TREATMENT OF ACUTE MYELOID LEUKEMIA WITH INDOLINONE COMPOUNDS

Related Applications

This application claims priority to U.S. Provisional Patent Application Serial No. 60/330,623, which is hereby incorporated in its entirety by reference.

Field of the Invention

The invention relates to a method of treating acute myeloid leukemia by administering an indolinone compound. Acute myeloid leukemia (AML) is a disease in which cancerous cells develop in the blood and bone marrow. Untreated AML is a fatal disease with median survival time of 3 months. Patients with AML that are FLT-3-ITD (internal tandem duplication) positive typically exhibit poor response to traditional chemotherapy. The present invention is directed to treating AML patients and preferably patients positive for FLT-3-ITD but not restricted to FLT-3-ITD by administering indolinone compounds of Formula I or II. The present invention also is directed to a method of inhibiting phosphorylation of FLT-3.

Background of the Invention

Acute myeloid leukemia, also called acute non-lymphocytic leukemia, is a form of cancer in which too many immature white blood cells are found in the blood and bone marrow. These immature cells, also called blasts, have failed to develop into mature infection-fighting cells.

Advances in the treatment of AML have resulted in substantially improved complete remission rates. Treatment is aggressive to achieve complete remission because partial remission offers no substantial survival benefit. Approximately 60% to 70% of adults with AML can be expected to attain complete remission status following appropriate induction therapy. More than 15% of adults with AML (about 25% of those who attain complete remission) can be expected to survive 3 or more years and may be cured. Remission rates in adult AML are inversely related to age, with an expected remission rate of greater than 65% for those younger than 60 years of age. Data suggest that once attained, duration of remission may be shorter in older patients. Increased morbidity and mortality during induction appear to be directly related to age. Other adverse prognostic factors include central nervous system

involvement with leukemia, systemic infection at diagnosis, elevated white blood cell count (>100,000 per cubic millimeter), treatment-induced AML, and history of myelodysplastic syndrome. The 5-year disease-free survival for relapsed patients who do not receive hematopoietic stem cells transplantation is less than 5%.

Mutations of receptor tyrosine kinases (RTK), including cKIT, PDGFRβ and FLT-3, have been found in human leukemia. Mutations of FLT-3 include any changes to any FLT-3 gene sequence including point mutations, deletions, insertions, internal tandem duplications, polymorphisms. An example of a known mutation in FLT-3 is a point mutation at amino acid residue 835 in human FLT-3, identified in approximately 7% of patients as reported in Abu-Duhier et al (Br J Haematol 2001 Jun; 113(4):983-8. Identification of novel FLT-3 Asp835 mutations in adult acute myeloid leukaemia. Abu-Duhier FM, Goodeve AC, Wilson GA, Care RS, Peake IR, Reilly JT). This mutation is in the activating loop of FLT-3 and is likely to result in constitutive activation based on homology to other tyrosine kinase receptors such as c-kit.

An internal tandem duplication (ITD) of the juxtamembrane (JM) domain-coding sequence of the FLT-3 gene is one of the most frequent mutations (25%-30% of AML patients). ITD are internal tandem duplications, mutations found in the juxtamembrane domain, repeats range in size but the duplicated sequence appears always to be in frame. The FLT-3 mutant is found in some patients with acute myeloid leukemia (AML) and 3% of myelodysplastic syndrome cases, whereas it appears more rare in chronic myeloid leukemia and lymphoid malignancies. The presence of the FLT-3 gene mutation is related to high peripheral white blood cell counts. The ITD of the FLT-3 gene sometimes emerged during progression of MDS or at relapse of AML which had no ITD at first diagnosis. This suggests that FLT-3 mutation promotes leukemia progression. See Zhao et al., *Leukemia*, vol. 14, pages 374-378 (2000).

FLT-3 (fms like tyrosine kinase 3) is a member of the class III receptor tyrosine kinases. Those of skill in the art will recognize that FLT-3 has also been called "flk2" in the scientific literature. "FLT-3" as used herein, refers to a polypeptide having, for example, the sequence set forth in accession number gi|4758396|ref|NP_004110.1| fms-related tyrosine kinase 3 [Homo sapiens], or gi|544320|sp|P36888|FLT-3_HUMAN FL CYTOKINE RECEPTOR PRECURSOR (TYROSINE-PROTEIN KINASE RECEPTOR FLT-3) (STEM CELL TYROSINE KINASE 1) (STK-1) (CD135 ANTIGEN), or gi|409573|gb|AAA18947.1| (U02687) serine/threonine protein kinase [Homo sapiens]. Corresponding mRNA accessions

for the first two sequences are gi|4758395|ref|NM_004119.1| Homo sapiens fms-related tyrosine kinase 3 (FLT-3), mRNA gi|406322|emb|Z26652.1|HSFLT-3RTK H.sapiens FLT-3 mRNA for FLT-3 receptor tyrosine kinase. For a review of FLT-3, see Gilliland, Current Opin. Hematol. 9 (4) 276-281 July 2002.

Zhao et al., *Leukemia* (2000), further discloses *in vivo* treatment of mutant FLT-3 transformed murine leukemia with a tyrosine kinase inhibitor. In developing the therapeutic protocol, Zhao investigated the use of tyrosine kinase inhibitors for in vitro growth suppression of transformed 32D cells (an IL-3 dependent murine cell line).

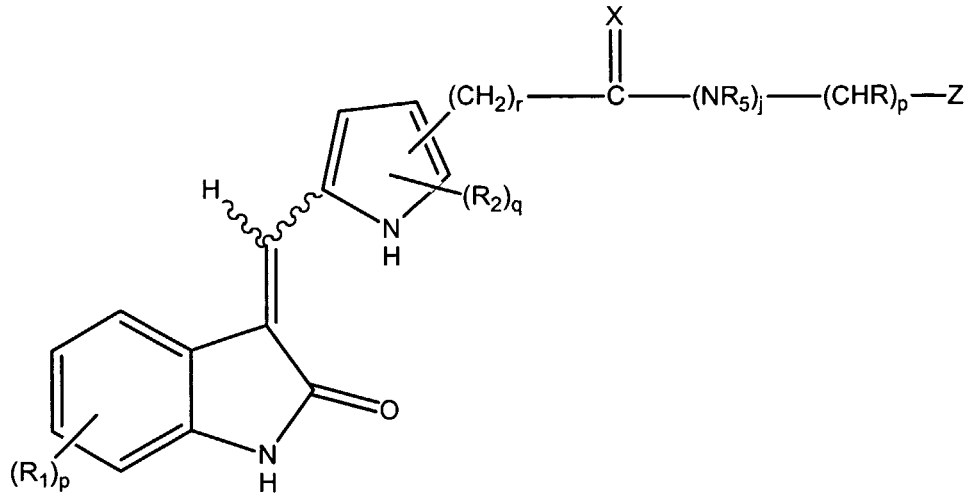
Internal tandem duplication (ITD) mutations of the receptor tyrosine kinase FLT-3 have been found in 20-30% of patients in with acute myeloid leukemia (AML), see e.g., Levis et al., *Blood*, vol. 98, pages 885-887 (2001). One of skill in the art will recognize that diagnosing FLT-3-ITD positive patients is readily made using PCR and gel electrophoresis testing of genomic DNA from an AML patient. See Abu-Duhier et al., British J. of Heamotology, Vol. 11, pages 190-195 (2000). The FLT-3 gene encodes a tyrosine kinase receptor that regulates proliferation and differentiation of hematopoietic stem cells. Levis discloses that these mutations constitutively activate the receptor and appear to be associated with a poor response to chemotherapy. Evidence suggests that this constitutive activation is leukemogenic, rendering this receptor a potential target for specific therapy.

Patients bearing ITD mutant FLT-3 are known to have poor prognosis, high relapse rate and decreased overall survival after conventional treatment, relative to non ITD mutant patients. Current therapies for AML have poor patient response rates and poor toxicity profiles. Therapies are generally nonspecific and not targeted exclusively to the diseased cells or to the mechanism which drives the malignancy. Inhibition of FLT-3 which mediates cell survival and proliferation signals would directly target the leukemic cells, inhibit signaling resulting in elimination of leukemic cell population.

Based on the need for improved prognosis for patients afflicted with ITD-AML, the present inventors developed a method of treating acute myeloid leukemia by administering an effective amount of a tyrosine kinase inhibitor of formula I or II.

Summary of the Invention

One embodiment of the invention relates to a method of treating acute myeloid leukemia (AML) comprising administering an effective amount of a compound of Formula I:



(I)

wherein

R is independently H, OH, alkyl, aryl, cycloalkyl, heteroaryl, alkoxy, heterocyclic and amino;

each R_1 is independently selected from the group consisting of alkyl, halo, aryl, alkoxy, haloalkyl, haloalkoxy, cycloalkyl, heteroaryl, heterocyclic, hydroxy, -C(O)-R_8, -NR_9R_{10}, -$NR_9C(O)$-R_{12} and –$C(O)NR_9R_{10}$;

each R_2 is independently selected from the group consisting of alkyl, aryl, heteroaryl, –C(O)-R_8, and SO_2R'', where R'' is alkyl, aryl, heteroaryl, NR_9N_{10} or alkoxy;

each R_5 is independently selected from the group consisting of hydrogen, alkyl, aryl, haloalkyl, cycloalkyl, heteroaryl, heterocyclic, hydroxy, -C(O)-R_8 and $(CHR)_rR_{11}$;

X is O or S;

j is 0-1

p is 0-3;

q is 0-2;

r is 0-3;

R_8 is selected from the group consisting of –OH, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

R_9 and R_{10} are independently selected from the group consisting of H, alkyl, aryl, aminoalkyl, heteroaryl, cycloalkyl and heterocyclic, or R_9 and R_{10} together with N may form a ring, where the ring atoms are selected from the group consisting of C, N, O and S;

R_{11} is selected from the group consisting of –OH, amino, monosubstituted amino, disubstituted amino, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic

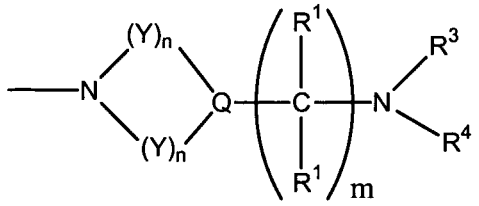
R_{12} is selected from the group consisting of alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

Z is –OH;

-Oalkyl;

$-NR_3R_4$, where R_3 and R_4 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, and heterocyclic, or R_3 and R_4 may combine with N to form a ring where the ring atoms are selected from the group consisting of CH_2, N, O and S

or



wherein Y is independently CH_2, O, N or S,

Q is C or N;

n is independently 0-4; and

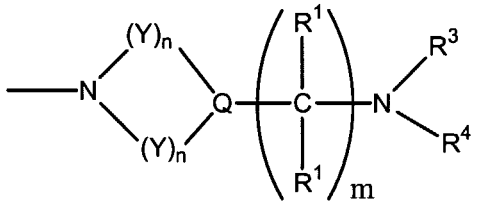
m is 0-3;

or a salt thereof, to a patient in need of such treatment.

In one embodiment of the invention, R_1 is halo (e.g., F and Cl) and p is 1 in Formula I or II as administered to a patient in need thereof.

In another embodiment, Z of Formula I or II is $-NR_3R_4$ wherein R_3 and R_4 form a morpholine ring.

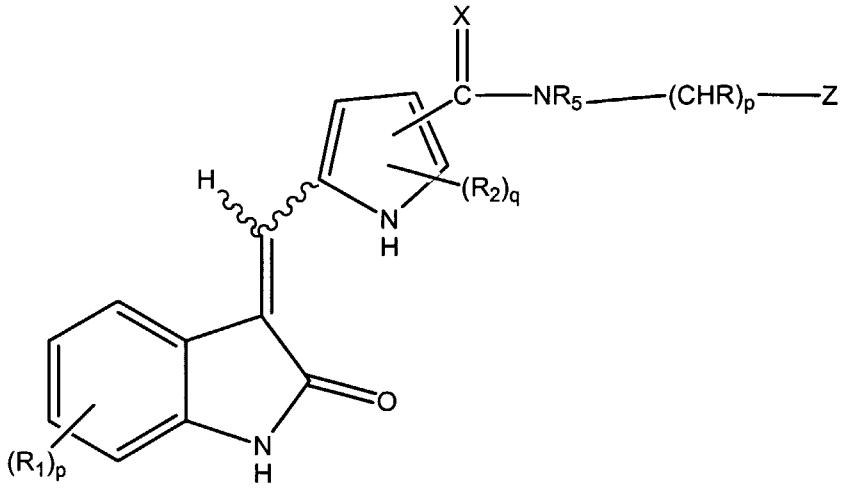
In another embodiment, Z of Formula I or II is:



wherein each Y is CH_2, each n is 2, m is 0 and R_3 and R_4 form a morpholine ring.

In any of the previously recited embodiments, R_2 is methyl and q is 2, wherein the methyls are bonded at the 3 and 5 positions of Formula I or II.

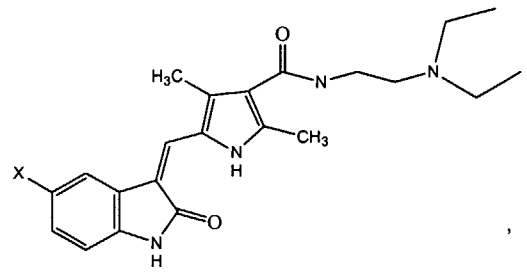
In a preferred embodiment, the compound administered to the patient is a compound of Formula II:



(II),

where the variables are as previously defined.

In a particular embodiment of the invention, the compound administered is selected from the group consisting of



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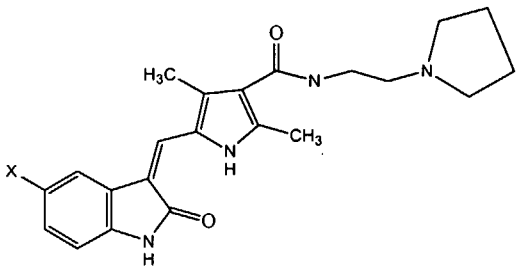

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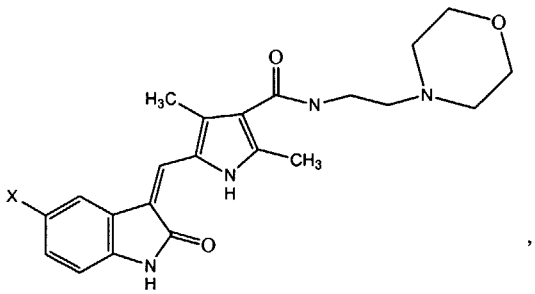

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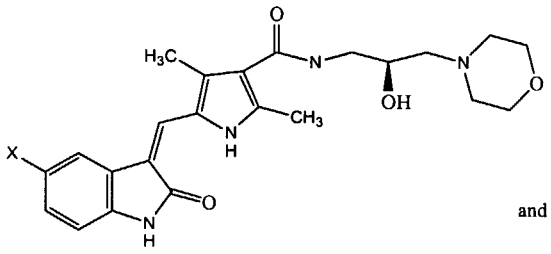
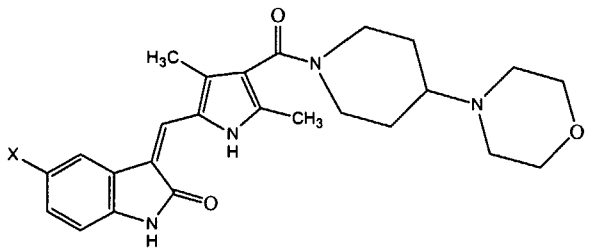


and

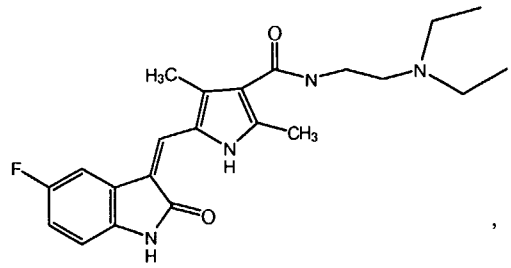


.

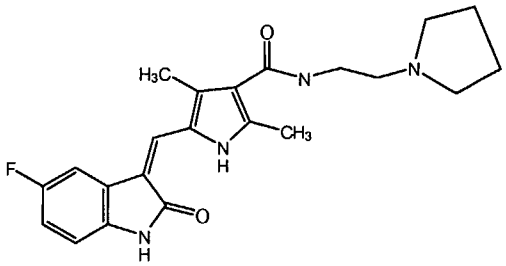
wherein X is F, Cl, I or Br. In a preferred embodiment, X is F.

In one embodiment of the invention, the patient population comprises human patients that are FLT-3-ITD positive or FLT-3 wild-type positive or other FLT-3 mutations.

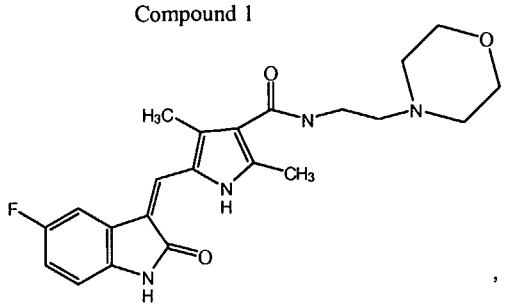
In a particular embodiment of the invention, the compound of formula I is selected from the group consisting of:



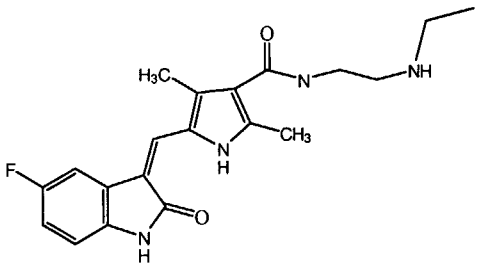
Compound 1



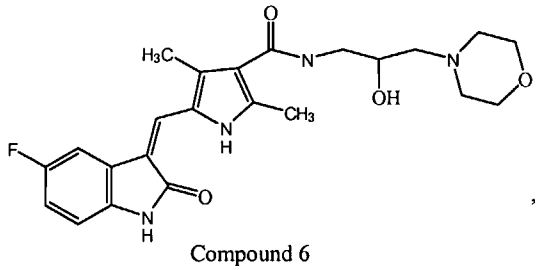
Compound 2



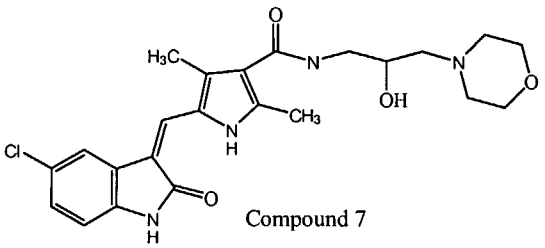
Compound 3



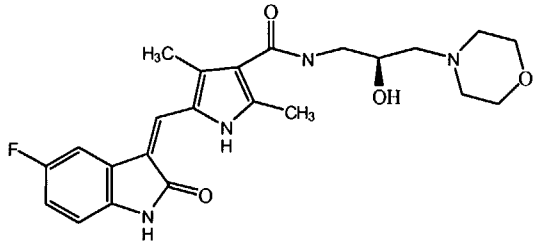
Compound 8



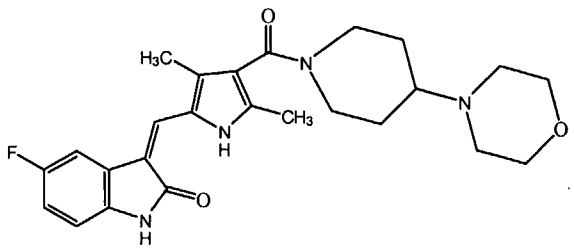
Compound 6



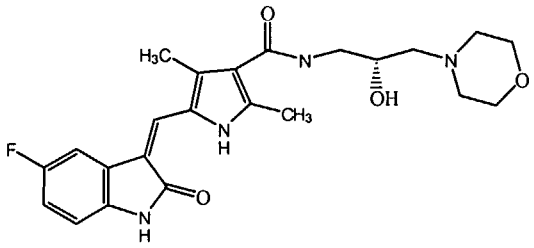
Compound 7



Compound 4

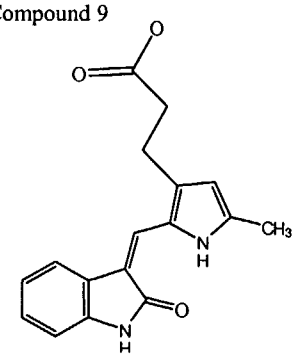


Compound 9



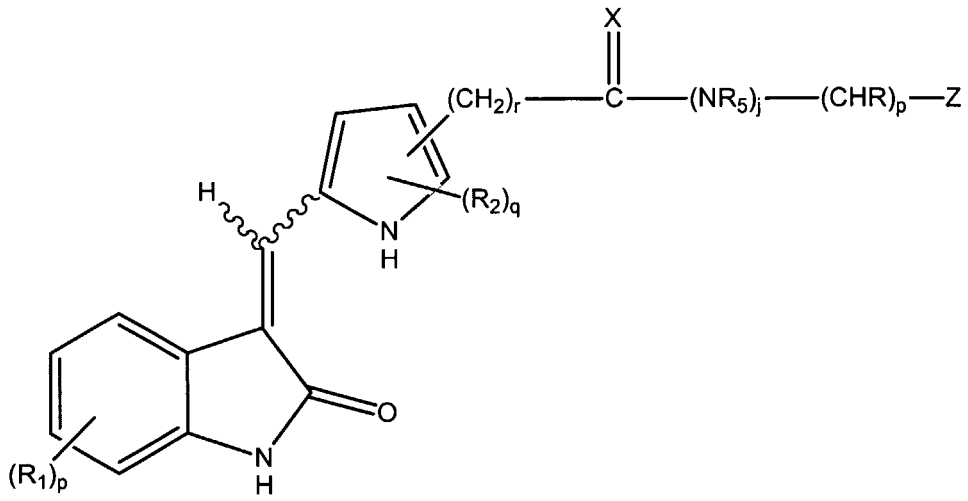
Compound 5

and



Compound 10

Another embodiment of the invention relates to a method of inhibiting phosphorylation of FLT-3 comprising administering an inhibitory amount of a compound of Formula I:



