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(54) Title: NOVEL PYRAZOLO[1,5-a]PYRROLO[3,2-e]PYRIMIDINE DERIVATIVES AS mTOR INHIBITORS

(57) Abstract: The present invention relates to certain pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidine compounds of Formula (I) as inhibitors of mammalian Target of Rapamycin (mTOR) kinase, which is also known as FRAP, RAFT, RAPT or SEP. The compounds may be used in the treatment of cancer and other disorders where mTOR is deregulated. The present invention further provides pharmaceutical compositions comprising the pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidine compounds.

TITLE OF THE INVENTION

NOVEL PYRAZOLO [1,5-a]PYRROLO[3,2-e]PYRIMIDINE DERIVATIVES AS mTOR INHIBITORS

FIELD OF THE INVENTION

This invention is directed to certain pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidine compounds of Formula (I) as inhibitors of mammalian Target Of Rapamycin (mTOR) kinase, which is also known as FRAP, RAFT, RAPT or SEP. The compounds may be useful in the treatment of cancer and other disorders where mTOR is deregulated.

BACKGROUND OF THE INVENTION

The mammalian target of rapamycin (mTOR) is a central regulator of cell growth and proliferation and plays a gatekeeper role in the control of cell cycle progression. The mTOR signaling pathway, which integrates both extracellular and intracellular signals, is activated in certain cellular processes such as tumor formation, angiogenesis, insulin resistance, adipogenesis, and T-lymphocyte activation. In addition, the mTOR signaling pathway is deregulated in diseases such as cancer and type 2 diabetes. See Laplante et al., J. Cell Science 122, pp 3589-3593 (2009).

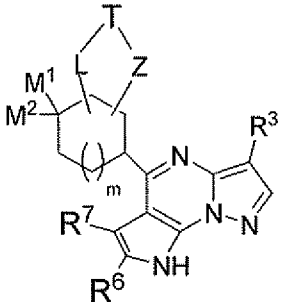
mTOR mediates mitogenic signals from PI3K/AKT through to the downstream targets S6K1 (ribosomal S6 kinase 1), 4E-BP1 (eukaryotic translation initiation factor 4E-binding protein) and AKT. Recently, it has been shown that mTOR exists in two complexes. Raptor-mTOR complex (mTORC1) is a rapamycin-sensitive complex that phosphorylates S6K1 and 4E-BP1. Rictor-mTOR complex (mTORC2) is a rapamycin-insensitive complex that phosphorylates AKT at Ser473. Although the precise mechanism by which rapamycin inhibits mTOR function is not well understood, rapamycin partially inhibits mTOR function through mTORC1. Since mTORC2 is involved in the regulation of cell survival, metabolism, proliferation, and cytoskeletal organization in a rapamycin-independent manner, complete inhibition of mTOR function through inhibition of both mTORC1 and mTORC2 may lead to a broader spectrum antitumor activity in the treatment of cancer or better efficacy. In addition, inhibition of both mTORC1 and mTORC2 may lead to better efficacy in treating other diseases than through inhibition of mTORC1 alone.

There exists a need in the art for small-molecule compounds having desirable physicochemical properties that are useful for treating cancer and other disorders associated with deregulated mTOR activity. Specifically, there exists a need for small molecule inhibitors of mTOR kinase that block signaling through mTORC1 and mTORC2 for treating cancer and other disorders.

SUMMARY OF THE INVENTION

The present invention relates to certain pyrazolopyrrolopyrimidine compounds of Formula (I) as inhibitors of mammalian Target Of Rapamycin (mTOR) kinase, which is also known as FRAP, RAFT, RAPT or SEP. The compounds may be used in the treatment of cancer and other disorders where mTOR is deregulated. The present invention further provides pharmaceutical compositions comprising the pyrazolopyrrolopyrimidine compounds.

The present invention thus relates to compounds of Formula I and pharmaceutically acceptable salts thereof, as detailed herein:



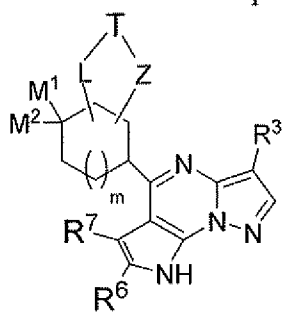
I

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides Pyrazolopyrrolopyrimidine Compounds, pharmaceutical compositions comprising a Pyrazolopyrrolopyrimidine Compound, and methods of using the Pyrazolopyrrolopyrimidine Compounds for treating cancer in a patient. In addition, the present invention provides methods of using the Pyrazolopyrrolopyrimidine Compounds for treating a disease or disorder associated with deregulated mTOR activity in a patient.

COMPOUNDS

The present invention provides compounds of Formula I



I

Wherein

M^1 and M^2 are independently selected from the group consisting of CN, $-(CR^aR^b)_nOR^1$, $-(CR^aR^b)_nNR^1R^2$, $-(CR^aR^b)_nR^1$, $-(CR^aR^b)_nSR^1$, $-(CR^aR^b)_nS(O)_2R^1$, $-(CR^aR^b)_nS(O)R^1$,

$-(CR^aR^b)_nS(O)_2NR^1R^2$, $-(CR^aR^b)_nNR^1S(O)_2R^4$, $-(CR^aR^b)_nC(O)NR^1S(O)_2R^2$, $-(CR^aR^b)_nC(O)R^1$, $-(CR^aR^b)_nC(O)OR^1$, $-(CR^aR^b)_nC(O)NR^1R^2$, $-(CR^aR^b)_nC(=NR^4)NR^1R^2$, $-(CR^aR^b)_nNR^1C(O)R^4$, $-(CR^aR^b)_nNR^1C(O)OR^4$, $-(CR^aR^b)_nNR^4C(O)NR^1R^2$, $-(CR^aR^b)_nOR^1$ and $-(CR^aR^b)_nO(CR^cR^d)_qOR^4$;

L and Z are not present, or

L and Z are bonded to any two carbons of the ring which M^1 and M^2 are not attached and are independently selected from the group consisting of CH_2, $C(H)(R^{10})$,$C(R^{10})(R^{11})$, $N(R^{10})$, C(O), O, S, S(O) and $S(O)_2$;

T is not present such that L is bonded directly to Z, or T is selected from the group consisting of CH_2, $C(H)(R^{10})$,$C(R^{10})(R^{11})$, $N(R^{10})$, C(O), O, S, S(O) and $S(O)_2$ and C_1-C_4 alkylene, wherein said alkylene of T is unsubstituted or substituted with 1 to two substituents selected from the group consisting of C_1-C_3 alkyl, halo, hydroxyl, C_1-C_3 alkoxy, amino, C_1-C_3 alkylamino and C_1-C_3 dialkylamino;

R^a, R^b, R^c and R^d are independently selected from H, halogen and C_1-C_6 alkyl;

R^1, R^2 and R^4 are independently selected from H, OH, halogen, NH_2, $-(CR^aR^b)_nO(CR^cR^d)_qR^8$, C_1-C_6alkyl, C_3-C_8cycloalkyl, C_3-C_8cycloalkylC_1-C_6alkyl, C_6-C_{10}arylC_1-C_6alkyl, C_6-C_{10}aryl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-membered heterocyclenylC_1-C_6alkyl, 5- to 10-membered heterocyclyl and 5- to 10-membered heterocyclenyl, wherein the alkyl, cycloalkyl, cycloalkylalkyl, arylalkyl, aryl, heteroarylalkyl, heteroaryl, heterocyclylalkyl, heterocyclenylalkyl, heterocyclyl or heterocyclenyl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, $-CF_3$, -CN, -C(O)OH, $-(CR^aR^b)_nC(O)OH$, $-OCF_3$, $-OR^9$, $-C(O)R^9$, $-NR^8R^9$, $-C(O)O$-C_1-C_6alkyl, $-C(O)NR^8R^9$, $-NR^8C(O)R^9$, $-S(O_2)NR^8R^9$, $-NR^8S(O_2)R^9$, $-SR^9$, $-S(O_2)R^9$, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl and 5- to 10-membered heterocyclyl;

Or R^1 and R^2 form a 3- to 8- membered cycloalkyl, 5- to 6-membered heterocyclyl or 5- to 6-membered heterocyclenyl;

R^3 is selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, halo-C_1-C_6alkyl, $-CF_3$, $-C(O)R^9$, C_6-C_{10}aryl, C_3-C_8cycloalkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclyl, 5- to 10-membered heterocyclenyl, C_6-C_{10}arylC_1-C_6alkyl, C_3-C_8cycloalkylC_1-C_6alkyl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heterocyclylC_1-C_6alkyl and 5- to 10-membered heterocyclenylC_1-C_6alkyl, wherein each of said aryl, cycloalkyl, heteroaryl, heterocyclyl, heterocyclenyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, heterocyclylalkyl and heterocyclenylalkyl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, $-CF_3$, -CN, -

C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -$(CR^aR^b)_nOR^9$, -$(CR^aR^b)_nC(O)R^9$, -$(CR^aR^b)_nNR^8R^9$, -$(CR^aR^b)_nNR^8$,
-NR^8R^9, -$(CR^aR^b)_nC(O)O$-C_1-C_6alkyl, -O-haloC_1-C_6alkyl, -$(CR^aR^b)_nC(O)NR^8R^9$, -$(CR^aR^b)_n$ $C(O)NR^8S(O)_2R^9$, -$(CR^aR^b)_nNR^8C(O)R^9$, -$(CR^aR^b)_nNR^8C(O)OR^9$,
-$(CR^aR^b)_nNR^8C(O)NR^8R^9$, -$(CR^aR^b)_nS(O_2)NR^8R^9$, -$(CR^aR^b)_nS(O_2)NR^8C(O)R^9$, -$(CR^aR^b)_n$ $NR^8S(O_2)R^9$, -$(CR^aR^b)_nSR^9$, -$(CR^aR^b)_nS(O_2)R^9$, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl, 5- to 10-membered heterocyclyl, C_6-C_{10}arylalkyl, 5- to 10-membered heteroarylalkyl, 5- to 10-membered heterocyclenylalkyl and 5- to 10-membered heterocyclylalkyl wherein each of said alkyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, heterocyclenyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, heterocyclylalkyl and heterocyclenylalkyl is unsubstituted or substituted with one to five moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^9, -$C(O)R^9$, -NR^8R^9, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^8R^9$, -$NR^8C(O)R^9$, -$S(O_2)NR^8R^9$, -$NR^8S(O_2)R^9$, -SR^9, and -$S(O_2)R^9$;

R^6 and R^7 are independently selected from the group consisting of H, halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -OR^9, -$C(O)R^9$, -NR^8R^9, -$C(O)O$-C_1-C_6alkyl, -CR^aR^b, -OR^a, -$S(O)R^a$, -$C(O)OR^a$, -$S(O_2)NR^aR^b$, -$NR^aC(O)R^b$, -$NR^aS(O_2)R^b$,-$C(O)NR^8R^9$, -SR^9, and -$S(O_2)R^9$;

R^8 and R^9 are independently selected from the group consisting of H, OH, C_1-C_6alkyl, C_3-C_8cycloalkyl, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl, 5- to 10-membered heterocyclyl, C_3-C_8cycloalkylC_1-C_6alkyl, C_6-C_{10}arylC_1-C_6alkyl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-membered heterocyclenylC_1-C_6alkyl, and said alkyl, cycloalkyl, aryl, heteroaryl, heterocyclenyl, heterocyclyl, cycloalkylalkyl, arylalkyl, heteroarylalkyl, heterocyclenylalkyl or heterocyclylalkyl is optionally substituted with halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -OR^a, -C(O), amino, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^aR^b$, -SR^a, and -$S(O_2)R^a$; or R^8 and R^9 together with the nitrogen atom to which they are attached form a 3- to 6-membered heterocyclyl ring;

R^{10} and R^{11} are independently selected from the group consisting of H, C_1-C_3alkyl, halo, hydroxyl, C_1-C_3alkoxy, amino, C_1-C_3alkylamino and C_1-C_3dialkylamino;

n is independently 0, 1, 2, 3 or 4;

m is independently 0, 1, 2, 3 or 4;

q is independently 0, 1, 2, 3 or 4;

or a pharmaceutically acceptable salt thereof.

In another embodiment,

M^1 and M^2 are independently selected from the group consisting of CN, -$(CR^aR^b)_nOR^1$, -$(CR^aR^b)_nNR^1R^2$, -$(CR^aR^b)_nR^1$, -$(CR^aR^b)_nSR^1$, -$(CR^aR^b)_nS(O)_2R^1$, -$(CR^aR^b)_nS(O)R^1$,

$-(CR^aR^b)_nS(O)_2NR^1R^2$, $-(CR^aR^b)_nNR^1S(O)_2R^4$, $-(CR^aR^b)_nC(O)NR^1S(O)_2R^2$, $-(CR^aR^b)_nC(O)R^1$, $-(CR^aR^b)_nC(O)OR^1$, $-(CR^aR^b)_nC(O)NR^1R^2$, $-(CR^aR^b)_nC(=NR^4)NR^1R^2$, $-(CR^aR^b)_nNR^1C(O)R^4$, $-(CR^aR^b)_nNR^1C(O)OR^4$, $-(CR^aR^b)_nNR^4C(O)NR^1R^2$, $-(CR^aR^b)_nOR^1$ and $-(CR^aR^b)_nO(CR^cR^d)_qOR^4$;

L, T and Z are not present, or

L and Z are bonded to any two carbons of the ring which are not attached to M^1 and M^2 and are both CH_2, and T is not present;

R^a, R^b, R^c and R^d are independently selected from H and C_1-C_6 alkyl;

R^1, R^2 and R^4 are independently selected from H, OH, halogen, $-(CR^aR^b)_nO(CR^cR^d)_qR^8$, C_1-C_6alkyl, C_3-C_8cycloalkyl, C_3-C_8cycloalkylC_1-C_6alkyl, C_6-C_{10}arylC_1-C_6alkyl, C_6-C_{10}aryl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-membered heterocyclyl, 5- to 10-membered heterocyclenylC_1-C_6alkyl and 5- to 10-membered heterocyclenyl, wherein the alkyl, cycloalkyl, cycloalkylalkyl, arylalkyl, aryl, heteroarylalkyl, heteroaryl, heterocyclylalkyl, heterocyclyl, heterocyclenylalkyl or heterocyclenyl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, $-CF_3$, -CN, -C(O)OH, $-(CR^aR^b)_nC(O)OH$, $-OCF_3$, $-OR^a$, $-C(O)R^a$, $-NR^aR^b$, -C(O)O-alkyl, $-C(O)NR^aR^b$, $-NR^aC(O)R^b$, $-S(O_2)NR^aR^b$, $-NR^aS(O_2)R^b$, $-SR^a$, $-S(O_2)R^a$, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl and 5- to 10-membered heterocyclyl;

R^3 is selected from the group consisting of C_6-C_{10}aryl, 5- to 10-membered heteroaryl, wherein each of said aryl or heteroaryl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, $-CF_3$, -CN, -C(O)OH, $-(CR^aR^b)_nC(O)OH$, $-OCF_3$, -O-haloC_1-C_6alkyl, $-OR^8$, $-C(O)R^8$, $-NR^8R^9$, -C(O)O-C_1-C_6alkyl, $-C(O)NR^8R^9$, $-NR^8C(O)R^9$, $-S(O_2)NR^8R^9$, $-NR^8S(O_2)R^9$, $-SR^8$, and $-S(O_2)R^8$, wherein each of said heteroaryl or aryl is unsubstituted or substituted with one to three moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, $-CF_3$, -CN, -C(O)OH, $-(CR^aR^b)_nC(O)OH$, $-OCF_3$, -O-haloC_1-C_6alkyl, $-OR^a$, $-C(O)R^a$, $-NR^aR^b$, -C(O)O-C_1-C_6alkyl, $-C(O)NR^aR^b$, $-NR^aC(O)R^b$, $-S(O_2)NR^aR^b$, $-NR^aS(O_2)R^b$, $-SR^a$, and $-S(O_2)R^a$;

R^6 and R^7 are independently selected from the group consisting of H, $-OR^a$, $-NR^aR^b$, $-SR^a$, $-S(O)R^a$, $-S(O_2)R^a$, $-C(O)C_1$-C_6alkyl, $-C(O)NR^aR^b$, $-C(O)OR^a$, $-S(O_2)NR^aR^b$, $-NR^aC(O)R^b$, $-NR^aS(O_2)R^b$, C_1-C_6alkyl, C_1-C_6alkoxy, halogen, hydroxyl, amino and -CN;

R^8 and R^9 are independently selected from the group consisting of H, OH, C_1-C_6alkyl, C_3-C_8cycloalkyl, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl, 5- to 10-membered heterocyclyl, C_3-C_8cycloalkylC_1-C_6alkyl, C_6-C_{10}arylC_1-C_6alkyl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-

membered heterocyclenylC_1-C_6alkyl, and said alkyl, cycloalkyl, aryl, heteroaryl, heterocyclenyl, heterocyclyl, cycloalkylalkyl, arylalkyl, heteroarylalkyl, heterocyclenylalkyl or heterocyclylalkyl is optionally substituted with halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -OR^a, -C(O), amino, -C(O)O-C_1-C_6alkyl, -$C(O)NR^aR^b$,-SR^a, and -$S(O_2)R^a$;

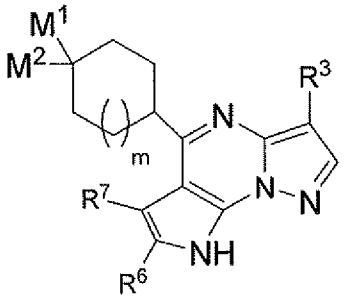
n is independently 0, 1 or 2;

m is 1;

q is independently 0, 1, or 2;

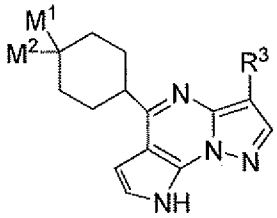
or a pharmaceutically acceptable salt thereof.

In a further embodiment, the compounds of the invention are under formula II or III



II

or



III

M^1 and M^2 are independently selected from the group consisting of CN, -$(CR^aR^b)_nOR^1$, -$(CR^aR^b)_nNR^1R^2$, -$(CR^aR^b)_nR^1$, -$(CR^aR^b)_nSR^1$, -$(CR^aR^b)_nS(O)_2R^1$, -$(CR^aR^b)_nS(O)R^1$, -$(CR^aR^b)_nS(O)_2NR^1R^2$, -$(CR^aR^b)_nNR^1S(O)_2R^4$, -$(CR^aR^b)_nC(O)NR^1S(O)_2R^2$, -$(CR^aR^b)_nC(O)R^1$, -$(CR^aR^b)_nC(O)OR^1$,-$(CR^aR^b)_nC(O)NR^1R^2$, -$(CR^aR^b)_nC(=NR^4)NR^1R^2$, -$(CR^aR^b)_nNR^1C(O)R^4$, -$(CR^aR^b)_nNR^1C(O)OR^4$, -$(CR^aR^b)_nNR^4C(O)NR^1R^2$, -$(CR^aR^b)_nOR^1$ and -$(CR^aR^b)_nO(CR^cR^d)_qOR^4$;

R^a, R^b, R^c and R^d are independently selected from H and C_1-C_3 alkyl;

R^1, R^2 and R^4 are independently selected from H, OH, NH_2, -$(CR^aR^b)_nO(CR^cR^d)_qR^8$, C_1-C_3alkyl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-membered heterocyclyl, 5- to 10-membered heterocyclenylC_1-C_6alkyl and 5- to 10-membered heterocyclenyl, wherein the alkyl, heteroarylalkyl, heteroaryl, heterocyclylalkyl, heterocyclyl, heterocyclenylalkyl or heterocyclenyl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -OR^a, -$C(O)R^a$, -NR^aR^b, -C(O)O-C_1-C_6alkyl, -$C(O)NR^aR^b$, -$NR^aC(O)R^b$, -$S(O_2)NR^aR^b$, -$NR^aS(O_2)R^b$,-SR^a and -$S(O_2)R^a$; wherein all other substituents are as defined above.

In another embodiment, M^1 is -$(CR^aR^b)_nO(CR^cR^d)_qOC_1$-$C_3$alkyl, -$(CR^aR^b)_nO(CR^cR^d)_qOH$, COOH, or -$(CR^aR^b)_nC(O)OC_1$-$C_3$alkyl;

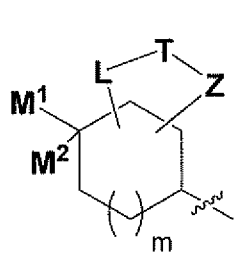
M^2 is selected from the group consisting of CN, $-(CR^aR^b)_nOR^1$, $-(CR^aR^b)_nNR^1R^2$, $-(CR^aR^b)_nR^1$, $-(CR^aR^b)_nSR^1$, $-(CR^aR^b)_nS(O)_2R^1$, $-(CR^aR^b)_nS(O)R^1$, $-(CR^aR^b)_nS(O)_2NR^1R^2$, $-(CR^aR^b)_nNR^1S(O)_2R^4$, $-(CR^aR^b)_nC(O)NR^1S(O)_2R^2$, $-(CR^aR^b)_nC(O)R^1$, $-(CR^aR^b)_nC(O)OR^1$, $-(CR^aR^b)_nC(O)NR^1R^2$, $-(CR^aR^b)_nC(=NR^4)NR^1R^2$, $-(CR^aR^b)_nNR^1C(O)R^4$, $-(CR^aR^b)_nNR^1C(O)OR^4$, $-(CR^aR^b)_nNR^4C(O)NR^1R^2$, $-(CR^aR^b)_nOR^1$ and $-(CR^aR^b)_nO(CR^cR^d)_qOR^4$;

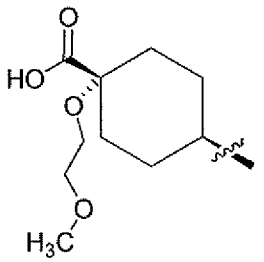
Wherein all other substituents are as defined above.

In a further embodiment, M^1 and M^2 are independently selected from the group consisting of halo, $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, $-OCH_3$, $-SCH_3$, -OH, -C(O)OH, $-C(O)NHOCH_3$, -C(O)NHOH, $-C(O)NHCH_2CH_2OH$, $-CONH_2$ and $-CH_3$, and all other substituents are as defined above.

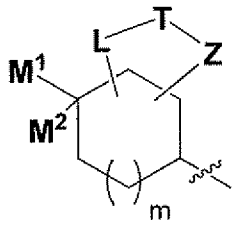
In yet a further embodiment, M^1 and M^2 are independently selected from the group consisting of halo, CN, NH_2, $-OCH_3$, $-CH_2OCH_3$, $-SCH_3$, -OH, $-CH_2OH$, $-CH_2CH_2OH$, $-C(O)OH$, $-C(O)CH_2OH$, $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, $-C(O)N(CH_3)_2$, $-CONH_2$, $C(=NH)NH_2$, $C(O)NH\text{-}NH_2$, $-CONHCH_3$, $-C(O)NHOCH_3$, $-C(O)N(CH_3)OCH_3$, -C(O)NHOH, $-C(O)NHCH_2CH_2OH$, $-CH_3$, $-SO_2CH_3$, $-CH_2SO_2CH_3$, $-CH_2NHSO_2CH_3$, $-C(O)NHS(O)_2CH_3$, $-OCH_2CH_2$-morpholinyl, triazolyl, tetrazolyl, oxadiazolyl, wherein said triazolyl, tetrazolyl or oxadiazolyl are optionally substituted with methyl or halo, and all other substituents are as defined above.

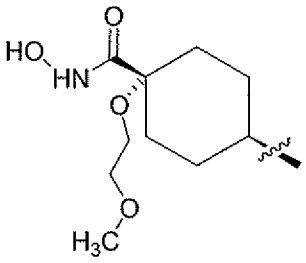
In one embodiment, M^1 is $-OCH_2CH_2OCH_3$, and M^2 is selected from the group consisting of halo, CN, $-OCH_3$, $-SCH_3$, -OH, $-CH_2OH$, -C(O)OH, $-C(O)CH_2OH$, $-C(O)N(CH_3)_2$, $-CONH_2$, $C(=NH)NH_2$, $C(O)NH\text{-}NH_2$, $-CONHCH_3$, $-C(O)NHOCH_3$, $-C(O)NHS(O)_2CH_3$, $-C(O)N(CH_3)OCH_3$, -C(O)NHOH, $-C(O)NHCH_2CH_2OH$, $-CH_3$, triazolyl, tetrazolyl, oxadiazolyl, wherein said triazolyl, tetrazolyl or oxadiazolyl are optionally substituted with methyl or halo, and all other substituents are as defined above.

In another embodiment, 

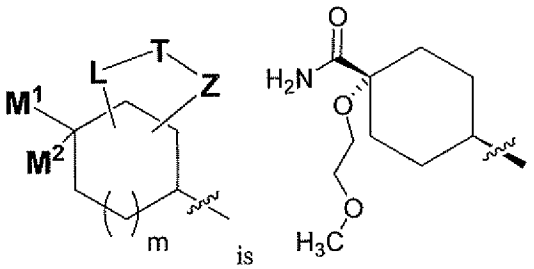
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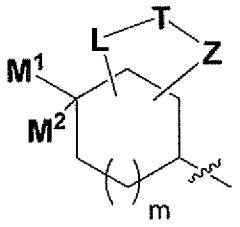
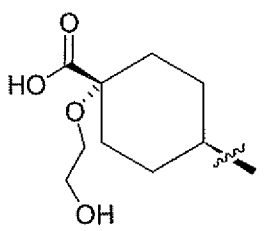
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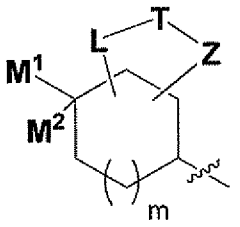
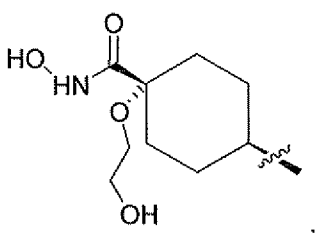
In a further embodiment, 

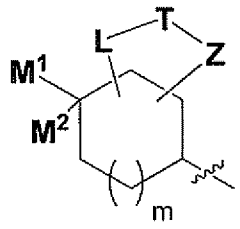
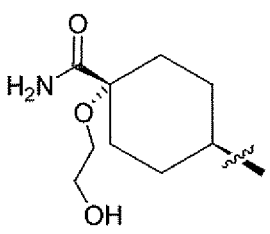
is 

.

In yet a further embodiment,  is .

In another embodiment,  is .

In a further embodiment,  is .

In yet a further embodiment,  is .

In one embodiment, M^1 is $-OCH_2CH_2OCH_3$, and M^2 is selected from the group consisting of -C(O)OH, $-CONH_2$ and $-C(O)NHOH$, and all other substituents are as defined above.

In one embodiment, M^1 is $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, –OH, $-CH_2OH$ or $-CH_2CH_2OH$, and M^2 is selected from the group consisting of halo, CN, $-OCH_3$, $-CH_2OCH_3$, $-SCH_3$, $-OCH_2CH_2OCH_3$, -OH, $-CH_2OH$, $-CH_2CH_2OH$, -C(O)OH, $-C(O)CH_2OH$, $-C(O)N(CH_3)_2$, $-CONH_2$, $C(=NH)NH_2$, $C(O)NH\text{-}NH_2$, $-CONHCH_3$, $-C(O)NHOCH_3$, $-C(O)N(CH_3)OCH_3$, -C(O)NHOH, $-C(O)NHCH_2CH_2OH$, $-CH_3$, $-CH_2SO_2CH_3$, $CH_2NHSO_2CH_3$, $-C(O)NHS(O)_2CH_3$, triazolyl, tetrazolyl, oxadiazolyl, wherein said triazolyl, tetrazolyl or oxadiazolyl are optionally substituted with methyl or halo, and all other substituents are as defined above.

In one embodiment, M^1 is –OH, $-CH_2OH$ or $-CH_2CH_2OH$, and M^2 is selected from the group consisting of halo, CN, $-OCH_3$, $-CH_2OCH_3$, $-SCH_3$, $-OCH_2CH_2OCH_3$, -OH, $-CH_2OH$, $-CH_2CH_2OH$, -C(O)OH, $-C(O)CH_2OH$, $-C(O)N(CH_3)_2$, $-CONH_2$, $-CONHCH_3$, $-C(O)NHOCH_3$, $-C(O)N(CH_3)OCH_3$, -C(O)NHOH, $-C(O)NHCH_2CH_2OH$, $-CH_3$, $-CH_2SO_2CH_3$, $CH_2NHSO_2CH_3$,

triazolyl, and oxadiazolyl, wherein said triazolyl, or oxadiazolyl are optionally substituted with methyl or halo; and all other substituents are as defined above.

In another embodiment, M^1 is –OH, -CH_2OH or -CH_2CH_2OH, and M^2 is selected from the group consisting of CN, -OCH_3, -CH_2OCH_3, -SCH_3, –$OCH_2CH_2OCH_3$, -OH, -CH_2OH, -CH_2CH_2OH, -C(O)OH, -$C(O)CH_2OH$, -CH_3, -$CH_2SO_2CH_3$, $CH_2NHSO_2CH_3$; and all other substituents are as defined above.

In one embodiment, M^1 is –C(O)OH, and M^2 is selected from the group consisting of halo, CN, NH_2, -OCH_3, -CH_2OCH_3, -SCH_3, –$OCH_2CH_2OCH_3$, -OH, -CH_2OH, -CH_2CH_2OH, -C(O)OH, -$C(O)CH_2OH$, -$C(O)N(CH_3)_2$, -$CONH_2$, -$CONHCH_3$, -$C(O)NHOCH_3$, -$C(O)N(CH_3)OCH_3$, -C(O)NHOH, -$C(O)NHCH_2CH_2OH$, -CH_3, -SO_2CH_3, -$CH_2SO_2CH_3$, $CH_2NHSO_2CH_3$, -OCH_2CH_2-morpholinyl, triazolyl and oxadiazolyl, wherein said triazolyl or oxadiazolyl are optionally substituted with methyl or halo; and all other substituents are as defined above.

In another embodiment, M^1 is –C(O)OH, and M^2 is selected from the group consisting of halo, CN, NH_2, -OCH_3, -SCH_3, -CH_3, -SO_2CH_3, -OCH_2CH_2-morpholinyl; and all other substituents are as defined above.

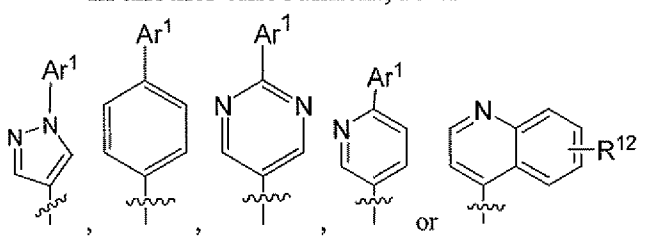
In a further embodiment, R^3 is a 5- to 6-membered heteroaryl or phenyl unsubstituted or substituted with one to three moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, phenyl, 5- to 6-membered heteroaryl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^a, -$C(O)R^a$, -NR^aR^b, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^aR^b$, -$NR^aC(O)R^b$, -$S(O_2)NR^aR^b$, -$NR^aS(O_2)R^b$, -SR^a, and

-$S(O_2)R^a$, wherein the alkyl, phenyl or heteroaryl is optionally substituted with one to three moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^a, -$C(O)R^a$, -NR^aR^b, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^aR^b$, -$NR^aC(O)R^b$, -$S(O_2)NR^aR^b$, -$NR^aS(O_2)R^b$, -SR^a, and -$S(O_2)R^a$; and all other substituents are as defined above.

In one embodiment, R^3 is pyrazolyl, isoquinolinyl, pyrimidinyl, phenyl or pyridyl, unsubstituted or substituted with one to three moieties as defined above.

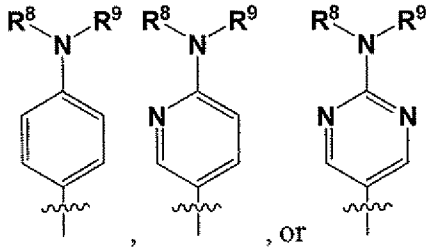
In one embodiment, R^3 is unsubstituted or substituted pyrazolyl or pyridyl as defined above.

