

THIAZOLIDINEDIONE DERIVATIVES AS PI3 KINASE INHIBITORS**Field of the Invention**

This invention relates to the use of thiazolidinedione derivatives for the modulation, notably the inhibition of the activity or function of the phosphoinositide 3' OH kinase family (hereinafter PI3 kinases), suitably, PI3K α , PI3K δ , PI3K β , and/or PI3K γ . Suitably, the present invention relates to the use of thiazolidinediones in the treatment of one or more disease states selected from: autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan failure, kidney diseases, platelet aggregation, cancer, sperm motility, transplantation rejection, graft rejection and lung injuries.

Background of the Invention

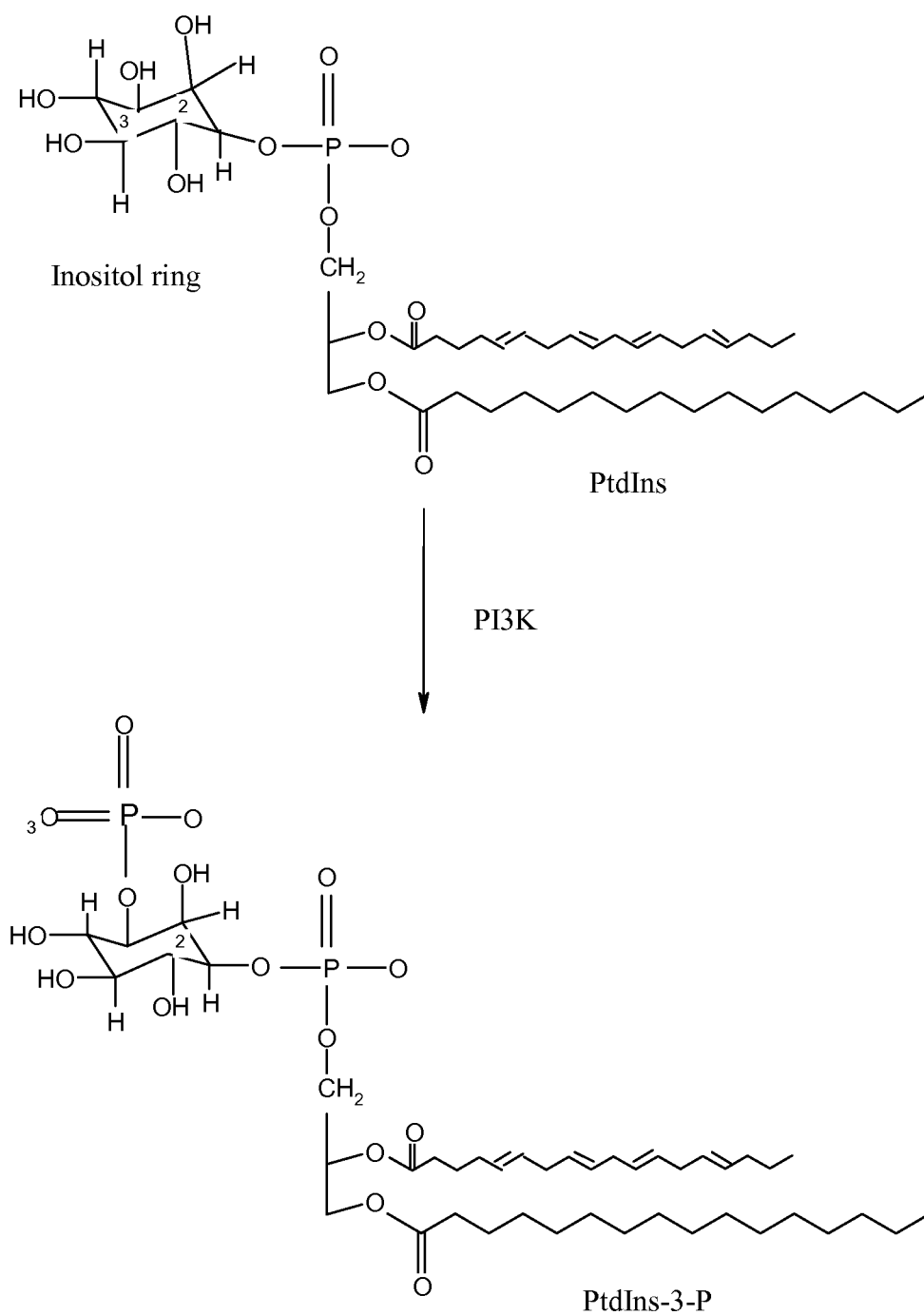
Cellular membranes represent a large store of second messengers that can be enlisted in a variety of signal transduction pathways. In regards function and regulation of effector enzymes in phospholipids signaling pathways, these enzymes generate second messengers from the membrane phospholipid pools (class I PI3 kinases (e.g. PI3K α)) are dual-specificity kinase enzymes, meaning they display both: lipid kinase (phosphorylation of phosphoinositides) as well as protein kinase activity, shown to be capable of phosphorylation of protein as substrate, including auto-phosphorylation as intramolecular regulatory mechanism. These enzymes of phospholipids signaling are activated in response to a variety of extracellular signals such as growth factors, mitogens, integrins (cell-cell interactions) hormones, cytokines, viruses and neurotransmitters such as described in Scheme A hereinafter and also by intracellular regulation by other signaling molecules (cross-talk, where the original signal can activate some parallel pathways that in a second step transmit signals to PI3Ks by intra-cellular signaling events), such as small GTPases, kinases or phosphatases for example. Intracellular regulation can also occur as a result of aberrant expression or lack of expression of cellular oncogenes or tumor suppressors. The inositol phospholipid (phosphoinositides) intracellular signaling pathways begin with activation of a signaling molecules (extra cellular ligands, stimuli, receptor dimerization, transactivation by heterologous receptor (e.g. receptor tyrosine kinase)) the recruitment and activation of PI3K

including the involvement of G-protein linked transmembrane receptor integrated into the plasma membrane.

PI3K converts the membrane phospholipids PI(4,5)P₂ into PI(3,4,5)P₃ that functions as a second messenger. PI and PI(4)P are also substrates of PI3K and
 5 can be phosphorylated and converted into PI3P and PI(3,4)P₂, respectively. In addition, these phosphoinositides can be converted into other phosphoinositides by 5'-specific and 3'-specific phosphatases, thus PI3K enzymatic activity results either directly or indirectly in the generation of two 3' -phosphoinositide subtypes that function as 2nd messengers in intra-cellular signal transduction pathways (Trends
 10 Biochem. Sci. 22(7) p.267-72 (1997) by Vanhaesebroeck et al.; Chem. Rev. 101(8) p.2365-80 (2001) by Leslie et al (2001); Annu. Rev. Cell.Dev. Biol. 17p, 615-75 (2001) by Katso et al. and Cell. Mol. Life Sci. 59(5) p.761-79 (2002) by Toker et al.). Multiple PI3K isoforms categorized by their catalytic subunits, their regulation by corresponding regulatory subunits, expression patterns and signaling-specific
 15 functions (p110α, β, δ and γ) perform this enzymatic reaction (Exp. Cell. Res. 25 (1) p. 239-54 (1999) by Vanhaesebroeck and Katso et al., 2001, above).

The closely related isoforms p110α and β are ubiquitously expressed, while δ and γ are more specifically expressed in the haematopoietic cell system, smooth muscle cells, myocytes and endothelial cells (Trends Biochem. Sci. 22(7) p.267-72
 20 (1997) by Vanhaesebroeck et al.). Their expression might also be regulated in an inducible manner depending on the cellular, tissue type and stimuli as well as disease context. Inducibility of protein expression includes synthesis of protein as well as protein stabilization that is in part regulated by association with regulatory subunits.

25 To date, eight mammalian PI3Ks have been identified, divided into three main classes (I, II, and III) on the basis of sequence homology, structure, binding partners, mode of activation, and substrate preference. In vitro, class I PI3Ks can phosphorylate phosphatidylinositol (PI), phosphatidylinositol-4-phosphate (PI4P), and phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂) to produce
 30 phosphatidylinositol-3-phosphate (PI3P), phosphatidylinositol-3,4-bisphosphate (PI(3,4)P₂), and phosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5)P₃), respectively. Class II PI3Ks phosphorylate PI and phosphatidylinositol-4-phosphate. Class III PI3Ks can only phosphorylate PI (Vanhaesebroeck et al., 1997, above; Vanhaesebroeck et al., 1999, above and Leslie et al, 2001, above)

Scheme A: Conversion of PI(4,5)P₂ to PIP₃

- 5 As illustrated in Scheme A above, phosphoinositide 3-kinases (PI3Ks) phosphorylate the hydroxyl of the third carbon of the inositol ring. The phosphorylation of phosphoinositides that generate PtdIns to 3,4,5-trisphosphate (PtdIns(3,4,5)P₃), PtdIns(3,4)P₂ and PtdIns(3)P produce second messengers for a

variety of signal transduction pathways, including those essential to cell proliferation, cell differentiation, cell growth, cell size, cell survival, apoptosis, adhesion, cell motility, cell migration, chemotaxis, invasion, cytoskeletal rearrangement, cell shape changes, vesicle trafficking and metabolic pathway (Katso et al., 2001, above and Mol. Med. Today 6(9) p. 347-57 (2000) by Stein). G-protein coupled receptors mediated phosphoinositide 3'OH-kinase activation via small GTPases such as G $\beta\gamma$ and Ras, and consequently PI3K signaling plays a central role in establishing and coordinating cell polarity and dynamic organization of the cytoskeleton – which together provides the driving force of cells to move.

- 10 Chemotaxis – the directed movement of cells toward a concentration gradient of chemical attractants, also called chemokines is involved in many important diseases such as inflammation/auto-immunity, neurodegeneration, angiogenesis, invasion/metastasis and wound healing (Immunol. Today 21(6) p. 260-4 (2000) by Wyman et al.; Science 287(5455) p. 1049-53 (2000) by Hirsch et al.; FASEB J. 15(11) p. 2019-21 (2001) by Hirsch et al. and Nat. Immunol. 2(2) p. 108-15 (2001) by Gerard et al.).

- Recent advances using genetic approaches and pharmacological tools have provided insights into signalling and molecular pathways that mediate chemotaxis in response to chemoattractant activated G-protein coupled receptors PI3-Kinase, responsible for generating these phosphorylated signalling products, was originally identified as an activity associated with viral oncoproteins and growth factor receptor tyrosine kinases that phosphorylates phosphatidylinositol (PI) and its phosphorylated derivatives at the 3'-hydroxyl of the inositol ring (Panayotou et al., Trends Cell Biol. 2 p. 358-60 (1992)). However, more recent biochemical studies revealed that, class I PI3 kinases (e.g. class IB isoform PI3K γ) are dual-specific kinase enzymes, means they display both: lipid kinase (phosphorylation of phospho-inositides) as well as protein kinase activity, shown to be capable of phosphorylation of other protein as substrates, including auto-phosphorylation as intra-molecular regulatory mechanism.

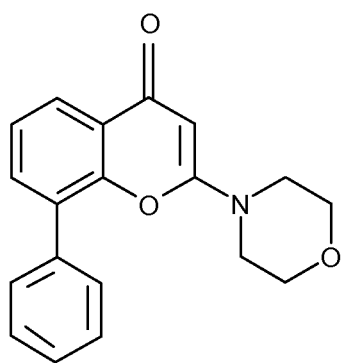
- 30 PI3-kinase activation, is therefore believe to be involved in a range of cellular responses including cell growth, differentiation, and apoptosis (Parker et al., Current Biology, 5 p. 577-99 (1995); Yao et al., Science, 267 p. 2003-05 (1995)). PI3-kinase appears to be involved in a number of aspects of leukocyte activation. A p85-associated PI3-kinase activity has been shown to physically associate with the cytoplasmic domain of CD28, which is an important costimulatory molecule for the

activation of T-cells in response to antigen (Pages et al., *Nature*, 369 p. 327-29 (1994); Rudd, *Immunity* 4 p. 527-34 (1996)). Activation of T cells through CD28 lowers the threshold for activation by antigen and increases the magnitude and duration of the proliferative response. These effects are linked to increases in the transcription of a number of genes including interleukin-2 (IL2), an important T cell growth factor (Fraser et al., *Science* 251 p. 313-16 (1991)). Mutation of CD28 such that it can longer interact with PI3-kinase leads to a failure to initiate IL2 production, suggesting a critical role for PI3-kinase in T cell activation. PI3K γ has been identified as a mediator of G beta-gamma-dependent regulation of JNK activity, and G beta-gamma are subunits of heterotrimeric G proteins (Lopez-Illasaca et al., *J. Biol. Chem.* 273(5) p. 2505-8 (1998)). Cellular processes in which PI3Ks play an essential role include suppression of apoptosis, reorganization of the actin skeleton, cardiac myocyte growth, glycogen synthase stimulation by insulin, TNF α -mediated neutrophil priming and superoxide generation, and leukocyte migration and adhesion to endothelial cells.

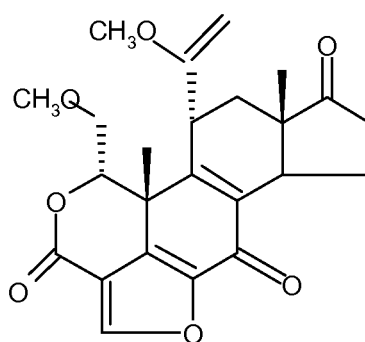
Recently, (Laffargue et al., *Immunity* 16(3) p. 441-51 (2002)) it has been described that PI3K γ relays inflammatory signals through various G(i)-coupled receptors and its central to mast cell function, stimuli in context of leukocytes, immunology includes cytokines, chemokines, adenosines, antibodies, integrins, aggregation factors, growth factors, viruses or hormones for example (*J. Cell. Sci.* 114(Pt 16) p. 2903-10 (2001) by Lawlor et al.; Laffargue et al., 2002, above and *Curr. Opin Cell Biol.* 14(2) p. 203-13 (2002) by Stephens et al.).

