Benzodiazepine Derivative Hydrochloride and Crystal Form, Preparation Method and Application thereof

Cross Reference to Related Application

This application claims the priority of Chinese Patent Application No. 201810151979.0 filed on February 13, 2018, the content of which is incorporated herein by reference.

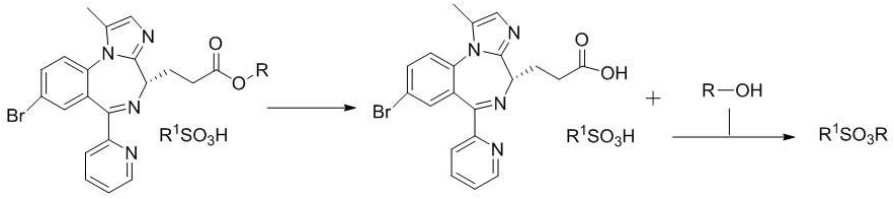
Technical Field

The present invention relates to hydrochloride of benzodiazepine derivatives, and their crystal forms, preparation method and use thereof.

Background of the Invention

Remimazolam (CNS 7056) is a new generation of benzodiazepine derivatives improved on the basis of midazolam. It has attracted attention due to its fast onset of action and fast recovery. With the deepening of research, the shortcomings of remimazolam gradually emerged. In the Phase II clinical trial of ICU sedation, Ono Company found that the hemodynamics of the patients was unstable after receiving remimazolam, and the plasma concentration in 10% of the patients was higher than the normal range (PAION AG Analyst call Oct 14 2014).

WO 0069836 disclosed remimazolam and pharmaceutically acceptable salt thereof, but did not disclose the preparation method of the pharmaceutically acceptable salt. CN 104059071 and CN 103221414 disclosed preparation methods and crystal forms of remimazolam besylate and p-toluenesulfonate. PCT/CN2015/084770 disclosed a series of methods for preparing benzodiazepine derivatives and their sulfonates. These derivatives have a good intravenous anesthesia effect. In the published references, the salt of these compounds is formed by employing an organic sulfonic acid (such as ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, etc.) with a basic group of the benzodiazepines to increase their solubility in water. However, employing an organic sulfonic acid to form a salt has the following disadvantages: it is necessary to use a corresponding alcohol as a solvent for the benzodiazepine derivative during the salification. If an organic sulfonic acid is employed, there is a possibility to form an organic sulfonate ester. For example, see the following reaction scheme:

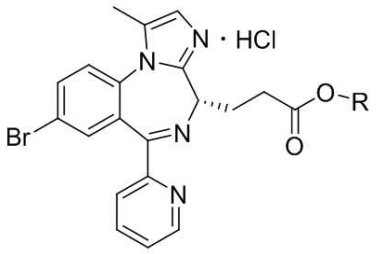


wherein R is methyl or ethyl; R^1 is methyl, ethyl, phenyl, 4-methylphenyl, 4-hydroxyphenyl and the like.

The organic sulfonate ester thus produced has a strong genotoxicity (ICH Harmonised Tripartite Guideline, Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk, Current Step 4 version, 23 June 2014). Therefore, these organic sulfonates of the benzodiazepine derivatives have the risk of forming potentially genotoxic substances during their production, storage and application. The genotoxic substances are characterized in that they may cause damage to human genetic materials at a very low concentration, and then may lead to gene mutations and promote tumorigenesis. Because of their strong toxicity, genotoxic substances pose a strong threat to the drug safety. In recent years, more and more serious medical accidents are occurred due to traces of genotoxic impurities found in the marketed drugs. Therefore, regulatory agencies in various countries, such as ICH, FDA, EMA, etc., have more specific requirements for genotoxic impurities, and more and more pharmaceutical companies are focusing on the control and testing of genotoxic impurities in the development of new drugs. In order to avoid the risk of genotoxicity caused by an organic sulfonate ester, it is preferable to replace the sulfonate with an acid radical with no or only a small risk of genotoxicity, such as Cl^-. However, there are multiple basic centers in the free base molecules of such benzodiazepine derivatives, using a general method--single amino group to form a salt with a strong acid-hydrochloric acid, which forms a mixture of single and multiple salts, making it difficult to obtain monohydrochloride, and leading to difficulty in crystallization, strong hygroscopicity and poor stability.

Summary of the Invention

According to an aspect of the present invention, it provides a crystalline form of hydrochloride of a benzodiazepine derivative of Formula I or its ethanolate,



Formula I

wherein R is methyl or ethyl; wherein when R is methyl, the crystal form has the following cell parameters: a=7.6929(6) Å, b=11.9174(10) Å, c=13.2096(11) Å, α= 90°, β= 96.904(1)°, γ=90°; and when R is ethyl, the crystal form has the following cell parameters: a=7.3774(1) Å, b=12.7332(2) Å, c=27.1779(4) Å, α= 90°, β= 90°, γ=90°.

In an embodiment according to this aspect, R is methyl, and its crystal form has a structure substantially as shown in FIG. 1, or may be characterized by one or more parameters substantially as shown in Tables 1-6. In another embodiment, R is ethyl, and its crystal form has a structure substantially as shown in FIG. 2, or may be characterized by one or more parameters substantially as shown in Tables 7-12.

In an embodiment according to this aspect, R is methyl, and the compound of Formula I has a content of chloride ion of 6.71-7.52% (w/w). In another embodiment, R is ethyl, and the compound of Formula I has a content of chloride ion of 6.51-7.31% (w/w).

In one embodiment, the crystal form of the compound of Formula I wherein R is methyl has an X-ray powder diffraction pattern with the following 2θ values measured by using CuKα radiation: about 6.81, 8.93, 13.39, 19.38, 21.23, 22.42, 24.20, 27.31±0.2 degrees. The X-ray powder diffraction pattern may also have the following 2θ values measured by using CuKα radiation: about 8.11, 9.86, 14.73, 17.47, 23.03, 25.94, 28.31±0.2 degrees. In addition, the crystal form has an X-ray powder diffraction pattern substantially as shown in FIG. 3.

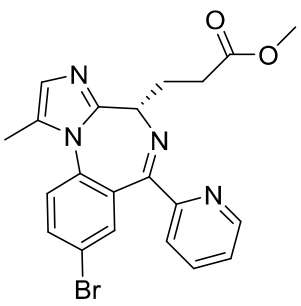
In another embodiment, the crystal form of the compound of Formula I wherein R is methyl, has an X-ray powder diffraction pattern with the following 2θ values measured by using CuKα radiation: about 6.80, 8.93, 9.87, 13.37, 14.69, 19.36, 20.76, 21.25, 22.19, 22.38, 23.06, 24.21, 25.93, 27.73±0.2 degrees. The X-ray powder diffraction pattern may also have the following 2θ values measured by using CuKα radiation: about 16.14, 17.48, 20.02, 25.17, 26.36, 28.30, 34.13±0.2 degrees. In addition, the crystal form has an X-ray powder diffraction pattern substantially as shown in FIG. 4.

In an embodiment, the crystal form of the compound of Formula I wherein R is ethyl has an X-ray powder diffraction pattern with the following 2θ values measured by using CuKα radiation: about 6.87, 7.38, 9.53, 13.65, 18.71, 22.13, 22.67, 25.10, 27.25, 29.30±0.2 degrees. The X-ray powder diffraction pattern may also have the following 2θ values measured by using CuKα radiation: about 14.96, 15.43, 20.23, 20.67, 21.13, 23.52, 28.22, 31.26±0.2 degrees. In addition, the crystal form has an X-ray powder diffraction pattern substantially as shown in any one of FIGs. 5-8.

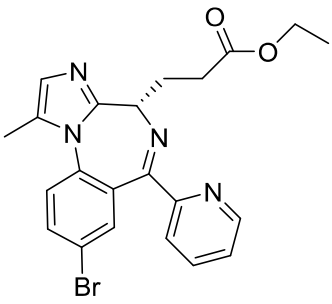
In an embodiment, the crystal form of the compound of Formula I wherein R is ethyl has an X-ray powder diffraction pattern with the following 2θ values measured by using CuKα radiation: about 7.41, 9.24, 12.71, 13.64, 15.06, 18.30, 18.72, 21.59, 22.18, 25.74±0.2 degrees. The X-ray powder diffraction pattern may also have the following 2θ values measured by using CuKα radiation: about 9.52, 11.69, 20.90, 22.60, 23.65, 24.26, 26.40, 28.43, 29.35±0.2 degrees. In addition, the crystal form has an X-ray powder diffraction pattern substantially as shown in FIG. 9.

In another embodiment, the crystal form of the compound of Formula I wherein R is ethyl has an X-ray powder diffraction pattern with the following 2θ values measured by using CuKα radiation: about 6.84, 7.37, 9.53, 13.66, 22.63, 25.57, 29.28, 31.26±0.2 degrees. The X-ray powder diffraction pattern may also have the following 2θ values measured by using CuKα radiation: about 15.43, 19.07, 22.16, 34.25±0.2 degrees. In addition, the crystal form has an X-ray powder diffraction pattern substantially as shown in any one of FIGs. 10-13.

According to another aspect of the present invention, it provides a method for preparing the above-mentioned crystal forms of the hydrochloride of the benzodiazepine derivative of Formula I according to the present invention, comprising the following steps: dissolving the free base of the benzodiazepine derivative of the following Formula II-1 or II-2 in an organic solvent 1, adding HCl donor A wherein $[H^+]$ is equimolar to the free base, to form a salt at a temperature of -20 to 60°C, preferably -10 to 30°C, after decolorizing the crude salt, crystallizing it in a crystallization solvent 1 at a temperature of -60 to 80°C, preferably -20 to 60°C, to obtain the crystal form of the hydrochloride of the benzodiazepine derivative of Formula I.



Formula II-1



Formula II-2

In an embodiment according to this aspect, the organic solvent 1 is an alcohol solvent, such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol; an ester solvent, such as methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate; a ketone solvent, such as acetone and butanone; or a mixture thereof.

In an embodiment according to this aspect, the HCl donor A is an amino acid hydrochloride, such as glycine hydrochloride, alanine hydrochloride, valine hydrochloride; a HCl-anhydrous alcohol solution, that is, an alcohol solution of dry HCl, such as dry HCl-methanol solution, dry HCl-ethanol solution, dry HCl-isopropanol solution; or a solution B that can generate HCl, such as acetyl chloride-methanol solution, acetyl chloride-ethanol solution, propionyl chloride-ethanol solution, acetyl chloride-isopropanol solution.

In an embodiment according to this aspect, the HCl donor A is an amino acid hydrochloride, and the crystal form of the hydrochloride of the benzodiazepine derivative has an amount of amino acid of 0%-8% (w/w).

In an embodiment according to this aspect, the HCl donor A is a HCl-anhydrous alcohol solution or a solution B that can generate HCl, and the ratio of amount of substance (molar ratio) of the free base of the benzodiazepine derivative to the HCl donor A (calculated by $[H^+]$) is 1:0.4 - 1; the HCl donor A is an amino acid hydrochloride, and the amount ratio (molar ratio) of the free base of the benzodiazepine derivative to the amino acid hydrochloride is 1:1-10.

