

STEREoisomer AND DEUTERATED DERIVATIVE OF PYRAZOLOQUINAZOLINE COMPOUND AND USE

This application claims priority to Chinese Patent Application No. 202310083163.X filed on January 17, 2023 and Chinese Patent Application No. 202310430237.2 filed on April 20, 2023, the entire contents of which are incorporated herein by reference.

TECHNICAL FIELD

The present disclosure relates to stereoisomers, deuterated derivatives or stereoisomeric deuterated derivatives of pyrazoloquinazoline-based compounds, or pharmaceutically acceptable salts, solvates thereof. The present disclosure also relates to pharmaceutical compositions comprising one or more of such stereoisomers and derivatives, or pharmaceutically acceptable salts, solvates thereof, as active ingredients. Furthermore, the present disclosure relates to uses of such stereoisomers and derivatives, or pharmaceutically acceptable salts or solvates thereof, as well as pharmaceutical compositions comprising such stereoisomeric derivatives or pharmaceutically acceptable salts or solvates thereof, as polo-like kinase inhibitors.

BACKGROUND

Mammalian polo-like kinases (PLKs) consist of a family of five serine/threonine kinases (PLKs1-5) with different functions and expression patterns in mammalian cells. Most PLKs have a C-terminal polo-box domain (PBD) for substrate recognition, and an N-terminal kinase domain for substrate phosphorylation. Among PLKs, PLK1 is the most extensively studied family member and plays critical roles in cell cycle progression, especially at the G2-M checkpoint, mitosis, and cytokinesis. PLK1 activity is tightly regulated during cell cycle through interactions with other phosphorylated scaffold proteins.

To date, PLK1 has been extensively studied as an attractive target in cancer therapy, and several small molecule PLK1 inhibitors have been developed over the past decade or more. These inhibitors target both liquid tumors such as leukemia and lymphoma, as well as solid tumors such as pancreatic cancer, breast cancer, and prostate cancer. However, although PLK1 inhibitors have shown anti-tumor effects in preclinical cancer models and have also shown preliminary promising results in clinical trials, they have not yet achieved satisfactory therapeutic effects due to dose-limiting toxicity (Translational Oncology Volume 16, February 2022, 101332). Among clinically investigated ATP-competitive inhibitors primarily targeting PLK1, onvansertib (NMS-P937, NMS1286937) exhibits high selectivity and holds significant clinical development prospects. However, among the onvansertib derivative series (Bioorg Med Chem Lett. 2011 May 15;21(10):2969-74, Bioorg Med Chem Lett. 2010 Nov 15;20(22):6489-94), most derivative molecules show poor oral absorption and bioavailability in mice. The literature (Mol Cancer Ther; 11(4) April 2012, Supplementary table S4) further reveal substantial interspecies variability in onvansertib's absorption and bioavailability, with no discernible pattern, posing challenges for developing selective PLK1 inhibitors.

The researchers of this study unexpectedly discovered that parameters such as oral absorption, bioavailability, and tissue distribution of onvansertib can be effectively modulated by stereocyclization modification, deuteration, and stereocyclization plus deuteration at the N-methylpiperazine of onvansertib. Multiple animal experiments have shown that the modified molecules have lower toxicity and better efficacy than onvansertib, providing a broader selection

pharmaceutically acceptable salt, solvate thereof for use in treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1.

In a further aspect, the present disclosure relates to the use of a stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I) as provided herein, or a pharmaceutically acceptable salt, solvate thereof, in the preparation of medicaments for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1.

In a further aspect, the present disclosure relates to a kit for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1, the kit comprising: a stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I) as provided herein, or a pharmaceutically acceptable salt, solvate thereof, or a pharmaceutical composition comprising a stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I) as provided herein, or a pharmaceutically acceptable salt, solvate thereof and a pharmaceutically acceptable carrier or excipient, a container and optionally a package insert or label indicating treatment.

The diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 include, but are not limited to, leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, sepsis, and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the IC₅₀ values of the compounds of the Examples of the present disclosure and a positive control compound for growth inhibition effects on pancreatic cancer cell line (PSN1). Among them, A represents CPD02 of the present disclosure; B represents CPD03 of the present disclosure; C represents the positive control compound NMS-P937; D represents CPD04 of the present disclosure; and E represents CPD06 of the present disclosure.

FIG. 2 shows a graph of tumor growth of tested drugs in a HCT116 subcutaneous tumor xenograft mouse model after treatment initiation with the compounds of the present disclosure.

FIG3 shows a graph of body weight changes of experimental animals in the HCT116 subcutaneous xenograft mouse model after treatment initiation with the compounds of the present disclosure.

FIG. 4 shows a graph of tumor growth of tested drugs in a BxPC-3 subcutaneous xenograft mouse model after treatment initiation with the compounds of the present disclosure.

FIG. 5 shows a graph of body weight changes of experimental animals in the BxPC-3 subcutaneous xenograft mouse model after treatment initiation with the compounds of the present disclosure.

FIG. 6 shows a graph of tumor growth of tested drugs in a PSN1 subcutaneous xenograft mouse model after initiation of treatment with a compound of the present disclosure.

FIG. 7 shows a graph of body weight changes of experimental animals in the PSN1 subcutaneous xenograft mouse model after treatment initiation with the compounds of the present disclosure.

DETAILED DESCRIPTION

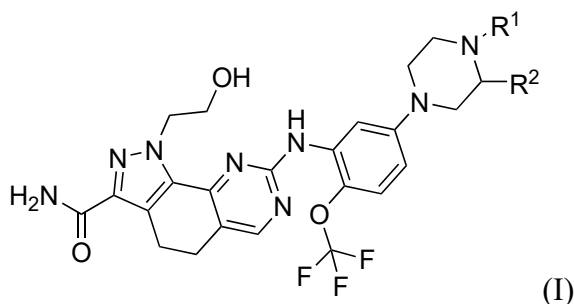
Certain embodiments will now be described in detail, examples of which are illustrated in the accompanying detailed description. Although the enumerated embodiments will be described,

it should be understood that they are not intended to limit the present disclosure to these embodiments. On the contrary, the present disclosure is intended to cover all alternatives, modifications and equivalents, which may fall within the scope of the present disclosure as defined by the claims. Those skilled in the art will recognize that many methods and materials similar or equivalent to those described herein that could be used in the implementation of the present disclosure. The present disclosure is by no means limited to the methods and materials described. If one or more incorporated references and similar materials are different or contradictory from the present disclosure, including but not limited to defined terms, term usage, described technology, etc., the present disclosure shall control.

It is to be understood that certain features of the present disclosure, which are described in the context of separate embodiments for clarity, may also be provided in combination in a single embodiment. Conversely, various features of the present disclosure that are described in the context of a single embodiment for conciseness may also be provided separately or in any suitable subcombination.

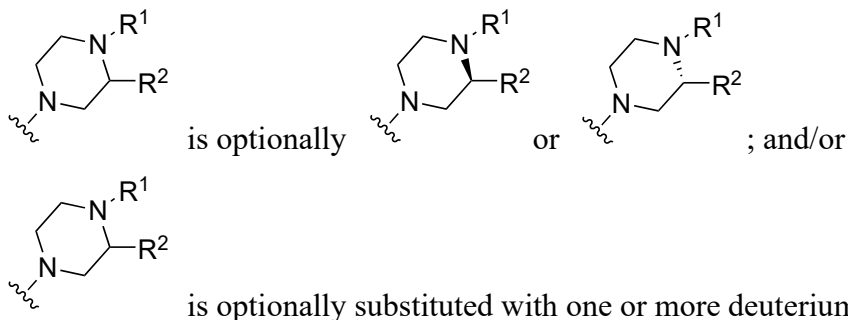
Provides herein are the following.

Item 1. A stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I):



or a pharmaceutically acceptable salt, solvate thereof,

wherein R^1 and R^2 are each independently selected from hydrogen, deuterium, deuterated or non-deuterated C_{1-6} alkyl; or R^1 and R^2 together with the N atom and the C atom to which they are attached form an optionally substituted, deuterated or non-deuterated 5-membered heterocyclic ring;



Item 2. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to Item 1, wherein

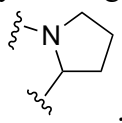
R^1 is a deuterated or non-deuterated C_{1-6} alkyl, preferably methyl or deuterated methyl, more preferably perdeuterated methyl;

R^2 is hydrogen or deuterium.

Item 3. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a

compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to Item 1, wherein

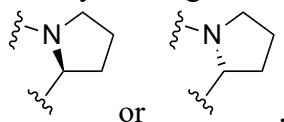
R¹ and R² together with the N atom and C atom to which they are attached form an optionally substituted 5-membered heterocyclic ring as follows:



wherein the 5-membered heterocyclic ring is optionally substituted with 1-7 deuterium atoms.

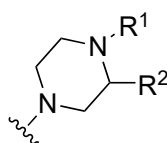
Item 4. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to Item 3, wherein

R¹ and R² together with the N atom and C atom to which they are attached form an optionally substituted 5-membered heterocyclic ring selected from:



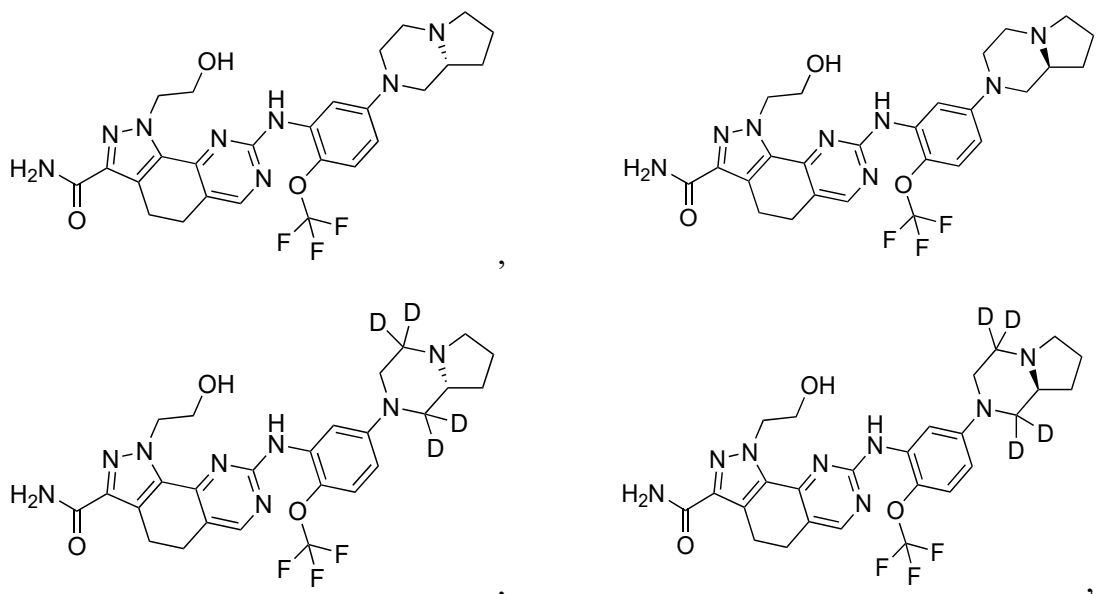
wherein the 5-membered heterocyclic ring is optionally substituted with 1-7 deuterium atoms.

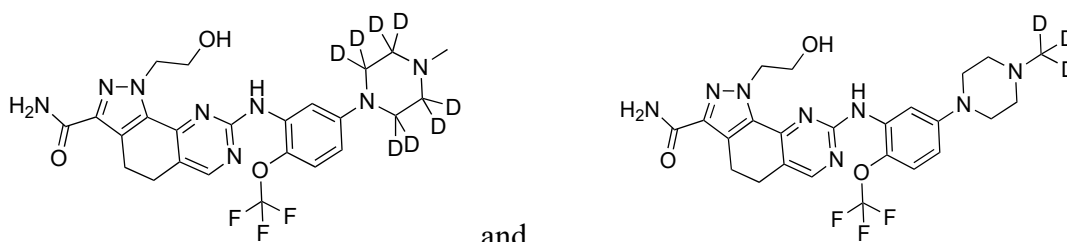
Item 5. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of the preceding Items, wherein



is optionally substituted with 1-8 deuterium atoms.

Item 6. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to Item 1, wherein the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I) is selected from:





and

Item 7. A pharmaceutical composition, comprising the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of Items 1 to 6, and a pharmaceutically acceptable carrier or excipient.

Item 8. A method for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of Items 1 to 6.

Item 9. The method according to Item 8, wherein the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are selected from the group consisting of leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, and sepsis.

Item 10. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of Items 1 to 6, for use in treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1.

Item 11. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof, for use in treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 according to Item 10, wherein the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are selected from the group consisting of leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, and sepsis.

Item 12. Use of the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of Items 1 to 6, in the preparation of a medicament for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1.

Item 13. The use according to Item 12, wherein the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are selected from the group consisting of leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, and sepsis.

Item 14. A kit for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1, the kit comprising
the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a

compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of Items 1 to 6, or the pharmaceutical composition according to Item 7;

a container; and

optionally a package insert or label indicating treatment.

Item 15. A kit according to Item 14, wherein the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are selected from the group consisting of leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, and sepsis.

Definitions

Terms used but not defined herein have their ordinary meanings, and the meaning of such terms is independent at each occurrence. However, unless otherwise stated, the following definitions apply throughout the specification and claims.

As used herein, the terms “comprise” and “include” are intended to specify the presence of stated features, integers, components or steps, but do not exclude the presence or addition of one or more other features, integers, components, steps or groups thereof.

The definitions of specific functional groups and chemical terms are described in more detail below. For the purposes of the present disclosure, chemical elements are identified according to the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th edition, inside cover, and specific functional groups are generally defined as described therein. In addition, the general principles of organic chemistry and specific functional moieties and reactivity are described in *Organic Chemistry*, Thomas Sorrell, University Science Books, Sausalito, 1999; Smith and March, *March's Advanced Organic Chemistry*, 5th edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd edition, Cambridge University Press, Cambridge, 1987.

All ranges cited herein are inclusive unless otherwise expressly indicated.

When a range of values is listed, it is intended to encompass every value and subrange within that range. For example, “C₁₋₆” is intended to encompass C₁, C₂, C₃, C₄, C₅, C₆, C₁₋₆, C₁₋₅, C₁₋₄, C₁₋₃, C₁₋₂, C₂₋₆, C₂₋₅, C₂₋₄, C₂₋₃, C₃₋₆, C₃₋₅, C₃₋₄, C₄₋₆, C₄₋₅ and C₅₋₆.

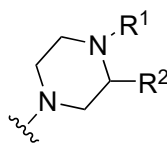
When any variable occurs more than once in any constituent or in Formula (I) or in any other formula depicting and describing compounds of the present disclosure, its definition at each occurrence is independent of its definition at every other occurrence. Furthermore, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

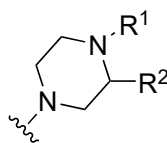
As used herein, the term “deuterated” refers to one or more hydrogen atoms on a compound or group being substituted with deuterium. When a compound or group is deuterated, one, two, three or even more hydrogen atoms on the compound or group may be substituted with deuterium, up to the substitution of all hydrogen atoms on the compound or group with deuterium, at which point it may be referred to as a “perdeuterated compound or group”.

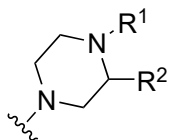
In some embodiments, at the deuterated positions, the deuterium isotope abundance is greater than the natural deuterium isotope abundance (0.015%), preferably greater than 50%, more preferably greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, greater than

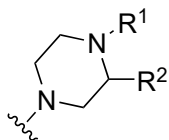
99.5%, or 100%.

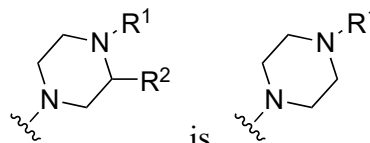
In some cases, for example, when “hydrogen” and “deuterium” appear in a statement as parallel alternatives or when the description states that “hydrogen” is substituted with “deuterium”, the term “hydrogen” represents the hydrogen isotope “¹hydrogen (¹H)”, and the term “deuterium” represents the hydrogen isotope “²hydrogen (²H)”; or it is understood that at the specified position in the compound, the state in which hydrogen exists with the natural abundance of its isotopes is replaced by a state in which deuterium exists at an abundance greater than natural deuterium isotopic abundance (for example, deuterium abundance of greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, greater than 99.5%, or 100%).