In another embodiment, R^3 is



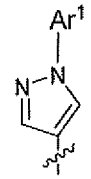
;

Ar^1 is C_6-C_{10}aryl or a 5- to 10-membered heteroaryl optionally substituted with one to three of R^{12}, which can be the same or different, each R^{12} being selected from the group consisting of halogen, C_1-C_6alkyl, $-CF_3$, -CN, -C(O)OH, $-(CR^aR^b)_nC(O)OH$, $-OCF_3$, -O-haloC_1-C_6alkyl, $-OR^a$, $-C(O)R^a$, $-NR^aR^b$, $-C(O)O-C_1$-C_6alkyl, $-C(O)NR^aR^b$, $-NR^aC(O)R^b$, $-S(O_2)NR^aR^b$, $-NR^aS(O_2)R^b$, $-SR^a$, and $-S(O_2)R^a$. In one embodiment, Ar^1 is phenyl or a 5- to 6-membered heteroaryl optionally substituted.

In a another embodiment, R^3 is  , wherein R^8 and R^9 are as defined above. In one embodiment, R^8 is H and R^9 is cyclopropyl.

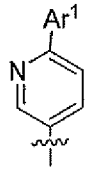
In a further embodiment, Ar^1 is phenyl, pyrazolyl, pyrimidinyl, pyridyl, imidazolyl, pyrazinyl or thiazolyl optionally substituted with one to three of R^{12}. In a another embodiment, Ar^1 is phenyl, pyridyl or imidazolyl optionally substituted with one to three of R^{12}.

In another embodiment, R^3 is

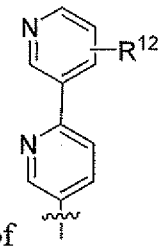
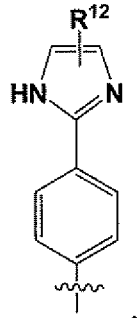
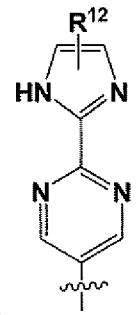
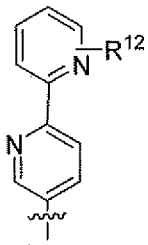
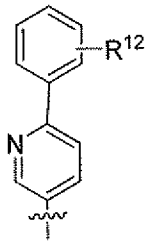
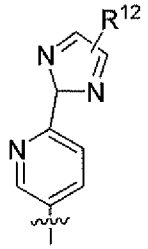
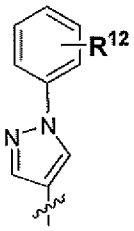
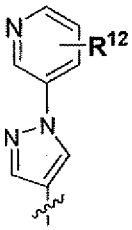
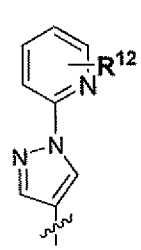
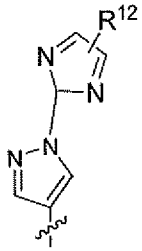
 ;

Ar^1 is is phenyl , pyridyl, pyrazinyl or imidazolyl optionally substituted with one to three of R^{12} as defined above.

In another embodiment, R^3 is

 ;

Ar^1 is is phenyl , pyridyl, pyrazinyl or imidazolyl optionally substituted with one to three of R^{12} as defined above.

In another embodiment, R^3 is selected from the group consisting of , , , , , , , , , and , and R^{12} is defined above.

In one embodiment, in the foregoing embodiments, R^{12} is selected from the group consisting of halogen, C_1-C_6alkyl, -CF_3, and -OCF_3. In another embodiment, R^{12} is selected from the group consisting of F and methyl.

In one embodiment,

R^6 and $R^7$are independently selected from the group consisting of H, halo, -$C(O)C_1$-C_6alkyl, -$S(O)_2C_1$-C_6alkyl and CN; and all other substituents are as defined above.

Specific embodiments depicting non-limiting Examples of of the above Formulas are provided in the Experimental Section hereinbelow.

Specific examples of the compounds of the instant invention include:

(1*R*,4*R*)-4-(3-(6-fluoroquinolin-3-yl)-8*H*-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methylcyclohexanecarboxylic acid;

(1*R*,4*R*)-4-(3-(6-fluoroquinolin-3-yl)-8*H*-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methylcyclohexanecarboxylic acid;

(1R,4R)-1-methyl-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;

(1S,4S)-1-methyl-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;

(1R,4R)-1-(methoxymethyl)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;

(1S,4S)-1-(methoxymethyl)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;

(1R,4R)-1-(methoxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-(methoxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-(methoxymethyl)cyclohexanecarboxylic acid;
(1S,4S)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-(methoxymethyl)cyclohexanecarboxylic acid;
(1R,4R)-1-(methylthio)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-(methylthio)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
1-((2-methoxyethoxy)methyl)-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-methoxy-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-1-methoxy-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylic acid;
(1S,4S)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylic acid;
((1R,4R)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexyl)methanol;
((1S,4S)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexyl)methanol;
1-(hydroxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8*H*-pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidin-5-yl)cyclohexanol; and
4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-(hydroxymethyl)cyclohexanol;
Or a stereoisomer thereof;
Or a pharmaceutically acceptable salt thereof;
Or a pharmaceutically acceptable salt of the stereoisomer thereof.

Chemical Definitions

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. For example, C_1-C_{10}, as in "C_1-C_{10} alkyl" is defined to include groups having 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbons in a linear or branched arrangement. For example, "C_1-C_{10} alkyl" specifically includes methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *t*-butyl, *i*-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, and so on.

When used in the phrases "alkylaryl", "alkylcycloalkyl" and "alkylheterocyclyl" the term "alkyl" refers to the alkyl portion of the moiety and does not describe the number of atoms in the heterocyclyl portion of the moiety. In an embodiment, if the number of carbon atoms is not specified, the "alkyl" of "alkylaryl", "alkylcycloalkyl" and "alkylheterocyclyl" refers to C_1-C_{12} alkyl and in a further embodiment, refers to C_1-C_6 alkyl.

The term "cycloalkyl" means a monocyclic saturated or unsaturated aliphatic hydrocarbon group having the specified number of carbon atoms. The cycloalkyl is optionally bridged (i.e., forming a bicyclic moiety), for example with a methylene, ethylene or propylene bridge. The cycloalkyl may be fused with an aryl group such as phenyl, and it is understood that the cycloalkyl substituent is attached via the cycloalkyl group. For example, "cycloalkyl" includes cyclopropyl, methyl-cyclopropyl, 2,2-dimethyl-cyclobutyl, 2-ethyl-cyclopentyl, cyclohexyl, cyclopentenyl, cyclobutenyl and so on.

In an embodiment, if the number of carbon atoms is not specified, "alkyl" refers to C_1-C_{12} alkyl and in a further embodiment, "alkyl" refers to C_1-C_6 alkyl. In an embodiment, if the number of carbon atoms is not specified, "cycloalkyl" refers to C_3-C_{10} cycloalkyl and in a further embodiment, "cycloalkyl" refers to C_3-C_7 cycloalkyl. In an embodiment, examples of "alkyl" include methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *t*-butyl and *i*-butyl.

The term "alkylene" means a hydrocarbon diradical group having the specified number of carbon atoms. For example, "alkylene" includes -CH_2-, -CH_2CH_2- and the like. In an embodiment, if the number of carbon atoms is not specified, "alkylene" refers to C_1-C_{12} alkylene and in a further embodiment, "alkylene" refers to C_1-C_6 alkylene.

If no number of carbon atoms is specified, the term "alkenyl" refers to a non-aromatic hydrocarbon radical, straight, branched or cyclic, containing from 2 to 10 carbon atoms and at least one carbon to carbon double bond. Preferably one carbon to carbon double bond is present, and up to four non-aromatic carbon-carbon double bonds may be present. Thus, "C_2-C_6 alkenyl" means an alkenyl radical having from 2 to 6 carbon atoms. Alkenyl groups include ethenyl, propenyl, butenyl, 2-methylbutenyl and cyclohexenyl. The straight, branched or cyclic portion of the alkenyl group may contain double bonds and may be substituted if a substituted alkenyl group is indicated.

The term "alkynyl" refers to a hydrocarbon radical straight, branched or cyclic, containing from 2 to 10 carbon atoms and at least one carbon to carbon triple bond. Up to three carbon-carbon triple bonds may be present. Thus, "C_2-C_6 alkynyl" means an alkynyl radical having from 2 to 6 carbon atoms. Alkynyl groups include ethynyl, propynyl, butynyl, 3-methylbutynyl and so on. The straight, branched or cyclic portion of the alkynyl group may contain triple bonds and may be substituted if a substituted alkynyl group is indicated.

In certain instances, substituents may be defined with a range of carbons that includes zero, such as (C_0-C_6)alkylene-aryl. If aryl is taken to be phenyl, this definition would include phenyl itself as well as -CH_2Ph, -CH_2CH_2Ph, CH(CH_3)CH_2CH(CH_3)Ph, and so on.

"Aryl" is intended to mean any stable monocyclic, bicyclic or tricyclic carbon ring of up to 7 atoms in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include phenyl, naphthyl, tetrahydronaphthyl, indanyl and biphenyl. In cases where the aryl substituent is bicyclic and one ring is non-aromatic, it is understood that attachment is via the aromatic ring.

In one embodiment, "aryl" is an aromatic ring of 6 to 14 carbons atoms, and includes a carbocyclic aromatic group fused with a 5-or 6-membered cycloalkyl group such as indan. Examples of carbocyclic aromatic groups include, but are not limited to, phenyl, naphthyl, e.g. 1-naphthyl and 2-naphthyl; anthracenyl, e.g. 1-anthracenyl, 2-anthracenyl; phenanthrenyl; fluorenonyl, e.g. 9-fluorenonyl, indanyl and the like.

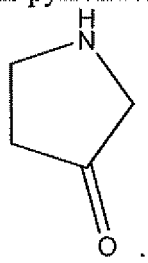
The term heteroaryl, as used herein, represents a stable monocyclic, bicyclic or tricyclic ring of up to 7 atoms in each ring, wherein at least one ring is aromatic and contains carbon and from 1 to 4 heteroatoms selected from the group consisting of O, N and S. In another embodiment, the term heteroaryl refers to a monocyclic, bicyclic or tricyclic aromatic ring of 5- to 14-ring atoms of carbon and from one to four heteroatoms selected from O, N, or S. As with the definition of heterocycle below, "heteroaryl" is also understood to include the N-oxide derivative of any nitrogen-containing heteroaryl. In cases where the heteroaryl substituent is bicyclic and one ring is non-aromatic or contains no heteroatoms, it is understood that attachment is via the aromatic ring or via the heteroatom containing ring, respectively.

Heteroaryl groups within the scope of this definition include but are not limited to acridinyl, carbazolyl, cinnolinyl, quinoxalinyl, pyrrazolyl, indolyl, benzotriazolyl, furanyl, thienyl, benzothienyl, benzofuranyl, quinolinyl, isoquinolinyl, oxazolyl, isoxazolyl, indolyl, pyrazinyl, pyridazinyl, pyridinyl, pyrimidinyl, pyrrolyl, tetrahydroquinoline. Additional examples of heteroaryl include, but are not limited to pyridyl, e.g., 2-pyridyl (also referred to as α-pyridyl), 3-pyridyl (also referred to as β-pyridyl) and 4-pyridyl (also referred to as (γ-pyridyl); thienyl, e.g., 2-thienyl and 3-thienyl; furanyl, e.g., 2-furanyl and 3-furanyl; pyrimidyl, e.g., 2-pyrimidyl and 4-pyrimidyl; imidazolyl, e.g., 2-imidazolyl; pyranyl, e.g., 2-pyranyl and 3-pyranyl; pyrazolyl, e.g., 4-pyrazolyl and 5-pyrazolyl; thiazolyl, e.g., 2-thiazolyl, 4-thiazolyl

and 5-thiazolyl; thiadiazolyl; isothiazolyl; oxazolyl, e.g., 2-oxazoyl, 4-oxazoyl and 5-oxazoyl; isoxazoyl; pyrrolyl; pyridazinyl; pyrazinyl and the like.

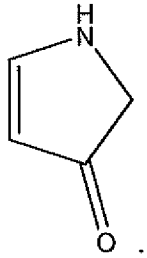
In an embodiment, "heteroaryl" may also include a "fused polycyclic aromatic", which is a heteroaryl fused with one or more other heteroaryl or nonaromatic heterocyclic ring. Examples include, quinolinyl and isoquinolinyl, e.g. 2-quinolinyl, 3-quinolinyl, 4-quinolinyl, 5-quinolinyl, 6-quinolinyl, 7-quinolinyl and 8-quinolinyl, 1-isoquinolinyl, 3-quinolinyl, 4-isoquinolinyl, 5-isoquinolinyl, 6-isoquinolinyl, 7-isoquinolinyl and 8-isoquinolinyl; benzofuranyl, e.g. 2-benzofuranyl and 3-benzofuranyl; dibenzofuranyl, e.g. 2,3-dihydrobenzofuranyl; dibenzothiophenyl; benzothienyl, e.g. 2-benzothienyl and 3-benzothienyl; indolyl, e.g. 2-indolyl and 3-indolyl; benzothiazolyl, e.g., 2-benzothiazolyl; benzooxazolyl, e.g., 2-benzooxazolyl; benzimidazolyl, e.g. 2-benzoimidazolyl; isoindolyl, e.g. 1-isoindolyl and 3-isoindolyl; benzotriazolyl; purinyl; thianaphthenyl, pyrazinyland the like.

"Heterocyclyl" means a non-aromatic saturated monocyclic, bicyclic, tricyclic or spirocyclic ring system comprising up to 7 atoms in each ring. Preferably, the heterocyclyl contains 3 to 14, or 5 to 10 ring atoms, in which one or more of the atoms in the ring system is an element other than carbon, for example, nitrogen, oxygen, phosphor or sulfur, alone or in combination. There are no adjacent oxygen and/or sulfur atoms present in the ring system. Preferred heterocyclyls contain about 5 to about 6 ring atoms. The heterocycle may be fused with an aromatic aryl group such as phenyl or heterocyclenyl. The prefix aza, oxa or thia before the heterocyclyl root name means that at least a nitrogen, oxygen or sulfur atom, respectively, is present as a ring atom. The nitrogen or sulfur atom of the heterocyclyl can be optionally oxidized to the corresponding N-oxide, S-oxide or S,S-dioxide. Non-limiting examples of suitable monocyclic heterocyclyl rings include piperidyl, pyrrolidinyl, piperazinyl, morpholinyl, thiomorpholinyl, thiazolidinyl, 1,4-dioxanyl, tetrahydrofuranyl, tetrahydrothiophenyl, lactam, lactone, and the like. "Heterocyclyl" also includes heterocyclyl rings as described above wherein =O replaces two available hydrogens on the same ring carbon atom. An example of such a moiety is pyrrolidone:



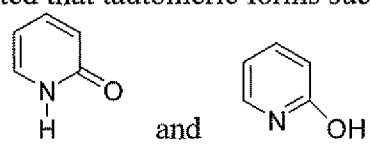
In describing the heteroatoms contained in a specified heterocyclyl group, the expression, "having one to x heteroatoms selected from the group of N, O, P and S" (wherein x is an a specified integer), for example, means that each heteroatom in the specified heterocyclyl is independently selected from the specified selection of heteroatoms. Attachment of a heterocyclyl substituent can occur via a carbon atom or via a heteroatom.

"Heterocyclenyl" means a non-aromatic monocyclic, bicyclic, tricyclic or spirocyclic ring system comprising up to 7 atoms in each ring. Preferably, the heterocyclenyl contains 3 to 14, or 5 to 10 ring atoms, in which one or more of the atoms in the ring system is an element other than carbon, for example nitrogen, oxygen or sulfur atom, alone or in combination, and which contains at least one carbon-carbon double bond or carbon-nitrogen double bond. There are no adjacent oxygen and/or sulfur atoms present in the ring system. Preferred heterocyclenyl rings contain about 5 to about 6 ring atoms. The prefix aza, oxa or thia before the heterocyclenyl root name means that at least a nitrogen, oxygen, phosphor or sulfur atom respectively is present as a ring atom. The nitrogen or sulfur atom of the heterocyclenyl can be optionally oxidized to the corresponding N-oxide, S-oxide or S,S-dioxide. Non-limiting examples of suitable heterocyclenyl groups include 1,2,3,4-tetrahydropyridinyl, 1,2-dihydropyridinyl, 1,4-dihydropyridinyl, 1,2,3,6-tetrahydropyridinyl, 1,4,5,6-tetrahydropyrimidinyl, 2-pyrrolinyl, 3-pyrrolinyl, 2-imidazolinyl, 2-pyrazolinyl, dihydroimidazolyl, dihydrooxazolyl, dihydrooxadiazolyl, dihydrothiazolyl, 3,4-dihydro-2H-pyranyl, dihydrofuranyl, fluorodihydrofuranyl, 7-oxabicyclo[2.2.1]heptenyl, dihydrothiophenyl, dihydrothiopyranyl, and the like. "Heterocyclenyl" also includes heterocyclenyl rings as described above wherein =O replaces two available hydrogens on the same ring carbon atom. An example of such a moiety is pyrrolidinone:



In describing the heteroatoms contained in a specified heterocyclenyl group, the expression, "having one to x heteroatoms selected from the group of N, O, P and S" (wherein x is an a specified integer), for example, means that each heteroatom in the specified heterocyclenyl is independently selected from the specified selection of heteroatoms.

It should also be noted that tautomeric forms such as, for example, the moieties:



are considered equivalent in certain embodiments of this invention.

An "alkylaryl group" is an alkyl group substituted with an aryl group, for example, a phenyl group. Suitable aryl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the aryl group.

An "alkylheteroaryl group" is an alkyl group substituted with a heteroaryl group. Suitable heteroaryl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the heteroaryl group.

An "alkylheterocyclyl group" is an alkyl group substituted with a heterocyclyl group. Suitable heterocyclyl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the heterocyclyl group.

An "alkylheterocyclenyl group" is an alkyl group substituted with a heterocyclenyl group. Suitable heterocyclenyl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the heterocyclenyl group.

An "alkylcycloalkyl group" is an alkyl group substituted with a cycloalkyl group. Suitable cycloalkyl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the cycloalkyl group.

An "arylalkyl group" is an aryl group substituted with an alkyl group, for example, a phenyl group. Suitable aryl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the alkyl group.

A "heteroarylalkyl group" is a heteroaryl group substituted with an alkyl group. Suitable heteroaryl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the alkyl group.

A "heterocyclylalkyl group" is a heterocyclyl group substituted with an alkyl group. Suitable heterocyclyl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the alkyl group.

A "heterocyclenylalkyl group" is a heterocyclenyl group substituted with an alkyl group. Suitable heterocyclenyl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the alkyl group.

A "cycloalkylalkyl group" is a cycloalkyl group substituted with an alkyl group. Suitable cycloalkyl groups are described herein and suitable alkyl groups are described herein. The bond to the parent moiety is through the alkyl group.

An "aryloxy group" is an aryl group that is attached to a compound via an oxygen (e.g., phenoxy).

An "alkoxy group" (alkyloxy), as used herein, is a straight chain or branched C_1-C_{12} or cyclic C_3-C_{12} alkyl group that is connected to a compound via an oxygen atom. Examples of alkoxy groups include but are not limited to methoxy, ethoxy and propoxy.

An "arylalkoxy group" (arylalkyloxy) is an arylalkyl group that is attached to a compound via an oxygen on the alkyl portion of the arylalkyl (e.g., phenylmethoxy).

An "arylamino group" as used herein, is an aryl group that is attached to a compound via a nitrogen.

An "alkylamino group" as used herein, is an alkyl group that is attached to a compound via a nitrogen.

As used herein, an "arylalkylamino group" is an arylalkyl group that is attached to a compound via a nitrogen on the alkyl portion of the arylalkyl.

An "alkylsulfonyl group" as used herein, is an alkyl group that is attached to a compound via the sulfur of a sulfonyl group.

When a moiety is referred to as "unsubstituted" or not referred to as "substituted" or "optionally substituted", it means that the moiety does not have any substituents. When a moiety is referred to as substituted, it denotes that any portion of the moiety that is known to one skilled in the art as being available for substitution can be substituted. The phrase "optionally substituted with one or more substituents" means, in one embodiment, one substituent, two substituents, three substituents, four substituents or five substituents. For example, the substitutable group can be a hydrogen atom that is replaced with a group other than hydrogen (i.e., a substituent group). Multiple substituent groups can be present. When multiple substituents are present, the substituents can be the same or different and substitution can be at any of the substitutable sites. Such means for substitution are well known in the art. For purposes of exemplification, which should not be construed as limiting the scope of this invention, some examples of groups that are substituents are: alkyl, alkenyl or alkynyl groups (which can also be substituted, with one or more substituents), alkoxy groups (which can be substituted), a halogen or halo group (F, Cl, Br, I), hydroxy, nitro, oxo, -CN, -COH, -COOH, amino, azido, N-alkylamino or N,N-dialkylamino (in which the alkyl groups can also be substituted), N-arylamino or N,N-diarylamino (in which the aryl groups can also be substituted), esters (-C(O)-OR, where R can be a group such as alkyl, aryl, etc., which can be substituted), ureas (-NHC(O)-NHR, where R can be a group such as alkyl, aryl, etc., which can be substituted), carbamates (-NHC(O)-OR, where R can be a group such as alkyl, aryl, etc., which can be substituted), sulfonamides (-NHS(O)$_2$R, where R can be a group such as alkyl, aryl, etc., which can be substituted), alkylsulfonyl (which can be substituted), aryl (which can be substituted), cycloalkyl (which can be substituted) alkylaryl (which can be substituted), alkylheterocyclyl (which can be substituted), alkylcycloalkyl (which can be substituted), and aryloxy.

In the compounds of generic Formula I, the atoms may exhibit their natural isotopic abundances, or one or more of the atoms may be artificially enriched in a particular isotope having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number predominantly found in nature. The present invention is meant to include all suitable isotopic variations of the compounds of generic Formula I. For example, different isotopic forms of hydrogen (H) include protium (1H) and deuterium (2H). Protium is the predominant hydrogen isotope found in nature. Enriching for deuterium may afford certain therapeutic advantages, such as increasing in vivo half-life or reducing dosage requirements, or

may provide a compound useful as a standard for characterization of biological samples. Isotopically-enriched compounds within generic Formula I can be prepared without undue experimentation by conventional techniques well known to those skilled in the art or by processes analogous to those described in the Schemes and Examples herein using appropriate isotopically-enriched reagents and/or intermediates.

Certain isotopically-labelled compounds of Formula (I) (e.g., those labeled with ^{3}H and ^{14}C) are useful in compound and/or substrate tissue distribution assays. Tritiated (i.e., ^{3}H) and carbon-14 (i.e., ^{14}C) isotopes are particularly preferred for their ease of preparation and detectability. Certain isotopically-labelled compounds of Formula (I) can be useful for medical imaging purposes. For instance those compounds labeled with positron-emitting isotopes like ^{11}C or ^{18}F can be useful for application in Positron Emission Tomography (PET) and those labeled with gamma ray emitting isotopes like ^{123}I can be useful for application in Single Photon Emission Computed Tomography (SPECT). Additionally, isotopic substitution of a compound at a site where epimerization occurs may slow or reduce the epimerization process and thereby retain the more active or efficacious form of the compound for a longer period of time.

It is also possible that the compounds of Formula (I) may exist in different tautomeric forms, and all such forms are embraced within the scope of the invention. Also, for example, all keto-enol and imine-enamine forms of the compounds are included in the invention.

Stereochemistry

When structures of the same constitution differ in respect to the spatial arrangement of certain atoms or groups, they are stereoisomers, and the considerations that are significant in analyzing their interrelationships are topological. If the relationship between two stereoisomers is that of an object and its nonsuperimposable mirror image, the two structures are enantiomeric, and each structure is said to be chiral. Stereoisomers also include diastereomers, cis-trans isomers and conformational isomers. Diastereoisomers can be chiral or achiral, and are not mirror images of one another. Cis-trans isomers differ only in the positions of atoms relative to a specified planes in cases where these atoms are, or are considered as if they were, parts of a rigid structure. Conformational isomers are isomers that can be interconverted by rotations about formally single bonds. Examples of such conformational isomers include cyclohexane conformations with chair and boat conformers, carbohydrates, linear alkane conformations with staggered, eclipsed and gauche confomers, etc. See J. Org. Chem. 35, 2849 (1970)

Many organic compounds exist in optically active forms having the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule

about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, enantiomers are identical except that they are non-superimposable mirror images of one another. A mixture of enantiomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture. Many of the compounds described herein can have one or more chiral centers and therefore can exist in different enantiomeric forms. If desired, a chiral carbon can be designated with an asterisk (*). When bonds to the chiral carbon are depicted as straight lines in the Formulas of the invention, it is understood that both the (R) and (S) configurations of the chiral carbon, and hence both enantiomers and mixtures thereof, are embraced within the Formula. As is used in the art, when it is desired to specify the absolute configuration about a chiral carbon, one of the bonds to the chiral carbon can be depicted as a wedge (bonds to atoms above the plane) and the other can be depicted as a series or wedge of short parallel lines is (bonds to atoms below the plane). The Cahn-Inglod-Prelog system can be used to assign the (R) or (S) configuration to a chiral carbon.

When the compounds of the present invention contain one chiral center, the compounds exist in two enantiomeric forms and the present invention includes both enantiomers and mixtures of enantiomers, such as the specific 50:50 mixture referred to as a racemic mixtures. The enantiomers can be resolved by methods known to those skilled in the art, such as formation of diastereoisomeric salts which may be separated, for example, by crystallization (see, CRC Handbook of Optical Resolutions via Diastereomeric Salt Formation by David Kozma (CRC Press, 2001)); formation of diastereoisomeric derivatives or complexes which may be separated, for example, by crystallization, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an enantiomer-specific reagent, for example enzymatic esterification; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support for example silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where the desired enantiomer is converted into another chemical entity by one of the separation procedures described above, a further step is required to liberate the desired enantiomeric form. Alternatively, specific enantiomers may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer into the other by asymmetric transformation.

Designation of a specific absolute configuration at a chiral carbon of the compounds of the invention is understood to mean that the designated enantiomeric form of the compounds is in enantiomeric excess (ee) or in other words is substantially free from the other enantiomer. For example, the "R" forms of the compounds are substantially free from the "S" forms of the compounds and are, thus, in enantiomeric excess of the "S" forms. Conversely, "S" forms of the compounds are substantially free of "R" forms of the compounds and are, thus, in enantiomeric excess of the "R" forms. Enantiomeric excess, as used herein, is the

presence of a particular enantiomer at greater than 50%. In a particular embodiment when a specific absolute configuration is designated, the enantiomeric excess of depicted compounds is at least about 90%.

When a compound of the present invention has two or more chiral carbons it can have more than two optical isomers and can exist in diastereoisomeric forms. For example, when there are two chiral carbons, the compound can have up to 4 optical isomers and 2 pairs of enantiomers ((S,S)/(R,R) and (R,S)/(S,R)). The pairs of enantiomers (e.g., (S,S)/(R,R)) are mirror image stereoisomers of one another. The stereoisomers that are not mirror-images (e.g., (S,S) and (R,S)) are diastereomers. The diastereoisomeric pairs may be separated by methods known to those skilled in the art, for example chromatography or crystallization and the individual enantiomers within each pair may be separated as described above. The present invention includes each diastereoisomer of such compounds and mixtures thereof.

As used herein, "a," an" and "the" include singular and plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an active agent" or "a pharmacologically active agent" includes a single active agent as well a two or more different active agents in combination, reference to "a carrier" includes mixtures of two or more carriers as well as a single carrier, and the like.

This invention is also intended to encompass pro-drugs of the pyrazolopyrrolopyrimidine compounds disclosed herein. A prodrug of any of the compounds can be made using well-known pharmacological techniques.

Pharmaceutically acceptable salts

The pyrazolopyrrolopyrimidine compounds described herein can, as noted above, be prepared in the form of their pharmaceutically acceptable salts. Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects. Examples of such salts are (a) acid addition salts organic and inorganic acids, for example, acid addition salts which may, for example, be hydrochloric acid, sulphuric acid, methanesulphonic acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, oxalic acid, citric acid, tartaric acid, carbonic acid, phosphoric acid, trifluoroacetic acid, formic acid and the like. Pharmaceutically acceptable salts can also be prepared from by treatment with inorganic bases, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like. Pharmaceutically acceptable salts can also be formed from elemental anions such as chlorine, bromine and iodine.

The active compounds disclosed can, as noted above, also be prepared in the form of their hydrates. The term "hydrate" includes but is not limited to hemihydrate, monohydrate, dihydrate, trihydrate, tetrahydrate and the like.

The active compounds disclosed can, as noted above, also be prepared in the form of a solvate with any organic or inorganic solvent, for example alcohols such as methanol, ethanol, propanol and isopropanol, ketones such as acetone, aromatic solvents and the like.

The active compounds disclosed can also be prepared in any solid or liquid physical form. For example, the compound can be in a crystalline form, in amorphous form, and have any particle size. Furthermore, the compound particles may be micronized, or may be agglomerated, particulate granules, powders, oils, oily suspensions or any other form of solid or liquid physical form.

The compounds of the present invention may also exhibit polymorphism. This invention further includes different polymorphs of the compounds of the present invention. The term "polymorph" refers to a particular crystalline state of a substance, having particular physical properties such as X-ray diffraction, IR spectra, melting point, and the like.

As used herein, "a," an" and "the" include singular and plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an active agent" or "a pharmacologically active agent" includes a single active agent as well a two or more different active agents in combination, reference to "a carrier" includes mixtures of two or more carriers as well as a single carrier, and the like.

METHODS OF TREATMENT

The Pyrazolopyrrolopyrimidine Compounds may be useful in human and veterinary medicine in the therapy of proliferative diseases such as cancer, autoimmune diseases, viral diseases, fungal diseases, neurological/neurodegenerative disorders, arthritis, inflammation, anti-proliferative (e.g., ocular retinopathy), neuronal, alopecia and cardiovascular disease. Many of these diseases and disorders are listed in U.S. 6,413,974, which is hereby incorporated by reference.

While not being bound by any specific theory it is believed that the Pyrazolopyrrolopyrimidine Compounds may be useful in the treatment of proliferative diseases such as cancer, autoimmune diseases, viral diseases, fungal diseases, neurological/neurodegenerative disorders, arthritis, inflammation, anti-proliferative (e.g., ocular retinopathy), neuronal, alopecia and cardiovascular disease because of their mTOR inhibitory activity.

The general value of the compounds of the invention in inhibiting mTOR can be determined, for example, using the assay described in Example 9. In addition, the general value in inhibiting mTORC1 or mTORC2 function can be evaluated using the assays described

in Example 10.

More specifically, the Pyrazolopyrrolopyrimidine Compounds can be useful in the treatment of a variety of cancers, including (but not limited to) the following:
tumor of the bladder, breast (including BRCA-mutated breast cancer), colorectal, colon, kidney, liver, lung, small cell lung cancer, non-small cell lung cancer, head and neck, esophagus, bladder, gall bladder, ovary, pancreas, stomach, cervix, thyroid, prostate, and skin, including squamous cell carcinoma;
leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma, mantle cell lymphoma, myeloma and Burkett's lymphoma;
chronic lymphocytic leukemia ("CLL"),
acute and chronic myelogenous leukemia, myelodysplastic syndrome and promyelocytic leukemia;
fibrosarcoma, rhabdomyosarcoma;
head and neck, mantle cell lymphoma, myeloma;
astrocytoma, neuroblastoma, glioma, glioblastoma, malignant glial tumors, astrocytoma, hepatocellular carcinoma, gastrointestinal stromal tumors ("GIST") and schwannomas;
melanoma, multiple myeloma, seminoma, teratocarcinoma, osteosarcoma, xenoderma pigmentosum, keratoctanthoma, thyroid follicular cancer, endometrial cancer, gastrointestinal tract cancer and Kaposi's sarcoma.

