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CA 2812245 C 2016/07/19

(11)(21) **2 812 245**

(12) BREVET CANADIEN / CANADIAN PATENT

(13) **C**

(22) **Date de dépôt/Filing Date:** 2013/04/10

(41) **Mise à la disp. pub./Open to Public Insp.:** 2014/03/21

(45) **Date de délivrance/Issue Date:** 2016/07/19

(30) **Priorité/Priority:** 2012/09/21 (US61/704,053)

(51) **Cl.Int./Int.Cl.** ***A61K 31/4709*** (2006.01),
A61P 35/00 (2006.01)

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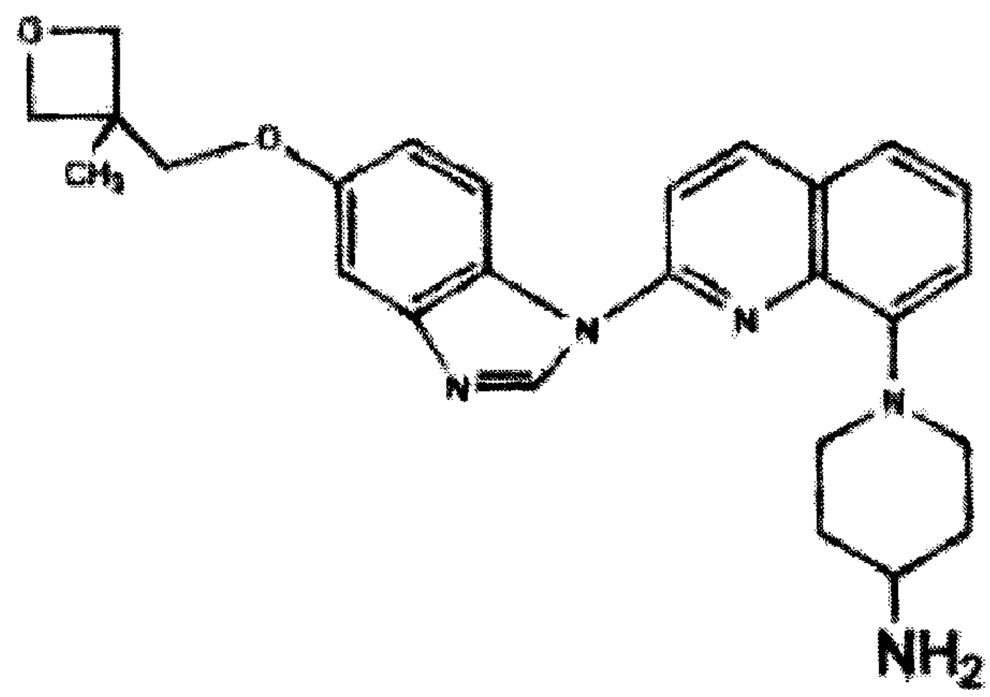
(54) **Titre : PROCEDE D'INHIBITION DE LA KINASE FLT3 PHOSPHORYLEE CONSTITUTIONNELLEMENT ACTIVE**
(54) **Title: METHOD OF INHIBITING CONSTITUTIVELY ACTIVE PHOSPHORYLATED FLT3 KINASE**



(57) **Abrégé/Abstract:**
The present invention includes a method of reducing or inhibiting the kinase activity of normal and mutated FLT3 in a cell or a subject, and the use of such compound for preventing or treating cell proliferative disorder(s) in a subject related to using a

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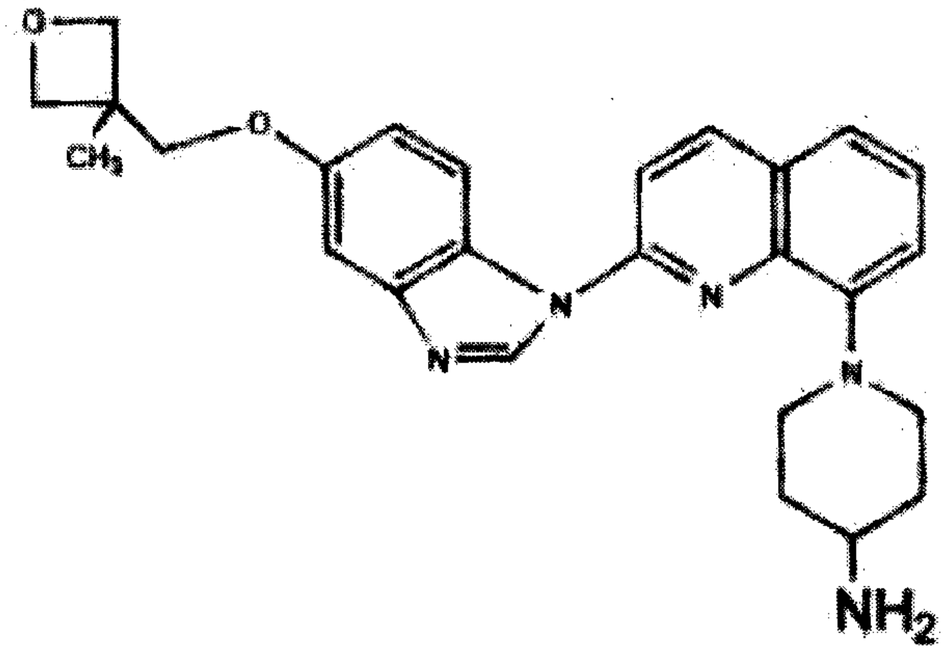
(57) **Abrégé(suite)/Abstract(continued):**
compound of the present invention:

(see above formula)

or pharmaceutically acceptable salt thereof.

ABSTRACT OF THE INVENTION

The present invention includes a method of reducing or inhibiting the kinase activity of normal and mutated FLT3 in a cell or a subject, and the use of such compound for preventing or treating cell proliferative disorder(s) in a subject related to using a compound of the present invention:



or pharmaceutically acceptable salt thereof.

METHOD OF INHIBITING CONSTITUTIVELY ACTIVE PHOSPHORYLATED FLT3 KINASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] None.

TECHNICAL FIELD OF THE INVENTION

[0002] The present invention relates to methods of reducing or inhibiting the kinase activity of normal and mutated FLT3 in a cell or a subject, and the use of such methods for preventing or treating cell proliferative disorder (s) related to FLT3.

STATEMENT OF FEDERALLY FUNDED RESEARCH

[0003] None.

INCORPORATION-BY-REFERENCE OF MATERIALS FILED ON COMPACT DISC

[0004] None.

BACKGROUND OF THE INVENTION

[0005] Without limiting the scope of the invention, its background is described in connection with protein kinases.

[0006] Protein kinases are enzymes that chemically modify other proteins by catalyzing the transfer of gamma phosphates from nucleotide triphosphates, often adenosine triphosphate (ATP), and covalently attaching them to a free hydroxyl group of amino acid residues serine, threonine and tyrosine.

[0007] Approximately 30% of all human proteins may be modified by kinase activity. Protein kinases direct the enzymatic activity, cellular location and primary function/association of substrate proteins and regulate cell signal transduction and cell function coordination.

[0008] Research studies have shown that aberrant expression of normal or mutated protein kinases are frequently associated with the formation and propagation of a number of diseases. Studies have shown that overexpression or inappropriate protein kinase expression is associated with cancer, cardiovascular disease, rheumatoid arthritis, diabetes, ocular disease, neurologic

disorders and autoimmune disease. Thus, investigating compounds that potently inhibit the activity and function of protein kinases will allow for a greater understanding of the physiological roles of protein kinases.

[0009] The FMS-like tyrosine kinase 3 (FLT3) gene encodes a membrane bound receptor tyrosine kinase that affects hematopoiesis leading to hematological disorders and malignancies. See Drexler, HG et al. Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. Leukemia. 1996; 10:588-599; Gilliland, DG and JD Griffin. The roles of FLT3 in hematopoiesis and leukemia. Blood. 2002;100:1532-1542; Stirewalt, DL and JP Radich. The role of FLT3 in hematopoietic malignancies. Nat Rev Cancer. 2003;3:650-665. Activation of FLT3 receptor tyrosine kinases is initiated through the binding of the FLT3 ligand (FLT3L) to the FLT3 receptor, also known as *S*tem cell *t*yrosine *k*inase-1(STK-1) and fetal liver kinase-2 (flk-2), which is expressed on hematopoietic progenitor and stem cells.

[0010] FLT3 is one of the most frequently mutated genes in hematological malignancies, present in approximately 30% of adult acute myeloid leukemias (AML). See Nakao M, S Yokota and T Iwai.Internal tandem duplication of the FLT3 gene found in acute myeloid leukemia. Leukemia. 1996;10:1911-1918; H Kiyoi, M Towatari and S Yokota. Internal Tandem duplication of the FLT3 gene is a novel modality of elongation mutation, which causes constitutive activation of the product. Leukemia.1998;12:1333-1337;PD Kottaridis, RE Gale, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood. 2001;98:1742-1759; Yamamoto Y, Kiyoi H, Nakano Y. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood. 2001;97:2434-2439; Thiede C, C Steudel, Mohr B. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroupswith poor prognosis. Blood. 2002;99:4326-4335. FLT3 mutations have been detected in approximately 2% of patients diagnosed with intermediate and high risk myelodysplastic syndrome (MDS). See S Bains, Luthra R, Medeiros LJ and Zuo Z. FLT3 and NPM1 mutations in myelodysplastic syndromes: Frequency and potential value for predicting progression to acute myeloid leukemia. American Journal of Clinical Pathology. January 2011;135:62-69;PK Bhamidipati, Daver NG, Kantarjian H, et al. FLT3 mutations in

myelodysplastic syndromes(MDS) and chronic myelomonocytic leukemia (CMML). 2012. Journal of Clinical Oncology. Suppl; abstract 6597. Like MDS, the number of FLT3 mutations in patients with acute promyelocytic leukemia (APL) is small. The most common FLT3 mutations are internal tandem duplications (ITDs) that lead to in-frame insertions within the juxtamembrane domain of the FLT3 receptor. FLT3-ITD mutations have been reported in 15-35% of adult AML patients. See Nakao M, S Yokota and T Iwai. Internal tandem duplication of the FLT3 gene found in acute myeloid leukemia. Leukemia. 1996;10:1911-1918; H Kiyoi, M Towatari and S Yokota. Internal Tandem duplication of the FLT3 gene is a novel modality of elongation mutation, which causes constitutive activation of the product. Leukemia.1998;12:1333-1337; H Kiyoi, T Naoe and S Yokota. Internal tandem duplication of FLT3 associated with leukocytosis in acute promyelocytic leukemia. Leukemia Study Group of the Ministry of Health and Welfare (Kohseisho). Leukemia.1997;11:1447-1452;S Schnittger, C Schoch and M Duga. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood. 2002;100:59-66. A FLT3-ITD mutation is an independent predictor of poor patient prognosis and is associated with increased relapse risk after standard chemotherapy, and decreased disease free and overall survival. See FM Abu-Duhier, Goodeve AC, Wilson GA, et al. FLT3 internal tandem duplication mutations in adult acute myeloid leukemia define a high risk group. British Journal of Haematology. 2000;111:190-195; H Kiyoi, T Naoe, Y Nakano, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood. 1999;93:3074-3080. Less frequent are FLT3 point mutations that arise in the activation loop of the FLT3 receptor. The most commonly affected codon is aspartate 835 (D835). Nucleotide substitutions of the D835 residue occur in approximately 5-10% of adult acute myeloid leukemia patients. See DL Stirewalt and JP Radich. The role of FLT3 in haematopoietic malignancies. Nature Reviews Cancer. 2003;3:650-665;Y Yamamoto, H Kiyoi and Y Nakano, et al. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood. 2001;97:2434-2439; C Thiede, Steudal C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 2002;99:4326-4335;U Bacher,

Haferlach C, W Kern, et al. Prognostic relevance of FLT3-TKD mutations in AML: the combination matters-an analysis of 3082 patients. Blood. 2008;111:2527-2537.