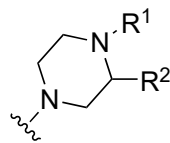
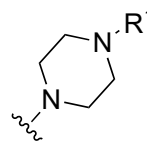


In some embodiments, when  is optionally substituted with one deuterium atom, the deuterium atom may substitute on any ring carbon atom. In some embodiments, when

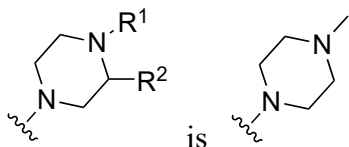


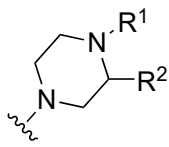
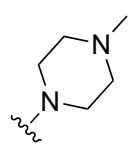
 is optionally substituted with two or more deuterium atoms, these two or more deuterium atoms may substitute on the same ring carbon atom or on different ring carbon atoms.

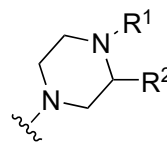


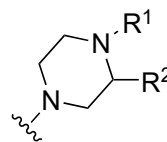
In some embodiments, when R² is hydrogen, the  is . wherein hydrogens on the ring carbon atoms including R² may be substituted with 1 to 8 deuterium atoms, for example 1, 2, 3, 4, 5, 6, 7, or 8 deuterium atoms. These deuterium atoms may substitute on the same or different ring carbon atoms.

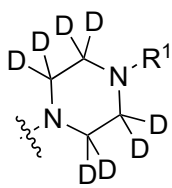
In some embodiments, when R¹ is non-deuterated methyl and R² is hydrogen, the

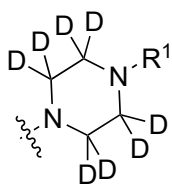


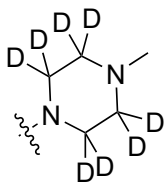
 is , wherein at least one hydrogen on the ring carbon atoms including R² is substituted with deuterium, for example, substituted with 1, 2, 3, 4, 5, 6, 7 or 8 deuterium atoms. These deuterium atoms may substitute on the same or different ring carbon atoms.



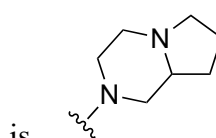
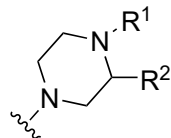
In preferred embodiments, optionally deuterated  is selected from



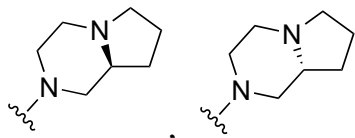
, R¹ is optionally non-deuterated, such as non-deuterated methyl, or deuterated,



such as perdeuterated methyl, for example

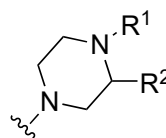


In some embodiments, when is or its stereoisomers

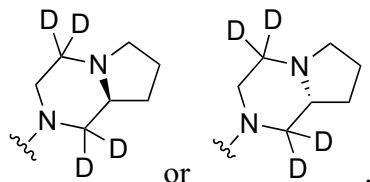


one or more deuterium atoms may substitute on any ring carbon

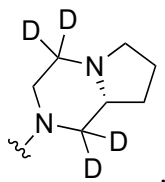
atom; when there are two or more deuterium atoms, these two or more deuterium atoms may substitute on the same ring carbon atom, or may substitute on different ring carbon atoms. In some embodiments, when there are two or more deuterium atoms, these two or more deuterium atoms may substitute on one ring of a fused ring structure, or may substitute on two rings of a fused ring structure. In some embodiments, one or more deuterium atoms may substitute on a carbon atom common to two rings in a fused ring structure.



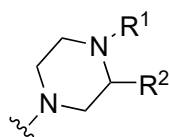
In a preferred embodiment, the optionally deuterated is selected from



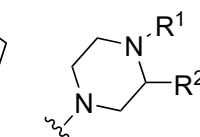
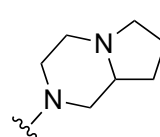
or



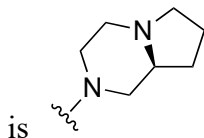
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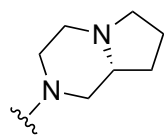
is non-deuterated



In some embodiments, when



or



is

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As used herein, the term “alkyl” refers to a straight or branched saturated hydrocarbon group. The term “C_{i-j} alkyl” refers to an alkyl group having i to j carbon atoms. Unless otherwise indicated, an alkyl group may contain 1 to 10 carbon atoms. In certain embodiments, the alkyl group contains 1 to 6 carbon atoms, such as 1 to 5 carbon atoms, 1 to 4 carbon atoms, 1 to 3 carbon atoms, or 1 to 2 carbon atoms. Non-limiting examples of alkyl groups include methyl, ethyl, n-propyl and isopropyl, n-butyl, sec-butyl, isobutyl and tert-butyl, neopentyl, etc.

As used herein, the term “deuterated alkyl” refers to a substituent obtained by replacing one or more hydrogens on an alkyl group with deuterium. When an alkyl group is deuterated, one, two, three or even more hydrogen atoms on the alkyl group may be substituted with deuterium,

up to the substitution of all hydrogen atoms on the alkyl group with deuterium, at which point it may be referred to as a “perdeuterated alkyl group”. In some embodiments, non-limiting examples of deuterated alkyl groups include deuterated methyl groups, such as monodeuterated methyl, dideuterated methyl, trideuterated methyl (perdeuterated methyl), monodeuterated ethyl, dideuterated ethyl, trideuterated ethyl, tetra deuterated ethyl, pentadeuterated ethyl (perdeuterated ethyl) etc.

As used herein, the term “cycloalkyl” refers to non-aromatic, saturated monocyclic and polycyclic ring systems, wherein all ring-forming atoms are carbon. Unless otherwise indicated, a cycloalkyl group may contain 3 to 10 ring carbon atoms (i.e., C₃₋₁₀ cycloalkyl). In certain embodiments, the cycloalkyl group can include 3 to 9, 3 to 8, 3 to 7, 3 to 6, 4 to 10, 4 to 9, 4 to 8, 4 to 7, 4 to 6, 4 to 5, 5 to 10, 5 to 9, 5 to 8, 5 to 7, 5 to 6 ring carbon atoms, etc. In particular, cycloalkyl may be monocyclic or bicyclic. Alternatively, bicyclic cycloalkyl groups can include fused, spiro and bridged cycloalkyl structures.

In another aspect, also included are cycloalkyl rings wherein 1, 2, or 3 ring carbon atoms are replaced with heteroatoms. Such groups are referred to as “heterocyclyl” or “heterocycle”, which refers to a cycloalkyl group as defined above but having at least one heteroatom selected from N, O, and S as ring-forming atoms. Unless otherwise indicated, a heterocyclyl group may contain 3 to 10 ring-forming atoms (i.e. 3- to 10-membered heterocyclyl). In certain embodiments, a heterocyclyl group may contain 3 to 9, 3 to 8, 3 to 7, 3 to 6, 4 to 10, 4 to 9, 4 to 8, 4 to 7, 4 to 6, 4 to 5, 5 to 10, 5 to 9, 5 to 8, 5 to 7, 5 to 6 ring-forming atoms etc. Especially, the heterocyclyl group can be monocyclic or bicyclic. Optionally, bicyclic heterocyclyl groups may include fused, spiro, and bridged heterocyclyl structures. Non-limiting examples of heterocyclyl groups include oxiranyl, pyrrolidinyl, piperidinyl, tetrahydropyranyl, piperazinyl, pyrrolidinyl, and morpholinyl. Heterocyclyl groups may also be described by the number of carbon atoms. For example, C₃₋₆ heterocyclyl refers to a heterocyclyl group containing three to six ring-forming carbon atoms, and may further contain at least one heteroatom, such as 1, 2 or 3 heteroatoms as ring-forming atoms. In certain embodiments, the heterocyclyl groups or heterocycles contain 1 or 2 heteroatoms as ring-forming atoms. In certain embodiments, the heterocyclyl groups can be monocyclic or bicyclic, such as fused bicyclic and spiro bicyclic. In the context of the present disclosure, the terms “heterocyclyl” and “heterocycle” can be used interchangeably.

As used herein, the term “deuterated heterocyclyl” refers to a substituent obtained by substituting one or more hydrogen atoms on a heterocyclyl with deuterium. When a heterocyclyl is deuterated, one, two, three or even more hydrogen atoms on the heterocyclyl may be substituted with deuterium, up to the substitution of all hydrogen atoms on the heterocyclyl group with deuterium, at which point it may be referred to as a “perdeuterated heterocyclyl group”.

As used herein, the term “halo” or “halogen” refers to fluoride, chloride, bromide and iodide. In certain embodiments, non-limiting examples of halo include fluoride, chloride and bromide, more particularly fluoride and chloride.

As used herein, the term “heteroatom” refers to nitrogen (N), oxygen (O), and sulfur (S), and may include any oxidized forms of nitrogen and sulfur, and any quaternized forms of basic nitrogen, unless otherwise specified.

As used herein, the term “substituted” when referring to a chemical group means that the chemical group has one or more hydrogen atoms that are removed and replaced with a substituent. As used herein, the term “substituent” has its ordinary meaning known in the art and refers to a chemical moiety covalently attached to a parent group or, where appropriate, fused to the parent

group. It is understood that the substitution of a given atom is limited by valence. It should be understood that substituents may be further substituted.

When it is indicated in Formula (I) or any embodiment thereof that a moiety is “optionally” substituted, this means that Formula (I) or its embodiments encompasses compounds wherein the moiety is substituted with the indicated substituent(s), and compounds wherein the moiety does not contain the indicated substituent(s) (i.e., wherein the moiety is unsubstituted)..

The compounds provided herein are described with reference to generic formulae and specific compounds. In addition, the compounds of the present disclosure may exist in a variety of different forms or derivatives, all of which are within the scope of the present disclosure. These include, for example, pharmaceutically acceptable salts, tautomers, stereoisomers, racemic mixtures, positional isomers, prodrugs, solvated forms, different crystalline forms or polymorphs, and active metabolites, etc.

As used herein, unless otherwise indicated, the term “pharmaceutically acceptable salt” includes salts that maintain the biological effectiveness of the free acid/base form of the specific compound and are not undesirable biologically or otherwise. Pharmaceutically acceptable salts may include salts formed with inorganic bases or acids and organic bases or acids. In the case where the compounds of the present disclosure contain one or more acidic or basic groups, the disclosure also includes their corresponding pharmaceutically acceptable salts. Therefore, compounds of the present disclosure containing acidic groups (e.g., carboxyl groups) may exist in salt form and may be used according to the disclosure as, for example, as alkali metal salts, alkaline earth metal salts, aluminum salts or ammonium salts. Further non-limiting examples of such salts include lithium salts, sodium salts, potassium salts, calcium salts, magnesium salts, barium salts or salts with ammonia or organic amines (e.g., ethylamine, ethanolamine, diethanolamine, triethanolamine, piperidine, N-methylglutamine, or amino acids). For example, these salts are readily obtained by reacting compounds having acidic groups with suitable bases (e.g., lithium hydroxide, sodium hydroxide, sodium propoxide, potassium hydroxide, potassium ethoxide, magnesium hydroxide, calcium hydroxide, or barium hydroxide). Other basic salts of compounds of the present disclosure include, but are not limited to, copper (I), copper (II), iron (II), iron (III), manganese (II), and zinc salts. Compounds of the present disclosure contain one or more basic groups, such as groups that can be protonated, they may exist in the form of salts, and may be used in the form of addition salts thereof with inorganic or organic acids according to the present disclosure. Examples of suitable acids include hydrochloric acid, hydrobromic acid, hydroiodic acid, phosphoric acid, sulfuric acid, nitric acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, sulfoacetic acid, trifluoroacetic acid, oxalic acid, acetic acid, tartaric acid, lactic acid, salicylic acid, benzoic acid, carbonic acid, formic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, malonic acid, maleic acid, malic acid, pamoic acid, mandelic acid, sulfamic acid, phenylpropionic acid, gluconic acid, ascorbic acid, isonicotinic acid, citric acid, adipic acid, taurocholic acid, glutaric acid, stearic acid, glutamic acid or aspartic acid, as well as other acids known to those skilled in the art. The salts formed are particularly hydrochlorides, chlorides, hydrobromides, bromides, iodides, sulfates, phosphates, methanesulfonates (mesylates), tosylates, carbonates, bicarbonates, formates, acetates, sulfoacetates, triflates, oxalates, malonates, maleates, succinates, tartrates, malates, pamoates, mandelates, fumarates, lactates, citrates, glutarates, stearates, aspartates and glutamates. Furthermore, the stoichiometry of salts formed from the compounds of the present disclosure may be integer or non-integer multiples of

1.

Compounds of the present disclosure containing basic nitrogen-containing groups can be quaternized using agents such as C₁₋₄ alkyl halides, for example, methyl, ethyl, isopropyl and tert-butyl chlorides, bromines and iodines; di-C₁₋₄ alkyl sulfates, for example, dimethyl, diethyl and diamyl sulfates; C₁₀₋₁₈ alkyl halides, for example, decyl, dodecyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; and aryl C₁₋₄ alkyl halides, for example, benzyl chloride and phenethyl bromide.

If the compounds of the present disclosure contain both acidic and basic groups in the molecule, the present disclosure also includes inner salts or betaines (zwitterions) in addition to the above-mentioned salt forms. The corresponding salts may be obtained by conventional methods known to those skilled in the art, for example, by contacting them with organic or inorganic acids or bases in solvents or dispersants, or via anion exchange or cation exchange with other salts. The present disclosure further includes all salts of the compounds of the present disclosure, which are not directly suitable for use as pharmaceuticals due to low physiological compatibility, but can be used, for example, as intermediates for chemical reactions or for the preparation of pharmaceutically acceptable salts. For a review of more suitable salts, see Stahl and Wermuth, *Handbook of Pharmaceutical Salts: Properties, Selection and Use* (Wiley-VCH, 2002).

The compound of Formula (I) and its pharmaceutically acceptable salt may exist in both unsolvated and solvated forms. As used herein, the term “solvate” refers to a molecular complex comprising a stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable solvent molecules. For example, the term “hydrate” is used when the solvent is water.

The compound of Formula (I) may have one or more chiral (asymmetric) centers. The present disclosure encompasses all stereoisomeric forms of compounds of Formula (I). The asymmetric centers present in the compound of Formula (I) may each independently have (*R*) or (*S*) configuration. When bonds to chiral carbons are depicted as straight lines in the structural formulae of the present disclosure, or when compound names are described without (*R*) or (*S*) chiral designations for chiral carbons, it is understood that both (*R*) and (*S*) configurations of each such chiral carbon and therefore each enantiomer or diastereomer and mixtures thereof are encompassed by said formula or name. The production of specific stereoisomers or mixtures thereof may be identified in examples where such stereoisomers or mixtures are obtained, but this in no way limits the inclusion of all stereoisomers and mixtures thereof within the scope of the present disclosure. When bonds to chiral carbons are depicted as solid or dashed wedges in the structural formulae of the present disclosure, or when compound names are described with (*R*) or (*S*) chiral designations for chiral carbons, it should be understood that the compound represented by the structural formula or name at this time has a definite stereoconfiguration at the chiral carbon position and will be distinguished from other stereoisomers or enantiomers or diastereomers or mixtures thereof.

The present disclosure includes all possible enantiomers and diastereomers as well as mixtures of two or more stereoisomers, for example mixtures of enantiomers and/or diastereomers in all ratios. Therefore, the enantiomers in the subject matter of the present disclosure are in enantiomerically pure forms (as levorotatory and dextrorotatory enantiomers), in racemic forms, and as mixtures of the two enantiomers in all proportions. In the case of

cis/trans isomers, the present disclosure includes the cis form and trans form, as well as mixtures of these forms in all proportions. In the case of cis/trans isomers, the present disclosure includes the cis form and trans form, as well as mixtures of these forms in all proportions. If required, individual stereoisomers may be prepared by conventional methods (for example, through chromatography or crystallization, by using stereochemically homogeneous starting materials for synthesis, or via stereoselective synthesis) to separate the mixtures. Optionally, derivatization may be performed prior to separation of the stereoisomers. The separation of stereoisomer mixtures may be carried out in an intermediate step during the synthesis of the compound of Formula (I), or may be carried out on the final racemic product. Absolute stereochemistry may be determined by X-ray crystallography of crystalline products or crystalline intermediates, if necessary by derivatizing these crystalline products or intermediates with reagents containing stereocenters of known configuration. Alternatively, absolute stereochemistry may be determined by vibrational circular dichroism (VCD) spectroscopy analysis.

