



(51) International Patent Classification:

A61K 31/4439 (2006.01) A61K 31/506 (2006.01)

A61K 31/496 (2006.01)

MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(21) International Application Number:

PCT/US2018/037221

(22) International Filing Date:

13 June 2018 (13.06.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/518,855 13 June 2017 (13.06.2017) US

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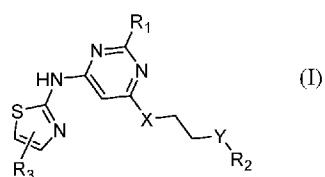
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: AMINOTHIAZOLE COMPOUNDS AS PROTEIN KINASE INHIBITORS



(57) Abstract: Aminothiazole compounds of Formula (I) shown below and pharmaceutical compositions containing one of such compounds. Also disclosed are methods of inhibiting a tyrosine kinase and treating cancer associated with a tyrosine kinase with one of the aminothiazole compounds.



AMINOTHIAZOLE COMPOUNDS AS PROTEIN KINASE INHIBITORS

BACKGROUND

Protein kinases are important in cellular signal pathways that regulate various cell functions, including differentiation, proliferation, migration, and apoptosis. Deregulation of protein kinases is implicated in cancer and a number of other diseases.

Tyrosine kinases, a subclass of protein kinases, regulate target protein function through transfer of phosphate from ATP to the hydroxyl group of a target protein tyrosine. FMS-like tyrosine kinase 3 (“FLT3”), vascular endothelial growth factor receptor (“VEGFR”), and tyrosine-protein kinase Kit (“c-Kit”) are three tyrosine kinases that have been studied as attractive therapeutic targets in cancer treatment.

Mutations of FLT3, a receptor tyrosine kinase, can lead to development of cancer, e.g., acute myeloid leukemia. See Pratz et al., *Current Drug Targets*, 2010, 11(7), 781-9.

By binding to VEGFR and activating it via transphosphorylation, vascular endothelial growth factor, a signal protein, stimulates growth of new blood vessels. VEGFR has been identified as the predominant regulator of tumor angiogenesis. See Hicklin et al., *J Clin Oncol.*, 2005, 23, 1011-1027.

c-Kit, also a receptor tyrosine kinase, is involved in intracellular signaling. The mutated form of c-Kit plays a crucial role in occurrence of some cancers. Inhibition of c-Kit has proved to be effective in treating gastrointestinal stromal tumor, acute myeloid leukemia, and melanoma. See Babaei et al., *Drug Des Devel Ther.*, 2016 10, 2443-2459.

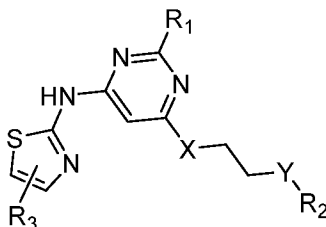
Aminothiazoles compounds, extensively explored as potent tyrosine kinase inhibitors, present several challenges as drug candidates. They possess poor kinase selectivity, often cause animal death in toxicity studies, and generally lack adequate *in vivo* exposure to exert desirable efficacy in pre-clinical or clinical studies.

There is a need to develop new aminothiazole compounds that specifically inhibit certain tyrosine kinases, demonstrate desirable safety profiles, and exert sufficient *in vivo* efficacy in treating target cancers.

SUMMARY

The present invention is based on unexpected discoveries that certain aminothiazole compounds effectively inhibit multiple tyrosine kinases, e.g., FLT3, VEGFR, and c-Kit.

In one aspect, this invention relates to aminothiazole compounds of Formula (I):



(I),

in which R_1 is C_{1-6} alkyl or C_{1-6} thioalkyl; X is O or NR_a , in which R_a is H or C_{1-6} alkyl; Y is CR_bR_c or NR_d , in which each of R_b and R_c , independently, is H , halo, C_{1-6} alkyl, C_{1-6} alkoxy, or amino, or R_b , together with R_a , the carbon atom bonded to R_b , and the nitrogen atom bonded to R_a , is C_{3-10} heterocycloalkyl, and R_d is H or C_{1-6} alkyl, or R_d , together with R_a and the nitrogen atoms bonded to R_d and R_a , is C_{3-10} heterocycloalkyl; R_2 is $-CH_2CH_2R_e$ or NR_fR_g , in which R_e is H , halo, C_{1-6} alkyl, or OR_h and each of R_f and R_g , independently, is C_{1-6} alkyl or C_{3-8} cycloalkyl, R_h being H or C_{1-6} alkyl, or R_h , together with R_d , the oxygen atom bonded to R_h , and the nitrogen atom bonded to R_d , being C_{3-10} heterocycloalkyl; and R_3 is heteroaryl.

The term “alkyl” herein refers to a saturated, linear or branched hydrocarbon moiety, such as $-CH_3$ or branched $-C_3H_7$. The term “cycloalkyl” refers to a non-aromatic, monocyclic, bicyclic, tricyclic, or tetracyclic hydrocarbon moiety, such as cyclohexyl, cyclohexen-3-yl, or adamantyl. The term “alkoxy” refers to an $-O$ -alkyl radical. Examples of alkoxy include, but are not limited to, methoxy, ethoxy, *n*-propoxy, isopropoxy, *n*-butoxy, iso-butoxy, sec-butoxy, and tert-butoxy. The term “thioalkyl” refers to an $-S$ -alkyl radical. Examples of thioalkyl include, but are not limited to, methylthiol, ethylthiol, and benzylthiol. The term “heterocycloalkyl” refers to a non-aromatic, monocyclic, bicyclic, tricyclic, or tetracyclic moiety having one or more ring heteroatoms (e.g., N , O , or S). Examples of heterocycloalkyl include, but are not limited to, 4-morpholinyl, 1-piperazinyl, 4-tetrahydropyranyl, and 4-pyranyl. The term “heteroaryl” refers to a moiety having one or more aromatic rings that contain at least one heteroatom (e.g., N , O , or S). Examples of heteroaryl moieties include furyl, furylene, fluorenyl, pyrrolyl, thienyl, oxazolyl, imidazolyl, thiazolyl, pyridyl, pyrimidinyl, quinazolinyl, quinolyl, isoquinolyl, and indolyl.

Alkyl, thioalkyl, alkoxy, cycloalkyl, heterocycloalkyl, and heteroaryl mentioned herein include both substituted and unsubstituted moieties, unless specified otherwise. Possible substituents on cycloalkyl, heterocycloalkyl, and heteroaryl include C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₂₀ cycloalkyl, C₃₋₂₀ cycloalkenyl, C₁₋₂₀ heterocycloalkyl, C₁₋₂₀ heterocycloalkenyl, C₁₋₁₀ alkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, amino, C₁₋₁₀ alkylamino, C₁₋₂₀ dialkylamino, arylamino, diarylamino, hydroxyl, halogen, thio, C₁₋₁₀ alkylthio, arylthio, C₁₋₁₀ alkylsulfonyl, arylsulfonyl, acylamino, aminoacyl, aminothioacyl, amidino, guanidine, ureido, cyano, nitro, acyl, thioacyl, acyloxy, carboxyl, and carboxylic ester. On the other hand, possible substituents on alkyl include all of the above-recited substituents except C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, and C₂₋₁₀ alkynyl. Cycloalkyl, heterocycloalkyl, aryl, and heteroaryl can also be fused with each other.

The aminothiazole compounds described above include the compounds themselves, as well as their salts, prodrugs, and solvates, if applicable. A salt, for example, can be formed between an anion and a positively charged group (e.g., amino) on an aminothiazole compound. Suitable anions include chloride, bromide, iodide, sulfate, nitrate, phosphate, citrate, methanesulfonate, trifluoroacetate, acetate, malate, tosylate, tartrate, fumurate, glutamate, glucuronate, lactate, glutarate, and maleate. Likewise, a salt can also be formed between a cation and a negatively charged group (e.g., carboxylate) on an aminothiazole compound. Suitable cations include sodium ion, potassium ion, magnesium ion, calcium ion, and an ammonium cation such as tetramethylammonium ion. The aminothiazole compounds also include those salts containing quaternary nitrogen atoms. Examples of prodrugs include esters and other pharmaceutically acceptable derivatives, which, upon administering to a subject, are capable of providing active aminothiazole compounds. A solvate refers to a complex formed between an active aminothiazole compound and a pharmaceutically acceptable solvent. Examples of a pharmaceutically acceptable solvent include water, ethanol, isopropanol, ethyl acetate, acetic acid, and ethanolamine.

In another aspect, this invention relates to a method for inhibiting a tyrosine kinase, e.g., FLT3, VEGFR, and c-Kit. The method includes contacting the tyrosine kinase with an effective amount of one or more of the above-described aminothiazole compounds.

Also within the scope of this invention is a method for treating cancer associated with a tyrosine kinase. The method includes administering to a subject in need thereof an effective amount of one or more of the aminothiazole compounds of Formula (I) described above.

The tyrosine kinase associated to a cancer can be a wild type or mutant. Examples of the tyrosine kinase include, but are not limited to, FLT3, FLT4, VEGFR, platelet-derived growth factor receptor (PDGFR) A, PDGFR B, c-Kit, c-Src (SRC), tyrosine-protein kinase Lyn (LYN) A, LYN B, rearranged during transfection tyrosine kinase (RET), lymphocyte-specific protein tyrosine kinase, Gardner-Rasheed feline sarcoma viral oncogene homolog, discoidin domain receptor 1, kinase insert domain receptor, B lymphocyte kinase, tyrosine-protein kinase Yes, Abelson murine leukemia viral oncogene homolog 1 (ABL1), tyrosine-protein kinase Tek, RET V804L, RET Y791F, FLT3 D835Y, PDGFR A V561D, or ABL1 T315I.

In an exemplary method, the aminothiazole compounds of Formula (I) are used for treating cancer associated with FLT3, VEGFR, or c-Kit.

Examples of the cancer include acute myeloid leukemia, chloroma, chronic myelogenous leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, B-cell lymphoma, multiple myeloma, Waldenström's macroglobulinemia, myelodysplastic syndrome, pancreatic cancer, bladder cancer, colorectal cancer, breast cancer, male genital tract cancer, renal cancer, hepatocellular cancer, lung cancer, ovarian cancer, cervical cancer, uterus cancer, gestational trophoblastic disease, gastric cancer, bile duct cancer, gallbladder cancer, small intestine cancer, esophageal cancer, oropharyngeal cancer, hypopharyngeal cancer, eye cancer, nerve cancer, head and neck cancer, melanoma, plasmacytoma, endocrine gland neoplasm, neuroendocrine cancer, brain tumor, bone cancer, and sarcoma (e.g., gastrointestinal stromal tumor or GIST).

Further within the scope of this invention is a pharmaceutical composition containing one or more of the above-described aminothiazole compounds of Formula (I). The pharmaceutical composition can be used for treating cancer.

This invention also encompasses use of one or more of the above-described aminothiazole compounds of Formula (I) for the manufacture of a medicament for treating cancer.

The term "treating" or "treatment" refers to administering one or more of the aminothiazole compounds to a subject, who has an above-described disease, i.e., cancer, a

symptom of such a disease, or a predisposition toward such a disease, with the purpose to confer a therapeutic effect, e.g., to cure, relieve, alter, affect, ameliorate, or prevent the above-described disease, the symptom of it, or the predisposition toward it. "An effective amount" refers to the amount of an active compound that is required to confer the therapeutic effect. Effective doses will vary, as recognized by those skilled in the art, depending on the types of disease treated, route of administration, excipient usage, and the possibility of co-usage with other therapeutic treatment.

To practice the method of the present invention, a composition having one or more of the above-described aminothiazole compounds can be administered parenterally, orally, nasally, rectally, topically, or buccally. The term "parenteral" as used herein refers to subcutaneous, intracutaneous, intravenous, intraperitoneal, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, or intracranial injection, as well as any suitable infusion technique.

A sterile injectable composition can be a solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are mannitol, water, Ringer's solution, and isotonic sodium chloride solution. In addition, fixed oils are conventionally employed as a solvent or suspending medium (e.g., synthetic mono- or di-glycerides). Fatty acid, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils, such as olive oil and castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions can also contain a long chain alcohol diluent or dispersant, carboxymethyl cellulose, or similar dispersing agents. Other commonly used surfactants such as Tweens and Spans or other similar emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms can also be used for the purpose of formulation.

A composition for oral administration can be any orally acceptable dosage form including capsules, tablets, emulsions and aqueous suspensions, dispersions, and solutions. In the case of tablets, commonly used carriers include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions or emulsions are administered orally, the active ingredient can be suspended or dissolved in an oily phase

combined with emulsifying or suspending agents. If desired, certain sweetening, flavoring, or coloring agents can be added.

A nasal aerosol or inhalation composition can be prepared according to techniques well known in the art of pharmaceutical formulation. For example, such a composition can be prepared as a solution in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

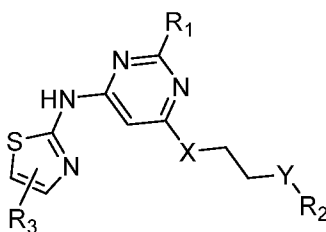
A composition having one or more of the above-described aminothiazole compounds can also be administered in the form of suppositories for rectal administration.

The carrier in the pharmaceutical composition must be “acceptable” in the sense that it is compatible with the active ingredient of the composition (and preferably, capable of stabilizing the active ingredient) and not deleterious to the subject to be treated. One or more solubilizing agents can be utilized as pharmaceutical excipients for delivery of an active 1,5-diphenyl-penta-1,4-dien-3-one compound. Examples of other carriers include colloidal silicon oxide, magnesium stearate, cellulose, sodium lauryl sulfate, and D&C Yellow # 10.

The details of one or more embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

DETAILED DESCRIPTION

Disclosed in detail are aminothiazole compounds of Formula (I):



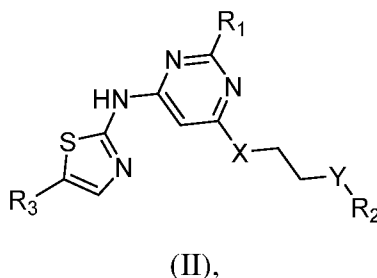
(I),

in which variables R₁, R₂, R₃, X, and Y are defined in the SUMMARY section above.

Typically, compounds of Formula (I) have R₃ being 5- or 6-membered heteroaryl substituted with one or more (CH₂)_nZ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF₃, C₁₋₆ alkyl, or C₁₋₆ alkoxy; or have R₃ being 5- or 6-membered heteroaryl fused with a phenyl ring substituted with one or more substituents independently selected from

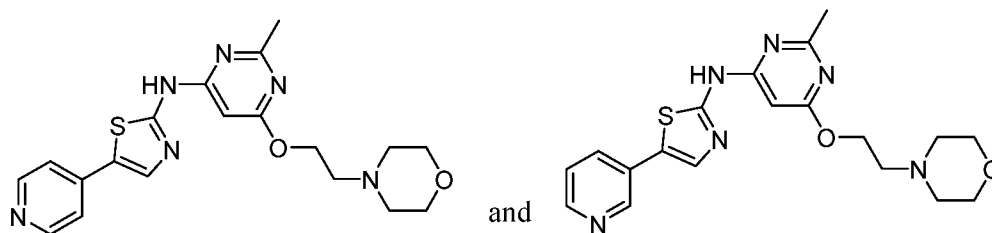
the group consisting of H, halo, CN, OH, CF₃, C₁₋₆ alkyl, and C₁₋₆ alkoxy. Exemplary compounds have R₃ being 6-membered heteroaryl substituted with one or more (CH₂)_nZ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF₃, C₁₋₆ alkyl, or C₁₋₆ alkoxy. Two examples of R₃ are pyridyl and pyrimidyl.

A group of the above-described novel aminothiazole compounds are compounds of Formula (II):



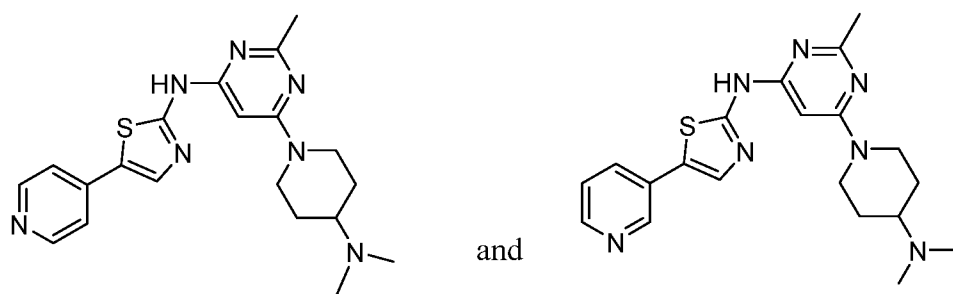
in which R₁ is C₁₋₆ alkyl.

In one subset, compounds of Formula (II) have X being O, Y being NR_d, and R₂ being –CH₂CH₂R_e, in which R_e is OR_h, R_h, together with R_d, the oxygen atom bonded to R_h, and the nitrogen atom bonded to R_d, being C₃₋₁₀ heterocycloalkyl. Compounds of this subset can have R₃ being 5- or 6-membered heteroaryl substituted with one or more (CH₂)_nZ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF₃, C₁₋₆ alkyl, or C₁₋₆ alkoxy; or is 5- or 6-membered heteroaryl fused with a phenyl ring substituted with one or more substituents independently selected from H, halo, CN, OH, CF₃, C₁₋₆ alkyl, and C₁₋₆ alkoxy. For example, R₃ can be pyridyl or pyrimidyl. Exemplary compounds include, but are not limited to, the following compounds:

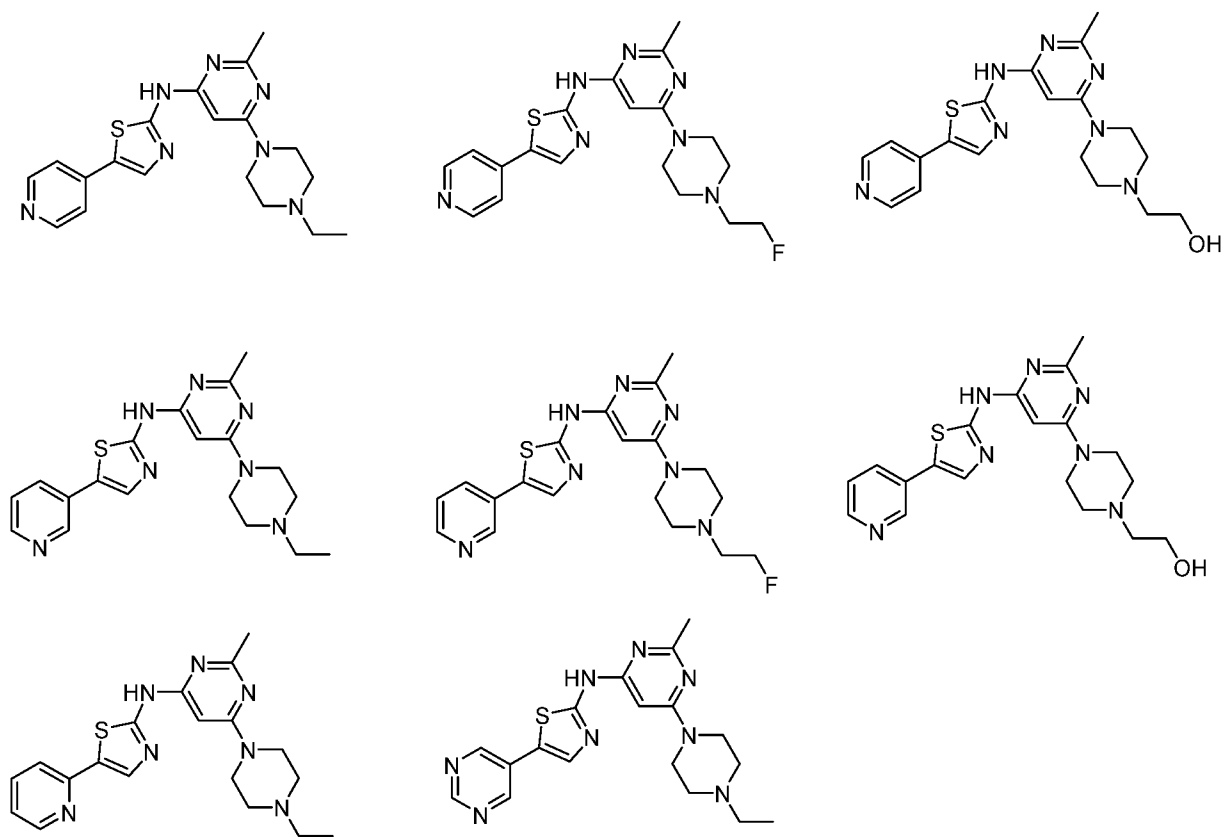


In another subset, compounds of Formula (II) have X being NR_a and Y being CR_bR_c or NR_d, in which R_a, together with R_b, the nitrogen atom bonded to R_a, and the carbon atom bonded to R_b, is C₃₋₁₀ heterocycloalkyl; R_c is H, halo, C₁₋₆ alkyl, C₁₋₆ alkoxy, or amino; and R_d, together with R_a and the nitrogen atoms bonded to R_a and R_d, is C₃₋₁₀ heterocycloalkyl.

