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(54) Benævnelse: **4-(3H-INDOL-5-YL)-N-(PYRIDIN-2-YL)PYRIMIDIN-2-AMINDERIVATER SOM PROTEINKINASEINHIBITORER, FREMSTILLINGSFREMGANGSMÅDE OG MEDICINSK ANVENDELSE DERAF**

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WO-A2-2005/076854
WO-A2-2013/173506
CN-A- 102 007 124
CN-A- 102 264 725
US-A1- 2010 160 340

DESCRIPTION

Cross -reference to related applications

[0001] This application claims the priority of Chinese invention patent application No. CN201510856641.1, filed on November 30, 2015.

Technical Field

[0002] The present invention belongs to the field of medicine, and particularly relates to a series of substituted 2-(pyridin-2-yl)aminopyrimidines having the activity of inhibiting protein kinase, and preparation method and pharmaceutical use thereof.

Background Art

[0003] Cell cycle is an important part of cell vital activity. In normal cell growth, the achievement of cell cycle progression depends on precise and tight regulation of cell cycle by various levels of regulatory factors. The core of these regulatory factors is Cyclin Dependent Kinase (CDK) and its positive and negative regulators, i.e. cyclin and Cyclin Dependent Kinase Inhibitors (CDI). The CDK-Cyclin complex formed by cyclin-dependent protein kinase and cyclin is involved in the growth, proliferation, dormancy, or apoptosis of cells. During the process of cell cycle, cyclin periodically and continuously expresses and degrades, and binds to CDKs that are transiently activated by them, respectively. The phosphorylation of different substrates is catalyzed by CDK activity to realize promotion and conversion of different phases of cell cycle.

[0004] Currently, 13 members of the CDK family have been found, and they are CDK1-CDK13, respectively, in which CDK1, CDK2, CDK3, CDK4 and CDK6 are involved in the regulation of cell proliferation, and CDK7, CDK8, CDK9, CDK11, CDK12 and CDK13 are involved in the regulation of transcription.

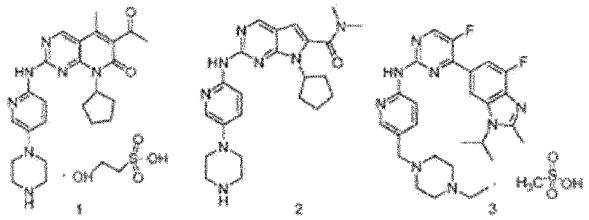
[0005] Cyclin is divided into A-L, and different CDKs connect different subtypes of Cyclin. Among them, the Cyclin D family (Cyclin D1, D2, D3) starts to express in the G1 phase, binds and activates CDK4 and CDK6 to form a CDK4/6-Cyclin D complex, so as to phosphorylate a series of substrates including Retinoblastomaprotein (Rb). After phosphorylation, Rb releases the proteins that are bound to it and are inhibited by it, mainly including transcription factor E2F which activates and transcripts some genes necessary for entering S phase (MA Ke, Advance in Anti-tumor Effect of CDK4/6 Inhibitors, World Notes on Antibiotics, 2013, 34 (5):197-202). If the balance is broken due to various factors, whether signal for promoting cell proliferation being enhanced or signal for inhibiting cell proliferation being decreased to some extent, cell proliferation will be out of control, and then tumor occurs. It has been found in the study that abnormality of the Cyclin D-CDK4/6-INK4-Rb pathway is present in approximately 80% of human cancers (1. Malumbres M, Barbacid M., To cycle or not to cycle: a critical decision in cancer [J]. Nature Reviews Cancer, 2001, 1 (3): 222; 2. Shapiro GI., Cyclin-dependent kinase pathways as targets for cancer treatment [J]. J Clinical Oncology, 2006, 24

(11):1770). The change of this pathway accelerates the process of the G1 phase, such that tumor cells are accelerated in proliferation and gain survival advantage. Therefore, intervention to the pathway has become a therapeutic strategy and thus CDK4/6 has become one of the potential anti-tumor targets.

[0006] The advantages of CDK4/6 as an anti-tumor target lie in that: (1) most proliferating cells rely on CDK2 or CDK4/6 proliferation, but CDK4/6 inhibitors do not exhibit cytotoxicity as "pan-CDK inhibitors", such as myelosuppression and intestinal reaction; and (2) Preclinical experiments show that if the level of Cyclin D in cells is increased or p16INK4a is inactivated, the sensitivity of cells to drug can be increased. Since tumor cells exhibit the aforementioned phenomenon relative to normal cells, targeting of drugs is increased to some extent.

[0007] In addition to the inhibition of tumor growth, CDK inhibitors are also used in the treatment of other disorders, for example, cardiovascular disorders, including atherosclerosis, restenosis after implantation of a vascular stent, and other cardiovascular disorders caused by abnormal cellular proliferation; for example, in the treatment of diseases caused by fungi, protozoan parasites (such as Plasmodium falciparum) and DNA and RNA virus infections, including malaria, AIDS and so on. In addition, it has been further found in the studies that CDK inhibitors can also be used for treating autoimmune diseases (such as psoriasis, rheumatoid arthritis, glomerulonephritis and lupus erythematosus, etc.), and inhibiting the proliferation of inflammatory cells.

[0008] Since WO9811095 discloses a series of 2-pyrimidinamine compounds having cytokine inhibitory activity, a lot of compounds based on such a core structure and having CDK4/6 inhibitory activity have successively appeared in the prior art, and some have become promising candidate drugs, and even entered the phase III clinical trials. For example, compound PD0332991, also known as Palbociclib, which has been disclosed in WO2003062236, is represented by structural formula 1, and developed by Pfizer. PD0332991 has IC_{50}s of 11 nmol/L and 15 nmol/L for inhibiting CDK4 and CDK6, respectively; and its IC_{50} for inhibiting CDK2, CDK1 and CDK5 is greater than 10 μmol/ L (Fry DW, Harvey PJ, Keller PR, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts [J]. Molecular Cancer Therapeutics, 2004, 3 (11):1427). Compound LEE011 that is being developed by Novartis (disclosed by WO2011101409) is represented by structural formula 2. Compound LY2835219 (disclosed by WO2010075074), also known as Bemaciclib, is represented by structural formula 3; it has been reported that its IC_{50}s for inhibiting CDK4 and CDK6 are 2 nmol/L and 9.9 nmol/L, respectively (Lawrence MG, S.F.Cai, X. Lin et al. Preclinical characterization of the CDK4/6 inhibitor LY2835219: in-vivo cell cycle-dependent/independent anti-tumor activities alone/in combination with gemcitabine [J]. Invest New Drugs, (2014), 32: 825). Currently LY2835219 is in phase III clinical trial by Eli Lilly Company.



[0009] Due to the emergence of these compounds, CDK4/6 has become a clear anti-tumor target.

The applicant has also filed a patent application (No. 201510708487.3, filed on October 27, 2015) for a series of new substituted 2-(pyridin-2-yl) aminopyrimidines which exhibit the activity of selectively inhibiting CDK4/6.

[0010] Malignant tumors are still a serious threat to human health. Therefore, it is necessary and urgent to develop CDK4/6 inhibitors with higher activity, selectivity and bioavailability so as to provide more clinical options for the treatment of diseases associated with abnormal cell proliferation, such as cancer.

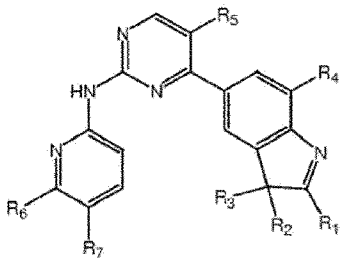
Summary of the Invention

[0011] The invention is set out in the appended set of claims.

[0012] The references to methods of treatment in the subsequent paragraphs of this description are to be interpreted as references to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the human or animal body by therapy.

[0013] In view of the above problems, an object of the present invention is to provide a substituted 2-(pyridin-2-yl)aminopyrimidine compound. The compound provided in the invention can selectively inhibit the cyclin kinase CDK4/6 and stop the cell in G1 phase, and thus can be used for treating cell proliferative disorder.

[0014] In order to achieve the above technical effect, the present invention provides the following technical solutions:
In one aspect, the present invention provides a compound of structural formula I, or a tautomer, a mesomer, a racemate, an enantiomer, a diastereomer, a deuterated compound, a prodrug, or a mixture thereof; or pharmaceutically acceptable salts or solvates of the compound of structural formula I or its tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or a mixture thereof,



I

wherein R_1 is selected from a hydrogen atom, unsubstituted linear or branched C1-C4 alkyl;

wherein R_2 and R_3 are each independently selected from unsubstituted linear or branched C1-C4 alkyl,

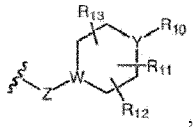
or R_2 and R_3, together with the C atoms to which they are attached respectively, form a saturated or unsaturated 3 to 7 membered ring;

R_4 and R_5 are each independently selected from the group consisting of hydrogen and halogen, and

at least one of R_4 and R_5 is halogen;

R_6 is selected from the group consisting of a hydrogen atom, C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxyl or halogen;

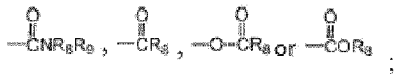
R_7 is



wherein Z is carbonyl, O, S, imino, sulfonyl or

n is an integer from 0 to 4; W and Y are each independently C, N, O or S, but W and Y cannot both be C at the same time, and when Z is O or S, W is C; R_{10}, R_{11}, R_{12} and R_{13} are each independently selected from a hydrogen atom, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, C_1-C_6 hydroxyalkyl, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, hydroxyl, halogen; or

R_6 and R_7 together with the C atoms to which they are attached form a 5 to 7 membered heterocycle containing one or more atoms selected from N, O or S, and the 5 to 7 membered heterocycle is substituted by one or more substituents selected from C_1-C_6 alkyl, C_3-C_6 cycloalkyl, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, C_1-C_6 hydroxyalkyl, hydroxyl, halogen, cyano, $-NH_2$, $-NHR_8$, $-NR_8R_9$,



;

wherein R_5 and R_9 are each independently selected from the group consisting of a hydrogen atom, C_1-C_6 alkyl and C_1-C_6 hydroxyalkyl.

[0015] Most preferably, R_1, R_2 and R_3 are each independently selected from a hydrogen atom, unsubstituted linear or branched C_1-C_4 alkyl.

[0016] As another preferred embodiment, R_2 and R_3, together with the C atoms to which they are both attached, form a saturated or unsaturated 3 to 7 membered ring.

[0017] More preferably, R_2 and R_3, together with the C atoms to which they are both attached, form a saturated 3 to 7 membered ring.

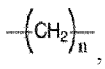
[0018] Preferably, R_4 and R_5 are each independently selected from hydrogen, fluorine or chlorine, and at least one of R_4 and R_5 is fluorine or chlorine.

[0019] More preferably, R_4 and R_5 are each independently hydrogen or fluorine, and at least one of R_4 and R_5 is fluorine.

[0020] Most preferably, R_4 is hydrogen or fluorine, and R_5 is fluorine.

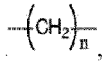
[0021] Preferably, R_6 is selected from a hydrogen atom or C_1-C_6 alkyl.

[0022] Preferably, Z is a carbonyl group, O or



n is an integer from 0 to 4.

[0023] More preferably, Z is



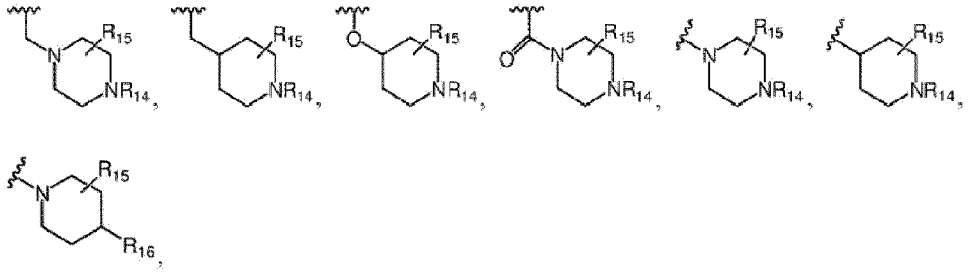
n is an integer from 0 to 2, more preferably, n= 0 or 1.

[0024] Preferably, W and Y are each independently selected from C or N, but W and Y cannot both be C at the same time.

[0025] Preferably, R_{10}, R_{11}, R_{12} and R_{13} are each independently selected from a hydrogen atom, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, C_1-C_6 hydroxyalkyl, C_1-C_6 haloalkyl, C_1-C_6 alkoxy, hydroxyl, or - NR_8R_9, and when Y = N, R_{10} cannot be -NR_8R_9, wherein R_8 and R_9 are each independently selected from a hydrogen atom and C_1-C_4 alkyl.

[0026] More preferably, R_{10}, R_{11}, R_{12} and R_{13} are each independently selected from a hydrogen atom, C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl, C_1-C_6 haloalkyl, C_1-C_6 alkoxy or -NR_8R_9, wherein R_8 and R_9 are independently selected from a hydrogen atom and C_1-C_4 alkyl.

[0027] More preferably, R_7 is selected from the substituents having the following structures:



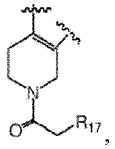
wherein R_{14} and R_{15} are each independently selected from a hydrogen atom, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, C_1-C_6 haloalkyl, C_1-C_6 hydroxyalkyl, C_1-C_6 alkoxy or hydroxyl; R_{16} is selected from a hydrogen atom, C_3-C_6 cycloalkyl, C_1-C_6 haloalkyl, C_1-C_6 hydroxyalkyl, C_1-C_6 alkoxy or hydroxyl.

[0028] More preferably, R_{14} and R_{15} are each independently selected from the group consisting of a hydrogen atom, C_1-C_6 alkyl, C_3-C_6 cycloalkyl, and C_1-C_6 hydroxyalkyl; R_{16} is selected from a hydrogen atom, C_1-C_6 alkyl, C_3-C_6 cycloalkyl or C_1-C_6 hydroxyalkyl.

[0029] As another preferred embodiment, R_6 and R_7 together with the C atoms to which they are attached form a 6-membered heterocycle containing one or more atoms selected from N, O or S.

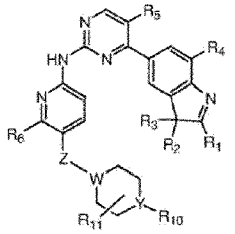
[0030] More preferably, R_6 and R_7 together with the C atoms to which they are attached form a 6-membered heterocycle containing N.

[0031] More preferably, R_6 and R_7 together with the C atoms to which they are attached form the following chemical structure:

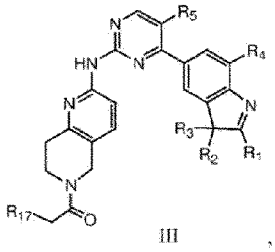


wherein R_{17} is selected from hydroxyl or C_1-C_3 alkoxy; further preferably, R_{17} is hydroxyl.

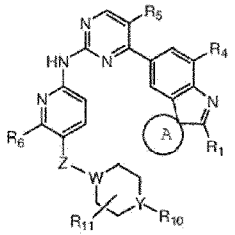
[0032] As a preferred embodiment, the present invention further provides the compounds of structural formula II, III, IV or V, or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug, or mixture thereof; or pharmaceutically acceptable salts or solvates of the compounds of formula II, III, IV or V or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture thereof,



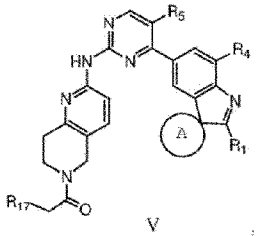
II



III



IV

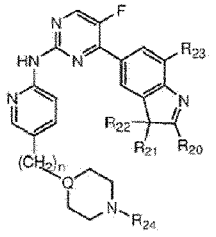


V

wherein Z, W, Y, R_1, R_2, R_3, R_4, R_5, R_6, R_{10}, R_{11} and R_{17} are defined as above, ring A is a saturated 3 to 7 membered ring.

[0033] Preferably, ring A is a saturated 3 to 6 membered ring.

[0034] More preferably, the present invention provides a compound of structural formula VIII, or a tautomer, a mesomer, a racemate, an enantiomer, a diastereomer, a deuterated compound, a prodrug, or a mixture thereof; or pharmaceutically acceptable salts or solvates of the compound of structural formula VIII or its tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture thereof,

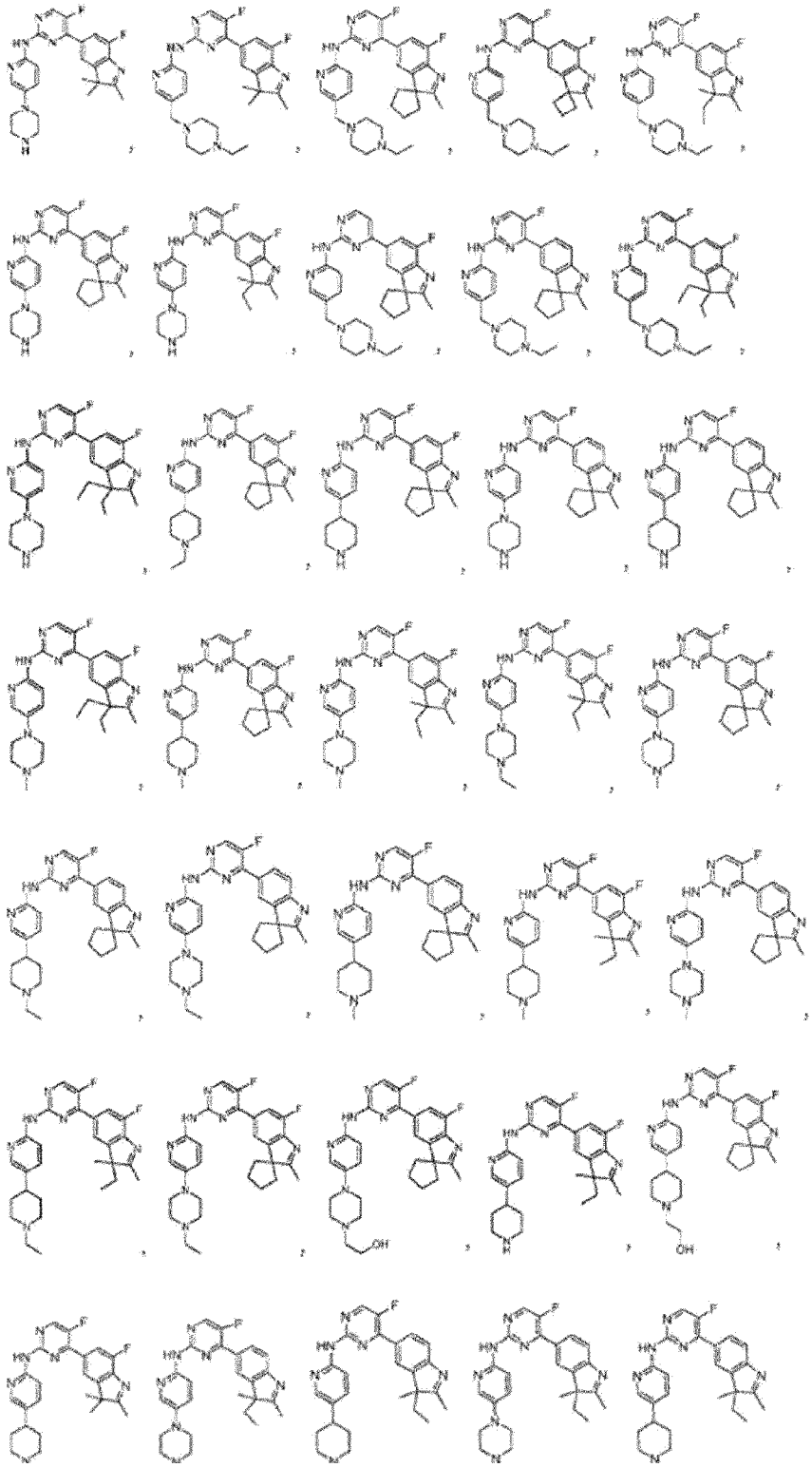


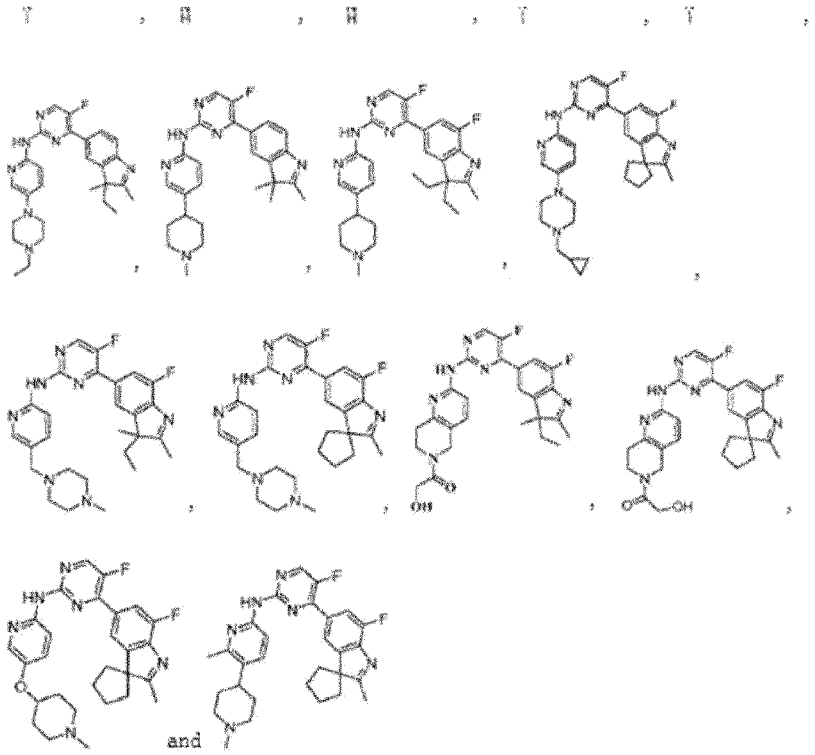
VIII

wherein R_{20}, R_{21}, R_{22} are each independently selected from C_1-C_4 alkyl, or R_{20} is C_1-C_4 alkyl, and R_{21} and R_{22} together with the C atom to which they are attached form a saturated 5 to 6 membered

ring; R_{23} is selected from hydrogen or fluorine; n = 0 or 1; R_{24} is selected from the group consisting of hydrogen, C_1-C_4 alkyl or C_1-C_4 hydroxyalkyl, Q is C or N.

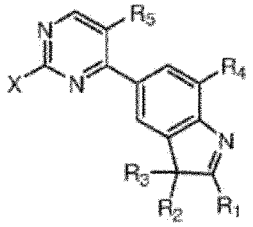
[0035] As a more preferable embodiment, the present invention provides compounds of the following structures, or a tautomer, a mesomer, a racemate, an enantiomer, a diastereomer, a deuterated compound, a prodrug, or a mixture thereof; or pharmaceutically acceptable salts or solvates of said compounds of the structures or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture thereof,





[0036] The compounds according to the present invention also include all the above-mentioned compounds that are isotopically-labeled.

[0037] In another aspect, the present invention further provides a compound of structural formula VI, or a tautomer, a mesomer, a racemate, an enantiomer, a diastereomer, a deuterated compound, a prodrug, or a mixture thereof; or pharmaceutically acceptable salts or solvates of the compound of structural formula VI or its tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug, or a mixture thereof,

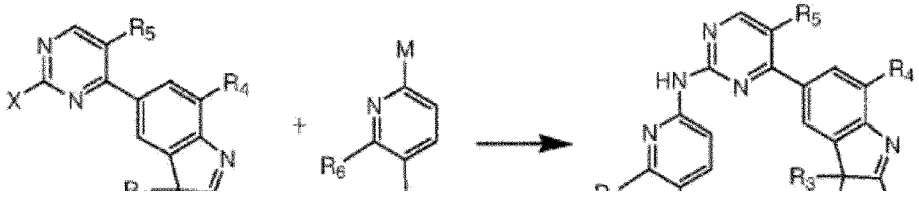


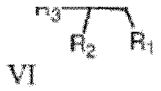
VI

wherein R_1, R_2, R_3, R_4 and R_5 are defined as above, X is a leaving group or an amino group.

