# CRYSTALLINE FORM OF PDE4B INHIBITOR AND PHARMACEUTICAL USE THEREOF

## Technical Field

[1] The present invention belongs to the field of pharmaceutical chemistry and relates to a crystalline form of a compound and a salt thereof, a preparation method therefor, and the use thereof, in particular to a crystalline form of a PDE4B inhibitor compound and a salt thereof, a preparation method therefor, and the use thereof in the preparation of a medicament for treating a related disease mediated by PDE4B.

## Background Art

[2] PDE4 inhibitors produce antidepressant effects in humans and animals by enhancing cAMP signaling in the brain. PDE4 inhibitors also play an important role in the treatment of other central nervous system diseases, including diseases such as Alzheimer's disease, Parkinson's disease, schizophrenia, stroke, and Huntington's chorea. In addition, the research and development of PDE4 inhibitors have also made great progress in the treatment of respiratory diseases such as asthma and chronic obstructive pulmonary disease. The principle of research and development of such drugs stems from the role of PDE4 in inhibiting the function of a range of inflammatory cells and resident cells, which is believed to be associated with the pathogenesis of such diseases. A large number of clinical studies have shown that cyclic adenosine monophosphate (cAMP) can block the proliferation and chemotaxis of inflammatory cells and inhibit the release of inflammatory and cytotoxic mediators in lungs. In addition, PDE4 is especially abundant in immune cells, inflammatory cells, and smooth muscle cells.

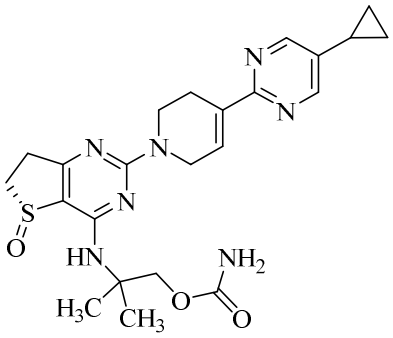
[3] PDE4 inhibitors play an anti-inflammatory role mainly by inhibiting the hydrolysis function of PDE4 so as to increase the cAMP level *in vivo*, thereby inhibiting the release of inflammatory factors and at the same time promoting the production of anti-inflammatory mediators. Roflumilast is used clinically for the treatment of COPD with a significant anti-inflammatory effect and can inhibit the release of inflammatory mediators such as TNF-α, interleukins, and chemokines from monocytes, macrophages, T cells, etc. However, such inhibitors generally have serious side effects such as nausea and vomiting, which limits the clinical application of PDE4

inhibitors. A large number of studies have shown that phosphodiesterase 4 subtype B (PDE4B), which is associated with inflammatory responses in the human body, participates in the release of various inflammatory mediators *in vivo*, while subtype D is closely related to the production of side effects such as nausea and vomiting, which provides a new idea for finding PDE4 inhibitors with low side effects, i.e., designing PDE4B inhibitors that may have reduced side effects, thereby promoting further clinical application.

[4] Phosphodiesterase 4 is highly selective for cAMP and has four subtypes, namely PDE4A, 4B, 4C, and 4D, with at least 25 splice variants. The protein sequences of the catalytic domains of the four subtypes of PDE4 are highly homologous; therefore, inhibitors that act on the catalytic domain do not exhibit subtype selectivity. Most of the classical PDE4 inhibitors have been reported to act on the catalytic domain. A new mode of action of PDE4 inhibitors has been reported in recent years, in which the inhibitor acts on both the catalytic domain and a regulatory sequence, so that the regulatory sequence can stabilize the closed conformation of the protein, thereby preventing cAMP from entering, thus exerting an inhibitory effect. However, it has been found from research that there are amino acid differences between PDE4B and 4D in this regulatory sequence, so designing inhibitors based on such differences can produce subtype selectivity. Therefore, based on the difference of the two amino acids PDE4B Leu674/PDE4D Gln594 in CR3 (Conserved Region 3) of the downstream regulatory sequence, it is expected to achieve the selectivity to subtype B and reduce the side effects of the inhibitors while maintaining the activity.

[5] Polymorphs or salt forms of drugs are of vital pharmaceutical and pharmacological significance. Polymorphs or salt forms possess different physical properties, which influence pharmaceutical parameters such as storage stability, compressibility, and density (which are important for formulations and product manufacturing) and dissolution rate (a key factor determining bio-availability). Therefore, the discovery of crystalline forms/salt forms with good activity, high safety, and few side effects shows promising prospects for clinical development.

[6] PCT/CN2023/112061 discloses a compound as shown in formula (I) that has good inhibitory effect on PDE4B.



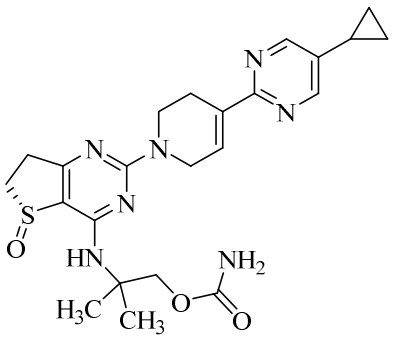
(I).

## Summary of the Invention

[7] The present invention provides a crystalline form of a small molecule compound with PDE4B inhibitory activity or a pharmaceutically acceptable salt thereof, wherein the compound is as shown in formula (I), and has a high activity, relatively low toxic and side effects, excellent pharmacokinetic characteristics and high bio-availability.

[8] The advantages of the crystalline form of the compound as shown in formula (I) or the pharmaceutically acceptable salt thereof include, but are not limited to, easy processing and crystallization, convenient handling, easy purification, easy industrialization, good flowability, easy micronization, relatively high solubility, relatively good pharmacokinetic characteristics, and good stability, and are suitable for preparing pharmaceutical preparations.

[9] The present invention provides a crystalline form of a compound as shown in formula (I) or a pharmaceutically acceptable salt thereof,



(I).

[10]In some embodiments, the pharmaceutically acceptable salt is selected from maleate, 2-naphthalenesulfonate, 1,5-naphthalenedisulfonate, fumarate, hydrohalide (preferably hydrobromide and hydrochloride), sulfate, phosphate, L-tartrate, citrate, L-malate, hippurate, D-glucuronate, glycolate, mucate, succinate, lactate, orotate, pamoate, glycinate, alaninate, argininate, cinnamate, benzoate, benzenesulfonate, p-toluenesulfonate, acetate, propionate,

embodiments, the crystalline form B has an X-ray powder diffraction pattern with characteristic diffraction peaks at the following 2θ positions by using Cu-Kα radiation: 6.11°±0.2°, 9.71°±0.2°, 14.90°±0.2°, 19.51°±0.2°, 19.87°±0.2°, 20.85°±0.2°, 22.53°±0.2° and 23.71°±0.2°. In some embodiments, the crystalline form B has an X-ray powder diffraction pattern with characteristic diffraction peaks at the following 2θ positions by using Cu-Kα radiation: 6.11°±0.2°, 9.71°±0.2°, 14.90°±0.2°, 18.33°±0.2°, 19.51°±0.2°, 19.87°±0.2°, 20.33°±0.2°, 20.85°±0.2°, 22.53°±0.2°, 23.71°±0.2°, 25.06°±0.2°, 26.42°±0.2° and 29.71°±0.2°. In some embodiments, the crystalline form B has an X-ray powder diffraction pattern as shown in Figure 20 by using Cu-Kα radiation. In some embodiments, the crystalline form B has a differential scanning calorimetry (DSC) curve showing peak temperatures of 47.47°C, 115.97°C and 154.87°C, respectively; and a thermogravimetric analysis (TGA) curve showing weight loss of about 1.89% before the temperature reaches 90.13°C and about 2.84% before the temperature is in the range of 90.13°C-150.12°C. The differential scanning calorimetry curve and thermogravimetric analysis curve thereof are as shown in Figures 21-22, respectively.