(I)

wherein

R is independently H, OH, alkyl, aryl, cycloalkyl, heteroaryl, alkoxy, heterocyclic and amino;

each R_1 is independently selected from the group consisting of alkyl, halo, aryl, alkoxy, haloalkyl, haloalkoxy, cycloalkyl, heteroaryl, heterocyclic, hydroxy, -C(O)-R_8, -NR_9R_{10}, -$NR_9C(O)$-R_{12} and –$C(O)NR_9R_{10}$;

each R_2 is independently selected from the group consisting of alkyl, aryl, heteroaryl, –C(O)-R_8, and SO_2R'', where R'' is alkyl, aryl, heteroaryl, NR_9N_{10} or alkoxy;

each R_5 is independently selected from the group consisting of hydrogen, alkyl, aryl, haloalkyl, cycloalkyl, heteroaryl, heterocyclic, hydroxy, -C(O)-R_8 and $(CHR)_rR_{11}$;

X is O or S;

j is 0-1

p is 0-3;

q is 0-2;

r is 0-3;

R_8 is selected from the group consisting of –OH, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

R_9 and R_{10} are independently selected from the group consisting of H, alkyl, aryl, aminoalkyl, heteroaryl, cycloalkyl and heterocyclic, or R_9 and R_{10} together with N may form a ring, where the ring atoms are selected from the group consisting of C, N, O and S;

R_{11} is selected from the group consisting of –OH, amino, monosubstituted amino, disubstituted amino, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic

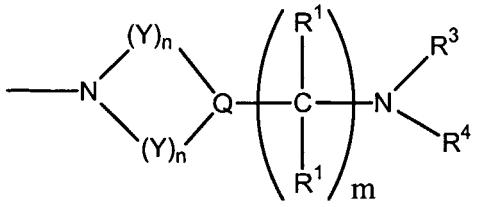
R_{12} is selected from the group consisting of alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

Z is –OH;

-Oalkyl;

$-NR_3R_4$, where R_3 and R_4 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, and heterocyclic, or R_3 and R_4 may combine with N to form a ring where the ring atoms are selected from the group consisting of CH_2, N, O and S

or



wherein Y is independently CH_2, O, N or S,

Q is C or N;

n is independently 0-4; and

m is 0-3;

or a salt thereof, to a patient in need of such treatment.

In an embodiment of the invention the FLT-3 is mutant FLT-3 or wild-type FLT-3. A particular FLT-3 mutant is FLT-3-ITD.

Brief Description of the Drawings

Figure 1 is a FACS profile of caspase 3 stained cell line.

Figure 2 shows a Western blot for PARP cleavage indicating that FLT-3-ITD mutant cells are more susceptible to compound 1 induced apoptosis than wildtype.

Figure 3 shows a Western blot of phosphotyrosine following FLT-3 immunoprecipitation indicating compound 1 inhibits both wildtype and mutant-ITD FLT-3.

Figure 4a shows a Western blot of phosphotyrosine following FLT-3 immunoprecipitation shows that compound 1 inhibits FLT-3-ITD phosphorylation in xenograft models and

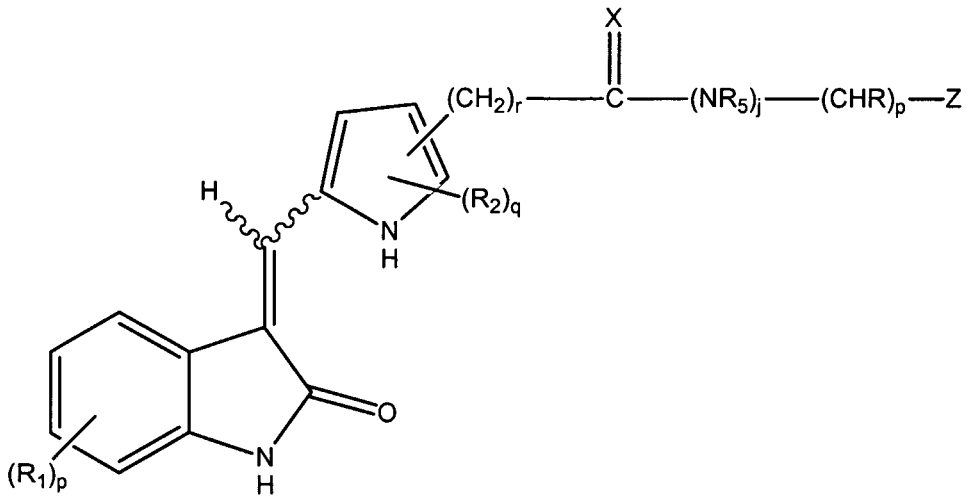
Figure 4b shows a graph indicating tumor size versus time after drug treatment.

Figure 5 shows the percent survival after varying dosages of compound 1.

Detailed Description of the Invention

The compounds of formula I and II are useful in the treatment of patients with AML. In particular, they are useful in the treatment of patients with AML who are FLT-3-ITD positive. In addition, patients diagnosed with sarcomas, melanomas, and solid tumors where the pathophysiology indicates that FLT-3-ITD or FLT-3 is associated with the malignancy may be treated by administering the compounds of Formula I or II.

An embodiment of the invention relates to a method of treating acute myeloid leukemia (AML) comprising administering an effective amount of a compound of Formula I:



(I)

wherein

R is independently H, OH, alkyl, aryl, cycloalkyl, heteroaryl, alkoxy, heterocyclic and amino;

each R_1 is independently selected from the group consisting of alkyl, halo, aryl, alkoxy, haloalkyl, haloalkoxy, cycloalkyl, heteroaryl, heterocyclic, hydroxy, $-C(O)-R_8$, $-NR_9R_{10}$, $-NR_9C(O)-R_{12}$ and $-C(O)NR_9R_{10}$;

each R_2 is independently selected from the group consisting of alkyl, aryl, heteroaryl, $-C(O)-R_8$ and SO_2R'', where R'' is alkyl, aryl, heteroaryl, NR_9N_{10} or alkoxy;

each R_5 is independently selected from the group consisting of hydrogen, alkyl, aryl, haloalkyl, cycloalkyl, heteroaryl, heterocyclic, hydroxy, $-C(O)-R_8$ and $(CHR)_rR_{11}$;

X is O or S;

j is 0-1

p is 0-3;

q is 0-2;

r is 0-3;

R_8 is selected from the group consisting of –OH, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

R_9 and R_{10} are independently selected from the group consisting of H, alkyl, aryl, aminoalkyl, heteroaryl, cycloalkyl and heterocyclic, or R_9 and R_{10} together with N may form a ring, where the ring atoms are selected from the group consisting of C, N, O and S;

R_{11} is selected from the group consisting of –OH, amino, monosubstituted amino, disubstituted amino, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic

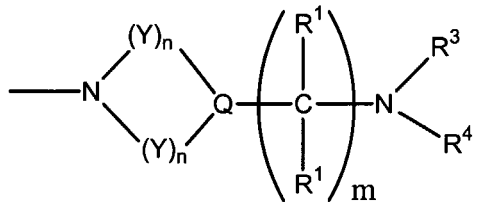
R_{12} is selected from the group consisting of alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

Z is –OH;

-Oalkyl;

$-NR_3R_4$, where R_3 and R_4 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, and heterocyclic, or R_3 and R_4 may combine with N to form a ring where the ring atoms are selected from the group consisting of CH_2, N, O and S

or



wherein Y is independently CH_2, O, N or S,

Q is C or N;

n is independently 0-4; and

m is 0-3;

or a salt thereof, to a patient in need of such treatment.

In an alternative embodiment of the invention, a compound of Formula I is administered to a patient in need of treatment of AML, provided that the compound is not 3-[2,4-Dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid.

In another embodiment of the invention, the therapeutic method involves administering to an AML patient an effective amount of a compound selected from the group consisting of:

5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide (compound 1);

5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide (compound 2);

5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (compound 3);

(S)-5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (compound 4);

(R)-5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (compound 5);

5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (compound 6);

5-(5-Chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (compound 7);

5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-ethylamino-ethyl)-amide (compound 8);

3-[3,5-dimethyl-4-(4-morpholin-4-yl-piperidine-1-carbonyl)-1H-pyrrol-2-methylene]-5-fluoro-1,3-dihydro-indol-2-one (compound 9); and

3-[5-methyl-2-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid (compound 10).

In order to clearly set forth the compounds of Formula I and II, useful in the inventive method, the following definitions are provided.

"Alkyl" refers to a saturated aliphatic hydrocarbon radical including straight chain and branched chain groups of 1 to 20 carbon atoms (whenever a numerical range; e.g. "1-20", is stated herein, it means that the group, in this case the alkyl group, may contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, etc. up to and including 20 carbon atoms). Alkyl groups containing from 1 to 4 carbon atoms are referred to as lower alkyl groups. When said lower alkyl groups lack substituents, they are referred to as unsubstituted lower alkyl groups. More preferably, an alkyl group is a medium size alkyl having 1 to 10 carbon atoms e.g., methyl, ethyl, propyl, 2-propyl, n-butyl, iso-butyl, tert-butyl, pentyl, and the like. Most preferably, it is a lower alkyl having 1 to 4 carbon atoms e.g., methyl, ethyl, propyl, 2-propyl, n-butyl, iso-butyl, or tert-butyl, and the like. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more, more preferably one to three, even more preferably one or two substituent(s) independently selected from the group consisting of halo, hydroxy, unsubstituted lower alkoxy, aryl

optionally substituted with one or more groups, preferably one, two or three groups which are independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more groups, preferably one, two or three groups which are independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more groups, preferably one, two or three groups which are independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and the nitrogen atoms in the group being optionally substituted with one or more groups, preferably one, two or three groups which are independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5- or 6-member heterocyclic group having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitrogen (if present) atoms in the group being optionally substituted with one or more groups, preferably one, two or three groups which are independently of each other halo , hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, mercapto, (unsubstituted lower alkyl)thio, arylthio optionally substituted with one or more groups, preferably one, two or three groups which are independently of each other halo, hydroxy, unsubstituted lower alkyl or alkoxy groups, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, RS(O)-, $RS(O)_2$-, -C(O)OR, RC(O)O-, and $-NR_{13}R_{14}$, wherein R_{13} and R_{14} are independently selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, cycloalkyl, heterocyclic and aryl optionally substituted with one or more, groups, preferably one, two or three groups which are independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups.

Preferably, the alkyl group is substituted with one or two substituents independently selected from the group consisting of hydroxy, 5- or 6-member heterocyclic group having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitrogen (if present) atoms in the group being optionally substituted with one or more groups, preferably one, two or three groups which are independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen,

oxygen and sulfur, the carbon and the nitrogen atoms in the group being optionally substituted with one or more groups, preferably one, two or three groups which are independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more groups, preferably one, two or three groups which are independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, or $-NR_{13}R_{14}$, wherein R_{13} and R_{14} are independently selected from the group consisting of hydrogen and alkyl. Even more preferably the alkyl group is substituted with one or two substituents which are independently of each other hydroxy, dimethylamino, ethylamino, diethylamino, dipropylamino, pyrrolidino, piperidino, morpholino, piperazino, 4-lower alkylpiperazino, phenyl, imidazolyl, pyridinyl, pyridazinyl, pyrimidinyl, oxazolyl, triazinyl, and the like.

"Cycloalkyl" refers to a 3 to 8 member all-carbon monocyclic ring, an all-carbon 5-member/6-member or 6-member/6-member fused bicyclic ring or a multicyclic fused ring (a "fused" ring system means that each ring in the system shares an adjacent pair of carbon atoms with each other ring in the system) group wherein one or more of the rings may contain one or more double bonds but none of the rings has a completely conjugated pi-electron system.

Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, adamantane, cycloheptane, cycloheptatriene, and the like. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more, more preferably one or two substituents, independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, aryl optionally substituted with one or more, preferably one or two groups independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, aryloxy optionally substituted with one or more, preferably one or two groups independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 6-member heteroaryl having from 1 to 3 nitrogen atoms in the ring, the carbons in the ring being optionally substituted with one or more, preferably one or two groups independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5-member heteroaryl having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitrogen atoms of the group being optionally

substituted with one or more, preferably one or two groups independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, 5- or 6-member heterocyclic group having from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, the carbon and nitogen (if present)atoms in the group being optionally substituted with one or more, preferably one or two groups independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, mercapto,(unsubstituted lower alkyl)thio, arylthio optionally substituted with one or more, preferably one or two groups independently of each other halo, hydroxy, unsubstituted lower alkyl or unsubstituted lower alkoxy groups, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, RS(O)-, $RS(O)_2$-, -C(O)OR, RC(O)O-, and $-NR_{13}R_{14}$ are as defined above.

"Alkenyl" refers to a lower alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon double bond. Representative examples include, but are not limited to, ethenyl, 1-propenyl, 2-propenyl, 1-, 2-, or 3-butenyl, and the like.

"Alkynyl" refers to a lower alkyl group, as defined herein, consisting of at least two carbon atoms and at least one carbon-carbon triple bond. Representative examples include, but are not limited to, ethynyl, 1-propynyl, 2-propynyl, 1-, 2-, or 3-butynyl, and the like.

"Aryl" refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups of 1 to 12 carbon atoms having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more, more preferably one, two or three, even more preferably one or two, independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto,(unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, RS(O)-, $RS(O)_2$-, -C(O)OR, RC(O)O-, and $-NR_{13}R_{14}$, with R_{13} and R_{14} as defined above. Preferably, the aryl group is optionally substituted with one or two substituents independently selected from halo, unsubstituted lower alkyl, trihaloalkyl, hydroxy, mercapto, cyano, N-amido, mono or dialkylamino, carboxy, or N-sulfonamido.

"Heteroaryl" refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group of 5 to 12 ring atoms containing one, two, or three ring heteroatoms

selected from N, O, or S, the remaining ring atoms being C, and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of unsubstituted heteroaryl groups are pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline, purine and carbazole. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more, more preferably one, two, or three, even more preferably one or two, independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto,(unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, RS(O)-, $RS(O)_2$-, -C(O)OR, RC(O)O-, and $-NR_{13}R_{14}$, with R_{13} and R_{14} as defined above. Preferably, the heteroaryl group is optionally substituted with one or two substituents independently selected from halo, unsubstituted lower alkyl, trihaloalkyl, hydroxy, mercapto, cyano, N-amido, mono or dialkylamino, carboxy, or N-sulfonamido.

"Heterocyclic" refers to a monocyclic or fused ring group having in the ring(s) of 5 to 9 ring atoms in which one or two ring atoms are heteroatoms selected from N, O, or S(O)n (where n is an integer from 0 to 2), the remaining ring atoms being C. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. Examples, without limitation, of unsubstituted heterocyclic groups are pyrrolidino, piperidino, piperazino, morpholino, thiomorpholino, homopiperazino, and the like. The heterocyclic ring may be substituted or unsubstituted. When substituted, the substituted group(s) is preferably one or more, more preferably one, two or three, even more preferably one or two, independently selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, halo, hydroxy, unsubstituted lower alkoxy, mercapto,(unsubstituted lower alkyl)thio, cyano, acyl, thioacyl, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, nitro, N-sulfonamido, S-sulfonamido, RS(O)-, $RS(O)_2$-, -C(O)OR, RC(O)O-, and $-NR_{13}R_{14}$, with R_{13} and R_{14} as defined above. Preferably, the heterocyclic group is optionally substituted with one or two substituents independently selected from halo, unsubstituted lower alkyl, trihaloalkyl, hydroxy, mercapto, cyano, N-amido, mono or dialkylamino, carboxy, or N-sulfonamido.

Preferably, the heterocyclic group is optionally substituted with one or two substituents independently selected from halo, unsubstituted lower alkyl, trihaloalkyl, hydroxy, mercapto, cyano, N-amido, mono or dialkylamino, carboxy, or N-sulfonamido.

"Hydroxy" refers to an -OH group.

"Alkoxy" refers to both an -O-(unsubstituted alkyl) and an -O-(unsubstituted cycloalkyl) group. Representative examples include, but are not limited to, e.g., methoxy, ethoxy, propoxy, butoxy, cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, cyclohexyloxy, and the like.

"Aryloxy" refers to both an -O-aryl and an -O-heteroaryl group, as defined herein. Representative examples include, but are not limited to, phenoxy, pyridinyloxy, furanyloxy, thienyloxy, pyrimidinyloxy, pyrazinyloxy, and the like, and derivatives thereof.

"Mercapto" refers to an -SH group.

"Alkylthio" refers to both an -S-(unsubstituted alkyl) and an -S-(unsubstituted cycloalkyl) group. Representative examples include, but are not limited to, e.g., methylthio, ethylthio, propylthio, butylthio, cyclopropylthio, cyclobutylthio, cyclopentylthio, cyclohexylthio, and the like.

"Arylthio" refers to both an -S-aryl and an -S-heteroaryl group, as defined herein. Representative examples include, but are not limited to, phenylthio, pyridinylthio, furanylthio, thientylthio, pyrimidinylthio, and the like and derivatives thereof.

"Acyl" refers to a -C(O)-R" group, where R" is selected from the group consisting of hydrogen, unsubstituted lower alkyl, trihalomethyl, unsubstituted cycloalkyl, aryl optionally substituted with one or more, preferably one, two, or three substituents selected from the group consisting of unsubstituted lower alkyl, trihalomethyl, unsubstituted lower alkoxy, halo and $-NR_{13}R_{14}$ groups, heteroaryl (bonded through a ring carbon) optionally substituted with one or more, preferably one, two, or three substitutents selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR_{13}R_{14}$ groups and heterocyclic (bonded through a ring carbon) optionally substituted with one or more, preferably one, two, or three substituents selected from the group consisting of unsubstituted lower alkyl, trihaloalkyl, unsubstituted lower alkoxy, halo and $-NR_{13}R_{14}$ groups. Representative acyl groups include, but are not limited to, acetyl, trifluoroacetyl, benzoyl, and the like.

"Aldehyde" refers to an acyl group in which R" is hydrogen.

"Thioacyl" refers to a -C(S)-R" group, with R" as defined herein.

"Ester" refers to a -C(O)O-R" group with R" as defined herein except that R" cannot be hydrogen.

"Acetyl" group refers to a $-C(O)CH_3$ group.

"Halo" group refers to fluorine, chlorine, bromine or iodine, preferably fluorine or chlorine.

"Trihalomethyl" group refers to a $-CX_3$ group wherein X is a halo group as defined herein.

"Methylenedioxy" refers to a $-OCH_2O-$ group where the two oxygen atoms are bonded to adjacent carbon atoms.

"Ethylenedioxy" group refers to a $-OCH_2CH_2O-$ where the two oxygen atoms are bonded to adjacent carbon atoms.

"S-sulfonamido" refers to a $-S(O)_2NR_{13}R_{14}$ group, with R_{13} and R_{14} as defined herein.

"N-sulfonamido" refers to a $-NR_{13}S(O)_2R$ group, with R_{13} and R as defined herein.

"O-carbamyl" group refers to a $-OC(O)NR_{13}R_{14}$ group with R_{13} and R_{14} as defined herein.

"N-carbamyl" refers to an $ROC(O)NR_{14}-$ group, with R and R_{14} as defined herein.