[0038] Preferably, X is halogen or amino, more preferably fluorine, bromine, chlorine or amino.

[0039] In another aspect, the present invention provides a method for preparing the compound of structural formula I, comprising carrying out a palladium-catalyzed coupling reaction between a compound of formula VI and a compound of formula VII in a solvent to give a compound of formula I,




VI


VII


I

wherein R_1, R_2, R_3, R_4, R_5, R_6 and R_7 are defined as above; X and M are each independently a leaving group or amino, only one of X and M is amino and one of the two must be amino;

preferably, the leaving group is halogen;

more preferably, the leaving group is fluorine, bromine or chlorine.

[0040] Wherein, the above preparation method may further comprises removing the protective group.

[0041] Wherein, the above preparation method may further comprise product separation and/or purification, and the separation and/or purification may be performed by a method generally used in organic synthesis, for example, a suitable combination of the methods of filtration, extraction, washing, concentration, chromatography and the like.

[0042] In another aspect, the present invention provides use of the compounds of structural formulas I-V and VIII, or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug, or mixture thereof; or pharmaceutically acceptable salts or solvates of the compounds of formulas I-V and VIII or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture thereof, in the manufacture of a pharmaceutical formulation for the treatment of a cell proliferative disorder.

[0043] Preferably, the pharmaceutical formulation comprises a pharmaceutically acceptable excipient.

[0044] Preferably, the cell proliferative disorder refers to cancer of mammal or human, more preferably refers to human cancer, including malignant solid tumors and malignant non-solid tumors, specifically including but not limited to breast cancer, lung cancer, prostate cancer, leukemia, brain cancer, gastric cancer, and glioma.

[0045] Preferably, the cell proliferative disorder may also be AIDS, atherosclerosis, and restenosis after implantation of a vascular stent.

[0046] Preferably, said use refers to the use of the compounds of structural formulas I-V and VIII, or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug, or mixture thereof; or pharmaceutically acceptable salts or solvates of the compounds of formulas I-V and VIII or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture thereof, as the sole active ingredient or in combination with other biologically active substances, in the manufacture of a pharmaceutical formulation for the treatment of a cell proliferative disorder.

[0047] The other biologically active substances include but not limited to anticancer agents, immunosuppressive agents and anti-viral agents; wherein the anticancer agent is selected from

alkylating agent (such as cyclophosphamide, ifosfamide, thiotepa, semustine, mechlorethamine hydrochloride, busulfan, chlorambucil, melphalan, nitrocaphane, formylmelphalan, carmustine, lomustine, altretamine, dibromomannitol, temozolomide, and the like), antimetabolite antineoplastic drugs (such as cytarabine, fluorouracil, methotrexate, hydroxyurea, tegafur, meisoindigo, mercaptopurine and the like), platinum complexing agent (such as cisplatin, carboplatin, oxaliplatin and the like), antibiotic antineoplastic drugs (actinomycin D, mitomycin, doxorubicin, pingyangmycin, epirubicin, pirarubicin, daunorubicin, bleomycin, and the like), naturally-derived antineoplastic drugs (homoharringtonine and its derivatives, vincristine and its derivatives, hydroxycamptothecin and its derivatives, etoposide and its derivatives, vindesine and its derivatives, vinblastine and its derivatives, vinorelbine bitartrate, taxol and its derivatives, colchicine and its derivatives, elemene and its derivatives and the like), hormonal antineoplastic drugs (such as aminoglutethimide, tamoxifen, dexamethasone, dutasteride, flutamide, gonadorelin, leuprolide acetate, letrozole and the like), VEGFR or EGFR inhibitors (such as sunitinib, sorafenib, imatinib, gefitinib, erlotinib, vandetanib, pazopanib, lapatinib, canertinib, afatinib, mubritinib, dasatinib, neratinib and the like), antibody antineoplastic drugs (such as trastuzumab, pertuzumab, rituximab, panitumumab, bevacizumab, ipilimumab, ofatumumab, ramucirumab and the like), mTOR inhibitors (such as everolimus, sirolimus, zotarolimus and the like), and the drugs for treating brain tumor, such as temozolomide and the like.

[0048] In yet another aspect, the present invention provides combination product for treating a cell proliferative disorder, wherein the combination product comprises one or more compounds selected from the compounds of structural formulas I-V and VIII, or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug, or mixture thereof; or pharmaceutically acceptable salts or solvates of the compounds of formulas I-V and VIII or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture thereof.

[0049] Preferably, the combination product further includes pharmaceutically acceptable excipients, and/or the combination product is a kit.

[0050] In another aspect, the present invention further provides a method for treating a cell proliferative disorder, comprising administering to a patient in need thereof, orally or non-orally, an effective amount of the compounds of the present invention or the above-mentioned combination product.

[0051] Preferably, the above method for treating a cell proliferative disorder comprises administering to a patient, orally or non-orally, an effective amount of the compounds of the present invention and said other biologically active substances. Said other biologically active substances include, but not limited to, anticancer agents, immunosuppressive agents and antiviral agents; wherein the anticancer agent is selected from alkylating agent (such as cyclophosphamide, ifosfamide, thiotepa, semustine, mechlorethamine hydrochloride, busulfan, chlorambucil, melphalan, nitrocaphane, formylmelphalan, carmustine, lomustine, altretamine, dibromomannitol, temozolomide and the like), antimetabolite antineoplastic drugs (such as cytarabine, fluorouracil, methotrexate, hydroxyurea, tegafur, meisoindigo, mercaptopurine and the like), platinum complexing agent (such as cisplatin, carboplatin, and oxaliplatin), antibiotic antineoplastic drugs (actinomycin D, mitomycin, doxorubicin, pingyangmycin, epirubicin, pirarubicin, daunorubicin, bleomycin and the like), naturally-derived antineoplastic drugs (homoharringtonine and its derivatives, vincristine and its derivatives,

hydroxycamptothecin and its derivatives, etoposide and its derivatives, vindesine and its derivatives, vinblastine and its derivatives, vinorelbine bitartrate, taxol and its derivatives, colchicine and its derivatives, elemene and its derivatives and the like), hormonal antineoplastic drugs (such as aminoglutethimide, tamoxifen, dexamethasone, dutasteride, flutamide, gonadorelin, leuprolide acetate, letrozole and the like), VEGFR or EGFR inhibitors (such as sunitinib, sorafenib, imatinib, gefitinib, erlotinib, vandetanib, pazopanib, lapatinib, canertinib, afatinib, mubritinib, dasatinib, neratinib and the like), antibody antineoplastic drugs (such as trastuzumab, pertuzumab, rituximab, panitumumab, bevacizumab, ipilimumab, ofatumumab, ramucirumab and the like), mTOR inhibitors (such as everolimus, sirolimus zotarolimus and the like), and the drugs for treating brain tumor, such as temozolomide and the like.

[0052] The oral or non-oral route can be delivery to the patient orally or by injection, patch, spray, and one or more other known routes. The effective amount can include an amount effective to treat, reduce, moderate, alleviate, eliminate one or more symptoms of a condition sought to be treated or alternatively sought to be avoided, or an amount effective to additionally generate clinically identifiable advantageous changes in the condition or its effect.

[0053] In another aspect, the present invention provides a compound for the treatment of a cell proliferative disorder or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug, or mixture thereof; or pharmaceutically acceptable salts or solvates of the compounds of formulas I-V and VIII or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture thereof, wherein the structural formula of the compound is one or more structural formulas selected from the group consisting of the structural formulas I-V and VIII;

preferably, the cell proliferative disorder refers to cancer of mammal or human, more preferably refers to human cancer, including malignant solid tumors and malignant non-solid tumors, specifically including but not limited to breast cancer, lung cancer, prostate cancer, leukemia, brain cancer, glioma, and gastric cancer; and/or

the cell proliferative disorder is one or more diseases selected from the group consisting of AIDS, atherosclerosis, and restenosis after implantation of a vascular stent.

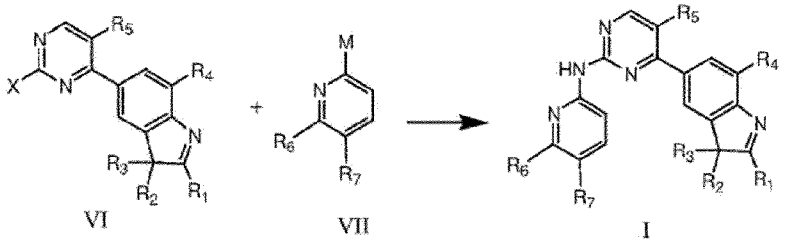
[0054] In the description of the present invention, unless otherwise specified, the "C_1-C_6 alkyl" refers to a linear or branched C_1-C_6 alkyl; the "C_1-C_4 alkyl" refers to a linear or branched C_1-C_4 alkyl, preferably methyl, ethyl, propyl or isopropyl. The "C_1-C_6 alkoxy" refers to a C_1-C_6 linear or branched alkoxy, preferably a C_1-C_4 linear or branched alkoxy, more preferably methoxy, ethoxy, propoxy or 2-methylethoxy. The "C_3-C_6 cycloalkyl" refers to an unsubstituted C_3-C_6 cycloalkyl or C_3-C_6 cycloalkyl substituted by C_1-C_4 alkyl and/or C_1-C_4 alkoxy, preferably unsubstituted C_3-C_6 cycloalkyl or C_3-C_6 cycloalkyl substituted by C_1-C_4 alkyl and/or C_1-C_4 alkoxy, more preferably cyclopropyl, cyclobutyl, methylcyclopropyl, cyclopentyl or cyclohexyl. The "halogen" refers to bromine, chlorine or fluorine. The "C_1-C_6 haloalkyl" refers to linear or branched C_1-C_6 alkyl substituted with bromine, chlorine, or fluorine, preferably linear or branched C_1-C_4 alkyl substituted with chlorine or fluorine, more preferably monofluoromethyl, difluoromethyl, trifluoromethyl, monochloromethyl, dichloromethyl, trichloromethyl, 1-fluoroethyl, 1-chloropropyl, 1-chloroethyl, and 1-chloropropyl.

[0055] Existing research suggests that the toxicity of inhibitors of CDKs is mainly related to their inhibition of CDK1 and other protein kinases, such as Pim-1, a threonine/serine kinase encoded by a protooncogene of the same name. Therefore, as CDK inhibitor compounds, they are expected to have more significant difference between the effect on CDK4/CDK6 and that on CDK1 and other kinases, that is, selective inhibition of CDK4/CDK6. The compounds provided by the present invention are superior to or comparable in activity to LY2835219, a candidate currently being in phase III clinical trials, and some compounds exhibit better kinase selectivity. Moreover, the preferred compound (prepared in Example 17) is well absorbed orally and has good blood-brain distribution. The above results indicate that the compounds of the present invention are promising to be developed into new drugs for the treatment of diseases associated with cell proliferation, especially malignant tumors, especially brain cancer.

Detailed Description of the Invention

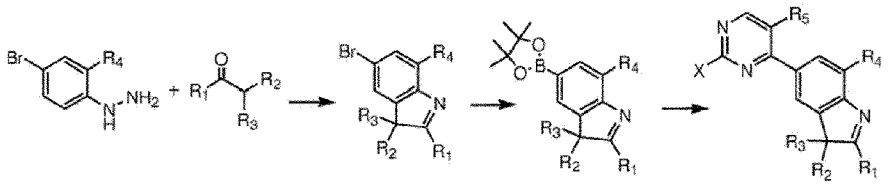
[0056] The present invention is described below with reference to specific examples. It will be understood by a person skilled in the art that these examples are merely illustrative of the invention and are not intended to limit the scope of the invention in any way.

[0057] The compounds of formula VI of the invention are key intermediates for the synthesis of the compounds of formula I, and they are subjected to a palladium-catalyzed coupling reaction with the compounds of formula VII in a solvent to give the compounds of formula I.



VI VII I

[0058] The compounds of formula VI can be synthesized by the following reaction scheme:



VI

wherein R_1, R_2, R_3, R_4, R_5, R_6 and R_7 are defined as above; X is a leaving group or amino group.

[0059] Preferably, R_1, R_2 and R_3 are each independently selected from a hydrogen atom, unsubstituted C_1-C_6 hydrocarbon group, or a C_1-C_6 hydrocarbon group substituted by one or more substituents selected from C_1-C_6 hydrocarbon group, hydroxyl, or halogen.

[0060] More preferably, R_1, R_2 and R_3 are each independently selected from a hydrogen atom, unsubstituted linear or branched C_1-C_6 alkyl, unsubstituted linear or branched C_2-C_4 alkenyl.

[0061] Most preferably, R_1, R_2 and R_3 are each independently selected from a hydrogen atom, unsubstituted linear or branched C_1-C_4 alkyl.

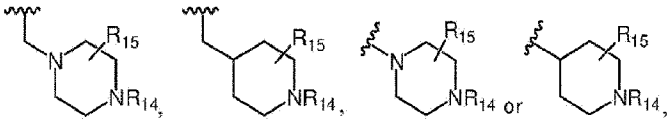
[0062] Alternatively, as another preferred mode, R_1 is defined as above, and R_2 and R_3 together with the C atom to which they are attached form a saturated or unsaturated 3 to 7 membered ring; more preferably, R_2 and R_3 together with the C atom to which they are attached form a saturated 3 to 7 membered ring.

[0063] Preferably, R_4 and R_5 are each independently hydrogen or fluorine, and at least one of R_4 and R_5 is fluorine.

[0064] X is preferably halogen or amino, more preferably fluorine, bromine, chlorine or amino.

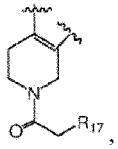
[0065] R_6 is preferably a hydrogen atom or C_1-C_4 alkyl.

[0066] R_7 is preferably a substituent of the following structure:



[0067] Wherein, R_{14} and R_{15} are each independently selected from the group consisting of a hydrogen atom, C_1-C_4 alkyl and C_1-C_4 hydroxyalkyl.

[0068] Alternatively, as another preferred embodiment, R_6 and R_7 together with the C atom to which they are attached form a chemical structure as follows:



wherein, R_{17} is selected from hydroxyl or C_1-C_3 alkoxy; more preferably, hydroxyl.

[0069] Unless otherwise specified, all of the experimental methods in the following examples are conventional methods. Unless otherwise specified, the chemical raw materials, reagents and the like used in the following examples are commercially available products.

[0070] Abbreviations and their meanings appearing in the examples of the present invention are given as follows:

PE: petroleum ether

EA: Ethyl acetate

DCM: dichloromethane

MeOH: methanol

Pd (dppf)Cl_2: [1,1'-bis (diphenylphosphino) ferrocene]palladium dichloride

Pd (PPh_3)$_4$: tetrakis (triphenylphosphine) palladium

$Pd_2(dba)_3$: Tris(dibenzylideneacetone)dipalladium

$NaHB(OAc)_3$: sodium triacetoxyborohydride

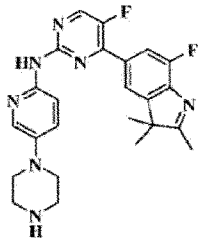
LHMDS: lithium hexamethyldisilazide

DAPI: DAPI fluorescent dye

Example 1

5-fluoro-4-(7-fluoro-2,3,3-trimethyl-3H-indol-5-yl)-N-(5-(piperazin-1-yl)pyridin-2-yl)pyri midine-2-amino

[0071]



Step 1: 4-(6-nitropyridin-3-yl)piperazine-1-carboxylic acid t-butyl ester

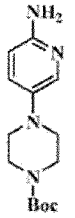
[0072]

[0073] A reaction flask was charged with 5-bromo-2-nitropyridine (5.0 g, 24.63 mmol), piperazine-1-carboxylic acid t-butyl ester (5.04 g, 27.09 mol), acetonitrile (30 mL) and diisopropylethylamine (4.77 g, 36.94 mmol). The mixture was allowed to react under refluxing for 2 h. The reaction product was subjected to rotary evaporation to remove solvent, and separated by column chromatography (PE/EA = 1:1 to DCM/MeOH = 20:1) to obtain the titled compound (3.8 g, yellow solid).

[0074] MS (ESI): mass calcd. for $C_{14}H_{20}N_4O_4$ 308.1, m/z found 309.1 $[M+H]^+$.

Step 2: 4-(6-aminopyridin-3-yl) piperazine-1-carboxylic acid t-butyl ester

[0075]

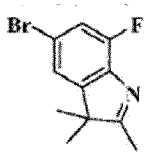


[0076] A reaction flask was charged with 4-(6-nitropyridin-3-yl)piperazine-1-carboxylic acid t-butyl ester (0.92 g, 3.0 mmol) prepared in Step 1, ethyl acetate/methanol (10 mL/10 mL) and Pd/C (0.1 g), and introduced with hydrogen gas. The reaction was carried out at room temperature for 2 h. The reaction product was filtered, and concentrated to obtain the titled compound (792 mg, off-white solid).

[0077] MS (ESI): mass calcd. for $C_{14}H_{22}N_4O_2$ 278.2, m/z found 279.2 $[M+H]^+$.

Step 3: Preparation of 5-bromo-7-fluoro-2,3,3-trimethyl-3H-indole

[0078]



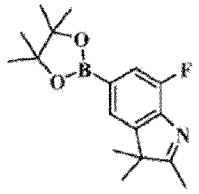
[0079] A reaction flask was charged with (4-bromo-2-fluorophenyl)hydrazine hydrochloride (1.0 g, 4.14 mmol), acetic acid (10 ml), and 3-methyl-2-butanone (0.32 g, 4.14 mmol). The mixture was allowed to react under refluxing for 5 h. The reaction product was subjected to rotary evaporation to remove solvent, added with 20 ml of water, and extracted with ethyl acetate three times (20 ml for each time). The combined organic phase was washed once with 25 ml of saturated sodium chloride aqueous solution, dried with anhydrous sodium sulfate, filtered, subjected to rotary evaporation, and separated by column chromatography (DCM: MeOH = 50:1 to 25:1) to obtain the titled compound (420 mg, yellow solid).

[0080] ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.23-7.21 (m, 2H), 2.30 (s, 3H), 1.32 (s, 6H).

[0081] MS (ESI): m/z 258.0 $[M+H]^+$.

Step 4: Preparation of 7-fluoro-2,3,3-trimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan -2-yl)-3H-indole

[0082]

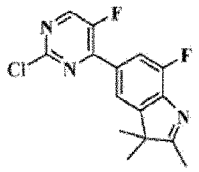


[0083] A reaction flask was charged with 5-bromo-7-fluoro-2,3,3-trimethyl-3H-indole (400.0 mg, 1.56 mmol) prepared in Step 3, 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bis(1,3,2-dioxaborolane) (436.5 mg, 1.71 mmol), potassium acetate (306.3 mg, 3.12 mmol), dixoane (10 ml), and $Pd(dppf)Cl_2$ (228.7mg,0.32mmol). The mixture was heated to 90°C under protection of nitrogen gas, and allowed to react overnight. The reaction product was cooled to room temperature, filtered, added with 10 ml of water, and extracted with ethyl acetate three times (20 ml for each time). The organic phase of ethyl acetate was combined, washed with 25 ml of saturated salt solution once, dried with anhydrous sodium sulfate, filtered, concentrated and separated by silica gel column chromatography (DCM: MeOH = 50:1-30:1) to obtain the titled compound (306.5 mg, yellow oil).

[0084] MS (ESI): m/z 304.1 $[M+H]^+$.

Step 5: Preparation of 5-(2-chloro-5-fluoropyrimidin-4-yl)-7-fluoro-2,3,3-trimethyl-3H-indole

[0085]

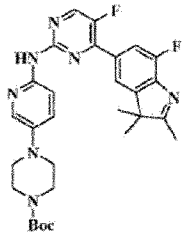


[0086] A microwave reaction flask was charged with 7-fluoro-2,3,3-trimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-indole (300 mg, 0.99 mmol) prepared in step 4, 2,4-dichloro-5-fluoropyrimidine (181.8 mg, 1.08 mmol), potassium phosphate (419.8 mg, 1.98 mmol), dioxane/water (4 mL/1 mL) and Pd $(PPh_3)_4$ (114.5 mg, 0.09 mmol). Microwave reaction was carried out under the protection of nitrogen gas at 130°C for 1 h. The reaction mixture was cooled to room temperature, filtered, added with 10 mL of water, and extracted with dichloromethane three times (15 ml for each time). The organic phases were combined, washed with 20 ml of saturated sodium chloride aqueous solution once, then dried with anhydrous sodium sulfate, filtered, subjected to rotary evaporation, and separated by silica gel column chromatography (DCM: MeOH = 100:1-50:1) to obtain the titled compound (301.2 mg, yellow solid).

[0087] MS (ESI): mass calcd. for $C_{15}H_{12}ClF_2N_3$ 307.1, m/z found 308.1 $[M+H]^+$.

Step 6: Preparation of 4-(6-((5-fluoro-4-(7-fluoro-2,3,3-trimethyl-3H-indol-5-yl)pyrimidin -2-yl)amino)pyridin-3-yl)piperazine-1-carboxylic acid t-butyl ester

[0088]

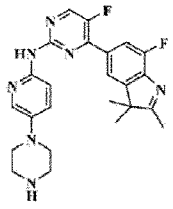


[0089] A reaction flask was charged with 5-(2-chloro-5-fluoropyrimidin-4-yl) -7-fluoro-2,3,3-trimethyl-3H-indole (150.0 mg, 0.48 mmol) prepared in Step 5, 4-(6-aminopyridin-3-yl) piperazine-1-carboxylic acid t-butyl ester (135.8 mg, 0.48 mmol) prepared in Step 2, cesium carbonate (371.6 mg, 0.96 mmol), dioxane (3 ml), $Pd_2(dba)_3$ (44.7 mg, 0.05 mmol), and 4,5-bis (diphenylphosphino)-9,9-dimethylxanthene (30.4 mg, 0.05 mmol). The mixture was heated to 150°C under the protection of nitrogen gas to conduct microwave reaction for 1h. The reaction product was cooled to room temperature, filtered, added with 10 ml of water, and extracted with dichloromethane three times (10 ml for each time). The organic phases were combined, washed with 30 ml of saturated sodium chloride aqueous solution once, dried with anhydrous sodium sulfate, filtered, concentrated and separated by silica gel column chromatography (DCM/MeOH = 50:1) to obtain the titled compound (53.4 mg, yellow solid).

[0090] MS (ESI): mass calcd. for $C_{29}H_{33}F_2N_7O_2$ 549.3, m/z found 550.3 $[M+H]^+$.

Step 7: Preparation of 5-fluoro-4-(7-fluoro-2,3,3-trimethyl-3H-indol-5-yl)-N-(5-(piperazin -1-yl)pyridin-2-yl)pyrimidine-2-amino trifluoroacetate

[0091]



[0092] A reaction flask was charged with 4-(6-((5-fluoro-4-(7-fluoro-2,3,3-trimethyl-3H-indol -5-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazine-1-carboxylic acid t-butyl ester (30.0 mg, 0.054 mmol) prepared in Step 6, dichloromethane (4 ml) and trifluoroacetic acid (1 ml), and the mixture was stirred at room temperature for 2 h. The reaction product was subjected to rotary evaporation to

remove the solvent, adjusted to pH 8 with saturated sodium bicarbonate aqueous solution, and extracted with dichloromethane three times (5 ml for each time). The organic phases were combined, washed with 10 ml saturated sodium chloride aqueous solution once, dried with anhydrous sodium sulfate, filtered, subjected to rotary evaporation, and separated by silica gel column chromatography (DCM/MeOH = 20:1) to obtain the titled compound (10.5 mg, yellow solid).

