TITLE OF THE INVENTION

INHIBITORS OF AKT ACTIVITY

BACKGROUND OF THE INVENTION

The present invention relates to substituted naphthyridine compounds which are inhibitors of the activity of one or more of the isoforms of the serine/threonine kinase, Akt (also known as PKB; hereinafter referred to as "Akt"). The present invention also relates to pharmaceutical compositions comprising such compounds and methods of using the instant compounds in the treatment of cancer.

Apoptosis (programmed cell death) plays essential roles in embryonic development and pathogenesis of various diseases, such as degenerative neuronal diseases, cardiovascular diseases and cancer. Recent work has led to the identification of various pro- and anti-apoptotic gene products that are involved in the regulation or execution of programmed cell death. Expression of anti-apoptotic genes, such as Bcl2 or Bcl-x_L, inhibits apoptotic cell death induced by various stimuli. On the other hand, expression of pro-apoptotic genes, such as Bax or Bad, leads to programmed cell death (Adams et al. *Science*, 281:1322-1326 (1998)). The execution of programmed cell death is mediated by caspase-1 related proteinases, including caspase-3, caspase-7, caspase-8 and caspase-9 etc (Thornberry et al. *Science*, 281:1312-1316 (1998)).

The phosphatidylinositol 3'-OH kinase (PI3K)/Akt pathway appears important for regulating cell survival/cell death (Kulik et al. *Mol. Cell. Biol.* 17:1595-1606 (1997); Franke et al, *Cell*, 88:435-437 (1997); Kauffmann-Zeh et al. *Nature* 385:544-548 (1997) Hemmings *Science*, 275:628-630 (1997); Dudek et al., *Science*, 275:661-665 (1997)). Survival factors, such as platelet derived growth factor (PDGF), nerve growth factor (NGF) and insulin-like growth factor-1 (IGF-1), promote cell survival under various conditions by inducing the activity of PI3K (Kulik et al. 1997, Hemmings 1997). Activated PI3K leads to the production of phosphatidylinositol (3,4,5)-triphosphate (PtdIns(3,4,5)-P3), which in turn binds to, and promotes the activation of, the serine/threonine kinase Akt, which contains a pleckstrin homology (PH)-domain (Franke et al *Cell*, 81:727-736 (1995); Hemmings *Science*, 277:534 (1997); Downward, *Curr. Opin. Cell Biol.* 10:262-267 (1998), Alessi et al., *EMBO J.* 15: 6541-6551 (1996)). Specific inhibitors of PI3K or dominant negative Akt mutants abolish survival-promoting activities of these growth factors or cytokines. It has been previously disclosed that inhibitors of PI3K (LY294002 or wortmannin) blocked the activation of Akt by upstream kinases. In addition, introduction of constitutively active PI3K or Akt mutants promotes cell survival under conditions in which cells normally undergo apoptotic cell death (Kulik et al. 1997, Dudek et al. 1997).

Three members of the Akt subfamily of second-messenger regulated serine/threonine protein kinases have been identified and termed Akt1/ PKBα, Akt2/PKBβ, and

Akt3/PKBγ (hereinafter referred to as "Akt1", "Akt2" and "Akt3"), respectively. The isoforms are homologous, particularly in regions encoding the catalytic domains. Akts are activated by phosphorylation events occurring in response to PI3K signaling. PI3K phosphorylates membrane inositol phospholipids, generating the second messengers phosphatidyl-inositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate, which have been shown to bind to the PH domain of Akt. The current model of Akt activation proposes recruitment of the enzyme to the membrane by 3'-phosphorylated phosphoinositides, where phosphorylation of the regulatory sites of Akt by the upstream kinases occurs (B.A. Hemmings, *Science* 275:628-630 (1997); B.A. Hemmings, *Science* 276:534 (1997); J. Downward, *Science* 279:673-674 (1998)).

Phosphorylation of Akt1 occurs on two regulatory sites, Thr^{308} in the catalytic domain activation loop and on Ser^{473} near the carboxy terminus (D. R. Alessi *et al. EMBO J.* 15:6541-6551 (1996) and R. Meier *et al. J. Biol. Chem.* 272:30491-30497 (1997)). Equivalent regulatory phosphorylation sites occur in Akt2 and Akt3. The upstream kinase, which phosphorylates Akt at the activation loop site has been cloned and termed 3'-phosphoinositide - dependent protein kinase 1 (PDK1). PDK1 phosphorylates not only Akt, but also p70 ribosomal S6 kinase, p90RSK, serum and glucocorticoid-regulated kinase (SGK), and protein kinase C. The upstream kinase phosphorylating the regulatory site of Akt near the carboxy terminus has not been identified yet, but recent reports imply a role for the integrin-linked kinase (ILK-1), a serine/threonine protein kinase, or autophosphorylation.

Analysis of Akt levels in human tumors showed that Akt2 is overexpressed in a significant number of ovarian (J. Q. Cheng *et al. Proc. Natl. Acad. Sci. U.S.A.* 89:9267-9271(1992)) and pancreatic cancers (J. Q. Cheng *et al. Proc. Natl. Acad. Sci. U.S.A.* 93:3636-3641 (1996)). Similarly, Akt3 was found to be overexpressed in breast and prostate cancer cell lines (Nakatani et al. *J. Biol. Chem.* 274:21528-21532 (1999).

The tumor suppressor PTEN, a protein and lipid phosphatase that specifically removes the 3' phosphate of PtdIns(3,4,5)-P3, is a negative regulator of the PI3K/Akt pathway (Li et al. *Science* 275:1943-1947 (1997), Stambolic et al. *Cell* 95:29-39 (1998), Sun et al. *Proc. Natl. Acad. Sci. U.S.A.* 96:6199-6204 (1999)). Germline mutations of PTEN are responsible for human cancer syndromes such as Cowden disease (Liaw et al. *Nature Genetics* 16:64-67 (1997)). PTEN is deleted in a large percentage of human tumors and tumor cell lines without functional PTEN show elevated levels of activated Akt (Li et al. supra, Guldberg et al. *Cancer Research* 57:3660-3663 (1997), Risinger et al. *Cancer Research* 57:4736-4738 (1997)).

These observations demonstrate that the PI3K/Akt pathway plays important roles for regulating cell survival or apoptosis in tumorigenesis.

Inhibition of Akt activation and activity can be achieved by inhibiting PI3K with inhibitors such as LY294002 and wortmannin. However, PI3K inhibition has the potential to indiscriminately affect not just all three Akt isozymes but also other PH domain-containing signaling molecules that are dependent on PdtIns(3,4,5)-P3, such as the Tec family of tyrosine

kinases. Furthermore, it has been disclosed that Akt can be activated by growth signals that are independent of PI3K.

Alternatively, Akt activity can be inhibited by blocking the activity of the upstream kinase PDK1. No specific PDK1 inhibitors have been disclosed. Again, inhibition of PDK1 would result in inhibition of multiple protein kinases whose activities depend on PDK1, such as atypical PKC isoforms, SGK, and S6 kinases (Williams et al. *Curr. Biol.* 10:439-448 (2000).

The compounds of the instant invention have unexpected advantageous properties over the cyclopropyl substituted naphthyridine compounds specifically described in WO 2006/135627.

It is an object of the instant invention to provide novel compounds that are inhibitors of Akt.

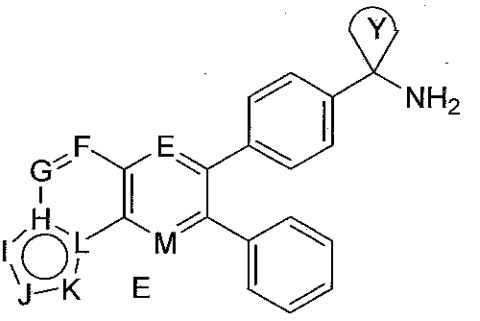
It is also an object of the present invention to provide pharmaceutical compositions that comprise the novel compounds that are inhibitors of Akt.

It is also an object of the present invention to provide a method for treating cancer that comprises administering such inhibitors of Akt activity.

SUMMARY

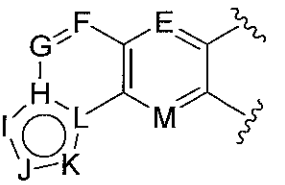
The disclosure provides for substituted naphthyridine compounds that inhibit Akt activity. In particular, the compounds disclosed selectively inhibit one or two of the Akt isoforms. The disclosure also provides for compositions comprising such inhibitory compounds and methods of inhibiting Akt activity by administering the compound to a patient in need of treatment of cancer.

In a first aspect, there is provided a compound according to Formula E:

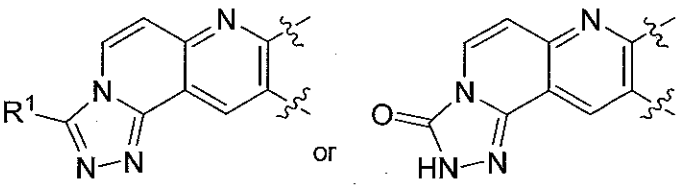


E

wherein:



is



Ring Y is cyclobutyl;

R^1 is H, pyrimidyl, methylimidazole, OH, methyl or cyclopropyl;

or a pharmaceutically acceptable salt thereof.

In a second aspect, there is provided a compound which is:

1-{4-[3-(1-methyl-1*H*-imidazol-4-yl)-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl]phenyl}cyclobutanamine; or a pharmaceutically acceptable salt thereof.

In a third aspect, there is provided a compound which is:

tert-butyl {1-[4-(9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl} carbamate

or a pharmaceutically acceptable salt thereof.

In a fourth aspect, there is provided a compound which is:

1-[4-(9-phenyl-3-pyrimidin-2-yl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine;

or a pharmaceutically acceptable salt thereof.

In a fifth aspect, there is provided a compound which is:

8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-3(2*H*)-one;

or a pharmaceutically acceptable salt thereof.

In a sixth aspect, there is provided a compound which is:

1-[4-(3-methyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine;

or a pharmaceutically acceptable salt thereof.

In a seventh aspect, there is provided a compound which is:

1-[4-(3-cyclopropyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine;

or a pharmaceutically acceptable salt thereof.

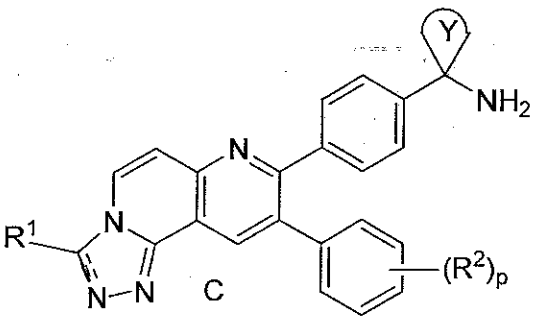
In an eighth aspect, there is provided a pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of the first aspect.

In a ninth aspect, there is provided the use of the compound according to the first aspect for the preparation of a medicament useful in the treatment or prevention of cancer in a mammal in need of such treatment.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the instant invention are useful in the inhibition of the activity of the serine/threonine kinase Akt.

In an embodiment the inhibitors of the instant invention are illustrated by the Formula C:



wherein:

a is 0 or 1; b is 0 or 1; m is 0, 1 or 2; p is 0, 1 or 2;

the dashed line represents an optional double bond;

R^2 is independently selected from: $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, CO_2H, halo, OH and NH_2;

Ring Y is $(C_4\text{-}C_7)$cycloalkyl;

R^1 is selected from: H, oxo, $(C{=}O)_aO_b(C_1\text{-}C_{10})$alkyl, $(C{=}O)_aO_b$-aryl, $(C{=}O)_aO_b(C_2\text{-}C_{10})$alkenyl, $(C{=}O)_aO_b(C_2\text{-}C_{10})$alkynyl, CO_2H, halo, OH, $O_b(C_1\text{-}C_6)$perfluoroalkyl, $(C{=}O)_aNR^7R^8$, CN, $(C{=}O)_aO_b(C_3\text{-}C_8)$cycloalkyl, $S(O)_mNR^7R^8$, SH, $S(O)_m\text{-}(C_1\text{-}C_{10})$alkyl and $(C{=}O)_aO_b$-heterocyclyl, said alkyl, aryl, alkenyl, alkynyl, cycloalkyl, and heterocyclyl is optionally substituted with one or more substituents selected from R^6;

R^6 is: $(C{=}O)_aO_bC_1\text{-}C_{10}$ alkyl, $(C{=}O)_aO_b$aryl, $C_2\text{-}C_{10}$ alkenyl, $C_2\text{-}C_{10}$ alkynyl, $(C{=}O)_aO_b$ heterocyclyl, CO_2H, halo, CN, OH, $O_bC_1\text{-}C_6$ perfluoroalkyl, $O_a(C{=}O)_bNR^7R^8$, oxo, CHO, $(N{=}O)R^7R^8$, $S(O)_mNR^7R^8$, SH, $S(O)_m\text{-}(C_1\text{-}C_{10})$alkyl or $(C{=}O)_aO_bC_3\text{-}C_8$ cycloalkyl, said alkyl, aryl, alkenyl, alkynyl, heterocyclyl, and cycloalkyl optionally substituted with one or more substituents selected from R^{6a};

R^{6a} is selected from: $(C{=}O)_aO_b(C_1\text{-}C_{10})$alkyl, $O_a(C_1\text{-}C_3)$perfluoroalkyl, $(C_0\text{-}C_6)$alkylene-$S(O)_mR^a$, SH, oxo, OH, halo, CN, $(C_2\text{-}C_{10})$alkenyl, $(C_2\text{-}C_{10})$alkynyl, $(C_3\text{-}C_6)$cycloalkyl, $(C_0\text{-}C_6)$alkylene-aryl, $(C_0\text{-}C_6)$alkylene-heterocyclyl, $(C_0\text{-}C_6)$alkylene-$N(R^b)_2$, $C(O)R^a$, $(C_0\text{-}C_6)$alkylene-CO_2R^a, C(O)H, and $(C_0\text{-}C_6)$alkylene-CO_2H, said alkyl, alkenyl, alkynyl, cycloalkyl, aryl, and heterocyclyl is optionally substituted with up to three substituents selected from R^b, OH, $(C_1\text{-}C_6)$alkoxy, halogen, CO_2H, CN, $O_a(C{=}O)_b(C_1\text{-}C_6)$alkyl, oxo, and $N(R^b)_2$;

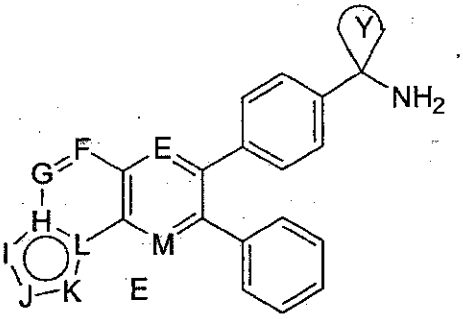
R^7 and R^8 are independently selected from: H, $(C{=}O)_aO_b(C_1\text{-}C_{10})$alkyl, $(C{=}O)_aO_b(C_3\text{-}C_8)$cycloalkyl, $(C{=}O)_aO_b$-aryl, $(C{=}O)_aO_b$-heterocyclyl, $(C_2\text{-}C_{10})$alkenyl, $(C_2\text{-}C_{10})$alkynyl, SH, SO_2R^a, and $(C{=}O)_aNR^b{}_2$, said alkyl, cycloalkyl, aryl, heterocylyl, alkenyl, and alkynyl is optionally substituted with one or more substituents selected from R^{6a}, or R^7 and R^8 can be taken together with the nitrogen to which they are attached to form a monocyclic or bicyclic heterocycle with 3-7 members in each ring and optionally containing, in addition to the nitrogen, one or two additional heteroatoms selected from N, O and S, said monocylcic or bicyclic heterocycle optionally substituted with one or more substituents selected from R^{6a};

R^a is $(C_1\text{-}C_6)$alkyl, $(C_3\text{-}C_6)$cycloalkyl, aryl, or heterocyclyl; and

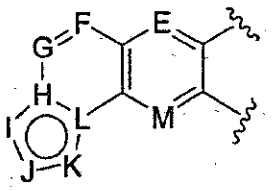
R^b is independently: H, $(C_1\text{-}C_6)$alkyl, aryl, heterocyclyl, $(C_3\text{-}C_6)$cycloalkyl, $(C{=}O)_aO_b(C_1\text{-}C_6)$alkyl, or $S(O)_mR^a$;

or a pharmaceutically acceptable salt, a tautomer or a stereoisomer thereof.

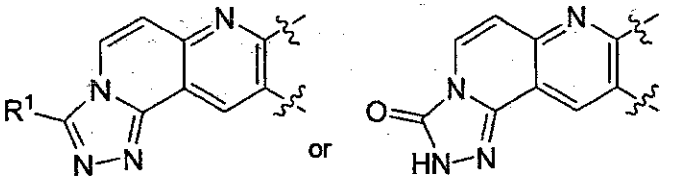
In another embodiment of this invention, the inhibitors of Akt activity are illustrated by the Formula E:



wherein:



is



Ring Y is cyclobutyl;

R^1 is H, pyrimidyl, methylimidazole, OH, methyl or cyclopropyl;

or a pharmaceutically acceptable salt thereof.

A compound of the instant invention is:

1-{4-[3-(1-methyl-1*H*-imidazol-4-yl)-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl]phenyl}cyclobutanamine dihydrochloride (**1-9**);

or a pharmaceutically acceptable salt thereof.

A compound of the instant invention is:

tert-butyl {1-[4-(9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl} carbamate (**1-15**);

or a pharmaceutically acceptable salt thereof.

A compound of the instant invention is:

1-[4-(9-phenyl-3-pyrimidin-2-yl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine (**1-10**);

or a pharmaceutically acceptable salt thereof.