[26]The present invention provides a crystalline form A of a compound as shown in formula (I). In some embodiments, the crystalline form A has an X-ray powder diffraction pattern with characteristic diffraction peaks at the following 2θ positions by using Cu-Kα radiation: 14.96°±0.2°, 17.88°±0.2°, 20.12°±0.2°, 20.54°±0.2° and 27.07°±0.2°. In some embodiments, the crystalline form A has an X-ray powder diffraction pattern with characteristic diffraction peaks at the following 2θ positions by using Cu-Kα radiation: 10.60°±0.2°, 12.79°±0.2°, 14.96°±0.2°, 17.88°±0.2°, 20.12°±0.2°, 20.54°±0.2°, 23.34°±0.2° and 27.07°±0.2°. In some embodiments, the crystalline form A has an X-ray powder diffraction pattern with characteristic diffraction peaks at the following 2θ positions by using Cu-Kα radiation: 10.60°±0.2°, 11.08°±0.2°, 12.79°±0.2°, 14.60°±0.2°, 14.96°±0.2°, 17.88°±0.2°, 19.85°±0.2°, 20.12°±0.2°, 20.54°±0.2°, 23.34±0.2°, 24.03±0.2°, 27.07±0.2° and 29.54±0.2°. In some embodiments, the crystalline form A has an X-ray powder diffraction pattern as shown in Figure 23 by using Cu-Kα radiation. In some embodiments, the crystalline form A has a differential scanning calorimetry (DSC) curve showing a peak temperature of 220.37°C; and a thermogravimetric analysis (TGA) curve showing a weight loss of about 1.28% before the temperature reaches 250°C. The differential scanning calorimetry curve and thermogravimetric analysis curve thereof are as shown in Figures 24-25, respectively.

[44]It can be understood that the numerical values described and claimed in the present invention are approximate values. Variations in values may be attributed to device calibration, device errors, crystal purity, crystal size, sample size and other factors.

[45]The structure of the crystalline form of the present invention can be analyzed by using various analytical techniques known to those skilled in the art, including but not limited to, X-ray powder diffraction (XRD), ion chromatography (IC), differential scanning calorimetry (DSC) and/or thermogravimetric analysis (TGA), also known as thermogravimetry (TG).

[46]It can be understood that the crystalline forms of the present invention are not limited to characteristic patterns such as XRD, DSC, and TGA, which are completely identical to those described in the drawings disclosed in the present invention, and any crystalline form having a characteristic pattern which is substantially or essentially the same as those described in the drawings falls within the scope of the present invention.

[47]It can be understood that, as is well known in the field of differential scanning calorimetry (DSC), a melting peak height of a DSC curve depends on many factors related to sample preparation and geometric shapes of instruments, and a peak position is relatively insensitive to experiment details. Therefore, in some embodiments, the crystallized compounds of the present invention are characterized in that DSC patterns comprising characteristic peak positions have substantially the same properties as the DSC patterns provided in the drawings of the present invention, with an error tolerance of ± 3°C.

[48]Unless otherwise specified, the technical and scientific terms used herein have the same meanings as commonly understood by those skilled in the art to which the present invention pertains. In case of conflict, the definitions provided in the present application will control. When an amount, concentration, or other value or parameter is expressed in the form of a range, a preferred range, or a preferred upper numerical limit and a preferred lower numerical limit, it should be understood as specifically disclosing any range formed by combining any upper limit or preferred value with any lower limit or preferred value, regardless of whether the range is specifically enumerated. Unless otherwise specified, all numerical ranges listed herein are intended to include the endpoints of the range and all integers and fractions (decimals) within the range.

[49]Unless otherwise stated, the terms used in the description and the claims have the following meanings.

gelatin, vegetable oil, polyethylene glycols, diluents, granulating agents, lubricants, binders, disintegrants, etc.

[59]The term "IC50" in the present invention refers to the half maximal inhibitory concentration, i.e., a concentration where half of the maximum inhibitory effect is achieved.

[60]The term "ether solvent" in the present invention refers to a chain compound or a cyclic compound containing an ether bond -O- and having 1-10 carbon atoms, and the specific examples thereof include, but are not limited to: tetrahydrofuran, diethyl ether, propylene glycol methyl ether, methyl tert-butyl ether, isopropyl ether or 1,4-dioxane.

[61]The term "alcohol solvent" in the present invention refers to a group derived from "$C_{1-6}$ alkyl" on which one or more hydrogen atoms are substituted with one or more "hydroxyl groups", wherein the "hydroxyl group" and "$C_{1-6}$ alkyl" are as defined above, and the specific examples thereof include, but are not limited to: methanol, ethanol, isopropanol, n-propanol, isopentanol or trifluoroethanol.

[62]The term "ester solvent" of the present invention refers to a combination of a lower organic acid containing 1-4 carbon atoms and a lower alcohol containing 1-6 carbon atoms, and the specific examples thereof include, but are not limited to: ethyl acetate, isopropyl acetate or butyl acetate.

[63]The term "ketone solvent" in the present invention refers to a compound in which a carbonyl group (-C(O)-) is connected to two hydrocarbon groups. According to different hydrocarbon groups in molecules, ketones can be classified into aliphatic ketones, alicyclic ketones, aromatic ketones, saturated ketones and unsaturated ketones, and the specific examples thereof include, but are not limited to: acetone, acetophenone and 4-methyl-2-pentanone.

[64]The term "nitrile solvent" in the present invention refers to a group derived from "$C_{1-6}$ alkyl" on which one or more hydrogen atoms are substituted with one or more "cyano groups", wherein the "cyano group" and "$C_{1-6}$ alkyl" are as defined above, and the specific examples thereof include, but are not limited to: acetonitrile or propionitrile.

[65]The term "halogenated hydrocarbon solvent" in the present invention refers to a group derived from "$C_{1-6}$ alkyl" on which one or more hydrogen atoms are substituted with one or more "halogen atoms", wherein the "halogen atom" and "$C_{1-6}$ alkyl" are as defined above, and the specific examples thereof include, but are not limited to: dichloromethane, 1,2-dichloroethane, chloroform or carbon tetrachloride.

[92]Figure 18 shows the differential scanning calorimetry pattern of the crystalline form A of methanesulfonate of the compound as shown in formula (I).

[93]Figure 19 shows the thermogravimetric analysis curve of the crystalline form A of methanesulfonate of the compound as shown in formula (I).

[94]Figure 20 shows the X-ray powder diffraction pattern of the crystalline form B of methanesulfonate of the compound as shown in formula (I).

[95]Figure 21 shows the differential scanning calorimetry pattern of the crystalline form B of methanesulfonate of the compound as shown in formula (I).

[96]Figure 22 shows the thermogravimetric analysis curve of the crystalline form B of methanesulfonate of the compound as shown in formula (I).

[97]Figure 23 shows the X-ray powder diffraction pattern of the crystalline form A of the compound as shown in formula (I).

[98]Figure 24 shows the differential scanning calorimetry pattern of the crystalline form A of the compound as shown in formula (I).

[99]Figure 25 shows the thermogravimetric analysis curve of the crystalline form A of the compound as shown in formula (I).