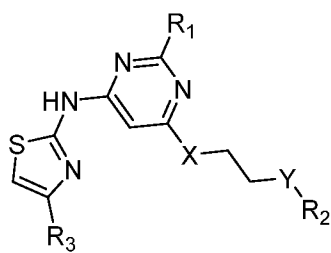
Of note, these compounds can have X being NR_a , Y being CR_bR_c , and R_2 being NR_fR_g , in which R_a , together with R_b , the nitrogen atom bonded to R_a , and the carbon atom bonded to R_b , is C_{3-10} heterocycloalkyl; R_c is H, halo, C_{1-6} alkyl, C_{1-6} alkoxy, or amino; and each of R_f and R_g is C_{1-6} alkyl. They typically have R_3 being 5- or 6-membered heteroaryl substituted with one or more $(\text{CH}_2)_n\text{Z}$ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF_3 , C_{1-6} alkyl, or C_{1-6} alkoxy; or is 5- or 6-membered heteroaryl fused with a phenyl ring substituted with one or more substituents independently selected from H, halo, CN, OH, CF_3 , C_{1-6} alkyl, and C_{1-6} alkoxy. R_3 can be pyridyl or pyrimidyl. Exemplary compounds include, but are not limited to, the following compounds:



On the other hand, the compounds in this subset can have X being NR_a , Y being NR_d , and R_2 being $-\text{CH}_2\text{CH}_2\text{R}_e$, in which R_a , together with R_d and the nitrogen atoms bonded to R_a and R_d , is C_{3-10} heterocycloalkyl; and R_e is H, halo, or OR_h , R_h being H or C_{1-6} alkyl. In general, these compounds have R_3 being 5- or 6-membered heteroaryl substituted with one or more $(\text{CH}_2)_n\text{Z}$ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF_3 , C_{1-6} alkyl, or C_{1-6} alkoxy; or is 5- or 6-membered heteroaryl fused with a phenyl ring substituted with one or more substituents independently selected from H, halo, CN, OH, CF_3 , C_{1-6} alkyl, and C_{1-6} alkoxy. For instance, R_3 is pyridyl or pyrimidyl. Exemplary compounds include, but are not limited to, the following compounds:



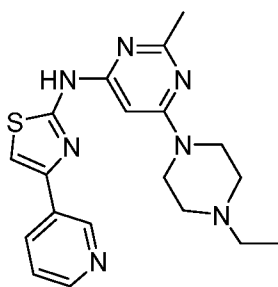
Another group of the novel aminothiazole compounds set forth above are compounds of Formula (III):



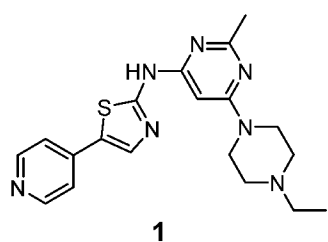
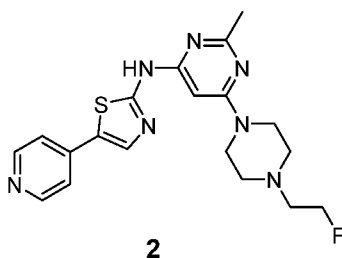
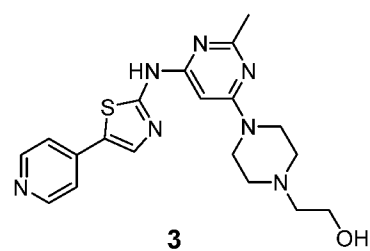
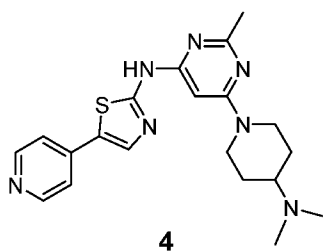
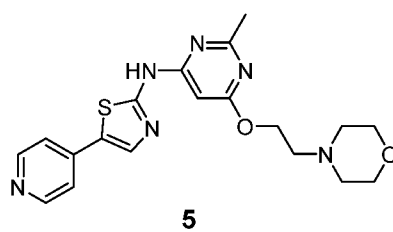
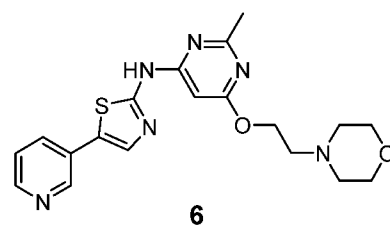
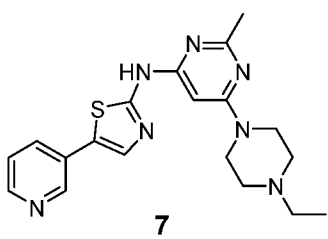
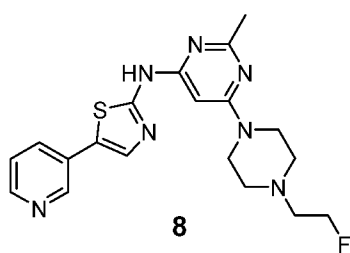
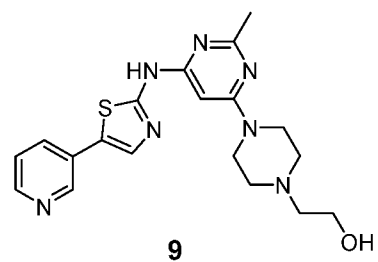
(III),

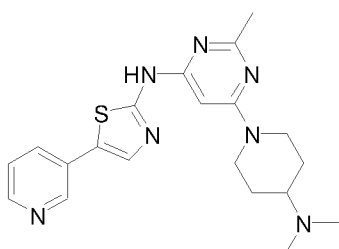
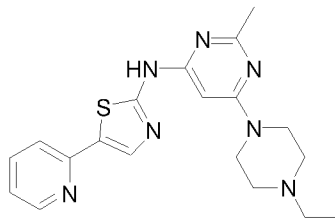
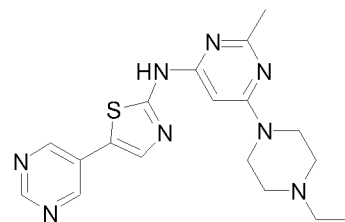
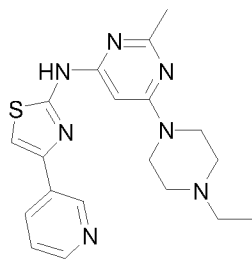
in which R_1 is C_{1-6} alkyl.

An exemplary compound of formula (III) is



Listed below are exemplary compounds of this invention, each assigned a compound number.



**10****11****12****13**

Also within this invention is a pharmaceutical composition containing one or more of the aminothiazole compounds of Formula (I) for treating cancer.

Further covered by this invention is a method for treating cancer, the method including administering to a subject in need thereof an effective amount of a compound of Formula (I).

Synthetic chemistry transformations and protecting group methodologies (protection and de-protection) used for synthesizing the compounds of Formula (I) are well known in the art. See, for example, R. Larock, *Comprehensive Organic Transformations* (2nd Ed., VCH Publishers 1999); P. G. M. Wuts and T. W. Greene, *Greene's Protective Groups in Organic Synthesis* (4th Ed., John Wiley and Sons 2007); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis* (John Wiley and Sons 1994); L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis* (2nd ed., John Wiley and Sons 2009); and G. J. Yu *et al.*, *J. Med. Chem.* 2008, *51*, 6044-6054.

The compounds of Formula (I) thus prepared can be initially screened using biochemical assays, e.g., the kinase assays described in EXAMPLES 2-4 below, or cellular assays, e.g., the *in vitro* anticancer activity assay described in EXAMPLE 5 below, for their potency in inhibiting tyrosine kinases or inhibiting the growth of cancer cells expressing certain tyrosine kinases. They can be subsequently evaluated using *in vivo* assays, e.g., a xenograft animal model assay, for their activity in suppressing tumor growth in a mammal. The selected compounds can be

further tested to verify their efficacy in treating cancer. For example, a compound can be administered to an animal (e.g., a mouse) having cancer and its therapeutic effect is then assessed. Based on the results, appropriate dosage ranges and administration routes can be investigated and determined.

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific examples are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference in their entirety.

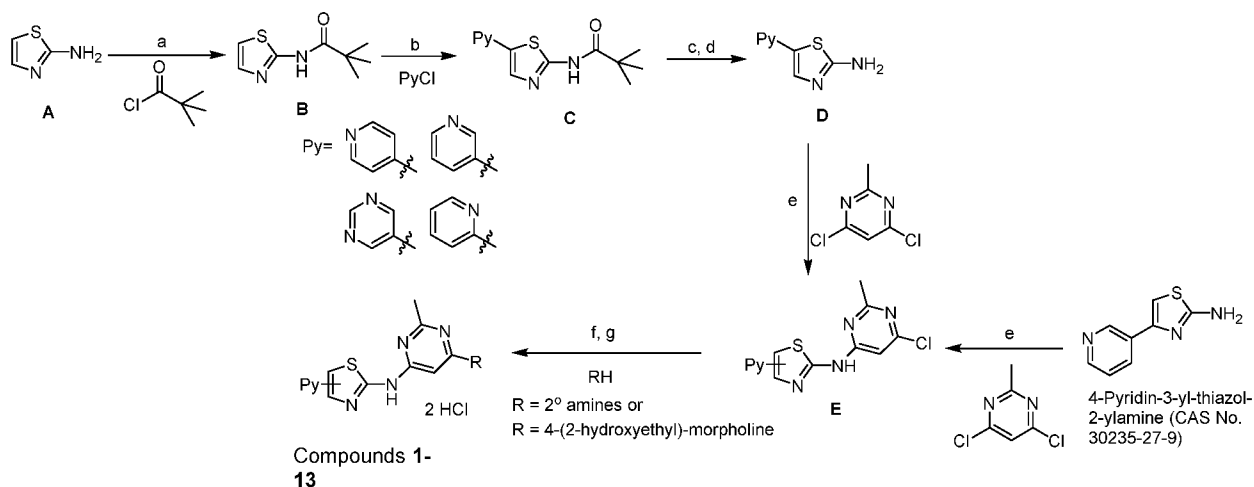
Shown in EXAMPLE 1 below are the synthesis and characterization of 13 exemplary compounds of Formula (I). The analytical data for the compounds thus prepared are also set forth in EXAMPLE 1 and the procedures for testing these compounds are described in EXAMPLES 2-5 that follow.

All chemicals and solvents were purchased from commercial suppliers and used as received. All reactions were carried out under an atmosphere of dry nitrogen. Reactions were monitored by TLC using Merck 60 F254 silica gel glass backed plates (5 × 10 cm); and zones were detected visually under ultraviolet irradiation (254 nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80°C. All flash column chromatography was performed with Merck Kieselgel 60, No. 9385, 230-400 mesh ASTM silica gel as the stationary phase. Proton (¹H) nuclear magnetic resonance spectra were measured on a Varian Mercury-300 or Varian Mercury-400 spectrometer. Chemical shifts were recorded in parts per million (ppm) on the delta (δ) scale relative to the resonance of the solvent peak. The following abbreviations were used to describe coupling: s = singlet; d = doublet; t = triplet; q = quartet; quin = quintet; br = broad; and m = multiplet. LCMS data were measured on an Agilent MSD-1100 ESI-MS/MS, Agilent 1200 series LC/MSD VL, and Waters Acquity UPLC-ESI-MS/MS system.

EXAMPLE 1: Synthesis of Compounds 1-13

Compounds **1-13** were prepared according to the synthetic route shown in Scheme 1 below. Among the listed reagents, TEA is triethylamine, KOAc is potassium acetate, Pd(PPh₃)₄ is tetrakis(triphenylphosphine)palladium(0), DMAc is *N,N*-dimethylacetamide, CsF is cesium

fluoride, HCl is hydrochloric acid, NaHCO₃ is sodium bicarbonate, NaH is sodium hydride, NMP is 1-methyl-2-pyrrolidinone, KOH potassium hydroxide, and DMSO is dimethyl sulfoxide.



Scheme 1. Reagents and conditions: (a) TEA, CH₂Cl₂, 0 °C to rt; (b) KOAc, Pd(PPh₃)₄, DMAc, 150 °C (KOAc is replaced by CsF for Py = pyridin-2-yl); (c) 12N HCl, H₂O, reflux; (d) NaHCO₃, H₂O, rt; (e) NaH, NMP, 0 °C; (f) DMSO, RH, 100 °C or diglyme, KOH, 160 °C for RH = 4-(2-hydroxyethyl)-morpholine; and (g) 6N HCl, 0 °C.

Step I. Synthesis of 2,2-dimethyl-N-thiazol-2-yl-propionamide **B**

To a mixture of 2-aminothiazole **A** (300 mmol) and triethylamine (330 mmol) in anhydrous CH₂Cl₂ (250 mL) at 0 °C was added trimethylacetyl chloride (310 mmol) and the mixture was stirred at room temperature under an argon atmosphere for 1 h. The mixture was washed with 6 N HCl (60 mL) and the organic layer was separated, dried over magnesium sulfate (MgSO₄), and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (20% EtOAc/hexane) to give the titled product **B** as an off-white solid (72%). ¹H NMR (300 MHz, DMSO-*d*₆): δ 11.75 (s, 1H), 7.46 (d, *J* = 6.0 Hz, 1H), 7.17 (d, *J* = 6.0 Hz, 1H), 1.22 (s, 9H); MS (ES⁺) *m/z* calcd. for C₈H₁₂N₂OS: 184.07; found: 185.1 (M+H⁺).

Step II. Synthesis of Compound **C** (Py = pyridin-3-yl, pyridin-4-yl, and pyrimidin-5-yl)

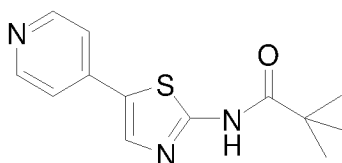
A mixture of 2,2-dimethyl-N-thiazol-2-yl-propionamide **B** (30 mmol), chloropyridine (30 mmol), potassium acetate (120 mmol) and tetrakis(triphenylphosphine)palladium(0) (1.5 mmol) in *N,N*-dimethylacetamide (60 mL) was heated at 150 °C under an argon atmosphere for 24 h. Most of solvent was removed by distillation (120 °C/160 mm Hg) and the residue was

washed with water (250 mL). The precipitate was collected by filtration, redissolved in 10% CH₃OH/CH₂Cl₂ (200 mL) and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure and purified by chromatography on silica gel (1% MeOH/CH₂Cl₂) to give the desired product as an off-white solid (40-85%).

Step II. Synthesis of Compound C (Py= pyridin-2-yl)

A mixture of 2,2-dimethyl-*N*-thiazol-2-yl-propionamide B (10 mmol), chloropyridine (10 mmol), cesium fluoride (20 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.5 mmol) in dimethyl sulfoxide (20 mL) was heated at 160 °C under an argon atmosphere for 16 h. The resultant mixture was partitioned with 0.5N HCl (150 mL) and CH₂Cl₂ (150 mL). The organic layer was separated, dried over MgSO₄, concentrated under reduced pressure and purified by chromatography on silica gel (3% acetone /CH₂Cl₂) to give the desired product as a pale brown solid (20%)

Only one of **C** was selected to show its NMR spectrum and Mass.



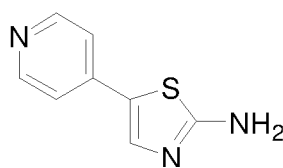
2,2-Dimethyl-*N*-(5-pyridin-4-yl-thiazol-2-yl)-propionamide. ¹H NMR (400 MHz, CDCl₃): δ 9.28 (bs, 1H), 8.61 (dd, *J* = 4.8, 1.6 Hz, 2H), 7.84 (s, 1H), 7.42 (dd, *J* = 4.8, 1.6 Hz, 2H), 1.38 (s, 9H); MS (ES⁺) *m/z* calcd. for C₁₃H₁₅N₃OS: 261.09; found: 262.1 (M+H⁺).

Step III. Synthesis of Compound D

A mixture of **C** (5 mmol) and 12 N HCl (5 mL) in water (5 mL) was heated to reflux for 2 h. Most of solvent was removed under reduced pressure and the residue was diluted with CH₃OH (15 mL). Most of solvent was removed by distillation and the residue was dried in vacuo to give **D** hydrochloride as a pale brown solid.

To a stirred suspension of the above solid in water (30 mL) at room temperature was adjusted to pH = 7 with sodium bicarbonate and the mixture was stirred at 50 °C for 2 h. The precipitate was collected by filtration and dried in vacuo to give the desired product **D** as a pale brown solid (85-90%).

Only one of **D** was selected to show its NMR spectrum and Mass.

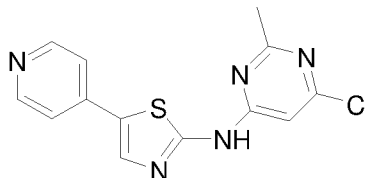


5-Pyridin-4-yl-thiazol-2-ylamine. ^1H NMR (300 MHz, DMSO- d_6): δ 8.41 (dd, $J = 4.8$, 1.5 Hz, 2H), 7.73 (s, 1H), 7.48 (s, 2H), 7.35 (dd, $J = 4.8$, 1.5 Hz, 2H); MS (ES^+) m/z calcd. for $\text{C}_8\text{H}_7\text{N}_3\text{S}$: 177.04; found: 178.1 ($\text{M}+\text{H}^+$).

Step IV. Synthesis of Compound E

To a mixture of **D** or commercially available 4-pyridin-3-yl-thiazol-2-ylamine (4 mmol) and 4,6-dichloro-2-methylpyrimidine (8 mmol) in 1-methyl-2-pyrrolidinone (20 mL) at 0 °C was added sodium hydride (60% in oil, 10 mmol) and the mixture was stirred at 0 °C under an argon atmosphere for 1 h. The reaction was quenched with water (100 mL) at 0 °C and was adjusted to pH = 2 with 6 N HCl. The slurry was adjusted to pH = 7 with sodium bicarbonate and the precipitate was collected by filtration, washed with water (50 mL) and dried in vacuo. The residue was purified by chromatography on silica gel (20% EtOAc/ CH_2Cl_2 , then 5% to 10% MeOH/ CH_2Cl_2 gradient) to give the desired product **E** as a brown solid (45-60%).

Only one of **E** was selected to show its NMR spectrum and Mass.



(6-Chloro-2-methyl-pyrimidin-4-yl)-(5-pyridin-4-yl-thiazol-2-yl)-amine. ^1H NMR (300 MHz, DMSO- d_6): δ 12.15 (s, 1H), 8.53 (dd, $J = 4.5$, 1.5 Hz, 2H), 8.18 (s, 1H), 7.59 (dd, $J = 4.5$, 1.5 Hz, 2H), 6.90 (s, 1H), 2.59 (s, 3H); MS (ES^+) m/z calcd. for $\text{C}_{13}\text{H}_{10}\text{ClN}_5\text{S}$: 303.03; found: 304.1 ($\text{M}+\text{H}^+$).

Step V. Synthesis of Compounds 1-4 and 7-13

A mixture of Compound **E** (2 mmol) and 1-ethylpiperazine (8 mmol) in dimethyl sulfoxide (2 mL) was heated at 100 °C for 1 h. After cooling to room temperature, the mixture was diluted with water (50 mL). The precipitate was collected by filtration, washed with water (10 mL) and dried in vacuo. The residue was purified by chromatography on aluminium oxide

(0.5% to 1.5% MeOH/CH₂Cl₂ gradient) to give freebase of each Compounds **1-4** and **7-13** as an off-white solid.

To a stirred 6 N HCl (10 mL) at 0 °C was added the above solid and the solution was filtered through a 0.45 µm PVDF membrane. To the stirred filtrate was added acetone (40 mL) dropwise over the course of 1 h and was stirred for an additional 1 h at 0 °C. The precipitate was collected by filtration, washed with acetone (15 mL) and dried in vacuo to give the HCl salt of each Compounds **1-4** and **7-13** as a yellow solid (90-95%).

[6-(4-Ethyl-piperazin-1-yl)-2-methyl-pyrimidin-4-yl]-(5-pyridin-4-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 1). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.55 (bs, 1H), 8.72 (d, *J* = 5.6 Hz, 2H), 8.61 (s, 1H), 8.14 (d, *J* = 5.2 Hz, 2H), 6.27 (s, 1H), 4.35 (d, *J* = 13.2 Hz, 2H), 3.55 (d, *J* = 12.0 Hz, 2H), 3.45 (t, *J* = 13.0 Hz, 2H), 3.13 (t, *J* = 5.8 Hz, 2H), 3.02 (q, *J* = 10.0 Hz, 2H), 2.50 (s, 3H), 1.28 (t, *J* = 6.8 Hz, 3H); MS (ES⁺) *m/z* calcd. for C₁₉H₂₃N₇S: 381.17; found: 382.2 (M+H⁺).