A compound of the instant invention is:

8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-3(2*H*)-one **(1-16)**;

or a pharmaceutically acceptable salt thereof.

A compound of the instant invention is:

1-[4-(3-methyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine **(1-17)**;

or a pharmaceutically acceptable salt thereof.

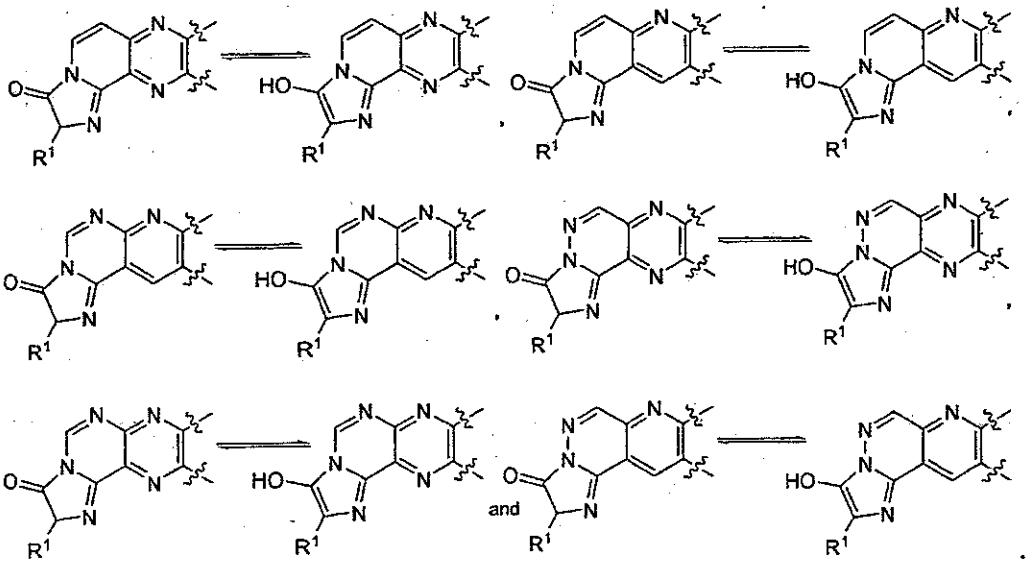
A compound of the instant invention is:

1-[4-(3-cyclopropyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine **(1-23)**;

or a pharmaceutically acceptable salt thereof.

The compounds of the present invention may have asymmetric centers, chiral axes, and chiral planes (as described in: E.L. Eliel and S.H. Wilen, *Stereochemistry of Carbon Compounds*, John Wiley & Sons, New York, 1994, pages 1119-1190), and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers and mixtures thereof, including optical isomers, all such stereoisomers being included in the present invention.

In addition, the compounds disclosed herein may exist as tautomers and both tautomeric forms are intended to be encompassed by the scope of the invention, even though only one tautomeric structure is depicted. For example:



Tetrazoles exist as a mixture of 1H/2H tautomers. The tautomeric forms of the tetrazol moiety are also within the scope of the instant invention.

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

When any variable (e.g. R^2, etc.) occurs more than one time in any constituent, its definition on each occurrence is independent at every other occurrence. Also, combinations of substituents and variables are permissible only if such combinations result in stable compounds. Lines drawn into the ring systems from substituents represent that the indicated bond may be attached to any of the substitutable ring atoms. If the ring system is bicyclic, it is intended that the bond be attached to any of the suitable atoms on either ring of the bicyclic moiety.

It is understood that one or more silicon (Si) atoms can be incorporated into the compounds of the instant invention in place of one or more carbon atoms by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art from readily available starting materials. Carbon and silicon differ in their covalent radius leading to differences in bond distance and the steric arrangement when comparing analogous C-element and Si-element bonds. These differences lead to subtle changes in the size and shape of silicon-containing compounds when compared to carbon. One of ordinary skill in the art would understand that size and shape differences can lead to subtle or dramatic changes in potency, solubility, lack of off target activity, packaging properties, and so on. (Diass, J. O. *et al.* Organometallics (2006) 5:1188-1198; Showell, G.A. et al. Bioorganic & Medicinal Chemistry Letters (2006) 16:2555-2558).

It is understood that substituents and substitution patterns on the compounds of the instant invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials. If a substituent is itself substituted with more than one group, it is understood that these multiple groups may be on the same carbon or on different carbons, so long as a stable structure results. The phrase "optionally substituted with one or more substituents" should be taken to be equivalent to the phrase "optionally substituted with at least one substituent" and in such cases the preferred embodiment will have from zero to four substituents, and the more preferred embodiment will have from zero to three substituents.

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 arrange-ment. 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.

The term "cycloalkyl" means a monocyclic saturated aliphatic hydrocarbon group having the specified number of carbon atoms. For example, "cycloalkyl" includes cyclopropyl, methyl-cyclopropyl, 2,2-dimethyl-cyclobutyl, 2-ethyl-cyclopentyl, cyclohexyl, and so on.

"Alkoxy" represents either a cyclic or non-cyclic alkyl group of indicated number of carbon atoms attached through an oxygen bridge. "Alkoxy" therefore encompasses the definitions of alkyl and cycloalkyl above.

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_{10})alkenyl" means an alkenyl radical having from 2 to 10 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_{10})alkynyl" means an alkynyl radical having from 2 to 10 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.

As used herein, "aryl" is intended to mean any stable monocyclic or bicyclic 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, tetrahydro-naphthyl, 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.

The term heteroaryl, as used herein, represents a stable monocyclic or bicyclic ring of up to 7 atoms in each ring, wherein at least one ring is aromatic and contains from 1 to 4 heteroatoms selected from the group consisting of O, N and S. 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. 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. Such heteraoaryl moieties for substituent Q include but are not limited to: 2-benzimidazolyl, 2-quinolinyl, 3-quinolinyl, 4-quinolinyl, 1-isoquinolinyl, 3-isoquinolinyl and 4-isoquinolinyl.

The term "heterocycle" or "heterocyclyl" as used herein is intended to mean a 3- to 10-membered aromatic or nonaromatic heterocycle containing from 1 to 4 heteroatoms selected from the group consisting of O, N and S, and includes bicyclic groups. "Heterocyclyl" therefore includes the above mentioned heteroaryls, as well as dihydro and tetrathydro analogs thereof. Further examples of "heterocyclyl" include, but are not limited to the following: benzoimidazolyl, benzoimidazolonyl, benzofuranyl, benzofurazanyl, benzopyrazolyl, benzotriazolyl, benzothiophenyl, benzoxazolyl, carbazolyl, carbolinyl, cinnolinyl, furanyl, imidazolyl, indolinyl, indolyl, indolazinyl, indazolyl, isobenzofuranyl, isoindolyl, isoquinolyl, isothiazolyl, isoxazolyl, naphthpyridinyl, oxadiazolyl, oxazolyl, oxazoline, isoxazoline, oxetanyl, pyranyl, pyrazinyl, pyrazolyl, pyridazinyl, pyridopyridinyl, pyridazinyl, pyridyl, pyrimidyl, pyrrolyl, quinazolinyl, quinolyl, quinoxalinyl, tetrahydropyranyl, tetrazolyl, tetrazolopyridyl, thiadiazolyl, thiazolyl, thienyl, triazolyl, azetidinyl, 1,4-dioxanyl, hexahydroazepinyl, piperazinyl, piperidinyl, pyridin-2-onyl, pyrrolidinyl, morpholinyl, thiomorpholinyl, dihydrobenzoimidazolyl, dihydrobenzofuranyl, dihydrobenzothiophenyl, dihydrobenzoxazolyl, dihydrofuranyl, dihydroimidazolyl, dihydroindolyl, dihydroisooxazolyl, dihydroisothiazolyl, dihydrooxadiazolyl, dihydrooxazolyl, dihydropyrazinyl, dihydropyrazolyl, dihydropyridinyl, dihydropyrimidinyl, dihydropyrrolyl, dihydroquinolinyl, dihydrotetrazolyl, dihydrothiadiazolyl, dihydrothiazolyl, dihydrothienyl, dihydrotriazolyl, dihydroazetidinyl, methylenedioxybenzoyl, tetrahydrofuranyl, and tetrahydrothienyl, and N-oxides thereof. Attachment of a heterocyclyl substituent can occur via a carbon atom or via a heteroatom.

As appreciated by those of skill in the art, "halo" or "halogen" as used herein is intended to include chloro (Cl), fluoro (F), bromo (Br) and iodo (I).

In an embodiment, R^1 is selected from: oxo, NH_2, OH, SH, $O_a(C_1\text{-}C_6)$alkyl, said alkyl is optionally substituted with R^{6a}.

In an embodiment R^b is independently selected from H and $(C_1\text{-}C_6)$alkyl.

In an embodiment of Formula C, R^1 is selected from: oxo, $(C{=}O)_aO_b(C_1\text{-}C_{10})$alkyl, $(C{=}O)_aO_b$-aryl, $(C{=}O)_aO_b(C_2\text{-}C_{10})$alkenyl, $(C{=}O)_aO_b$ $(C_2\text{-}C_{10})$alkynyl, CO_2H, halo, OH, $O_b(C_1\text{-}C_6)$perfluoroalkyl, $(C{=}O)_aNR^7R^8$, CN, $(C{=}O)_aO_b(C_3\text{-}C_8)$cycloalkyl, $S(O)_2NR^7R^8$, SH, $S(O)_2\text{-}(C_1\text{-}C_{10})$alkyl and $(C{=}O)_aO_b$-heterocyclyl, said alkyl, aryl, alkenyl, alkynyl, cycloalkyl, and heterocyclyl is optionally substituted with R^{6a}.

In another embodiment of Formula C, R^1 is selected from: oxo, $(C{=}O)_aO_b(C_1\text{-}C_{10})$alkyl, CO_2H, halo, OH, CN, $(C_1\text{-}C_6)$alkoxy, $S(O)_2NR^7R^8$, SH, $S(O)_2\text{-}(C_1\text{-}C_{10})$alkyl, $O(C{=}O)(C_1\text{-}C_6)$alkyl and $N(R^b)_2$, said alkyl is optionally substituted with R^{6a}.

In another embodiment of Formula C, R^1 is selected from: oxo, NH_2, OH, SH, $O_a(C_1\text{-}C_6)$alkyl, said alkyl is optionally substituted with R^{6a}.

In another embodiment of Formula C, R^2 is selected from: oxo, $(C{=}O)_aO_b(C_1\text{-}C_{10})$alkyl, CO_2H, halo, OH, CN, $(C_1\text{-}C_6)$alkoxy, $O(C{=}O)(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_{10})$alkenyl and

$N(R^b)_2$, said alkyl is optionally substituted with R^b, OH, $(C_1$-$C_6)$alkoxy, halogen, CO_2H, CN, $O(C=O)(C_1$-$C_6)$alkyl, oxo, and $N(R^b)_2$.

In another embodiment of Formula C, R^1 is selected from: H, heterocyclyl, $(C_3$-$C_6)$cycloalkyl, OH, $(C_1$-$C_6)$alkyl, $NH(C_1$-$C_6)$alkyl, NH-heterocyclyl, NH-cycloalkyl, said heterocyclyl, cycloalkyl and alkyl is optionally substituted with: halo, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkyl-NH_2, OH, $O(C_1$-$C_6)$alkyl and R^2 is selected from: H and halogen.

In another embodiment of Formula C, Ring Y is cyclobutyl; R^1 is selected from: H, heterocyclyl, $(C_3$-$C_6)$cycloalkyl, OH, $(C_1$-$C_6)$alkyl, $NH(C_1$-$C_6)$alkyl, NH-heterocyclyl, NH-cycloalkyl, said heterocyclyl, cycloalkyl and alkyl is optionally substituted with: halo, $(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkyl-NH_2, OH, $O(C_1$-$C_6)$alkyl and R^2 is H or F.

In another embodiment of Formula C, Ring Y is cyclobutyl; R^1 is: H or $(C_1$-$C_6)$alkyl; and R^2 is H or F.

Included in the instant invention is the free form of compounds of Formula C or E, as well as the pharmaceutically acceptable salts and stereoisomers thereof. Some of the isolated specific compounds exemplified herein are the protonated salts of amine compounds. The term "free form" refers to the amine compounds in non-salt form. The encompassed pharmaceutically acceptable salts not only include the isolated salts exemplified for the specific compounds described herein, but also all the typical pharmaceutically acceptable salts of the free form of compounds of Formula C or E. The free form of the specific salt compounds described may be isolated using techniques known in the art. For example, the free form may be regenerated by treating the salt with a suitable dilute aqueous base solution such as dilute aqueous NaOH, potassium carbonate, ammonia and sodium bicarbonate. The free forms may differ from their respective salt forms somewhat in certain physical properties, such as solubility in polar solvents, but the acid and base salts are otherwise pharmaceutically equivalent to their respective free forms for purposes of the invention.

The pharmaceutically acceptable salts of the instant compounds can be synthesized from the compounds of this invention which contain a basic or acidic moiety by conventional chemical methods. Generally, the salts of the basic compounds are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents. Similarly, the salts of the acidic compounds are formed by reactions with the appropriate inorganic or organic base.

Thus, pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed by reacting a basic instant compound with an inorganic or organic acid. For example, conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like, as well as salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic,

pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic (TFA) and the like.

When the compound of the present invention is acidic, suitable "pharmaceutically acceptable salts" refers to salts prepared form pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as arginine, betaine caffeine, choline, N,N^1-dibenzylethylenediamine, diethylamin, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine tripropylamine, tromethamine and the like.

The preparation of the pharmaceutically acceptable salts described above and other typical pharmaceutically acceptable salts is more fully described by Berg *et al.*, "Pharmaceutical Salts," *J. Pharm. Sci.*, 1977:66:1-19.

It will also be noted that the compounds of the present invention are potentially internal salts or zwitterions, since under physiological conditions a deprotonated acidic moiety in the compound, such as a carboxyl group, may be anionic, and this electronic charge might then be balanced off internally against the cationic charge of a protonated or alkylated basic moiety, such as a quaternary nitrogen atom.

UTILITY

The compounds of the instant invention are inhibitors of the activity of Akt and are thus useful in the treatment of cancer, in particular cancers associated with irregularities in the activity of Akt and downstream cellular targets of Akt. Such cancers include, but are not limited to, ovarian, pancreatic, breast and prostate cancer, as well as cancers (including glioblastoma) where the tumor suppressor PTEN is mutated (Cheng et al., *Proc. Natl. Acad. Sci.* (1992) 89:9267-9271; Cheng et al., *Proc. Natl. Acad. Sci.* (1996) 93:3636-3641; Bellacosa et al., *Int. J. Cancer* (1995) 64:280-285; Nakatani et al., *J. Biol. Chem.* (1999) 274:21528-21532; Graff, *Expert. Opin. Ther. Targets* (2002) 6(1):103-113; and Yamada and Araki, *J. Cell Science.* (2001) 114:2375-2382; Mischel and Cloughesy, *Brain Pathol.* (2003) 13(1):52-61).

The compounds, compositions and methods provided herein are particularly deemed useful for the treatment of cancer. Cancers that may be treated by the compounds, compositions and methods of the invention include, but are not limited to: Cardiac: sarcoma

(angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma; Lung: non-small cel lung, bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma; Gastrointestinal: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma), colon, colorectal, rectal; Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma); Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma; Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors; Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, meningioma, glioma, sarcoma); Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma], granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), fallopian tubes (carcinoma); Hematologic: blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma [malignant lymphoma]; Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and Adrenal glands: neuroblastoma. Thus, the term "cancerous cell" as provided herein, includes a cell afflicted by any one of the above-identified conditions.

Cancers that may be treated by the compounds, compositions and methods of the invention include, but are not limited to: breast, prostate, colon, colorectal, lung, non-small cell lung, brain, testicular, stomach, pancrease, skin, small intestine, large intestine, throat, head and neck, oral, bone, liver, bladder, kidney, thyroid and blood.

Cancers that may be treated by the compounds, compositions and methods of the invention include: breast, prostate, colon, ovarian, colorectal, lung and non-small cell lung.

Cancers that may be treated by the compounds, compositions and methods of the invention include: breast, colon, (colorectal) and lung (non-small cell lung).

Cancers that may be treated by the compounds, compositions and methods of the invention include: lymphoma and leukemia.

The compounds of the instant invention are useful for the treatment of breast cancer.

The compounds of the instant invention are useful for the treatment of prostate cancer.

Akt signaling regulates multiple critical steps in angiogenesis. Shiojima and Walsh, *Circ. Res.* (2002) 90:1243-1250. The utility of angiogenesis inhibitors in the treatment of cancer is known in the literature, see J. Rak et al. *Cancer Research*, 55:4575-4580, 1995 and Dredge et al., *Expert Opin. Biol. Ther.* (2002) 2(8):953-966, for example. The role of angiogenesis in cancer has been shown in numerous types of cancer and tissues: breast carcinoma (G. Gasparini and A.L. Harris, *J. Clin. Oncol.*, 1995, 13:765-782; M. Toi et al., *Japan. J. Cancer Res.*, 1994, 85:1045-1049); bladder carcinomas (A.J. Dickinson et al., *Br. J. Urol.*, 1994, 74:762-766); colon carcinomas (L.M. Ellis et al., *Surgery*, 1996, 120(5):871-878); and oral cavity tumors (J.K. Williams et al., *Am. J. Surg.*, 1994, 168:373-380). Other cancers include, advanced tumors, hairy cell leukemia, melanoma, advanced head and neck, metastatic renal cell, non-Hodgkin's lymphoma, metastatic breast, breast adenocarcinoma, advanced melanoma, pancreatic, gastric, glioblastoma, lung, ovarian, non-small cell lung, prostate, small cell lung, renal cell carcinoma, various solid tumors, multiple myeloma, metastatic prostate, malignant glioma, renal cancer, lymphoma, refractory metastatic disease, refractory multiple myeloma, cervical cancer, Kaposi's sarcoma, recurrent anaplastic glioma, and metastatic colon cancer (Dredge et al., *Expert Opin. Biol. Ther.* (2002) 2(8):953-966). Thus, the Akt inhibitors disclosed in the instant application are also useful in the treatment of these angiogenesis related cancers.

