Title: CANCER COMBINATION THERAPY USING IMIDAZO[4,5-C]QUINOLINE DERIVATIVES

Abstract: The present invention relates to a pharmaceutical combination comprising a PI3K/mTOR inhibitor selected from the compounds of Formula (I) (as described herein) or pharmaceutically acceptable salts or solvates thereof; and one or more anti-proliferative agents; pharmaceutical compositions containing said the compounds of Formula (I) and one or more anti-proliferative agents; and use of the said combination in the treatment of proliferative diseases or disorders.
CANCER COMBINATION THERAPY USING MIDAZO[4,5-C]QUINOLINE DERIVATIVES

FIELD OF THE INVENTION

The present invention relates to a pharmaceutical combination comprising a PI3K/mTOR inhibitor selected from the compounds of Formula (I) (as described herein) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-proliferative agents; pharmaceutical compositions containing said combination; and use of the said combination in the treatment of proliferative diseases or disorders.

BACKGROUND OF THE INVENTION

Phosphatidylinositol-3-kinase or phosphoinositide-3-kinase (PI3K)/Akt pathway aberrations are commonly seen in various malignancies. PI3K is a lipid kinase, divided into three classes viz.; class I, II and III. The classification is based on primary structure, regulation and in vitro lipid substrate specificity. Class III PI3K enzymes phosphorylate PI (phosphatidylinositol) alone, while, Class II PI3K enzymes phosphorylate both PI and PI 4-phosphate (PI(4)P). Class I PI3Ks are divided into two groups, class IA and class IB, in terms of their activation mechanism. Class 1B is composed of p110γ alone, whereas Class IA PI3K is composed of p110α, p110β and p110δ subtypes which are generally activated in response to growth factor-stimulation of receptor tyrosine kinases. p110α protein is encoded by the PIK3CA gene. Mutations in PIK3CA oncogene is implicated in various cancers including colon, lung, ovarian, liver, brain, stomach, cervical and breast cancer (Br. J. Cancer., 2006, 94(4), 455-459).

Class I PI3K enzymes phosphorylate PI, PI(4)P and PI 4,5-biphosphate (PIP2). Phosphorylation of PIP2 generates phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 acts as a ligand for the PH (Pleckstrin Homology) domain containing protein, Akt. The protein, Akt is a serine-threonine kinase and acts downstream of PI3K to regulate many biological processes. It plays a critical role in cell growth, proliferation and cell survival. Akt is activated by growth factors and the activation depends on the integrity of the PH domain and phosphorylation of Thr308 and Ser473. Activated Akt, phosphorylated at Ser473 is found elevated in patients with multiple myeloma (Blood, 2002, 99, 2278-2279),

The PI3K/Akt/mTOR pathway plays a very important role in cancer cell survival, cell proliferation, angiogenesis and metastasis (Nat. Rev. Drug Discov., 2005, 4, 988-1004). The PI3K pathway has gained importance in cancer therapy due to its unique nature, in that, all the key factors of this pathway have been found mutated or amplified in a broad range of cancers. Thus, PI3K/Akt/mTOR pathway is an attractive target for the development of anticancer agents.

PI3K/Akt/mTOR pathway is inhibited by PTEN (phosphatase and tensin homolog deleted on chromosome ten), a tumor suppressor under normal circumstances. However, in certain cancers such as brain, head and neck, thyroid, breast, lung, liver, prostate, endometrial and bladder, PTEN is seen to be mutated. Therefore, PI3K/Akt/mTOR inhibitors can be effective in the treatment of cancers having PTEN aberrations.

Inhibition of PI3K-Akt pathway suppresses coagulation and inflammation (Arteriosclerosis, Thrombosis, and Vascular Biology, 2004, 24,1963). Thus, PI3K/mTOR inhibitors are also useful in the treatment of autoimmune and inflammatory diseases and disorders.