{6-[4-(2-Fluoro-ethyl)-piperazin-1-yl]-2-methyl-pyrimidin-4-yl}-(5-pyridin-4-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 2). ¹H NMR (300 MHz, DMSO-*d*₆): δ 11.89 (bs, 1H), 8.73 (d, *J* = 6.3 Hz, 2H), 8.62 (s, 1H), 8.15 (d, *J* = 5.7 Hz, 2H), 6.26 (s, 1H), 4.95 (d, *J* = 47.4 Hz, 2H), 4.38 (s, 2H, overlapping with water peak), 3.70-3.35 (m, 6H), 3.18 (bs, 2H), 2.50 (s, 3H); MS (ES⁺) *m/z* calcd. for C₁₉H₂₂FN₇S: 399.16; found: 400.1 (M+H⁺).

2-{4-[2-Methyl-6-(5-pyridin-4-yl-thiazol-2-ylamino)-pyrimidin-4-yl]-piperazin-1-yl}-ethanol hydrochloride salt (Compound 3). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.03 (s, 1H), 8.73 (d, *J* = 7.2 Hz, 2H), 8.63 (s, 1H), 8.15 (d, *J* = 7.2 Hz, 2H), 6.26 (s, 1H), 4.34 (d, *J* = 12.4 Hz, 2H), 3.82 (t, *J* = 5.2 Hz, 2H), 3.62 (d, *J* = 12.0 Hz, 2H), 3.43 (t, *J* = 12.4 Hz, 2H), 3.30-3.09 (m, 4H), 2.49 (s, 3H); MS (ES⁺) *m/z* calcd. for C₁₉H₂₃N₇OS: 397.17; found: 398.1 (M+H⁺).

[6-(4-Dimethylamino-piperidin-1-yl)-2-methyl-pyrimidin-4-yl]-(5-pyridin-4-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 4). ¹H NMR (300 MHz, DMSO-*d*₆): δ 11.07 (s, 1H), 8.73 (d, *J* = 6.9 Hz, 2H), 8.62 (s, 1H), 8.15 (d, *J* = 6.9 Hz, 2H), 6.25 (s, 1H), 4.43 (d, *J* = 12.9 Hz, 2H), 3.44 (quin, *J* = 5.2 Hz, 1H), 2.94 (t, *J* = 12.5 Hz, 2H), 2.69 (d, *J* = 4.5 Hz, 6H), 2.49 (s, 3H), 2.15 (d, *J* = 10.5 Hz, 2H), 1.60 (q, *J* = 11.0 Hz, 2H); MS (ES⁺) *m/z* calcd. for C₂₀H₂₅N₇S: 395.19; found: 396.1 (M+H⁺).

[6-(4-Ethyl-piperazin-1-yl)-2-methyl-pyrimidin-4-yl]-(5-pyridin-3-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 7). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.23 (bs, 1H),

9.15 (s, 1H), 8.68 (d, $J = 5.2$ Hz, 1H), 8.60 (d, $J = 8.0$ Hz, 1H), 8.19 (s, 1H), 7.93 (t, $J = 6.2$ Hz, 1H), 6.21 (s, 1H), 4.35 (d, $J = 14.4$ Hz, 2H), 3.55 (d, $J = 11.6$ Hz, 2H), 3.40 (t, $J = 13.2$ Hz, 2H), 3.13 (t, $J = 5.8$ Hz, 2H), 3.01 (q, $J = 6.9$ Hz, 2H), 2.50 (s, 3H), 1.28 (t, $J = 6.6$ Hz, 3H); MS (ES⁺) m/z calcd. for C₁₉H₂₃N₇S: 381.17; found: 382.2 (M+H⁺).

{6-[4-(2-Fluoro-ethyl)-piperazin-1-yl]-2-methyl-pyrimidin-4-yl}-(5-pyridin-3-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 8). ¹H NMR (400 MHz, DMSO-*d*6): δ 11.80 (bs, 1H), 9.16 (s, 1H), 8.68 (d, $J = 5.6$ Hz, 1H), 8.62 (d, $J = 8.0$ Hz, 1H), 8.20 (s, 1H), 7.95 (t, $J = 6.8$ Hz, 1H), 6.22 (s, 1H), 4.98 (d, $J = 46.8$ Hz, 2H), 4.34 (bs, 2H), 3.70-3.35 (m, 6H), 3.16 (bs, 2H), 2.49 (s, 3H); MS (ES⁺) m/z calcd. for C₁₉H₂₂FN₇S: 399.16; found: 400.1 (M+H⁺).

2-{4-[2-Methyl-6-(5-pyridin-3-yl-thiazol-2-ylamino)-pyrimidin-4-yl]-piperazin-1-yl}-ethanol hydrochloride salt (Compound 9). ¹H NMR (400 MHz, DMSO-*d*6): δ 11.02 (bs, 1H), 9.18 (s, 1H), 8.69 (s, 1H), 8.64 (d, $J = 7.6$ Hz, 1H), 8.22 (s, 1H), 7.96 (d, $J = 5.2$ Hz, 1H), 6.25 (s, 1H), 4.33 (d, $J = 11.2$ Hz, 2H), 3.80 (s, 1H), 3.60 (d, $J = 11.6$ Hz, 2H), 3.19 (s, 2H), 3.13 (s, 2H), 2.48 (s, 3H); MS (ES⁺) m/z calcd. for C₁₉H₂₃N₇OS: 397.17; found: 398.1 (M+H⁺).

[6-(4-Dimethylamino-piperidin-1-yl)-2-methyl-pyrimidin-4-yl]-(5-pyridin-3-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 10). ¹H NMR (400 MHz, DMSO-*d*6): δ 11.27 (bs, 1H), 9.18 (s, 1H), 8.69 (d, $J = 5.2$ Hz, 1H), 8.64 (d, $J = 8.0$ Hz, 1H), 8.23 (s, 1H), 7.97 (t, $J = 6.8$ Hz, 1H), 6.36 (bs, 1H), 4.42 (d, $J = 8.8$ Hz, 2H), 3.43 (bs, 1H), 2.99 (t, $J = 12.4$ Hz, 2H), 2.68 (s, 3H), 2.67 (s, 3H), 2.55 (s, 3H), 2.17 (d, $J = 10.8$ Hz, 2H), 1.64 (q, $J = 9.2$ Hz, 2H); MS (ES⁺) m/z calcd. for C₂₀H₂₅N₇S: 395.19; found: 396.2 (M+H⁺).

[6-(4-Ethyl-piperazin-1-yl)-2-methyl-pyrimidin-4-yl]-(5-pyridin-2-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 11). ¹H NMR (300 MHz, DMSO-*d*6): δ 11.34 (s, 1H), 8.54 (d, $J = 4.8$ Hz, 1H), 8.32 (s, 1H), 8.01-7.96 (m, 2H), 7.40-7.34 (m, 1H), 6.31 (s, 1H), 4.37 (d, $J = 13.2$ Hz, 2H), 3.62-3.38 (m, 4H), 3.20-2.90 (m, 4H), 2.47 (s, 3H), 1.26 (t, $J = 7.4$ Hz, 3H); MS (ES⁺) m/z calcd. for C₁₉H₂₃N₇S: 381.17; found: 382.2 (M+H⁺).

[6-(4-Ethyl-piperazin-1-yl)-2-methyl-pyrimidin-4-yl]-(5-pyrimidin-5-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 12). ¹H NMR (400 MHz, DMSO-*d*6): δ 11.35 (bs, 1H), 9.20-9.03 (m, 3H), 8.09 (s, 1H), 6.28 (s, 1H), 4.40 (s, 2H), 3.56 (d, $J = 12.4$ Hz, 2H), 3.44 (d, $J = 7.4$ Hz, 2H), 3.13 (bs, 2H), 3.02 (d, $J = 8.0$ Hz, 2H), 1.28 (bs, 3H); MS (ES⁺) m/z calcd. for C₁₈H₂₂N₈S: 382.17; found: 383.3 (M+H⁺).

[6-(4-Ethyl-piperazin-1-yl)-2-methyl-pyrimidin-4-yl]-(4-pyridin-3-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 13). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.80 (bs, 1H), 11.54 (bs, 1H), 9.28 (s, 1H), 8.94 (d, *J* = 8.4 Hz, 1H), 8.84 (d, *J* = 5.2 Hz, 1H), 8.15-8.07 (m, 2H), 6.31 (bs, 2H), 4.35 (d, *J* = 14.0 Hz, 2H), 3.55 (d, *J* = 12.0 Hz, 2H), 3.45 (t, *J* = 13.0 Hz, 2H), 3.15-3.07 (m, 2H), 3.00 (q, *J* = 10.0 Hz, 2H), 2.49 (s, 3H), 1.27 (t, *J* = 7.4 Hz, 3H); MS (ES⁺) *m/z* calcd. for C₁₉H₂₃N₇S: 381.17; found: 382.1 (M+H⁺).

Step V. Synthesis of Compounds 5 and 6

To a mixture of Compound **E** (1 mmol) and 4-(2-hydroxyethyl)-morpholine (4 mmol) in diglyme (1 mL) at 100 °C was added potassium hydroxide (10 mmol) and the mixture was stirred at 160 °C under an argon atmosphere for 10 min. The reaction was quenched with water (20 mL) at 0 °C and was adjusted to pH = 2 with 6 N HCl. The slurry was adjusted to pH = 7 with sodium bicarbonate and the precipitate was collected by filtration, washed with water (10 mL) and dried in vacuo. The residue was purified by chromatography on aluminium oxide (0.5% to 1.5% MeOH/CH₂Cl₂ gradient) to give freebase of Compound **6** as an off-white solid.

To a suspension of the above solid in MeOH (10 mL) at 0 °C was added 6 N HCl (1 mL) with stirring. Most of solvent was removed under reduced pressure and the residue was treated with EtOH (10 mL). The precipitate was collected by filtration, washed with acetone (10 mL) and dried in vacuo to give the HCl salt of each compounds **5-6** as a yellow solid (45-50%).

[2-Methyl-6-(2-morpholin-4-yl-ethoxy)-pyrimidin-4-yl]-(5-pyridin-4-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 5). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.61 (s, 1H), 8.74 (d, *J* = 5.2 Hz, 2H), 8.64 (s, 1H), 8.17 (d, *J* = 5.2 Hz, 2H), 6.37 (s, 1H), 4.72 (s, 2H), 4.00-3.80 (m, 4H), 3.64-3.42 (m, 4H), 3.16 (bs, 2H), 2.59 (s, 3H); MS (ES⁺) *m/z* calcd. for C₁₉H₂₂N₆O₂S: 398.15; found: 399.2 (M+H⁺).

[2-Methyl-6-(2-morpholin-4-yl-ethoxy)-pyrimidin-4-yl]-(5-pyridin-3-yl-thiazol-2-yl)-amine hydrochloride salt (Compound 6). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.51 (bs, 1H), 9.20 (s, 1H), 8.71 (d, *J* = 5.6 Hz, 1H), 8.66 (d, *J* = 8.4 Hz, 1H), 8.24 (s, 1H), 7.98 (dd, *J* = 8.0, 5.6 Hz, 1H), 6.35 (s, 1H), 4.72 (t, *J* = 4.8 Hz, 2H), 3.96 (d, *J* = 10.8 Hz, 2H), 3.85 (t, *J* = 12.0 Hz, 2H), 3.55 (bs, 2H), 3.47 (d, *J* = 12.4 Hz, 2H), 3.19 (bs, 2H), 2.59 (s, 3H); MS (ES⁺) *m/z* calcd. for C₁₉H₂₂N₆O₂S: 398.15; found: 399.1 (M+H⁺).

EXAMPLE 2: Inhibiting FLT3 activity

A study was carried out as follows to test certain compounds prepared according to EXAMPLE 1 in inhibiting FLT3 activity.

GST-FLT3-KD^{WT} containing the FLT3 kinase catalytic domain (residues Y567-S993) was expressed in Sf9 insect cells transfected the baculovirus containing pBac-PAK8-GST-FLT3-KD plasmid. An FLT3^{WT} Kinase-Glo assay was carried out in 96-well plates at 30 °C for 4 hrs to test compound in a final volume of 50 µl including the following components: 75 ng GST-FLT3-KD^{WT} proteins, 25 mM HEPES, pH 7.4, 4 mM MnCl₂, 10 mM MgCl₂, 2 mM DTT, 0.02% Triton X-100, 0.1 mg/ml bovine serum albumin, 25 µM Her2 peptide substrate, 0.5 mM Na₃VO₄, and 1 µM ATP. Following incubation, 50 µl Kinase-Glo Plus Reagent (Promega, Madison, WI, USA) was added to each well and the mixture was incubated at 25 °C for 20 min. A 70-µL aliquot of each reaction mixture was transferred to a black microtiter plate and the luminescence was measured on Wallac Vector 1420 multilabel counter (PerkinElmer, Shelton, CT, USA).

Multiple compounds were tested. Unexpectedly, Compounds **1-11** showed IC₅₀ (the concentration of an inhibitor where the response is reduced by half) values lower than 100 nM.

EXAMPLE 3: Inhibiting VEGFR2 activity

A study was carried out as follows to test certain compounds prepared according to EXAMPLE 1 in inhibiting VEGFR2 activity. Note that VEGFR2 is one of the three main subtypes of VEGFR.

The recombinant GST-VEGFR2 (residues V789-V1356) containing kinase domain was expressed in Sf9 insect cells. The kinase assay was carried out in 96-well plates with tested compound in a final volume of 50 µl reaction at 30°C for 120 minutes with following components: 25 mM HEPES pH 7.4, 10 mM MgCl₂, 4 mM MnCl₂, 0.5 mM Na₃VO₄, 2 mM DTT, 0.02% Triton X100, 0.01% BSA, 1 µM ATP, 2 µM polyGlu4:Tyr peptide, 50~100 ng recombinant VEGFR2. Following incubation, 50 µl Kinase-Glo Plus Reagent (Promega, Madison, WI, USA) was added to each well and the mixture was incubated at 25 °C for 20 min. A 70-µL aliquot of each reaction mixture was transferred to a black microtiter plate and the luminescence was measured on Wallac Vector 1420 multilabel counter (PerkinElmer, Shelton, CT, USA).

Multiple compounds were tested in the VEGFR2 assay. Compounds **1**, **9** and **10** unexpectedly showed IC₅₀ values lower than 30 nM.

EXAMPLE 4: Inhibiting c-Kit activity

A study was carried out as follows to test certain compounds prepared according to EXAMPLE 1 in inhibiting c-Kit activity.

The N-terminal His-tagged human c-KIT (residues T544-V976) recombinant proteins, expressed in Sf9 baculovirus-insect cell expression systems, were purified for c-KIT ADP Kinase-Glo assay. A c-Kit-ADP Kinase-Glo assay was carried out in 96-well plates at 30 °C for 150 mins with a final volume of 10 µl, including 40 mM Tris pH 7.4, 20 mM MgCl₂, 2 mM MnCl₂, 2 mM DTT, 0.01% BSA, 20 µM ATP, 20 µM poly(Glu,Tyr) 4:1 peptide, 0.1 mM Na₃VO₄, 250 ng of recombinant c-Kit proteins, and a tested compound at the indicated concentration. The reactions were stopped by the addition of 5 µl ADP-Glo™ Reagent (Promega, Madison, WI, USA) at 25 °C with 40 min incubation, followed by 10 µl of kinase detection reagent for a further 30 min. Finally, a 30 µl aliquot of each reaction mixture was transferred to a black microtiter plate and the luminescence was measured on Wallac Vector 1420 multilabel counter (Perkin-Elmer, Shelton, CT, USA).

Multiple compounds were tested. Unexpectedly, Compounds **1-7** and **11-12** showed IC₅₀ values lower than 100 nM.

EXAMPLE 5: *In vitro* anticancer activity

A study was carried out as follows to evaluate *in vitro* anticancer activity of certain compounds prepared according to EXAMPLE 1 using cell lines and MTS cell viability assays (MTS represents 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium).

Leukemia cell lines MOLM-13, MV4:11, and Kasumin-1 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The human gastrointestinal stromal tumor GIST-T1 cell line was purchased from Cosmo Bio Co., LTD (Tokyo, Japan). All leukemia cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 10 U/ml penicillin, and 10 g/ml streptomycin at 37°C and 5% CO₂. The cell line

GIST-T1 was cultured in DMEM medium supplemented with 10% FBS, 0.01% nonessential amino acids, 10 U/ml penicillin, and 10 g/ml streptomycin.

GIST882, GIST48 and GIST430 cells were all cultured in incubators maintained at 37 °C and 5% CO₂. GIST882 was cultured in RPMI-1640 supplemented with 20% fetal bovine serum (FBS). GIST48 was cultured with F10 supplemented with 20% FBS, 0.5% Mito, serum extender (BD Bioscience, 355006) and 1% pituitary extract bovine (BD Bioscience 354123). GIST430 was cultured in IMDM supplemented with 20% FBS. GIST882, GIST430 and GIST48 cells were provided by Dr. Jonathan A. Fletcher (Harvard Medical School, US).

MOLM-13, MV4:11, and Kasumin-1 MTS assays

Cells were seeded in 96-well culture plates at a density of 1×10^4 cells/100 μ l/well in triplicates and were treated for 72 hours with an indicated concentration of test compound ranging from 1 nM to 10 μ M. Colorimetric CellTiter 96® Aqueous One Solution Cell Proliferation assay (MTS assay; Promega, Madison, WI, USA) was used to determine the cytotoxicity. The optical density at 492 nm was measured using a microplate photometer (Victor2; Perkin-Elmer, Waltham, MA, USA). IC₅₀ values were determined by MTS assay when cells were treated with test compound for 72 hours and calculated with GraphPad Prism 6. Each experiment was in triplicate.

GIST-T1 MTS assay

GIST-T1 cells were seeded in 96-well culture plates at a density of 8000 cells/100 μ l/well in triplicates and were treated for 72 hours with an indicated concentration of test compound ranging from 1 nM to 10 μ M. Colorimetric CellTiter 96® Aqueous One Solution Cell Proliferation assay (MTS assay; Promega, Madison, WI, USA) was used to determine the cytotoxicity. The optical density at 492 nm was measured using a microplate photometer (Victor2; Perkin-Elmer, Waltham, MA, USA). IC₅₀ values were determined by MTS assay when cells were treated with test compound for 72 hours and calculated with GraphPad Prism 6. Each experiment was in triplicate.

GIST882, GIST48, and GIST430 MTS assays

GIST cells (4×10^4) were treated with different dosage of compounds. The treated GIST882 cells were incubated for 144 hours and GIST48 and GIST430 cells were incubated for 120 hours at 37°C in 5% CO₂. Cell proliferation was determined by incubating the cells with

methylene blue (Clontech, CA, US) for 1 hour. The absorbance was measured at 450 nm using SpectraMax M5 microplate reader (Molecular Devices, US).

The GI₅₀ (the concentration for 50% of maximal inhibition of cell proliferation) values of certain compounds of Formula (I) are shown in the table below:

Compound	GI ₅₀ (nM)						
	MOLM-13	MV4:11	Kasumi-1	GIST-T1	GIST430	GIST48	GIST882
1	10	13	11	11	3.8	19	5.0
7	62	34	266	53	60	820	20
12	211	132	349	119	ND	ND	ND

ND, not determined.

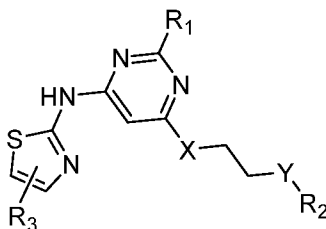
OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A compound of formula (I) below or a pharmaceutically acceptable salt thereof:



(I),

wherein

R_1 is C_{1-6} alkyl or C_{1-6} thioalkyl;

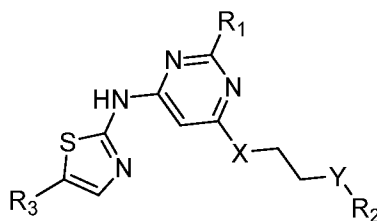
X is O or NR_a , in which R_a is H or C_{1-6} alkyl;

Y is CR_bR_c or NR_d , in which each of R_b and R_c , independently, is H, halo, C_{1-6} alkyl, C_{1-6} alkoxy, or amino, or R_b , together with R_a , the carbon atom bonded to R_b , and the nitrogen atom bonded to R_a , is C_{3-10} heterocycloalkyl; and R_d is H or C_{1-6} alkyl, or R_d , together with R_a and the nitrogen atoms bonded to R_d and R_a , is C_{3-10} heterocycloalkyl;

R_2 is $-CH_2CH_2R_e$ or NR_fR_g , in which R_e is H, halo, C_{1-6} alkyl, or OR_h and each of R_f and R_g , independently, is C_{1-6} alkyl or C_{3-8} cycloalkyl, R_h being H or C_{1-6} alkyl, or R_h , together with R_d , the oxygen atom bonded to R_h , and the nitrogen atom bonded to R_d , being C_{3-10} heterocycloalkyl; and

R_3 is heteroaryl.