[0011] The heightened frequency of constitutively activated mutant FLT3 in adult AML has made the FLT3 gene a highly attractive drug target in this tumor type. Several FLT3 inhibitors with varying degrees of potency and selectivity for the target have been or are currently being investigated and examined in AML patients. See T Kindler, Lipka DB, and Fischer T. FLT3 as a therapeutic target in AML: still challenging after all these years. Blood.2010;116:5089-102.

[0012] FLT3 kinase inhibitors known in the art include Lestaurtinib (also known as CEP 701, formerly KT-555, Kyowa Hakko, licensed to Cephalon); CHIR-258 (Chiron Corp.); EB10 and IMC-EB10 (ImClone Systems Inc.); Midostauirn (also known as PKC412, Novartis AG); Tandutinib (also known as MLN-518, formerly CT53518, COR Therapeutics Inc., licensed to Millennium Pharmaceuticals Inc.); Sunitinib (also known as SU11248, Pfizer USA); Quizartinib (also known as AC220, Ambit Biosciences); XL 999 (Exelixis USA, licensed to Symphony Evolution, Inc.); GTP 14564 (Merck Biosciences UK); AG1295 and AG1296; CEP-5214 and CEP-7055 (Cephalon). The following PCT International Applications and U.S. patent applications disclose additional kinase modulators, including modulators of FLT3: WO 2002032861, WO 2002092599, WO 2003035009, WO 2003024931, WO 2003037347, WO 2003057690, WO 2003099771, WO 2004005281, WO 2004016597, WO 2004018419, WO 2004039782, WO 2004043389, WO 2004046120, WO 2004058749, WO 2004058749, WO 2003024969 and U.S Patent Application No, 20040049032. See also Levis M, KF Tse, et al. 2001 "A FLT3 tyrosine kinase inhibitor is selectively cytotoxic to acute myeloid leukemia blasts harboring FLT3 internal tandem duplication mutations." Blood 98(3): 885-887; Tse K F, et al., Inhibition of FLT3-mediated transformation by use of a tyrosine kinase inhibitor. Leukemia. July 2001; 15 (7): 1001-1010; Smith, B. Douglas et al., Singlet agent CEP-701, a novel FLT3 inhibitor, shows biologic and clinical activity in patients with relapsed or refractory acute myeloid leukemia Blood, May 2004; 103: 3669-3676; Griswold, Ian J. et al., Effects of MLN518, A Dual FLT3 and KIT Inhibitor, on Normal and Malignant Hematopoiesis. Blood, Nov 2004; 104 (9): 2912-2918 [Epub ahead of print Jul 8]; Yee, Kevin W.H. et al., SU5416 and SU5614 inhibit kinase activity of wild-type and mutant FLT3 receptor tyrosine kinase. Blood, Oct 2002; 100(8): 2941-2949.O'Farrell, Anne-Marie et al., SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. Blood, May 2003; 101(9): 3597-3605;

Stone, R. M et al., PKC-412 FLT3 inhibitor therapy in AML: results of a phase II trials. Ann. Hematol. 2004; 83 Suppl 1:S89-90; and Murata, K. et al., Selective cytotoxic mechanism of GTP-14564, a novel tyrosine kinase inhibitor in leukemia cells expressing a constitutively active Fms-like tyrosine kinase 3 (FLT3). J Biol Chem. Aug. 29, 2003; 278 (35): 32892-32898 [Epub 2003 Jun 18]; Levis, Mark et al., Small Molecule FLT3 Tyrosine Kinase Inhibitors. Current Pharmaceutical Design, 2004, 10, 1183-1193.

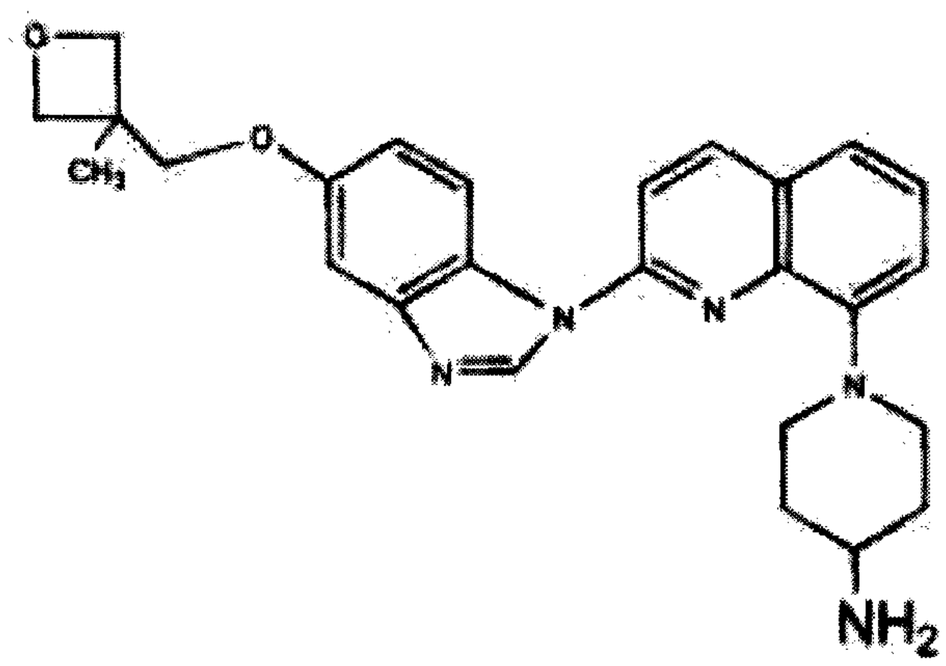
[0013] FLT3 inhibitors are classified as Type I or Type II inhibitors. These two classifications are distinguished based on their relative affinities and mechanism of binding to phosphorylated and non-phosphorylated receptor sites. Type I inhibitors recognize the active conformation of kinases. This conformation is conducive to phosphotransfer.Type I inhibitors are generally composed of a heterocyclc ring system. See Liu, Y and N Gray. Rational design of inhibitors that bind to inactive kinase conformations. Nature Chem. Biol. 2006;2:358-354. Examples of Type I FLT3 inhbitiors include Crenolanib and Midostaurin. See A Ramachandran, Marshall H and Jain V. Crenolanib, a novel type I, mutant specific inhibitor of class III receptor tyrosine kinases, preferentially binds to phosphorylated kinases. Cancer Res.2012;72 (8 supplement): 368; J Cools, et al. Prediction of resistance to small molecule FLT3 inhibitors: implications for molecularly targeted therapy of acute leukemia. Cancer Res. 2004;64:6385-6389. Resistant mutations that render the kinase of the receptor tyrosine kinase constitutively phosphorylated could potentially be sensitive to type I inhibitors that have greater affinity for the phosphorylated kinase.

[0014] By contrast, Type II inhibitors prefer to bind to the inactive conformation of kinases. This conformation is typically referred to as 'DFG-out' owing to the rearrangement of the motif. See J Zhang, Yang PL, and Gray NS. Targeting cancer with small molecule kinase inhibitors. Nature Reviews Cancer.2009;9:28-39. Inhibitors such as imatinib, sorafenib and nilotinib bind in the type II conformation. See PW Manley, Cowan-Jacob SW, Mestan J. Advances in the structural biology, design and clinical development of Bcr-Abl kinase inhibitors for the treatment of chronic myeloid leukaemia. Biochim. Biophis. Acta. 2005;1754:3-13; PT Wan, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell. 2004;116:855-867. Resistant mutations to Type II inhibitors are mutations that render the kinase domain of the receptor tyrosine kinase constitutively phosphorylated. Type I inhibitors that target the phosphorylated kinase can overcome the resistance resulting from the

treatment with Type II inhibitors, and therefore have potential use in treating diseases that harbor these resistance mutations.

SUMMARY OF THE INVENTION

[0015] The present invention includes a method of inhibiting or reducing deregulated FLT3 tyrosine kinase activity or expression in a subject suffering from a proliferative disease which comprises administering to the subject having or suspected to have the proliferative disease, a therapeutically or prophylactically effective amount of the compound of Formula I:



or a pharmaceutically acceptable salt or solvate thereof. In one aspect, the proliferative disease is selected from at least one of a leukemia, myeloma, myeloproliferative disease, myelodysplastic syndrome, idiopathic hypereosinophilic syndrome (HES), bladder cancer, breast cancer, cervical cancer, CNS cancer, colon cancer, esophageal cancer, head and neck cancer, liver cancer, lung cancer, nasopharyngeal cancer, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, salivary gland cancer, small cell lung cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, uterine cancer, and hematologic malignancy. In another aspect, the therapeutically and prophylactically effective amounts are from about 50 to 500 mg per day. In another aspect, the compound is administered at least one of continuously, intermittently, systemically, or locally. In another aspect, the deregulated FLT3 is defined further as a mutated FLT3 is constitutively active. In another aspect, the compound is administered orally, intravenously, or intraperitoneally. In another aspect, the Crenolanib is Crenolanib Besylate, Crenolanib Phosphate, Crenolanib Lactate, Crenolanib Hydrochloride, Crenolanib Citrate, Crenolanib Acetate, Crenolanib Toluenesulphonate and Crenolanib Succinate. In another aspect, the FLT3 is at least one of FLT-ITD, FLT-TKD, FLT3-D835Y, FLT3-D835H, FLT3-K663Q, or FLT-R834Q. In another

aspect, the therapeutically or prophylactically effective amount of the compound is administered up to three times or more a day for as long as the subject is in need of treatment for the proliferative disease. In another aspect, the composition is provided at least one of sequentially or concomitantly, with another pharmaceutical agent in a newly diagnosed proliferative disease patient, to maintain remission, or a relapsed/refractory proliferative disease patient. In another aspect, the compound is provided as a single agent or in combination with another pharmaceutical agent in a newly diagnosed proliferative disease patient, to maintain remission, or a relapsed/refractory proliferative disease patient. In another aspect, the compound is provided as a single agent or in combination with another pharmaceutical agent in a newly diagnosed proliferative disease pediatric patient, to maintain remission, or a relapsed/refractory proliferative disease pediatric patient. In another aspect, the patient is relapsed/refractory to a Type II tyrosine kinase inhibitor.

