats distributed body weight unequally on both SNI-induced and contra-lateral paws, which were measured by weight balance changing instrument (YLS-11A, JinanYiYan Science

and technology development co., LTD). The animals were tested to register the weight load exerted by the hind paws by means of force plate inserted in the floor. The mean weight bearing (in g) between SNI-induce paw and contra-lateral paw were determined in 10 seconds.

[00284] The difference of weight bearing of Groups 1~6 were measured five times including baseline prior surgery, baseline prior dosing, and 1, 2 and 4 hours post dosing on the Day 10.

[00285] Blood Collection

[00286] Blood samples were collected at 4 hours after last measurement from Groups 1~6 animals. The blood samples were collected into a tube. Leave the blood for 30 min, and then blood samples were centrifuged at 4°C for 10 min at 8000 g to collect the serum samples, total 60 serum samples were stored at -80°C.

[00287] Data Analysis

[00288] All statistical analysis was conducted, and the level of significance was set at $P < 0.05$. The group means, standard errors were calculated for all measurement parameters as study designed. One way ANOVA comparisons among the groups were performed with software SPSS 16.0.

[00289] RESULT

[00290] In this study, test compound TLZ-16-CL administered orally as a single dose at 10.8 mg/kg and 5.4 mg/kg showed a significantly antagonistic effect base on Von Frey and Weight bearing test at 1, 2 and 4 hours post dosing; Test compound TLZ-16-CL administered orally as a single dose at 2.7 mg/kg showed a significantly antagonistic effect base on Von Frey and Weight bearing test at 2 hours and showed a significantly antagonistic effect base on only Von Frey test at 4 hours post doing.

Table 12. Von Frey test results of study groups (Mean $\pm$ SD)

Group	Von Frey (g)			
	Baseline	1 hour	2 hours	4 hours
SNI+ Vehicle 1	6.3 $\pm$ 0.4	6.3 $\pm$ 0.2	6.4 $\pm$ 0.2	6.2 $\pm$ 0.5
SNI+ Pregabalin (30 mg/kg)	6.2 $\pm$ 0.5	8.0 $\pm$ 0.7**	8.4 $\pm$ 0.6**	8.3 $\pm$ 0.8**
SNI+ Morphine Hydrochloride (3.2 mg/kg)	6.3 $\pm$ 0.3	9.8 $\pm$ 1.1**	8.1 $\pm$ 0.5**	6.7 $\pm$ 0.7
SNI+ TLZ-16-CL (2.7 mg/kg)	6.2 $\pm$ 0.4	6.5 $\pm$ 0.3	7.6 $\pm$ 0.4**	7.2 $\pm$ 0.5**
SNI+ TLZ-16-CL (5.4 mg/kg)	6.2 $\pm$ 0.4	7.5 $\pm$ 0.7**	8.5 $\pm$ 0.7**	8.4 $\pm$ 0.6**

SNI+ TLZ-16-CL (10.8 mg/kg) 6.1±0.4 8.4±0.4** 9.1±0.7** 8.8±0.9**

Note: Compared with Group 1 (SNI+ Vehicle 1), ** P<0.01. The Von Frey of animals from groups 1~6 were measured five times including baseline prior surgery, baseline prior dosing, and 1, 2 and 4 hours post dosing on Day 10. The baseline of Von Frey prior SNI surgery on day 0 was: 13.2 ± 0.4.