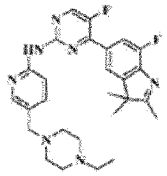
[0093] ^{1}H-NMR(400MHz,DMSO-d_6) δ9.75(br s, 1H), 8.65(d, 1H, J=3.2Hz), 8.02-7.94(m, 3H), 7.82(d, 1H, J=10.8Hz), 7.43(d, 1H, J=8.8Hz), 3.09-3.02(m, 4H), 2.84-2.83(m, 4H), 2.31(s, 3H), 1.34(s, 6H).

[0094] MS(ESI): mass calcd.for $C_{24}H_{25}F_2N_7$ 449.50,m/z found 450.2$[M+H]^+$.

Example 2

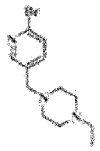
N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(7-fluoro-2,3,3-trimethyl-3H-indol-5-yl)pyrimidine-2-amino

[0095]



Step 1: 1-((6-bromopyridin-3-yl)methyl)-4-ethylpiperazine

[0096]

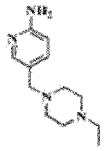


[0097] 2-bromo-5-formylpyridine (1.5 g, 8.15 mmol), 1-ethylpiperazine (0.93 g, 8.15 mmol), and dichloromethane (15 mL) were added to the reaction flask, and then $NaHB(OAc)_3$ (2.58 g, 12.23 mmol) was added in batches. Reaction was carried out at room temperature overnight. The reaction product was filtered, concentrated and separated by column chromatography (DCM/MeOH = 100:1 to 10:1) to obtain the titled product (1.64g, yellow oil).

[0098] MS (ESI): mass calcd. for $C_{12}H_{18}BrN_3$ 285.1, m/z found 286.1 $[M+H]^+$.

Step 2: 5-((4-ethylpiperazin-1-yl)methyl)pyridine-2-amino

[0099]

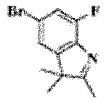


[0100] A reaction flask was charged with 1-((6-bromopyridin-3-yl)methyl)-4-ethylpiperazine (2.84 g, 10 mmol) prepared in Step 1, 2-(dicyclohexylphosphino)biphenyl (700mg, 2mmol), $Pd_2(dba)_3$ (915 mg, 1 mmol) and toluene (30 mL), LHMDS (1 N) (20 ml, 20 mmol) was added under the protection of nitrogen gas. The mixture was heated to 80°C and allowed to react overnight, then cooled to room temperature, filtered, concentrated and separated by column chromatography (DCM/MeOH = 100:1-10:1) to give 1.52 g of the titled product (brown solid).

[0101] MS (ESI): mass calcd. for $C_{13}H_{21}N_3$ 220.2, m/z found 221.2 $[M+H]^+$.

Step 3: Preparation of 5-bromo-7-fluoro-2,3,3-trimethyl-3H-indole

[0102]

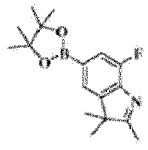


[0103] (4-Bromo-2-fluorobenzene)hydrazine (900.0 mg, 3.73 mmol), acetic acid (5 mL) and 3-methylbutan-2-one (353.3 mg, 4.09 mmol) were added to the reaction flask. The mixture was allowed to react under refluxing for 5 h. The reaction product was subjected to rotary evaporation to remove solvent, added with 10 ml of water, and extracted with ethyl acetate three times (20 ml for each time). The organic phases were combined, washed with 25 ml of saturated sodium chloride aqueous solution once, dried with anhydrous sodium sulfate, filtered and subjected to rotary evaporation. The residue was separated by silica gel column chromatography (ethyl acetate: petroleum ether = 1: 50-1: 25) to give 910 mg of the titled compound (yellow solid).

[0104] MS (ESI): m/z 258.0 $[M+H]^+$.

Step 4: Preparation of 7-fluoro-2,3,3-trimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan -2-yl)-3H-indole

[0105]

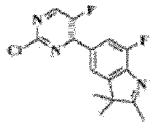


[0106] 5-Bromo-7-fluoro-2,3,3-trimethyl-3H-indole (1.0 g, 3.91 mmol) prepared in Step 3, 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bis(1,3,2-dioxaborolane) (1.09 g, 4.29 mmol), potassium acetate (770 mg, 7.82 mmol), dioxane (10ml), $Pd(dppf)Cl_2$ (570 mg, 0.78 mmol) were added to a reaction flask, and heated to 90°C under the protection of nitrogen gas to react overnight. The reaction product was cooled to room temperature, filtered, diluted with 10 ml of water, and extracted with ethyl acetate three times (20ml for each time). The organic phases were combined, washed once with 25 ml of saturated salt solution, dried with sodium sulfate, filtered, subjected to rotary evaporation, and separated by silica gel column chromatography (EA: PE = 1:100-1:20) to give 1.02 g of the titled compound (yellow oil).

[0107] MS (ESI): m/z 304.2 $[M+H]^+$.

Step 5: Preparation of 5-(2-chloro-5-fluoropyrimidin-4-yl)-7-fluoro-2,3,3-trimethyl-3H -indole

[0108]

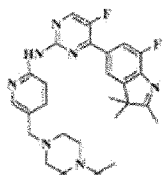


[0109] A microwave reaction flask was charged with 7-fluoro-2,3,3-trimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-indole (1.0 g, 3.30 mmol) prepared in Step 4, 2,4-dichloro-5-fluoropyrimidine (610 mg, 3.63 mmol), potassium phosphate (1.39 g, 6.60 mmol), dioxane/water (8mL/2mL), and $Pd(PPh_3)_4$ (380mg, 0.33mmol). Microwave reaction was carried out at 130°C under the protection of nitrogen gas for 1 h. The reaction product was cooled to room temperature, filtered, added with 10ml of water, extracted three times with dichloromethane (15 ml for each time). The organic phases were combined, washed once with 20 ml of saturated salt solution, dried with anhydrous sodium sulfate, filtered, concentrated and separated by silica gel column chromatography (EA: PE = 1:50 to 1:10) to give the titled compound (290.0 mg, yellow solid).

[0110] MS (ESI): m/z 308.1 $[M+H]^+$.

Step 6: Preparation of N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(7-fluoro-2,3,3-trimethyl-3H-indol-5-yl)pyrimidine-2-amino

[0111]



[0112] A reaction flask was charged with 5-(2-chloro-5-fluoropyrimidin-4-yl)-7-fluoro -2,3,3-trimethyl-3H-indole (290.0 mg, 0.94 mmol) prepared in step 5, 5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-amino (228.6 mg, 1.04 mmol) prepared in Step 2, potassium phosphate (400.5 mg, 1.88 mmol), 10 ml of dioxane, $Pd_2(dba)_3$ (86.4 mg, 0.09 mmol), and 4,5-bis (diphenylphosphino)-9,9-dimethylxanthene (109.2 mg, 0.19 mmol). Microwave reaction was carried out at 150°C under the protection of nitrogen gas for 1 h. The reaction product was cooled to room temperature, filtered, added with 10ml of water, extracted three times with dichloromethane (10 ml for each time). The organic phases were combined, washed once with 30 ml of saturated salt solution, dried with anhydrous sodium sulfate, filtered, subjected to rotary evaporation to remove solvent, and separated by silica gel column chromatography (dichloromethane: methanol = 30:1) to give the titled compound (140.3 mg, y ellow solid).

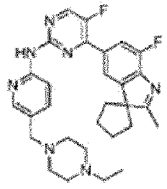
[0113] ^{1}H-NMR(400MHz,$CDCl_3$) δ8.72(s,1H), 8.49(d,1H,J=3.2Hz), 8.38(d,1H,J=8.4Hz), 8.31(s,1H), 7.92(s,1H), 7.89(s,1H), 7.73(d,1H,J=8.4Hz), 3.52(s,2H), 2.54-2.41(m,10H), 2.38(s,3H), 1.40(s,6H), 1.10(t,3H,J=7.2Hz).

[0114] MS(ESI):m/z 492.2$[M+H]^+$.

Example 3

N-(5-((4-ethylpiperazin-1-yl)methylpyridin-2-yl)-5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1, 3'-indol]-5'-yl)pyrimidine-2-amino

[0115]



[0116] The titled compound was obtained by the steps similar to those of Example 2.

[0117] ^{1}H-NMR(40OMHz,$CDCl_3$) δ9.75(s,1H), 8.54(d, 1H, J=3.2Hz), 8.38-8.37(m, 2H), 7.94(s, 1H), 7.87(d, 1H, J=10.8Hz), 7.68(d, 1H, J=8.4Hz), 3.49(s, 2H), 2.99-2.39(m, 10H), 2.37(s, 3H), 2.14-2.08(m, 6H), 1.87-1.84(m, 2H), 1.06(t,3H,J = 6.4Hz).

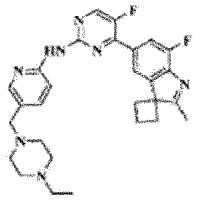
[0118] MS(ESI):m/z 518.3$[M+H]^+$.

Example 4

N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(7'-fluoro-2'-methylspiro[cycl

obutane-1,3'-indol]-5'-yl) aminopyrimidine-2-amino

[0119]



[0120] The titled compound was obtained by the steps similar to those of Example 2.

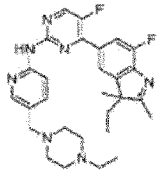
[0121] ^{1}H-NMR(400MHz,$CDCl_3$) δ8.36-8.32(m, 2H), 8.23(s, 1H), 8.04(s, 1H), 7.78-7.75(m, 2H), 7.71(d, 1H, J=8.4Hz), 5.84-5.83(m, 1H), 5.63-5.62(m, 1H), 4.39-4.38(m, 1H), 3.66-3.64(m, 1H), 3.51(s, 2H), 3.06-3.00(m, 1H), 2.71-2.38(m, 11H), 1.55(s, 3H), 1.11(t, 3H, J=7.2Hz).

[0122] MS(ESI):m/z 504.3$[M+H]^+$

Example 5

4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-N-(5-((4-ethylpiperazin-1-yl)methyl)pyrid in-2-yl)-5-fluoropyrimidine-2-amino

[0123]



[0124] The titled compound was obtained by the steps similar to those of Example 2.

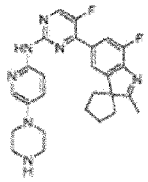
[0125] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.71(s, 1H), 8.49(d, 1H, J=3.6Hz), 8.38(d, 1H, J=8.4Hz), 8.31(s, 1H), 7.94(d, 1H, J=11.2Hz), 7.86(s, 1H), 7.72(dd, 1H, J=8.0, 1.2Hz), 3.52(s, 2H), 2.53-2.41(m, 10H), 2.34(s, 3H), 2.06-1.93(m, 1H), 1.91-1.84(m, 1H), 1.39(s, 3H), 1.09(t, 3H, J=7.2Hz), 0.50(t, 3H, J=7.2Hz).

[0126] MS(ESI):m/z 506.3$[M+H]^+$.

Example 6

5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimidine-2-amino

[0127]



[0128] The titled compound was obtained by the steps similar to those of Example 1.

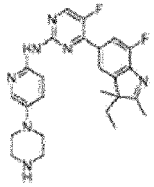
[0129] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.67(br s, 1H), 8.44(d, 1H, J=3.6Hz), 8.29(d, 1H, J=9.2Hz), 8.11(d, 1H, J=2.4Hz), 7.95(s, 1H), 7.89(d, 1H, J=10.8Hz), 7.36(dd, 1H, J=9.2, 2.8Hz), 3.12-3.06(m, 8H), 2.39(s, 3H), 2.16-2.10(m, 6H), 1.89-1.86(m, 2H).

[0130] MS(ESI):m/z 476.2$[M+H]^+$

Example 7

4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimidine-2-amino

[0131]



[0132] The titled compound was obtained by the steps similar to those of Example 1.

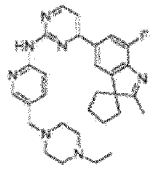
[0133] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.33(br s, 1H), 8.46(d, 1H, J=3.6Hz), 8.28(d, 1H, J=8.8Hz), 8.16(d, 1H, J=2.4Hz), 7.90(d, 1H, J=10.8Hz), 7.82(s, 1H), 7.35(dd, 1H, J=8.8Hz, 2.8Hz), 3.10-3.03(m, 8H), 2.31(s, 3H), 2.03-1.80(m, 3H), 1.36(s, 3H), 0.47(t, 6H, J=7.6Hz).

[0134] MS(ESI):m/z 464.2$[M+H]^+$.

Example 8

N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)pyrimidine-2-amino

[0135]



[0136] The titled compound was obtained by the steps similar to those of Example 2.

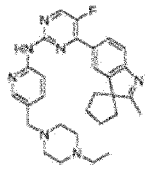
[0137] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.83(s, 1H), 8.61(d, 1H, J=5.2Hz), 8.49(d, 1H, J=8.8Hz), 8.32(d, 1H, J=1.2Hz), 7.91(d, 1H, J=1.2Hz), 7.78(d, 1H, J=10.8Hz), 7.78(dd, 1H, J=8.4, 1.6Hz), 7.21(d, 1H, J=5.2Hz), 3.52(s, 2H), 2.54-2.41(m, 10H), 2.38(s, 3H), 2.19-2.08(m, 6H), 1.90-1.87(m, 2H), 1.10(t, 3H, J=6.8Hz).

[0138] MS(ESI):m/z 500.3$[M+H]^+$.

Example 9

N-(5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-yl)-5-fluoro-4-(2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)pyrimidine-2-amino

[0139]



[0140] The titled compound was obtained by the steps similar to those of Example 2.

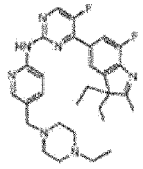
[0141] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.73(br s, 1H), 8.47(d, 1H, J=3.6Hz), 8.43(d, 1H, J=8.4Hz), 8.30(s, 1H), 8.15-8.13(m, 2H), 7.70-7.64(m, 2H), 3.52(s, 2H), 2.53-2.37(m, 10H), 2.31(s, 3H), 2.21-2.06(m, 6H), 1.89-1.86(m, 2H), 1.10(t, 3H, J=7.2Hz).

[0142] MS(ESI):m/z 500.3$[M+H]^+$.

Example 10

4-(3,3-diethyl-7-fluoro-2-methyl-3H-indol-5-yl)-N-(5-((4-ethylpiperidin-1-yl)methyl)pyrid in-2-yl)-5-fluoropyrimidine-2-amino

[0143]



[0144] The titled compound was obtained by the steps similar to those of Example 2.

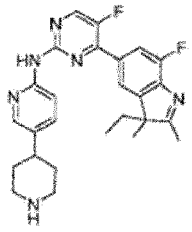
[0145] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.19(br s, 1H), 8.53(s, 1H), 8.39-8.34(m, 2H), 7.95(d, 1H, J=11.2Hz), 7.83(s, 1H), 7.72(d, 1H, J=8.0Hz), 3.51(s, 2H), 2.52-2.41(m, 10H), 2.31(s, 3H), 2.06-2.01(m, 2H), 1.90-1.85(m, 2H), 1.08(t, 3H, J=6.8Hz), 0.46(t, 6H, J=6.8Hz).

[0146] MS(ESI):m/z 520.3$[M+H]^+$.

Example 11

4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-(piperidin-1-yl)pyridin-2-yl)pyrimidine-2-amino

[0147]



<u>Step 1:</u> 6-Nitro-3',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylic acid t-butyl ester

[0148]

[0149] A reaction flask was charged with 5-bromo-2-nitropyridine (20.3 g, 0.1 mol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylic acid t-butyl ester (31 g, 0.1 mol), dioxane/water (250 mL/30 mL), cesium carbonate (66 g, 0.2 mol) and $Pd(dppf)Cl_2$ (7.33 g, 0.01 mol), and protected by nitrogen gas. The mixture was heated to 85°C for 12 h. The reaction product was cooled to room temperature, concentrated and separated by column chromatography (PE/EA = 1:1 to DCM/MeOH = 20:1) to give the title product (11 g, yellow solid).

[0150] MS (ESI): mass calcd. for $C_{15}H_{19}N_3O_4$ 305.1, m/z found 306.1 $[M+H]^+$.

Step 2: 4-(6-Aminopyridin-3-yl)piperidine-1-carboxylic acid t-butyl ester

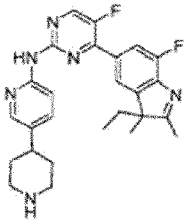
[0151]

[0152] A reaction flask was charged with 6-nitro-3',6'-dihydro-[3,4'-bipyridine]-1'(2'H)-carboxylic acid t-butyl ester (0.9 g, 3.0 mmol) prepared in Step 1, ethyl acetate/methanol (10 mL/10 mL) and Pd/C (0.1 g). Hydrogen was introduced thereinto and the reaction was carried out at room temperature for 2 h. The reaction product was filtered and concentrated to obtain the titled product (790 mg, off-white solid).

[0153] MS (ESI): mass calcd. for $C_{15}H_{23}N_3O_2$ 277.2, m/z found 278.2 $[M+H]^+$.

4-(3-Ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-(piperidin-1-yl)pyridin-2-yl)pyrimidine-2-amino

[0154]



[0155] Other steps were carried out according to the steps similar to Steps 3-7 of Example 1 to obtain the titled compound of this example.

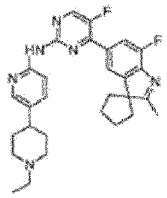
[0156] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.61(br s, 1H), 8.46(d, 1H, J=3.6Hz), 8.34(d, 1H, J=8.8Hz), 8.26(d, 1H, J=1.6Hz), 7.93(d, 1H, J=11.2Hz), 7.64(d, 1H, J=8.0Hz), 7.86(s, 1H), 7.61(dd, 1H, J=8.4Hz, 2.0Hz), 3.24-3.21(m, 2H), 2.77(t, 1H, J=10.8Hz), 2.64-2.61(m, 1H), 2.34(s, 3H), 2.06-1.91(m, 4H), 1.89-1.84(m, 3H), 1.72-1.63(m, 2H), 1.39(s, 3H), 0.50(t, 1H, J=7.2Hz).

[0157] MS(ESI):m/z found 463.3$[M+H]^+$.

Example 12

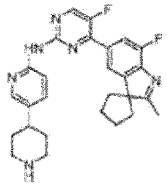
N-(5-(1-ethylpiperidin-4-yl)pyridin-2-yl)-5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane -1,3'-indol]-5'-yl)pyrimidine-2-amino

[0158]



5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(piperidin-4-yl)pyridin-2-yl)pyrimidine-2-amino

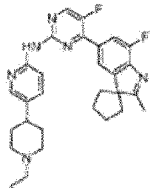
[0159]



[0160] This intermediate was obtained according to a step similar to that of Example 11.

N-(5-(1-ethylpiperidin-4-yl)pyridin-2-yl)-5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane -1,3'-indol]-5'-yl)pyrimidine-2-amino

[0161]



[0162] A reaction flask was charged with 5-fluoro-4-(7'-fluoro-2'-methylspiro [cyclopentane-1,3'-indol]-5'-yl)-N-(5-(piperidin-4-yl)pyridin-2-yl)pyrimidine-2-amino 50 mg (0.1mmol) prepared in the above step, acetaldehyde 26 mg (0.6 mmol) and dichloromethane 5 ml, and reaction was carried out at room temperature for 0.5 h. Then sodium triethylborohydride 60 mg (0.28 mmol) was added, and reaction was carried out at room temperature for 2 h. The reaction solution was added with 20ml of saturated sodium carbonate aqueous solution, and then extracted three times with dichloromethane (10mL for each time). The organic phases were combined, washed once with saturated salt solution, dried with anhydrous sodium sulfate, filtered, concentrated under reduced pressure, and separated by silica gel column chromatography (PE/EA= 5:1) to give the titled compound (11 mg, yield 22%).

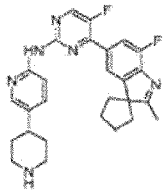
[0163] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.45(d, 1H, J=3.6Hz), 8.38(s, 1H), 8.33(d, 1H, J=8.8Hz), 8.25(s, 1H), 7.97(s, 1H), 7.90(d, 1H, J=11.2Hz), 7.62(dd, 1H, J=8.8, 2.0Hz), 3.14-3.11(m, 2H), 2.55-2.46(m, 3H), 2.40(s, 3H), 2.18-2.03(m, 8H), 1.90-1.82(m, 6H), 1.15(t, 3H, J=7.2Hz).

[0164] MS(ESI):m/z 503.3$[M+H]^+$.

Example 13

5-Fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(piperidin-4-yl)pyridin-2-yl)pyrimidine-2-amino

[0165]



[0166] The titled compound was obtained by the steps similar to those of Example 11.

[0167] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.14(br s, 1H), 8.48(d, 1H, J=3.2Hz), 8.34-8.30(m, 2H), 7.96(s, 1H), 7.89(d, 1H, J=10.8Hz), 7.58(d, 1H, J=8.4Hz), 3.22-3.19(m, 2H), 2.76(t, 2H, J=11.6Hz), 2.66-2.60(m, 1H), 2.38(s, 3H), 2.16-2.02(m, 6H), 1.88-1.83(m, 4H), 1.69-1.61(m, 2H).

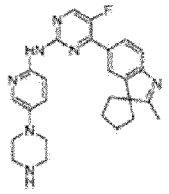
[0168] MS(ESI):m/z 475.3$[M+H]^+$.

Example 14

5-fluoro-4-(2'-methylspiro [cyclopentane-1,3'-indol]-5'-yl)-N-(5-(piperazin-1-yl)pyridin-2-y

l)pyrimidine-2-amino

[0169]



[0170] The titled compound was obtained by the steps similar to those of Example 11.

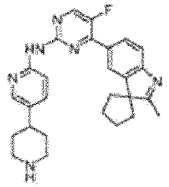
[0171] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.28(br s, 1H), 8.43(d, 1H, J=3.6Hz), 8.33(d, 1H, J=9.2Hz), 8.16-8.09(m, 3H), 7.63(d, 1H, J=8.0Hz), 7.32(dd, 1H, J=9.2Hz, 2.8Hz), 3.08-3.06(m, 4H), 3.03-3.02(m, 4H), 2.34(s, 3H), 2.29-2.04(m, 7H), 1.86-1.83(m, 2H).

[0172] MS(ESI):m/z 458.3$[M+H]^+$.

Example 15

5-fluoro-4-(2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(piperidin-4-yl)pyridin-2-yl)pyrimidine-2-amino

[0173]



[0174] The titled compound was obtained by the steps similar to those of Example 11.

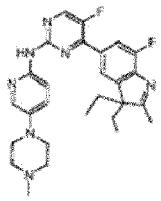
[0175] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.99(br s, 1H), 8.46(d, 1H, J=3.6Hz), 8.39(d, 1H, J=8.4Hz), 8.29(d, 1H, J=1.6Hz), 8.15-8.11(m, 2H), 7.65(d, 1H, J=8.0Hz), 7.58(dd, 1H, J=8.8Hz, 2.0Hz), 3.22-3.19(m, 2H), 2.76(t, 2H, J=10.4Hz), 2.65-2.59(m, 1H), 2.36(s, 3H), 2.18-2.05(m, 7H), 1.88-1.83(m, 4H), 1.70-1.60(m, 2H).

[0176] MS(ESI):m/z 457.3$[M+H]^+$.