There are several PI3K inhibitors in the early phase of the clinical trials, including GDC-0941 (Piramed Ltd. and Genentech Inc.), BEZ-235 and BGT-226 (Novartis AG) and XL-765 (Exelixis Inc.). PI3K inhibitors are also disclosed in

Imidazoquinoline derivatives represented herein by the compounds of Formula (I) that act through the inhibition of PI3K/mTOR and ALK1 pathways are disclosed in PCT Published Application WO2012007926. In the context of the present invention the compounds of Formula (I) are referred to as PI3K/m-TOR inhibitors.

Although, the compounds of Formula (I) (as described herein) as a single agent can be effective in the treatment of cancers owing to the fact that the compounds act through inhibition of PI3K/mTOR and ALK1 pathways. However, it is apparent that in spite of the efforts made to date, there is still a need to explore different approaches for the treatment of cancers which will not only provide improvement in efficacy but will also provide added survival benefits to the patients suffering from different types of cancers. One such approach is directed to a protocol involving combining different anticancer agents having different biological mechanism. An optimal combination chemotherapy protocol may not only result in increased therapeutic efficacy but also may result in decreasing host toxicity, and minimal or delayed drug resistance. The present inventors have directed their efforts to provide pharmaceutical combination comprising a PI3K/mTOR inhibitor selected from the compounds represented by Formula I (as described herein) and one or more anti-proliferative agents for use in the treatment of cancers.

SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a pharmaceutical combination comprising a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof; and one or more anti-proliferative agents (as described herein).

In another aspect, the present invention relates to pharmaceutical composition comprising a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof; and one or
more anti-proliferative agents and at least one pharmaceutically acceptable carrier or excipient.

In another aspect, the pharmaceutical combination of the present invention is provided for use in the treatment of a disease or a disorder mediated by mTOR kinase and/or PI3K.

In another further aspect, the pharmaceutical combination of the present invention is provided for use in the treatment of a proliferative disease or a disorder.

In another aspect, the present invention relates to a method of treating a disease or a disorder mediated by mTOR kinase and/or PI3K comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof; in combination with a therapeutically effective amount of one or more anti-proliferative agents.

In yet another aspect, the present invention relates to a method of treating a proliferative disease or a disorder comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof; in combination with one or more anti-proliferative agents.

In further aspect, the present invention relates to use of a pharmaceutical combination comprising of a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof; and one or more anti-proliferative agents in the treatment of a proliferative disease or a disorder.

In further aspect, the present invention relates to use of a pharmaceutical combination comprising a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof; and one or more anti-proliferative agents in the manufacture of a medicament for the treatment of a proliferative disease or disorder.

In still further aspect, the present invention relates to a pharmaceutical kit comprising: (a) a pharmaceutical combination comprising a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts
or solvates thereof; and one or more anti-proliferative agent; and (b) optionally a package insert comprising instructions for using the pharmaceutical combination.

These and other objectives and advantages of the present invention will be apparent to those skilled in the art from the following description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1aa is a graphical representation comparing cytotoxicity of Compound A (a representative example of the compound of Formula (I)) and metformin at different concentrations, when used alone and in combination in breast cancer cell line-MDAMB453. The combination is administered sequentially.

FIG. 1ab is a graphical representation comparing cytotoxicity of Compound A and metformin at different concentrations, when used alone and in combination in breast cancer cell line-MDAMB231. The combination is administered sequentially.

FIG. 1ac is a graphical representation comparing cytotoxicity of Compound A and metformin at different concentrations, when used alone and in combination in breast cancer cell line-BT549. The combination compounds are administered sequentially.

FIG. 1ba is a graphical representation comparing cytotoxicity of Compound A and metformin at different concentrations, when used alone and in combination in breast cancer cell line-BT549. The combination compounds are administered simultaneously.

FIG. 1bb is a graphical representation comparing cytotoxicity of Compound A and metformin at different concentrations, when used alone and in combination in breast cancer cell line-MDAMB231. The combination compounds are administered simultaneously.