In an embodiment according to this aspect, the crystallization solvent 1 comprises an alcohol solvent, such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol; an ether solvent, such as ethyl ether, isopropyl ether, dioxane, methyl tert-butyl ether; an ester solvent, such as methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate; a ketone solvent, such as acetone and butanone; an alkane solvent, such as n-pentane, hexane, heptane, petroleum ether; a halogenated alkane, such as dichloromethane, chloroform, 1,2-dichloroethane; and a

combination thereof.

According to another aspect of the present invention, it provides a pharmaceutical composition comprising the above-mentioned crystal form of the hydrochloride of the benzodiazepine derivative of Formula I according to the present invention, and a pharmaceutically acceptable excipient, carrier and/or other auxiliary materials.

The crystal form and the pharmaceutical composition according to the present invention may be used as intravenous anesthetics.

According to yet another aspect of the present invention, it provides a method of anesthesia, comprising intravenously administering an effective amount of the crystal form of the hydrochloride of the benzodiazepine derivative of Formula I according to the present invention, or a pharmaceutical composition comprising the crystal form to a subject in need thereof.

The crystal form of the hydrochloride of the benzodiazepine derivative provided by the present invention can not only improve the stability of the benzodiazepine derivative, but also eliminate the possibility of forming sulfonate ester impurities with strong genotoxicity during production and storage of the benzodiazepine derivative sulfonate, and has a more excellent anesthetic effect, which is more conducive to clinical use.

In addition, the present invention provides a hydrochloride of the benzodiazepine derivative of Formula I, which, compared to the corresponding sulfonate, 1) has good stability and is less prone to produce a hydrolysis product; 2) does not produce sulfonate ester impurities with strong genotoxicity during production or long-term storage; 3) has a shorter duration of anesthesia and a shorter interval of time to start walking after awakening, less individual differences, which is of great clinical significance.

The present invention will be described in more detail below with reference to the drawings.

Brief Description of the Drawings

FIG. 1: The monocrystal molecular structure of an ethanolate of a compound of Formula I wherein R is methyl;

FIG. 2: The monocrystal molecular structure of an ethanolate of a compound of Formula I wherein R is ethyl;

FIG. 3: An X-ray powder diffraction pattern of the crystal of a compound of

Formula I wherein R=CH_3 (CNS-7056A2017120401);

FIG. 4: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_3 (CNS-7056AG20171225);

FIG. 5: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_2CH_3 (EL-001A2017120401);

FIG. 6: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_2CH_3 (EL-001A2017120801);

FIG. 7: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_2CH_3 (EL-001A20180105);

FIG. 8: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_2CH_3 (EL-001A2018010801);

FIG. 9: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_2CH_3 (EL-001A20180130);

FIG. 10: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_2CH_3 (EL-001AG2017121801);

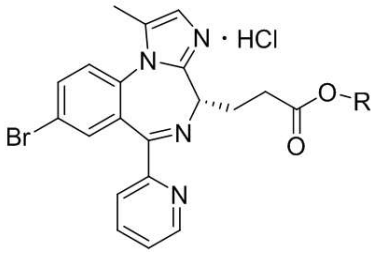
FIG. 11: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_2CH_3 (EL-001AG2017122101);

FIG. 12: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_2CH_3 (EL-001AG2017122702LJ) ; and

FIG. 13: An X-ray powder diffraction pattern of the crystal of a compound of Formula I wherein R=CH_2CH_3 (EL-001AG2018010201).

Detailed Description of the Invention

The present invention provides a crystal structure of a hydrochloride of a benzodiazepine derivative of the following general Formula I and its ethanolate,



Formula I

wherein R is methyl or ethyl.

According to one embodiment of the present invention, when R is methyl, the crystal of the ethanolate of the benzodiazepine hydrochloride provided by the present invention has the following cell parameters: a=7.6929(6) Å, b=11.9174(10) Å,

c=13.2096(11) Å, α= 90°, β= 96.904(1)°, γ=90°. It may also be further characterized by its structure as shown in Fig. 1, the parameters as shown in Table 1, the structural coordinates as shown in Table 2, Table 3, and Table 4, and the bond lengths and angles as shown in Table 5 and Table 6.

According to one embodiment of the present invention, when R is ethyl, the crystal of the ethanolate of the benzodiazepine hydrochloride provided by the present invention has the following cell parameters: a=7.3774(1) Å, b=12.7332(2) Å, c=27.1779(4) Å, α= 90°, β= 90°, γ=90°. It may also be further characterized by its structure as shown in Figure 2, the structural parameters as shown in Table 7, the structural coordinates as shown in Table 8, Table 9, and Table 10, and the bond lengths and angles as shown in Table 11 and Table 12.

According to an embodiment of the present invention, when R is methyl, the compound of Formula I has a content of chloride ion of 6.71-7.52% (w/w).

In an embodiment of the present invention, when R is ethyl, the compound of Formula I has a content of chloride ion of 6.51-7.31% (w/w).

The hydrochloride of the benzodiazepine derivative provided by the present invention is a crystalline salt, and their crystal structures and X-ray powder diffraction data and patterns are also provided.

According to an embodiment of the present invention, R is methyl, and it has an X-ray powder diffraction pattern expressed in 2θ degrees by using Cu-Kα radiation with characteristic absorption at about 6.81, 8.93, 13.39, 19.38, 21.23, 22.42, 24.20, 27.31±0.2, and can be further characterized by an X-ray powder diffraction pattern at 2θ of about 8.11, 9.86, 14.73, 17.47, 23.03, 25.94, 28.31±0.2 degrees, or the X-ray powder diffraction pattern as shown in FIG. 3.

According to an embodiment of the present invention, R is methyl, and it has an X-ray powder diffraction pattern expressed in 2θ degrees by using Cu-Kα radiation with characteristic absorption at about 6.80, 8.93, 9.87, 13.37, 14.69, 19.36, 20.76, 21.25, 22.19, 22.38, 23.06, 24.21, 25.93, 27.73±0.2, and can be further characterized by an X-ray powder diffraction pattern at 2θ of about 16.14, 17.48, 20.02, 25.17, 26.36, 28.30, 34.13±0.2 degrees, or the X-ray powder diffraction pattern as shown in FIG. 4.

According to an embodiment of the present invention, R is ethyl, and it has an X-ray powder diffraction pattern expressed in 2θ degrees by using Cu-Kα radiation with characteristic absorption at about 6.87, 7.38, 9.53, 13.65, 18.71, 22.13, 22.67, 25.10, 27.25, 29.30±0.2, and can be further characterized by an X-ray powder

diffraction pattern at 2θ of about 14.96, 15.43, 20.23, 20.67, 21.13, 23.52, 28.22, 31.26±0.2 degrees, or the X-ray powder diffraction patterns as shown in FIGs. 5-8.

According to an embodiment of the present invention, R is ethyl, and it has an X-ray powder diffraction pattern expressed in 2θ degrees by using Cu-Kα radiation with characteristic absorption at about 7.41, 9.24, 12.71, 13.64, 15.06, 18.30, 18.72, 21.59, 22.18, 25.74±0.2, and can be further characterized by an X-ray powder diffraction pattern at 2θ of about 9.52, 11.69, 20.90, 22.60, 23.65, 24.26, 26.40, 28.43, 29.35±0.2 degrees, or the X-ray powder diffraction pattern as shown in FIG. 9.

According to an embodiment of the present invention, R is ethyl, and it has an X-ray powder diffraction pattern expressed in 2θ degrees by using Cu-Kα radiation with characteristic absorption at about 6.84, 7.37, 9.53, 13.66, 22.63, 25.57, 29.28, 31.26±0.2, and can be further characterized by an X-ray powder diffraction pattern at 2θ of about 15.43, 19.07, 22.16, 34.25±0.2 degrees, or the X-ray powder diffraction patterns as shown in FIGs. 10-13

According to a second aspect of the present invention, it provides a method for preparing the hydrochloride of the benzodiazepine derivative and its crystal form: dissolving the free base of the benzodiazepine derivative in an organic solvent 1; adding HCl donor A equimolar to the free base of the benzodiazepine derivative, to form a salt at -20-60℃ to obtain a crude product; and after decolorizing, crystallizing the crude product in a crystallization solvent 1 at -60-80℃ to obtain hydrochloride of the benzodiazepine derivative.

According to an embodiment of the present invention, the organic solvent 1 is an alcohol solvent (such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, etc.), an ester solvent (such as methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, etc.), a ketone solvent (such as acetone, butanone, etc.), or mixtures thereof.

According to an embodiment of the present invention, the HCl donor A is an amino acid hydrochloride (such as glycine hydrochloride, alanine hydrochloride, valine hydrochloride, etc.), a HCl-anhydrous alcohol solution (i.e., alcohol solution of dry HCl gas, such as dry HCl-methanol solution, dry HCl-ethanol solution), a solution B that can generate HCl (such as acetyl chloride-methanol solution, acetyl chloride-ethanol solution, etc.).

According to an embodiment of the present invention, when the HCl donor A is an amino acid hydrochloride, the hydrochloride of the benzodiazepine derivative has an amount of amino acid of 0%-8% (w/w).

According to an embodiment of the present invention, when the HCl donor A is an amino acid hydrochloride, the ratio of amount of substance of the benzodiazepine derivative (calculated by free base) to the amino acid hydrochloride is 1:1-10; and when the HCl donor A is a HCl-anhydrous alcohol solution or a solution B that can generate HCl, the amount ratio of the benzodiazepine derivative (calculated by free base) to acid (calculated by HCl) is 1:0.4-1.

According to an embodiment of the present invention, the temperature for forming a salt is -20 to 60°C, preferably -10 to 30°C; and the crystallization temperature is -60-80°C, preferably -20-60°C.

According to an embodiment of the present invention, the crystallization solvent 1 comprises an alcohol solvent (such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, etc.), an ether solvent (such as diethyl ether, isopropyl ether, dioxane, methyl tert-butyl ether, isopropyl ether, etc.), an ester solvent (such as methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, etc.), a ketone solvent (such as acetone, butanone, etc.), an alkane solvent (such as pentane, hexane, heptane, petroleum ether, etc.), a halogenated alkane (such as dichloromethane, chloroform, 1,2-dichloroethane, etc.) and combinations thereof.

According to a third aspect of the present invention, it provides the hydrochloride of the benzodiazepine derivative and the pharmaceutical composition of the present invention, which can be used as intravenous anesthetics.