Specific inhibitors against individual members of a family of enzymes provide invaluable tools for deciphering functions of each enzyme. Two compounds, LY294002 and wortmannin (cf. hereinafter), have been widely used as PI3-kinase inhibitors. These compounds are non-specific PI3K inhibitors, as they do not distinguish among the four members of Class I PI3-kinases. For example, the IC₅₀ values of wortmannin against each of the various Class I PI3-kinases are in the range of 1-10 nM. Similarly, the IC₅₀ values for LY294002 against each of these PI3-kinases is about 15-20 μ M (Fruman et al., *Ann. Rev. Biochem.*, 67, p. 481-507 (1998)), also 5-10 microM on CK2 protein kinase and some inhibitory activity on phospholipases. Wortmannin is a fungal metabolite which irreversibly inhibits PI3K activity by binding covalently to the catalytic domain of this enzyme. Inhibition of PI3K activity by wortmannin eliminates subsequent cellular response to the extracellular factor. For example, neutrophils respond to the chemokine fMet-

Leu-Phe (fMLP) by stimulating PI3K and synthesizing PtdIns (3, 4, 5)P₃. This synthesis correlates with activation of the respirators burst involved in neutrophil destruction of invading microorganisms. Treatment of neutrophils with wortmannin prevents the fMLP-induced respiratory burst response (Thelen et al., Proc. Natl. Acad. Sci. USA, 91, p. 4960-64 (1994)). Indeed, these experiments with wortmannin, as well as other experimental evidence, shows that PI3K activity in cells of hematopoietic lineage, particularly neutrophils, monocytes, and other types of leukocytes, is involved in many of the non-memory immune response associated with acute and chronic inflammation.



LY294002



WORTMANNIN

Based on studies using wortmannin, there is evidence that PI3-kinase function is also required for some aspects of leukocyte signaling through G-protein coupled receptors (Thelen et al., 1994, above). Moreover, it has been shown that wortmannin and LY294002 block neutrophil migration and superoxide release. Cyclooxygenase inhibiting benzofuran derivatives are disclosed by John M. Janusz et al., in J. Med. Chem. 1998; Vol. 41, No. 18.

It is now well understood that deregulation of oncogenes and tumour-suppressor genes contributes to the formation of malignant tumours, for example by way of increase cell growth and proliferation or increased cell survival. It is also now known that signaling pathways mediated by the PI3K family have a central role in a number of cell processes including proliferation and survival, and deregulation of these pathways is a causative factor a wide spectrum of human cancers and other diseases (Katso et al., *Annual Rev. Cell Dev. Biol.*, 2001, 17: 615-617 and Foster et

al., J. Cell Science, 2003, 116: 3037-3040).

Class I PI3K is a heterodimer consisting of a p110 catalytic subunit and a regulatory subunit, and the family is further divided into class Ia and Class Ib enzymes on the basis of regulatory partners and mechanism of regulation. Class Ia enzymes consist of three distinct catalytic subunits (p110 α , p110 β , and p110 δ) that dimerise with five distinct regulatory subunits (p85 α , p55 α , p50 α , p85 β , and p55 γ), with all catalytic subunits being able to interact with all regulatory subunits to form a variety of heterodimers. Class Ia PI3K are generally activated in response to growth factor-stimulation of receptor tyrosine kinases, via interaction of the regulatory subunit SH2 domains with specific phospho-tyrosine residues of the activated receptor or adaptor proteins such as IRS-1. Small GTPases (ras as an example) are also involved in the activation of PI3K in conjunction with receptor tyrosine kinase activation. Both p110 α and p110 β are constitutively expressed in all cell types, whereas p110 δ expression is more restricted to leukocyte populations and some epithelial cells. In contrast, the single Class Ib enzyme consists of a p110 γ catalytic subunit that interacts with a p101 regulatory subunit. Furthermore, the Class Ib enzyme is activated in response to G-protein coupled receptor (GPCR) systems and its expression appears to be limited to leukocytes.

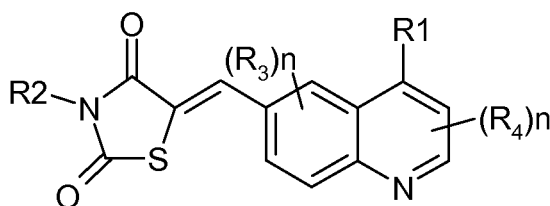
There is now considerable evidence indicating that Class Ia PI3K enzymes contribute to tumourigenesis in a wide variety of human cancers, either directly or indirectly (Vivanco and Sawyers, Nature Reviews Cancer, 2002, 2, 489-501). For example, the p110 α subunit is amplified in some tumours such as those of the ovary (Shayesteh, *et al.*, Nature Genetics, 1999, 21: 99-102) and cervix (Ma *et al.*, Oncogene, 2000, 19: 2739-2744). More recently, activating mutations within p110 α (PIK3CA gene) have been associated with various other tumors such as those of the colon and of the breast and lung (Samuels, *et al.*, Science, 2004, 304, 554). Tumor-related mutations in p85 α have also been identified in cancers such as those of the ovary and colon (Philp *et al.*, Cancer Research, 2001, 61, 7426-7429). In addition to direct effects, it is believed that activation of Class Ia PI3K contributes to tumourigenic events that occur upstream in signaling pathways, for example by way of ligand-dependent or ligand-independent activation of receptor tyrosine kinases, GPCR systems or integrins (Vara *et al.*, Cancer Treatment Reviews, 2004, 30, 193-204). Examples of such upstream signaling pathways include over-expression of the receptor tyrosine kinase Erb2 in a variety of tumors leading to activation of PI3K-mediated pathways (Harari *et al.*, Oncogene, 2000, 19, 6102-

6114) and over-expression of the oncogene Ras (Kauffmann-Zeh *et al.*, Nature, 1997, 385, 544-548). In addition, Class Ia PI3Ks may contribute indirectly to tumourigenesis caused by various downstream signaling events. For example, loss of function of the PTEN tumor-suppressor phosphatase that catalyses conversion of PI(3,4,5)P3 back to PI(4,5)P2 is associated with a very broad range of tumors via deregulation of PI3K-mediated production of PI(3,4,5)P3 (Simpson and Parsons, Exp. Cell Res., 2001, 264, 29-41). Furthermore, augmentation of the effects of other PI3K-mediated signaling events is believed to contribute to a variety of cancers, for example by activation of AKT (Nicholson and Andeson, Cellular Signaling, 2002, 14, 381-395).

In addition to a role in mediating proliferative and survival signaling in tumor cells, there is also good evidence that class Ia PI3K enzymes also contributes to tumourigenesis via its function in tumor-associated stromal cells. For examples, PI3K signaling is known to play an important role in mediating angiogenic events in endothelial cells in response to pro-angiogenic factors such as VEGF (abid *et al.*, Arterioscler. Thromb. Vasc. Biol., 2004, 24, 294-300). As Class I PI3K enzymes are also involved in motility and migration (Sawyer, Expert Opinion investing. Drugs, 2004, 13, 1-19), PI3K inhibitors are anticipated to provide therapeutic benefit via inhibition of tumor cell invasion and metastasis.

Summary of the Invention

This invention relates to novel compounds of Formula (I):



(I)

in which

R1 is heteroaryl or substituted heteroaryl;

R2 is selected from: hydrogen, C1-C6alkyl, substituted C1-C6alkyl, -COOH, aryl, substituted aryl, arylalkyl, substituted arylalkyl;

each R3 and R4 is independently selected from: hydrogen, halogen, acyl, amino,

substituted amino, C1-6alkyl, substituted C1-6alkyl, C3-7cycloalkyl, substituted C3-7cycloalkyl, C3-7heterocycloalkyl, substituted C3-7heterocycloalkyl, alkylcarboxy, aminoalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, arylcycloalkyl, substituted arylcycloalkyl, heteroarylalkyl, substituted heteroarylalkyl, cyano, hydroxyl, alkoxy, nitro, acyloxy, and aryloxy;
 5 n is 0-2;
 and/or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof.

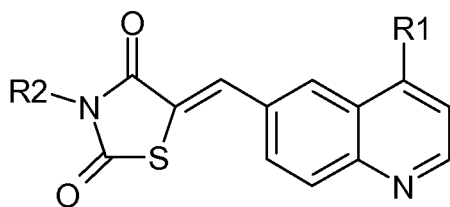
This invention also relates to a method of treating cancer, which comprises
 10 administering to a subject in need thereof an effective amount of a compound of Formula (I).

This invention also relates to a method of treating one or more disease states selected from: autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan
 15 failure, kidney diseases, platelet aggregation, sperm motility, transplantation rejection, graft rejection and lung injuries, which comprises administering to a subject in need thereof an effective amount of a compound of Formula (I).

Included in the present invention are methods of co-administering the present PI3 kinase inhibiting compounds with further active ingredients.
 20

Detailed Description of the Invention

Present compounds of Formula (I) inhibit one or more PI3 kinases. Suitably, the compounds of Formula (I) inhibit one or more PI3 kinases selected from: PI3K α , PI3K δ , PI3K β and PI3K γ .
 25 Suitably, among the compounds of Formula (I) that are active as inhibitors of PI3 kinase activity are those having Formula (II):



II

30 in which

R1 is heteroaryl or substituted heteroaryl;

R2 is selected from: hydrogen, C1-6alkyl, substituted C1-C6alkyl, -COOH, aryl, substituted aryl, arylalkyl, substituted arylalkyl; and/or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof.

Suitably, present invention includes compounds of formula (I) or (II), wherein
5 R1 is a monocyclic heteroaryl or substituted monocyclic heteroaryl.

Suitably, the present invention includes compounds of formula (I) or (II), wherein R2 is hydrogen, and R1 is a monocyclic heteroaryl or substituted monocyclic heteroaryl.

Suitably, the present invention includes compounds of formula (I) or (II),
10 wherein R1 is a optionally substituted monocyclic heteroaryl containing 1 to 2 nitrogens.

Suitably, the present invention includes compounds of formula (I) or (II), wherein R2 is hydrogen, and R1 is a optionally substituted monocyclic heteroaryl containing 1 to 2 nitrogens.

15 Suitably, the present invention includes compounds of formula (I) or (II), wherein R1 is a monocyclic heteroaryl optionally substituted with one to three substituents selected from a group consisting of: hydrogen, halogen, C1-C6alkyl, acyl, trifluoromethyl, $-(CH_2)_nCOOH$, cyano, amino, alkyl amino, nitro, hydroxyl, alkoxy, acyloxy, aryloxy, acylamino, arylamino; n is 0 to 6.

20 Suitably, the present invention includes compounds of formula (I) or (II), wherein R2 is hydrogen, and R1 is a monocyclic heteroaryl optionally substituted with one to three substituents selected from a group consisting of: hydrogen, halogen, C1-C6alkyl, acyl, trifluoromethyl, $-(CH_2)_nCOOH$, cyano, amino, alkyl amino, nitro, hydroxyl, alkoxy, acyloxy, aryloxy, acylamino, arylamino; n is 0 to 6.

25 Suitably, the present invention includes compounds of formula (I) or (II), wherein R1 is a monocyclic heteroaryl containing 1 or 2 nitrogens, optionally substituted with one to three substituents selected from a group consisting of: hydrogen, halogen, C1-C6alkyl, acyl, trifluoromethyl, $-(CH_2)_nCOOH$, cyano, amino, alkyl amino, nitro, hydroxyl, alkoxy, acyloxy, aryloxy, acylamino, arylamino; n is 0 to
30 6.

Suitably, the present invention includes compounds of formula (I) or (II), wherein R2 is hydrogen, and R1 is a monocyclic heteroaryl containing 1 or 2 nitrogens, optionally substituted with one to three substituents selected from a group consisting of: hydrogen, halogen, C1-C6alkyl, acyl, trifluoromethyl, -
35 $(CH_2)_nCOOH$, cyano, amino, alkyl amino, nitro, hydroxyl, alkoxy, acyloxy, aryloxy,

acylamino, arylamino; n is 0 to 6.

Suitably, the present invention includes compounds of formula (I) or (II), wherein R₂ is hydrogen, and R₁ is a monocyclic heteroaryl containing one nitrogen, optionally substituted with one to three substituents selected from a group
 5 consisting of: hydrogen, halogen, C₁-C₆alkyl, acyl, trifluoromethyl, -(CH₂)_nCOOH, cyano, amino, alkyl amino, nitro, hydroxyl, alkoxy, acyloxy, aryloxy, acylamino, arylamino; n is 0 to 6.

Suitably, the present invention includes compounds of formula (I) or (II), wherein R₂ is hydrogen or C₁-6alkyl, and/or a pharmaceutically acceptable salt,
 10 hydrate, solvate or pro-drug thereof.

Suitably, among the compounds of the present invention that are useful as inhibitors of PI3 kinase activity are :

(5Z)-5-{{4-(3-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-(2-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 15 (5Z)-5-{{4-[2-(methyloxy)-5-pyrimidinyl]-6-quinolinyl}methylidene)-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-[2-(methyloxy)-4-pyridinyl]-6-quinolinyl}methylidene)-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-(6-amino-3-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-
 20 2,4-dione;
 (5Z)-5-{{4-(2-oxo-1,2-dihydro-4-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-[6-(4-morpholinyl)-3-pyridinyl]-6-quinolinyl}methylidene)-1,3-thiazolidine-2,4-dione;
 25 (5Z)-5-{{4-[6-(4-methyl-1-piperazinyl)-3-pyridinyl]-6-quinolinyl}methylidene)-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-(3-pyridazinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-[2-(methyloxy)-5-pyrimidinyl]-6-quinolinyl}methylidene)-1,3-
 30 thiazolidine-2,4-dione;
 (5Z)-5-{{4-(4-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 and
 (5Z)-3-methyl-5-{{4-(4-pyridinyl)-6-quinolinyl}methylidene)-1,3-thiazolidine-2,4-dione; and/or pharmaceutically acceptable salts, hydrates, solvates or pro-
 35 drugs thereof.

This invention also relates to a method of treating cancer, which comprises co-administering to a subject in need thereof an effective amount of a compound of Formula (I), and/or a pharmaceutically acceptable salt thereof; and at least one anti-neoplastic agent such as one selected from the group consisting of: anti-
5 microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors, immunotherapeutic agents, proapoptotic agents, and cell cycle signaling inhibitors.

10 This invention also relates to a method of treating cancer, which comprises co-administering to a subject in need thereof an effective amount of a compound of Formula (I), and/or a pharmaceutically acceptable salt thereof; and at least one signal transduction pathway inhibitor such as one selected from the group consisting of: receptor tyrosine kinase inhibitor, non-receptor tyrosine kinase
15 inhibitor, SH2/SH3 domain blocker, serine/threonine kinase inhibitor, phosphatidylinositol-3 kinase inhibitor, myo-inositol signaling inhibitor, and Ras oncogene inhibitor.

As used herein, the term "effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue,
20 system, animal or human that is being sought, for instance, by a researcher or clinician. Furthermore, the term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a
25 disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function.

Compounds of Formula (I) are included in the pharmaceutical compositions of the invention.

By the term substituted amino as used herein, is meant $-NR_{30}R_{40}$ wherein
30 each R_{30} and R_{40} is independently selected from a group including hydrogen, C1-6alkyl, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, C3-C7cycloalkyl, and substituted cycloalkyl.