"O-thiocarbamyl" refers to a $-OC(S)NR_{13}R_{14}$ group with R_{13} and R_{14} as defined herein.

"N-thiocarbamyl" refers to a $ROC(S)NR_{14}-$ group, with R and R_{14} as defined herein.

"Amino" refers to an $-NR_{13}R_{14}$ group, wherein R_{13} and R_{14} are both hydrogen.

"C-amido" refers to a $-C(O)NR_{13}R_{14}$ group with R_{13} and R_{14} as defined herein.

"N-amido" refers to a $RC(O)NR_{14}-$ group, with R and R_{14} as defined herein.

"Nitro" refers to a $-NO_2$ group.

"Haloalkyl" means an unsubstituted alkyl, preferably unsubstituted lower alkyl as defined above that is substituted with one or more same or different halo atoms, e.g., $-CH_2Cl$, $-CF_3$, $-CH_2CF_3$, $-CH_2CCl_3$, and the like.

"Aralkyl" means unsubstituted alkyl, preferably unsubstituted lower alkyl as defined above which is substituted with an aryl group as defined above, e.g., $-CH_2$phenyl, $-(CH_2)_2$phenyl, $-(CH_2)_3$phenyl, $CH_3CH(CH_3)CH_2$phenyl, and the like and derivatives thereof.

"Heteroaralkyl" group means unsubstituted alkyl, preferably unsubstituted lower alkyl as defined above which is substituted with a heteroaryl group, e.g., -CH_2pyridinyl, -$(CH_2)_2$pyrimidinyl, -$(CH_2)_3$imidazolyl, and the like, and derivatives thereof.

"Monoalkylamino" means a radical –NHR' where R' is an unsubstitued alkyl or unsubstituted cycloalkyl group as defined above, e.g., methylamino, (1-methylethyl)amino, cyclohexylamino, and the like.

"Dialkylamino" means a radical –NR'R' where each R' is independently an unsubstitued alkyl or unsubstituted cycloalkyl group as defined above, e.g., dimethylamino, diethylamino, (1-methylethyl)-ethylamino, cyclohexylmethylamino, cyclopentylmethylamino, and the like.

"Cyanoalkyl" means unsubstituted alkyl, preferably unsubstituted lower alkyl as defined above, which is substituted with 1 or 2 cyano groups.

"Optional" or "optionally" means that the subsequently described event or circumstance may but need not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For example, "heterocycle group optionally substituted with an alkyl group" means that the alkyl may but need not be present, and the description includes situations where the heterocycle group is substituted with an alkyl group and situations where the heterocyclo group is not substituted with the alkyl group.

A "pharmaceutical composition" refers to a mixture of one or more of the compounds described herein, or physiologically/pharmaceutically acceptable salts or prodrugs thereof, with other chemical components, such as physiologically/pharmaceutically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

The compound of Formula (I) or (II) may also act as a prodrug. A "prodrug" refers to an agent which is converted into the parent drug in vivo. Prodrugs are often useful because, in some situations, they may be easier to administer than the parent drug. They may, for instance, be bioavailable by oral administration whereas the parent drug is not. The prodrug may also have improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a compound of the present invention which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but then is metabolically

hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water solubility is beneficial.

A further example of a prodrug might be a short polypeptide, for example, without limitation, a 2 - 10 amino acid polypeptide, bonded through a terminal amino group to a carboxy group of a compound of this invention wherein the polypeptide is hydrolyzed or metabolized in vivo to release the active molecule. The prodrugs of a compound of Formula (I) or (II) are within the scope of this invention.

Additionally, it is contemplated that a compound of Formula (I) or (II) would be metabolized by enzymes in the body of the organism such as human being to generate a metabolite that can modulate the activity of the protein kinases. Such metabolites are within the scope of the present invention.

As used herein, a "physiologically/pharmaceutically acceptable carrier" refers to a carrier or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound.

An "pharmaceutically acceptable excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which retain the biological effectiveness and properties of the parent compound. Such salts include:

(i) acid addition salt which is obtained by reaction of the free base of the parent compound with inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, phosphoric acid, sulfuric acid, and perhcloric acid and the like, or with organic acids such as acetic acid, oxalic acid, (D) or (L) malic acid, maleic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, tartaric acid, citric acid, succinic acid or malonic acid and the like, preferably hydrochloric acid or (L)-malic acid such as the L-malate salt of 5-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid(2-diethylaminoethyl)amide; or

(2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or

coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like.

"Method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by, practitioners of the chemical, pharmaceutical, biological, biochemical and medical arts.

"In vivo" refers to procedures performed within a living organism such as, without limitation, a mouse, rat or rabbit.

"Treat", "treating" and "treatment" refer to a method of alleviating or abrogating acute myeloid leukemia, other leukemias, FLT-3 related cancers and/or their attendant symptoms. Leukemias treatable with the compounds of Formula I or II include acute myelogenous leukemia (AML), Acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), acute myelomonoblastic leukemia (AMMOL), and acute monoblastic leukemia (AMOL). In addition, other types of cancers associated with FLT-3, include without limitation leukemias, lymphomas, carcinomas, myelomas, neural crest derived cancers, sarcomas and gliomas may be treatable by administration of a compound of Formula (I) or (II). The term "treat" simply mean that the life expectancy of an individual affected with AML or a FLT-3 related cancer will be increased or that one or more of the symptoms of the disease will be reduced.

"FLT-3 related cancer" includes but is not limited to acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), chronic myeloid leukemia (CLL), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), acute myelomonoblastic leukemia (AMMOL), and acute monoblastic leukemia (AMOL).

"Patient" refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single eukariotic cell or as complex as a mammal, including a human being.

"Therapeutically effective amount" refers to that amount of the compound being administered which will prevent, alleviate, ameliorate or relieve to some extent, one or more of the symptoms of the disorder being treated. In reference to the treatment of cancer, a therapeutically effective amount refers to that amount which has the effect of:

(1) reducing the size of the tumor;

(2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis;

(3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth,

(4) reducing blast cell counts, and/or

(5) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer.

ADMINISTRATION AND PHARMACEUTICAL COMPOSITION

The claimed methods involve administration of a compound of formula I or II or a pharmaceutically acceptable salt thereof, to a human patient. Alternatively, the compounds of Formula I or II can be administered in pharmaceutical compositions in which the foregoing materials are mixed with suitable carriers or excipient(s). Techniques for formulation and administration of drugs may be found in "Remington's Pharmacological Sciences," Mack Publishing Co., Easton, PA., latest edition.

As used herein, "administer" or "administration" refers to the delivery of a compound of Formula (I) or (II) or a pharmaceutically acceptable salt thereof or of a pharmaceutical composition containing a compound of Formula (I) or (II) or a pharmaceutically acceptable salt thereof of this invention to an organism for the purpose of treatment of AML.

Suitable routes of administration may include, without limitation, oral, rectal, transmucosal or intestinal administration or intramuscular, subcutaneous, intramedullary, intrathecal, direct intraventricular, intravenous, intravitreal, intraperitoneal, intranasal, or intraocular injections. The preferred routes of administration are oral and parenteral.

Alternatively, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a solid tumor, often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with tumor-specific antibody. The liposomes will be targeted to and taken up selectively by the tumor.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving,

granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the compounds of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, lozenges, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient. Pharmaceutical preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding other suitable auxiliaries if desired, to obtain tablets or dragee cores. Useful excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol, cellulose preparations such as, for example, maize starch, wheat starch, rice starch and potato starch and other materials such as gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinyl- pyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid. A salt such as sodium alginate may also be used.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with a filler such as lactose, a binder such as starch, and/or a lubricant such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. Stabilizers may be added in these formulations, also.

Pharmaceutical compositions which may also be used include hard gelatin capsules. As a non-limiting example, compound 1 in a capsule oral drug product formulation may be as 50 and 200 mg dose strengths. The two dose strengths are made from the same granules by filling into different size hard gelatin capsules, size 3 for the 50 mg capsule and size 0 for the 200 mg capsule.

The capsules may be packaged into brown glass or plastic bottles to protect the active compound from light. The containers containing the active compound capsule formulation must be stored at controlled room temperature (15-30°C).

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray using a pressurized pack or a nebulizer and a suitable propellant, e.g., without limitation, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetra- fluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be controlled by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may also be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulating materials such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of a water soluble form, such as, without limitation, a salt, of the active compound. Additionally, suspensions of the active compounds may be prepared in a lipophilic vehicle.

Suitable lipophilic vehicles include fatty oils such as sesame oil, synthetic fatty acid esters such as ethyl oleate and triglycerides, or materials such as liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers and/or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

In addition to the fomulations described previously, the compounds may also be formulated as depot preparations. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. A compound of this invention may be formulated for this route of administration with suitable polymeric or hydrophobic materials (for instance, in an emulsion with a pharamcologically acceptable oil), with ion exchange resins, or as a sparingly soluble derivative such as, without limitation, a sparingly soluble salt.

A non-limiting example of a pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer and an aqueous phase such as the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:D5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of such a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of Polysorbate 80, the fraction size of polyethylene glycol may be varied, other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone, and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. In addtion, certain organic solvents such as dimethylsulfoxide also may be employed, although often at the cost of greater toxicity.

Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions herein also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Examples of formulations for use in the present invention are in Tables 1-3:

TABLE 1

COMPOSITION OF 5-(5-FLUORO-2-OXO-1,2-DIHYDRO-INDOL-3-YLIDENEMETHYL)-2,4-DIMETHYL-1H-PYRROLE-3-CARBOXYLIC ACID (2-DIETHYLAMINO-ETHYL)-AMIDE HARD GELATIN CAPSULES				
INGREDIENT NAME	CONCENTRATION IN GRANULATION (% W/W)	AMOUNT IN 50 MG CAPSULE (MG)	AMOUNT IN 75 MG CAPSULE (MG)	AMOUNT IN 200 MG CAPSULE (MG)
API	65.0	50.0	75.0	200.0
MANNITOL	23.5	18.1	27.2	72.4
CROSCARAMELLOSE SODIUM[E]	6.0	4.6	6.9	18.4
POVIDONE (K-25)	5.0	3.8	5.7	15.2
MAGNESIUM STEARATE	0.5	0.38	0.57	1.52
CAPSULE	-	SIZE 1	SIZE 3	SIZE 0

TABLE 2

COMPOSITION OF 5-(5-FLUORO-2-OXO-1,2-DIHYDRO-INDOL-3-YLIDENEMETHYL)-2,4-DIMETHYL-1H-PYRROLE-3-CARBOXYLIC ACID (2-DIETHYLAMINO-ETHYL)-AMIDE L-MALATE HARD GELATIN CAPSULES		
INGREDIENT NAME/GRADE	CONCENTRATION IN GRANULATION (% W/W)	AMOUNT IN 50 MG CAPSULE (MG)
API	75.0	66.800[C]
MANNITOL	13.5	12.024
CROSCARAMELLOSE SODIUM[E]	6.0	5.344
POVIDONE (K-25)	5.0	4.453
MAGNESIUM STEARATE	0.5	1.445
CAPSULE	-	SIZE 3

TABLE 3

COMPOSITION OF 5-(5-FLUORO-2-OXO-1,2-DIHYDRO-INDOL-3-YLIDENEMETHYL)-2,4-DIMETHYL-1H-PYRROLE-3-CARBOXYLIC ACID (2-DIETHYLAMINO-ETHYL)-AMIDE L-MALATE HARD GELATIN CAPSULES				
INGREDIENT NAME/GRADE	CONCENTRATION IN GRANULATION (% W/W)	AMOUNT IN 25 MG CAPSULE (MG)	AMOUNT IN 50 MG CAPSULE (MG)	AMOUNT IN 100 MG CAPSULE (MG)
API[A]	40.0	33.400[D]	66.800[C]	200.0[B]
MANNITOL	47.5	39.663	79.326	158.652
CROSCARAMELLOSE SODIUM[E]	6.0	5.010	10.020	20.04
POVIDONE (K-25)	5.0	4.175	8.350	16.700
MAGNESIUM STEARATE	1.5	1.252	2.504	5.008
CAPSULE	-	SIZE 3	SIZE 1	SIZE 0

[A] DRUG SUBSTANCE QUANTITY REQUIRED FOR THE BATCH WILL BE AJUSTED TO HAVE 100% OF LABELED STRENGTH FOR CAPSULES. APPROPRIATE ADJUSTMENT WILL BE MADE TO MANNITOL QUANTITY TO KEEP THE SAME FILL WEIGHT FOR EACH STRENGTH.
[B] QUANTITY EQUIVALENT TO 100 MG FREE BASE.
[C] QUANTITY EQUIVALENT TO 50 MG FREE BASE.
[D] QUANTITY EQUIVALENT TO 25 MG FREE BASE.
[E] HALF INTRAGANULAR HALF EXTRAGRANULAR.

which can be found in U.S. Patent Application Serial No. 10/237,966, filed September 10, 2002, which is expressly incorporated in its entirety by reference.

Many of the compounds of the Formula I and II may be provided as physiologically acceptable salts wherein the compound may form the negatively or the positively charged species. Examples of salts in which the compound forms the positively charged moiety include, without limitation, quaternary ammonium, salts such as the hydrochloride, sulfate, carbonate, lactate, tartrate, malate, maleate, succinate wherein the nitrogen atom of the quaternary ammonium group is a nitrogen of the selected compound of this invention which has reacted with the appropriate acid. Salts in which a compound of this invention forms the negatively charged species include, without limitation, the sodium, potassium, calcium and magnesium salts formed by the reaction of a carboxylic acid group in the compound with an appropriate base (e.g. sodium hydroxide (NaOH), potassium hydroxide (KOH), Calcium hydroxide ($Ca(OH)_2$), etc.).

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an amount sufficient to achieve the intended purpose, e.g., treatment of AML in FLT-3-ITD positive patients.

More specifically, a "therapeutically effective amount" means an amount of compound effective to prevent, alleviate or ameliorate symptoms of AML or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from cell culture assays. Then, the dosage can be formulated for use in animal models so as to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (i.e., the concentration of the test compound

which achieves a half-maximal inhibition of phosphorylation of FLT-3). Such information can then be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the compounds described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC_{50} and the LD_{50}, wherein the LD_{50} is the concentration of test compound which achieves a half-maximal inhibition of lethality, for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active species which are sufficient to maintain the kinase modulating effects. These plasma levels are referred to as minimal effective concentrations (MECs). The MEC will vary for each compound but can be estimated from in vitro data, e.g., the concentration necessary to achieve 50-90% inhibition of a kinase may be ascertained using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen that maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

At present, the therapeutically effective amounts of compounds of Formula (I) or (II) may range from approximately 25 mg/m2 to 1500 mg/m2 per day; preferably about 3 mg/m2/day. Even more preferably 50mg/qm qd till 400 mg/qd.

In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration and other procedures known in the art may be employed to determine the correct dosage amount and interval.

The amount of a composition administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

It is contemplated that the inventive method could be used in combination with other cancer therapies, incuding radiation and bone marrow transplantation.

Finally, it is also contemplated that the combination of a compound of this invention will be effective in combination with ENDOSTATIN©, GLEEVEC©, CAMPTOSAR©, HERCEPTIN©, IMCLONE C225©, mitoxantrone, daunorubicin, cytarabine, methotrexate, vincristine, 6-thioguanine, 6-mercaptopurine or paclitaxel for the treatment of solid cancers or leukemias, including but not limited to AML. Additionally, the inventive method can involve combination thereapy with an anti-angiogenic agent, such as, but not limited to a cyclooxygenase inhibitor such as celecoxib.

For the combination therapies and pharmaceutical compositions described herein, the effective amounts of the compound of the invention and of the chemotherapeutic or other agent useful for inhibiting abnormal cell growth (e.g., other antiproliferative agent, antiangiogenic, signal transduction inhibitor or immune system enhancer) can be determined by those of ordinary skill in the art, based on the effective amounts for the compounds described herein and those known or described for the chemotherapeutic or other agent. The formulations and route of administration for such therapies and composition can be based on the information described herein for compositions and therapies comprising the compound of the invention as the sole active agent and on information provided for the chemotherapeutic and other agent in combination therewith.

GENERAL SYNTHETIC PROCEDURE

The following general methodology may be employed to prepare the compounds of this invention:

The appropriately substituted 2-oxindole (1 equiv.), the appropriately substituted aldehyde (1.2 equiv.) and a base (0.1 equiv.) are mixed in a solvent (1-2 ml/mmol 2-oxindole) and the mixture is then heated for from about 2 to about 12 hours. After cooling, the precipitate that forms is filtered, washed with cold ethanol or ether and vacuum dried to give the solid product. If no precipitate forms, the reaction mixture is concentrated and the residue is triturated with dichloromethane/ether, the resulting solid is collected by filtration and then dried. The product may optionally be further purified by chromatography.

The base may be an organic or an inorganic base. If an organic base is used, preferably it is a nitrogen base. Examples of organic nitrogen bases include, but are not

limited to, diisopropylamine, trimethylamine, triethylamine, aniline, pyridine, 1,8-diazabicyclo[5.4.1]undec-7-ene, pyrrolidine and piperidine.

Examples of inorganic bases are, without limitation, ammonia, alkali metal or alkaline earth hydroxides, phosphates, carbonates, bicarbonates, bisulfates and amides. The alkali metals include, lithium, sodium and potassium while the alkaline earths include calcium, magnesium and barium.

In a presently preferred embodiment of this invention, when the solvent is a protic solvent, such as water or alcohol, the base is an alkali metal or an alkaline earth inorganic base, preferably, a alkali metal or an alkaline earth hydroxide.

It will be clear to those skilled in the art, based both on known general principles of organic synthesis and on the disclosures herein which base would be most appropriate for the reaction contemplated.

The solvent in which the reaction is carried out may be a protic or an aprotic solvent, preferably it is a protic solvent. A "protic solvent" is a solvent which has hydrogen atom(s) covalently bonded to oxygen or nitrogen atoms which renders the hydrogen atoms appreciably acidic and thus capable of being "shared" with a solute through hydrogen bonding. Examples of protic solvents include, without limitation, water and alcohols.

An "aprotic solvent" may be polar or non-polar but, in either case, does not contain acidic hydrogens and therefore is not capable of hydrogen bonding with solutes. Examples, without limitation, of non-polar aprotic solvents, are pentane, hexane, benzene, toluene, methylene chloride and carbon tetrachloride. Examples of polar aprotic solvents are chloroform, tetrahydro- furan, dimethylsulfoxide and dimethylformamide.

In a presently preferred embodiment of this invention, the solvent is a protic solvent, preferably water or an alcohol such as ethanol.

The reaction is carried out at temperatures greater than room temperature. The temperature is generally from about 30°C to about 150°C, preferably about 80°C to about 100°C, most preferable about 75°C to about 85°C, which is about the boiling point of ethanol. By "about" is meant that the temperature range is preferably within 10 degrees Celsius of the indicated temperature, more preferably within 5 degrees Celsius of the indicated temperature and, most preferably, within 2 degrees Celsius of the indicated

temperature. Thus, for example, by "about 75°C" is meant 75°C ± 10°C, preferably 75°C ± 5°C and most preferably, 75°C ± 2°C.

2-Oxindoles and aldehydes, may be readily synthesized using techniques well known in the chemical arts. It will be appreciated by those skilled in the art that other synthetic pathways for forming the compounds of the invention are available and that the following is offered by way of example and not limitation.

Compounds of the present invention are prepared according to the following methodologies and as described, e.g., in U.S. Patent Application Serial No. 09/783,264 and WO 01/60814, WO 00/08202, U.S Provisional Application No. 60/312,353, filed August 15, 2001, now U.S. Patent Application Serial No. 10/281,985, filed August 13, 2002, U.S. Provisional Application No. 60/411,732, filed September 18, 2002,U.S. Provisional Application No. 60/328,226, filed Octobter 10, 2001, now U.S. Patent Application Serial No. _____ filed October 10, 2002 and U.S. Patent Application Serial No. 10/076,140, filed February 15, 2002, all of which are incorporated by reference in their entirety.

SYNTHETIC METHODOLOGIES

Method A: Formylation of pyrroles

$POCl_3$ (1.1 equiv.) is added dropwise to dimethylformamide (3 equiv.)at –10°C followed by addition of the appropriate pyrrole dissolved in dimethylformamide. After stirring for two hours, the reaction mixture is diluted with H_2O and basified to pH 11 with 10 N KOH. The precipitate which forms is collected by filtration, washed with H_2O and dried in a vacuum oven to give the desired aldehyde.

Method B: Saponification of pyrrolecarboxylic acid esters

A mixture of a pyrrolecarboxylic acid ester and KOH (2 – 4 equiv.) in EtOH is refluxed until reaction completion is indicated by thin layer chromatography (TLC). The cooled reaction mixture is acidified to pH 3 with 1 N HCl. The precipitate which forms is collected by filtration, washed with H_2O and dried in a vacuum oven to give the desired pyrrolecarboxylic acid.