Tumors which have undergone neovascularization show an increased potential for metastasis. In fact, angiogenesis is essential for tumor growth and metastasis. (S.P. Cunningham, et al., *Can. Research*, 61: 3206-3211 (2001)). The Akt inhibitors disclosed in the present application are therefore also useful to prevent or decrease tumor cell metastasis.

Further included within the scope of the invention is a method of treating or preventing a disease in which angiogenesis is implicated, which is comprised of administering to a mammal in need of such treatment a therapeutically effective amount of a compound of the

present invention. Ocular neovascular diseases are an example of conditions where much of the resulting tissue damage can be attributed to aberrant infiltration of blood vessels in the eye (see WO 00/30651, published 2 June 2000). The undesireable infiltration can be triggered by ischemic retinopathy, such as that resulting from diabetic retinopathy, retinopathy of prematurity, retinal vein occlusions, etc., or by degenerative diseases, such as the choroidal neovascularization observed in age-related macular degeneration. Inhibiting the growth of blood vessels by administration of the present compounds would therefore prevent the infiltration of blood vessels and prevent or treat diseases where angiogenesis is implicated, such as ocular diseases like retinal vascularization, diabetic retinopathy, age-related macular degeneration, and the like.

Further included within the scope of the invention is a method of treating or preventing a non-malignant disease in which angiogenesis is implicated, including but not limited to: ocular diseases (such as, retinal vascularization, diabetic retinopathy and age-related macular degeneration), atherosclerosis, arthritis, psoriasis, obesity and Alzheimer's disease (Dredge et al., *Expert Opin. Biol. Ther.* (2002) 2(8):953-966). In another embodiment, a method of treating or preventing a disease in which angiogenesis is implicated includes: ocular diseases (such as, retinal vascularization, diabetic retinopathy and age-related macular degeneration), atherosclerosis, arthritis and psoriasis.

Further included within the scope of the invention is a method of treating hyperproliferative disorders such as restenosis, inflammation, autoimmune diseases and allergy/asthma.

Further included within the scope of the instant invention is the use of the instant compounds to coat stents and therefore the use of the instant compounds on coated stents for the treatment and/or prevention of restenosis (WO03/032809).

Further included within the scope of the instant invention is the use of the instant compounds for the treatment and/or prevention of osteoarthritis (WO03/035048).

Further included within the scope of the invention is a method of treating hyperinsulinism.

The compounds of the invention are also useful in preparing a medicament that is useful in treating the diseases described above, in particular cancer.

In an embodiment of the invention, the instant compound is a selective inhibitor whose inhibitory efficacy is dependent on the PH domain. In this embodiment, the compound exhibits a decrease in *in vitro* inhibitory activity or no *in vitro* inhibitory activity against truncated Akt proteins lacking the PH domain.

In a further embodiment, the instant compound is selected from the group of a selective inhibitor of Akt1, a selective inhibitor of Akt2 and a selective inhibitor of both Akt1 and Akt2.

In another embodiment, the instant compound is selected from the group of a selective inhibitor of Akt1, a selective inhibitor of Akt2, a selective inhibitor of Akt3 and a selective inhibitor of two of the three Akt isoforms.

In another embodiment, the instant compound is a selective inhibitor of all three Akt isoforms, but is not an inhibitor of one, two or all of such Akt isoforms that have been modified to delete the PH domain, the hinge region or both the PH domain and the hinge region.

The present invention is further directed to a method of inhibiting Akt activity which comprises administering to a mammal in need thereof a pharmaceutically effective amount of the instant compound.

The compounds of this invention may be administered to mammals, including humans, either alone or, in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, microcrystalline cellulose, sodium crosscarmellose, corn starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to mask the unpleasant taste of the drug or delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a water soluble taste masking material such as hydroxypropylmethyl-cellulose or hydroxypropylcellulose, or a time delay material such as ethyl cellulose, cellulose acetate buryrate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as polyethyleneglycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as butylated hydroxyanisol or alpha-tocopherol.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

The pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsion. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring agents, preservatives and antioxidants.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

The pharmaceutical compositions may be in the form of sterile injectable aqueous solutions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution.

The sterile injectable preparation may also be a sterile injectable oil-in-water microemulsion where the active ingredient is dissolved in the oily phase. For example, the active ingredient may be first dissolved in a mixture of soybean oil and lecithin. The oil solution then introduced into a water and glycerol mixture and processed to form a microemulation.

The injectable solutions or microemulsions may be introduced into a patient's blood-stream by local bolus injection. Alternatively, it may be advantageous to administer the solution or microemulsion in such a way as to maintain a constant circulating concentration of the instant compound. In order to maintain such a constant concentration, a continuous intravenous delivery device may be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous pump.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of Formula C or E may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of Formula C or E are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

The compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen. Compounds of the present invention may also be delivered as a suppository employing bases such as cocoa butter,

glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

When a composition according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, and response of the individual patient, as well as the severity of the patient's symptoms.

The dosage regimen utilizing the compounds of the instant 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 cancer 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. For example, compounds of the instant invention can be administered in a total daily dose of up to 10,000 mg. Compounds of the instant invention can be administered once daily (QD), or divided into multiple daily doses such as twice daily (BID), and three times daily (TID). Compounds of the instant invention can be administered at a total daily dosage of up to 10,000 mg, e.g., 2,000 mg, 3,000 mg, 4,000 mg, 6,000 mg, 8,000 mg or 10,000 mg, which can be administered in one daily dose or can be divided into multiple daily doses as described above.

For example, compounds of the instant invention can be administered in a total daily dose of up to 1,000 mg. Compounds of the instant invention can be administered once daily (QD), or divided into multiple daily doses such as twice daily (BID), and three times daily (TID). Compounds of the instant invention can be administered at a total daily dosage of up to 1,000 mg, e.g., 200 mg, 300 mg, 400 mg, 600 mg, 800 mg or 1,000 mg, which can be administered in one daily dose or can be divided into multiple daily doses as described above.

In addition, the administration can be continuous, i.e., every day, or intermittently. The terms "intermittent" or "intermittently" as used herein means stopping and starting at either regular or irregular intervals. For example, intermittent administration of a compound of the instant invention may be administration one to six days per week or it may mean administration in cycles (e.g. daily administration for two to eight consecutive weeks, then a rest period with no administration for up to one week) or it may mean administration on alternate days.

In addition, the compounds of the instant invention may be administered according to any of the schedules described above, consecutively for a few weeks, followed by a rest period. For example, the compounds of the instant invention may be administered according to any one of the schedules described above from two to eight weeks, followed by a rest period of one week, or twice daily at a dose of 100 - 500 mg for three to five days a week. In another particular embodiment, the compounds of the instant invention may be administered three times daily for two consecutive weeks, followed by one week of rest.

Any one or more of the specific dosages and dosage schedules of the compounds of the instant invention, may also be applicable to any one or more of the therapeutic agents to be used in the combination treatment (hereinafter refered to as the "second therapeutic agent").

Moreover, the specific dosage and dosage schedule of this second therapeutic agent can further vary, and the optimal dose, dosing schedule and route of administration will be determined based upon the specific second therapeutic agent that is being used.

Of course, the route of administration of the compounds of the instant invention is independent of the route of administration of the second therapeutic agent. In an embodiment, the administration for a compound of the instant invention is oral administration. In another embodiment, the administration for a compound of the instant invention is intravenous administration. Thus, in accordance with these embodiments, a compound of the instant invention is administered orally or intravenously, and the second therapeutic agent can be administered orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraoccularly, via local delivery by catheter or stent, subcutaneously, intraadiposally, intraarticularly, intrathecally, or in a slow release dosage form.

In addition, a compound of the instant invention and second therapeutic agent may be administered by the same mode of administration, i.e. both agents administered e.g. orally, by IV. However, it is also within the scope of the present invention to administer a compound of the instant invention by one mode of administration, e.g. oral, and to administer the second therapeutic agent by another mode of administration, e.g. IV or any other ones of the administration modes described hereinabove.

The first treatment procedure, administration of a compound of the instant invention, can take place prior to the second treatment procedure, i.e., the second therapeutic agent, after the treatment with the second therapeutic agent, at the same time as the treatment with the second therapeutic agent, or a combination thereof. For example, a total treatment period can be decided for a compound of the instant invention. The second therapeutic agent can be administered prior to onset of treatment with a compound of the instant invention or following treatment with a compound of the instant invention. In addition, anti-cancer treatment can be administered during the period of administration of a compound of the instant invention but does not need to occur over the entire treatment period of a compound of the instant invention.

The instant compounds are also useful in combination with therapeutic, chemotherapeutic and anti-cancer agents. Combinations of the presently disclosed compounds with therapeutic, chemotherapeutic and anti-cancer agents are within the scope of the invention. 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 agents include 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, HIV protease inhibitors, reverse transcriptase inhibitors, inhibitors of cell proliferation and survival signaling, bisphosphonates, aromatase inhibitors, siRNA therapeutics, γ-secretase inhibitors, agents that interfere with receptor tyrosine kinases (RTKs) and agents that interfere with cell cycle checkpoints. The instant compounds are particularly useful when co-administered with radiation therapy.

A compound of the instant invention may also be useful for treating cancer 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®); 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®); Darbepoetin alfa (Aranesp®); daunorubicin liposomal (DanuoXome®); daunorubicin, daunomycin (Daunorubicin®); daunorubicin, daunomycin (Cerubidine®); Denileukin diftitox (Ontak®); dexrazoxane (Zinecard®); docetaxel (Taxotere®); doxorubicin (Adriamycin PFS®); doxorubicin (Adriamycin®, Rubex®); doxorubicin (Adriamycin PFS Injection®); doxorubicin liposomal (Doxil®); dromostanolone propionate (dromostanolone®); dromostanolone propionate (masterone injection®); Elliott's B Solution (Elliott's B Solution®); epirubicin (Ellence®); Epoetin alfa (epogen®); erlotinib (Tarceva®); estramustine (Emcyt®); etoposide phosphate (Etopophos®); etoposide, VP-16 (Vepesid®); exemestane (Aromasin®); 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®); irinotecan (Camptosar®); 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®); Nofetumomab (Verluma®); Oprelvekin (Neumega®); oxaliplatin (Eloxatin®); paclitaxel (Paxene®); paclitaxel (Taxol®); paclitaxel protein-bound particles (Abraxane®); palifermin (Kepivance®); pamidronate (Aredia®); pegademase (Adagen (Pegademase Bovine)®); pegaspargase (Oncaspar®); Pegfilgrastim (Neulasta®); pemetrexed disodium (Alimta®); pentostatin (Nipent®); pipobroman (Vercyte®); plicamycin, mithramycin (Mithracin®); porfimer sodium (Photofrin®); procarbazine (Matulane®); quinacrine (Atabrine®); Rasburicase (Elitek®); Rituximab (Rituxan®); sargramostim (Leukine®); Sargramostim (Prokine®); sorafenib (Nexavar®); streptozocin (Zanosar®); sunitinib maleate (Sutent®); talc (Sclerosol®); tamoxifen (Nolvadex®); temozolomide (Temodar®); 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®); zoledronate (Zometa®) and vorinostat (Zolinza®).

The compounds of the instant invention are useful for treating cancer in combination with taxanes.

The compounds of the instant invention are useful for treating cancer in combination with docetaxel (Taxotere®).

The compounds of the instant invention are useful for treating cancer in combination with vorinostat (Zolinza®).

The compounds of the instant invention are useful for treating cancer in combination with the aurora kinase inhibitor, MK-0457.

The compounds of the instant invention are useful for treating cancer in combination with the mTor inhibitor, AP 23573.

The compounds of the instant invention are useful for treating cancer in combination with the IGF1R inhibitor, MK-0646.

The compounds of the instant invention are useful for treating cancer in combination with satraplatin.

The compounds of the instant invention are useful for treating cancer in combination with lapatinib (Tykerb®).

Thus, the scope of the instant invention encompasses the use of the instantly claimed compounds in combination with 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, 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), an agent that interferes with a cell cycle checkpoint and any of the therapeutic agents listed above.

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.

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

The term "therapeutically effective amount" as used herein 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 term "treating cancer" or "treatment of cancer" refers to administration to a mammal afflicted with a cancerous condition and refers to an effect that alleviates the cancerous condition by killing the cancerous cells, but also to an effect that results in the inhibition of growth and/or metastasis of the cancer.

In an embodiment, the angiogenesis inhibitor to be used as the second compound is selected from a tyrosine kinase inhibitor, an inhibitor of epidermal-derived growth factor, an inhibitor of fibroblast-derived growth factor, an inhibitor of platelet derived growth factor, an MMP (matrix metalloprotease) inhibitor, an integrin blocker, interferon-α, interleukin-12, pentosan polysulfate, a cyclooxygenase inhibitor, carboxyamidotriazole, combretastatin A-4, squalamine, 6-O-chloroacetyl-carbonyl)-fumagillol, thalidomide, angiostatin, troponin-1, or an antibody to VEGF. In an embodiment, the estrogen receptor modulator is tamoxifen or raloxifene.

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 the instant invention 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), an agent that interferes with a cell cycle checkpoint and any of the therapeutic agents listed above.

The instant invention also includes a pharmaceutical composition useful for treating or preventing cancer that comprises a therapeutically effective amount of a compound of the instant invention 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), an agent that interferes with a cell cycle checkpoint and any of the therapeutic agents listed above.

All patents, publications and pending patent applications identified are hereby incorporated by reference.

Abbreviations used in the description of the chemistry and in the Examples that follow are: AEBSF (p-aminoethylbenzenesulfonyl fluoride); BSA (bovine serum albumin); BuLi (n-Butyl lithium); $CDCl_3$ (chloroform-d); CuI (copper iodide); $CuSO_4$ (copper sulfate); DCE (dichloroethane); DCM (dichloromethane); DEAD (diethyl azodicarboxylate); DMF (N,N-dimethylformamide); DMSO (dimethyl sulfoxide); DTT (dithiothreitol); EDTA (ethylene-diamine-tetra-acetic acid); EGTA (ethylene-glycol-tetra-acetic acid); EtOAc (ethyl acetate); EtOH (ethanol); HOAc (acetic acid); HPLC (high-performance liquid chromatography); HRMS (high resolution mass spectrum); IPA (isopropyl alcohol); LCMS (liquid chromatograph-mass spectrometer); LHMDS (lithium bis(trimethylsilyl)amide); LRMS (low resolution mass spectrum); MeOH (methanol); MP-B(CN)H3 (Macroporous cyanoborohydride); $NaHCO_3$ (sodium bicarbonate); Na_2SO_4 (sodium sulfate); Na(OAc)3BH (sodium triacetoxyborohydride); NH_4OAc (ammonium acetate); NBS (N-bromosuccinamide); NMR (nuclear magnetic resonance); PBS (phosphate buffered saline); PCR (polymerase chain reaction); Pd(dppf) ([1,1'-bis(diphenylphosphino)ferrocene] palladium); Pd(Ph3)4 (palladium(0) tetrakis-triphenylphosphine); $POCl_3$ (phosphorous oxychloride); PS-DIEA (polystyrene diisopropylethylamine); PS-PPh3 (polystyrene-triphenyl phosphine); TBAB (tetrabutylammonium bromide); TBAF (tetrabutylammonium fluoride); THF (tetrahydrofuran);

TFA (trifluoroacteic acid); $TMSCH_2N_2$ (trimethylsilyldiazomethane) and Ac (acetyl); BOC (t-butoxycarbonyl); Bu (butyl); Cal (calculated); Calc'd (calculated); DIEA (diisopropylethylamine); DMAP (4-dimethylaminopyridine); EDC (N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide); Eq (equivalents); Et (ethyl); HOBT (hydroxybenzotriazole); IPA (isopropanol); LC/MS (liquid chromatograph-mass spectrometer); Me (methyl); MeCN (acetonitrile); NMP (N-methylpyrrolidinone); Pr (propyl); Pyr (pyridine); Sat (saturated), Tosic (p-toluenesulfonic acid) and Bn (benzyl); t-Bu (tert-butyl); dba (dibenzylideneacetone); DIPEA (diisopropylethylamine); IPAC (isopropyl acetate); MTBE (tert-butyl methyl ether); OAc (acetate); RT (room temperature); Wt (weight); and XRPD (x-ray powder diffraction).

The compounds of this invention may be prepared by employing reactions as shown in the following Reaction Schemes, in addition to other standard manipulations that are known in the literature or exemplified in the experimental procedures. The illustrative Reaction Schemes below, therefore, are not limited by the compounds listed or by any particular substituents employed for illustrative purposes. Substituent numbering as shown in the Reaction Schemes does not necessarily correlate to that used in the claims and often, for clarity, a single substituent is shown attached to the compound where multiple substituents are allowed under the definitions of Formula C or E hereinabove.

Synopsis of Reaction Schemes

The following Reaction Schemes, Reaction Schemes I - III, provide useful details for preparing the instant compounds. The requisite intermediates are in some cases commercially available or can be prepared according to literature procedures.