The pharmaceutical composition comprises the above-mentioned crystal form of the hydrochloride of the benzodiazepine derivative of Formula I according to the present invention, and optionally a pharmaceutically acceptable excipient, carrier and/or other auxiliary materials. The excipient and/or carrier include, for example, one or more of mannitol, sorbitol, xylitol, sucrose, lactose, glucose, dextrin, maltose, maltitol, maltodextrin, erythritol, trehalose, calcium gluconate, calcium sulfate, sodium chloride, glycine, hydrolyzed gelatin, human albumin, etc. The composition may optionally include other auxiliary materials, such as a pH adjusting agent, stabilizer, analgesic, bacteriostatic agent, and the like. The pH adjusting agent includes, for example, one or more of hydrochloric acid, sulfuric acid, phosphoric acid, citric acid, acetic acid, sodium dihydrogen phosphate, potassium dihydrogen phosphate, ammonium dihydrogen phosphate, disodium hydrogen phosphate, dipotassium hydrogen phosphate, diammonium hydrogen phosphate, sodium phosphate, potassium phosphate, ammonium phosphate, sodium bisulfate, potassium bisulfate, ammonium bisulfate, sodium bicarbonate, potassium bicarbonate, sodium

carbonate, potassium carbonate, sodium hydroxide, potassium hydroxide, ammonia water, citric acid, sodium dihydrogen citrate, potassium dihydrogen citrate, ammonium dihydrogen citrate, disodium hydrogen citrate, dipotassium hydrogen citrate, diammonium hydrogen citrate, potassium sodium hydrogen citrate, sodium citrate, potassium citrate, ammonium citrate, lactic acid, sodium lactate, potassium lactate, ammonium lactate, malic acid, sodium malate, potassium malate, malic acid, sodium hydrogen malate, potassium hydrogen malate, ammonium hydrogen malate, potassium sodium malate, tartaric acid, sodium hydrogen tartrate, potassium hydrogen tartrate, ammonium hydrogen tartrate, potassium sodium tartrate, vitamin C, sodium vitamin C, alginic acid, sodium alginate, succinic acid, sodium succinate, potassium succinate, ammonium succinate, sodium hydrogen succinate, potassium hydrogen succinate, ammonium hydrogen succinate, potassium sodium succinate, acetic acid, sodium acetate, potassium acetate, ammonium acetate, amino acids and their salts. The stabilizer includes, for example, one or more of sodium bisulfite, sodium metabisulfite, sodium sulfite, sodium thiosulfate, vitamin C, sodium thioglycolate, glycine, cysteine, disodium edetate, sodium calcium edetate, etc. The analgesic include, for example: one or more of benzyl alcohol, 1,1,1-trichloro-2-methyl-2-propanol and the like. The bacteriostatic agent includes, for example, one or more of benzyl alcohol, 1,1,1-trichloro-2-methyl-2-propanol, benzoic acid and its salts, sorbic acid and its salts, parabens and the like.

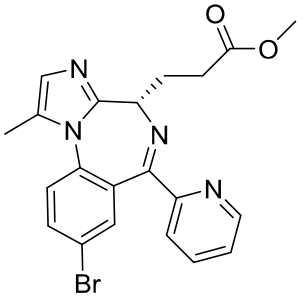
According to a fourth aspect of the present invention, it provides a method of anesthesia, comprising intravenously administering a certain dose of the hydrochloride of the benzodiazepine derivative and the pharmaceutical composition of the present invention to a patient.

According to a fifth aspect of the present invention, it provides the use of the hydrochloride of the benzodiazepine derivative of the present invention in the preparation of intravenous anesthetics.

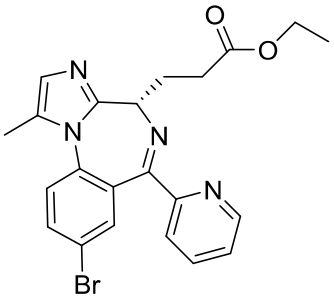
In order to better illustrate the objective and technical solution of the present invention, examples of the present invention are described in detail below. It should be noted that the following examples are only used to further illustrate the present invention, and cannot be understood as limiting the scope of protection of the present invention. Some non-essential improvements and adjustments made by those skilled in the art based on the above content of the present invention fall into the protection scope of the present invention.

The preparation of the free base of the benzodiazepine derivative (Formula II-1,

Formula II-2) involved in the preparation method according to the present invention is disclosed in PCT/CN2015/084770 and WO0069836, which are incorporated herein as a reference in their entirety.



Formula II-1



Formula II-2

Test Instruments Used in the Experiment

X-ray powder diffraction pattern: Instrument model: Bruker D8 FOCUS X-ray powder diffractometer; X-ray: Cu target; Scanning method: θ/2θ; Scanning range: 3-60°; Voltage: 40KV; Current: 40mA.

A. Preparation of the hydrochloride of the compound of Formula II-1 (the compound of Formula I wherein R is methyl)

A-1: Use of a HCl-anhydrous alcohol solution as HCl donor A

Example 1: Using a HCl-anhydrous methanol solution

The compound of Formula II-1 (1.8 g, 4 mmol) was dissolved in anhydrous methanol (6 ml) at 13°C, and then 1.57 g anhydrous methanol-HCl (HCl content of 9.29%) (with HCl molar quantity of 4 mmol) was added dropwise thereto. The mixture reacted for 0.5h, then MTBE (54ml) was added dropwise and reacted for another 0.5h. The reaction mixture was filtered, and the filter cake was dissolved in 30 ml of anhydrous methanol, decolorized at 50° C for 0.5 h, and then filtered. The filtrate was concentrated, and the residue was dissolved in anhydrous methanol (14 ml) at 50℃. Methyl tert-butyl ether (7 ml) was added dropwise. The solution became turbid, and was stirred for 0.5h. MTBE (98ml) was added dropwise. Then, the solution was cooled to -10°C and stirred for 1 hour and filtered. The filter cake was subjected to slurrying with ether (30 ml) for 1.5h, and then filtered. The filter cake was dried to obtain 1.62 g of a white solid, with a yield of 90%, purity: 99.57%, m.p: 173-175°C. The theoretical value of chloride ion content was 7.45% (w/w), and the measured value was 7.42% (w/w). See FIG. 3 for the X-ray powder diffraction pattern.

A-2: Use of amino acid hydrochloride as HCl donor A

Example 2: Using Glycine Hydrochloride

Glycine hydrochloride (2.46 g, 22 mmol) was added in anhydrous methanol (50 ml) at 60°C. An anhydrous methanol solution (15 ml) containing the compound of Formula II-1 (5 g, 11 mmol) was added dropwise to the above mixture within 5 min, and allowed to react for 0.5 h. The reaction mixture was cooled to -20°C and maintained at this temperature overnight, and then filtered. The filtrate was concentrated, and the residue was dissolved in anhydrous methanol (50 ml), decolorized at 55-60°C for 0.5h, and then filtered. The filtrate was concentrated, and the residue was dissolved in anhydrous methanol (20 ml) at 60°C. Methyl tert-butyl ether (140 ml) was added dropwise thereto. Then, it was cooled to room temperature and stirred overnight, and then filtered. The obtained solid was dried to obtain the target product. The theoretical value of chloride ion content was 7.45% (w/w), and the measured value was 7.38% (w/w). See FIG. 4 for the X-ray powder diffraction pattern.

Example 3: Using Valine Hydrochloride

With reference to the operation of Example 2, the target compound was prepared with the compound of Formula II-1 and valine hydrochloride as starting materials (with a molar ratio of 1:1.5). The theoretical value of chloride ion content was 7.45% (w/w), and the measured value was 6.94% (w/w).

Example 4: Using Alanine Hydrochloride

With reference to the operation of Example 2, the target compound was prepared with the compound of Formula II-1 and alanine hydrochloride as starting materials (with a molar ratio of 1:3). The theoretical value of chloride ion content was 7.45% (w/w), and the measured value was 6.81% (w/w).

A-3: Use of a solution B that can generate HCl as HCl donor A

Example 5: Using acetyl chloride-anhydrous methanol solution

With reference to the operation of Example 1, the target compound was prepared by crystallizing at 20℃, with the compound of Formula II-1 and acetyl chloride-anhydrous methanol solution as starting materials (with a molar ratio of acetyl chloride to the compound of Formula II-1 of 1:1). Theoretical value of chloride

ion content was 7.45% (w/w), and the measured value was 7.52% (w/w).

Example 6: Preparation and structural characterization of a single crystal of an ethanolate of a compound of Formula I wherein R is methyl

The compound of Formula I prepared in Example 1 was recrystallized with ethanol and methyl tert-butyl ether, and allowed to stand at room temperature for 4 days. The crystals were then collected. The obtained crystal was subjected to an X-ray single crystal diffraction experiment, and its crystal parameters are shown in Tables 1-6 below.

Table 1: Data and structure refinement data of the crystal of the ethanolate of the compound of Formula I wherein R is methyl

```
Bond precision:  C-C = 0.0032 A          Wavelength=0.71073

Cell:            a=7.6929(6)    b=11.9174(10)      c=13.2096(11)
                 alpha=90       beta=96.904(1)     gamma=90
Temperature:     293 K

                 Calculated                 Reported
Volume           1202.27(17)                1202.27(17)
Space group      P 21                       P 1 21 1
Hall group       P 2yb                      P 2yb
Moiety formula   C21 H20 Br N4 O2, C2 H6 O, C21 H20 Br N4 O2, Cl, C2
                 Cl                         H6 O
Sum formula      C23 H26 Br Cl N4 O3        C23 H26 Br Cl N4 O3
Mr               521.83                     521.83
Dx,g cm-3        1.441                      1.441
Z                2                          2
Mu (mm-1)        1.852                      1.852
F000             536.0                      536.0
F000'            535.91
h,k,lmax         10,15,17                   10,15,17
Nref             5607[ 2937]                4078
Tmin,Tmax                                   0.565,0.746
Tmin'

Correction method= # Reported T Limits: Tmin=0.565 Tmax=0.746
AbsCorr = MULTI-SCAN

Data completeness= 1.39/0.73      Theta(max)= 27.651

R(reflections)= 0.0204( 3828)     wR2(reflections)= 0.0473( 4078)

S = 0.856                 Npar= 293
```

Table 2: Non-hydrogen atomic coordinates (x 10^4) and equivalent isotropic shift parameter (Å2x 10^3) data of the ethanolate of the compound of Formula I wherein R is methyl

No. of Atom	x	y	z	U(eq)
Br(1)	-4202(1)	8268(1)	6312(1)	20(1)
O(1)	1797(2)	1562(2)	6318(1)	24(1)
O(2)	3608(2)	1616(2)	7788(1)	24(1)
N(1)	3120(2)	7198(2)	8336(1)	11(1)
N(2)	5345(3)	6118(2)	8788(1)	14(1)
N(3)	2920(3)	5605(2)	6571(1)	12(1)
N(4)	2033(3)	7931(2)	4955(1)	16(1)
C(1)	-1864(3)	8033(2)	6918(2)	15(1)
C(2)	-744(3)	7443(2)	6369(2)	15(1)
C(3)	940(3)	7172(2)	6826(2)	12(1)
C(4)	1444(3)	7520(2)	7839(2)	12(1)