In another embodiment, the present invention includes a method for treating a patient suffering from a proliferative disease comprising: administering to the patient in need of such treatment a therapeutically effective amount of Crenolanib or a salt thereof, wherein the cell proliferative disorder is characterized by deregulated FLT3 receptor tyrosine kinase activity, proliferative disease is selected from at least one of a leukemia, myeloma, myeloproliferative disease, myelodysplastic syndrome, idiopathic hypereosinophilic syndrome (HES), bladder cancer, breast cancer, cervical cancer, CNS cancer, colon cancer, esophageal cancer, head and neck cancer, liver cancer, lung cancer, nasopharyngeal cancer, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, salivary gland cancer, small cell lung cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, uterine cancer, and hematologic malignancy. In one aspect, the compound is administered orally, intravenously, or intraperitoneally. In another aspect, the Crenolanib is Crenolanib Besylate, Crenolanib Phosphate, Crenolanib Lactate, Crenolanib Hydrochloride, Crenolanib Citrate, Crenolanib Acetate, Crenolanib Toluenesulphonate and Crenolanib Succinate. In another aspect, the FLT3 is at least one of FLT-ITD, FLT-TKD, FLT3-D835Y, FLT3-D835H, FLT3-K663Q, or FLT-R834Q. In another aspect, the Crenolanib is provided at least one of sequentially or concomitantly, with chemotherapy, radiotherapy, or surgery in a newly diagnosed proliferative disease, to maintain remission, or a relapsed/refractory proliferative disease. In another aspect, the Crenolanib is provided as a single agent or in combination with chemotherapy, radiotherapy

or surgery for treatment of pediatric patient with the proliferative disease. In another aspect, the Crenolanib is provided as a single agent to at least one of post standard induction therapy, or high dose induction therapy, in newly diagnosed proliferative disease. In another aspect, the Crenolanib is provided as a single agent in treatment of patients with the proliferative disease that is either refractory to, or has relapsed after, standard or high dose chemotherapy, radiotherapy or surgery. In another aspect, the patient is relapsed/refractory to at least one other tyrosine kinase inhibitor, including sorafenib, quizartinib, axitinib, sunitinib, pazopanib, Midostaurin, or Lestaurtinib.

Yet another embodiment of the present invention includes a method for treating a patient suffering from leukemia comprising: obtaining a sample from the patient suspected of having an leukemia; determining from the patient sample that the patient has a deregulated FLT3 receptor tyrosine kinase; and administering to the patient in need of such treatment a therapeutically effective amount of Crenolanib or a salt thereof, wherein the leukemia is characterized by deregulated FLT3 receptor tyrosine kinase activity.

Another embodiment of the present invention includes a method for specifically inhibiting a deregulated receptor tyrosine kinase comprising: obtaining a patient sample and determining which receptor tyrosine kinases are deregulated; and administering to a mammal in need of such treatment a therapeutically effective amount of Crenolanib or a salt thereof, wherein the deregulated receptor tyrosine kinase is a FLT3 receptor tyrosine kinase. In one aspect, the therapeutically effective amount of Crenolanib or a salt thereof is provided in an amount that does not downregulate c-Kit activity sufficient to prevent its physiological activity. In another aspect, the proliferative disease is selected from at least one of a leukemia, myeloma, myeloproliferative disease, myelodysplastic syndrome, idiopathic hypereosinophilic syndrome (HES), bladder cancer, breast cancer, cervical cancer, CNS cancer, colon cancer, esophageal cancer, head and neck cancer, liver cancer, lung cancer, nasopharyngeal cancer, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, salivary gland cancer, small cell lung cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, uterine cancer, and hematologic malignancy. In another aspect, the therapeutically and prophylactically effective amounts are from about 50 to 500 mg per day. In another aspect, the compound is administered at least one of continuously, intermittently, systemically, or locally. In another aspect, the deregulated FLT3 is defined further as a mutated FLT3 is constitutively active. In

another aspect, the compound is administered orally, intravenously, or intraperitoneally. In another aspect, the Crenolanib is Crenolanib Besylate, Crenolanib Phosphate, Crenolanib Lactate, Crenolanib Hydrochloride, Crenolanib Citrate, Crenolanib Acetate, Crenolanib Toluenesulphonate and Crenolanib Succinate. In another aspect, the FLT3 is at least one of FLT-ITD, FLT-TKD, FLT3-D835Y, FLT3-D835H, FLT3-K663Q, or FLT-R834Q. In another aspect, the therapeutically or prophylactically effective amount of the compound is administered up to three times or more a day for as long as the subject is in need of treatment for the proliferative disease. In one aspect, the patient is provided treatment, one or more patient samples are obtained to determine the effect of the treatment, and treatment is continued until the proliferative disease is reduced or eliminated. In another aspect, the compound is provided at least one of sequentially or concomitantly, with another pharmaceutical agent in a newly diagnosed proliferative disease patient, to maintain remission of an existing patient, or a relapsed/refractory proliferative disease patient. In another aspect, the present invention is provided as a single agent or in combination with another pharmaceutical agent in a newly diagnosed proliferative disease patient, to maintain remission, or a relapsed/refractory proliferative disease patient. In another aspect, the present invention is provided as a single agent or in combination with another pharmaceutical agent in a newly diagnosed proliferative disease pediatric patient, to maintain remission, or a relapsed/refractory proliferative disease pediatric patient. In another aspect, the patient is relapsed/refractory to a Type II tyrosine kinase inhibitor.

Yet another embodiment of the present invention includes a method for treating a patient with cancer comprising: obtaining a sample suspected of having cancer from the patient; determining if the patient that has become resistant to Type II protein tyrosine kinase inhibitors; and administering a therapeutically effective amount of Crenolanib or a salt thereof to overcome the resistance to the type II protein tyrosine kinase inhibitors. In another aspect, the therapeutically effective amount of Crenolanib or a salt thereof does not inhibit a c-Kit kinase after uptake of the drug at physiological levels.

[0016] The present invention provides methods of reducing or inhibiting the kinase activity of FLT3 in a cell or a subject, and the use of such methods for preventing or treating cell proliferative disorder (s) related to FLT3. Other features and advantages of the invention will be apparent from the following detailed description of the invention and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] For a more complete understanding of the features and advantages of the present invention, reference is now made to the detailed description of the invention along with the accompanying figures and in which:

[0018] Figure 1 shows the specificity of the besylate salt of the present invention for class III receptor tyrosine kinases including FLT3, PDGFRA, PDGFRB, CSF1R and KIT;

[0019] Figure 2 shows the affinity of the besylate salt of the present invention for the non-autoinhibited and autoinhibited states of FLT3, left panel: non-autoinhibited state of FLT3; right panel: autoinhibited stated of FLT3;

[0020] Figure 3 shows the binding constants of the besylate salt of the present invention compared to other FLT3 tyrosine kinase inhibitors for wild type FLT3;

[0021] Figure 4 shows the binding constants of the besylate salt of the present invention compared to other FLT3 tyrosine kinase inhibitors for the constitutively active FLT3 ITD mutation;

[0022] Figure 5 shows the binding constants of the besylate salt of the present invention compared to other FLT3 tyrosine kinase inhibitors for the constitutively active FLT3 D835Y mutation;

[0023] Figure 6 shows the binding constants of the besylate salt of the present invention compared to other FLT3 tyrosine kinase inhibitors for the constitutively active FLT3 D835H mutation;

[0024] Figure 7 shows the binding constants of the besylate salt of the present invention for phosphorylated ABL1, non-phosphorylated ABL1, phosphorylated ABL1 (T315I) and non-phosphorylated ABL1 (T315I).

DETAILED DESCRIPTION OF THE INVENTION

[0025] While the making and using of various embodiments of the present invention are discussed in detail below, it should be appreciated that the present invention provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the invention and do not delimit the scope of the invention.

[0026] To facilitate the understanding of this invention, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present invention. Terms such as "a", "an" and "the" are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the invention, but their usage does not delimit the invention, except as outlined in the claims.

[0027] The present invention comprises the use of the compounds of the present invention to inhibit FLT3 kinase activity in a cell or a subject, or to treat disorders related to FLT3 kinase activity or expression in a subject.

[0028] In one embodiment to this aspect, the present invention provides a method for reducing or inhibiting the kinase activity of FLT3 in a cell comprising the step of contacting the cell with a compound of the present invention. The present invention also provides a method for reducing or inhibiting the kinase activity of FLT3 in a subject comprising the step of administering a compound of the present invention to the subject. The present invention further provides a method of inhibiting cell proliferation in a cell comprising the step of contacting the cell with a compound of the present invention.

[0029] The term "subject" refers to an animal, such as a mammal or a human, who has been the object of treatment, observation or experiment.

[0030] The term "contacting" refers to the addition of the present invention or pharmaceutically acceptable salt to cells such that the compound is taken up by the cell.

[0031] In other embodiments to this aspect, the present invention provides both prophylactic and therapeutic methods for treating a subject at risk or susceptible to developing a cell proliferative disorder driven by aberrant kinase activity of FLT3. In one example, the invention provides methods for preventing a cell proliferative disorder related to FLT3, comprising administration of a prophylactically effective amount of a pharmaceutical composition comprising a compound of the present invention in a subject. Administration of said prophylactic agent can occur prior to the manifestation of symptoms characteristic of the FLT3 driven cell proliferative disorder, such that a disease or disorder is prevented or, alternatively, delayed in its progression.

[0032] The term "prophylactically effective amount" refers to an amount of active compound or pharmaceutical salt that inhibits or delays in a subject the onset of a disorder as being sought by a researcher, veterinarian, medical doctor or other clinician.

[0033] The term "therapeutically effective amount" as used herein, refers to an amount of active compound or pharmaceutical salt that elicits the biological or medicinal response in a subject that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated.

[0034] Methods for determining therapeutically and prophylactically effective doses for pharmaceutical compositions comprising a compound of the present invention are known in the art.

[0035] As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combinations of the specified ingredients in the specified amounts.

[0036] As used herein, the terms “disorder related to FLT3,” or “disorders related to FLT3 receptor,” or “disorders related to FLT3 receptor tyrosine kinase,” or “FLT3 driven cell proliferative disorder” includes diseases associated with or implicating FLT3 activity, for example, mutations leading to constitutive activation of FLT3. Examples of “disorders related to FLT3” include disorders resulting from over stimulation of FLT3 due to mutations in FLT3, or disorders resulting from abnormally high amount of FLT3 activity due to abnormally high amount of mutations in FLT3. It is known that over-activity of FLT3 has been implicated in the pathogenesis of many diseases, including the following listed cell proliferative disorders, neoplastic disorders and cancers.

[0037] The term “cell proliferative disorders” refers to excess cell proliferation of one or more subset of cells in a multicellular organism resulting in harm (i.e. discomfort or decreased life expectancy) to the multicellular organism. Cell proliferative disorders can occur in different types of animals and humans. As used herein, “cell proliferative disorders” include neoplastic disorders.

[0038] The term “neoplastic disorder’ as used herein, refers to a tumor resulting from abnormal or uncontrolled cellular growth. Examples of neoplastic disorders include, but are not limited to

the following disorders, for instance: the myeloproliferative disorders, such as thrombocytopenia, essential thrombocytosis (ET), agnogenic myeloid metaplasia, myelofibrosis (MF), myelofibrosis with myeloid metaplasia (MMM), chronic idiopathic myelofibrosis (UIMF), and polycythemia vera (PV), the cytopenias, and pre-malignant myelodysplastic syndromes; cancerse such as glioma cancers, lung cancers, breast cancers, colorectal cancers, prostate cancers, gastric cancers, esophageal cancers, colon cancers, pancreatic cancers, ovarian cances, and hematological malignancies, including myelodysplasia, multiple myeloma, leukemias, and lymphomas. Examples of hematological malignancies include, for instance, leukemias, lymphomas, Hodgkin's disease, and myeloma. Also, acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), acute promyelocytic leukemia (APL), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), acute undifferentiated leukemia (AUL), anaplastic large-cell lymphoma (ALCL), prolymphocytic leukemia (PML), juvenile myelomonocytic leukemia (JMML), adult T-cell ALL, AML, with trilineage myelodysplasia (AMLITMDS), mixed lineage leukemia (MLL), myelodysplastic syndoromes (MDSs), myeloproliferative disorders (MPD), and multiple myeloma (MM).

[0039] In a further embodiment, the present invention can be combined with another therapy as a combination therapy for treating or inhibiting the onset of a cell proliferative disorder related to FLT3 in a subject. The combination therapy comprises the administration of a prophylactically and therapeutically effective amount of a compound of the present invention and one or more other anti-cell proliferation therapies including, but not limited to, chemotherapy and radiation therapy.