FIG. 2a is a graphical representation comparing cytotoxicity of Compound A and lapatinib at different concentrations, when used alone and in combination in breast cancer cell line-HCC 1569. The combination compounds are administered simultaneously.
FIG. 2b is a graphical representation comparing cytotoxicity of Compound A and lapatinib at different concentrations, when used alone and in combination in breast cancer cell line-HCC 1954. The combination compounds are administered simultaneously.

FIG. 2c is a graphical representation comparing cytotoxicity of Compound A and lapatinib at different concentrations, when used alone and in combination in breast cancer cell line-SKBR3. The combination compounds are administered simultaneously.

FIG. 3a is a graphical representation comparing cytotoxicity of Compound A and cisplatin at different concentrations, when used alone and in combination in breast cancer cell line-MDAMB231. The combination compounds are administered simultaneously.

FIG. 3b is a graphical representation comparing cytotoxicity of Compound A and cisplatin at different concentrations, when used alone and in combination in breast cancer cell line-BT549. The combination compounds are administered simultaneously.

FIG. 3c is a graphical representation comparing cytotoxicity of Compound A and cisplatin at different concentrations, when used alone and in combination in breast cancer cell line-MDAMB453. The combination compounds are administered simultaneously.

FIG. 3d is a graphical representation comparing cytotoxicity of Compound A and cisplatin at different concentrations, when used alone and in combination in breast cancer cell line-MDAMB468. The combination compounds are administered simultaneously.

FIG. 4a is a graphical representation comparing cytotoxicity of Compound A and doxorubicin at different concentrations, when used alone and in combination in breast cancer cell line-BT549. The combination compounds are administered simultaneously.
FIG. 4b is a graphical representation comparing cytotoxicity of Compound A and doxorubicin at different concentrations, when used alone and in combination in breast cancer cell line- MDAMB468. The combination compounds are administered simultaneously.

FIG. 4c is a graphical representation comparing cytotoxicity of Compound A and doxorubicin at different concentrations, when used alone and in combination in breast cancer cell line- MDAMB453. The combination compounds are administered simultaneously.

FIG. 5a is a graphical representation comparing cytotoxicity of Compound A and paclitaxel at different concentrations, when used alone and in combination in breast cancer cell line-BT549. The combination compounds are administered simultaneously.

FIG. 5b is a graphical representation comparing cytotoxicity of Compound A and paclitaxel at different concentrations, when used alone and in combination in breast cancer cell line- MDAMB453. The combination compounds are administered simultaneously.

FIG. 5c is a graphical representation comparing cytotoxicity of Compound A and paclitaxel at different concentrations, when used alone and in combination in breast cancer cell line- MDAMB468. The combination compounds are administered simultaneously.

FIG. 6a is a graphical representation comparing cytotoxicity of Compound A and 5-Fluorouracil at different concentrations, when used alone and in combination in breast cancer cell line-BT549. The combination compounds are administered simultaneously.
FIG. 6b is a graphical representation comparing cytotoxicity of Compound A and 5-Fluorouracil at different concentrations, when used alone and in combination in breast cancer cell line- MDAMB453. The combination compounds are administered simultaneously.

FIG. 6c is a graphical representation comparing cytotoxicity of Compound A and 5-Fluorouracil at different concentrations, when used alone and in combination in breast cancer cell line- MDAMB468. The combination compounds are administered simultaneously.

FIG. 7 is a graphical representation comparing cytotoxicity of Compound A and PL225B at different concentrations, when used alone and in combination in breast cancer cell line- HCC1569. The combination compounds are administered simultaneously.

FIG. 8a is a graphical representation comparing effect of Compound A alone and in combination with erlotinib at different concentrations, in lung cancer cell line- A549. The combination compounds are administered simultaneously.

FIG. 8b is a graphical representation comparing effect of Compound A alone and in combination with erlotinib at different concentrations in lung cancer cell line- H358. The combination compounds are administered simultaneously.

FIG. 8c is a graphical representation comparing effect of Compound A alone and in combination with erlotinib at different concentrations in lung cancer cell line- Hi 975. The combination compounds are administered simultaneously.

