

SARCOMA TREATMENTS USING CSF-1R/PDGFR MULTI-TARGET INHIBITORS**Technical Field**

[0001] The present disclosure relates to the field of pharmaceutical technology, specifically to the application of a colony-stimulating factor 1 receptor (CSF-1R)/platelet-derived growth factor receptor (PDGFR) multi-target inhibitor in the treatment of sarcoma.

Background

[0002] Tumor-associated macrophages (TAMs) constitute a large portion of all tumor-infiltrating immune cells within the tumor microenvironment (TME). As participants in tumor-associated inflammation, TAMs play a dominant role in tumor growth and progression, making them a highly promising new direction for therapeutic intervention. The pro-tumor functions of TAMs are based on their ability to secrete pro-angiogenic factors and growth factors, as well as their capacity to effectively suppress T-cell effector functions through the release of immunosuppressive cytokines. The regulation of TAMs is highly complex, influenced by various mechanisms and the microenvironment in which macrophages reside. Among these, colony-stimulating factor 1 (CSF-1) is one of the primary regulators of the survival, proliferation, and differentiation of mononuclear phagocytes/macrophages, and it maintains the pro-tumorigenic functions of TAMs. CSF-1 recruits peripheral blood monocytes to the TME, where monocytes differentiate into macrophages and bind to the CSF-1 receptor, polarizing macrophages into TAMs. TAMs play a crucial role in tumor progression, immune evasion, and resistance to immune checkpoint blockade therapy. Vascular endothelial growth factor (VEGF) not only regulates vascular normalization but also significantly influences the morphological distribution of TAMs, as well as the infiltration of regulatory T cells and myeloid-derived suppressor cells into tumor tissues. Preclinical studies have shown that blocking CSF-1R can inhibit tumor growth in various cancer models by eliminating or polarizing TAMs.

[0003] Due to the complexity of the TME, from the perspective of drug development, it is difficult to regulate the TME through a single mechanism. Multiple mechanisms are needed to work synergistically. While small molecule drugs may have a disadvantage compared to monoclonal antibodies in terms of target specificity, this disadvantage can become an advantage in this context, as small molecules may achieve multi-mechanism regulation with a single drug.

[0004] Osteosarcoma, also known as osteogenic sarcoma, is a common primary bone tumor (primary bone tumors refer to cancers that originate in the bone). The annual incidence rate is 2-3

per million, accounting for 0.2% of all human malignant tumors and 11.7% of primary bone tumors. It is more commonly seen in individuals aged 15-25, with a median age of 20. It is rare in individuals under 6 years old or over 60 years old. Osteosarcoma is highly malignant, prone to lung metastasis, and has a poor prognosis. The first-line treatment for osteosarcoma primarily involves surgery, radiotherapy, and chemotherapy. Currently, there is no second-line treatment strategy that provides overall survival benefits, leaving an unmet clinical need. After the failure of first-line treatment for osteosarcoma, it is recommended to participate in clinical trials to gain access to the latest treatment opportunities, or to consider oral anti-tumor angiogenesis drugs such as regorafenib or sorafenib. However, their efficacy remains relatively low, with an overall objective response rate of around 8%-14%, and median progression-free survival and overall survival of approximately 4 months and 10 months, respectively.

[0005] Clinical and preclinical evidence demonstrates that TAMs, as key regulatory immune cells, can promote tumor development. Macrophages primarily exist in two polarized states. Alternatively activated M2 subtype TAMs promote tumor progression by secreting anti-inflammatory cytokines (e.g., interleukin (IL)-10, transforming growth factor- β), whereas activated M1 subtype TAMs enhance immune-mediated tumor killing by producing pro-inflammatory cytokines. To overcome the immunosuppressive and tumor-promoting functions of TAMs, current therapeutic strategies focus on the depletion of TAMs in the tumor microenvironment and the reprogramming of TAMs to promote anti-tumor functions (shifting M2 polarization to M1). The CSF-1R kinase is a product encoded by the proto-oncogene *c-fms* and belongs to the class III receptor tyrosine kinase family, which also includes FMS-like tyrosine kinase 3, stem cell factor receptor, FDGFR, and PDGFR. CSF-1R binds to its ligands, CSF-1 and IL-34, to activate CSF-1R, thereby playing a crucial role in the proliferation, differentiation, and growth of mononuclear phagocytes. In the tumor microenvironment, a large number of macrophages (TAMs) are present, expressing the CSF-1R on their cell surface. When CSF-1 binds to this receptor, macrophages are converted to a pro-tumor state, driving immune suppression and promoting tumor cell growth. Furthermore, high expression of CSF-1R on tumor cells is associated with lower survival rates in certain cancer patients, indicating tumor dependency, making it a potential therapeutic target. By targeting CSF-1R, the development of CSF-1R/CSF-1 inhibitors can effectively reduce the number of TAMs in tumor tissues, promote the generation of anti-tumor macrophages, and help relieve immune suppression. Additionally, it facilitates the infiltration of various immune cells, including T cells and lymphocytes, into the tumor tissue.

[0006] PDGFR is a transmembrane glycoprotein with tyrosine kinase activity, primarily expressed on mesenchymal-derived cells such as fibroblasts, pericytes, vascular smooth muscle cells, and stromal stem/progenitor cells. PDGFR consists of α and β subunits. Typically, PDGFR α and PDGFR β exist as monomers in an autoinhibited state. Upon binding with platelet-derived growth factor (PDGF), PDGFR monomers dimerize, forming isomers PDGFR- $\alpha\alpha$, PDGFR- $\alpha\beta$, and PDGFR- $\beta\beta$, which mediate a series of downstream signaling responses. The PDGF/PDGFR primarily promotes angiogenesis and vascular maturation, playing a role in growth and development, and is also closely related to the occurrence and progression of various diseases. Due to the characteristics of tumor microvasculature, such as rapid but immature growth, irregularity, and high permeability, the transport and distribution of chemotherapeutic drugs and oxygen to tumor tissues are hindered, making it easy for tumor cells to metastasize and invade other areas through the leaky vessels. The abnormal vascular system creates a malignant tumor microenvironment characterized by hypoxia, low pH, and elevated interstitial fluid pressure, which interferes with the function of immune cells within the tumor and reduces the efficacy of radiation therapy and chemotherapy. Therefore, the focus of current anti-angiogenic therapy has shifted from simply destroying blood vessels to “normalizing” tumor microvasculature, in order to increase oxygen supply and alter the direction of drug delivery. The root cause of abnormal vascular growth in tumor tissues lies in the disruption of various growth factors within the microenvironment. The PDGF/PDGFR pathway, as a key factor in regulating vascular growth and recruiting pericytes to promote vascular maturation, plays an indispensable role in this process. PDGF/PDGFR are signaling molecules that play an important role in the proliferation and differentiation of osteoblasts and osteoclasts. The overexpression of PDGFR is associated with disease progression and poor prognosis. Since the growth and metastasis of osteosarcoma depend on neovascularization, inhibiting or blocking PDGF/PDGFR signaling with novel PDGFR inhibitors may open new avenues for osteosarcoma treatment.