5

Table 13. Weight Bearing of Study Groups (Mean ± SD)

Group	Weight Bearing (g)			
	Baseline	1 hour	2 hours	4 hours
G1=SNI+ Vehicle 1	45.4±1.8	46.9±1.0	47.5±0.5	46.7±1.0
G2=SNI+ Pregabalin (30 mg/kg)	45.8±1.7	44.0±1.5**	45.7±0.5**	44.1±1.2**
G3=SNI+ Morphine Hydrochloride (3.2 mg/kg)	45.3±1.8	38.5±1.1**	42.9±2.4**	45.8±1.1
G4=SNI+ TLZ-16-CL (2.7 mg/kg)	44.7±1.9	45.4±2.2	45.8±1.4*	46.0±1.0
G5=SNI+ TLZ-16-CL (5.4 mg/kg)	45.3±1.4	43.8±1.0**	44.1±1.6**	44.1±1.3**
G6=SNI+ TLZ-16-CL (10.8 mg/kg)	45.7±1.4	42.7±2.8*	42.1±0.6**	43.6±1.5**

Note: Compared with Group 1 (SNI + Vehicle 1), * P<0.05, ** P<0.01.

The baseline of Weight Bearing prior SNI surgery on day 0 was: 6.0±1.4

10 Example 4. Analgesic effect of TLZ-16-CL in a rat Chronic Constriction Injury (CCI) Model

[00291] **Study Objective:** To evaluate the analgesic effect of TLZ-16-CL on rat CCI model.

[00292] This study is a non-GLP study. All the procedures related to animal handling, care, and the treatment in this study were performed according to guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Pharmaron following the guidance of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

15

[00293] **Study Design**

[00294] **Animals**

Species & Strain: SD rat, supplied by Beijing Vital River Laboratory Animal Technology Co., Ltd.

20

Age: 6-8 weeks

Body weight: 257-349 g

Sex: Male

[00295] Housing: General procedures for animals and housing were in accordance with the standard, Commission on Life Sciences, National Research Council, Standard operating procedures (SOPs) of Pharmaron, Inc. The animals were kept in laminar flow rooms at constant temperature and humidity with 3 animals in each cage. Animals were housed in polycarbonate cage and in an environmentally monitored, well-ventilated room maintained at a temperature of (22±3°C) and a relative humidity of 40%-80%. Fluorescent lighting provided illumination approximately 12 hours per day. The bedding material was soft wood, which was changed once per week.

[00296] Animal ID: Each animal was assigned an identification number; the following identification method was applied. Each cage card was labeled with such information as study number, sex, animal number, initiation date, study director and telephone number. Individual animals were identified by ear coding.

[00297] Diet: Animals had free access to irradiation sterilized dry granule food (Beijing Keaoxili Feed Co., Ltd., Beijing, China) during the entire study period except for time periods specified by the protocol.

[00298] Water: Sterile drinking water in a bottle was available to all animals ad libitum during the quarantine and study periods. The bottle and the stopper with attached sipper tube were autoclaved prior to use. Samples of water from the animal facility were analyzed and results of water analysis were retained in the facility records and were reviewed by the veterinarian, or designee, to assure that no known contaminants were present that could interfere with or affect the outcome of the studies.

[00299] Groups and Treatments

[00300] Based on the body weight, baseline of Von Frey and Weight bearing on Day13, animals were randomly assigned to respective groups using a computer-generated randomization procedure (see the following table).

Table. Groups and Treatments

Group	Compound	Dose (mg/kg)	Route	CCI	N	Dosing Volume (mL/kg)	Von Frey& Weight bearing
1	Vehicle	-	PO	Yes	10	5	* (1) Baseline prior CCI surgery
2	Pregabalin	30	PO	Yes	10	5	(2) Baseline prior dosing on Day13 and 1, 2 and 4 hours post
3	Morphine Hydrochloride	5	PO	Yes	10	5	

4	TLZ-16-CL	2.7	PO	Yes	10	5	dosing on day14
5	TLZ-16-CL	5.4	PO	Yes	10	5	(3) Baseline prior dosing and 1, 2 and 4 hours post dosing on day20
6	TLZ-16-CL	10.8	PO	Yes	10	5	

*Note: 10 animals were randomly selected to test and record the baseline of Von Frey and weight bearing prior CCI surgery.

[00301] Vehicle: 0.9% Physiological saline, supplied by Shandong Hualu Pharmaceutical Co., Inc.