Unless otherwise indicated, structures described herein are also intended to include compounds that differ only in the presence of one or more isotopically enriched atoms, in other words, compounds in which one or more atoms are replaced by atoms with the same atomic number, but the atomic mass or mass number is different from the atomic mass or mass number that prevails in nature. Such compounds are referred to as “isotopic variants”. The present disclosure is intended to include all pharmaceutically acceptable isotopic variants of compounds of Formula (I). Examples of isotopes suitable for inclusion in the compounds of the present disclosure include, but are not limited to, isotopes of hydrogen, such as ^2H (i.e., D, deuterium) and ^3H (i.e., tritium); carbon, such as ^{11}C , ^{13}C , and ^{14}C ; chlorine, such as ^{36}Cl ; fluorine, such as ^{18}F ; iodine, such as ^{123}I and ^{125}I ; nitrogen, such as ^{13}N and ^{15}N ; oxygen, such as ^{15}O , ^{17}O , and ^{18}O ; phosphorus, such as ^{32}P ; and sulfur, such as ^{35}S . Certain isotopic variants of compounds of Formula (I), such as those incorporating radioactive isotopes, can be used for drug and/or substrate tissue distribution studies. In particular, compounds having depicted structures that differ only in substitution with heavier isotopes (e.g., substitution of hydrogen with deuterium (^2H or D)) can provide certain therapeutic advantages, for example, due to greater metabolic stability, increased *in vivo* half-life or reduced dosage requirements, and therefore can be used in some specific cases. Isotopic variants of compounds of Formula (I) can generally be prepared by conventional techniques known to those skilled in the art or by methods analogous to those described in the accompanying Examples, using appropriate isotopically labeled reagents in place of the non-labeled reagents previously employed for synthesis.

When describing the structure of a compound herein, for hydrogen atoms not designated as D (i.e., ^2H) in the structural formula shown, it shall be generally understood that the hydrogen at this position exists either in the form of the hydrogen isotope “ ^1H hydrogen (^1H)” or in a form with the natural isotopic abundance in the nature. For hydrogen atoms explicitly designated as D (i.e., ^2H , deuterium) in the structure shown, it is understood that the hydrogen at this position exists either in the form of the hydrogen isotope “ ^2H hydrogen (^2H , D, deuterium)” or in a form in which deuterium exists in a form with a deuterium isotopic abundance greater than the natural deuterium isotopic abundance (e.g., the deuterium abundance of greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, greater than 99.5%, or 100%).

Pharmaceutically acceptable solvates according to the present disclosure may include those wherein the solvent of crystallization may be isotopically substituted, for example D_2O , d_6 -

acetone, d₆-DMSO.

One way to implement the present disclosure is to administer the compound of Formula (I) in the form of a prodrug. Therefore, certain derivatives of the compound of Formula (I) may have little or no pharmacological activity by themselves, and when administered in or on the body, for example through hydrolytic cleavage, particularly hydrolytic cleavage promoted by esterases or peptidases, are converted into the compound of Formula (I) with the desired activity. Such derivatives are referred to as “prodrugs”. Further information on the use of prodrugs can be found in, for example, T. Higuchi and W. Stella, “Pro-drugs as Novel Delivery Systems”, Vol. 14, ACS Symposium Series, and EB Roche (Ed.), “Bioreversible Carriers in Drug Design”, Pergamon Press, 1987, American Pharmaceutical Association. Reference can also be made to Nature Reviews/Drug Discovery, 2008, 7, 355, and Current Opinion in Drug Discovery and Development, 2007, 10, 550.

Prodrugs according to the present disclosure can be prepared, for example, by replacing appropriate functional groups present in compounds of Formula (I) with certain moieties known to those skilled in the art, for example, the “promoieties” described in H. Bundgaard, “Design of Prodrugs”, Elsevier, 1985, and Y. M. Choi-Sledeski and C. G. Wermuth, “Designing Prodrugs and Bioprecursors”, Practice of Medicinal Chemistry, 4th edition, Chapter 28, 657-696, Elsevier, 2015. Thus, prodrugs according to the present disclosure can include, but are not limited to, (a) ester or amide derivatives of carboxylic acids in compounds of Formula (I), if any; (b) amide, imine, carbamate or amine derivatives of amino groups in compounds of Formula (I); (c) oxime or imine derivatives of carbonyl groups in compounds of Formula (I), if any; or (d) methyl, primary alcohol or aldehyde groups in compounds of Formula (I) which can be metabolically oxidized to carboxylic acids, if any.

References to the compounds of Formula (I) include the compounds themselves and prodrugs thereof. The present disclosure includes such compounds of Formula (I) as well as pharmaceutically acceptable salts of such compounds and pharmaceutically acceptable solvates of said compounds and salts.

Use and Administration

The compounds of the present disclosure (stereoisomers, deuterated derivatives or stereoisomeric deuterated derivatives of the compounds of Formula (I)) - or pharmaceutically acceptable salts, solvates thereof, including mixtures thereof in all ratios - can be used as medicaments. They have been found to exhibit pharmacological activity that inhibits PLK1. It is hypothesized that through this activity, the compounds of the present disclosure may prevent or reverse dysregulation of PLK1 activity. By preventing such dysregulation of PLK1 activity, it is able to exert its effect as a tumor suppressor. In addition to preventing or reversing dysregulation of PLK1 activity, the pharmacological activity of the compounds of the present disclosure may also be used in other pathophysiological conditions where inhibition of PLK1 would be beneficial.

Therefore, the compounds of the present disclosure as PLK1 inhibitors are particularly useful for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1, such as cancer. The diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 include but not limited to cancers such as leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer,

melanoma, multiple myeloma, and sepsis, etc. Without wishing to be bound to any particular theory or explanation, it may be hypothesized that these compounds may be able to achieve this objective through acting directly on cancer cells and/or indirectly by modulating the immune system's response to tumors.

The compounds of the present disclosure may be administered in an amount effective to treat the diseases or conditions described herein. The compounds of the present disclosure may be administered as the compounds themselves, or alternatively, as pharmaceutically acceptable salts. For administration and dosing purposes, the compounds of the present disclosure themselves (stereoisomers, deuterated derivatives or stereoisomeric deuterated derivatives of compounds of Formula (I)) or their pharmaceutically acceptable salts, solvates will be referred to collectively as the compounds of the present disclosure.

The compounds of the present disclosure are administered by any suitable route in the form of pharmaceutical compositions appropriate for such routes, and at doses effective for the intended treatment. The compounds of the present disclosure may be administered orally, rectally, vaginally, parenterally or topically.

As used herein, the term "administer" refers to absorbing, ingesting, injecting, inhaling, implanting or other means of introducing a compound of the present disclosure or pharmaceutical composition thereof. The term "treatment" refers to reversing, alleviating, delaying the onset of, or inhibiting the progression of "pathological conditions" (e.g., diseases, disorders or conditions, or one or more signs or symptoms thereof) described herein. In certain embodiments, treatment may be administered after one or more signs or symptoms of the disease or condition have developed or have been observed. In other embodiments, treatment may be administered in the absence of signs or symptoms of the disease or condition. For example, susceptible individuals may be treated prior to the onset of symptoms (e.g., according to a history of symptoms and/or according to genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, such as to delay or prevent recurrence. As used herein, the terms "disease", "disorder", "condition", and "pathological condition" are used interchangeably.

Those skilled in the art can determine the dosage level of administration by routine experiments. The dosage regimen of the compounds of the present disclosure and/or compositions containing said compounds is based on multiple factors including: the type, age, weight, sex and medical condition of the patient; the severity of the condition; the route of administration; and the activity of the specific compound used. Therefore, the dosage regimen may vary widely. For example, the dosage level of the compounds of the present disclosure may range from about 0.001 to about 100 mg/kg (i.e., mg per kg of body weight) per day. In certain embodiments, the total daily dose of the compounds of the present disclosure administered in a single or divided dose may be about 0.001 to about 10 mg/kg. It is not uncommon that the administration of the compounds disclosed herein is repeated multiple times in one day.

Pharmaceutical Composition

In some aspects, the present disclosure relates to a pharmaceutical composition comprising the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I) as provided herein, or a pharmaceutically acceptable salt, solvate thereof, and at least one pharmaceutically acceptable carrier or excipient.

As used herein, the term "pharmaceutically acceptable carrier or excipient" refers to a carrier or excipient that can be used to prepare a pharmaceutical composition, which is generally

safe, non-toxic and not biologically or otherwise undesirable, and includes carriers or excipients that are acceptable for veterinary use as well as human pharmaceutical use. Pharmaceutically acceptable carriers or excipients as used herein include one and more than one such carrier or excipient. The specific carrier or excipient used will depend on the manner and purpose of applying the compounds of the present disclosure. Suitable carriers and excipients are well known to those skilled in the art and are described in detail in, for example, Ansel, Howard C et al., *Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems*. Philadelphia: Lippincott, Williams & Wilkins, 2004; Gennaro, Alfonso R. et al., *Remington: The Science and Practice of Pharmacy*. Philadelphia: Lippincott, Williams & Wilkins, 2000; and Rowe, Raymond C. *Handbook of Pharmaceutical Excipients*. Chicago, Pharmaceutical Press, 2005. One or more of buffers, stabilizers, surfactants, wetting agents, lubricants, emulsifiers, suspending agents, preservatives, antioxidants, opacifiers, glidants, processing aids, colorants, sweeteners, aromatizing agent, flavorants, diluents and other known additives may also be included to provide a refined presentation of the drug (i.e., the compound or pharmaceutical composition provided herein) or to aid in the production of a pharmaceutical product (i.e., the medicament).

The compositions of the present disclosure can be formulated into a variety of forms. These include, for example, liquid, semisolid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes, suppositories, etc. The form depends on the intended route of administration and therapeutic application.

The pharmaceutical compositions of the present disclosure can be prepared by any well-known pharmaceutical techniques (e.g., effective formulations and administration procedures). The above considerations on effective formulations and administration procedures are well known in the art and are described in standard textbooks. For example, the preparation of the pharmaceutical product is discussed in Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania, 1975; Liberman et al., ed., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, NY, 1980; and Kibbe et al., ed., *Handbook of Pharmaceutical Excipients*, 3rd edition, American Pharmaceutical Association, Washington, 1999.

In yet another aspect, the present disclosure relates to a kit for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1, comprising the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof as provided herein, or the pharmaceutical composition comprising the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof as provided herein, a container, and optionally a package insert or label indicating treatment.

Treatment Methods

In yet another aspect, the present disclosure relates to a method for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof as provided herein, due to the PLK1 inhibitory activity of the compounds of the present disclosure.

As used herein, the term “subject in need thereof” is a subject suffering from a disease and condition caused by dysregulated PLK1 activity and/or a disease and condition associated with PLK1, or a subject having an increased risk of developing a PLK1-related disease or condition relative to the population as a whole. In certain embodiments, the subject is a warm-blooded animal. In certain embodiments, the warm-blooded animal is a mammal. In certain embodiments, the warm-blooded animal is a human.

As used herein, the term “diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1” refers to any pathophysiological condition in which inhibition of PLK1 would be beneficial. In certain embodiments, the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are cancers. In certain embodiments, the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are diseases and conditions selected from the group consisting of leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, sepsis, and the like.

In another aspect, the present disclosure relates to the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof as provided herein for use in treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1.

In yet another aspect, the present disclosure relates to the use of the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof as provided herein, in the preparation of medicaments for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1.

Synthesis

The compounds of the present disclosure can be prepared by the general and specific methods described below using the common knowledge of those skilled in the art of synthetic organic chemistry. Such common knowledge can be found in standard reference books, for example, Barton and Ollis (eds.), *Comprehensive Organic Chemistry*, Elsevier; Richard Larock, *Comprehensive Organic Transformations: A Guide to Functional Group Preparation*, John Wiley and Sons; and *Compendium of Organic Synthesis Methods*, Volumes I-XII, Wiley-Interscience.

The schemes described below are intended to provide a general description of methods for preparing compounds of the present disclosure. Some compounds of the present disclosure may contain single or multiple chiral centers with stereochemical designations (*R*) or (*S*). It will be apparent to those skilled in the art that all synthetic transformations can be performed in a similar manner, regardless of whether the material is enantiomerically enriched or racemic. In addition, the resolution of the desired optically active material can be performed at any desired point in the procedure using well-known methods, such as those described herein and in the chemical literature.

Examples

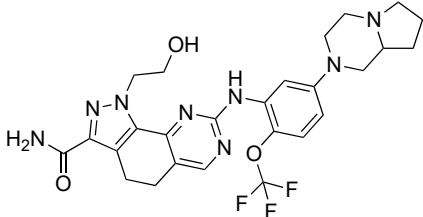
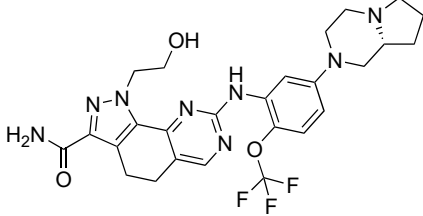
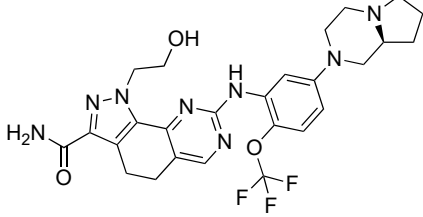
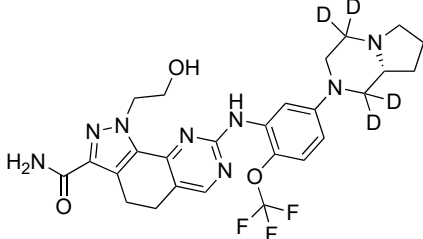
To describe the present disclosure in more detail, the following examples are provided. The examples described herein are intended to illustrate the compounds, methods, and compositions provided herein, and should not be construed in any way as limiting their scope.

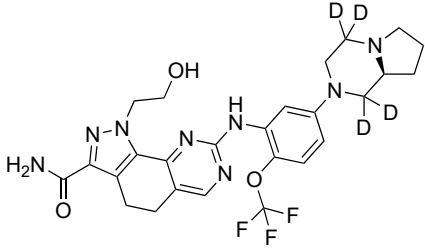
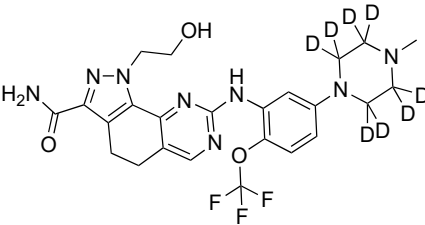
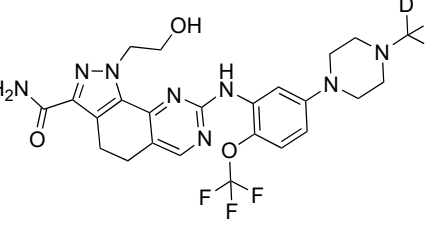
During the synthesis process, it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules involved. This can be achieved through conventional protecting groups, such as those described in T.W. Greene and P.G.M. Wuts, *Protective Groups in Organic Synthesis*, 4th Edition, John Wiley & Sons. The protective groups are optionally removed at a convenient subsequent stage using methods well known in the art.

The compounds of the present disclosure can be readily prepared according to the following reaction schemes and examples or modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, variants known to those skilled in the art but not mentioned in more detail may also be used. In addition, according to the reaction schemes and examples described herein, other methods for preparing the compounds of the present disclosure will be apparent to those skilled in the art. Unless otherwise indicated, all variables are defined as above. In general, in chemical procedures, all reagents and starting materials can be purchased from commercial suppliers or can be easily prepared by those skilled in the art.