[0007] The present disclosure provides an innovative CSF-1R/PDGFR multi-target small molecule inhibitor, which achieves optimal remodeling of the tumor immune microenvironment through multiple mechanisms, turning “cold” tumors into “hot” tumors. By inhibiting CSF-1R, it suppresses and polarizes TAMs, downregulates TAM levels, and promotes the conversion of macrophages into M1-like TAMs, thereby relieving the suppression of T cells. At the same time, through appropriate inhibition of PDGFR, it not only suppresses tumor angiogenesis but also “normalizes” the formation of tumor microvasculature, increasing oxygen supply and altering the direction of drug delivery. This, in turn, modulates the immune microenvironment surrounding osteosarcoma,

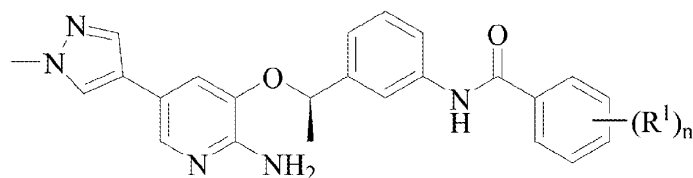
alleviates immune suppression, and, through the synergistic effects of other targets, achieves better tumor suppression.

Summary of the Invention

[0008] One aspect of the present disclosure provides a use of a CSF-1R/PDGFR multi-target inhibitor in the treatment of sarcoma.

[0009] Another aspect of the present disclosure provides a method for preventing and/or treating sarcoma, comprising administering an effective amount of a CSF-1R/PDGFR multi-target inhibitor to an individual in need thereof.

[0010] In some embodiments of the present disclosure, the CSF-1R/PDGFR multi-target inhibitor comprises a compound having the structure as shown in Formula (I), or a pharmaceutically acceptable salt or isotopic derivative thereof:



Formula (I)

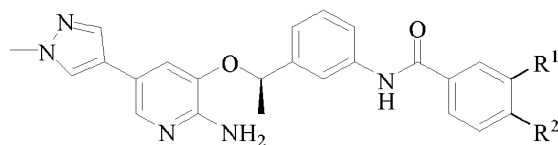
[0011] wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, C_3 - C_6 cycloalkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0012] wherein, n represents 0, 1, 2, 3, or 4;

[0013] or, when n is 2, the two R^1 groups in the ortho position together with the carbon atom to which they are attached form a 5-6 membered saturated or unsaturated ring, and the ring may optionally contain 0, 1, or 2 heteroatoms selected from O, N, and S; and further, the ring may optionally be substituted by 0, 1, or 2 substituents selected from C_1 - C_6 alkyl or halogen;

[0014] wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

[0015] In some embodiments of the present disclosure, the compound has a structure of Formula (II), or a pharmaceutically acceptable salt or isotopic derivative thereof:



Formula (II)

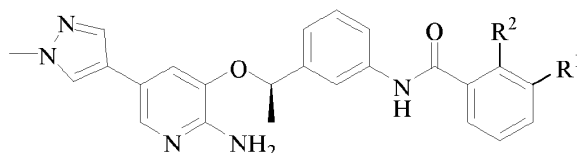
[0016] wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0017] Wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0018] or, R^1 and R^2 together with the carbon atom to which they are attached form a 5-6 membered saturated or unsaturated ring, and the ring may optionally contain 0, 1, or 2 heteroatoms selected from O, N, and S; and further, the ring may optionally be substituted by 0, 1, or 2 substituents selected from C_1 - C_6 alkyl or halogen;

[0019] wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

[0020] In some embodiments of the present disclosure, the compound has a structure of Formula (III), or a pharmaceutically acceptable salt or isotopic derivative thereof:



Formula (III)

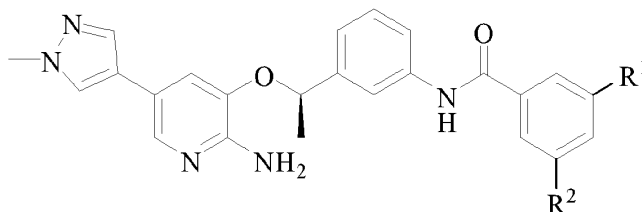
[0021] wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0022] Wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0023] or, R^1 and R^2 together with the carbon atom to which they are attached form a 5-6 membered saturated or unsaturated ring, and the ring may optionally contain 0, 1, or 2 heteroatoms selected from O, N, and S; and further, the ring may optionally be substituted by 0, 1, or 2 substituents selected from C_1 - C_6 alkyl or halogen;

[0024] wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

[0025] In some embodiments of the present disclosure, the compound has a structure of Formula (IV), or a pharmaceutically acceptable salt or isotopic derivative thereof:



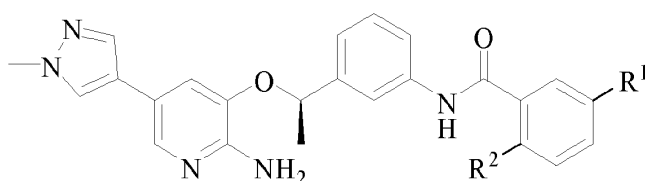
Formula (IV)

[0026] wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0027] wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0028] wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

[0029] In some embodiments of the present disclosure, the compound has a structure of Formula (V), or a pharmaceutically acceptable salt or isotopic derivative thereof:



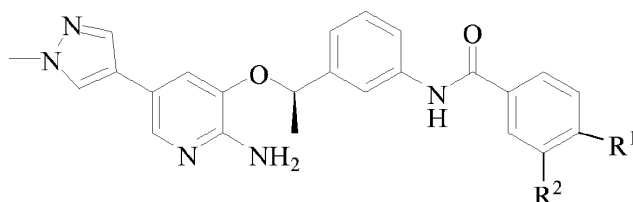
Formula (V)

[0030] wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0031] wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0032] wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

[0033] In some embodiments of the present disclosure, the compound has a structure of Formula (VI), or a pharmaceutically acceptable salt or isotopic derivative thereof:



Formula (VI)

[0034] wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

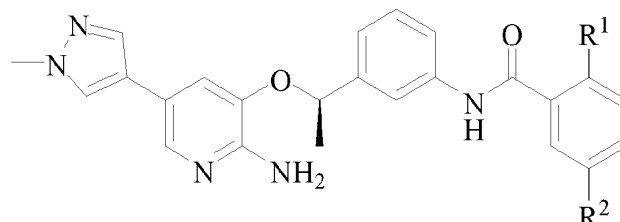
[0035] Wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

[0036] or, R^1 and R^2 together with the carbon atom to which they are attached form a 5-6 membered saturated or unsaturated ring, and the ring may optionally contain 0, 1, or 2 heteroatoms selected from O, N, and S; and further, the ring may optionally be substituted by 0, 1, or 2 substituents

selected from C₁-C₆ alkyl or halogen;

[0037] wherein, R^a represents hydrogen, C₁-C₆ alkyl, or halogenated C₁-C₆ alkyl.