As illustrated in Reaction Scheme I, a cycloalkyl(phenyl)acetic acid derivative, in this case cyclobutyl, is first reacted and rearranged under Curtius-type conditions to give the carbamate **I-1**. Cyanation, in this case catalysed by palladium, gives nitrile **I-2**. Deprotonation of **I-2** followed by reaction with a nucleophilic benzyl Grignard reagent and hydrolytic work-up gives ketone **I-3**. Condensation of **I-3** with aldehyde **I-4** under basic conditions gives the chloronaphthyridine **I-5**. Displacement of the chlorine with hydrazine gives hydrazide **I-6**. Acylation gives acycl hydrazide **I-7** which is cyclized under acidic conditions gives the triazolonaphthyridine **I-8**. Deprotection of the amine, in this case with HCl, generates **I-9**.

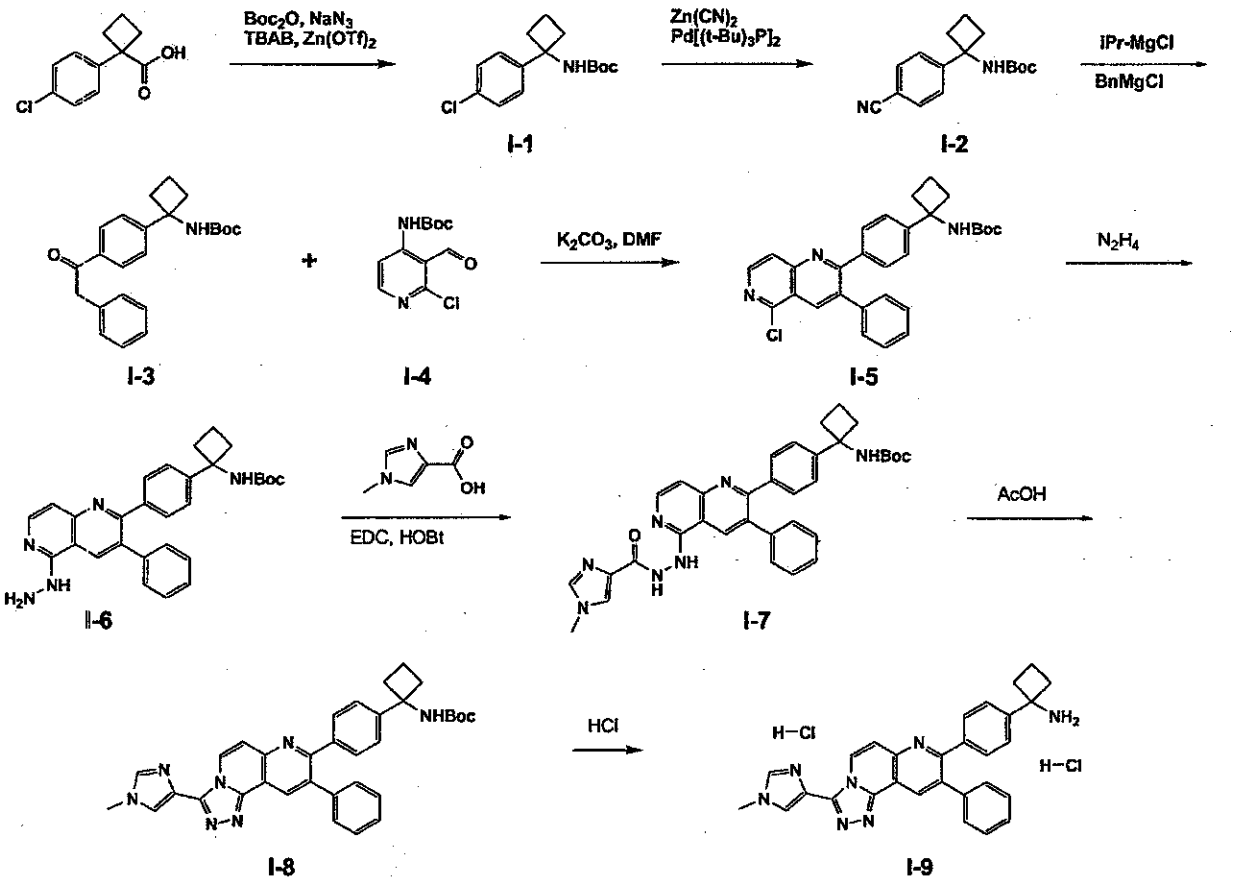
The fused triazole may be prepared by acyclating hydrazide **I-6** with an activated carbonyl equivalent such as carbonyldiimidazole or with formic acid in the presence of a carbodiimide, without the need for a cyclization step. Alternatively chloronaphthyridine **I-5** may be reacted with a derivatized hydrazine such as an alkyl hydrazine carboxylate under acid catalysis to give the hydrazine adduct which is cyclized to the triazole under basic conditions.

Compounds of the instant invention in which R^1 is an aminoalkyl group may be prepared according to the procedures outlined in reaction Scheme II. Hydrazide **I-6** is reacted

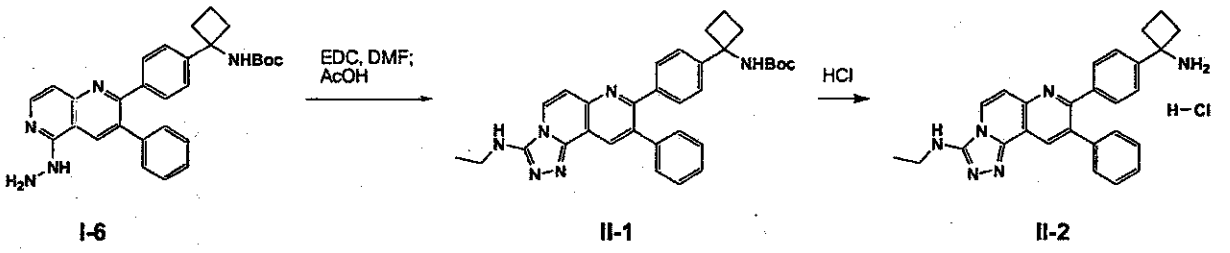
with a carbodiimide to generate a urea which is cyclized in situ under acidic conditions to give the alkylaminotriazole **II-1**. Deprotection then gives **II-2**.

An alternative synthesis of chloronaphthyridine **I-5** is illustrated in Reaction Scheme III. A (4-halophenyl)acetonitrile derivative **III-1**, in this case bromo, is first alkylated under basic conditions to give the cyclobutane **III-2** which is hydrated with hydrogen peroxide in the presence of base to give amide **III-3**. The amide is then oxidatively rearranged in the presence of t-butanol to give the carbamate **III-4**. Cyanation, in this case catalysed by palladium, gives nitrile **I-2**. Reaction of **I-2** with an excess of nucleophilic benzyl Grignard reagent and hydrolytic work-up gives ketone **I-3**. Deprotection of aldehyde **I-4** with an acid such as TFA gives the aminopyridine **III-5**. Condensation of **I-3** with aldehyde **III-5** under basic conditions gives the chloronaphthyridine **I-5**.

Reaction Scheme I

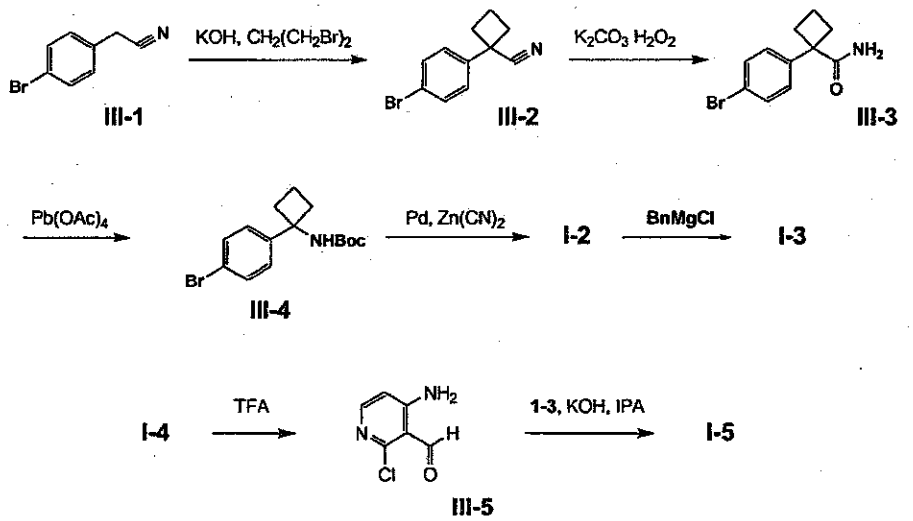


Reaction Scheme II



I-6 II-1 II-2

Reaction Scheme III

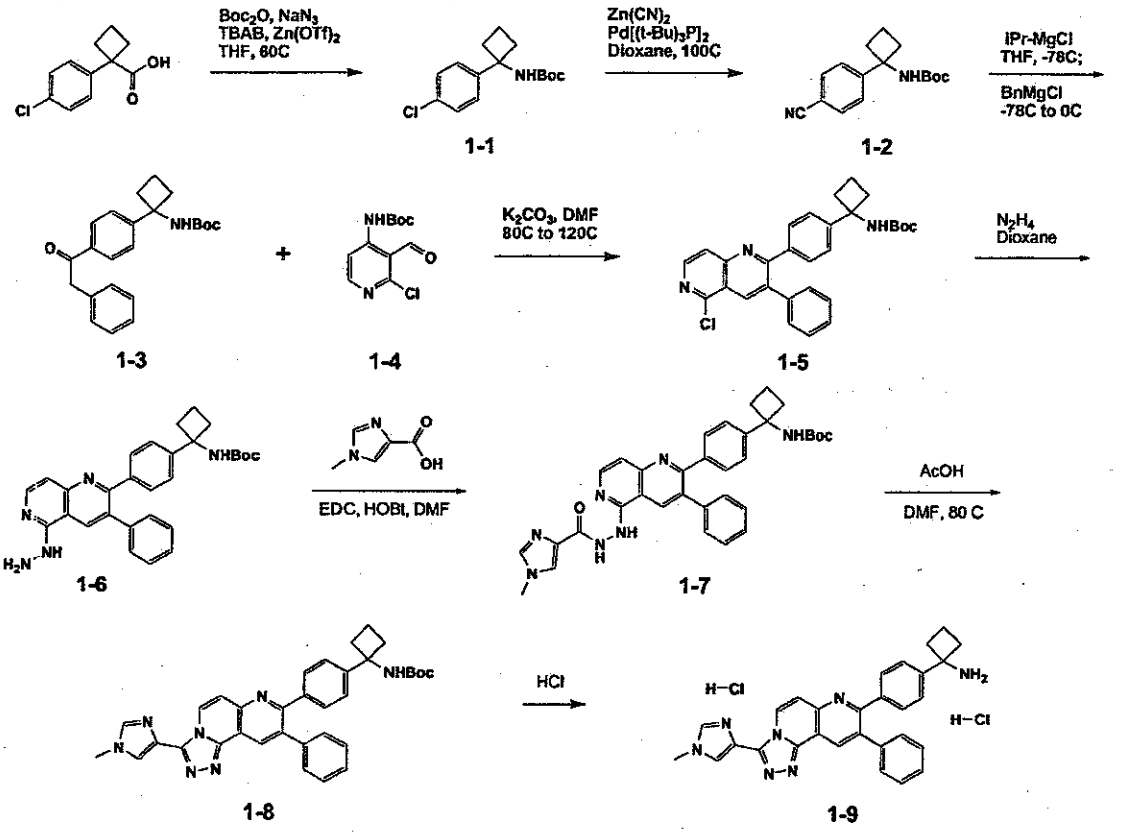


III-1 III-2 III-3 III-4 III-5

EXAMPLES

Examples and schemes provided are intended to assist in a further understanding of the invention. Particular materials employed, species and conditions are intended to be further illustrative of the invention and do not limit the reasonable scope thereof.

SCHEME 1



tert-butyl [1-(4-chlorophenyl)cyclobutyl]carbamate (**1-1**)

To a round bottom flask was added 1-(4-chlorophenyl)cyclobutanecarboxylic acid (40.4 g, 192 mmol), di-tert-butyl dicarbonate (46.0 g, 211 mmol), sodium azide (43.6 g, 671 mmol), tetrabutylammonium bromide (9.27 g, 28.7 mmol), zinc trifluoromethanesulfonate (2.30 g, 6.32 mmol), and THF (1L). The reaction mixture was heated to 60°C while stirring in a hot oil bath with a water cooled reflux condenser attached under an atmosphere of nitrogen for 18 hours. To the crude reaction mixture was added a saturated solution of sodium bicarbonate, suspended in ethyl acetate and washed with a saturated solution of sodium bicarbonate, followed by water, brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was purified by silica gel chromatography (0-3% IPA/DCM) to give *tert*-butyl [1-(4-chlorophenyl)cyclobutyl]carbamate (**1-1**) as a white solid. HRMS $(M+Na)^+$: observed = 304.1075, calculated = 304.1075.

tert-butyl [1-(4-cyanophenyl)cyclobutyl]carbamate (**1-2**)

To a solution of *tert*-butyl [1-(4-chlorophenyl)cyclobutyl]carbamate (**1-1**) (5.32 g, 18.9 mmol) in anhydrous 1,4-dioxane (70 mL) was added zinc cyanide (2.44 g, 20.8 mmol), followed by bis(tri-t-butylphosphine)palladium(0) (0.965 g, 1.89 mmol). The reaction mixture was heated to 100°C while stirring in a hot oil bath with a water cooled reflux condenser attached under an atmosphere of nitrogen for 1.5 hours. The reaction mixture was filtered, and

concentrated in vacuo. The resulting residue was purified by silica gel chromatography (0-5% IPA/DCM) to give *tert*-butyl [1-(4-cyanophenyl)cyclobutyl] (**1-2**) as a waxy off-white/ yellow solid. HRMS $(M+H)^+$: observed = 273.1598, calculated = 273.1597.

tert-butyl {1-[4-(phenylacetyl)phenyl]cyclobutyl}carbamate (**1-3**)

A solution of *tert*-butyl [1-(4-cyanophenyl)cyclobutyl]carbamate (**1-2**) (16.6 g, 61.0 mmol) in anhydrous THF (300 mL) was cooled to -78°C while stirring under an atmosphere of nitrogen. Then a 2.0 M solution isopropylmagnesium chloride in THF (30.5 mL, 61.0 mmol) was added dropwise over 5 minutes. The reaction mixture was permitted to stir at -78°C under an atmosphere of nitrogen for 10 minutes. Then a 2.0 M solution of benzylmagnesium chloride in THF (116 mL, 232 mmol) was added dropwise over 10 minutes. The reaction mixture was permitted to warm to 0°C. After 2 hours the reaction mixture was quenched at 0°C by addition of a saturated solution of ammonium chloride. The reaction was permitted to warm to room temperature, suspended in ethyl acetate, washed with a saturated solution of ammonium chloride, a saturated solution of sodium bicarbonate, followed by water, brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (1-40% EtOAc/5% DCM/Hexane) to give *tert*-butyl {1-[4-(phenylacetyl)phenyl]cyclobutyl}carbamate (**1-3**) as a waxy off-white solid. HRMS $(M+Na)^+$: observed = 388.1892, calculated = 388.1883.

tert-butyl {1-[4-(5-chloro-3-phenyl-1,6-naphthyridin-2-yl)phenyl] cyclobutyl} carbamate (**1-5**)

To a round bottom flask was added *tert*-butyl {1-[4-(phenylacetyl)phenyl]cyclobutyl}carbamate (**1-3**) (2.7 g, 6.1 mmol), *tert*-butyl (2-chloro-3-formylpyridin-4-yl)carbamate (**1-4**) (1.6 g, 6.1 mmol), potassium carbonate (5.0 g, 6.0 mmol), and DMF (20 mL). The reaction mixture was heated to 80°C while stirring in a hot oil bath under an atmosphere of nitrogen for 15 hours. Then the reaction mixture was warmed to 120°C for 1 hour. The reaction mixture was permitted to cool to room temperature, added water, suspended in ethyl acetate, washed with a saturated solution of sodium bicarbonate, followed by water, brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (5-50% EtOAc/5%DCM/Hexane) to give *tert*-butyl {1-[4-(5-chloro-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-5**) as an off-white solid. HRMS $(M+H)^+$: observed = 486.1954, calculated = 486.1943.

tert-butyl {1-[4-(5-hydrazino-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl} carbamate (**1-6**)

To a microwave vial was added *tert*-butyl {1-[4-(5-chloro-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-5**) (4.05 g, 8.34 mmol), anhydrous hydrazine (5.24 mL, 167 mmol), and 1,4-dioxane (15 mL). The reaction mixture was then heated under microwave irradiation for 5 minutes at 100°C (high absorption). The reaction mixture was permitted to cool to room temperature, suspended in ethyl acetate, washed with a saturated

solution of sodium bicarbonate, followed by water, brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give *tert*-butyl {1-[4-(5-hydrazino-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-6**) as an orange solid/foam. MS $(M+H)^+$: observed = 482.3, calculated = 482.59.

tert-butyl {1-[4-(5-{2-[(1-methyl-1*H*-imidazol-4-yl)carbonyl]hydrazino}-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate.(**1-7**)

To a round bottom flask was added *tert*-butyl {1-[4-(5-hydrazino-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-6**) (3.94 g, 8.18 mmol), EDC (1.34 g, 10.6 mmol), HOBt (1.44 g, 10.6 mmol), 1-methyl-1*H*-imidazole-4-carboxylic acid (1.34 g, 10.6 mmol), and DMF (40 mL). The reaction mixture was heated to 60°C while stirring under an atmosphere of nitrogen in a hot oil bath. After 45 minutes the reaction mixture was permitted to cool to room temperature, suspended in ethyl acetate, washed with a saturated solution of sodium bicarbonate, followed by water, brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give *tert*-butyl {1-[4-(5-{2-[(1-methyl-1*H*-imidazol-4-yl)carbonyl]hydrazino}-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-7**) as red foam. MS $(M+H)^+$: observed = 590.3, calculated = 590.69.

tert-butyl (1-{4-[3-(1-methyl-1*H*-imidazol-4-yl)-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl]phenyl}cyclobutyl)carbamate (**1-8**)

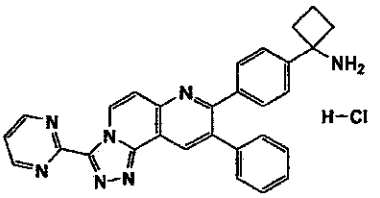
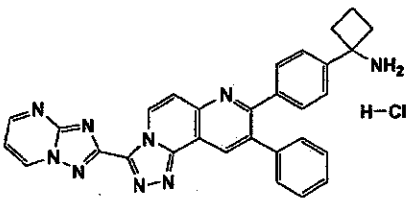
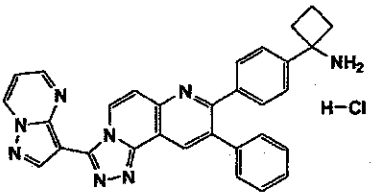
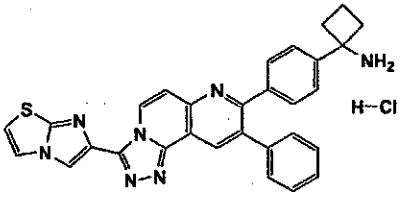
To a round bottom flask was added *tert*-butyl {1-[4-(5-{2-[(1-methyl-1*H*-imidazol-4-yl)carbonyl]hydrazino}-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-7**) (2.73 g, 4.63 mmol), acetic acid (5.30 mL, 93 mmol), and 1,4-dioxane (20 mL). The reaction mixture was heated to 80°C while stirring open to the atmosphere (capped) in a hot oil bath. After 3 hours the reaction mixture was permitted to cool to room temperature, suspended in ethyl acetate, washed with a saturated solution of sodium bicarbonate, followed by water, brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography 1-15% IPA/DCM. The appropriate fractions were combined and the solvent was removed in vacuo. The resulting residue was repurified by reverse phase column chromatography (Sunfire C18) eluting with 5 to 95% acetonitrile / (0.1% TFA / water) gradient. The appropriate fractions were free based by suspending in ethyl acetate, washed with a saturated solution of sodium bicarbonate, followed by water, brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give *tert*-butyl (1-{4-[3-(1-methyl-1*H*-imidazol-4-yl)-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl]phenyl}cyclobutyl)carbamate (**1-8**) as an off-white solid. MS $(M+H)^+$: observed = 572.3, calculated = 572.7.