Method C: Amidation

To a stirred solution of a pyrrolecarboxylic acid dissolved in dimethylformamide(0.3M) is added 1-ethyl-3-(3-dimethylamino- propyl)carbodiimide (1.2 equiv.), 1-hydroxybenzotriazole (1.2 equiv.), and triethylamine (2 equiv.). The appropriate

amine is added (1 equiv.) and the reaction stirred until completion is indicated by TLC. Ethyl acetate is then added to the reaction mixture and the solution washed with saturated $NaHCO_3$ and brine (with extra salt), dried over anhydrous $MgSO_4$ and concentrated to afford the desired amide.

Method D: Condensation of aldehydes and oxindoles containing carboxylic acid substituents

A mixture of the oxindole (1 equivalent), 1 equivalent of the aldehyde and 1 – 3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100°C until reaction completion is indicated by TLC. The mixture is then concentrated and the residue acidified with 2N HCl. The precipitate that forms is washed with H_2O and EtOH and then dried in a vacuum oven to give the product.

Method E: Condensation of aldehydes and oxindoles not containing carboxylic acid substituents

A mixture of the oxindole (1 equivalent), 1 equivalent of the aldehyde and 1 – 3 equivalents of piperidine (or pyrrolidine) in ethanol (0.4 M) is stirred at 90-100°C until reaction completion is indicated by TLC. The mixture is cooled to room temperature and the solid which forms is collected by vacuum filtration, washed with ethanol and dried to give the product. If a precipitate does not form upon cooling of the reaction mixture, the mixture is concentrated and purified by column chromatography.

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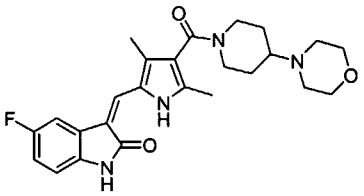
The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available documents are specifically incorporated into this patent application by reference.

SYNTHETIC EXAMPLES

Example 1-Synthesis of (3Z)-3-{[3,5-dimethyl-4-(morpholin-4-yl)piperidin-1-ylcarbonyl]-1H-pyrrol-2-ylmethylidene}-5-fluoro-1,3-dihydro-2H-indol-2-one (Compound 9)



Step 1

To a stirred mixture of 4-amino-1-benzylpiperidine (Aldrich, 1.53 mL, 7.5 mmol), K_2CO_3 (2.28 g, 16.5 mmol), and DMF (15 mL) heated at 50 °C was added dropwise over 60 min bis(2-bromoethyl) ether (Aldrich, tech. 90%, 0.962 mL, 7.65 mmol). After stirring 6 h at 80 °C, TLC (90:10:1 chloroform/MeOH/aq. conc NH_4OH) indicated formation of a new spot. Heating was continued as the solvent was evaporated by blowing with a stream of nitrogen over 2 h. The crude material was relatively pure, but subjected to a relatively short silica gel column (1% to 6% gradient of 9:1 MeOH/aq. NH_4OH in chloroform). Evaporation of the pure fractions gave ~1.7 g of the diamine 4-(morpholin-4-yl)-1-benzylpiperidine as a waxy solid.

^{1}HNMR (400 MHz, d_6-DMSO) δ 7.31 (m, 4H), 7.26 (m 1H), 3.72 (t, J = 4.7 Hz, 4H), 3.49 (s, 2H), 2.94 (br d, J = 5.9 Hz, 2H), 2.54 (t, J = 4.7 Hz, 4H), 2.19 (tt, J = 11.5, 3.9 Hz, 1H), 1.96 (td, J = 11.7, 2.2 Hz, 2H), 1.78 (br d, J = 12.5 Hz, 2H), 1.55 (m, 2H).

Step 2

A stirred mixture of $Pd(OH)_2$ (20% on carbon (<50% wet), 390 mg, 25 wt%), methanol (50 mL), and ≤1.7 M HCl (3 eq, ~10.6 mL – including water added later when ppt was seen) under nitrogen was exchanged to 1 atm. hydrogen atmosphere by flushing (~20 sec) using a balloon of nitrogen into the vessel and out through an oil bubbler. After 20 min. the reaction mixture under hydrogen was heated to 50 °C and 4-(morpholin-4-yl)-1-benzylpiperidine (1.56 g, 6.0 mmol) in methanol (8 mL) was added dropwise over 30 min. After 10 h, tlc indicated all starting amine was consumed to a more polar spot (ninhydrin active). The reaction mixture was then filtered through Celite and evaporated to yield the 4-(morpholin-4-yl)piperidine dihydrochloride as an off-white solid. This material was subjected to free-basing using excess basic resin (>16 g, Bio-Rad Laboratories, AG 1-X8, 20-50 mesh, hydroxide form, methanol washed two times) and a methanol mixture of the amine hydrochloride. After swirling with the resin for 30 min., the methanol solution was decanted and evaporated to yield 932 mg of 4-(morpholin-4-yl)piperidine free base as a waxy crystalline solid.

^{1}HNMR (400 MHz, d_6-DMSO) δ 3.53 (br s, 4H), 3.30 (v br s, 1H(+H_2O)), 2.92 (br d, J = 11.7 Hz, 1H), 2.41 (s, 4H), 2.35 (~obscd t, J = 11.7 Hz, 2H), 2.12 (br t, 1H), 1.65 (br d, J = 11.7 Hz, 2H), 1.18 (br q, J = 10.9 Hz, 2H); LCMS-APCI *m/z* 171 $[M+1]^+$.

Step 3

(3Z)-3-(3,5-Dimethyl-4-carboxy-1H-pyrrol-2-ylmethylidene)-5-fluoro-1,3-dihydro-2H-indol-2-one (120 mg, 0.40 mmol), prepared as described in PCT Publication No 01/60814, and BOP (221 mg, 0.50 mmol) were suspended in DMF (5 mL) with good stirring at room temperature and triethylamine (134 μL, 0.96 mmol) was added. After 10-15 min., to the homogeneous reaction mixture was added the 4-(morpholin-4-yl)piperidine (85 mg, 0.50 mmol) all at once. The reaction mixture was stirred for 48 h (might be done much earlier), then transferred to a funnel containing chloroform-isopropanol (5/1) and 5% aq. LiCl. The cloudy-orange organic phase was separated, washed with additional 5% aq LiCl (2X), 1 M aq NaOH (3X), satd aq NaCl (1X), and then dried (Na_2SO_4) and evaporated to yield the crude product (96.3% pure; trace HMPA by ^{1}HNMR). This crude product was then further purified by passage through a very short column (3 cm) of silica gel (5 to 15% gradient of MeOH in DCM) where a trace of faster moving 3E-isomer was removed. The pure fractions were evaporated and recrystallized overnight from a satd EtOAc soln which was diluted with Et_2O (~3-fold) and chilled at 0 °C. The mother liquor was decanted to yield after full vacuum the desired compound as orange crystals (153 mg 85%).

^{1}HNMR (400 MHz, d_6-DMSO) δ 13.60 (s, 1H), 10.87 (s, 1H), 7.72 (dd, J = 9.4, 2.7 Hz, 1H), 7.68 (s, 1H), 6.91 (td, J = 9.3, 2.6 Hz, 1H), 6.82 (dd, J = 8.6, 4.7 Hz, 1H), 3.54 (app br t, J = 4.3 Hz, 4H), 3.31 (2x s, 3H+3H), 2.43 (br s, 4H), 2.36 (m, 1H), 2.25 (br m, 6H), 1.79 (br s, 2H), 1.22 (br s, 2H); LCMS *m/z* 453 $[M+1]^+$.

Proceeding as described in Example 1 above but substituting (3Z)-3-(3,5-dimethyl-4-carboxy-1H-pyrrol-2-ylmethylidene)-5-fluoro-1,3-dihydro-2H-indol-2-one for (3Z)-3-(3,5-dimethyl-4-carboxy-1H-pyrrol-2-ylmethylidene)-1,3-dihydro-2H-indol-2-one gave (3Z)-3-{[3,5-dimethyl-4-(morpholin-4-yl)piperidin-1-ylcarbonyl]-1H-pyrrol-2-ylmethylidene}-,3-dihydro-2H-indol-2-one. ^{1}HNMR (400 MHz, d_6-DMSO) δ 13.55 (s, 1H), 10.87 (s, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.59 (s, 1H), 7.11 (t, J = 7.6 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 6.86 (d, J = 7.4 Hz, 1H), 3.54 (app br t, J = 4.3 Hz, 4H), 3.31 (2x s, 3H+3H), 2.43 (br s, 4H), 2.35 (m, 1H), 2.28 (br m, 6H), 1.79 (br s, 2H), 1.22 (br s, 2H); LCMS *m/z* 435 $[M+1]^+$.

Proceeding as described in Example 1 above but substituting (3Z)-3-(3,5-dimethyl-4-carboxy-1H-pyrrol-2-ylmethylidene)-5-fluoro-1,3-dihydro-2H-indol-2-one for (3Z)-3-(3,5-dimethyl-4-carboxy-1H-pyrrol-2-ylmethylidene)-5-chloro-1,3-dihydro-2H-indol-2-one gave (3Z)-3-{[3,5-dimethyl-4-(morpholin-4-yl)piperidin-1-ylcarbonyl]-1H-pyrrol-2-ylmethylidene}-5-chloro-1,3-dihydro-2H-indol-2-one.

^{1}HNMR (400 MHz, d_6-DMSO) δ 13.56 (s, 1H), 10.97 (s, 1H), 7.95 (d, J = 2.0 Hz, 1H), 7.74 (s, 1H), 7.11 (dd, J = 8.2, 2.0 Hz, 1H), 6.85 (d, J = 8.2 Hz, 1H), 3.54 (app br t, J = ~4 Hz, 4H), 3.31 (2x s, 3H+3H), 2.43 (br s, 4H), 2.37 (m, 1H), 2.25 (br m, 6H), 1.79 (br s, 2H), 1.23 (br s, 2H); LCMS *m/z* 470 $[M+1]^+$.

Proceeding as described in Example 1 above but substituting 4-(morpholin-4-yl)-piperidine with commercially available 4-(1-pyrrolidinyl)-piperidine gave (3Z)-3-{[3,5-dimethyl-4-[4-(pyrrolidin-1-yl)piperidin-1-ylcarbonyl]-1H-pyrrol-2-yl)methylidene]-5-fluoro-1,3-dihydro-2H-indol-2-one.

^{1}HNMR (400 MHz, d_6-DMSO) δ E/Z isomer mixture; LCMS *m/z* 437 $[M+1]^+$.

Synthesis of the above examples can proceed according to the procedure of U.S. Provisional Application No. 60/328,226, filed October 10, 2001 and U.S. Patent Application Serial No. _____, filed October 10, 2002, incorporated by reference in its entirety.

Example 2-Synthesis of (3Z)-3-{[3,5-dimethyl-4-(morpholin-4-yl)azetidin-1-ylcarbonyl]-1H-pyrrol-2-ylmethylidene}-5-fluoro-1,3-dihydro-2H-indol-2-one

Step 1

A solution of 1-azabicyclo[1.1.0]butane, prepared from 2,3-dibromopropylamine hydrobromide (58.8 mmol) according to a known procedure described in Tetrahedron Letters 40 (1999) 3761-64, was slowly added to a solution of morpholine (15.7 ml; 180 mmol) and sulfuric acid (3.3 g of 96% soln.) in anhydrous non-denaturated ethanol (250 ml) at 0 °C. The reaction mixture was stirred on ice bath for 30 min., then at room temperature for 8 h. Calcium hydroxide (5.5 g) and 100 ml of water was added and the obtained slurry was stirred for 1 h and then filtered through a pad of cellite. The filtrate was concentrated and distilled at reduced pressure (20 mm Hg) to remove water and an excess of morpholine. The distillation residue was re-distilled at high vacuum using a Kugelrohr apparatus to obtain a pure 4-(azetidin-3-yl)morpholine in 33% yield (2.759 g) as a colorless oily liquid.

^{13}C-NMR ($CDCl_3$, 100 MHz): 66.71(2C), 59.37 (1C), 51.46 (2C), 49.95(2C) ^{1}H ($CDCl_3$, 400 MHz): 3.727 (t, J=4.4 Hz, 4H), 3.619 (t, J=8Hz, 2H), 3.566 (t, J=8Hz, 2H), 3.227 (m, J=7Hz, 1H), 2.895 (br s, 1H), 2.329 (br s, 4H)

Step 2

1-(8-Azabenztriazolyl)-ester of (3Z)-3-({3,5-dimethyl-4-carboxy]1-H-pyrrol-2-yl}methylene)-5-fluoro-1.3-dihydro-2H-indol-2-one (0.5 mmol, 210 mg) [prepared by activating (3Z)-3-(3,3-dimethyl-4-carboxy-1-H-pyrrol-2-ylmethylene)-5-fluoro-1.3-dihydro-2H-indol-2-one (480 mg; 1.6 mmol) with the HATU reagent (570 mg, 1.5 mmol) in the presence of Hunig base (3.0 mmol, 0.525 ml) in DMF (5ml) and isolated in pure form by precipitation with chloroform (5ml) and drying on high vacuum in 92% yield (579 mg)] was suspended in anhydrous DMA (1.0 ml). A solution of 4-(azetidin-3-yl)-morpholine; (142.5 mg, 1 mmol) in anhydrous DMA (1.0 ml) was added in one portion and the obtained solution was stirred at room temperature for 20 min. The reaction mixture was evaporated at room temperature using an oil pump, the thick residue was diluted with 6 ml of a mixture of methanol plus diethyl amine (20:1; v/v), inoculated mechanically and placed into a refrigerator (+3 °C) for 8 hours. The precipitates were filtered (with a brief wash with an ice-cold methanol) and dried on high vacuum to give the desired product. 71.5% yield (152 mg of an orange solid)

LC/MS: +APCI: M+1=425; -APCI: M-1=423

^{19}F-NMR (d-DMSO, 376.5 MHz): -122.94 (m, 1F)

^{1}H (d-DMSO, 400 MHz): 13.651 (s, 1H), 10.907 (s, 1H), 7.754 (dd, J=9.4 Hz, J=2.4 Hz, 1H), 7.700 (s, 1H), 6.935 (dt, J=8.2 Hz, J=2.4 Hz, 1H), 6.841 (dd, J=8.6 Hz, J=3.9Hz; 1H), 3.963 (br s, 2H), 3.793 (br s, 2H), 3.581 (br t, J=4.3 Hz, 4H), 3.133 (m, 1H), 2.367 (s, 3H), 2.340 (s, 3H), 2.295 (br s, 4H)

Proceeding as described in Example 2 above but substituting (3Z)-3-(3,5-dimethyl-4-carboxy-1H-pyrrol-2-ylmethylidene)-5-fluoro-1,3-dihydro-2H-indol-2-one with (3Z)-3-(3,5-dimethyl-4-carboxy-1H-pyrrol-2-ylmethylidene)-5-chloro-1,3-dihydro-2H-indol-2-one gave (3Z)-3-{[3,5-dimethyl-4-(morpholin-4-yl)azetidin-1-ylcarbonyl]-1H-pyrrol-2-ylmethylidene}-5-chloro-1,3-dihydro-2H-indol-2-one as an orange solid.

LC/MS: +APCI: M+1=441; -APCI: M-1=440,441

^{1}H (d-DMSO, 400 MHz): 13.607 (s, 1H), 11.006 (s,1H), 7.976 (d, J=2.0Hz, 1H), 7.756 (s, 1H), 7.136 (dd, J=8.2 Hz, J=2.0 Hz, 1H), 6.869 (d, J=8.2 Hz, 1H), 3.964 (br s, 2H), 3.793 (br s, 2H), 3.582 (br t, J=4.3 Hz, 4H), 3.134 (m,1H), 2.369 (s, 3H), 2.347 (s, 3H), 2.296 (br s, 4H)

Proceeding as described in Example 2 above but substituting 4-(azetidin-3-yl)morpholine with 4-(azetidin-3-yl)-cis-3,5-dimethylmorpholine (prepared in a procedure analogous to the preparation of 4-(azetidin-3-yl)-morpholine but using cis-3,5-dimethylmorpholine (20.7g; 180 mmol) in place of morpholine) gave (3Z)-3-{[3,5-dimethyl-4-(2,5-dimethylmorpholin-4-yl)azetidin-1-ylcarbonyl]-1H-pyrrol-2-ylmethylidene}-5-fluoro-1,3-dihydro-2H-indol-2-one as an orange solid

LC/MS: +APCI: M+1=453; -APCI: M-1=451

^{19}F-NMR (d-DMSO, 376.5 MHz): -122.94 (m, 1F)

^{1}H (d-DMSO, 400 MHz): 13.651 (s, 1H), 10.907 (s; 1H), 7.758 (dd, J=9.4 Hz, J=2.3 Hz; 1H), 7.700 (s, 1H), 6.935 (dt, J=8.6 Hz, J=2.7 Hz, 1H), 6.842 (dd, J=8.2 Hz, J=4.3 Hz, 1H), 3.961 (br s, 2H), 3.790 (br s, 2H), 3.546 (br m, 2H), 3.092 (m, 1H), 2.690 (br s; 2H), 2.364 (s, 3H), 2.338 (s, 3H), 1.492 (br m, 2H), 1.038 (br s, 6H)

Proceeding as described in Example 2 above but substituting (3Z)-3-(3,5-dimethyl-4-carboxy-1H-pyrrol-2-ylmethylidene)-5-fluoro-1,3-dihydro-2H-indol-2-one with (3Z)-3-(3,5-dimethyl-4-carboxy-1H-pyrrol-2-ylmethylidene)-5-chloro-1,3-dihydro-2H-indol-2-one and 4-(azetidin-3-yl)morpholine with 4-(azetidin-3-yl)-cis-3,5-dimethylmorpholine gave (3Z)-3-{[3,5-dimethyl-4-(3,5-dimethylmorpholin-4-yl)azetidin-1-ylcarbonyl]-1H-pyrrol-2-ylmethylidene}-5-chloro-1,3-dihydro-2H-indol-2-one as an orange solid.

LC/MS: +APCI: M+1=469, 470; -APCI: M-1=468,469

^{1}H (d-DMSO, 400 MHz): 13.606 (s, 1H), 11.008 (s, 1H), 7.979 (d, J=2.0Hz, 1H), 7.758 (s, 1H), 7.138 (dd, J=8.2Hz, J=2.0Hz, 1H), 6.870 (d, J=8.2Hz, 1H), 3.964 (br s, 2H), 3.790 (br s, 2H), 3.547 (br m, 2H), 3.095 (m, 1H), 2.691 (br s, 2H), 2.366 (s, 3H), 2.345 (s, 3H), 1.494 (br m, 2H), 1.039 (br s, 6H)

Proceeding as described in Example 1 above, but substituting 4-(morpholin-4-yl)-piperidine with 2-(R)-pyrrolidin-1-ylmethylpyrrolidine prepared as described below provided (3Z)-3-{[3,5-dimethyl-2R-(pyrrolidin-1-ylmethyl)pyrrolidin-1-ylcarbonyl]-1H-pyrrol-2-ylmethylidene}-5-fluoro-1,3-dihydro-2H-indol-2-one

Synthesis of 2(R)-pyrrolidin-1-ylmethylpyrrolidine

Step 1

To a solution of (+)-Carbobenzyloxy-D-proline (1.5 g, 6.0 mmol), EDC (2.3 g, 12.0 mmol) and HOBt (800 mg, 12.9 mmol) in DMF (20 ml) was added trietylamine (1.5 ml) and pyrrolidine (1.0 ml, 12.0 mmol). It was stirred for 18 h at rt. Sat. $NaHCO_3$ was added, it was extracted with CH2CL2 (three times). The organic layers were separated and dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (EtOAc) to give 1-(R)-[N-(benzyloxycarbonyl)-pyrolyl]pyrrolidine as a white solid (94%).

^1H NMR (400 MHz, $CDCl_3$, all rotamers) 1.57-1.66 (m, 1H), 1.71-2.02 (m, 5H), 2.04-2.19 (m, 2H), 3.26-3.43 (m, 3H), 3.44-3.78 (m, 3H), 4.41 (dd, J = 4.5, 7.6 Hz, 0.5H), 4.52 (dd, J = 3.7, 7.6 Hz, 0.5H), 4.99 (d, J = 12.1 Hz, 0.5H), 5.05 (d, J = 12.5 Hz, 0.5H), 5.13 (d, J = 12.1 Hz, 0.5H), 5.20 (d, J = 12.5 Hz, 0.5H), 7.27-7.38 (m, 5H).