[0038] In some embodiments of the present disclosure, the compound has a structure of Formula (VII), or a pharmaceutically acceptable salt or isotopic derivative thereof:



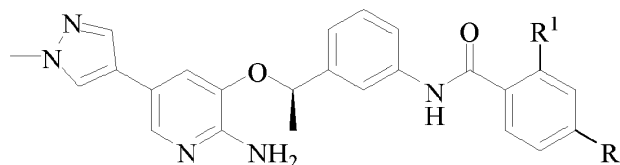
Formula (VII)

[0039] wherein, R¹ represents hydrogen, halogen, C₁-C₆ alkyl, -OR^a, -SR^a, halogenated C₁-C₆ alkyl, or cyano;

[0040] wherein, R² represents hydrogen, halogen, C₁-C₆ alkyl, -OR^a, -SR^a, halogenated C₁-C₆ alkyl, or cyano;

[0041] wherein, R^a represents hydrogen, C₁-C₆ alkyl, or halogenated C₁-C₆ alkyl.

[0042] In some embodiments of the present disclosure, the compound has a structure of Formula (VIII), or a pharmaceutically acceptable salt or isotopic derivative thereof:



Formula (VIII)

[0043] wherein, R¹ represents hydrogen, halogen, C₁-C₆ alkyl, -OR^a, -SR^a, halogenated C₁-C₆ alkyl, or cyano;

[0044] wherein, R² represents hydrogen, halogen, C₁-C₆ alkyl, -OR^a, -SR^a, halogenated C₁-C₆ alkyl, or cyano;

[0045] wherein, R^a represents hydrogen, C₁-C₆ alkyl, or halogenated C₁-C₆ alkyl.

[0046] In some embodiments of the present disclosure: R¹ represents hydrogen, halogen, C₁-C₃ alkyl, fluoromethyl, -OR^a, -SR^a; wherein R^a represents hydrogen or C₁-C₃ alkyl.

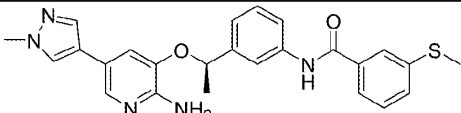
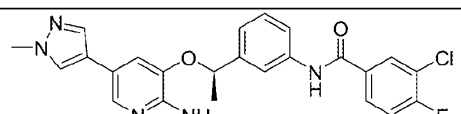
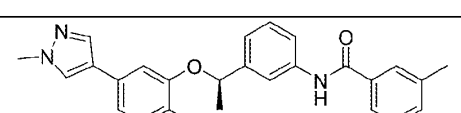
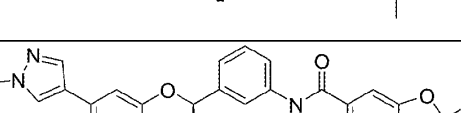
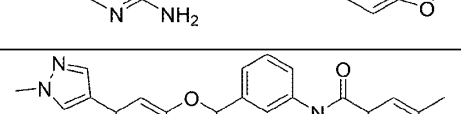
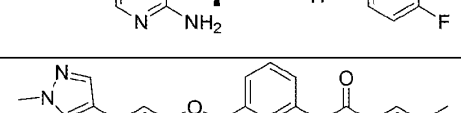
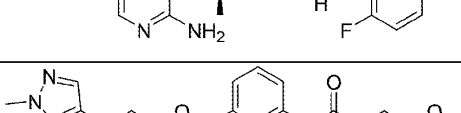
[0047] In some embodiments of the present disclosure: R² represents hydrogen, halogen, C₁-C₃ alkyl, fluoromethyl, -OR^a, -SR^a; wherein R^a represents hydrogen or C₁-C₃ alkyl.

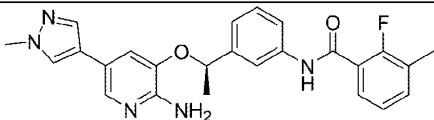
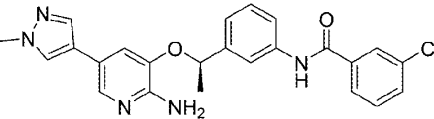
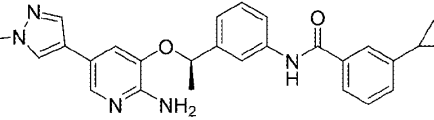
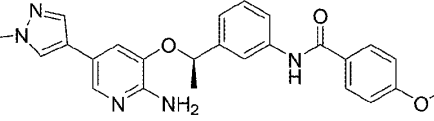
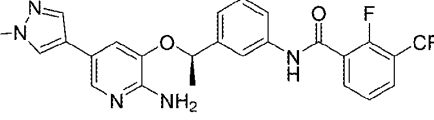
[0048] In some embodiments of the present disclosure: R¹ represents hydrogen, halogen, methyl, trifluoromethyl, methoxy, or -SCH₃.

[0049] In some embodiments of the present disclosure: R¹ represents hydrogen, methyl, trifluoromethyl, fluorine, chlorine, or -SCH₃.

[0050] In some embodiments of the present disclosure: R² represents hydrogen, methyl, fluorine, or chlorine.

[0051] In some embodiments of the present disclosure: the described compound or its pharmaceutically acceptable salt or isotopic derivative thereof is selected from the following:

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[0052] In some embodiments of the present disclosure: the described sarcoma is selected from one or more of osteosarcoma, soft tissue sarcoma, and hemangioma.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0053] As used herein, if the chemical structure and chemical name conflict, the chemical structure is determinative of the identity of the compound. The compounds of the present disclosure may contain one or more chiral centers and/or double bonds and therefore, may exist as stereoisomers, such as double-bond isomers (i.e., geometric isomers), enantiomers, or diastereomers. Accordingly, any chemical structures within the scope of the specification depicted, in whole or in part, with a relative configuration encompass all possible enantiomers and stereoisomers of the illustrated compounds including the stereoisomerically pure form (e.g., geometrically pure, enantiomerically pure or diastereomerically pure) and enantiomeric and stereoisomeric mixtures. Enantiomeric and stereoisomeric mixtures can be resolved into the component enantiomers or stereoisomers using separation techniques or chiral synthesis techniques well known to the skilled artisan.

[0054] Compounds of the present disclosure include, but are not limited to, compounds of Formula (I), Formula (II), Formula (III), Formula (IV), Formula (V), Formula (VI), Formula (VII) and Formula (VIII) and all pharmaceutically acceptable forms thereof. The disclosure includes

pharmaceutically acceptable forms of the compounds pharmaceutically acceptable salts, solvates, chelates, non-covalent complexes, prodrugs, and mixtures thereof. The invention discloses compounds, and a pharmaceutically acceptable salt thereof, a solvate thereof, a chelate thereof, a non-covalent complex thereof, a prodrug thereof, and ester prodrugs and mixtures of any of the foregoing.