2. The compound or salt of claim 1, wherein the compound is of formula (II):



(II),

in which R_1 is C_{1-6} alkyl.

3. The compound or salt of claim 2, wherein X is O, Y is NR_d, and R₂ is –CH₂CH₂R_e, in which R_e is OR_h, R_h, together with R_d, the oxygen atom bonded to R_h, and the nitrogen atom bonded to R_d, being C₃₋₁₀ heterocycloalkyl.

4. The compound or salt of claim 2, wherein X is NR_a and Y is CR_bR_c or NR_d, in which R_a, together with R_b, the nitrogen atom bonded to R_a, and the carbon atom bonded to R_b, is C₃₋₁₀ heterocycloalkyl; R_c is H, halo, C₁₋₆ alkyl, C₁₋₆ alkoxy, or amino; and R_d, together with R_a and the nitrogen atoms bonded to R_a and R_d, is C₃₋₁₀ heterocycloalkyl.

5. The compound or salt of claim 4, wherein X is NR_a, Y is CR_bR_c, and R₂ is NR_fR_g, in which R_a, together with R_b, the nitrogen atom bonded to R_a, and the carbon atom bonded to R_b, is C₃₋₁₀ heterocycloalkyl; R_c is H, halo, C₁₋₆ alkyl, C₁₋₆ alkoxy, or amino; and each of R_f and R_g is C₁₋₆ alkyl.

6. The compound or salt of claim 4, wherein X is NR_a, Y is NR_d, and R₂ is –CH₂CH₂R_e, in which R_a, together with R_d and the nitrogen atoms bonded to R_a and R_d, is C₃₋₁₀ heterocycloalkyl; and R_e is H, halo, or OR_h, R_h being H or C₁₋₆ alkyl.

7. The compound or salt of claim 1, wherein R₃ is 5- or 6-membered heteroaryl substituted with one or more (CH₂)_nZ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF₃, C₁₋₆ alkyl, or C₁₋₆ alkoxy; or is 5- or 6-membered heteroaryl fused with a phenyl ring substituted with one or more substituents independently selected from the group consisting of H, halo, CN, OH, CF₃, C₁₋₆ alkyl, and C₁₋₆ alkoxy.

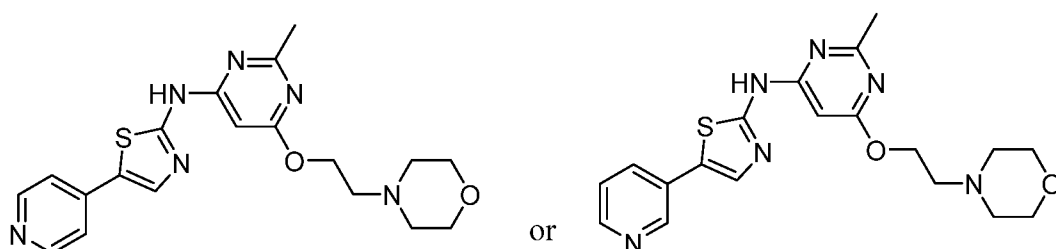
8. The compound or salt of claim 7, wherein R₃ is 6-membered heteroaryl substituted with one or more (CH₂)_nZ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF₃, C₁₋₆ alkyl, or C₁₋₆ alkoxy.

9. The compound or salt of claim 8, wherein R₃ is pyridyl or pyrimidyl.

10. The compound or salt of claim 3, wherein R_3 is 5- or 6-membered heteroaryl substituted with one or more $(CH_2)_nZ$ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF_3 , C_{1-6} alkyl, or C_{1-6} alkoxy; or is 5- or 6-membered heteroaryl fused with a phenyl ring substituted with one or more substituents independently selected from the group consisting of H, halo, CN, OH, CF_3 , C_{1-6} alkyl, and C_{1-6} alkoxy.

11. The compound or salt of claim 10, wherein R_3 is pyridyl or pyrimidyl.

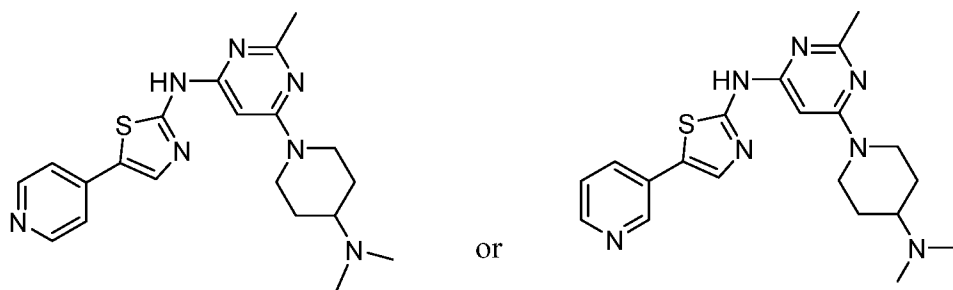
12. The compound or salt of claim 11, wherein the compound is



13. The compound or salt of claim 5, wherein R_3 is 5- or 6-membered heteroaryl substituted with one or more $(CH_2)_nZ$ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF_3 , C_{1-6} alkyl, or C_{1-6} alkoxy; or is 5- or 6-membered heteroaryl fused with a phenyl ring substituted with one or more substituents independently selected from the group consisting of H, halo, CN, OH, CF_3 , C_{1-6} alkyl, and C_{1-6} alkoxy.

14. The compound or salt of claim 13, wherein R_3 is pyridyl or pyrimidyl.

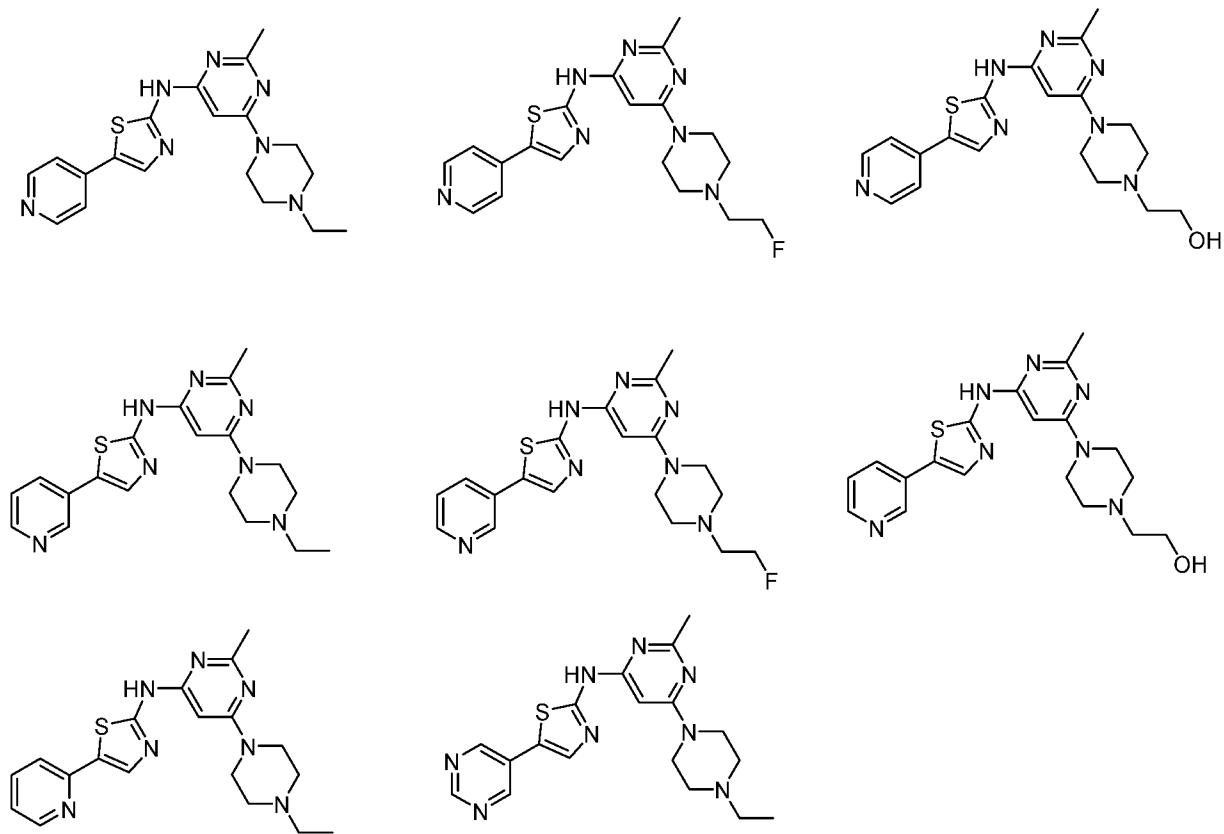
15. The compound or salt of claim 14, wherein the compound is

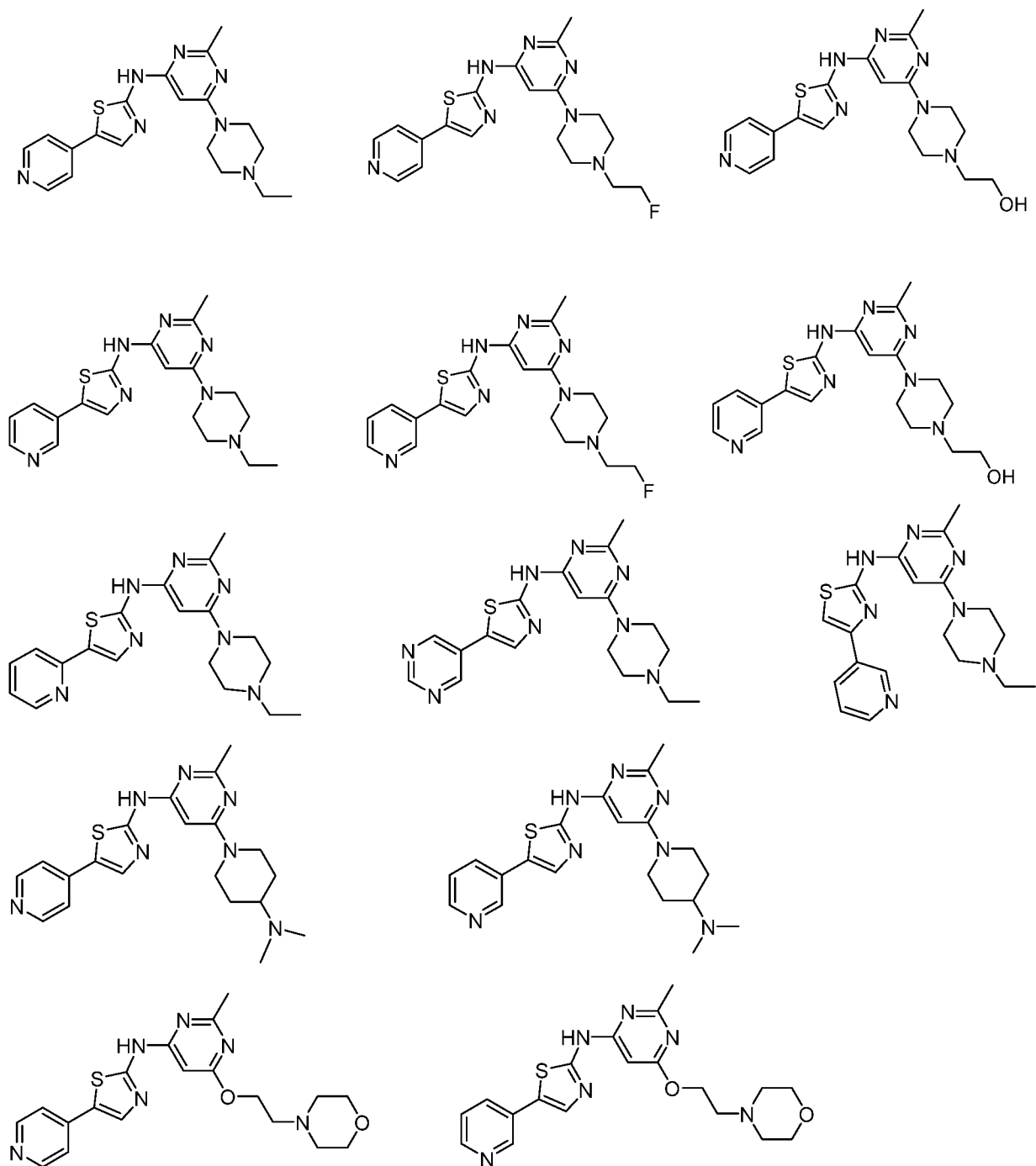


16. The compound or salt of claim 6, wherein R_3 is 5- or 6-membered heteroaryl substituted with one or more $(CH_2)_nZ$ moieties independently, in which n is 0 or 1 and Z is H, halo, CN, OH, CF_3 , C_{1-6} alkyl, or C_{1-6} alkoxy; or is 5- or 6-membered heteroaryl fused with a phenyl ring substituted with one or more substituents independently selected from the group consisting of H, halo, CN, OH, CF_3 , C_{1-6} alkyl, and C_{1-6} alkoxy.

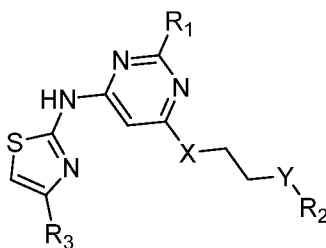
17. The compound or salt of claim 16, wherein R_3 is pyridyl or pyrimidyl.

18. The compound or salt of claim 17, wherein the compound is one of the following compounds:





20. The compound or salt of claim 1, wherein the compound is of formula (III):



(III),

in which R₁ is C₁₋₆ alkyl.

21. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound or salt of claim 1.

22. A method of inhibiting a tyrosine kinase, comprising contacting the tyrosine kinase with an effective amount of a compound or salt of claim 1.

23. A method of treating cancer associated with a tyrosine kinase, the method comprising administering to a subject in need thereof an effective amount of a compound or salt of claim 1.

24. The method of claim 23, wherein the tyrosine kinase is FMS-like tyrosine kinase 3 (FLT3), FMS-like tyrosine kinase 4, vascular endothelial growth factor receptor (VEGFR), colony stimulating factor 1 receptor (CSF1R), platelet-derived growth factor receptor (PDGFR) A, PDGFR B, tyrosine-protein kinase Kit (c-KIT), c-Src (SRC), tyrosine-protein kinase Lyn (LYN) A, LYN B, rearranged during transfection tyrosine kinase (RET), lymphocyte-specific protein tyrosine kinase, Gardner-Rasheed feline sarcoma viral oncogene homolog, discoidin domain receptor 1, kinase insert domain receptor, B lymphocyte kinase, tyrosine-protein kinase Yes, Abelson murine leukemia viral oncogene homolog 1 (ABL1), tyrosine receptor kinase TRKA, TRKB, TRKC, ZAK/MLTK, IRAK4, RET V804L, RET Y791F, FLT3 D835Y, PDGFR A V561D, or ABL1 T315I.

25. The method of claim 24, wherein the tyrosine kinase is FLT3, VEGFR, or c-KIT.

26. The method of claim 25, wherein the cancer is acute myeloid leukemia, chloroma, chronic myelogenous leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, B-cell lymphoma, multiple myeloma, Waldenstrom's macroglobulinemia, myelodysplastic syndrome, pancreatic cancer, bladder cancer, colorectal cancer, breast cancer, male genital tract cancer, renal cancer, hepatocellular cancer, lung cancer, ovarian cancer, cervical cancer, uterus cancer, gestational trophoblastic disease, gastric cancer, bile duct cancer, gallbladder cancer, small intestine cancer, esophageal cancer, oropharyngeal cancer, hypopharyngeal cancer, eye cancer, nerve cancer, head and neck cancer, melanoma, plasmacytoma, endocrine gland neoplasm, neuroendocrine cancer, brain tumor, bone cancer, or sarcoma.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/37221

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/4439, A61K 31/496, A61K 31/506 (2018.01)
CPC - A61K 31/4439, A61K 31/496, A61K 31/506

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2008/0125440 A1 (Cai et al.) 29 May 2008 (29.05.2008); p10	1-3, 7-11, 20-21
A	Pubmed Compound Summary for CID 108777084, 'RYLSWZAXLWZDPI-UHFFFAOYSA-N', U.S. National Library of Medicine, 15 January 2016 (15.01.2016), page1-9; p3 (https://pubchem.ncbi.nlm.nih.gov/compound/108777084)	1-3, 7-11, 20-21
A	WO 2008/150446 A1 (Liang) 11 December 2008 (11.12.2008); p23	1-3, 7-11, 20-21
A	US 2010/0009987 A1 (Bettati et al.) 14 January 2010 (14.01.2010); entire document	1-3, 7-11, 20-21
A	CN 106279143 A (Tianjin Int Joint Acad Of Biomedicine) 04 January 2017 (04.01.2017); entire document	1-3, 7-11, 20-21
P/A	WO 2018/106643 A1 (Vertex Pharmaceuticals Incorporated) 14 June 2018 (14.06.2018); entire document	1-3, 7-11, 20-21

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 September 2018

Date of mailing of the international search report

07 NOV 2018

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
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Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/37221

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 7-11, 20-21

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/37221

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-21 directed to a compound of formula (I) or a pharmaceutically acceptable salt thereof. The compound of formula (I) will be searched to the extent that it encompasses the compound of formula (I) or a pharmaceutically acceptable salt thereof, wherein: R1 is C1-6 alkyl; X is O; Y is CRbRc, in which each of Rb and Rc, independently, is H; R2 is -CH2CH2Re, in which Re is H; and R3 is heteroaryl. It is believed that claims 1-3(in part), 7-11, 20-21 read on this first named invention, and thus these claims will be searched without fee. Applicant is invited to elect additional compounds of claim 1, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be the compound of formula (I) or a pharmaceutically acceptable salt thereof, wherein; R1 is C1-6 thioalkyl; X is NRa, in which Ra is H; Y is NRd, in which Rd is H; R2 is NRfRg, in which Rf and Rg are C1-6alkyl; and Re is heteroaryl (i.e., claims 1, 7-11, and 21).

Group II: claims 22-26 directed to a method of inhibiting a tyrosine kinase or treating cancer associated with a tyrosine kinase, comprising contacting the tyrosine kinase with an effective amount of a compound or salt of claim 1.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique compound of formula (I) in claim 1 containing the same, which is not required by any other invention of Group I+.

Group II includes the technical feature of a method of inhibiting a tyrosine kinase or treating cancer associated with a tyrosine kinase, not required by Group I+.

Common technical features:

The inventions of Group I+ share the technical feature of a compound of formula (I) containing the same.

Groups I+ and II share the technical feature of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by CN 106279143 A to Tianjin Int Joint Acad Of Biomedicine (hereinafter 'Tianjin'). Tianjin discloses a compound of formula (I) wherein R1 is C1 alkyl; X is NRa, in which Ra is C2 alkyl; Y is NRd, in which Rd, together with Ra and the nitrogen atoms bonded to Rd and Ra, is C6 heterocycloalkyl; R2 is -CH2CH2Re, in which Re is ORh, Rh being H; and R3 is heteroaryl (para[0149] "Compound 13a").

As said compound and compositions were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions of Groups I+ and II. The inventions of Group I+ and II thus lack unity under PCT Rule 13.



(12)发明专利申请

(10)申请公布号 CN 110831596 A

(43)申请公布日 2020.02.21

(21)申请号 201880039827.X

(22)申请日 2018.06.13

(30)优先权数据

62/518,855 2017.06.13 US

(85)PCT国际申请进入国家阶段日

2019.12.13

(86)PCT国际申请的申请数据

PCT/US2018/037221 2018.06.13

(87)PCT国际申请的公布数据

W02018/231910 EN 2018.12.20

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(51)Int.Cl.