Methylcellulose, supplied by Sigma-Aldrich

[00302] Control Compound 1: Pregabalin, supplied by JiuDing chemistry (Shanghai), Inc.

[00303] Control Compound 2: Morphine Hydrochloride, supplied by Beijing Taizhou Lize Pharmaceutical Technology co., LTD

[00304] Test Compound: TLZ-16-CL, supplied by Beijing Taizhou Lize Pharmaceutical Technology co., LTD

[00305] Dosing Procedure and Formulation

Route: P.O.

Volume: 5 mL/kg

Frequency: QD*7

Dosing Time: From Day 14 and Day 20 post CCI surgery.

[00306] Formulation

[00307] All test compounds were freshly prepared before dosing. The preparation of vehicle and compounds were listed in the following table.

Table. Preparation of Vehicle and Compounds

Treatment	Dose (mg/kg)	Preparation	Concentration (mg/mL)	Storage
Vehicle 1	-	0.9% Physiological saline	-	Room temperature
Vehicle 2	-	5.0000 g of Methylcellulose was dissolved in 1000 mL of water. Stirred until a solution of 0.5%MC was achieved.	-	4 °C
Pregabalin	30	70.7 mg of Pregabalin (99%) was dissolved in 11.666 mL of 0.5%MC. vortex until a solution was achieved. The final concentration was 6 mg/mL.	6	Prepared freshly

Morphine Hydrochloride	5	11.7 mg of Morphine Hydrochloride (100%) was dissolved in 11.700 mL of physiological saline. vortex until a solution was achieved. The final concentration was 1 mg/mL.	1	Prepared freshly
TLZ-16-CL	10.8	44.0 mg of TLZ-16-CL (99.3%) was added to 20.228 mL physiological saline, vortex until a solution was achieved. The final concentration was 2.16 mg/mL.	2.16	Prepared freshly
TLZ-16-CL	5.4	Take out 9.000 mL liquid of TLZ-16-CL (2.16 mg/mL) into 9.000 mL physiological saline by vortex until a solution was achieved. The final concentration was 1.08 mg/mL.	1.08	Prepared freshly
TLZ-16-CL	2.7	Take out 6.000 mL liquid of TLZ-16-CL (1.08 mg/mL) into 6.000 mL physiological saline by vortex until a solution was achieved. The final concentration was 0.54 mg/mL.	0.54	Prepared freshly

[00308] Experimental Method and Measurement Parameters

[00309] Body Weight

[00310] Body weight of all animals was recorded prior dosing during the course of the study.

5 [00311] Chronic Constriction Injury (CCI)

[00312] Rats were anesthetized with isoflurane. The left sciatic nerve was exposed by blunt dissection at the mid-thigh level, and the adhering tissue was dissected for an approximately 10 mm section of the nerve. Attach 10 mm of chrome catgut to the nerve, and the constriction cuffs were applied around the nerve proximal part of the trifurcation with an approximate 1 mm distance between the cuffs. Wounds were closed with 4.0 sutures for the dissected muscle, followed by applying wound-clips to the skin.

[00313] Von Frey Test

[00314] Mechanical allodynia of the left hind paw was measured during the course of study by determining withdrawal thresholds to an electronic Von Frey filament (Bioseb, France). The filament was applied perpendicularly to the plantar surface of the paw with increasing force. The force required to induce a reflex paw withdrawal were calculated by taking the average of 2-3 repeated stimuli (in g), which induced a reflex paw withdrawal.

[00315] The Von Frey test of group 1-6 was performed nine times during the course of the study including baseline prior CCI surgery, baseline prior dosing on day13, 1, 2 and 4 hours post dosing on day14, baseline prior dosing and 1, 2 and 4 hours post dosing on day 20.

[00316] Difference of Weight Bearing Test

[00317] Rats distributed body weight unequally on CCI-induction and contra-lateral paws, which were measured by weight balance changing instrument. (YLS-11A, JinanYiYan Science and technology development co., LTD). The animals were tested to register the weight load exerted by the hind paws by means of force plate inserted in the floor. The mean weight bearing (in g) between dose-infection paw and contra-lateral paw were determined in 10 seconds.