Step 2

A mixture of 1-(R)-[*N*-(benzyloxycarbonyl)prolyl]pyrrolidine (2.7 g, 8.9 mmol) and 5% Pd-C catalyst (270 mg) in methanol (15 ml) were stirred under a hydrogen atmosphere for 20 h. The reaction mixture was filtered through celite and the solvent was removed yielding 2(R)-prolylpyrrolidine as a viscous oil (80%), which was used without further purification for the next step.

^1H NMR (400 MHz, d_6-DMSO) δ 1.52-1.78 (m, 5H), 1.82-1.89 (m, 2H), 1.97-2.04 (m, 1H), 2.63-2.71 (m, 1H), 2.97-3.02 (m, 1H), 3.22-3.35 (m, 3H), 3.48-3.54 (m, 1H), 3.72 (dd, J = 6.1, 8.0 Hz, 1H).

Step 3

2-(R)-Prolylpyrrolidine (1.2 g, 7.1 mmol) was dissolved in THF (10 ml). The reaction mixture was cooled to 0° C and BH_3, 1M in THF (10 ml, 10 mmol) was dropwise at 0 C. The reaction mixture was refluxed for 16 h, 3 M HCl (4.7 ml). 2 M NaOH solution was added until pH 10 was reached. The product was extracted with 5% MeOH in CH_2Cl_2 (three times). The organic layers were dried over Na_2SO_4 and the solvent was removed to provide the title compound as a slightly yellow liquid (73%), which was used without further purification for the next step.

^{1}H NMR (400 MHz, d_6-DMSO) δ 1.22-1.30 (m, 1H), 1.55-1.69 (m, 6H), 1.71-1.79 (m, 1H), 2.26-2.30 (m, 1H), 2.33-2.38 (m, 1H), 2.40-2.45 (m, 4H), 2.65-2.71 (m, 1H), 2.78-2.84 (m, 1H), 3.02-3.09 (m, 1H).

Proceeding as described in Example 1 above, but substituting 4-(morpholin-4-yl)-piperidine with 2-(S)-pyrrolidin-1-ylmethylpyrrolidine (prepared as described above, by substituting (+)-carbobenzyloxy-D-proline with carbobenzyloxy-L-proline) provided (3Z)-3-{[3,5-dimethyl-2S-(pyrrolidin-1-ylmethyl)pyrrolidin-1-ylcarbonyl]-1H-pyrrol-2-ylmethylidene}-5-fluoro-1,3-dihydro-2H-indol-2-one.

Example 3-Synthesis of 5-[5-fluoro-2-oxo-1,2-dihydro-indol-(3Z)-ylidene-methyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid

Step 1

Dimethylformamide (25 mL, 3 eq.) was cooled with stirring in an ice bath. To this was added $POCl_3$ (1.1 eq., 10.8 mL). After 30 minutes, a solution of the 3,5-dimethyl-4-ethylester pyrrole (17.7g, 105.8mmol) in DMF (2M, 40 mL) was added to the reaction and stirring continued. After 2 hour, the reaction was diluted with water (250 mL) and basified to pH=11 with 1N aqueous NaOH. The white solid was removed by filtration, rinsing with water and then hexanes and dried to afford 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (19.75 g, 95%) as a tan solid.

^{1}H NMR (360 MHz, DMSO-d6) δ 12.11 (br s, 1H, NH), 9.59 (s, 1H, CHO), 4.17 (q, J = 6.7Hz, 2H, OCH_2CH_3), 2.44 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 1.26 (d, J = 6.7Hz, 3H, OCH_2CH_3).

Step 2

5-Formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (2 g, 10 mmol) was added to a solution of potassium hydroxide (3 g, 53 mmol) dissolved in methanol (3 mL) and water (10 mL). The mixture was refluxed for 3 hours, cooled to room temperature and acidified with 6 N hydrochloric acid to pH 3. The solid was collected by filtration, washed with water and dried in a vacuum oven overnight to give 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (1.6 g , 93%).

^{1}H NMR (300 MHz, DMSO-d6) δ 12.09 (s, br, 2H, NH & COOH), 9.59 (s, 1H, CHO), 2.44 (s, 3H, CH_3), 2.40 (s, 3H, CH_3).

Step 3

5-Fluoroisatin (8.2 g, 49.7 mmol) was dissolved in 50 mL of hydrazine hydrate and refluxed for 1 hour. The reaction mixtures were then poured in ice water. The precipitate was then filtered, washed with water and dried under vacuum oven to give 5-fluoro-2-oxindole (7.5 g).

Step 4

The reaction mixture of 5- fluorooxindole (100 mg, 0.66 mmol), 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (133 mg, 0.79 mmol), and 10 drops of piperidine in ethanol (3 mL) was stirred at 60 °C overnight and filtered. The solid was washed with 1 M of aqueous hydrochloride solution, water, and dried to afford 5-(5-fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (201 mg, quantitative) as a yellow solid. MS *m/z* (relative intensity, %) 299 ($[M-1]^{+\cdot}$, 100).

Example 4-Synthesis of 5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidene-methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-diethylamino-2-hydroxy-propyl)-amide

Step 1

To 2-chloromethyloxirane (95 g, 1.03 mole) was added a mixture of water (3.08 g, 0.17 mole) and diethylamine (106.2 mL, 1.03 mole) at 30 °C. The reaction mixture was then stirred at 28-35 °C for 6 hour and cooled to 20-25 °C to give 1-chloro-3-diethylamino-propan-2-ol.

Step 2

A solution of sodium hydroxide (47.9 g, 1.2 mole) in 78 mL water was added 1-chloro-3-diethylamino-propan-2-ol. The resultant was stirred at 20-25 °C for 1 hour, diluted with 178 mL of water and extracted with ether twice. The combined ether solution was dried with solid potassium hydroxide and evaporated to give 135 g of crude product which was purified by fraction distillation to give pure glycidyldiethylamine (98 g, 76%) as an oil.

Step 3

To the ice-cold solution of ammonium hydroxide (25 mL, 159 mmole) of 25% (w/w) was added glycidyldiethylamine dropwise (3.2 g, 24.8 mmol) over 10 minutes. The reaction mixture was stirred at 0 – 5 °C for 1 hour and then room temperature for 14 hours. The resulting reaction mixture was evaporated and distilled (84-90 °C at 500-600 mT) to yield 1-amino-3-diethylamino-propan-2-ol (3.3 g, 92%). MS *m/z* 147 ($[M+1]^{+\cdot}$).

Step 4

To the solution of 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (100 mg, 0.43 mmol), EDC (122.7 mg, 0.64 mmol) and HOBt (86.5 mg, 0.64 mmol) in 1.0 mL of DMF was added 1-amino-3-diethylamino-propan-2-ol (93.2 mg, 0.64 mmol). The resulting reaction solution was stirred at room temperature overnight and evaporated. The residue was suspended in 10 mL of water and filtered. The solid was washed with saturated sodium bicarbonate and water and dried in a high vaccum oven overnight to give crude procuct which was purified on column chromatography eluting with 6% methanol-dichlormethane containing triethylamine (2 drops/ 100mL of 6% methanol-dichloromethane) to give 5-(5-fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (3-diethylamino-2-hydroxy-propyl)-amide (62 mg, 34%) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d6) δ 13.70 (s, 1H, NH-1'), 10.90 (s, 1H, NH-1), 7.76 (dd, *J* = 2.38, 9.33 Hz, 1H, H-4), 7.72 (s, 1H, vinyl-H), 7.60 (m, br., 1H, $CONHCH_2CH(OH)$-$CH_2N(C_2H_5)_2$-4'), 6.93 (dt, *J* = 2.38, 8.99 Hz, 1H, H-5), 6.85 (dd, *J* = 4.55, 8.99 Hz, 1H, H-6), 3.83 (m, br, 1H, OH), 3.33 (m, 4H), 2.67 (m, br, 5H), 2.46 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 1.04 (m, br, 6H, CH_3x2). MS *m/z* (relative intensity, %) 427 ($[M+1]^{+\cdot}$, 100).

Example 5-Synthesis of 5-[5-Fluoro-2-oxo-1,2-dihydro-indol-(3Z)-ylidene-methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (R), (S) and (R/S) (Compounds 4, 5 and 6)

Step 1

A mixture of morpholine (2.6 mL, 30 mmol) and epichlorohydrin (2.35 ml, 30 mmol) in ethanol (50 mL) was stirred at 70 °C overnight. After removing the solvent, the residue was diluted with methylene chloride (50 mL). The clear solid precipitated was collected by vacuum filtration to give 1-chloro-3-morpholin-4-yl-propan-2-ol (2.0g, 37%). ^{1}H NMR (DMSO-d_6) δ 3.49 (t, J=4.8 Hz, 2H), 3.60 (t, J=4.6Hz, 2H), 3.75 (m, 4H, 2xCH_2), 4.20 (dd, J=5.2, 12 Hz, 2H), 4.54 (m, 2H), 4.62 (m, 1H, CH), 6.64 (d, J=6.4 Hz, 1H, OH). MS (m/z) 180.2 (M+1).

Step 2

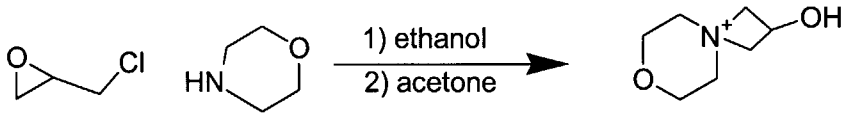
1-Chloro-3-morpholin-4-yl-propan-2-ol (2.0g, 11 mmol) was treated with the solution of NH_3 in methanol (25% by weight, 20 mL) at room temperature. Nitrogen was bulbbed into the reaction mixture to remove the ammonia. Evaporation of solvent gave the hydrogen chloride salt of 1-amino-3-morpholin-4-yl-propan-2-ol (2.0g, 91%). ^{1}H NMR (DMSO-d_6) δ

2.30 (d, J=6.0Hz, 2H), 2.36 (m, 4H, NCH_2), 2.65 (dd, J=8.4, 12.8Hz, 1H), 2.91 (dd, J=3.6, 12.8Hz, 1H), 3.52 (m, 4H, OCH_2), 3.87 (m, 1H, CH), 5.32 (s, 1H, OH), 8.02 (brs., 3H, NH_3^+). MS (m/z) 161.1 (M+1).

Step 3

5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (120 mg, 0.4 mmol) was condensed with 1-amino-3-morpholin-4-yl-propan-2-ol(74 mg, 0.48 mmol) to precipitate 5-[5-fluoro-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (65 mg, 36%). The mother liquid was evaporated to dryness and the residue was purified by flash chromatography to give additional 2N (70 mg, 39%). ^{1}H NMR (DMSO-d_6) δ 2.28 (m, 1H), 2.32 (m, 1H), 2.40 (m, 4H), 2.40, 2.42 (2xs, 6H, 2xCH_3), 3.15 (s, 1H), 3.31 (m, 1H), 3.55 (m, 4H), 3.78 (m, 1H), 4.73 (brs, 1H, OH), 6.82 (dd, J=4.5, 8.4Hz, 1H), 6.90 (td, ^{2}J=2.8, ^{3}J=10.0Hz, 1H), 7.53 (m, 1H), 7.70 (s, 1H), 7.74 (dd, J=2.0, 9.6Hz, 1H) (aromatic and vinyl), 10.87 (s, 1H, CONH), 13.66 (s, 1H, NH). LC-MS (m/z) 441.4 (M-1).

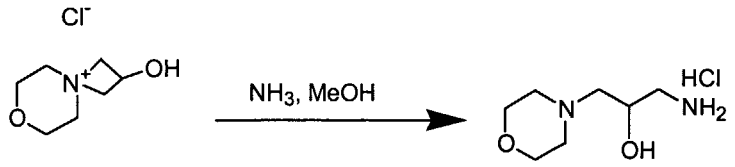
Synthesis of 2-hydroxy-7-oxa-4-azoniaspiro[3.5]nonane chloride



To a 1L 3-neck round bottom flask, fitted with a thermocouple, nitrogen inlet and a 250ml addition funnel, was charged morpholine (91.5g, 91.5 ml, 1.05 mole, 1.0 eq.) and 100ml of ethanol. The solution was stirred rapidly while adding epichlorohydrin (100g, 84.5 ml, 1.08 mole, 1.03 eq.) from the addition funnel over about 30 minutes. The temperature was monitored and when the pot temperature reached 27°C, the reaction was cooled with an ice water bath. The clear solution was stirred for 18 hours. The reaction was assayed by GC (dilute 5 drops of reaction mixture into 1 ml of ethanol and inject onto a 15m DB-5 capillary GC column with the following run parameters, Injector 250°C, detector 250°C, initial oven temperature 28°C warming to 250°C at 10°C per minute.) The reaction was complete with less than 3% morpholine remaining. The reaction was concentrated on the rotoevaporated at 50°C with full house vacuum until no more distillate could be condensed. The resulting oil was stored at room temperature for 24-48 hours or until a significant mass of crystals was observed (seeded will speed up the process). The slurry was diluted with 250ml of acetone and filtered. The solids were dried in the vacuum oven at 60°C for 18-24 hours. This

provided 84g of crystalline product. The mother liquors could be concentrated and the crystallization process repeated in increase recovery. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.55 (d, 1 H), 4.64 (m, 1 H), 4.53 (m, 2 H), 4.18 (m, 2 H), 3.74 (m, 4 H), 3.60 (m, 2 H), 3.48 (m, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 70.9, 61.39, 61.04, 60.25, 58.54, 57.80.

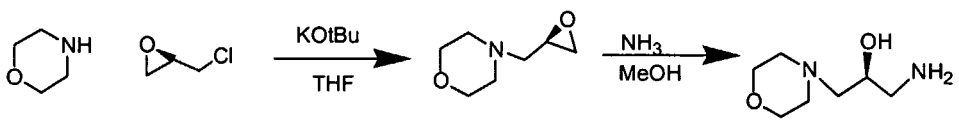
Synthesis of 1-amino-3-(4-morpholinyl)-2-propanol (Racemic)



To a 3L 1-neck round bottom flask with a magnetic stir bas was charged 2-hydroxy-7-oxa-4-azoniaspiro[3.5]nonane chloride (150g, 835mmole) followed by 23 wt. % anhydrous ammonia in methanol (2120ml). The flask was stoppered and the resulting clear solution was stirred at 20-23°C for 18 hours. GC under the conditions above showed no remaining starting material. The stopper was removed and the ammonia allowed to bubble out of the solution for 30 minutes. The flask was then transferred to a rotoevaporated and concentrated to a white solid with 45°C bath and full house vacuum. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.57 (dd, 2H), 3.3-3.5 (m, 6 H), 2.59 (m, 2 H), 2.2-2.4 (m, 6 H); ^{13}C NMR (100 MHz DMSO-d_6) δ 70.8, 67.1, 60.1, 53.8, 48.1.

Following the procedure described in Example 3 above but substituting 2-(RS)-1-amino-3-morpholin-4-yl-propan-2-ol with 2-(S)-1-amino-3-morpholin-4-yl-propan-2-ol prepared as described below the desired compound 5-[5-fluoro-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-(S)-hydroxy-3-morpholin-4-yl-propyl)-amide was obtained.

Synthesis of 1-amino-3-(4-morpholinyl)-2-propanol (Non-Racemic)



To 1L 3-neck round bottom flask, fitted with mechanical stirring, thermocouple and addition funnel, was charged morpholine (91.5g, 91.5 ml, 1.05 mole, 1.0 eq.) and 45 ml of t-butanol. The solution was stirred rapidly while adding R-epichlorohydrin (100g, 84.5 ml, 1.08 mole. 1.03 eq.) from the addition funnel over about 30 minutes. The temperature was

monitored and when the pot temperature reached 27°C, the reaction was cooled with an ice water bath. The clear solution was stirred for 18 hours. The reaction was assayed by GC (dilute 5 drops of reaction mixture into 1 ml of ethanol and inject onto a 15m DB-5 capillary GC column with the following run parameters, Injector 250°C, detector 250°C, initial oven temperature 28°C warming to 250°C at 10°C per minute). The reaction was complete with less than 3% morpholine remaining. The solution was cooled to 10°C and a 20 wt% solution of potassium t-butoxide in THF (576g) was added dropwise keeping the temperature less than 15°C. The resulting white slurry was stirred at 10-15°C for 2 hours and checked by GC using the above conditions. None of the chlorohydrin could be observed. The mixture was concentrated on the rotoevaporated using 50°C bath and full house vacuum. The resulting mixture was diluted with water (500ml) and methylene chloride. The phases were separated and the aqueous phase washed with methylene chloride (500ml). The combined organic layers were dried over sodium sulfate and concentrated to a clear, colorless oil. This provided 145g, 97% yield of the epoxide. ^{1}H NMR (400 MH$_z$, DMSO-d_6) δ 3.3 (dd, 4 H), 3.1 (m, 1 H), 2.6 (dd, 1 H), 2.5 (dd, 1 H), 2.4 (m, 4 H), 2.2 (dd, 2 H); ^{13}C NMR (100 MH$_z$, DMSO- d_6) δ 65.4, 60.1, 53.1, 48.9, 43.4.

The above crude epoxide was charged to a 3L 1-neck round bottom flask with a magnetic stir bar. Anhydrous ammonia in methanol (24% w/w 2.5L) was added, the flask was stoppered and the mixture stirred at room temperature for 24 hours. GC under the conditions above showed no remaining starting material. The stopper was removed and the ammonia allowed to bubble out of the solution for 30 minutes. The flask was then transferred to a rotoevaporated and concentrated to a clear colorless oil with 45°C bath and full house vacuum. This provided 124g of product. ^{1}H NMR (400 MH$_z$, DMSO-d_6) δ 3.57 (dd, 2H), 3.3-3.5 (m, 6 H), 2.59 (m, 2 H), 2.2-2.4 (m, 6 H); ^{13}C NMR (100 MH$_z$, DMSO- d_6) δ 70.8, 67.1, 60.1, 53.8, 48.1.

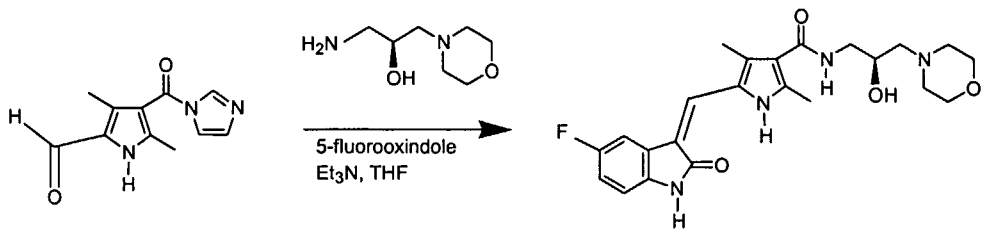
Synthesis of 1-amino-3-(4-morpholinyl)-2-(S)-propanol

To 1L 3-neck round bottom flask, fitted with mechanical stirring, thermocouple and addition funnel, was charged morpholine (91.5g, 91.5 ml, 1.05 mole, 1.0 eq.) and 200 ml of methanol. The solution was stirred rapidly while adding R-epichlorohydrin (100g, 84.5 ml, 1.08 mole, 1.03 eq.) from the addition funnel over about 30 minutes. The temperature was monitored and when the pot temperature reached 27°C, the reaction was cooled with an ice water bath. The clear solution was stirred for 18 hours. The reaction was assayed by GC

(dilute 5 drops of reaction mixture into1 ml of ethanol and inject onto a 15m DB-5 capillary GC column with the following run parameters, Injector 250°C, detector 250°C, initial oven temperature 28°C warming to 250°C at 10°C per minute.) The reaction was complete with less than 3% morpholine remaining. The solution was cooled to 10°C and a 25 wt. %solution of sodium methoxide in methanol (233g, 1.08 mole, 247 ml) was added dropwise keeping the temperature less than 15°C. The resulting white slurry was stirred at 10-15°C for 2 hours and checked by GC using the above conditions. None of the chlorohydrin could be observed. The mixture was concentrated on the rotoevaporator using 50°C bath and full house vacuum. The resulting mixture was diluted with water (500ml) and methylene chloride. The phases were separated and the aqueous phase washed with methylene chloride (500ml). The combined organic layers were dried over sodium sulfate and concentrated to a clear, colorless oil. This provided 145g, 97% yield of 1,2-epoxy-3-morpholin-4-ylpropane. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.3 (dd, 4 H), 3.1 (m, 1 H), 2.6 (dd, 1 H), 2.5 (dd, 1 H), 2.4 (m, 4 H), 2.2 (dd, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 65.4, 60.1, 53.1, 48.9, 43.4.