[0040] In an embodiment of the present invention, a compound of the present invention may be administered in combination with chemotherapy. Used herein, chemotherapy refers to a therapy involving a chemotherapeutic agent. A variety of chemotherapeutic agents may be used in combination with the present invention. By way of example only, taxane compounds, specifically docetaxel, is safely administered in combination with a compound of the present invention in a dosage of 75 mg per square meter (mg/m^2) of body surface area.

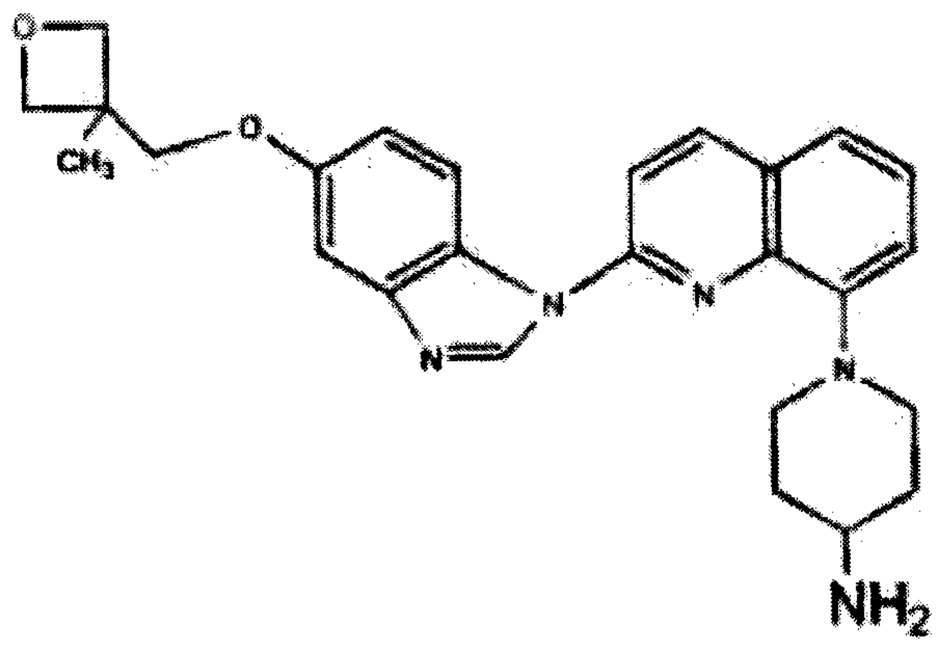
[0041] Chemotherapy is known to those skilled in the art. The appropriate dosage and scheme for chemotherapy will be similar to those already employed in clinical therapies wherein the chemotherapy is delivered in combination with other therapies or used alone.

[0042] In another embodiment of the present invention, compounds of the present invention may be administered in combination with radiation therapy. Used herein, "radiation therapy" refers to a therapy that comprises the exposure of a subject in need to radiation. Radiation therapy is known to those skilled in the art. The appropriate dosage and scheme for radiation therapy will be similar to those already employed in clinical therapies wherein the radiation therapy is delivered in combination with other therapies or used alone.

[0043] In another embodiment of the present invention, the compounds of the present invention may be administered in combination with a targeted therapy. As used herein, "targeted therapy" refers to a therapy targeting a particular class of proteins involved in tumor development or oncogenic signaling. For example, tyrosine kinase inhibitors against vascular endothelial growth factor have been used in treating cancers.

[0044] The present invention also includes methods that include the use of a second pharmaceutical agent in addition to compounds of the present invention, the two may be administered simultaneously or sequentially (in either order).

[0045] In one embodiment, the present invention therapeutically effective amounts of the compound having formula I:



[0046] or a pharmaceutically acceptable salt or solvate thereof, in a therapeutically or prophylactically effective amount against a proliferative disease is selected from at least one of a leukemia, myeloma, myeloproliferative disease, myelodysplastic syndrome, idiopathic

hypereosinophilic syndrome (HES), bladder cancer, breast cancer, cervical cancer, CNS cancer, colon cancer, esophageal cancer, head and neck cancer, liver cancer, lung cancer, nasopharyngeal cancer, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, salivary gland cancer, small cell lung cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, uterine cancer, and hematologic malignancy. Pharmaceutically acceptable salts including hydrochloride, phosphate and lactate are prepared in a manner similar to the benzenesulfonate salt and are well known to those of moderate skill in the art.

[0047] Compounds of the present invention may be administered to a subject systemically, for example, orally, intravenously, subcutaneously, intramuscular, intradermal or parenterally. The compounds of the present invention can also be administered to a subject locally.

[0048] Compounds of the present invention may be formulated for slow-release or fast-release with the objective of maintaining contact of compounds of the present invention with targeted tissues for a desired range of time.

[0049] Compositions suitable for oral administration include solid forms, such as pills, tablets, caplets, capsules, granules, and powders, liquid forms, such as solutions, emulsions, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions and suspensions.

[0050] The daily dosage of the compounds of the present invention may be varied over a wide range from 50 to 500 mg per adult human per day. For oral administration, the compositions are preferably provided in the form of tablets containing 20 and 100 milligrams. The compounds of the present invention may be administered on a regimen up to three times or more per day. Preferably three times per day. Optimal doses to be administered may be determined by those skilled in the art, and will vary with the compound of the present invention used, the mode of administration, the time of administration, the strength of the preparation, the details of the disease condition. Factors associated with patient characteristics, such as age, weight, and diet will call for dosage adjustments.

[0051] Preparation of the compounds of the present invention. General synthetic methods which may be referred to for preparing the compounds of formula I are provided in U.S. Pat. No. 5,990,146 (issued Nov. 23, 1999) (Warner-Lambert Co.) and PCT published application numbers WO 99/16755 (published Apr. 8, 1999) (Merck & Co.) WO 01/40217 (published Jul.

7, 2001) (Pfizer, Inc.), US Patent Application No. US 2005/0124599 (Pfizer, Inc.) and U.S. Patent No. 7,183,414 (Pfizer, Inc.).

[0052] Pharmaceutically acceptable salts such as hydrochloride, phosphate and lactate are prepared in a manner similar to the benzenesulfonate salt and are well known to those of moderate skill in the art. The following representative compounds of the present invention are for exemplary purposes only and are in no way meant to limit the invention, including Crenolanib as Crenolanib Besylate, Crenolanib Phosphate, Crenolanib Lactate, Crenolanib Hydrochloride, Crenolanib Citrate, Crenolanib Acetate, Crenolanib Toluenesulphonate and Crenolanib Succinate.

[0053] Biological Activity.

[0054] In Vitro Assays. The following representative in vitro assays were performed in determining the FLT3 biological activity of the present invention. These are given to illustrate the invention in a non-limiting fashion.

[0055] Inhibition of wild type and mutated FLT3 enzyme activity and specificity for the inhibition of the phosphorylated form of FLT3 exemplify the specific inhibition of the FLT3 enzyme and cellular processes that are dependent on FLT3 activity. All of the examples herein show significant and specific inhibition of the FLT3 kinase and FLT3-dependent cellular responses.

[0056] Competitive binding assay. To determine the activity of the present invention in an in vitro kinase assay. Inhibition of the kinase domain of the human FLT3 receptor was performed using the KINOMEscan Kdelect assay protocol. The KINOMEscan platform utilizes a high-throughput competitive binding technology. The assay was performed by combining DNA-tagged kinase, immobilized ligand, and the present invention. The ability of the present invention to compete with immobilized ligand was measured using quantitative PCR of the DNA tag. The competition binding assay was used to evaluate the present invention against a panel of 96 human protein kinases.

[0057] Kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an-E.coli host derived from the BL21 strain. E. coli were grown to log phase and infected with T7 phage from a frozen stock and incubated with shaking at 32 degrees Celsius until lysis. The lysates were then centrifuged and filtered. The remaining kinases were produced in HEK-293 cells and

tagged with DNA for quantitative PCR detection. Affinity resins for the kinase assay were generated by treating streptavidin-coated magnetic beads with biotinylated small molecule ligands for 30 minutes at room temperature. The liganded beads were blocked with excess biotin and washed with blocking buffer consisting of Sea Block, 1% Bovine Serum Albumin (BSA) 0.05% Tween* 20, 1 mM Dithithreitol (DTT) in order to reduce non-specific phage binding. An 11-point 3-fold serial dilution of the present invention was prepared as a 40x stock in 100% Dimethyl sulfoxide (DMSO) and diluted to 1x directly into the assay.

[0058] Binding reactions were initiated by combining the liganded affinity beads, kinases, and the present invention in 1x binding buffer consisting of 20% Sea Block, 0.17 Phosphate Buffered Saline (PBS), 0.05% Tween 20, 6 mM DTT. All reactions were performed in polypropylene 384-well plates in a final volume of 0.04 mL. The plates were incubated for 1 hour while shaking at room temperature. The affinity beads were washed with 1x PBS and 0.05% Tween 20 buffer, then re-suspended in elution buffer consisting of 1x PBS, 0.05% Tween 20, 0.5 uM non-biotinylated affinity ligand. Following re-suspension, the affinity beads were incubated at room temperature with shaking. The elutant kinase concentration was then measured by quantitative PCR.

[0059] Binding constants (Kds) were calculated with a standard dose-response curve using the Hill equation. Curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm. Kds of the present invention were compared to both a negative DMSO control and a positive control compound. The binding affinity of the present invention was visualized using the compound profile visualization interaction map, TREEspot.

[0060] Direct enzyme phosphorylation assay. The Millipore Kinase IC50 Profiler assay was used to screen the present invention against a panel of normal FLT3 and mutated FLT3 kinases. For assays of both kinases, the FLT3 enzyme was incubated with 8 mM of 3-(N-morpholino)propanesulfonic acid (MOPS) at a pH of 7.0, 0.2 mM Ethylenediaminetetraacetic acid (EDTA), 50 uM, a synthetic Abl peptide substrate EAIYAAPFAKKK, 10 mM MgAcetate and [γ-33P-ATP]. The reaction was initiated by the addition of MgATp mix. The reaction mixture was incubated for 40 minutes at room temperature and halted by the addition of 3% phosphoric acid solution. 10 uL of the reaction solution was spotted on P30 filtermat and washed three times in 75 mM phosphoric acid for 5 minutes and then once in methanol prior to

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drying and scintillation counting. The scintillation values for each replicate, including positive and negative controls, were analyzed using XLFit version 5.1 to determine the IC50 values for the present invention against normal and mutated FLT3.

[0061] Biological data for wild type FLT3. The activity of the besylate salt of the present invention against wild type FLT3 is presented in Figure No. 3. All binding constants are presented in nanomolar concentration. In figure No.3, the activity of the present invention for wild type FLT3 is compared against other inhibitors known in the art. See Davis MI, Hunt JP, Herrgard S, et al. Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnol 2011;29:1046-51. The binding constant (Kd) of the besylate salt of the present invention is 0.74 nM. When comparing the Kd of the besylate salt of the present invention for wild type FLT3 and another inhibitor in the art, AST-487, the besylate form of the present invention had one time greater affinity for FLT3 wild type than AST-487 (Kd=0.79nM).When comparing the Kd value of the besylate salt of the present invention for wild type FLT3 and another inhibitor in the art, Quizartinib, the besylate form of the present invention had two times greater affinity for FLT3 wild type than Quizartinib (Kd=1.3 nM). When comparing the Kd of the besylate salt of the present invention for wild type FLT3 and another inhibitor in the art, MLN-518, the besylate form of the present invention had four times greater affinity for FLT3 wild type than MLN-518 (Kd=3 nM). When comparing the Kd of the besylate salt of the present invention for wild type FLT3 and another inhibitor in the art, Lestaurtinib, the besylate form of the present invention had four times greater affinity for FLT3 wild type than Lestaurtinib (Kd=8.5 nM). When comparing the Kd of the besylate salt of the present invention for wild type FLT3 and another inhibitor in the art, Midostaurin, the besylate form of the present invention had approximately fifteen times greater affinity for FLT3 wild type than Midostaurin (Kd=11 nM). When comparing the Kd of the besylate salt of the present invention for wild type FLT3 and another inhibitor in the art, Sorafenib, the besylate form of the present invention had approximately eighteen times greater affinity for FLT3 wild type than Midostaurin (Kd=13 nM).