[0055] The disclosure discloses compounds which may also contain naturally occurring or unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (H), iodine-125 (¹²⁵I) or carbon-14 (¹⁴C). Radiolabeled compounds are useful as therapeutic or prophylactic agents, research reagents, e.g., assay reagents, and diagnostic agents, e.g., in vivo imaging agents. All isotopic variations of the compounds of the invention, whether radioactive or not, are intended to be encompassed within the scope of the invention. For example, the invention also includes deuterium (D) or tritium (T) containing compounds.

[0056] “Alkyl” refers to a saturated branched or straight-chain monovalent hydrocarbon group derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane. Typical alkyl groups include, but are not limited to, methyl, ethyl, propyls such as propan-1-yl and propan-2-yl, butyls such as butan-1-yl, butan-2-yl, 2-methyl-propan-1-yl, 2-methyl-propan-2-yl, tert-butyl, and the like. In certain embodiments, an alkyl group comprises 1 to 20 carbon atoms. In some embodiments, alkyl groups include 1 to 10 carbon atoms or 1 to 6 carbon atoms whereas in other embodiments, alkyl groups include 1 to 4 carbon atoms. In still other embodiments, an alkyl group includes 1 or 2 carbon atoms. Branched chain alkyl groups include at least 3 carbon atoms and typically include 3 to 7, or in some embodiments, 3 to 6 carbon atoms. An alkyl group having 1 to 6 carbon atoms may be referred to as a (C₁-C₆)alkyl group and an alkyl group having 1 to 4 carbon atoms may be referred to as a (C₁-C₄)alkyl. This nomenclature may also be used for alkyl groups with differing numbers of carbon atoms.

[0057] “Alkoxy” refers to a radical -OR where R represents an alkyl group as defined herein. Representative examples include, but are not limited to, methoxy, ethoxy, propoxy, butoxy, and the like. Typical alkoxy groups include 1 to 10 carbon atoms, 1 to 6 carbon atoms or 1 to 4 carbon atoms in the R group. Alkoxy groups that include 1 to 6 carbon atoms may be designated as -O(C₁-C₆) alkyl or as -O(C₁-C₆ alkyl) groups. In some embodiments, an alkoxy group may include 1 to 4 carbon atoms and may be designated as -O(C₁-C₄) alkyl or as -O(C₁-C₄ alkyl) groups group.

[0058] The term “alkylthio” refers to -S-alkyl.

[0059] The term “cycloalkyl”, “carbocyclic” and “carbocyclyl” as used herein, refers to a group derived from a non-aromatic monocyclic or polycyclic hydrocarbon molecule by removal of one hydrogen atom from a saturated ring carbon atom. Representative examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclopentyl, and cyclohexyl. When numbers appear in a subscript after the symbol “C”, the subscript defines with more specificity the number of carbon atoms that a particular cycloalkyl group may contain. For example, “C₃-C₆ cycloalkyl” denotes cycloalkyl groups with three to six carbon atoms.

[0060] “Cyano” refers to the radical -CN.

[0061] “Hydroxyl” refers to the radical -OH.

[0062] “Sulfhydryl” refers to the radical -SH.

[0063] “Halo” or “halogen” refers to a fluoro, chloro, bromo, or iodo group.

[0064] The term “heteroatom” refers to an atom other than carbon or hydrogen. Heteroatoms are typically independently selected from among oxygen, sulfur, nitrogen, silicon and phosphorus, but are not limited to these atoms. In embodiments in which two or more heteroatoms are present, the two or more heteroatoms can all be the same as one another, or some or all of the two or more heteroatoms can each be different from the others.

[0065] “Haloalkyl” or “halogenated alkyl” refers to an alkyl group in which at least one hydrogen is replaced with a halogen. Thus, the term “haloalkyl” includes monohaloalkyl (alkyl substituted with one halogen atom) and polyhaloalkyl (alkyl substituted with two or more halogen atoms). Representative “haloalkyl” groups include difluoromethyl, 2,2,2-trifluoroethyl, 2,2,2-trichloroethyl, and the like. The term “perhaloalkyl” means, unless otherwise stated, an alkyl group in which each of the hydrogen atoms is replaced with a halogen atom. For example, the term “perhaloalkyl”, includes, but is not limited to, trifluoromethyl, pentachloroethyl, 1,1,1-trifluoro-2-bromo-2-chloroethyl, and the like.

[0066] The term “comprising” or “comprise” is meant to be open ended, i.e., all-encompassing and

non-limiting. It may be used herein synonymously with “having” or “including”. Comprising is intended to include each and every indicated or recited component or element(s) while not excluding any other components or elements.

[0067] “Pharmaceutically acceptable” refers to generally recognized for use in animals, and more particularly in humans.

[0068] “Pharmaceutically acceptable salt” refers to a salt of a compound that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound.

[0069] “Therapeutically effective amount” refers to the amount of a compound that, when administered to a subject for treating a disease, or at least one of the clinical symptoms of a disease or disorder, is sufficient to affect such treatment for the disease, disorder, or symptom. As those skilled in the art will recognize, this amount is typically not limited to a single dose but may comprise multiple dosages over a significant period of time as required to bring about a therapeutic or prophylactic response in the subject. Thus, a “therapeutically effective amount” is not limited to the amount in a single capsule or tablet, but may include more than one capsule or tablet, which is the dose prescribed by a qualified physician or medical care provider. The “therapeutically effective amount” can vary depending on the compound, the disease, disorder, and/or symptoms of the disease or disorder, severity of the disease, disorder, and/or symptoms of the disease or disorder, the age of the subject to be treated, and/or the weight of the subject to be treated. An appropriate amount in any given instance can be readily apparent to those skilled in the art or capable of determination by routine experimentation.

[0070] “Treating” or “treatment” of any disease or disorder refers to arresting or ameliorating a disease, disorder, or at least one of the clinical symptoms of a disease or disorder, reducing the risk of acquiring a disease, disorder, or at least one of the clinical symptoms of a disease or disorder, reducing the development of a disease, disorder or at least one of the clinical symptoms of the disease or disorder, or reducing the risk of developing a disease or disorder or at least one of the clinical symptoms of a disease or disorder. “Treating” or “treatment” also refers to inhibiting the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both, or inhibiting at least one physical parameter which may not be discernible to the subject. Further, “treating” or “treatment” refers to delaying the onset of the disease or disorder or at least symptoms thereof in a subject which may be exposed to or predisposed to a disease or disorder even though that subject does not yet experience or display

symptoms of the disease or disorder.

[0071] The disclosure is further described by reference to the following examples, which are intended to exemplify the claimed invention but not to limit it in any way.