[00318] The difference of weight bearing of Group 1-6 was performed nine times during the course of the study including baseline prior CCI surgery, baseline prior to dosing on day13, 1, 2 and 4 hours post dosing on day14, baseline prior dose, 1, 2 and 4 hours post dosing on day 20.

[00319] Data Analysis

[00320] One-way analysis of variance (ANOVA) following multiple comparisons testing was applied among the groups with software SPSS 16.0, and $P < 0.05$ was accepted as significant.

[00321] Results

[00322] TLZ-16-CL at 5.4 mg/kg and 10.8 mg/kg showed analgesic effect at 1, 2 and 4 hours post dosing, and at 2.7 mg/kg also showed analgesic effect at 2 and 4 hours post dosing based on Von Frey and Weight bearing measurement on day 14 and day 20.

Table 14. Von Frey test results of study groups (Mean $\pm$ SD)

Group	Von Frey (g)			
	Day 13-Baseline	Day 14 - 1 h	Day 14 - 2 h	Day 14 - 4 h
CCI + Vehicle1	6.3 $\pm$ 0.4	6.2 $\pm$ 0.3	6.3 $\pm$ 0.3	6.4 $\pm$ 0.3
CCI+ Pregabalin (30 mg/kg)	6.2 $\pm$ 0.4	8.3 $\pm$ 0.4**	8.5 $\pm$ 0.8**	7.8 $\pm$ 0.3**
CCI + Morphine Hydrochloride (5 mg/kg)	6.2 $\pm$ 0.5	7.3 $\pm$ 0.3**	8.5 $\pm$ 0.5**	7.3 $\pm$ 0.4**
CCI+ TLZ-16-CL (2.7 mg/kg)	6.2 $\pm$ 0.3	6.6 $\pm$ 0.5*	7.6 $\pm$ 0.5**	7.3 $\pm$ 0.5**
CCI+ TLZ-16-CL (5.4 mg/kg)	6.2 $\pm$ 0.4	7.3 $\pm$ 0.4**	8.7 $\pm$ 0.4**	8.0 $\pm$ 0.4**
CCI+ TLZ-16-CL (10.8 mg/kg)	6.2 $\pm$ 0.4	8.1 $\pm$ 0.4**	9.1 $\pm$ 0.4**	8.3 $\pm$ 0.4**

Note: Compared with Vehicle group (G1), * $P < 0.05$, ** $P < 0.01$. The Von Frey test of groups 1-6 was performed nine times during the course of the study including baseline prior CCI surgery, baseline prior dosing on day 13, 1, 2 and 4 hours post dosing on day14, baseline prior dosing and 1, 2 and 4 hours post dosing on day 20. The baseline of Von Frey prior CCI surgery on day 0 was: 13.1 $\pm$ 0.3.

Table 15. Von Frey of Groups (Mean $\pm$ SD)

Group	Von Frey (g)
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	Day 20-Baseline	Day 20-1 h	Day 20-2 h	Day 20-4 h
G1=CCI+ Vehicle 1	6.4±0.3	6.3±0.2	6.4±0.2	6.4±0.2
G2=CCI+ Pregabalin (30 mg/kg)	6.4±0.2	8.3±0.4**	8.6±0.3**	8.1±0.4**
G3=CCI+ Morphine Hydrochloride (5 mg/kg)	6.5±0.2	7.4±0.3**	8.5±0.3**	8.1±0.7**
G4=CCI+ TLZ-16-CL (2.7 mg/kg)	6.4±0.3	7.1±0.3**	7.5±0.1**	7.3±0.3**
G5=CCI+ TLZ-16-CL (5.4 mg/kg)	6.3±0.2	7.9±0.4**	8.4±0.3**	8.0±0.2**
G6=CCI+ TLZ-16-CL (10.8 mg/kg)	6.3±0.2	8.1±0.3**	8.9±0.4**	8.4±0.3**

Note: Compared with Vehicle group (G1), * P<0.05, ** P<0.01.