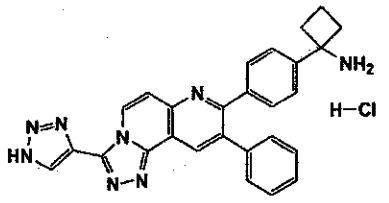
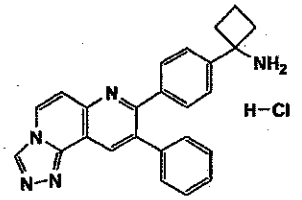
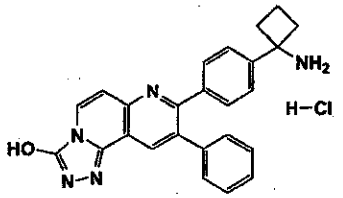
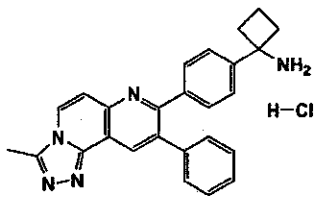
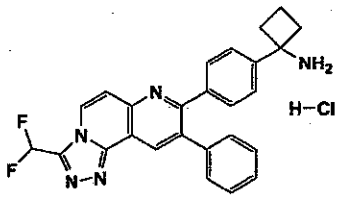
1-{4-[3-(1-methyl-1*H*-imidazol-4-yl)-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl]phenyl}cyclobutanamine dihydrochloride (**1-9**)

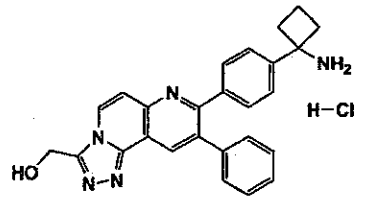
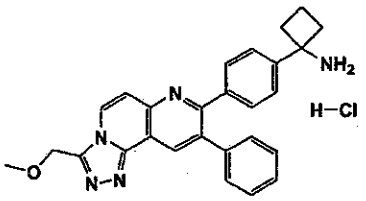
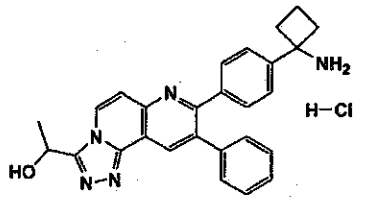
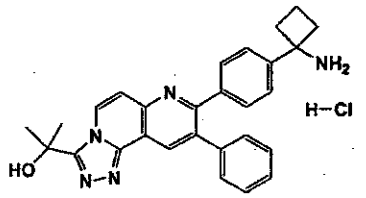
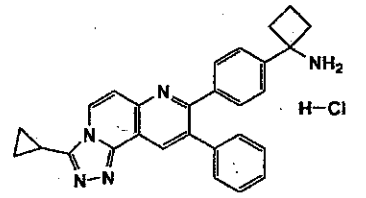
To a solution of *tert*-butyl (1-{4-[3-(1-methyl-1*H*-imidazol-4-yl)-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl]phenyl}cyclobutyl)carbamate (**1-8**) (2.52 g, 4.41 mmol) in MeOH (5 mL) and DCM (15 mL) was added a 4N solution of HCl in EtOAc (22

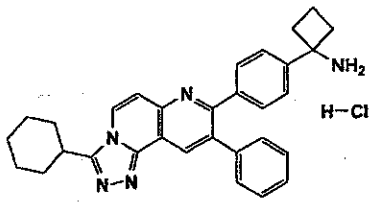
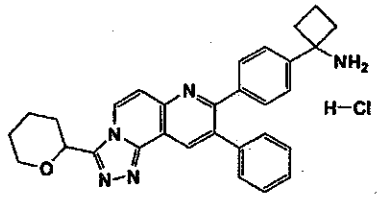
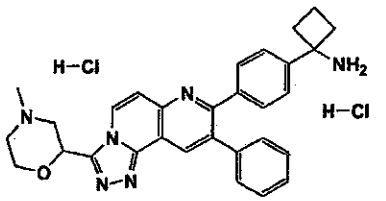
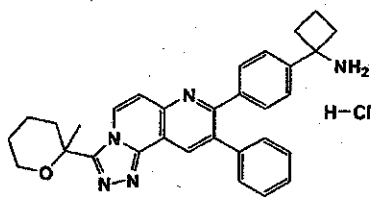
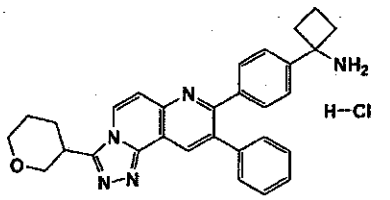
mL, 88 mmol). The sealed reaction mixture was permitted to stir at room temperature. After 4 hours the reaction mixture was concentrated in vacuo to give 1-{4-[3-(1-methyl-1*H*-imidazol-4-yl)-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl]phenyl}cyclobutanamine dihydrochloride (**1-9**) as a yellow solid. HRMS $(M+H)^+$: observed = 472.2249, calculated = 472.2244.

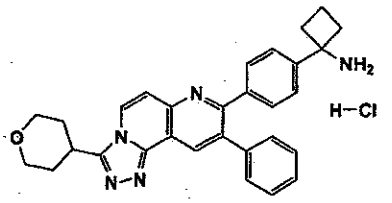
The following compounds were prepared in a similar fashion to Example **1-9**:

Cmp	Structure	Calculated HRMS m/z $(M+H)^+$	Observed HRMS m/z $(M+H)^+$
1-10		470.2088	470.2068
1-11		510.2149	510.2124
1-12		509.2197	509.2192
1-13		514.1809	514.1799

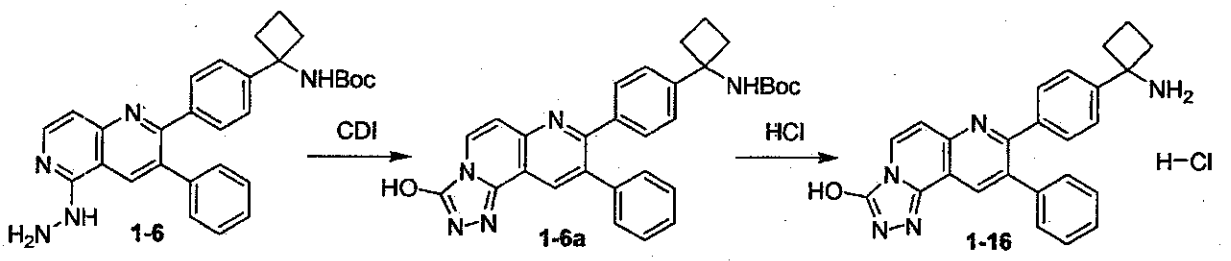
1-14		459.2040	459.2081
1-15		392.1870	392.1856
1-16		408.1819	408.1803
1-17		406.2026	406.2006
1-18		442.1838	442.1820

1-19		422.1976	422.1988
1-20		436.2132	436.2130
1-21		436.2132	436.2116
1-22		450.2289	450.2303
1-23		432.2183	432.2190

1-24		474.2652	474.2674
1-25		476.2445	476.2454
1-26		491.2554	491.2564
1-27		490.2602	490.2616
1-28		476.2445	476.2469

1-29		476.2445	476.2471

SCHEME 1A

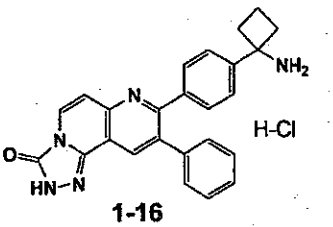


8-[4-(1-aminocyclobutyl)phenyl]-9-phenyl[1,2,4]triazolo[3,4-f]-1,6-naphthyridin-3-ol (**1-16**)

To a round bottom flask was added *tert*-butyl {1-[4-(5-hydrazino-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-6**) (2.77 g, 5.75 mmol), and 1,1'-carbonylbis(1*H*-imidazole) (4.10 g, 4.40 mmol), in 1,4 Dioxane (30 mL). The sealed reaction mixture was then heated at 95°C under an atmosphere of nitrogen. To the crude reaction mixture was added a saturated solution of sodium bicarbonate (100 mL) and ethyl acetate. The organic layer was washed with a saturated solution of sodium bicarbonate, followed by water, then brine, dried over sodium sulfate, filtered, and concentrated. The resulting residue was then purified by silica gel chromatography eluting with 0-10% IPA/DCM. The appropriate fractions were then combined and the solvent was removed in vacuo to give *tert*-butyl {1-[4-(3-hydroxy-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6a**) as a tan solid. MS $(M+H)^+$: observed =508.1, calculated = 508.6.

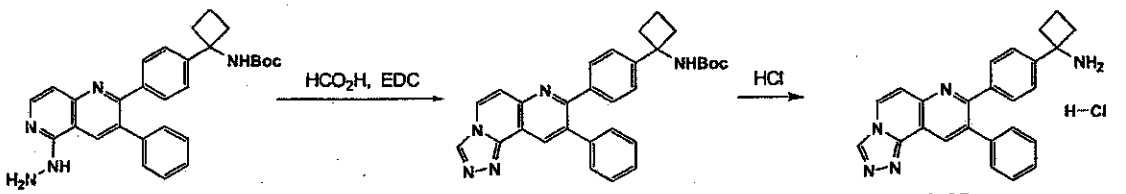
To a solution of *tert*-butyl {1-[4-(3-hydroxy-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6a**) (1.14 g, 2.25 mmol) in MeOH (5 mL) and DCM (15 mL) was added a 4N solution of HCl in EtOAc (22 mL, 88 mmol) at room temperature. After 4 hours the reaction mixture was concentrated in vacuo to give 8-[4-(1-aminocyclobutyl)phenyl]-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-3-ol hydrochloride (**1-16**) as a yellow solid. HRMS $(M+H)^+$: observed = 408.1803, calculated = 408.1819.

Subsequent, X-ray crystallographic analysis of the yellow solid indicates that solid Compound **1-16** exists as the following chemical structure:
8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-3(2*H*)-one hydrocloride



1-16

SCHEME 1B



1-6 1-6b 1-15

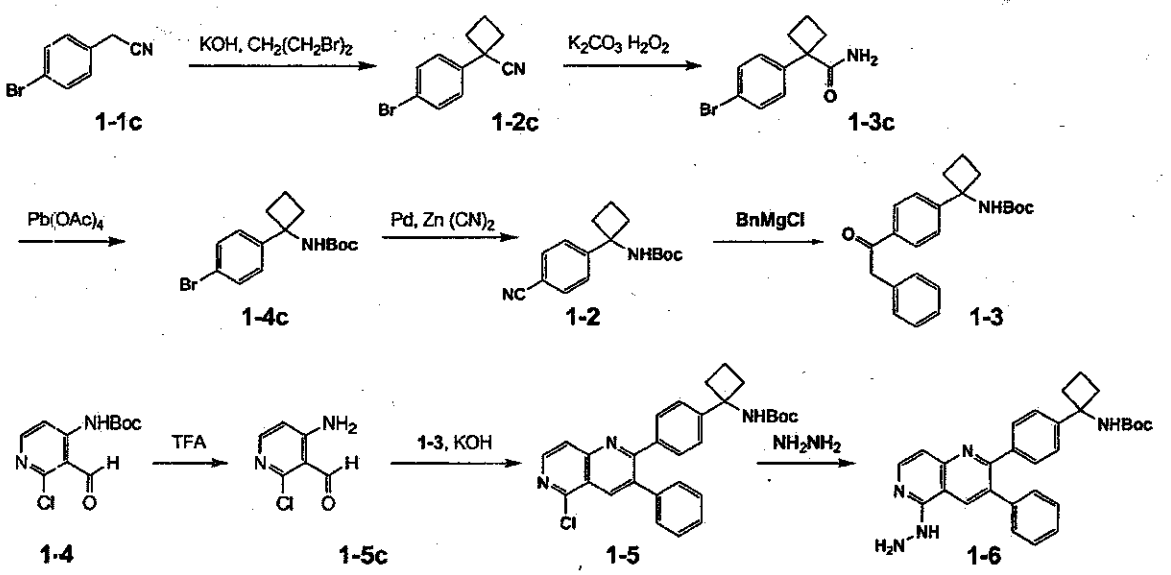
tert-butyl {1-[4-(9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-15**)

To a round bottom flask was added EDC (569 mg, 2.97 mmol), HOBt (401 mg, 10.6 mmol), formic acid (248 mg, 5.40 mmol), DCM (20 mL), & NMP (2.5 mL). The reaction mixture was then stirred at room temperature under an atmosphere of nitrogen for 30 minutes. Then added added *tert*-butyl {1-[4-(5-hydrazino-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-6**) (1.30 g, 2.70 mmol). The reaction mixture was then permitted to stir overnight at room temperature under an atmosphere of nitrogen. The reaction mixture was then suspended in ethyl acetate, washed with a saturated solution of sodium bicarbonate, followed by water, brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography 50-90% EtOAc/DCM, then 1-15% IPA/DCM. The appropriate fractions were combined and the solvent was removed in vacuo to give *tert*-butyl {1-[4-(9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6b**) as a tan solid. MS $(M+H)^+$: observed = 492.2, calculated = 492.6.

1-[4-(9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine hydrochloride (**1-15**)

To a solution of *tert*-butyl {1-[4-(9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6b**) (1.07 g, 2.177 mmol) in MeOH (5 mL) and DCM (15 mL) was added a 4N solution of HCl in EtOAc (5.44 mL, 21.77 mmol). The sealed reaction mixture was permitted to stir at room temperature. After 4 hours the reaction mixture was filtered to give 1-[4-(9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutanamine hydrochloride (**1-15**) as a tan solid. HRMS $(M+H)^+$: observed = 392.1872, calculated = 392.1870.

SCHEME 1C



1-(4-bromophenyl)cyclobutanecarbonitrile (1-2c)

TBAB (1.61 g, 0.5 mmol), dibromopropane (22.2 g, 110 mmol), and nitrile **1-1c** (19.6 g, 100 mmol) were added to a stirred solution of KOH (31.17 g, 500 mmol) in a mixture of 15 mL of water and 200 mL of toluene (temperature maintained between 72 and 79 °C). The mixture was heated by steam and was stirred at 99-108 °C for 2.5 h. The mixture was cooled to 80 °C and 200 mL of heptane was added. After the resulting mixture was cooled to RT with stirring, the top clear solution was filtered, washed with water (3X30 mL) and concentrated in vacuo to give oily product **1-2c**.