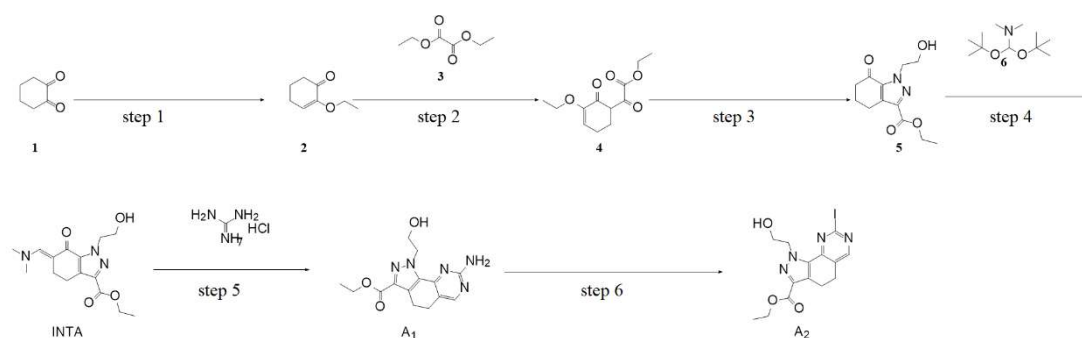
Exemplary compounds are shown in Table 1.

Table 1

Compound ID	Structure
CPD01	
CPD02	
CPD03	
CPD04	

CPD05	
CPD06	
CPD07	

Preparation of Intermediates



Step 1. Synthesis of Compound 2

At room temperature (RT), Compound 1 (40.0 g, 408.2 mmol, 1.0 eq) was dissolved in EtOH (40 mL), p-toluenesulfonic acid PTSA (7.0 g, 40.8 mmol, 0.1 eq) was added, and the mixture was stirred and refluxed overnight. After TLC showed that no Compound 1 remained, the mixture was concentrated. The residue was dissolved in EtOAc (200 mL), washed with saturated sodium bicarbonate solution (100 mL $\times$ 3), dried over anhydrous Na_2SO_4 , and concentrated to give a crude product. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc = 100:1) to obtain Compound 2 (30.0 g, 60.1%) as a yellow oil. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ ppm 5.98 (t, J = 4.5 Hz, 1H), 3.67 (q, J = 6.9 Hz, 2H), 2.46 - 2.24 (m, 4H), 1.95 - 1.77 (m, 2H), 1.22 (t, J = 6.9 Hz, 3H). LCMS: 141.1 ($[\text{M}+\text{H}]^+$).

Step 2. Synthesis of Compound 4

Compound 2 (20.0 g, 142.7 mmol, 1.0 eq) was dissolved in THF (250 mL), and LiHMDS (171 mL, 171.2 mmol, 1.2 eq, 1 M in THF) was added dropwise at -50°C under nitrogen protection. After the addition was completed, stirring was continued at this temperature for 30 minutes. Compound 3 (22.2 mL, 157.0 mmol, 1.1 eq) was added dropwise at -50°C . After the

addition was completed, the mixture was allowed to warm to room temperature and stirred overnight. After LC-MS showed complete consumption of Compound 2, 1 N HCl (about 180 mL) was used to adjust the pH to 4 under an ice-water bath. The mixture was extracted with ethyl acetate (300 mL $\times$ 3). The organic phases were combined and dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporation to obtain a residue, which was further purified by column chromatography to obtain Compound 4 (20.0 g, 58.3%) as a yellow oil. ^1H NMR (300 MHz, CDCl_3): δ ppm 14.93 (s, 1H), 5.88 (d, J = 4.8 Hz, 1H), 4.34 (q, J = 6.9 Hz, 2H), 3.82 (q, J = 6.9 Hz, 2H), 2.89 (t, J = 6.9 Hz, 2H), 2.40 (q, J = 5.1 Hz, 2H), 1.50 - 1.32 (m, 6H).

Step 3. Synthesis of Compound 5

Compound 4 (20.0 g, 83.3 mmol, 1.0 eq) was dissolved in acetic acid (200 mL), and 2-hydroxyethylhydrazine (6.3 g, 83.3 mmol, 1.0 eq) was added under stirring at room temperature. The mixture was stirred for 2 hours at room temperature until LCMS monitoring showed complete consumption of Compound 4. The reaction solution was concentrated, and the residue was dissolved in 500 mL of ethyl acetate, washed with water, saturated sodium bicarbonate three times, and saturated brine three times. After drying over anhydrous sodium sulfate, the desiccant was filtered out, and the solvent was removed by rotary evaporation to obtain a light yellow Compound 5 (16.0 g, 76.2%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ ppm 4.86 (t, J = 5.7 Hz, 1H), 4.54 (t, J = 5.7 Hz, 2H), 4.29 (q, J = 6.9 Hz, 2H), 3.72 (q, J = 5.7 Hz, 2H), 2.93 (t, J = 6.0 Hz, 2H), 2.62 - 2.42 (m, 2H), 2.13 - 1.95 (m, 2H), 1.30 (t, J = 7.2 Hz, 3H). LCMS: 253.1 ($[\text{M}+\text{H}]^+$).

Step 4. Synthesis of Intermediate Int-A

Compound 5 (20.0 g, 79.3 mmol, 1.0 eq) was dissolved in DMF (180 mL) and Compound 6 (48.3 g, 237.9 mmol, 3.0 eq) was added, and the mixture was stirred at 60°C for 2 hours. After LCMS monitoring showed complete consumption of Compound 5, the mixture was allowed to cool to room temperature, the reaction solution was poured into 1000 mL of water, extracted with ethyl acetate 6 times, the organic phases were combined, washed with saturated brine, dried over anhydrous sodium sulfate, the desiccant was filtered out, and the solvent was removed by rotary evaporation to obtain a light yellow Intermediate Int-A (13.0 g, 66.7%) without further purification. ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ ppm 7.48 (s, 1H), 4.84 (brs, 1H), 4.60 (t, J = 6.0 Hz, 2H), 4.27 (q, J = 7.2 Hz, 2H), 3.78 - 3.65 (m, 2H), 3.11 (s, 6H), 2.96 - 2.86 (m, 2H), 2.86 - 2.76 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H).

Step 5. Synthesis of Intermediate A1

The Intermediate Int-A (6.0 g, 0.7 mmol, 1.0 eq) was dissolved in DMF (60 mL), and Compound 7 (4.7 g, 48.8 mmol, 2.5 eq) was added. The mixture was reacted at 110°C for 12 hours under N_2 protection. The reaction was monitored by LCMS until no INT-A remained. The mixture was allowed to cool to room temperature, the reaction solution was poured into 180 mL of water, extracted with ethyl acetate three times, the organic phases were combined, washed with saturated brine, dried over anhydrous sodium sulfate, the desiccant was filtered out, and the solvent was removed by rotary evaporation to obtain a crude product, which was purified by column chromatography to obtain a light yellow intermediate (3.6g, 60.8%). ^1H NMR (400 MHz, CDCl_3): δ ppm 8.20 (s, 1H), 5.10 (s, 2H), 4.93 (t, J = 4.8 Hz, 2H), 4.42 (q, J = 7.2 Hz, 2H), 4.13 (t, J = 4.8 Hz, 2H), 3.06 (t, J = 7.6 Hz, 2H), 2.81 (t, J = 7.6 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H). LCMS: 304.1 ($[\text{M}+\text{H}]^+$).

Step 6. Synthesis of Intermediate A2

Intermediate A1 (4.0 g, 13.2 mmol, 1.0 eq) was dissolved in DME (320 mL), and CsI (6.9

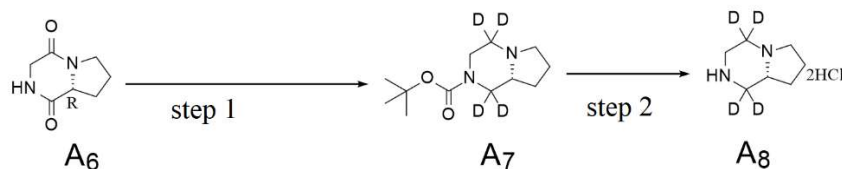
g, 26.4 mmol, 2.0 eq), I₂ (3.3 g, 13.2 mmol, 1.0 eq), CuI (1.0 g, 5.3 mmol, 0.4 eq) and isoamyl nitrite (4.6 g, 39.6 mmol, 3.0 eq) were added. The reaction solution was reacted at 80°C for 3 hours, allowed to cool to room temperature, and a white solid was precipitated. The white solid was filtered out and dissolved in 200 mL of dichloromethane. The solution was washed with aqueous ammonia, sodium thiosulfate solution, and saturated brine once, and dried over anhydrous sodium sulfate. The desiccant was filtered out, and the solvent was removed by rotary evaporation to obtain a crude product, which was purified by column chromatography to obtain a yellow Intermediate A2 (2.0 g, 36.6%). ¹H NMR (400 MHz, CDCl₃): δ ppm 8.30 (s, 1H), 4.92 (t, *J* = 4.8 Hz, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 4.14 (t, *J* = 4.8 Hz, 2H), 3.15 (t, *J* = 7.2 Hz, 2H), 2.94 (t, *J* = 8.0 Hz, 2H), 1.41 (t, *J* = 7.2 Hz, 3H). LCMS: 415.1 ([M+H]⁺).

Synthesis of Intermediate A4



HOBT (4.0 g, 29.6 mmol, 1.0 eq) was dissolved in methanol (40 mL), and aqueous ammonia (2.4 mL) was added and stirred at room temperature for 5 hours to precipitate a solid. The solid was filtered to obtain a white solid, which was washed with petroleum ether and dried to obtain Intermediate A4 (3.6 g, 79.9%).

Synthesis of Intermediate A8



Step 1. Synthesis of A7 ((R)-tert-butyl hexahydropyrrolo[1,2-a]pyrazine-2(1H)-carboxylate-1,1,4,4-d4)

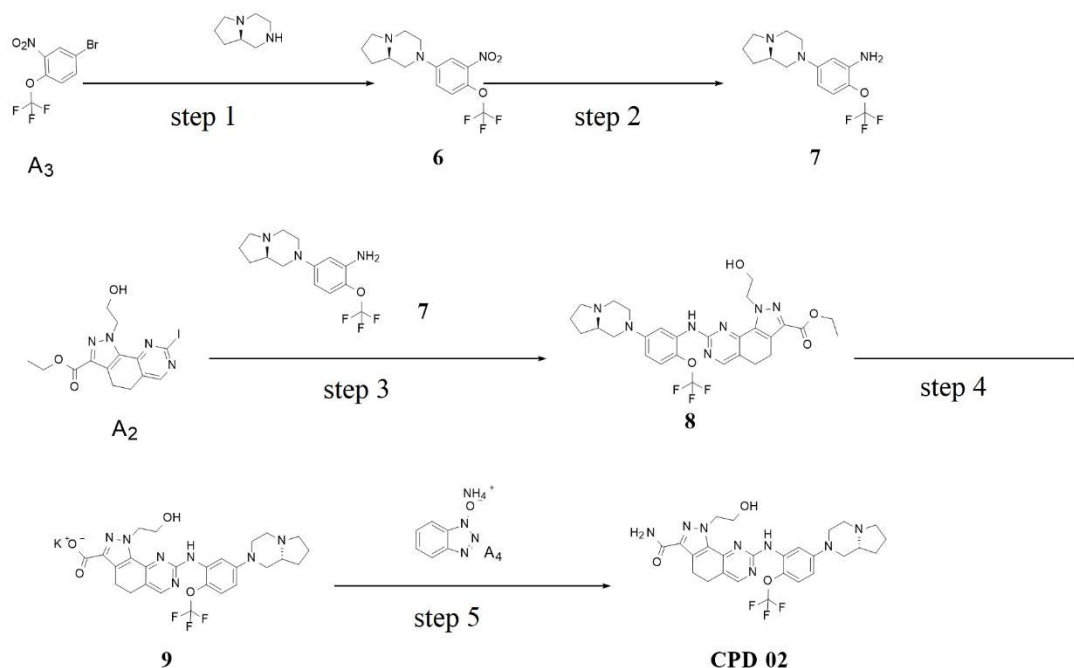
A6 (12 g, 77.4 mmol) was dissolved in a mixed solution of CD₃OD (25 mL) and DCM (500 mL), heated to reflux for 8 h, and the solvent was removed by rotary evaporation. The residue was redissolved in CD₃OD (25 mL) and DCM (500 mL), refluxed for 8 h, and the solvent was removed to obtain a white solid. The solid was dissolved in THF (200 mL), and LiAlD₄ (8.13 g, 193.5 mmol) was added at room temperature. The mixture was refluxed for 1 h, allowed to cool to 0°C, and DCM (500 mL), water (8.13 mL), and 15% NaOH (8.13 mL) were added. (Boc)₂O (33.75 g, 154.9 mmol) and TEA (23.46 g, 232.2 mmol) were added to the reaction solution, stirred at room temperature for 8 hours, the insoluble matter was filtered off, the filtrate was concentrated, and purified by silica gel column chromatography to obtain A7 as a brown oil, crude product (12.7 g, 71%), LCMS: 231.2 [M+H]⁺.

Step 2. Synthesis of A8

The Intermediate A7 was dissolved in MeOH (10 mL), and 4N HCl in dioxane (10 mL) was added to react at room temperature for 16 hours. The solvent was removed without further

purification to obtain a crude product A8 (1.26 g).

Synthesis Example 1. Synthesis of Compounds CPD02 and CPD03



Step 1. Synthesis of Compound 6

1,4-Dioxane (55 mL) was added to a mixture of Intermediate A3 (550.0 mg, 1.9 mmol, 1.0 eq), X-phos (190.7 mg, 0.4 mmol, 0.2 eq), (R)-octahydropyrrolo[1,2-A]pyrazine (718 mg, 5.7 mmol, 3 eq) and Cs₂CO₃ (1.2 g, 3.8 mmol, 2.0 eq), and Pd₂(dba)₃ (183.1 mg, 0.2 mmol, 0.1 eq) was added at room temperature. The reaction system gas was purged with N₂ three times, and the reaction was heated to 90°C under N₂ protection for 6 hours. The reaction solution was allowed to cool to room temperature, concentrated, and 15 mL of water was added. The product was extracted with dichloromethane three times, and the organic phase was washed with saturated brine three times and dried over anhydrous sodium sulfate. The desiccant was filtered off, and the solvent was removed by rotary evaporation to obtain a crude product, which was purified by column chromatography to obtain Compound 6 (370 mg, 58.1%) as a light yellow solid, LCMS: 332.1 ([M+H]⁺).

Step 2. Synthesis of Compound 7

Compound 6 (370.0 mg, 1.1 mmol, 1.0 eq) was dissolved in ethyl acetate (7.4 mL) and Pd/C (148.0 mg, 40% wt) was added. The mixture was reacted at room temperature overnight under H₂, the catalyst was filtered off, and the mixture was concentrated to give a crude product of Compound 7 (300 mg), which was used directly in subsequent reactions without purification. LCMS: 302.7 ([M+H]⁺).

Step 3. Synthesis of Compound 8

In a glove box, DMF (2 mL) was added to a mixture of Intermediate A2 (120.0 mg, 0.29 mmol, 1.0 eq), Compound 7 (174.6 mg, 0.58 mmol, 2.0 eq), K₂CO₃ (120.1 mg, 0.87 mmol, 3.0 eq), BINAP (72.2 mg, 0.12 mmol, 0.4 eq) and Pd(OAc)₂ (9.7 mg, 0.06 mmol, 0.2 eq). After the addition, the mixture was reacted at 120°C under N₂ protection for 4 hours. After LCMS monitoring showed complete consumption of Intermediate A2, the reaction temperature was allowed to cool to room temperature, water (10 mL) was added, and the mixture was extracted

with EtOAc three times. The organic phase was washed with saturated brine three times and dried over anhydrous sodium sulfate. The desiccant was filtered off, and the solvent was removed by rotary evaporation to obtain a crude product, which was purified by column chromatography to obtain Compound 8 (100 mg, 58.7%) as a brown oil. LCMS: 588.2 ($[M+H]^+$).