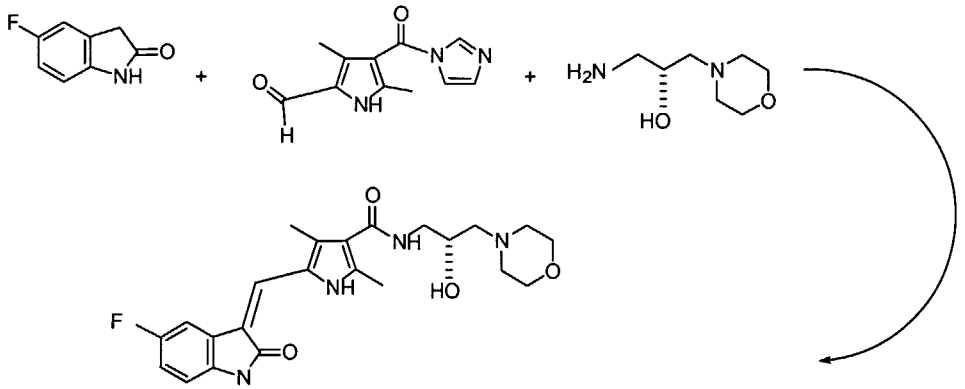
The above crude 1,2-epoxy-3-morpholin-4-ylpropane was charged to a 3L 1-neck round bottom flask with a magnetic stir bar. Anhydrous ammonia in methanol (24% w/w 2.5L) was added, the flask was stoppered and the mixture stirred at room temperature for 24 hours. GC under the conditions above showed no remaining starting material. The stopper was removed and the ammonia allowed to bubble out of the solution for 30 minutes. The flask was then transferred to a rotoevaporated and concentrated to a clear colorless oil with 45°C bath and full house vacuum. This provided 124g of 1-amino-3-(4-morpholinyl)-2-(S)-propanol.

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.57 (dd,2H), 3.3-3.5 (m, 6 H), 2.59 (m, 2 H), 2.2-2.4 (m, 6 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 70.8, 67.1, 60.1, 53.8, 48.1.



Imidazole amide (7.0 g, 32.3 mmol), amine (15.0 g, 64.6 mmol), 5-fluorooxindole (4.93 g, 32.6 mmol), triethylamine (9.79 g, 96.9 mmol), and THF (88 ml) were mixed and heated to 60°C. A brown solution formed. After stirring for 24 h at 60°C, the yellow slurry

was cooled to rt (room temperature) and filtered. The cake was washed with 80 ml THF and dried overnight at 50°C under house vacuum. A brown solid (23.2 g) was obtained. The solid was slurried in 350 ml water for 5 h at rt and filtered. The cake was washed with 100 ml water and dried at 50°C under house vacuum overnight. 8.31 g were obtained with 56% chemical yield.



A 0.25L flask fitted with a thermometer, condenser, magnetic stirring, and nitrogen inlet was charged with 4.92g 5-Fluorooxindole, 7.0g Imidazole amide, 15.5g (R)-1-Amino-3-(4-morpholinyl)-2-propanol, 9.78g Triethylamine and 88ml Tetrahydrofuran. The mixture was heated to 60° C for 16.5 hours. The reaction is cooled to ambient temperature and filtered. The solids obtained are slurried (3) three successive times in acetonitrile at 11ml/g, dried in vacuo for 3.6g (25.25%). [HPLC, Hypersil BDS, C-18, 5μ, (6:4), Acetonitrile:0.1M Ammonium Chloride, PHA-571437 = 4.05 min.] H^1NMR (DMSO): δ 10.86 (1H,bs); 7.75 (1H,d); 7.70 (1H,s); 7.50 (1H,m); 6.88 (2H,m); 4.72 (1H,bs); 3.78 (1H,bs); 3.56 (4H,m); 3.32 (6H,m); 3.15 (1H,m); 2.43 (8H,bm).

Example 6-Synthesis of 2,4-dimethyl-5-[2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide

5-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (113 mg, 0.4 mmol) was condensed with 1-amino-3-morpholin-4-yl-propan-2-ol (74 mg, 0.48 mmol) to precipitate 2,4-dimethyl-5-[2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (77 mg, 45.3%).

^{1}H NMR (DMSO-d_6) δ 2.27 (m, 1H), 2.32 (m, 1H), 2.40 (m, 4H), 2.40, 2.42 (2xs, 6H, 2xCH_3), 3.15 (s, 1H), 3.32 (m, 1H), 3.55 (m, 4H), 3.77 (m, 1H), 4.74 (d, J=4.8Hz, 1H, OH), 6.86 (d, J=7.6Hz, 1H), 6.96 (t, J=7.2 Hz, 1H), 7.10 (t, J=7.6Hz, 1H), 7.49 (t, J=5.6 Hz, 1H), 7.61 (s, 1H), 7.77 (d, J=8.0 Hz, 1H) (aromatic and vinyl), 10.88 (s, 1H, CONH), 13.62 (s, 1H, NH). LC-MS (m/z) 425.4 (M+1).

Example 7-Synthesis of 5-[5-chloro-2-oxo-1,2-dihydro-indol-(3Z)-ylidene-methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (Compound 7)

5-(5-Chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (126.6 mg, 0.4 mmol) was condensed with 1-amino-3-morpholin-4-yl-propan-2-ol (74 mg, 0.48 mmol) to precipitate 5-[5-Chloro-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (107 mg, 58%).

^{1}H NMR (DMSO-d_6) δ 2.29 (m, 1H), 2.33 (m, 1H), 2.39(m, 4H), 2.40, 2.42 (2xs, 6H, 2xCH_3), 3.15 (s, 1H), 3.37 (m, 1H), 3.55 (m, 4H), 3.77 (m, 1H), 4.74 (d, J=4.8Hz, 1H, OH), 6.85 (d, J=8.4Hz, 1H), 7.11 (dd, J=2.0, 8.0Hz, 1H), 7.53 (t, J=5.6Hz, 1H), 7.75 (s, 1H), 7.97 (d, J=2.0Hz, 1H) (aromatic and vinyl), 10.99 (s, 1H, CONH), 13.62 (s, 1H, NH). LC-MS (m/z) 457.4 (M-1).

Example 8-Synthesis of 5-[5-bromo-2-oxo-1,2-dihydro-indol-(3Z)-ylidene-methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide

5-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (72.2 mg, 0.2 mmol) was condensed with 1-amino-3-morpholin-4-yl-propan-2-ol (38mg, 0.24 mmol) to precipitate 5-[5-Bromo-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-propyl)-amide (55 mg, 55%).

^{1}H NMR (DMSO-d_6) δ 2.27 (m, 1H), 2.32 (m, 1H), 2.39(m, 4H), 2.41, 2.42 (2xs, 6H, 2xCH_3), 3.13 (s, 1H), 3.35 (m, 1H), 3.55 (m, 4H), 3.77 (m, 1H), 4.74 (d, J=4.4Hz, 1H, OH), 6.80 (d, J=8.4Hz, 1H), 7.24 (dd, J=2.0, 8.0Hz, 1H), 7.51 (t, J=5.6Hz, 1H), 7.76 (s, 1H), 8.09 (d, J=2.0Hz, 1H) (aromatic and vinyl), 10.99 (s, 1H, CONH), 13.62 (s, 1H, NH). LC-MS (m/z) 503.4 (M-1).

Example 9-Synthesis of 2,4-dimethyl-5-[2-oxo-1,2-dihydro-indol-(3Z)-ylidene-methyl]-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-[1,2,3]triazol-1-yl-propyl)-amide

Step 1

A mixture of 3-[1,2,3]triazole (2.0 g, 29 mmol), epichlorohydrin (3.4 ml, 43.5 mmol) and N, N-diisopropyl-ethylamine (2.6 mL, 15 mmol) in ethanol (50 mL) was stirred at room temperature overnight. After removing the solvents, the residue was purified by flash chromatography (CH_2Cl_2/CH_3OH=100/1-100/2-100/4) to give 1-chloro-3-(1,2,3)-triazol-2-ylpropan-2-ol (2.1 g, 45%). ^{1}H NMR ($CDCl_3$) δ 3.52 (m, 2H, OH and CH_2), 3.60 (dd, J=5.2,

11.2 Hz, 1H), 4.36 (m, 1H, CH), 4.68 (m, 2H), 7.67 (s, 2H). MS (m/z) 162.1 (M+1) and 1-chloro-3-(1,2,3)triazol-1-ylpropan-2-ol (2.3 g, 49%). ^{1}H NMR ($CDCl_3$) δ 3.56 (s, 1H), 3.57 (s, 1H), 4.35 (m, 1H), 4.53 (dd, J=7.2, 14 Hz, 1H), 4.67 (dd, J=3.8, 14Hz, 1H), 7.67 (s, 1H), 7.71 (s, 1H). MS (m/z) 162.1 (M+1).

Step 2

1-Chloro-3(1,2,3)triazol-1-ylpropan-2-ol (2.3g, 13 mmol) was treated with the solution of NH_3 in methanol (25% by weight, 20 mL) at 60 °C overnight in a sealed pressure vessel. After cooling to room temperature, nitrogen was bulbbed into the reaction mixture to remove the ammonia. Evaporation of solvent gave the hydrogen chloride salt of 1-amino-3-(1,2,3)triazol-1-ylpropan-2-ol (2.57g, 100%).

^{1}H NMR (DMSO-d_6) δ 2.68 (dd, J=8.8, 12.8Hz, 1H), 2.97 (dd, J=3.6, 12.8Hz, 1H), 4.15 (m, 1H), 4.44 (dd, J=6.4, 14Hz, 1H), 4.57 (dd, J=4.6, 14Hz, 1H), 5.95 (d, J=5.2Hz, 1H, OH), 7.77 (s, 1H), 8.01 (brs., 3H, NH_3^+), 8.12 (s, 1H). MS (m/z) 143.1 (M+1).

Step 3

5-(2-Oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (113 mg, 0.4 mmol) was condensed with 1-amino-3(1,2,3)triazole-1-yl-propan-2-ol (85 mg, 0. 48mmol) to precipitate 2,4-dimethyl-5-[2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-[1,2,3]triazol-1-yl-propyl)-amide (70 mg, 41%).

^{1}H NMR (DMSO-d_6) δ 2.45, 2.48 (2xs, 6H, 2xCH_3), 3.35 (m, 2H), 4.02 (m, 1H), 4.32 (dd, J=7.6, 14 Hz,1H), 4.53 (dd, J=3.4, 14 Hz,1H), 5.43 (d, J=5.6Hz, 1H, OH), 6.91 (d, J=7.6Hz, 1H), 7.01 (t, J=7.6 Hz, 1H), 7.15 (t, J=8.0Hz, 1H), 7.66 (s, 1H), 7.12 (t, J=5.6 Hz, 1H), 7.74 (s, 1H), 7.77 (d, J=7.6 Hz, 1H), 8.11 (s, 1H), 10.93 (s, 1H, CONH), 13.68 (s, 1H, NH). LC-MS (m/z) 405.4 (M-1).

Example 10-Synthesis of 5-[5-fluoro-2-oxo-1,2-dihydro-indol-(3Z)-ylidene-methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-[1,2,3]triazol-1-yl-propyl)-amide

5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (120 mg, 0.4 mmol) was condensed with 1-amino-3(1,2,3)triazol-1-yl-propan-2-ol (85 mg, 0. 48mmol) to precipitate 5-[5-fluoro-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-[1,2,3]triazol-1-yl-propyl)-amide (100 mg, 62%).

^{1}H NMR (DMSO-d_6) δ 2.42, 2.44 (2xs, 6H, 2xCH_3), 3.27 (m, 2H), 3.98 (m, 1H), 4.27 (dd, J=7.6, 14 Hz,1H), 4.50 (dd, J=3.4, 13.6 Hz,1H), 5.38 (d, J=5.6Hz, 1H, OH), 6.82 (dd, J=4.4, 8.4Hz, 1H), 6.91 (td, ^{2}J=2.4, ^{3}J=9.0Hz, 1H), 7.70 (m, 3H), 7.75 (dd, J=2.4, 9.2Hz, 1H), 8.11 (s. 1H), 10.93 (s, 1H, CONH), 13.73 (s, 1H, NH). LC-MS (m/z) 423.4 (M-1).

Example 11-Synthesis of 5-[5-chloro-2-oxo-1,2-dihydro-indol-(3Z)-ylidene-methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-[1,2,3]triazol-1-yl-propyl)-amide

5-(5-Chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (126.6 mg, 0.4 mmol) was condensed with 1-amino-3(1,2,3)triazole-1-yl-propan-2-ol (85 mg, 0. 48mmol) to precipitate 5-[5-Chloro-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-[1,2,3]triazol-1-yl-propyl)-amide (48 mg, 27%).

^{1}H NMR (DMSO-d_6) δ 2.42, 2.44 (2xs, 6H, 2xCH_3), 3.27 (m, 2H), 3.99 (m, 1H), 4.28 (dd, J=7.8, 14 Hz,1H), 4.51 (dd, J=3.2, 14 Hz,1H), 5.39 (d, J=6.0Hz, 1H, OH), 6.85 (d, J=8.4Hz, 1H), 7.12 (dd, J=2.0, 8.2Hz, 1H), 7.70 (m, 2H), 7.74 (s, 1H), 7.97 (d, J=2.0Hz, 1H), 8.07 (s, 1H), 10.99 (s, 1H, CONH), 13.65 (s, 1H, NH). LC-MS (m/z) 439.4 (M-1).

Example 12-Synthesis of 5-[5-bromo-2-oxo-1,2-dihydro-indol-(3Z)-ylidene-methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-[1,2,3]triazol-1-yl-propyl)-amide

5-(5-Bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (144.4 mg, 0.4 mmol) was condensed with 1-amino-3(1,2,3)triazole-1-yl-propan-2-ol (85 mg, 0.48mmol) to precipitate 5-[5-bromo-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid (2-hydroxy-3-[1,2,3]triazol-1-yl-propyl)-amide (130 mg, 67%).

^{1}H NMR (DMSO-d_6) δ 2.41, 2.44 (2xs, 6H, 2xCH_3), 3.27 (m, 2H), 3.99 (m, 1H), 4.28 (dd, J=7.6, 14 Hz,1H), 4.50 (dd, J=3.6, 14 Hz,1H), 5.40 (d, J=5.6Hz, 1H, OH), 6.81 (d, J=8.4Hz, 1H), 7.24 (dd, J=2.0, 8.0Hz, 1H), 7.70 (m, 2H), 7.77 (s, 1H), 8.07 (s. 1H), 8.10 (d, J=1.6Hz, 1H), 11.0 (s, 1H, CONH), 13.64 (s, 1H, NH). LC-MS (m/z) 485.4 (M-1).

Example 13-5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide (Compound 1)

5-Fluoro-1,3-dihydroindol-2-one (0.54 g, 3.8 mmol) was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide to give 0.83 g (55%) of the title compound as a yellow green solid.

^{1}HNMR (360 MHz, DMSO-d_6) δ 13.66 (s, 1H, NH), 10.83 (s, br, 1H, NH), 7.73 (dd, J = 2.5 & 9.4 Hz, 1H), 7.69 (s, 1H, H-vinyl), 7.37 (t, 1H, $CONHCH_2CH_2$), 6.91 (m, 1H), 6.81-6.85 (m, 1H), 3.27 (m, 2H, CH_2), 2.51 (m, 6H, 3xCH_2), 2.43 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 0.96 (t, J = 6.9 Hz, 6H, $N(CH_2CH_3)_2$).

MS-EI m/z 398 [M+].

Alternative synthesis of 5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide

Hydrazine hydrate (55 %, 3000 mL) and 5-fluoroisatin (300 g) were heated to 100 °C. An additional 5-fluoro-isatin (500 g) was added in portions (100 g) over 120 minutes with stirring. The mixture was heated to 110 °C and stirred for 4 hours. The mixture was cooled to room temperature and the solids collected by vacuum filtration to give crude (2-amino-5-fluoro-phenyl)-acetic acid hydrazide (748 g). The hydrazide was suspended in water (700 mL) and the pH of the mixture adjusted to < pH 3 with 12 N hydrochloric acid. The mixture was stirred for 12 hours at room temperature. The solids were collected by vacuum filtration and washed twice with water. The product was dried under vacuum to give 5-fluoro-1,3-dihydro-indol-2-one (600 g, 73 % yield) as a brown powder. ^{1}H-NMR (dimethylsulfoxide-d_6) δ 3.46 (s, 2H, CH_2), 6.75, 6.95, 7.05 (3 x m, 3H, aromatic), 10.35 (s, 1H, NH). MS m/z 152 [M+1].

3,5-Dimethyl-1H-pyrrole-2,4-dicarboxylic acid 2-tert-butyl ester 4-ethyl ester (2600 g) and ethanol (7800 mL) were stirred vigorously while 10 N hydrochloric acid (3650 mL) was slowly added. The temperature increased from 25 °C to 35 °C and gas evolution began. The mixture was warmed to 54 °C and stirred with further heating for one hour at which time the temperature was 67 °C. The mixture was cooled to 5 °C and 32 L of ice and water were slowly added with stirring. The solid was collected by vacuum filtration and washed three times with water. The solid was air dried to constant weight to give of 2,4-dimethyl-1H-pyrrole-3-carboxylic acid ethyl ester (1418 g, 87 % yield) as a pinkish solid. ^{1}H-NMR (dimethylsulfoxide-d_6) δ 2.10, 2.35 (2xs, 2x3H, 2xCH_3), 4.13 (q, 2H, CH_2), 6.37 (s, 1H, CH), 10.85 (s, 1H, NH). MS m/z 167 [M+1].

Dimethylformamide (322 g) and dichloromethane (3700 mL) were cooled in an ice bath to 4 °C and phosphorus oxychloride (684 g) was added with stirring. Solid 2,4-dimethyl-1H-pyrrole-3-carboxylic acid ethyl ester (670 g) was slowly added in aliquots over 15 minutes. The maximum temperature reached was 18 °C. The mixture was heated to

reflux for one hour, cooled to 10 °C in an ice bath and 1.6 L of ice water was rapidly added with vigorous stirring. The temperature increased to 15 °C. 10 N Hydrochloric acid (1.6 L) was added with vigorous stirring. The temperature increased to 22 °C. The mixture was allowed to stand for 30 minutes and the layers allowed to separate. The temperature reached a maximum of 40 °C. The aqueous layer was adjusted to pH 12-13 with 10 N potassium hydroxide (3.8 L) at a rate that allowed the temperature to reach and remain at 55 °C during the addition. After the addition was complete the mixture was cooled to 10 °C and stirred for 1 hour. The solid was collected by vacuum filtration and washed four times with water to give 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid ethyl ester (778 g, 100 % yield) as a yellow solid. ^{1}H-NMR (DMSO-d_6) δ 1.25 (t, 3H, CH_3), 2.44, 2.48 (2xs, 2x3H, 2xCH_3), 4.16 (q, 2H, CH_2), 9.59 (s, 1H, CHO), 12.15 (br s, 1H, NH). MS m/z 195 [M+1].

5-Formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid ethyl ester (806 g), potassium hydroxide (548 g), water (2400 mL) and methanol (300 mL) were refluxed for two hours with stirring and then cooled to 8 °C. The mixture was extracted twice with dichloromethane. The aqueous layer was adjusted to pH 4 with 1000 mL of 10 N hydrochloric acid keeping the temperature under 15 °C. Water was added to facilitate stirring. The solid was collected by vacuum filtration, washed three times with water and dried under vacuum at 50 °C to give 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic (645 g, 93.5 % yield) acid as a yellow solid. ^{1}H-NMR (DMSO-d_6) δ 2.40, 2.43 (2xs, 2x3H, 2xCH_3), 9.57 (s, 1H, CHO), 12.07 (br s, 2H, NH+COOH). MS m/z 168 [M+1].

5-Formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (1204 g) and 6020 mL of dimethylformamide were stirred at room temperature while 1-(3-dimethyl-aminopropyl-3-ethylcarbodiimide hydrochloride (2071 g), hydroxybenzotriazole (1460 g), triethylamine (2016 mL) and diethylethylenediamine (1215 mL) were added. The mixture was stirred for 20 hours at room temperature. The mixture was diluted with 3000 mL of water, 2000 mL of brine and 3000 mL of saturated sodium bicarbonate solution and the pH adjusted to greater than 10 with 10 N sodium hydroxide. The mixture was extracted twice with 5000 mL each time of 10 % methanol in dichloromethane and the extracts combined, dried over anhydrous magnesium sulfate and rotary evaporated to dryness. The mixture was with diluted with 1950 mL of toluene and rotary evaporated again to dryness. The residue was triturated with 3:1 hexane:diethyl ether (4000 mL). The solids were collected by vacuum filtration, washed twice with 400 mL of ethyl acetate and dried under vacuum at 34 °C for 21 hours to give 5-

formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide (819 g, 43 % yield) as a light brown solid. ^{1}H-NMR (dimethylsulfoxide-d_6) δ 0.96 (t, 6H, 2xCH_3), 2.31, 2.38 (2xs, 2 x CH_3), 2.51 (m, 6H 3xCH_2), 3.28 (m, 2H, CH_2), 7.34 (m, 1H, amide NH), 9.56 (s, 1H, CHO), 11.86 (s, 1H, pyrrole NH). MS m/z 266 [M+1].