[0062] The activity of the besylate salt of the present invention was determined using a direct enzymatic Millipore IC50 profiler assay. All IC50 values are presented in nanomolar concentration. In the direct enzymatic measurement assay, the IC50 of the besylate salt of the current invention against wild type FLT3 was 3 nM. Figure 1 shows the specificity of the

besylate salt of the present invention for class III receptor tyrosine kinases including FLT3, PDGFRA, PDGFRB, CSF1R and KIT.

[0063] Biological data for the FLT3-ITD mutation. The activity of the besylate salt of the present invention against FLT3 with an internal tandem duplication mutation (ITD) is presented in Figure No. 4. All binding constants are presented in nanomolar concentration. In figure No.4, the activity of the present invention for the FLT3-ITD mutation is compared against other inhibitors known in the art. See Davis MI, Hunt JP, Herrgard S, et al. Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnol 2011;29:1046-51. The Kd of the besylate salt of the present invention is 0.43 nM. When comparing the Kd of the besylate salt of the present invention for the FLT3-ITD mutation and another inhibitor in the art, Sunitinib, the besylate form of the present invention had more than two times greater affinity for the FLT3-ITD mutation than sunitinib (Kd=0.99 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3-ITD mutation and another inhibitor in the art, Lestaurtinib, the besylate form of the present invention had more than three times greater affinity for the FLT3-ITD mutation than sunitinib (Kd=1.5 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3-ITD mutation and another inhibitor in the art, Quizartinib, the besylate form of the present invention had more than twenty times greater affinity for the FLT3-ITD mutation than Quizartinib (Kd=8.8 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3-ITD mutation and another inhibitor in the art, MLN-518, the besylate form of the present invention had more than twenty-three times greater affinity for the FLT3-ITD mutation than MLN-518 (Kd=9.1 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3-ITD mutation and another inhibitor in the art, PKC-412, the besylate form of the present invention had more than twenty-five times greater affinity for the FLT3-ITD mutation than PKC-412 (Kd=11 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3-ITD mutation and another inhibitor in the art, AST-487, the besylate form of the present invention had more than twenty-five times greater affinity for the FLT3-ITD mutation than AST-487 (Kd=11 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3-ITD mutation and another inhibitor in the art, Sorafenib, the besylate form of the present invention had more than one-hundred eighty-three times greater affinity for the FLT3-ITD mutation than AST-487 (Kd=79 nM).

[0064] Biological data for the FLT3-D835 mutation. The activity of the besylate salt of the present invention against FLT3 tyrosine kinase domain mutations D835Y and D835H is presented in Figures No. 5 and No.6. All binding constants are presented in nanomolar concentration. In both figures No. 5 and No. 6, the activity of the present invention for the FLT3 D835 mutations is compared against other inhibitors known in the art. See Davis MI, Hunt JP, Herrgard S, et al. Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnol 2011;29:1046-51. The binding constant (Kd) of the besylate salt of the present invention for the FLT3 D835Y mutation is 0.18nM and 0.4nM for the FLT3 D835H mutation. When comparing the Kd of the besylate salt of the present invention for the FLT3 D835Y and D835H mutations and another inhibitor in the art, Lestaurtinib, the besylate form of the present invention had three times greater affinity for the FLT3 D835Y mutation and one time greater for the FLT3 D835H mutation than Lestaurtinib(D835Y Kd=0.57nm and D835H Kd=0.66 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3 D835Y and D835H mutations and another inhibitor in the art, Sunitinib, the besylate form of the present invention had twelve times greater affinity for the FLT3 D835Y mutation and ten times greater for the FLT3 D835H mutation than Sunitinib (D835Y Kd=2.3nM and D835H Kd=4.3 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3 D835Y and D835H mutations and another inhibitor in the art, Quizartinib, the besylate form of the present invention had thirty nine times greater affinity for the FLT3 D835Y mutation and nine times greater for the FLT3 D835H mutation than Quizartinib(D835Y Kd=7.1nM and D835H Kd=3.7 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3 D835Y and D835H mutations and another inhibitor in the art, AST-487, the besylate form of the present invention had sixty-one times greater affinity for the FLT3 D835Y mutation and twelve times greater for the FLT3 D835H mutation than AST-487 (D835Y Kd=11 nM and D835H Kd=4.9 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3 D835Y and D835H mutations and another inhibitor in the art, PKC-412, the besylate form of the present invention had eighty-three times greater affinity for the FLT3 D835Y mutation and nine times greater for the FLT3 D835H mutation than PKC-412 (D835Y Kd=15 nM and D835H Kd=6.8 nM). When comparing the Kd of the besylate salt of the present invention for the FLT3 D835Y and D835H mutations and another inhibitor in the art, Sorafenib, the besylate form of the present invention had four hundred fifty-five times greater affinity for the FLT3 D835Y

mutation and seventy-five times greater for the FLT3 D835H mutation than Sorafenib (D835Y Kd=82 nM and D835H Kd=30 nM).

[0065] The activity of the besylate salt of the present invention was determined using a direct enzymatic Millipore IC50 profiler assay. All IC50 values are presented in nanomolar concentration. In the direct enzymatic measurement assay, the IC50 of the besylate salt of the current invention against the FLT3 TKD mutation D835Y was 2 nM.

[0066] Biological data for phosphorylated kinase affinity.

[0067] Results of the effect of ABL1 A loop phosphorylation on the affinity of the present invention are presented in Figure No. 7. Analysis of the affinity of the besylate salt of the present invention for kinases ABL1 and ABL(T315I) demonstrated that the molecule exhibits the characteristic mechanism of a type I inhibitor. The binding constants for phosphorylated ABL1 (Kd=88 nM) and ABL(T315I) (Kd=760 nM) for the present invention were 7 and 15-fold lower than its binding constants for non-phosphorylated ABL1 (Kd=600 nM) and ABL(T315I) (Kd=12000 nM), respectively. Though the present invention is not active against ABL, the besylate salt of the invention significantly greater affinity for the phosphorylated kinase suggests that crenolanib is a type I TKI.

[0068] The difference in binding affinities of the besylate salt of the present invention for the non-autoinhibited and autoinhibited states of FLT3 also indicate that the molecule functions as a type I inhibitor. As is shown in Figure No. 2, the besylate salt of the present invention has a Kd value of 0.61nM for non-autoinhibited FLT3 and a Kd value of 6.7nM for autoinhibited FLT3. The besylate salt of the present invention thus has an approximately 10-fold affinity shift between the non-autoinhibited and autoinhibited states of FLT3. This value is within the range of affinity shifts reported for other type I tyrosine kinase inhibitors and is far outside the range of 100- to 1000-fold affinity shifts reported for type II TKIs. Davis, MI et al., Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnology. 2011; 29 (10): 1046-1051; Zhang, J et al., Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer. 2009; 9(1): 28-39; Liu, Y et al., Rational design of inhibitors that bind to inactive kinase conformations. Nat Chem Biol. 2006; 2(7): 358-364.

[0069] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the invention, and vice

versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

[0070] It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims.

[0071] All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains.

[0072] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0073] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0074] The term "or combinations thereof" as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is

important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, MB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

[0075] As used herein, words of approximation such as, without limitation, "about", "substantial" or "substantially" refers to a condition that when so modified is understood to not necessarily be absolute or perfect but would be considered close enough to those of ordinary skill in the art to warrant designating the condition as being present. The extent to which the description may vary will depend on how great a change can be instituted and still have one of ordinary skilled in the art recognize the modified feature as still having the required characteristics and capabilities of the unmodified feature. In general, but subject to the preceding discussion, a numerical value herein that is modified by a word of approximation such as "about" may vary from the stated value by at least ±1, 2, 3, 4, 5, 6, 7, 10, 12 or 15%.

[0076] All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein and that the scope of the claims is not to be limited by any preferred embodiment, but should be given the broadest interpretation consistent with the description as a whole.

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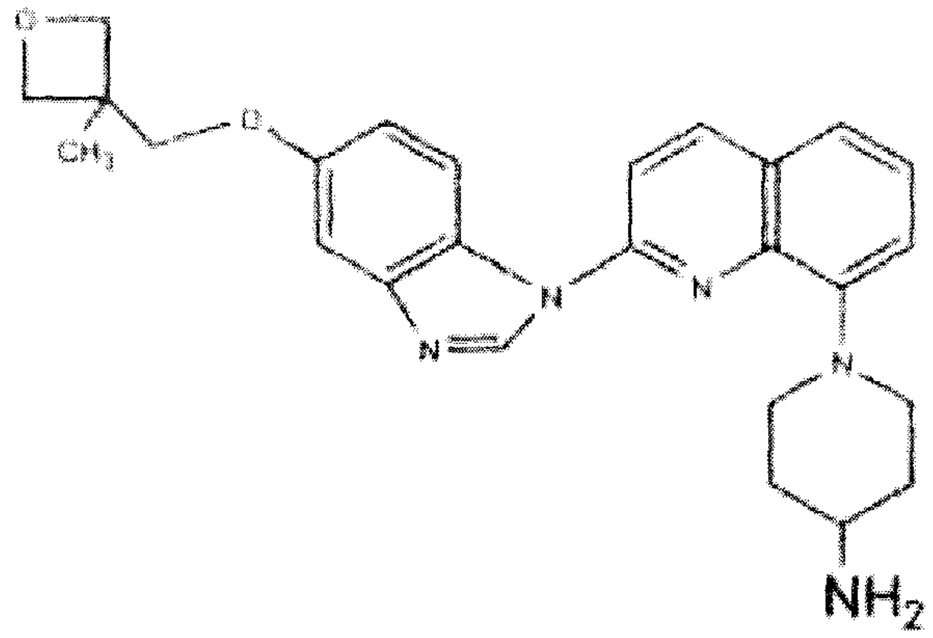
What is claimed is:

1. A use of a therapeutically or prophylactically effective amount of the Crenolanib compound of Formula I:



or a pharmaceutically acceptable salt or solvate thereof, for inhibiting or reducing deregulated FLT3 tyrosine kinase activity or expression in a subject suffering from or suspected to have a proliferative disease.

2. The use of claim 1, wherein the proliferative disease is at least one of a leukemia, myeloma, myeloproliferative disease, myelodysplastic syndrome, idiopathic hypereosinophilic syndrome (HES), bladder cancer, breast cancer, cervical cancer, CNS cancer, colon cancer, esophageal cancer, head and neck cancer, liver cancer, lung cancer, nasopharyngeal cancer, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, salivary gland cancer, small cell lung cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, uterine cancer, or hematologic malignancy.

3. The use of claim 1, wherein the therapeutically and prophylactically effective amounts are from about 50 to 500 mg per day.