A61K 31/4439(2006.01)

A61K 31/496(2006.01)

A61K 31/506(2006.01)

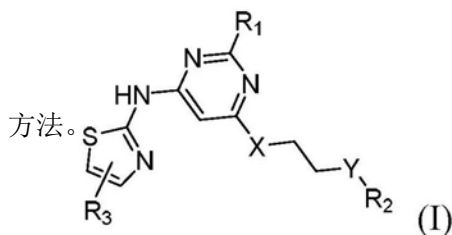
权利要求书5页 说明书16页

(54)发明名称

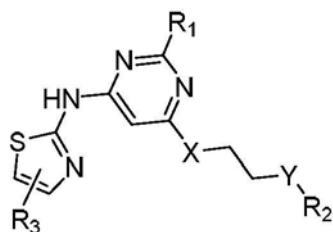
作为蛋白激酶抑制剂的氨基噻唑化合物

(57)摘要

本发明公开了下式(I)的氨基噻唑化合物及含有这些化合物中的一者的药物组合物。本发明还公开了利用氨基噻唑化合物中的一者抑制酪氨酸激酶和治疗与酪氨酸激酶相关的癌症的



1. 一种下式 (I) 的化合物或其药用盐:



(I),

其中,

R₁为C₁₋₆烷基或C₁₋₆硫代烷基;

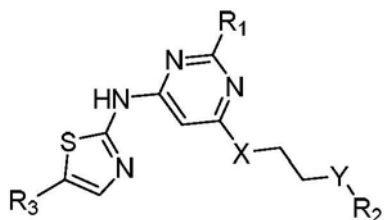
X为O或NR_a, 其中R_a为H或C₁₋₆烷基;

Y为CR_bR_c或NR_d, 其中R_b和R_c各自独立地为H、卤素、C₁₋₆烷基、C₁₋₆烷氧基或氨基, 或者R_b与R_a、键接到R_b的碳原子和键接到R_a的氮原子一起为C₃₋₁₀杂环烷基; 并且R_d为H或C₁₋₆烷基, 或者R_d与R_a和键接到R_d和R_a的氮原子一起为C₃₋₁₀杂环烷基;

R₂为-CH₂CH₂R_e或NR_fR_g, 其中R_e为H、卤素、C₁₋₆烷基或OR_h, 并且R_f和R_g各自独立地为C₁₋₆烷基或C₃₋₈环烷基, R_h为H或C₁₋₆烷基, 或者R_h与R_d、键接到R_h的氧原子和键接到R_d的氮原子一起为C₃₋₁₀杂环烷基; 并且

R₃为杂芳基。

2. 根据权利要求1所述的化合物或盐, 其中所述化合物为式 (II) 的化合物:



(II),

其中R₁为C₁₋₆烷基。

3. 根据权利要求2所述的化合物或盐, 其中X为O, Y为NR_d, 并且R₂为-CH₂CH₂R_e, 其中R_e为OR_h, R_h与R_d、键接到R_h的氧原子和键接到R_d的氮原子一起为C₃₋₁₀杂环烷基。

4. 根据权利要求2所述的化合物或盐, 其中X为NR_a, 并且Y为CR_bR_c或NR_d, 其中R_a与R_b、键接到R_a的氮原子和键接到R_b的碳原子一起为C₃₋₁₀杂环烷基; R_c为H、卤素、C₁₋₆烷基、C₁₋₆烷氧基或氨基; 并且R_d与R_a和键接到R_a和R_d的氮原子一起为C₃₋₁₀杂环烷基。

5. 根据权利要求4所述的化合物或盐, 其中X为NR_a, Y为CR_bR_c, 并且R₂为NR_fR_g, 其中R_a与R_b、键接到R_a的氮原子和键接到R_b的碳原子一起为C₃₋₁₀杂环烷基; R_c为H、卤素、C₁₋₆烷基、C₁₋₆烷氧基或氨基; 并且R_f和R_g各自为C₁₋₆烷基。

6. 根据权利要求4所述的化合物或盐, 其中X为NR_a, Y为NR_d, 并且R₂为-CH₂CH₂R_e, 其中R_a与R_d和键接到R_a和R_d的氮原子一起为C₃₋₁₀杂环烷基; 并且R_e为H、卤素或OR_h, R_h为H或C₁₋₆烷基。

7. 根据权利要求1所述的化合物或盐, 其中R₃为独立地被一个或多个(CH₂)_nZ部分取代的5-或6-元杂芳基, 其中n为0或1, 并且Z为H、卤素、CN、OH、CF₃、C₁₋₆烷基或C₁₋₆烷氧基; 或者R₃

为与被一个或多个取代基取代的苯环稠合的5-或6-元杂芳基,所述取代基独立地选自H、卤素、CN、OH、CF₃、C₁₋₆烷基和C₁₋₆烷氧基组成的组。

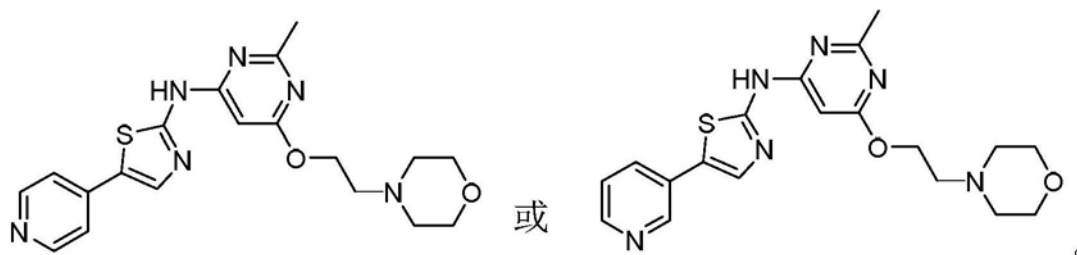
8. 根据权利要求7所述的化合物或盐,其中R₃为独立地被一个或多个(CH₂)_nZ部分取代的6-元杂芳基,其中n为0或1,并且Z为H、卤素、CN、OH、CF₃、C₁₋₆烷基或C₁₋₆烷氧基。

9. 根据权利要求8所述的化合物或盐,其中R₃为吡啶基或嘧啶基。

10. 根据权利要求3所述的化合物或盐,其中R₃为独立地被一个或多个(CH₂)_nZ部分取代的5-或6-元杂芳基,其中n为0或1,并且Z为H、卤素、CN、OH、CF₃、C₁₋₆烷基或C₁₋₆烷氧基;或者R₃为与被一个或多个取代基取代的苯环稠合的5-或6-元杂芳基,所述取代基独立地选自H、卤素、CN、OH、CF₃、C₁₋₆烷基和C₁₋₆烷氧基组成的组。

11. 根据权利要求10所述的化合物或盐,其中R₃为吡啶基或嘧啶基。

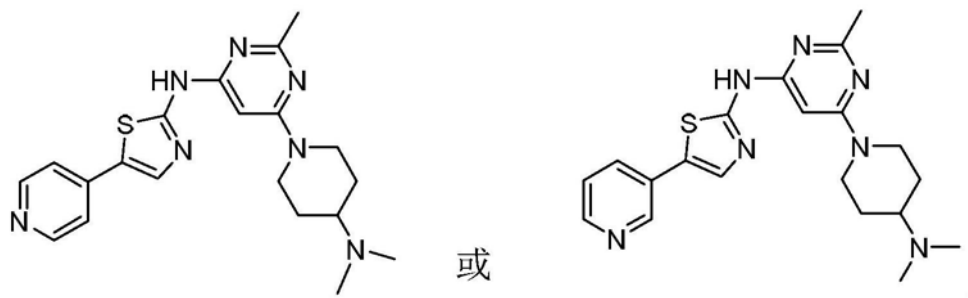
12. 根据权利要求11所述的化合物或盐,其中所述化合物为



13. 根据权利要求5所述的化合物或盐,其中R₃为独立地被一个或多个(CH₂)_nZ部分取代的5-或6-元杂芳基,其中n为0或1,并且Z为H、卤素、CN、OH、CF₃、C₁₋₆烷基或C₁₋₆烷氧基;或者R₃为与被一个或多个取代基取代的苯环稠合的5-或6-元杂芳基,所述取代基独立地选自H、卤素、CN、OH、CF₃、C₁₋₆烷基和C₁₋₆烷氧基组成的组。

14. 根据权利要求13所述的化合物或盐,其中R₃为吡啶基或嘧啶基。

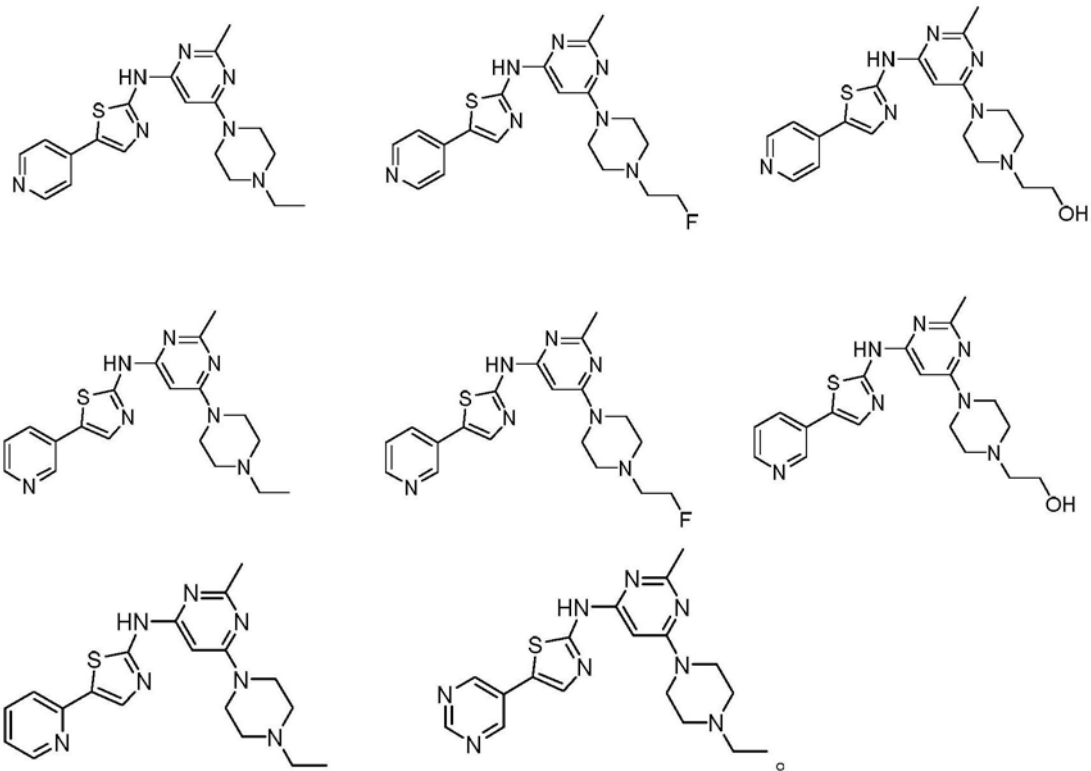
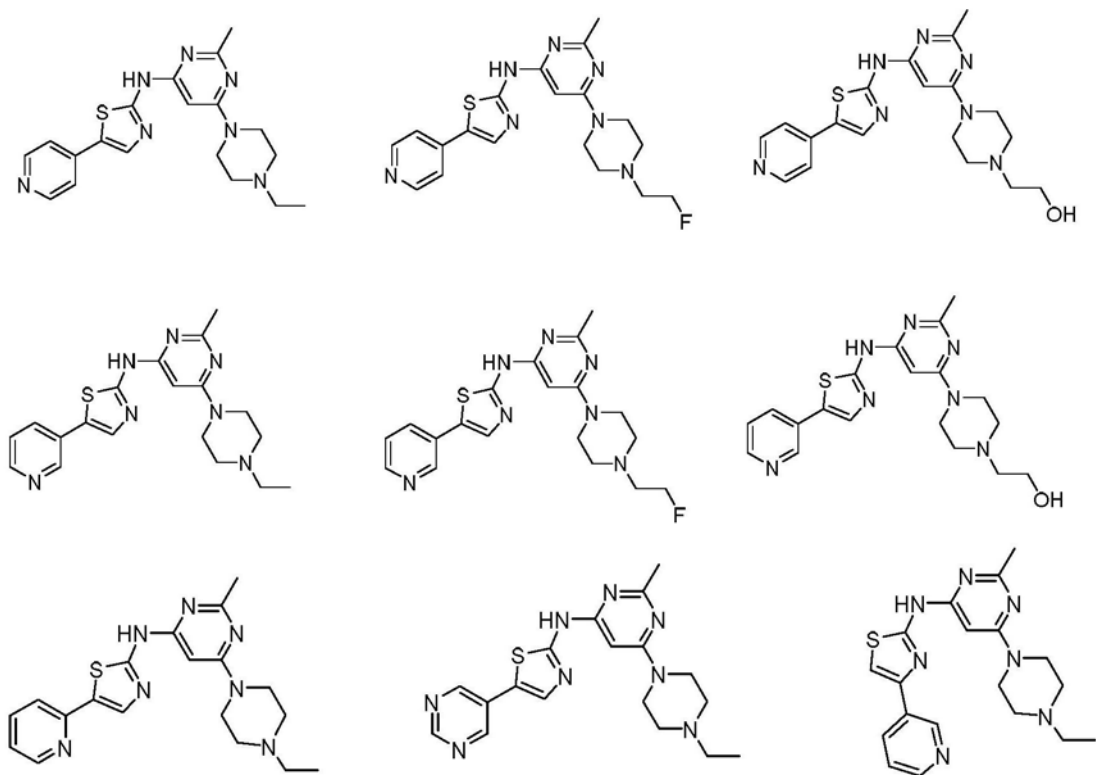
15. 根据权利要求14所述的化合物或盐,其中所述化合物为

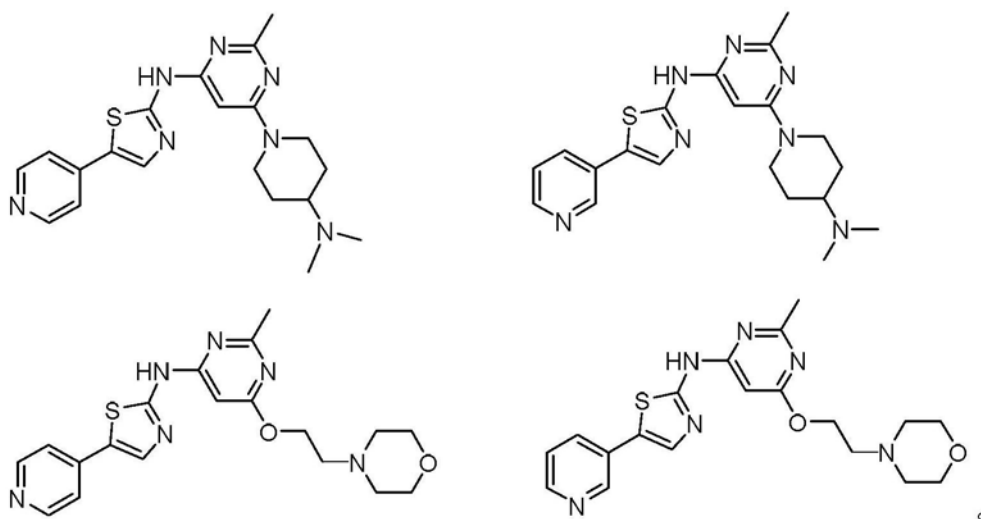


16. 根据权利要求6所述的化合物或盐,其中R₃为独立地被一个或多个(CH₂)_nZ部分取代的5-或6-元杂芳基,其中n为0或1,并且Z为H、卤素、CN、OH、CF₃、C₁₋₆烷基或C₁₋₆烷氧基;或者R₃为与被一个或多个取代基取代的苯环稠合的5-或6-元杂芳基,所述取代基独立地选自H、卤素、CN、OH、CF₃、C₁₋₆烷基和C₁₋₆烷氧基组成的组。

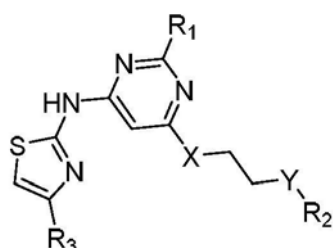
17. 根据权利要求16所述的化合物或盐,其中R₃为吡啶基或嘧啶基。

18. 根据权利要求17所述的化合物或盐,其中所述化合物为以下化合物中的一者:





20. 根据权利要求1所述的化合物或盐,其中所述化合物为式(III)的化合物:



(III),

其中R₁为C₁₋₆烷基。

21. 一种药物组合物,所述药物组合物包含药用载体以及根据权利要求1所述的化合物或盐。

22. 一种抑制酪氨酸激酶的方法,所述方法包括使所述酪氨酸激酶与有效量的根据权利要求1所述的化合物或盐接触。

23. 一种治疗与酪氨酸激酶相关的癌症的方法,所述方法包括向有需要的受试者施用有效量的根据权利要求1所述的化合物或盐。

24. 根据权利要求23所述的方法,其中所述酪氨酸激酶为FMS样酪氨酸激酶3 (FLT3)、FMS样酪氨酸激酶4、血管内皮生长因子受体 (VEGFR)、集落刺激因子1受体 (CSF1R)、血小板衍生生长因子受体 (PDGFR) A、PDGFR B、酪氨酸蛋白激酶Kit (c-KIT)、c-Src (SRC)、酪氨酸蛋白激酶Lyn (LYN) A、LYN B、在转染期间重排的酪氨酸激酶 (RET)、淋巴细胞特异性蛋白质酪氨酸激酶、加德纳-拉希德猫肉瘤病毒致癌基因同源物、盘状结构域受体1、激酶插入结构域受体、B淋巴细胞激酶、酪氨酸蛋白激酶Yes、爱柏森鼠类白血病病毒致癌基因同源物1 (ABL1)、酪氨酸受体激酶TRKA、TRKB、TRKC、ZAK/MLTK、IRAK4、RET V804L、RET Y791F、FLT3 D835Y、PDGFR A V561D、或ABL1 T315I。

25. 根据权利要求24所述的方法,其中所述酪氨酸激酶为FLT3、VEGFR或c-KIT。

26. 根据权利要求25所述的方法,其中所述癌症为急性髓细胞白血病、绿色瘤、慢性骨髓性白血病、急性淋巴母细胞性白血病、慢性淋巴细胞性白血病、霍奇金病、非霍奇金淋巴瘤、B细胞淋巴瘤、多发性骨髓瘤、瓦尔登斯特伦巨球蛋白血症、骨髓增生异常综合征、胰腺

癌、膀胱癌、结直肠癌、乳癌、男性生殖道癌、肾癌、肝细胞癌、肺癌、卵巢癌、宫颈癌、子宫癌、妊娠滋养细胞疾病、胃癌、胆管癌、胆囊癌、小肠癌、食管癌、口咽癌、下咽癌、眼癌、神经癌、头颈癌、黑色素瘤、浆细胞瘤、内分泌腺肿瘤、神经内分泌癌、脑瘤、骨癌或肉瘤。

作为蛋白激酶抑制剂的氨基噻唑化合物

背景技术

[0001] 蛋白激酶在调节各种细胞功能的细胞信号通路中是重要的,包括分化、增殖、迁移和凋亡。蛋白激酶的失调涉及癌症和许多其他疾病。

[0002] 酪氨酸激酶为蛋白激酶的一种亚类,其通过将磷酸根从ATP中转移至目标蛋白酪氨酸的羟基基团来调节目标蛋白功能。FMS样酪氨酸激酶3(FMS-like tyrosine kinase 3,“FLT3”)、血管内皮生长因子受体(vascular endothelial growth factor receptor,“VEGFR”)和酪氨酸蛋白激酶Kit(tyrosine-protein kinase Kit,“c-Kit”)是作为癌症治疗中引人注目的治疗靶标而已被研究的三种酪氨酸激酶。

[0003] FLT3为一种受体酪氨酸激酶,其突变可以导致癌症的发展,例如急性髓细胞白血病(acute myeloid leukemia)。参见Pratz等人,Current Drug Targets,2010,11(7),781-9。

[0004] 血管内皮生长因子是一种信号蛋白,通过结合至VEGFR和经由转磷酸作用(transphosphorylation)活化血管内皮生长因子,其刺激新血管的生长。VEGFR已被确认为肿瘤血管新生(angiogenesis)的主要调节因子。参见Hicklin等人,J Clin Oncol.,2005,23,1011-1027。

[0005] c-Kit也是一种受体酪氨酸激酶,其参与细胞内信号传导(intracellular signaling)。c-Kit的突变形式在一些癌症的发生中起到关键作用。已证实抑制c-Kit可以有效治疗胃肠间质瘤(gastrointestinal stromal tumor)、急性髓细胞白血病和黑色素瘤。参见Babaei等人,Drug Des Devel Ther.,2016 10,2443-2459。

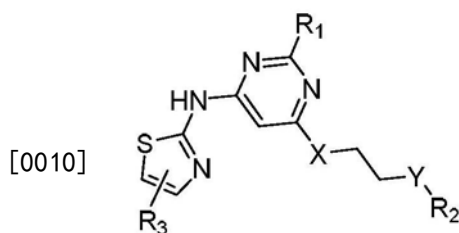
[0006] 作为有效的酪氨酸激酶抑制剂,已经对氨基噻唑化合物进行了广泛的探索,其作为候选药物存在若干挑战。它们具有差的激酶选择性,在毒性研究中经常引起动物死亡,并且通常缺乏为了在临床前或临床研究中发挥所需功效的足够的体内暴露(in vivo exposure)。