While not being bound by any specific theory, due to the key role of kinases in the regulation of cellular proliferation in general, inhibitors of kinases could act as reversible cytostatic agents which may be useful in the treatment of any disease process which features abnormal cellular proliferation, e.g., benign prostate hyperplasia, familial adenomatosis polyposis, neuro-fibromatosis, atherosclerosis, pulmonary fibrosis, arthritis, psoriasis, glomerulonephritis, restenosis following angioplasty or vascular surgery, hypertrophic scar formation, inflammatory bowel disease, transplantation rejection, endotoxic shock, and fungal infections.

The Pyrazolopyrrolopyrimidine Compounds may induce or inhibit apoptosis. The apoptotic response is aberrant in a variety of human diseases. The Pyrazolopyrrolopyrimidine Compounds, as modulators of apoptosis, can be useful in the treatment of cancer (including but not limited to those types mentioned hereinabove), viral infections (including, but not limited to, herpevirus, poxvirus, Epstein- Barr virus, Sindbis virus and adenovirus), prevention of AIDS development in HIV-infected individuals, autoimmune diseases (including but not limited to systemic lupus, erythematosus, autoimmune mediated glomerulonephritis, rheumatoid arthritis, psoriasis, inflammatory bowel disease, and autoimmune diabetes mellitus), neurodegenerative disorders (including but not limited to Alzheimer's disease, AIDS-related dementia, Parkinson's disease, amyotrophic lateral sclerosis,

retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration), myelodysplastic syndromes, aplastic anemia, ischemic injury associated with myocardial infarctions, stroke and reperfusion injury, arrhythmia, atherosclerosis, toxin-induced or alcohol related liver diseases, hematological diseases (including but not limited to chronic anemia and aplastic anemia), degenerative diseases of the musculoskeletal system (including but not limited to osteoporosis and arthritis) aspirin-sensitive rhinosinusitis, cystic fibrosis, multiple sclerosis, kidney diseases and cancer pain.

While not being bound by any specific theory, the Pyrazolopyrrolopyrimidine Compounds, as inhibitors of kinases, can modulate the level of cellular RNA and DNA synthesis. These compounds would therefore be useful in the treatment of viral infections (including but not limited to HIV, human papilloma virus, herpesvirus, poxvirus, Epstein-Barr virus, Sindbis virus and adenovirus).

In particular embodiments of the invention, Pyrazolopyrrolopyrimidine Compounds, as inhibitors of mTOR kinase could act in diseases or disorders other than cancer that are associated with dysregulated mTOR activity such as viral infections (including, but not limited to, herpevirus, poxvirus, Epstein- Barr virus, Sindbis virus and adenovirus), prevention of AIDS development in HIV-infected individuals, autoimmune diseases (including but not limited to systemic lupus, erythematosus, autoimmune mediated glomerulonephritis, rheumatoid arthritis, psoriasis, inflammatory bowel disease, and autoimmune diabetes mellitus), neurodegenerative disorders (including but not limited to Alzheimer's disease, AIDS-related dementia, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, spinal muscular atrophy and cerebellar degeneration), myelodysplastic syndromes, aplastic anemia, ischemic injury associated with myocardial infarctions, stroke and reperfusion injury, arrhythmia, atherosclerosis, toxin-induced or alcohol related liver diseases, hematological diseases (including but not limited to chronic anemia and aplastic anemia), degenerative diseases of the musculoskeletal system (including but not limited to osteoporosis and arthritis) aspirin-sensitive rhinosinusitis, cystic fibrosis, multiple sclerosis, kidney diseases and cancer pain. .

The Pyrazolopyrrolopyrimidine Compounds may also be useful in the chemoprevention of cancer. Chemoprevention is defined as inhibiting the development of invasive cancer by either blocking the initiating mutagenic event or by blocking the progression of pre-malignant cells that have already suffered an insult or inhibiting tumor relapse.

The Pyrazolopyrrolopyrimidine Compounds may also be useful in inhibiting tumor angiogenesis and metastasis.

Another aspect of this invention is a method of treating a patient (e.g., human) having a disease or condition associated with mTOR kinases by administering a therapeutically effective amount of a Pyrazolopyrrolopyrimidine Compound, or a pharmaceutically acceptable

salt of said compound to the patient.

The present invention provides a method of treating cancer comprising the step of administering to a subject a therapeutically effective amount of the Pyrazolopyrrolopyrimidine Compounds. The present invention also provides the Use of the Pyrazolopyrrolopyrimidine Compounds for the preparation of a medicament for the treatment of cancer. The invention also provides the Pyrazolopyrrolopyrimidine Compounds for use in the treatment of cancer.

In the therapies described above, an example dosage for administration to a patient is about 0.001 to 1000 mg/kg of body weight/day of the Pyrazolopyrrolopyrimidine Compound. Another example dosage is about 0.01 to 25 mg/kg of body weight/day of the Pyrazolopyrrolopyrimidine Compound, or a pharmaceutically acceptable salt of said compound.

The dosage regimen utilizing the compounds of the present invention can be selected in accordance with a variety of factors including type, species, age, weight, sex and the type of cancer being treated; the severity (i.e., stage) of the disease to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to treat, for example, to prevent, inhibit (fully or partially) or arrest the progress of the disease.

Definitions:

As used herein, the term "therapeutically effective amount" means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician. The therapeutic effect is dependent upon the disease or disorder being treated or the biological effect desired. As such, the therapeutic effect can be a decrease in the severity of symptoms associated with the disease or disorder and/or inhibition (partial or complete) of progression of the disease. The amount needed to elicit the therapeutic response can be determined based on the age, health, size and sex of the subject. Optimal amounts can also be determined based on monitoring of the subject's response to treatment.

Further, a therapeutically effective amount, can be an amount that selectively induces terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, or an amount that induces terminal differentiation of tumor cells.

The method of the present invention is intended for the treatment or chemoprevention of human patients with cancer. However, it is also likely that the method would be effective in the treatment of cancer in other subjects. "Subject", as used herein, refers to animals such as mammals, including, but not limited to, primates (e.g., humans),

cows, sheep, goats, horses, pigs, dogs, cats, rabbits, guinea pigs, rats, mice or other bovine, ovine, equine, canine, feline, rodent or murine species.

The term "administration" and variants thereof (e.g., "administering" a compound) in reference to a compound of the invention means introducing the compound or a prodrug of the compound into the system of the animal in need of treatment. When a compound of the invention or prodrug thereof is provided in combination with one or more other active agents (e.g., a cytotoxic agent, etc.), "administration" and its variants are each understood to include concurrent and sequential introduction of the compound or prodrug thereof and other agents.

COMBINATION THERAPY

The compounds of the present invention can be administered alone or in combination with other therapies suitable for the disease or disorder being treated. Where separate dosage formulations are used, the compound and the other therapeutic agent can be administered at essentially the same time (concurrently) or at separately staggered times (sequentially). The pharmaceutical combination is understood to include all these regimens. Administration in these various ways are suitable for the present invention as long as the beneficial therapeutic effect of the compound and the other therapeutic agent are realized by the patient at substantially the same time. In an embodiment, such beneficial effect is achieved when the target blood level concentrations of each active drug are maintained at substantially the same time.

The instant compounds are also useful in combination with known therapeutic agents and anti-cancer agents. For example, instant compounds are useful in combination with known anti-cancer agents. Combinations of the presently disclosed compounds with other anti-cancer or chemotherapeutic agents are within the scope of the invention. Therefore, the present invention encompasses pharmaceutical compositions comprising a therapeutically effective amount of the compound of the invention and a pharmaceutically acceptable carrier and optionally other threrapeutic ingredients, such as an anti-cancer agent. Examples of such agents can be found in *Cancer Principles and Practice of Oncology* by V.T. Devita and S. Hellman (editors), 6th edition (February 15, 2001), Lippincott Williams & Wilkins Publishers. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the cancer involved. Such anti-cancer agents include, but are not limited to, the following: estrogen receptor modulators, androgen receptor modulators, retinoid receptor modulators, cytotoxic/cytostatic agents, antiproliferative agents, prenyl-protein transferase inhibitors, HMG-CoA reductase inhibitors and other angiogenesis inhibitors, inhibitors of cell proliferation and survival signaling, apoptosis inducing agents, agents that interfere with cell cycle checkpoints, agents

that interfere with receptor tyrosine kinases (RTKs) and cancer vaccines. The instant compounds are particularly useful when co-administered with radiation therapy.

In an embodiment, the instant compounds are also useful in combination with known anti-cancer agents including the following: estrogen receptor modulators, androgen receptor modulators, retinoid receptor modulators, cytotoxic agents, antiproliferative agents, prenyl-protein transferase inhibitors, HMG-CoA reductase inhibitors, HIV protease inhibitors, reverse transcriptase inhibitors, and other angiogenesis inhibitors.

"Estrogen receptor modulators" refers to compounds that interfere with or inhibit the binding of estrogen to the receptor, regardless of mechanism. Examples of estrogen receptor modulators include, but are not limited to, diethylstibestral, tamoxifen, raloxifene, idoxifene, LY353381, LY117081, toremifene, fluoxymestero, lfulvestrant, 4-[7-(2,2-dimethyl-1-oxopropoxy-4-methyl-2-[4-[2-(1-piperidinyl)ethoxy]phenyl]-2H-1-benzopyran-3-yl]-phenyl-2,2-dimethylpropanoate, 4,4'-dihydroxybenzophenone-2,4-dinitrophenyl-hydrazone, and SH646.

Other hormonal agents include: aromatase inhibitors (e.g., aminoglutethimide, anastrozole and tetrazole), luteinizing hormone release hormone (LHRH) analogues, ketoconazole, goserelin acetate, leuprolide, megestrol acetate and mifepristone.

"Androgen receptor modulators" refers to compounds which interfere or inhibit the binding of androgens to the receptor, regardless of mechanism. Examples of androgen receptor modulators include finasteride and other 5α-reductase inhibitors, nilutamide, flutamide, bicalutamide, liarozole, and abiraterone acetate.

"Retinoid receptor modulators" refers to compounds which interfere or inhibit the binding of retinoids to the receptor, regardless of mechanism. Examples of such retinoid receptor modulators include bexarotene, tretinoin, 13-cis-retinoic acid, 9-cis-retinoic acid, α-difluoromethylornithine, ILX23-7553, trans-N-(4'-hydroxyphenyl) retinamide, and N-4-carboxyphenyl retinamide.

"Cytotoxic/cytostatic agents" refer to compounds which cause cell death or inhibit cell proliferation primarily by interfering directly with the cell's functioning or inhibit or interfere with cell mytosis, including alkylating agents, tumor necrosis factors, intercalators, hypoxia activatable compounds, microtubule inhibitors/microtubule-stabilizing agents, inhibitors of mitotic kinesins, inhibitors of histone deacetylase, inhibitors of kinases involved in mitotic progression, antimetabolites; biological response modifiers; hormonal/anti-hormonal therapeutic agents, haematopoietic growth factors, monoclonal antibody targeted therapeutic agents, topoisomerase inhibitors, proteasome inhibitors and ubiquitin ligase inhibitors.

Examples of cytotoxic agents include, but are not limited to, sertenef, cachectin, chlorambucil, cyclophosphamide, ifosfamide, mechlorethamine, melphalan, uracil mustard, thiotepa, busulfan, carmustine, lomustine, streptozocin, tasonermin, lonidamine, carboplatin, altretamine, dacarbazine, procarbazine, prednimustine, dibromodulcitol, ranimustine,

fotemustine, nedaplatin, oxaliplatin, temozolomide, heptaplatin, estramustine, improsulfan tosilate, trofosfamide, nimustine, dibrospidium chloride, pumitepa, lobaplatin, satraplatin, profiromycin, cisplatin, irofulven, dexifosfamide, cis-aminedichloro(2-methyl-pyridine)platinum, benzylguanine, glufosfamide, GPX100, (trans, trans, trans)-bis-mu-(hexane-1,6-diamine)-mu-[diamine-platinum(II)]bis[diamine(chloro)platinum (II)]tetrachloride, diarizidinylspermine, arsenic trioxide, 1-(11-dodecylamino-10-hydroxyundecyl)-3,7-dimethylxanthine, zorubicin, doxorubicin, daunorubicin, idarubicin, anthracenedione, bleomycin, mitomycin C, dactinomycin, plicatomycin, bisantrene, mitoxantrone, pirarubicin, pinafide, valrubicin, amrubicin, antineoplaston, 3'-deamino-3'-morpholino-13-deoxo-10-hydroxycarminomycin, annamycin, galarubicin, elinafide, MEN10755, and 4-demethoxy-3-deamino-3-aziridinyl-4-methylsulphonyl-daunorubicin (see WO 00/50032).

An example of a hypoxia activatable compound is tirapazamine.

Examples of proteasome inhibitors include but are not limited to lactacystin and bortezomib.

Examples of microtubule inhibitors/microtubule-stabilising agents include vincristine, vinblastine, vindesine, vinzolidine, vinorelbine, vindesine sulfate, 3',4'-didehydro-4'-deoxy-8'-norvincaleukoblastine, podophyllotoxins (e.g., etoposide (VP-16) and teniposide (VM-26)), paclitaxel, docetaxol, rhizoxin, dolastatin, mivobulin isethionate, auristatin, cemadotin, RPR109881, BMS184476, vinflunine, cryptophycin, 2,3,4,5,6-pentafluoro-N-(3-fluoro-4-methoxyphenyl) benzene sulfonamide, anhydrovinblastine, N,N-dimethyl-L-valyl-L-valyl-N-methyl-L-valyl-L-prolyl-L-proline-t-butylamide, TDX258, the epothilones (see for example U.S. Pat. Nos. 6,284,781 and 6,288,237) and BMS188797.

Some examples of topoisomerase inhibitors are topotecan, hycaptamine, irinotecan, rubitecan, 6-ethoxypropionyl-3',4'-O-exo-benzylidene-chartreusin, 9-methoxy-N,N-dimethyl-5-nitropyrazolo[3,4,5-kl]acridine-2-(6H) propanamine, 1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':b,7]-indolizino[1,2b]quinoline-10,13(9H,15H)dione, lurtotecan, 7-[2-(N-isopropylamino)ethyl]-(20S)camptothecin, BNP1350, BNPI1100, BN80915, BN80942, etoposide phosphate, teniposide, sobuzoxane, 2'-dimethylamino-2'-deoxy-etoposide, GL331, N-[2-(dimethylamino)ethyl]-9-hydroxy-5,6-dimethyl-6H-pyrido[4,3-b]carbazole-1-carboxamide, asulacrine, (5a, 5aB, 8aa,9b)-9-[2-[N-[2-(dimethylamino)ethyl]-N-methylamino]ethyl]-5-[4-hydro0xy-3,5-dimethoxyphenyl]-5,5a,6,8,8a,9-hexohydrofuro(3',4':6,7)naphtho(2,3-d)-1,3-dioxol-6-one, 2,3-(methylenedioxy)-5-methyl-7-hydroxy-8-methoxybenzo[c]-phenanthridinium, 6,9-bis[(2-aminoethyl)amino]benzo[g]isoguinoline-5,10-dione, 5-(3-aminopropylamino)-7,10-dihydroxy-2-(2-hydroxyethylaminomethyl)-6H-pyrazolo[4,5,1-de]acridin-6-one, N-[1-[2(diethylamino)ethylamino]-7-methoxy-9-oxo-9H-thioxanthen-4-

ylmethyl]formamide, N-(2-(dimethylamino)ethyl)acridine-4-carboxamide, 6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7H-indeno[2,1-c] quinolin-7-one, and dimesna.

Examples of inhibitors of mitotic kinesins, and in particular the human mitotic kinesin KSP, are described in PCT Publications WO 01/30768, WO 01/98278, WO 03/050,064, WO 03/050,122, WO 03/049,527, WO 03/049,679, WO 03/049,678, WO 03/39460 and WO2003/079973, WO2003/099211, WO2004/039774, WO2003/105855, WO2003/106417. In an embodiment inhibitors of mitotic kinesins include, but are not limited to inhibitors of KSP, inhibitors of MKLP1, inhibitors of CENP-E, inhibitors of MCAK, inhibitors of Kif14, inhibitors of Mphosph1 and inhibitors of Rab6-KIFL.

Examples of "histone deacetylase inhibitors" include, but are not limited to, SAHA, TSA, oxamflatin, PXD101, MG98, valproic acid and scriptaid. Further reference to other histone deacetylase inhibitors may be found in the following manuscript; Miller, T.A. et al. J. Med. Chem. 46(24):5097-5116 (2003).

"Inhibitors of kinases involved in mitotic progression" include, but are not limited to, inhibitors of aurora kinase, inhibitors of Polo-like kinases (PLK; in particular inhibitors of PLK-1), inhibitors of bub-1 and inhibitors of bub-R1. An example of an "aurora kinase inhibitor" is VX-680.

"Antiproliferative agents" includes antisense RNA and DNA oligonucleotides such as G3139, ODN698, RVASKRAS, GEM231, and INX3001, and antimetabolites such as enocitabine, carmofur, tegafur, pentostatin, doxifluridine, trimetrexate, fludarabine, capecitabine, galocitabine, cytarabine ocfosfate, fosteabine sodium hydrate, raltitrexed, paltitrexid, emitefur, tiazofurin, decitabine, nolatrexed, pemetrexed, nelzarabine, 2'-deoxy-2'-methylidenecytidine, 2'-fluoromethylene-2'-deoxycytidine, N-[5-(2,3-dihydro-benzofuryl)sulfonyl]-N'-(3,4-dichlorophenyl)urea, N6-[4-deoxy-4-[N2-[2(E),4(E)-tetradecadienoyl]glycylamino]-L-glycero-B-L-manno-heptopyranosyl]adenine, aplidine, ecteinascidin, troxacitabine, 4-[2-amino-4-oxo-4,6,7,8-tetrahydro-3H-pyrimidino[5,4-b][1,4]thiazin-6-yl-(S)-ethyl]-2,5-thienoyl-L-glutamic acid, aminopterin, 5-flurouracil, floxuridine, methotrexate, leucovarin, hydroxyurea, thioguanine (6-TG), mercaptopurine (6-MP), cytarabine, pentostatin, fludarabine phosphate, cladribine (2-CDA), asparaginase, gemcitabine, alanosine, 11-acetyl-8-(carbamoyloxymethyl)-4-formyl-6-methoxy-14-oxa-1,11-diazatetracyclo(7.4.1.0.0)-tetradeca-2,4,6-trien-9-yl acetic acid ester, swainsonine, lometrexol, dexrazoxane, methioninase, 2'-cyano-2'-deoxy-N4-palmitoyl-1-B-D-arabino furanosyl cytosine and 3-aminopyridine-2-carboxaldehyde thiosemicarbazone.

Examples of monoclonal antibody targeted therapeutic agents include those therapeutic agents which have cytotoxic agents or radioisotopes attached to a cancer cell specific or target cell specific monoclonal antibody. Examples include Bexxar.

"HMG-CoA reductase inhibitors" refers to inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase. Examples of HMG-CoA reductase inhibitors that may be used

include but are not limited to lovastatin (MEVACOR®; see U.S. Pat. Nos. 4,231,938, 4,294,926 and 4,319,039), simvastatin (ZOCOR®; see U.S. Pat. Nos. 4,444,784, 4,820,850 and 4,916,239), pravastatin (PRAVACHOL®; see U.S. Pat. Nos. 4,346,227, 4,537,859, 4,410,629, 5,030,447 and 5,180,589), fluvastatin (LESCOL®; see U.S. Pat. Nos. 5,354,772, 4,911,165, 4,929,437, 5,189,164, 5,118,853, 5,290,946 and 5,356,896) and atorvastatin (LIPITOR®; see U.S. Pat. Nos. 5,273,995, 4,681,893, 5,489,691 and 5,342,952). The structural formulas of these and additional HMG-CoA reductase inhibitors that may be used in the instant methods are described at page 87 of M. Yalpani, "Cholesterol Lowering Drugs", *Chemistry & Industry*, pp. 85-89 (5 February 1996) and US Patent Nos. 4,782,084 and 4,885,314. The term HMG-CoA reductase inhibitor as used herein includes all pharmaceutically acceptable lactone and open-acid forms (i.e., where the lactone ring is opened to form the free acid) as well as salt and ester forms of compounds which have HMG-CoA reductase inhibitory activity, and therefor the use of such salts, esters, open-acid and lactone forms is included within the scope of this invention.

"Prenyl-protein transferase inhibitor" refers to a compound which inhibits any one or any combination of the prenyl-protein transferase enzymes, including farnesyl-protein transferase (FPTase), geranylgeranyl-protein transferase type I (GGPTase-I), and geranylgeranyl-protein transferase type-II (GGPTase-II, also called Rab GGPTase).

Examples of prenyl-protein transferase inhibitors can be found in the following publications and patents: WO 96/30343, WO 97/18813, WO 97/21701, WO 97/23478, WO 97/38665, WO 98/28980, WO 98/29119, WO 95/32987, U.S. Pat. No. 5,420,245, U.S. Pat. No. 5,523,430, U.S. Pat. No. 5,532,359, U.S. Pat. No. 5,510,510, U.S. Pat. No. 5,589,485, U.S. Pat. No. 5,602,098, European Patent Publ. 0 618 221, European Patent Publ. 0 675 112, European Patent Publ. 0 604 181, European Patent Publ. 0 696 593, WO 94/19357, WO 95/08542, WO 95/11917, WO 95/12612, WO 95/12572, WO 95/10514, U.S. Pat. No. 5,661,152, WO 95/10515, WO 95/10516, WO 95/24612, WO 95/34535, WO 95/25086, WO 96/05529, WO 96/06138, WO 96/06193, WO 96/16443, WO 96/21701, WO 96/21456, WO 96/22278, WO 96/24611, WO 96/24612, WO 96/05168, WO 96/05169, WO 96/00736, U.S. Pat. No. 5,571,792, WO 96/17861, WO 96/33159, WO 96/34850, WO 96/34851, WO 96/30017, WO 96/30018, WO 96/30362, WO 96/30363, WO 96/31111, WO 96/31477, WO 96/31478, WO 96/31501, WO 97/00252, WO 97/03047, WO 97/03050, WO 97/04785, WO 97/02920, WO 97/17070, WO 97/23478, WO 97/26246, WO 97/30053, WO 97/44350, WO 98/02436, and U.S. Pat. No. 5,532,359. For an example of the role of a prenyl-protein transferase inhibitor on angiogenesis see *European J. of Cancer*, Vol. 35, No. 9, pp.1394-1401 (1999).

"Angiogenesis inhibitors" refers to compounds that inhibit the formation of new blood vessels, regardless of mechanism. Examples of angiogenesis inhibitors include, but are not limited to, tyrosine kinase inhibitors, such as inhibitors of the tyrosine kinase receptors Flt-1 (VEGFR1) and Flk-1/KDR (VEGFR2), inhibitors of epidermal-derived, fibroblast-derived,

or platelet derived growth factors, MMP (matrix metalloprotease) inhibitors, integrin blockers, interferon-α, interleukin-12, erythropoietin (epoietin-α), granulocyte-CSF (filgrastin), granulocyte, macrophage-CSF (sargramostim), pentosan polysulfate, cyclooxygenase inhibitors, including nonsteroidal anti-inflammatories (NSAIDs) like aspirin and ibuprofen as well as selective cyclooxy-genase-2 inhibitors like celecoxib and rofecoxib (*PNAS*, Vol. 89, p. 7384 (1992); *JNCI*, Vol. 69, p. 475 (1982); *Arch. Opthalmol.*, Vol. 108, p.573 (1990); *Anat. Rec.*, Vol. 238, p. 68 (1994); *FEBS Letters*, Vol. 372, p. 83 (1995); *Clin, Orthop.* Vol. 313, p. 76 (1995); *J. Mol. Endocrinol.*, Vol. 16, p.107 (1996); *Jpn. J. Pharmacol.*, Vol. 75, p. 105 (1997); *Cancer Res.*, Vol. 57, p. 1625 (1997); *Cell*, Vol. 93, p. 705 (1998); *Intl. J. Mol. Med.*, Vol. 2, p. 715 (1998); *J. Biol. Chem.*, Vol. 274, p. 9116 (1999)), steroidal anti-inflammatories (such as corticosteroids, mineralocorticoids, dexamethasone, prednisone, prednisolone, methylpred, betamethasone), carboxyamidotriazole, combretastatin A-4, squalamine, 6-O-chloroacetyl-carbonyl)-fumagillol, thalidomide, angiostatin, troponin-1, angiotensin II antagonists (see Fernandez et al., *J. Lab. Clin. Med.* 105:141-145 (1985)), and antibodies to VEGF (see, *Nature Biotechnology*, Vol. 17, pp.963-968 (October 1999); Kim et al., *Nature*, *362*, 841-844 (1993); WO 00/44777; and WO 00/61186).

Other therapeutic agents that modulate or inhibit angiogenesis and may also be used in combination with the compounds of the instant invention include agents that modulate or inhibit the coagulation and fibrinolysis systems (see review in *Clin. Chem. La. Med.* 38:679-692 (2000)). Examples of such agents that modulate or inhibit the coagulation and fibrinolysis pathways include, but are not limited to, heparin (see *Thromb. Haemost.* 80:10-23 (1998)), low molecular weight heparins and carboxypeptidase U inhibitors (also known as inhibitors of active thrombin activatable fibrinolysis inhibitor [TAFIa]) (see *Thrombosis Res.* 101:329-354 (2001)). TAFIa inhibitors have been described in PCT Publication WO 03/013,526 and U.S. Ser. No. 60/349,925 (filed January 18, 2002).

"Agents that interfere with cell cycle checkpoints" refer to compounds that inhibit protein kinases that transduce cell cycle checkpoint signals, thereby sensitizing the cancer cell to DNA damaging agents. Such agents include inhibitors of ATR, ATM, the Chk1 and Chk2 kinases and cdk and cdc kinase inhibitors and are specifically exemplified by 7-hydroxystaurosporin, flavopiridol, CYC202 (Cyclacel) and BMS-387032.

"Agents that interfere with receptor tyrosine kinases (RTKs)" refer to compounds that inhibit RTKs and therefore mechanisms involved in oncogenesis and tumor progression. Such agents include inhibitors of c-Kit, Eph, PDGF, Flt3 and c-Met. Further agents include inhibitors of RTKs shown as described by Bume-Jensen and Hunter, Nature, 411:355-365, 2001.

"Inhibitors of cell proliferation and survival signaling pathway" refer to pharmaceutical agents that inhibit cell surface receptors and signal transduction cascades downstream of those surface receptors. Such agents include inhibitors of inhibitors of EGFR

(for example gefitinib and erlotinib), inhibitors of ERB-2 (for example trastuzumab), inhibitors of IGFR, inhibitors of CD20 (rituximab), inhibitors of cytokine receptors, inhibitors of MET, inhibitors of PI3K family kinase (for example LY294002), serine/threonine kinases (including but not limited to inhibitors of Akt such as described in (WO 03/086404, WO 03/086403, WO 03/086394, WO 03/086279, WO 02/083675, WO 02/083139, WO 02/083140 and WO 02/083138), inhibitors of Raf kinase (for example BAY-43-9006), inhibitors of MEK (for example CI-1040 and PD-098059) and inhibitors of mTOR (for example Wyeth CCI-779 and Ariad AP23573). Such agents include small molecule inhibitor compounds and antibody antagonists.

Examples of mTOR inhibitors include ridaforolimus, temsirolimus, everolimus, a rapamycin-analog. Ridaforolimus, also known as AP 23573, MK-8669 and deforolimus, is a unique, non-prodrug analog of rapmycin that has antiproliferative activity in a broad range of human tumor cell lines in vitro and in murine tumor xenograft models utilizing human tumor cell lines. Ridaforolimus has been administered to patients with advanced cancer and is currently in clinical development for various advanced malignancies, including studies in patients with advanced soft tissue or bone sarcomas. Thus far, these trials have demonstrated that ridaforolimus is generally well-tolerated with a predictable and manageable adverse even profile, and possess anti-tumor activity in a broad range of cancers. A description and preparation of ridaforolimus is described in U.S. Patent No. 7,091,213 to Ariad Gene Therapeutics, Inc.

Temsirolimus, also known as Torisel®, is currently marketed for the treatment of renal cell carcinoma. A description and preparation of temsirolimus is described in U.S. Patent No. 5,362,718 to American Home Products Corporation. Everolimus, also known as Certican® or RAD001, marketed by Novartis, has greater stability and enhanced solubility in organic solvents, as well as more favorable pharmokinetics with fewer side effects than rapamycin (sirolimus). Everolimus has been used in conjunction with microemulsion cyclosporin (Neoral®, Novartis) to increase the efficacy of the immunosuppressive regime.

"Apoptosis inducing agents" include activators of TNF receptor family members (including the TRAIL receptors).

The invention also encompasses combinations with NSAID's which are selective COX-2 inhibitors. For purposes of this specification NSAID's which are selective inhibitors of COX-2 are defined as those which possess a specificity for inhibiting COX-2 over COX-1 of at least 100 fold as measured by the ratio of IC_{50} for COX-2 over IC_{50} for COX-1 evaluated by cell or microsomal assays. Such compounds include, but are not limited to those disclosed in U.S. Pat. 5,474,995, U.S. Pat. 5,861,419, U.S. Pat. 6,001,843, U.S. Pat. 6,020,343, U.S. Pat. 5,409,944, U.S. Pat. 5,436,265, U.S. Pat. 5,536,752, U.S. Pat. 5,550,142, U.S. Pat. 5,604,260, U.S. 5,698,584, U.S. Pat. 5,710,140, WO 94/15932, U.S. Pat. 5,344,991, U.S. Pat.

5,134,142, U.S. Pat. 5,380,738, U.S. Pat. 5,393,790, U.S. Pat. 5,466,823, U.S. Pat. 5,633,272, and U.S. Pat. 5,932,598.

Inhibitors of COX-2 that are particularly useful in the instant method of treatment are: 3-phenyl-4-(4-(methylsulfonyl)phenyl)-2-(*5H*)-furanone; and 5-chloro-3-(4-methylsulfonyl)phenyl-2-(2-methyl-5-pyridinyl)pyridine; or a pharmaceutically acceptable salt thereof.

Compounds that have been described as specific inhibitors of COX-2 and are therefore useful in the present invention include, but are not limited to: parecoxib, CELEBREX® and BEXTRA® or a pharmaceutically acceptable salt thereof.

Other examples of angiogenesis inhibitors include, but are not limited to, endostatin, ukrain, ranpirnase, IM862, 5-methoxy-4-[2-methyl-3-(3-methyl-2-butenyl)oxiranyl]-1-oxaspiro[2,5]oct-6-yl(chloroacetyl)carbamate, acetyldinanaline, 5-amino-1-[[3,5-dichloro-4-(4-chlorobenzoyl)phenyl]methyl]-1H-1,2,3-triazole-4-carboxamide,CM101, squalamine, combretastatin, RPI4610, NX31838, sulfated mannopentaose phosphate, 7,7-(carbonyl-bis[imino-N-methyl-4,2-pyrrolocarbonylimino[N-methyl-4,2-pyrrole]-carbonylimino]-bis-(1,3-naphthalene disulfonate), and 3-[(2,4-dimethylpyrrol-5-yl)methylene]-2-indolinone (SU5416).

As used above, "integrin blockers" refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha_v\beta_3$ integrin, to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v\beta 5$ integrin, to compounds which antagonize, inhibit or counteract binding of a physiological ligand to both the $\alpha_v\beta_3$ integrin and the $\alpha_v\beta_5$ integrin, and to compounds which antagonize, inhibit or counteract the activity of the particular integrin(s) expressed on capillary endothelial cells. The term also refers to antagonists of the $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$ and $\alpha_6\beta_4$ integrins. The term also refers to antagonists of any combination of $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$ and $\alpha_6\beta_4$ integrins.