Examples

Example 1: Inhibitory Effect of Compound on Proliferation Activity of 143B Cells

1. Experimental Purpose

[0072] To determine the inhibitory effect of the compound from the present disclosure on the *in vitro* proliferation activity of 143B cells.

2. Materials

- [0073] 143B human osteosarcoma cell line (AnWei-sci, Cat. No.: AW-CELLS-H0002)
- [0074] MEM medium (HyClone, Cat. No.: SH30024.01, Lot No.: AH30006415)
- [0075] PAN/Serum (BioTECH, Cat. No.: ST30-2602, Lot No.: ST200801ES)
- [0076] Trypsin/0.05% Trypsin-EDTA (Gibco, Cat. No.: 25300-062, Lot No.: 2458459)
- [0077] 0.4% Trypan Blue (Sigma, Cat. No.: T8154, Lot No.: RNBL1028)
- [0078] Penicillin-Streptomycin (Hyclone, Cat. No.: SV30010)
- [0079] Dimethyl sulfoxide (DMSO) (Solarbio, Cat. No.: D8371-50ml, Lot No.: 710N0312JH)
- [0080] Phosphate-buffered saline (PBS) (Solarbio, Cat. No.: P1020, Lot No.: 20230303)
- [0081] Cell counting kit (CCK) (APExBIO, Cat. No.: k1018, Lot No.: Batch5)

3. Instruments

Table 1

1 Biological safety cabinet	Thermo Fisher Scientific
2 371 direct-heated CO ₂ incubator	Thermo Fisher Scientific
3 Electronic digital display constant temperature water bath	Changzhou Guohua Electric Appliance Co., Ltd.
4 Inverted microscope	OLYMPUS
5 Hemocytometer	Shanghai Qiujiang Biochemical Reagent and Instrument Co., Ltd.
6 Shaker	IKA
7 4°C refrigerator	Thermo Fisher Scientific
8 -20°C refrigerator	Haier
9 L-500 centrifuge	Hunan Xiangyi Centrifuge
10 -80°C ultra-low temperature refrigerator	Thermo Fisher Scientific
11 Liquid nitrogen storage tank	Thermo Fisher Scientific
12 Full-wavelength reader/microplate reader	Thermo Fisher Scientific

13 Microplate centrifuge	Hunan Xiangyi Centrifuge
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4. Experimental Content

[0082] 4.1 Reagent Preparation

[0083] 1) Complete medium: Dulbecco's Modified Eagle Medium (DMEM) (Gibco) + 10% fetal bovine serum (FBS) + 1% Penicillin-Streptomycin.

[0084] 2) Compound preparation: Dissolve the compound in DMSO to prepare a 30 mM stock solution for future use. If the compound has poor solubility, reduce the concentration.

[0085] 4.2 Experimental Procedure

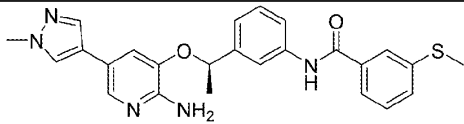
[0086] Cells in the logarithmic growth phase were collected after digestion and seeded into 384-well white transparent plates, 32 μL /well, with 1200 cells per well. After seeding, the plates were placed in a CO_2 incubator and incubated for 24 hours. The compound was precisely weighed and dissolved in DMSO to prepare a 30 mM stock solution. First, a 250 $\times$ intermediate stock solution was prepared using DMSO, and then a 5 $\times$ working solution was prepared using cell culture medium. The working solution was added to the 384-well plates, 8 μL /well, with the final concentration of the compound starting at 50 μM , followed by a 3-fold serial dilution, resulting in a total of 7 concentration points. For the normal control wells and DMSO control wells, 8 μL of culture medium was added. Additionally, a medium-only control (blank control, 40 μL /well) was set up, with 4 replicates for each group. After incubation in the CO_2 incubator for 48 hours, 4 μL of CCK8 detection reagent was added to each well and incubated in a 37 $^\circ\text{C}$ incubator for 1 hour. The optical density (OD) values were measured at 450 nm using a microplate reader, and the inhibition rate was calculated.

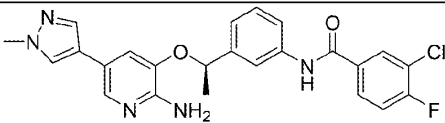
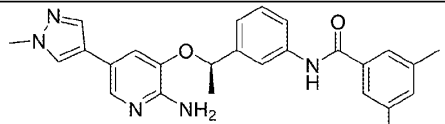
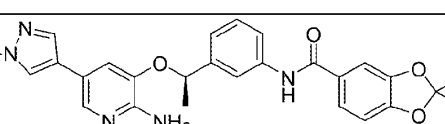
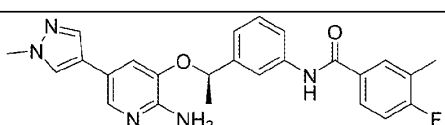
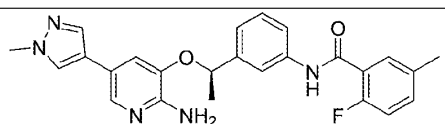
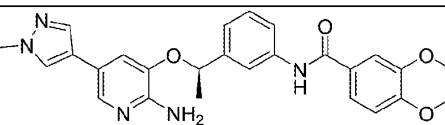
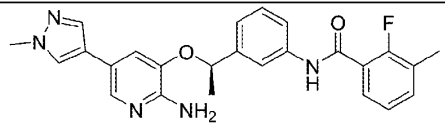
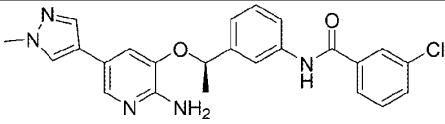
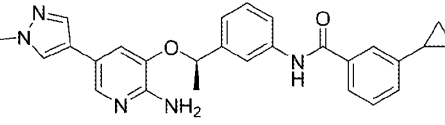
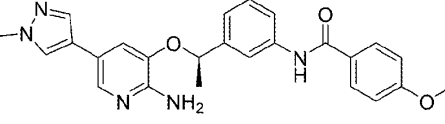
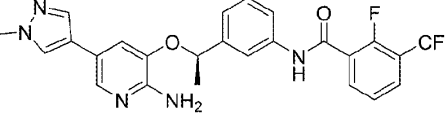
[0087] 4.3 Results Calculation

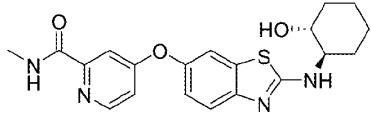
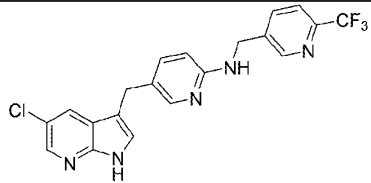
[0088] Inhibition rate % = $1 - (\text{OD value of the test well} - \text{OD value of the blank control}) / (\text{OD value of the DMSO well} - \text{OD value of the blank control}) \times 100\%$.