[100] Figure 26 shows the detection results of the inhibitory activity on TNF-α secretion of the compound as shown in formula (I) in an LPS-induced mouse model of lung inflammation.

[101] Figure 27 is a microscopic image of the crystalline form A of hydrochloride of the compound as shown in formula (I).

[102] Figure 28 is a microscopic image of the crystalline form A of the compound as shown in formula (I).

## Detailed Description of Embodiments

[103] The structures of the compounds are determined by nuclear magnetic resonance (NMR) or (and) mass spectrometry (MS). The NMR shift (δ) is given in the unit of 10-6 (ppm). NMR is determined with Bruker Avance III 400 and Bruker Avance 300 NMR spectrometer; the solvents for determination are deuterated dimethyl sulfoxide (DMSO-d6), deuterated chloroform (CDCl3) and deuterated methanol (CD3OD); and the internal standard is tetramethylsilane (TMS).

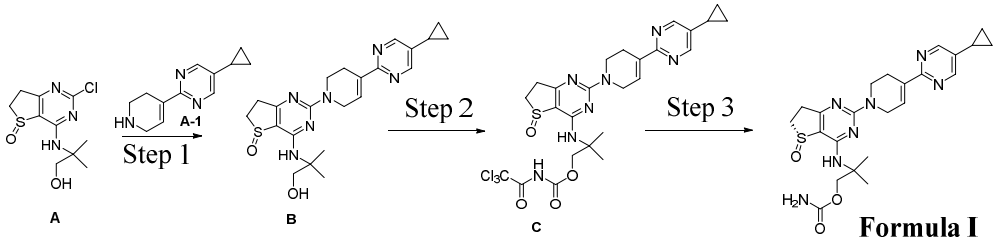
[104] MS is determined with Agilent 6120B (ESI) and Agilent 6120B (APCI).

[105] HPLC is determined with Agilent 1260DAD high-pressure liquid chromatograph (Eclipse Plus C18, 150×4.6 mm).

[106] The known starting materials of the present invention can be synthesized by or according to methods known in the art, or can be purchased from Titan Technology Co., Ltd., Energy Chemical Co., Ltd., Shanghai Demo Co., Ltd., Chengdu Kelong Chemical Co., Ltd., Accela ChemBio Co., Ltd., J&K Scientific Co., Ltd. and other companies.

[107] The implementation process and beneficial effects of the present invention are described in detail below through specific examples, which are intended to help readers better understand the essence and characteristics of the present invention, and are not intended to limit the scope of implementation of the present invention.

**Example 1: Preparation of compound as shown in formula (I)**




[108] Step 1: Compound **A** (1.0 g, 3.63 mmol) was dissolved in 1,4-dioxane (30 mL), compound **A-1** (880 mg, 4.36 mmol) and N,N-diisopropylethylamine (1.40 g, 10.86 mmol) were added, and the mixture was stirred at 90°C overnight under nitrogen atmosphere. After the reaction was complete, the reaction solution was concentrated and subjected to silica gel column chromatography (dichloromethane/methanol (v/v) = 95/5) to obtain compound **B** (1.6 g, 94%).

[109] LC-MS (ESI): m/z = 441.6 $[M+H]^+$

[110] Step 2: Compound **B** (1.6 g, 3.63 mmol) was dissolved in dichloromethane (5 mL), trichloroacetyl isocyanate (820 mg, 4.36 mmol) was added in an ice bath and the mixture was stirred in the ice bath for one hour. The reaction solution was concentrated to obtain compound **C** (2.28 g, 100%).

[111] Step 3: Compound **C** (2.28 g, 3.63 mmol) was dissolved in methanol (20 mL), potassium carbonate (1.51 g, 10.89 mmol) and water (20 mL) were added in an ice bath, and the mixture was stirred at room temperature for 2.5 hours. The reaction solution was diluted with water and extracted with dichloromethane, and the organic phases were combined, dried, filtered, concentrated, and subjected to SFC chiral resolution to obtain the compound as shown in formula (I) (1.3 g, 74%).

form B has an X-ray powder diffraction pattern as shown in Figure 20 by using Cu-Kα radiation. The crystalline form B has a differential scanning calorimetry (DSC) curve showing peak temperatures of 47.47°C, 115.97°C and 154.87°C, respectively; and a thermogravimetric analysis (TGA) curve showing weight loss of about 1.89% before the temperature reaches 90.13°C and about 2.84% before the temperature is in the range of 90.13°C-150.12°C. The differential scanning calorimetry curve and thermogravimetric analysis curve thereof are as shown in Figures 21-22, respectively.

[122] Based on the peak shift analysis of 1H NMR (400 MHz, DMSO) δ, the chemical shift at 8.56 (2H) of the compound as shown in formula (I) corresponds to the -Ch peak at positions 28 and 30; the chemical shift thereof at 2.35 (4H) corresponds to the -CH3 peak of methanesulfonic acid, and their ratio is 1:2. Therefore, the ratio of the compound as shown in formula (I) to methanesulfonic acid can be determined to be 1:0.7.

**Example 9: Preparation of crystalline form A of compound as shown in formula (I)**

[123] 50 mg of the compound as shown in formula (I) was placed in a 2 mL sample vial. 200 μL of an acetonitrile solution was added, and the mixture was slurried at room temperature for suspension for 3 days. The resulting mixture was centrifuged, and the solid was collected and dried under vacuum at 50°C to obtain the crystalline form A of the compound as shown in formula (I). The crystalline form A has an X-ray powder diffraction pattern as shown in Figure 23 by using Cu-Kα radiation. The crystalline form A has a differential scanning calorimetry (DSC) curve showing a peak temperature of 220.37°C; and a thermogravimetric analysis (TGA) curve showing a weight loss of about 1.28% before the temperature reaches 250°C. The differential scanning calorimetry curve and thermogravimetric analysis curve thereof are as shown in Figures 24-25, respectively.

**X-ray powder diffraction (XRD)/DSC/TGA/DVS/IC tests**

[124] The test parameters of XRD/DSC/TGA/DVS are detailed in Table 1. The XRD data of various relevant crystalline forms are shown in Tables 2-8.

| DSC | |
|---|---|
| Instrument brand | TA |
| Instrument model | DSC 25 |
| Atmosphere | $N_2$ |
| Gas flow rate | Protective gas: 50 ml/min Purge gas: 40 ml/min |
| Crucible type | Perforated lid aluminum crucible |
| Sample preparation method | A sample was spread on the bottom of the aluminum crucible and sealed tightly with a perforated aluminum lid |
| Heating rate | 10°C/min |
| TGA | |
| Instrument brand | TA |
| Instrument model | TGA 550 |
| Atmosphere | $N_2$ |
| Gas flow rate | Protective gas: 60 ml/min Purge gas: 30 ml/min |
| Crucible type | Open-top (or perforated lid) alumina (or aluminum) crucible |
| Sample preparation method | A sample was spread on the bottom of the crucible (sealed tightly with a perforated alumina lid/a perforated aluminum lid) |
| Heating rate | 10°C/min |

| Instrument | Model | Waters ACQULTY (JT-Y-J0617) | | |
|---|---|---|---|---|
| Parameter and method | Chromatographic column | Adamantyl-bonded silica gel as a filler (CAPCELL PAK ADME4.6*250 mm, 5 μm) | | |
| | Trap column | Welch Ghost-Buster Column 4.5*50 mm | | |
| | Column temperature | 30°C | | |
| | Flow rate | 1.0 mL/min | | |
| | Detection wavelength | 260 nm | | |
| | Injection volume | 10 μL | | |
| | Run time | 75 min | | |
| | Mobile phase | Mobile phase A: 0.01M sodium dihydrogen phosphate solution, pH adjusted to 3.0 with phosphoric acid<br>Mobile phase B: acetonitrile | | |
| | Running gradient | Time (min) | A (%) | B (%) |
| | | 0 | 85 | 15 |
| | | 20 | 70 | 30 |
| | | 50 | 70 | 30 |
| | | 65 | 20 | 80 |
| | | 66 | 85 | 15 |
| | | 75 | 85 | 15 |