Some specific examples of tyrosine kinase inhibitors include N-(trifluoromethylphenyl)-5-methylisoxazol-4-carboxamide, 3-[(2,4-dimethylpyrrol-5-yl)methylidenyl)indolin-2-one, 17-(allylamino)-17-demethoxygeldanamycin, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-[3-(4-morpholinyl)propoxyl]quinazoline, N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine, BIBX1382, 2,3,9,10,11,12-hexahydro-10-(hydroxymethyl)-10-hydroxy-9-methyl-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i][1,6]benzodiazocin-1-one, SH268, genistein, imatinib (STI571), CEP2563, 4-(3-chlorophenylamino)-5,6-dimethyl-7H-pyrrolo[2,3-d]pyrimidinemethane sulfonate, 4-(3-bromo-4-hydroxyphenyl)amino-6,7-dimethoxyquinazoline, 4-(4'-hydroxyphenyl)amino-6,7-dimethoxyquinazoline, SU6668, STI571A, N-4-chlorophenyl-4-(4-pyridylmethyl)-1-phthalazinamine, and EMD121974.

Combinations with compounds other than anti-cancer compounds are also encompassed in the instant methods. For example, combinations of the instantly claimed compounds with PPAR-γ (i.e., PPAR-gamma) agonists and PPAR-δ (i.e., PPAR-delta) agonists are useful in the treatment of certain malingnancies. PPAR-γ and PPAR-δ are the nuclear peroxisome proliferator-activated receptors γ and δ. The expression of PPAR-γ on endothelial cells and its involvement in angiogenesis has been reported in the literature (see *J. Cardiovasc. Pharmacol.* 1998; 31:909-913; *J. Biol. Chem.* 1999; 274:9116-9121; *Invest. Ophthalmol Vis. Sci.* 2000; 41:2309-2317). More recently, PPAR-γ agonists have been shown to inhibit the angiogenic response to VEGF in vitro; both troglitazone and rosiglitazone maleate inhibit the development of retinal neovascularization in mice. (*Arch. Ophthamol.* 2001; 119:709-717). Examples of PPAR-γ agonists and PPAR- γ/α agonists include, but are not limited to, thiazolidinediones (such as DRF2725, CS-011, troglitazone, rosiglitazone, and pioglitazone), fenofibrate, gemfibrozil, clofibrate, GW2570, SB219994, AR-H039242, JTT-501, MCC-555, GW2331, GW409544, NN2344, KRP297, NP0110, DRF4158, NN622, GI262570, PNU182716, DRF552926, 2-[(5,7-dipropyl-3-trifluoromethyl-1,2-benzisoxazol-6-yl)oxy]-2-methylpropionic acid (disclosed in USSN 09/782,856), and 2(R)-7-(3-(2-chloro-4-(4-fluorophenoxy) phenoxy)propoxy)-2-ethylchromane-2-carboxylic acid (disclosed in USSN 60/235,708 and 60/244,697).

Another embodiment of the instant invention is the use of the presently disclosed compounds in combination with gene therapy for the treatment of cancer. For an overview of genetic strategies to treating cancer see Hall et al (*Am J Hum Genet* 61:785-789, 1997) and Kufe et al (*Cancer Medicine*, 5th Ed, pp 876-889, BC Decker, Hamilton 2000). Gene therapy can be used to deliver any tumor suppressing gene. Examples of such genes include, but are not limited to, p53, which can be delivered via recombinant virus-mediated gene transfer (see U.S. Pat. No. 6,069,134, for example), Duc-4, NF-1, NF-2, RB, WT1, BRCA1, BRCA2, a uPA/uPAR antagonist ("Adenovirus-Mediated Delivery of a uPA/uPAR Antagonist Suppresses Angiogenesis-Dependent Tumor Growth and Dissemination in Mice," *Gene Therapy*, August 1998; 5(8):1105-13), and interferon gamma (*J. Immunol.* 2000; 164:217-222).

The compounds of the instant invention may also be administered in combination with an inhibitor of inherent multidrug resistance (MDR), in particular MDR associated with high levels of expression of transporter proteins. Such MDR inhibitors include inhibitors of p-glycoprotein (P-gp), such as LY335979, XR9576, OC144-093, R101922, VX853 and PSC833 (valspodar).

A compound of the present invention may be employed in conjunction with anti-emetic agents to treat nausea or emesis, including acute, delayed, late-phase, and anticipatory emesis, which may result from the use of a compound of the present invention, alone or with radiation therapy. For the prevention or treatment of emesis, a compound of the

present invention may be used in conjunction with other anti-emetic agents, especially neurokinin-1 receptor antagonists, 5HT3 receptor antagonists, such as ondansetron, granisetron, tropisetron, and zatisetron, GABAB receptor agonists, such as baclofen, a corticosteroid such as Decadron (dexamethasone), Kenalog, Aristocort, Nasalide, Preferid, Benecorten or others such as disclosed in U.S.Patent Nos. 2,789,118, 2,990,401, 3,048,581, 3,126,375, 3,929,768, 3,996,359, 3,928,326 and 3,749,712, an antidopaminergic, such as the phenothiazines (for example prochlorperazine, fluphenazine, thioridazine and mesoridazine), metoclopramide or dronabinol. In an embodiment, an anti-emesis agent selected from a neurokinin-1 receptor antagonist, a 5HT3 receptor antagonist and a corticosteroid is administered as an adjuvant for the treatment or prevention of emesis that may result upon administration of the instant compounds.

Neurokinin-1 receptor antagonists of use in conjunction with the compounds of the present invention are fully described, for example, in U.S. Pat. Nos. 5,162,339, 5,232,929, 5,242,930, 5,373,003, 5,387,595, 5,459,270, 5,494,926, 5,496,833, 5,637,699, 5,719,147; European Patent Publication Nos. EP 0 360 390, 0 394 989, 0 428 434, 0 429 366, 0 430 771, 0 436 334, 0 443 132, 0 482 539, 0 498 069, 0 499 313, 0 512 901, 0 512 902, 0 514 273, 0 514 274, 0 514 275, 0 514 276, 0 515 681, 0 517 589, 0 520 555, 0 522 808, 0 528 495, 0 532 456, 0 533 280, 0 536 817, 0 545 478, 0 558 156, 0 577 394, 0 585 913,0 590 152, 0 599 538, 0 610 793, 0 634 402, 0 686 629, 0 693 489, 0 694 535, 0 699 655, 0 699 674, 0 707 006, 0 708 101, 0 709 375, 0 709 376, 0 714 891, 0 723 959, 0 733 632 and 0 776 893; PCT International Patent Publication Nos. WO 90/05525, 90/05729, 91/09844, 91/18899, 92/01688, 92/06079, 92/12151, 92/15585, 92/17449, 92/20661, 92/20676, 92/21677, 92/22569, 93/00330, 93/00331, 93/01159, 93/01165, 93/01169, 93/01170, 93/06099, 93/09116, 93/10073, 93/14084, 93/14113, 93/18023, 93/19064, 93/21155, 93/21181, 93/23380, 93/24465, 94/00440, 94/01402, 94/02461, 94/02595, 94/03429, 94/03445, 94/04494, 94/04496, 94/05625, 94/07843, 94/08997, 94/10165, 94/10167, 94/10168, 94/10170, 94/11368, 94/13639, 94/13663, 94/14767, 94/15903, 94/19320, 94/19323, 94/20500, 94/26735, 94/26740, 94/29309, 95/02595, 95/04040, 95/04042, 95/06645, 95/07886, 95/07908, 95/08549, 95/11880, 95/14017, 95/15311, 95/16679, 95/17382, 95/18124, 95/18129, 95/19344, 95/20575, 95/21819, 95/22525, 95/23798, 95/26338, 95/28418, 95/30674, 95/30687, 95/33744, 96/05181, 96/05193, 96/05203, 96/06094, 96/07649, 96/10562, 96/16939, 96/18643, 96/20197, 96/21661, 96/29304, 96/29317, 96/29326, 96/29328, 96/31214, 96/32385, 96/37489, 97/01553, 97/01554, 97/03066, 97/08144, 97/14671, 97/17362, 97/18206, 97/19084, 97/19942 and 97/21702; and in British Patent Publication Nos. 2 266 529, 2 268 931, 2 269 170, 2 269 590, 2 271 774, 2 292 144, 2 293 168, 2 293 169, and 2 302 689. The preparation of such compounds is fully described in the aforementioned patents and publications.

In an embodiment, the neurokinin-1 receptor antagonist for use in conjunction with the compounds of the present invention is selected from: 2-(R)-(1-(R)-(3,5-bis(trifluoromethyl)phenyl)ethoxy)-3-(S)-(4-fluorophenyl)-4-(3-(5-oxo-1H,4H-1,2,4-

triazolo)methyl)morpholine, or a pharmaceutically acceptable salt thereof, which is described in U.S. Pat. No. 5,719,147.

A compound of the instant invention may also be administered with an agent useful in the treatment of anemia. Such an anemia treatment agent is, for example, a continuous eythropoiesis receptor activator (such as epoetin alfa).

A compound of the instant invention may also be administered with an agent useful in the treatment of neutropenia. Such a neutropenia treatment agent is, for example, a hematopoietic growth factor which regulates the production and function of neutrophils such as a human granulocyte colony stimulating factor, (G-CSF). Examples of a G-CSF include filgrastim.

A compound of the instant invention may also be administered with an immunologic-enhancing drug, such as levamisole, bacillus Calmette-Guerin, octreotide, isoprinosine and Zadaxin.

A compound of the instant invention may also be useful for treating or preventing cancer, including bone cancer, in combination with bisphosphonates (understood to include bisphosphonates, diphosphonates, bisphosphonic acids and diphosphonic acids). Examples of bisphosphonates include but are not limited to: etidronate (Didronel), pamidronate (Aredia), alendronate (Fosamax), risedronate (Actonel), zoledronate (Zometa), ibandronate (Boniva), incadronate or cimadronate, clodronate, EB-1053, minodronate, neridronate, piridronate and tiludronate including any and all pharmaceutically acceptable salts, derivatives, hydrates and mixtures thereof.

A compound of the instant invention may also be useful for treating or preventing breast cancer in combination with aromatase inhibitors. Examples of aromatase inhibitors include but are not limited to anastrozole, letrozole and exemestane.

A compound of the instant invention may also be useful for treating or preventing cancer in combination with siRNA therapeutics.

A compound of the instant invention may also be useful for treating or preventing cancer in combination withcompounds which induce terminal differentiation of the neoplastic cells. Suitable differentiation agents include the compounds disclosed in any one or more of the following references.

a) Polar compounds (Marks et al (1987); Friend, C., Scher, W., Holland, J. W., and Sato, T. (1971) *Proc. Natl. Acad. Sci.* (USA) 68: 378-382; Tanaka, M., Levy, J., Terada, M., Breslow, R., Rifkind, R. A., and Marks, P. A. (1975) *Proc. Natl. Acad. Sci.* (USA) 72: 1003-1006; Reuben, R. C., Wife, R. L., Breslow, R., Rifkind, R. A., and Marks, P. A. (1976) *Proc. Natl. Acad. Sci.* (USA) 73: 862-866);

b) Derivatives of vitamin D and retinoic acid (Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshika, S., and Suda, T. (1981) *Proc. Natl. Acad. Sci. (*USA) 78: 4990-4994; Schwartz, E. L., Snoddy, J. R., Kreutter, D., Rasmussen, H., and

Sartorelli, A. C. (1983) *Proc. Am. Assoc. Cancer Res.* 24: 18; Tanenaga, K., Hozumi, M., and Sakagami, Y. (1980) *Cancer Res.* 40: 914-919);

c) Steroid hormones (Lotem, J. and Sachs, L. (1975) *Int. J. Cancer 15:* 731-740);

d) Growth factors (Sachs, L. (1978) *Nature (Lond.)* 274: 535, Metcalf, D. (1985) *Science*, 229: 16-22);

e) Proteases (Scher, W., Scher, B. M., and Waxman, S. (1983) *Exp. Hematol.* 11: 490-498; Scher, W., Scher, B. M., and Waxman, S. (1982) *Biochem. & Biophys. Res. Comm.* 109: 348-354);

f) Tumor promoters (Huberman, E. and Callaham, M. F. (1979) *Proc. Natl. Acad. Sci.* (USA) 76: 1293-1297; Lottem, J. and Sachs, L. (1979) *Proc. Natl. Acad. Sci.* (USA) 76: 5158-5162); and

g) inhibitors of DNA or RNA synthesis (Schwartz, E. L. and Sartorelli, A. C. (1982) *Cancer Res. 42:* 2651-2655, Terada, M., Epner, E., Nudel, U., Salmon, J., Fibach, E., Rifkind, R. A., and Marks, P. A. (1978) *Proc. Natl. Acad. Sci.* (USA) 75: 2795-2799; Morin, M. J. and Sartorelli, A. C. (1984) *Cancer Res 44*: 2807-2812; Schwartz, E. L., Brown, B. J., Nierenberg, M., Marsh, J. C., and Sartorelli, A. C. (1983) *Cancer Res.* 43: 2725-2730; Sugano, H., Furusawa, M., Kawaguchi, T., and Ikawa, Y. (1973) *Bibl. Hematol.* 39: 943-954; Ebert, P. S., Wars, I., and Buell, D. N. (1976) *Cancer Res.* 36: 1809-1813; Hayashi, M., Okabe, J., and Hozumi, M. (1979) Gann 70: 235-238).

A compound of the instant invention may also be useful for treating or preventing cancer in combination with γ-secretase inhibitors.

Also included in the scope of the claims is a method of treating cancer that comprises administering a therapeutically effective amount of a compound of Formula I in combination with radiation therapy and/or in combination with a second compound selected from: an estrogen receptor modulator, an androgen receptor modulator, a retinoid receptor modulator, a cytotoxiccytostatic agent, an antiproliferative agent, a prenyl-protein transferase inhibitor, an HMG-CoA reductase inhibitor, an HIV protease inhibitor, a reverse transcriptase inhibitor, an angiogenesis inhibitor, PPAR-γ agonists, PPAR-δ agonists, an inhibitor of inherent multidrug resistance, an anti-emetic agent, an agent useful in the treatment of anemia, an agent useful in the treatment of neutropenia, an immunologic-enhancing drug, an inhibitor of cell proliferation and survival signaling, a bisphosphonate, an aromatase inhibitor, an siRNA therapeutic, γ-secretase inhibitors, agents that interfere with receptor tyrosine kinases (RTKs) and an agent that interferes with a cell cycle checkpoint.

The compounds of the instant invention are useful in combination with the following therapeutic agents: abarelix (Plenaxis depot®); aldesleukin (Prokine®); Aldesleukin (Proleukin®); Alemtuzumabb (Campath®); alitretinoin (Panretin®); allopurinol (Zyloprim®); altretamine (Hexalen®); amifostine (Ethyol®); anastrozole (Arimidex®); arsenic trioxide

(Trisenox®); asparaginase (Elspar®); azacitidine (Vidaza®); bendamustine hydrochloride (Treanda®); bevacuzimab (Avastin®); bexarotene capsules (Targretin®); bexarotene gel (Targretin®); bleomycin (Blenoxane®); bortezomib (Velcade®); busulfan intravenous (Busulfex®); busulfan oral (Myleran®); calusterone (Methosarb®); capecitabine (Xeloda®); carboplatin (Paraplatin®); carmustine (BCNU®, BiCNU®); carmustine (Gliadel®); carmustine with Polifeprosan 20 Implant (Gliadel Wafer®); celecoxib (Celebrex®); cetuximab (Erbitux®); chlorambucil (Leukeran®); cisplatin (Platinol®); cladribine (Leustatin®, 2-CdA®); clofarabine (Clolar®); cyclophosphamide (Cytoxan®, Neosar®); cyclophosphamide (Cytoxan Injection®); cyclophosphamide (Cytoxan Tablet®); cytarabine (Cytosar-U®); cytarabine liposomal (DepoCyt®); dacarbazine (DTIC-Dome®); dactinomycin, actinomycin D (Cosmegen®); dalteparin sodium injection (Fragmin®); Darbepoetin alfa (Aranesp®); dasatinib (Sprycel®); daunorubicin liposomal (DanuoXome®); daunorubicin, daunomycin (Daunorubicin®); daunorubicin, daunomycin (Cerubidine®); degarelix (Firmagon®); Denileukin diftitox (Ontak®); dexrazoxane (Zinecard®); dexrazoxane hydrochloride (Totect®); docetaxel (Taxotere®); doxorubicin (Adriamycin PFS®); doxorubicin (Adriamycin®, Rubex®); doxorubicin (Adriamycin PFS Injection®); doxorubicin liposomal (Doxil®); dromostanolone propionate (Dromostanolone ®); dromostanolone propionate (Masterone Injection®); eculizumab injection (Soliris®); Elliott's B Solution (Elliott's B Solution®); eltrombopag (Promacta®); epirubicin (Ellence®); Epoetin alfa (epogen®); erlotinib (Tarceva®); estramustine (Emcyt®); etoposide phosphate (Etopophos®); etoposide, VP-16 (Vepesid®); everolimus tablets (Afinitor®); exemestane (Aromasin®); ferumoxytol (Feraheme Injection®); Filgrastim (Neupogen®); floxuridine (intraarterial) (FUDR®); fludarabine (Fludara®); fluorouracil, 5-FU (Adrucil®); fulvestrant (Faslodex®); gefitinib (Iressa®); gemcitabine (Gemzar®); gemtuzumab ozogamicin (Mylotarg®); goserelin acetate (Zoladex Implant®); goserelin acetate (Zoladex®); histrelin acetate (Histrelin implant®); hydroxyurea (Hydrea®); Ibritumomab Tiuxetan (Zevalin®); idarubicin (Idamycin®); ifosfamide (IFEX®); imatinib mesylate (Gleevec®); interferon alfa 2a (Roferon A®); Interferon alfa-2b (Intron A®); iobenguane I 123 injection (AdreView®); irinotecan (Camptosar®); ixabepilone (Ixempra®); lapatinib tablets (Tykerb®); lenalidomide (Revlimid®); letrozole (Femara®); leucovorin (Wellcovorin®, Leucovorin®); Leuprolide Acetate (Eligard®); levamisole (Ergamisol®); lomustine, CCNU (CeeBU®); meclorethamine, nitrogen mustard (Mustargen®); megestrol acetate (Megace®); melphalan, L-PAM (Alkeran®); mercaptopurine, 6-MP (Purinethol®); mesna (Mesnex®); mesna (Mesnex tabs®); methotrexate (Methotrexate®); methoxsalen (Uvadex®); mitomycin C (Mutamycin®); mitotane (Lysodren®); mitoxantrone (Novantrone®); nandrolone phenpropionate (Durabolin-50®); nelarabine (Arranon®); nilotinib (Tasigna®); Nofetumomab (Verluma®); ofatumumab (Arzerra®); Oprelvekin (Neumega®); oxaliplatin (Eloxatin®); paclitaxel (Paxene®); paclitaxel (Taxol®); paclitaxel protein-bound particles (Abraxane®); palifermin (Kepivance®);

pamidronate (Aredia®); panitumumab (Vectibix®); pazopanib tablets (Votrienttm®); pegademase (Adagen (Pegademase Bovine)®); pegaspargase (Oncaspar®); Pegfilgrastim (Neulasta®); pemetrexed disodium (Alimta®); pentostatin (Nipent®); pipobroman (Vercyte®); plerixafor (Mozobil®); plicamycin, mithramycin (Mithracin®); porfimer sodium (Photofrin®); pralatrexate injection (Folotyn®); procarbazine (Matulane®); quinacrine (Atabrine®); Rasburicase (Elitek®); raloxifene hydrochloride (Evista®); Rituximab (Rituxan®); romidepsin (Istodax®); romiplostim (Nplate®); sargramostim (Leukine®); Sargramostim (Prokine®); sorafenib (Nexavar®); streptozocin (Zanosar®); sunitinib maleate (Sutent®); talc (Sclerosol®); tamoxifen (Nolvadex®); temozolomide (Temodar®); temsirolimus (Torisel®); teniposide, VM-26 (Vumon®); testolactone (Teslac®); thioguanine, 6-TG (Thioguanine®); thiotepa (Thioplex®); topotecan (Hycamtin®); toremifene (Fareston®); Tositumomab (Bexxar®); Tositumomab/I-131 tositumomab (Bexxar®); Trastuzumab (Herceptin®); tretinoin, ATRA (Vesanoid®); Uracil Mustard (Uracil Mustard Capsules®); valrubicin (Valstar®); vinblastine (Velban®); vincristine (Oncovin®); vinorelbine (Navelbine®); vorinostat (Zolinza®); and zoledronate (Zometa®).

Non-limiting examples of other suitable anti-cancer agents for combination with the instant compounds are selected from the group consisting of a Cytostatic agent, Cisplatin, Deforolimus (described in PCT publication No. 2003/064383), Doxorubicin, liposomal doxorubicin (e.g., Caelyx®, Myocet®, Doxil®), Taxotere, Taxol, Etoposide, Irinotecan, Camptostar, Topotecan, Paclitaxel, Docetaxel, Epothilones, Tamoxifen, 5-Fluorouracil, Methoxtrexate, Temozolomide, cyclophosphamide, SCH 66336, R115777®, L778,123®, BMS 214662®, Iressa®, Tarceva®, Antibodies to EGFR, antibodies to IGFR (including, for example, those published in US 2005/0136063 published June 23, 2005), ESK inhibitors, KSP inhibitors (such as, for example, those published in WO 2006/098962 and WO 2006/098961; ispinesib, SB-743921 from Cytokinetics), Centrosome associated protein E ("CENP-E") inhibitors (e.g., GSK-923295), Gleevec®, Intron, Ara-C, Adriamycin, Cytoxan, Gemcitabine, Uracil mustard, Chlormethine, Ifosfamide, Melphalan, Chlorambucil, Pipobroman, Triethylenemelamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine, Streptozocin, Dacarbazine, Floxuridine, Cytarabine, 6 Mercaptopurine, 6 Thioguanine, Fludarabine phosphate, Oxaliplatin, Leucovirin, ELOXATINTM, Vinblastine, Vincristine, Vindesine, Bleomycin, Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Mithramycin, Deoxycoformycin, Mitomycin C, L Asparaginase, Teniposide 17α-Ethinylestradiol, Diethylstilbestrol, Testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, Testolactone, Megestrolacetate, Methylprednisolone, Methyltestosterone, Prednisolone, Triamcinolone, Chlorotrianisene, Hydroxyprogesterone, Aminoglutethimide, Estramustine, Medroxyprogesteroneacetate, Leuprolide, Flutamide, Toremifene, Goserelin, Cisplatin, Carboplatin, Hydroxyurea, Amsacrine, Procarbazine, Mitotane, Mitoxantrone, Levamisole, Navelbene, Anastrazole, Letrazole, Capecitabine,

Reloxafine, Droloxafine, Hexamethylmelamine, Avastin, herceptin, Bexxar, bortezomib ("Velcade"), Zevalin, Trisenox, Xeloda, Vinorelbine, Porfimer, Erbitux, Liposomal, Thiotepa, Altretamine, Melphalan, Trastuzumab, Lerozole, Fulvestrant, Exemestane, Fulvestrant, Ifosfomide, Rituximab, C225®, Satriplatin, mylotarg, Avastin, Rituxan, Panitubimab, Sutent, Sorafinib, Sprycel (dastinib), Nilotinib, Tykerb (Lapatinib) and Campath.

In one embodiment, the invention provides a method of treating cancer, the method comprising administering an amount of a Pyrazolopyrrolopyrimidine Compound or a pharmaceutically acceptable salt thereof, and an amount of one additional anticancer agent selected from the group consisting of Adriamycin, Altretamine, Amidox, Aminoglutethimide, Amsacrine, Anastrazole, Antibodies to EGFR, 3-AP, Aphidicolon, Ara-C, Arsenic trioxide, L Asparaginase, Bevacizumab, Bleomycin, BMS 214662, Bortezomib, Busulfan, Campath, Camptostar, Capecitabine, Carboplatin, Carmustine, Centrosome associated protein E ("CENP-E") inhibitors, Cetuximab, Cladribine, Chlorambucil, Chlormethine, Chlorotrianisene, Cisplatin, Clofarabine, cyclophosphamide, Cytarabine, a Cytostatic agent, Cytoxan, Dacarbazine, Dactinomycin, Daunorubicin, Dasatinib, Deforolimus, Deoxycoformycin, Didox, Diethylstilbestrol, Docetaxel, Doxorubicin, Dromostanolone, Droloxafine, Epirubicin, Epothilones, ERK inhibitors, Erlotinib, Etoposide, 17α-Ethinylestradiol, Estramustine, Exemestane, Floxuridine, Fludarabine, Fludarabine phosphate, 5-Fluorouracil, Fluoxymesterone, Flutamide, Fulvestrant, Gefitinib, Gemcitabine, Gemtuzumab ozogamcicin, Goserelin, GSK-923295, Hexamethylmelamine, Hydroxyprogesterone, Hydroxyurea, Ibritumomab Tiuxetan, Idarubicin, Ifosfamide, Imatinib mesylate, Intron, Irinotecan, ispinesib, KSP inhibitors, L778,123, Lapatinib, Leucovirin, Leuprolide, Lerozole, Letrazole, Levamisole, Liposomal Doxorubicin, Liposomal, Lomustine, Lonafarnib, Medroxyprogesteroneacetate, Megestrolacetate, Melphalan, 6 Mercaptopurine, Methoxtrexate, Methylprednisolone, Methyltestosterone, Mithramycin, Mitomycin C, Mitotane, Mitoxantrone, Navelbene, Nilotinib, Oxaliplatin, Paclitaxel, Panitubimab, Pentostatin, Pipobroman, Porfimer, Prednisolone, Prednisone propionate, Procarbazine, Reloxafine, Rituximab, Satriplatin, SB-743921, Sml1, Sorafinib, Streptozocin, Sunitinib, Tamoxifen, Taxotere, Taxol, Temozolomide, Teniposide, Testolactone, Testosterone, Tezacitabine, 6 Thioguanine, Thiotepa, Tipifarnib, Topotecan, Toremifene, Tositumomab, Trastuzumab, Triamcinolone, Triapine, Triethylenemelamine, Triethylenethiophosphoramine, Trimidox, Uracil mustard, Vinblastine, Vincristine, Vindesine, and Vinorelbine.

In one embodiment, the invention provides a method of treating cancer, the method comprising administering an amount of a Pyrazolopyrrolopyrimidine Compound or a pharmaceutically acceptable salt thereof, and an amount of one or more of a MAP Kinase pathway inhibitor such as bRaf, MEK, or ERK inhibitors to a patient in need thereof.

In another embodiment, the invention provides a method of treating cancer, the method comprising administering an amount of a Pyrazolopyrrolopyrimidine Compound or a

pharmaceutically acceptable salt thereof, and an amount of one or more of ERK inhibitors (for example, compounds described in WO2008/156739, WO2007/070398, WO 2008/156739 and US publication 2007/0232610) to a patient in need thereof.

In one embodiment, the invention provides a method of treating cancer, the method comprising administering an amount of a Pyrazolopyrrolopyrimidine Compound or a pharmaceutically acceptable salt thereof, and an amount of one or more of an anti-IGF-1R antibody. Specific anti-IGF-1R antibodies include, but are not limited to, dalotuzumab, figitumumab, cixutumumab, SHC 717454, Roche R1507, EM164 or Amgen AMG479.

The instant invention also includes a pharmaceutical composition useful for treating or preventing cancer that comprises a therapeutically effective amount of a compound of Formula I and a second compound selected from: an estrogen receptor modulator, an androgen receptor modulator, a retinoid receptor modulator, a cytotoxic/cytostatic agent, an antiproliferative agent, a prenyl-protein transferase inhibitor, an HMG-CoA reductase inhibitor, an HIV protease inhibitor, a reverse transcriptase inhibitor, an angiogenesis inhibitor, a PPAR-γ agonist, a PPAR-δ agonist, an inhibitor of cell proliferation and survival signaling, a bisphosphonate, an aromatase inhibitor, an siRNA therapeutic, γ-secretase inhibitors, agents that interfere with receptor tyrosine kinases (RTKs) and an agent that interferes with a cell cycle checkpoint.

The use of all of these approaches in combination with the instant compounds described herein are within the scope of the present invention.

Compositions and Administration

This invention is also directed to pharmaceutical compositions which comprise at least one Pyrazolopyrrolopyrimidine Compound, or a pharmaceutically acceptable salt of said compound and at least one pharmaceutically acceptable carrier.

When administered to a patient, the Pyrazolopyrrolopyrimidine Compounds can be administered as a component of a composition that comprises a pharmaceutically acceptable carrier or vehicle. The present invention provides pharmaceutical compositions comprising an effective amount of at least one Pyrazolopyrrolopyrimidine Compound and a pharmaceutically acceptable carrier. In the pharmaceutical compositions and methods of the present invention, the active ingredients will typically be administered in admixture with suitable carrier materials suitably selected with respect to the intended form of administration, *i.e.*, oral tablets, capsules (either solid-filled, semi-solid filled or liquid filled), powders for constitution, oral gels, elixirs, dispersible granules, syrups, suspensions, and the like, and consistent with conventional pharmaceutical practices. Examples of pharmaceutically acceptable carriers and methods of manufacture for various compositions may be found in A. Gennaro (ed.), *Remington's Pharmaceutical Sciences,* 18th Edition, (1990), Mack Publishing Co., Easton, Pennsylvania. For example, for oral administration in the form of tablets or capsules, the active drug

component may be combined with any oral non-toxic pharmaceutically acceptable inert carrier, such as lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, talc, mannitol, ethyl alcohol (liquid forms) and the like. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets and suppositories. Powders and tablets may be comprised of from about 0.5 to about 95 percent inventive composition. Tablets, powders, cachets and capsules can be used as solid dosage forms suitable for oral administration.

Moreover, when desired or needed, suitable binders, lubricants, disintegrating agents and coloring agents may also be incorporated in the mixture. Suitable binders include starch, gelatin, natural sugars, corn sweeteners, natural and synthetic gums such as acacia, sodium alginate, carboxymethylcellulose, polyethylene glycol and waxes. Among the lubricants there may be mentioned for use in these dosage forms, boric acid, sodium benzoate, sodium acetate, sodium chloride, and the like. Disintegrants include starch, methylcellulose, guar gum, and the like. Sweetening and flavoring agents and preservatives may also be included where appropriate.

Liquid form preparations include solutions, suspensions and emulsions and may include water or water-propylene glycol solutions for parenteral injection.

Liquid form preparations may also include solutions for intranasal administration.

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be in combination with a pharmaceutically acceptable carrier, such as an inert compressed gas.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions and emulsions.

For preparing suppositories, a low melting wax such as a mixture of fatty acid glycerides or cocoa butter is first melted, and the active ingredient is dispersed homogeneously therein as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool and thereby solidify.

The Pyrazolopyrrolopyrimidine Compounds of the present invention may also be delivered transdermally. The transdermal compositions can take the form of creams, lotions, aerosols and/or emulsions and can be included in a transdermal patch of the matrix or reservoir type as are conventional in the art for this purpose.

Additionally, the compositions of the present invention may be formulated in sustained release form to provide the rate controlled release of any one or more of the components or active ingredients to optimize therapeutic effects, *i.e.*, anti-cancer activity and the like. Suitable dosage forms for sustained release include layered tablets containing layers of varying disintegration rates or controlled release polymeric matrices impregnated with the

active components and shaped in tablet form or capsules containing such impregnated or encapsulated porous polymeric matrices.

In one embodiment, the Pyrazolopyrrolopyrimidine Compound is administered orally.

In another embodiment, the Pyrazolopyrrolopyrimidine Compound is administered intravenously.

In another embodiment, the Pyrazolopyrrolopyrimidine Compound is administered topically.

In still another embodiment, the Pyrazolopyrrolopyrimidine Compounds is administered sublingually.

In one embodiment, a pharmaceutical preparation comprising at least one Pyrazolopyrrolopyrimidine Compound is in unit dosage form. In such form, the preparation is subdivided into unit doses containing effective amounts of the active components.

Compositions can be prepared according to conventional mixing, granulating or coating methods, respectively, and the present compositions can contain, in one embodiment, from about 0.1% to about 99% of the Pyrazolopyrrolopyrimidine Compound(s) by weight or volume. In various embodiments, the present compositions can contain, in one embodiment, from about 1% to about 70% or from about 5% to about 60% of the Pyrazolopyrrolopyrimidine Compound(s) by weight or volume.

The quantity of Pyrazolopyrrolopyrimidine Compound in a unit dose of preparation may be varied or adjusted from about 0.1 mg to about 5000 mg. In various embodiments, the quantity is from about 10 mg to about 5000 mg, about 10 mg to about 1000 mg, 1 mg to about 500 mg, 1 mg to about 100 mg, and 1 mg to about 50 mg.