[0007] 有需要开发新的氨基噻唑化合物,其特异性地抑制特定酪氨酸激酶,展现出期望的安全性,并且在治疗目标癌症中发挥足够的体内功效。

发明内容

[0008] 本发明基于意想不到的发现:特定氨基噻唑化合物有效地抑制多种酪氨酸激酶,例如FLT3、VEGFR和c-Kit。

[0009] 在一个方面中,本发明涉及式(I)的氨基噻唑化合物:



(I),

[0011] 其中R₁为C₁₋₆烷基或C₁₋₆硫代烷基;X为O或NR_a,其中R_a为H或C₁₋₆烷基;Y为CR_bR_c或NR_d,其中R_b和R_c各自独立地为H、卤素、C₁₋₆烷基、C₁₋₆烷氧基或氨基,或者R_b与R_a、键接到R_b的碳原子和键接到R_a的氮原子一起为C₃₋₁₀杂环烷基,并且R_d为H或C₁₋₆烷基,或者R_d与R_a和键接到R_d和R_a的氮原子一起为C₃₋₁₀杂环烷基;R₂为-CH₂CH₂R_e或NR_fR_g,其中R_e为H、卤素、C₁₋₆烷基或OR_h,并且R_f和R_g各自独立地为C₁₋₆烷基或C₃₋₈环烷基,R_h为H或C₁₋₆烷基,或者R_h与R_d、键接到R_h的氧原子和键接到R_d的氮原子一起为C₃₋₁₀杂环烷基;并且R₃为杂芳基。

[0012] 本文中的术语“烷基”是指饱和的直链或支链烃部分(moiety),诸如-CH₃或支链的-C₃H₇。术语“环烷基”是指非芳族的单环、双环、三环或四环的烃部分,诸如环己基、环己烯-3-基或金刚烷基(adamantyl)。术语“烷氧基”是指-O-烷基基团。烷氧基的实例包括但不限于甲氧基、乙氧基、正丙氧基、异丙氧基、正丁氧基、异丁氧基、仲丁氧基和叔丁氧基。术语“硫代烷基”是指-S-烷基基团。硫代烷基的实例包括但不限于甲硫醇、乙硫醇和苄硫醇(benzylthiol)。术语“杂环烷基”是指具有一个或多个环杂原子(例如N、O或S)的非芳族的单环、双环、三环、或四环的部分。杂环烷基的实例包括但不限于4-吗啉基(4-morpholinyl)、1-哌嗪基(1-piperazinyl)、4-四氢吡喃基(4-tetrahydropyranyl)和4-吡喃基(4-pyranyl)。术语“杂芳基”是指具有含有至少一个杂原子(例如N、O或S)的一个或多个芳族环的部分。杂芳基部分的实例包括呋喃基(furyl)、亚呋喃基(furylene)、茱基(fluorenyl)、吡咯基(pyrrolyl)、噻吩基(thienyl)、噁唑基(oxazolyl)、咪唑基(imidazolyl)、噻唑基(thiazolyl)、吡啶基(pyridyl)、嘧啶基(pyrimidinyl)、喹唑啉基(quinazolinyl)、喹啉基(quinolyl)、异喹啉基(isoquinolyl)和吲哚基(indolyl)。

[0013] 除非另有说明,本文提及的烷基、硫代烷基、烷氧基、环烷基、杂环烷基和杂芳基包括经取代的和未经取代的部分两者。环烷基、杂环烷基和杂芳基上可能的取代基包括C₁₋₁₀烷基、C₂₋₁₀烯基、C₂₋₁₀炔基、C₃₋₂₀环烷基、C₃₋₂₀环烯基、C₁₋₂₀杂环烷基、C₁₋₂₀杂环烯基、C₁₋₁₀烷氧基、芳基、芳氧基、杂芳基、杂芳氧基、氨基、C₁₋₁₀烷基氨基、C₁₋₂₀二烷基氨基、芳基氨基、二芳基氨基、羟基、卤素、硫代(thio)、C₁₋₁₀烷硫基(alkylthio)、芳硫基(arylthio)、C₁₋₁₀烷基磺酰基(alkylsulfonyl)、芳基磺酰基(arylsulfonyl)、酰氨基(acylamino)、氨酰基(aminoacyl)、氨基硫代酰基(aminothioacyl)、脒基(amidino)、胍(guanidine)、脲基(ureido)、氰基、硝基、酰基、硫酰基(thioacyl)、酰氧基(acyloxy)、羧基和羧酸酯(carboxylic ester)。另一方面,烷基上可能的取代基包括除C₁₋₁₀烷基、C₂₋₁₀烯基和C₂₋₁₀炔基以外的所有上述取代基。环烷基、杂环烷基、芳基和杂芳基也可以彼此耦合。

[0014] 如果适用,上述氨基噻唑化合物包括化合物本身,以及它们的盐、前药和溶剂化物。例如,可以在阴离子与氨基噻唑化合物上带正电的基团(如氨基)之间形成盐。合适的阴离子包含氯、溴、碘、硫酸根、硝酸根、磷酸根、柠檬酸根(citrate)、甲磺酸根

(methanesulfonate)、三氟乙酸根(trifluoroacetate)、乙酸根、苹果酸根(malate)、甲苯磺酸根(tosylate)、酒石酸根(tartrate)、延胡索酸根(fumurate)、谷氨酸根(glutamate)、葡萄糖醛酸根(glucuronate)、乳酸根(lactate)、戊二酸根(glutarate)和马来酸根(maleate)。同样地,也可以在阳离子和氨基噻唑化合物上带负电的基团(如羧酸根)之间形成盐。合适的阳离子包含钠离子、钾离子、镁离子、钙离子和铵阳离子诸如四甲基铵离子。氨基噻唑化合物也包括含有季氮原子的那些盐。前药的实例包括酯类和其他药用衍生物,其在施用至受试者时能够提供活性氨基噻唑化合物。溶剂化物是指在活性氨基噻唑化合物和药用溶剂之间形成的复合物。药用溶剂的实例包括水、乙醇、异丙醇、乙酸乙酯、乙酸和乙醇胺。

[0015] 在另一个方面中,本发明涉及一种抑制酪氨酸激酶(例如FLT3、VEGFR和c-Kit)的方法。该方法包括使酪氨酸激酶与有效量的一种或多种上述氨基噻唑化合物接触。

[0016] 治疗与酪氨酸激酶相关的癌症的方法也在本发明的范围内。该方法包括向有需要的受试者施用有效量的一种或多种上述式(I)的氨基噻唑化合物。

[0017] 与癌症相关的酪氨酸激酶可以是野生型或突变体。酪氨酸激酶的实例包括但不限于FLT3、FLT4、VEGFR、血小板衍生生长因子受体(platelet-derived growth factor receptor,PDGFR)A、PDGFR B、c-Kit、c-Src(SRC)、酪氨酸蛋白激酶Lyn(tyrosine-protein kinase Lyn,LYN)A、LYN B、在转染期间重排的酪氨酸激酶(rearranged during transfection tyrosine kinase,RET)、淋巴细胞特异性蛋白质酪氨酸激酶(lymphocyte-specific protein tyrosine kinase)、加德纳-拉希德猫肉瘤病毒致癌基因同源物(Gardner-Rasheed feline sarcoma viral oncogene homolog)、盘状结构域受体1(discoidin domain receptor 1)、激酶插入结构域受体(kinase insert domain receptor)、B淋巴细胞激酶(B lymphocyte kinase)、酪氨酸蛋白激酶Yes(tyrosine-protein kinase Yes)、爱柏森鼠类白血病病毒致癌基因同源物1(Abelson murine leukemia viral oncogene homolog 1,ABL1)、酪氨酸蛋白激酶Tek(tyrosine-protein kinase Tek)、RET V804L、RET Y791F、FLT3 D835Y、PDGFR A V561D或ABL1 T315I。

[0018] 在一个示例性方法中,式(I)的氨基噻唑化合物用于治疗与FLT3、VEGFR或c-Kit相关的癌症。

[0019] 癌症的实例包括急性髓细胞白血病(acute myeloid leukemia)、绿色瘤(chloroma)、慢性骨髓性白血病(chronic myelogenous leukemia)、急性淋巴母细胞性白血病(acute lymphoblastic leukemia)、慢性淋巴细胞性白血病(chronic lymphocytic leukemia)、霍奇金病(Hodgkin's disease)、非霍奇金淋巴瘤(non-Hodgkin's lymphoma)、B细胞淋巴瘤(B-cell lymphoma)、多发性骨髓瘤(multiple myeloma)、瓦尔登斯特伦巨球蛋白血症(Waldenstrom's macroglobulinemia)、骨髓增生异常综合征(myelodysplastic syndrome)、胰腺癌(pancreatic cancer)、膀胱癌(bladder cancer)、结直肠癌(colorectal cancer)、乳癌(breast cancer)、男性生殖道癌(male genital tract cancer)、肾癌(renal cancer)、肝细胞癌(hepatocellular cancer)、肺癌(lung cancer)、卵巢癌(ovarian cancer)、宫颈癌(cervical cancer)、子宫癌(uterus cancer)、妊娠滋养细胞疾病(gestational trophoblastic disease)、胃癌(gastric cancer)、胆管癌(bile duct cancer)、胆囊癌(gallbladder cancer)、小肠癌(small intestine cancer)、食管癌

(esophageal cancer)、口咽癌(oralpharyngeal cancer)、下咽癌(hypopharyngeal cancer)、眼癌(eye cancer)、神经癌(nerve cancer)、头颈癌(head and neck cancer)、黑色素瘤(melanoma)、浆细胞瘤(plasmacytoma)、内分泌腺肿瘤(endocrine gland neoplasm)、神经内分泌癌(neuroendocrine cancer)、脑瘤(brain tumor)、骨癌(bone cancer)和肉瘤(sarcoma)(例如胃肠间质瘤(gastrointestinal stromal tumor或GIST))。

[0020] 含有一种或多种上述式(I)的氨基噻唑化合物的药物组合物也在本发明的范围内。该药物组合物可以用于治疗癌症。

[0021] 本发明还包括一种或多种上述式(I)的氨基噻唑化合物用于制备治疗癌症的药物的用途。

[0022] 术语“治疗”(treating或treatment)是指将一种或多种氨基噻唑化合物施用至受试者,该受试者患有上述疾病(即癌症)、具有这样的疾病的症状或具有易患这样的疾病的倾向,目的是给予治愈效果,例如治愈、缓解、改变、影响、改善或预防上述疾病、其症状或易患其的倾向。术语“有效量”是指给予治疗效果所需的活性化合物的量。如本领域技术人员所认识到的,有效剂量将依据治疗疾病的类型、施用途径、赋形剂用法以及与其他治疗性治疗共同使用的可能性而变化。

[0023] 为了实施本发明的方法,具有一种或多种上述氨基噻唑化合物的组合物可以肠胃外、口服、经鼻、直肠、局部或经颊施用。本文所使用的术语“肠胃外”是指皮下、皮内、静脉内、腹膜内、肌内、关节内、动脉内、滑膜内、胸骨内、鞘内、病灶内或颅内注射,以及任何合适的灌注技术。

[0024] 无菌可注射组合物可以是在无毒的肠胃外可接受的稀释剂或溶剂中的溶液或悬浮液,例如在1,3-丁二醇中的溶液。其中可以使用的可接受的载体和溶剂为甘露醇、水、林格氏溶液(Ringer's solution)和等渗氯化钠溶液。此外,不挥发性油(fixed oils)通常用作溶剂或悬浮介质(例如合成的单或双甘油酯)。脂肪酸如油酸及其甘油酯衍生物可用于注射剂的制备,天然药用油如橄榄油、蓖麻油,特别是它们的聚氧乙烯化(polyoxyethylated)形式,也可用于注射剂的制备。这些油溶液或悬浮液也可以含有长链醇稀释剂或分散剂、羧甲基纤维素或类似的分散剂。为了配制的目的,还可以使用其他常用的表面活性剂,例如吐温类(Tweens)和司盘类(Spans)或其他类似的乳化剂或生物利用度增强剂,其通常用于制备药用固体、液体或其他剂型。

[0025] 用于口服施用的组合物可以是任何口服可接受的剂型,包括胶囊、片剂、乳剂以及水性悬浮液、分散体和溶液。在片剂的情况下,常用的载体包括乳糖和玉米淀粉。通常也添加润滑剂,例如硬脂酸镁。对于以胶囊形式的口服施用,可用的稀释剂包括乳糖和干燥玉米淀粉。当口服施用水性悬浮液或乳剂时,活性成分可以悬浮或溶解在与乳化剂或悬浮剂结合的油相中。如有需要,可以添加某些甜味剂、调味剂或着色剂。

[0026] 可以根据药物制剂领域公知的技术制备鼻用气雾剂或吸入剂组合物。例如,采用苯甲醇或其他合适的防腐剂、增强生物利用度的吸收促进剂、碳氟化合物(fluorocarbon)和/或本领域已知的其他增溶剂或分散剂,这样的组合物可以制备成盐水中的溶液。

[0027] 具有一种或多种上述氨基噻唑化合物的组合物也可以以用于直肠施用的栓剂形式施用。

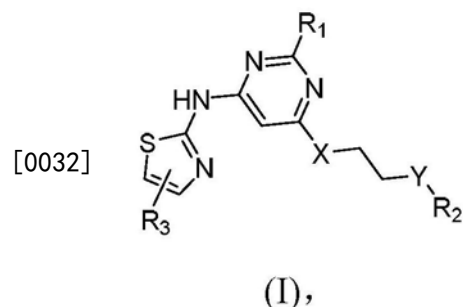
[0028] 药物组合物中的载体必须是“可接受的”,在此意义上,其与组合物的活性成分相

容(并且优选地能够稳定该活形成分),并且对待治疗的受试者无害。一种或多种增溶剂可以用作药物赋形剂,用来递送活性的1,5-二苯基-戊-1,4-二烯-3-酮化合物。其他载体的实例包括胶体二氧化硅、硬脂酸镁、纤维素、十二烷基硫酸钠和D&C黄色#10(D&C Yellow#10)。

[0029] 在下面的描述中阐述了本发明的一个或多个实施方案的细节。本发明的其他特征、目的和优点根据说明书和权利要求书将是显而易见的。

[0030] 发明详述

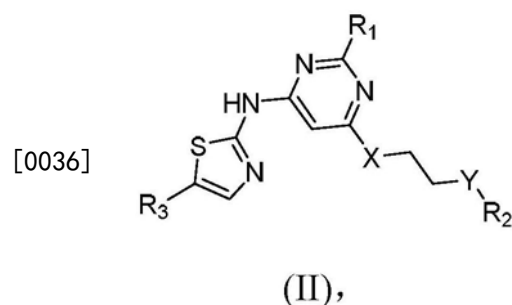
[0031] 详细公开了式(I)的氨基噻唑化合物:



[0033] 其中变量 R_1 、 R_2 、 R_3 、X和Y如在上述发明内容部分中定义的。

[0034] 通常,式(I)的化合物具有的 R_3 为独立地被一个或多个 $(CH_2)_nZ$ 部分取代的5-或6-元杂芳基,其中n为0或1,并且Z为H、卤素、CN、OH、 CF_3 、 C_{1-6} 烷基或 C_{1-6} 烷氧基;或者式(I)的化合物具有的 R_3 为与被一个或多个取代基取代的苯环稠合的5-或6-元杂芳基,所述取代基独立地选自H、卤素、CN、OH、 CF_3 、 C_{1-6} 烷基和 C_{1-6} 烷氧基组成的组。示例性化合物具有的 R_3 为独立地被一个或多个 $(CH_2)_nZ$ 部分取代的6-元杂芳基,其中n为0或1,并且Z为H、卤素、CN、OH、 CF_3 、 C_{1-6} 烷基或 C_{1-6} 烷氧基。 R_3 的两个实例为吡啶基或嘧啶基。

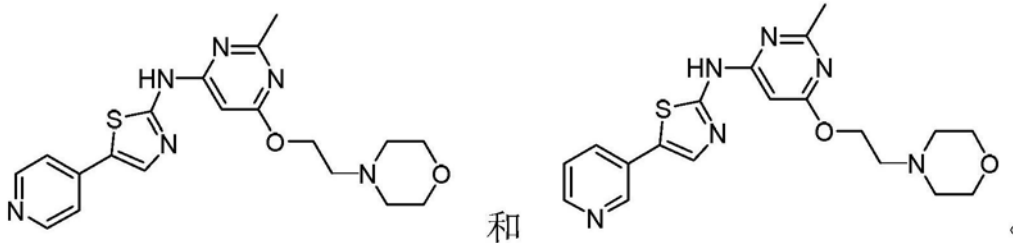
[0035] 一组上述新颖氨基噻唑化合物为式(II)的化合物:



[0037] 其中 R_1 为 C_{1-6} 烷基。

[0038] 在一个子集中,式(II)的化合物具有的X为O,Y为 NR_d ,并且 R_2 为 $-CH_2CH_2R_e$,其中 R_e 为 OR_h , R_h 与 R_d 、键接到 R_h 的氧原子和键接到 R_d 的氮原子一起为 C_{3-10} 杂环烷基。该子集的化合物可以具有 R_3 ,其为独立地被一个或多个 $(CH_2)_nZ$ 部分取代的5-或6-元杂芳基,其中n为0或1,并且Z为H、卤素、CN、OH、 CF_3 、 C_{1-6} 烷基或 C_{1-6} 烷氧基;或者 R_3 为与被一个或多个取代基取代的苯环稠合的5-或6-元杂芳基,所述取代基独立地选自H、卤素、CN、OH、 CF_3 、 C_{1-6} 烷基和 C_{1-6} 烷氧基。例如, R_3 可以是吡啶基或嘧啶基。示例性化合物包括但不限于以下化合物:

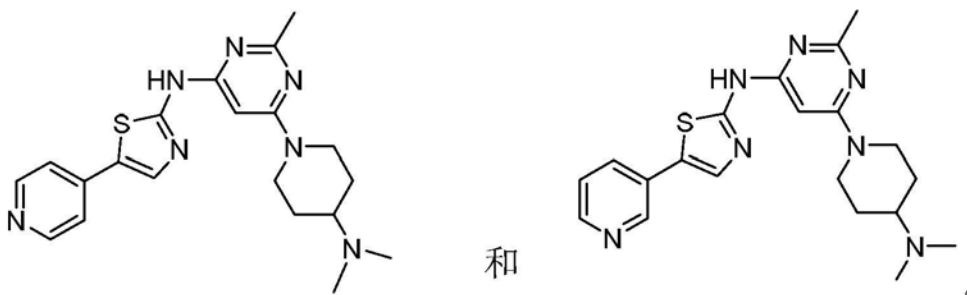
[0039]



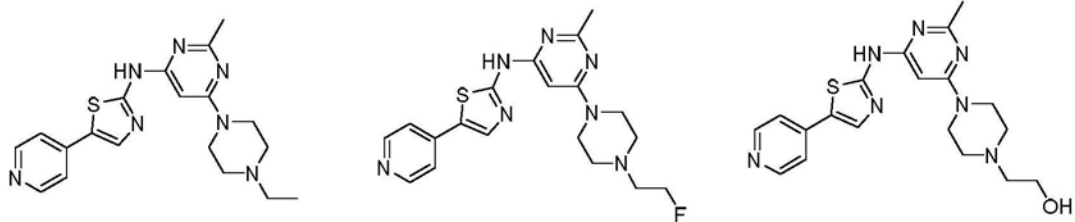
[0040] 在另一个子集中, 式(II)的化合物具有的X为NR_a, 并且Y为CR_bR_c或NR_d, 其中R_a与R_b、键接到R_a的氮原子和键接到R_b的碳原子一起为C₃₋₁₀杂环烷基; R_c为H、卤素、C₁₋₆烷基、C₁₋₆烷氧基或氨基; 并且R_d与R_a和键接到R_a和R_d的氮原子一起为C₃₋₁₀杂环烷基。

[0041] 值得注意的是,这些化合物可以具有的X为NR_a,Y为CR_bR_c,并且R₂为NR_fR_g,其中R_a与R_b、键接到R_a的氮原子和键接到R_b的碳原子一起为C₃₋₁₀杂环烷基;R_c为H、卤素、C₁₋₆烷基、C₁₋₆烷氧基或氨基;并且R_f和R_g各自为C₁₋₆烷基。它们通常具有的R₃为独立地被一个或多个(CH₂)_nZ部分取代的5-或6-元杂芳基,其中n为0或1,并且Z为H、卤素、CN、OH、CF₃、C₁₋₆烷基或C₁₋₆烷氧基;或者R₃为与被一个或多个取代基取代的苯环稠合的5-或6-元杂芳基,所述取代基独立地选自H、卤素、CN、OH、CF₃、C₁₋₆烷基和C₁₋₆烷氧基。R₃可以是吡啶基或嘧啶基。示例性化合物包括但不限于以下化合物:

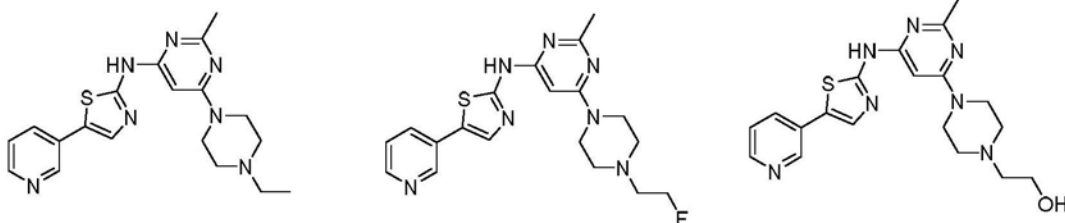
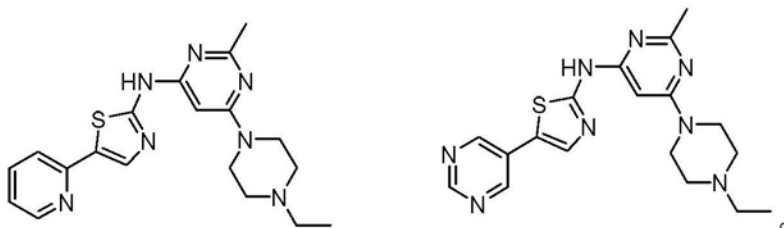
[0042]



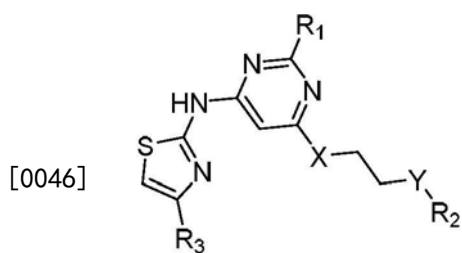
[0043] 另一方面,该子集中的化合物可以具有的X为NR_a,Y为NR_d,并且R₂为-CH₂CH₂R_e,其中R_a与R_d和键接到R_a和R_d的氮原子一起为C₃₋₁₀杂环烷基;并且R_e为H、卤素或OR_h,R_h为H或C₁₋₆烷基。通常,这些化合物具有的R₃为独立地被一个或多个(CH₂)_nZ部分取代的5-或6-元杂芳基,其中n为0或1,并且Z为H、卤素、CN、OH、CF₃、C₁₋₆烷基或C₁₋₆烷氧基;或者R₃为与被一个或多个取代基取代的苯环稠合的5-或6-元杂芳基,所述取代基独立地选自H、卤素、CN、OH、CF₃、C₁₋₆烷基和C₁₋₆烷氧基。例如,R₃为吡啶基或噻啶基。示例性化合物包括但不限于以下化合物:



[0044]



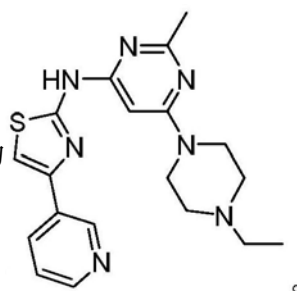
[0045] 另一组上述新颖氨基噻唑化合物为式(III)的化合物:



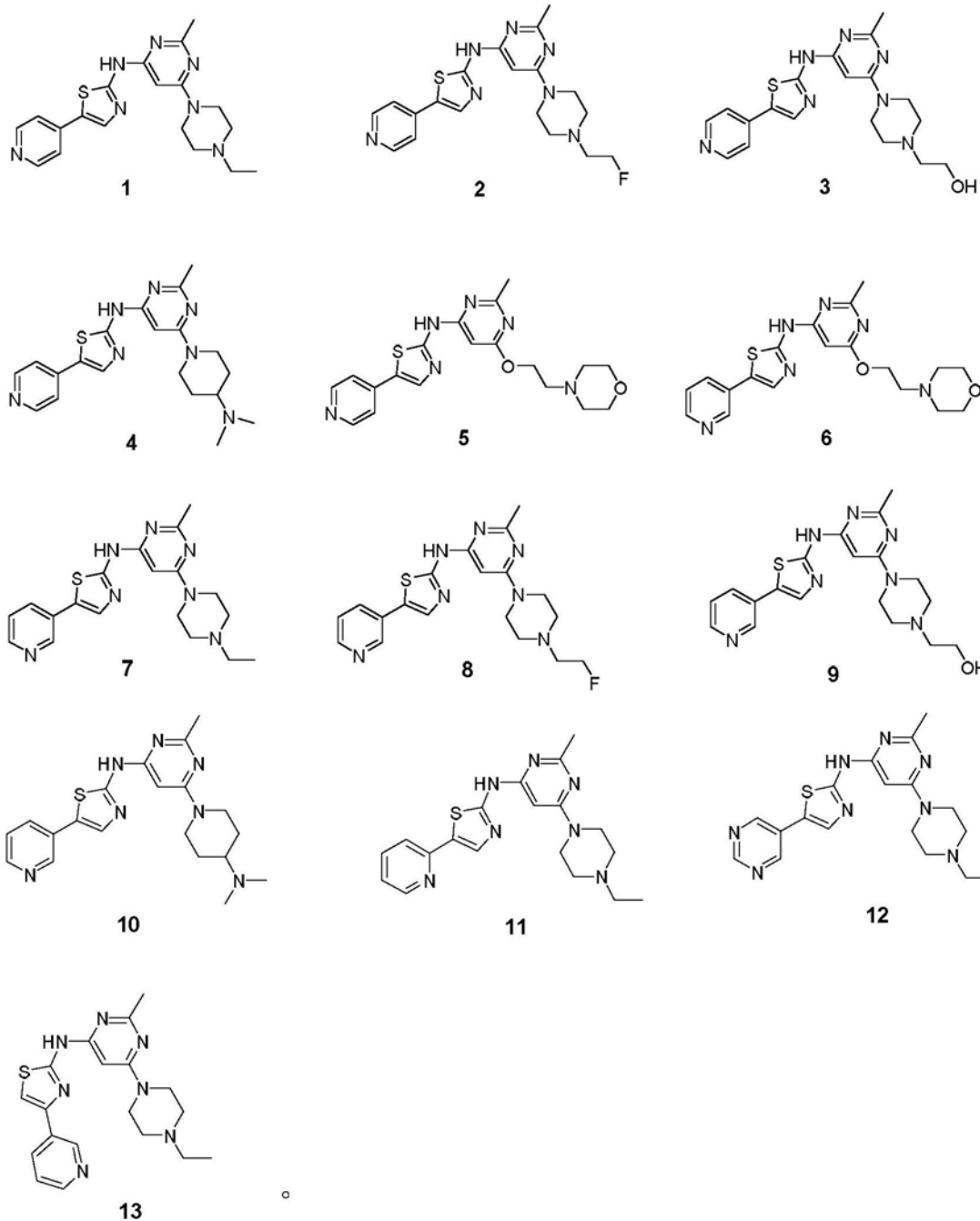
(III),

[0047] 其中R₁为C₁₋₆烷基。

[0048] 示例性的式(III)的化合物为



[0049] 下面列出的是本发明的示例性化合物,每个化合物分配一个化合物编号。



[0050]

[0051] 用于治疗癌症的含有一种或多种式 (I) 的氨基噻唑化合物的药物组合物也在本发明的范围内。

[0052] 本发明还涵盖一种治疗癌症的方法,该方法包括向有需要的受试者施用有效量的式 (I) 的化合物。

[0053] 用于合成式 (I) 的化合物的合成化学转化和保护基团方法(保护和去保护)为本领域公知的。参见例如R.Larock,Comprehensive Organic Transformations(第2版,VCH Publishers 1999);P.G.M.Wuts和T.W.Greene,Greenes' Protective Groups in Organic Synthesis(第4版,John Wiley and Sons 2007);L.Fieser和M.Fieser,Fieser and Fieser's Reagents for Organic Synthesis(John Wiley and Sons 1994);L.Paquette 编,Encyclopedia of Reagents for Organic Synthesis(第2版,John Wiley and Sons

2009);以及G.J.Yu等人,J.Med.Chem.2008,51,6044-6054。

[0054] 如此制备的式(I)的化合物可以使用生化测定(例如以下实施例2-4中描述的激酶测定)或细胞测定(例如以下实施例5中描述的体外抗癌活性测定)来初步筛选它们在抑制酪氨酸激酶或抑制表达特定酪氨酸激酶的癌细胞的生长的潜力。随后可以使用体内测定(例如异种移植动物模型测定)来评价它们在哺乳动物中抑制肿瘤生长的活性。可以进一步测试所选择的化合物以验证它们在治疗癌症中的功效。例如,可以将化合物施用至患有癌症的动物(例如小鼠),然后评价其治疗效果。基于结果,可以研究和确定适当的剂量范围和施用途径。

[0055] 不需要进一步说明,相信本领域技术人员可以基于以上描述最大程度地利用本发明。因此,以下的具体实施例应被解释为仅仅是说明性的,而不以任何方式限制本公开的其余部分。本文引用的所有出版物均通过引用整体结合于此。

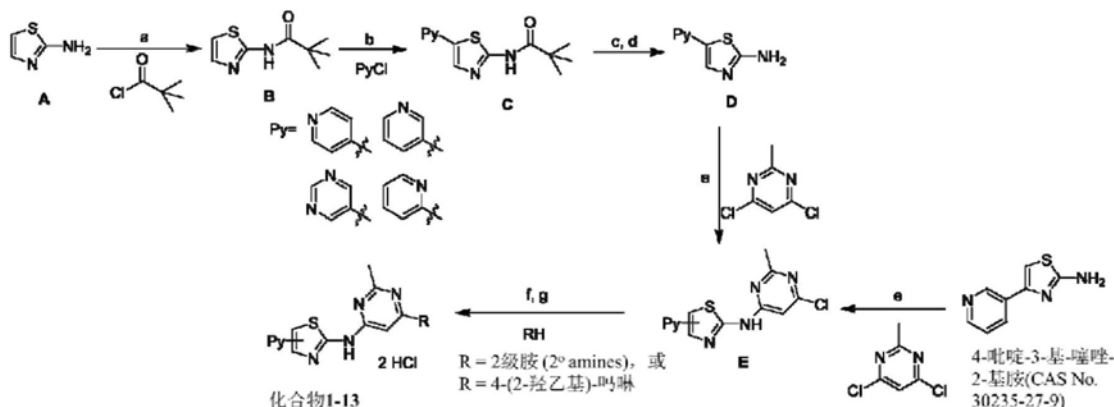
[0056] 以下实施例1示出了13种示例性式(I)化合物的合成和表征。由此制备的化合物的分析数据也列在实施例1中,并且测试这些化合物的程序在以下实施例2-5中描述。

[0057] 所有化学品和溶剂从商业供货商购买,并且按原样使用。所有反应在干燥氮气气氛下进行。通过使用Merck 60F254硅胶玻璃背板(5x 10cm)的TLC监测反应;并且在紫外线照射下(254nm)或通过喷洒磷钼酸试剂(Aldrich)接着在80℃下加热来目视检测区域。所有快速柱层析使用Merck Kieselgel 60, No.9385, 230-400目ASTM硅胶作为固定相进行。质子(¹H)核磁共振光谱在Varian Mercury-300或Varian Mercury-400光谱仪上测量。相对于溶剂峰的共振,在δ量度上以每百万分之几(ppm)纪录化学位移。以下缩写用于描述耦合:s=单峰;d=双重峰;t=三重峰;q=四重峰;quin=五重峰;br=宽;以及m=多重峰。LCMS数据在Agilent MSD-1100ESI-MS/MS、Agilent 1200系列LC/MSD VL和Waters Acquity UPLC-ESI-MS/MS系统上测量。

[0058] 实施例1:化合物1-13的合成

[0059] 根据以下流程1所示的合成路线制备化合物1-13。在所列出的试剂中,TEA为三乙胺,KOAc为乙酸钾,Pd(PPh₃)₄为四(三苯基膦)钯(0),DMAc为N,N-二甲基乙酰胺,CsF为氟化铯,HCl为盐酸,NaHCO₃为碳酸氢钠,NaH为氢化钠,NMP为1-甲基-2-吡咯烷酮,KOH为氢氧化钾,并且DMSO为二甲亚砜。

[0060]



[0061] 流程1. 试剂和条件: (a) TEA, CH₂Cl₂, 0℃至室温; (b) KOAc, Pd(PPh₃)₄, DMAc, 150℃(对于Py=吡啶-2-基, 用CsF替代KOAc); (c) 12N HCl, H₂O, 回流; (d) NaHCO₃, H₂O, 室温; (e) NaH, NMP, 0℃; (f) DMSO, RH, 100℃, 或二甘醇二甲醚, KOH, 160℃(对于RH=4-(2-羟乙基)-吗

啉;以及(g) 6N HCl, 0°C。

[0062] 步骤I. 2,2-二甲基-N-噻唑-2-基-丙酰胺B的合成

[0063] 在0°C向2-氨基噻唑A (300mmol) 和三乙胺 (330mmol) 在无水CH₂Cl₂ (250mL) 中的混合物中加入三甲基乙酰氯 (310mmol), 并且将混合物在氩气气氛下在室温搅拌1小时。将混合物用6N HCl (60mL) 洗涤, 并且分离有机层, 用硫酸镁 (MgSO₄) 干燥, 并且在减压下浓缩。残余物通过硅胶上的层析纯化 (20% EtOAc/己烷), 以得到标题产物B, 其为灰白色固体 (72%)。¹H NMR (300MHz, DMSO-d₆): δ 11.75 (s, 1H), 7.46 (d, J=6.0Hz, 1H), 7.17 (d, J=6.0Hz, 1H), 1.22 (s, 9H); MS (ES⁺) m/z C₈H₁₂N₂OS 计算值: 184.07; 实测值: 185.1 (M+H⁺)。

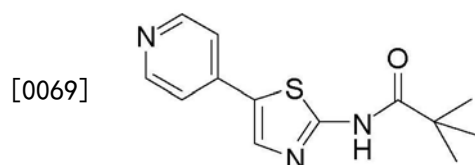
[0064] 步骤II. 化合物C的合成 (Py=吡啶-3-基、吡啶-4-基和嘧啶-5-基)

[0065] 将2,2-二甲基-N-噻唑-2-基-丙酰胺B (30mmol)、氯吡啶 (30mmol)、乙酸钾 (120mmol) 和四(三苯基膦) 钯 (0) (1.5mmol) 在N,N-二甲基乙酰胺 (60mL) 中的混合物在氩气气氛下在150°C加热24小时。通过蒸馏 (120°C/160mm Hg) 移除大部分溶剂, 并且将残余物用水 (250mL) 洗涤。通过过滤收集沉淀, 再溶解于CH₃OH/CH₂Cl₂ (200mL) 中, 并且通过硅藻土 (Celite) 垫过滤。将滤液在减压下浓缩, 并且通过硅胶上的层析纯化 (1% MeOH/CH₂Cl₂), 以得到所需产物, 其为灰白色固体 (40-85%)。

[0066] 步骤II. 化合物C的合成 (Py=吡啶-2-基)

[0067] 将2,2-二甲基-N-噻唑-2-基-丙酰胺B (10mmol)、氯吡啶 (10mmol)、氟化铯 (20mmol) 和四(三苯基膦) 钯 (0) (0.5mmol) 在二甲亚砜 (20mL) 中的混合物在氩气气氛下在160°C加热16小时。所得混合物用0.5N HCl (150mL) 和CH₂Cl₂ (150mL) 分配。将有机层分离, 用MgSO₄干燥, 在减压下浓缩, 并且通过硅胶上的层析纯化 (3% 丙酮/CH₂Cl₂), 以得到所需产物, 其为淡棕色固体 (20%)。

[0068] 仅选择C中的一个来示出其NMR光谱和质谱。



[0070] 2,2-二甲基-N-(5-吡啶-4-基-噻唑-2-基)-丙酰胺。¹H NMR (400MHz, CDCl₃): δ 9.28 (bs, 1H), 8.61 (dd, J=4.8, 1.6Hz, 2H), 7.84 (s, 1H), 7.42 (dd, J=4.8, 1.6Hz, 2H), 1.38 (s, 9H); MS (ES⁺) m/z C₁₃H₁₅N₃OS 计算值: 261.09; 实测值: 262.1 (M+H⁺)。

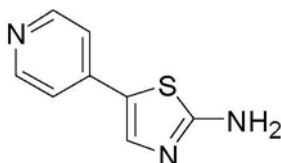
[0071] 步骤III. 化合物D的合成

[0072] 将C (5mmol) 和12N HCl (5mL) 在水 (5mL) 中的混合物加热至回流达2小时。在减压下移除大部分溶剂, 并且用CH₃OH (15mL) 稀释残余物。通过蒸馏移除大部分溶剂, 并且将残余物在真空中干燥, 以得到D盐酸盐, 其为淡棕色固体。

[0073] 在室温下将上述固体在水 (30mL) 中的搅拌悬浮液用碳酸氢钠调整至pH=7, 并且将混合物在50°C搅拌2小时。通过过滤收集沉淀, 并且在真空中干燥, 以得到所需产物D, 其为淡棕色固体 (85-90%)。

[0074] 仅选择D中的一个来示出其NMR光谱和质谱。

[0075]



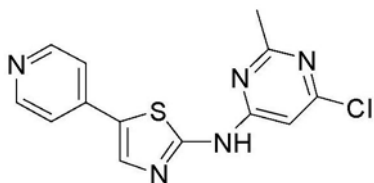
[0076] 5-吡啶-4-基-噻唑-2-基胺。¹H NMR (300MHz, DMSO-d₆): δ 8.41 (dd, J=4.8, 1.5Hz, 2H), 7.73 (s, 1H), 7.48 (s, 2H), 7.35 (dd, J=4.8, 1.5Hz, 2H); MS (ES⁺) m/z C₈H₇N₃S 计算值: 177.04; 实测值: 178.1 (M+H⁺)。

[0077] 步骤IV. 化合物E的合成

[0078] 在0℃向D或市售的4-吡啶-3-基-噻唑-2-基胺 (4mmol) 和4,6-二氯-2-甲基嘧啶 (8mmol) 在1-甲基-2-吡咯烷酮 (20mL) 中的混合物中加入氢化钠 (在油中的60%, 10mmol), 并且将混合物在氩气气氛下在0℃搅拌1小时。在0℃用水 (100mL) 猝灭反应, 并且将反应用6N HCl调整至pH=2。将浆料用碳酸氢钠调整至pH=7, 并且通过过滤收集沉淀, 用水 (50mL) 洗涤, 并且在真空下干燥。将残余物通过硅胶上的层析纯化 (20% EtOAc/CH₂Cl₂, 然后是5%至10% MeOH/CH₂Cl₂梯度), 以得到所需产物E, 其为棕色固体 (45-60%)。

[0079] 仅选择E中的一个来示出其NMR光谱和质谱。

[0080]



[0081] (6-氯-2-甲基-嘧啶-4-基)-(5-吡啶-4-基-噻唑-2-基)-胺。¹H NMR (300MHz, DMSO-d₆): δ 12.15 (s, 1H), 8.53 (dd, J=4.5, 1.5Hz, 2H), 8.18 (s, 1H), 7.59 (dd, J=4.5, 1.5Hz, 2H), 6.90 (s, 1H), 2.59 (s, 3H); MS (ES⁺) m/z C₁₃H₁₀ClN₅S 计算值: 303.03; 实测值: 304.1 (M+H⁺)。

[0082] 步骤V. 化合物1-4和7-13的合成

[0083] 将化合物E (2mmol) 和1-乙基哌嗪 (8mmol) 在二甲亚砜 (2mL) 中的混合物在100℃加热1小时。在冷却至室温后, 将混合物用水 (50mL) 稀释。通过过滤收集沉淀, 用水 (10mL) 洗涤并且在真空下干燥。将残余物通过氧化铝上的层析纯化 (0.5%至1.5% MeOH/CH₂Cl₂梯度), 以得到每个化合物1-4和7-13的游离碱, 其为灰白色固体。

[0084] 在0℃向搅拌的6N HCl (10mL) 中加入上述固体, 并且将溶液通过0.45μm PVDF膜过滤。在1小时的过程中向搅拌的滤液中逐滴加入丙酮 (40mL), 并且在0℃搅拌另外1小时。通过过滤收集沉淀, 用丙酮 (15mL) 洗涤, 并且在真空下干燥, 以得到每个化合物1-4和7-13的盐酸盐, 其为黄色固体 (90-95%)。