Table 2: XRD peak list of crystalline form A of hydrochloride of compound as shown in formula (I)

| Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] | Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] |
|---|---|---|---|---|---|---|---|
| 7.42 | 141.97 | 11.91 | 8.49 | 23.04 | 302.34 | 3.86 | 18.08 |
| 9.44 | 458.26 | 9.37 | 27.41 | 23.63 | 365.18 | 3.77 | 21.84 |
| 11.21 | 1672.04 | 7.89 | 100.00 | 25.23 | 370.74 | 3.53 | 22.17 |
| 15.85 | 83.58 | 5.59 | 5.00 | 26.50 | 1172.29 | 3.36 | 70.11 |
| 16.42 | 53.59 | 5.40 | 3.21 | 28.21 | 227.98 | 3.16 | 13.63 |
| 18.76 | 204.39 | 4.73 | 12.22 | 28.53 | 194.19 | 3.13 | 11.61 |
| 19.06 | 347.40 | 4.66 | 20.78 | 28.98 | 168.35 | 3.08 | 10.07 |
| 19.48 | 223.02 | 4.56 | 13.34 | 30.35 | 137.47 | 2.94 | 8.22 |
| 19.84 | 379.28 | 4.48 | 22.68 | 31.70 | 88.60 | 2.82 | 5.30 |
| 20.27 | 509.84 | 4.38 | 30.49 | 33.29 | 68.84 | 2.69 | 4.12 |
| 21.45 | 270.14 | 4.14 | 16.16 | 34.33 | 68.89 | 2.61 | 4.12 |
| 21.82 | 1135.52 | 4.07 | 67.91 | 36.45 | 93.04 | 2.47 | 5.56 |
| 22.65 | 379.31 | 3.93 | 22.69 | 38.33 | 92.73 | 2.35 | 5.55 |

Table 3: XRD peak list of crystalline form B of hydrochloride of compound as shown in formula (I)

| Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] | Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] |
|---|---|---|---|---|---|---|---|
| 4.46 | 4053.15 | 19.80 | 90.11 | 23.37 | 1383.04 | 3.81 | 30.75 |
| 7.85 | 2755.97 | 11.27 | 61.27 | 24.24 | 276.78 | 3.67 | 6.15 |
| 8.92 | 139.68 | 9.91 | 3.11 | 25.06 | 1678.72 | 3.55 | 37.32 |
| 10.13 | 1001.30 | 8.73 | 22.26 | 25.80 | 440.86 | 3.45 | 9.80 |
| 10.62 | 50.81 | 8.33 | 1.13 | 26.94 | 143.80 | 3.31 | 3.20 |
| 13.39 | 904.07 | 6.61 | 20.10 | 27.52 | 216.45 | 3.24 | 4.81 |
| 15.07 | 4498.14 | 5.88 | 100.00 | 28.06 | 204.09 | 3.18 | 4.54 |
| 15.67 | 503.14 | 5.66 | 11.19 | 28.49 | 290.58 | 3.13 | 6.46 |
| 16.51 | 145.93 | 5.37 | 3.24 | 29.14 | 535.49 | 3.06 | 11.90 |
| 17.46 | 1028.19 | 5.08 | 22.86 | 29.83 | 253.64 | 3.00 | 5.64 |
| 17.64 | 1099.00 | 5.03 | 24.43 | 30.42 | 343.32 | 2.94 | 7.63 |
| 18.09 | 463.97 | 4.90 | 10.31 | 31.23 | 201.15 | 2.86 | 4.47 |
| 19.35 | 1420.92 | 4.59 | 31.59 | 32.75 | 169.25 | 2.73 | 3.76 |
| 20.33 | 615.83 | 4.37 | 13.69 | 33.43 | 216.33 | 2.68 | 4.81 |
| 20.58 | 449.89 | 4.32 | 10.00 | 35.64 | 137.39 | 2.52 | 3.05 |
| 21.00 | 249.15 | 4.23 | 5.54 | 36.38 | 92.20 | 2.47 | 2.05 |
| 21.45 | 511.24 | 4.14 | 11.37 | 38.22 | 141.21 | 2.35 | 3.14 |
| 21.85 | 2165.84 | 4.07 | 48.15 | 38.83 | 155.42 | 2.32 | 3.46 |
| 22.03 | 2151.30 | 4.03 | 47.83 | 39.29 | 188.39 | 2.29 | 4.19 |
| 22.79 | 1355.88 | 3.90 | 30.14 | | | | |

Table 4: XRD peak list of crystalline form C of hydrochloride of compound as shown in formula (I)

| Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] | Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] |
|---|---|---|---|---|---|---|---|
| 3.78 | 152.24 | 23.39 | 7.76 | 19.45 | 305.57 | 4.56 | 15.57 |
| 4.96 | 1962.30 | 17.83 | 100.00 | 19.93 | 651.85 | 4.46 | 33.22 |
| 7.01 | 206.85 | 12.62 | 10.54 | 20.60 | 1093.06 | 4.31 | 55.70 |
| 8.05 | 175.23 | 10.98 | 8.93 | 21.22 | 1019.14 | 4.19 | 51.94 |
| 8.86 | 1138.00 | 9.98 | 57.99 | 21.88 | 1083.96 | 4.06 | 55.24 |
| 9.55 | 213.68 | 9.27 | 10.89 | 22.69 | 279.57 | 3.92 | 14.25 |
| 9.90 | 331.63 | 8.93 | 16.90 | 24.38 | 1201.80 | 3.65 | 61.24 |
| 10.64 | 225.37 | 8.31 | 11.49 | 25.08 | 506.52 | 3.55 | 25.81 |
| 11.19 | 119.96 | 7.91 | 6.11 | 25.72 | 620.35 | 3.46 | 31.61 |
| 11.70 | 130.51 | 7.57 | 6.65 | 26.26 | 368.02 | 3.39 | 18.75 |
| 12.52 | 353.14 | 7.07 | 18.00 | 26.89 | 310.60 | 3.32 | 15.83 |
| 12.83 | 137.29 | 6.90 | 7.00 | 28.06 | 135.79 | 3.18 | 6.92 |
| 14.96 | 581.62 | 5.92 | 29.64 | 28.95 | 171.28 | 3.08 | 8.73 |
| 16.01 | 373.15 | 5.54 | 19.02 | 30.08 | 194.20 | 2.97 | 9.90 |
| 17.23 | 195.41 | 5.15 | 9.96 | 31.77 | 168.96 | 2.82 | 8.61 |
| 17.80 | 394.20 | 4.98 | 20.09 | 34.63 | 76.14 | 2.59 | 3.88 |
| 18.36 | 210.27 | 4.83 | 10.72 | 37.11 | 36.03 | 2.42 | 1.84 |
| 18.90 | 439.09 | 4.70 | 22.38 | 38.41 | 30.45 | 2.34 | 1.55 |