Step 4. Synthesis of Compound 9

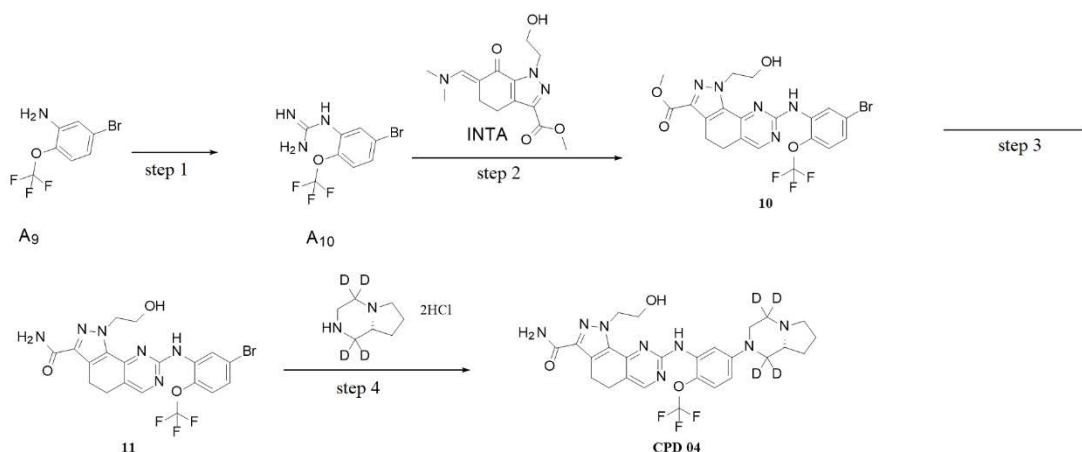
Compound 8 (100 mg, 0.17 mmol, 1.0 eq) was dissolved in MeOH (1 mL), THF (1 mL) and H₂O (1 mL), KOH (19.0 mg, 0.34 mmol, 2.0 eq) was added, and the mixture was stirred at room temperature for 2 hours. The reaction solution was concentrated, 5 mL of water was added to the residue, and the mixture was extracted with ethyl acetate three times. The aqueous phase was lyophilized to obtain a gray crude product of Compound 9 (80 mg). LCMS: 560.2 ($[M-K+H+H]^+$, signal of the acid form).

Step 5. Synthesis of Compound CPD02

The above Compound 9 (80 mg, 0.14 mmol, 1.0 eq) was dissolved in DMF (2 mL), and EDCI (80.2 mg, 0.42 mmol, 3.0 eq) and Intermediate A4 (63.8 mg, 0.42 mmol, 3.0 eq) were added. The mixture was stirred at room temperature for 4 hours. After HPLC monitoring showed complete consumption of Compound 9, 10 mL of water was added. The mixture was extracted with ethyl acetate three times. The organic phases were combined, and dried over anhydrous sodium sulfate. The desiccant was filtered out, and the solvent was removed by rotary evaporation to obtain a crude product. The crude product was purified by preparative HPLC (Prep-HPLC) (mobile phase: acetonitrile, 0.1% formic acid and water from 5% to 35%) and lyophilized to obtain a yellow solid powder CPD02 (10.0 mg, 2-step comprehensive yield 10.5%). ¹H NMR (400 MHz, DMSO-*d*₆): δ ppm 8.85 (s, 1H), 8.33 (s, 1H), 7.43 (s, 1H), 7.31-7.23 (m, 3H), 6.80 (dd, *J* = 9.2, 2.1 Hz, 1H), 4.62 (t, *J* = 5.2 Hz, 2H), 3.76 (d, *J* = 9.6 Hz, 1H), 3.70-3.58 (m, 3H), 3.10-2.90 (m, 4H), 2.85-2.69 (m, 3H), 2.42 (t, *J* = 10.4 Hz, 1H), 2.21 (td, *J* = 11.2, 3.2 Hz, 1H), 2.13-1.95 (m, 2H), 1.90-1.76 (m, 1H), 1.76-1.60 (m, 2H), 1.45-1.27 (m, 1H). LCMS: 559.1 ($[M+H]^+$).

The synthesis of Compound CPD03 is similar to that of CPD02. The structural spectrum is ¹H NMR (300 MHz, DMSO-*d*₆): δ ppm 8.86 (brs, 1H), 8.34 (s, 1H), 7.44 (s, 1H), 7.29-7.15 (m, 3H), 6.80 (dd, *J* = 9.1, 2.9 Hz, 1H), 4.66-4.54 (m, 3H), 3.80-3.70 (m, 1H), 3.68-3.54 (m, 3H), 3.08-2.93 (m, 4H), 2.83-2.69 (m, 3H), 2.31-2.22 (m, 1H), 2.22-2.14 (m, 1H), 2.09-1.96 (m, 2H), 1.86-1.64 (m, 3H), 1.43-1.29 (m, 1H). LCMS: 559.3 ($[M+H]^+$).

Synthesis Example 2. Synthesis of Compound CPD04



Step 1. Synthesis of A10

5-Bromo-2-trifluoromethoxyaniline A9 (10.0 g, 39.0 mmol) was dissolved in EtOH (30 mL) and added dropwise to a solution containing cyanamide (3.28 g, 78 mmol), EtOH (10 mL) and H₂O (2 mL). A mixture of 37% HCl (6.5 mL) and EtOH (20 mL) was added dropwise to the mixture and refluxed for 5 days. The reaction solution was allowed to cool to room temperature, concentrated and diluted with water; 1N NaOH was added until the reaction solution became alkaline, extracted with ethyl acetate several times, dried over sodium sulfate and concentrated to obtain A10 (3.2 g, 27%).

Step 2. Synthesis of Intermediate 10

A10 (29.3 g, 98.3 mmol, 1.2 eq) was dissolved in DMF (400 mL), and a solution of the Intermediate INT-A (24 g, 81.9 mmol, 1.0 eq) in DMF (200 mL) was added. The reaction solution was reacted at 110°C for 16 hours, allowed to cool to room temperature, poured into ice water, and the insoluble matter was filtered to obtain Compound 10 (21.2 g, 50%) as a white solid. LCMS: 528.2, 530.2 ([M+H]⁺).

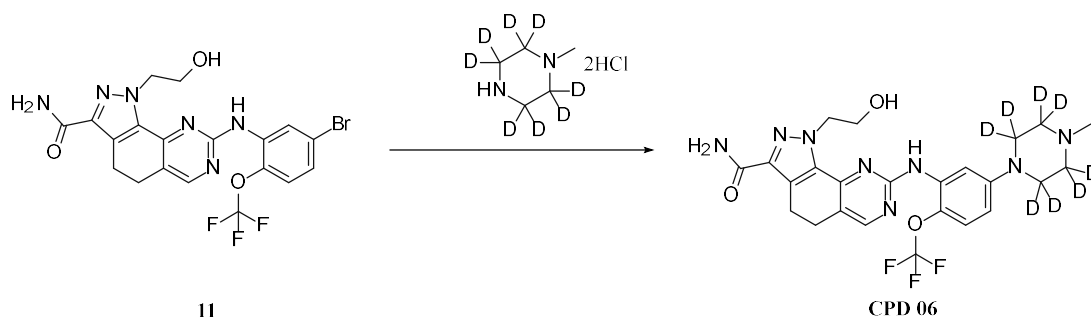
Step 3. Synthesis of Intermediate Compound 11

Compound 10 (21.2 g, 40.1 mmol, 1.0 eq) was dissolved in THF (500 mL), and NH₄Cl (6.44 g, 120 mmol, 3.0 eq) and LiHMDS (240 mmol, 6.0 eq) were added, and stirred at room temperature for 30 minutes. The solvent was removed by rotary evaporation, and the residue was washed with water and dried to obtain Compound 11 (16.8 g, 81.8%) as a white solid, LCMS: 513.1, 515.1 ([M+H]⁺).

Step 4. Synthesis of CPD04

A mixture of Compound 11 (2.0 g, 3.9 mmol, 1.0 eq), Intermediate A8 (1.26 g, 6.25 mmol, 1.6 eq), Pd₂(dba)₃ (285 mg, 0.312 mmol, 0.08 eq) and Davephos (123 mg, 0.312 mmol, 0.08 eq) were added with THF (100 mL) and LiHMDS (40 mL, 40 mmol). The mixture was reacted at 100°C for 2 hours, and water was added to quench the reaction. The reaction solution was extracted with EtOAc (50 mL × 3). The organic phases were combined and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography to obtain the target product CPD04 (1 g, 45.6%) as a white solid. LCMS: 563.3 [M+H]⁺. ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.30 (s, 1H), 7.57 (d, 1H), 7.26 (dd, 1H), 6.85 (dd, 1H), 4.79 (t, 2H), 3.84 (t, 2H), 3.69 (d, 1H), 3.47 (dt, 1H), 3.23 (d, 1H), 3.07 (q, 3H), 2.88 (t, 2H), 2.20 (dt, 1H), 2.14-2.05 (m, 2H), 1.97 (s, 1H), 1.85 (q, 1H).

Synthesis Example 3. Synthesis of Compound CPD06



A mixture of Compound 11 (2.0 g, 3.9 mmol, 1.0 eq), N-methylpiperazine-2,2,3,3,5,5,6,6-D₈ hydrochloride (1.13g, 6.25mmol, 1.6 eq), Pd₂(dba)₃ (285 mg, 0.312 mmol, 0.08 eq) and Davephos (123mg, 0.312mmol, 0.08 eq) were added with THF (100 mL) and LiHMDS (40 mL, 40 mmol). The mixture was reacted at 100°C for 2 hours, water was added to quench the reaction,

the reaction solution was extracted with EtOAc (50 mL×3), the organic phases were combined, the solvent was removed by rotary evaporation, and the residue was purified by silica gel column chromatography to obtain the target product CPD06 (1g, 47.4%) as a white solid. LCMS: 541.3 [M+H]⁺, ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 8.92 (s, 1H), 8.34 (s, 1H), 7.45 (bs, 1H), 7.27-7.21 (m, 3H), 6.80 (dd, 1H), 4.62 (q, 3H), 3.62 (q, 2H), 2.97 (t, 2H), 2.79 (t, 2H), 2.49-2.32 (m, 3H).

Test Example 1. Pharmacokinetic Study in Mice

The objective of this study is to investigate the pharmacokinetic properties of the disclosed compounds after tail vein intravenous injection (IV) and oral administration (PO) in female CD1 mice. Plasma was sampled at 0, 0.083, 0.25, 0.5, 1, 2, 4, 7 and 24 hours post-dosing, and the drug concentrations in plasma at different time points were detected by LC-MS/MS, and the relevant pharmacokinetic parameters were calculated using WinNonlin software.

1.1 Test Materials

1.1.1 The reagents and instruments used in this example are as follows:

Table 2. Reagent Information

Reagents	Catalog No.	Batch No.	Brand
DMSO	A00878	KDGLA04	Innochem
SBE-β-CD	C830138	C14707121	Macklin
MC	M0262	SLCL7954	Sigma-Aldrich
Tween-20	P1379	WXBD5553V	Sigma-Aldrich
NMS-P937	S81189	A03IS21133	Shanghai yuanye Bio-Technology Co., Ltd.

Table 3. Instrument Information

Instrument	Brand	Model
Liquid Chromatograph	Waters	ACQUITY SHIMADZU LC-30AD
Mass Spectrometer	Waters	Xevo TQ-S Micro Sciex Triple Quad 5500
Micro Analytical Balance	Sartorius Scientific Instruments (Beijing) Co., Ltd.	SQP
Vortex Mixer	IKA	IKA MS3 basic
High-Speed Refrigerated Centrifuge	Eppendorf China Co., Ltd.	5810R

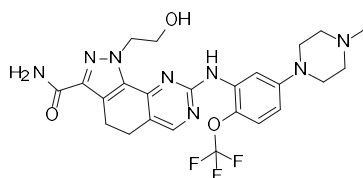
1.1.2 Experimental Animals

Experimental female CD1 mice were purchased from SPF Laboratory Animal Technology Co., Ltd., aged 6-8 weeks with body weights of 20-30 g. The female CD1 mice were housed in a controlled environment (set temperature at 20-25°C and relative humidity at 40-70%). A 12-hour light/12-hour dark cycle was maintained unless interrupted by study-related events.

1.2 Experimental Procedures

1.2.1 Preparation of Experimental Compound Solutions

The positive control compound used in this example is NMS-P937, which has the structure shown in the following formula:



NMS-P937

Compound solutions are prepared in an ultra-clean workbench and prepared immediately before use, and mixed thoroughly prior to use to ensure formulation homogeneity. The specific compound preparation method is shown in the table below:

Preparation of 5 mg/mL stock solutions of compounds CPD02 and CPD03

Test Compound	Concentration (mg/mL)	Mass (mg)	DMSO (mL)
CPD02	5	1.18	0.234
CPD03	5	1.37	0.271
NMS-P937	5	1.33	0.263

Preparation of 20 mg/mL stock solutions of compounds CPD04, CPD06 and NMS-P937

Test Compound	Concentration (mg/mL)	Mass(mg)	DMSO (mL)
CPD04	20	1.79	0.090
CPD06	20	1.87	0.091
NMS-P937	20	2.06	1.03

1.2.2 Administration

Administration method: IV

Vehicle composition: 10% DMSO+90% (20%SBE-β-CD)

Table 4. Doses Used in IV Administration

Test Compound	Dose (mg/kg)	Volume (mL/kg)	Concentration (mg/mL)	Stock Solution (mL)	DMSO (mL)	20% SBE-β-CD (mL)
CPD02	2	5	0.4	0.080	0.020	0.900
CPD03	2	5	0.4	0.096	0.024	1.080
NMS-P937	2	5	0.4	0.096	0.024	1.080
CPD04	10	5	2	0.935	0.094	0.842
CPD06	10	5	2	0.895	0.090	0.806
NMS-P937	10	5	2	1.030	0.103	0.773

Administration method: PO

Vehicle composition: 10% DMSO + 90% (20%SBE-β-CD)

Table 5. Doses Used in PO Administration

Test Compound	Dose (mg/kg)	Volume (mL/kg)	Concentration (mg/mL)	Stock Solution (mL)	20% SBE-β-CD (mL)
CPD02	5	10	0.5	0.100	0.900
CPD03	5	10	0.5	0.120	1.080
NMS-P937	5	10	0.5	0.120	1.080

Vehicle composition: 0.5% MC + 1% Tween-20

Table 6. Doses Used in PO Administration

Test Compound	Dose (mg/kg)	Volume (mL/kg)	concentration (mg/mL)	Stock solution (mL)	0.5% MC +1% Tween-20 (mL)
CPD04	10	10	1	1.510	1.510
CPD06	10	10	1	1.430	1.430
NMS-P937	10	10	1	1.440	1.440

1.2.3 Drug administration and blood sample collection protocol

Prior to the experiment, the animals were randomly grouped. The mice were fasted for 12 hours before drug administration, and free feeding was resumed 4 hours after administration. The animal grouping and blood sampling schedule are shown in the table below.

Table 7. Animal Grouping and Blood Sampling Schedule

Compound	Administration Method	Dose (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)	Number of animals (n)	Blood sampling time points
CPD 02, CPD 03 and NMS-P937	IV	2	0.4	5	N=3	0 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 7 h, 24 h
	PO	5	0.5	10	N=3	0 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 7 h, 24 h
CPD 04, CPD 06 and NMS-P937	IV	10	1	10	N=3	0 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 7 h, 24 h
	PO	10	1	10	N=3	0 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 7 h, 24 h

All blood samples were transferred into plastic microcentrifuge tubes containing anticoagulant (EDTA-K₂), and then centrifuged at 4000 g for 5 minutes at 4°C. The supernatant was transferred into a microcentrifuge tube without anticoagulant, and the plasma was stored in a -75±15°C refrigerator until detection.

1.3 Data and Results

The blood samples were purified by HPLC and analyzed by mass spectrometry to obtain the plasma concentration-time curve of the disclosed compound.