FIG. 8d is a graphical representation comparing effect of Compound A alone and in combination with erlotinib at different concentrations in lung cancer cell line- H1650. The combination compounds are administered simultaneously.
FIG. 9a is a graphical representation comparing effect of Compound A alone and in combination with PLX4032 at different concentrations in melanoma cell line-A375. The combination compounds are administered sequentially.

FIG. 9b is a graphical representation comparing effect of Compound A alone and in combination with PLX4032 at different concentrations in melanoma cell line-G361. The combination compounds are administered sequentially.

FIG. 9c is a graphical representation comparing effect of Compound A alone and in combination with PLX4032 at different concentrations in melanoma cell line-SKME-3. The combination compounds are administered sequentially.

FIG. 9d is a graphical representation comparing effect of Compound A alone and in combination with PLX4032 at different concentrations in melanoma cell line-A2058. The combination compounds are administered simultaneously.

FIG. 10a is a graphical representation comparing effect of Compound A alone and in combination with sorafenib at different concentrations in non-small-cell lung cancer cell line- H460. The combination compounds are administered sequentially.

FIG. 10b is a graphical representation comparing effect of Compound A alone and in combination with sorafenib at different concentrations in lung cancer cell line- A549. The combination compounds are administered sequentially.

FIG. 11a is a graphical representation comparing effect of Compound A alone and in combination with BMS7081 63 and cyclopamine at different concentrations in breast cancer cell line- MDAMB231. The combination compounds are administered simultaneously.

FIG. 11b is a graphical representation comparing effect of Compound A alone and in combination with BMS7081 63 and cyclopamine at different concentrations in breast cancer cell line- MDAMB453. The combination compounds are administered simultaneously.
FIG. 11c is a graphical representation comparing effect of Compound A alone and in combination with BMS7081 63 and cyclopamine at different concentrations in breast cancer cell line- MDAMB468. The combination compounds are administered simultaneously.

FIG. 11d is a graphical representation comparing effect of Compound A alone and in combination with BMS7081 63 and cyclopamine at different concentrations in breast cancer cell line- BT549. The combination compounds are administered simultaneously.

FIG. 11e is a graphical representation comparing effect of Compound A alone and in combination with BMS7081 63 and cyclopamine at different concentrations in breast cancer cell line- HCC1 937. The combination compounds are administered simultaneously.

FIG. 12a is a graphical representation comparing effect of Compound A alone and in combination with everolimus at different concentrations in breast cancer cell line- MCF7. The combination compounds are administered simultaneously.

FIG. 12b is a graphical representation comparing effect of Compound A alone and in combination with everolimus at different concentrations in pancreatic cancer cell line- Panel. The combination compounds are administered simultaneously.

FIG. 13 is a graphical representation of tumor growth profile in triple negative breast cancer (MDA-MB-231) xenograft model after administration of Compound A and metformin alone and in combination.

FIG. 14 is a graphical representation of tumor growth profile in Her 2 overexpressing breast cancer (HCC 1569) xenograft model after administration of Compound A and lapatinib alone and in combination.

FIG. 15 is a graphical representation of tumor growth profile in EGFR mutated non-small cell lung cancer (H1650) xenograft model after administration of Compound A and erlotinib alone and in combination.
FIG. 16 is a graphical representation of tumor growth profile in EGFR mutated non-small cell lung cancer (H1975) xenograft model after administration of Compound A and erlotinib alone and in combination.

FIG. 17a and FIG. 17b are graphical representations of tumor growth profile in K-ras mutated colon cancer (HCT116) xenograft model after administration of Compound A and irinoitecan alone and in combination.

FIG. 18a is a graphical representation of tumor growth profile in triple negative breast cancer (MDAMB231) xenograft model after administration of Compound A and PL225B alone and in combination.

FIG. 18b is a graphical representation of tumor growth profile in erlotinib resistant lung cancer (T790M) xenograft model after administration of low dose of Compound A and PL225B alone and in combination.