Example 16

4-(3,3-diethyl-7-fluoro-2-methyl-3H-indol-5-yl)-5-fluoro-N-(5-(4-methylpiperazin-1-yl)py ridin-2-yl)pyrimidine-2-amino

[0177]



Step 1: Preparation of 5-bromo-3,3-diethyl-7-fluoro-2-methyl-3H-indole

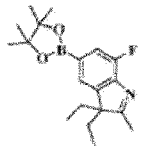
[0178]

[0179] 10 ml of acetic acid, 5.0 g (20.75 mmol) of (4-bromo-2-fluorophenyl)hydrazine hydrochloride and 2.35 g (20.75 mmol) of 3-ethylpentan-2-one were added to a reaction flask, and the mixture was allowed to react under refluxing for 5 h. The reaction product was subjected to rotary evaporation to remove solvent, added with 50 ml of water, and extracted with ethyl acetate three times (50 ml for each time). The organic phases were combined, washed once with 50 ml of salt solution, dried with sodium sulfate, filtered, subjected to rotary evaporation, and separated by column chromatography (EA: PE = 1:100-1:10) to obtain the titled compound (2.5 g, yellow oil), yield 87.1%.

[0180] MS (ESI): m/z 286.1 $[M+H]^+$.

Step 2: Preparation of 3,3-Diethyl-7-fluoro-2-methyl-5-(4,4,5,5-tetramethyl-1,3,2 -dioxaborolan-2-yl)-3H-indole

[0181]



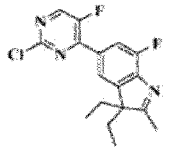
[0182] 2.0 g (7.07 mol) of 5-bromo-3,3-diethyl-7-fluoro-2-methyl-3H-indole prepared in Step 1, 1.97 g (7.77 mmol) of 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bis(1,3,2-dioxaborolane), 1.38 g (1.41 mmol) of potassium acetate, 10 ml of 1,4-dioxane and 1.03 g (1.41 mmo) of $Pd(dppf)Cl_2$ were added to the

reaction flask, and the mixture was heated to 90°C under the protection of nitrogen gas to conduct reaction overnight. The reaction product was cooled to room temperature, filtered, diluted with 10 mL of water and extracted three times with ethyl acetate (10 mL for each time). The organic phases were combined, washed once with 15 ml of salt solution, dried with anhydrous sodium sulfate, filtered, concentrated and separated by silica gel column chromatography (EA: PE = 1:50 to 1:10) to give the titled compound (2.0g, yellow oil), yield 86.96%.

[0183] MS (ESI): m/z 332.3 $[M+H]^+$.

Step 3: 5-(2-Chloro-5-fluoropyrimidin-4-yl)-3,3-diethyl-7-fluoro-2-methyl-3H-indole

[0184]

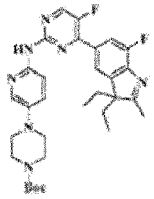


[0185] A reaction flask was charged with 2.0 g (6.05 mmol) of 3,3-diethyl-7-fluoro-2-methyl-5-(4,4,5,5-tetramethyl-l,3,2-dioxaborolan-2-yl)-3H-indole prepared in Step 2, 1.10 g (6.65 mmol) of 2,4-dichloro-5-fluoropyrimidine, 2.56 g (12.1 mmol) of potassium phosphate, 20 mL/5 mL of dioxane/water, 0.69 g (0.61 mmol) of Pd $(PPh_3)_4$, and the mixture was heated to 120°C under the protection of nitrogen gas and allowed to react for 2 h. The reaction product was cooled to room temperature, filtered, diluted with 10 mL of water and extracted three times with 50 mL of dichloromethane. The organic phases were combined, washed once with 20 ml of salt solution, dried with anhydrous sodium sulfate, filtered, concentrated under reduced pressure, and separated by silica gel column chromatography (EA: PE = 1:100-1:10) to give the titled compound (1.2 g, yellow solid), yield 59.4%.

[0186] MS (ESI): m/z 336.1 $[M+H]^+$.

Step 4: Preparation of 4-(6-((4-(3,3-diethyl-7-fluoro-2-methyl-3H-indol-5-yl) -5-fluoropyrimidin-2-yl)amino)pyridin-3-yl)piperazine-1-carboxylic acid t-butyl ester

[0187]



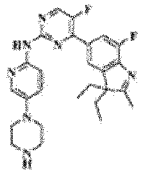
[0188] A reaction flask was charged with 400.0 mg (1.19 mmol) of 5-(2-chloro-5-fluoropyrimidin-4-yl)-3,3-diethyl-7-fluoro-2-methyl-3H-indole prepared in Step 3, 331.9 mg (1.19 mmol) of t-butyl 4-(6-

aminopyridin-3-yl)piperazine-1-carboxylate prepared according to Steps 1-2 of Example 1, 776.2 mg (2.38 mmol) of cesium carbonate, 10 ml of 1,4-dioxane, 109.5 mg (0.12 mmol) of $Pd_2(dba)_3$, and 69.0 mg (0.12 mmol) of 4,5-bis (diphenylphosphino)-9,9-dimethylxanthene, and protected by nitrogen. The mixture was allowed to conduct microwave reaction at 130°C for 1 h. The reaction product is cooled to room temperature, filtered, diluted with 10 ml of water, extracted three times with 10 ml of dichloromethane. The organic phases were combined, washed once with 30 ml of salt solution, dried with anhydrous sodium sulfate, filtered, concentrated and separated by TLC to obtain the titled compound (253.1 mg, yellow solid), yield 36.74%.

[0189] MS (ESI): m/z 578.3 $[M+H]^+$.

Step 5: 4-(3,3-diethyl-7-fluoro-2-methyl-3H-indol-5-yl)-5-fluoro-N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimidine-2-amino

[0190]

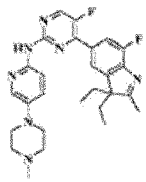


[0191] A reaction flask was charged with 4-(6-((4-(3,3-diethyl-7-fluoro-2-methyl-3H-indol-5-yl)-5-fluoropyrimidin-2-yl)amino)pyridin-3-yl)piperazine-1-carboxylic acid t-butyl ester 250.0 mg(0.43 mmol) prepared in step 4, dichloromethane 4 mL, and TFA 1 ml. The mixture was stirred at room temperature for 2 h, and the solvent was removed. The residue was adjusted to pH8 with 5 ml of saturated sodium bicarbonate solution, and extracted three times with dichloromethane (5 ml for each time). The organic phases were combined, washed once with 10 ml of saturated salt solution, dried with anhydrous sodium sulfate, filtered, concentrated under reduced pressure and separated by TLC to give the titled compound (201.2 mg, yellow solid), yield 97.6%.

[0192] MS (ESI): m/z 478.3 $[M+H]^+$.

Step 6: 4-(3,3-diethyl-7-fluoro-2-methyl-3H-indol-5-yl)-5-fluoro-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidine-2-amino

[0193]



[0194] A reaction flask was charged with 50.0 mg (0.10 mmol) of 4-(3,3-diethyl-7-fluoro-2-methyl-3H-

indol-5-yl)-5-fluoro-N-(5-(piperazin-1-yl)pyridin-2-yl)pyri midine-2-amino, 30.8 mg (1.0 mmol) of formaldehyde, 2 ml of 1-ethyl-(3-dimethylaminopropyl) carbodiimide, 65.3mg (0.3mmol) of sodium triethylborohydride, and the mixture was allowed to react overnight. 2 ml of methanol was added to quench the reaction, and the reaction product was extracted three times with dichloromethane (5 ml for each time). The organic phase was washed once with 10 ml of saturated salt solution, dried with anhydrous sodium sulfate, filtered, concentrated and separated by TLC to obtain the titled compound (20.3 mg, yellow solid), yield 39.4%.

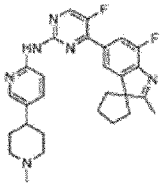
[0195] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.57(br s, 1H), 8.47(d, 1H, J=3.2Hz), 8.28(d, 1H, J=8.8Hz), 8.18(d, 1H, J=2.0Hz), 7.91(d, 1H, J=11.2Hz), 7.78(s, 1H), 7.33(d, 1H, J=8.8Hz), 3.16-3.15(m, 4H), 2.58-2.57(m, 4H), 2.33(s, 3H), 2.26(s, 3H), 2.04-1.95(m, 2H), 1.85-1.78(m, 7H), 0.43(t, 6H, J=7.2Hz).

[0196] MS(ESI):m/z 492.3$[M+H]^+$.

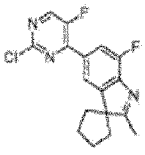
Example 17

5-Fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(1-methylpiperidi n-4-yl)pyridin-2-yl)pyrimidine-2-amino

[0197]

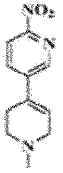


[0198] The intermediate 5'-(2-chloro-5-fluoropyrimidin-4-yl)-7'-fluoro-2'-methylspiro [cyclopentane-1,3'-indole] was obtained by the steps similar to those of Example 1.



<u>Step 1:</u> 1'-Methyl-6-nitro-1',2',3',6'-tetrahydro-3,4'-bipyridine

[0199]



[0200] A reaction flask was charged with 5-bromo-2-nitropyridine (20.3 g, 0.1 mol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine (22.3 g, 0.1 mol), dioxane/water (250 mL/30 mL), cesium carbonate (66 g, 0.2 mol) and $Pd(dppf)Cl_2$ (7.33 g, 0.01 mol). The mixture was stirred to react at 85°C under the protection of nitrogen gas for 12 h. The reaction product was cooled to room temperature, concentrated and separated by column chromatography (PE/EA = 1:1 to DCM/MeOH = 20:1) to give the titled product (5.7 g, white solid).

[0201] MS (ESI): mass calcd. for $C_{11}H_{13}N_3O_2$ 219.1, m/z found 220.1 $[M+H]^+$.

Step 2: 5-(1-Methylpiperidin-4-yl) pyridin-2-amino

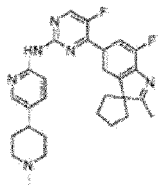
[0202]

[0203] A reaction flask was charged with 1'-methyl-6-nitro-1',2',3',6'-tetrahydro-3,4'-bipyridine (657 mg, 3.0 mmol) prepared in Step 1, ethyl acetate/methanol (10 mL/10 mL), and Pd/C (0.1 g). Hydrogen gas was introduced into the mixture , and the mixture was stirred to react for 2 h, filtered and concentrated to give the titled product (550 mg, white solid).

[0204] MS (ESI): mass calcd. for $C_{11}H_{17}N_3$ 191.1, m/z found 192.2 $[M+H]^+$.

Step 3 5-Fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(1-methylpiperidi n-4-yl)pyridin-2-yl)pyrimidine-2-amino

[0205]



[0206] A reaction flask was charged with the intermediate 5'-(2-chloro-5-fluoropyrimidin-4-yl)-7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indole] (150.0mg,0.45mmol), 5-(1-methylpiperidin-4-yl)pyridin-2-amino (86.6 mg, 0.45 mmol) prepared in Step 2, cesium carbonate (293.2 mg, 0.9 mmol), dioxane (3 ml), $Pd_2(dba)_3$ (44.7 mg, 0.05 mmol), 4,5-bis (diphenylphosphino)-9,9-dimethylxanthene (30.4 mg, 0.05 mmol). The mixture was heated to 150°C under the protection of

nitrogen gas to conduct microwave reaction for 1 h. The reaction product was cooled to room temperature, filtered, added with 10 ml of water, and extracted three times with dichloromethane (10 ml for each time). The combined organic phase was washed once with 30 ml of saturated sodium chloride aqueous solution, dried with anhydrous sodium sulfate, filtered, concentrated and separated by silica gel column chromatography (dichloromethane/methanol = 50:1) to give the titled compound (51.1 mg, yellow solid).

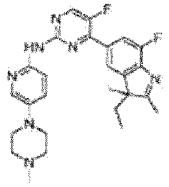
[0207] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.38(br s, 1H), 8.49(d, 1H, J=3.2Hz), 8.34-8.33(m, 2H), 7.96(s, 1H), 7.88(d, 1H, J=11.2Hz), 7.58(dd, 1H, J=8.8Hz, 1.6Hz), 3.01-2.98(m, 2H), 2.52-2.44(m, 1H), 2.38(s, 3H), 2.34(s, 3H), 2.25-2.04(m, 8H), 1.87-1.76(m, 6H).

[0208] MS(ESI):m/z 489.3$[M+H]^+$.

Example 18

4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-(4-methylpiperazin-1-yl)py ridin-2-yl) pyrimidine-2-amino

[0209]



[0210] The titled compound was obtained by the steps similar to those of Example 16.

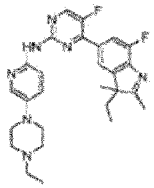
[0211] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.48(br s, 1H), 8.47(d, 1H, J=3.2Hz), 8.28(d, 1H, J=9.2Hz), 8.17(s, 1H), 7.90(d, 1H, J=10.8Hz), 7.82(s, 1H), 7.34(d, 1H, J=8.4Hz), 3.17-3.16(m, 4H), 2.59-2.58(m, 4H), 2.35(s, 3H), 2.31(s, 3H), 2.01-1.96(m, 1H), 1.87-1.82(m, 1H), 1.35(s, 3H), 0.47(t, 3H, J=7.2Hz).

[0212] MS(ESI):m/z 478.3$[M+H]^+$.

Example 19

4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-N-(5-(4-ethylpiperazin-1-yl)pyridin-2-yl)-5-fluoropyrimidine-2-amino

[0213]



Step 1: Preparation of 1-ethyl-4-(6-nitropyridin-3-yl) piperazine

[0214]

[0215] 4.00 g (19.7 mmol) of 5-bromo-2-nitropyridine, 3.40 g (2.98 mmol) of 1-ethylpiperazine, 4.10 g (29.6 mmol) of potassium carbonate, 0.4 g (1.2 mmol) of tetrabutylammonium iodide, and 40mL of DMSO were added to a reaction flask, and reacted at 80°C for 16 h. The reaction solution was then poured into ice-water and extracted three times with dichloromethane (20 ml for each time). The organic phases were combined, dried with anhydrous sodium sulfate, filtered, concentrated and separated by column chromatography (DCM/MeOH = 100:1-10:1) to give 3.59 g of yellow solid, yield 56.1%.

[0216] MS (ESI): m/z 237.2 $[M+H]^+$.

Step 2: Preparation of 5-(4-ethylpiperazin-1-yl)pyridin-2-amino

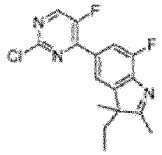
[0217]

[0218] 650 mg (2.13 mmol) of 1-ethyl-4-(6-nitropyridin-3-yl)piperazine prepared in Step 1 was dissolved in 45 ml of methanol, 10% palladium carbon (250 mg, cat) was added, the atmosphere was replaced three times with hydrogen gas, and the reaction was carried out at room temperature for 12h in the atmosphere of hydrogen gas under 3 atmos. After the reaction stopped, the reaction product was filtered with a small amount of diatomite, and the filter cake was washed once with 20 ml of a mixed solvent of dichloromethane and methanol (V/V = 10:1). Then, the filtrate was collected and concentrated under reduced pressure to give 559 mg of crude product of the titled compound (transparent and viscous material) which was used in the subsequent reaction directly without further purification.

[0219] MS (ESI): m/z 207.1 $[M+H]^+$.

Step 3: Preparation of 5-(2-chloro-5-fluoropyrimidin-4-yl)-3-ethyl-7-fluoro-2,3-dimethyl -3H-indole

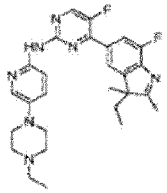
[0220]



[0221] This intermediate was prepared by the same method as Steps 1-3 of Example 16.

Step 4: Preparation of 4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-N-(5-(4-ethylpiperazin-1-yl)pyridin -2-yl)-5-fluoropyrimidine-2-amino

[0222]



[0223] A reaction flask was charged with 321 mg (1 mmol) of 5-(2-chloro-5-fluoropyrimidin-4-yl)-3-ethyl-7-fluoro-2,3-dimethyl-3H-indole prepared in Step 3, 206 mg (1 mmol) of 5-(4-ethylpiperazin-1-yl)pyridin-2-amino obtained in Step 2, 2 ml of 1,4-dioxane, 650 mg (2 mmol) of Cs_2CO_3, 91mg (0.1mmol) of $Pd_2(dba)_3$, and 58mg (0.1mmol) of diphenylphosphine. The mixture was heated to 120°C to conduct microwave reaction for 1 h. The reaction product was cooled to room temperature, added with 10ml of water and then extracted with ethyl acetate three times (40ml for each time). The organic phases were combined, washed once with 40 ml of saturated salt solution, dried with sodium sulfate, filtered, concentrated under reduced pressure and separated by silica gel column chromatography (DCM/MeOH = 10:1) to give the titled compound (49 mg, yellow solid), yield 10%.

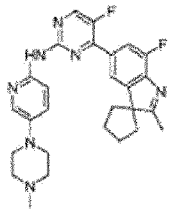
[0224] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.25(br s, 1H), 8.46(d, 1H, J=3.2Hz), 8.28(d, 1H, J=9.2Hz), 8.17(d, 1H, J=2.0Hz), 7.90(d, 1H, J=11.2Hz), 7.82(s, 1H), 7.35(dd, 1H, J=9.2Hz, 2.8Hz), 3.20-3.18(m, 4H), 2.64-2.61(m, 4H), 2.51(q, 2H, J=6.8Hz), 2.31(s, 3H), 2.03-1.94(m, 1H), 1.89-1.82(m, 1H), 1.36(s, 3H), 1.13(t, 3H, J=7.2Hz), 0.48(t, 3H, J=7.2Hz).

[0225] MS(ESI):m/z 492.3$[M+H]^+$.

Example 20

5-Fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(4-methylpiperaz in-1-yl)pyridin-2-yl)pyrimidine-2-amino

[0226]



[0227] The titled compound was obtained by the steps similar to those of Example 16.

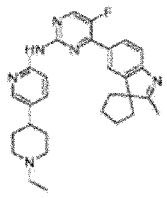
[0228] ^{1}H-NMR(400MHz, DMSO-d_6) δ9.77(br s, 1H), 8.63(d, 1H, J=4.0Hz), 8.02-7.98(m, 3H), 7.82(d, 1H, J=11.2Hz), 7.41(dd, 1H, J=8.8Hz, 2.8Hz), 3.13-3.11(m, 4H), 2.49-2.46(m, 4H), 2.33(s, 3H), 2.26(s, 3H), 2.23-2.07(m, 6H), 1.76-1.74(m, 2H).

[0229] MS(ESI):m/z 490.3$[M+H]^+$.

Example 21

N-(5-(1-ethylpiperidin-4-yl)pyridin-2-yl)-5-fluoro-4-(2'-methylspiro[cyclopentane-1,3'-ind ol]-5'-yl)pyrimidine-2-amino

[0230]



[0231] The titled compound was obtained by the steps similar to those of Example 12.

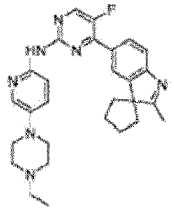
[0232] ^{1}H-NMR(400MHz, DMSO-d_6) δ9.91(br s, 1H), 8.65(d, 1H, J=4.0Hz), 8.28-8.14(m, 3H), 8.04(d, 1H, J=8.4Hz), 7.43(dd, 1H, J=8.4Hz, 2.0Hz), 7.60(d, 1H, J=8.0Hz), 3.00-2.97(m, 4H), 2.38-2.33(m, 2H), 2.30(s, 3H), 2.08-2.07(m, 6H), 1.99-1.94(m, 2H), 1.77-1.64(m, 6H), 1.02(t, 3H, J=7.2Hz).

[0233] MS(ESI):m/z 485.3$[M+H]^+$.

Example 22

N-(5-(4-ethylpiperazin-1-yl)pyridin-2-yl)-5-fluoro-4-(2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)pyrimidine-2-amino

[0234]



[0235] The titled compound was obtained by the steps similar to those of Example 19.

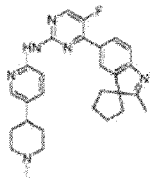
[0236] ^{1}H-NMR(400MHz,$CDCl_3$) δ8.87(br s, 1H), 8.42(d, 1H, J=3.6Hz), 8.33(d, 1H, J=9.2Hz), 8.13-8.10(m, 3H), 7.65(d, 1H, J=8.0Hz), 7.35(dd, 1H, J=8.8Hz, 2.4Hz), 3.19-3.18(m, 4H), 2.64-2.63(m, 4H), 2.53(q, 2H, J=6.8Hz), 2.35(s, 3H), 2.27-2.06(m, 6H), 1.88-1.85(m, 2H), 1.14(t, 3H, J=6.8Hz).

[0237] MS(ESI):m/z 486.4$[M+H]^+$.

Example 23

N-(5-(4-methylpiperidin-1-yl)pyridin-2-yl)-5-fluoro-4-(2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)pyrimidine-2-amino

[0238]



[0239] The titled compound was obtained by the steps similar to those of Example 17.

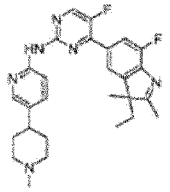
[0240] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.24(br s, 1H), 8.47(d, 1H, J=3.2Hz), 8.38(d, 1H, J=8.8Hz), 8.31(s, 1H), 8.14-8.09(m, 2H), 7.64(d, 1H, J=8.4Hz), 7.57(dd, 1H, J=8.4Hz, 2.4Hz), 3.00-2.98(m, 2H), 2.49-2.46(m, 1H), 2.35(s, 3H), 2.33(s, 3H), 2.16-1.99(m, 8H), 1.85-1.76(m, 6H).

[0241] MS(ESI):m/z 471.3$[M+H]^+$.

Example 24

4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-(l-methylpiperidin-4-yl)pyr idin-2-yl)pyrimidine-2-amino

[0242]



[0243] The titled compound was obtained by the steps similar to those of Example 17.

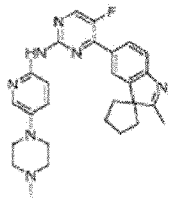
[0244] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.74(br s, 1H), 8.50(d, 1H, J=3.6Hz), 8.35-8.32(m, 2H), 7.88-7.82(m, 2H), 7.57(d, 1H, J=8.8Hz), 2.97-2.95(m, 2H), 2.49-2.42(m, 1H), 2.30(s, 6H), 2.24-1.93(m, 3H), 1.88-1.74(m, 5H), 1.35(s, 3H), 0.47(t, 3H, J=7.2Hz).

[0245] MS(ESI):m/z 477.3$[M+H]^+$.

Example 25

5-Fluoro-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)-4-(2'-methylspiro[cyclopentane-1,3'-i ndol]-5'-yl) pyrimidine-2-amino

[0246]



[0247] The titled compound was obtained by the steps similar to those of Example 16.

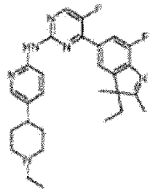
[0248] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.28(br s, 1H), 8.44(d, 1H, J=3.6Hz), 8.33(d, 1H, J=9.2Hz), 8.18(d, 1H, J=2.0Hz), 8.12-8.09(m, 2H), 7.64(d, 1H, J=8.0Hz), 7.33(dd, 1H, J=9.2Hz, 2.4Hz), 3.16-3.14(m, 4H), 2.59-2.58(m, 4H), 2.35(s, 3H), 2.34(s, 3H), 2.15-2.06(m, 6H), 1.87-1.83(m, 2H).

[0249] MS(ESI):m/z 472.3$[M+H]^+$.