1-(4-bromophenyl)cyclobutanecarboxamide (1-3c)

H_2O_2 (30% 11.3 mL, 118 mmol) was added over 3 h to a stirred mixture of nitrile **1-2c** (13.88 g, ~58.9 mmol) and K_2CO_3 (1.62 g, 11.8 mol) in 59 mL of DMSO at 40-87 °C, cooling with a water bath. The resulting mixture was cooled to 27 °C and water (100 mL) was added over 30 min. Crystalline product **1-3c** formed. More water (100 mL) was added over 1 h. The resulting slurry was aged at RT for 16 h before filtration. The cake was rinsed with 100 mL of water and then with 100 mL of heptane. After drying in a vacuum oven at 50 °C, product **1-3c** was obtained as a white solid.

tert-butyl [1-(4-bromophenyl)cyclobutyl]carbamate (1-4c)

Pb(OAc)4 (25.7 g, 25.7 mmol) was added to a stirred solution of amide **1-3c** (12.7 g, 50 mmol) in 64 mL of t-BuOH at 57 °C to 86 °C cooling with a water bath. The resulting mixture was stirred at 65-86 °C for 0.5 h. The mixture was cooled to 26 °C and 12.7 g of Na_2CO_3 were added followed by 65 mL MTBE. After 10 min, the mixture was filtered. The cake was rinsed with 10 L of MTBE and the combined filtrate was washed with 20 mL of water and the organic layer was then washed with 3 X 10 mL of 10% KHCO3 (caution: bubbling) dried

over Na_2SO_4 and concentrated in vacuo. The resulting solid was rinsed with 8 mL of IPAc and 8 mL of heptane and dried in a vacuum oven at 40 °C to give product **1-4c** as a grey solid.

tert-butyl [1-(4-cyanophenyl)cyclobutyl]carbamate (**1-2**)

A stirred slurry of Pd_2dba_3 (101 mg; 1 mol%) and dppf (122 mg; 2 mole%) in DMF (25 mL) was sparged with nitrogen for 5 min and then warmed to 65 °C and aged for 30 min. At this temp was added the aryl bromide **1-4c** (3.6 g, 11 mmol), zinc powder (51 mg; 6 mol%) and the zinc cyanide (777 mg; 0.60 equiv) rinsing with DMF (5 mL). The solution was heated to 92-95 °C and aged for 4 h. The solution was cooled to RT overnight and filtered through a pad of Solka Floc, rinsing the cake with DMF (5 mL). Water (30 mL) was added over 3.5 h at 25-33 °C, along with seed. After aging overnight at RT, the resulting crystalline solution was filtered and washed with aqueous methanol and dried overnight to yield **1-2** as a yellow solid.

tert-butyl {1-[4-phenylacetyl)phenyl]cyclobutyl}carbamate (**1-3**)

Benzyl Grignard (19 mL, 38.5 mmol) was added to a stirred, slightly cloudy solution of nitrile **1-2** (3 g, 11 mmol) in THF (25 mL) cooled to ca. -20 °C at a rate such that the reaction temperature did not warm above -10 °C. The solution was aged for 3-4 hours keeping the reaction temperature between -10 °C and -20 °C. The stirred solution was cooled to -30 °C and added to a 15 wt% aqueous citric acid solution (60 mL) which was previously cooled to 5-10 °C, maintaining the temperature below 15 °C. The layers were separated and the aqueous layer was washed with MTBE.

The organic layers were combined, washed with half saturated brine (60 mL), and concentrated under reduced pressure. Heptane was added and the mixture was concentrated to a slurry which was filtered, washed with heptane (15 mL) and dried under nitrogen to give **1-3**.

4-Amino-2-chloronicotinaldehyde (**1-5c**)

Trifluoroacetic acid (17.4 mL, 234 mmol) was added carefully to a stirred mixture of Boc aldehyde **1-4** (20 g, 78.1 mmol) and dichloromethane (60 mL) keeping the temperature below 25 °C. The solution was warmed to 35 °C, aged overnight (vigorous off-gassing) and then cooled to room temperature. 25 mL of MTBE was added and the resulting white slurry was aged for one hour, filtered, and the filter cake rinsed with MTBE (10 mL x 2). Solid **1-5c** TFA salt was dried under vacuum.

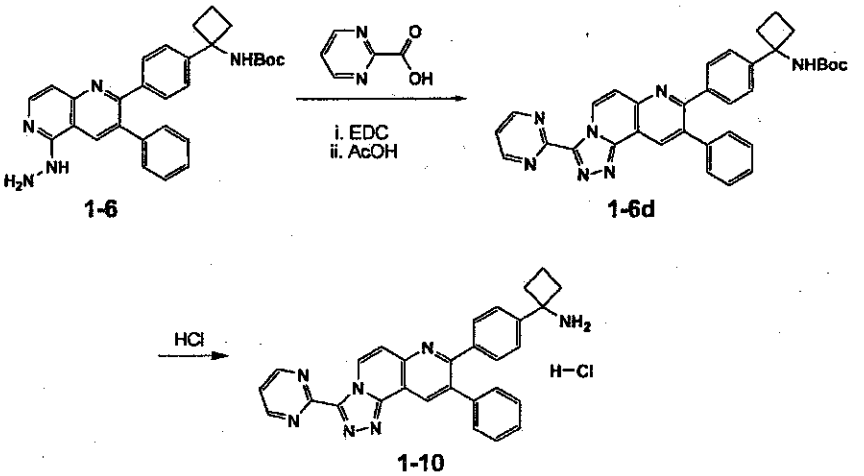
tert-butyl {1-[4-(5-chloro-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl} carbamate (**1-5**)

45wt% Potassium hydroxide solution (18 mL; 5 equiv) was added dropwise over 20 minutes to a stirred mixture of chloropyridine TFA salt **1-5c** (19.5 g), cyclobutylamino ketone **1-3** (26 g) and isopropanol (200 mL) keeping the temperature below 24 °C. After 1 h, water (100 mL) was added and after a further 1 h the resulting slurry was filtered, washing with 2:1 IPA/water (30 mL, then 24 mL) then with water (80 mL then 2 x 60 mL). The solid was dried under nitrogen flow to afford **1-5** as an off-white solid.

tert-butyl {1-[4-(5-hydrazino-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl} carbamate (**1-6**)

Anhydrous hydrazine (25 mL, 797 mmol) was added to a stirred solution of chloronaphthyridine (**1-5**) (25.12 g, 51.7 mmol) in 1,4-dioxane (200 mL) under an atmosphere of nitrogen and the reaction mixture was heated to 95°C. After 30 min the solution was cooled to room temperature, ethyl acetate (400 mL) was added and the solution was washed with a saturated solution of sodium bicarbonate, followed by water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give (**1-6**) as an orange solid.

SCHEME 1D



1-6 1-6d 1-10

tert-butyl {1-[4-(9-phenyl-3-pyrimidin-2-yl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6d**)

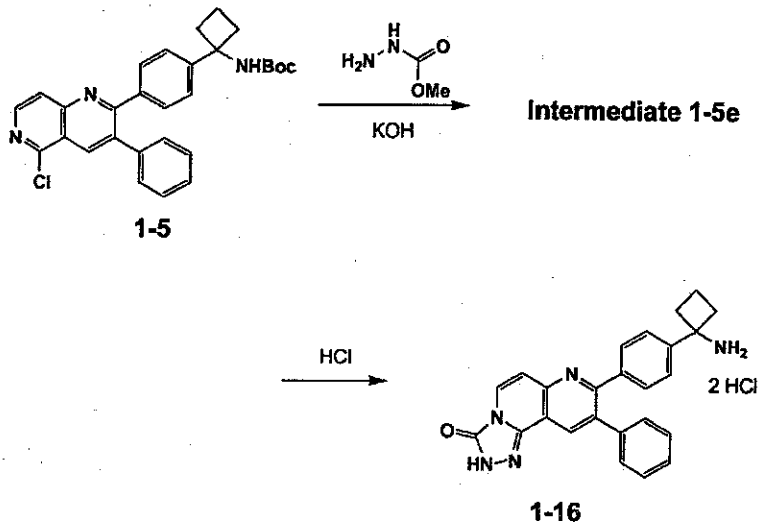
To a round bottom flask was added *tert*-butyl {1-[4-(5-hydrazino-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-6**) (267 mg, 0.554 mmol), EDC (138 mg, 0.721 mmol), HOBt (97 mg, 0.721 mmol), pyrimidine-2-carboxylic acid (89 mg, 0.721 mmol), DIPEA (0.367 mL, 2.218 mmol), and DMF (3 mL). The reaction mixture was then capped & heated under microwave irradiation at 100°C for 5 minutes. The mixture was cooled, acetic acid (0.476 mL, 8.32 mmol) was added and the reaction was then capped & heated under microwave irradiation at 80°C for 5 minutes. The reaction mixture was permitted to cool to room temperature, suspended in ethyl acetate, washed with a saturated solution of sodium bicarbonate, followed by water, brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography 0-10% IPA/DCM. The appropriate fractions were combined and the solvent was removed in vacuo to give *tert*-butyl {1-[4-(9-phenyl-3-pyrimidin-2-yl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6d**) as an orange solid. MS $(M+H)^+$: observed = 570.2, calculated = 570.7.

1-[4-(9-phenyl-3-pyrimidin-2-yl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine hydrochloride (**1-10**)

To a solution of *tert*-butyl {1-[4-(9-phenyl-3-pyrimidin-2-yl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6d**) (71 mg, 0.125 mmol) in MeOH (2 mL) and DCM (3 mL) was added a 4N solution of HCl in EtOAc (3 mL, 12 mmol). The sealed reaction mixture was permitted to stir at room temperature. After 4 hours the reaction mixture was concentrated. After 4 hours the reaction mixture was concentrated in vacuo. The resulting residue was purified by reverse phase column chromatography (Sunfire C18) eluting with 1 to 50% acetonitrile / (0.1% TFA / water) gradient. The appropriate fractions were concentrated in vacuo. The resulting residue was then dissolved in DCM (5 mL) & MeOH (5 mL), then added a 4N solution of HCl in EtOAc (5 mL, 20 mmol), then concentrated in vacuo to give 1-[4-(9-phenyl-3-pyrimidin-2-yl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutanamine hydrochloride (**1-10**) as a tan solid. HRMS $(M+H)^+$: observed = 470.2098, calculated = 470.2088.

SCHEME 1E

(ALTERNATE SYNTHESIS FOR COMPOUND 1-16)



Intermediate (1-5e)

A stirred slurry of chloronapthyridine **1-5** (1.8 g), methyl hydrazine carboxylate (0.318 g) and isopropanol (20 L) is warmed to 66 °C before becoming homogeneous. 5-6 N HCl in IPA (0.05 ml) is added and the temperature is increased to 70 °C for 16 hours and then is cooled to RT. After cooling to RT, 45wt% potassium hydroxide solution (0.52 mL) is mixed with water (5.5 mL) and added over 15 minutes. After 30 minutes, aqueous acetic acid (0.7 mL in 6 mL water) is added followed by water (2 mL). The resulting slurry is aged at RT for three hours, filtered and washed with 1:1 IPA/water (2 x 2.4 mL). The product is dried under nitrogen flow then slurried in methylene chloride at 20 °C for 4 hours, filtered and dried under nitrogen flow to afford **1-5e** as an off-white solid.

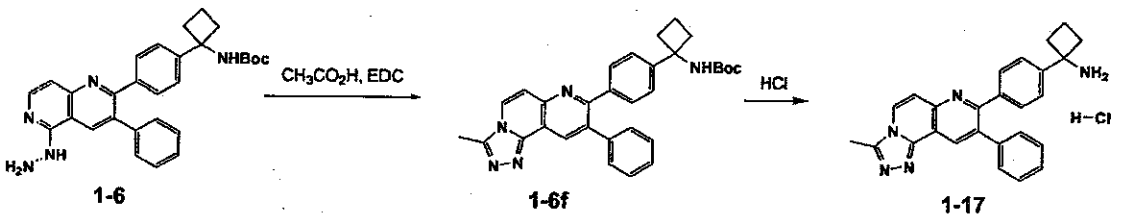
8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-3(2*H*)-one (**1-16**)

A solution of aqueous concentrated HCl (12.1 M, 1.64 mL) in ethanol (2.0 mL) was added dropwise over 30 min to a stirred slurry of **1-5e** (500 mg, 0.985 mol) in ethanol (1.7 mL) and water (0.2 mL) at 50°C. After 3 hours following acid addition, the mixture was seeded and aged overnight at 50°C, cooled to room temperature and filtered. Acetyl chloride (0.5 g, 7 mmol) was added over 1 h to ethanol (2 mL) at 0°C. The solution was then cooled to room temperature and aged for 30 minutes. The filter cake was washed with this solution (1 mL x 2), then with ethyl acetate (4 mL x 2) and dried, finally in a vacuum oven at 75.0°C with nitrogen sweep (50 torr) until complete conversion from **1-16** Form IV to Form II is seen by XRPD.

The method employed in Scheme 1A gave a crystalline bis-HCl salt **1-16** denoted Form I. The procedures described in Scheme 1E produced three new polymorphs of the bis-HCl salt **1-16**, denoted Forms II, III and IV. It was found that drying either Form III or Form IV under reduced pressure with a nitrogen sweep converted them entirely to Form II. Slurrying a mixture of Form I and II in acidic ethanol produced Form III in the slurry. Isolating Form III and drying as described above produces Form II.

A mono-HCl version of **1-16** was also produced via dissolution in water. After 6 hours, the aqueous slurry turns light yellow and is filtered. Silver chloride titration of this solid reveals the presence of one equivalent of chloride. Finally, treating the bis-HCl salt **1-16** with aqueous KOH (2 equiv) produces neutral **1-16** as determined by chloride titration.

SCHEME 1F



tert-butyl {1-[4-(3-methyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6f**)

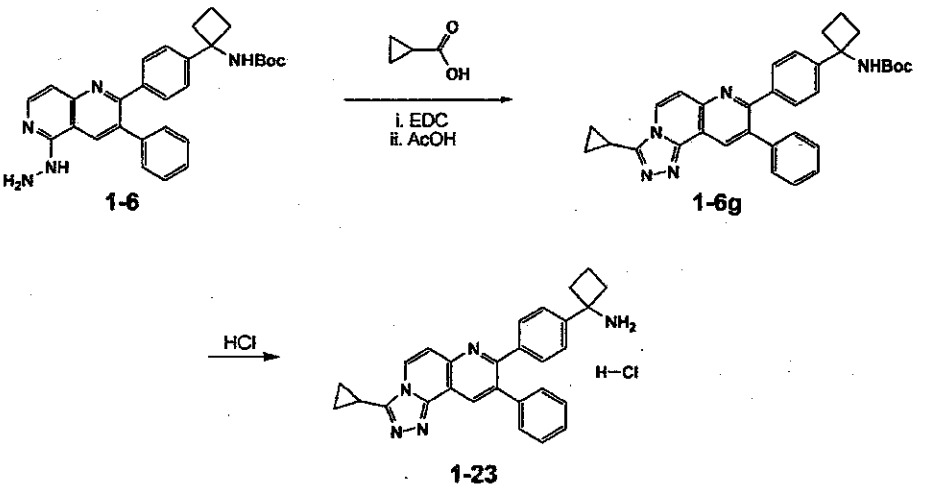
To a round bottom flask was added EDC (4.63 g, 24.17 mmol), HOBt (3.27 g, 24.17 mmol), anhydrous DCM (50 mL) and acetic acid (13.2 mL, 13.86 mmol). The reaction mixture was then stirred at room temperature under an atmosphere of nitrogen for 30 minutes. Then added added *tert*-butyl {1-[4-(5-hydrazino-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-6**) (10.58 g, 21.97 mmol). The reaction mixture was then permitted to stir overnight (18 hours) at room temperature under an atmosphere of nitrogen, then heated to 80°C for 2 hours. The reaction mixture was then suspended in ethyl acetate, slowly poured into a saturated solution of sodium bicarbonate, followed by water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography 1-20% IPA/DCM. The appropriate fractions were combined and the solvent was removed in vacuo to give *tert*-butyl {1-[4-(3-methyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-

naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6f**) as a tan solid. MS $(M+H)^+$: observed = 506.2, calculated = 506.6.

1-[4-(3-methyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine hydrochloride (**1-17**)

To a solution of *tert*-butyl {1-[4-(3-methyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6f**) (9.23 g, 18.26 mmol) in MeOH (20 mL) and DCM (100 mL) was added a 4N solution of HCl in EtOAc (91 mL, 365 mmol). The sealed reaction mixture was permitted to stir at room temperature. After 18 hours the reaction mixture was filtered to give 1-[4-(3-methyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutanamine hydrochloride (**1-17**) as an off-white solid. HRMS $(M+H)^+$: observed = 406.2047, calculated = 406.2026.

SCHEME 1G



tert-butyl {1-[4-(3-cyclopropyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (**1-6g**)

To a round bottom flask was added EDC (535 mg, 2.79 mmol), HOBt (377 mg, 2.79 mmol), anhydrous DCM (17.5 mL), anhydrous NMP (.2.5 mL), and cyclopropanecarboxylic acid (248 mg, 5.40 mmol). The reaction mixture was then stirred at room temperature under an atmosphere of nitrogen for 30 minutes. Then added added *tert*-butyl {1-[4-(5-hydrazino-3-phenyl-1,6-naphthyridin-2-yl)phenyl]cyclobutyl}carbamate (**1-6**) (1.221 g, 2.54 mmol). The reaction mixture was then permitted to stir overnight (18 hours) at room temperature under an atmosphere of nitrogen. Acetic acid (2.178 mL, 38.0 mmol) was added and the reaction mixture was heated in a hot oil bath at 80°C for 2 hours. The reaction mixture was permitted to cool to room temperature and ethyl acetate was added and the mixture was washed with a saturated solution of sodium bicarbonate, water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography 90% EtOAc/DCM (isocratic). The appropriate fractions were combined and the solvent was removed in vacuo to give *tert*-butyl {1-[4-(3-cyclopropyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-

8-yl)phenyl]cyclobutyl}carbamate (**1-6g**) as a pink solid. HRMS $(M+H)^+$: observed = 532.2729, calculated = 532.2707.

1-[4-(3-cyclopropyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutanamine hydrochloride (**1-23**)

To a solution of *tert*-butyl {1-[4-(3-cyclopropyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl}carbamate (MJK-9) (899.4 mg, 18.26 mmol) in MeOH (5 mL) and DCM (15 mL) was added a 4N solution of HCl in EtOAc (4.23 mL, 16.92 mmol). The sealed reaction mixture was permitted to stir at room temperature. After 18 hours the reaction mixture was filtered to give 1-[4-(3-methyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutanamine hydrochloride (**1-23**) as a yellow solid. HRMS $(M+H)^+$: observed = 432.2186, calculated = 432.2183.