By the term "aryl" as used herein, unless otherwise defined, is meant
35 aromatic, hydrocarbon, ring system. The ring system may be monocyclic or fused

polycyclic (e.g. bicyclic, tricyclic, etc.). In various embodiments, the monocyclic aryl ring is C5-C10, or C5-C7, or C5-C6, where these carbon numbers refer to the number of carbon atoms that form the ring system. A C6 ring system, i.e. a phenyl ring is a suitable aryl group. In various embodiments, the polycyclic ring is a bicyclic aryl group, where suitable bicyclic aryl groups are C8-C12, or C9-C10. A naphthyl ring, which has 10 carbon atoms, is a suitable polycyclic aryl group.

By the term "heteroaryl" as used herein, unless otherwise defined, is meant an aromatic ring system containing carbon(s) and at least one heteroatom. Heteroaryl may be monocyclic or polycyclic. The monocyclic heteroaryl group may have 1 to 4 heteroatoms in the ring, where the polycyclic ring may contain fused, spiro or bridged ring junctions. Monocyclic heteroaryl rings may contain from 5 to 8 member atoms (carbons and heteroatoms). Bicyclic heteroaryl rings may contain from 8 to 12 member atoms. Exemplary heteroaryl groups include benzofuran, benzothiophene, furan, imidazole, indole, isothiazole, oxazole, piperazine, pyrazine, pyrazole, pyridazine, pyridine, pyrimidine, prrole, quinoline, quinazoline, quinoxaline, thiazole, and thiophene.

By the term "monocyclic heteroaryl" as used herein, unless otherwise defined, is meant a monocyclic heteroaryl ring containing 1-5 carbon atoms and 1-3 hetero atoms.

By the term "alkoxy" as used herein is meant -O(alkyl) where alkyl is as described herein including -OCH₃, -OCH₂CH₃ and -OC(CH₃)₃.

The term "cycloalkyl" as used herein unless otherwise defined, is meant a nonaromatic, unsaturated or saturated, cyclic or polycyclic C₃-C₁₂.

Examples of cycloalkyl and substituted cycloalkyl substituents as used herein include: cyclohexyl, aminocyclohexyl, cyclobutyl, aminocyclobutyl, 4-hydroxy-cyclohexyl, 2-ethylcyclohexyl, propyl4-methoxycyclohexyl, 4-methoxycyclohexyl, 4-carboxycyclohexyl, cyclopropyl, aminocyclopentyl, and cyclopentyl.

By the term "heterocycloalkyl" as used herein is meant a non-aromatic, unsaturated or saturated, monocyclic or polycyclic, heterocyclic ring containing at least one carbon and at least one heteroatom. Exemplary monocyclic heterocyclic rings include: piperidine, piperazine, pyrrolidine, and morpholine. Exemplary polycyclic heterocyclic rings include quinuclidine.

By the term "substituted" as used herein, unless otherwise defined, is meant that the subject chemical moiety has one or more substituents, suitably from one to

five substituents, suitably from one to three, selected from the group consisting of: hydrogen, halogen, C1-C6alkyl, amino, trifluoromethyl, $-(CH_2)_nCOOH$, C3-C7cycloalkyl, aminoalkyl, aryl, heteroaryl, arylalkyl, arylcycloalkyl, heteroarylalkyl, heterocycloalkyl, cyano, hydroxyl, alkoxy, aryloxy, acyloxy, acylamino, arylamino,
 5 nitro, oxo, $-CO_2R_{50}$, and $-CONR_{55}R_{60}$, wherein R_{50} , R_{55} and R_{60} are each independently selected from hydrogen, and alkyl; n is 0 to 6.

By the term "acyloxy" as used herein is meant $-OC(O)alkyl$ where alkyl is as described herein. Examples of acyloxy substituents as used herein include: $-OC(O)CH_3$, $-OC(O)CH(CH_3)_2$ and $-OC(O)(CH_2)_3CH_3$.

10 By the term "acylamino" as used herein is meant $-N(H)C(O)alkyl$, where alkyl is as described herein. Examples of N-acylamino substituents as used herein include: $-N(H)C(O)CH_3$, $-N(H)C(O)CH(CH_3)_2$ and $-N(H)C(O)(CH_2)_3CH_3$.

By the term "aryloxy" as used herein is meant $-O(aryl)$, $-O(substituted\ aryl)$, $-O(heteroaryl)$ or $-O(substituted\ heteroaryl)$.

15 By the term "arylamino" as used herein is meant $-NH(aryl)$, $-NH(substituted\ aryl)$, $-NH(heteroaryl)$ or $-NH(substituted\ heteroaryl)$.

By the term "heteroatom" as used herein is meant oxygen, nitrogen or sulfur.

20 By the term "halogen" as used herein is meant a substituent selected from bromide, iodide, chloride and fluoride.

By the term "alkyl" and derivatives thereof and in all carbon chains as used herein, including alkyl chains defined by the term " $-(CH_2)_n$ ", " $-(CH_2)_m$ " and the like, is meant a linear or branched, saturated or unsaturated hydrocarbon chain, and unless otherwise defined, the carbon chain will contain from 1 to 12 carbon atoms.

25 Examples of alkyl and substituted alkyl substituents as used herein include:

$-CH_3$, $-CH_2-CH_3$, $-CH_2-CH_2-CH_3$, $-CH(CH_3)_2$, $-CH_2-CH_2-C(CH_3)_3$, $-CH_2-CF_3$, $-C\equiv C-C(CH_3)_3$, $-C\equiv C-CH_2-OH$, cyclopropylmethyl, $-CH_2-C(CH_3)_2-CH_2-NH_2$, $-C\equiv C-C_6H_5$, $-C\equiv C-C(CH_3)_2-OH$, $-CH_2-CH(OH)-CH(OH)-CH(OH)-CH(OH)-CH_2-OH$, piperidinylmethyl, methoxyphenylethyl, $-C(CH_3)_3$, $-(CH_2)_3-CH_3$, $-CH_2-CH(CH_3)_2$,
 30 $-CH(CH_3)-CH_2-CH_3$, $-CH=CH_2$, and $-C\equiv C-CH_3$.

By the term "treating" and derivatives thereof as used herein, is meant prophylactic and therapeutic therapy.

By the term "co-administering" and derivatives thereof as used herein is meant either simultaneous administration or any manner of separate sequential administration of a PI3 kinase inhibiting compound, as described herein, and a further active ingredient or ingredients, known to be useful in the treatment of cancer, including chemotherapy and radiation treatment. The term further active ingredient or ingredients, as used herein, includes any compound or therapeutic agent known to or that demonstrates advantageous properties when administered to a patient in need of treatment for cancer. Suitably, if the administration is not simultaneous, the compounds are administered in a close time proximity to each other. Furthermore, it does not matter if the compounds are administered in the same dosage form, e.g. one compound may be administered topically and another compound may be administered orally.

The term "compound" as used herein includes all isomers of the compound. Examples of such isomers include: enantiomers, tautomers, rotamers.

Certain compounds described herein may contain one or more chiral atoms, or may otherwise be capable of existing as two enantiomers, or two or more diastereoisomers. Accordingly, the compounds of this invention include mixtures of enantiomers/diastereoisomers as well as purified enantiomers/diastereoisomers or enantiomerically/diastereoisomerically enriched mixtures. Also included within the scope of the invention are the individual isomers of the compounds represented by formula I or II above as well as any wholly or partially equilibrated mixtures thereof. The present invention also covers the individual isomers of the compounds represented by the formulas above as mixtures with isomers thereof in which one or more chiral centers are inverted. Further, an example of a possible tautomer is an oxo substituent in place of a hydroxy substituent. Also, as stated above, it is understood that all tautomers and mixtures of tautomers are included within the scope of the compounds of Formula I or II.

Compounds of Formula (I) are included in the pharmaceutical compositions of the invention. Where a -COOH or -OH group is present, pharmaceutically acceptable esters can be employed, for example methyl, ethyl, pivaloyloxymethyl, and the like for -COOH, and acetate maleate and the like for -OH, and those esters known in the art for modifying solubility or hydrolysis characteristics, for use as sustained release or prodrug formulations.

It has now been found that compounds of the present invention are inhibitors of the Phosphatoinositides 3-kinases (PI3Ks). When the

phosphatoinositides 3-kinase (PI3K) enzyme is inhibited by a compound of the present invention, PI3K is unable to exert its enzymatic, biological and/or pharmacological effects. The compounds of the present invention are therefore useful in the treatment of autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan failure, kidney diseases, platelet aggregation, cancer, sperm motility, transplantation rejection, graft rejection and lung injuries.

The compounds of Formula (I) are useful as medicaments in particular for the treatment of autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan failure, kidney diseases, platelet aggregation, cancer, sperm motility, transplantation rejection, graft rejection and lung injuries. According to one embodiment of the present invention, the compounds of Formula (I) are inhibitors of one or more phosphatoinositides 3-kinases (PI3Ks), suitably, Phosphatoinositides 3-kinase γ (PI3K γ), Phosphatoinositides 3-kinase α (PI3K α), Phosphatoinositides 3-kinase β (PI3K β), and/or Phosphatoinositides 3-kinase δ (PI3K δ).

Compounds according to Formula (I) are suitable for the modulation, notably the inhibition of the activity of phosphatoinositides 3-kinases (PI3K), suitably phosphatoinositides 3-kinase (PI3K α). Therefore the compounds of the present invention are also useful for the treatment of disorders which are mediated by PI3Ks. Said treatment involves the modulation – notably the inhibition or the down regulation – of the phosphatoinositides 3-kinases.

Suitably, the compounds of the present invention are used for the preparation of a medicament for the treatment of a disorder selected from multiple sclerosis, psoriasis, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, lung inflammation, thrombosis or brain infection/inflammation, such as meningitis or encephalitis, Alzheimer's disease, Huntington's disease, CNS trauma, stroke or ischemic conditions, cardiovascular diseases such as athero-sclerosis, heart hypertrophy, cardiac myocyte dysfunction, elevated blood pressure or vasoconstriction.

Suitably, the compounds of Formula (I) are useful for the treatment of autoimmune diseases or inflammatory diseases such as multiple sclerosis, psoriasis, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, lung inflammation, thrombosis or brain infection/inflammation such as meningitis or encephalitis.

Suitably, the compounds of Formula (I) are useful for the treatment of neurodegenerative diseases including multiple sclerosis, Alzheimer's disease, Huntington's disease, CNS trauma, stroke or ischemic conditions.

5 Suitably, the compounds of Formula (I) are useful for the treatment of cardiovascular diseases such as atherosclerosis, heart hypertrophy, cardiac myocyte dysfunction, elevated blood pressure or vasoconstriction.

Suitably, the compounds of Formula (I) are useful for the treatment of chronic obstructive pulmonary disease, anaphylactic shock fibrosis, psoriasis, allergic diseases, asthma, stroke, ischemic conditions, ischemia-reperfusion,
10 platelets aggregation/activation, skeletal muscle atrophy/hypertrophy, leukocyte recruitment in cancer tissue, angiogenesis, invasion metastasis, in particular melanoma, Karposi's sarcoma, acute and chronic bacterial and viral infections, sepsis, transplantation rejection, graft rejection, glomerulo sclerosis, glomerulo nephritis, progressive renal fibrosis, endothelial and epithelial injuries in the lung,
15 and lung airway inflammation.

Because the pharmaceutically active compounds of the present invention are active as PI3 kinase inhibitors, particularly the compounds that inhibit PI3K α , either selectively or in conjunction with one or more of PI3K δ , PI3K β , and/or PI3K γ , they exhibit therapeutic utility in treating cancer.

20 Suitably, the invention relates to a method of treating cancer in a mammal, including a human, wherein the cancer is selected from: brain (gliomas), glioblastomas, leukemias, Bannayan-Zonana syndrome, Cowden disease, Lhermitte-Duclos disease, breast, inflammatory breast cancer, Wilm's tumor, Ewing's sarcoma, Rhabdomyosarcoma, ependymoma, medulloblastoma, colon,
25 head and neck, kidney, lung, liver, melanoma, ovarian, pancreatic, prostate, sarcoma, osteosarcoma, giant cell tumor of bone and thyroid.

Suitably, the invention relates to a method of treating cancer in a mammal, including a human, wherein the cancer is selected from: Lymphoblastic T cell leukemia, Chronic myelogenous leukemia, Chronic lymphocytic leukemia, Hairy-cell
30 leukemia, acute lymphoblastic leukemia, acute myelogenous leukemia, Chronic neutrophilic leukemia, Acute lymphoblastic T cell leukemia, Plasmacytoma, Immunoblastic large cell leukemia, Mantle cell leukemia, Multiple myeloma Megakaryoblastic leukemia, multiple myeloma, Acute megakaryocytic leukemia, promyelocytic leukemia and Erythroleukemia.

35 Suitably, the invention relates to a method of treating cancer in a mammal,

including a human, wherein the cancer is selected from: malignant lymphoma, hodgkins lymphoma, non-hodgkins lymphoma, lymphoblastic T cell lymphoma, Burkitt's lymphoma and follicular lymphoma.

Suitably, the invention relates to a method of treating cancer in a mammal,
5 including a human, wherein the cancer is selected from: neuroblastoma, bladder cancer, urothelial cancer, lung cancer, vulval cancer, cervical cancer, endometrial cancer, renal cancer, mesothelioma, esophageal cancer, salivary gland cancer, hepatocellular cancer, gastric cancer, nasopharyngeal cancer, buccal cancer, cancer of the mouth, GIST (gastrointestinal stromal tumor) and testicular cancer.

10 When a compound of Formula (I) is administered for the treatment of cancer, the term "co-administering" and derivatives thereof as used herein is meant either simultaneous administration or any manner of separate sequential administration of a PI3 kinase inhibiting compound, as described herein, and a further active ingredient or ingredients, known to be useful in the treatment of
15 cancer, including chemotherapy and radiation treatment. The term further active ingredient or ingredients, as used herein, includes any compound or therapeutic agent known to or that demonstrates advantageous properties when administered to a patient in need of treatment for cancer. Preferably, if the administration is not simultaneous, the compounds are administered in a close time proximity to each
20 other. Furthermore, it does not matter if the compounds are administered in the same dosage form, e.g. one compound may be administered topically and another compound may be administered orally.