[0089] The final inhibition rate of the compound was compared with the inhibition rate of the positive compound. The experimental results are shown in Table 2.

Table 2

NO.	Compound structure	143B IC_{50}
1		++++

2		++++
3		++++
4		++++
5		++++
6		++++
7		++++
8		++++
9		++++
10		++++
11		++++
12		++++

Comparative Example 1		+
Comparative Example 2		++

[0090] Where, ++++ indicates half maximal inhibitory concentration (IC₅₀) ≤ 2 μM; +++ indicates IC₅₀ > 2 μM, ≤ 15 μM; ++ indicates IC₅₀ > 15 μM, ≤ 35 μM; + indicates IC₅₀ > 35 μM.

Example 2: *In Vitro* Inhibitory Activity of the Compound of the Present disclosure on CSF-1R and PDGFR-α Kinases

1. Materials and Instruments

- [0091] ENVISION (Perkinelmer, 2104)
- [0092] White 384-well MicroPlate(Cat#264706, Nunc)
- [0093] HTRF kinEASE TK kit main reagents (Cat #62TKOPEC, Cisbio)
- [0094] TK-biotin substrate
- [0095] Streptavidin-XL665
- [0096] TK Antibody-Cryptate
- [0097] 5× Enzymatic buffer (5×KB)
- [0098] Staphylococcal enterotoxin B (SEB)
- [0099] HTRF Detection buffer
- [0100] CSF1R (Cat # PV3249, Invitrogen)
- [0101] PDGFR-α (Cat # PV3811, Invitrogen)
- [0102] Adenosine triphosphate (ATP) 10 mM (Cat #PV3227, Invitrogen)
- [0103] Dithiothreitol (DTT) 1 M (Cat #D5545, Sigma)
- [0104] MgCl₂ 1 M (Cat #M8266, Sigma)
- [0105] MnCl₂ 1 M (Cat #244589, Sigma)

2. Experimental Procedure

[0106] 2.1 Reagent Preparation

Table 3 Composition and Concentration Table of Each Component in the Kinase Reaction System

	Final Concentration in the Kinase Reaction Step (10	Final Concentration in the Total
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	μL				Reaction (20 μL)	
	Enzyme Concentration	ATP	TK-substrate	Enzyme Reaction Time	Streptavidin-XL665	TK Antibody-Cryptate
CSF-1R	0.12 ng/well	0.46 μM	2.5 μM	40 min	28.75 nM	1:100 dilution
PDGFR- α	1.2 ng/well	2 μM	1 μM	30 min	100 nM	1:100 dilution

Table 4 Components of 1 mL 1 $\times$ Kinase Buffer (μL):

Kinase	5 $\times$ Enzyme buffer	MgCl ₂	MnCl ₂	DTT	SEB	ddH ₂ O
CSF-1R	200	5	1	1	20	773
PDGFR- α	200	5	1	1	8	785

[0107] 5 $\times$ Substrate-TK and ATP Working Solution

[0108] The specific concentrations of Substrate-TK and ATP are shown in Table 3.

[0109] Dilute Substrate-TK and ATP to 5 $\times$ of the reaction concentration using 1 $\times$ Kinase Buffer.

[0110] 5 $\times$ Enzyme Working Solution

[0111] The concentrations of CSF-1R and PDGFR- α kinases are shown in Table 3. Prepare the 5 $\times$ enzyme working solution using 1 $\times$ Kinase Buffer.

[0112] 4 $\times$ Sa-XL665 Working Solution

[0113] The concentration of Sa-XL665 in the reaction is shown in Table 3. Prepare the 4 $\times$ Sa-XL665 working solution using Detection Buffer.

[0114] 4 $\times$ TK-Ab-Cryptate Working Solution

[0115] Dilute TK-Ab-Cryptate 100 times using Detection Buffer to prepare the working solution.

2.2 Experimental Procedure

[0116] After all reagents were prepared according to the methods described above, except for the enzyme, they were equilibrated to room temperature before starting the sample addition.

[0117] a) First, 2.5% DMSO solutions were prepared separately using the pre-prepared 1 $\times$ kinase buffer (DMSO concentrations that were too high could affect the reaction, so the final concentration of DMSO was controlled at 1%). Then, the corresponding 2.5% DMSO solution for the enzyme was used to dilute the test compounds. The screening concentration of the compounds started at 1000 nM, followed by a 4-fold serial dilution, with 8 concentrations in total. Except for the control wells, 4 μL of the diluted test compound solution was added to the reaction wells, and 4 μL of the pre-prepared 2.5% DMSO solution corresponding to the CSF-1R kinase was added to the control wells.

[0118] b) 2 μL of the pre-prepared TK-biotin substrate solution at the corresponding concentration

for the CSF-1R kinase was added to all reaction wells.

[0119] c) 2 μ L of the pre-prepared enzyme solution at the corresponding concentration was added to all reaction wells except the negative control wells. For the negative control wells, 2 μ L of 1 $\times$ kinase buffer corresponding to the enzyme was added to make up the volume. The plate was sealed with sealing film, mixed gently, and incubated at room temperature for 10 min to allow the compounds to fully interact with the enzyme.

[0120] d) 2 μ L of the ATP solution at the corresponding concentration for the enzyme was added to all reaction wells to initiate the kinase reaction. The enzyme reaction time was shown in Table 3.

[0121] e) Five minutes before the end of the kinase reaction, the detection solution was prepared. The detection buffer from the kit was used to prepare detection solutions for Streptavidin-XL665 and TK antibody europium cryptate (1:100) at the appropriate concentrations for the two enzymes.

[0122] f) After the kinase reaction was completed, 5 μ L of the diluted Streptavidin-XL665 solution was added to all reaction wells. After mixing, the diluted TK antibody europium cryptate detection solution was immediately added.

[0123] g) The plate was sealed and mixed, incubated at room temperature for 1 hour, and the fluorescence signal was detected using the ENVISION (PerkinElmer) instrument (excitation at 320 nm, emission at 665 nm and 615 nm). The inhibition rate for each well was calculated based on the full-activity control wells and background signal wells, and the average value of the replicates was taken. The IC₅₀ values for each test compound were fitted using professional graphing and analysis software PRISM 5.0.