EXAMPLE 1

Cloning of the human Akt isoforms and ΔPH-Akt1

The pS2neo vector (deposited in the ATCC on April 3, 2001 as ATCC PTA-3253) was prepared as follows: The pRmHA3 vector (prepared as described in *Nucl. Acid Res.* 16:1043-1061 (1988)) was cut with BglII and a 2734 bp fragment was isolated. The pUChsneo vector (prepared as described in *EMBO J.* 4:167-171 (1985)) was also cut with BglII and a 4029 bp band was isolated. These two isolated fragments were ligated together to generate a vector termed pS2neo-1. This plasmid contains a polylinker between a metallothionine promoter and an alcohol dehydrogenase poly A addition site. It also has a neo resistance gene driven by a heat shock promoter. The pS2neo-1 vector was cut with Psp5II and BsiWI. Two complementary oligonucleotides were synthesized and then annealed (CTGCGGCCGC (SEQ.ID.NO.: 1) and GTACGCGGCCGCAG (SEQ.ID.NO.: 2)). The cut pS2neo-1 and the annealed oligonucleotides were ligated together to generate a second vector, pS2neo. Added in this conversion was a NotI site to aid in the linearization prior to transfection into S2 cells.

Human Akt1 gene was amplified by PCR (Clontech) out of a human spleen cDNA (Clontech) using the 5' primer: 5'CGCGAATTCAGATCTACCATGAGCGACGTGGCTATTGTG 3' (SEQ.ID.NO.: 3), and the 3' primer: 5'CGCTCTAGAGGATCCTCAGGCCGTGCTGCTGGC3' (SEQ.ID.NO.: 4). The 5' primer included an EcoRI and BglII site. The 3' primer included an XbaI and BamHI site for cloning purposes. The resultant PCR product was subcloned into pGEM3Z (Promega) as an EcoRI/Xba I fragment. For expression/purification purposes, a middle T tag was added to the 5' end of the full length Akt1 gene using the PCR primer: 5'GTACGATGCTGAACGATATCTTCG 3' (SEQ.ID.NO.: 5). The resulting PCR product encompassed a 5' KpnI site and a 3' BamHI site which were used to subclone the fragment in frame with a biotin tag containing insect cell expression vector, pS2neo.

For the expression of a pleckstrin homology domain (PH) deleted (Δaa 4-129, which includes deletion of a portion of the Akt1 hinge region) version of Akt1, PCR deletion

mutagenesis was done using the full length Akt1 gene in the pS2neo vector as template. The PCR was carried out in 2 steps using overlapping internal primers (5'GAATACATGCCGATGGAAAGCGACGGGGCTGAAGAGATGGAGGTG 3' (SEQ.ID.NO.: 6), and 5'CCCCTCCATCTCTTCAGCCCCGTCGCTTTCCATCGGCATG TATTC 3' (SEQ.ID.NO.: 7)) which encompassed the deletion and 5' and 3' flanking primers which encompassed the KpnI site and middle T tag on the 5' end. The final PCR product was digested with KpnI and SmaI and ligated into the pS2neo full length Akt1 KpnI/SmaI cut vector, effectively replacing the 5' end of the clone with the deleted version.

Human Akt3 gene was amplified by PCR of adult brain cDNA (Clontech) using the amino terminal oligo primer:
5' GAATTCAGATCTACCATGAGCGATGTTACCATTGTG 3' (SEQ.ID.NO.: 8); and the carboxy terminal oligo primer :
5' TCTAGATCTTATTCTCGTCCACTTGCAGAG 3'(SEQ.ID.NO.: 9).
These primers included a 5' EcoRI/BglII site and a 3' XbaI/BglII site for cloning purposes. The resultant PCR product was cloned into the EcoRI and XbaI sites of pGEM4Z (Promega). For expression/purification purposes, a middle T tag was added to the 5' end of the full length Akt3 clone using the PCR primer:
5'GGTACCATGGAATACATGCCGATGGAAAGCGATGTTACCATTGTGAAG 3'(SEQ.ID.NO.: 10). The resultant PCR product encompassed a 5' KpnI site which allowed in frame cloning with the biotin tag containing insect cell expression vector, pS2neo.

Human Akt2 gene was amplified by PCR from human thymus cDNA (Clontech) using the amino terminal oligo primer:
5' AAGCTTAGATCTACCATGAATGAGGTGTCTGTC 3' (SEQ.ID.NO.: 11); and the carboxy terminal oligo primer: 5'GAATTCGGATCCTCACTCGCGGATGCTGGC 3' (SEQ.ID.NO.: 12). These primers included a 5' HindIII/BglII site and a 3' EcoRI/BamHI site for cloning purposes. The resultant PCR product was subcloned into the HindIII/EcoRI sites of pGem3Z (Promega). For expression/purification purposes, a middle T tag was added to the 5' end of the full length Akt2 using the PCR primer:
5'GGTACCATGGAATACATGCCGATGGAAAATGAGGTGTCTGTCATCAAAG 3' (SEQ.ID.NO.: 13). The resultant PCR product was subcloned into the pS2neo vector as described above.

EXAMPLE 2

Expression of human Akt isoforms and ΔPH-Akt1

The DNA containing the cloned Akt1, Akt2, Akt3 and ΔPH-Akt1 genes in the pS2neo expression vector was purified and used to transfect *Drosophila* S2 cells (ATCC) by the calcium phosphate method. Pools of antibiotic (G418, 500 μg/ml) resistant cells were selected. Cell were expanded to a 1.0 L volume (~7.0 x 10^6 / ml), biotin and $CuSO_4$ were added to a final

concentration of 50 μM and 50 mM respectively. Cells were grown for 72 h at 27°C and harvested by centrifugation. The cell paste was frozen at –70°C until needed.

EXAMPLE 3

Purification of human Akt isoforms and ΔPH-Akt1

Cell paste from one liter of S2 cells, described in Example 2, was lysed by sonication with 50 mls 1% CHAPS in buffer A: (50mM Tris pH 7.4, 1mM EDTA, 1mM EGTA, 0.2mM AEBSF, 10μg/ml benzamidine, 5μg/ml of leupeptin, aprotinin and pepstatin each, 10% glycerol and 1mM DTT). The soluble fraction was purified on a Protein G Sepharose fast flow (Pharmacia) column loaded with 9mg/ml anti-middle T monoclonal antibody and eluted with 75 μM EYMPME (SEQ.ID.NO.: 14) peptide in buffer A containing 25% glycerol. Akt/PKB containing fractions were pooled and the protein purity evaluated by SDS-PAGE. The purified protein was quantitated using a standard Bradford protocol. Purified protein was flash frozen on liquid nitrogen and stored at -70°C.

Akt and Akt pleckstrin homology domain deletions purified from S2 cells required activation. Akt and Akt pleckstrin homology domain deletions were activated (Alessi et al. *Current Biology* 7:261-269) in a reaction containing 10 nM PDK1 (Upstate Biotechnology, Inc.), lipid vesicles (10 μM phosphatidylinositol-3,4,5-trisphosphate – Metreya, Inc, 100 μM phosphatidylcholine and 100 μM phosphatidylserine – Avanti Polar lipids, Inc.) and activation buffer (50 mM Tris pH7.4, 1.0 mM DTT, 0.1 mM EGTA, 1.0 μM Microcystin–LR, 0.1 mM ATP, 10 mM $MgCl_2$, 333 μg/ml BSA and 0.1mM EDTA). The reaction was incubated at 22°C for 4 hours. Aliquots were flash frozen in liquid nitrogen.

EXAMPLE 4

Akt Kinase Assays

Activated Akt isoforms and pleckstrin homology domain deletion constructs were assayed utilizing a GSK-derived biotinylated peptide substrate. The extent of peptide phosphorylation was determined by Homogeneous Time Resolved Fluorescence (HTRF) using a lanthanide chelate(Lance)-coupled monoclonal antibody specific for the phosphopeptide in combination with a streptavidin-linked allophycocyanin (SA-APC) fluorophore which will bind to the biotin moiety on the peptide. When the Lance and APC are in proximity (i.e. bound to the same phosphopeptide molecule), a non-radiative energy transfer takes place from the Lance to the APC, followed by emission of light from APC at 665 nm.

Materials required for the assay:

A. Activated Akt isozyme or pleckstrin homology domain deleted construct

B. Akt peptide substrate: GSK3α (S21) Peptide #3928 biotin-GGRARTSSFAEPG (SEQ.ID.NO.:15), Macromolecular Resources.

C. Lance labeled anti-phospho GSK3α monoclonal antibody (Cell Signaling Technology, clone # 27).

D. SA-APC (Prozyme catalog no. PJ25S lot # 896067).

E. Microfluor®B U Bottom Microtiter Plates (Dynex Technologies, Catalog no. 7205).

F. Discovery® HTRF Microplate Analyzer, Packard Instrument Company.

G. 100 X Protease Inhibitor Cocktail (PIC): 1 mg/ml benzamidine, 0.5 mg/ml pepstatin, 0.5 mg/ml leupeptin, 0.5 mg/ml aprotinin.

H. 10X Assay Buffer: 500 mM HEPES, pH 7.5, 1% PEG, mM EDTA, 1 mM EGTA, 1% BSA, 20 mM 9-Glycerol phosphate.

I. Quench Buffer: 50 mM HEPES pH 7.3, 16.6 mM EDTA, 0.1% BSA, 0.1% Triton X-100, 0.17 nM Lance labeled monoclonal antibody clone # 27, 0.0067 mg/ml SA-APC

J. ATP/$MgCl_2$ working solution: 1X Assay buffer, 1 mM DTT, 1X PIC, 125 mM KCl, 5% Glycerol, 25 mM $MgCl_2$, 375 TM ATP

K. Enzyme working solution: 1X Assay buffer, 1 mM DTT, 1X PIC, 5% Glycerol, active Akt. The final enzyme concentrations were selected so that the assay was in a linear response range.

L. Peptide working solution: 1X Assay buffer, 1 mM DTT, 1X PIC, 5% Glycerol, 2 TM GSK3 biotinylated peptide # 3928

The reaction is assembled by adding 16 TL of the ATP/$MgCl_2$ working solution to the appropriate wells of a 96-well microtiter plate. Inhibitor or vehicle (1.0 Tl) is added followed by 10 Tl of peptide working solution. The reaction is started by adding 13 Tl of the enzyme working solution and mixing. The reaction is allowed to proceed for 50 min and then stopped by the addition of 60 Tl HTRF quench buffer. The stopped reactions were incubated at room temperature for at least 30 min and then read on the Discovery instrument.

Procedure for Streptavidin Flash Plate Assay:

Step 1:

A 1 µl solution of the test compound in 100% DMSO was added to 20 µl of 2X substrate solution (20 uM GSK3 Peptide, 300 µM ATP, 20 mM $MgCl_2$, 20 µCi / ml [γ^{33}P] ATP, 1X Assay Buffer, 5% glycerol, 1 mM DTT, 1X PIC, 0.1% BSA and 100 mM KCl). Phosphorylation reactions were initiated by adding 19 µl of 2X Enzyme solution (6.4 nM active Akt/PKB, 1X Assay Buffer, 5% glycerol, 1 mM DTT, 1X PIC and 0.1% BSA). The reactions were then incubated at room temperature for 45 minutes.

Step 2:

The reaction was stopped by adding 170 µl of 125 mM EDTA. 200 µl of stopped reaction was transferred to a Streptavidin Flashplate® PLUS (NEN Life Sciences, catalog no. SMP103). The plate was incubated for ≥10 minutes at room temperature on a plate shaker. The contents of each well was aspirated, and the wells rinsed 2 times with 200 µl TBS per well. The wells were then washed 3 times for 5 minutes with 200 µl TBS per well with the plates incubated at room temperature on a platform shaker during wash steps.

The plates were covered with sealing tape and counted using the Packard TopCount with the appropriate settings for counting [^{33}P] in Flashplates.

Procedure for Streptavidin Filter Plate Assay:

Step 1:

The enzymatic reactions as described in Step 1 of the Streptavidin Flash Plate Assay above were performed.

Step 2:

The reaction was stopped by adding 20 µl of 7.5M Guanidine Hydrochloride. 50 µl of the stopped reaction was transferred to the Streptavidin filter plate (SAM$^{2™}$ Biotin Capture Plate, Promega, catalog no. V7542) and the reaction was incubated on the filter for 1-2 minutes before applying vacuum.

The plate was then washed using a vacuum manifold as follows: 1) 4 x 200 µl/well of 2M NaCl; 2) 6 x 200 µl/well of 2M NaCl with 1% H_3PO_4; 3) 2 x 200 µl/well of di$H_2$0; and 4) 2 x 100 µl/well of 95% Ethanol. The membranes were then allowed to air dry completely before adding scintillant.

The bottom of the plate was sealed with white backing tape, 30 µl/well of Microscint 20 (Packard Instruments, catalog no. 6013621) was added. The top of the plate was sealed with clear sealing tape, and the plate then counted using the Packard TopCount with the appropriate settings for [^{33}P] with liquid scintillant.

Procedure for Phosphocellulose Filter Plate Assay:

Step 1:

The enzymatic reactions were performed as described in Step 1 of the Streptavidin Flash Plate Assay (above) utilizing KKGGRARTSSFAEPG (SEQ.ID.NO.: 16) as the substrate in place of biotin-GGRARTSSFAEPG.

Step 2:

The reaction was stopped by adding 20 µl of 0.75% H_3PO_4. 50 µl of stopped reaction was transferred to the filter plate (UNIFILTER™, Whatman P81 Strong Cation Exchanger, White Polystyrene 96 Well Plates, Polyfiltronics, catalog no. 7700-3312) and the reaction incubated on the filter for 1-2 minutes before applying vacuum.

The plate was then washed using a vacuum manifold as follows: 1) 9 x 200 µl/well of 0.75% H_3PO_4; and 2) 2 x 200 µl/well of di$H_2$0. The bottom of the plate was sealed with white backing tape, then 30 µl/well of Microscint 20 was added. The top of the plate was sealed with clear sealing tape, and the plate counted using the Packard TopCount with the appropriate settings for [^{33}P] and liquid scintillant.

PKA assay:

Each individual PKA assay consists of the following components:

A. 5X PKA assay buffer (200 mM Tris pH7.5, 100 mM $MgCl_2$, 5mM β-mercaptoethanol, 0.5 mM EDTA)

B. 50 μM stock of Kemptide (Sigma) diluted in water

C. ^{33}P-ATP prepared by diluting 1.0 μl ^{33}P-ATP [10 mCi/ml] into 200 Tl of a 50 μM stock of unlabeled ATP

D. 10 μl of a 70 nM stock of PKA catalytic subunit (UBI catalog # 14-114) diluted in 0.5 mg/ml BSA

E. PKA/Kemptide working solution: equal volumes of 5X PKA assay buffer, Kemptide solution and PKA catalytic subunit.

The reaction is assembled in a 96 deep-well assay plate. The inhibitor or vehicle (10 Tl) is added to 10 Tl of the ^{33}P-ATP solution. The reaction is initiated by adding 30 Tl of the PKA/Kemptide working solution to each well. The reactions were mixed and incubated at room temperature for 20 min. The reactions were stopped by adding 50 Tl of 100 mM EDTA and 100 mM sodium pyrophosphate and mixing.

The enzyme reaction product (phosphorylated Kemptide) was collected on p81 phosphocellulose 96 well filter plates (Millipore). To prepare the plate, each well of a p81 filter plate was filled with 75 mM phosphoric acid. The wells were emptied through the filter by applying a vacuum to the bottom of the plate. Phosphoric acid (75 mM, 170 μl) was added to each well. A 30 μl aliquot from each stopped PKA reaction was added to corresponding wells on the filter plate containing the phosphoric acid. The peptide was trapped on the filter following the application of a vacuum and the filters were washed 5 times with 75 mM phosphoric acid. After the final wash, the filters were allowed to air dry. Scintillation fluid (30 μl) was added to each well and the filters counted on a TopCount (Packard).

PKC assay:

Each PKC assay consists of the following components:

A. 10X PKC co-activation buffer: 2.5 mM EGTA, 4mM $CaCl_2$

B. 5X PKC activation buffer: 1.6 mg/ml phosphatidylserine, 0.16 mg/ml diacylglycerol, 100 mM Tris pH 7.5, 50 mM $MgCl_2$, 5 mM β-mercaptoethanol

C. ^{33}P-ATP prepared by diluting 1.0 μl ^{33}P-ATP [10 mCi/ml] into 100μl of a 100 μM stock of unlabeled ATP

D. Myelin basic protein (350 μg/ml, UBI) diluted in water

E. PKC (50ng/ml, UBI catalog # 14-115) diluted into 0.5 mg/ml BSA

F. PKC/Myelin Basic Protein working solution: Prepared by mixing 5 volumes each of PKC co-activation buffer and Myelin Basic protein with 10 volumes each of PKC activation buffer and PKC.

The assays were assembled in 96 deep-well assay plates. Inhibitor or vehicle (10 Tl) was added to 5.0 ul of ^{33}P-ATP. Reactions were initiated with the addition of the PKC/Myelin Basic Protein working solution and mixing. Reactions were incubated at 30°C for 20 min. The reactions were stopped by adding 50 Tl of 100 mM EDTA and 100 mM sodium

pyrophosphate and mixing. Phosphorylated Mylein Basic Protein was collected on PVDF membranes in 96 well filter plates and quantitated by scintillation counting.