C(5)	3750(3)	6139(2)	8272(2)	12(1)
C(6)	5737(3)	7165(2)	9197(2)	17(1)
C(7)	4366(3)	7865(2)	8929(2)	14(1)
C(8)	4159(3)	9076(2)	9132(2)	19(1)
C(9)	2771(3)	5267(2)	7635(2)	12(1)
C(10)	2080(3)	6484(2)	6240(2)	12(1)
C(11)	2264(3)	6836(2)	5168(2)	13(1)
C(12)	2297(3)	8259(3)	4006(2)	19(1)
C(13)	2764(3)	7536(2)	3271(2)	20(1)
C(14)	2999(3)	6408(2)	3507(2)	22(1)
C(15)	2745(3)	6053(2)	4475(2)	19(1)
C(16)	3417(3)	4067(2)	7808(2)	14(1)
C(17)	2268(3)	3324(3)	7070(2)	20(1)
C(18)	2680(3)	2085(2)	7127(2)	17(1)
C(19)	1984(4)	359(2)	6277(3)	29(1)
C(20)	324(3)	8166(2)	8354(2)	15(1)
C(21)	-1338(3)	8426(2)	7891(2)	16(1)
Cl(1)	8473(1)	4573(1)	8670(1)	25(1)
O(3)	11847(2)	5057(2)	10155(1)	30(1)
C(22)	11733(4)	6161(3)	10571(2)	29(1)
C(23)	10127(4)	6328(3)	11098(2)	27(1)

Note: U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Table 3: Non-hydrogen atom anisotropic shift parameter (Å2x 10^3) data of the ethanolate of the compound of Formula I wherein R is methyl

No. of Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	10(1)	18(1)	30(1)	5(1)	-2(1)	1(1)
O(1)	33(1)	10(1)	28(1)	-4(1)	-4(1)	2(1)
O(2)	25(1)	15(1)	30(1)	4(1)	-2(1)	1(1)
N(1)	11(1)	10(1)	12(1)	-1(1)	2(1)	0(1)
N(2)	12(1)	14(1)	16(1)	2(1)	0(1)	3(1)
N(3)	14(1)	10(1)	12(1)	1(1)	1(1)	-1(1)
N(4)	17(1)	15(1)	16(1)	2(1)	-1(1)	-1(1)
C(1)	9(1)	14(2)	22(1)	2(1)	0(1)	0(1)
C(2)	16(1)	11(1)	16(1)	1(1)	-1(1)	-1(1)
C(3)	13(1)	8(1)	14(1)	0(1)	1(1)	-1(1)
C(4)	10(1)	10(1)	14(1)	1(1)	1(1)	0(1)
C(5)	13(1)	10(1)	12(1)	1(1)	3(1)	1(1)
C(6)	16(1)	18(1)	15(1)	0(1)	-2(1)	-4(1)
C(7)	14(1)	16(1)	10(1)	-1(1)	0(1)	-2(1)
C(8)	20(1)	13(1)	22(1)	-3(1)	-1(1)	-2(1)
C(9)	14(1)	11(1)	12(1)	1(1)	1(1)	1(1)
C(10)	12(1)	10(1)	14(1)	-1(1)	-1(1)	-4(1)
C(11)	12(1)	13(1)	13(1)	0(1)	-1(1)	0(1)
C(12)	19(1)	18(1)	21(1)	9(1)	-1(1)	-4(2)
C(13)	19(1)	26(1)	14(1)	6(1)	2(1)	-2(1)
C(14)	26(1)	23(1)	16(1)	-2(1)	4(1)	3(1)
C(15)	24(1)	16(1)	18(1)	1(1)	2(1)	3(1)
C(16)	16(1)	11(1)	16(1)	3(1)	1(1)	1(1)
C(17)	25(1)	9(1)	25(1)	0(1)	-5(1)	3(1)
C(18)	16(1)	11(1)	24(1)	1(1)	5(1)	-2(1)
C(19)	31(2)	11(1)	45(2)	-5(1)	-1(1)	3(1)
C(20)	17(1)	12(1)	15(1)	-1(1)	3(1)	-2(1)

C(21)	14(1)	11(1)	23(1)	-2(1)	7(1)	2(1)
Cl(1)	16(1)	26(1)	32(1)	-12(1)	-1(1)	3(1)
O(3)	22(1)	40(1)	26(1)	-5(1)	-3(1)	9(1)
C(22)	34(2)	31(2)	22(1)	2(1)	7(1)	-3(1)
C(23)	27(1)	26(2)	27(1)	-3(1)	1(1)	2(1)

Table 4: Hydrogen atomic coordinates (x 10^4) and equivalent isotropic shift parameter (Å2x 10^3) data of the ethanolate of the compound of Formula I wherein R is methyl

No. of hydrogen atom	x	y	z	U(eq)
H(2)	6024	5542	8856	17
H(2A)	-1108	7227	5701	18
H(6)	6775	7361	9593	20
H(8A)	3702	9449	8512	28
H(8B)	5276	9392	9382	28
H(8C)	3364	9174	9632	28
H(9)	1536	5300	7748	15
H(12)	2155	9015	3840	23
H(13)	2919	7800	2625	24
H(14)	3320	5903	3026	26
H(15)	2893	5302	4658	23
H(16A)	4631	4008	7684	17
H(16B)	3331	3840	8506	17
H(17A)	1060	3427	7193	24
H(17B)	2364	3580	6382	24
H(19A)	1305	76	5673	44
H(19B)	1577	28	6868	44
H(19C)	3194	171	6262	44
H(20)	693	8423	9010	18
H(21)	-2090	8859	8230	19
H(3)	10946	4916	9780	45
H(22A)	11727	6706	10026	35
H(22B)	12763	6299	11054	35
H(23A)	9102	6175	10629	40
H(23B)	10090	7089	11333	40
H(23C)	10164	5827	11669	40

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Table 5: Bond length (Å) and bond angle (°) data of the ethanolate of the compound of Formula I wherein R is methyl

Bond	Bond length (bond angle°)	Bond	Bond length Å (bond angle°)
Br(1)-C(1)	1.899(2)	N(1)-C(5)	1.359(3)
O(1)-C(18)	1.348(3)	N(1)-C(7)	1.408(3)
O(1)-C(19)	1.443(3)	N(2)-C(5)	1.330(3)
O(2)-C(18)	1.198(3)	N(2)-C(6)	1.379(3)
N(1)-C(4)	1.427(3)	N(3)-C(9)	1.480(3)
N(3)-C(10)	1.281(3)	C(2)-C(3)	1.400(3)
N(4)-C(11)	1.342(3)	C(3)-C(4)	1.410(3)
N(4)-C(12)	1.352(3)	C(3)-C(10)	1.484(3)
C(1)-C(2)	1.382(3)	C(4)-C(20)	1.393(3)
C(1)-C(21)	1.383(3)	C(5)-C(9)	1.484(3)

C(6)-C(7)	1.358(4)	C(12)-C(13)	1.379(4)
C(7)-C(8)	1.481(4)	C(13)-C(14)	1.386(4)
C(9)-C(16)	1.522(3)	C(14)-C(15)	1.383(3)
C(10)-C(11)	1.498(3)	C(16)-C(17)	1.520(3)
C(11)-C(15)	1.390(3)	C(17)-C(18)	1.510(4)
C(20)-C(21)	1.385(3)	C(18)-O(1)-C(19)	116.5(2)
O(3)-C(22)	1.432(4)	C(5)-N(1)-C(4)	122.0(2)
C(22)-C(23)	1.502(4)	C(5)-N(1)-C(7)	109.57(19)
C(7)-N(1)-C(4)	128.4(2)	C(2)-C(1)-C(21)	121.9(2)
C(5)-N(2)-C(6)	109.2(2)	C(21)-C(1)-Br(1)	119.90(17)
C(10)-N(3)-C(9)	117.0(2)	C(1)-C(2)-C(3)	119.7(2)
C(11)-N(4)-C(12)	116.5(2)	C(2)-C(3)-C(4)	118.4(2)
C(2)-C(1)-Br(1)	118.20(17)	C(2)-C(3)-C(10)	118.5(2)
C(4)-C(3)-C(10)	123.0(2)	N(2)-C(5)-N(1)	107.4(2)
C(3)-C(4)-N(1)	119.2(2)	N(2)-C(5)-C(9)	130.7(2)
C(20)-C(4)-N(1)	120.1(2)	C(7)-C(6)-N(2)	109.1(2)
C(20)-C(4)-C(3)	120.7(2)	N(1)-C(7)-C(8)	124.7(2)
N(1)-C(5)-C(9)	121.7(2)	C(6)-C(7)-N(1)	104.7(2)
C(6)-C(7)-C(8)	130.4(2)	N(3)-C(10)-C(11)	116.8(2)
N(3)-C(9)-C(5)	104.79(18)	C(3)-C(10)-C(11)	117.8(2)
N(3)-C(9)-C(16)	109.69(19)	N(4)-C(11)-C(10)	116.4(2)
C(5)-C(9)-C(16)	116.00(19)	N(4)-C(11)-C(15)	123.6(2)
N(3)-C(10)-C(3)	125.3(2)	C(15)-C(11)-C(10)	119.9(2)
N(4)-C(12)-C(13)	123.6(3)	C(18)-C(17)-C(16)	115.8(2)
C(12)-C(13)-C(14)	119.1(2)	O(1)-C(18)-C(17)	109.3(2)
C(15)-C(14)-C(13)	118.4(2)	O(2)-C(18)-O(1)	124.2(2)
C(14)-C(15)-C(11)	118.8(2)	O(2)-C(18)-C(17)	126.6(2)
C(17)-C(16)-C(9)	107.3(2)	C(21)-C(20)-C(4)	120.0(2)
C(1)-C(21)-C(20)	119.1(2)	O(3)-C(22)-C(23)	113.0(2)

Table 6: Bond torsion angle (°) data of the ethanolate of the compound of Formula I wherein R is methyl

Bond	Torsion angle (°)	Bond	Torsion angle (°)
Br(1)-C(1)-C(2)-C(3)	-174.59(17)	N(2)-C(5)-C(9)-N(3)	-102.1(3)
Br(1)-C(1)-C(21)-C(20)	174.62(19)	N(2)-C(5)-C(9)-C(16)	19.0(3)
N(1)-C(4)-C(20)-C(21)	-176.5(2)	N(2)-C(6)-C(7)-N(1)	-0.1(3)
N(1)-C(5)-C(9)-N(3)	71.7(3)	N(2)-C(6)-C(7)-C(8)	-176.9(2)
N(1)-C(5)-C(9)-C(16)	-167.2(2)	N(3)-C(9)-C(16)-C(17)	-60.3(2)
N(3)-C(10)-C(11)-N(4)	-153.5(2)	C(1)-C(2)-C(3)-C(10)	176.7(2)
N(3)-C(10)-C(11)-C(15)	23.0(3)	C(2)-C(1)-C(21)-C(20)	-3.8(4)
N(4)-C(11)-C(15)-C(14)	-0.2(4)	C(2)-C(3)-C(4)-N(1)	176.6(2)
N(4)-C(12)-C(13)-C(14)	-0.7(4)	C(2)-C(3)-C(4)-C(20)	-3.2(4)
C(1)-C(2)-C(3)-C(4)	-0.4(3)	C(2)-C(3)-C(10)-N(3)	-130.2(3)
C(2)-C(3)-C(10)-C(11)	49.3(3)	C(4)-N(1)-C(5)-C(9)	3.2(3)
C(3)-C(4)-C(20)-C(21)	3.3(4)	C(4)-N(1)-C(7)-C(6)	-178.4(2)
C(3)-C(10)-C(11)-N(4)	26.9(3)	C(4)-N(1)-C(7)-C(8)	-1.4(4)
C(3)-C(10)-C(11)-C(15)	-156.5(2)	C(4)-C(3)-C(10)-N(3)	46.7(4)
C(4)-N(1)-C(5)-N(2)	178.27(19)	C(4)-C(3)-C(10)-C(11)	-133.8(2)
C(4)-C(20)-C(21)-C(1)	0.2(4)	C(5)-N(2)-C(6)-C(7)	-0.4(3)
C(5)-N(1)-C(4)-C(3)	-44.1(3)	C(5)-C(9)-C(16)-C(17)	-178.74(19)
C(5)-N(1)-C(4)-C(20)	135.7(2)	C(6)-N(2)-C(5)-N(1)	0.7(3)
C(5)-N(1)-C(7)-C(6)	0.6(3)	C(6)-N(2)-C(5)-C(9)	175.1(2)
C(5)-N(1)-C(7)-C(8)	177.6(2)	C(7)-N(1)-C(4)-C(3)	134.8(2)
C(7)-N(1)-C(4)-C(20)	-45.4(3)	C(9)-C(16)-C(17)-C(18)	-179.3(2)