Typically, any anti-neoplastic agent that has activity versus a susceptible tumor being treated may be co-administered in the treatment of cancer in the
25 present 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. Typical anti-
30 neoplastic agents useful in the present invention include, but are not limited to, anti-microtubule agents such as diterpenoids and vinca alkaloids; platinum coordination complexes; alkylating agents such as nitrogen mustards, oxazaphosphorines, alkylsulfonates, nitrosoureas, and triazenes; antibiotic agents such as anthracyclins, actinomycins and bleomycins; topoisomerase II inhibitors such as
35 epipodophyllotoxins; antimetabolites such as purine and pyrimidine analogues and

anti-folate compounds; topoisomerase I inhibitors such as camptothecins; hormones and hormonal analogues; signal transduction pathway inhibitors; non-receptor tyrosine kinase angiogenesis inhibitors; immunotherapeutic agents; proapoptotic agents; and cell cycle signaling inhibitors.

5 Examples of a further active ingredient or ingredients for use in combination or co-administered with the present PI3 kinase inhibiting compounds are chemotherapeutic agents.

 Anti-microtubule or anti-mitotic agents are phase specific agents active against the microtubules of tumor cells during M or the mitosis phase of the cell
10 cycle. Examples of anti-microtubule agents include, but are not limited to, diterpenoids and vinca alkaloids.

 Diterpenoids, which are derived from natural sources, are phase specific anti-cancer agents that operate at the G₂/M phases of the cell cycle. It is believed that the diterpenoids stabilize the β -tubulin subunit of the microtubules, by binding
15 with this protein. Disassembly of the protein appears then to be inhibited with mitosis being arrested and cell death following. Examples of diterpenoids include, but are not limited to, paclitaxel and its analog docetaxel.

 Paclitaxel, 5 β ,20-epoxy-1,2 α ,4,7 β ,10 β ,13 α -hexa-hydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine; is a
20 natural diterpene product isolated from the Pacific yew tree *Taxus brevifolia* and is commercially available as an injectable solution TAXOL®. It is a member of the taxane family of terpenes. It was first isolated in 1971 by Wani et al. J. Am. Chem. Soc., 93:2325. 1971), who characterized its structure by chemical and X-ray crystallographic methods. One mechanism for its activity relates to paclitaxel's
25 capacity to bind tubulin, thereby inhibiting cancer cell growth. Schiff et al., Proc. Natl. Acad. Sci. USA, 77:1561-1565 (1980); Schiff et al., Nature, 277:665-667 (1979); Kumar, J. Biol. Chem, 256: 10435-10441 (1981). For a review of synthesis and anticancer activity of some paclitaxel derivatives see: D. G. I. Kingston *et al.*, Studies in Organic Chemistry vol. 26, entitled "New trends in Natural Products
30 Chemistry 1986", Attaur-Rahman, P.W. Le Quesne, Eds. (Elsevier, Amsterdam, 1986) pp 219-235.

 Paclitaxel has been approved for clinical use in the treatment of refractory ovarian cancer in the United States (Markman et al., Yale Journal of Biology and
35 Medicine, 64:583, 1991; McGuire et al., Ann. Intern. Med., 111:273,1989) and for

the treatment of breast cancer (Holmes et al., J. Nat. Cancer Inst., 83:1797,1991.) It is a potential candidate for treatment of neoplasms in the skin (Einzig et. al., Proc. Am. Soc. Clin. Oncol., 20:46) and head and neck carcinomas (Forastire et. al., Sem. Oncol., 20:56, 1990). The compound also shows potential for the treatment of polycystic kidney disease (Woo et. al., Nature, 368:750. 1994), lung cancer and malaria. Treatment of patients with paclitaxel results in bone marrow suppression (multiple cell lineages, Ignoff, R.J. et. al, Cancer Chemotherapy Pocket Guide, 1998) related to the duration of dosing above a threshold concentration (50nM) (Kearns, C.M. et. al., Seminars in Oncology, 3(6) p.16-23, 1995).

Docetaxel, (2R,3S)- N-carboxy-3-phenylisoserine,N-*tert*-butyl ester, 13-ester with 5 β -20-epoxy-1,2 α ,4,7 β ,10 β ,13 α -hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate; is commercially available as an injectable solution as TAXOTERE®. Docetaxel is indicated for the treatment of breast cancer. Docetaxel is a semisynthetic derivative of paclitaxel *q.v.*, prepared using a natural precursor, 10-deacetyl-baccatin III, extracted from the needle of the European Yew tree. The dose limiting toxicity of docetaxel is neutropenia.

Vinca alkaloids are phase specific anti-neoplastic agents derived from the periwinkle plant. Vinca alkaloids act at the M phase (mitosis) of the cell cycle by binding specifically to tubulin. Consequently, the bound tubulin molecule is unable to polymerize into microtubules. Mitosis is believed to be arrested in metaphase with cell death following. Examples of vinca alkaloids include, but are not limited to, vinblastine, vincristine, and vinorelbine.

Vinblastine, vincaleukoblastine sulfate, is commercially available as VELBAN® as an injectable solution. Although, it has possible indication as a second line therapy of various solid tumors, it is primarily indicated in the treatment of testicular cancer and various lymphomas including Hodgkin's Disease; and lymphocytic and histiocytic lymphomas. Myelosuppression is the dose limiting side effect of vinblastine.

Vincristine, vincaleukoblastine, 22-oxo-, sulfate, is commercially available as ONCOVIN® as an injectable solution. Vincristine is indicated for the treatment of acute leukemias and has also found use in treatment regimens for Hodgkin's and non-Hodgkin's malignant lymphomas. Alopecia and neurologic effects are the most common side effect of vincristine and to a lesser extent myelosuppression and gastrointestinal mucositis effects occur.

Vinorelbine, 3',4'-didehydro -4'-deoxy-C'-norvincaleukoblastine [R-(R*,R*)-

2,3-dihydroxybutanedioate (1:2)(salt)], commercially available as an injectable solution of vinorelbine tartrate (NAVELBINE®), is a semisynthetic vinca alkaloid. Vinorelbine is indicated as a single agent or in combination with other chemotherapeutic agents, such as cisplatin, in the treatment of various solid tumors, particularly non-small cell lung, advanced breast, and hormone refractory prostate cancers. Myelosuppression is the most common dose limiting side effect of vinorelbine.

Platinum coordination complexes are non-phase specific anti-cancer agents, which are interactive with DNA. The platinum complexes enter tumor cells, undergo, aquation and form intra- and interstrand crosslinks with DNA causing adverse biological effects to the tumor. Examples of platinum coordination complexes include, but are not limited to, cisplatin and carboplatin.

Cisplatin, cis-diamminedichloroplatinum, is commercially available as PLATINOL® as an injectable solution. Cisplatin is primarily indicated in the treatment of metastatic testicular and ovarian cancer and advanced bladder cancer. The primary dose limiting side effects of cisplatin are nephrotoxicity, which may be controlled by hydration and diuresis, and ototoxicity.

Carboplatin, platinum, diammine [1,1-cyclobutane-dicarboxylate(2-)-O,O'], is commercially available as PARAPLATIN® as an injectable solution. Carboplatin is primarily indicated in the first and second line treatment of advanced ovarian carcinoma. Bone marrow suppression is the dose limiting toxicity of carboplatin.

Alkylating agents are non-phase anti-cancer specific agents and strong electrophiles. Typically, alkylating agents form covalent linkages, by alkylation, to DNA through nucleophilic moieties of the DNA molecule such as phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups. Such alkylation disrupts nucleic acid function leading to cell death. Examples of alkylating agents include, but are not limited to, nitrogen mustards such as cyclophosphamide, melphalan, and chlorambucil; alkyl sulfonates such as busulfan; nitrosoureas such as carmustine; and triazenes such as dacarbazine.

Cyclophosphamide, 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate, is commercially available as an injectable solution or tablets as CYTOXAN®. Cyclophosphamide is indicated as a single agent or in combination with other chemotherapeutic agents, in the treatment of malignant lymphomas, multiple myeloma, and leukemias. Alopecia, nausea,

vomiting and leukopenia are the most common dose limiting side effects of cyclophosphamide.

Melphalan, 4-[bis(2-chloroethyl)amino]-L-phenylalanine, is commercially available as an injectable solution or tablets as ALKERAN®. Melphalan is indicated for the palliative treatment of multiple myeloma and non-resectable epithelial carcinoma of the ovary. Bone marrow suppression is the most common dose limiting side effect of melphalan.

Chlorambucil, 4-[bis(2-chloroethyl)amino]benzenebutanoic acid, is commercially available as LEUKERAN® tablets. Chlorambucil is indicated for the palliative treatment of chronic lymphatic leukemia, and malignant lymphomas such as lymphosarcoma, giant follicular lymphoma, and Hodgkin's disease. Bone marrow suppression is the most common dose limiting side effect of chlorambucil.

Busulfan, 1,4-butanediol dimethanesulfonate, is commercially available as MYLERAN® TABLETS. Busulfan is indicated for the palliative treatment of chronic myelogenous leukemia. Bone marrow suppression is the most common dose limiting side effects of busulfan.

Carmustine, 1,3-[bis(2-chloroethyl)-1-nitrosourea, is commercially available as single vials of lyophilized material as BiCNU®. Carmustine is indicated for the palliative treatment as a single agent or in combination with other agents for brain tumors, multiple myeloma, Hodgkin's disease, and non-Hodgkin's lymphomas. Delayed myelosuppression is the most common dose limiting side effects of carmustine.

Dacarbazine, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, is commercially available as single vials of material as DTIC-Dome®. Dacarbazine is indicated for the treatment of metastatic malignant melanoma and in combination with other agents for the second line treatment of Hodgkin's Disease. Nausea, vomiting, and anorexia are the most common dose limiting side effects of dacarbazine.

Antibiotic anti-neoplastics are non-phase specific agents, which bind or intercalate with DNA. Typically, such action results in stable DNA complexes or strand breakage, which disrupts ordinary function of the nucleic acids leading to cell death. Examples of antibiotic anti-neoplastic agents include, but are not limited to, actinomycins such as dactinomycin, anthrocyclins such as daunorubicin and doxorubicin; and bleomycins.

Dactinomycin, also known as Actinomycin D, is commercially available in injectable form as COSMEGEN®. Dactinomycin is indicated for the treatment of Wilm's tumor and rhabdomyosarcoma. Nausea, vomiting, and anorexia are the most common dose limiting side effects of dactinomycin.

- 5 Daunorubicin, (8S-cis-)-8-acetyl-10-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as a liposomal injectable form as DAUNOXOME® or as an injectable as CERUBIDINE®. Daunorubicin is indicated for remission induction in the treatment of acute
10 nonlymphocytic leukemia and advanced HIV associated Kaposi's sarcoma. Myelosuppression is the most common dose limiting side effect of daunorubicin.

- Doxorubicin, (8S, 10S)-10-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-8-glycoloyl, 7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as an injectable
15 form as RUBEX® or ADRIAMYCIN RDF®. Doxorubicin is primarily indicated for the treatment of acute lymphoblastic leukemia and acute myeloblastic leukemia, but is also a useful component in the treatment of some solid tumors and lymphomas. Myelosuppression is the most common dose limiting side effect of doxorubicin.

- Bleomycin, a mixture of cytotoxic glycopeptide antibiotics isolated from a
20 strain of *Streptomyces verticillus*, is commercially available as BLENOXANE®. Bleomycin is indicated as a palliative treatment, as a single agent or in combination with other agents, of squamous cell carcinoma, lymphomas, and testicular carcinomas. Pulmonary and cutaneous toxicities are the most common dose limiting side effects of bleomycin.

- 25 Topoisomerase II inhibitors include, but are not limited to, epipodophyllotoxins.

- Epipodophyllotoxins are phase specific anti-neoplastic agents derived from the mandrake plant. Epipodophyllotoxins typically affect cells in the S and G₂
30 phases of the cell cycle by forming a ternary complex with topoisomerase II and DNA causing DNA strand breaks. The strand breaks accumulate and cell death follows. Examples of epipodophyllotoxins include, but are not limited to, etoposide and teniposide.

- Etoposide, 4'-demethyl-epipodophyllotoxin 9[4,6-O-(R)-ethylidene- β -D-glucopyranoside], is commercially available as an injectable solution or capsules as
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VePESID® and is commonly known as VP-16. Etoposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of testicular and non-small cell lung cancers. Myelosuppression is the most common side effect of etoposide. The incidence of leucopenia tends to be more severe than thrombocytopenia.

Teniposide, 4'-demethyl-epipodophyllotoxin 9[4,6-O-(R)-thenylidene-β-D-glucopyranoside], is commercially available as an injectable solution as VUMON® and is commonly known as VM-26. Teniposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia in children. Myelosuppression is the most common dose limiting side effect of teniposide. Teniposide can induce both leucopenia and thrombocytopenia.

Antimetabolite neoplastic agents are phase specific anti-neoplastic agents that act at S phase (DNA synthesis) of the cell cycle by inhibiting DNA synthesis or by inhibiting purine or pyrimidine base synthesis and thereby limiting DNA synthesis. Consequently, S phase does not proceed and cell death follows. Examples of antimetabolite anti-neoplastic agents include, but are not limited to, fluorouracil, methotrexate, cytarabine, mercaptopurine, thioguanine, and gemcitabine.

5-fluorouracil, 5-fluoro-2,4-(1H,3H) pyrimidinedione, is commercially available as fluorouracil. Administration of 5-fluorouracil leads to inhibition of thymidylate synthesis and is also incorporated into both RNA and DNA. The result typically is cell death. 5-fluorouracil is indicated as a single agent or in combination with other chemotherapy agents in the treatment of carcinomas of the breast, colon, rectum, stomach and pancreas. Myelosuppression and mucositis are dose limiting side effects of 5-fluorouracil. Other fluoropyrimidine analogs include 5-fluoro deoxyuridine (floxuridine) and 5-fluorodeoxyuridine monophosphate.

Cytarabine, 4-amino-1-β-D-arabinofuranosyl-2 (1H)-pyrimidinone, is commercially available as CYTOSAR-U® and is commonly known as Ara-C. It is believed that cytarabine exhibits cell phase specificity at S-phase by inhibiting DNA chain elongation by terminal incorporation of cytarabine into the growing DNA chain. Cytarabine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Other cytidine analogs include 5-azacytidine and 2',2'-difluorodeoxycytidine (gemcitabine). Cytarabine induces leucopenia, thrombocytopenia, and mucositis.

Mercaptopurine, 1,7-dihydro-6H-purine-6-thione monohydrate, is

commercially available as PURINETHOL®. Mercaptopurine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Mercaptopurine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Myelosuppression and gastrointestinal mucositis are expected side effects of mercaptopurine at high doses. A useful mercaptopurine analog is azathioprine.