For convenience, the total daily dosage may be divided and administered in portions during the day if desired. In one embodiment, the daily dosage is administered in one portion. In another embodiment, the total daily dosage is administered in two divided doses over a 24 hour period. In another embodiment, the total daily dosage is administered in three divided doses over a 24 hour period. In still another embodiment, the total daily dosage is administered in four divided doses over a 24 hour period.

For administration to human patients, the amount and frequency of administration of the Pyrazolopyrrolopyrimidine Compounds will be regulated according to the judgment of the attending clinician considering such factors as age, condition and size of the patient as well as severity of the symptoms being treated. Generally, a total daily dosage of the Pyrazolopyrrolopyrimidine Compounds range from about 0.1 to about 5000 mg per day, although variations will necessarily occur depending on the target of therapy, the patient and the route of administration. In one embodiment, the dosage is from about 1 to about 200 mg/day, administered in a single dose or in 2-4 divided doses. In another embodiment, the dosage is from about 10 to about 5000 mg/day, administered in a single dose or in 2-4 divided

doses. In another embodiment, the dosage is from about 100 to about 5000 mg/day, administered in a single dose or in 2-4 divided doses. In still another embodiment, the dosage is from about 500 to about 5000 mg/day, administered in a single dose or in 2-4 divided doses.

The compositions of the invention can further comprise one or more additional therapeutic agents, selected from those listed above herein. Accordingly, in one embodiment, the present invention provides compositions comprising: (i) at least one Pyrazolopyrrolopyrimidine Compound or a pharmaceutically acceptable salt thereof; (ii) one or more additional therapeutic agents that are not a Pyrazolopyrrolopyrimidine Compound; and (iii) a pharmaceutically acceptable carrier, wherein the amounts in the composition are together effective to treat disease or disorder associated with dysregulated mTOR activity, such as a cancer.

In Vitro and In Vivo METHODS:

The present invention also provides methods of using the pyrazolopyrrolopyrimidine compounds of the present invention for inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells thereby inhibiting the proliferation of such cells. The methods can be practiced *in vivo* or *in vitro*.

In one embodiment, the present invention provides *in vitro* methods for selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, thereby inhibiting proliferation of such cells, by contacting the cells with an effective amount of any one or more of the pyrazolopyrrolopyrimidine compounds described herein.

In a particular embodiment, the present invention relates to an *in vitro* method of selectively inducing terminal differentiation of neoplastic cells and thereby inhibiting proliferation of such cells. The method comprises contacting the cells under suitable conditions with an effective amount of one or more of the pyrazolopyrrolopyrimidine compounds described herein.

In another embodiment, the invention relates to an *in vitro* method of selectively inducing cell growth arrest of neoplastic cells and thereby inhibiting proliferation of such cells. The method comprises contacting the cells under suitable conditions with an effective amount of one or more of the pyrazolopyrrolopyrimidine compounds described herein.

In another embodiment, the invention relates to an *in vitro* method of selectively inducing apoptosis of neoplastic cells and thereby inhibiting proliferation of such cells. The method comprises contacting the cells under suitable conditions with an effective amount of one or more of the pyrazolopyrrolopyrimidine compounds described herein.

In another embodiment, the invention relates to an *in vitro* method of inducing terminal differentiation of tumor cells in a tumor comprising contacting the cells with an effective amount of any one or more of the pyrazolopyrrolopyrimidine compounds described herein.

Although the methods of the present invention can be practiced *in vitro*, it is contemplated that the preferred embodiment for the methods of selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells, and of inhibiting mTor will comprise contacting the cells *in vivo*, i.e., by administering the compounds to a subject harboring neoplastic cells or tumor cells in need of treatment.

Thus, the present invention provides *in vivo* methods for selectively inducing terminal differentiation, cell growth arrest and/or apoptosis of neoplastic cells in a subject, thereby inhibiting proliferation of such cells in the subject, by administering to the subject an effective amount of any one or more of the pyrazolopyrrolopyrimidine compounds described herein.

In a particular embodiment, the present invention relates to a method of selectively inducing terminal differentiation of neoplastic cells and thereby inhibiting proliferation of such cells in a subject. The method comprises administering to the subject an effective amount of one or more of the pyrazolopyrrolopyrimidine compounds described herein.

In another embodiment, the invention relates to a method of selectively inducing cell growth arrest of neoplastic cells and thereby inhibiting proliferation of such cells in a subject. The method comprises administering to the subject an effective amount of one or more of the pyrazolopyrrolopyrimidine compounds described herein.

In another embodiment, the invention relates to a method of selectively inducing apoptosis of neoplastic cells and thereby inhibiting proliferation of such cells in a subject. The method comprises administering to the subject an effective amount of one or more of the pyrazolopyrrolopyrimidine compounds described herein.

In another embodiment, the invention relates to a method of treating a patient having a tumor characterized by proliferation of neoplastic cells. The method comprises administering to the patient one or more of the pyrazolopyrrolopyrimidine compounds described herein. The amount of compound is effective to selectively induce terminal differentiation, induce cell growth arrest and/or induce apoptosis of such neoplastic cells and thereby inhibit their proliferation.

Kits

Another aspect of this invention is a kit comprising a therapeutically effective amount of at least one Pyrazolopyrrolopyrimidine Compound, or a pharmaceutically acceptable salt of said compound, and a pharmaceutically acceptable carrier, vehicle or diluent.

Yet another aspect of this invention is a kit comprising an amount of at least one Pyrazolopyrrolopyrimidine Compound, or a pharmaceutically acceptable salt of said compound and an amount of at least one additional anti-cancer agent listed above, wherein the amounts of the two or more active ingredients result in a desired therapeutic effect. In one embodiment,

the at least one Pyrazolopyrrolopyrimidine Compound and the at least one additional anti-cancer agent are provided in the same container. In one embodiment, the at least one Pyrazolopyrrolopyrimidine Compound and the at least one additional anti-cancer agent are provided in separate containers.

The invention is illustrated in the examples in the Experimental Details Section that follows. This section is set forth to aid in an understanding of the invention but is not intended to, and should not be construed to limit in any way the invention as set forth in the claims which follow thereafter.

EXPERIMENTAL DETAILS SECTION

Solvents, reagents, and intermediates that are commercially available were used as received. Reagents and intermediates that are not commercially available were prepared in the manner as described below. ^{1}H NMR spectra were obtained on a Varian spectrometer (400 MHz and 500 MHz) are reported as ppm down field from Me_4Si with number of protons, multiplicities, and coupling constants, in Hertz indicated parenthetically. Where LC/MS data are presented, analyses was performed using an Agilent 1100 Series LC w/ MicroMass Quattro MS Varian Pursuit XRs C18, 5micron, 150mm x 4.6mm ID gradient flow (0.1% TFA or 0.2% FA): 0 min – 5% ACN, 7.5 min – 100% ACN, 8.5 min -100 ACN, 8.51 min – 5% ACN, 10 min – stop 3 ml/min. The retention time and observed parent ion are given. Where the description indicates the reaction mixture was purified by HPLC, the description refers to using a preparative Agilent 1100 Series LC/MSD SL system: Column Reverse Phase- Varian Pursuit XRs 10□C-18 250 X 21.2mm; elution with gradient Acetonitrile/water with 0.1%TFA or 0.2% formic acid. The desired product was detected and collected by a mass-triggered automatic sample collector. Flash column chromatography was performed using pre-packed normal phase silica from Biotage, Inc.

The following solvents, reagents and reaction conditions may be referred to by their abbreviations:

Aq: aqueous

g or gm: grams

psi: pounds per square inch

pH: concentration of hydronium ions in a solution

°C: degrees Celsius

h: hours

THF: Tetrahydrofuran

Et_2O: diethyl ether

SEM: 2-(trimethylsilyl)ethoxymethyl

LC-MS: Liquid chromatography mass spectrometry

DCM: dichloromethane

N: Normal

ml: milliliter

NBS: N-Bromosuccinimide

NCS: N-Chlorosuccinimide

NIS: N-iodosuccinimide

r.t.: room temperature

MeOH: methanol

DIEA: diisopropylethylamine

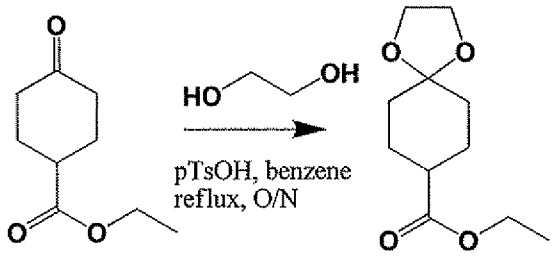
EtOAc: ethyl acetate
EtOH: ethanol
DMF: dimethylformamide
wt%: weight percent
m/z: mass per charge
LiOH: lithium hydroxide
DMSO: dimethylsulfoxide
HPLC: high performance liquid chromatography
IPA: isopropanol
Ret: retention
Rt: retention time
RP: reverse phase
ACN: acetonitrile
CH_3CN: acetonitrile
MeCN: acetonitrile
MeI: iodomethane
r.t.: room temperature
pTSA: para-toluene sulfonic acid
CDI: N,N'-carbonyldiimidazole
mg: milligram
PMA: phosphomolybdic acid
LiHMDS: Lithium bis(trimethylsilyl)amide
HMDS: hexamethyldisilazane
Pd/C: palladium on carbon
H2: hydrogen gas
$PdCl_2$(dppf): [1,1'-bis(diphenylphosphino)ferrocene] dichloropalladium(II)
μmol: micromole
TFA: trifluoroacetic acid
NMP: N-methyl-2-pyrrolidone
min: minute
DME: dimethylethane
AcOH: acetic acid
BBN: 9-borabicyclo[3.3.1]nonane
BOC: tertiary-butyloxycarbonyl
M: Molar
mmol: millimolar
DIEA: diisopropylethylamine
Bu3SnCN: tributyltin cyanide

$Pd[P(t\text{-}Bu)_3]_2$: bis(tributyl)Phosphine) palladium
$Pd(PPh_3)_4$: tetrakis(triphenylphosphine) palladium
EDCI: 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
UV: ultraviolet
LDA: lithium diisopropylamide
Tf: trifluoromethanesulfonyl

Example 1

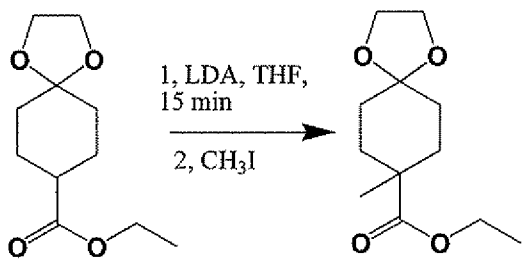
Preparation of (1*R*,4*R*)-4-(3-(6-fluoroquinolin-3-yl)-8*H*-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methylcyclohexanecarboxylic acid (**Compound 1**) and (1*S*,4*S*)-4-(3-(6-fluoroquinolin-3-yl)-8*H*-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methylcyclohexanecarboxylic acid (**Compound 2**)

Step 1: Preparation of ethyl 1,4-dioxaspiro[4.5]decane-8-carboxylate



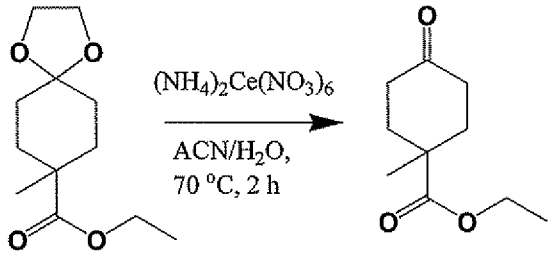
The ketone (25 g, 146.9 mmol) was mixed together with p-TsOH (2.85 g, 15 mmol) and ethylene glycol (25 mL) in benzene (300 mL). The mixture was refluxed with D-M trap and stirred overnight. After the concentration to remove the solvent, the residue was taken up with EtOAc (250 mL) and washed with $NaHCO_3$ (aq.) and brine. The organic was dried over Na_2SO_4 and concentrated. The crude product was purified with column (silica gel, 0~50% EtOAc/Hexane) to give the product (27.0 g).

Step 2: Preparation of ethyl 8-methyl-1,4-dioxaspiro[4.5]decane-8-carboxylate



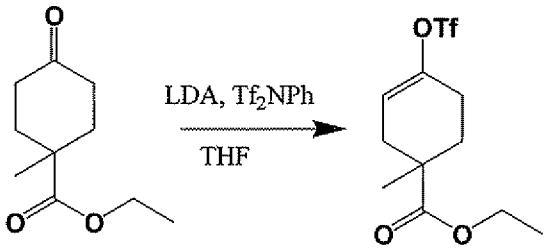
The ester (2.14 g, 10 mmol) was dissolved in dry THF (20 mL) and cooled to -78 °C. LDA (1.8 M, 6.6 mL, 12 mmol) was added dropwise and the mixture was stirred at -78 °C for 15 min. Then CH_3I (1.87 mL, 30 mmol) was added at this temperature and the resulting mixture was allowed to warm to room temperature and stirred overnight. NH_4Cl (aq.) was added to quench the reaction and extracted with EtOAc. The organics was dried over Na_2SO_4, concentrated and purified with column (0~50% EtOAc/Hexane) to give the product (2.09 g). HPLC-MS t_R = 1.68 min ($UV_{254\ nm}$); mass calculated for formula $C_{12}H_{20}O_4$ 228.1, observed LCMS m/z 229.2 (M+H).

Step 3: Preparation of ethyl 1-methyl-4-oxocyclohexanecarboxylate



The ketal compound (2.09 g, 9.17 mmol) was dissolved in CAN (100 mL) and water (50 mL). $(NH_4)_2Ce(NO_3)_6$ (503 mg, 0.92 mmol) was added in water (50 mL) was added and the mixture was heated up to 70 °C and stirred for 1 hour. After cooling down to room temperature, water (100 mL) was added and extracted with Et_2O (100 mL x 3) and the organics was dried over Na_2SO_4. After concentration, the crude was purified with column (0~30% EtOAc/Hexane) to give the product (1.72 g).

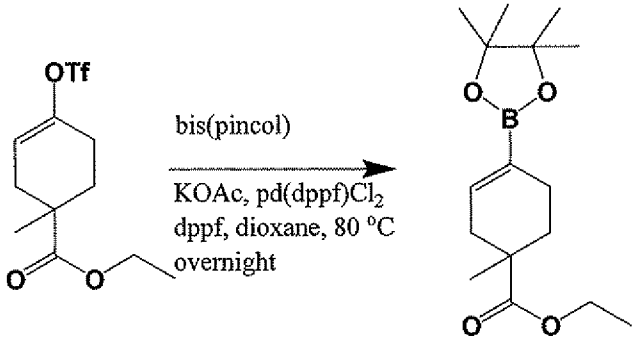
Step 4: Preparation of ethyl 1-methyl-4-(trifluoromethylsulfonyloxy)cyclohex-3-enecarboxylate



LDA (17.28 mmol) in THF (20 mL) was cooled to -78 °C and the ketone (2.65 g, 14.39 mmol) in THF (10 mL) was added dropwise and stirred for 30 min. Then N-phenylbis(trifluoromethanesulfonimide) (5.66 g, 15.8 mmol) in THF (10 mL) was added. The resulting mixture was allowed to warm up to room temperature and stirred overnight. The

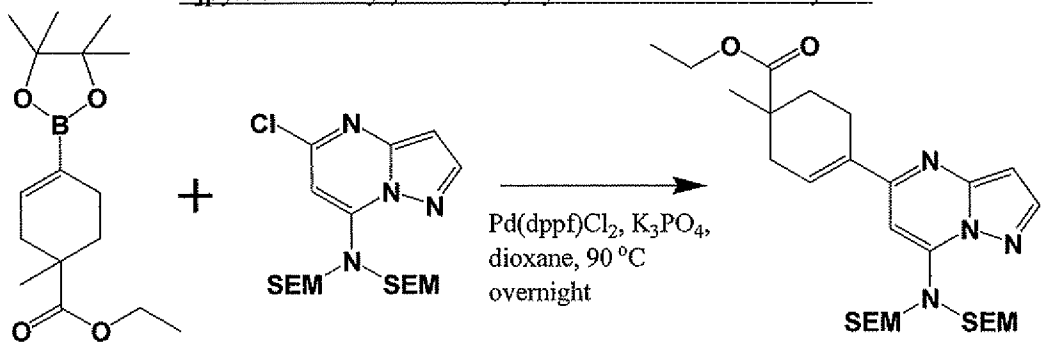
NH_4Cl(aq.) was added to quench the reaction and extracted with EtOAc. The organics was dried over Na_2SO_4, concentrated and purified with column (0~30%) to give the product (3.36 g).

Step 5: Preparation of ethyl 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)cyclohex-3-enecarboxylate



Under Ar, TfO-compound (3.36 g, 10.63 mmol) was mixed with $Pd(dppf)Cl_2$ (815 mg, 1.0 mmol), DPPF (554 mg, 1.0 mmol), KOAc (3.2 g, 33 mmol), bis(pinacolato)diboron (3.24 g, 12.76 mmol) and dioxane (20 mL). The resulting mixture was heated at 80 °C and stirred overnight. After cooling to room temperature, the mixture was diluted with EtOAc (60 mL) and filtered through celite. After concentration, the crude was purified with column (silica gel, 0~30% EtOAc/Hexane) to give the product (2.03 g). HPLC-MS t_R = 2.35 min ($UV_{254\ nm}$); mass calculated for formula $C_{16}H_{27}BO_4$ 294.2, observed LCMS m/z 295.3 (M+H).

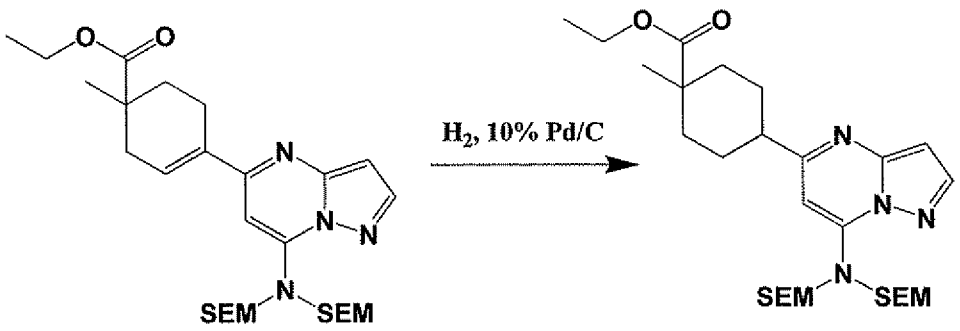
Step 6: Preparation of ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methylcyclohex-3-enecarboxylate



Under Ar, the chloro-compound (2.14 g, 5.0 mmol) was mixed with $Pd(dppf)Cl_2$ (400 mg, 0.5 mmol), K_3PO_4 (2.12 g, 10.0 mmol), bornated (1.7 g, 5.78 mmol) and dioxane (20 mL with 2 ml water). The resulting mixture was heated at 90 °C and stirred overnight. After cooling to room temperature, the mixture was diluted with EtOAc (60 mL) and filtered through celite. After concentration, the crude was purified with column (silica gel, 0~30% EtOAc/Hexane) to give

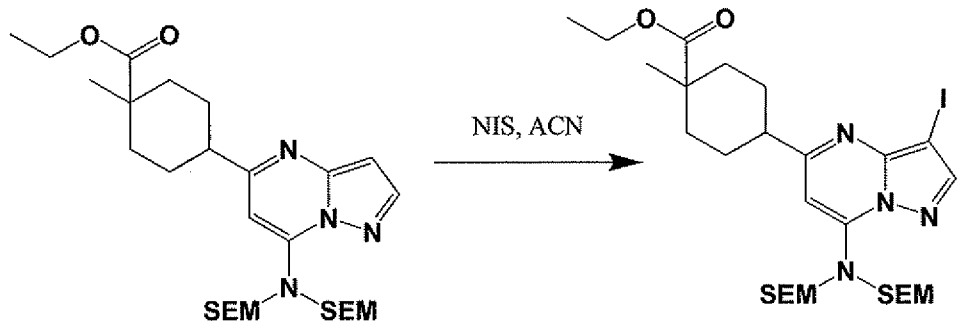
the product (2.44 g). HPLC-MS t_R = 2.67 min ($UV_{254\ nm}$); mass calculated for formula $C_{28}H_{48}N_4O_4Si_2$ 560.3, observed LCMS m/z 561.3 (M+H).

Step 7: Preparation of ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate



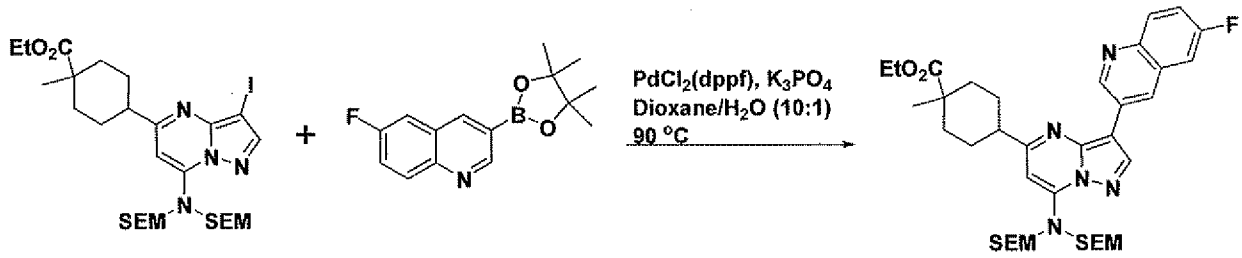
The double bond compound (2.44 g, 4.33 mmol) was dissolved in EtOAc (50 mL) under Ar and 10% Pd/C (500 mg) was added carefully. The sealed flask was switched between vacuum and H_2 three times and the mixture was heated to 45 °C and stirred overnight under the H_2. After cooling to room temperature, the reaction was filtered through celite and washed with EtOAc. The organics was concentrated and the crude product (2.48 g) was used in the next step directly without further purification. HPLC-MS t_R = 2.61 min ($UV_{254\ nm}$); mass calculated for formula $C_{28}H_{50}N_4O_4Si_2$ 562.3, observed LCMS m/z 563.3 (M+H).

Step 8: Preparation of ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-iodopyrazolo[1,5-a]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate



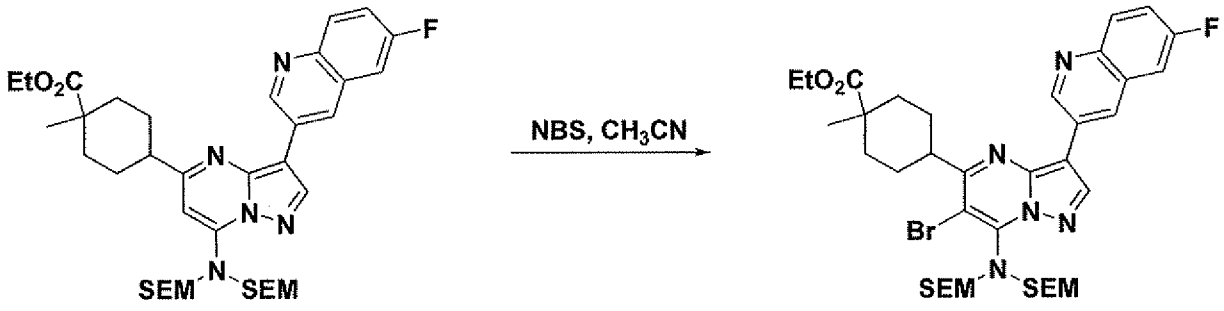
Compound (2.44 g, 4.33 mmol) was dissolved in ACN (20 mL). NIS (975 mg, 4.33 mmol) was added. The mixture was stirred at room temperature for 1 hour and concentrated. The residue was purified with column (silica gel, 0-30% EtOAc/Hexane) to give the iodo-product (2.71 mg). HPLC-MS t_R = 3.03 min ($UV_{254\ nm}$); mass calculated for formula $C_{28}H_{47}IN_4O4Si_2$ 686.2, observed LCMS m/z 687.1 (M+H).

Step 9: Preparation of ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-(6-fluoroquinolin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate



A mixture of ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-iodopyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate (1.08 g, 1.57 mmol), 6-fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (642 mg, 2.35 mmol), $PdCl_2(dppf)CH_2Cl_2$ (128 mg, 0.16 mmol), and K_3PO_4 (1.00 g, 4.71 mmol) in dioxane/H_2O (15/1.5 mL) was degassed and then heated at 90 °C for 16 h. After cooling, the reaction mixture was diluted with EtOAc and washed with H_2O and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by a SiO_2 column (0-30% EtOAc/Hexanes, Rf = 0.2 in 20% EtOAc/Hexanes) to afford ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-(6-fluoroquinolin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate as a pale yellow oil (1.06 g). HPLC-MS T_R = 3.39 min (UV 254 nm, 5 min method); mass calculated for formula $C_{37}H_{54}FN_5O_4Si_2$ 707.4, observed LCMS m/z 708.3 (M+H).

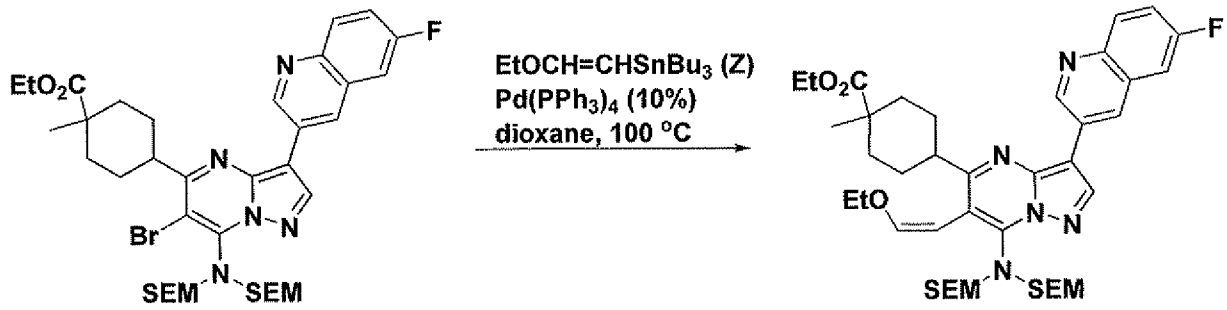
Step 10: Preparation of ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-bromo-3-(6-fluoroquinolin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate



To a solution of ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-(6-fluoroquinolin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate (1.06 g, 1.50 mmol) in CH_3CN (15 mL) was added NBS (320 mg, 1.80 mmol) and stirred at rt for 30 min. TLC showed complete conversion. All the volatiles were removed under reduced pressure and the

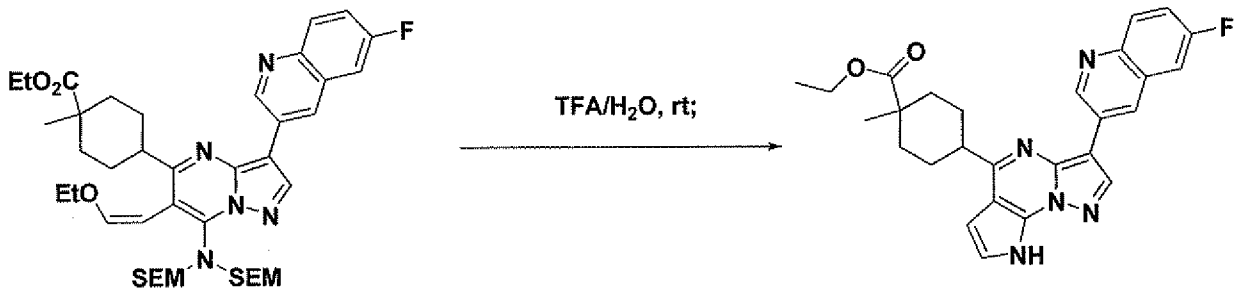
residue was purified by a SiO_2 column (0-20% EtOAc/Hexanes, R_f = 0.3 in 20% EtOAc) to afford ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-bromo-3-(6-fluoroquinolin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate as a pale yellow oil (965 mg).

Step 11: Preparation of (*Z*)-ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-(2-ethoxyvinyl)-3-(6-fluoroquinolin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate



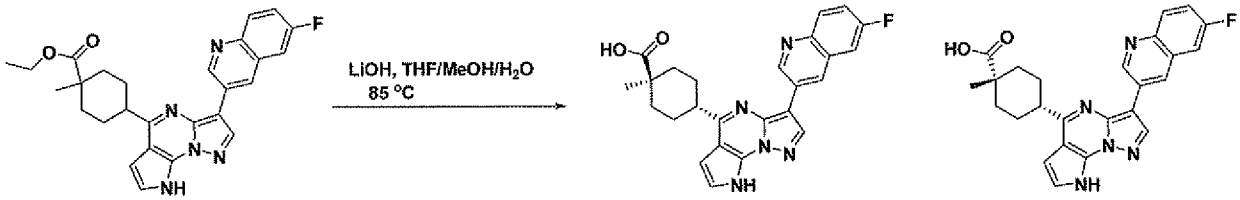
A mixture of ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-bromo-3-(6-fluoroquinolin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate (482 mg, 0.613 mmol), (*Z*)-1-ethoxy-2-(tributylstannyl)ethane (664 mg, 1.84 mmol), $Pd(PPh_3)_4$ (70.8 mg, 0.0613 mmol) in dioxane was stirred at 100 °C under Ar_2 for 12 h. The reaction mixture was passed through a short SiO_2/KF (9:1) plug to removed majority of the Sn species, and then purified by a SiO_2 column (0-20% EtOAc/Hexanes, R_f = 0.3 in 20% EtOAc) to afford (*Z*)-ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-(2-ethoxyvinyl)-3-(6-fluoroquinolin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate as a pale yellow oil (461 mg). HPLC-MS T_R = 3.20 min (UV 254 nm, 5 min method); mass calculated for formula $C_{41}H_{60}FN_5O_5Si_2$ 777.4, observed LCMS m/z 778.3 (M+H).

Step 12: Preparation of ethyl 4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate



(*Z*)-ethyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-(2-ethoxyvinyl)-3-(6-fluoroquinolin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylate (461 mg, 0.592 mmol) was treated with a mixture of TFA/H_2O (2/2 mL) at rt overnight. All the volatiles were removed and the residue was used in the next step directly without further purification.

Step 13: Preparation of (1*R*,4*R*)-4-(3-(6-fluoroquinolin-3-yl)-8*H*-pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylic acid (**Compound 1**) and (1*S*,4*S*)-4-(3-(6-fluoroquinolin-3-yl)-8*H*-pyrazolo[1,5-a]pyrrolo[3,2-*e*]pyrimidin-5-yl)-1-methylcyclohexanecarboxylic acid (**Compound 2**)

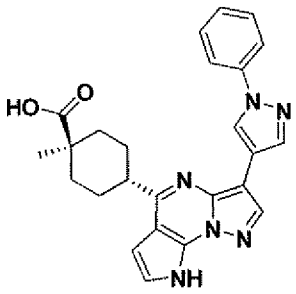
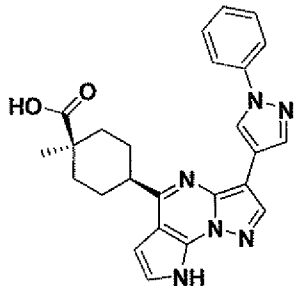
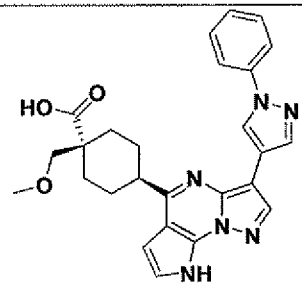


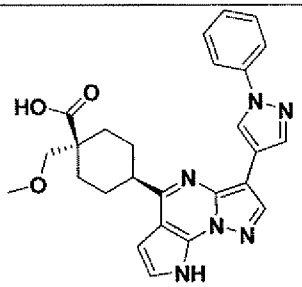
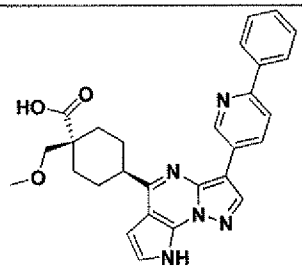
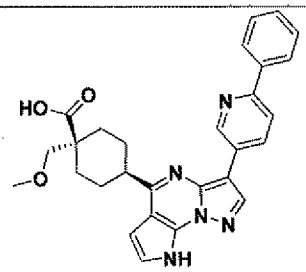
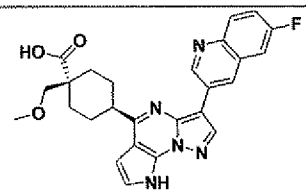
The residue from Step 12 was treated with excess of LiOH (10 eq) in a mixture of THF/MeOH/H_2O (4/2/1 mL) at 85 °C overnight. All the volatiles were removed and the residue was taken with water (3 mL) and the pH was adjusted to ~6 with 1N HCl. The solid was collected with filtration and dried under air. The crude product was dissolved in DMSO, filtered and purified by a reverse phase HPLC to afford *trans*-isomer of (**Compound 1**) as a pale yellow solid (30.2 mg), HPLC-MS T_R = 4.41 min (UV 254 nm, 10 min method); mass calculated for formula $C_{25}H_{22}FN_5O_2$ 443.2, observed LCMS m/z 444 (M+H); and *cis*-isomer of (**Compound 2**) as a pale yellow solid (45.3 mg), HPLC-MS T_R = 4.62 min (UV 254 nm, 10 min method); mass calculated for formula $C_{25}H_{22}FN_5O_2$ 443.2, observed LCMS m/z 444 (M+H).