Table 5: XRD peak list of crystalline form A of p-toluenesulfonate of compound as shown in formula (I)

| Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] | Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] |
|---|---|---|---|---|---|---|---|
| 5.50 | 4634.96 | 16.08 | 100.00 | 20.58 | 1266.50 | 4.32 | 27.32 |
| 6.33 | 1559.62 | 13.95 | 33.65 | 20.92 | 1037.60 | 4.25 | 22.39 |
| 7.77 | 2337.42 | 11.38 | 50.43 | 21.22 | 693.86 | 4.19 | 14.97 |
| 8.97 | 446.80 | 9.85 | 9.64 | 21.84 | 933.11 | 4.07 | 20.13 |
| 9.55 | 276.08 | 9.26 | 5.96 | 22.21 | 1114.73 | 4.00 | 24.05 |
| 10.48 | 263.42 | 8.44 | 5.68 | 22.45 | 890.56 | 3.96 | 19.21 |
| 11.01 | 522.30 | 8.04 | 11.27 | 22.79 | 1143.72 | 3.90 | 24.68 |
| 12.69 | 1485.72 | 6.97 | 32.05 | 24.15 | 900.02 | 3.69 | 19.42 |
| 13.11 | 778.32 | 6.75 | 16.79 | 25.57 | 525.37 | 3.48 | 11.34 |
| 13.46 | 419.37 | 6.58 | 9.05 | 26.00 | 836.48 | 3.43 | 18.05 |
| 13.91 | 368.63 | 6.36 | 7.95 | 27.18 | 350.67 | 3.28 | 7.57 |
| 14.38 | 205.90 | 6.16 | 4.44 | 28.30 | 171.65 | 3.15 | 3.70 |
| 15.05 | 793.53 | 5.89 | 17.12 | 28.94 | 243.35 | 3.08 | 5.25 |
| 15.57 | 1055.09 | 5.69 | 22.76 | 29.45 | 339.97 | 3.03 | 7.33 |
| 16.52 | 2237.03 | 5.37 | 48.26 | 30.01 | 278.07 | 2.98 | 6.00 |
| 17.55 | 443.53 | 5.05 | 9.57 | 30.53 | 145.99 | 2.93 | 3.15 |
| 17.90 | 719.52 | 4.95 | 15.52 | 32.39 | 194.40 | 2.76 | 4.19 |
| 18.23 | 609.53 | 4.87 | 13.15 | 33.14 | 116.44 | 2.70 | 2.51 |
| 18.59 | 706.44 | 4.77 | 15.24 | 34.71 | 108.28 | 2.58 | 2.34 |
| 19.12 | 493.46 | 4.64 | 10.65 | 35.72 | 160.55 | 2.51 | 3.46 |
| 19.63 | 3718.19 | 4.52 | 80.22 | 36.74 | 94.91 | 2.45 | 2.05 |
| 19.95 | 898.81 | 4.45 | 19.39 | 37.57 | 58.51 | 2.39 | 1.26 |
| 20.15 | 838.51 | 4.41 | 18.09 | 38.47 | 104.35 | 2.34 | 2.25 |

Table 6: XRD peak list of crystalline form B of p-toluenesulfonate of compound as shown in formula (I)

| Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] | Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] |
|---|---|---|---|---|---|---|---|
| 6.64 | 334.63 | 13.31 | 8.68 | 21.24 | 332.50 | 4.18 | 8.63 |
| 7.62 | 2053.47 | 11.61 | 53.29 | 22.33 | 509.28 | 3.98 | 13.22 |
| 8.00 | 2813.78 | 11.05 | 73.02 | 22.59 | 821.80 | 3.94 | 21.33 |
| 9.70 | 3023.29 | 9.12 | 78.45 | 23.15 | 3853.58 | 3.84 | 100.00 |
| 10.57 | 852.48 | 8.37 | 22.12 | 23.85 | 406.67 | 3.73 | 10.55 |
| 11.10 | 1455.94 | 7.97 | 37.78 | 24.63 | 755.70 | 3.61 | 19.61 |
| 11.91 | 999.30 | 7.43 | 25.93 | 25.01 | 1614.66 | 3.56 | 41.90 |
| 12.79 | 195.53 | 6.92 | 5.07 | 25.67 | 801.96 | 3.47 | 20.81 |
| 13.83 | 518.22 | 6.40 | 13.45 | 26.38 | 1416.58 | 3.38 | 36.76 |
| 14.97 | 262.04 | 5.92 | 6.80 | 27.09 | 560.87 | 3.29 | 14.55 |
| 15.30 | 552.25 | 5.79 | 14.33 | 27.77 | 443.84 | 3.21 | 11.52 |
| 16.13 | 626.78 | 5.49 | 16.26 | 28.45 | 520.04 | 3.14 | 13.50 |
| 16.48 | 997.67 | 5.38 | 25.89 | 29.36 | 320.89 | 3.04 | 8.33 |
| 17.24 | 765.64 | 5.14 | 19.87 | 29.64 | 405.80 | 3.01 | 10.53 |
| 18.16 | 2611.98 | 4.88 | 67.78 | 30.96 | 194.74 | 2.89 | 5.05 |
| 18.39 | 2094.59 | 4.82 | 54.35 | 32.53 | 156.36 | 2.75 | 4.06 |
| 19.01 | 1418.93 | 4.67 | 36.82 | 33.25 | 84.13 | 2.69 | 2.18 |
| 19.50 | 2144.78 | 4.55 | 55.66 | 34.55 | 58.24 | 2.60 | 1.51 |
| 20.14 | 784.67 | 4.41 | 20.36 | 35.18 | 42.66 | 2.55 | 1.11 |
| 20.56 | 1574.15 | 4.32 | 40.85 | 38.53 | 154.91 | 2.34 | 4.02 |
| 20.83 | 303.31 | 4.26 | 7.87 | | | | |

Table 7: XRD peak list of crystalline form A of methanesulfonate of compound as shown in formula (I)

| Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] | Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] |
|---|---|---|---|---|---|---|---|
| 5.12 | 729.74 | 17.25 | 31.89 | 19.85 | 796.23 | 4.47 | 34.80 |
| 6.23 | 1061.69 | 14.20 | 46.40 | 20.58 | 1997.75 | 4.32 | 87.31 |
| 7.28 | 2288.02 | 12.14 | 100.00 | 20.81 | 928.16 | 4.27 | 40.57 |
| 9.21 | 416.61 | 9.60 | 18.21 | 21.55 | 1150.29 | 4.12 | 50.27 |
| 9.69 | 64.02 | 9.13 | 2.80 | 21.96 | 836.29 | 4.05 | 36.55 |
| 10.63 | 364.37 | 8.33 | 15.92 | 22.53 | 482.98 | 3.95 | 21.11 |
| 11.06 | 204.37 | 8.00 | 8.93 | 23.19 | 905.34 | 3.84 | 39.57 |
| 12.49 | 240.32 | 7.09 | 10.50 | 24.06 | 1237.30 | 3.70 | 54.08 |
| 12.80 | 189.49 | 6.92 | 8.28 | 25.37 | 311.49 | 3.51 | 13.61 |
| 13.20 | 496.57 | 6.71 | 21.70 | 26.41 | 322.48 | 3.38 | 14.09 |
| 13.77 | 299.07 | 6.43 | 13.07 | 27.11 | 407.65 | 3.29 | 17.82 |
| 14.61 | 790.90 | 6.06 | 34.57 | 27.49 | 283.99 | 3.24 | 12.41 |
| 14.98 | 401.83 | 5.92 | 17.56 | 28.93 | 318.62 | 3.09 | 13.93 |
| 15.54 | 483.56 | 5.70 | 21.13 | 29.48 | 220.85 | 3.03 | 9.65 |
| 16.32 | 337.64 | 5.43 | 14.76 | 30.25 | 189.33 | 2.95 | 8.27 |
| 16.82 | 261.21 | 5.27 | 11.42 | 32.31 | 100.70 | 2.77 | 4.40 |
| 17.32 | 320.38 | 5.12 | 14.00 | 32.76 | 124.23 | 2.73 | 5.43 |
| 17.93 | 1975.04 | 4.95 | 86.32 | 34.88 | 85.47 | 2.57 | 3.74 |
| 18.51 | 2176.38 | 4.79 | 95.12 | 36.02 | 117.79 | 2.49 | 5.15 |
| 19.53 | 758.08 | 4.55 | 33.13 | 37.77 | 55.30 | 2.38 | 2.42 |