Compounds of the instant invention described in the Schemes and Tables were tested in the assay described above and were found to have IC_{50} of $\leq$ 50 μM against one or more of Akt1, Akt2 and Akt3.

EXAMPLE 5

Cell based Assays to Determine Inhibition of Akt/PKB

Cells (for example LnCaP or a $PTEN^{(-/-)}$tumor cell line with activated Akt/PKB) were plated in 100 mM dishes. When the cells were approximately 70 to 80% confluent, the cells were refed with 5 mls of fresh media and the test compound added in solution. Controls included untreated cells, vehicle treated cells and cells treated with either LY294002 (Sigma) or wortmanin (Sigma) at 20 μM or 200 nM, respectively. The cells were incubated for 2, 4 or 6 hrs, and the media removed, the cells were washed with PBS, scraped and transferred to a centrifuge tube. They were pelleted and washed again with PBS. Finally, the cell pellet was resuspended in lysis buffer (20 mM Tris pH8, 140 mM NaCl, 2 mM EDTA, 1% Triton, 1 mM Na Pyrophosphate, 10 mM β-Glycerol Phosphate, 10 mM NaF, 0.5 mm $NaVO_4$, 1 μM Microsystine, and 1x Protease Inhibitor Cocktail), placed on ice for 15 minutes and gently vortexed to lyse the cells. The lysate was spun in a Beckman tabletop ultra centrifuge at 100,000 x g at 4°C for 20min. The supernatant protein was quantitated by a standard Bradford protocol (BioRad) and stored at –70°C until needed.

Proteins were immunoprecipitated (IP) from cleared lysates as follows: For Akt1/PKBα, lysates are mixed with Santa Cruz sc-7126 (D-17) in NETN (100mM NaCl, 20mM Tris pH 8.0, 1mM EDTA, 0.5% NP-40) and Protein A/G Agarose (Santa Cruz sc-2003) was added. For Akt2/PKBβ, lysates were mixed in NETN with anti-Akt2 agarose (Upstate Biotechnology #16-174) and for Akt3/PKBγ, lysates were mixed in NETN with anti-Akt3 agarose (Upstate Biotechnology #16-175). The IPs were incubated overnight at 4°C, washed and seperated by SDS-PAGE.

Western blots were used to analyze total Akt, pThr308 Akt1, pSer473 Akt1, and corresponding phosphorylation sites on Akt2 and Akt3, and downstream targets of Akt using specific antibodies (Cell Signaling Technology): Anti-Total Akt (cat. no. 9272), Anti-Phopho Akt Serine 473 (cat. no. 9271), and Anti-Phospho Akt Threonine 308 (cat. no. 9275). After incubating with the appropriate primary antibody diluted in PBS + 0.5% non-fat dry milk (NFDM) at 4 °C overnight, blots were washed, incubated with Horseradish peroxidase (HRP)-tagged secondary antibody in PBS + 0.5% NFDM for 1 hour at room temperature. Proteins were detected with ECL Reagents (Amersham/Pharmacia Biotech RPN2134).

EXAMPLE 6

Heregulin Stimulated Akt Activation

MCF7 cells (a human breast cancer line that is $PTEN^{+/+}$) were plated at $1x10^6$ cells per 100mM plate. When the cells were 70 – 80% confluent, they were refed with 5 ml of serum free media and incubated overnight. The following morning, compound was added and the cells were incubated for 1 – 2 hrs, after which time heregulin was added (to induce the activation of Akt) for 30 minutes and the cells were analyzed as described above.

EXAMPLE 7

Inhibition Of Tumor Growth

In vivo efficacy of an inhibitor of the growth of cancer cells may be confirmed by several protocols well known in the art.

Human tumor cell lines which exhibit a deregulation of the PI3K pathway (such as LnCaP, PC3, C33a, OVCAR-3, MDA-MB-468, A2780 or the like) are injected subcutaneously into the left flank of 6-10 week old female nude (also male mice [age 10-14 weeks] are used for prostate tumor xenografts [LnCaP and PC3]) mice (Harlan) on day 0. The mice are randomly assigned to a vehicle, compound or combination treatment group. Daily subcutaneous administration begins on day 1 and continues for the duration of the experiment. Alternatively, the inhibitor test compound may be administered by a continuous infusion pump. Compound, compound combination or vehicle is delivered in a total volume of 0.2 ml. Tumors are excised and weighed when all of the vehicle-treated animals exhibited lesions of 0.5 - 1.0 cm in diameter, typically 4 to 5.5 weeks after the cells were injected. The average weight of the tumors in each treatment group for each cell line is calculated.

EXAMPLE 8

Spot Multiplex Assay

This procedure describes a sandwich immunoassay used to detect multiple phosphorylated proteins in the same well of a 96 well format plate. Cell lysates are incubated in 96-well plates on which different capture antibodies are placed on spatially distinct spots in the same well. Phoshorylation-specific rabbit polyclonal antibodies are added and the complex is detected by an anti-rabbit antibody labeled with an electrochemiluminescent tag.

96-Well LNCaP plates +/- Compounds:

Spin in Beckman J6 1200 rpm 10 min, aspirate media. Add 50µl/well: TBS (Pierce #28376-20mM Tris pH 7.5, 150mM NaCl) + 1% Triton X-100 + Protease and Phosphatase Inhibitors. Wrap in plastic wrap, place in -70°C freezer until completely frozen. Block Multiplex Plates (Meso Scale Discovery, Gaithersburg, MD) with 3% Blocker A in 1X Tris Wash Buffer, 150µl/well. Cover with plate sealer, incubate on Micromix shaker RT 2h (minimum). Wash with 1X RCM 51 (TTBS). Thaw cell lysate plates on ice, add 40µl lysate/well into blocked plates. Cover with plate sealer, incubate on Micromix shaker 4°C O/N, Wash with 1X RCM 51. Dilute Secondary Antibodies in 1% Blocker A in 1X Tris Wash Buffer: Anti phospho AKT (T308), Anti phospho Tuberin (T1462), alone or in combination. Add 25µl/well, cover with plate sealer, incubate on Micromix shaker RT 3h. Wash with 1X RCM 51.

Dilute Ru-GAR in 1% Blocker A in 1X Tris Wash Buffer. Add 25µl/well, cover with plate sealer, incubate on Micromix shaker RT 1h. Wash with 1X RCM 51. Dilute 4X Read Buffer T to 1X with Water, add 200µl diluted Read Buffer/well

Read on Sector 6000 Imager.

Protease and Phosphatase Inhibitors:

Microcystin-LR, Calbiochem # 475815 to 1 µM final concentration (stock=500µM)

Calbiochem # 524624, 100X Set I

Calbiochem # 524625, 100X Set II

Calbiochem # 539134, 100X Set III

Anti Phospho AKT (T308):

Cell Signaling Technologies # 9275

Anti Phospho Tuberin (T1462):

Covance Affinity Purified (Rabbits MS 2731/2732)

Ru-GAR = Ruthenylated Goat anti Rabbit

10X Tris Wash Buffer, Blocker A and 4X Read Buffer T

10X RCM 51 (10X TTBS, RCM 51)

1X = 20mM Tris pH 7.5, 140mM NaCl, 0.1% Tween-20

EXAMPLE 9

Cell-Based (In-vivo) Assay

This procedure describes a cell-based (in vivo) activity assay for the Akt serine/threonine kinase. Activated endogenous Akt is capable of phosphorylatinga specific Akt substrate (GSK3β) peptide which is biotinylated. Detection is performed by Homogeneous Time Resolved Fluorescence (HTRF) using a Europium Kryptate [Eu(K)] coupled antibody specific for the phosphopeptide and streptavidin linked XL665 fluorophore which will bind to the biotin moiety on the peptide. When the [Eu(K)] and XL665 are in proximity (i.e. bound to the same phosphopeptide molecule) a non-radiative energy transfer takes place from the Eu(K) to the XL665, followed by emission of light from XL665 at 665 nm.

The assay can be used to detect inhibitors of all three Akt isozymes (Akt1, Akt2, and Akt3) from multiple different species if specific antibodies to each exist.

MATERIALS AND REAGENTS

A. Cell Culture Microtiter Flat Bottom 96 well plates, Corning Costar, Catalog no. 3598

B. Reacti-Bind Protein A Coated 96-well plates, Pierce, Catalog no 15130.

C. Reacti-Bind Protein G Coated 96-well plates, Pierce, Catalog no 15131.

D. Micromix 5 Shaker.

E. Microfluor®B U Bottom Microtiter Plates, Dynex Technologies, Catalog no. 7205.

F. 96 Well Plate Washer, Bio-Tek Instruments, Catalog no. EL 404.

G. Discovery® HTRF Microplate Analyzer, Packard Instrument Company.

BUFFER SOLUTIONS

A. IP Kinase Cell Lysis Buffer: 1X TBS; 0.2% Tween 20; 1X Protease Inhibitor Cocktail III (Stock is 100X, Calbiochem, 539134); 1X Phosphatase Inhibitor Cocktail I (Stock is 100X, Calbiochem, 524624); and 1X Phosphatase Inhibitor Cocktail II (Stock is 100X, Calbiochem, 524625).

B. 10X Assay Buffer: 500 mM Hepes pH 7.5; 1% PEG; 1 mM EDTA; 1 mM EGTA; and 20 mM β-glycerophosphate.

C. IP Kinase Assay Buffer: 1X Assay Buffer; 50 mM KCl; 150 μM ATP; 10 mM $MgCl_2$; 5% Glycerol; 1 mM DTT; 1 Tablet Protease Inhibitor Cocktail per 50 ml Assay Buffer; and 0.1% BSA

D. GSK3β Substrate Solution: IP Kinase Assay Buffer; and 500 nM Biotinylated GSK3β peptide.

E. Lance Buffer: 50 mM Hepes pH 7.5; 0.1% BSA; and 0.1%Triton X-100.

F. Lance Stop Buffer: Lance Buffer; and 33.3 mM EDTA.

G. Lance Detection Buffer: Lance Buffer; 13.3 μg/ml SA-APC; and 0.665 nM EuK Ab a-phospho (Ser-21) GSK3ß

Multi-Step Immunoprecipitation Akt Kinase Assay

Day1

A. Seed C33a cells Step: Plate 60,000 C33a cells/well in 96 well microtiter plate.

B. Incubate cells overnight at 37°C.

Day 2

D. Compound Addition Step: Add compounds in fresh media (alpha-MEM/10% FBS, room temp) to 96 well plate from above and incubate for 5 hrs in tissue culture incubator.

E. Cell Lysis Step: Aspirate media and add 100 μl of IP Kinase Cell Lysis Buffer.

F. Freeze 96 well microtiter plate at -70°C (NOTE: This step can be done for a minimum of 1 hour or overnight.)

Day 3

G. Coat Protein A/G 96 well plate Step: Add appropriate concentration of α-Akt antibody (Akt1, Akt2, or Akt3) in a 100 μl of PBS to the following wells:

α-Akt 1 (20 ng/well/100ul)	B2 >>>>>> B10 / rows B – G / Akt1 plate
α-Akt 2 (50 ng/well/100ul)	B2 >>>>>> B10 / rows B – G / Akt2 plate
Rabbit-IgG (150 ng/well/100 ul):	B11 – G11 on every plate (Akt1 and Akt2)

H. Incubate in the cold room (+4°C) for 4 hours on the Micromix 5 (Form 20; Attitude 2) (NOTE: Attitude depends on which Micromix 5 machine).

I. Aspirate off α-Akt antibody solution and add 100 μl of PBS to each well.

J. Akt Immunoprecipitation Step: To the 100 μl of PBS from Step(I) add 5 μl of thawed cell lystate for Akt1 plates and 10 μl of thawed cell lysate for Akt2 plates. NOTE: Thaw cell lysate on ice. Mix thawed lysate by pipetting up & down 10X before transferring to antibody plates.

Keep the cell lysate plates on ice. After transfer of cell lysate to the antibody plates refreeze the cell lysate plates at -70°C.

K. Incubate in the cold room (+4°C) overnight on Micromix 5 shaker (form 20, attitude 3).

Day 4

L. Immunoprecipitation Plate Wash Step: Wash 96 well plates 1X with TTBS (RCM 51, 1X = 2 cycles) using the 96-Well Plate Washer. Fill wells with TTBS and incubate for 10 minutes. Wash 96 well plates 2X with TTBS. (NOTE: Prime plate washer before use: 1. Check buffer reservoirs, making sure they are full and 2. empty waste containers.

M. Manual Plate Wash Step: Add 180 µl of IP Kinase Assay buffer.

N. Start Akt Enzyme Reaction: Aspirate supernatant. Add 60 µl of GSK3β Substrate Solution.

O. Incubate for 2.5 hours on Micromix 5 shaker @ RT. NOTE: Time of incubation should be adjusted so that the ratio of Column 10 /Column 11 is not >10.

P. Combine 30 µl of Lance Detection Buffer with 30 µl of Lance Stop Buffer (60 µl total/well) and add to Microfluor U bottom 96 well black plates.

Q. Stop Akt Enzyme Reaction: Transfer 40 µl of Akt Enzyme Reaction Mix from Protein A/G 96 well plate from Step (O) to Microfluor U bottom 96 well black plates from Step (P).

U. Incubate at room temperature for 1-2 hrs on Micromix 5 shaker (form 20, attitude 3), then read with the Discovery HTRF Microplate Analyzer using Akt program.

IP Kinase Cell Lysis Buffer

100 µl per well

	8 ml (1 Plate)		45 ml (6 Plates)
1X TBS	7744 µl		NA
Tween 20	20 µl		NA
1X Protease Inhibitor Cocktail III	80 µl		NA
1X Phosphatase Inhibitor Cocktail I	80 µl		450 µl 450 µl
1X Phosphatase Inhibitor Cocktail II	80 µl		450 µl
Microcystin LR (500X)			90 µl

IP Kinase Assay Buffer

100 µl per well

	8 ml (1 Plate)		50 ml (3 Plates)

10X Assay Buffer	800 µl		5 ml
1 M KCl	400 µl		2.5 ml
250 mM ATP	4.8 µl		30 µl
1M $MgCl_2$	80 µl		500 µl
Glycerol	400 µl		2.5 ml
1M DTT	8 µl		50 µl
Protease Inhibitor Cocktail	1 tablet/50 ml		1
10% BSA	80 µl		500 µl
di dH_20	6227.2 µl		38.9 ml

GSK3ß Substrate Solution

60 µl per well

	5 ml (1 Plate)		7 ml
IP Kinase Assay Buffer	5 ml		-
1 mM GSK3ß peptide	2.5 µl		3.5 µl

Lance Stop Buffer

30 µl per well

	3 ml (1 Plate)	5 ml	5 ml
1X Lance Buffer	2800.2 µl		
EDTA 0.5 M	199.8 µl		

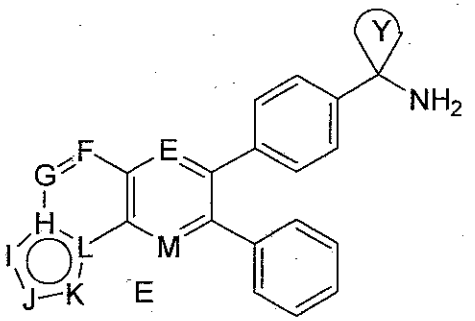
Lance Detection Buffer

30 µl per well

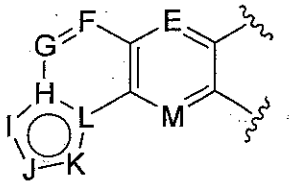
	3 ml (1 Plate)		5 ml
SA-APC (1 mg/ml in ddH2O, dilute 1/75.2 in Lance Buffer)	40 µl		66.7 µl
EuK Ab a-phospho (Ser 21)GSK3ß (680 nM, dilute 1/1133 in Lance Buffer)	2.7 µl		4.5 µl

WHAT IS CLAIMED IS:

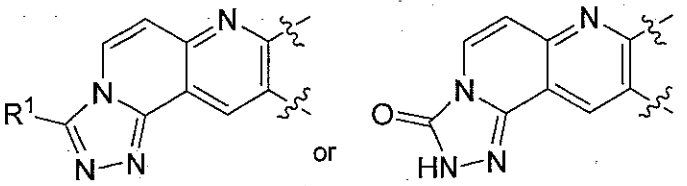
1. A compound according to Formula E:



wherein:



is



Ring Y is cyclobutyl;
R1 is H, pyrimidyl, methylimidazole, OH, methyl or cyclopropyl;
or a pharmaceutically acceptable salt thereof.

2. A compound which is:

1-{4-[3-(1-methyl-1*H*-imidazol-4-yl)-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl]phenyl}cyclobutanamine;

or a pharmaceutically acceptable salt thereof.

3. The compound of claim 2, wherein said pharmaceutically acceptable salt is 1-{4-[3-(1-methyl-1*H*-imidazol-4-yl)-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl]phenyl}cyclobutanamine dihydrochloride.

4. A compound which is:

tert-butyl {1-[4-(9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl]cyclobutyl} carbamate;

or a pharmaceutically acceptable salt thereof.

5. A compound which is:

1-[4-(9-phenyl-3-pyrimidin-2-yl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine;

or a pharmaceutically acceptable salt thereof.

6. A compound which is:

8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-3(2*H*)-one;

or a pharmaceutically acceptable salt thereof.

7. A compound which is:

1-[4-(3-methyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine;

or a pharmaceutically acceptable salt thereof.

8. A compound which is:

1-[4-(3-cyclopropyl-9-phenyl[1,2,4]triazolo[3,4-*f*]-1,6-naphthyridin-8-yl)phenyl] cyclobutanamine;

or a pharmaceutically acceptable salt thereof.

9. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 1.

10. The use of the compound according to Claim 1 for the preparation of a medicament useful in the treatment or prevention of cancer in a mammal in need of such treatment.