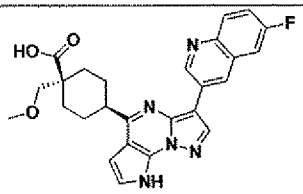
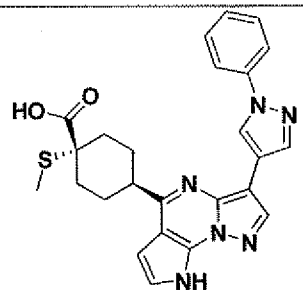
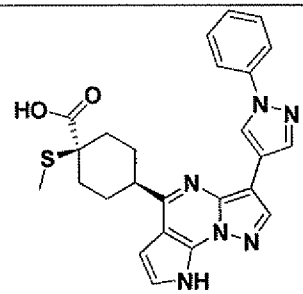
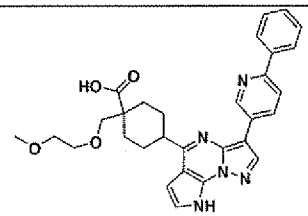
Example 2

By essentially the same procedure in Preparative Example 1, compounds in Column 2 of Table 1 can be prepared when the reaction is quenched with dimethyl disulfide, chloromethyl methyl ether and 2-methoxyethoxymethyl chloride, respectively, in example 1 Step 2 instead of with iodomethane, or reacted with a different boronic acid in the Suzuki coupling reaction in example 1 Step 9.

TABLE 1

Compound	*Chemical name*	*Structure*	*Exact mass*	*MS m/z (M+H)*	*HPLC MS t_R*
3	(1R,4R)-1-methyl-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		440.2	441.0	4.68 (10 min method)
4	(1S,4S)-1-methyl-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		440.2	441.0	4.88 (10 min method)
5	(1R,4R)-1-(methoxymethyl)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		470.2	471.1	1.98

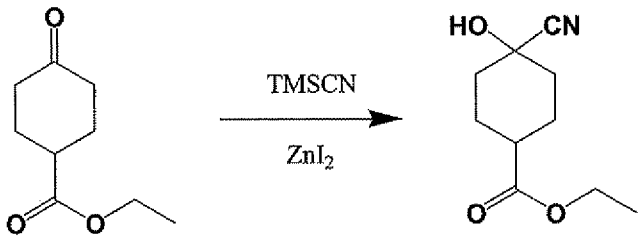
6	(1S,4S)-1-(methoxymethyl)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		470.2	471.1	2.06
7	(1R,4R)-1-(methoxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		481.2	482.2	1.56
8	(1S,4S)-1-(methoxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		481.2	482.2	1.64
9	(1R,4R)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-(methoxymethyl)cyclohexanecarboxylic acid		473.2	474.1	1.61

10	(1S,4S)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-(methoxymethyl)cyclohexanecarboxylic acid		473.2	474.1	1.72
11	(1R,4R)-1-(methylthio)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		472.2	473.2	1.92
12	(1S,4S)-1-(methylthio)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		472.2	473.2	1.83
13	1-((2-methoxyethoxy)methyl)-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		525.2	526.2	1.58

Example 3

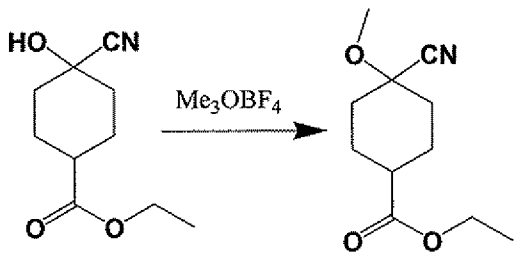
Preparation of (1S,4S)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid (**Compound 14**)

Step 1: Preparation of ethyl 4-cyano-4-hydroxycyclohexanecarboxylate



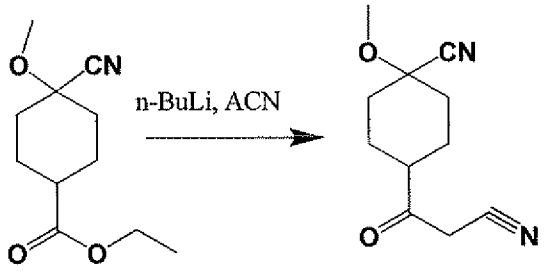
The starting material (25 g, 146.88 mmol) and TMSCN (29.14 g, 293.76 mmol) were dissolved in DCM (400 mL). ZnI_2 (4.686 g, 14.7 mmol) was added to the reaction and the resulting mixture was stirred at room temperature for 3 hours. Then HCl (1N, 147 mL) was added and stirred for another 30 min. The mixture was extracted with EtOAc (200 ml x 3) and the combined organics was dried over Na_2SO_4. The crude was purified with column after concentration (silica gel, 0-40%) to give the product (28.9 g). HPLC-MS t_R = 1.25 min ($UV_{254\ nm}$); mass calculated for formula $C_{10}H_{15}NO_3$ 197.1, observed LCMS m/z 198.2 (M+H).

Step 2: Preparation of ethyl 4-cyano-4-methoxycyclohexanecarboxylate



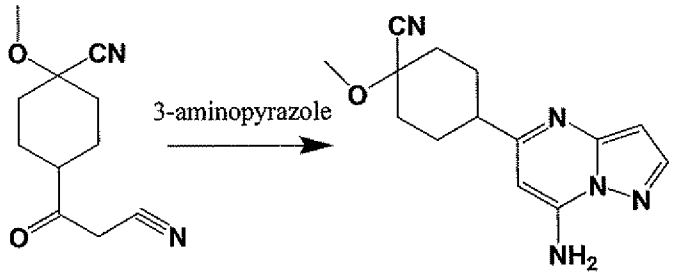
The hydroxyl compound (28.9 g, 146.7 mmol), trimethyloxonium tetrafluoroborate (26.62 g, 180 mmol) and 1,8-bis(dimethylamino)naphthalene (38.58 g, 180 mmol) were mixed in dry DCM (500 mL). The resulting mixture was stirred at room temperature overnight and then filtered through celite. The organics was washed with water, brine and dried over Na_2SO_4. After concentration, the crude was purified with column (silica gel, 0~30%) to give the product (19 g). HPLC-MS t_R = 1.64 min ($UV_{254\ nm}$); mass calculated for formula $C_{11}H_{17}NO_3$ 211.1, observed LCMS m/z 212.1 (M+H).

Step 3: Preparation of 4-(2-cyanoacetyl)-1-methoxycyclohexanecarbonitrile



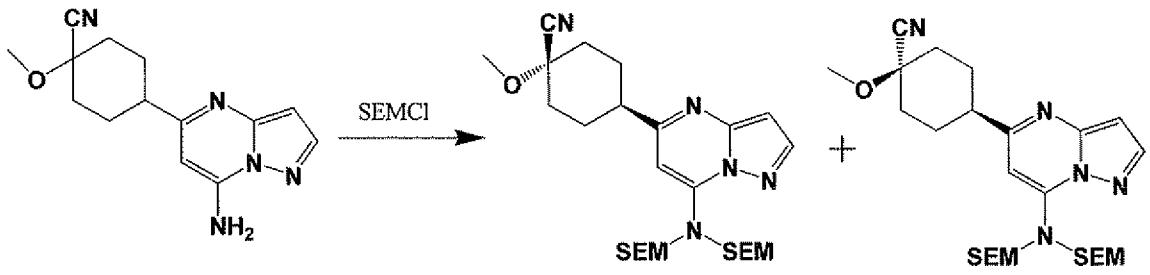
The ACN (9.4 mL, 180 mmol) in dry THF (300 mL) was cooled to -78°C and n-BuLi (2.5 M in hexane, 72 mL, 180 mmol) was added carefully. The resulting mixture was stirred at -78 °C for 1 hour and the ester (19.0 g, 90 mmol) in THF (50 mL) was added slowly. The reaction was stirred at -78°C for another 30 min after the addition then was allowed to warm to room temperature and stirred for another 30 min at room temperature. NH_4Cl (aq.) was added to quench the reaction and HCl (1N, 90 mL) was added. The mixture was extracted with EtOAc (300 mL x 3) and the organics was washed with brine and dried over Na_2SO_4. After concentration, the crude was used in the next step directly without further purification.

Step 4: Preparation of 4-(7-aminopyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexanecarbonitrile



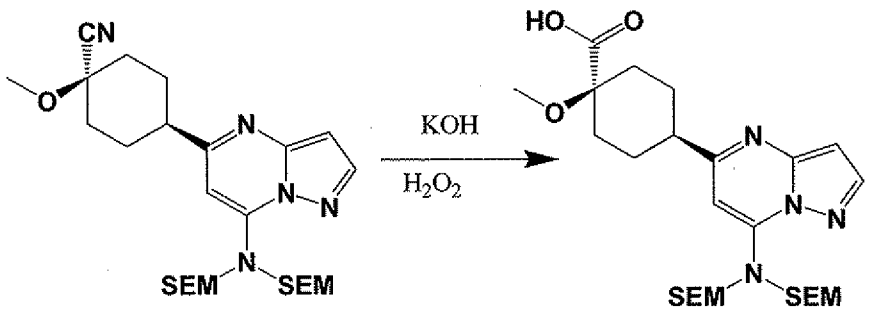
The cyanoketone from above was dissolved in HOAc (50 mL) and 3-aminopyrazole (7.5 g, 90 mmol) was added. The mixture was heated to reflux and stirred overnight. After concentration to remove the solvent, $NaHCO_3$ (aq.) was added carefully. The solid was collected and washed with water and dried under air. The crude product (24.3 g) was used in the next step directly without further purification. HPLC-MS t_R = 0.81 min ($UV_{254\ nm}$); mass calculated for formula $C_{14}H_{17}N_5O$ 271.1, observed LCMS m/z 272.2 (M+H).

Step 5: Preparation of (1R,4R)-4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexanecarbonitrile and (1S,4S)-4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexanecarbonitrile



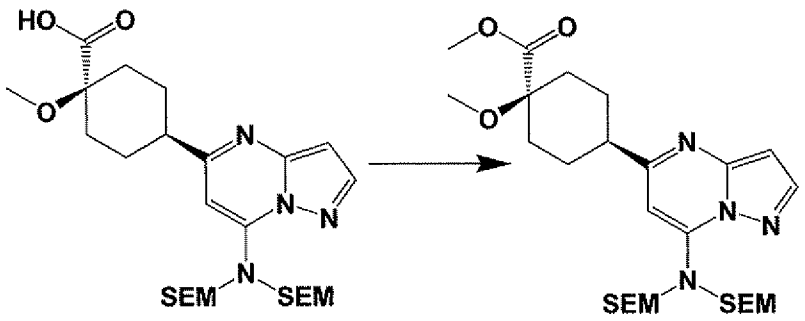
The crude compound from step 4 (~90 mmol) and DIEA (47 mL, 270 mmol) mixed in DCM (300 mL). To the mixture, SEMCl (45g, 270 mmol) in DCM (100 mL) was added dropwise. After addition, the mixture was heated up to 50°C and stirred for 6 h. After cooling to room temperature, $NaHCO_3$ (aq.) was added. The aqueous was extracted with EtOAc (100 mL x 3). The combined organics was dried, concentrated and purified with column (silica gel, 0-30%) to give two isomers. Isomer 1, (13 g): HPLC-MS t_R = 3.55 min ($UV_{254\ nm}$); mass calculated for formula $C_{26}H_{45}N_5O_3Si_2$ 531.3, observed LCMS m/z 532.3 (M+H). Isomer 2 (13 g): HPLC-MS t_R = 3.60 min ($UV_{254\ nm}$); mass calculated for formula $C_{26}H_{45}N_5O_3Si_2$ 531.3, observed LCMS m/z 532.3 (M+H).

Step 6: Preparation of (1S,4S)-4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylic acid



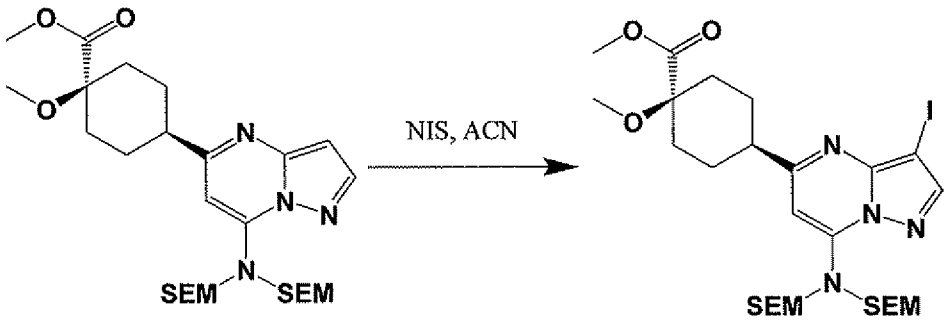
The cyano compound isomer 1 (13 g, 24.4 mmol) was dissolved in EtOH (50 mL) and KOH (20%, 30 mL) was added followed by H_2O_2 (30%, 3.0 mL). The resulting mixture was heated up to 100°C and stirred overnight. After cooling to room temperature, the solvent was removed under reduced pressure. The aqueous pH value was adjusted to ~6 with 6N HCl and extracted with EtOAc (300 mL x 3). The combined organics was dried over Na_2SO_4 and concentrated to give the crude product (9.7 g) and be used in the next step without further purification. HPLC-MS t_R = 2.17 min ($UV_{254\ nm}$); mass calculated for formula $C_{26}H_{46}N_4O_5Si_2$ 550.3, observed LCMS m/z 551.3 (M+H).

Step 7: Preparation of (1S,4S)-methyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylate



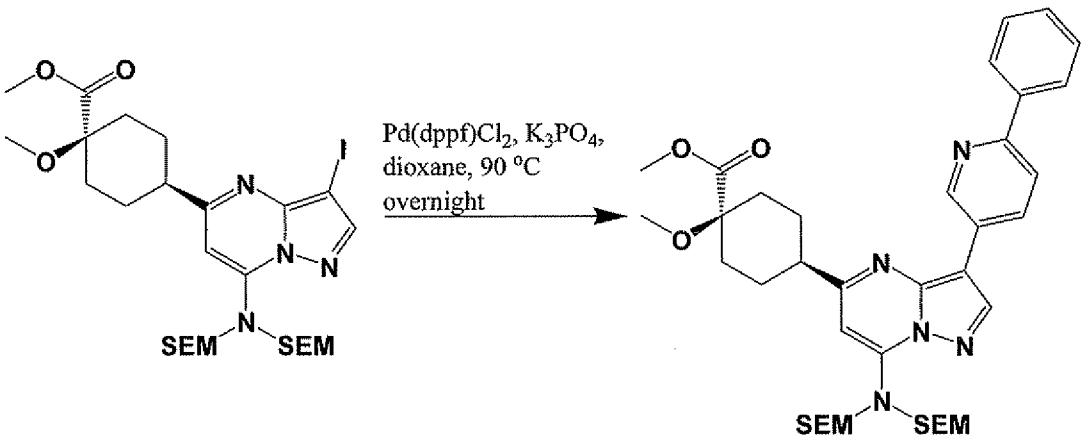
The carboxylic acid compound (9.7 g, 17.6 mmol) was dissolved in DCM (150 mL) and $TMSCHN_2$ (2.0 M in hexane, 24.4 mL) was added. The mixture was stirred at room temperature overnight and concentrated. The crude was purified with column (silica gel, 0-30% EtOAc/Hexane) to give the product (9.9 g, mixture of methyl ester and TMS-methyl ester). HPLC-MS t_R = 2.38 min ($UV_{254\ nm}$); mass calculated for formula $C_{27}H_{48}N_4O_5Si_2$ 564.3, observed LCMS m/z 565.3 (M+H).

Step 8: Preparation of (1S,4S)-methyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-iodopyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylate



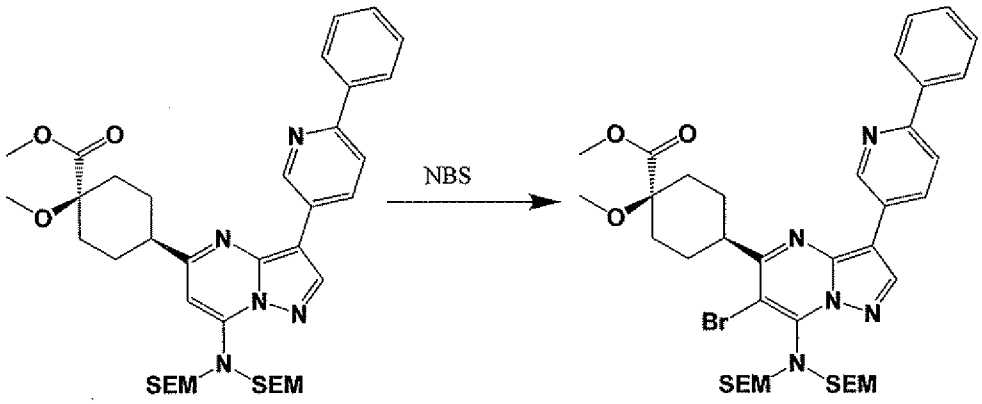
The iodo compound was prepared with the same condition described in example 1 Step 8. HPLC-MS t_R = 2.81 min ($UV_{254\ nm}$); mass calculated for formula $C_{27}H_{47}IN_4O_5Si_2$ 690.2, observed LCMS m/z 691.2 (M+H).

Step 9: Preparation of (1S,4S)-methyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-(6-phenylpyridin-3-yl)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylate



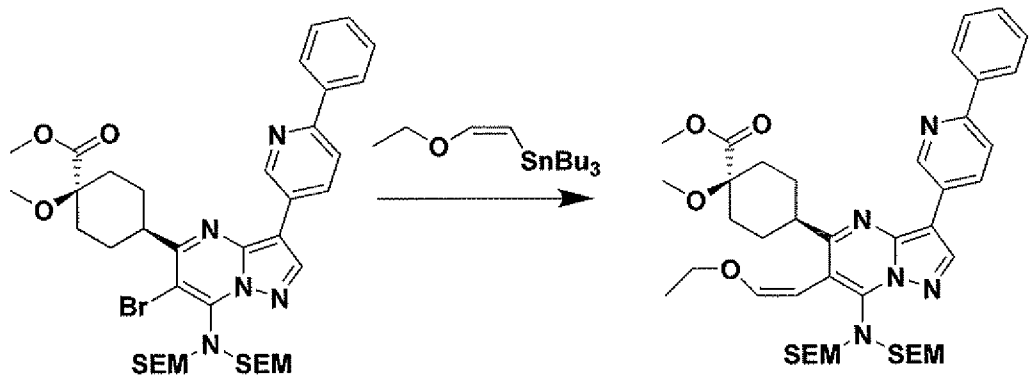
The 3-phenylpyridyl compound was prepared with with the same condition described in example 1 Step 9. HPLC-MS t_R = 2.77 min ($UV_{254\ nm}$); mass calculated for formula $C_{38}H_{55}N_5O_5Si_2$ 717.4, observed LCMS m/z 718.3 (M+H).

Step 10: Preparation of (1S,4S)-methyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-bromo-3-(6-phenylpyridin-3-yl)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylate



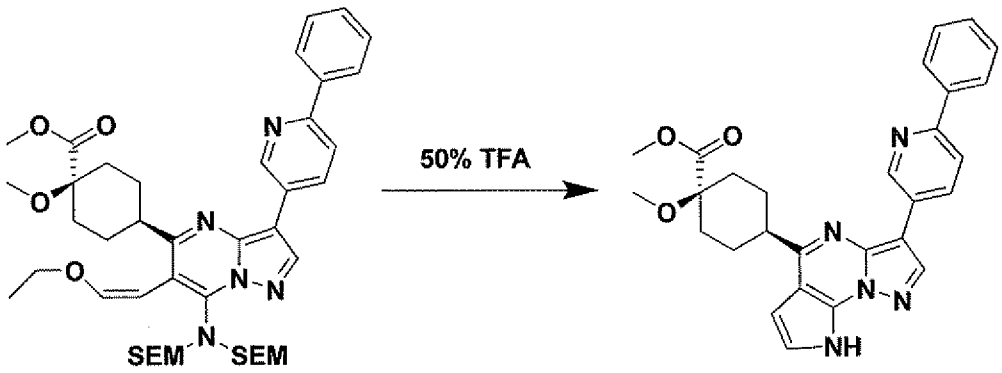
The bromo compound was prepared with the same condition described in example 1 step 10. HPLC-MS t_R = 2.76 min ($UV_{254\ nm}$); mass calculated for formula $C_{38}H_{54}BrN_5O_5Si_2$ 795.3, observed LCMS m/z 796.2 (M+H).

Step 11: Preparation of (1S,4S)-methyl 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-((Z)-2-ethoxyvinyl)-3-(6-phenylpyridin-3-yl)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylate



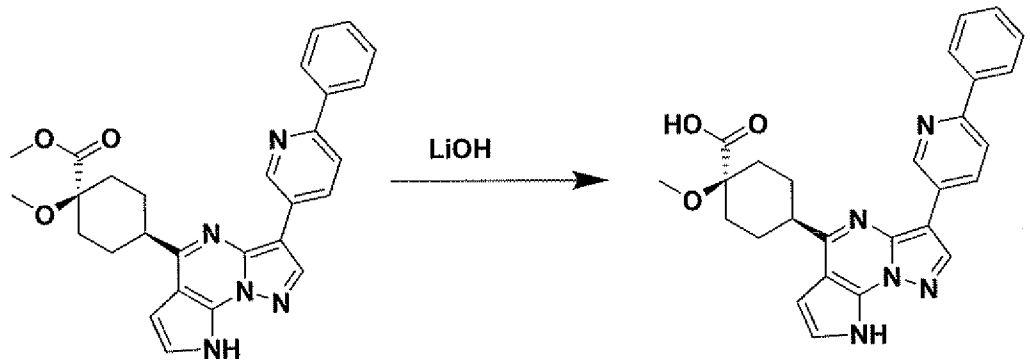
The Stille coupling reaction was replace with the same condition described in example 1 step 11. HPLC-MS t_R = 2.77 min ($UV_{254\ nm}$); mass calculated for formula $C_{42}H_{61}N_5O_6Si_2$ 787.4, observed LCMS m/z 788.3 (M+H).

Step 12: Preparation of (1S,4S)-methyl 1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylate



The cyclization of the vinyl ether was replace with the same condition described in example 1 Step 12. HPLC-MS t_R = 1.61 min ($UV_{254\ nm}$); mass calculated for formula $C_{28}H_{27}N_5O_3$ 481.2, observed LCMS m/z 482.2 (M+H).

Step 12: Preparation of (1S,4S)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid (**Compound 14**)

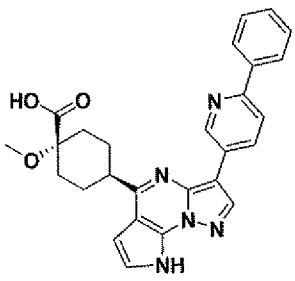


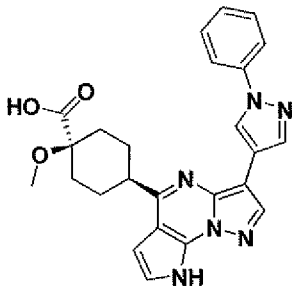
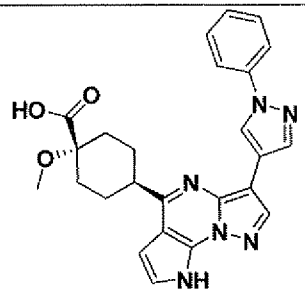
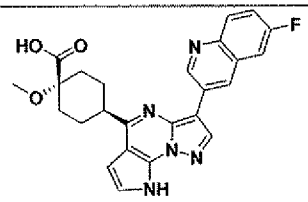
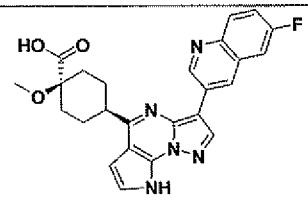
The hydrolysis of the ester was replace with the same conditions described in example 1 Step 13. . HPLC-MS t_R = 1.38 min ($UV_{254\ nm}$); mass calculated for formula $C_{27}H_{25}N_5O_3$ 467.2, observed LCMS m/z 468.2 (M+H).

Example 4

By essentially the same procedure in Preparative Example 3, compounds in Column 2 of Table 2 can be prepared when reacted with different boronic acid in Suzuki coupling reaction in example 1 Step 9.

TABLE 2

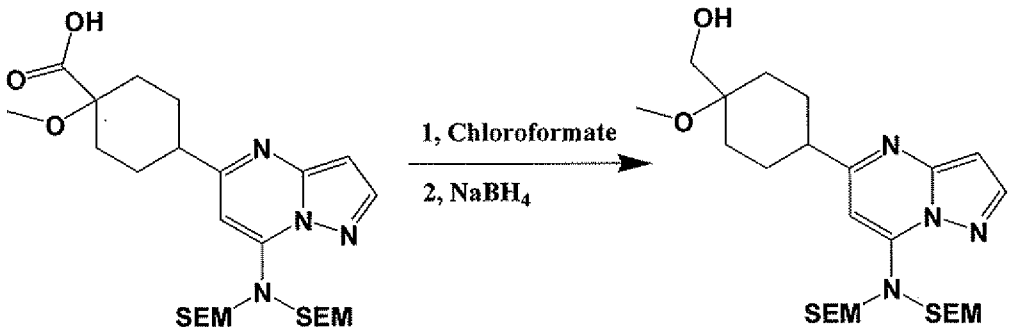
Compound	*Chemical name*	*Structure*	*Exact mass*	*MS m/z (M+H)*	*HPLC MS t_R*
15	(1R,4R)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		467.2	468.2	1.39

16	(1S,4S)-1-methoxy-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		456.2	457.1	4.52
17	(1R,4R)-1-methoxy-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid		456.2	457.1	4.52 (10 min method)
18	(1R,4R)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylic acid		459.2	460.2	1.52
19	(1S,4S)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylic acid		459.2	460.2	1.51

Example 5

Preparation of ((1R,4R)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexyl)methanol (**Compound 20**)

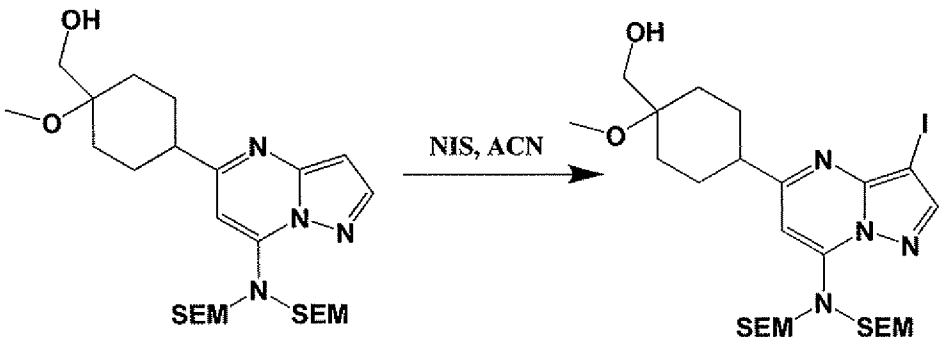
Step 1: Preparation of (4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexyl)methanol



The carboxylic acid was made in example 3 Step 6.

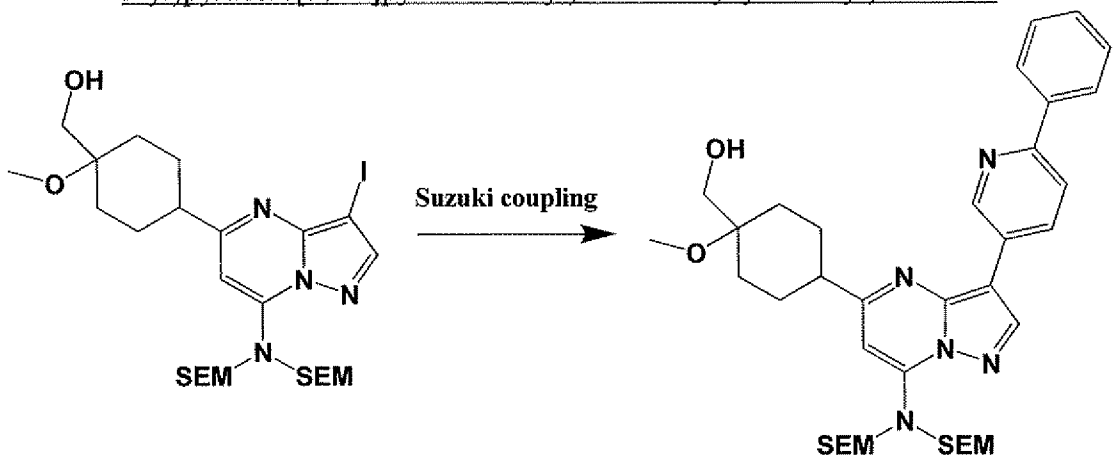
The carboxylic acid (3.35 g, 6.1 mmol) was dissolved in dry THF (50 mL) and cooled to 0°C. The N-methylmorpholine (657 mg, 6.5 mmol) was added followed by the addition of iso-butyl chloroformate (888 mg, 6.5 mmol). The mixture was stirred at 0°C for 2 hours and $NaBH_4$ (495 mg, 13 mmol) in water (5 mL) was added carefully. The resulting mixture was stirred at 0 °C for 30 min followed by room temperature for another 30 min. The solvent was removed under reduced pressure and extracted with EtOAc. After concentration, the crude was purified with column (silica gel, 0~40% EtOAc/hexane) to give the product (2.32 g). HPLC-MS t_R = 2.07 min ($UV_{254\ nm}$); mass calculated for formula $C_{26}H_{48}N_4O_4Si_2$ 536.3, observed LCMS m/z 537.4 (M+H).

Step 2: Preparation of (4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-iodopyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexyl)methanol



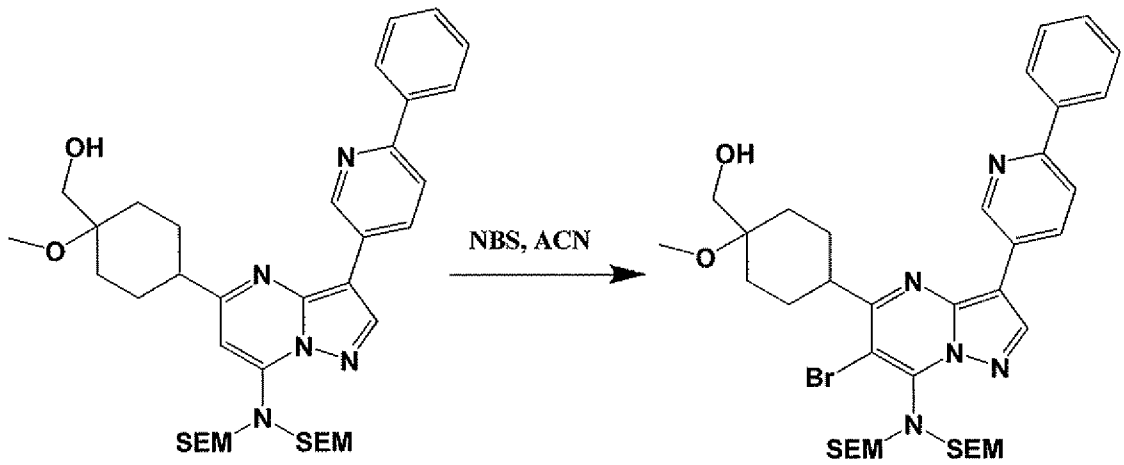
The iodo compound was prepared with the same condition described in example 1 Step 8. HPLC-MS t_R = 2.62 min ($UV_{254\ nm}$); mass calculated for formula $C_{26}H_{47}IN_4O_4Si_2$ 662.2, observed LCMS m/z 663.3 (M+H).