Table 8: XRD peak list of crystalline form B of methanesulfonate of compound as shown in formula (I)

| Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] | Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] |
|---|---|---|---|---|---|---|---|
| 6.11 | 12088.62 | 14.46 | 100.00 | 25.06 | 851.83 | 3.55 | 7.05 |
| 7.43 | 296.21 | 11.90 | 2.45 | 26.42 | 786.80 | 3.37 | 6.51 |
| 9.71 | 2408.61 | 9.11 | 19.92 | 26.87 | 377.27 | 3.32 | 3.12 |
| 12.19 | 421.60 | 7.26 | 3.49 | 27.55 | 605.34 | 3.24 | 5.01 |
| 14.40 | 277.36 | 6.15 | 2.29 | 28.32 | 274.74 | 3.15 | 2.27 |
| 14.90 | 1981.36 | 5.95 | 16.39 | 29.44 | 382.55 | 3.03 | 3.16 |
| 15.75 | 323.10 | 5.63 | 2.67 | 29.71 | 708.59 | 3.01 | 5.86 |
| 16.39 | 184.86 | 5.41 | 1.53 | 30.40 | 261.58 | 2.94 | 2.16 |
| 18.33 | 768.68 | 4.84 | 6.36 | 31.52 | 109.96 | 2.84 | 0.91 |
| 19.51 | 9034.80 | 4.55 | 74.74 | 32.02 | 71.97 | 2.80 | 0.60 |
| 19.87 | 2421.19 | 4.47 | 20.03 | 32.81 | 232.36 | 2.73 | 1.92 |
| 20.33 | 1714.14 | 4.37 | 14.18 | 33.77 | 99.06 | 2.65 | 0.82 |
| 20.85 | 2110.91 | 4.26 | 17.46 | 34.57 | 125.75 | 2.59 | 1.04 |
| 22.53 | 2753.97 | 3.95 | 22.78 | 36.33 | 74.48 | 2.47 | 0.62 |
| 23.21 | 441.91 | 3.83 | 3.66 | 37.24 | 307.07 | 2.41 | 2.54 |
| 23.71 | 2835.04 | 3.75 | 23.45 | 37.81 | 270.15 | 2.38 | 2.23 |
| 24.62 | 293.25 | 3.62 | 2.43 | 39.24 | 132.96 | 2.30 | 1.10 |

Table 9: XRD peak list of crystalline form A of compound as shown in formula (I)

| Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] | Pos. [°2Th.] | Height [cts] | d-spacing | Rel. Int. [%] |
|---|---|---|---|---|---|---|---|
| 3.28 | 90.18 | 26.95 | 1.20 | 25.05 | 49.78 | 3.55 | 0.66 |
| 7.33 | 102.91 | 12.06 | 1.37 | 25.46 | 44.08 | 3.50 | 0.58 |
| 10.60 | 919.10 | 8.34 | 12.19 | 26.12 | 195.88 | 3.41 | 2.60 |
| 11.08 | 514.71 | 7.98 | 6.83 | 26.54 | 132.65 | 3.36 | 1.76 |
| 12.43 | 167.14 | 7.12 | 2.22 | 27.07 | 1577.77 | 3.29 | 20.93 |
| 12.79 | 811.49 | 6.92 | 10.77 | 27.48 | 214.26 | 3.25 | 2.84 |
| 14.60 | 350.46 | 6.07 | 4.65 | 27.79 | 80.82 | 3.21 | 1.07 |
| 14.96 | 1099.83 | 5.92 | 14.59 | 28.93 | 64.33 | 3.09 | 0.85 |
| 15.94 | 45.31 | 5.56 | 0.60 | 29.54 | 285.80 | 3.02 | 3.79 |
| 16.48 | 75.75 | 5.38 | 1.01 | 30.26 | 172.67 | 2.95 | 2.29 |
| 17.28 | 230.43 | 5.13 | 3.06 | 30.69 | 194.97 | 2.91 | 2.59 |
| 17.88 | 1614.80 | 4.96 | 21.42 | 31.03 | 136.76 | 2.88 | 1.81 |
| 18.88 | 128.01 | 4.70 | 1.70 | 31.54 | 139.04 | 2.84 | 1.84 |
| 19.85 | 602.39 | 4.47 | 7.99 | 32.25 | 212.19 | 2.78 | 2.82 |
| 20.12 | 2973.29 | 4.41 | 39.45 | 32.74 | 103.45 | 2.74 | 1.37 |
| 20.54 | 7537.06 | 4.32 | 100.00 | 33.98 | 103.51 | 2.64 | 1.37 |
| 21.47 | 208.75 | 4.14 | 2.77 | 34.54 | 154.10 | 2.60 | 2.04 |
| 22.25 | 56.81 | 4.00 | 0.75 | 34.84 | 186.78 | 2.58 | 2.48 |
| 22.79 | 259.85 | 3.90 | 3.45 | 35.54 | 66.08 | 2.53 | 0.88 |
| 23.03 | 181.90 | 3.86 | 2.41 | 36.30 | 73.55 | 2.48 | 0.98 |
| 23.34 | 719.42 | 3.81 | 9.55 | 36.90 | 18.90 | 2.44 | 0.25 |
| 24.03 | 322.38 | 3.70 | 4.28 | 37.49 | 60.90 | 2.40 | 0.81 |

**Thermal stability study**

[125] Two samples of the crystalline form A of hydrochloride of the compound as shown in formula (I) (40 mg each) were taken and 1 mL of ethanol was added. The mixtures were heated to internal temperatures of 50°C and 70°C, respectively, and samples were collected at regular intervals and delivered for HPLC detection to investigate the stability thereof. Specific data are

shown in the table below. The detection results showed that the hydrochloride had good thermal stability.

Table 10. Stability data of crystalline form A of hydrochloride at 50°C

| RT | Deg-30min | Deg-1 h | Deg-3 h |
|---|---|---|---|
| Compound as shown in formula (I) | 99.77 | 99.71 | 99.76 |

Table 11. Stability data of crystalline form A of hydrochloride at 70°C

| RT | Deg-10min | Deg-30min | Deg-1 h | Deg-3 h |
|---|---|---|---|---|
| Compound as shown in formula (I) | 99.77 | 99.81 | 99.80 | 99.79 |

**Aqueous solution stability study**

[126] 100 mg of the crystalline form A of hydrochloride of the compound as shown in formula (I) was added to 4 mL of water to prepare a suspension. The suspension was stirred at room temperature, and samples were collected at regular intervals and delivered for HPLC and XRPD detection to investigate the stability thereof. Specific data are shown in the table below. The detection results showed that the XRD pattern of the hydrochloride of the compound as shown in formula (I) in aqueous solution was consistent with that of the starting material. No significant related substances (i.e., impurities) were generated, indicating good stability in aqueous solution.