Among them, for compounds CPD02 and CPD03, the Waters ACQUITY HPLC apparatus was used, and the HPLC purification conditions are shown in the table below:

Table 8. HPLC Purification Conditions

Chromatographic column:	XSelect Hss T3 2.5µm (2.1 × 50 mm) Column XP		
Column temperature:	40°C		
Run time:	2 min		
Mobile phase A:	Water (containing 0.1% formic acid)		
Mobile phase B:	Acetonitrile (containing 0.1% formic acid)		
Injection volume:	10µL		
Time (min)	Flow rate (mL/min)	Water (containing 0.1% FA)	Acetonitrile (containing 0.1% FA)
Initial	0.5	95.0	5.00
0.30	0.5	95.0	5.00

0.80	0.5	5.00	95.0
1.20	0.5	5.00	95.0
1.50	0.5	95.0	5.00
2.00	0.5	95.0	5.00

Among them, for compounds CPD 04 and CPD06, AB SHIMADZU LC-30AD HPLC apparatus was used, and the HPLC purification conditions are shown in the table below:

Table 9. HPLC Purification Conditions

Chromatographic column:	XSelect Hss T3 2.5 μ m (2.1 $\times$ 50 mm) Column XP		
Column temperature:	40°C		
Run time:	2 min		
Mobile phase A:	Water (containing 0.1% formic acid)		
Mobile phase B:	Acetonitrile (containing 0.1% formic acid)		
Injection volume:	10 μ L		

Time (min)	Flow rate (mL/min)	Water (containing 0.1% FA)	Acetonitrile (containing 0.1% FA)
initial	0.8	95.0	5.00
0.30	0.8	95.0	5.00
1.00	0.8	2.00	98.0
1.48	0.8	2.00	98.0
1.51	0.8	95.0	5.00
2.00	0.8	95.0	5.00

The pharmacokinetic parameters were estimated by non-compartmental model method (calculated by Phoenix WinNonlin software). IV pharmacokinetic parameters included CL, $T_{1/2}$, C_0 , AUC, MRT, Vss; PO pharmacokinetic parameters included $T_{1/2}$, T_{max} , C_{max} , AUC, MRT, etc.

The formula for calculating bioavailability F is as follows:

$$F = \left[\frac{AUC_{PO} \times Dose_{IV}}{AUC_{IV} \times Dose_{PO}} \right] \times 100\%$$

The pharmacokinetic parameters of the disclosed compounds obtained in this example are summarized as follows:

Table 10. Pharmacokinetic Parameters of SD Mice After Intravenous Administration

Compound	Dose (mg/kg)	$t_{1/2}$ (hr)	AUC _{last} (hr*ng/mL)	Vss (L/kg)	CL (mL/min/kg)
CPD02	2	1.36	404	3.05	81.7
CPD03	2	3.76	433	4.29	79.1
NMS-P937	2	1.52	353	5.71	95.7
CPD 04	10	1.06	3341	2.31	50.1
CPD 06	10	1.05	3051	3.02	55.1
NMS-P937	10	3.52	4539	3.22	36.9

Table 11. Pharmacokinetic Parameters of SD Mice After Oral Administration

Compound	Dose (mg/kg)	t _{1/2} (hr)	t _{max} (hr)	C _{max} (ng/mL)	AUC _{last} (hr*ng/mL)	F (%)
CPD02	5	2.71	0.500	169	359	38.0
CPD03	5	3.03	0.417	62.7	144	16.2
NMS-P937	5	2.62	0.833	55	167	20.5
CPD 04	10	1.44	0.667	613	1397	42.8
CPD 06	10	1.16	0.333	510	1288	43.1
NMS-P937	10	1.14	0.333	599	1212	27.1

The pharmacokinetic data show that the disclosed compounds have a suitable half-life in mice, the oral bioavailability of the stereospecific design R-configuration molecule CPD 02 is significantly higher than that of the S-configuration CPD 03, and higher than that of NMS-P937, and the bioavailability of the deuterated compound is significantly higher than that of the non-deuterated molecule (CPD 04 vs. CPD 02, CPD 06 vs. NMS-P937).

Test Example 2. Proliferation Inhibitory Activity on Cell Line PSN1

2.1 Test Materials

The reagents and instrument information used in this Example are as follows:

Table 12 Cell Line Information

Cell lines	Manufacturer	Culture Medium
PSN1	Nanjing Cobioer	RPMI 1640 + 10% FBS

Table 13. Main Reagents and Consumables Information

Name	Supplier	Catalog No.
CellTiter-Glo®	Promega	G7572
RPMI-1640	VivaCell	C3010-0500
DMEM	VivaCell	C3113-0500
IMDM	ATCC	ATCC®30-2005
Fetal bovine serum	ExCellBio	FND500
Insulin	BasalMedia	S454T0
Trypsin (2.5%)	Gibco	15090-046
Horse serum	LIFE	16050122
96-well plate	Agilent Technologies	204626-100

Table 14. Instrument and Software Information

Catalog	Supplier	Catalog No.
Incubator	RADOBIO	Herocell 180
Biological Safety Cabinets	AIRTECH	BSC-1304 II A2
Automated Cell Counter	Thermo	Countess III
Multimode Reader	PE	Ensign
GraphPad Prism 8.0 Software	/	/

Table 15. Compound Information and Stock Solution Preparation

Compound	MW	Mass (mg)	Stock Concentration (mM)	DMSO Volume (μL)
CPD02	558.57	1.10	20	98.5
CPD03	558.57	1.08	20	96.7
CPD04	562.59	2.01	20	178.6
CPD06	540.58	2.1	20	194.2
NMS-P937	532.53	2	20	187.8

2.2 Test Methods

Day 0: Plating

- Adherent cells need to be digested and centrifuged, while suspension cells can be directly centrifuged;
- Resuspend with complete culture medium and count using an automatic cell counter;
- Plate 6,000 cells per well for PSN1, and dilute the cell suspension to the required density;
- Add 100 μL of cells per well, and culture overnight at 37°C in a 5% CO₂ incubator to stabilize and balance the cells.

Day 1: Compound Preparation and Addition

- Prepare the compound with DMSO as a solution of 200-fold final concentration;
- Dilute the 200-fold compound to a 3-fold final concentration with complete culture medium;
- Add 50 μL of the compound per well, use wells with the same volume of culture medium as controls, and continue culturing in a 37°C, 5% CO₂ incubator for 72 hours.

Day 4: Detection

- Equilibrate the cell plate and CTG reagent to room temperature;
- Add 30 μL of CellTiter-Glo[®] reagent per well;
- Incubate at room temperature for 15 minutes;
- Detect with a microplate reader.

Data Analysis

- Calculate IC₅₀ using GraphPad Prism 8;
- %Inh = (1-(Raw data - Mean_{min}) / (Mean_{max} - Mean_{min}))×100;
- Mean_{max} is the positive control well with only cells and the same volume of culture medium;
- Mean_{min} is the negative control well with only culture medium;
- Raw data refers to the signal values of wells with added compounds. For two replicate wells, they are denoted as Raw1 and Raw2.

2.3 Test Results

The half maximal inhibitory concentration (IC₅₀) of the compounds of the present disclosure on PSN1 cell proliferation is shown in Table 16.

Table 16. IC₅₀ of the Compounds of the Present Disclosure on PSN1 Cell Proliferation

Compound No.	Max Inhibition Percentage	Max Inhibition Percentage	IC ₅₀ (nM)
CPD02	80.8	6.7	67.6
CPD03	73.6	-3.1	255.9
CPD04	87.6	1.3	105.0
CPD06	93.7	13.7	57.0

NMS-P937	85.9	2.3	53.7
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It can be concluded from the cell proliferation inhibition test that the compounds of the present disclosure exhibit significant growth inhibitory effects on the pancreatic cancer cell line (PSN1). The stereospecific design R-configured molecule CPD02 shows higher sensitivity than the S-configured CPD03. Deuterated compounds and non-deuterated molecules show no significant difference in biological activity (PSN1) (e.g., CPD04 vs. CPD02, CPD06 vs. NMS-P937).

Test Example 3. Metabolic Stability Evaluation in Liver Microsomes

3.1 Test materials

The materials used in this example are shown in Tables 17-19 below:

Table 17. Compound Information

Compound Name	Mass	Molecular Weight	Concentration	Solvent (μL) -DMSO	Preparation Date
CPD 04	1.80 mg	562.59	10 mM	320	20230510
CPD 06	3.13 mg	540.58	10 mM	579	20230510
NMS-P937	2.65 mg	523.53	10 mM	498	20230510
CPD 02	1.73 mg	558.57	10 mM	310	20230510

Table 18. Microsome Information

Name	Brand or supplier	Catalog No.	Batch No.
Human Liver Microsomes, Male and Female	Gentest	452117	38297
Male Mouse Liver Microsomes, Male CD-1	Gentest	452701	1313002

Table 19. Reagent Information

Name	Brand or supplier	Catalog No.	Batch No.
NADPH	Solarbio	N8100	812P021
Ketoprofen	MCE	HY-B0227	105978
Labetalol hydrochloride	MCE	HY-B1108	108780
Alprazolam	Sigma	A-903-1ML	FE12172003
MeOH	Fisher	A452-4	213476
ACN	Honeywell	LC015-1-1	EB072-CN
DMSO	Solarbio	D8372	1121C0310
MgCl ₂	Solarbio	M8161	111D031
UDPGA	yuanye	S18073	O11GS162290
NADPH*	solarbio	N8100	812P021
Alamethicin	MCE	HY-N6708	133878

3.2 Test Methods

3.2.1 Reagent Preparation

Name	Stock Concentration	Volume (μL)	Final Concentration
Phosphate Buffer Saline	100 mM	266	100 mM
MgCl ₂	50 mM	40	5 mM
NADPH	10 mM	40	1 mM
UDPGA	20 mM	40	2 mM
Liver Microsomes	20 mg/mL	10	0.5 mg/mL
Alamethicin	5 mg/mL	2	0.025 mg/mL
Test compound	400 μM	2	2 μM
Total		400	

3.2.2 Experimental Procedures

- 1) Prepare a microsome solution at 0.6289 mg/mL using PBS.
- 2) Add 318 μL of the 0.6289 mg/mL microsome solution to each well of a 96-well incubation plate.
- 3) Add 40μL of 10mM NADPH solution and 40μL of 20mM UDPGA solution to the incubation plate. For negative samples, add 80μL of PBS (100mM, pH = 7.4) solution. Vortex at 800 rpm for 10 seconds, then pre-incubate in a 37°C water bath for 10 minutes.
- 4) After pre-incubation, add 2 μL of control drug or test compound to each well to initiate the reaction. The reaction is carried out in a 37°C water bath at 60 rpm.
- 5) At 0.5, 15, 30, 45, and 60 minutes, take 50 μL of the reaction system from each well and add it to 200 μL of cold methanol solution containing the internal standard to terminate the reaction.
- 6) Mix thoroughly and centrifuge at 4000 rpm at 4°C for 30 minutes.
- 7) Take 100μL of supernatant and mix with 100μL ultrapure water for liquid-mass analysis.

*Internal standards: 50nM alprazolam, 50nM labetalol and 100nM ketoprofen

3.2.3 Data Processing

The internal standard method is adopted, and the ratio of the sample peak area to the internal standard peak area is used to calculate the residual percentage (taking the ratio of the sample peak area to the internal standard peak area at 0.5 minutes as 100%).

The natural logarithm of the residual percentage is plotted against the incubation time, and the slope of the straight line is the elimination rate constant k.

$$t_{1/2}(\text{min}) = -\frac{0.693}{k}$$

$$CL_{\text{int}}(\mu\text{L}/\text{min}/\text{mg protein}) = \frac{\text{Incubation Volume } (\mu\text{L})}{\text{Incubation Protein (mg)}} \times \frac{0.693}{t_{1/2}}$$

3.3 Test Results

The evaluation results of the metabolic stability of the disclosed compound in liver microsomes are shown in Table 20.

Table 20. Summary of Liver Microsomal Metabolic Stability Data

Compound	Species	Residual Amount (%)		CL _{int}
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		0.5 min	15 min	30 min	45 min	60 min	T _{1/2} (min)	(μL/min/mg protein)
Diclofenac	Human	100.00	10.78	5.62	3.65	2.93	7.13	194.50
		100.00	105.89	102.20	104.38	91.57		
Diclofenac	Mouse	100.00	61.24	56.66	51.87	45.26	20.49	67.63
		100.00	114.45	108.86	109.75	114.70		
CPD04	Human	100.00	52.72	28.62	21.10	13.41	20.95	66.17
		100.00	86.71	108.16	107.82	102.03		
CPD04	Mouse	100.00	61.30	39.18	34.10	26.56	31.93	43.41
		100.00	103.15	96.04	101.86	109.33		
CPD06	Human	100.00	86.05	73.64	68.37	63.15	89.92	15.41
		100.00	93.25	90.15	89.75	91.37		
CPD06	Mouse	100.00	81.80	68.21	64.96	57.01	76.33	18.16
		100.00	87.75	92.85	96.22	89.24		
NMS-P937	Human	100.00	80.23	73.16	69.74	58.94	86.35	16.05
		100.00	102.54	96.00	101.77	90.35		
NMS-P937	Mouse	100.00	82.56	69.31	61.60	53.17	66.38	20.88
		100.00	93.89	92.17	88.22	85.68		
CPD02	Human	100.00	54.16	32.53	22.97	13.71	21.38	64.82
		100.00	103.64	103.22	102.41	97.08		
CPD02	Mouse	100.00	56.76	39.33	30.29	26.56	31.53	43.95
		100.00	103.87	96.01	95.65	88.91		

Conclusion: The disclosed compound shows moderate *in vitro* clearance.

Test Example 4. Evaluation of Test Substance Permeability and Transporter Substrate Test

4.1 Test Materials

4.1.1 Compound Information

Compound Name	Mass	Batch No.	Molecular Weight	Concentration	Solvent (μL) DMSO	Preparation Date
CPD 04	1.80 mg	-	562.59	10 mM	320	2023/05/10
CPD 06	3.13 mg	-	540.58	10 mM	579	2023/05/10
NMS-P937	2.65 mg	-	532.53	10 mM	498	2023/05/10
CPD02	1.73 mg	-	558.57	10 mM	310	2023/05/10

4.1.2 Positive Control Drug Information

Compound	Catalog No.	Batch No.	Source	Molecular Weight	Working Fluid Concentration
Propranolol	HY-B0573	26496	MCE	295.80	10 mM
Digoxin	HY-B1049	121301	MCE	780.94	10 mM
Prazosin	HY-B0193A	15874	MCE	419.86	10 mM

4.2 Test Methods

4.2.1 Caco-2 Cell Culture

- Add 50 μL and 25 mL of cell culture medium to the upper chamber and lower chamber of each well of the Transwell plate, respectively;
- Pre-incubate the Transwell plate at 37°C, 5% CO₂ for 1 hour before seeding the cells;

- c) Seed 50 μL of cell suspension at a density of 2×10^5 cells/mL in the upper chamber, and culture in a cell culture incubator at 37°C , 5% CO_2 , and 95% relative humidity for 14-21 days, replacing the medium every two days;
- d) Replace the cell culture medium every other day within 7 days, and daily after 7 days.
- e) Measure the electrical resistance (TEER) of the monolayer using an EVOM3.

4.2.2 ABBA Testing Process

- a) Wash the transwell plate twice with pre-warmed HBSS (10 mM HEPES, pH 7.4) and then incubate at 37°C for 30 minutes;
- b) Prepare a 1 mM DMSO stock solution of the test compound and dilute it 200-fold to 5 μM with buffer (pH 7.2-7.4);
- c) A to B direction: add 75 μL of 5 μM working solution to the upper chamber and 235 μL of HBSS (10mM HEPES, pH 7.4) to the lower chamber;
- d) B to A direction: add 235 μL 5 μM working solution to the lower chamber and 75 μL HBSS (10mM HEPES, pH 7.4) to the upper chamber;
- e) Transfer 50 μL of the 5 μM working solution to the sample plate containing 200 μL of pre-chilled methanol internal standard as the zero point;
- f) Incubate at 37°C , 5% CO_2 , 95% relative humidity for 2 hours;
- g) After 2 hours of incubation, transfer 50 μL from each well to the sample plate containing 200 μL of methanol internal standard;
- h) Centrifuge the sample plate at 4000 rpm for 30 minutes;
- i) Transfer 100 μL of the supernatant to an assay plate containing 100 μL of water and centrifuge for 10 min before LC-MS/MS analysis.