C(7)-N(1)-C(5)-N(2)	-0.8(2)	C(10)-N(3)-C(9)-C(5)	-70.0(2)
C(7)-N(1)-C(5)-C(9)	-175.84(19)	C(10)-N(3)-C(9)-C(16)	164.8(2)
C(9)-N(3)-C(10)-C(3)	-1.6(4)	C(10)-C(3)-C(4)-N(1)	-0.3(3)
C(9)-N(3)-C(10)-C(11)	178.83(19)	C(10)-C(3)-C(4)-C(20)	179.9(2)
C(10)-C(11)-C(15)-C(14)	-176.5(2)	C(13)-C(14)-C(15)-C(11)	0.1(4)
C(11)-N(4)-C(12)-C(13)	0.5(3)	C(16)-C(17)-C(18)-O(1)	-168.7(2)
C(12)-N(4)-C(11)-C(10)	176.34(19)	C(16)-C(17)-C(18)-O(2)	12.9(4)
C(12)-N(4)-C(11)-C(15)	-0.1(3)	C(19)-O(1)-C(18)-O(2)	1.1(4)
C(12)-C(13)-C(14)-C(15)	0.3(4)	C(19)-O(1)-C(18)-C(17)	-177.4(2)
C(21)-C(1)-C(2)-C(3)	3.9(4)		

B: Preparation of the hydrochloride of the compound of Formula II-2 (the compound of Formula I wherein R is ethyl)

B-1: Use of a solution B that can generate HCl as HCl donor A

Example 7: Using acetyl chloride-absolute ethanol solution

The compound of Formula II-2 (1.38 g, 3 mmol) was dissolved in absolute ethanol (5 ml) at 13°C, and then an absolute ethanol solution (5 ml) containing acetyl chloride (3 mmol) was added dropwise, and reacted overnight. Then, MTBE (45ml) was added dropwise to the above reaction mixture, reacted for 0.5h, and then filtered. The filter cake was dissolved in 30 ml absolute ethanol, decolorized at 50°C for 0.5h, and then filtered. The filtrate was concentrated, and the residue was dissolved with absolute ethanol (12 ml) at 50°C, and then MTBE (6ml) was added dropwise to it. The solution became turbid and was stirred for 0.5 h. MTBE (82 ml) was added dropwise to the above mixture. Then, it was cooled to -8°C and stirred for 1h, and then filtered. The filter cake was subjected to pulping with ether (25 ml) for 1.5h, and then filtered. The filter cake was dried to obtain 1.3 g of a white solid, with a yield of 92%, purity: 99.73%, m.p: 160-163℃. Theoretical value of chloride ion content was 7.24% (w/w), and the measured value was 7.31% (w/w). See FIG. 5 for the X-ray powder diffraction pattern of the crystal.

Example 8: Using acetyl chloride-isopropanol solution

With reference to the operation of Example 7, the target compound was prepared by crystallizing at 20℃, with the compound of Formula II-2 and acetyl chloride-anhydrous isopropanol solution as starting materials (with a molar ratio of 1:1). See FIG. 6 for the X-ray powder diffraction pattern of the crystal. Theoretical value of chloride ion content was 7.24% (w/w), and the measured value was 7.21% (w/w).

B-2: Use of a HCl-anhydrous alcohol solution as HCl donor A

Example 9: Using HCl-anhydrous ethanol solution

The compound of Formula II-2 (1.38 g, 3 mmol) was dissolved in absolute ethanol (5 ml) at 13°C, and then 1.2 g absolute ethanol-HCl (HCl content of 8.87%) (with HCl molar quantity of 3 mmol) was added dropwise thereto, and reacted for 0.5 h. Then, MTBE (45 ml) was added dropwise to the above reaction mixture, reacted for 0.5 h, and then filtered. The filter cake was dissolved in 30 ml absolute ethanol, decolorized at 50°C for 0.5h, and then filtered. The filtrate was concentrated, and the residue was dissolved with absolute ethanol (12 ml) at 50°C, and then MTBE (60 ml) was added dropwise thereto. The solution became turbid and was stirred for 0.5 h. MTBE (82 ml) was then added dropwise. Then, it was cooled to -8°C and stirred for 1 h, the mixture was filtered, and the filter cake was subjected to slurrying with ether (25 ml) for 1.5 h, and then filtered. The filter cake was dried to obtain 1.3 g of a white solid, with a yield of 92%, purity: 99.89%, m.p: 162-165℃. Theoretical value of chloride ion content was 7.24% (w/w), and the measured value was 7.15% (w/w). See FIG. 7 for the X-ray powder diffraction pattern of the crystal.

With reference to the operation of Example 9, another batch of the crystal of the compound of Formula I wherein R is ethyl was obtained. The X-ray powder diffraction pattern of the crystal is shown in FIG. 8.

Example 10: Using HCl-anhydrous ethanol solution

With reference to the operation of Example 9, the crystallization solvent ethanol: methyl tert-butyl ether=1:7 (v/v) was used, and the crystal of the compound of Formula I wherein R is ethyl was obtained. Theoretical value of chloride ion content was 7.24% (w/w), and the measured value was 7.19% (w/w). See FIG. 9 for the X-ray powder diffraction pattern of the crystal.

B-3: Use of amino acid hydrochloride as HCl donor A

Example 11: Using Glycine Hydrochloride

Glycine hydrochloride (2.46 g, 22 mmol) was added in absolute ethanol (50 ml) at 60°C, and then an absolute ethanol solution (15 ml) containing the compound of Formula II-2 (5 g, 11 mmol) was added dropwise within 5 minutes, and allowed to react for 0.5h. The reaction mixture was cooled to -20°C and maintained overnight, and then filtered. The filtrate was concentrated, and the residue was dissolved in absolute ethanol (50 ml), decolorized at 55-60°C for 0.5h, and filtered. The filtrate was concentrated. The residue was dissolved in absolute ethanol (20 ml) at 60°C.

Butyl tert-butyl ether (140 ml) was added dropwise. Then, it was cooled to room temperature, stirred overnight, and filtered. The filter cake was dried to obtain the target product. Theoretical value of chloride ion content was 7.24% (w/w), and the measured value was 6.82% (w/w). See FIG. 10 for the X-ray powder diffraction pattern of the crystal.

With reference to the operation of Example 11, another batch of the crystal of the compound of Formula I wherein R is ethyl was obtained. The X-ray powder diffraction pattern of the crystal is shown in FIG. 11.

Example 12: Using Glycine Hydrochloride

Glycine hydrochloride (2.46 g, 22 mmol) was added in absolute ethanol (50 ml) at 60°C, and then an absolute ethanol solution (15 ml) containing the compound of Formula II-2 (5 g, 11 mmol) was added dropwise within 5 minutes, and allowed to react for 0.5h. It was cooled to -20°C and maintained overnight, then filtered. The filtrate was concentrated, and the residue was dissolved in absolute ethanol (50 ml), decolorized at 55-60°C for 0.5h, and then filtered. The filtrate was concentrated, and the residue was dissolved with absolute ethanol (25 ml) at 60°C, and ethyl acetate (240 ml) was added dropwise thereto. Then, it was cooled to -40°C, stirred for 2h, and filtered. The residue was dissolved in absolute ethanol (25 ml) at 50°C, and methyl tert-butyl ether (150ml) was added dropwise thereto. Then, it was cooled to room temperature, stirred for 1h, and filtered. The filter cake was dried to obtain the target product. Theoretical value of chloride ion content was 7.24% (w/w), and the measured value was 7.02% (w/w). See FIG. 12 for the X-ray powder diffraction pattern of the crystal.

With reference to the operation of Example 12, another batch of the crystal of the compound of Formula I wherein R is ethyl was obtained. The X-ray powder diffraction pattern of the crystal is shown in FIG. 13.

Example 13: Preparation of the compound of Formula I wherein R is ethyl using valine hydrochloride

With reference to the operation of Example 11, the target product was obtained by crystallizing at -10°C with the compound of Formula II-2 and valine hydrochloride as starting materials (with a molar ratio of 1:1.5), and ethanol and isopropyl ether as the crystallization solvent. Theoretical value of chloride ion content was 7.24% (w/w), and the measured value was 6.74% (w/w).

Example 14: Preparation of the compound of Formula I wherein R is ethyl

With reference to the operation of Example 11, the target compound was prepared with the compound of Formula II-2 and alanine hydrochloride as starting materials (with a molar ratio of 1:3). Theoretical value of chloride ion content was 7.24% (w/w), and the measured value was 6.63% (w/w).

Example 15: Preparation and structural characterization of the compound of Formula I wherein R is ethyl

The II-2 hydrochloride prepared in Example 9 was recrystallized with ethanol and methyl tert-butyl ether, and allowed to stand at room temperature for 4 days. The crystals were collected and subjected to an X-ray single crystal diffraction experiment. The crystal parameters are shown in Tables 7 -12 below.