Table 12. Stability of crystalline form A of hydrochloride in aqueous solution

| RT | 1 h | 2 h | 24 h | 48 h |
|---|---|---|---|---|
| Compound as shown in formula (I) | 99.78 | 99.80 | 99.71 | 99.84 |

**Study on hygroscopicity of crystalline forms**

Table 13. Comparison of hygroscopicity of compound as shown in formula (I)

| Salt form | Characterization of crystalline forms | Hygroscopicity (0%RH to 80%RH) |
|---|---|---|
| Crystalline form A of hydrochloride | Crystalline form A of hydrochloride | 0.431% (w/w), slightly hygroscopic, crystalline form unchanged |
| Crystalline form A of p-toluenesulfonate | Nearly amorphous | 14.323% (w/w), hygroscopic, crystallinity of the crystalline form deteriorated |
| Crystalline form A of methanesulfonate | Nearly amorphous | 6.201% (w/w), hygroscopic, crystallinity of the crystalline form deteriorated |
| Crystalline form A | Crystalline form A | 0.013% (w/w), practically non-hygroscopic, crystalline form unchanged |

**Comparative study of crystallinity**

Table 14. Comparison of crystallinity of compound as shown in formula (I)

| Salt form | Crystallinity |
|---|---|
| Crystalline form A of hydrochloride | Medium |
| Crystalline form A of p-toluenesulfonate | Low |
| Crystalline form A of methanesulfonate | Low |
| Crystalline form A of compound as shown in formula (I) | High |

**Experimental study on hydrochloride competition**

[127] 100 mg samples of crystalline form A of hydrochloride, crystalline form B of hydrochloride and crystalline form C of hydrochloride were taken and mixed uniformly, and samples were collected for XRPD characterization. The mixture was divided into three equal portions, and 0.5 mL of solvent (acetone, methanol, or acetonitrile) was added to each portion to form a suspension. The suspension was stirred at room temperature for 3 days, and samples were collected for XRPD characterization. The results are shown in Table 15. It could be seen

that the mixtures of the three crystalline forms of hydrochloride in different solvents were ultimately converted to the crystalline form A.

Table 15. Comparison of crystallinity of compound as shown in formula (I)

| Condition | Acetone | Methanol | acetonitrile |
|---|---|---|---|
| Crystalline form result | Crystalline form A of hydrochloride | Crystalline form A of hydrochloride | Crystalline form A of hydrochloride |

**Study on solid-state property**

[128] The sample of the crystalline form A of hydrochloride of the compound as shown in formula (I) was irregular, non-uniform in size, and difficult to prepare and exhibited poor flowability. The sample of the crystalline form A of the compound as shown in formula (I) was block-shaped agglomerates with uniform size and easy to prepare and exhibited good flowability.

[129] Microscopic images of the crystalline form A of hydrochloride of the compound as shown in formula (I) and the crystalline form A of the compound as shown in formula (I) are shown in Figure 27 and Figure 28, respectively.

Table 16. Study data table of solid-state property of compound as shown in formula (I)

| | Crystalline form A of compound as shown in formula (I) | Crystalline form A of hydrochloride of compound as shown in formula (I) |
|---|---|---|
| Bulk density | 0.38 g/ml | 0.29 g/ml |
| Microscopic image | Figure 28 | Figure 27 |

**Solid stability study**

[130] 100 mg of the crystalline form A of the compound as shown in formula (I) was taken and left to stand under various conditions, and relevant data thereof were measured at 1, 2, 3, 6, and 9 months, respectively. The specific conditions and results regarding related substances are shown in the table below. The detection results showed that the crystalline form A of the compound as shown in formula (I) exhibited good stability under various conditions.

Table 17. Stability of crystalline form A of compound as shown in formula (I)

| Storage condition | Storage duration | Purity (%) |
|---|---|---|
| Accelerated condition (40°C/75%RH) | 0 days | 99.0 |
| | 1 month | 99.0 |
| Accelerated condition (40°C/75%RH) | 2 months | 99.0 |
| Accelerated condition (40°C/75%RH) | 3 months | 99.0 |
| Accelerated condition (40°C/75%RH) | 6 months | 99.1 |
| Long-term condition (30°C/65%RH) | 1 month | 99.0 |
| Long-term condition (30°C/65%RH) | 2 months | 99.0 |
| Long-term condition (30°C/65%RH) | 3 months | 99.0 |
| Long-term condition (30°C/65%RH) | 6 months | 99.1 |
| Long-term condition (30°C/65%RH) | 9 months | 99.1 |

**Biological test:**

1. Effect of compounds on PDE4B2 activity

[131] The effect of compounds on PDE4B2 activity was detected using a fluorescence polarization kit (BPS Bioscience, Catalog #60343). According to the instructions of the kit, FAM-Cyclic-3',5'-AMP at a final concentration of 0.1 μM, 1 ng/well of PDE4B2 (for a negative control, a PDE buffer was added), and serially diluted compounds (for a positive control well, a PDE buffer containing 10% of DMSO was added) were added to each well, fully mixed, and then reacted at room temperature for 1 h. The Binding Agent was diluted with the Binding Agent Diluent (cAMP) at a ratio of 1:100 for later use, and the Binding Agent dilution was taken and

added to the test plate at 50 µl/well and incubated at room temperature with slow shaking for 20 minutes. After the incubation was complete, Envision was used for FP detection, with Excitation at 480 nm and Emission at 535 nm. FP was usually represented by mP value.

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000$$

$I_{//}$ (S535): fluorescence intensity in parallel direction

$I_{\perp}$ (P535): fluorescence intensity in perpendicular direction

G: G factor = 1

Calculation of inhibition rate (% Inhibition):

% Inhibition = [1 - ($mP_{(sample)}$ - $mP_{(negative\ control)}$) / ($mP_{(positive\ control)}$ - $mP_{(negative\ control)}$)] x 100%

$mP_{(sample)}$: mP of the test compound in a reaction well

$mP_{(negative\ control)}$: mP in the negative control well

$mP_{(positive\ control)}$: mP in the positive control well

[132] According to the calculated inhibition rate at each concentration, the $IC_{50}$ value of each compound was calculated by GraphPad Prism 8 software.

[133] The compounds of the present invention had an $IC_{50}$ value < 300 nM against PDE4B2; preferably, some compounds had an $IC_{50}$ value < 100 nM; more preferably, some compounds had an $IC_{50}$ value < 50 nM; and further preferably, some compounds had an $IC_{50}$ value < 10 nM.

[134] The compounds of the present invention had an $IC_{50}$ value of less than 300 nM against PDE4B2, preferably less than 100 nM, more preferably less than 50 nM, and further preferably less than 10 nM. The $IC_{50}$ values of some specific compounds are as shown in Table 18 below, wherein A < 10 nM, 10 nM ≤ B < 50 nM, and 50 nM ≤ C < 100 nM.