4.2.3 Data Analysis

Apparent permeability coefficient (P_{app} , cm/s) and efflux ratio were calculated using Microsoft Excel.

$$P_{\text{app}} = \frac{V_A \times C_{\text{acceptor}} \times 100}{\text{area} \times \text{time} \times C_{\text{initial,donor}}}$$

$$\text{Efflux ratio} = \frac{P_{\text{app(B-A)}}}{P_{\text{app(A-B)}}$$

$$\text{Recovery}\% = \frac{V_{\text{acceptor}} \times C_{\text{acceptor}} + V_{\text{donor}} \times C_{\text{donor}}}{V_{\text{donor}} \times C_{\text{initial,donor}}} \times 100$$

$V_{\text{acceptor}} = 0.235$ mL (for A $\rightarrow$ B) and 0.075 mL (for B $\rightarrow$ A)

$V_{\text{donor}} = 0.075$ mL (for A $\rightarrow$ B) and 0.235 mL (for B $\rightarrow$ A)

Area = membrane surface area, cm^2 (0.143 for Corning 3391)

Time = total transport time, s

4.3 Classification Criteria

Parameter	Classification	Criteria
Permeability	Low permeability	$P_{\text{app}} (\text{A-B}) < 2\text{E-}6$ cm/s
	Moderate Permeability	$2\text{E-}6$ cm/s $< P_{\text{app}} (\text{A-B}) < 20\text{E-}6$ cm/s
	High Permeability	$P_{\text{app}} (\text{A-B}) > 20\text{E-}6$ cm/s
Efflux Transporter Substrate	Probable	$\text{ERa} \geq 2$
	Unlikely or Not	$\text{ERa} < 2$

4.4 Test Results

The permeability results of the disclosed compounds in Caco-2 cells are shown in Table 21.

Table 21. Permeability Results in Caco-2 cells

	Average P _{app} (1E-6 cm/s)				Classification
Compound	P _{appA-B}	P _{appB-A}	Efflux Rate (ER _a)	Permeability	Efflux Transporter Substrate
Propranolol	22.32	14.73	0.66	61.61	Unlikely or Not
Digoxin	0.94	26.07	27.84	78.46	Probable
Prazosin	8.97	29.57	3.30	71.80	Probable
CPD 04	4.20	25.15	5.98	53.35	Probable
CPD 06	5.18	34.74	6.71	79.65	Probable
NMS-P937	6.09	34.16	5.61	71.57	Probable
CPD 02	7.83	23.78	3.04	67.07	Probable

Test Example 5. Evaluation of Solubility Test for Test Substances

5.1 Test Materials

Name	Brand or Supplier	Catalog No.	Batch No.
PBS	ICE	-	20230509
Progesterone	MCE	57-83-0	16163
FaSSIF/FeSSIF/FaSSGF Powder	Biorelevant	FFF02	FFF-0421-E
1.5mL MTP System-(Topas)	JG Finneran	9915LC-812FT	436021308MTP
DMSO	Solarbio	D8372	1121C0310

5.2 Test Methods

5.2.1 Preparation of Solution (Buffer)

Weigh 7.098 g Na₂HPO₄ (Solution A) and dissolve in 500 mL ultrapure water via sonication; weigh 3.40 g KH₂PO₄ (Solution B) and dissolve in 250 mL ultrapure water via sonication. Slowly add Solution B dropwise to Solution A and adjust the pH to 7.40 ± 0.05.

5.2.2 Test Procedures

- Weigh approximately 1.0 mg of compound into three separate 1.5 mL glass vials (one vial for preparing the standard solution and the other two for testing solubility);
- Add an appropriate volume (approximately 1000 µL) of assay buffer to each solubility sample vial to a final concentration of 1 mg/mL;
- Place a PTFE-encapsulated stainless steel rod stirrer into each vial and seal the vials using PTDE/SIL silicone stoppers;
- Transfer the solubility sample plate to a Thermomixer Comfort plate shaker and shake at 1100 rpm, 25°C for 24 hours;
- After 24 hours, remove the stirrers using a magnet;
- Pipette 200 µL of sample into the filter plate, perform vacuum filtration, and collect the filtrate;
- Add 10 µL of the filtered sample and 10 µL of DMSO to 980 µL of methanol, then further dilute 10-fold with methanol:water (1:1) as the filtered sample for LC-MS/MS analysis;

- h) For the standard sample, add DMSO to a final concentration of 1.0 mg/mL. Shake at 1100 rpm, 25°C for 5 minutes;
- i) Add 10 µL of the 1 mg/mL DMSO sample and 10 µL of buffer to 980 µL of methanol, then dilute 10-fold with methanol:water (1:1) as the standard sample for LC-MS/MS analysis.

5.2.3 Data Processing

Solubility was calculated using Excel.

$$\text{Solubility } (\mu\text{M}) = \frac{\text{Area}_{\text{filtered}} \times DF_{\text{filtered}}}{\text{Area}_{\text{std}} \times DF_{\text{std}}} \times [\text{std}] \times DF_{\text{std}}$$

5.3 Test Results

The solubility results of the disclosed compounds are shown in Table 22.

Table 22 Summary of Thermodynamic Solubility Data

Compound	Solvents	Solubility (mg/mL)
CPD04	pH 7.4	0.0030
CPD06	pH 7.4	0.0226
NMS-P937	pH 7.4	0.0248
CPD 02	pH 7.4	0.0021

5.4 Test Conclusion

The compounds of the present disclosure exhibit solubility comparable to that of NMS-P937.

Test Example 6. Pharmacodynamic Study of Compound on Colorectal Cancer in Mice

6.1 Test Materials

Balb/c nude mice: SPF grade, female, 6-8 weeks old, weighing 16-20 g, purchased from SPF (Beijing) Biotechnology Co., Ltd.

HCT116 cells: purchased from ATCC;

The reagents and instruments used in the *in vivo* pharmacodynamic experiments are shown in Tables 23 and 24.

Table 23. Reagents Used in *In Vivo* Pharmacodynamic Experiments

Name	Specification	Manufacturer	Batch No.
PBS (Phosphate Buffered Saline)	500 mL/bottle	Dalian Meilun Biotechnology Co., Ltd.	MA0015-Sep-27G
RPMI1640 Medium	500 mL/bottle	Dalian Meilun Biotechnology Co., Ltd.	MA0215-Dec-17G
Fetal Bovine Serum (FBS)	500mL/bottle	ExcellBio	12A222
Trypsin-EDTA Digestion Solution (0.25%)	100 mL/bottle	Solarbio	20220328
Penicillin-Streptomycin	100 mL/bottle	Solarbio	20211027
Sterile Water for Injection	500 mL/bottle	Dalian Meilun Biotechnology Co., Ltd.	PWL093-Mar-25H

Table 24. Instruments and Equipment Used in *In Vivo* Pharmacodynamic Experiments

Name	Model	Manufacturer	Application
CO ₂ Incubator	3111	Thermo	Cell culture
Microscope	IX53	Olympus	Cell culture
Ultra-Clean Workbench	YT-CJ-1N	Beijing Yatai Kelong Instrument Technology Co., Ltd.	Cell culture
Ultra-Clean Workbench	SW-CJ-1FD	Suzhou Purification Equipment Co., Ltd.	Dispensing
Ultra-Clean Workbench	YT-CJ-2NH	Beijing Yatai Kelong Instrument Technology Co., Ltd.	<i>In vivo</i> experimental operation
Electronic Balance	EJ-1201C	Beijing Langke Xingye Weighing Equipment Co., Ltd.	<i>In vivo</i> experimental operation

6.2 Test Methods

6.2.1 Cell Culture

HCT116 cells were cultured in RPMI-1640 medium containing inactivated 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin and 2 mM glutamine in an incubator at 37°C with 5% CO₂. Once the cells reached confluence, they were subcultured into new flasks. The tumor cells in the logarithmic growth phase were used for inoculation of tumors *in vivo*.

6.2.2 Tumor Cell Inoculation and Grouping

The tumor cells were washed twice with PBS, and the cell concentration was adjusted to 5×10⁷/ml. The resuspended tumor cells were inoculated subcutaneously in the right flank of the experimental animals at 100 µL/mouse, i.e. 5×10⁶/mouse. When the tumor grew to an average tumor volume of 100-150 mm³, the animals were grouped for drug administration according to the tumor volume, with a total of 7 groups, 8 animals in each group. The specific administration schedule is shown in Table 25. The first day of group drug administration was recorded as PG-D0.

Table 25. Administration Schedule

Group	Number of Animals	Treatment	Dose (mg/kg)	Route of Administration	Dosing Frequency	Dosing Cycle
1	8	Vehicle	--	p.o.	q.d.	10 consecutive days
2	8	CPD04	30	p.o.	q.d.	10 consecutive days
3	8	CPD04	60	p.o.	q.d.	10 consecutive days
4	8	CPD06	30	p.o.	q.d.	10 consecutive days
5	8	CPD06	60	p.o.	q.d.	10 consecutive days
6	8	NMS-P937	30	p.o.	q.d.	10 consecutive days
7	8	NMS-P937	60	p.o.	q.d.	10 consecutive days

Note: The administration volume is 10 µl /g. p.o.: administration by oral gavage. q.d.: administration once daily for 10 consecutive days.

6.2.3 Dose Adjustment or Suspension

During the administration, the health status of the animals is monitored. If any of the following conditions occur, the administration will be suspended:

- 1) If the animal's body weight decreases to 85% or less of the weight at the start of drug treatment, administration shall be stopped until the body weight recovers to 90% of that

at the start of drug treatment, after which administration may resume;

2) If the animal exhibits progressive slowness or abnormality in behavior after administration (such as hypothermia, dyspnea, diarrhea, hunched posture, convulsions, etc.), administration shall be suspended until the animal's behavior and general condition restore to normal, after which administration may resume.

6.2.4 Humane Endpoints for Experimental Animals

During the experiment, animals shall be euthanized with CO₂ if any of the following conditions occur:

1) The animal exhibits abnormal behavior or becomes paralyzed and is unable to eat or drink spontaneously;

2) The animal's body weight decreases by more than 20% of the weight at the start of drug treatment, and the veterinarian believes that it seriously affects the animal's welfare and/or the normal progress of the experiment .

6.2.5 Euthanasia of Experimental Animals

At the end of the experiment or when a humane endpoint is achieved, animals are euthanized using CO₂.

6.2.6 Detection Indicators

General Observations

From grouping until the end of the experiment, observations were conducted once daily, including but were not limited to the tumor inoculation site, animal mental state, food intake, motor activity, etc.

Body Weight Measurement

From grouping until the end of the experiment, mouse body weight was measured every 3 days using an electronic balance.

Tumor Volume

From grouping until the end of the experiment, the long and short diameters of the mouse tumors were measured every 3 days using an electronic vernier caliper, and the volume was calculated using the formula: Volume (TV) = $0.5 \times \text{long diameter} \times (\text{short diameter})^2$.

Tumor Photography

At the end of the experiment, the mice were euthanized, the tumors were excised, and the excised tumors from the control group and the test group were neatly arranged for photography.

6.2.7 Drug Evaluation Indicators

Tumor Growth Inhibition Rate TGI_{TV} (%)

Tumor growth inhibition rate (%) = $(1 - T/C) \times 100\%$

T/C = Mean RTV of the treatment group / Mean RTV of the control group

RTV is the relative tumor volume, that is, the ratio of the tumor volume after administration to that before administration.

The tumor growth inhibition rate $\geq 30\%$ and statistical analysis $p < 0.05$ was considered effective.

Tumor Weight Inhibition Rate TGIT_W (%)

Tumor weight inhibition rate (%) = $(1 - T/C) \times 100\%$

T/C = Mean TW of the treatment group / Mean TW of the control group

TW is tumor weight

The tumor weight inhibition rate $\geq 30\%$ and statistical analysis $p < 0.05$ was considered effective.

6.2.8 Statistical Analysis

Graphpad prism 8 statistical software was used to perform statistical analysis of tumor volume and tumor weight among groups using the One-Way ANOVA test (homogeneity of variance), and $p < 0.05$ was considered statistically significant.

6.3 Test Results

6.3.1 Tumor Inhibitory Effect of Test Drugs on HCT116 Subcutaneous Tumor Xenograft Mouse Model

As shown in Figure 2: After oral administration for 10 days, at the end of the experiment (PG-D22), compared with the vehicle group, the 60 mg/kg CPD04 administration group, and the 30 mg/kg and 60 mg/kg CPD06 and NMS-P937 administration groups could significantly inhibit the growth of mouse tumors. Each dosage group of CPD04, CPD06 and NMS-P937 showed a dose-dependent effect of tumor inhibition. The tumor growth inhibition rates (TGI_{TV}) of each group are shown in Table 26

Table 26. Tumor Growth Inhibition Rates (TGI_{TV}) of Each Group at Test Endpoint

Treatment	Dose (mg/kg)	TGI (%)	Significance (p-value)
CPD04	30	7.6	$p < 0.01$
CPD04	60	30.5	$p < 0.0001$
CPD06	30	42.4	$p < 0.0001$
CPD06	60	75.6	$p < 0.0001$
NMS-P937	30	44.1	$p < 0.0001$
NMS-P937	60	64.2	$p < 0.0001$

6.3.2 Safety of the Test Drugs in HCT116 Subcutaneous Tumor Xenograft Mouse Model

As shown in Figure 3, during the treatment, three mice in the NMS-P937 60 mg/kg group died from PG-D12 and PG-D16, respectively. Mice in the NMS-P937 group showed greater body weight fluctuations. The weight loss may be due to the poor tolerance of tumor-bearing mice to NMS-P937. After drug withdrawal, the condition of some animals failed to improve in time and led to death. In other groups, the weight loss during the treatment period was less than 10%.

6.4 Test conclusion

In summary, in the HCT116 subcutaneous tumor xenograft mouse model, each dose group of CPD04, CPD06 and NMS-P937 inhibited mouse tumor growth in a dose-dependent manner. The CPD06 60mg/kg administration group had a significantly better tumor growth inhibition effect than other treatment groups, with a TGI_{TV} of 75.6%, and showed a significant toxicity advantage over the same dose of NMS-P937 (3 out of 8 mice died in the same dose of NMS-P937 group).

Test Example 7. Pharmacodynamic Study of Compounds on Pancreatic Cancer in Mice

7.1 Test Materials

Pancreatic cancer cell line BxPC-3: purchased from ATCC, other test materials and instruments are the same as in Example 6.

7.2 Test Methods

The administration schedule is shown in Table 27. Other test methods are the same as those

in Example 9.

Table 27. Administration Schedule

Group	Number of Animals	Treatment	Dose (mg/kg)	Route of Administration	Dosing Frequency	Dosing Cycle
1	8	Vehicle	--	p.o.	q.d.	10 consecutive days
2	8	CPD04	90	p.o.	q.d.	10 consecutive days
3	8	CPD06	30	p.o.	q.d.	10 consecutive days
4	8	CPD06	60	p.o.	q.d.	10 consecutive days
5	8	NMS-P937	60	p.o.	q.d.	10 consecutive days

7.3 Test Results

7.3.1 Tumor Inhibitory Effect of Test Drugs on BxPC-3 Subcutaneous Tumor Xenograft Mouse Model

As shown in Figure 4: After oral administration for 10 days, at the end of the experiment, compared with the vehicle group, each administration group was able to significantly ($p < 0.0001$) inhibit the growth of mouse tumors. Different doses of CPD06 showed a dose-dependent effect on pancreatic tumor inhibition. The tumor growth inhibition rates (TGI_{TV}) of each group are shown in Table 28.

Table 28. Tumor Growth Inhibition Rates (TGI_{TV}) of Each Group at Test Endpoint

Treatment	Dose (mg/kg)	TGI (%)	Mouse Mortality Rate (%)
CPD04	90	53	0
CPD06	30	33	0
CPD06	60	71	0
NMS-P937	60	61	25

7.3.2 Safety of the Test Drugs in the BxPC-3 Subcutaneous Tumor Xenograft Mouse Model

As shown in Figure 5, during the treatment, 2 mice in the NMS-P937 60 mg/kg group died from PG-D9 and PG-D19, respectively. Mice in the NMS-P937 60 mg/kg group showed greater body weight fluctuations. The weight loss may be due to the poor tolerance of tumor-bearing mice to NMS-P937. After drug withdrawal, the condition of some animals failed to improve in time and led to death. However, the weight of other groups was relatively stable during the treatment, with a decrease of less than 10%, and the weight recovered quickly after drug withdrawal.