Step 3: Preparation of (4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-(6-phenylpyridin-3-yl)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexyl)methanol



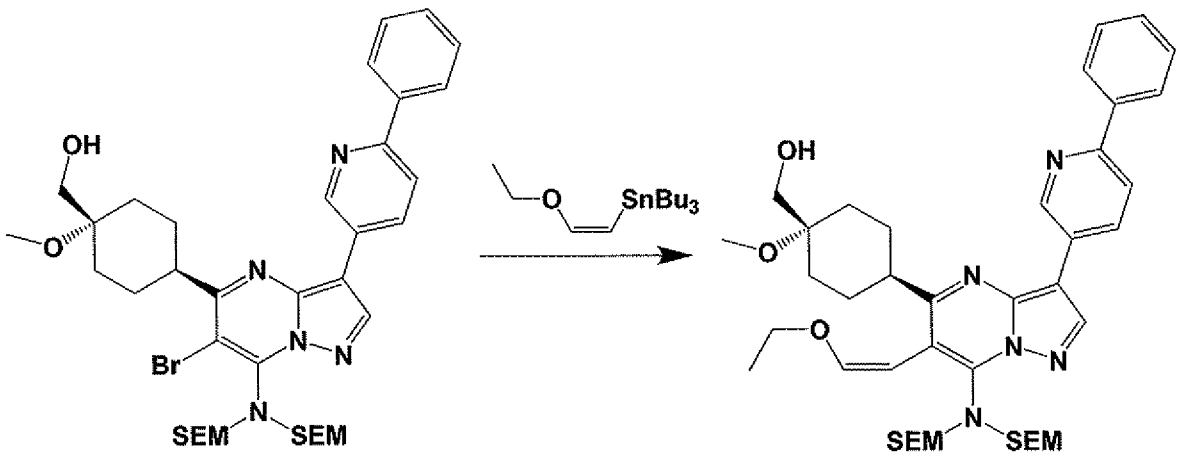
The 3-phenylpyridyl compound was prepared with the same condition described in example 1 Step 9. HPLC-MS t_R = 2.29 min ($UV_{254\ nm}$); mass calculated for formula $C_{37}H_{55}N_5O_4Si_2$ 689.4, observed LCMS m/z 690.4 (M+H).

Step 4: Preparation of (4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-bromo-3-(6-phenylpyridin-3-yl)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexyl)methanol



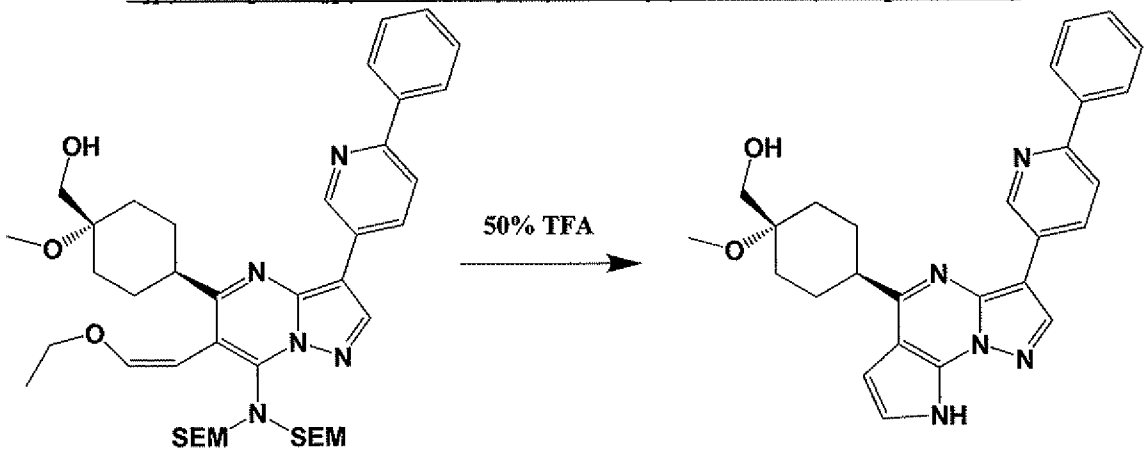
The bromo compound was prepared with the same condition described in example 1 Step 10. HPLC-MS t_R = 2.69 min ($UV_{254\ nm}$); mass calculated for formula $C_{37}H_{54}BrN_5O_4Si_2$ 767.3, observed LCMS m/z 768.2 (M+H).

Step 5: Preparation of ((1R,4R)-4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-((Z)-2-ethoxyvinyl)-3-(6-phenylpyridin-3-yl)pyrazolo[1,5-a]pyrimidin-5-yl)-1-methoxycyclohexyl)methanol



The Stille coupling reaction was replace with the same conditions described in example 1 Step 11. HPLC-MS t_R = 2.43 min ($UV_{254\ nm}$); mass calculated for formula $C_{41}H_{61}N_5O_5Si_2$ 759.4, observed LCMS m/z 760.5 (M+H).

Step 6: Preparation of ((1R,4R)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexyl)methanol (**Compound 20**)

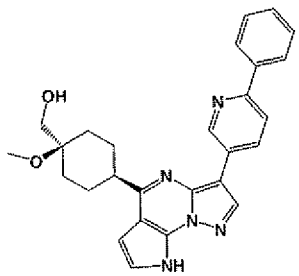


The vinyl-compound was de-protected with the same condition described in example 1 Step 12. HPLC-MS t_R = 1.37 min ($UV_{254\ nm}$); mass calculated for formula $C_{27}H_{27}N_5O_2$ 453.2, observed LCMS m/z 454.3 (M+H).

Example 6

By essentially the same procedure in Preparative Example 5, compounds in Column 2 of Table 3 can be prepared with another carboxylic acid isomer started from example 5 Step 1.

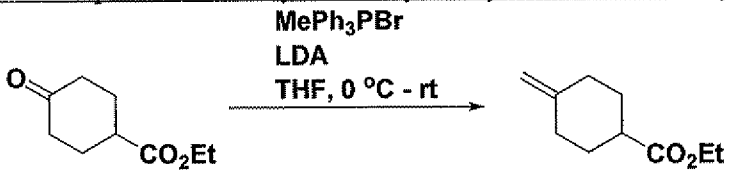
TABLE 3

Compound	*Chemical name*	*Structure*	*Exact mass*	*MS m/z (M+H)*	*HPLC MS t_R*
21	((1S,4S)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexyl)methanol		453.2	454.3	1.37

Example 7

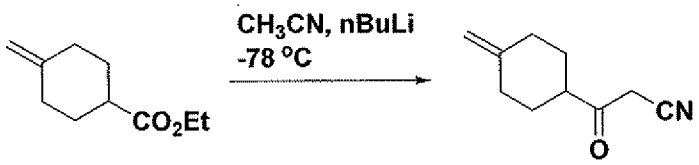
Preparation of 1-(hydroxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8*H*-pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidin-5-yl)cyclohexanol (**Compound 22**)

Step 1: Preparation of ethyl 4-methylenecyclohexanecarboxylate



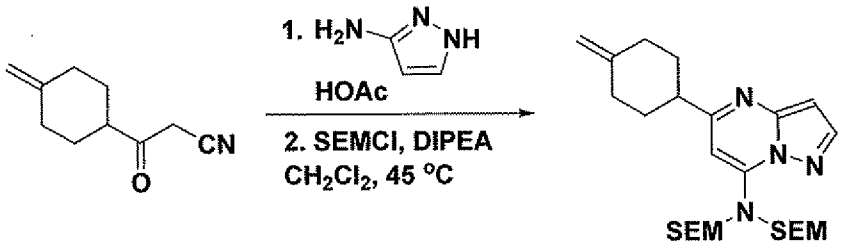
To a suspension of $MePh_3PBr$ (37.1 g, 104 mmol) in THF (500 mL) at 0°C was added slowly LDA (1.2 eq) over 1 h. The resulting orange solution was stirred for 30 min before ethyl 4-oxocyclohexanecarboxylate (16.1 g, 94.4 mmol) was added dropwise. The resulting suspension was warmed to rt and stirred overnight. A saturated NH_4Cl (aq) was added and THF was removed. The aqueous residue was extracted with EtOAc (100mlx3). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by passing through a short silica gel plug (hexanes/EtOAc 7:1). After being concentrated, ethyl 4-methylenecyclohexanecarboxylate was obtained as a pale yellow oil (12.1 g).

Step 2: Preparation of 3-(4-methylenecyclohexyl)-3-oxopropanenitrile



CH_3CN (2.55 mL, 48.8 mmol. 2.2 eq) was added dropwise to a solution of nBuLi (48.8 mmol) in 180 mL of THF at -78°C. After stirring for 1 h at -78 °C, a solution of ethyl 4-methylenecyclohexanecarboxylate (3.73 g, 22.2 mmol) in 20 mL of THF was added dropwise and the resulting reaction mixture (turned into nearly clear solution after addition) was stirred at -78°C for 1 h, then slowly warmed to 0°C before being quenched with sat. NH_4Cl. THF was removed and the residue was diluted with EtOAc. The organic layer was separated and washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by a SiO_2 column (0-30% EtOAc/Hexanes, R_f = 0.2 in 20% EtOAc) to afford 3-(4-methylenecyclohexyl)-3-oxopropanenitrile as a colorless oil (3.25 g).

Step 3: Preparation of 5-(4-methylenecyclohexyl)-N,N-bis((2-(trimethylsilyl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidin-7-amine

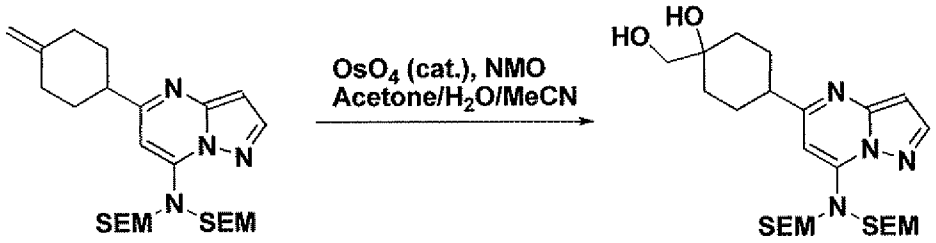


A mixture of 3-aminopyrazole (1.82 g, 21.9 mmol) and 3-(4-methylenecyclohexyl)-3-oxopropanenitrile (3.25 g, 19.9 mmol) in HOAc (10 mL) was heated at 100°C in a sealed tube overnight (18 h). LCMS showed very good conversion to the desired product (229) and no SM (164) left. TLC also showed no SM left. After cooling to rt, all the volatiles were removed under reduced pressure to afford a light brown oil, which was used without further purification.

To a slurry of the above crude material in CH_2Cl_2 (100 mL) was added SEMCl (14.0 mL, 79.6 mmol), followed by DIPEA (27.7 mL, 159 mmol). The resulting reaction mixture was stirred at 45°C for 1 h. LCMS showed nearly complete conversion. After cooling to rt, all the volatiles were removed under reduced pressure. The residue was diluted with EtOAc, washed with H_2O and brine, and concentrated. The crude product was purified by a SiO_2 column (0-15% EtOAc/Hexane, R_f = 0.65 in 20% EtOAc) to afford 5-(4-methylenecyclohexyl)-N,N-bis((2-(trimethylsilyl)ethoxy)methyl)pyrazolo[1,5-a]pyrimidin-7-amine as a pale yellow oil

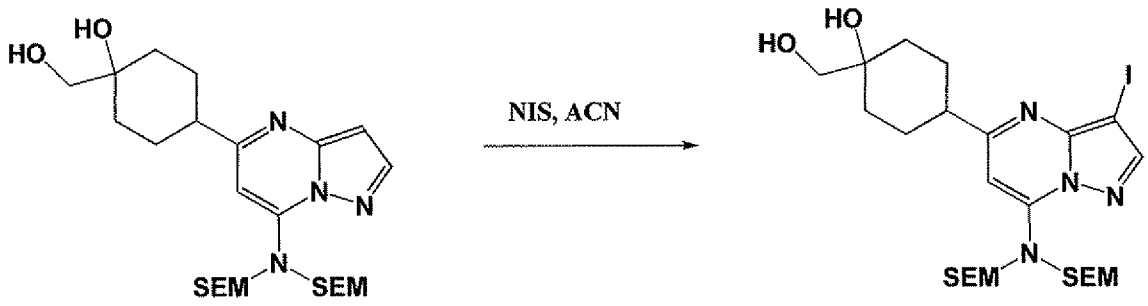
(10.3 g). HPLC-MS T_R = 3.12 min (UV 254 nm, 5 min method); mass calculated for formula $C_{25}H_{44}N_4O_2Si_2$ 488.3, observed LCMS m/z 489.3 (M+H).

Step 4: Preparation of 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-(hydroxymethyl)cyclohexanol



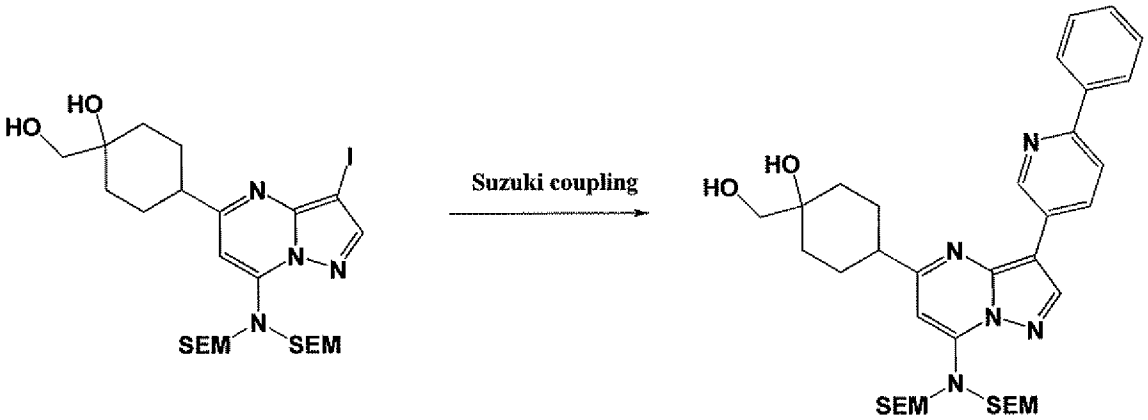
To a slurry of 5-(4-methylenecyclohexyl)-*N*,*N*-bis((2-(trimethylsilyl)ethoxy)methyl)pyrazolo[1,5-*a*]pyrimidin-7-amine (4.89 g, 10.0 mmol) in Acetone/H_2O/MeCN (60/20/20) was added NMO (4.15 mL, 20 mmol), followed by OsO_4 (2.5 wt. % in ^{t}BuOH) (6.27 mL, 0.50 mmol) at rt. The resulting reaction mixture was stirred at rt overnight. Organic solvent were evaporated and the aqueous residue was extracted with EtOAc three times, dried over Na_2SO_4 and concentrated to afford 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-(hydroxymethyl)cyclohexanol (**37**) as a pale brownish oil (5.52 g), which was used without further purification. HPLC-MS T_R = 2.48 min (UV 254 nm, 5 min method); mass calculated for formula $C_{25}H_{46}N_4O_4Si_2$ 522.3, observed LCMS m/z 523.3 (M+H).

Step 5: Preparation of 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-3-iodopyrazolo[1,5-a]pyrimidin-5-yl)-1-(hydroxymethyl)cyclohexanol



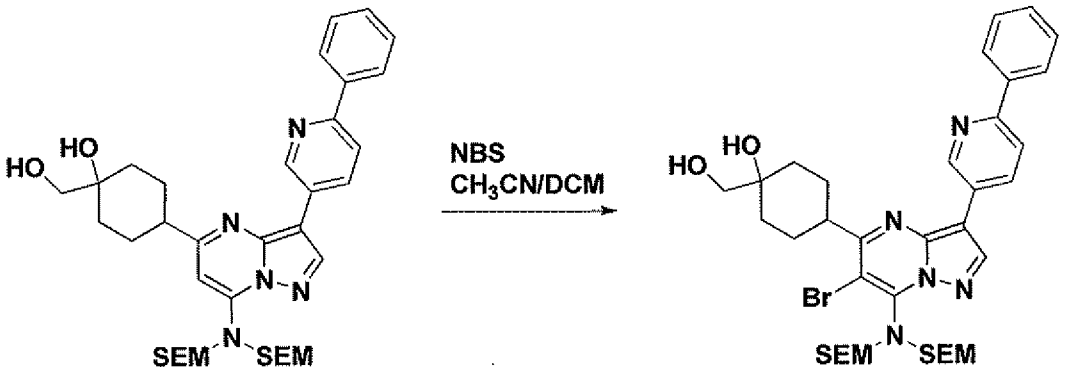
The iodo compound was prepared with the same condition described in example 1 Step 8. HPLC-MS t_R = 2.56 min ($UV_{254\ nm}$); mass calculated for formula $C_{25}H_{45}IN_4O_4Si_2$ 648.2, observed LCMS m/z 649.3 (M+H).

Step 6: Preparation of 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino) -3-(6-phenylpyridin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-(hydroxymethyl)cyclohexanol



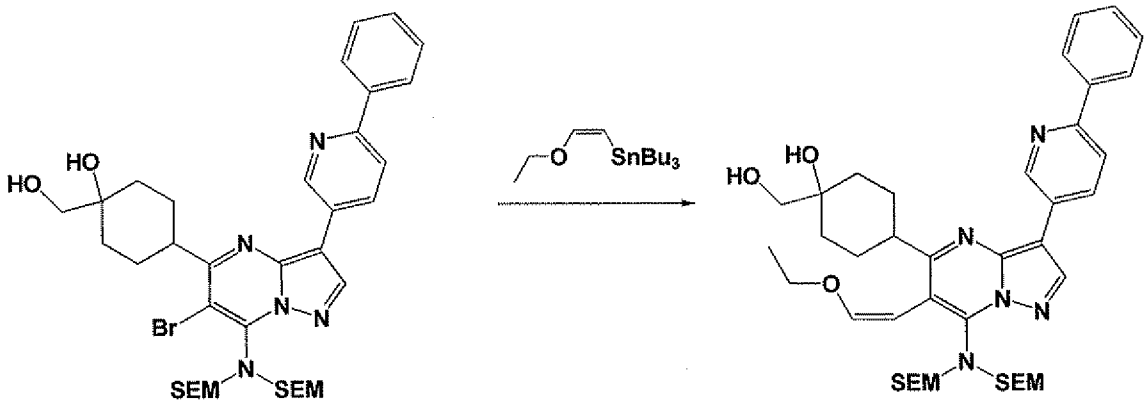
The 3-phenylpyridyl compound was prepared with the same condition described in example 1 Step 9. HPLC-MS T_R = 2.14 min (UV 254 nm); mass calculated for formula $C_{36}H_{53}N_5O_4Si_2$ 675.4, observed LCMS m/z 676.3 (M+H).

Step 7: Preparation of 4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-bromo-3-(6-phenylpyridin-3-yl)pyrazolo[1,5-*a*]pyrimidin-5-yl)-1-(hydroxymethyl)cyclohexanol



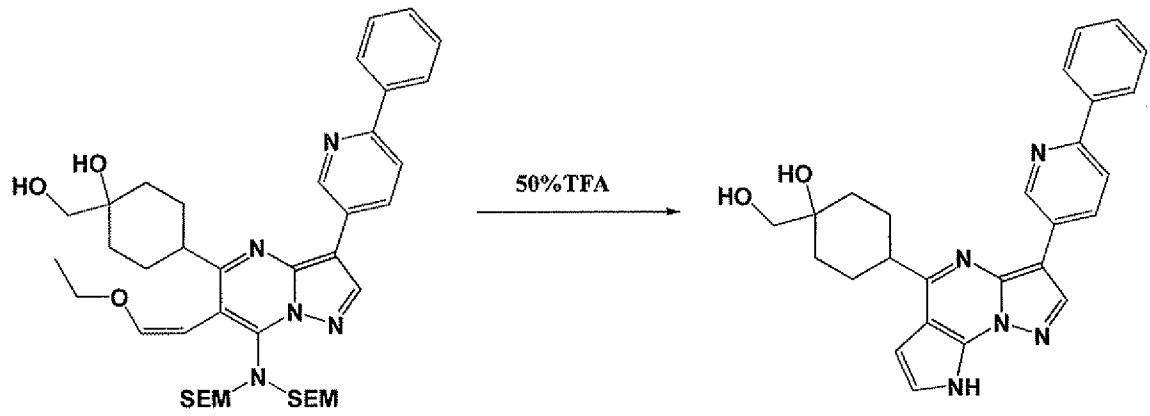
The bromo compound was prepared with the same condition described in example 1 Step 10. HPLC-MS T_R = 3.06 min (UV 254 nm); mass calculated for formula $C_{36}H_{52}BrN_5O_4Si_2$ 753.3, observed LCMS m/z 754.2 (M+H).

Step 8: Preparation of (Z)-4-(7-(bis((2-(trimethylsilyl)ethoxy)methyl)amino)-6-(2-ethoxyvinyl)-3-(6-phenylpyridin-3-yl)pyrazolo[1,5-a]pyrimidin-5-yl)-1-(hydroxymethyl)cyclohexanol



The Stille coupling reaction was replace with the same conditions described in example 1 Step 11. HPLC-MS t_R = 2.41 min ($UV_{254\ nm}$); mass calculated for formula $C_{40}H_{59}N_5O_5Si_2$ 745.4, observed LCMS m/z 746.5 (M+H).

Step 9: Preparation of 1-(hydroxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8*H*-pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidin-5-yl)cyclohexanol (**Compound 22**)

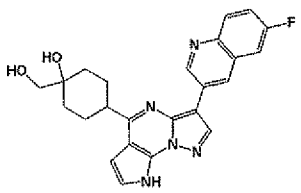


The vinyl-compound was de-protected with the same condition described in example 1 Step 12. HPLC-MS T_R = 3.25 min (UV 254 nm, 10 min method); mass calculated for formula $C_{26}H_{25}N_5O_2$ 439.2, observed LCMS m/z 440.0 (M+H).

Example 8

By essentially the same procedure in Preparative Example 7 compounds in Column 2 of Table 4 can be prepared from example 7 Step 6 with 6-fluoroquinolin-3-ylboronic acid.

TABLE 4

Example	*Chemical name*	*Column 2*	*Exact mass*	*MS m/z (M+H)*	*HPLC MS t_R*
23	4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-(hydroxymethyl)cyclohexanol		431.2	432.0	3.15 (10 min method)

EXAMPLE 9

mTOR Kinase Assay

Methods: An HTRF mTOR enzyme assay was developed to assess the compounds' inhibitory activity. The mTOR assay buffer contained 10 mM Hepes (pH 7.4), 50 mM NaCl, 100 µg/ml BSA, 50 mM µ-glycerophosphate, 10 mM $MnCl_2$ and 0.5 mM DTT. An active truncated mTOR enzyme was prepared similarly to that reported by Toral-Barza et al., Biochemical and Biophysical Research Communications 332, pp 304–310 (2005). 20 ng of human mTOR enzyme (< 5 % pure was preincubated with the compound for 10 minutes followed by the addition of 5 µM ATP and 0.2 µM GST-S6K (Zhang et al., Protein Expression and Purification 46, pp 414–420 (2006)). The reaction was incubated for one hour at 30°C. Anti phospho p70-S6K(Thr389) (~1.7 ng/well, anti-phospho-p70S6K-cryptate (Pho-p70S6-Kin-K cat# 64CUSKAY, from Cisbio)) and anti GST-XL665 (1:1 Ratio with the substrate GST-S6K, anti GST-XL665, cat# 61GSTXLB) Cisbio) were added after the reaction was stopped. The plates were read (PHERAstar, BMG) at least 2 hours after adding the anti phospho p70-S6K and the anti GST-XL665.

IC_{50} DETERMINATIONS: Dose-response curves were plotted from the inhibition data generated, each in duplicate, from 8 point serial dilutions of inhibitory compounds. Concentration of compound was plotted against the HTRF em665/em590 ratio signal. To generate IC_{50} values, the dose-response curves were fitted to a standard sigmoidal curve and IC_{50} values were derived by nonlinear regression analysis.

EXAMPLE 10

mTOR Target Engagement Assay

The target engagement of mTOR kinase inhibitors was evaluated using an immunofluorescent cell-based assay. In this assay, inhibition of mTORC1 activity was measured by the reduction in the level of phosphorylated 4E-BP1Thr37/46 (p4E-BP1 Thr37/46), and inhibition of mTORC2 activity was measured by the reduction of phosphorylated AKTSer473 (pAKT S473).

PC3 cells (prostate tumor cell-line that contains a mutation in the tumor suppressor PTEN, that promotes the phosphorylation and activation of AKT and 4E-BP1) were used in the immunofluorescence assay. PC3 cells were seeded on 384 well plates (black clear bottom, Matrix #4332) overnight. PC3 cells were then treated with 40 µl of the serially diluted test compounds (in 5% fetal bovine serum, F12 medium containing 0.25% DMSO) for ninety minutes at 37°C. The test compound solution was removed, and the plates were washed gently two times with 25 µl phosphate buffered saline (PBS). The cells were fixed by adding 25 µl of Prefer reagent (from Anatech LTD, Cat#414, a glyoxal fixative for fixing proteins within a cell) for sixty minutes followed by three washes with PBS. 5% Goat serum in PBS/0.3% Triton was used to block non-specific binding (60 minutes).

The primary antibodies targeting pAKT S473 and p4E-BP1 Thr37/46 were diluted into PBS/0.3% Triton and incubated with the cells overnight at 4°C. The antibodies targeting pAKTS473 (Cat# 4085, Cell signaling) and p4E-BP1 Thr37/46 (Cat#2855, Cell signaling) were used at a 1:100 dilution. Plates were washed three times with PBS/0.1% Tween 20 before adding the secondary antibody at a 1:200 dilution. (goat anti-rabbit containing a fluorescent label, Alexa Fluor 488, Cat# A11008, Invitrogen) in PBS/0.3% Triton for 60 minutes. Finally, the plates were washed three times with PBS/0.1% Tween 20 and the fluorescent intensity was read using an Analyst HT from Molecular Devices. The fluorescent intensity values from the serially diluted compound treatment group were analyzed using the Xlfit 4 program (Microsoft) (Formula 205: Y=Bottom+(Top-Bottom)/(1+(IC_{50}/X)^Hillslope) to generate the IC_{50} value. Where Top is the maximum signal without Compound (+ DMSO only) and Bottom represents maximum inhibition. Y is the fluorescence at some compound concentration. The control used to determine the fluorescent intensities for 100% pAKT S473 or 100% phosphorylated p4E-BP1 Thr37/46 were measured from untreated wells that contained only DMSO, instead of test compound.

Table 5 below lists representative compounds of the invention with activity data whereby the IC50 values are rated "A", "B," "C," or "D." The IC50 values are rated "A" for IC_{50} values in the range of 1 nM to 100 nM, "B" for IC_{50} values in the range from 100 nM to 1000 nM, "C" for IC_{50} values in the range from 1000 nM to 2000 nM, "D" for IC_{50} values in the range from 2000 nM to 5000 nM and "E" for IC_{50} values of 5000 nM to 15 µM.

TABLE 5

Compound	hMTOR IC50, nM	pAKT IC50 nM	P4EBP1 IC50 nM
1	1.54	222.88	
2	A		
3	A	B	
4	A		
5	A		
6	A	B	
7	A	B	
8	A	B	
9	A	B	
10	B		
11	A		
12	A	C	
13	2.17	906.39	
14	A	B	B
15	A	B	B
16	A	B	B
17	A	B	B
18	A	D	
19	A	D	
20	A	B	
21	A	B	
22	1.53	240.16	
23	A	B	

EXAMPLE 11

CHK1 in vitro Kinase Assay

This *in vitro* assay utilizes recombinant His-CHK1 expressed in the baculovirus expression system as an enzyme source and a biotinylated peptide based on CDC25C as substrate (biotin-RSGLYRSPSMPENLNRPR).

Materials and Reagents:

1) CDC25C Ser 216 C-term Biotinylated peptide substrate (25 mg), stored at -20 ^{o}C, Custom Synthesis by Research Genetics: biotin-RSGLYRSPSMPENLNRPR 2595.4 MW

2) His-CHK1 In House lot P976, 235 µg/mL, stored at -80°C.
3) D-PBS (without CaCl and MgCl): GIBCO, Cat.# 14190-144
4) SPA beads: Amersham, Cat.# SPQ0032: 500 mg/vial
   Add 10 mL of D-PBS to 500 mg of SPA beads to make a working concentration of 50 mg/mL. Store at 4 °C. Use within 2 week after hydration.
5) 96-Well White Microplate with Bonded GF/B filter: Packard, Cat.# 6005177
6) Top seal-A 96 well Adhesive Film: Perkin Elmer, Cat.# 6005185
7) 96-well Non-Binding White Polystyrene Plate: Corning, Cat. # 6005177
8) $MgCl_2$: Sigma, Cat.# M-8266
9) DTT: Promega, Cat.# V3155
10) ATP, stored at 4 °C: Sigma, Cat.# A-5394
11) γ33P-ATP, 1000-3000 Ci/mMol: Amersham, Cat.# AH9968
12) NaCl: Fisher Scientific, Cat.# BP358-212
13) H_3PO_4 85% Fisher, Cat.#A242-500
14) Tris-HCL pH 8.0: Bio-Whittaker, Cat. # 16-015V
15) Staurosporine, 100 µg: CALBIOCHEM, Cat. # 569397
16) Hypure Cell Culture Grade Water, 500 mL: HyClone, Cat.# SH30529.02

Reaction Mixtures:

1) Kinase Buffer: 50 mM Tris pH 8.0; 10 mM $MgCl_2$; 1 mM DTT
2) His-CHK1, In House Lot P976, MW ~30KDa, stored at -80 °C.

6 nM is required to yield positive controls of ~5,000 CPM. For 1 plate (100 reaction): dilute 8 µL of 235 µg/mL (7.83 µM) stock in 2 mL Kinase Buffer. This makes a 31 nM mixture. Add 20 µL/well. This makes a final reaction concentration of 6 nM.

3) CDC25C Biotinylated peptide.

Dilute CDC25C to 1 mg/mL (385 µM) stock and store at -20 °C. For 1 plate (100 reactions): dilute 10 µL of 1 mg/mL peptide stock in 2 mL Kinase Buffer. This gives a 1.925 µM mix. Add 20 µL/reaction. This makes a final reaction concentration of 385 nM.

4) ATP Mix.

For 1 plate (100 reactions): dilute 10 µL of 1 mM ATP (cold) stock and 2 µL fresh P33-ATP (20 µCi) in 5 mL Kinase Buffer. This gives a 2 µM ATP (cold) solution; add 50 µL/well to start the reaction. Final volume is 100 µL/reaction so the final reaction concentrations will be 1 µM ATP (cold) and 0.2µCi/reaction.

5) Stop Solution:
   For 1 plate add: To 10 mL Wash Buffer 2 (2M NaCl 1% H_3PO_4) : 1mL SPA bead slurry (50 mg); Add 100 µL/well
6) Wash buffer 1: 2 M NaCl
7) Wash buffer 2: 2 M NaCl, 1% H_3PO_4

Assay Procedure:

Assay Component	Final Concentration	Volume
CHK1	6nM	20 µl/rxn
Compound (10% DMSO)	- -	10 µl/rxn
CDC25C	0.385 µM	20 µl/rxn
^{33}P-ATP Cold ATP	0.2 µCi/rxn 1 µM	50µl/rxn
Stop solution SPA beads	0.5 mg/rxn	**100 µl/rxn***
		200 µl/rxn**

* Total reaction volume for assay.** Final reaction volume at termination of reaction (after addition of stop solution).