[0124] Experimental Procedure as follows:

Table 5

	Kinase Assay	Control	
Enzyme Step (10 μ L)	Sample	Negative	Positive
Compound	4 μ L	4 μ L 2.5% DMSO/kinase buffer	4 μ L 2.5% DMSO/kinase buffer
TK Substrate-biotin	2 μ L	2 μ L	2 μ L
Kinase	2 μ L	2 μ L Kinase buffer	2 μ L
Seal the plate, incubate at room temperature for 10 min			
ATP	2 μ L	2 μ L	2 μ L
Seal the plate, incubate at room temperature for the time shown in Table 3			
Detection Step (10 μ L)			

Sa-XL665	5 μL	5 μL	5 μL
TK Ab-Cryptate	5 μL	5 μL	5 μL
Seal the plate, incubate at room temperature for 1 hour			
Excitation at 320 nm, emission at 665 nm and 615 nm			

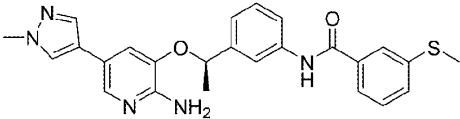
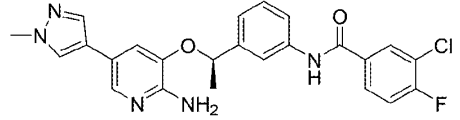
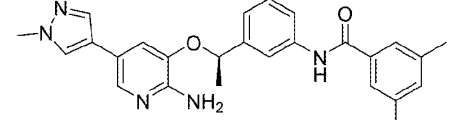
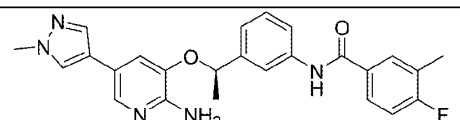
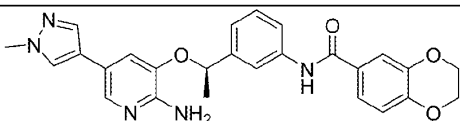
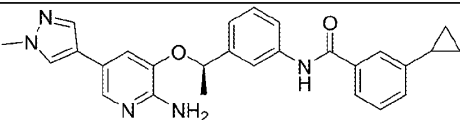
[0125] 2.3 Data Analysis

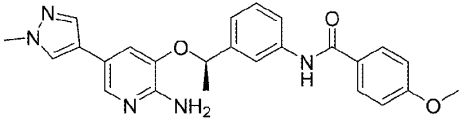
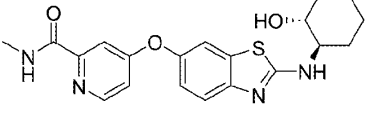
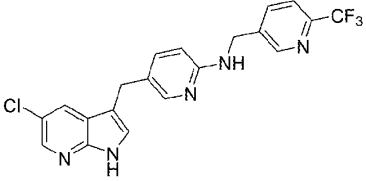
[0126] Emission Ratio (ER)= 665 nm Emission signal/ 615 nm Emission signal

[0127] Inhibition rate = (ER_{positive}-ER_{sample})/(ER_{positive}-ER_{negative})*100%

[0128] IC₅₀ fitting was performed using statistical software.

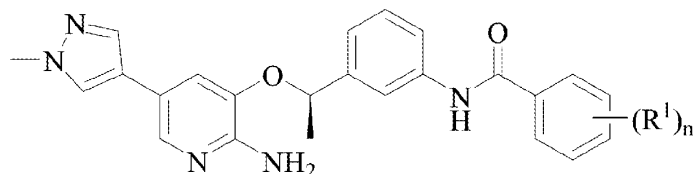
Table 6

NO.	Compound structure	%Inhibition Rate of RTKs			
		CSF-1R		PDGFRα	
		100 nM	10 nM	100 nM	10 nM
1		99.6	79.2	100.2	75.3
2		95.0	72.0	92.1	31.1
3		101.3	70.4	98.8	72.5
4		93.9	68.0	96.2	40.9
5		101.3	96.2	94.3	32.7
6		96.8	69.7	100.5	71.7

7		99.6	80.8	74.3	40.3
Comparative Example 1		IC ₅₀ 1 nM		> 1μM	
Comparative Example 2		IC ₅₀ 20 nM		> 1μM	

What is claimed is:

1. A method for preventing and/or treating sarcoma, comprising administering an effective amount of a CSF-1R/PDGFR multi-target inhibitor to an individual in need thereof.
2. The method according to claim 1, wherein the CSF-1R/PDGFR multi-target inhibitor comprises a compound having the structure as shown in Formula (I), or a pharmaceutically acceptable salt or isotopic derivative thereof:

**Formula (I)**

wherein,

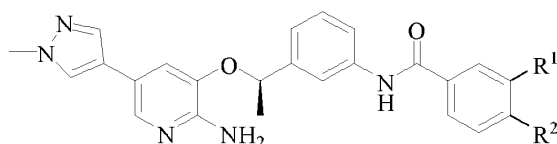
R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, C_3 - C_6 cycloalkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

wherein, n represents 0, 1, 2, 3, or 4;

or when n is 2, the two R^1 groups in the ortho position together with the carbon atom to which they are attached form a 5-6 membered saturated or unsaturated ring, and the ring may optionally contain 0, 1, or 2 heteroatoms selected from O, N, and S; and further, the ring may optionally be substituted by 0, 1, or 2 substituents selected from C_1 - C_6 alkyl or halogen;

wherein, R^a represents hydrogen or C_1 - C_6 alkyl.

3. The method according to claim 1, wherein the compound has a structure as shown below, or a pharmaceutically acceptable salt or isotopic derivative thereof:



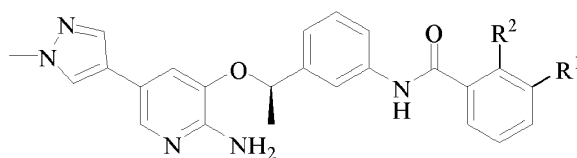
wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;

or R^1 and R^2 together with the carbon atom to which they are attached form a 5-6 membered saturated or unsaturated ring, and the ring may optionally contain 0, 1, or 2 heteroatoms selected from O, N, and S; and further, the ring may optionally be substituted by 0, 1, or 2 substituents selected from C_1 - C_6 alkyl or halogen;

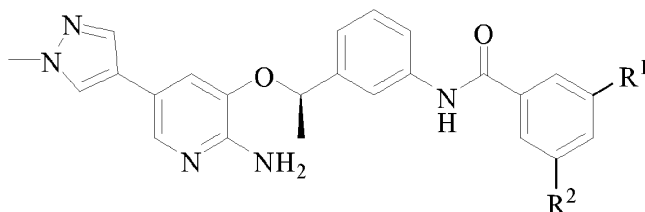
wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

4. The method according to claim 1, wherein the compound has a structure as shown below, or a pharmaceutically acceptable salt or isotopic derivative thereof:



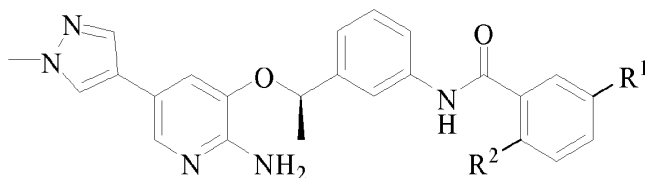
wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 or R^1 and R^2 together with the carbon atom to which they are attached form a 5-6 membered saturated or unsaturated ring, and the ring may optionally contain 0, 1, or 2 heteroatoms selected from O, N, and S; and further, the ring may optionally be substituted by 0, 1, or 2 substituents selected from C_1 - C_6 alkyl or halogen;
 wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