Thioguanine, 2-amino-1,7-dihydro-6H-purine-6-thione, is commercially available as TABLOID®. Thioguanine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Thioguanine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Myelosuppression, including leucopenia, thrombocytopenia, and anemia, is the most common dose limiting side effect of thioguanine administration. However, gastrointestinal side effects occur and can be dose limiting. Other purine analogs include pentostatin, erythrohydroxynonyladenine, fludarabine phosphate, and cladribine.

Gemcitabine, 2'-deoxy-2', 2'-difluorocytidine monohydrochloride (β -isomer), is commercially available as GEMZAR®. Gemcitabine exhibits cell phase specificity at S-phase and by blocking progression of cells through the G1/S boundary. Gemcitabine is indicated in combination with cisplatin in the treatment of locally advanced non-small cell lung cancer and alone in the treatment of locally advanced pancreatic cancer. Myelosuppression, including leucopenia, thrombocytopenia, and anemia, is the most common dose limiting side effect of gemcitabine administration.

Methotrexate, N-[4[[[(2,4-diamino-6-pteridiny) methyl]methylamino] benzoyl]-L-glutamic acid, is commercially available as methotrexate sodium. Methotrexate exhibits cell phase effects specifically at S-phase by inhibiting DNA synthesis, repair and/or replication through the inhibition of dihydrofolic acid reductase which is required for synthesis of purine nucleotides and thymidylate. Methotrexate is indicated as a single agent or in combination with other chemotherapy agents in the treatment of choriocarcinoma, meningeal leukemia, non-Hodgkin's lymphoma, and carcinomas of the breast, head, neck, ovary and bladder. Myelosuppression (leucopenia, thrombocytopenia, and anemia) and mucositis are expected side effect of methotrexate administration.

Camptothecins, including, camptothecin and camptothecin derivatives are available or under development as Topoisomerase I inhibitors. Camptothecins

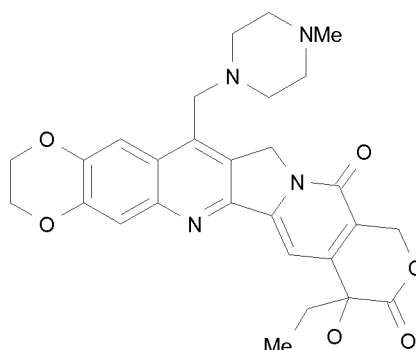
cytotoxic activity is believed to be related to its Topoisomerase I inhibitory activity. Examples of camptothecins include, but are not limited to irinotecan, topotecan, and the various optical forms of 7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20-camptothecin described below.

- 5 Irinotecan HCl, (4S)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino) carbonyloxy]-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione hydrochloride, is commercially available as the injectable solution CAMPTOSAR®.

Irinotecan is a derivative of camptothecin which binds, along with its active metabolite SN-38, to the topoisomerase I – DNA complex. It is believed that
10 cytotoxicity occurs as a result of irreparable double strand breaks caused by interaction of the topoisomerase I : DNA : irinotecan or SN-38 ternary complex with replication enzymes. Irinotecan is indicated for treatment of metastatic cancer of the colon or rectum. The dose limiting side effects of irinotecan HCl are myelosuppression, including neutropenia, and GI effects, including diarrhea.

- 15 Topotecan HCl, (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14-(4H,12H)-dione monohydrochloride, is commercially available as the injectable solution Hycamtin®. Topotecan is a derivative of camptothecin which binds to the topoisomerase I – DNA complex and prevents religation of single strand breaks caused by Topoisomerase I in response
20 to torsional strain of the DNA molecule. Topotecan is indicated for second line treatment of metastatic carcinoma of the ovary and small cell lung cancer. The dose limiting side effect of topotecan HCl is myelosuppression, primarily neutropenia.

- Also of interest, is the camptothecin derivative of formula A following,
25 currently under development, including the racemic mixture (R,S) form as well as the R and S enantiomers:



known by the chemical name "7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20(R,S)-camptothecin (racemic mixture) or "7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20(R)-camptothecin (R enantiomer) or "7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20(S)-camptothecin (S enantiomer). Such compound as well as related compounds are described, including methods of making, in U.S. Patent Nos. 6,063,923; 5,342,947; 5,559,235; 5,491,237 and pending U.S. patent Application No. 08/977,217 filed November 24, 1997.

Hormones and hormonal analogues are useful compounds for treating cancers in which there is a relationship between the hormone(s) and growth and/or lack of growth of the cancer. Examples of hormones and hormonal analogues useful in cancer treatment include, but are not limited to, adrenocorticosteroids such as prednisone and prednisolone which are useful in the treatment of malignant lymphoma and acute leukemia in children ; aminoglutethimide and other aromatase inhibitors such as anastrozole, letrozole, vorazole, and exemestane useful in the treatment of adrenocortical carcinoma and hormone dependent breast carcinoma containing estrogen receptors; progestrins such as megestrol acetate useful in the treatment of hormone dependent breast cancer and endometrial carcinoma; estrogens, androgens, and anti-androgens such as flutamide, nilutamide, bicalutamide, cyproterone acetate and 5 α -reductases such as finasteride and dutasteride, useful in the treatment of prostatic carcinoma and benign prostatic hypertrophy; anti-estrogens such as tamoxifen, toremifene, raloxifene, droloxifene, idoxifene, as well as selective estrogen receptor modulators (SERMS) such those described in U.S. Patent Nos. 5,681,835, 5,877,219, and 6,207,716, useful in the treatment of hormone dependent breast carcinoma and other susceptible cancers; and gonadotropin-releasing hormone (GnRH) and analogues thereof which stimulate the release of leutinizing hormone (LH) and/or follicle stimulating hormone (FSH) for the treatment prostatic carcinoma, for instance, LHRH agonists and antagagonists such as goserelin acetate and luprolide.

Signal transduction pathway inhibitors are those inhibitors, which block or inhibit a chemical process which evokes an intracellular change. As used herein this change is cell proliferation or differentiation. Signal transduction inhibitors useful in the present invention include inhibitors of receptor tyrosine kinases, non-receptor tyrosine kinases, SH2/SH3domain blockers, serine/threonine kinases, phosphatidylinositol-3 kinases, myo-inositol signaling, and Ras oncogenes.

Several protein tyrosine kinases catalyse the phosphorylation of specific tyrosyl residues in various proteins involved in the regulation of cell growth. Such protein tyrosine kinases can be broadly classified as receptor or non-receptor kinases.

5 Receptor tyrosine kinases are transmembrane proteins having an extracellular ligand binding domain, a transmembrane domain, and a tyrosine kinase domain. Receptor tyrosine kinases are involved in the regulation of cell growth and are generally termed growth factor receptors. Inappropriate or uncontrolled activation of many of these kinases, i.e. aberrant kinase growth factor
10 receptor activity, for example by over-expression or mutation, has been shown to result in uncontrolled cell growth. Accordingly, the aberrant activity of such kinases has been linked to malignant tissue growth. Consequently, inhibitors of such kinases could provide cancer treatment methods. Growth factor receptors include, for example, epidermal growth factor receptor (EGFr), platelet derived growth factor
15 receptor (PDGFr), erbB2, erbB4, vascular endothelial growth factor receptor (VEGFr), tyrosine kinase with immunoglobulin-like and epidermal growth factor homology domains (TIE-2), insulin growth factor -I (IGFI) receptor, macrophage colony stimulating factor (cfms), BTK, ckit, cmet, fibroblast growth factor (FGF) receptors, Trk receptors (TrkA, TrkB, and TrkC), ephrin (eph) receptors, and the
20 RET protooncogene. Several inhibitors of growth receptors are under development and include ligand antagonists, antibodies, tyrosine kinase inhibitors and anti-sense oligonucleotides. Growth factor receptors and agents that inhibit growth factor receptor function are described, for instance, in Kath, John C., Exp. Opin. Ther. Patents (2000) 10(6):803-818; Shawver et al DDT Vol 2, No. 2 February 1997; and
25 Lofts, F. J. et al, "Growth factor receptors as targets", New Molecular Targets for Cancer Chemotherapy, ed. Workman, Paul and Kerr, David, CRC press 1994, London.

 Tyrosine kinases, which are not growth factor receptor kinases are termed non-receptor tyrosine kinases. Non-receptor tyrosine kinases useful in the present
30 invention, which are targets or potential targets of anti-cancer drugs, include cSrc, Lck, Fyn, Yes, Jak, cAbl, FAK (Focal adhesion kinase), Brutons tyrosine kinase, and Bcr-Abl. Such non-receptor kinases and agents which inhibit non-receptor tyrosine kinase function are described in Sinh, S. and Corey, S.J., (1999) Journal of Hematotherapy and Stem Cell Research 8 (5): 465 – 80; and Bolen, J.B., Brugge, J.S., (1997) Annual review of Immunology. 15: 371-404.
35

SH2/SH3 domain blockers are agents that disrupt SH2 or SH3 domain binding in a variety of enzymes or adaptor proteins including, PI3-K p85 subunit, Src family kinases, adaptor molecules (Shc, Crk, Nck, Grb2) and Ras-GAP. SH2/SH3 domains as targets for anti-cancer drugs are discussed in Smithgall, T.E.

5 (1995), *Journal of Pharmacological and Toxicological Methods*. 34(3) 125-32.

Inhibitors of Serine/Threonine Kinases including MAP kinase cascade blockers which include blockers of Raf kinases (rafk), Mitogen or Extracellular Regulated Kinase (MEKs), and Extracellular Regulated Kinases (ERKs); and Protein kinase C family member blockers including blockers of PKCs (alpha, beta, 10 gamma, epsilon, mu, lambda, iota, zeta). Ikb kinase family (IKKa, IKKb), PKB family kinases, AKT kinase family members, and TGF beta receptor kinases. Such Serine/Threonine kinases and inhibitors thereof are described in Yamamoto, T., Taya, S., Kaibuchi, K., (1999), *Journal of Biochemistry*. 126 (5) 799-803; Brodt, P, Samani, A., and Navab, R. (2000), *Biochemical Pharmacology*, 60. 1101-1107; 15 Massague, J., Weis-Garcia, F. (1996) *Cancer Surveys*. 27:41-64; Philip, P.A., and Harris, A.L. (1995), *Cancer Treatment and Research*. 78: 3-27, Lackey, K. et al *Bioorganic and Medicinal Chemistry Letters*, (10), 2000, 223-226; U.S. Patent No. 6,268,391; and Martinez-lacaci, L., et al, *Int. J. Cancer* (2000), 88(1), 44-52.

Inhibitors of Phosphotidyl inositol-3 Kinase family members including 20 blockers of PI3-kinase, ATM, DNA-PK, and Ku are also useful in the present invention. Such kinases are discussed in Abraham, R.T. (1996), *Current Opinion in Immunology*. 8 (3) 412-8; Canman, C.E., Lim, D.S. (1998), *Oncogene* 17 (25) 3301-3308; Jackson, S.P. (1997), *International Journal of Biochemistry and Cell Biology*. 29 (7):935-8; and Zhong, H. et al, *Cancer res*, (2000) 60(6), 1541-1545.

25 Also useful in the present invention are Myo-inositol signaling inhibitors such as phospholipase C blockers and Myoinositol analogues. Such signal inhibitors are described in Powis, G., and Kozikowski A., (1994) *New Molecular Targets for Cancer Chemotherapy* ed., Paul Workman and David Kerr, CRC press 1994, London.

30 Another group of signal transduction pathway inhibitors are inhibitors of Ras Oncogene. Such inhibitors include inhibitors of farnesyltransferase, geranyl-geranyl transferase, and CAAX proteases as well as anti-sense oligonucleotides, ribozymes and immunotherapy. Such inhibitors have been shown to block ras activation in cells containing wild type mutant ras , thereby acting as antiproliferation agents. 35 Ras oncogene inhibition is discussed in Scharovsky, O.G., Rozados, V.R.,

Gervasoni, S.I. Matar, P. (2000), Journal of Biomedical Science. 7(4) 292-8; Ashby, M.N. (1998), Current Opinion in Lipidology. 9 (2) 99 – 102; and BioChim. Biophys. Acta, (1989) 1423(3):19-30.

As mentioned above, antibody antagonists to receptor kinase ligand binding may also serve as signal transduction inhibitors. This group of signal transduction pathway inhibitors includes the use of humanized antibodies to the extracellular ligand binding domain of receptor tyrosine kinases. For example Imclone C225 EGFR specific antibody (see Green, M.C. et al, Monoclonal Antibody Therapy for Solid Tumors, Cancer Treat. Rev., (2000), 26(4), 269-286); Herceptin® erbB2 antibody (see Tyrosine Kinase Signalling in Breast cancer:erbB Family Receptor Tyrosine Kinases, Breast cancer Res., 2000, 2(3), 176-183); and 2CB VEGFR2 specific antibody (see Brekken, R.A. et al, Selective Inhibition of VEGFR2 Activity by a monoclonal Anti-VEGF antibody blocks tumor growth in mice, Cancer Res. (2000) 60, 5117-5124).

Non-receptor kinase angiogenesis inhibitors may also find use in the present invention. Inhibitors of angiogenesis related VEGFR and TIE2 are discussed above in regard to signal transduction inhibitors (both receptors are receptor tyrosine kinases). Angiogenesis in general is linked to erbB2/EGFR signaling since inhibitors of erbB2 and EGFR have been shown to inhibit angiogenesis, primarily VEGF expression. Thus, the combination of an erbB2/EGFR inhibitor with an inhibitor of angiogenesis makes sense. Accordingly, non-receptor tyrosine kinase inhibitors may be used in combination with the EGFR/erbB2 inhibitors of the present invention. For example, anti-VEGF antibodies, which do not recognize VEGFR (the receptor tyrosine kinase), but bind to the ligand; small molecule inhibitors of integrin ($\alpha_v\beta_3$) that will inhibit angiogenesis; endostatin and angiostatin (non-RTK) may also prove useful in combination with the disclosed erb family inhibitors. (See Bruns CJ et al (2000), Cancer Res., 60: 2926-2935; Schreiber AB, Winkler ME, and Derynck R. (1986), Science, 232: 1250-1253; Yen L et al. (2000), Oncogene 19: 3460-3469).

Agents used in immunotherapeutic regimens may also be useful in combination with the compounds of formula (I). There are a number of immunologic strategies to generate an immune response against erbB2 or EGFR. These strategies are generally in the realm of tumor vaccinations. The efficacy of immunologic approaches may be greatly enhanced through combined inhibition of erbB2/EGFR signaling pathways using a small molecule inhibitor. Discussion of the

immunologic/tumor vaccine approach against erbB2/EGFR are found in Reilly RT et al. (2000), *Cancer Res.* 60: 3569-3576; and Chen Y, Hu D, Eling DJ, Robbins J, and Kipps TJ. (1998), *Cancer Res.* 58: 1965-1971.