Table 7: Crystal data and structure refinement data of the ethanolate of the compound of Formula I wherein R is ethyl

Bond precision: C-C = 0.0051 A		Wavelength=1.54184	
Cell:	a=7.3774(1)	b=12.7332(2)	c=27.1779(4)
	alpha=90	beta=90	gamma=90
Temperature:	150 K		
	Calculated		Reported
Volume	2553.04(6)		2553.04(6)
Space group	P 21 21 21		P 21 21 21
Hall group	P 2ac 2ab		P 2ac 2ab
Moiety formula	C22 H22 Br N4 O2, C2 H6 O, Cl		C22 H22 Br N4 O2, C2 H6 O, Cl
Sum formula	C24 H28 Br Cl N4 O3		C24 H28 Br Cl N4 O3
Mr	535.85		535.85
Dx,g cm-3	1.394		1.394
Z	4		4
Mu (mm-1)	3.419		3.419
F000	1104.0		1104.0
F000'	1105.42		
h,k,lmax	9,15,33		8,15,33
Nref	5191[2973]		4625
Tmin,Tmax			0.276,1.000
Tmin'			

Correction method= # Reported T Limits: Tmin=0.276 Tmax=1.000
AbsCorr = MULTI-SCAN

Data completeness= 1.56/0.89 Theta(max)= 74.052

R(reflections)= 0.0303(4503) wR2(reflections)= 0.0795(4625)

S = 1.035 Npar= 302

Table 8: Data of non-hydrogen atom coordinates (x 10^4) and equivalent isotropic shift parameter (Å2x 10^3) of the ethanate of the compound of Formula I wherein R is ethyl

No. of Atom	x	y	z	U(eq)
Br(1)	-3552(1)	10601(1)	5747(1)	37(1)
O(1)	4576(6)	4196(2)	6487(2)	73(1)
N(1)	3579(4)	9375(2)	6716(1)	24(1)
N(2)	5735(4)	8340(2)	6948(1)	28(1)
O(3)	2760(4)	4013(2)	5838(1)	46(1)

N(4)	2822(4)	9849(2)	5048(1)	31(1)
N(3)	3572(4)	7810(2)	5886(1)	24(1)
C(11)	2919(4)	8840(3)	5178(1)	23(1)
C(7)	4778(5)	9987(3)	6995(1)	28(1)
C(2)	-125(4)	9623(2)	5794(1)	24(1)
C(22)	-855(5)	10674(3)	6501(1)	29(1)
C(16)	3957(5)	6435(3)	6506(1)	28(1)
C(3)	1528(4)	9324(2)	6006(1)	24(1)
C(6)	6102(5)	9324(3)	7136(1)	31(1)
C(4)	1938(4)	9694(2)	6478(1)	23(1)
C(1)	-1287(5)	10272(2)	6046(1)	27(1)
C(5)	4197(5)	8375(3)	6699(1)	25(1)
C(10)	2729(4)	8615(2)	5715(1)	24(1)
C(21)	759(5)	10376(3)	6721(1)	27(1)
C(8)	4561(5)	11133(3)	7077(1)	34(1)
C(15)	3156(5)	8029(3)	4848(1)	33(1)
C(14)	3266(6)	8272(3)	4351(1)	39(1)
C(9)	3279(4)	7547(2)	6409(1)	24(1)
C(12)	2931(5)	10065(3)	4565(1)	34(1)
C(18)	3569(6)	4567(3)	6194(1)	38(1)
C(13)	3142(5)	9305(3)	4208(1)	34(1)
C(17)	2968(5)	5690(3)	6167(1)	36(1)
C(19)	3152(8)	2898(3)	5820(2)	60(1)
C(20)	1904(10)	2421(3)	5460(2)	74(2)
O(2)	12010(4)	7592(3)	7610(1)	50(1)
C(23)	11583(7)	8592(3)	7804(2)	48(1)
C(24)	10366(10)	8548(4)	8231(2)	83(2)
Cl(1)	8848(1)	6748(1)	6988(1)	34(1)

Note：U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

Table 9: Data of non-hydrogen atom isotropic shift parameter (Å2x 10^3) of the ethanate of the compound of Formula I wherein R is ethyl

No. of Atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	30(1)	42(1)	39(1)	4(1)	-2(1)	9(1)
O(1)	88(3)	35(2)	96(3)	4(2)	-51(2)	17(2)
N(1)	25(1)	27(1)	21(1)	-2(1)	-1(1)	-6(1)
N(2)	25(1)	33(2)	27(1)	4(1)	-2(1)	0(1)
O(3)	68(2)	21(1)	49(2)	-3(1)	-9(2)	12(1)
N(4)	42(2)	25(1)	25(1)	1(1)	2(1)	-1(1)
N(3)	28(1)	23(1)	22(1)	-1(1)	1(1)	2(1)
C(11)	21(2)	25(2)	23(1)	-2(1)	0(1)	1(1)
C(7)	29(2)	36(2)	21(1)	-5(1)	2(1)	-8(2)
C(2)	26(2)	20(1)	26(1)	1(1)	1(1)	-3(1)
C(22)	31(2)	23(2)	34(2)	-3(1)	9(1)	0(1)
C(16)	26(2)	29(2)	30(2)	2(1)	-3(1)	1(1)
C(3)	26(2)	21(1)	25(1)	-2(1)	2(1)	-2(1)
C(6)	27(2)	39(2)	27(1)	-3(1)	-4(1)	-6(2)
C(4)	22(2)	24(2)	23(1)	-2(1)	1(1)	-2(1)
C(1)	24(2)	24(1)	34(2)	4(1)	4(1)	-4(1)
C(5)	27(2)	28(2)	21(1)	0(1)	2(1)	-1(1)
C(10)	26(2)	22(1)	24(1)	-3(1)	1(1)	-2(1)
C(21)	28(2)	27(2)	25(1)	-5(1)	3(1)	-3(1)

C(8)	32(2)	36(2)	34(2)	-13(2)	2(2)	-8(2)
C(15)	46(2)	27(2)	27(2)	-4(1)	3(2)	8(2)
C(14)	50(2)	40(2)	26(2)	-9(1)	1(2)	8(2)
C(9)	24(2)	25(2)	24(1)	2(1)	2(1)	2(1)
C(12)	44(2)	31(2)	27(2)	6(1)	0(2)	-1(2)
C(18)	43(2)	25(2)	46(2)	3(2)	-6(2)	5(2)
C(13)	33(2)	45(2)	24(1)	2(2)	0(1)	-2(2)
C(17)	42(2)	22(2)	45(2)	2(2)	-13(2)	3(2)
C(19)	85(4)	21(2)	73(3)	-7(2)	-4(3)	17(2)
C(20)	127(5)	24(2)	72(3)	-10(2)	-6(4)	4(3)
O(2)	37(2)	65(2)	47(2)	-18(1)	-12(1)	14(1)
C(23)	49(2)	43(2)	50(2)	4(2)	2(2)	-7(2)
C(24)	116(5)	50(3)	81(4)	-28(3)	49(4)	-19(3)
Cl(1)	28(1)	42(1)	30(1)	-4(1)	0(1)	0(1)

Table 10: Data of hydrogen atom coordinates (x 10^4) and equivalent isotropic shift parameter (Å2x 10^3) of the ethanate of the compound of Formula I wherein R is ethyl

No. of hydrogen atom	x	y	z	U(eq)
H(2)	6416	7778	6987	34
H(2A)	-443	9376	5476	29
H(22)	-1652	11149	6662	35
H(16A)	5278	6396	6444	34
H(16B)	3731	6240	6853	34
H(6)	7121	9503	7333	37
H(21)	1064	10636	7038	32
H(8A)	4204	11472	6768	51
H(8B)	5713	11430	7191	51
H(8C)	3623	11253	7326	51
H(15)	3242	7322	4958	40
H(14)	3424	7733	4113	47
H(9)	1950	7574	6480	29
H(12)	2860	10778	4464	41
H(13)	3200	9492	3870	41
H(17A)	1658	5721	6245	44
H(17B)	3123	5938	5825	44
H(19A)	2972	2579	6148	71
H(19B)	4425	2782	5718	71
H(20A)	2128	2722	5134	112
H(20B)	649	2563	5558	112
H(20C)	2105	1660	5449	112
H(2B)	11129	7368	7445	74
H(23A)	11005	9020	7544	57
H(23B)	12720	8948	7902	57
H(24A)	9274	8150	8145	124
H(24B)	10026	9262	8328	124
H(24C)	10986	8201	8506	124

Table 11: Data of bond length (Å) and bond angle (°) of the ethanolate of the compound of Formula I in which R is ethyl

Bond	Bond length Å (bond angle°)	Bond	Bond length Å (bond angle°)
Br(1)-C(1)	1.904(4)	O(1)-C(18)	1.186(5)
N(1)-C(7)	1.402(4)	N(1)-C(4)	1.431(4)

N(1)-C(5)	1.354(4)	N(2)-C(6)	1.380(4)
N(2)-C(5)	1.321(4)	O(3)-C(18)	1.340(5)
O(3)-C(19)	1.450(4)	N(4)-C(11)	1.334(4)
N(4)-C(12)	1.345(4)	N(3)-C(10)	1.286(4)
N(3)-C(9)	1.478(4)	C(11)-C(10)	1.493(4)
C(11)-C(15)	1.380(5)	C(7)-C(6)	1.347(5)
C(7)-C(8)	1.485(5)	C(2)-C(3)	1.401(4)
C(2)-C(1)	1.373(4)	C(22)-C(1)	1.376(5)
C(22)-C(21)	1.386(5)	C(16)-C(9)	1.523(4)
C(16)-C(17)	1.509(5)	C(3)-C(4)	1.401(4)
C(3)-C(10)	1.491(4)	C(4)-C(21)	1.395(4)
C(5)-C(9)	1.481(4)	C(15)-C(14)	1.390(5)
C(14)-C(13)	1.374(5)	C(12)-C(13)	1.379(5)
C(18)-C(17)	1.498(5)	C(19)-C(20)	1.474(7)
O(2)-C(23)	1.415(5)	C(23)-C(24)	1.468(7)
C(7)-N(1)-C(4)	128.3(3)	C(18)-O(3)-C(19)	116.8(3)
C(5)-N(1)-C(7)	109.2(3)	C(11)-N(4)-C(12)	116.9(3)
C(5)-N(1)-C(4)	122.5(3)	C(10)-N(3)-C(9)	117.2(3)
C(5)-N(2)-C(6)	109.1(3)	N(4)-C(11)-C(10)	116.1(3)
N(4)-C(11)-C(15)	123.7(3)	C(6)-C(7)-C(8)	130.7(3)
C(15)-C(11)-C(10)	120.2(3)	C(1)-C(2)-C(3)	120.2(3)
N(1)-C(7)-C(8)	124.0(3)	C(1)-C(22)-C(21)	119.0(3)
C(6)-C(7)-N(1)	105.2(3)	C(17)-C(16)-C(9)	108.7(3)
C(2)-C(3)-C(4)	118.2(3)	C(3)-C(4)-N(1)	120.1(3)
C(2)-C(3)-C(10)	117.7(3)	C(21)-C(4)-N(1)	119.4(3)
C(4)-C(3)-C(10)	124.1(3)	C(21)-C(4)-C(3)	120.5(3)
C(7)-C(6)-N(2)	108.8(3)	C(2)-C(1)-Br(1)	117.9(2)
C(2)-C(1)-C(22)	121.8(3)	N(2)-C(5)-C(9)	129.9(3)
C(22)-C(1)-Br(1)	120.3(3)	N(3)-C(10)-C(11)	117.4(3)
N(1)-C(5)-C(9)	122.2(3)	N(3)-C(10)-C(3)	125.4(3)
N(2)-C(5)-N(1)	107.7(3)	C(3)-C(10)-C(11)	117.2(3)
C(22)-C(21)-C(4)	120.2(3)	C(22)-C(21)-C(4)	120.2(3)
C(11)-C(15)-C(14)	118.2(3)	C(11)-C(15)-C(14)	118.2(3)
C(13)-C(14)-C(15)	119.0(3)	C(13)-C(14)-C(15)	119.0(3)
N(3)-C(9)-C(16)	109.1(2)	N(3)-C(9)-C(16)	109.1(2)
C(22)-C(21)-C(4)	120.2(3)	N(3)-C(9)-C(5)	106.5(3)
C(11)-C(15)-C(14)	118.2(3)	C(5)-C(9)-C(16)	114.8(3)
C(13)-C(14)-C(15)	119.0(3)	N(4)-C(12)-C(13)	123.4(3)
N(3)-C(9)-C(16)	109.1(2)	O(1)-C(18)-O(3)	123.6(3)
O(1)-C(18)-C(17)	126.8(4)	C(18)-C(17)-C(16)	115.3(3)
O(3)-C(18)-C(17)	109.6(3)	O(3)-C(19)-C(20)	107.5(4)
C(14)-C(13)-C(12)	118.7(3)	O(2)-C(23)-C(24)	113.4(4)