Table 18. PDE4B2 activity

| Compound | PDE4B2 $IC_{50}$ (nM) |
|---|---|
| Compound as shown in formula (I) | A |

2. Effect of compounds on PDE4D2 activity

[135] The effect of compounds on PDE4D2 activity was detected using a fluorescence polarization kit (BPS Bioscience, Catalog #60345). During the reaction, 12.5 µL of 0.272 ng/well of an enzyme (final concentration: 0.068 ng/well) and 12.5 µL of the serially diluted compound (DMSO concentration: 4%) were first pre-incubated at room temperature for 15 minutes, the

same volume of the enzyme at the same concentration and 12.5 μL of PDE buffer containing 4% DMSO were added to the positive control well, and 25 μL of PDE buffer containing 2% DMSO was added to the negative control well. After completion, 25 μL of 0.2 μM FAM-Cyclic-3',5'-AMP (final concentration: 0.1 μM) was added to each well, fully mixed, and then incubated at room temperature with slow shaking for 30 minutes. The Binding Agent was diluted with the Binding Agent Diluent (cAMP) at a ratio of 1:100 for later use, and the Binding Agent dilution was taken and added to all the wells of the test plate at 100 μl/well and incubated at room temperature with slow shaking for 1 hour. After the incubation was complete, BMG LRBTECH microplate reader was used for FP detection, with Excitation at 485 nm and Emission at 520 nm. FP was usually represented by mP value.

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000$$

$I_{//}$ (S520): fluorescence intensity in parallel direction

$I_{\perp}$ (P520): fluorescence intensity in perpendicular direction

G: G factor = 1

Calculation of inhibition rate (% Inhibition):

% Inhibition = [1 - ($mP_{(sample)}$ - $mP_{(negative\ control)}$) / ($mP_{(positive\ control)}$ - $mP_{(negative\ control)}$)] x 100%

$mP_{(sample)}$: mP of the test compound in a reaction well

$mP_{(negative\ control)}$: mP in the negative control well

$mP_{(positive\ control)}$: mP in the positive control well

[136] According to the calculated inhibition rate at each concentration, the IC50 value of each compound was calculated by GraphPad Prism 8 software.

[137] The $IC_{50}$ values of some specific compounds of the present invention are as shown in Table 19 below, wherein A < 10 nM, 10 nM ≤ B < 50 nM, and 50 nM ≤ C < 100 nM.

Table 19. PDE4D2 activity

| Compound | PDE4D2 $IC_{50}$ (nM) |
|---|---|
| Compound as shown in formula (I) | A |

**3.** Detection of inhibitory activity of compounds on the release of tumor necrosis factor-α (TNF-α) from human peripheral blood mononuclear cells induced by lipopolysaccharide (LPS) *in vitro*

[138] Normal human peripheral blood which was anticoagulated (citrate anticoagulation) was collected, and hPBMCs were prepared by Ficoll-Paque PLUS (Cytiva, Cat#17144002, density 1.077 g/mL). The concentration of hPBMC cells was adjusted to 0.25 x $10^6$ cells/mL with RPMI1640 medium and inoculated into a 96-well plate at 50000 cells per well. Subsequently, different concentrations of drugs were added and pre-incubated for 1 h (final DMSO concentration: 0.1%, and the positive and negative control wells had the same volume of RPMI1640 containing 0.1% DMSO). After the pre-incubation was completed, 100 ng/mL LPS (SIGMA, L2630) was added to the compound and positive control wells, and the negative control well had the same volume of RPMI1640 containing 0.1% DMSO. The plate was incubated in an incubator at 37°C with 5% $CO_2$ for 4 h. The cell supernatant was collected, and the content of TNF-α in the supernatant sample was detected by human TNF-α Elisa quantitative test kit (Sino Biological, Cat#KIT10602). The $IC_{50}$ value was calculated using GraphPad Prism software. The $IC_{50}$ values of some specific compounds of the present invention are as shown in Table 20 below, wherein A < 10 nM, 10 nM ≤ B < 50 nM, and 50 nM ≤ C < 100 nM.

Table 20. Inhibitory activity of compounds on TNF-α induced by LPS *in vitro*

| Test compound | **Re** $IC_{50}$ (nM) | **Max inhibition (%)** |
|---|---|---|
| **Compound as shown in formula (I)** | A | 68.04 |

Re $IC_{50}$: relative $IC_{50}$.

**4 Pharmacokinetic test in rats**

**[139]** **4.1 Experimental animals:** Male SD rats, about 220 g, 6-8 weeks old, 6 rats/compound, purchased from Chengdu Dossy Experimental Animals Co., Ltd.

[140] **4.2 Experimental design:** On the day of the experiment, 6 SD rats were randomly grouped according to their body weights. The animals were fasted with water available for 12-14 h one day before the administration, and were fed 4 h after the administration.

Table 21. Administration information

| Group | Number | Administration information | | | | | |
|---|---|---|---|---|---|---|---|
| | Male | Test compound | Administration dosage (mg/kg) | Administration concentration (mg/mL) | Administration volume (mL/kg) | Collected sample | Administration mode |
| G1 | 3 | Compound as shown in formula (I) | 2.5 | 0.5 | 5 | Plasma | Intravenous administration |
| G2 | 3 | | 10 | 1 | 10 | Plasma | Intragastric administration |

[141] Note: vehicle for intravenous administration: 10% DMA+10% Solutol+80% Saline; vehicle for intragastric administration: 0.5% MC

(DMA: dimethylacetamide; Solutol: polyethylene glycol-15-hydroxystearate; Saline: physiological saline; MC: methylcellulose)

[142] Before and after administration, 0.15 ml of blood was taken from the orbit under isoflurane anesthesia and placed in an EDTAK2 centrifuge tube. The tube was centrifuged at 5000 rpm at 4°C for 10 min, and plasma was collected. The blood collection time points for the intravenous administration group and intragastric administration group were: 0, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h. Before analysis and detection, all samples were stored at -80°C, and the samples were quantitatively analyzed by LC-MS/MS.

Table 22. Pharmacokinetic parameters of test compound in plasma of rats

| Test compound | Mode of administration | CL (mL/min/kg) | $Vd_{ss}$ (L/kg) | $AUC_{0-t}$ (hr*ng/mL) | F (%) |
|---|---|---|---|---|---|
| Compound as shown in formula (I) | i.v. (2.5 mg/kg) | 2.54±0.32 | 0.324±0.012 | 16531±1991 | - |
| | i.g. (10 mg/kg) | - | - | 52179±21323 | 78.9±32 |

-: not applicable.

[143] Conclusion: The compound as shown in formula (I) of the present invention exhibits excellent pharmacokinetic properties in the PK test in rats.

**5 Detection of inhibitory activity on TNF-α secretion in LPS-induced mouse model of lung inflammation**

[144] In mice, intratracheal administration of lipopolysaccharides (LPS, Sigma, L2880) might induce increased TNF-α concentration in lung tissue. In this experiment, a given number of mice (BABL/c, male, weighing 18-22 g) were randomly divided into groups, with 8 mice in each group, and orally administered with a given dose of the compound as shown in formula (I) (the compound as shown in formula (I) was suspended with 0.5% MC and prepared into a given concentration, and the suspension was orally administered at a volume of 10 ml/kg body weight). After 0.5 h, based on 2.5 uL/g animal body weight, LPS with a concentration of 0.8 mg/mL was intratracheally administered via a lung quantitative atomizing needle. 24 h later, the mice were intraperitoneally anesthetized with 20% urethane (10 mL/kg), and then sacrificed by cervical dislocation. Subsequently, lung tissue was taken and the lower part of the left lobe was fully homogenized, and the content of TNF-α in the supernatant of the homogenate was detected by Mouse TNFα ELISA Kit (RD, SMTA00B).

[145] Inhibition rate of test compound on TNF-α level (% Inh) = (model group - test group) / model group * 100%.

[146] Results and conclusion: The results are shown in Figure 26 and demonstrate that the compound as shown in formula (I) of the present invention has significant inhibitory activity on TNF-α secretion.