[0085] [6-(4-乙基-哌嗪-1-基)-2-甲基-嘧啶-4-基]-(5-吡啶-4-基-噻唑-2-基)-胺盐酸盐。¹H NMR (400MHz, DMSO-d₆): δ 11.55 (bs, 1H), 8.72 (d, J=5.6Hz, 2H), 8.61 (s, 1H), 8.14 (d, J=5.2Hz, 2H), 6.27 (s, 1H), 4.35 (d, J=13.2Hz, 2H), 3.55 (d, J=12.0Hz, 2H), 3.45 (t, J=13.0Hz, 2H), 3.13 (t, J=5.8Hz, 2H), 3.02 (q, J=10.0Hz, 2H), 2.50 (s, 3H), 1.28 (t, J=6.8Hz, 3H); MS (ES⁺) m/z C₁₉H₂₃N₇S 计算值: 381.17; 实测值: 382.2 (M+H⁺)。

[0086] {6-[4-(2-氟-乙基)-哌嗪-1-基]-2-甲基-嘧啶-4-基}-(5-吡啶-4-基-噻唑-2-基)-胺盐酸盐 (化合物2)。¹H NMR (300MHz, DMSO-d₆): δ 11.89 (bs, 1H), 8.73 (d, J=6.3Hz,

2H), 8.62 (s, 1H), 8.15 (d, J=5.7Hz, 2H), 6.26 (s, 1H), 4.95 (d, J=47.4Hz, 2H), 4.38 (s, 2H, 与水峰重叠), 3.70-3.35 (m, 6H), 3.18 (bs, 2H), 2.50 (s, 3H); MS (ES⁺) m/z C₁₉H₂₂FN₇S 计算值: 399.16; 实测值: 400.1 (M+H⁺)。

[0087] 2-{4-[2-甲基-6-(5-吡啶-4-基-噻唑-2-基氨基)-嘧啶-4-基]-哌嗪-1-基}-乙醇盐酸盐(化合物3)。¹H NMR (400MHz, DMSO-d₆): δ11.03 (s, 1H), 8.73 (d, J=7.2Hz, 2H), 8.63 (s, 1H), 8.15 (d, J=7.2Hz, 2H), 6.26 (s, 1H), 4.34 (d, J=12.4Hz, 2H), 3.82 (t, J=5.2Hz, 2H), 3.62 (d, J=12.0Hz, 2H), 3.43 (t, J=12.4Hz, 2H), 3.30-3.09 (m, 4H), 2.49 (s, 3H); MS (ES⁺) m/z C₁₉H₂₃N₇OS 计算值: 397.17; 实测值: 398.1 (M+H⁺)。

[0088] [6-(4-二甲基氨基-哌啶-1-基)-2-甲基-嘧啶-4-基]-(5-吡啶-4-基-噻唑-2-基)-胺盐酸盐(化合物4)。¹H NMR (300MHz, DMSO-d₆): δ11.07 (s, 1H), 8.73 (d, J=6.9Hz, 2H), 8.62 (s, 1H), 8.15 (d, J=6.9Hz, 2H), 6.25 (s, 1H), 4.43 (d, J=12.9Hz, 2H), 3.44 (quin, J=5.2Hz, 1H), 2.94 (t, J=12.5Hz, 2H), 2.69 (d, J=4.5Hz, 6H), 2.49 (s, 3H), 2.15 (d, J=10.5Hz, 2H), 1.60 (q, J=11.0Hz, 2H); MS (ES⁺) m/z C₂₀H₂₅N₇S 计算值: 395.19; 实测值: 396.1 (M+H⁺)。

[0089] [6-(4-乙基-哌嗪-1-基)-2-甲基-嘧啶-4-基]-(5-吡啶-3-基-噻唑-2-基)-胺盐酸盐(化合物7)。¹H NMR (400MHz, DMSO-d₆): δ11.23 (bs, 1H), 9.15 (s, 1H), 8.68 (d, J=5.2Hz, 1H), 8.60 (d, J=8.0Hz, 1H), 8.19 (s, 1H), 7.93 (t, J=6.2Hz, 1H), 6.21 (s, 1H), 4.35 (d, J=14.4Hz, 2H), 3.55 (d, J=11.6Hz, 2H), 3.40 (t, J=13.2Hz, 2H), 3.13 (t, J=5.8Hz, 2H), 3.01 (q, J=6.9Hz, 2H), 2.50 (s, 3H), 1.28 (t, J=6.6Hz, 3H); MS (ES⁺) m/z C₁₉H₂₃N₇S 计算值: 381.17; 实测值: 382.2 (M+H⁺)。

[0090] {6-[4-(2-氟-乙基)-哌嗪-1-基]-2-甲基-嘧啶-4-基}-(5-吡啶-3-基-噻唑-2-基)-胺盐酸盐(化合物8)。¹H NMR (400MHz, DMSO-d₆): δ11.80 (bs, 1H), 9.16 (s, 1H), 8.68 (d, J=5.6Hz, 1H), 8.62 (d, J=8.0Hz, 1H), 8.20 (s, 1H), 7.95 (t, J=6.8Hz, 1H), 6.22 (s, 1H), 4.98 (d, J=46.8Hz, 2H), 4.34 (bs, 2H), 3.70-3.35 (m, 6H), 3.16 (bs, 2H), 2.49 (s, 3H); MS (ES⁺) m/z C₁₉H₂₂FN₇S 计算值: 399.16; 实测值: 400.1 (M+H⁺)。

[0091] 2-{4-[2-甲基-6-(5-吡啶-3-基-噻唑-2-基氨基)-嘧啶-4-基]-哌嗪-1-基}-乙醇盐酸盐(化合物9)。¹H NMR (400MHz, DMSO-d₆): δ11.02 (bs, 1H), 9.18 (s, 1H), 8.69 (s, 1H), 8.64 (d, J=7.6Hz, 1H), 8.22 (s, 1H), 7.96 (d, J=5.2Hz, 1H), 6.25 (s, 1H), 4.33 (d, J=11.2Hz, 2H), 3.80 (s, 1H), 3.60 (d, J=11.6Hz, 2H), 3.19 (s, 2H), 3.13 (s, 2H), 2.48 (s, 3H); MS (ES⁺) m/z C₁₉H₂₃N₇OS 计算值: 397.17; 实测值: 398.1 (M+H⁺)。

[0092] [6-(4-二甲基氨基-哌啶-1-基)-2-甲基-嘧啶-4-基]-(5-吡啶-3-基-噻唑-2-基)-胺盐酸盐(化合物10)。¹H NMR (400MHz, DMSO-d₆): δ11.27 (bs, 1H), 9.18 (s, 1H), 8.69 (d, J=5.2Hz, 1H), 8.64 (d, J=8.0Hz, 1H), 8.23 (s, 1H), 7.97 (t, J=6.8Hz, 1H), 6.36 (bs, 1H), 4.42 (d, J=8.8Hz, 2H), 3.43 (bs, 1H), 2.99 (t, J=12.4Hz, 2H), 2.68 (s, 3H), 2.67 (s, 3H), 2.55 (s, 3H), 2.17 (d, J=10.8Hz, 2H), 1.64 (q, J=9.2Hz, 2H); MS (ES⁺) m/z C₂₀H₂₅N₇S 计算值: 395.19; 实测值: 396.2 (M+H⁺)。

[0093] [6-(4-乙基-哌嗪-1-基)-2-甲基-嘧啶-4-基]-(5-吡啶-2-基-噻唑-2-基)-胺盐酸盐(化合物11)。¹H NMR (300MHz, DMSO-d₆): δ11.34 (s, 1H), 8.54 (d, J=4.8Hz, 1H), 8.32 (s, 1H), 8.01-7.96 (m, 2H), 7.40-7.34 (m, 1H), 6.31 (s, 1H), 4.37 (d, J=13.2Hz, 2H), 3.62-

3.38 (m, 4H), 3.20–2.90 (m, 4H), 2.47 (s, 3H), 1.26 (t, J=7.4Hz, 3H); MS (ES⁺) m/z C₁₉H₂₃N₇S 计算值: 381.17; 实测值: 382.2 (M+H⁺)。

[0094] [6-(4-乙基-哌嗪-1-基)-2-甲基-嘧啶-4-基]-(5-嘧啶-5-基-噻唑-2-基)-胺盐酸盐(化合物12)。¹H NMR (400MHz, DMSO-d₆): δ11.35 (bs, 1H), 9.20–9.03 (m, 3H), 8.09 (s, 1H), 6.28 (s, 1H), 4.40 (s, 2H), 3.56 (d, J=12.4Hz, 2H), 3.44 (d, J=7.4Hz, 2H), 3.13 (bs, 2H), 3.02 (d, J=8.0Hz, 2H), 1.28 (bs, 3H); MS (ES⁺) m/z C₁₈H₂₂N₈S 计算值: 382.17; 实测值: 383.3 (M+H⁺)。

[0095] [6-(4-乙基-哌嗪-1-基)-2-甲基-嘧啶-4-基]-(4-吡啶-3-基-噻唑-2-基)-胺盐酸盐(化合物13)。¹H NMR (400MHz, DMSO-d₆): δ11.80 (bs, 1H), 11.54 (bs, 1H), 9.28 (s, 1H), 8.94 (d, J=8.4Hz, 1H), 8.84 (d, J=5.2Hz, 1H), 8.15–8.07 (m, 2H), 6.31 (bs, 2H), 4.35 (d, J=14.0Hz, 2H), 3.55 (d, J=12.0Hz, 2H), 3.45 (t, J=13.0Hz, 2H), 3.15–3.07 (m, 2H), 3.00 (q, J=10.0Hz, 2H), 2.49 (s, 3H), 1.27 (t, J=7.4Hz, 3H); MS (ES⁺) m/z C₁₉H₂₃N₇S 计算值: 381.17; 实测值: 382.1 (M+H⁺)。

[0096] 步骤V. 化合物5和6的合成

[0097] 在100℃向化合物E (1mmol) 和4-(2-羟乙基)-吗啉 (4mmol) 在二甘醇二甲醚 (1mL) 中的混合物中加入氢氧化钾 (10mmol), 并且将混合物在氩气氛下在160℃搅拌10分钟。将反应在0℃用水 (20mL) 猝灭, 并且用6N HCl 调整至pH=2。用碳酸氢钠将浆料调整至pH=7, 并且通过过滤收集沉淀, 用水 (10mL) 洗涤并且在真空下干燥。将残余物通过氧化铝上的层析纯化 (0.5%至1.5% MeOH/CH₂Cl₂ 梯度), 以得到化合物6的游离碱, 其为灰白色固体。

[0098] 在0℃在搅拌下向上述固体在MeOH (10mL) 中的悬浮液中加入6N HCl (1mL)。在减压下移除大部分溶剂, 并且将残余物用EtOH (10mL) 处理。通过过滤收集沉淀, 用丙酮 (10mL) 洗涤并且在真空下干燥, 以得到每个化合物5-6的盐酸盐, 其为黄色固体 (45–50%)。

[0099] [2-甲基-6-(2-吗啉-4-基-乙氧基)-嘧啶-4-基]-(5-吡啶-4-基-噻唑-2-基)-胺盐酸盐(化合物5)。¹H NMR (400MHz, DMSO-d₆): δ11.61 (s, 1H), 8.74 (d, J=5.2Hz, 2H), 8.64 (s, 1H), 8.17 (d, J=5.2Hz, 2H), 6.37 (s, 1H), 4.72 (s, 2H), 4.00–3.80 (m, 4H), 3.64–3.42 (m, 4H), 3.16 (bs, 2H), 2.59 (s, 3H); MS (ES⁺) m/z C₁₉H₂₂N₆O₂S 计算值: 398.15; 实测值: 399.2 (M+H⁺)。

[0100] [2-甲基-6-(2-吗啉-4-基-乙氧基)-嘧啶-4-基]-(5-吡啶-3-基-噻唑-2-基)-胺盐酸盐(化合物6)。¹H NMR (400MHz, DMSO-d₆): δ11.51 (bs, 1H), 9.20 (s, 1H), 8.71 (d, J=5.6Hz, 1H), 8.66 (d, J=8.4Hz, 1H), 8.24 (s, 1H), 7.98 (dd, J=8.0, 5.6Hz, 1H), 6.35 (s, 1H), 4.72 (t, J=4.8Hz, 2H), 3.96 (d, J=10.8Hz, 2H), 3.85 (t, J=12.0Hz, 2H), 3.55 (bs, 2H), 3.47 (d, J=12.4Hz, 2H), 3.19 (bs, 2H), 2.59 (s, 3H); MS (ES⁺) m/z C₁₉H₂₂N₆O₂S 计算值: 398.15; 实测值: 399.1 (M+H⁺)。

[0101] 实施例2: 抑制FLT3活性

[0102] 如下进行研究以在抑制FLT3活性方面对根据实施例1制备的特定化合物进行测试。

[0103] 将含有FLT3激酶催化结构域(残基Y567–S993)的GST-FLT3-KD^{WT}在转染了含有pBac-PAK8-GST-FLT3-KD质粒的杆状病毒的Sf9昆虫细胞中表达。FLT3^{WT}激酶-Glo测定在96孔板中在30℃下进行4小时以测试化合物, 最终体积为50μl, 包含以下组分: 75ng GST-

FLT3-KD^{WT}蛋白、25mM HEPES, pH 7.4、4mM MnCl₂、10mM MgCl₂、2mM DTT、0.02% Triton X-100、0.1mg/ml牛血清白蛋白、25μM Her2肽底物、0.5mM Na₃VO₄和1μM ATP。温育后,向每个孔中加入50μl激酶-Glo Plus试剂(Promega, Madison, WI, USA),并且将混合物在25℃温育20分钟。将每个反应混合物的70-μL等分试样转移至黑色微量滴定板中,并且在Wallac Vector 1420多标记计数器(PerkinElmer, Shelton, CT, USA)上测量荧光。

[0104] 测试了多种化合物。出乎意料地,化合物1-11显示出低于100nM的IC₅₀(响应减少一半时抑制剂的浓度)值。

[0105] 实施例3:抑制VEGFR2活性

[0106] 如下进行研究以在抑制VEGFR2活性方面对根据实施例1制备的特定化合物进行测试。请注意VEGFR2为VEGFR的三种主要亚型之一。

[0107] 含有激酶结构域的重组GST-VEGFR2(残基V789-V1356)在Sf9昆虫细胞中表达。激酶测定在具有测试化合物的96孔板中在30℃进行120分钟,最终体积为50μl反应,具有以下组分:25mM HEPES pH 7.4、10mM MgCl₂、4mM MnCl₂、0.5mM Na₃VO₄、2mM DTT、0.02% Triton X100、0.01% BSA、1μM ATP、2μM聚Glu4:Tyr肽、50~100ng重组VEGFR2。温育后,向每个孔中加入50μl激酶-Glo Plus试剂(Promega, Madison, WI, USA),并且将混合物在25℃温育20分钟。将每个反应混合物的70-μL等分试样转移至黑色微量滴定板中,并且在Wallac Vector 1420多标记计数器(PerkinElmer, Shelton, CT, USA)上测量荧光。

[0108] 在VEGFR2测定中测试了多种化合物。化合物1、9和10出乎意料地显示出低于30nM的IC₅₀值。

[0109] 实施例4:抑制c-Kit活性

[0110] 如下进行研究以在抑制c-Kit活性方面对根据实施例1制备的特定化合物进行测试。

[0111] 纯化在Sf9杆状病毒-昆虫细胞表达系统中表达的N-末端His-标记的人类c-KIT(残基T544-V976)重组蛋白,以用于c-KIT ADP激酶-Glo测定。c-Kit-ADP激酶-Glo测定在96孔板中在30℃下进行150分钟,最终体积为10μl,包含40mM Tris pH 7.4、20mM MgCl₂、2mM MnCl₂、2mM DTT、0.01% BSA、20μM ATP、20μM聚(Glu, Tyr) 4:1肽、0.1mM Na₃VO₄、250ng的重组c-Kit蛋白和指定浓度的测试化合物。在25℃,在40分钟温育的情况下,通过加入5μl ADP-GloTM试剂(Promega, Madison, WI, USA)终止反应,然后加入10μl的激酶检测试剂温育另外30分钟。最后,将每个反应混合物的30μl等分试样转移至黑色微量滴定板中,并且在Wallac Vector 1420多标记计数器(Perkin-Elmer, Shelton, CT, USA)上测量荧光。

[0112] 测试了多种化合物。出乎意料地,化合物1-7和11-12显示出低于100nM的IC₅₀值。

[0113] 实施例5:体外抗癌活性

[0114] 如下进行研究以使用细胞系和MTS细胞存活测定(MTS代表3-(4,5-二甲基噻唑-2-基)-5-(3-羧基甲氧基苯基)-2-(4-磺苯基)-2H-四唑鎓)评价根据实施例1制备的特定化合物的体外抗癌活性。

[0115] 白血病细胞系MOLM-13、MV4:11和Kasumin-1购自美国模式培养物保藏中心(American Type Culture Collection)(ATCC, Manassas, VA, USA)。人类胃肠间质瘤GIST-T1细胞系购自Cosmo Bio Co., LTD(Tokyo, 日本)。在37℃和5%CO₂下,将所有白血病细胞系维持在补充有10%胎牛血清(FBS)、10U/ml青霉素(penicillin)和10g/ml链霉素

(streptomycin)的RPMI1640培养基中。细胞系GIST-T1在补充有10%FBS、0.01%非必需氨基酸、10U/ml青霉素和10g/ml链霉素的DMEM培养基中培养。

[0116] GIST882、GIST48和GIST430细胞均在维持在37℃和5%CO₂的培养箱中培养。GIST882在补充有20%胎牛血清(FBS)的RPMI-1640中培养。GIST48在补充有20%胎牛血清(FBS)、0.5%Mito、血清增充剂(serum extender)(BD Bioscience,355006)和1%牛垂体提取物(BD Bioscience354123)的F10中培养。GIST430在补充有20%FBS的IMDM中培养。GIST882、GIST430和GIST48细胞由Jonathan A.Fletcher博士(哈佛医学院(Harvard Medical School),US)提供。

[0117] MOLM-13、MV4:11和Kasumi-1MTS测定

[0118] 将细胞以 1×10^4 个细胞/100μl/孔的密度一式三份地接种在96孔培养板中,并且用范围为1nM至10μM的指定浓度的测试化合物处理72小时。比色法的CellTiter 96® Aqueous One Solution细胞增殖测定(MTS测定;Promega, Madison, WI, USA)用于测定细胞毒性。使用微孔板光度计(Victor2;Perkin-Elmer, Waltham, MA, USA)测量在492nm处的光密度。当细胞用测试化合物处理72小时时,通过MTS测定确定IC₅₀值并且使用GraphPad Prism 6进行计算。每个实验重复三次。

[0119] GIST-T1 MTS测定

[0120] 将GIST-T1细胞以8000个细胞/100μl/孔的密度一式三份地接种在96孔培养板中,并且用范围为1nM至10μM的指定浓度的测试化合物处理72小时。比色法的CellTiter 96® Aqueous One Solution细胞增殖测定(MTS测定;Promega, Madison, WI, USA)用于测定细胞毒性。使用微孔板光度计(Victor2;Perkin-Elmer, Waltham, MA, USA)测量在492nm处的光密度。当细胞用测试化合物处理72小时时,通过MTS测定确定IC₅₀值并且使用GraphPad Prism 6进行计算。每个实验重复三次。

[0121] GIST882、GIST48和GIST430 MTS测定

[0122] 将GIST细胞(4×10^4)用不同剂量的化合物处理。在37℃在5%CO₂中,将处理过的GIST882细胞培养144小时,并且将GIST48和GIST430细胞培养120小时。通过将细胞与亚甲蓝(Clontech, CA, US)温育1小时来测定细胞增殖。使用SpectraMax M5微孔板读数器(Molecular Devices, US)在450nm处测量吸光度。

[0123] 特定式(I)化合物的GI₅₀(细胞增殖的最大抑制的50%处的浓度)值显示于下表:

	化合物	GI ₅₀ (nM)						
		MOLM-13	MV4:11	Kasumi-1	GIST-T1	GIST430	GIST48	GIST882
[0124]	1	10	13	11	11	3.8	19	5.0
	7	62	34	266	53	60	820	20
	12	211	132	349	119	ND	ND	ND

[0125] ND: 未测量

[0126] 其他实施方案

[0127] 本说明书公开的所有特征可以以任何组合方式结合。本说明书公开的各个特征可以被具有相同、等价或相似目的的替代特征代替。因此,除非以其他方式明确指明,所公开

的各个特征仅为一系列等价或相似特征的实例。

[0128] 另外,根据前述描述,本领域技术人员能够容易地确定本发明的基本特征,且在不偏离本发明的精神及范围的情况下,本领域技术人员能够对本发明做出各种改变和修饰,以使其适应各种用途及情况。因此,其他实施方案也在以下权利要求书的范围中。