- Agents used in proapoptotic regimens (e.g., bcl-2 antisense oligonucleotides) may also be used in the combination of the present invention. Members of the Bcl-2 family of proteins block apoptosis. Upregulation of bcl-2 has therefore been linked to chemoresistance. Studies have shown that the epidermal growth factor (EGF) stimulates anti-apoptotic members of the bcl-2 family (i.e., mcl-1). Therefore, strategies designed to downregulate the expression of bcl-2 in tumors have demonstrated clinical benefit and are now in Phase II/III trials, namely Genta's G3139 bcl-2 antisense oligonucleotide. Such proapoptotic strategies using the antisense oligonucleotide strategy for bcl-2 are discussed in Water JS et al. (2000), *J. Clin. Oncol.* 18: 1812-1823; and Kitada S et al. (1994), *Antisense Res. Dev.* 4: 71-79.
- Cell cycle signalling inhibitors inhibit molecules involved in the control of the cell cycle. A family of protein kinases called cyclin dependent kinases (CDKs) and their interaction with a family of proteins termed cyclins controls progression through the eukaryotic cell cycle. The coordinate activation and inactivation of different cyclin/CDK complexes is necessary for normal progression through the cell cycle. Several inhibitors of cell cycle signalling are under development. For instance, examples of cyclin dependent kinases, including CDK2, CDK4, and CDK6 and inhibitors for the same are described in, for instance, Rosania et al, *Exp. Opin. Ther. Patents* (2000) 10(2):215-230.

In one embodiment, the cancer treatment method of the claimed invention includes the co-administration a compound of formula I and/or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof and at least one anti-neoplastic agent, such as one selected from the group consisting of anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors, immunotherapeutic agents, proapoptotic agents, and cell cycle signaling inhibitors.

Because the pharmaceutically active compounds of the present invention are active as PI3 kinase inhibitors, particularly the compounds that modulate/inhibit PI3K γ , either selectively or in conjunction with one or more of PI3K δ , PI3K β , and/or PI3K α , they exhibit therapeutic utility in treating a disease state selected from: autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan failure, kidney diseases, platelet aggregation, sperm motility, transplantation rejection, graft rejection and lung injuries.

When a compound of Formula (I) is administered for the treatment of a disease state selected from: autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan failure, kidney diseases, platelet aggregation, sperm motility, transplantation rejection, graft rejection or lung injuries, the term "co-administering" and derivatives thereof as used herein is meant either simultaneous administration or any manner of separate sequential administration of a PI3 kinase inhibiting compound, as described herein, and a further active ingredient or ingredients, known to be useful in the treatment of autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan failure, kidney diseases, platelet aggregation, sperm motility, transplantation rejection, graft rejection and/or lung injuries.

Biological assays

PI3K alpha Leadseeker SPA Assay

Compounds of the present invention were tested according to the following assays and found as inhibitors of PI3 kinases, particularly PI3K α . The activities (IC₅₀) range from 1 nM to 500 μ M against PI3K α .

The compound of example 1 was tested and found to have an IC₅₀ of 1 nM against PI3K α in the assays.

Assay principle

- 5 SPA imaging beads are microspheres containing scintillant which emit light in the red region of the visible spectrum. As a result, these beads are ideally suited to use with a CCD imager such as the Viewlux. The Leadseeker beads used in this system are polystyrene beads that have been coupled with polyethyleneimine. When added to the assay mixture, the beads absorb both the substrate (PIP₂) and
- 10 product (PIP₃). Adsorbed P³³-PIP₃ will cause an increase in signal, measured as ADUs (analog to digital units). This protocol details the use of the PEI-PS Leadseeker beads for assays using His-p110/p85 PI3K α .

Assay protocol

- 15 Solid compounds are typically plated with 0.1 μ l of 100% DMSO in all wells (except column 6 and 18) of a 384-well, flat bottom, low volume plate (Greiner 784075). The compounds are serially diluted (3-fold in 100% DMSO) across the plate from column 1 to column 12 and column 13 to column 24 and leave column 6 and 18 containing only DMSO to yield 11 concentrations for each test compound.
- 20 The assay buffer contains MOPS (pH 6.5), CHAPS, and DTT. PI3K α and PIP₂ (L- α -D-myo-Phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂]-3-O-phospho linked, D(+)-sn-1,2-di-O-octanoylglycerol, CellSignals # 901) are mixed and incubated in the plate with compound for 30min prior to starting the reaction with the addition of P³³-ATP and MgCl₂ (reagents added using Zoom). Enzyme-free
- 25 wells (column 18) are typically done to determine the low control. PEI-PS Leadseeker beads in PBS/EDTA/CHAPS are added (by Multidrop) to quench the reaction, and the plates are allowed to incubate for at least one hour (typically overnight) before centrifugation. The signal is determined using a Viewlux detector and is then imported into curve fitting software (Activity Base) for construction of
- 30 concentration response curves. The percent inhibition of activity was calculated relative to high controls (C1, 0.1 μ l DMSO in column 6, rows A-P) and low controls (C2, 5 μ l of 40 μ M PIP₂ in buffer in column 18, rows A-P) using, $100 \times (1 - (U1 - C2) / (C1 - C2))$. The concentration of test compound yielding 50% inhibition was determined using the equation, $y = ((V_{max} \times x) / (K + x)) + Y_2$, where "K" was equal to
- 35 the IC₅₀. The IC₅₀ values were converted to pIC₅₀ values, i.e., -log IC₅₀ in Molar

concentration.

Cellular assays:

DAY 1

- 5 Plate cells before noon
10K cells/well in clear flat-bottomed 96-well plates (*f.v. 105ul*)
Last four wells in last column receive media only
Place in 37degC incubator overnight
Compound plate
- 10 Prepare in polypropylene round-bottomed 96-well plates; 8 compounds per plate, 11-pt titrations of each (3x serial dilution), DMSO in last column (0.15% f.c. on cells)
15ul in first well, 10ul DMSO in the rest; take 5ul from first well and mix in next, continue across plate (*excluding last column*); seal with foil lid and place at 4degC

DAY 2

- 15 Take out Lysis buffer inhibitors (4degC/-20degC) and compound plates (4degC), thaw on bench top; make 1x Tris wash buffer (WB) to fill reservoir on plate washer and top off bench supply (use MiliQ), turn on centrifuge to allow it to cool
Block MSD plates
- 20 Make 20ml 3% blocking solution/plate (600mg blocker A in 20ml WB), add 150ul/well and incubate at RT for at least 1 hr
Add compound (while blocking)
Add 300ul growth media (RPMI w/ Q, 10% FBS) per well (682x dil of compound) to each compound plate
- 25 Add 5ul compound dilution into each well (*f.v. 110ul*) on duplicate plates
Place in 37degC incubator for 30min
Make lysates
Prepare MSD Lysis buffer; for 10ml add 200ul protease inhibitor solution, and 100ul each of Phosphatase inhibitors I & II (*Keep on ice until ready for use*)
- 30 Remove plates post-incubation, aspirate media with plate washer, wash 1x with cold PBS, and add 80ul MSD Lysis buffer per well; incubate on shaker at 4degC for ≥30min
Spin cold at 2500rpm for 10min; leave plates in 4degC centrifuge until ready for use
- #### **AKT duplex assay**
- 35 Wash plates (4x with 200ul/well WB in plate washer); tap plates on paper towel to

blot.

Add 60ul of lysates/well, incubate on shaker at RT for 1hr

During incubation prepare detection Ab (3ml/plate; 2ml WB and 1ml blocking solution w/ Ab at 10nM); repeat wash step as above

- 5 Add 25ul of Ab/well, incubate on shaker at RT for 1hr; repeat wash step as above
Add 150ul/well 1x Read Buffer (dilute 4x stock in ddH₂O, 20ml/plate), read immediately

Analysis

- 10 Observe all the data points at each compound concentration.
The data point from highest inhibitor concentration must be equal or greater than 70% of DMSO control.
IC₅₀ for duplicate runs must be within 2-fold of each other (not flagged in summary template).
- 15 Y min must be greater than zero; if both mins are red flagged (>35) then compound is listed as inactive (IC₅₀= > highest dose). If only one min is red flagged, but still ≤50 then call IC₅₀ as listed.
Any data points equal or greater than 30% off the curve will not be considered.

20 Cell Growth/Death Assay:

- BT474, HCC1954 and T-47D (human breast) were cultured in RPMI-1640 containing 10% fetal bovine serum at 37°C in 5% CO₂ incubator. Cells were split into T75 flask (Falcon #353136) two to three days prior to assay set up at density which yields approximately 70-80% confluence at time of harvest for assay. Cells
- 25 were harvested using 0.25% trypsin-EDTA (Sigma #4049). Cell counts were performed on cell suspension using Trypan Blue exclusion staining. Cells were then plated in 384 well black flat bottom polystyrene (Greiner #781086) in 48 µl of culture media per well at 1,000 cells/well. All plates were placed at 5% CO₂, 37°C overnight and test compounds were added the following day. One plate was treated with
- 30 CellTiter-Glo (Promega #G7573) for a day 0 (t=0) measurement and read as described below. The test compounds were prepared in clear bottom polypropylene 384 well plates (Greiner#781280) with consecutive two fold dilutions. 4 µl of these dilutions were added to 105 µl culture media, after mixing the solution, 2 µl of these dilutions were added into each well of the cell plates. The final concentration of
- 35 DMSO in all wells was 0.15%. Cells were incubated at 37°C, 5% CO₂ for 72 hours.

Following 72 hours of incubation with compounds each plate was developed and read. CellTiter-Glo reagent was added to assay plates using a volume equivalent to the cell culture volume in the wells. Plates were shaken for approximately two minutes and incubated at room temperature for approximately 30 minutes and
5 chemiluminescent signal was read on the Analyst GT (Molecular Devices) reader. Results were expressed as a percent of the t=0 and plotted against the compound concentration. Cell growth inhibition was determined for each compound by fitting the dose response with a 4 or 6 parameter curve fit using XLfit software and determining the concentration that inhibited 50% of the cell growth (glC50) with the
10 Y min as the t=0 and Y max as the DMSO control. Value from wells with no cells was subtracted from all samples for background correction.

Additional references:

The compounds of the present invention can also be tested to determine their
15 inhibitory activity at PI3K α , PI3K δ , PI3K β and PI3K γ according to the following references:

For all PI3K isoforms:

Cloning, expression, purification, and characterization of the human Class Ia
20 phosphoinositide 3-kinase isoforms: Meier, T.I.; Cook, J.A.; Thomas, J.E.; Radding, J.A.; Horn, C.; Lingaraj, T.; Smith, M.C. Protein Expr. Purif., **2004**, 35(2), 218.
Competitive fluorescence polarization assays for the detection of phosphoinositide kinase and phosphatase activity: Drees, B.E.; Weipert, A.; Hudson, H.; Ferguson, C.G.; Chakravarty, L.; Prestwich, G.D. Comb. Chem. High Throughput.Scrn.,
25 **2003**, 6(4), 321.

For PI3K γ : WO 2005/011686 A1

The pharmaceutically active compounds within the scope of this invention are useful as PI3 Kinase inhibitors in mammals, particularly humans, in need
30 thereof.

The present invention therefore provides a method of treating diseases associated with PI3 kinase inhibition, particularly: autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan failure, kidney diseases, platelet
35 aggregation, cancer, sperm motility, transplantation rejection, graft rejection and

lung injuries and other conditions requiring PI3 kinase modulation/inhibition, which comprises administering an effective compound of Formula (I) or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof. The compounds of Formula (I) also provide for a method of treating the above indicated disease states because of their ability to act as PI3 inhibitors. The drug may be administered to a patient in need thereof by any conventional route of administration, including, but not limited to, intravenous, intramuscular, oral, subcutaneous, intradermal, and parenteral.

The pharmaceutically active compounds of the present invention are incorporated into convenient dosage forms such as capsules, tablets, or injectable preparations. Solid or liquid pharmaceutical carriers are employed. Solid carriers include, starch, lactose, calcium sulfate dihydrate, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Liquid carriers include syrup, peanut oil, olive oil, saline, and water. Similarly, the carrier or diluent may include any prolonged release material, such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies widely but, preferably, will be from about 25 mg to about 1 g per dosage unit. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion, soft gelatin capsule, sterile injectable liquid such as an ampoule, or an aqueous or nonaqueous liquid suspension.

The pharmaceutical preparations are made following conventional techniques of a pharmaceutical chemist involving mixing, granulating, and compressing, when necessary, for tablet forms, or mixing, filling and dissolving the ingredients, as appropriate, to give the desired oral or parenteral products.

Doses of the presently invented pharmaceutically active compounds in a pharmaceutical dosage unit as described above will be an efficacious, nontoxic quantity preferably selected from the range of 0.001 - 100 mg/kg of active compound, preferably 0.001 - 50 mg/kg. When treating a human patient in need of a PI3K inhibitor, the selected dose is administered preferably from 1-6 times daily, orally or parenterally. Preferred forms of parenteral administration include topically, rectally, transdermally, by injection and continuously by infusion. Oral dosage units for human administration preferably contain from 0.05 to 3500 mg of active compound. Oral administration, which uses lower dosages is preferred. Parenteral administration, at high dosages, however, also can be used when safe and convenient for the patient.

Optimal dosages to be administered may be readily determined by those skilled in the art, and will vary with the particular PI3 kinase inhibitor in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition. Additional factors depending on the particular patient being
5 treated will result in a need to adjust dosages, including patient age, weight, diet, and time of administration.

The method of this invention of inducing PI3 kinase inhibitory activity in mammals, including humans, comprises administering to a subject in need of such activity an effective PI3 kinase modulating/inhibiting amount of a pharmaceutically
10 active compound of the present invention.

The invention also provides for the use of a compound of Formula (I) in the manufacture of a medicament for use as a PI3 kinase inhibitor.

The invention also provides for the use of a compound of Formula (I) in the manufacture of a medicament for use in therapy.

15 The invention also provides for the use of a compound of Formula (I) in the manufacture of a medicament for use in treating autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan failure, kidney diseases, platelet aggregation, cancer, sperm motility, transplantation rejection, graft rejection and
20 lung injuries.

The invention also provides for a pharmaceutical composition for use as a PI3 inhibitor which comprises a compound of Formula (I) and a pharmaceutically acceptable carrier.