4. The use of claim 1, wherein the use is at least one of continuously, intermittently, systemically, or locally.

5. The use of claim 1, wherein deregulated FLT3 is defined further as a mutated FLT3 constitutively active.

6. The use of claim 1, wherein the use is oral, intravenous, or intraperitoneal.

7. The use of claim 1, wherein the Crenolanib is at least one of Crenolanib Besylate, Crenolanib Phosphate, Crenolanib Lactate, Crenolanib Hydrochloride,

Crenolanib Citrate, Crenolanib Acetate, Crenolanib Toluenesulphonate or Crenolanib Succinate.

8. The use of claim 1, wherein the FLT3 is at least one of FLT-ITD, FLT-TKD, FLT3-D835Y, FLT3-D835H, FLT3-K663Q, or FLT-R834Q.

9. The use of claim 1, wherein the therapeutically or prophylactically effective amount of compound is provided up to three times or more a day, for as long as the subject is in need of treatment for the proliferative disease.

10. The use of claim 1, wherein the compound is provided at least one of sequentially or concomitantly, with another pharmaceutical agent, in a newly diagnosed proliferative disease patient, to maintain remission of an existing patient, or a relapsed/refractory proliferative disease patient.

11. The use of claim 1, wherein the compound is provided as a single agent or in combination with another pharmaceutical agent, in a newly diagnosed proliferative disease patient, to maintain remission, or a relapsed/refractory proliferative disease patient.

12. The use of claim 1, wherein the compound is provided as a single agent or in combination with another pharmaceutical agent, in a newly diagnosed proliferative disease pediatric patient, to maintain remission, or a relapsed/refractory proliferative disease pediatric patient.

13. The use of claim 1, wherein the patient is relapsed/refractory to a Type II tyrosine kinase inhibitor.

14. A use of a therapeutically effective amount of Crenolanib or a salt thereof, for treating a patient suffering from a proliferative disease, wherein the cell proliferative disease is characterized by deregulated FLT3 receptor tyrosine kinase activity, and said proliferative disease is at least one of a leukemia, myeloma, myeloproliferative disease, myelodysplastic syndrome, idiopathic hypereosinophilic syndrome (HES), bladder cancer, breast cancer, cervical cancer, CNS cancer, colon cancer, esophageal cancer, head and neck cancer, liver cancer, lung cancer, nasopharyngeal cancer, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, salivary gland cancer, small cell lung cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, uterine cancer, or hematologic malignancy.

15. The use of claim 14, wherein the compound is provided orally, intravenously, or intraperitoneally.

16. The use of claim 14, wherein the Crenolanib is at least one of Crenolanib Besylate, Crenolanib Phosphate, Crenolanib Lactate, Crenolanib Hydrochloride, Crenolanib Citrate, Crenolanib Acetate, Crenolanib Touluenesulphonate, or Crenolanib Succinate.

17. The use of claim 14, wherein the FLT3 is at least one of FLT-ITD, FLT-TKD, FLT3-D835Y, FLT3-D835H, FLT3-K663Q, or FLT-R834Q.

18. The use of claim 14, wherein the Crenolanib is provided at least one of sequentially or concomitantly, with chemotherapy, radiotherapy, or surgery in a newly diagnosed proliferative disease, to maintain remission, or a relapsed/refractory proliferative disease.

19. The use of claim 14, wherein the Crenolanib is provided as a single agent or in combination with chemotherapy, radiotherapy or surgery for treatment of pediatric patient with the proliferative disease.

20. The use of claim 14, wherein the Crenolanib is provided as a single agent to at least one of post standard induction therapy, or high dose induction therapy, in newly diagnosed proliferative disease.

21. The use of claim 14, wherein the Crenolanib is provided as a single agent in treatment of patients with the proliferative disease that is either refractory to, or has relapsed after, standard or high dose chemotherapy, radiotherapy or surgery.

22. The use of claim 14, wherein the patient is refractory to at least one other tyrosine kinase inhibitor.

23. A use of a therapeutically effective amount of Crenolanib or a salt thereof, for treating a patient suffering from leukemia, wherein from a patient sample it was determined that the patient has a deregulated FLT3 receptor tyrosine kinase; and wherein the leukemia is characterized by deregulated FLT3 receptor tyrosine kinase activity.

24. A use of a therapeutically effective amount of Crenolanib or a salt thereof, for specifically inhibiting a deregulated receptor tyrosine kinase in a mammal in need of such treatment, wherein from a patient sample has been determined which receptor

tyrosine kinases are deregulated, and wherein the deregulated receptor tyrosine kinase is a FLT3 receptor tyrosine kinase.

25. The use of claim 24, wherein the therapeutically effective amount of Crenolanib or a salt thereof is provided in an amount that does not downregulate c-Kit activity sufficient to prevent its physiological activity.

26. The use of claim 24, wherein the proliferative disease is at least one of a leukemia, myeloma, myeloproliferative disease, myelodysplastic syndrome, idiopathic hypereosinophilic syndrome (HES), bladder cancer, breast cancer, cervical cancer, CNS cancer, colon cancer, esophageal cancer, head and neck cancer, liver cancer, lung cancer, nasopharyngeal cancer, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, salivary gland cancer, small cell lung cancer, skin cancer, stomach cancer, testicular cancer, thyroid cancer, uterine cancer, or hematologic malignancy.

27. The use of claim 24, wherein the therapeutically effective amounts are from about 50 to 500 mg per day.

28. The use of claim 24, wherein the compound is provided at least one of continuously, intermittently, systemically, or locally.

29. The use of claim 24, wherein deregulated FLT3 is defined further as a mutated FLT3 constitutively active.

30. The use of claim 24, wherein the compound is provided orally, intravenously, or intraperitoneally.

31. The use of claim 24, wherein the Crenolanib is at least one of Crenolanib Besylate, Crenolanib Phosphate, Crenolanib Lactate, Crenolanib Hydrochloride, Crenolanib Citrate, Crenolanib Acetate, Crenolanib Touluenesulphonate or Crenolanib Succinate Crenolanib Besylate.

32. The use of claim 24, wherein the FLT3 is at least one of FLT-ITD, FLT-TKD, FLT3-D835Y, FLT3-D835H, FLT3-K663Q, or FLT-R834Q.

33. The use of claim 24, wherein the therapeutically or prophylactically effective amount of the compound is used up to three times or more a day for as long as the subject is in need of treatment for the proliferative disease.

34. The use of claim 24, wherein from one or more patient samples, the effect of the treatment is determined and the Crenolanib or salt thereof is provided until the proliferative disease is reduced or eliminated.

35. The use of claim 24, wherein the compound is provided at least one of sequentially or concomitantly, with another pharmaceutical agent, in a newly diagnosed proliferative disease patient, to maintain remission, or a relapsed/refractory proliferative disease patient.

36. The use of claim 24, wherein the compound is provided as a single agent or in combination with another pharmaceutical agent, in a newly diagnosed proliferative disease patient, to maintain remission, or a relapsed/refractory proliferative disease patient.

37. The use of claim 24, wherein the compound is provided as a single agent or in combination with another pharmaceutical agent, in a newly diagnosed proliferative disease pediatric patient, to maintain remission, or a relapsed/refractory proliferative disease pediatric patient.

38. The use of claim 24, wherein the patient is relapsed/refractory to a Type II tyrosine kinase inhibitor.

39. A use of a therapeutically effective amount of Crenolanib or a salt thereof, for treating a patient with cancer, wherein the cancer is characterized by deregulated FLT3 receptor tyrosine kinase activity or expression and wherein from a sample of the patient it has been determined that the patient has become resistant to Type II protein tyrosine kinase inhibitors, to overcome the resistance to the type II protein tyrosine kinase inhibitors.

40. The use of claim 39, wherein the therapeutically effective amount of Crenolanib or a salt thereof does not inhibit a c-Kit kinase after uptake of the drug at physiological levels.