5-Formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)-amide (809 g), 5-fluoro-1,3-dihydro-indol-2-one (438 g), ethanol (8000 mL) and pyrrolidine (13 mL) were heated at 78 °C for 3 hours. The mixture was cooled to room temperature and the solids collected by vacuum filtration and washed with ethanol. The solids were stirred with ethanol (5900 mL) at 72 °C for 30 minutes. The mixture was cooled to room temperature. The solids were collected by vacuum filtration, washed with ethanol and dried under vacuum at 54 °C for 130 hours to give 5-[5-fluoro-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)-amide (1013 g, 88 % yield) as an orange solid. ^{1}H-NMR (dimethylsulfoxide-d6) δ 0.98 (t, 6H, 2xCH3), 2.43, 2.44 (2xs, 6H, 2xCH3), 2.50 (m, 6H, 3xCH2), 3.28 (q, 2H, CH2), 6.84, 6.92, 7.42, 7.71, 7.50 (5xm, 5H, aromatic, vinyl, CONH), 10.88 (s, 1H, CONH), 13.68 (s, 1H, pyrrole NH). MS m/z 397 [M-1].

The malic salt of 5-(5-Fluoro-2-oxo-1,2-dihydroindol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylamino-ethyl)amide can be prepared according to the disclosure of U.S. Patent Application Serial No. 10/281,985, filed August 13, 2002, which claims priority to U.S. Patent Provisional Application No. 60/312,353, filed August 15, 2001, which is incorporated by reference in its entirety.

Synthesis of 5-(5-bromo-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid, 5-(5-chloro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid, 5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid is described in Serial No. 09/783,264 filed on February 14th, 2001, titled "PYRROLE SUBSTITUTED 2-INDOLINONE --PROTEIN KINASE INHIBITORS", the disclosure of which is incorporated herein in its entirety.

Example 14-5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide (Compound 2)

5-Fluoro-1,3-dihydro-indolin-2-one was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-pyrrolidin-1-yl-ethyl)-amide to yield the title compound.

MS + ve APCI 397 [M+1].

Exmaple 15-5-(5-Fluoro-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-ethylamino-ethyl)-amide (Compound 8)

5-Formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-ethylamino-ethyl)-amide (99g), ethanol (400 ml), 5-fluoro-2-oxindole (32 g) and pyrrolidine (1.5 g) were refluxed for 3 hours with stirring. The mixture was cooled to room temperature and the solids collected by vacuum filtration. The solids were stirred in ethanol at 60°C, cooled to room temperature and collected by vacuum filtration. The product was dried under vaccuum to give 5-(5-Fluoro-2-oxo-1,2-dihydro-indol-(3Z)-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-ethylamino-ethyl)-amide (75g, 95% yield). ^{1}H-NMR (dimethylsulfoxide-d_6) δ 1.03 (t, 3H, CH_3), 2.42, 2.44 (2xs, 6H, 2xCH_3), 2.56 (q, 2H, CH_2), 2.70, 3.30 (2xt, 4H, 2xCH_2), 6.85, 6.92, 7.58, 7.72, 7.76 (5xm, 5H, aromatic, vinyl, and CONH), 10.90 (br s, 1H, CONH), 13.65 (br s, 1H, pyrrole NH).
MS *m/z* 369 [M-1].

Example 16- 3-[5[Methyl-2-(2-oxo-1,2-dihydroindol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid (Compound 10)

1,3-dihydroindole-2-one was condensed with 3-(2-formyl-5-methyl-1H-pyrrol-3-yl)-propionic acid to give the title compound.

Example 17-5-(5-Fluoro-2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-morpholin-4-yl-ethyl)-amide (Compound 3)

5-Fluoro-1,3-dihydro-indolin-2-one was condensed with 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-morpholin-1-yl-ethyl)-amide to yield the title compound.

Biologic Examples

The first cell line used was the OC1-AML5 cell line known to express the FLT-3 tyrosine kinase. This cell line was maintained in conventional medium containing cytokines to maintain growth in liquid culture. This cell line provides a model to assess activation and inhibition of FLT-3 signaling by FLT-3 ligand and compounds which may inhibit FLT-3. The biological consequences of FLT-3 can be assessed with this cell line.

Example 1-Assessment of FLT-3 signaling

Cells were stimulated with FLT-3 ligand and lysed. FLT-3 was immunoprecipitated from lysates with a commercially available antibody. Proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to membranes and analyzed by Western blotting for phosphotyrosine and subsequently for total FLT-3 protein as control.

The OC1-AML5 cell line which express FLT-3-wild type was obtained (Pharmacia). First, the ability of FLT-3 ligand to stimulate and compound 1 to inhibit biological responses mediated via FLT-3 was assessed by analysis of cell viability (trypan blue assays) and cell proliferation (alamar blue assay). Data suggests that that the FLT-3 ligand increased cell numbers where some inhibition was apparent in response to compound 1, thereby suggesting that compound 1 inhibits FLT-3.

Example 2-FLT-3 expression and phosphorylation by Immunoprecipitation/Western Analysis

(i) OC1-AML5 cells

Using OC1-AML5 cells, it was observed that FLT-3 ligand stimulates phosphorylation of FLT-3. Phosphorylation was decreased by compound 1, confirming that compound 1 inhibits the FLT-3 receptor.

Activation of downstream pathways by FLT-3 ligand was also investigated, specifically for Stat5 and erk. Stat5 and Erk are downstream mediators of RTK signaling, and may provide readouts for FLT-3 signaling. Stat 5 is a transcription factor which regulates many genes involves in cell survival and proliferation. Erk1/2 are kinases on the Raf signaling pathway. Activation of Stat5 was observed in response to FLT-3 ligand by 3 approaches; IP/Western, direct Western using phospho-specific antibodies and gel shift analysis. Stat5 activity was inhibited by compound 1. Phosphorylation of erk1/2 was also activated by FLT-3 ligand and inhibited by compound 1, whereas IL-3 dependent erk activation was not inhibited, suggesting that the effect of compound 1 is specific.

(ii) normal PBMC

To investigate FLT-3 signaling in normal blood cells, peripheral blood mononuclear cells (PBMC) were isolated from normal donor blood and used for analysis of FLT-3 signaling. FLT-3 ligand stimulated Stat5 phosphorylation in PBMC and activated FLT-3 was weakly detected.

Example 3-Use of additional cell lines; MV411 (ITD mutant FLT-3) and RS411 (wild type FLT-3) to investigate effects of compound 1 on proliferation in vitro.

This examples was performed to determine if inhibition of FLT-3 signaling by compound 1, observed in OC1-AML5 cell lines, is also observed in wild-type (RS411) or mutant FLT-3 (MV411).

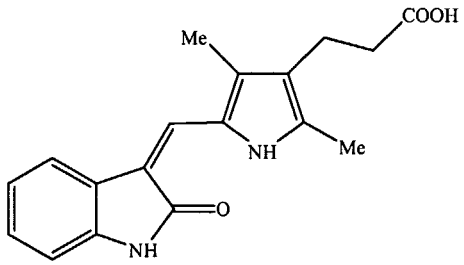
Cell lines were obtained from ATCC. Analysis of cell proliferation showed that compound 1 inhibited expansion of both RS411 (wild type FLT-3) and MV411. This indicated that compound 1 could potentially target ITD mutant FLT-3 in leukemias, in addition to targeting wild type FLT-3.

To address if ITD-mutant cells show increased sensitivity to compound 1 additional experiments were performed. Apoptosis was measured by analysis of PARP cleavage and by caspase 3 staining. Both methods indicated that compound 1 causes apoptosis, and that ITD-mutant cells appear more sensitive than wild type cells. See figures 1 and 2.

Example 4-Effect of compound 1 on FLT-3 phosphorylation in MV411 (ITD mutant FLT-3) and RS411 (wild type FLT-3)

FLT-3 was immunoprecipitated from lysates with a commercially available antibody. Proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to membranes and analyzed by Western blotting for phosphotyrosine and subsequently for total FLT-3 protein as control.

IP/W analysis showed that compound 1 inhibits FLT-3 phosphorylation in both MV411 (ITD mutant FLT-3) and RS411 (wild type FLT-3) cell lines. Approximate IC_{50}s for compound 1 on WT and ITD mutant FLT-3 are 250nM and 50nM respectively, supporting the possibility that ITD mutants have increased sensitivity to compound 1. See figure 3. The comparative example is a known protein kinase inhibitor having the following formula:



The comparative compound exhibited no inhibition of either wild-type FLT-3 or mutant FLT-3.

Compound	Wild-type FLT-3	Mutant FLT-3
1	++	+++
2	++	++
3	+/-	+
4	++	nd
5	++	nd

6	+ +	+ +
7	+ +	nd
8	+ +	nd
9	+ +	nd
10	+/-	-
Comparative	-	-

+ + + very strong inhibition

+ +: inhibition

+/-: weak inhibition

-: no inhibition

nd: not determined

Example 5-Establishment of blood spike model using MV411 (ITD mutant FLT-3) and RS411 (wild type FLT-3) to investigate effects of compound 1 *in vitro*

The blood spike model is an *ex-vivo* model, developed to help translate preclinical observations with *in vitro* models to the clinical situation. In patients with leukemia where targets are expressed on blood cells, it is desirable to monitor effects of drug by analysis of target (such as FLT-3) phosphorylation on blood cells or whole blood. In the blood spike model, cells expressing the receptor of interest are spiked into normal human blood donor blood (normal blood does not express high levels of target protein). Compound and ligand are added as necessary and cells are lysed and analyzed for protein phosphorylation and expression by immunoprecipitation and Western blot analysis. This mimics the clinical situation and enables prediction of the time and dose-dependence of compound needed to inhibit the target.

To predict the ability of compound 1 to inhibit FLT-3 phosphorylation in leukemia, cell lines expressing FLT-3 were added to normal human donor blood, and the kinetics and dose-dependence of inhibition of phosphorylation was measured. This method should provide a more accurate determination of compound exposure required for inhibition of target phosphorylation than conventional biochemical or cellular assays performed in synthetic media.

Example 6-Establishment of *in vivo* models using MV411 and RS411 cells and effect of Compound 1 on tumorigenesis

Tumor cells, MV411 in the example shown were implanted subcutaneously in the hindflank of athymic mice. Treatment with compound or vehicle control was started when

tumors had reached a specific size. For measurement of efficacy, tumor growth was measured at various subsequent time points using vernier calipers. For analysis of phosphorylation, tumors were resected following dosing (4 hours here), pulverized in liquid nitrogen and homogenized in lysis buffer. FLT-3 and Stat5 phosphorylation were measured by immunoprecipitation and Western blot analysis.

Athymic mice were injected subcutaneously with MV411 and RS411 cells to cause tumor formation. MV411 led to rapid tumor formation, while RS411 cells also formed tumors, though more slowly. Treatment with compound 1 dramatically reduced tumor size to almost undetectable within 4 days of treatment. In addition, activated FLT-3 was detectable in untreated tumors, and completely inhibited by a 4 hour treatment with compound 1. See figure 4a and 4b. This data provides evidence that compound has efficacy against FLT-3 driven tumors in vivo, consistent with inhibition of FLT-3 phosphorylation.

Example 7-*In vivo* Bone Marrow Model for VEGF production

NOD-SCID mice were pretreated with cyclophosphamide (Neosar, Pharmacia, Kalamazoo, MI) by intraperitoneal injection of 150 mg/kg/day for 2 days [46], followed by 24 hours of rest prior to intravenous (i.v.) injection of 5 X 10^6 cells via the tail vein. At experimental endpoints, mice were anesthetized, followed by terminal blood collection via intracardiac puncture. Bone marrow cell suspensions were prepared by flushing mouse femurs with cold, sterile PBS. A range of doses of compound 1 or its vehicle were orally administered once daily, as indicated in Figure and Table legends. For all studies, a paired Student's t test was used to assess differences between treated and control groups ($P < 0.05$ was considered significant).

	MV4;11		OC1-AML5		RS4;11	
CMPD 1 uM	mean sd	%	mean sd	%	mean sd	%
0	346.7 ±	100.	100.8 ±	100.	31.03 ±	100.
0.00	287.8 ±	83.0	92.5 ±	91.8	32.82 ±	105.
0.01	65.4 ±	18.9	35.6 ±	35.3	9.62 ±	31.0
0.1	31.2 ±	9.0	33.6±	33.3	1.24 ±	4.0
1	30.5 ±	8.8	28.3 ±	28.0	2.3 ±	7.4
10	23.3 ±	6.7	15.6 ±	15.5	2.94 ±	9.5

The data above indicate that treatment with compound 1 prolonged survival in a dose-dependent manner with highest efficacy at 20 mg/kg/day of compound 1.

Example 8-Detection of VEGF in NOD-SCID mice

Plasma from the NOD-SCID mice described above was analyzed by ELISA for VEGF protein levels using a commercially available kit. Consistent with in vitro data showing that FLT-3 activation (wild type or ITD) correlates with VEGF secretion (as seen in the table above) which is inhibited by compound 1, it was determined that VEGF was detectable in plasma of diseased mice (mean 49 pg/ml) in compound 1 treated mice. This data suggests that VEGF is a target of FLT-3 signaling and may be a biomarker for FLT-3 activity.

Example 9-*In vivo* human study of inhibition of phosphorylation of FLT-3

A phase I single dose clinical study in AML patients was conducted. The primary objective was to assess modulation (inhibition) of FLT-3 phosphorylation. All patients also had correlative pharmacokinetics and FLT-3 genotyping performed. FLT-3 phosphorylation was analyzed predose and at 4, 6, 8, 10, 12, 24, 48 hours after compound 1 administration. Methods of development showed that the optimal method to enable FLT-3 phosphorylation analysis was direct addition of whole blood, once drawn form the AML patient, to lysis buffer, prior to freezing on dry ice. Subsequently samples were thawed and analyzed for FLT-3 phosphorylation by immunosuppression using bead conjugated anti-FLT-3 antibodies, followed by Western blotting for phospho-tyrosine and FLT-3, as for the blood spike model (example 5). The primary endpoint, >50% inhibition of FLT-3 phosphorylation in 3/6 pts, was reached in 3 pts at each dose level >200mg, including both WT and mutant FLT-3 patients. Two patients are shown. The data generated in this study was consistent with preclinical in vitro and in vivo tumor model data and verifies that compound 1 inhibits FLT-3 in humans. This novel single dose study using whole peripheral blood analysis demonstrated that compound 1 modulates FLT-3 and downstream signaling pathways which mediate survival and proliferation of AML blasts in vivo.

Protocol for collection of blood for receptor target modulation studies

A. Lysis buffer supplied by Sugen (20 ml frozen aliquots, 1.5x stock,is prepared as detailed below):

i. Thaw lysis buffer (1.5X stock, contains protease/phosphatase inhibitors) at room temperature. 20 ml of lysis buffer is required for each 10 ml blood.

ii. Store thawed lysi buffer on ice.

iii. Draw blood and add 10 ml blood to 20 ml lysis buffer.

iv. Mix by inverting several times and place immediately on dry ice or at -70°C.

v. Store at -70°C and transport on dry ice.

(i) Lysis buffer composition-Composition yields 500 ml of 1.5x stock

Volume	Stock	Final Concentration
10 ml	1M Tris, pH 7.5	20mM
13.7 ml	5M NaCl	137 mM
50 ml	Glycerol	10%
5 ml	NP-40	1%
5 ml	10% SDS	0.1%
2 ml	0.5 EDTA	2mM

Deionized water is added to equal 500 ml. Then the mixture is filtered through a 0.2μM filter. The mixture is stored at 4°C or in aliquots at -20°C if protease inhibitors are added.

(ii) Addition of protease inhibitors

To 9 ml of 1.5x lysis buffer is added:

Volume	Stock	Final Concentration
0.5 ml	1M NaF	50mM
100 μl	100mM Na_3VO_4	1mM
200 μl	protease inhibitor cocktail	
200 μl	100mM (PefaBloc* or PMSF)	2mM

Protease inhbitor cocktail = 100 μM leupeptin, 200μM pepstatin, 60 μM aprotonin, 2mM bestatin.

*PefaBloc is a more stable water soluble form of PMSF, available from Boehringer Mannheim.

Method for analysis of FLT-3 phosphorylation in blood: Frozen samples were stored at -70°C until use. While blood lysate was rapidly thawed at 37°C and lysed in 2x volume of lysis buffer (20mM Tris, pH 7.5, 137 mM NaCl, 10% glycerol, 1% NP-40, 0.1% SDS, 2mM EDTA, 50mM NaF, 1mM Na_3VO_4, 2mM Pefabloc, 2μg/mL aprotonin, 3.5

μg/mL betstatin, 0.5 μg/ml E-64, 0.5 μg/ml leupeptin and 0.7 μg/ml pepstatin A). The amount of protein in each lysate was determined using the BCA Protein Assay (Pierce, Rockford, Il). Approximately 35 mg of lysate from each sample was immunoprecipitated for FLT-3, c-kit or Stat5.

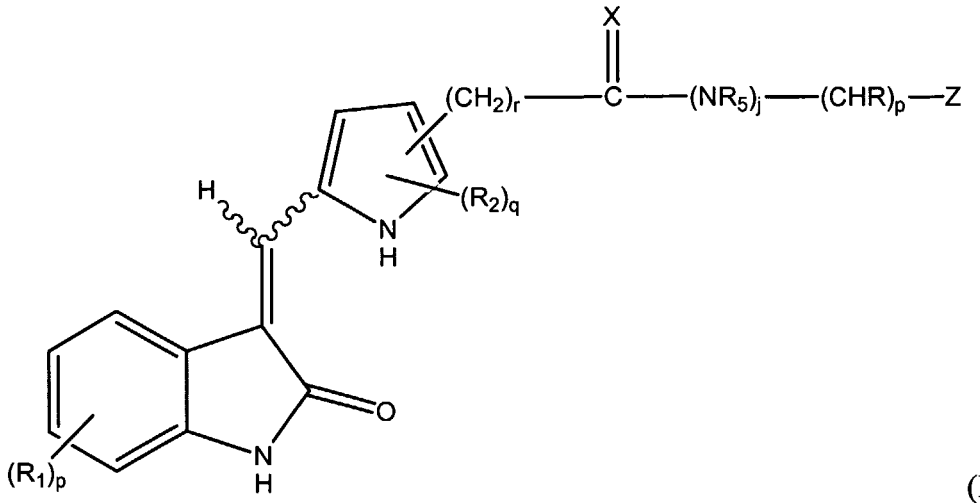
Immunoprecipitation and Western Blot (IP/W) analysis: Cells were lysed in lysis buffer (20 mM Tris, pH 7.5; 137 mM NaCl; 10% glycerol; 1% NP-40; 0.1% SDS; 2 mM EDTA) containing protease and phosphatase inhibitors (50 mM sodium fluoride, 1 mM sodium orthovanadate, 2 mM Pefabloc, 1.2 mM aprotinin, 40 mM bestatin, 5.6 mM E-64, 4 mM leupeptin, and 4 mM pepstatin A). Equivalent amounts of protein were separated by SDS-PAGE, then transferred to nitrocellulose membranes. For analysis of FLT3 phosphorylation, equivalent amounts of protein from each sample were immunoprecipitated overnight at 4*C with an agarose-conjugated anti-FLT3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Immune complexes were washed (150 mM NaCl, 1.5 mM MgCl2, 50 mM HEPES, pH 7.5, 10% glycerol, 0.1% Triton X-100, and 1 mM EGTA) and following SDS-PAGE, proteins were transferred to nitrocellulose membranes. Membranes were probed with an anti-phosphotyrosine antibody (Upstate, Lake Placid, NY or Transduction Laboratories, Lexington, KY) and then stripped with Restore Western Blot Stripping Buffer (Pierce, Rockford, IL). Membranes were reprobed with an anti-FLT3 antibody (Santa Cruz Biotechnology). Stat5 antibodies for immunprecipitation and Western blot analysis were from Upstate Biotechnology and Transduction labs respectively.