25 The invention also provides for a pharmaceutical composition for use in the treatment of autoimmune disorders, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan failure, kidney diseases, platelet aggregation, cancer, sperm motility, transplantation rejection, graft rejection and lung injuries, which comprises a compound of Formula (I) and a pharmaceutically acceptable carrier.

30 No unacceptable toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

In addition, the pharmaceutically active compounds of the present invention can be co-administered with further active ingredients, including compounds known
35 to have utility when used in combination with a PI3 kinase inhibitor.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are, therefore, to be construed as merely illustrative and not a limitation of the scope of the present invention in any way.

5

Experimental Details

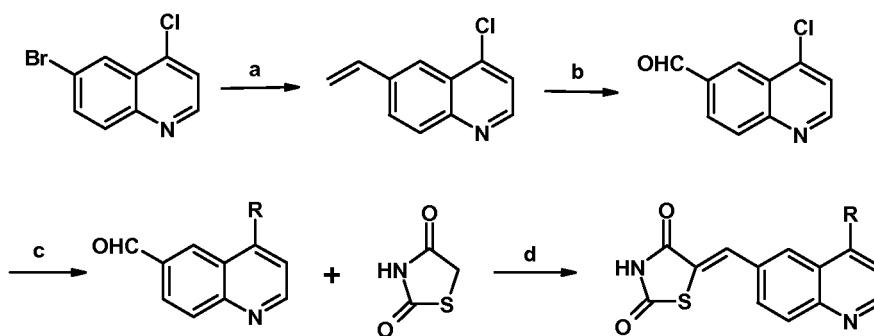
Preparation

The derivatives described herein are prepared by the general methods described below:

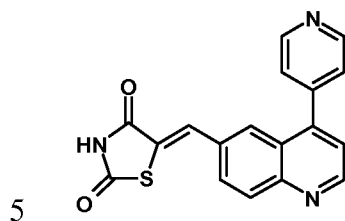
10

Schemes/Experimentals

Scheme I:



Conditions: a) Tributyl(vinyl)tin, Pd(PPh₃)₄, dioxane, reflux; b) OsO₄, NaIO₄, 2,6-lutidine, *t*-BuOH, dioxane, H₂O, rt; c) heteroaryl (**R**) boronic acid, Pd(PPh₃)₄, 2 M K₂CO₃, DMF, 100 °C; d) 2,4-thiazolidinedione, piperidine, AcOH, EtOH, μ wave, 150 °C.

Examples:**Example 1: (5Z)-5-{[4-(4-pyridinyl)-6-quinolinyl]methylidene}-1,3-thiazolidine-2,4-dione**

a) 4-chloro-6-ethenylquinoline

A mixture of 6-bromo-4-chloroquinoline (6.52 g, 26.88 mmol; see *J. Med. Chem.*, 21, 268 (1978)), tributyl(vinyl)tin (8.95 g, 28.22 mmol), and
 10 tetrakis(triphenyl)phosphine palladium (0) (0.62 g, 0.54 mmol) in 1,4-dioxane (150 mL) was refluxed for 2.0 h, cooled to room temperature, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (0-4% MeOH:CH₂Cl₂) to give the title compound (5.1 g) as a pale yellow solid. MS(ES)+ m/e 190 [M+H]⁺. This material was used directly in the next step.

15

b) 4-chloro-6-quinolinecarbaldehyde

A mixture of 4-chloro-6-ethenylquinoline (5.1 g, 26.88 mmol), 2,6-lutidine (5.76 g, 53.75 mmol), sodium (meta) periodate (22.99 g, 107.51 mmol), and osmium tetroxide (5.48 g of a 2.5% solution in tert-butanol, 0.538 mmol) in 1,4-
 20 dioxane:H₂O (350 mL of 3:1 mixture) was stirred for 3.5 h at room temperature and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (CH₂Cl₂) to give the title compound (4.26 g, 83% for 2 steps) as a pale yellow solid. MS(ES)+ m/e 192 [M+H]⁺.

25 c) 4-(4-pyridinyl)-6-quinolinecarbaldehyde

A mixture of 4-chloro-6-quinolinecarbaldehyde (3.24 g, 16.92 mmol), 4-pyridylboronic acid (3.12 g, 25.38 mmol), tetrakis(triphenyl)phosphine palladium (0) (0.978 g, 0.846 mmol), and 2M aqueous K₂CO₃ (7.02 g, 50.76 mmol, 25.4 mls of 2M solution) in DMF (100 mL) was heated at 100°C for 3.0 h and cooled to room
 30 temperature. The mixture was filtered through celite and the celite was washed with EtOAc. The filtrate was transferred to a separatory funnel, washed with water

and saturated NaCl, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (5% MeOH: CH_2Cl_2) to give the title compound (2.03 g, 51%) as a tan solid. MS(ES)+ m/e 235 $[\text{M}+\text{H}]^+$.

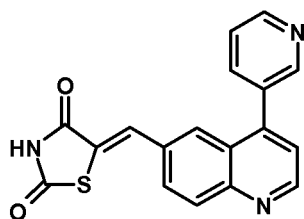
5 d) (5Z)-5- $\{[4-(4\text{-pyridinyl})-6\text{-quinolinyl}]\text{methylidene}\}$ -1,3-thiazolidine-2,4-dione

A mixture of 4-(4-pyridinyl)-6-quinolinecarbaldehyde (0.108 g, 0.463 mmol), 2,4-thiazolidinedione (0.0417 g, 0.356 mmol), piperidine (0.0303 g, 0.356 mmol), and acetic acid (0.0214 g, 0.356 mmol) in EtOH (5 mL) was heated at 150°C for 30 minutes in a microwave oven. The reaction was cooled to room temperature and

10 the resulting precipitate was filtered and dried in a Buchner funnel to give the title compound (0.0594 g, 50%) as a tan solid. MS(ES)+ m/e 334 $[\text{M}+\text{H}]^+$. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 9.08 (d, $J=4.42$ Hz, 1 H) 8.80 - 8.88 (m, 2 H) 8.25 (d, $J=8.72$ Hz, 1 H) 8.00 - 8.07 (m, 2 H) 7.98 (s, 1 H) 7.65 - 7.68 (m, 2 H) 7.63 (d, $J=4.42$ Hz, 1 H).

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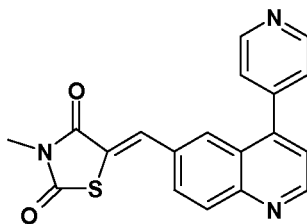
Example 2: (5Z)-5- $\{[4-(3\text{-pyridinyl})-6\text{-quinolinyl}]\text{methylidene}\}$ -1,3-thiazolidine-2,4-dione



20

Following the procedure used to prepare Example 1, the title compound was prepared by substituting 3-pyridinylboronic acid for 4-pyridinylboronic acid. MS(ES)+ m/e 334 $[\text{M}+\text{H}]^+$.

Example 3: (5Z)-3-methyl-5-[4-(4-pyridinyl)-6-quinolinyl]methyldiene}-1,3-thiazolidine-2,4-dione



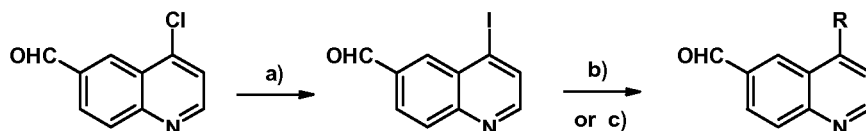
5

Following the procedure used to prepare Example 1, the title compound was prepared by substituting 3-methyl-1,3-thiazolidine-2,4-dione in place of 1,3-thiazolidine-2,4-dione, to give a light yellow solid. MS(ES)⁺ m/e 348 [M+H]⁺.

Alternatively, some examples were prepared via a palladium mediated Suzuki (or Stille) coupling of a heteroaryl boronic acid (or heteroaryl stannane) with 4-iodo-6-quinolinecarbaldehyde (Scheme II). The iodide intermediate was prepared by treating the corresponding chloride with 4 N HCl, followed by sodium iodide. The heteroaryl aldehydes were converted to the title compounds following the same procedure used to prepare Example 1.

15

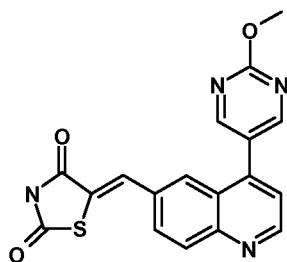
Scheme II:



Conditions: a) 4 N HCl in dioxane, rt, 5 min; then NaI, 105 ° C, 18 h; b) heteroaryl (R) boronic acid, Pd(PPh₃)₄, 2 M K₂CO₃, dioxane, 100 ° C or c) tributyl(heteroaryl)tin, Pd(PPh₃)₄, dioxane, reflux.

20

Example 4: (5Z)-5-({4-[2-(methyloxy)-5-pyrimidinyl]-6-quinolinyl}methyldene)-1,3-thiazolidine-2,4-dione



5 a) 4-iodo-6-quinolinecarbaldehyde

In a large reaction tube, to a solution of 4-chloro-6-quinolinecarbaldehyde (4.26 g, 22.2 mmol) in propionitrile (125 mL) was added 4 N HCl in dioxane (2 eq HCl, 11.1 mL, 44.4 mmol). The solution was stirred for 15 minutes and charged with 3 eq of sodium iodide (10 g, 66 mmol). The reaction tube was sealed and
10 heated at 105 °C overnight. The reaction was monitored by LCMS. The reaction was allowed to cool to ambient temperature and the product filtered off and washed with acetonitrile. The crude solid was then placed in a beaker and saturated sodium bicarbonate was added with stirring until pH 9.5. The solid was then extracted into methylene chloride. The methylene chloride layer was then
15 washed with water, dried over sodium sulfate and concentrated *in vacuo* to give the title compound in 85% yield.

b) 4-[6-(methyloxy)-3-pyridinyl]-6-quinolinecarbaldehyde

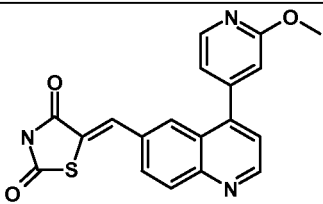
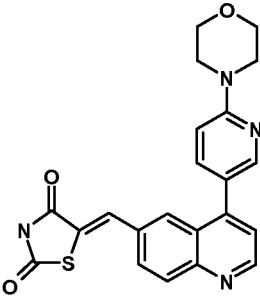
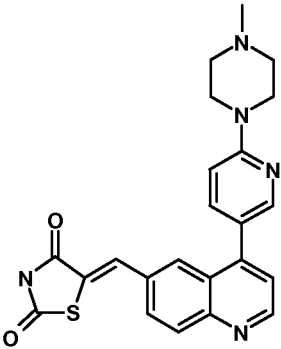
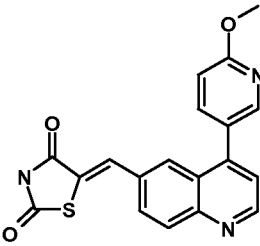
A mixture of 6-(methyloxy)-3-pyridinylboronic acid (225 mg, 1.5 mmol), 4-
20 iodo-6-quinolinecarbaldehyde (283 mg, 1 mmol), tetrakis(triphenylphosphine) palladium (0) (57 mg, 0.05 mmol), 2 M aqueous K₂CO₃ (2.5 mL of a 2 M solution), and dioxane (5 mL)) was heated at 100 °C for 8 h and cooled to room temperature. The dioxane was removed under reduced pressure and the residue dissolved in 2:1 mixture of methylene chloride/water and the solution filtered. The organic layer was
25 separated and dried with sodium sulfate and the crude product obtained by decanting the solution and evaporation of the methylene chloride. The crude product was purified by silica gel chromatography eluting with methylene chloride/methanol (0-1% methanol gradient) to give the title compound (250 mg, 95 %). MS(ES)⁺ m/e 265 [M+H]⁺.

30

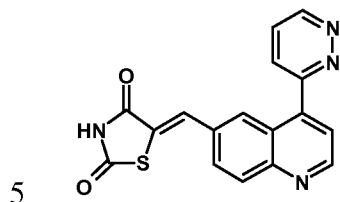
(5Z)-5-({4-[2-(methyloxy)-5-pyrimidinyl]-6-quinolinyl}methylidene)-1,3-thiazolidine-2,4-dione was then prepared following the procedure of Example 1d. MS(ES)⁺ m/e 365 [M+H]⁺.

5 Compound 5, 6, 7, and 8 are prepared following the procedure used to prepare

Example 4:

Example	Structure	MS(ES) [M+H] ⁺
5		364
6		419
7		432
8		364

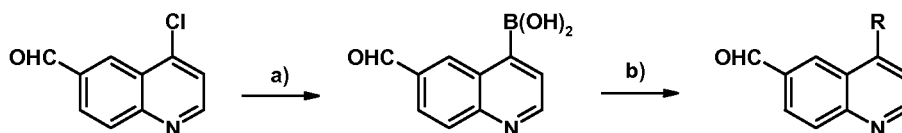
Example 9: (5Z)-5-[[4-(3-pyridazinyl)-6-quinolinyl]methylidene]-1,3-thiazolidine-2,4-dione



a) 4-(3-pyridazinyl)-6-quinolinecarbaldehyde

A mixture of 4-iodo-6-quinolinecarbaldehyde (142 mg, 0.5 mmol), 3-(tributylstannyl)pyridazine (220 mg, 0.6 mmol), and dichloro-
 10 [1,1'-bis(diphenylphosphino)ferrocene]palladium (II) dichloromethane adduct (12.2 mg, 0.015 mmol) in dioxane (3 mL) was heated at 100 °C for 5 h and cooled to room temperature. Three hundred milligrams of silica gel was added to the reaction and the dioxane was evaporated. The dry pack was loaded on top of a 10 gram silica column packed in methylene chloride and eluted with (0-3 % MeOH:CH₂Cl₂)
 15 to give the title compound (170 mg, 72%) as a solid. MS(ES)⁺ m/e 236 [M+H]⁺. (5Z)-5-[[4-(3-pyridazinyl)-6-quinolinyl]methylidene]-1,3-thiazolidine-2,4-dione was then prepared following the procedure of Example 1d. MS(ES)⁺ m/e 335 [M+H]⁺. Additional examples were prepared via a palladium mediated Suzuki coupling of a heteroaryl bromide with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6-
 20 quinolinecarbaldehyde (Scheme III). The boronate intermediate was prepared by a palladium (0) mediated reaction of the corresponding chloride. The heteroaryl aldehydes were converted to the title compounds following the same procedure used to prepare Example 1.