Example 26

4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-N-(5-(1-ethylpiperidin-4-yl)pyridin-2-yl) -5-fluoropyrimidine-2-amino

[0250]



[0251] The titled compound was obtained by the steps similar to those of Example 12.

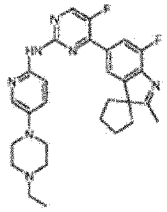
[0252] ^{1}H-NMR(400MHz, DMSO-d_6) δ9.99(br s, 1H), 8.70(d, 1H, J=3.2Hz), 8.19(s, 1H), 8.14(d, 1H, J=8.8Hz), 7.92(s, 1H), 7.85(d, 1H, J=11.2Hz), 7.69(d, 1H, J=8.4Hz), 3.04-3.02(m, 2H), 2.42-2.41(m, 2H), 2.28(s, 3H), 2.04-1.99(m, 3H), 1.89-1.84(m, 1H), 1.78-1.63(m, 4H), 1.33(s, 3H), 1.04(t, 3H, J=6.8Hz), 0.36(t, 3H, J=7.2Hz).

[0253] MS(ESI):m/z 491.3$[M+H]^+$.

Example 27

N-(5-(4-ethylpiperazin-1-yl)pyridin-2-yl)-5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentan e-1,3'-indol]-5'-yl)pyrimidine-2-amino

[0254]



[0255] The titled compound was obtained by the steps similar to those of Example 19.

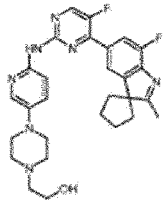
[0256] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.92(br s, 1H), 8.44(d, 1H, J=3.6Hz), 8.29(d, 1H, J=9.2Hz), 8.15(d, 1H, J=2.8Hz), 7.94(s, 1H), 7.89(d, 1H, J=11.2Hz), 7.36(dd, 1H, J=9.2Hz, 2.8Hz), 3.21-3.19(m, 4H), 2.65-2.63(m, 4H), 2.51(q, 2H, J=7.2Hz), 2.38(s, 3H), 2.22-2.07(m, 6H), 1.89-1.86(m, 2H), 1.15(t, 3H, J=7.2Hz).

[0257] MS(ESI):m/z 504.3$[M+H]^+$.

Example 28

2-(4-(6-((5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)ethanol

[0258]



[0259] A reaction flask was charged with 5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimidine-2-amino 20 mg (0.04 mmol, prepared by the same method as in Example 6), 16 mg (0.12 mmol) of 2-bromoethanol, 2 ml of DMF and 17 mg (0.12 mmol) of cesium carbonate. The mixture was heated to 80°C and reacted for 1 h, and then the reaction product was cooled to room temperature, added with 10 ml of water, and extracted three times with ethyl acetate (40 ml for each time). The organic phases were combined, washed once with 40 ml of saturated salt solution, dried with anhydrous sodium sulfate, filtered, concentrated under reduced pressure, and separated by silica gel column chromatography (DCM/MeOH = 10:1) to give the titled compound (10 mg, yellow solid), yield 55%.

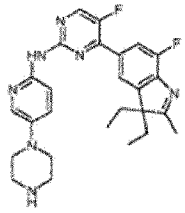
[0260] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.52(br s, 1H), 8.43(d, 1H, J=2.8Hz), 8.29(d, 1H, J=9.2Hz), 8.09(s, 1H), 7.94(s, 1H), 7.89(d, 1H, J=10.8Hz), 7.36(d, 1H, J=8.8Hz), 3.70-3.68(m, 2H), 3.19-3.18(m, 4H), 2.71-2.63(m, 7H), 2.39(s, 3H), 2.25-2.00(m, 6H), 1.90-1.87(m, 2H).

[0261] MS(ESI):m/z 520.3$[M+H]^+$.

Example 29

4-(3,3-diethyl-7-fluoro-2-methyl-3H-indol-5-yl)-5-fluoro-N-(5-(piperazin-4-yl)pyridin-2-yl)pyrimidine-2-amino

[0262]



[0263] The titled compound was obtained by the steps similar to those of Example 1.

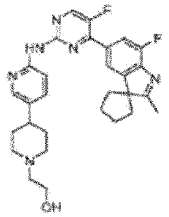
[0264] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.66(br s, 1H), 8.45(d, 1H, J=3.6Hz), 8.29(d, 1H, J=9.2Hz), 8.11(d, 1H, J=2.8Hz), 7.94(d, 1H, J=11.2Hz), 7.81(s, 1H), 7.37(dd, 1H, J=8.8Hz, 2.4Hz), 3.13-3.12(m, 4H), 3.08-3.07(m, 4H), 2.30(s, 3H), 2.08-1.99(m, 2H), 1.91-1.82(m, 3H), 0.46(t, 6H, J=7.6Hz).

[0265] MS(ESI):m/z 478.3$[M+H]^+$.

Example 30

2-(4-(6-((5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperidin-1-yl)ethanol

[0266]



[0267] A reaction flask was charged with 20 mg (0.04 mmol) of 5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(piperidin-4-yl)pyridin -2-yl)pyrimidine-2-amino prepared by the same methods as Example 13, 16 mg (0.12 mmol) of 2-bromoethanol, 2 mL of DMF, and 17 mg (0.12 mmol) of cesium carbonate, and the mixture was heated to 80°C and reacted for 1 h. Then, the reaction product was cooled to room temperature, added with 10 ml of water and extracted three times with ethyl acetate (40 ml for each time). The organic phases were combined, washed once with 40 ml of saturated salt solution, dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure, and separated by silica gel column chromatography (DCM/MeOH = 10:1) to give the titled compound (10 mg, yellow solid), yield 55%.

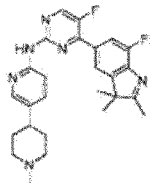
[0268] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.63(br s, 1H), 8.46(d, 1H, J=3.2Hz), 8.34(d, 1H, J=8.4Hz), 8.26(s, 1H), 7.96(s, 1H), 7.90(d, 1H, J=10.8Hz), 7.60(d, 1H, J=8.0Hz), 3.67-3.66(m, 2H), 3.08-3.06(m, 2H), 2.60-2.52(m, 4H), 2.39(s, 3H), 2.25-2.11(m, 8H), 1.89-1.83(m, 4H), 1.80-1.77(m, 2H).

[0269] MS(ESI):m/z 519.3$[M+H]^+$.

Example 31

5-Fluoro-4-(7-fluoro-2,3,3-trimethyl-3H-indol-5-yl)-N-(5-(1-methylpiperidin-4-yl)pyridin-2-yl)pyrimidine-2-amino

[0270]



[0271] The titled compound was obtained by the steps similar to those of Example 17.

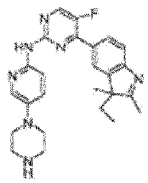
[0272] ^{1}H-NMR(400MHz, Methanol-d_4) δ8.57(br s, 1H), 8.37(d, 1H, J=3.6Hz), 8.18(s, 1H), 8.13(d, 1H, J=8.8Hz), 7.84(s, 1H), 7.70(d, 1H, J=11.2Hz), 7.51(d, 1H, J=7.2Hz), 3.21-3.18(m, 2H), 2.60-2.54(m, 4H), 2.50-2.44(m, 2H), 2.37(s, 3H), 1.90-1.79(m, 4H), 1.38(s, 6H).

[0273] MS(ESI):m/z 463.3$[M+H]^+$.

Example 32

4-(3-ethyl-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimid ine-2-amino

[0274]



[0275] The titled compound was obtained by the steps similar to those of Example 6.

[0276] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.68(br s, 1H), 8.44(d, 1H, J=3.6Hz), 8.32(d, 1H, J=8.8Hz), 8.18(d, 1H, J=2.4Hz), 8.11(d, 1H, J=8.0Hz), 7.98(s, 1H), 7.64(d, 1H, J=8.0Hz), 7.30(d, 1H, J=2.8Hz), 3.05-3.04(m, 4H), 3.00-2.98(m, 4H), 2.26(s, 3H), 2.00-1.90(m, 2H), 1.83-1.74(m, 1H), 1.32(s, 3H),

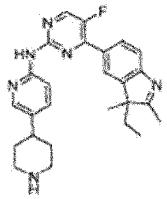
0.45(t, 1H, J=7.2Hz).

[0277] MS(ESI):m/z 446.3$[M+H]^+$.

Example 33

4-(3-ethyl-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-(piperidin-4-yl)pyridin-2-yl)pyrimidi ne-2-amino

[0278]



[0279] The titled compound was obtained by the steps similar to those of Example 13.

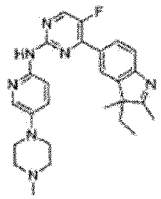
[0280] ^{1}H-NMR(400MHz, Methanol-d_4) δ8.33(d, 1H, J=2.8Hz), 8.16-8.13(m, 2H), 7.95-7.93(m, 2H), 7.48-7.41(m, 2H), 3.18-3.15(m, 2H), 2.73(t, 1H, J=11.6Hz), 2.59-2.53(m, 1H), 2.30(s, 3H), 2.01-1.96(m, 1H), 1.89-1.83(m, 1H), 1.79-1.76(m, 2H), 1.66-1.58(m, 2H), 1.32(s, 3H), 0.42(t, 1H, J=6.8Hz).

[0281] MS(ESI):m/z 445.3$[M+H]^+$.

Example 34

4-(3-ethyl-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-(4-methylpiperazin-1-yl)pyridin-2-yl)pyrimidine-2-amino

[0282]



[0283] The titled compound was obtained by the steps similar to those of Example 16.

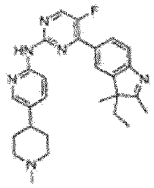
[0284] ^{1}H-NMR(400MHz, Methanol-d_4) δ8.42(d, 1H, J=3.2Hz), 8.18(d, 1H, J=8.8Hz), 8.09-8.01(m, 3H), 7.57(d, 1H, J=8.0Hz), 7.42-7.37(m, 1H), 3.22-3.15(m, 4H), 2.61-2.60(m, 4H), 2.35(s, 3H), 2.32(s, 3H), 2.07-2.02(m, 1H), 1.94-1.89(m, 1H), 1.38(s, 3H), 0.43(t, 1H, J=7.2Hz).

[0285] MS(ESI):m/z 460.3$[M+H]^+$.

Example 35

4-(3-ethyl-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-(1-methylpiperidin-4-yl)pyridin-2-yl)pyrimidine-2-amino

[0286]



[0287] The titled compound was obtained by the steps similar to those of Example 17.

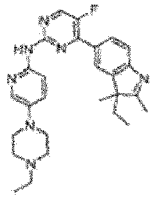
[0288] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.97(br s, 1H), 8.47(d, 1H, J=3.6Hz), 8.39(d, 1H, J=8.4Hz), 8.30(d, 1H, J=2.0Hz), 8.15(d, 1H, J=8.4Hz), 8.04(s, 1H), 7.68(d, 1H, J=8.0Hz), 7.59(dd, 1H, J=8.8Hz, 2.4Hz), 3.01-2.98(m, 4H), 2.51-2.45(m, 1H), 2.34(s, 3H), 2.31(s, 3H), 2.10-1.97(m, 3H), 1.85-1.81(m, 5H), 1.37(s, 3H), 0.49(t, 1H, J=7.2Hz).

[0289] MS(ESI):m/z 459.3$[M+H]^+$.

Example 36

4-(3-ethyl-2,3-dimethyl-3H-indol-5-yl)-N-(5-(4-ethylpiperazin-1-yl)pyridin-2-yl)-5-fluorop yrimidine-2-amino

[0290]



[0291] The titled compound was obtained by the steps similar to those of Example 19.

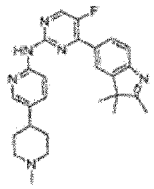
[0292] ^{1}H-NMR(400MHz, $CDCl_3$) δ8.93(br s, 1H), 8.44(d, 1H, J=3.6Hz), 8.33(d, 1H, J=9.2Hz), 8.15-8.13(m, 2H), 8.02(s, 1H), 7.67(d, 1H, J=8.0Hz), 7.35(d, 1H, J=8.0Hz), 3.19-3.18(m, 4H), 2.63-2.62(m, 4H), 2.52-2.47(m, 2H), 2.30(s, 3H), 2.02-1.97(m, 1H), 1.86-1.80(m, 1H), 1.36(s, 3H), 1.14(t, 3H, J=7.2Hz), 0.57(t, 1H, J=7.2Hz).

[0293] MS(ESI):m/z 474.3$[M+H]^+$.

Example 37

5-Fluoro-N-(5-(1-methylpiperidin-4-yl)pyridin-2-yl)-4-(2,3,3-trimethyl-3H-indol-5-yl)pyri midine-2-amino

[0294]



[0295] The titled compound was obtained by the steps similar to those of Example 17.

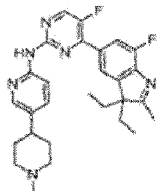
[0296] ^{1}H-NMR(400MHz, $CDCl_3$) δ9.03(br s, 1H), 8.46(d, 1H, J=3.2Hz), 8.38(d, 1H, J=8.4Hz), 8.29(s, 1H), 7.67(d, 1H, J=8.0Hz), 7.60(dd, 1H, J=8.8Hz, 1.6Hz), 3.00-2.97(m, 2H), 2.49-2.44(m, 1H), 2.34(s, 3H), 2.33(s, 3H), 2.10-2.03(m, 2H), 1.84-1.79(m, 4H), 1.37(s, 6H).

[0297] MS(ESI):m/z 445.3$[M+H]^+$.

Example 38

4-(3,3-diethyl-7-fluoro-2-methyl-3H-indol-5-yl)-5-fluoro-N-(5-(1-methylpiperidin-4-yl)pyr idin-2-yl)pyrimidine-2-amino

[0298]



[0299] The titled compound was obtained by the steps similar to those of Example 17.

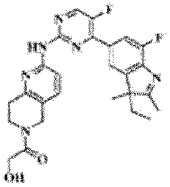
[0300] ^{1}H-NMR(400MHz, DMSO-d_6) δ9.95(s, 1H), 8.69(s, 1H), 8.19-8.11(m, 2H), 7.89-7.84(m, 2H), 7.67(d, 1H, J=7.6Hz), 2.87-2.85(m, 2H), 2.25(s, 3H), 2.19(s, 3H), 2.02-1.92(m, 6H), 1.90-1.65(m, 4H), 0.34(m, 6H).

[0301] MS(ESI):m/z 491.3$[M+H]^+$.

Example 39

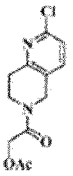
1-(2-((4-(3-Ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-5-fluoropyrimidin-2-yl)amino)-7,8-dihydro-1,6-naphthyridine-6 (5H)-yl)-2-hydroxyacetamide

[0302]



Step 1: 2-(2-Chloro-7,8-dihydro-1,6-naphthyridine-6 (5H)-yl)-2-acetoxyacetamide

[0303]

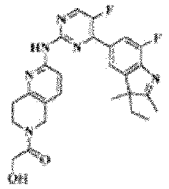


[0304] A reaction flask was charged with 1.0 g (5.95 mmol) of 2-chloro-5,6,7,8-tetrahydro-1,6-naphthyridine, 1.21 g (11.90 mmol) of triethylamine, and 5 ml of dichloromethane, and then 2-chloro-2-acetoxyacetyl chloride (1.22 g, 8.93 mmol) was slowly dropwise added. The mixture was allowed to react for 1 h at room temperature, and the reaction was quenched with 5 mL of water. The solvent was removed, and the residue was extracted three times with dichloromethane (15 mL for each time). The organic phases were combined, washed once with 10 ml of saturated salt solution, dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give crude product of the titled compound (1.09 g, off-white), yield 68.21%.

[0305] MS (ESI): m/z 269.1 $[M+H]^+$.

Step 2: **1-(2-((4-(3-Ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-5-fluoropyrimidin-2-yl)amino)-7,8-dihydro-1,6-naphthyridine-6(5H)-yl)-2-hydroxyacetamide**

[0306]



[0307] A reaction flask was charged with 101 mg (0.34 mmol) of 4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-5-fluoropyrimidine-2-amino prepared by the method similar to Steps 1-3 of Example 1, 85.2 mg (0.32 mmol) of 2-(2-chloro-7,8-dihydro-1,6-naphthyridine-6(5H)-yl)-2-acetoxyacetamide, 10 mL of dioxane, 65.3 mg (0.68 mmol) of sodium tert-butoxide, 31.2 mg (0.034 mmol) of $Pd_2(dba)_3$, 19.7 mg (0.034 mmol) of 4,5-bis (diphenylphosphino)-9,9-dimethylxanthene. The mixture was heated to 120°C and allowed to conduct microwave reaction for 1 h, and then the reaction product was cooled to room temperature, added with 50 ml of water, and extracted three times with ethyl acetate (50 ml for each time). The organic phases were combined, washed once with 50 ml of saturated salt solution, dried with anhydrous sodium sulfate, filtered, concentrated, and separated by silica gel column chromatography (dichloromethane/methanol = 10:1) to give the titled compound (20 mg, white solid), yield 12%.

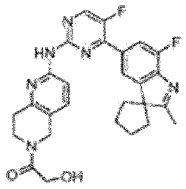
[0308] ^{1}H-NMR(400MHz, DMSO-d_6) δ9.98(s, 1H), 8.70(d, 1H, J=3.6Hz), 8.07(d, 1H, J=8.4Hz), 7.94(s, 1H), 7.87(d, 1H, J=11.2Hz), 7.64-7.57(m, 1H), 4.68-4.55(m, 3H), 4.22-4.21(m, 2H), 4.21-4.19(m, 2H), 2.89-2.81(m, 2H), 2.28(s, 3H), 2.04-1.84(m, 2H), 1.33(s, 3H), 0.37(t, 3H, J=7.2Hz).

[0309] MS(ESI):m/z 493.2$[M+H]^+$.

Example 40

1-(2-((5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)pyrimidin-2-yl)amino)-7,8-dihydro-1,6-naphthyridine-6 (5H)-yl) -2-hydroxyacetamide

[0310]



[0311] The titled compound was obtained by the steps similar to those of Example 39.

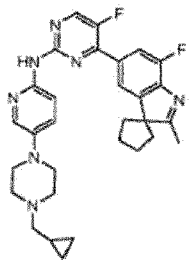
[0312] ^{1}H-NMR(400MHz, DMSO-d_6) δ10.07(brs, 1H), 8.69(d, 1H, J=3.6Hz), 8.53(s, 1H), 8.09-8.02(m, 1H), 7.98(s, 1H), 7.88(d, 1H, J=12.4Hz), 7.62-7.54(m, 1H), 4.73(brs, 1H), 4.62-4.61(m, 2H), 4.21-4.19(m, 2H), 2.89-2.81(m, 2H), 2.50(s, 2H), 2.34-2.11(m, 5H), 1.75-1.72(m, 2H).

[0313] MS(ESI):m/z 505.2$[M+H]^+$.

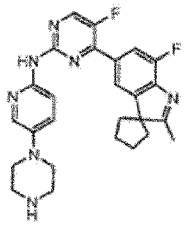
Example 41

N-(5-(4-(cyclopropylmethyl)piperazin-1-yl)pyridin-2-yl)-5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5 '-yl)pyrimidine-2-amino

[0314]



[0315] The intermediate 5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimidine-2-amino was obtained by the steps similar to those of Example 6.



[0316] The above intermediate 5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl) -N-(5-(piperazin-1-yl)pyridin-2-yl)pyrimidine-2-amino (150 mg, 0.32 mmol), bromomethyl cyclopropane, acetonitrile (5 ml), and potassium carbonate (130.0 mg, 0.96 mmol) were added to a reaction flask, and heated to 80°C and reacted for 4 h. The reaction product was cooled to room temperature, added with 50 ml of water, extracted three times with 10 ml of dichloromethane (10 ml for each time). The organic layers were combined, washed once with 15 mg of saturated salt solution, dried with anhydrous sodium sulfate, filtered, and separated by column chromatography(dichloromethane/Methanol = 10:1) to obtain the titled product of this example (42.1 mg, white solid).

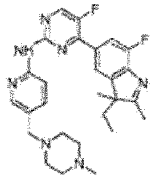
[0317] ^{1}H-NMR(400MHz, DMSO-d_6) δ9.89(s, 1H), 8.68(m, 1H), 8.64-8.01(m, 3H), 7.87(d, 1H, J=14.4Hz), 7.42-7.40(m, 1H), 3.14-3.13(m, 5H), 2.60-2.51(m, 5H), 2.33(s, 3H), 2.24-2.23(m, 2H), 2.09-2.02(m, 6H), 1.99-1.97(m, 2H), 1.75-1.74(m, 2H), 0.85-0.83(m, 2H), 0.48-0.47(m, 2H).

[0318] MS(ESI):m/z 530.3$[M+H]^+$.

Example 42

4-(3-ethyl-7-fluoro-2,3-dimethyl-3H-indol-5-yl)-5-fluoro-N-(5-((4-methylpiperazin-1-yl)methyl)pyridin-2-yl)pyrimidine-2-amino

[0319]



[0320] The titled compound was obtained by the steps similar to those of Example 2.

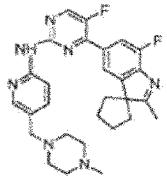
[0321] ^{1}H-NMR(400MHz, DMSO-d_6) δ10.06(brs, 1H), 8.72(d, 1H, J=3.2Hz), 8.18-8.15(m, 2H), 7.93(s, 1H), 7.86(d, 1H, J=11.6Hz), 7.67(d, 1H, J=5.2Hz), 3.42(s, 2H), 2.35-2.28(m, 8H), 2.14(s, 3H), 2.04-1.98(m, 1H), 1.89-1.84(m, 1H), 1.34(s, 3H), 0.36(t, 3H, J=7.2Hz).

[0322] MS(ESI):m/z 492.3$[M+H]^+$.

Example 43

5-Fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indol]-5'-yl)-N-(5-((4-methylpiperazin-1-yl)methyl)pyridin-2-yl)pyrimidine-2-amino

[0323]



[0324] The titled compound was obtained by the steps similar to those of Example 2.

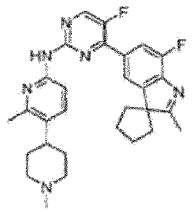
[0325] ^{1}H-NMR(400MHz, DMSO-d_6) δ10.09(s, 1H), 8.71(d, 1H, J=3.2Hz), 8.18-8.15(m, 2H), 8.02(s, 1H), 7.85(d, 1H, J=11.2Hz), 7.67(d, 1H, J=8.4Hz), 3.43(s, 2H), 2.50-2.34(m, 8H), 2.14(s, 3H), 2.14-2.08(m, 6H), 1.75-1.74(m, 2H), 1.75-1.72(m, 2H).

[0326] MS(ESI):m/z 504.2$[M+H]^+$.

Example 44

N-(5-(1-methylpiperidin-4-yl)-(6-methylpyridin)-2-yl)-5-fluoro-4-(7'-fluoro-2'-methylspiro [cyclopentane-1,3'-indol]-5'-yl)pyrimidine-2-amino

[0327]



[0328] The titled compound was obtained by the steps similar to those of Example 12.

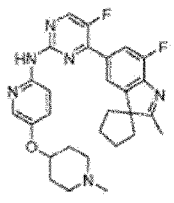
[0329] ^{1}HNMR(400MHz, DMSO-d_6), δ9.84(s, 1H), 8.70(s, 1H), 8.11(s, 1H), 7.98-8.01(d, 2H), 7.81-7.85(d, 1H), 2.87-2.90(d, 2H), 2.50-2.51(m, 1H), 2.20-2.34(m, 6H), 1.78-1.98(m, 8H), 1.69-1.75(m, 6H).

[0330] MS(ESI):m/z 503.3$[M+H]^+$.