5. The method according to claim 1, wherein the compound has a structure as shown below, or a pharmaceutically acceptable salt or isotopic derivative thereof:



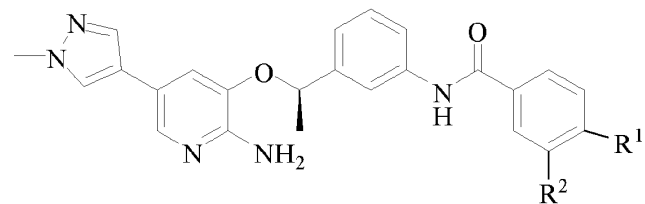
wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

6. The method according to claim 1, wherein the compound has a structure as shown below, or a pharmaceutically acceptable salt or isotopic derivative thereof:



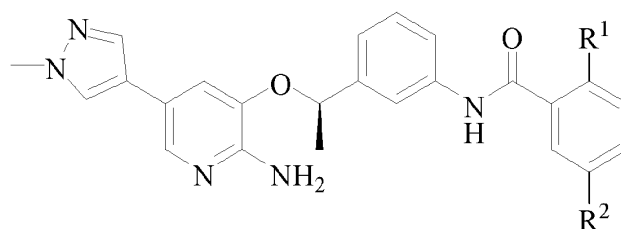
wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

7. The method according to claim 1, wherein the compound has a structure as shown below, or a pharmaceutically acceptable salt or isotopic derivative thereof:



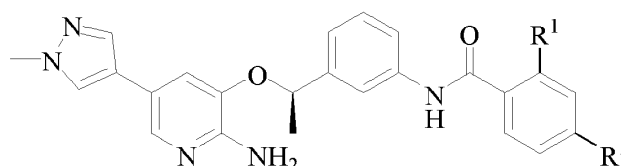
wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 or, R^1 and R^2 together with the carbon atom to which they are attached form a 5-6 membered saturated or unsaturated ring, and the ring may optionally contain 0, 1, or 2 heteroatoms selected from O, N, and S; and further, the ring may optionally be substituted by 0, 1, or 2 substituents selected from C_1 - C_6 alkyl or halogen;
 wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

8. The method according to claim 1, wherein the compound has a structure as shown below, or a pharmaceutically acceptable salt or isotopic derivative thereof:



wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

9. The method according to claim 1, wherein the compound has a structure as shown below, or a pharmaceutically acceptable salt or isotopic derivative thereof:



wherein, R^1 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^2 represents hydrogen, halogen, C_1 - C_6 alkyl, $-OR^a$, $-SR^a$, halogenated C_1 - C_6 alkyl, or cyano;
 wherein, R^a represents hydrogen, C_1 - C_6 alkyl, or halogenated C_1 - C_6 alkyl.

10. The method according to any one of claims 1 to 9, wherein R^1 represents hydrogen, halogen, C_1 - C_3 alkyl, fluoromethyl, $-OR^a$, $-SR^a$; wherein R^a represents hydrogen or C_1 - C_3 alkyl.

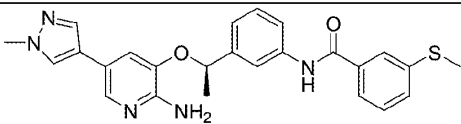
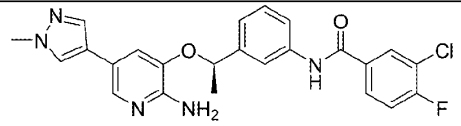
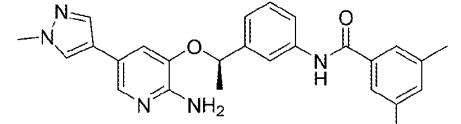
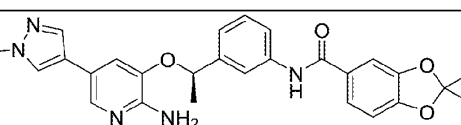
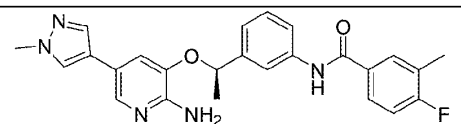
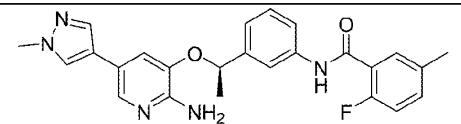
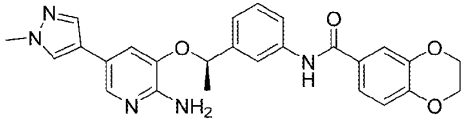
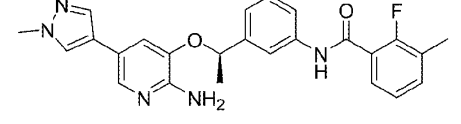
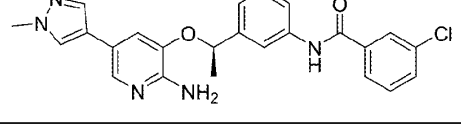
11. The method according to any one of claims 1 to 9, wherein R^2 represents hydrogen, halogen, C_1 - C_3 alkyl, fluoromethyl, $-OR^a$, $-SR^a$; wherein R^a represents hydrogen or C_1 - C_3 alkyl.

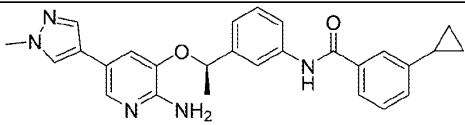
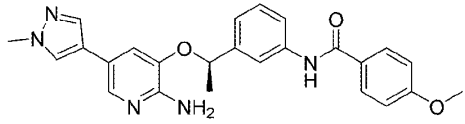
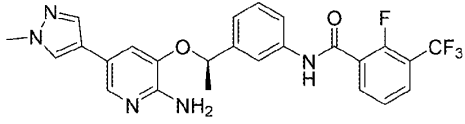
12. The method according to any one of claims 1 to 9, wherein R^1 represents hydrogen, halogen, methyl, trifluoromethyl, methoxy, or $-SCH_3$.

13. The method according to any one of claims 1 to 9, wherein R^1 represents hydrogen, methyl, trifluoromethyl, fluorine, chlorine, or $-SCH_3$.

14. The method according to any one of claims 1 to 9, wherein R² represents hydrogen, methyl, fluorine, or chlorine.

15. The method according to any one of claims 1 to 9, wherein the compound or its pharmaceutically acceptable salt or isotopic derivative thereof is selected from any of the following:

NO.	Structure
1	
2	
3	
4	
5	
6	
7	
8	
9	

10	
11	
12	

16. The method according to any one of claims 1 to 15, wherein the sarcoma is one or more selected from osteosarcoma, soft tissue sarcoma, and hemangioma.