1) Dilute test compounds to desired concentrations in water/10% DMSO - this will give a final DMSO concentration of 1% in the reaction. Dispense 10 µL/reaction to appropriate wells. Add 10 µL 10% DMSO to positive (CHK1+CDC25C+ATP) and negative (CHK1+ATP only) control wells.

2) Thaw enzyme on ice -- dilute enzyme to proper concentration in kinase buffer (see Reaction Mixtures) and dispense 20 µL to each well.

3) Thaw the Biotinylated substrate on ice and dilute in kinase buffer (see Reaction Mixtures). Add 20 µL/well except to negative control wells. Instead, add 20 µL Kinase Buffer to these wells.

4) Dilute ATP (cold) and P^{33}-ATP in kinase buffer (see Reaction Mixtures). Add 50 µL/well to start the reaction.

5) Allow the reaction to run for 2 hours at room temperature.

6) Stop reaction by adding 100 µL of the SPA beads/stop solution (see Reaction Mixtures) and leave to incubate for 15 minutes before harvest.

7) Place a blank Packard GF/B filter plate into the vacuum filter device (Packard plate harvester) and aspirate 200 mL water through to wet the system.

8) Take out the blank and put in the Packard GF/B filter plate.

9) Aspirate the reaction through the filter plate.

10) Wash: 200 mL each wash; 1X with 2M NaCl; 1X with 2M NaCl/ 1% H_3PO_4

11) Allow filter plate to dry 15 minutes.

12) Put TopSeal-A adhesive on top of filter plate.

13) Run filter plate in Top Count

Settings: Data mode: CPM

Radio nuclide: Manual SPA:P^{33}

Scintillator: Liq/plast
Energy Range: Low

IC_{50} DETERMINATIONS: Dose-response curves were plotted from inhibition data generated, each in duplicate, from 8 point serial dilutions of inhibitory compounds. Concentration of compound was plotted against % kinase activity, calculated by CPM of treated samples divided by CPM of untreated samples. To generate IC_{50} values, the dose-response curves were then fitted to a standard sigmoidal curve and IC_{50} values were derived by nonlinear regression analysis.

CDK2 Kinase Assay

BACULOVIRUS CONSTRUCTIONS: Cyclin E was cloned into pVL1393 (Pharmingen, La Jolla, California) by PCR, with the addition of 5 histidine residues at the amino-terminal end to allow purification on nickel resin. The expressed protein was approximately 45kDa. CDK2 was cloned into pVL1393 by PCR, with the addition of a haemaglutinin epitope tag at the carboxy-terminal end (YDVPDYAS). The expressed protein was approximately 34kDa in size.

ENZYME PRODUCTION: Recombinant baculoviruses expressing cyclin E and CDK2 were co-infected into SF9 cells at an equal multiplicity of infection (MOI=5), for 48 hrs. Cells were harvested by centrifugation at 1000 RPM for 10 minutes, then pellets lysed on ice for 30 minutes in five times the pellet volume of lysis buffer containing 50mM Tris pH 8.0, 150mM NaCl, 1% NP40, 1mM DTT and protease inhibitors (Roche Diagnostics GmbH, Mannheim, Germany). Lysates were spun down at 15000 RPM for 10 minutes and the supernatant retained. 5mL of nickel beads (for one liter of SF9 cells) were washed three times in lysis buffer (Qiagen GmbH, Germany). Imidazole was added to the baculovirus supernatant to a final concentration of 20mM, then incubated with the nickel beads for 45 minutes at 4 °C. Proteins were eluted with lysis buffer containing 250mM imidazole. Eluate was dialyzed about 15 hours in 2 liters of kinase buffer containing 50mM Tris pH 8.0, 1mM DTT, 10mM $MgCl_2$, 100μM sodium orthovanadate and 20% glycerol. Enzyme was stored in aliquots at -70°C.

In Vitro Cyclin E/CDK2 Kinase Assays

Cyclin E/CDK2 kinase assays can be performed as described below in low protein binding 96-well plates (Corning Inc, Corning, New York).

Enzyme is diluted to a final concentration of 50 μg/mL in kinase buffer containing 50mM Tris pH 8.0, 10 mM $MgCl_2$, 1 mM DTT, and 0.1 mM sodium orthovanadate. The substrate used in these reactions is a biotinylated peptide derived from Histone H1 (from Amersham, UK). The substrate is thawed on ice and diluted to 2 μM in kinase buffer. Test compounds are diluted in 10% DMSO to desirable concentrations. For each kinase reaction,

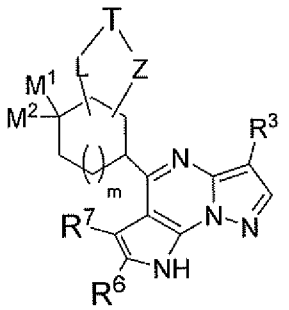
20 μL of the 50 μg/mL enzyme solution (1 μg of enzyme) and 20 μl of the 2 μM substrate solution are mixed, then combined with 10 μL of diluted compound in each well for testing. The kinase reaction is initiated by addition of 50 μL of 2 μM ATP and 0.1 μCi of ^{33}P-ATP (from Amersham, UK). The reaction iss allowed to run for 1 hour at room temperature, then is stopped by adding 200 μL of stop buffer containing 0.1% Triton X-100, 1 mM ATP, 5mM EDTA, and 5 mg/mL streptavidine coated SPA beads (from Amersham, UK) for 15 minutes. The SPA beads are then captured onto a 96-well GF/B filter plate (Packard/Perkin Elmer Life Sciences) using a Filtermate universal harvester (Packard/Perkin Elmer Life Sciences.). Non-specific signals are eliminated by washing the beads twice with 2M NaCl then twice with 2 M NaCl with 1% phosphoric acid. The radioactive signal can then be measured using, for example, a TopCount 96 well liquid scintillation counter (from Packard/Perkin Elmer Life Sciences).

IC_{50} DETERMINATIONS: Dose-response curves are plotted from inhibition data generated, each in duplicate, from 8 point serial dilutions of inhibitory compounds. Concentration of compound is plotted against % kinase activity, calculated by CPM of treated samples divided by CPM of untreated samples. To generate IC_{50} values, the dose-response curves are then fitted to a standard sigmoidal curve and IC_{50} values can be derived using nonlinear regression analysis.

While this invention has been particularly shown and described with references to embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the meaning of the invention described. Rather, the scope of the invention is defined by the claims that follow.

WHAT IS CLAIMED IS:

1. A compound of Formula I



I

M^1 and M^2 are independently selected from the group consisting of CN, $-(CR^aR^b)_nOR^1$, $-(CR^aR^b)_nNR^1R^2$, $-(CR^aR^b)_nR^1$, $-(CR^aR^b)_nSR^1$, $-(CR^aR^b)_nS(O)_2R^1$, $-(CR^aR^b)_nS(O)R^1$, $-(CR^aR^b)_nS(O)_2NR^1R^2$, $-(CR^aR^b)_nNR^1S(O)_2R^4$, $-(CR^aR^b)_nC(O)NR^1S(O)_2R^2$, $-(CR^aR^b)_nC(O)R^1$, $-(CR^aR^b)_nC(O)OR^1$, $-(CR^aR^b)_nC(O)NR^1R^2$, $-(CR^aR^b)_nC(=NR^4)NR^1R^2$, $-(CR^aR^b)_nNR^1C(O)R^4$, $-(CR^aR^b)_nNR^1C(O)OR^4$, $-(CR^aR^b)_nNR^4C(O)NR^1R^2$, $-(CR^aR^b)_nOR^1$ and $-(CR^aR^b)_nO(CR^cR^d)_qOR^4$;

L and Z are not present, or
L and Z are bonded to any two carbons of the ring which are not attached to M^1 and M^2 and are independently selected from the group consisting of CH_2, $C(H)(R^{10})$, $C(R^{10})(R^{11})$, $N(R^{10})$, C(O), O, S, S(O) and $S(O)_2$;

T is not present such that L is bonded directly to Z, or T is selected from the group consisting of CH_2, $C(H)(R^{10})$, $C(R^{10})(R^{11})$, $N(R^{10})$, C(O), O, S, S(O) and $S(O)_2$ and C_1-C_4 alkylene, wherein said alkylene of T is unsubstituted or substituted with 1 to two substituents selected from the group consisting of C_1-C_3 alkyl, halo, hydroxyl, C_1-C_3 alkoxy, amino, C_1-C_3 alkylamino and C_1-C_3 dialkylamino;

R^a, R^b, R^c and R^d are independently selected from H, halogen and C_1-C_6 alkyl;

R^1, R^2 and R^4 are independently selected from H, OH, NH_2, halogen, $-(CR^aR^b)_nO(CR^cR^d)_qR^8$, C_1-C_6alkyl, C_3-C_8cycloalkyl, C_3-C_8cycloalkylC_1-C_6alkyl, C_6-C_{10}arylC_1-C_6alkyl, C_6-C_{10}aryl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-membered heterocyclenylC_1-C_6alkyl, 5- to 10-membered heterocyclyl and 5- to 10-membered heterocyclenyl, wherein the alkyl, cycloalkyl, cycloalkylalkyl, arylalkyl, aryl, heteroarylalkyl, heteroaryl, heterocyclylalkyl, heterocyclenylalkyl, heterocyclyl or heterocyclenyl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group

consisting of halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -OR^9, -$C(O)R^9$, -NR^8R^9, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^8R^9$, -$NR^8C(O)R^9$, -$S(O_2)NR^8R^9$, -$NR^8S(O_2)R^9$, -SR^9, -$S(O_2)R^9$, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl and 5- to 10-membered heterocyclyl;
Or R^1 and R^2 form a 3- to 8- membered cycloalkyl, 5- to 6-membered heterocyclyl or 5- to 6-membered heterocyclenyl;

R^3 is selected from the group consisting of H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, halo-C_1-C_6alkyl, -CF_3, -$C(O)R^9$, C_6-C_{10}aryl, C_3-C_8cycloalkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclyl, 5- to 10-membered heterocyclenyl, C_6-C_{10}arylC_1-C_6alkyl, C_3-C_8cycloalkylC_1-C_6alkyl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heterocyclylC_1-C_6alkyl and 5- to 10-membered heterocyclenylC_1-C_6alkyl, wherein each of said aryl, cycloalkyl, heteroaryl, heterocyclyl, heterocyclenyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, heterocyclylalkyl and heterocyclenylalkyl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -$(CR^aR^b)_nOR^9$, -$(CR^aR^b)_nC(O)R^9$, -$(CR^aR^b)_nNR^8R^9$, -$(CR^aR^b)_nNR^8$, -NR^8R^9, -$(CR^aR^b)_nC(O)O$-C_1-C_6alkyl, -O-haloC_1-C_6alkyl, -$(CR^aR^b)_nC(O)NR^8R^9$, -$(CR^aR^b)_nC(O)NR^8S(O)_2R^9$, -$(CR^aR^b)_nNR^8C(O)R^9$, -$(CR^aR^b)_nNR^8C(O)OR^9$, -$(CR^aR^b)_nNR^8C(O)NR^8R^9$, -$(CR^aR^b)_nS(O_2)NR^8R^9$, -$(CR^aR^b)_nS(O_2)NR^8C(O)R^9$, -$(CR^aR^b)_nNR^8S(O_2)R^9$, -$(CR^aR^b)_nSR^9$, -$(CR^aR^b)_nS(O_2)R^9$, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl, 5- to 10-membered heterocyclyl, C_6-C_{10}arylalkyl, 5- to 10-membered heteroarylalkyl, 5- to 10-membered heterocyclenylalkyl and 5- to 10-membered heterocyclylalkyl wherein each of said alkyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, heterocyclenyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, heterocyclylalkyl and heterocyclenylalkyl is unsubstituted or substituted with one to five moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^9, -$C(O)R^9$, -NR^8R^9, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^8R^9$, -$NR^8C(O)R^9$, -$S(O_2)NR^8R^9$, -$NR^8S(O_2)R^9$, -SR^9, and -$S(O_2)R^9$;

R^6 and R^7 are independently selected from the group consisting of H, halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -OR^9, -$C(O)R^9$, -NR^8R^9, -$C(O)O$-C_1-C_6alkyl, -CR^aR^b, -OR^a, -$S(O)R^a$, -$C(O)OR^a$, -$S(O_2)NR^aR^b$, -$NR^aC(O)R^b$, -$NR^aS(O_2)R^b$,-$C(O)NR^8R^9$, -SR^9, and -$S(O_2)R^9$;

R^8 and R^9 are independently selected from the group consisting of H, OH, C_1-C_6alkyl, C_3-C_8cycloalkyl, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl, 5- to 10-membered heterocyclyl, C_3-C_8cycloalkylC_1-C_6alkyl, C_6-C_{10}arylC_1-C_6alkyl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-membered heterocyclenylC_1-C_6alkyl, and said alkyl, cycloalkyl, aryl, heteroaryl, heterocyclenyl, heterocyclyl, cycloalkylalkyl, arylalkyl, heteroarylalkyl, heterocyclenylalkyl or heterocyclylalkyl is optionally substituted with halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, $-CF_3$, -CN, $-(CR^aR^b)_nC(O)OH$, $-OCF_3$, $-OR^a$, -C(O), amino, $-C(O)O-C_1$-C_6alkyl, $-C(O)NR^aR^b$, $-SR^a$, and $-S(O_2)R^a$; or R^8 and R^9 together with the nitrogen atom to which they are attached form a 3- to 6-membered heterocyclyl ring;

R^{10} and R^{11} are independently selected from the group consisting of H, C_1-C_3alkyl, halo, hydroxyl, C_1-C_3alkoxy, amino, C_1-C_3alkylamino and C_1-C_3dialkylamino;

n is independently 0, 1, 2, 3 or 4;
m is independently 0, 1, 2, 3 or 4;
q is independently 0, 1, 2, 3 or 4;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein

M^1 and M^2 are independently selected from the group consisting of CN, $-(CR^aR^b)_nOR^1$, $-(CR^aR^b)_nNR^1R^2$, $-(CR^aR^b)_nR^1$, $-(CR^aR^b)_nSR^1$, $-(CR^aR^b)_nS(O)_2R^1$, $-(CR^aR^b)_nS(O)R^1$, $-(CR^aR^b)_nS(O)_2NR^1R^2$, $-(CR^aR^b)_nNR^1S(O)_2R^4$, $-(CR^aR^b)_nC(O)NR^1S(O)_2R^2$, $-(CR^aR^b)_nC(O)R^1$, $-(CR^aR^b)_nC(O)OR^1$, $-(CR^aR^b)_nC(O)NR^1R^2$, $-(CR^aR^b)_nC(=NR^4)NR^1R^2$, $-(CR^aR^b)_nNR^1C(O)R^4$, $-(CR^aR^b)_nNR^1C(O)OR^4$, $-(CR^aR^b)_nNR^4C(O)NR^1R^2$, $-(CR^aR^b)_nOR^1$ and $-(CR^aR^b)_nO(CR^cR^d)_qOR^4$;

L, T and Z are not present, or
L and Z are bonded to any two carbons of the ring which are not attached to M^1 and M^2 and are both CH_2, and T is not present;

R^a, R^b, R^c and R^d are independently selected from H and C_1-C_6 alkyl;

R^1, R^2 and R^4 are independently selected from H, OH, NH_2, halogen, $-(CR^aR^b)_nO(CR^cR^d)_qR^8$, C_1-C_6alkyl, C_3-C_8cycloalkyl, C_3-C_8cycloalkylC_1-C_6alkyl, C_6-C_{10}arylC_1-C_6alkyl, C_6-C_{10}aryl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-membered heterocyclyl, 5- to 10-membered heterocyclenylC_1-

C_6alkyl and 5- to 10-membered heterocyclenyl, wherein the alkyl, cycloalkyl, cycloalkylalkyl, arylalkyl, aryl, heteroarylalkyl, heteroaryl, heterocyclylalkyl, heterocyclyl, heterocyclenylalkyl or heterocyclenyl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -OR^a, -$C(O)R^a$, -NR^aR^b, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^aR^b$, -$NR^aC(O)R^b$, -$S(O_2)NR^aR^b$, -$NR^aS(O_2)R^b$, -SR^a, -$S(O_2)R^a$, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl and 5- to 10-membered heterocyclyl;

R^3 is selected from the group consisting of C_6-C_{10}aryl, 5- to 10-membered heteroaryl, wherein each of said aryl or heteroaryl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^8, -$C(O)R^8$, -NR^8R^9, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^8R^9$, -$NR^8C(O)R^9$, -$S(O_2)NR^8R^9$, -$NR^8S(O_2)R^9$, -SR^8, and -$S(O_2)R^8$, wherein each of said heteroaryl or aryl is unsubstituted or substituted with one to three moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^a, -$C(O)R^a$, -NR^aR^b, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^aR^b$, -$NR^aC(O)R^b$, -$S(O_2)NR^aR^b$, -$NR^aS(O_2)R^b$, -SR^a, and -$S(O_2)R^a$;

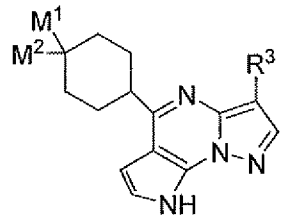
R^6 and R^7 are independently selected from the group consisting of H, -OR^a, -NR^aR^b, -SR^a, -$S(O)R^a$, -$S(O_2)R^a$, -$C(O)C_1$-C_6alkyl, -$C(O)NR^aR^b$, -$C(O)OR^a$, -$S(O_2)NR^aR^b$, -$NR^aC(O)R^b$, -$NR^aS(O_2)R^b$, C_1-C_6alkyl, C_1-C_6alkoxy, halogen, hydroxyl, amino and -CN;

R^8 and R^9 are independently selected from the group consisting of H, OH, C_1-C_6alkyl, C_3-C_8cycloalkyl, C_6-C_{10}aryl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclenyl, 5- to 10-membered heterocyclyl, C_3-C_8cycloalkylC_1-C_6alkyl, C_6-C_{10}arylC_1-C_6alkyl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-membered heterocyclenylC_1-C_6alkyl, and said alkyl, cycloalkyl, aryl, heteroaryl, heterocyclenyl, heterocyclyl, cycloalkylalkyl, arylalkyl, heteroarylalkyl, heterocyclenylalkyl or heterocyclylalkyl is optionally substituted with halogen, C_1-C_6alkyl, C_3-C_8cycloalkyl, -CF_3, -CN, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -OR^a, -C(O), amino, -$C(O)O$-C_1-C_6alkyl, -$C(O)NR^aR^b$,-SR^a, and -$S(O_2)R^a$;

n is independently 0, 1 or 2;
m is 1;
q is independently 0, 1, or 2;

or a pharmaceutically acceptable salt thereof.

3. The compound of claim 1 or 2, under Formula III:



III

M^1 and M^2 are independently selected from the group consisting of CN, $-(CR^aR^b)_nOR^1$, $-(CR^aR^b)_nNR^1R^2$, $-(CR^aR^b)_nR^1$, $-(CR^aR^b)_nSR^1$, $-(CR^aR^b)_nS(O)_2R^1$, $-(CR^aR^b)_nS(O)R^1$, $-(CR^aR^b)_nS(O)_2NR^1R^2$, $-(CR^aR^b)_nNR^1S(O)_2R^4$, $-(CR^aR^b)_nC(O)NR^1S(O)_2R^2$, $-(CR^aR^b)_nC(O)R^1$, $-(CR^aR^b)_nC(O)OR^1$, $-(CR^aR^b)_nC(O)NR^1R^2$, $-(CR^aR^b)_nC(=NR^4)NR^1R^2$, $-(CR^aR^b)_nNR^1C(O)R^4$, $-(CR^aR^b)_nNR^1C(O)OR^4$, $-(CR^aR^b)_nNR^4C(O)NR^1R^2$, $-(CR^aR^b)_nOR^1$ and $-(CR^aR^b)_nO(CR^cR^d)_qOR^4$;

R^a, R^b, R^c and R^d are independently selected from H and C_1-C_3 alkyl;

R^1, R^2 and R^4 are independently selected from H, OH, NH_2, $-(CR^aR^b)_nO(CR^cR^d)_qR^8$, C_1-C_3alkyl, 5- to 10-membered heteroarylC_1-C_6alkyl, 5- to 10-membered heteroaryl, 5- to 10-membered heterocyclylC_1-C_6alkyl, 5- to 10-membered heterocyclyl, 5- to 10-membered heterocyclenylC_1-C_6alkyl and 5- to 10-membered heterocyclenyl, wherein the alkyl, heteroarylalkyl, heteroaryl, heterocyclylalkyl, heterocyclyl, heterocyclenylalkyl or heterocyclenyl is unsubstituted or substituted with one to three moieties which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, $-CF_3$, -CN, -C(O)OH, $-(CR^aR^b)_nC(O)OH$, $-OCF_3$, $-OR^a$, $-C(O)R^a$, $-NR^aR^b$, $-C(O)O$-C_1-C_6alkyl, $-C(O)NR^aR^b$, $-NR^aC(O)R^b$, $-S(O_2)NR^aR^b$, $-NR^aS(O_2)R^b$, $-SR^a$ and $-S(O_2)R^a$;

wherein all other substituents are as defined in claim 1 or 2.

4. The compound of any one of claims 1 to 3, wherein
M^1 is $-(CR^aR^b)_nO(CR^cR^d)_qOC_1$-$C_3$alkyl, $-(CR^aR^b)_nO(CR^cR^d)_qOH$, COOH, or $-(CR^aR^b)_nC(O)OC_1$-C_3alkyl;
M^2 is selected from the group consisting of CN, $-(CR^aR^b)_nOR^1$, $-(CR^aR^b)_nNR^1R^2$, $-(CR^aR^b)_nR^1$, $-(CR^aR^b)_nSR^1$, $-(CR^aR^b)_nS(O)_2R^1$, $-(CR^aR^b)_nS(O)R^1$, $-(CR^aR^b)_nS(O)_2NR^1R^2$, $-(CR^aR^b)_nNR^1S(O)_2R^4$, $-(CR^aR^b)_nC(O)NR^1S(O)_2R^2$, $-(CR^aR^b)_nC(O)R^1$, $-(CR^aR^b)_nC(O)OR^1$, $-(CR^aR^b)_nC(O)NR^1R^2$, $-(CR^aR^b)_nC(=NR^4)NR^1R^2$, $-(CR^aR^b)_nNR^1C(O)R^4$,

$-(CR^aR^b)_nNR^1C(O)OR^4$, $-(CR^aR^b)_nNR^4C(O)NR^1R^2$, $-(CR^aR^b)_nOR^1$ and $-(CR^aR^b)_nO(CR^cR^d)_qOR^4$;
wherein all other substituents are as defined in any one of claims 1 to 3.

5. The compound of any one of claims 1 to 3, wherein
M^1 and M^2 are independently selected from the group consisting of halo, CN, NH_2, $-OCH_3$, $-CH_2OCH_3$, $-SCH_3$, -OH, $-CH_2OH$, $-CH_2CH_2OH$, -C(O)OH, $-C(O)CH_2OH$, $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, $-C(O)N(CH_3)_2$, $-CONH_2$, $C(=NH)NH_2$, $C(O)NH-NH_2$, $-CONHCH_3$, $-C(O)NHOCH_3$, $-C(O)N(CH_3)OCH_3$, -C(O)NHOH, $-C(O)NHCH_2CH_2OH$, $-CH_3$, $-SO_2CH_3$, $-CH_2SO_2CH_3$, $CH_2NHSO_2CH_3$, $-C(O)NHS(O)_2CH_3$, $-OCH_2CH_2$-morpholinyl, triazolyl, tetrazolyl, oxadiazolyl, wherein said triazolyl, tetrazolyl or oxadiazolyl are optionally substituted with methyl or halo, wherein all other substituents are as defined in any one of claims 1 to 3.

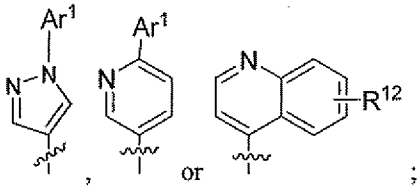
6. The compound of any one of claims 1 to 3, wherein
M^1 is $-OCH_2CH_2OCH_3$, $-OCH_2CH_2OH$, –OH, $-CH_2OH$ or $-CH_2CH_2OH$, and M^2 is selected from the group consisting of halo, CN, $-OCH_3$, $-CH_2OCH_3$, $-SCH_3$, $-OCH_2CH_2OCH_3$, -OH, $-CH_2OH$, $-CH_2CH_2OH$, -C(O)OH, $-C(O)CH_2OH$, $-C(O)N(CH_3)_2$, $-CONH_2$, $C(=NH)NH_2$, $C(O)NH-NH_2$, $-CONHCH_3$, $-C(O)NHOCH_3$, $-C(O)N(CH_3)OCH_3$, -C(O)NHOH, $-C(O)NHCH_2CH_2OH$, $-CH_3$, $-CH_2SO_2CH_3$, $CH_2NHSO_2CH_3$, $-C(O)NHS(O)_2CH_3$, triazolyl, tetrazolyl, oxadiazolyl, wherein said triazolyl, tetrazolyl or oxadiazolyl are optionally substituted with methyl or halo, and all other substituents are as defined in any one of claims 1 to 3.

7. The compound of any one of claims 1 to 6, wherein
R^3 is a 5- to 6-membered heteroaryl or phenyl unsubstituted or substituted with one to three moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, phenyl, 5- to 6-membered heteroaryl, $-CF_3$, -CN, -C(O)OH, $-(CR^aR^b)_nC(O)OH$, $-OCF_3$, -O-haloC_1-C_6alkyl, $-OR^a$, $-C(O)R^a$, $-NR^aR^b$, $-C(O)O-C_1$-C_6alkyl, $-C(O)NR^aR^b$, $-NR^aC(O)R^b$, $-S(O_2)NR^aR^b$, $-NR^aS(O_2)R^b$, $-SR^a$, and $-S(O_2)R^a$, wherein the alkyl, phenyl or heteroaryl is optionally substituted with one to three moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, $-CF_3$, -CN, -C(O)OH, $-(CR^aR^b)_nC(O)OH$, $-OCF_3$, -O-haloC_1-C_6alkyl, $-OR^a$, $-C(O)R^a$, $-NR^aR^b$, $-C(O)O-C_1$-C_6alkyl, $-C(O)NR^aR^b$, $-NR^aC(O)R^b$, $-S(O_2)NR^aR^b$, $-NR^aS(O_2)R^b$, $-SR^a$, and $-S(O_2)R^a$;
Wherein all other substituents are as defined in any one of claims 1 to 6.

8. The compound of claim 7, wherein R^3 is pyrazolyl, isoquinolinyl,

pyrimidinyl, phenyl or pyridyl, unsubstituted or substituted with one to three moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, phenyl, 5- to 6-membered heteroaryl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^a, -$C(O)R^a$, -NR^aR^b, -C(O)O-C_1-C_6alkyl, -$C(O)NR^aR^b$, -$NR^aC(O)R^b$, -$S(O_2)NR^aR^b$, -$NR^aS(O_2)R^b$, -SR^a, and -$S(O_2)R^a$, wherein the alkyl, phenyl or heteroaryl is optionally substituted with one to three moieties, which can be the same or different, each moiety being selected from the group consisting of halogen, C_1-C_6alkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^a, -$C(O)R^a$, -NR^aR^b, -C(O)O-C_1-C_6alkyl, -$C(O)NR^aR^b$, -$NR^aC(O)R^b$, -$S(O_2)NR^aR^b$, -$NR^aS(O_2)R^b$, -SR^a, and -$S(O_2)R^a$.

9. The compound of claim 7, wherein R^3 is



; Ar^1 is phenyl or a 5- to 6-membered heteroaryl optionally substituted with one to three of R^{12}, which can be the same or different, each R^{12} being selected from the group consisting of halogen, C_1-C_6alkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^a, -$C(O)R^a$, -NR^aR^b, -C(O)O-C_1-C_6alkyl, -$C(O)NR^aR^b$, -$NR^aC(O)R^b$, -$S(O_2)NR^aR^b$, -$NR^aS(O_2)R^b$, -SR^a, and -$S(O_2)R^a$.

10. The compound of claim 9 wherein Ar^1 is phenyl, pyridyl, pyrimidinyl, imidazolyl, pyrazinyl, pyrazolyl, or thiazolyl, optionally substituted with one to three of R^{12}, which can be the same or different, each R^{12} being selected from the group consisting of halogen, C_1-C_6alkyl, -CF_3, -CN, -C(O)OH, -$(CR^aR^b)_nC(O)OH$, -OCF_3, -O-haloC_1-C_6alkyl, -OR^a, -$C(O)R^a$, -NR^aR^b, -C(O)O-C_1-C_6alkyl, -$C(O)NR^aR^b$, -$NR^aC(O)R^b$, -$S(O_2)NR^aR^b$, -$NR^aS(O_2)R^b$, -SR^a, and -$S(O_2)R^a$.

11. The compound of claim 9 or 10, wherein R^{12} is selected from the group consisting of halogen, C_1-C_6alkyl, -CF_3, and -OCF_3.

12. A compound selected from the group consisting of:
(1*R*,4*R*)-4-(3-(6-fluoroquinolin-3-yl)-8*H*-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methylcyclohexanecarboxylic acid;
(1*S*,4*S*)-4-(3-(6-fluoroquinolin-3-yl)-8*H*-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methylcyclohexanecarboxylic acid;

(1R,4R)-1-methyl-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-methyl-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-1-(methoxymethyl)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-(methoxymethyl)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-1-(methoxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-(methoxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-(methoxymethyl)cyclohexanecarboxylic acid;
(1S,4S)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-(methoxymethyl)cyclohexanecarboxylic acid;
(1R,4R)-1-(methylthio)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-(methylthio)-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
1-((2-methoxyethoxy)methyl)-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1S,4S)-1-methoxy-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-1-methoxy-4-(3-(1-phenyl-1H-pyrazol-4-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexanecarboxylic acid;
(1R,4R)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylic acid;
(1S,4S)-4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-methoxycyclohexanecarboxylic acid;
((1R,4R)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexyl)methanol;
((1S,4S)-1-methoxy-4-(3-(6-phenylpyridin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)cyclohexyl)methanol;

1-(hydroxymethyl)-4-(3-(6-phenylpyridin-3-yl)-8*H*-pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidin-5-yl)cyclohexanol; and
4-(3-(6-fluoroquinolin-3-yl)-8H-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidin-5-yl)-1-(hydroxymethyl)cyclohexanol;
Or a stereoisomer thereof;
Or a pharmaceutically acceptable salt thereof;
Or a pharmaceutically acceptable salt of the stereoisomer thereof.

13. A pharmaceutical composition comprising a therapeutically effective amount of the compound of any one of claims 1-12 and a pharmaceutically acceptable carrier and optionally other therapeutic ingredients.

14. A compound according to any one of claims 1-12 for the treatment of cancer.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/48548

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/42; A61K 31/44 (2011.01)
USPC - 514/293-294
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/293-294

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/259.1, 288, 290 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST: US Patents full-text; US PGPubs full-text; EPO Abstracts; and JPO Abstracts; Google
Dialog: Chemical Engineering and General Research
Terms: Pyrazolopyrrolopyrimidine, pyrazolo, pyrrolo, pyrimidin, cycloalkyl, cylcohexane,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,055,479 A (TAKIGUCHI et al.) 08 October 1991 (08.10.1991) col 1-24	1-3 and 12
Y	US 2005/0004159 A1 (HIBI et al.) 06 January 2005 (06.01.2005) para [0039]-[0287].	1-3 and 12
A	US 6,057,330 A (NAMIKI et al.) 02 May 2000 (02.05.2000) cols 1-7	1-3 and 12

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 Dec. 2011 (29.12.2011)	09 JAN 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/48548

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

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

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

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

3. ☒ Claims Nos.: 4-11 and 13-14
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.