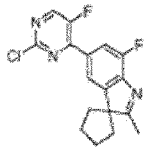
Example 45

N-(5-((1-methylpiperidin-4-yl)oxy)-pyridin-2-yl)-5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3 '-indol]-5 '-yl)pyrimidine-2-amino

[0331]

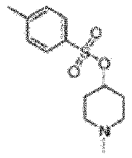


[0332] The intermediate 5'-(2-chloro-5-fluoropyrimidin-4-yl)-7'-fluoro-2'-methylspiro [cyclopentane-1,3'-indole] was prepared by the steps similar to those of Example 1.



Step 1: 1-Methylpiperidin-4-yl 4-methylbenzenesulfonate

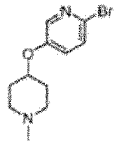
[0333]



[0334] 4-Hydroxy-1-methylpiperidine (1000 mg, 8.69 mmol), p-toluenesulfonyl chloride (3310 mg, 17.38 mmol), dichloromethane (50 ml) and triethylamine (1 mL) were added to a reaction flask, and allowed to react at room temperature for 2 h. Then, the reaction product was added with 50 ml of water and extracted three times with 30 ml of dichloromethane (30 ml for each time). The organic layers were combined, washed once with 50 mg of saturated salt solution, dried with anhydrous sodium sulfate, filtered and separated by column chromatography (dichloromethane/methanol = 50:1) to give 1.87 g of intermediate, yield 80.0% (pale yellow solid).

Step 2: 2-bromo-5 -((1-methylpiperidin-4-yl) oxy) pyridine

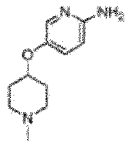
[0335]



[0336] Intermediate 1-methylpiperidin-4-yl 4-methylbenzenesulfonate (1000 mg, 3.70 mmol), 2-bromo-5-hydroxypyridine (637 mg, 3.70 mmol), and DMF (50 ml) were added to a reaction flask, and the mixture was heated to 90°C and reacted for 2h. The reaction product was added with 50ml of water and extracted three times with dichloromethane (30ml for each time). The organic layers were combined, washed once with 50mg of saturated salt solution, dried with anhydrous sodium sulfate, filtered and separated by column chromatography (dichloromethane/methanol = 40:1) to give 0.49 g of the intermediate (pale yellow solid), yield of 50.1%.

Step 3: 5 -((1-methylpiperidin-4-yl) oxy)-pyridin-2-amine

[0337]



[0338] A reaction flask was charged with the intermediate 2-bromo-5-((1-methylpiperidin-4-yl)oxy)pyridine (1000 mg, 3.70 mmol), sodium bis(trimethylsilyl)amide (618 mg, 3.70 mmol), tetrahydrofuran (50 ml), 2-(dicyclohexylphosphino) biphenyl (120 mg, 0.37 mmol), and tris(dibenzylideneindeneacetone) dipalladium (338mg, 0.37mmol), and the mixture was heated to 65°C and reacted for 12h. The reaction product was added with 50 ml of water, extracted three times with dichloromethane (30ml for each time). The organic layers were combined, washed with 50 ml of saturated salt solution, dried with anhydrous sodium sulfate, filtered, and separated by column chromatography (dichloromethane/methanol = 10:1) to give the intermediate (0.37 g, yellow solid), yield 49.3%.

Step 4 :

[0339] N-(5-((1-methylpiperidin-4-yl)oxy)-pyridin-2-yl)-5-fluoro-4-(7'-fluoro-2'-methylspiro[cyclopentane-1,3'-indole]-5'-yl)pyrimidine-2-amino was prepared by the method similar to Step 6 of Example 1.

[0340] ^{1}HNMR(400MHz, DMSO-d_6), δ9.69(s, 1H), 8.63(s, 1H), 7.93-7.99(d, 1H), 7.80-7.83(d, 1H), 7.81-7.85(d, 1H), 7.12-7.14(d, 1H), 5.76-5.82(m, 1H), 5.02-5.13(m, 2H), 3.79(s, 2H), 2.56-2.59(m, 13H), 2.28-2.33(m, 6H), 1.74-1.99(m, 2H).

[0341] MS(ESI):m/z 505.2$[M+H]^+$.

[0342] The control used in the following experimental examples with No. LY2835219 was prepared according to the preparation method of WO2010075074, and its structural formula was as shown in the structural formula 3 in the background.

Experimental Example 1

[0343] Measurement of CDK kinase inhibitory activity of the compounds of the present invention

[0344] *In vitro* inhibitory effect of the compounds of the present invention on CDK (CDK1, CDK4 and CDK6) kinase activity was tested by the following method.

1.1 Instrument and kit information

[0345]

Name	Type	Manufacturer
Plate shaker	MTS2/4	IKA
Microplate reader	M1000pro	TECAN
Centrifuge	Avanti J-26XP	Beckman Coulter
ADP-Glo™ Kinase Assay + CDK1/CyclinA2 Kinase Enzyme System	V9211	Promega
ADP-Glo™ Kinase Assay + CDK4/CyclinE1 Kinase Enzyme System	V4489	Promega
ADP-Glo™ Kinase Assay +	V4511	Promega
CDK6/CyclinD3 Kinase Enzyme System		
ADP-Glo™ Kinase Assay + CDK9/CyclinK Kinase Enzyme System	V4105	Promega
5×Reaction Buffer A	V307A-C	Promega

1.2 Experimental Preparation

[0346] 1.2.1 Preparation of Kinase Reaction Buffer I: The 5×Reaction Buffer A (Promega; V307A-C) provided in the kit was mixed and diluted with Milli Q H_2O and 0.1 M DTT (dithiothreitol) to give 4× kinase buffer, and then Milli Q H_2O was further added to finally formulate the solution into 1× Kinase buffer.

[0347] Preparation of kinase reaction buffer II: 0.5% DMSO (dimethyl sulfoxide) was added to the 1× kinase reaction buffer and mixed well.

[0348] 1.2.2 Preparation of kinase solution: Kinase solutions having the concentrations required for each reaction system were prepared from 100 ng/μl kinase stock solution and the 1× kinase reaction buffer.

[0349] 1.2.3 Preparation of the test compound solution and control LY2835219 solution:

1. (1) Preparation of control LY2835219 solution
    1. a. 1 μl of 10mM standard substance stock solution was taken and added to 9 μl of kinase reaction buffer I and mixed well; then 90 μl of kinase reaction buffer I was added and mixed well; then 100 μl of kinase reaction buffer I was added and mixed well. The final concentration was 50 μM.
    2. b. 40 μl of kinase reaction buffer II was added into B2 to B10 of a 96-well plate, and 50 μl of the above solution was added into B1;
    3. c. 10μl of solution was taken from well B1, added into B2, and mixed well; then 10μl of the resulting solution was taken and added into B3, and dilution was carried out in sequence to B9, so as to obtain control solutions that are diluted by 5 folds sequentially.
2. (2) Preparation of test compound solution:

1. a. Test compound solutions at a certain concentration were taken, respectively, diluted with kinase reaction buffer I to a final concentration of 50 μM compound solution;
2. b. 40 μl of kinase reaction buffer II was added into H2 to H10 of the 96-well plate; and 50 μl of the above solution was added into H1;
3. c. 10μl of solution was taken from well H1, added into H2, and mixed well; then 10μl of the resulting solution was taken and added into H3, and dilution was carried out in sequence to H9, so as to obtain test compound solutions that are diluted by 5 folds sequentially.

[0350] 1.2.4 Preparation of a mixed solution of reaction substrate and ATP:

1. a. Preparation of ATP solution:

   200 μl of 0.1 mM ATP solution: 2 μl of 10 mM ATP was added to 198 μl of kinase reaction buffer I;

   300 μl of 50 μM ATP solution: 150 μl of kinase reaction buffer I was added to 150 μl of the above 0.1 mM ATP solution;

2. b. Preparation of 300 μl of reaction substrate solution:
   150μl 1μg/μl reaction substrate stock solution was added to 150μl kinase reaction buffer I, and mixed well;
3. c. The above a/b solutions were mixed to obtain mixed solutions, respectively.

[0351] 1.3 Experimental process:

1.3.1: 2μl of compound solutions of various concentrations were taken, added to a 384-well plate, and centrifuged 3min;

1.3.2: 4 μl of kinase solution was added to each well, centrifuged at 5000rpm and 18°C for 10min, and shaken on a plate shaker for 10min;

1.3.3: 4 μl of mixed solution of substrate and ATP was added to each well, centrifuged at 5000rpm and 18°C for 10min, and shaken on a plate shaker at 37°C for 90 min;

1.3.4: The 384-well plate was taken out, and allowed to stand to room temperature;

1.3.5: 10μl of ADP-Glo reagent was added to each well, centrifuged at 5000rpm and 18°C for 18min, and shaken on a plate shaker at 25°C for 40min, and then the reaction was stopped;

1.3.6: 20 μl of kinase detection reagent was added to each well, centrifuged at 5000rpm and 18°C for 10min, and shaken on a plate shaker at 25°C for 30min; and

1.3.7: M1000pro MICROPLATE READER was used to read fluorescence values.

[0352] 1.4 Data processing:

The inhibition rate of each compound at each concentration point was calculated by the formula as follows and curve fitting was performed by GraphPad Prism 5 software to obtain the IC_{50} value.

$$\text{Inhibition rate at each concentration point (inh\%)} = \frac{\text{fluorescence value at zero point concentration} - \text{fluorescence value at each concentration point}}{\text{fluorescence value at zero concentration}} \times 100\%$$

[0353] 1.5 Test results:

The inhibitory effects of LY2835219 disclosed by WO2010075074 and the compounds of Examples 1-43 on CDK1/CyclinA2 and CDK6/CyclinD3 are expressed by IC_{50}, and the specific results are shown in Table 1.

Table 1: Detection results of inhibitory activity of test compounds on CDK1/CyclinA2 and CDK6/CyclinD3 (IC_{50}: nM)

Examples	CDK1/CyclinA2	CDK6/CyclinD3	CDK1/CDK6
LY2835219	319.43	3.81	83.83
1	2223.92	43.03	51.68
2		158.7	-
3	1678	79.06	21.22
4	-	2147	-
5	438.5	7.528	58.23
6	152.2	6.45	23.59
7	83.83	3.26	25.71
8	-	85.77	-
9	268.28	20.42	13.13
10	435.68	47.11	9.25
11	110.06	5.62	19.58
12	1071.23	3.78	283.33
13	327.00	0.39	838.46
14	233.64	4.07	57.40
15	26.92	4.31	6.25
16	95.70	9.02	10.61
17	2707.11	0.73	3708.37
18	189.86	2.74	69.29
19	64.82	65.49	0.99
20	1444.45	87.60	16.49
21	135.19	27.67	4.89
22	915.35	52.50	17.43
23	50.27	4.53	11.10
24	76.44	12.86.	5.94
25	417.16	8.78	47.51

Examples	CDK1/CyclinA2	CDK6/CyclinD3	CDK1/CDK6
26	216.08	12.51	17.27
27	1160.23	16.21	71.57
28	328.47	6.24	52.63
29	82.42	3.145	26.20
30	176.58	0.49	360.37
31	-	58.84	-
32	142.03	10.14	14.01
33	166.28	15.61	10.65
34	111.59	5.13	21.75
35	64.03	8.74	7.33
36	100.9	3.79	26.65
37	73.67	22.87	3.22
38	19.83	0.20	99.15
39	38.51	5.65	6.82
40	161.60	24.97	6.47
41		76.65	
42	3681.98	2.33	1580.25
43	268.90	0.90	298.78
44	-	37.5	-
45	-	43.9	-

[0354] The inhibitory effects of some representative compounds of the present invention on CDK9/CyclinD3, Pim-1 and CDK2/CyclinE1 and CDK4/CyclinE1 are shown in Table 2, Table 3 and Table 4, respectively.

Table 2: Detection results of inhibitory activity of some test compounds on CDK9/CyclinD3 (IC_{50}: nM)

Examples	CDKl/CyclinA2	CDK6/CyclinD3	CDK9/CyclinD3	CDK1/CDK6	CDK9/CDK6
LY2835219	319.43	3.81	5.08	83.83	1.33
1	2223.92	43.03	244.97	51.68	5.69
3	1678	79.06	50.02	21.22	0.63
6	152.2	6.45	0.42	23.59	0.06
7	83.83	3.26	4.58	25.71	1.40
9	268.28	20.42	14.43	13.13	0.71
10	435.68	47.11	27.04	9.25	0.57
12	1071.23	3.78	57.19	283.33	18.75
13	327.00	0.39	2.56	838.46	6.56
16	95.70	9.02	16.37	10.61	1.81
17	2707.11	0.73	5.36	3708.37	7.33

Examples	CDKl/CyclinA2	CDK6/CyclinD3	CDK9/CyclinD3	CDK1/CDK6	CDK9/CDK6
18	189.86	2.74	1.00	69.29	0.36
20	1444.45	87.60	0.24	16.49	0.003
22	915.35	52.50	0.50	17.43	0.009
24	76.44	12.86.	1.74	5.94	0.13
25	417.16	8.78	1.09	47.51	0.12
26	216.08	12.51	10.95	17.27	0.88
27	1160.23	16.21	2.83	71.57	0.17
28	328.47	6.24	0.96	52.63	0.15
30	176.58	0.49	0.43	360.37	0.88

Table 3: Detection results of inhibitory activity of some test compounds on Pim-1 (IC_{50}: nM)

Examples	CDK1/ CyclinA2	CDK6/ CyclinD3	CDK9/ CyclinD3	Pim-1	CDK1/ CDK6	CDK9/ CDK6	Pim-1/ CDK6
LY2835219	319.43	3.81	5.08	3.92	83.83	1.33	1.03
1	2223.92	43.03	244.97	220.42	51.68	5.69	5.12
3	1678	79.06	50.02	197.8	21.22	0.63	2.50
6	152.2	6.45	0.42	15.09	23.59	0.06	2.33
7	83.83	3.26	4.58	461.39	25.71	1.40	141.53
9	268.28	20.42	14.43	173.89	13.13	0.71	8.51
12	1071.23	3.78	57.19	15.11	283.33	18.75	3.99
13	327.00	0.39	2.56	2.22	838.46	6.56	5.69
14	233.64	4.07	-	28.65	57.40	-	7.03
16	95.70	9.02	16.37	686.40	10.61	1.81	76.10
17	2707.11	0.73	5.36	38.27	3708.37	7.33	52.43
24	76.44	12.86	1.74	72.81	5.94	0.13	5.66

Table 4: Inhibitor effect of a part of test compounds on CDK4 and CDK2 (IC_{50}: nM)

Examples	CDK1/ CyclinA2	CDK2/ CyclinEI	CDK4/ CyclinE1	CDK6/ CyclinD3	CDK9/ CyclinD3	Pim-1	CDK1/ CDK6	CDK9/ CDK6	Pim-1/ CDK6
LY2835219	319.43	769.22-	14.83	3.81	5.08	3.92	83.83	1.33	1.03
6	152.2	-	80.9	6.45	0.42	15.09	23.59	0.06	2.33
12	1071.23	394.21	4.46	3.78	57.19	15.11	283.33	18.75	3.99
17	2707.11	2320.88	2.62	0.73	5.36	38.27	3708.37	7.33	52.43

[0355] 1.6 Test Conclusion:

1. 1) The compounds of the present invention have significant inhibitory effect on CDK6 and CDK4.
2. 2) CDK1/CDK6, CDK9/CKD6 and Pim-1/CDK6 can reflect the selectivity of the compound for

protein kinases. The larger the number, the better the selectivity of the compound for CDK6, which indicates that the toxicity of compound for inhibiting pan-kinase may be smaller. The control compound (LY2835219) exhibits CDK1/CDK6 = 83.83, CDK9/CDK6 = 1.33, and Pim-1/CDK6 = 1.03; some of the compounds of the present invention show better selectivity than LY2835219, especially the compound prepared in Example 17 shows higher enzymatic activity for CDK6 and better selectivity for CDK1, CDK9 and Pim1.

Experimental Example 2

[0356] Measurement of Inhibitory Effect of Representative Compounds of the Present Invention on Proliferation of Human Breast Cancer Cell MDB-MA-231

[0357] 2.1 Experimental Materials: Human breast cancer cells MDA-MB-231 purchased from the Cell Resource Center of Peking Union Medical College, DAPI (5mg/mL, Beyotime, c1002), 4% paraformaldehyde (DINGGUO BIOTECHNOLOGY CO. LTD, AR-0211), 96-well plate with black transparent bottom (PE, 6005182), In Cell Analyzer 2200 (GE Healthcare).

[0358] 2.2 Experimental Preparation:

2.2.1 Preparation of human breast cancer cell MDA-MB-231 medium: RPIM1640 + 10% FBS + 1% penicillin/streptomycin

2.2.2 Preparation of test compound solutions and solutions of standard LY2835219:

1. (1) Preparation of solutions of standard LY2835219
    1. a. 3.6μl of 10mM standard stock solution was taken, added to 6.4μl medium, and mixed well; then 90μl of medium was added and mixed well; then 200μl of medium was added, and mixed well to give an initial concentration of 20 mM;
    2. b. 200 μl of medium containing 0.2% DMSO (dimethylsulfoxide) was added into B2 to B10 of a 96-well plate; 300 μl of the above solution was added into B1; and
    3. c. 100 μl of solution was taken from well B1, added into B2, and mixed well; then 100 μl of the resulting solution was taken and added into B3, and dilution was carried out in sequence to B9, so as to obtain standard solutions diluted by 3-fold sequentially.
2. (2) Preparation of test compound solutions
    1. a. Test compound solution at a certain concentration was taken, and diluted with a medium to give a compound solution with a final concentration of 20 μM;
    2. b. 200 μl of medium containing 0.2% DMSO (dimethylsulfoxide) was added into H2 to H10 of the 96-well plate; and 300 μl of the above solution was added into H1; and
    3. c. 100 μl of solution was taken from well H1, added into H2, and mixed well; then 100 μl of the resulting solution was taken and added into H3, and dilution was carried out in sequence to B9, so as to obtain test compound solutions diluted by 3-fold sequentially.

[0359] 2.3 Experimental process:

2.3.1: MDA-MB-231 cells were inoculated into a 96-well cell plate with black transparent bottom at 4000cells/100ul/well, and cultured overnight at 37°C;

2.3.2: The above samples were added at 100μl/well to a culture plate inoculated with cells, gently patted to mix well, and incubated at 37°C for 72h;

2.3.3: Fixation: the cell plate was taken out, the medium was removed, and 50μl of 4% paraformaldehyde was added per well to fix for 10 min;

2.3.4: 50 μl of 0.1M glycine was added to neutralize for 10 min;

2.3.5: 1× PBS (phosphate buffer pH7.2) was used to wash twice;

2.3.6: Permeabilization: 50 μl of 0.2% TritonX-100 (Triton) was added per well, and permeabilization was carried out at room temperature for 10min;

2.3.7: 1× PBS (Phosphate buffer pH 7.2) was used to wash twice;

2.3.8: 5 mg/mL DAPI stock solution was diluted at a ratio of 1: 5000 (final concentration of 1 μg/ml), and staining was performed at room temperature for 20 min;

2.3.9: 1× PBS (Phosphate buffer pH7.2) was used to wash three times; and

2.3.10: Scanning and analysis were performed by In cell analyzer.

[0360] 2.4 Data Processing:

The inhibition rate of each compound at each concentration point was calculated by the formula as follows and curve fitting was performed by GraphPad Prism 5 software to obtain the IC_{50} value.

$$\text{Inhibition rate at each concentration point (inh\%)} = \frac{\text{cell value at zero point concentration} - \text{cell value at each concentration point}}{\text{cell value at zero point concentration.}} \times 100\%$$

[0361] 2.5 Determination Results:

The detection results of the cytological activity of LY2835219 disclosed by WO2010075074 and the compounds of Examples 12 and 17 were expressed by IC_{50}, and the specific results were shown in Table 5.

Table 5: Inhibitory activity of the representative compounds of the present invention on the proliferation of human breast cancer cell MDB-MA-231 (IC_{50}: nM)

Examples	IC_{50}
LY2835219	229.05
12	182.72
17	109.82

[0362] 2.6 Experimental Conclusion:

The compounds of Examples 12 and 17 have significant inhibitory activity on proliferation of the

MDA-MB-231 cell line, and the representative compounds of the present invention have a higher proliferation inhibitory activity relative to the control compound LY2835219.

Experimental Example 3

Rat Pharmacokinetic Determination of Representative Compounds of the Present Invention

3.1 Experimental Summary

[0363] SD rats were used as the test animals, and the concentrations of the drugs in the plasma of rats at different time points after the intravenous administration and intragastric administration of the representative compounds were determined by LC/MS/MS, so as to study the pharmacokinetic behavior of the compounds of the present invention in rats and evaluate the pharmacokinetic characteristics thereof.

3.2 Experimental Scheme

[0364]
3.2.1 Test drugs:

The compound prepared in Example 17 of the present invention.

Control drug LY2835219, prepared by ourselves.

3.2.2 Test animals:
12 healthy adult SD rats, male, 6-8 weeks old, weighing 200-250g, purchased from Suzhou ZhaoYan New Drug Research Center Co., Ltd., Animal Production License No: SCXK (Su) 2013-0003
3.2.3 Preparation of the test drugs

Intragastric administration: An appropriate amount of sample was weighed, and added with 0.1% hydroxyethyl cellulose/0.5% Tween 80 to a final volume to give 1mg/ml solution.

Intravenous injection: An appropriate amount of sample was weighed, and added with 10% of N-methyl-2-pyrrolidone and 90% of 18% sulfobutyl-β-cyclodextrin to a final volume to give 0.4mg/ml solution for intravenous injection.

3.2.4 Administration of the test drugs

Intravenous administration: for each test compound, 3 male SD rats were administered intravenously at a dose of 2 mg/kg and administration volume of 1 ml/kg after fasting overnight.

Intragastric administration: for each test compound, 3 male SD rats were administrated

intragastrically at a dose of 5 mg/kg and an administration volume of 5 ml/kg after fasting overnight.

3.3 Experimental Operation

Before administration and 0.0833, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours after administration, blood was taken through the carotid artery cannula. Whole blood was anticoagulated with EDTA-K2 and centrifuged, the supernatant was removed, and the residue was frozen at -20°C until sample analysis. Plasma samples were analyzed by LC-MS/MS, sample pretreatment was performed using protein precipitation method, the linear range of sample analysis was 1-2000 ng/ml, and the lowest quantification limit was 1 ng/ml.

3.4 Pharmacokinetic data results

The pharmacokinetic parameters of the compounds of the present invention were shown in Table 6 and Table 7.

Table 6: PK parameters of compound 17 of the present invention in rats undergoing single intravenous administration (Mean ± SD)

PK parameters	LY2835219	Example 17
Half life T1/2(hr)	3.69±1.40	8.67±4.98
Area under curve AUC_{0-t}(ng·hr/mL)	1499±337.3	1018±239
Area under curve $AUC_{0-\infty}$(ng·hr/mL)	1535±346.9	1220±456
Apparent volume of distribution V_Z(L/Kg)	6.95±2.43	19.42±5.89
Clearance rate Cl (mL/min/kg)	22.4±4.49	30.0±11.1
Retention time MRT(hr)	3.82±1.44	7.78±1.30

Table 7: PK parameters of compound 17 of the present invention in rats undergoing single intragastric administration (Mean ± SD)

PK parameters	LY2835219	Example 17
Half life T1/2(hr)	4.07	2.00
Blood concentration C_{max}(ng/mL)	312±33.0	188±75
Area under curve AUC_{0-t}(ng·hr/mL)	3275±731	2608±1217.8
Area under curve $AUC_{0-\infty}$(ng·hr/mL)	3438	5256
Retention time MRT(hr)	7.97±1.17	10.21±0.27
Bioavailability(%)	87.4	102.5

3.5 Experimental Conclusion: The representative compound of the present invention (prepared in Example 17) has higher bioavailability in rats relative to compound LY2835219, and has a good oral absorbing effect.