25 **Scheme III:**

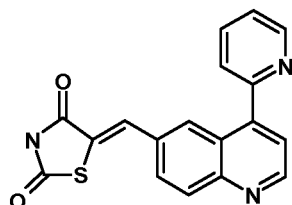


Conditions: a) 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi-1,3,2-dioxaborolane, potassium acetate, dichloro-[1,1'-bis(diphenylphosphino) ferrocene]palladium (II)
 30 dichloromethane adduct, dioxane, 100 °C, 18 h; b) heteroaryl (**R**) bromide (1.3 eq),

tetrakis(triphenyl)phosphine palladium (0), 2 M aqueous K₂CO₃, dioxane, 100 °C, 8 h.

Example 10: (5Z)-5-{[4-(2-pyridinyl)-6-quinolinyl]methylidene}-1,3-thiazolidine-2,4-dione

5



a) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6-quinolinecarbaldehyde

A mixture of 4-chloro-6-quinolinecarbaldehyde (2.5 g, 13 mmol),
 10 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi-1,3,2-dioxaborolane (7 g, 27 mmole), potassium acetate (5 g, 39 mmol), and dichloro-
 [1,1'-bis(diphenylphosphino)ferrocene]palladium (II) dichloromethane adduct (530 mg, 0.065 mmol) in dioxane (30 mL) was heated at 100 °C for 18 h and cooled to
 room temperature. The dioxane was removed under reduced pressure and the
 15 residue dissolved in 2:1 mixture of ethyl acetate/water and the solution filtered. The organic layer was separated and dried with sodium sulfate and the crude product
 obtained by decanting the solution and evaporation of the ethyl acetate. The crude
 product was purified by silica gel chromatography eluting with methylene chloride/
 methanol (0-2% methanol gradient) to give the title compound (2.9 g, 78 %).

20

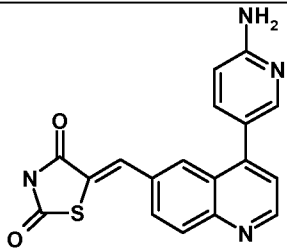
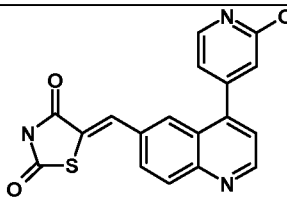
b) 4-(2-pyridinyl)-6-quinolinecarbaldehyde

A mixture of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6-
 quinolinecarbaldehyde (180 mg, 0.64 mmol), 2-bromopyridine (158 mg, 1.0 mmol),
), tetrakis(triphenyl)phosphine palladium (0) (38 mg, 0.0335 mmol), and 2 M aqueous
 25 K₂CO₃ (1 mL of a 2 M solution), and dioxane (4 mL) was heated at 100 °C for 8 h
 and cooled to room temperature. The dioxane was removed under reduced
 pressure and the residue dissolved in 2:1 mixture of methylene chloride/water and
 the solution filtered. The organic layer was separated and dried with sodium sulfate
 and the crude product obtained by decanting the solution and evaporation of the
 30 methylene chloride. The crude product was purified by silica gel chromatography
 eluting with methylene chloride/ methanol (0-2% methanol gradient) to give the title

compound (80mg, 65 %). MS(ES)+ m/e 235 $[M+H]^+$.

(5Z)-5-[[4-(2-pyridinyl)-6-quinolinyl]methylidene]-1,3-thiazolidine-2,4-dione was then prepared following the procedure of Example 1d. MS(ES)+ m/e 334 $[M+H]^+$.

- 5 Compound 11 and 12 are prepared following the procedure used to prepare Example 10:

Example	Structure	MS(ES) $[M+H]^+$
11		349
12		350

- The compounds of the following examples are readily made according to Schemes I or by analogous methods.
- 10

Exemplary capsule composition

- An oral dosage form for administering the present invention is produced by filing a standard two piece hard gelatin capsule with the ingredients in the proportions shown in Table I, below.
- 15

Table I

<u>INGREDIENTS</u>	<u>AMOUNTS</u>
(5Z)-5-[[4-(4-pyridinyl)-6-quinolinyl]methylidene]-1,3-thiazolidine-2,4-dione	25 mg
Lactose	55 mg
Talc	16 mg
Magnesium Stearate	4 mg

5 **Exemplary Injectable Parenteral Composition**

An injectable form for administering the present invention is produced by stirring 1.5% by weight of (5Z)-5-[[4-(4-pyridinyl)-6-quinolinyl]methylidene]-1,3-thiazolidine-2,4-dione in 10% by volume propylene glycol in water.

10 **Exemplary Tablet Composition**

The sucrose, calcium sulfate dihydrate and an PI3K inhibitor as shown in Table II below, are mixed and granulated in the proportions shown with a 10% gelatin solution. The wet granules are screened, dried, mixed with the starch, talc and stearic acid; screened and compressed into a tablet.

15

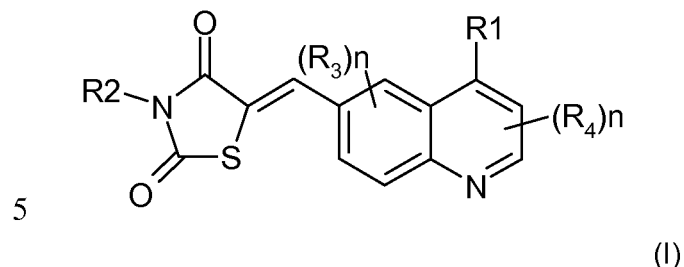
Table II

<u>INGREDIENTS</u>	<u>AMOUNTS</u>
(5Z)-5-[[4-(4-pyridinyl)-6-quinolinyl]methylidene]-1,3-thiazolidine-2,4-dione	20 mg
calcium sulfate dehydrate	30 mg
Sucrose	4 mg
Starch	2 mg
Talc	1 mg
stearic acid	0.5 mg

20 While the preferred embodiments of the invention are illustrated by the above, it is to be understood that the invention is not limited to the precise instructions herein disclosed and that the right to all modifications coming within the scope of the following claims is reserved.

What is claimed is:

1. A compound of Formula (I):



in which

R1 is heteroaryl or substituted heteroaryl;

- 10 R2 is selected from a group consisting of: hydrogen, C1-C6 alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, substituted arylalkyl;

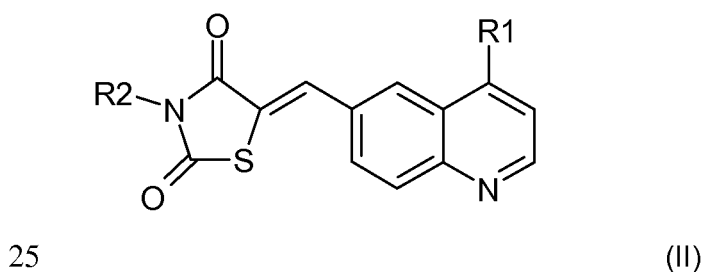
each R3 and R4 is independently selected from a group consisting of: hydrogen, halogen, acyl, amino, substituted amino, C1-6alkyl, substituted C1-6alkyl, C3-7cycloalkyl, substituted C3-7cycloalkyl, C3-7heterocycloalkyl, substituted C3-

- 15 7heterocycloalkyl, alkylcarboxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, arylalkyl, substituted arylalkyl, arylcycloalkyl, substituted, trifluoromethyl, arylcycloalkyl, heteroarylalkyl, substituted heteroarylalkyl, cyano, hydroxyl, alkoxy, nitro, acyloxy, acylamino, and aryloxy;

n is 0-2;

- 20 and/or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof.

2. A compound according to claim 1 selected from a compound of formula (II)



in which

R1 is heteroaryl or substituted heteroaryl;

R2 is selected from a group consisting of: hydrogen, C1-C6 alkyl, substituted alkyl, aryl, substituted aryl, arylalkyl, and substituted arylalkyl; and/or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof.

5 3. A compound according to claim 1 or 2 wherein R2 is hydrogen, and/or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof.

4. A compound according to claim 1 or 2, wherein R1 is a optionally substituted monocyclic heteroaryl; and/or a pharmaceutically acceptable salt,
10 hydrate, solvate or pro-drug thereof.

5. A compound of claim 1 selected from a group consisting of:
 (5Z)-5-{{4-(4-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-3-methyl-5-{{4-(4-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-
 15 2,4-dione;
 (5Z)-5-{{4-(3-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-(2-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-[2-(methyloxy)-5-pyrimidinyl]-6-quinolinyl}methylidene}-1,3-
 thiazolidine-2,4-dione;
 20 (5Z)-5-{{4-[2-(methyloxy)-4-pyridinyl]-6-quinolinyl}methylidene}-1,3-
 thiazolidine-2,4-dione;
 (5Z)-5-{{4-(6-amino-3-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-
 2,4-dione;
 (5Z)-5-{{4-(2-oxo-1,2-dihydro-4-pyridinyl)-6-quinolinyl}methylidene}-1,3-
 25 thiazolidine-2,4-dione;
 (5Z)-5-{{4-[6-(4-morpholinyl)-3-pyridinyl]-6-quinolinyl}methylidene}-1,3-
 thiazolidine-2,4-dione;
 (5Z)-5-{{4-[6-(4-methyl-1-piperazinyl)-3-pyridinyl]-6-quinolinyl}methylidene}-
 1,3-thiazolidine-2,4-dione;
 30 (5Z)-5-{{4-(3-pyridazinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-
 dione; and
 (5Z)-5-{{4-[2-(methyloxy)-5-pyrimidinyl]-6-quinolinyl}methylidene}-1,3-
 thiazolidine-2,4-dione;
 and/or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof.

35

6. A compound of formula (I) selected from a group consisting of:
(5Z)-5-{{[4-(4-pyridinyl)-6-quinolinyl]methylidene}-1,3-thiazolidine-2,4-dione, and/or a
pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof.

5

7. A pharmaceutical composition comprising a compound according to claim 1
to 2 and a pharmaceutically acceptable carrier.

8. A method of inhibiting one or more phosphatoinositides 3-kinases (PI3Ks) in
10 a mammal; comprising administering to the mammal a therapeutically effective
amount of a compound of Formula (I) and/or a pharmaceutically acceptable salt,
hydrate solvate or pro-drug thereof as defined in claim 1.

9. A method of treating one or more disease states selected from a group
15 consisting of: autoimmune disorders, inflammatory diseases, cardiovascular
diseases, neurodegenerative diseases, allergy, asthma, pancreatitis, multiorgan
failure, kidney diseases, platelet aggregation, cancer, sperm motility,
transplantation rejection, graft rejection and lung injuries, in a mammal, which
method comprises administering to such mammal, a therapeutically effective
20 amount of a compound according to claim 2.

10. A method of treating cancer comprises co-administration a compound
according to claim 1; and/or a pharmaceutically acceptable salt, hydrate, solvate or
pro-drug thereof; and at least one anti-neoplastic agent, such as one selected from
25 a group consisting of anti-microtubule agents, platinum coordination complexes,
alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites,
topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction
pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors,
immunotherapeutic agents, proapoptotic agents, and cell cycle signaling inhibitors.

30

11. A method of claim 9, wherein the disease state is selected from a group
consisting of: multiple sclerosis, psoriasis, rheumatoid arthritis, systemic lupus
erythematosus, inflammatory bowel disease, lung inflammation, thrombosis, brain
infection/inflammation, meningitis and encephalitis.

35

12. A method of claim 9, wherein the disease state is selected from a group consisting of: Alzheimer's disease, Huntington's disease, CNS trauma, stroke and ischemic conditions.
- 5 13. A method of claim 9, wherein the disease state is selected from a group consisting of: atherosclerosis, heart hypertrophy, cardiac myocyte dysfunction, elevated blood pressure and vasoconstriction.
- 10 14. A method of claim 9, wherein the disease state is selected from a group consisting of: chronic obstructive pulmonary disease, anaphylactic shock fibrosis, psoriasis, allergic diseases, asthma, stroke, ischemia-reperfusion, platelets aggregation/activation, skeletal muscle atrophy/hypertrophy, leukocyte recruitment in cancer tissue, angiogenesis, invasion metastasis, melanoma, Kaposi's sarcoma, acute and chronic bacterial and viral infections, sepsis, transplantation rejection,
- 15 graft rejection, glomerulo sclerosis, glomerulo nephritis, progressive renal fibrosis, endothelial and epithelial injuries in the lung, and lung airways inflammation.
15. A method of claim 9 wherein the disease is cancer.
- 20 16. A method of claim 15 wherein the cancer is selected from a group consisting of: brain (gliomas), glioblastomas, leukemias, Bannayan-Zonana syndrome, Cowden disease, Lhermitte-Duclos disease, breast, inflammatory breast cancer, Wilm's tumor, Ewing's sarcoma, Rhabdomyosarcoma, ependymoma, medulloblastoma, colon, head and neck, kidney, lung, liver, melanoma, ovarian,
- 25 pancreatic, prostate, sarcoma, osteosarcoma, giant cell tumor of bone, and thyroid,
17. A method of claim 15 wherein the disease is selected from a group consisting of: ovarian cancer, pancreatic cancer, breast cancer, prostate cancer and leukemia.
- 30 18. A method according to any one of claim 9 to 17, wherein the mammal is human.
19. A method of claim 8, wherein said PI3 kinase is a PI3 α .
- 35

20. A method of claim 8, wherein said PI3 kinase is a PI3 γ .

21. A method according to any one of claim 8 to 17, wherein said compound is selected from:

- 5 (5Z)-5-{{4-(4-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-3-methyl-5-{{4-(4-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-(3-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-(2-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 10 (5Z)-5-{{4-[2-(methyloxy)-5-pyrimidinyl]-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-[2-(methyloxy)-4-pyridinyl]-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-(6-amino-3-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 15 (5Z)-5-{{4-(2-oxo-1,2-dihydro-4-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-[6-(4-morpholinyl)-3-pyridinyl]-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 20 (5Z)-5-{{4-[6-(4-methyl-1-piperazinyl)-3-pyridinyl]-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 (5Z)-5-{{4-(3-pyridazinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione; and
 (5Z)-5-{{4-[2-(methyloxy)-5-pyrimidinyl]-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione;
 25 and/or a pharmaceutically acceptable salt, solvate, hydrate, or pro-drug thereof.

22. A method according to any one of claim 8 to 17, wherein said compound is selected from a group consisting of: (5Z)-5-{{4-(4-pyridinyl)-6-quinolinyl}methylidene}-1,3-thiazolidine-2,4-dione, and/or a pharmaceutically acceptable salt, solvate, hydrate, or pro-drug thereof.

23. A method of claim 8 wherein the compound according to claim 1, and/or a pharmaceutically acceptable salt, hydrate, solvate or pro-drug thereof, is administered in a pharmaceutical composition.