Table 12: Data of bond torsion angle (°) of the ethanolate of the compound of Formula I wherein R is ethyl

Bond	Torsion angle (°)	Bond	Torsion angle (°)
O(1)-C(18)-C(17)-C(16)	8.9(7)	N(1)-C(5)-C(9)-C(16)	-169.6(3)
N(1)-C(7)-C(6)-N(2)	0.3(4)	N(2)-C(5)-C(9)-N(3)	-105.1(4)
N(1)-C(4)-C(21)-C(22)	-178.5(3)	N(2)-C(5)-C(9)-C(16)	15.8(5)
N(1)-C(5)-C(9)-N(3)	69.5(4)	O(3)-C(18)-C(17)-C(16)	-172.3(3)
N(4)-C(11)-C(10)-N(3)	-149.5(3)	C(11)-N(4)-C(12)-C(13)	-0.3(6)
N(4)-C(11)-C(10)-C(3)	32.4(4)	C(11)-C(15)-C(14)-C(13)	0.3(6)
N(4)-C(11)-C(15)-C(14)	-1.3(6)	C(7)-N(1)-C(4)-C(3)	138.6(3)

N(4)-C(12)-C(13)-C(14)	-0.7(6)	C(7)-N(1)-C(4)-C(21)	-41.4(4)
C(7)-N(1)-C(5)-N(2)	-1.0(3)	C(2)-C(3)-C(10)-N(3)	-135.7(3)
C(7)-N(1)-C(5)-C(9)	-176.7(3)	C(2)-C(3)-C(10)-C(11)	42.2(4)
C(2)-C(3)-C(4)-N(1)	177.6(3)	C(3)-C(2)-C(1)-Br(1)	-177.5(2)
C(2)-C(3)-C(4)-C(21)	-2.5(4)	C(3)-C(2)-C(1)-C(22)	2.1(5)
C(3)-C(4)-C(21)-C(22)	1.6(5)	C(4)-N(1)-C(7)-C(8)	-2.6(5)
C(6)-N(2)-C(5)-N(1)	1.2(3)	C(4)-N(1)-C(5)-N(2)	179.4(2)
C(6)-N(2)-C(5)-C(9)	176.4(3)	C(4)-N(1)-C(5)-C(9)	3.7(4)
C(4)-N(1)-C(7)-C(6)	-180.0(3)	C(4)-C(3)-C(10)-N(3)	43.3(5)
C(4)-C(3)-C(10)-C(11)	-138.8(3)	C(5)-N(1)-C(7)-C(6)	0.4(3)
C(1)-C(2)-C(3)-C(4)	0.7(4)	C(5)-N(1)-C(7)-C(8)	177.8(3)
C(1)-C(2)-C(3)-C(10)	179.8(3)	C(5)-N(1)-C(4)-C(3)	-41.9(4)
C(1)-C(22)-C(21)-C(4)	1.2(5)	C(5)-N(1)-C(4)-C(21)	138.2(3)
C(5)-N(2)-C(6)-C(7)	-0.9(4)	C(10)-C(3)-C(4)-N(1)	-1.4(5)
C(10)-N(3)-C(9)-C(16)	165.0(3)	C(10)-C(3)-C(4)-C(21)	178.5(3)
C(10)-N(3)-C(9)-C(5)	-70.5(4)	C(21)-C(22)-C(1)-Br(1)	176.5(2)
C(10)-C(11)-C(15)-C(14)	177.9(3)	C(21)-C(22)-C(1)-C(2)	-3.1(5)
C(8)-C(7)-C(6)-N(2)	-176.9(3)	C(9)-N(3)-C(10)-C(11)	-175.9(3)
C(15)-C(11)-C(10)-N(3)	31.3(5)	C(9)-N(3)-C(10)-C(3)	2.0(5)
C(15)-C(11)-C(10)-C(3)	-146.8(3)	C(9)-C(16)-C(17)-C(18)	176.3(3)
C(15)-C(14)-C(13)-C(12)	0.6(6)	C(12)-N(4)-C(11)-C(10)	-177.9(3)
C(12)-N(4)-C(11)-C(15)	1.3(6)	C(17)-C(16)-C(9)-C(5)	-177.2(3)
C(18)-O(3)-C(19)-C(20)	170.6(4)	C(19)-O(3)-C(18)-O(1)	0.6(7)
C(17)-C(16)-C(9)-N(3)	-57.7(4)	C(19)-O(3)-C(18)-C(17)	-178.2(4)

Example 16: Stability test of the hydrochloride of the benzodiazepine derivative

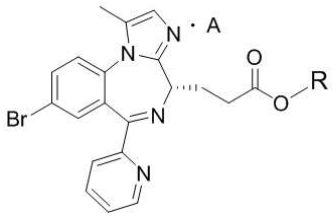
The compound of Formula I prepared in the above examples were selected, and placed under the conditions of 40℃, RH75% and 25℃, RH60% for an accelerated stability test and a long-term stability test after packaging. The changes in the degradation product (CNS-7054) in these compounds in 6 months were observed, and the results are shown in Table 13 below.

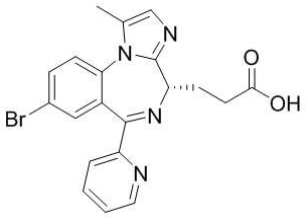
Table 13: Data of stability test of the hydrochloride of the benzodiazepine derivative

R	Amino acid content	Change in CNS-7054	
		Accelerated experiment (40℃, RH75%)	Long - term experiment (25℃, RH60%)
Methyl	None	Not increased	Not increased
Methyl	2% glycine	Not increased	Not increased
Methyl	3% glycine	Not increased	Not increased
Methyl	4% glycine	Increased by	Not increased
Methyl	5% alanine	Increased by 0.01%	Not increased
Methyl	4% valine	Increased by 0.01%	Not increased
Ethyl	None	Not increased	Not increased
Ethyl	1.7% glycine	Not increased	Not increased

Ethyl	2.8% glycine	Not increased	Not increased
Ethyl	4.5% glycine	Not increased	Not increased
Ethyl	4% alanine	Increased by 0.01%	Not increased
Ethyl	5% alanine	Increased by 0.01%	Not increased
Ethyl	4% valine	Increased by 0.01%	Not increased

In addition, a lyophilized preparation made of sulfonates according to the prior art were partially degraded into carboxylic acid (CNS-7054) and released alcohol in both the accelerated and long-term experiments. The changes are shown in Table 14 below.





Sulfonate of benzazepine derivative CNS-7054

wherein R is methyl or ethyl; A is benzenesulfonic acid or p-toluenesulfonic acid.

Table 14: Data of stability experiments of the sulfonate of the benzodiazepine derivative

R	A	Class	CNS-7054	
			Accelerated experiment (40℃, RH75%)	Long-term experiment (25℃, RH60%)
Methyl	Benzenesulfonic acid	Drug substance	Increased by 0.07% in 1 month	Increased by 0.04% in 3 months
Methyl	Benzenesulfonic acid	Lyophilized preparation	Increased by 0.37% in 1 month	Increased by 0.35% in 3 months
Methyl	P-toluenesulfonic acid	Drug substance	Increased by 0.1% in 1 month	Increased by 0.1% in 3 months
Methyl	P-toluenesulfonic acid	Lyophilized preparation	Increased by 0.25% in 1 month	Increased by 0.2% in 3 months
Ethyl	Benzenesulfonic acid	Drug substance	Increased by 0.05% in 1 month	Increased by 0.03% in 3 months
Ethyl	Benzenesulfonic acid	Lyophilized preparation	Increased by 0.3% in 1 month	Increased by 0.26% in 3 months
Ethyl	P-toluenesulfonic acid	Drug substance	Increased by 0.2% in 1 month	Increased by 0.1% in 3 months
Ethyl	P-toluenesulfonic acid	Lyophilized preparation	Increased by 0.25% in 1 month	Increased by 0.15% in 3 months

From the above data, it can be seen that the hydrochloride of the benzodiazepine derivative provided by the present invention has good stability, does not generate degradation products (CNS-7054), and does not generate genotoxic impurities.

Example 17: Determination of ED_{50} and LD_{50} in KM mice for the hydrochloride of the benzodiazepine derivative

Sequential method was used to determine the hypnotic ED_{50} and LD_{50} in KM mice for the hydrochloride of the benzodiazepine derivative. Healthy and qualified male KM mice were selected, n=10-20. The drug was injected through the tail vein at a constant rate in 5 seconds. After preliminary pre-test, the approximate dosage that may cause hypnosis (or death) of the animals was found as an intermediate dosage in the formal experiment. A group interval of 0.8 was used, and 2-3 dosage groups were set up and down respectively. The formal experiment started with the administration from the intermediate dosage. When the animal was narcotized (or died), the dosage was reduced by one dose. If the animal was not narcotized (or died), the dosage was increased by one until 3-4 repetitions occurred. The ED_{50} value and LD_{50} value were measured with the disappearance of righting reflex or death as indicators. The therapeutic index (TI index = ED_{50}/LD_{50}) was calculated through LD_{50} and ED_{50} values. The experimental results are shown in Table 15 below.

Table 15: ED_{50} and LD_{50} data in KM mice for the hydrochloride of the benzodiazepine derivative

No.	R	Acid	Amino acid content	ED_{50}(mg/kg, 95% confidence interval)	LD_{50}(mg/kg, 95% confidence interval)	TI
1	methyl	HCl	None	35.20（32.41~38.39）	217.48（192.55~245.23）	6.2
2	methyl	HCl	3% glycine	36.26（33.57~40.11）	224.82 (198.12~252.72)	6.2
3	ethyl	HCl	None	13.21（10.68~16.32）	205.78（187.43~226.14）	15.6
4	ethyl	HCl	2.8% glycine	13.53（11.11~16.95）	211.73 (193.08~232.18)	15.6
positive control 1	Methyl	Benzene sulfonic acid	None	40.64（37.21~44.40）	270.09 (237.72~306.88)	6.6
positive control 2	Ethyl	Benzene sulfonic acid	None	15.62（13.14~18.56）	263.14 (223.77~309.44)	16.8

From the above data, it can be seen that the therapeutic index of the

hydrochloride of the benzodiazepine derivative provided by the present invention is not significantly different from that of benzenesulfonate, and with good safety.