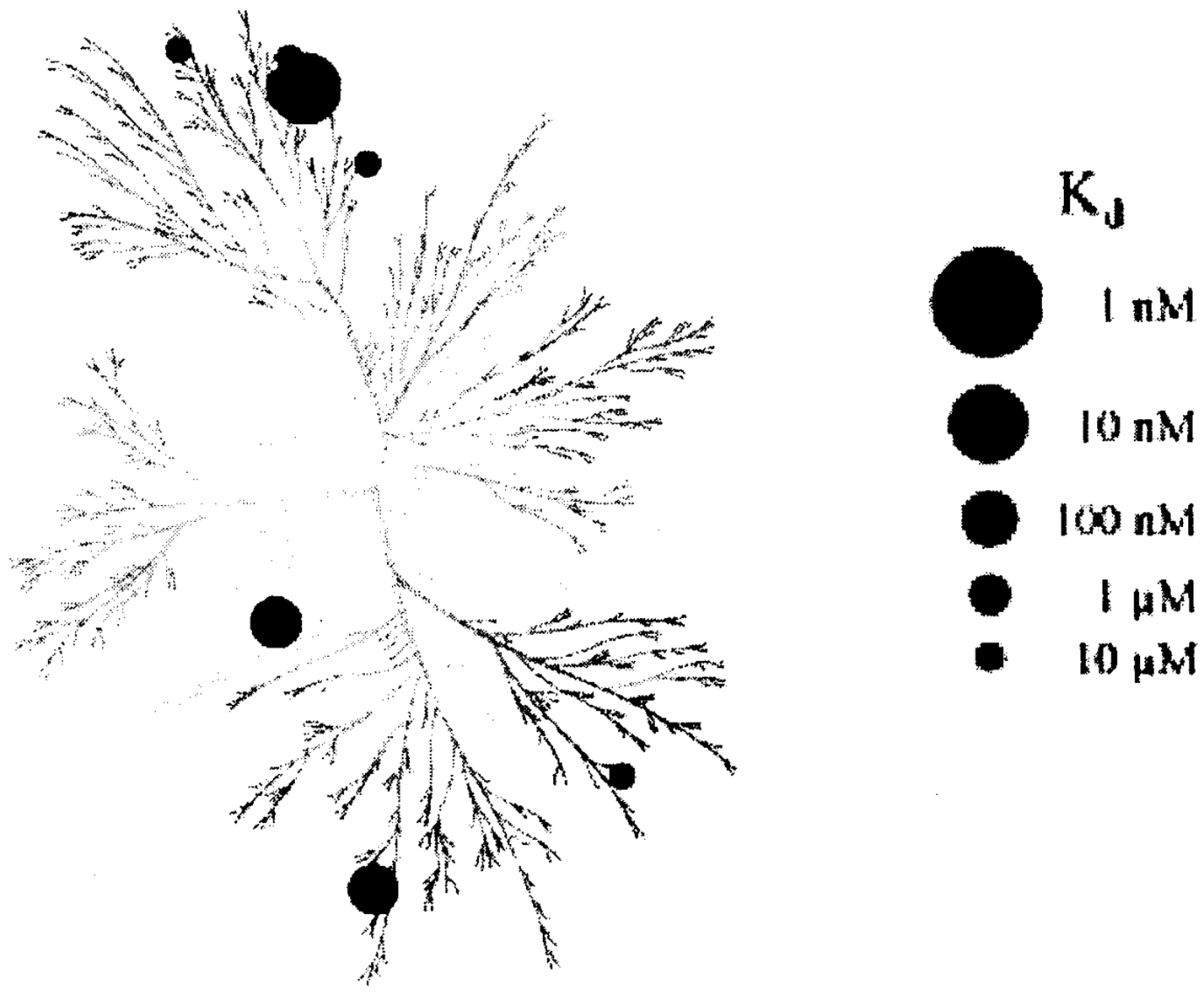


FIGURE 1

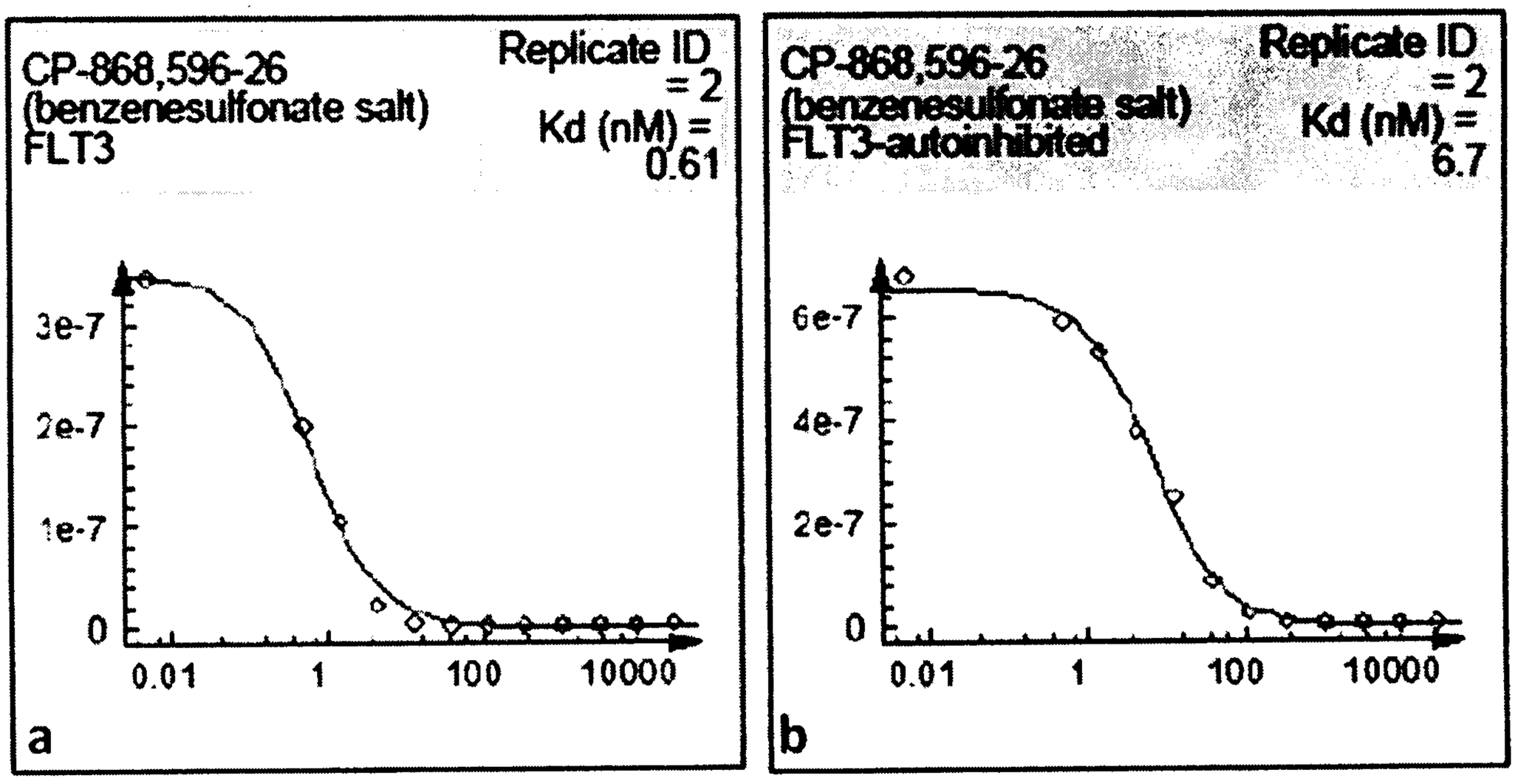


FIGURE 2

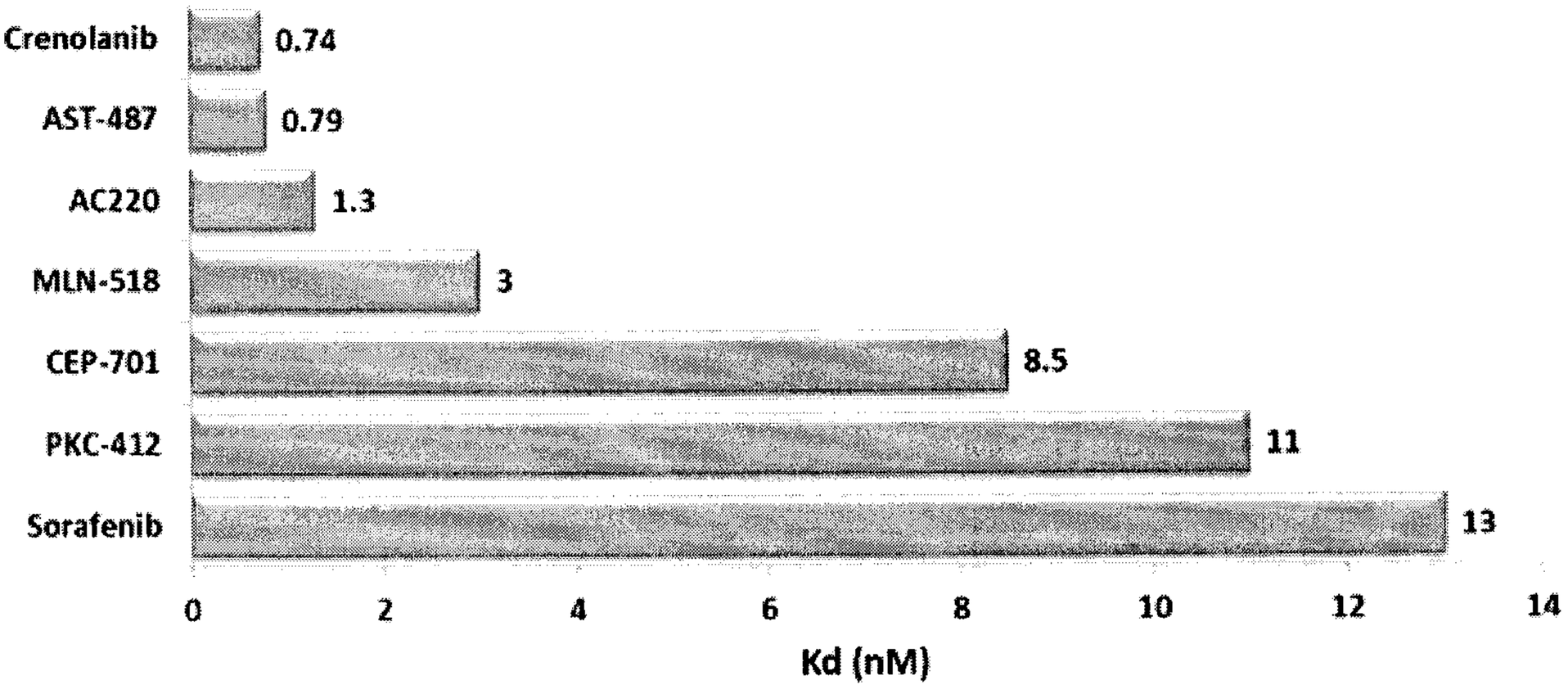


FIGURE 3

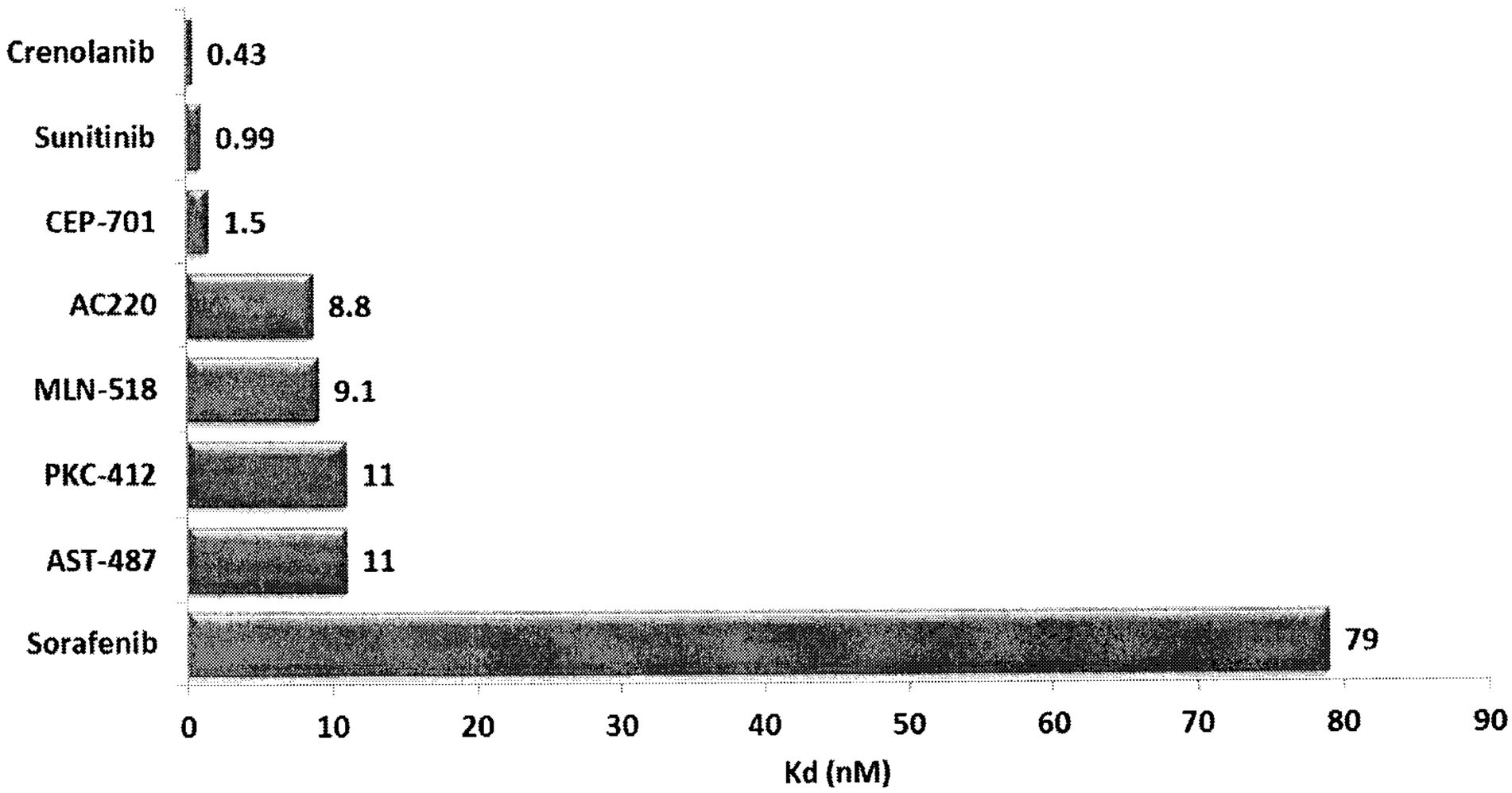


FIGURE 4

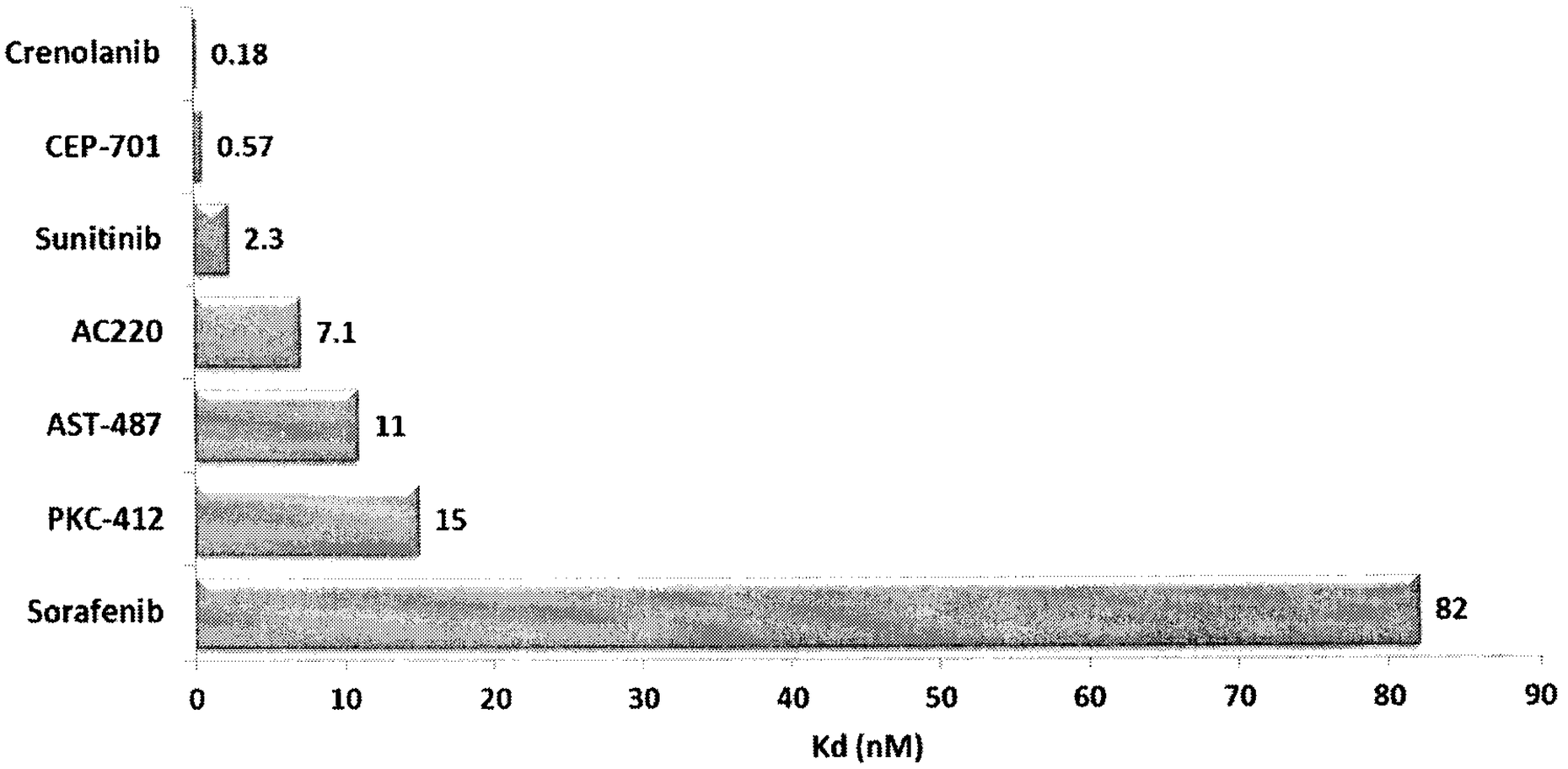


FIGURE 5

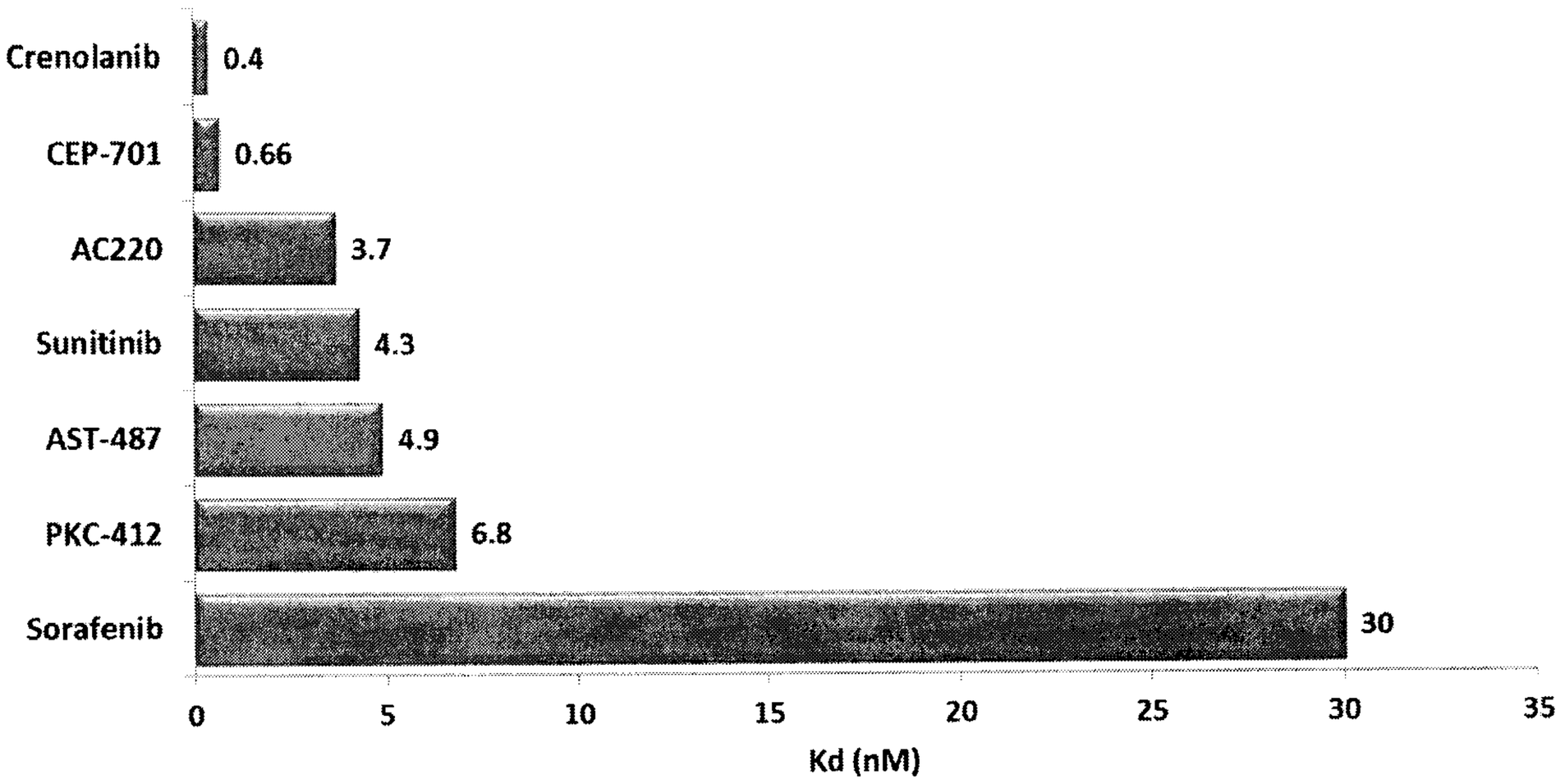