Experimental Example 4

Pharmacokinetic Determination of Representative Compounds of the Present Invention in Mouse

4.1 Experimental Summary

[0365] The ICR mice were used as the test animals, and the concentrations of the drugs in the plasma of mice at different time points after intragastric administration and intravenous administration of the representative compound of the present invention were determined by LC/MS/MS, so as to study the pharmacokinetic behavior of the compounds of the present invention in mice and evaluate the pharmacokinetic characteristics thereof.

4.2 Experimental Scheme

[0366]

4.2.1 Test drugs:

The compound prepared in Example 17 of the present invention.

Control drug LY2835219, prepared by ourselves.

4.2.2 Test animals:
12 healthy adult ICR mice, males, 6-8 weeks old, weighing 20-25 g, purchased from Suzhou ZhaoYan New Drug Research Center Co., Ltd. Animal Production License No. SCXK (Su) 2013-0003

4.2.3 Preparation of the test drugs

An appropriate amount of sample was weighed, and added with 0.1% hydroxyethyl cellulose/0.5% Tween 80 to a final volume to give 0.5mg/ml solution for intragastric administration.

An appropriate amount of sample was weighed, and added with 10% of N-methyl-2-pyrrolidone and 90% of 18% sulfobutyl-β-cyclodextrin to a final volume to give 0.2mg/ml solution for intravenous administration.

4.2.4 Administration of the test drugs

For each test drug, 3 male ICR mice were administered intragastrically at a dose of 5 mg/kg and administration volume of 10 ml/kg after fasting overnight.

For each test drug, 3 male ICR mice were administered intravenously at a dose of 2mg/kg and administration volume of 10ml/kg after fasting overnight.

4.3 Experimental operation

[0367] Before administration and 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours after administration, blood of the intragastrically administered group was taken through carotid artery cannula. Whole blood was anticoagulated with EDTA-K2 and centrifuged, the supernatant was removed, and the residue was frozen at -20°C until sample analysis. Before administration and 0.083, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours after administration, blood of the intravenously administered group was taken through carotid artery cannula. The plasma samples were treated in the same manner as that employed for plasma samples of intragastrically administered group. Plasma samples were analyzed by LC-MS/MS, sample pretreatment was performed using protein precipitation method, the linear range of sample analysis was 1-2000 ng/ml, and the lowest quantification limit was 1 ng/ml.

4.4 Pharmacokinetic data results: see Table 8 and Table 9.

[0368]

Table 8: PK parameters of compound 17 of the present invention in mice undergoing single intravenous administration (Mean ± SD)

PK parameters	LY2835219	Example 17
Half life T1/2(hr)	1.68±0.10	9.1±0.26
Area under curve AUC_{0-t}(ng·hr/mL)	674±82.1	1137±77.8
Area under curve $AUC_{0-\infty}$(ng·hr/mL)	679±81.0	1327±4
Apparent volume of distribution V_Z(L/Kg)	7.21±1.08	19.8±0.81
Clearance rate Cl (mL/min/kg)	49.6±5.72	25.2±1.72
Retention time MRT(hr)	1.64±0.17	7.51±0.28

Table 9: PK parameters of compound 17 of the present invention in mice undergoing single intragastric administration (Mean ± SD)

PK parameters	LY2835219	Example 17
Half life T1/2(hr)	1.70±0.02	8.38±3.16
Blood concentration C_{max}(ng/mL)	154±6.4	134±11.8
Area under curve AUC_{0-t}(ng·hr/mL)	756±34	2134±96.9
Area under curve $AUC_{0-\infty}$(ng·hr/mL)	765±34	2504±387
Retention time MRT(hr)	3.08±0.02	9.45±1.05
Bioavailability(%)	45.1	75.1

[0369] 4.5 Experimental Conclusion: The representative compound 17 of the present invention has higher bioavailability and longer half-life in mice relative to the compound LY2835219, and has a good oral absorbing effect.

Experimental Example 5

Determination of plasma and brain exposure levels of the representative compound 17 of the present invention

5.1 Experimental Summary

[0370] The CD-1 mice were used as the test animals, the concentrations of the drugs in the plasma and brain tissue of mice at different time points after single intragastric administration of the representative compound of the present invention were determined by LC/MS/MS, so as to study the plasma level and brain exposure level of the compounds of the present invention in mice.

5.2 Experimental Scheme

[0371]

5.2.1 Test drugs:

The compound prepared in Example 17 of the present invention.

Control drug LY2835219, prepared by ourselves.

5.2.2 Test animals:
24 healthy adult CD-1 mice, male, 6-8 weeks old, body weight 20-25g, purchased from Shanghai sippr BK Laboratory Animal Co., Ltd., Animal Production License No: SCXK (Shanghai) 2013-0016 .

5.2.3 Preparation of the test drugs
An appropriate amount of sample was weighed, and added with 0.1% hydroxyethylcellulose/0.5% Tween 80 to a final volume to give 1.0mg/ml solution.

5.2.4 Administration of test drug
For each test drug, 12 male CD-1 mice were administered intragastrically at a dose of 10mg/kg and an administration volume of 10ml/kg after fasting overnight.

5.3 Experimental operation

[0372] LY2835219: Before administration and 0.25, 1.5 and 6 hours after administration, blood was taken through carotid artery cannula, and three mice were killed at the same time. The whole brain was collected, mashed and frozen in liquid nitrogen. 10 hours after administration, the remaining animals were sacrificed, whole blood was collected by cardiac puncture, and the whole brain was collected, mashed and frozen in liquid nitrogen.

[0373] Example 17: Before administration and 2, 4 and 24 hours after administration, blood was taken through carotid artery cannula, and three mice were killed at the same time. The whole brain was collected, mashed and frozen in liquid nitrogen. 48 hours after administration, the remaining animals were sacrificed, whole blood was collected by cardiac puncture, and the whole brain was collected, mashed and frozen in liquid nitrogen.

[0374] Treatment of the whole blood sample: The collected whole blood was anticoagulated with EDTA-K2, and centrifuged, the supernatant was removed, and the residue was frozen and stored at -20°C until the sample was analyzed by LC-MS/MS.

[0375] Brain homogenate sampling: Brain homogenate were dispersed with PBS (pH = 7.4): MeOH (v: v, 2: 1) solution in a volume 5 times of the volume of the brain homogenate. 100μl of solution was taken, and the protein therein was precipitated with 600 μl IS. The mixture was centrifuged at 13,000 rpm and 20-25°C for 15 minutes. 50 μL of the supernatant was mixed with 150 μL of water containing 0.3% FA and centrifuged at 4°C. 5 μL of the sample was analyzed by LC-MS/MS.

[0376] The linear range of sample analysis was 1-2000 ng/ml, and the lowest quantification limit was 1ng/ml.

5.4 Measurement results of blood brain exposure level were shown in Table 10.

[0377]

Table 10: Mean exposure of test compound in plasma and brain of CD-1 mice

Parameters		LY2835219	17
Blood concentration C_{max}(ng/mL)	Plasma	836	639
	Brain	188	1270
	Brain/Plasmad	0.22	1.98
The time for blood concentration reaching peak T_{max} (h)	Plasma	1.50	4.00
	Brain	6.00	4.00
Area under curve $AUC_{0\text{-last}}$(ng·hr/mL)	Plasma	4247	7661
	Brain	1113	16786
	Brain/Plasma	0.28	2.19

[0378] 5.5 Experimental Conclusion: The representative compound of the present invention (prepared in Example 17) has better blood brain distribution, higher $AUC_{0\text{-last}}$ ratio (brain/plasma)

and higher C_{max} ratio (brain/plasma) relative to compound LY2835219, and the T_{max} in the brain equals to T_{max} in plasma, indicating that the drug has similar PK behavior in the brain and plasma. It is suggested that the compounds of the present invention can cross the blood-brain barrier to inhibit the growth of brain tumors (brain cancer) and treat brain cancer.

Experimental Example 6

[0379] Determination of inhibitory effect of the representative compound 17 of the present invention on proliferation of the U87 MG cell line.

[0380] 6.1 Experimental Materials: Human glioma cell line U87 MG was purchased from the Cell Bank of Chinese Academy of Sciences, Shanghai, DAPI (5mg/mL, Beyotime, c1002), 4% paraformaldehyde (DINGGUO BIOTECHNOLOGY CO. LTD AR-0211), 96-well plate with black transparent bottom (PE, 6005182), In Cell Analyzer 2200 (GE Healthcare).

[0381] 6.2 Experimental Preparation:

6.2.1 Preparation of U87 MG medium: RPIM1640 + 10% FBS + 1% penicillin/streptomycin

6.2.2 Preparation of test compound solutions and solutions of standard LY2835219:

1. (1) Preparation of solutions of standard LY2835219
    1. a. 3.6μl of 10mM standard stock solution was taken, added with to 6.4 μl medium, and mixed well; then 90μl of medium was added, and mixed well; then 200μl of medium was added, and mixed well to give an initial concentration of 20μM.
    2. b. 200 μl of medium containing 0.2% DMSO (dimethylsulfoxide) was added into oB2 to B10 of a 96-well plate; 300 μl of the above solution was added into B1;
    3. c. 100 μl of solution was taken from well B1, added into B2, and mixed well; then 100 μl of the resulting solution was taken and added into B3, and dilution was carried out in sequence to B9, so as to obtain standard solutions diluted by 3-fold sequentially.
2. (2) Preparation of test compound solutions:
    1. a. Test compound solution at a certain concentration was taken, and diluted with a medium to give a compound solution with a final concentration of 20 μM;
    2. b. 200 μl of medium containing 0.2% DMSO (dimethylsulfoxide) was added into H2 to H10 of the 96-well plate; and 300 μl of the above solution was added into H1;
    3. c. 100 μl of solution was taken from well H1, added into H2, and mixed well; then 100 μl of the resulting solution was taken and added into H3, and dilution was carried out in sequence to H9, so as to obtain test compound solutions diluted by 3-fold sequentially.

[0382] 6.3 Experimental process:

6.3.1: U87 MG cells were inoculated into a 96-well plate with black transparent bottom at 4000cells/100μl/well, and cultured at 37°C overnight;

6.3.2: The above samples were added at 100μl/well to a culture plate inoculated with cells, gently

patted to mix well, and incubated at 37°C for 72h;

6.3.3: Fixation: the cell plate was taken out, the medium was removed, and 50μl of 4% paraformaldehyde was added per well to fix for 10 min;

6.3.4: 50 μl of 0.1M glycine was added to neutralize for 10 min;

6.3.5: 1× PBS (phosphate buffer pH7.2) was used to wash twice;

6.3.6: Permeabilization: 50 μl of 0.2% TritonX-100 (Triton) was added per well, and permeabilization was carried out at room temperature for 10min;

6.3.7: 1× PBS (Phosphate buffer pH 7.2) was used to wash twice;

6.3.8: 5 mg/mL DAPI stock solution was diluted at a ratio of 1: 5000 (final concentration of 1 μg/ml), and staining was performed at room temperature for 20 min;

6.3.9: 1× PBS (Phosphate buffer pH7.2) was used to wash three times; and

6.3.10: Scanning and analysis were performed by In cell analyzer.

[0383] 6.4 Data Processing:

The inhibition rate of each compound at each concentration point was calculated by the formula as follows and curve fitting was performed by GraphPad Prism 5 software to obtain the IC_{50} value.

$$\text{Inhibition rate at each concentration point (inh\%)} = \frac{\text{cell value at zero point concentration} - \text{cell value at each concentration point}}{\text{cell value at zero point concentration.}} \times 100\%$$

[0384] 6.5 Determination Results:

The detection results of the cytological activity of LY2835219 disclosed by WO2010075074 and the compound of Example 17 were expressed by IC_{50}, and the specific results are shown in Table 11.

Table 11: Inhibitory activity of the representative compound of the present invention on the proliferation of U87MG Cell Line (IC_{50}: nM)

Examples	IC_{50}
LY2835219	150.70
17	35.43

[0385] 6.6 Experimental Conclusion:

The compound of Example 17 has significant inhibitory activity on proliferation of the U87MG cell line, and the representative compound of the present invention has a higher proliferation inhibitory activity relative to the control compound LY2835219.

Experimental Example 7

Pharmacodynamic study of the compound 17 of the present invention and the combination of compound 17 of the present invention and temozolomide in a U87-luc orthotopic brain xenograft model

7.1 Experimental Summary

[0386] Adult female BALB/c nude mice were used as test animals. A U87-luc orthotopic brain xenograft model was used to study the effect of representative compound 17 of the present invention on the median survival of female BALB/c nude mice after intragastric administration.

7.2 Experimental Scheme

[0387]

7.2.1 Test drugs:

The compound prepared in Example 17 of the present invention.

Control drug LY2835219, made by ourselves.

Temozolomide was purchased from selleck.

7.2.2 Test animals:
Healthy adult female BALB/c nude mice, 8 mice/group, 6-8 weeks old, weighing 18-22g, purchased from Shanghai sippr BK Laboratory Animal Co., Ltd., Animal Production License No: 2008001658261;2008001658263.

7.2.3 Preparation of the test compounds

An appropriate amount of compound 17 was weighed, and added with 0.1% hydroxyethyl cellulose/0.5% Tween 80 as vehicle to 0.3125 mg/ml;

An appropriate amount of Temozolomide sample was weighed, and added with 0.1% CMC-Na + 0.25% Tween 80 as vehicle to 0.3 mg/ml.

7.3 Orthotopic brain xenograft model

[0388] Adult female BALB/c nude mice were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 80 mg/kg. To relieve pain, animals were injected subcutaneously with buprenorphine at the time points 30 minutes before operation and 6 hours after operation at a dose of 0.1 mg/kg. Animals were observed after anesthesia until all animals had awakened.

[0389] The anesthetized animals were properly fixed, the animals' head skin was sterilized with 70% ethanol, and an approximately 10 mm long incision was made on the right side of the midline from the forehead to the ear line. 3×10^5 U87-luc cells (3 μl, mixture of PBS and Matrigel at a ratio of 4:1) were inoculated at the right frontal lobe that is 2 mm away from the right side of the bregma and is 1 mm away from the front side of the coronal suture in each animal. The incision was sutured with No. 6 suture, and sterilized with polyvinylpyrrolidone. Animals are kept warm until anabiosis. 6 Days after tumor cell transplantation, tumor animals were grouped by stratified randomization based on fluorescence signal intensity values, and the average bioluminescence reached 2.812E+07 photons/sec when the groups were administered in groups. Different animal groups were administered at different doses for a total of 35 days.

7.4 Median survival (days) of the compound 17 of the present invention and the combination of compound 17 of the present invention with Temozolomide in a U87-luc orthotopic brain xenograft model

[0390]

Table 12: Median survival caused by the compound 17 and the combination of compound 17 with temozolomide

Groups	Medium survival (days)	P value[b]	P value[c]
Vehicle	30(29-37)[a]	-	-
Compound 17 3.125 mg/kg QD	38.5(26-59)	0.0822	-
Compound 17 6.25 mg/kg QD	43.5(31-48)	0.0007	-
Compound 17 12.5 mg/kg QD	44.5(31-114)	0.0004	-
Compound 17 25 mg/kg QD	61(41-76)	<0.0001	-
Compound 17 50 mg/kg QD	78.5(63->114)	<0.0001	-
Temozolomide 3 mg/kg IP Day 0, 7, 14, 21 and 28	47(38-83)	<0.0001	-
Compound 17 6.25 mg/kg QD + Temozolomide 3 mg/kg IP Day 0, 7, 14, 21 and 28	57(42->114)	<0.0001	0.0454

a. Survival time range.
b. p values when each group was compared with Vehicle group.
c. p values when each group was compared with temozolomide single-dose group.

[0391] 7.5 Experimental Conclusion: The representative compound 17 of the present invention can significantly prolong the median survival of animals in a U87-luc orthotopic brain xenograft model in a dose-dependent manner. In the study on combined medication with temozolomide, the combined medication further prolongs the median survival of the animals compared to temozolomide used alone.

[0392] In summary, the present invention provides a series of compounds having selective CDK4/6

kinase inhibitory activity which is superior to, or comparable to, that of LY2835219, a candidate drug presently in Phase III clinical trials, with some of the compounds exhibiting better selectivity. Moreover, the preferred compound exhibits good oral absorbing effect, and good blood-brain distribution, and has significant pharmacological effect on the U87-luc orthotopic brain xenograft model, suggesting that the compounds of the present invention are promising to be developed into new drugs for the treatment of diseases related with cell proliferation, in particular malignant tumors, especially brain cancers, and to offer new options for clinicians and patients.

Kits

[0393] The present invention also provides a kit comprising the compounds of structural formulas I-V and VIII or their respective tautomer, mesomer, racemate, enantiomers, diastereomer, deuterated compound, prodrug, or mixture thereof, or pharmaceutically acceptable salts or solvates of the compounds of structural formulas I-V and VIII, or their respective tautomer, mesomers, racemate, enantiomer, diastereomer deuterated compound, prodrug or mixture thereof.

[0394] In addition, the kit may further comprise operation instruction.

Pharmaceutical Compositions

[0395] The present invention also relates to a combination product for treating a cell proliferative disorder, wherein the combination product comprises a pharmaceutically acceptable carrier, and the compounds of structural formulas I-V and VIII, or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug, or mixture thereof; or pharmaceutically acceptable salts or solvates of the compounds of formulas I-V and VIII or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture thereof. The compounds or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug, or mixture thereof; or pharmaceutically acceptable salts or solvates of the compounds of formulas I-V and VIII or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture may be in an effective amount or a therapeutically effective amount in the pharmaceutical composition.

[0396] As used herein, "an effective amount" refers to an amount that is functional and active to humans and/or animals and acceptable to humans and/or animals.

[0397] As used herein, "pharmaceutically acceptable" ingredients are suitable for use in humans and/or animals (such as mammals or birds) without undue adverse side effects (such as toxicity, irritation and allergy), i.e., a substance with reasonable benefit/risk ratio. "Pharmaceutically acceptable carrier" means a carrier for administration and may include various excipients and diluents and the like. Such carriers may include but not limited to water, normal saline, liposomes, lipids, proteins, protein-antibody conjugates, peptides, cellulose, nanogel, buffer, glucose, glycerol, ethanol, and combinations thereof. The choice of carrier should generally be compatible with the mode of administration, as is well known to a person skilled in the art.

[0398] The effective amount of the present invention may vary depending on the mode of administration and the severity of the disease to be treated. The preferred effective amount can be determined by a person skilled in the art based on various factors (eg, through clinical trials). Such factors include, but not limited to: the pharmacokinetic parameters of the active ingredient such as bioavailability, metabolism, half-life, etc.; the severity of the disease of the patient to be treated, the body weight of the patient, the immunological status of the patient, administration route and so on.

Treatment method

[0399] The references to methods of treatment in this paragraph are to be interpreted as references to the compounds, pharmaceutical compositions and medicaments of the present invention for use in a method for treatment of the human or animal body by therapy.

[0400] The present invention also provides a method for treating a cell proliferative disorder, comprising administering to a patient, orally or non-orally, an effective amount of the compounds of structural formulas I-V and VIII, or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug, or mixture thereof; or pharmaceutically acceptable salts or solvates of the compounds of formulas I-V and VIII or their respective tautomer, mesomer, racemate, enantiomer, diastereomer, deuterated compound, prodrug or mixture thereof, or the aforementioned pharmaceutical composition.

[0401] The oral or non-oral routes can be gastrointestinal administration, nasal administration, intratracheal administration, intrapulmonary administration, administration through veins or epidermis at non-lesional sites, intradermal administration, subcutaneous administration, intracardiac administration, intramuscular administration, intraosseous administration, intraperitoneal administration, epidural administration, buccal administration, sublingual administration, ophthalmic administration, rectal administration, vagina administration, urethral administration, ear canal administration and other ways. Preferred modes of administration include oral administration, respiratory tract administration, injection, transdermal administration, mucosal administration, or cavitary administration.

[0402] Wherein, oral administration includes swallowing, sublingual and the like. The respiratory tract administration mode includes inhalation, such as ultrasonic atomizing inhalation, oxygen atomizing aerosol inhalation, manual press atomizing inhalation and the like. The administration mode of injection includes arterial injection, intravenous injection, intramuscular injection, intracardiac injection, intradermal injection and the like. The transdermal administration methods include iontophoresis, electroporation and the like. The mucosal administration mode includes nasal mucosal administration, oral mucosal administration, ophthalmic mucosal administration, rectal mucosal administration, uterine mucosal administration and vaginal mucosal administration. The cavitary administration mode includes rectal administration, vaginal administration, urethral administration, nasal administration, and ear canal administration. It can be understood that the invention is not limited to the described embodiments, but defined by the scope of the claims.

REFERENCES CITED IN THE DESCRIPTION

Cited references

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- WO2003062236A [0008]
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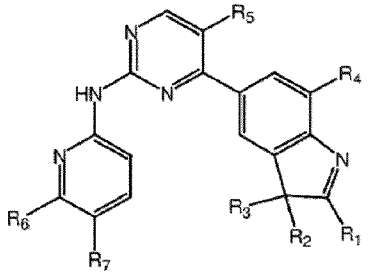
- **FRY DWHARVEY PJKELLER PR et al.**Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts [JMolecular Cancer Therapeutics, 2004, vol. 3, 111427- [0008]
- **LAWRENCE MGS.F.CAIX. LIN et al.**Preclinical characterization of the CDK4/6 inhibitor LY2835219: in-vivo cell cycle-dependent/independent anti-tumor activities alone/in combination with gemcitabine [JInvest New Drugs, 2014, vol. 32, 825- [0008]

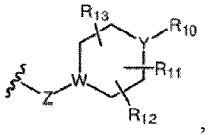
Patentkrav

1. Forbindelse med strukturformel I,



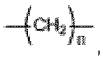
I

hvor R_1 er valgt fra et hydrogenatom, ikke-substitueret lineær eller forgrenet C_1-C_4-alkyl;
hvor R_2 og R_3 er hver uafhængigt valgt fra ikke-substitueret lineær eller forgrenet C_1-C_4-alkyl, eller R_2 og R_3, sammen med C-atomerne, til hvilke de er bundet respektivt, danner en mættet eller umættet 3- til 7-leddet ring;
R_4 og R_5 er hver uafhængigt valgt fra gruppen bestående af hydrogen og halogen, og mindst en af R_4 og R_5 er halogen;
R_6 er valgt fra et hydrogenatom, C_1-C_6-alkyl, C_1-C_6-alkoxy, hydroxyl eller halogen;
R_7 er



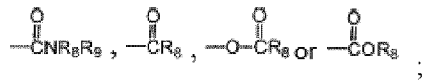
,

hvor Z er carbonyl, O, S, imino, sulfonyl eller



, og n er et heltal fra 0 til 4; W og Y er hver uafhængigt C, N, O eller S, men W og Y kan ikke begge samtidigt være C, og når Z er O eller S, er W C; R_{10}, R_{11}, R_{12} og R_{13} er hver uafhængigt valgt fra et hydrogenatom, C_1-C_6-alkyl, C_3-C_6-cycloalkyl, C_1-C_6-hydroxyalkyl, C_1-C_6-halogenalkyl, C_1-C_6-alkoxy, hydroxyl, halogen; eller
R_6 og R_7 sammen med C-atomerne, til hvilke de er bundet, danner en 5- til 7-leddet heterocyklus indeholdende et eller flere atomer valgt fra N, O eller S, og den 5- til 7-leddede heterocyklus er substitueret med en eller flere substituenter valgt fra C_1-C_6-alkyl, C_3-C_6-cycloalkyl, C_1-C_6-halogenalkyl, C_1-

C_6-alkoxy, C_1-C_6-hydroxyalkyl, hydroxyl, halogen, cyano, -NH_2, -NHR_8, -NR_8R_9,



;

hvor R_8 og R_9 er hver uafhængigt valgt fra gruppen bestående af et hydrogenatom, C_1-C_6-alkyl og C_1-C_6-hydroxyalkyl;
eller en tautomer, en mesomer, en racemat, en enantiomer, en diastereomer, en isotop-mærket forbindelse, eller en blanding deraf; eller farmaceutisk acceptable salte eller solvater af forbindelsen af strukturformel I eller dens tautomer, mesomer, racemat, enantiomer, diastereomer, isotop-mærket forbindelse, eller en blanding deraf.