Example 18: 2*ED_{50} anesthesia pharmacodynamics experiment in KM mice (n=20) for the hydrochloride and sulfonate of the benzodiazepine derivative

KM mice, half male and half female, 20 mice in each group. With a dosage of 2*ED_{50}, it was injected into the tail vein at a constant rate in 5 seconds. The time of loss of the righting reflex in mice (induction time), recovery time (duration) and walking time were recorded. The experimental results are shown in Table 16 below.

Table 16: Experimental data of 2*ED_{50} anesthesia pharmacodynamics in KM mice for the hydrochloride and sulfonate of the benzodiazepine derivative

No.	R	Acid	A*	Dosage (2*ED50)	B*	C*	D*	E*	F*
1	Methyl	HCl	None	70.40 mg/kg	17.8± 2.06	674.3±354.28	5/20	508.95± 510.89	8/20
2	Methyl	HCl	33% glycine	72.52 mg/kg	17.7± 2.12	673.3±348.53	5/20	510.41± 512.25	8/20
3	Ethyl	HCl	None	26.42 mg/kg	17.28± 2.04	462.75± 179.82	4/20	66.53 ± 149.76	3/20
4	Ethyl	HCl	2.8% glycine	27.06 mg/kg	17.35± 2.13	459.75± 184.15	4/20	67.75 ± 155.35	3/20
Positive control 1	Methyl	BSA*	None	81.28 mg/kg	17.6± 1.93	692.3 ± 399.15	9/20	514.95± 525.16	17/20
Positive control 2	Ethyl	BSA*	None	31.24 mg/kg	17.1± 2.00	443.75± 247.86	7/20	192.9 ± 268.98	10/20

Note: A* = amino acid content; B* = induction time (s); C* = duration time (s);
D* = Number of animals with duration time longer than 10 min; E* = Walking time (s);
F* = Number of animals with walking time longer than 1 min; and BSA* = Benzenesulfonic acid

From the above data, it can be seen that:

1. The benzodiazepine derivative wherein R is ethyl are better than that wherein R is methyl with respect to the duration time of anesthesia and walking time, with statistical differences.

2. With respect to the benzodiazepine derivative wherein R is ethyl, the incidence of the animal anesthesia time of more than 10 minutes is 35% for the

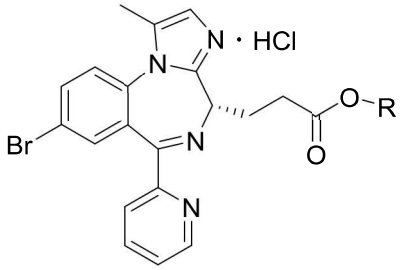
sulfonate, and 20% for the hydrochloride. The incidence of the animal walking time of more than 1 min is 50% for the sulfonate and 15% for the hydrochloride, which indicates that the pharmacokinetic properties of the hydrochloride are more stable than those of the sulfonate, and are less affected by individual differences.

3. With respect to the benzodiazepine derivative wherein R is methyl, the incidence of the animal anesthesia time of more than 10 minutes is 45% for the sulfonate, and 25% for the hydrochloride. The incidence of the animal walking time of more than 1 min is 85% for the sulfonate and 40% for the hydrochloride, which indicates that the pharmacokinetic properties of the hydrochloride are more stable than those of the sulfonate, and are less affected by individual differences.

Conclusion: The hydrochloride of the benzodiazepine derivative provided by the present invention has more stable pharmacokinetic properties than sulfonates, and is less affected by individual differences.

What is claimed is:

1. A crystalline form of the hydrochloride of a benzodiazepine derivative of Formula I or its ethanolate,



Formula I

wherein R is methyl or ethyl; wherein when R is methyl, the crystal form has the following cell parameters: a=7.6929(6) Å, b=11.9174(10) Å, c=13.2096(11) Å, α= 90°, β= 96.904(1)°, γ=90°; and when R is ethyl, the crystal form has the following cell parameters: a=7.3774(1) Å, b=12.7332(2) Å, c=27.1779(4) Å, α= 90°, β= 90°, γ=90°.

2. The crystal form according to claim 1, wherein R is methyl, and its crystal form has a structure substantially as shown in FIG. 1, or may be characterized by one or more parameters substantially as shown in Tables 1-6; and wherein R is ethyl, and its crystal form has a structure substantially as shown in FIG. 2, or may be characterized by one or more parameters substantially as shown in Tables 7-12.

3. The crystal form according to claim 1, wherein R is methyl, and the compound of Formula I has a content of chloride ion of 6.71-7.52% (w/w); and wherein R is ethyl, and the compound of Formula I has a content of chloride ion of 6.51-7.31% (w/w).

4. The crystal form according to claim 1, wherein R is methyl, and it has an X-ray powder diffraction pattern with the following 2θ values measured using CuKα radiation: about 6.81, 8.93, 13.39, 19.38, 21.23, 22.42, 24.20, 27.31±0.2 degrees; or the X-ray powder diffraction pattern shows the following 2θ values measured using CuKα radiation: about 8.11, 9.86, 14.73, 17.47, 23.03, 25.94, 28.31±0.2 degrees; or the crystal form has an X-ray powder diffraction pattern substantially as shown in FIG. 3.

5. The crystal form according to claim 1, wherein R is methyl, and it has an

X-ray powder diffraction pattern with the following 2θ values measured using CuKα radiation: about 6.80, 8.93, 9.87, 13.37, 14.69, 19.36, 20.76, 21.25, 22.19, 22.38, 23.06, 24.21, 25.93, 27.73±0.2 degrees; or the X-ray powder diffraction pattern shows the following 2θ values measured using CuKα radiation: about 16.14, 17.48, 20.02, 25.17, 26.36, 28.30, 34.13±0.2 degrees; or the crystal form has an X-ray powder diffraction pattern substantially as shown in FIG. 4.

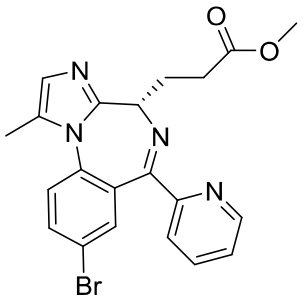
6. The crystal form according to claim 1, wherein R is ethyl, and it has an X-ray powder diffraction pattern with the following 2θ values measured using CuKα radiation: about 6.87, 7.38, 9.53, 13.65, 18.71, 22.13, 22.67, 25.10, 27.25, 29.30±0.2 degrees; or the X-ray powder diffraction pattern shows the following 2θ values measured using CuKα radiation: about 14.96, 15.43, 20.23, 20.67, 21.13, 23.52, 28.22, 31.26±0.2 degrees; or the crystal form has an X-ray powder diffraction pattern substantially as shown in any one of FIGs. 5-8.

7. The crystal form according to claim 1, wherein R is ethyl, and it has an X-ray powder diffraction pattern with the following 2θ values measured using CuKα radiation: about 7.41, 9.24, 12.71, 13.64, 15.06, 18.30, 18.72, 21.59, 22.18, 25.74±0.2 degrees; or the X-ray powder diffraction pattern shows the following 2θ values measured using CuKα radiation: about 9.52, 11.69, 20.90, 22.60, 23.65, 24.26, 26.40, 28.43, 29.35±0.2 degrees; or the crystal form has an X-ray powder diffraction pattern substantially as shown in FIG. 9.

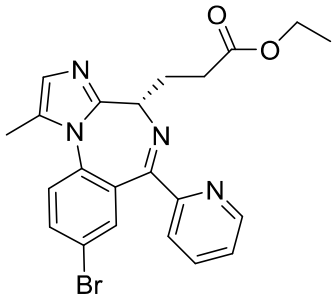
8. The crystal form according to claim 1, wherein R is ethyl, and it has an X-ray powder diffraction pattern with the following 2θ values measured using CuKα radiation: about 6.84, 7.37, 9.53, 13.66, 22.63, 25.57, 29.28, 31.26±0.2 degrees; or the X-ray powder diffraction pattern shows the following 2θ values measured using CuKα radiation: about 15.43, 19.07, 22.16, 34.25±0.2 degrees; or the crystal form has an X-ray powder diffraction pattern substantially as shown in any one of FIGs. 10-13.

9. A method for preparing the crystal form according to any one of claims 1 to 8, comprising the following steps: dissolving the free base of the benzodiazepine derivative of the following Formula II-1 or II-2 in an organic solvent 1, adding HCl donor A wherein $[H^+]$ is equimolar to the free base, to form a salt at a temperature of -20 to 60°C, after decolorizing the crude salt, crystallizing it in a crystallization

solvent 1 at a temperature of -60 to 80°C, to obtain the crystal form of the hydrochloride of the benzodiazepine derivative of Formula I.



Formula II-1



Formula II-2

10. The method according to claim 9, wherein the organic solvent 1 is an alcohol solvent, such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol; an ester solvent, such as methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate; a ketone solvent, such as acetone and butanone; or a mixture thereof.

11. The method according to claim 9, wherein the HCl donor A is an amino acid hydrochloride, such as glycine hydrochloride, alanine hydrochloride, valine hydrochloride; a HCl-anhydrous alcohol solution, that is, an alcohol solution of dry HCl gas, such as dry HCl-methanol solution, dry HCl-ethanol solution, dry HCl-isopropanol solution; or a solution B that can generate HCl, such as acetyl chloride-methanol solution, acetyl chloride-ethanol solution, propionyl chloride-ethanol solution, acetyl chloride-isopropanol solution.

12. The method according to claim 11, wherein the HCl donor A is an amino acid hydrochloride, and the crystal form of the hydrochloride of the benzodiazepine derivative has an amount of amino acid of 0%-8% (w/w).

13. The method according to claim 11, wherein the HCl donor A is a HCl-anhydrous alcohol solution or a solution B that can generate HCl, and the ratio of amount of substance (molar ratio) of the free base of the benzodiazepine derivative to the HCl donor A (calculated by $[H^+]$) is 1:0.4 - 1; the HCl donor A is an amino acid hydrochloride, and the ratio of amount of substance (molar ratio) of the free base of the benzodiazepine derivative to the amino acid hydrochloride is 1:1-10.

14. The method according to claim 9, wherein the temperature for forming a salt is -10 to 30°C, and the temperature for crystallizing is -20 to 60°C.

15. The method according to claim 9, wherein the crystallization solvent 1 comprises an alcohol solvent, such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol; an ether solvent, such as ethyl ether, isopropyl ether, dioxane, methyl tert-butyl ether; an ester solvent, such as methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate; a ketone solvent, such as acetone and butanone; an alkane solvent, such as n-pentane, hexane, heptane, petroleum ether; a halogenated alkane, such as dichloromethane, chloroform, 1,2-dichloroethane; and a combination thereof.

16. A pharmaceutical composition comprising the crystal form according to any one of claims 1 to 8, and a pharmaceutically acceptable excipient, carrier and/or other auxiliary materials.

17. The crystal form according to any one of claims 1 to 8 or the pharmaceutical composition according to claim 16 for use as intravenous anesthetics.

18. Use of the crystal form according to any one of claims 1 to 8 in manufacturing intravenous anesthetics.