Table 16. Weight bearing of Study Groups (Mean ± SD)

Group	Weight Bearing(g)			
	Day13-Baseline	Day14-1 h	Day14-2 h	Day14-4 h
G1=CCI+Vehicle1	49.0±0.6	48.9±0.6	49.0±0.3	48.8±0.7
G2=CCI+ Pregabalin (30 mg/kg)	49.3±0.7	45.8±1.1**	45.7±1.0**	46.8±0.7**
G3=CCI + Morphine Hydrochloride (5 mg/kg)	49.5±0.4	46.8±0.6**	45.7±1.0**	46.9±1.2**
G4=CCI+ TLZ-16-CL (2.7 mg/kg)	49.0±0.4	48.6±0.6	47.0±0.8**	47.7±0.7**
G5=CCI+ TLZ-16-CL (5.4 mg/kg)	49.1±0.5	46.8±0.6**	46.6±1.0**	46.5±0.8**
G6=CCI+ TLZ-16-CL (10.8mg/kg)	49.3±0.8	46.1±0.8**	45.5±0.8**	45.8±0.7**

Note: Compared with Vehicle group (G1), ** P<0.01.

- 5 The baseline of weight bearing prior CCI surgery on day0 was: 5.3±1.3.

Table 17. Weight bearing of Study Groups (Mean ± SD)

Group	Weight Bearing(g)			
	Day 20-Baseline	Day 20-1 h	Day 20-2 h	Day 20-4 h
G1=CCI+ Vehicle1	48.9±0.4	48.7±0.4	48.6±0.4	48.6±0.4
G2=CCI+ Pregabalin (30 mg/kg)	48.8±0.7	46.1±0.9**	45.9±0.6**	46.1±0.6**
G3=CCI+ Morphine Hydrochloride (5 mg/kg)	48.6±0.5	46.4±0.7**	45.9±1.1**	46.2±0.6**
G4=CCI+ TLZ-16-CL (2.7 mg/kg)	48.7±0.4	47.6±0.5**	47.2±0.5**	47.3±0.7**
G5=CCI+ TLZ-16-CL (5.4 mg/kg)	48.7±0.4	46.7±0.5**	46.0±0.6**	46.4±0.6**
G6=CCI+ TLZ-16-CL (10.8 mg/kg)	48.6±0.3	46.2±0.5**	45.6±0.7**	45.5±0.6**

Note: Compared with Vehicle group (G1), ** P<0.01.

- 10 [00323] Test compound TLZ-16-CL showed a dose-dependent analgesic effect in the rat chronic constriction injury (CCI) model. TLZ-16-CL at 5.4 mg/kg and 10.8 mg/kg showed

analgesic effect at 1, 2 and 4 hours post dosing, and at 2.7 mg/kg also showed analgesic effect at 2 and 4 hours post dosing based on Von Frey and Weight bearing measurement on day 14 and day 20.

- 5 [00324] From above, it can be seen that the test compound TLZ-16-CL (TLZ-16 HCl salt) exhibits significant analgesic effect in both of an inflammatory pain model and a neuropathic pain model.

EQUIVALENTS

- 10 [00325] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.
- [00326] It is understood that the detailed examples and embodiments described herein are given by way of example for illustrative purposes only, and are in no way considered to be
- 15 limiting to the disclosure. Various modifications or changes in light thereof will be suggested to persons skilled in the art and are included within the spirit and purview of this application and are considered within the scope of the appended claims. For example, the relative quantities of the ingredients may be varied to optimize the desired effects, additional ingredients may be added, and/or similar ingredients may be substituted for one or more of the ingredients described.
- 20 Additional advantageous features and functionalities associated with the systems, methods, and processes of the present disclosure will be apparent from the appended claims. Moreover, those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. Such equivalents are intended to be encompassed by the following claims.