2. Forbindelsen ifølge krav 1, hvor den isotop-mærkede forbindelse er en deutereret forbindelse.

3. Forbindelsen ifølge krav 1 eller 2, hvor R_2 og R_3, sammen med C-atomerne, til hvilke de begge er bundet, danner en mættet eller umættet 3- til 7-leddet ring;

4. Forbindelsen ifølge krav 3, hvor R_2 og R_3, sammen med C-atomerne, til hvilke de begge er bundet, danner en mættet 3- til 7-leddet ring.

5. Forbindelsen ifølge et hvilket som helst af kravene 1 til 4, hvor R_4 og R_5 er hver uafhængigt valgt fra hydrogen, fluor eller chlor, og mindst en af R_4 og R_5 er fluor eller chlor.

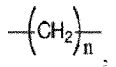
6. Forbindelsen ifølge krav 5, hvor R_4 og R_5 er hver uafhængigt hydrogen eller fluor, og mindst en af R_4 og R_5 er fluor.

7. Forbindelsen ifølge krav 6, hvor R_4 er hydrogen eller fluor, og R_5 er fluor.

8. Forbindelsen ifølge et hvilket som helst af kravene 1 til 7, hvor R_6 er valgt fra et hydrogenatom eller C_1-C_6-alkyl.

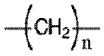
9. Forbindelsen med strukturformel I, eller en tautomer, en mesomer, en racemat, en enantiomer, en diastereomer, en isotop-mærket forbindelse, eller en

blanding deraf; eller farmaceutisk acceptable salte eller solvater af forbindelsen med strukturformel I eller dens tautomer, mesomer, racemat, enantiomer, diastereomer, isotop-mærket forbindelse, eller en blanding deraf ifølge et hvilket som helst af kravene 1 til 8, hvor Z er en carbonylgruppe, O eller



og n er et heltal fra 0 til 4, og/eller W og Y er hver uafhængigt valgt fra C eller N, men W og Y kan ikke begge samtidigt være C, og/eller R_{10}, R_{11}, R_{12} og R_{13} er hver uafhængigt valgt fra et hydrogenatom, C_1-C_6-alkyl, C_3-C_6-cycloalkyl, C_1-C_6-hydroxyalkyl, C_1-C_6-halogenalkyl, C_1-C_6-alkoxy eller hydroxyl.

10. Forbindelsen ifølge krav 9, hvor Z er

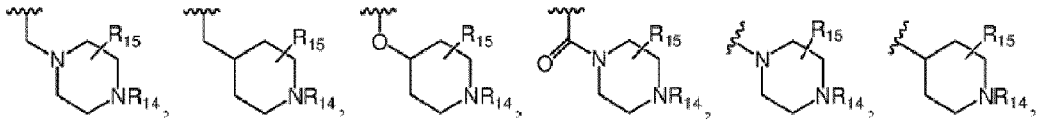


og n er et heltal fra 0 til 2.

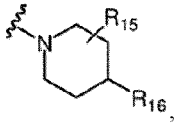
11. Forbindelsen ifølge krav 10, hvor n er 0 eller 1.

12. Forbindelsen ifølge et hvilket som helst af kravene 9 til 11, hvor R_{10}, R_{11}, R_{12} og R_{13} er hver uafhængigt valgt fra et hydrogenatom, C_1-C_6-alkyl, C_1-C_6-hydroxyalkyl, C_1- C_6-halogenalkyl eller C_1-C_6-alkoxy.

13. Forbindelsen ifølge et hvilket som helst af kravene 1 til 8, hvor R_7 er valgt fra substituenterne med følgende strukturer:



og



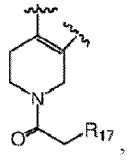
hvor R_{14} og R_{15} er hver uafhængigt valgt fra et hydrogenatom, C_1-C_6-alkyl, C_3-C_6-cycloalkyl, C_1-C_6-halogenalkyl, C_1-C_6-hydroxyalkyl, C_1-C_6-alkoxy eller hydroxyl; R_{16} er valgt fra et hydrogenatom, C_3-C_6-cycloalkyl, C_1-C_6-halogenalkyl, C_1-C_6-hydroxyalkyl, C_1-C_6-alkoxy eller hydroxyl.

14. Forbindelsen ifølge krav 13, hvor R_{14} og R_{15} er hver uafhængigt valgt fra gruppen bestående af et hydrogenatom, C_1-C_6-alkyl, C_3-C_6-cycloalkyl og C_1-C_6-hydroxyalkyl; R_{16} er valgt fra et hydrogenatom, C_1-C_6-alkyl, C_3-C_6-cycloalkyl eller C_1-C_6-hydroxyalkyl.

15. Forbindelsen ifølge et hvilket som helst af kravene 1 til 7, hvor R_6 og R_7 sammen med C-atomerne, til hvilke de er bundet, danner en 6-leddet heterocyklus indeholdende et eller flere atomer valgt fra N, O eller S.

16. Forbindelsen ifølge krav 15, hvor R_6 og R_7 sammen med C-atomerne, til hvilke de er bundet, danner en 6-leddet heterocyklus indeholdende N.

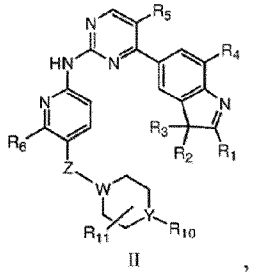
17. Forbindelsen ifølge krav 16, hvor R_6 og R_7 sammen med C-atomerne, til hvilke de er bundet, danner følgende kemiske struktur:



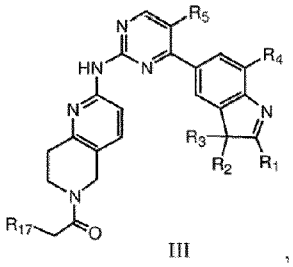
hvor R_{17} er valgt fra hydroxyl eller C_1-C_3-alkoxy.

18. Forbindelsen ifølge krav 17, hvor R_{17} er hydroxyl.

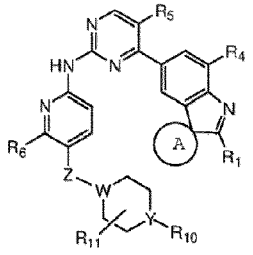
19. Forbindelsen ifølge krav 1 eller 2, hvor forbindelsen er repræsenteret med strukturformel II, III, IV eller V,



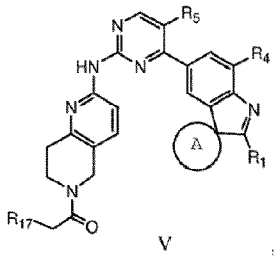
II ,



III ,



IV ,

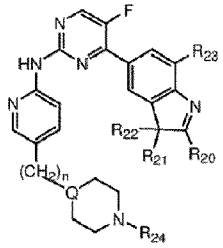


V ;

hvor R_1, R_2 og R_3 er som defineret i et hvilket som helst af kravene 1 til 4, R_4 og R_5 er som defineret i krav 1, 2, 5, 6 eller 7; R_6 er som defineret i krav 1, 2 eller 8; R_{10} og R_{11} er som defineret i krav 1, 2, 9 eller 12; R_{17} er som defineret i krav 17 eller 18; Z, W og Y er som defineret i krav 1, 2, 9, 10 eller 11;
ring A er en mættet 3- til 7-leddet ring.

20. Forbindelsen ifølge krav 19, hvor ring A er en mættet 3- til 6-leddet ring.

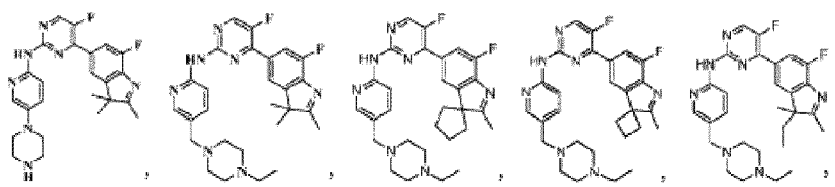
21. Forbindelsen ifølge krav 1 eller 2, hvor forbindelsen er repræsenteret med strukturformel VIII,

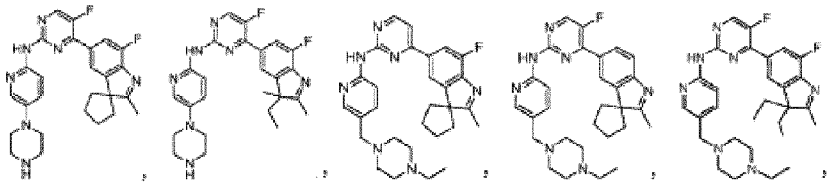


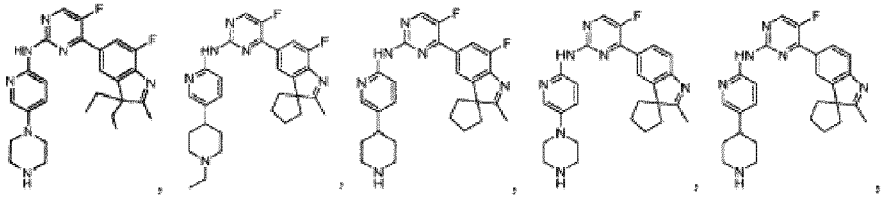
VIII

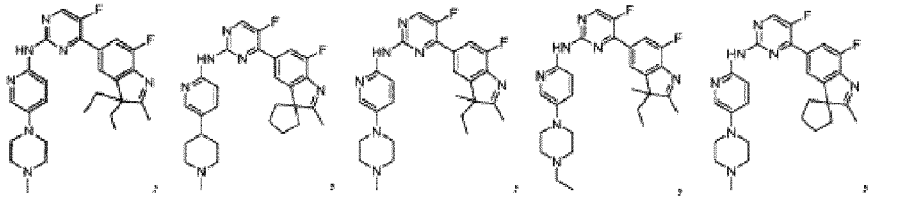
hvor R_{20}, R_{21}, R_{22} er hver uafhængigt valgt fra C_1-C_4-alkyl, eller R_{20} er C_1-C_4-alkyl, og R_{21} og R_{22} sammen med C-atomet, til hvilket de er bundet, danner en mættet 5- til 6-leddet ring; R_{23} er valgt fra hydrogen eller fluor; n = 0 eller 1; R_{24} er valgt fra hydrogen, C_1-C_4-alkyl eller C_1-C_4-hydroxyalkyl, og Q er C eller N.

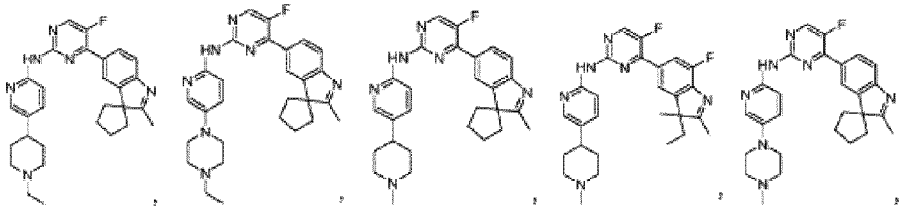
22. Forbindelsen ifølge krav 1 or 2, hvor forbindelsen er repræsenteret med en af følgende strukturer:

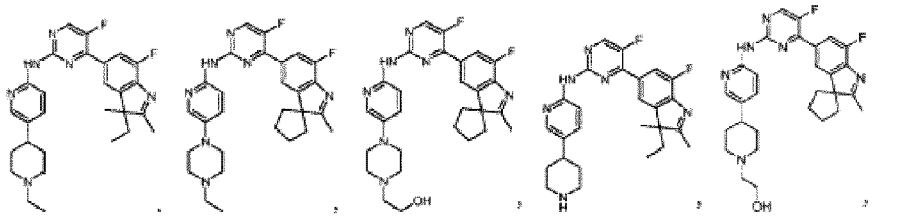


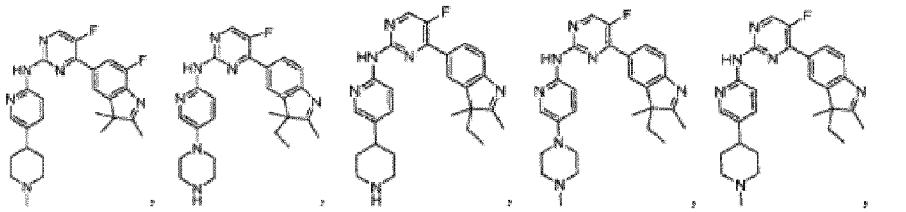


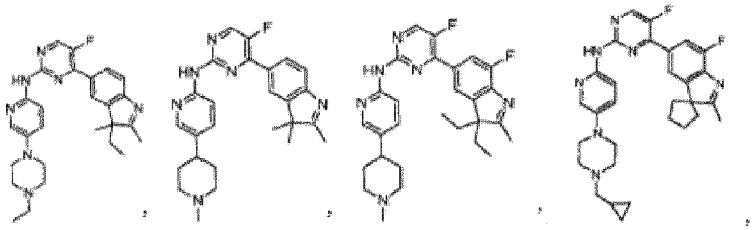


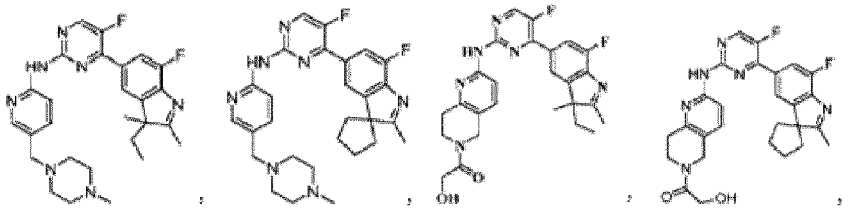


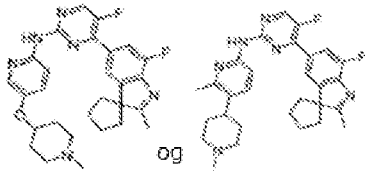






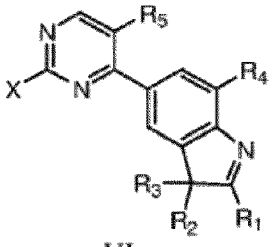






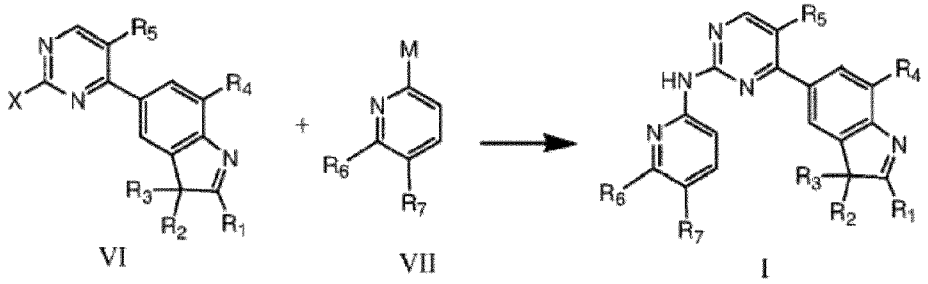
og

23. Forbindelse med strukturformel VI,



VI

hvor R_1, R_2 og R_3 er som defineret i et hvilket som helst af kravene 1 til 4; R_4 og R_5 er som defineret i krav 1, 5, 6 eller 7; X er en afgangsgruppe eller amino; hvor afgangsgruppen sætter forbindelsen med formel VI i stand til at blive reagere med en forbindelse med formel VII for at give forbindelsen med formel I ifølge krav 1; M er en afgangsgruppe eller amino, og kun en af X og M er amino, og en af de to skal være amino,



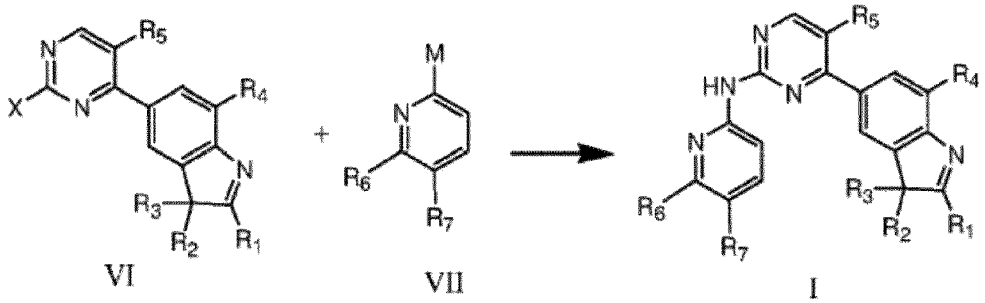
VI VII I

eller en tautomer, en mesomer, en racemat, en enantiomer, en diastereomer, en isotop-mærket forbindelse, eller en blanding deraf; eller farmaceutisk acceptable salte eller solvater af forbindelsen med strukturformel VI eller dens tautomer, mesomer, racemat, enantiomer, diastereomer, eller en blanding deraf.

24. Forbindelsen ifølge krav 23, hvor X er halogen eller amino,

25. Forbindelsen ifølge krav 24, hvor halogenet er fluor, brom eller chlor.

26. Fremgangsmåde til fremstilling af forbindelsen med strukturformel I, eller en tautomer, en mesomer, en racemat, en enantiomer, en diastereomer, en isotop-mærket forbindelse, eller en blanding deraf; eller farmaceutisk acceptable salte eller solvater af forbindelsen med strukturformel I eller dens tautomer, mesomer, racemat, enantiomer, diastereomer, isotop-mærkede forbindelse, eller en blanding deraf ifølge krav 1 eller 2, omfatter: udførelse af en palladium-katalyseret koblingsreaktion mellem en forbindelse med formel VI og en forbindelse med formel VII i et opløsningsmiddel til at give en forbindelse med formel I,



VI VII I

hvor R_1, R_2 og R_3 er som defineret i krav 1 eller 3, R_4 og R_5 er som defineret i krav 1, 5, 6 eller 7; R_6 er som defineret i krav 1, 8, 15, 16, 17 eller 18; R_7 er som defineret i et hvilket som helst af kravene 1, eller 9 til 18; X og M er hver uafhængigt en afgangsgruppe eller amino, og kun en af X og M er amino, og en af

de to skal være amino.

27. Fremgangsmåden ifølge krav 26, hvor afgangsgruppen er halogen.

28. Fremgangsmåden ifølge krav 27, hvor afgangsgruppen er fluor, brom eller chlor.

29. Forbindelser med strukturformlerne I-V og VIII ifølge et hvilket som helst af kravene 1 til 22, eller deres respektive tautomer, mesomer, racemat, enantiomer, diastereomer, isotop-mærkede forbindelse, eller en blanding deraf; eller farmaceutisk acceptable salte eller solvater af forbindelserne med formlerne I-V og VIII ifølge et hvilket som helst af kravene 1 til 22 eller deres respektive tautomer, mesomer, racemat, enantiomer, diastereomer, isotop-mærkede forbindelse, eller en blanding deraf, til anvendelse som et medikament.

30. Forbindelser med strukturformlerne I-V og VIII ifølge et hvilket som helst af kravene 1 til 22, eller deres respektive tautomer, mesomer, racemat, enantiomer, diastereomer, isotop-mærkede forbindelse, eller en blanding deraf; eller farmaceutisk acceptable salte eller solvater af forbindelserne af formlerne I-V og VIII ifølge et hvilket som helst af kravene 1 til 22 eller deres respektive tautomer, mesomer, racemat, enantiomer, diastereomer, isotop-mærkede forbindelse, eller en blanding deraf, til anvendelse i behandlingen af en celleproliferativ lidelse.

31. Forbindelsen til anvendelse ifølge krav 30, hvor den celleproliferative lidelse refererer til kræft hos et pattedyr eller menneske; og/eller

den celleproliferative lidelse er en eller flere sygdomme valgt fra gruppen bestående af

AIDS, atherosklerose, og restenose efter implantation af en vaskulær stent.

32. Forbindelsen til anvendelse ifølge krav 31, hvor den celleproliferative lidelse refererer til kræft hos mennesker, inklusiv ondartede faste tumorer og ondartede ikke-faste tumorer, specifikt inklusiv men ikke begrænset til brystkræft, lungekræft, prostatakræft, leukæmi, hjernekræft, gastrisk kræft og gliom.

33. Forbindelsen til anvendelse ifølge et hvilket som helst af kravene 30 til 32, hvor forbindelsen indgives til individet, som har behov derfor, som den eneste aktive ingrediens eller i kombination med andre biologisk aktive stoffer.

34. Forbindelsen til anvendelse ifølge krav 33, hvor de andre biologisk aktive stoffer inkluderer men er ikke begrænset til anti-kræftmidler, immunosuppressive midler og antivirusmidler; hvor anti-kræftmidlet er et eller flere midler valgt fra cyclophosphamid, ifosfamid, thiotepa, semustin, mechlorethamin hydrochlorid, busulfan, chlorambucil, melphalan, nitrocaphan, formylmelphalan, carmustin, lomustin, altretamin, dibrommannitol, cytarabin, fluoruracil, methotrexat, hydroxyurea, tegafur, meisoindigo, mercaptopurin, cisplatin, carboplatin, oxaliplatin, actinomycin D, mitomycin, doxorubicin, pingyangmycin, epirubicin, pirarubicin, daunorubicin, bleomycin, homoharringtonin og derivater deraf, vincristin og derivater deraf, hydroxycamptothecin og derivater deraf, etoposid og derivater deraf, vindesin og derivater deraf, vinblastin og derivater deraf, vinorelbinbitartrat, taxol og derivater deraf, colchicin og derivater deraf, elemen og derivater deraf, aminoglutethimid, tamoxifen, dexamethason, dutasterid, flutamid, gonadorelin, leuprolidacetat, letrozol, sunitinib, sorafenib, imatinib, gefitinib, erlotinib, vandetanib, pazopanib, lapatinib, canertinib, afatinib, mubritinib, dasatinib, neratinib, temozolomide, trastuzumab, pertuzumab, rituximab, panitumumab, bevacizumab, ipilimumab, ofatumumab, ramucirumab, everolimus, sirolimus og zotarolimus.

35. Kombinationsprodukt til behandling af en celleproliferativ lidelse, hvor kombinationsproduktet omfatter en eller flere forbindelser valgt fra forbindelserne med strukturformlerne I-V og VIII ifølge et hvilket som helst af kravene 1 til 22, eller deres respektive tautomer, mesomer, racemat, enantiomer, diastereomer, isotop-mærkede forbindelse, eller en blanding deraf; eller farmaceutisk acceptable salte eller solvater af forbindelserne med formlerne I-V og VIII ifølge et hvilket som helst af kravene 1 til 22 eller deres respektive tautomer, mesomer, racemat, enantiomer, diastereomer, isotop-mærkede forbindelse, eller en blanding deraf; og/eller kombinationsproduktet er et kit.

36. Kombinationen ifølge krav 35, hvor kombinationsproduktet yderligere inkluderer farmaceutisk acceptable excipienser.