* * * *

It will be apparent to those skilled in the arthat various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

WHAT IS CLAIMED IS:

1. A method of treating acute myeloid leukemia (AML) comprising administering an effective amount of a compound of Formula I:



(I)

wherein

R is independently H, OH, alkyl, aryl, cycloalkyl, heteroaryl, alkoxy, heterocyclic and amino;

each R_1 is independently selected from the group consisting of alkyl, halo, aryl, alkoxy, haloalkyl, haloalkoxy, cycloalkyl, heteroaryl, heterocyclic, hydroxy,

-C(O)-R_8, -NR_9R_{10}, -$NR_9C(O)$-R_{12} and –$C(O)NR_9R_{10}$;

each R_2 is independently selected from the group consisting of alkyl, aryl, heteroaryl, –C(O)-R_8 and SO_2R'', where R'' is alkyl, aryl, heteroaryl, NR_9N_{10} or alkoxy;

each R_5 is independently selected from the group consisting of hydrogen, alkyl, aryl, haloalkyl, cycloalkyl, heteroaryl, heterocyclic, hydroxy, -C(O)-R_8 and $(CHR)_rR_{11}$;

X is O or S;

j is 0-1

p is 0-3;

q is 0-2;

r is 0-3;

R_8 is selected from the group consisting of –OH, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

R_9 and R_{10} are independently selected from the group consisting of H, alkyl,

aryl, aminoalkyl, heteroaryl, cycloalkyl and heterocyclic, or R_9 and R_{10} together with N may form a ring, where the ring atoms are selected from the group consisting of C, N, O and S;

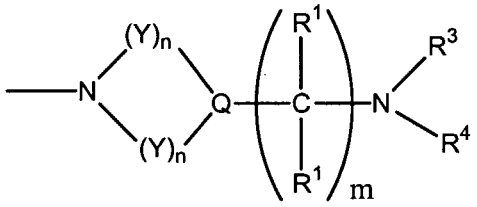
R_{11} is selected from the group consisting of –OH, amino, monosubstituted amino, disubstituted amino, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic

R_{12} is selected from the group consisting of alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

Z is –OH;

-Oalkyl;

$-NR_3R_4$, where R_3 and R_4 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, and heterocyclic, or R_3 and R_4 may combine with N to form a ring where the ring atoms are selected from the group consisting of CH_2, N, O and S or



wherein Y is independently CH_2, O, N or S,

Q is C or N;

n is independently 0-4; and

m is 0-3;

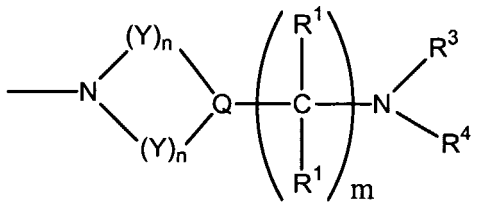
or a salt thereof, to a patient in need of such treatment.

2. The method of claim 1, wherein R_1 is halo and p is 1.

3. The method of claim 2, wherein halo is selected from F and Cl.

4. The method of claim 2, where Z is $-NR_3R_4$ wherein R_3 and R_4 form a morpholine ring.

5. The method of claim 1, wherein Z is:

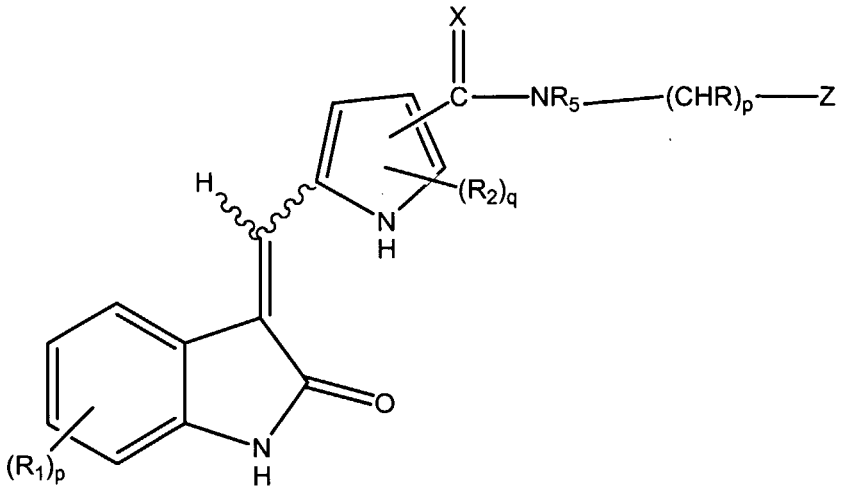


,

wherein each Y is CH_2, each n is 2, m is 0 and R_3 and R_4 form a morpholine ring.

6. The method of any of claims 1-4, wherein R_2 is methyl and q is 2, wherein the methyls are bonded at the 3 and 5 positions.

7. The method of claim 1, wherein the compound administered is a compound of Formula II:

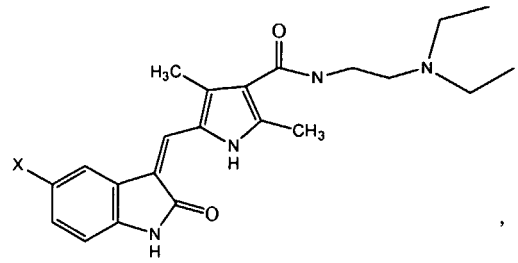


(II).

8. The method of claim 7, wherein R_5 is H.

9. The method of claim 7, wherein R_2 is methyl, q is 2, wherein the methyls are bonded at the 3 and 5 positions.

10. The method of claim 1, wherein the compound administered is selected from the group consisting of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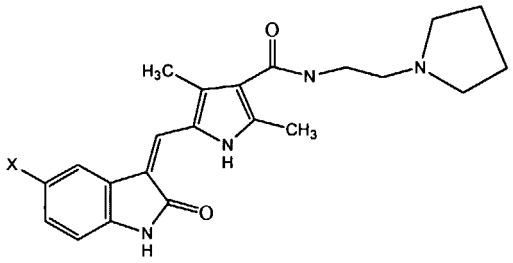
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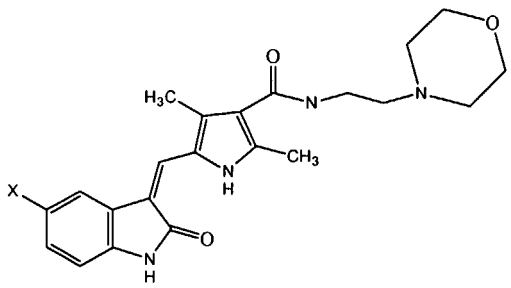
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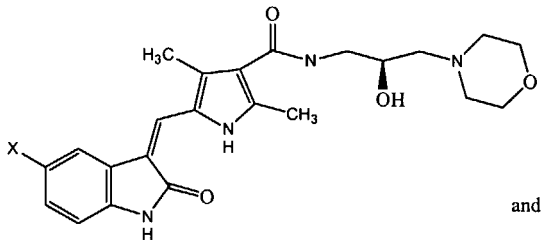
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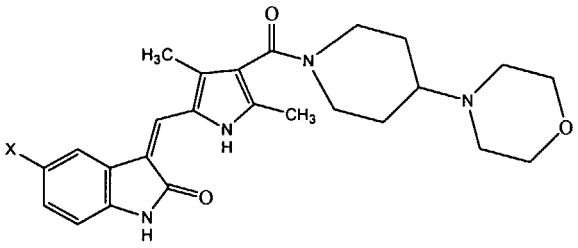
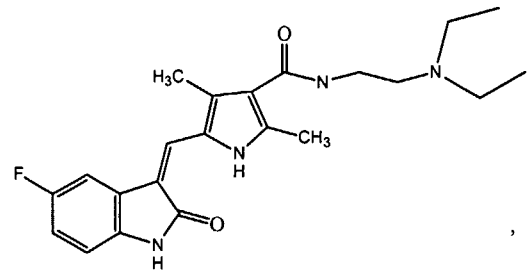


11. The method of claim 8, wherein the patient is FLT-3-ITD positive.

12. The method of claim 9, wherein the patient is FLT-3 wild-type positive.

13. The method of claim 1, wherein the compound of formula I is selected from the group consisting of: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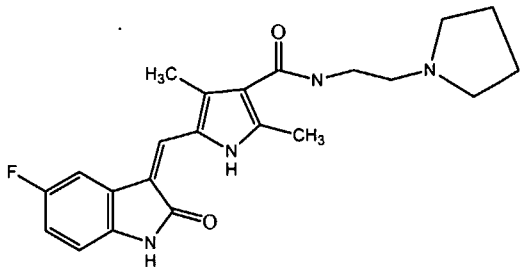
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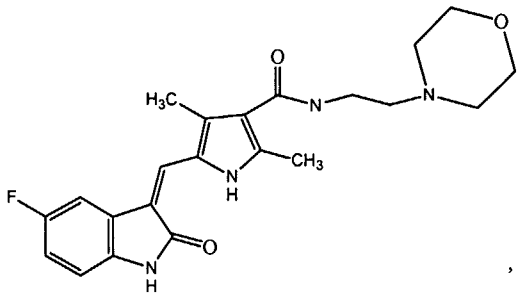
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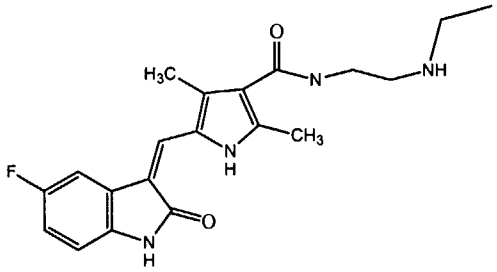
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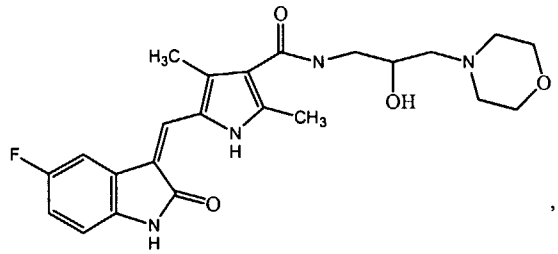
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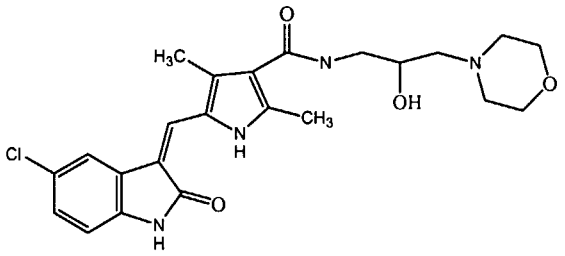
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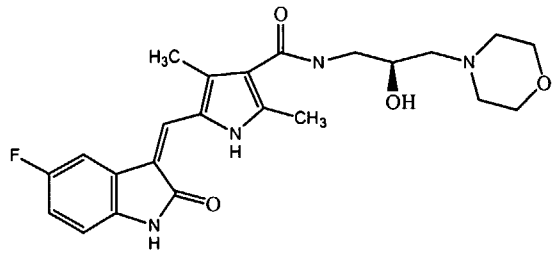
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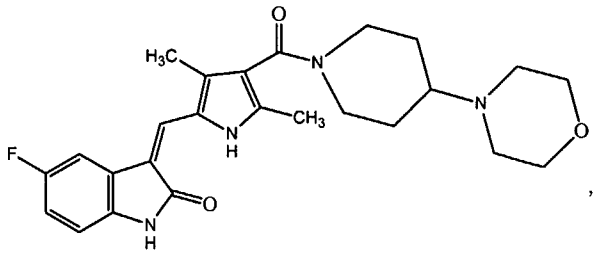
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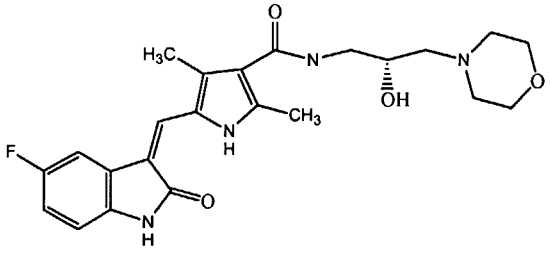
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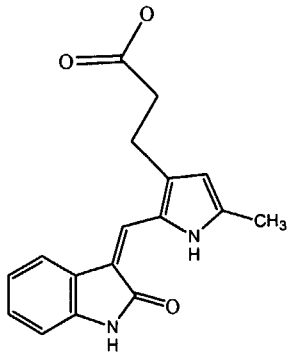
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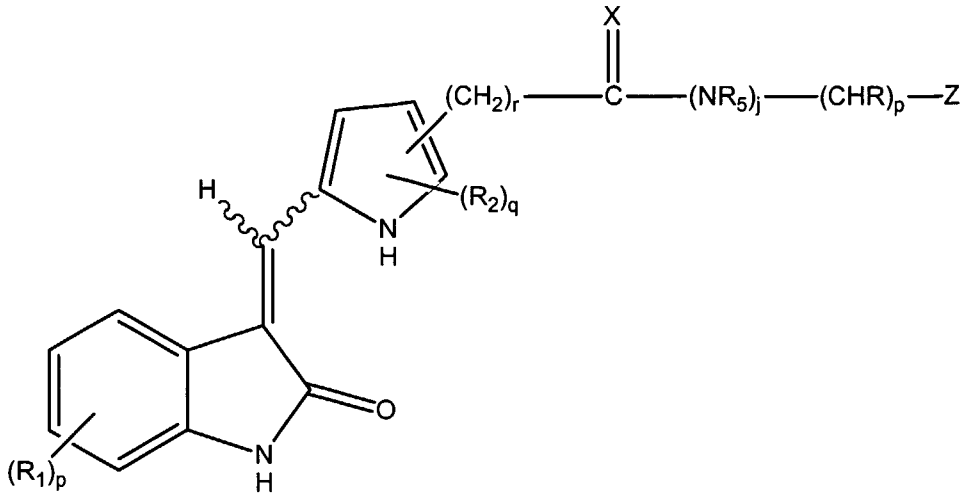
14. The method of claim 1, wherein the patient is a human.

15. A method to detect inhibition of phosphorylation of FLT-3 and analysis of phosphorylation in peripheral blood lysate comprising administering an inhibitory amount of a compound of Formula I:



(I)

wherein

R is independently H, OH, alkyl, aryl, cycloalkyl, heteroaryl, alkoxy, heterocyclic and amino;

each R_1 is independently selected from the group consisting of alkyl, halo, aryl, alkoxy, haloalkyl, haloalkoxy, cycloalkyl, heteroaryl, heterocyclic, hydroxy,

-C(O)-R_8, -NR_9R_{10}, -$NR_9C(O)$-R_{12} and –$C(O)NR_9R_{10}$;

each R_2 is independently selected from the group consisting of alkyl, aryl, heteroaryl, –C(O)-R_8 and SO_2R'', where R'' is alkyl, aryl, heteroaryl, NR_9N_{10} or alkoxy;

each R_5 is independently selected from the group consisting of hydrogen, alkyl, aryl, haloalkyl, cycloalkyl, heteroaryl, heterocyclic, hydroxy, -C(O)-R_8 and $(CHR)_rR_{11}$;

X is O or S;

j is 0-1

p is 0-3;

q is 0-2;

r is 0-3;

R_8 is selected from the group consisting of –OH, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

R_9 and R_{10} are independently selected from the group consisting of H, alkyl,

aryl, aminoalkyl, heteroaryl, cycloalkyl and heterocyclic, or R_9 and R_{10} together with N may form a ring, where the ring atoms are selected from the group consisting of C, N, O and S;

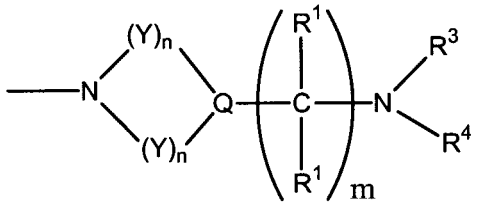
R_{11} is selected from the group consisting of –OH, amino, monosubstituted amino, disubstituted amino, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic

R_{12} is selected from the group consisting of alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

Z is –OH;

-Oalkyl;

$-NR_3R_4$, where R_3 and R_4 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, and heterocyclic, or R_3 and R_4 may combine with N to form a ring where the ring atoms are selected from the group consisting of CH_2, N, O and S or



wherein Y is independently CH_2, O, N or S,

Q is C or N

n is independently 0-4; and

m is 0-3;

to a patient in need of such treatment.

16. The method of claim 15, wherein FLT-3 is mutant FLT-3.

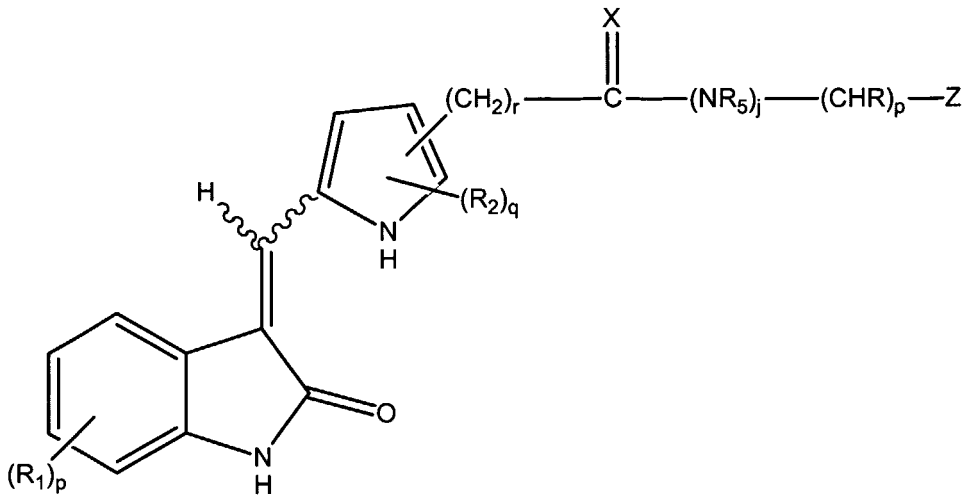
17. The method of claim 15, wherein FLT-3 is wild-type FLT-3.

18. The method of claim 16, wherein FLT-3 is FLT-3-ITD.

19. The method of claim 1, wherein prior to administration of the compound the acute myeloid leukemia is FLT-3-ITD AML.

20. The method of claim 15, wherein phosphorylation of FLT-3 is detected by measuring increased expression of VEGF protein.

21. A method of inhibiting phosphorylation of FLT-3 comprising administering an inhibitory amount of a compound of Formula I:



(I)

wherein

R is independently H, OH, alkyl, aryl, cycloalkyl, heteroaryl, alkoxy, heterocyclic and amino;

each R_1 is independently selected from the group consisting of alkyl, halo, aryl, alkoxy, haloalkyl, haloalkoxy, cycloalkyl, heteroaryl, heterocyclic, hydroxy,

-C(O)-R_8, -NR_9R_{10}, -$NR_9C(O)$-R_{12} and –$C(O)NR_9R_{10}$;

each R_2 is independently selected from the group consisting of alkyl, aryl, heteroaryl, –C(O)-R_8 and SO_2R'', where R'' is alkyl, aryl, heteroaryl, NR_9N_{10} or alkoxy;

each R_5 is independently selected from the group consisting of hydrogen, alkyl, aryl, haloalkyl, cycloalkyl, heteroaryl, heterocyclic, hydroxy, -C(O)-R_8 and $(CHR)_rR_{11}$;

X is O or S;

j is 0-1

p is 0-3;

q is 0-2;

r is 0-3;

R_8 is selected from the group consisting of –OH, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

R_9 and R_{10} are independently selected from the group consisting of H, alkyl, aryl, aminoalkyl, heteroaryl, cycloalkyl and heterocyclic, or R_9 and R_{10} together with N may form a ring, where the ring atoms are selected from the group consisting of C, N, O and S;

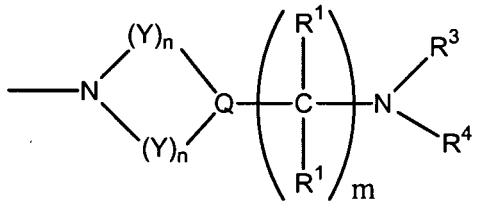
R_{11} is selected from the group consisting of –OH, amino, monosubstituted amino, disubstituted amino, alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic

R_{12} is selected from the group consisting of alkyl, aryl, heteroaryl, alkoxy, cycloalkyl and heterocyclic;

Z is –OH;

-Oalkyl;

$-NR_3R_4$, where R_3 and R_4 are independently selected from the group consisting of hydrogen, alkyl, aryl, heteroaryl, cycloalkyl, and heterocyclic, or R_3 and R_4 may combine with N to form a ring where the ring atoms are selected from the group consisting of CH_2, N, O and S or



wherein Y is independently CH_2, O, N or S,

Q is C or N

n is independently 0-4; and

m is 0-3;

to a patient in need of such treatment.

22. The method of claim 21, wherein FLT-3 is mutant FLT-3.

23. The method of claim 21, wherein FLT-3 is wild-type FLT-3.

24. The method of claim 22, wherein FLT-3 is FLT-3-ITD.