7.4 Test Conclusion

In summary, in the BxPC-3 subcutaneous tumor xenograft mouse model, each dose group of CPD06 inhibited mouse tumor growth in a dose-dependent manner. The CPD06 60mg/kg administration group had a significantly better tumor growth inhibition effect than other treatment groups, with the TGI_{TV} reached 71%, showing that the efficacy was significantly better than the same dose of NMS-P937, and the toxicity was significantly better than the same dose of NMS-P937 group. No obvious toxic symptoms were observed during the treatment of CPD06, but 2 of the 8 mice in the same dose of NMS-P937 group died.

Test Example 8. Pharmacodynamic Study Of Compound On Pancreatic Cancer In Mice

8.1 Test Materials

M-NSG mice: SPF grade, female, 6-8 weeks old, weighing 16-20 g, purchased from Shanghai Model Organisms Technology Co., Ltd.

PSN1 cells: purchased from ATCC;

The instruments used in the *in vivo* pharmacodynamic experiment are shown in Table 29.

Table 29. Reagents and Instruments

Reagent/Instrument Name	Supplier	Catalog No.	Storage Condition
Electronic Balance	Shanghai Jingtian Electronic Instrument Co., Ltd.	JT201N	RT
Vernier Caliper	Mitutoyo	500-171	RT
Disposable Syringe	Shanghai Kindly Enterprise Development Group Co., Ltd.	Batch K20190705	RT
PBS	BIOLOGICAL INDUSTRIES	C3580-0500	4°C

8.2 Test Methods

8.2.1 Drug Preparation

Sample Name	Preparation Method	Working Solution Concentration (Mg/ML)	Storage Conditions
NMS- P937 30mg/kg	Weigh 15.0 mg of NMS-P937 into a 5.0 mL EP tube, add 2.5 mL of solvent, and mix to obtain a solution with a concentration of 6 mg/mL.	6	2-8°C
CPD04 90mg/kg	Weigh 22.5 mg of CPD04 into a 5.0 mL EP tube, add 2.5 mL of solvent, and mix to obtain a solution with a concentration of 9 mg/mL.	9	2-8°C
CPD06 60mg/kg	Weigh 15.0 mg of CPD06 into a 5.0 mL EP tube, add 2.5 mL of solvent, and mix to obtain a solution with a concentration of 6 mg/mL.	6	2-8°C

8.2.2 Cell Culture

PSN1 cells were cultured in an incubator at 37°C and 5% CO₂ in RPMI-1640 medium containing 10% inactivated fetal bovine serum. Cells were subcultured by passaging into new culture dishes every 2 to 3 days when they reach confluent.

8.2.3 Tumor Cell Inoculation and Grouping

8.2.3.1 PSN1 cells in the logarithmic growth phase were taken and resuspended in PBS. The cell suspension was inoculated subcutaneously into the right flank of M-NSG mice using a 1mL syringe (inoculated cell count: 5E6/mouse + 30%M). The survival status of the mice and the subcutaneous tumor formation were observed every other day.

8.2.3.2 On the day of grouping, mice were randomly divided into 8 groups with 8 mice in each group based on the average tumor volume (~190 mm³) and mouse body weight;

8.2.3.3 The mice were dosed according to the grouping scheme, all by intragastric administration. The administration volume was 10 mL/kg, once a day for 6 times in total. The hair condition and status of the mice were observed, and the body weight and tumor size were recorded twice a week to calculate TGI%.

8.2.3.4 Grouping of Test Animals

When the average tumor volume reached ~190 mm³, mice with moderate individual tumor volume were selected for inclusion in the group. The animals were randomly assigned to

experimental groups according to tumor volume and mouse weight, with 8 mice in each group. Administration started one day after grouping. The specific administration regimen is shown in the table below:

Group	n	Treatment	Dose (mg/kg)	Administration Volume (μL/g)	Administration Route	Schedule
1	8	0.5% MC + 1% Tween20	-	--	<i>p.o.</i>	QD*6 times
2	8	CPD04	90	10	<i>p.o.</i>	QD*6 times
3	8	CPD06	60	10	<i>p.o.</i>	QD*6 times
4	8	NMS-P937	60	10	<i>p.o.</i>	QD*6 times

8.2.4 Calculation Formulae

Tumor volume (TV)

$$TV = 1/2 \times a \times b^2$$

wherein: a represents the long diameter of the tumor; b represents the short diameter of the tumor.

Relative tumor volume (RTV)

$$RTV = V_t / V_{initial} \times 100(\%)$$

wherein, $V_{initial}$ is the tumor volume measured at the time of group and administration (ie, $d_{initial}$), and V_t is the tumor volume at each measurement.

Relative tumor growth rate T/C (%)

$$T/C (\%) = (T_{RTV} / C_{RTV}) \times 100\%$$

wherein, T_{RTV} represents the relative tumor volume of the treatment group, and C_{RTV} represents the relative tumor volume of the vehicle group.

Tumor volume inhibition rate (TGI)

$$TGI = [1 - (TV_t - TV_{initial}) / (CV_t - CV_{initial})] \times 100\%$$

wherein, TV_t represents the tumor volume of the treatment group at each measurement; $TV_{initial}$ represents the tumor volume of the treatment group at the time of grouping and administration; CV_t represents the tumor volume of the control group at each measurement; $CV_{initial}$ represents the tumor volume of the control group at the time of grouping and administration.

8.2.5 Animal Body Weight Change Rate (BWC)

$$\text{Body Weight Change Rate} = (BW_{final} - BW_{initial}) / BW_{initial} \times 100\%$$

wherein, $BW_{initial}$ represents the animal body weight at the time of grouping and administration; BW_{final} represents the animal body weight at each measurement.

8.2.6 Data Analysis

The original data of measurement and observation must be recorded. The analysis and processing are based on the original data, and the results are presented as mean and standard error of the mean (Mean $\pm$ SEM). T-test is used to analyze the difference in tumor volume between the control group and the treatment group. $P < 0.05$ indicates statistical significance.

8.3 Test Results

8.3.1 Tumor Inhibitory Effect of Test Drugs on PSN1 Subcutaneous Tumor Xenograft Mouse Model

As shown in Figure 6, after oral administration for 6 days, at the end of the experiment (day 31), compared with the vehicle group, each administration group could significantly ($p < 0.0001$) inhibit the growth of mouse tumors. The body weight of the NMS-P937 60 mg/kg group decreased severely, with half of the mice died, resulting in the inability to statistically analyze

TGI. The tumor growth inhibition rate (TGI_{TV}) of each group is shown in Table 30.

Table 30. Tumor Growth Inhibition Rate (TGI_{TV}) of Each Group at the Endpoint of the Test

Treatment	Dose (mg/kg)	TGI (%)	Mouse Mortality Rate (%)
CPD04	90	40	0
CPD06	60	53	0
NMS-P937	60	NA	50%

8.3.2 Safety of Test Drugs in PSN1 Subcutaneous Tumor Xenograft Mouse Model

As shown in Figure 7, during the treatment, 4 mice in the NMS-P937 60mg/kg group died on D27 and D31, respectively. At the end of the test, only two mice in the NMS-P937 60mg/kg group showed slight body weight recovery. The weight loss may be due to the poor tolerance of tumor-bearing mice to NMS-P937. No mice died in the CPD06 60mg/kg group with body weight loss not exceeding 15% during administration, and the body weight recovered after drug withdrawal. In the CPD04 90mg/kg group, no mouse death was found, and no body weight loss was observed throughout the period.

8.4 Test Conclusion

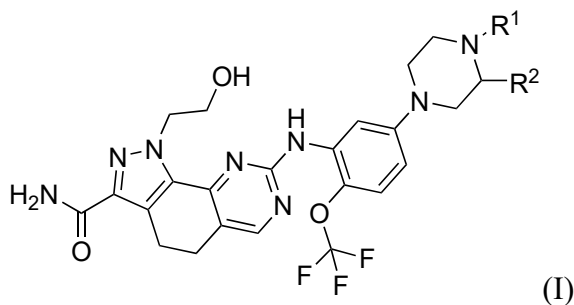
In conclusion, in the PSN1 subcutaneous tumor xenograft mouse model, CPD06 at 60 mg/kg and CPD04 at 90 mg/kg significantly inhibited tumor growth in mice. The tumor growth inhibition effect of the CPD06 60 mg/kg administration group was significantly superior to that of other treatment groups, with TGI_{TV} reaching 53%. The toxicity of NMS-P937 at 60 mg/kg in highly immune-deficient mice was far greater than that of CPD06 at 60 mg/kg and CPD04 at 90 mg/kg, with 4 out of 8 mice dying in the NMS-P937 60 mg/kg group.

The foregoing description is considered to be merely an illustration of the principles of the present disclosure. In addition, since many modifications and variations will be apparent to those skilled in the art, it is not intended to limit the present disclosure to the exact configuration and process as described above. Therefore, all suitable modifications and equivalents may be considered to fall within the scope of the present disclosure as defined by the appended claims.

All publications, patents, and patent applications cited herein are hereby incorporated by reference into this disclosure in their entirety.

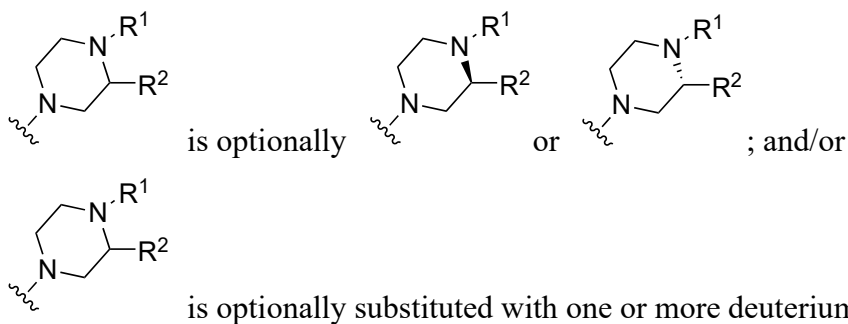
CLAIMS

1. A stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I):



or a pharmaceutically acceptable salt, solvate thereof,

wherein R^1 and R^2 are each independently selected from hydrogen, deuterium, deuterated or non-deuterated C_{1-6} alkyl; or R^1 and R^2 together with the N atom and the C atom to which they are attached form an optionally substituted, deuterated or non-deuterated 5-membered heterocyclic ring;



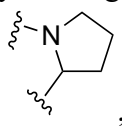
2. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to claim 1, wherein

R^1 is a deuterated or non-deuterated C_{1-6} alkyl, preferably methyl or deuterated methyl, more preferably perdeuterated methyl; and/or

R^2 is hydrogen or deuterium.

3. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to claim 1, wherein

R^1 and R^2 together with the N atom and C atom to which they are attached form an optionally substituted 5-membered heterocyclic ring as follows:

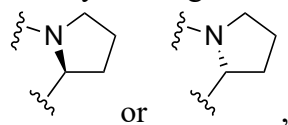


wherein the 5-membered heterocyclic ring is optionally substituted with 1-7 deuterium atoms.

4. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a

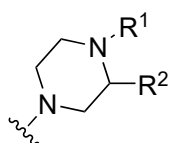
compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to claim 3, wherein

R¹ and R² together with the N atom and C atom to which they are attached form an optionally substituted 5-membered heterocyclic ring selected from:



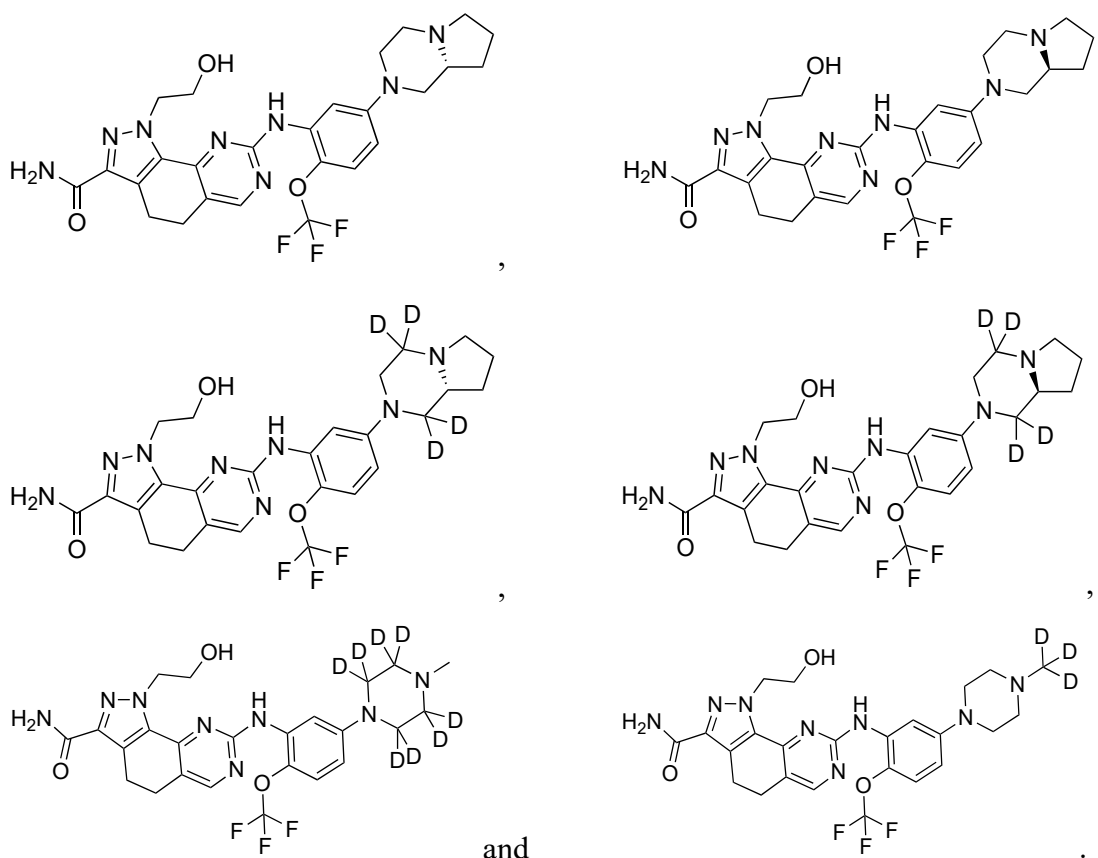
wherein the 5-membered heterocyclic ring is optionally substituted with 1-7 deuterium atoms.

5. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of the preceding claims, wherein



is optionally substituted with 1-8 deuterium atoms.

6. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to claim 1, wherein the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I) is selected from:



7. A pharmaceutical composition, comprising the stereoisomer, deuterated derivative or

stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of claims 1 to 6, and a pharmaceutically acceptable carrier or excipient.

8. A method for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of claims 1 to 6.

9. The method according to claim 8, wherein the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are selected from the group consisting of leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, and sepsis.

10. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of claims 1 to 6, for use in treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1.

11. The stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof, for use in treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 according to claim 10, wherein the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are selected from the group consisting of leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, and sepsis.

12. Use of the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of claims 1 to 6, in the preparation of a medicament for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1.

13. The use according to claim 12, wherein the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are selected from the group consisting of leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, and sepsis.

14. A kit for treating diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1, the kit comprising
the stereoisomer, deuterated derivative or stereoisomeric deuterated derivative of a

compound of Formula (I), or a pharmaceutically acceptable salt, solvate thereof according to any one of claims 1 to 6, or the pharmaceutical composition according to claim 7;
a container; and
optionally a package insert or label indicating treatment.

15. A kit according to claim 14, wherein the diseases and conditions caused by dysregulated PLK1 activity and/or diseases and conditions associated with PLK1 are selected from the group consisting of leukemia, lymphoma, pancreatic cancer, breast cancer, prostate cancer, lung cancer, ovarian cancer, colorectal cancer, liver cancer, gastric cancer, esophageal cancer, melanoma, multiple myeloma, and sepsis.