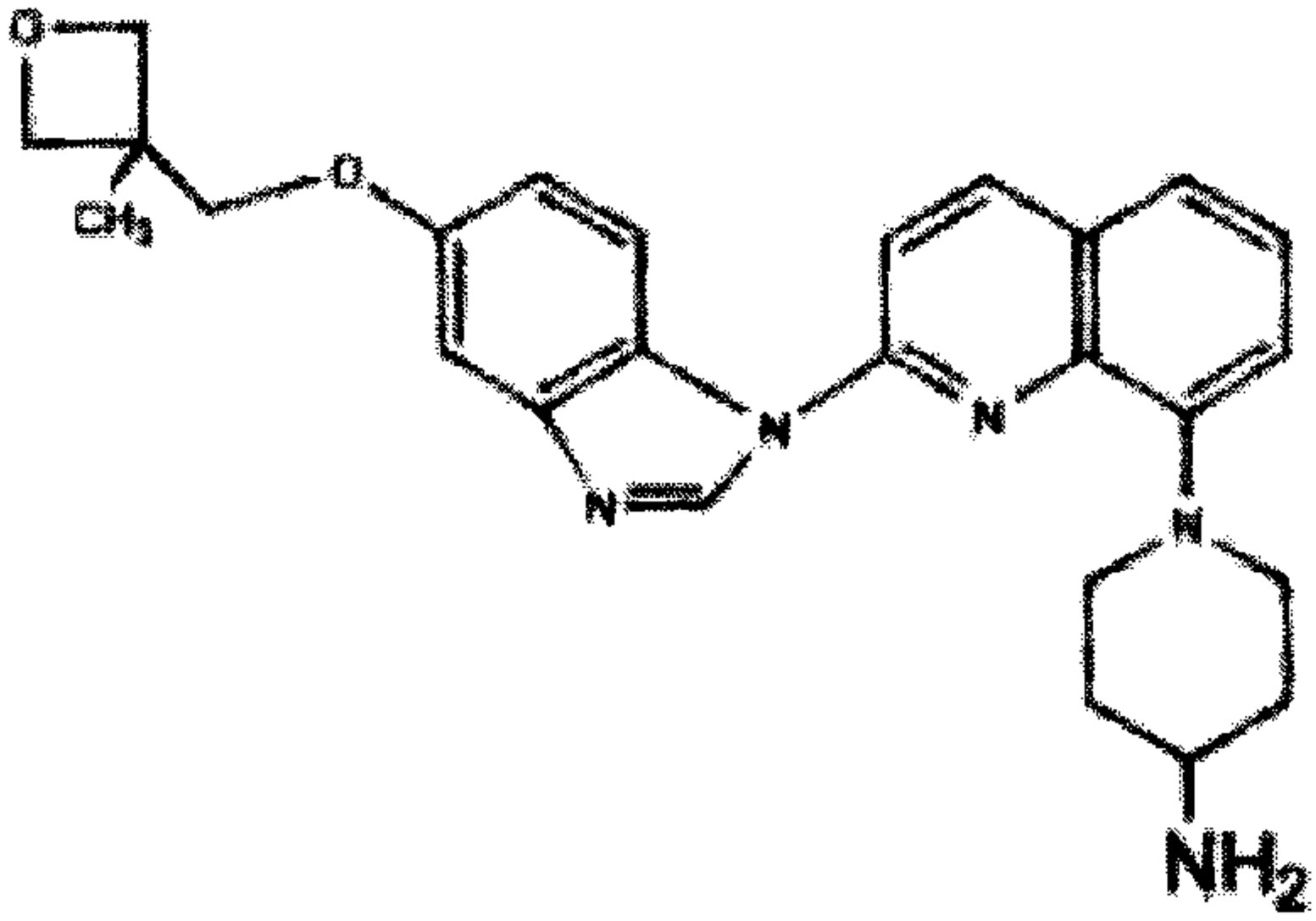


FIGURE 6

Kinase Target	CP-868,596-26 (benzenesulfonate salt)
KINOMEscan Gene Symbol	Kd (nM)
ABL1(T315I)-nonphosphorylated	12000
ABL1(T315I)-phosphorylated	760
ABL1-nonphosphorylated	600
ABL1-phosphorylated	88

FIGURE 7


O
CH3
O
N
N
N
N
NH2