FIG. 19a and FIG. 19b are graphical representations of tumor growth profile in lung cancer (H460) xenograft model after administration of Compound A and sorafenib alone and in combination.

FIG. 19c is graphical representation of tumor growth profile in colon cancer (HCT116) xenograft model after administration of Compound A and sorafenib alone and in combination.

FIG. 19d is graphical representation of tumor growth profile in liver cancer (Huh-7) xenograft model after administration of Compound A and sorafenib alone and in combination.

FIG. 20a and FIG. 20c are graphical representations of tumor growth profile in BRAF(V600E) mutant melanoma (A375) xenograft model after administration of Compound A and PLX4032 (vemurafenib) alone and in combination.
FIG. 20b and FIG. 20d are graphical representations of tumor growth profile in BRAF(V600E) mutant melanoma (A2058) xenograft model after administration of Compound A and PLX4032 (vemurafenib) alone and in combination.

5 DETAILED DESCRIPTION OF THE INVENTION

Definitions

Listed below are definitions, which apply to the terms as they are used throughout the specification and the appended claims (unless they are otherwise limited in specific instances), either individually or as part of a larger group. It will be understood that "substitution" or "substituted by" or "substituted with" includes the implicit proviso that such substitution is in accordance with the permitted valence of the substituted atom and the substituent, as well as represents a stable compound, which does not readily undergo transformation such as by rearrangement, cyclization, elimination, etc.

The term "halo" or "halogen" as used herein refers to an atom selected from F, Cl, Br and I.

The term "alkyl" whether used alone or as part of a substituent group, refers to the radical of saturated aliphatic groups, including straight or branched-chain containing from 1 to 8 carbon atoms, for example, 1 to 6 carbon atoms or 1 to 4 carbon atoms. Examples of alkyl groups include but are not limited to methyl, ethyl, propyl, butyl, isopropyl, isobutyl, 1-methylbutyl, sec-butyl, tert-butyl, pentyl, neo-pentyl, n-hexyl and the like.

The term "aryl" as used herein refers to a monocyclic or polycyclic hydrocarbon group having 6 to 14 ring carbon atoms, preferably up to 10 ring carbon atoms, more preferably up to 6 ring carbon atoms in which at least one carbocyclic ring is present that has a conjugated π electron system. Accordingly, the term "aryl" refers to C₆-C₁₄ aryl. Examples of aryl include but are not limited to phenyl, naphthyl, tetrahydropaphthyl and the like. Aryl residues can be bonded via any desired position, and in substituted aryl residues, the substituents can be located in any desired position.
In some embodiments, C\textsubscript{6}-C\textsubscript{14} aryl is selected from the group consisting of phenyl, naphthyl, anthracenyl and 1H-phenalenyl.

The term "heteroaryl" as used herein refers to an aromatic heterocyclic ring system containing 5 to 20 ring atoms, suitably 5 to 10 ring atoms, which may be a monocyclic or polycyclic, fused together or linked covalently. The rings may contain from 1 to 4 heteroatoms selected from N, O and S, wherein the N or S atom is optionally oxidized, or the N atom is optionally quaternized. Any suitable ring position of the heteroaryl moiety may be covalently linked to the defined chemical structure. Examples of heteroaryl include, but are not limited to, furanyl, thiophenyl, pyrrolyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, 1H-tetrazolyl, oxadiazolyl, triazolyl, pyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, benzoazolyl, benzothiazolyl, benzofuranyl, benzothienyl, phthalazinyl, dibenzofuranyl, benzimidazolyl, indolyl, isoindolyl, indazolyl, quinolinyl, isoquinolinyl, quinazolinyl, quinoxalinyl, purinyl, indolizyl, benzoisothiazolyl, benzoxazolyl, pyrrolopyridyl, furopyridinyl, benzothiadiazolyl, benzooxadiazolyl, benzotriazolyl, benzodiaxolyl, dibenzothienyl and the like.

The foregoing heteroaryl groups may be C-attached or N-attached (where such an attachment is possible). For instance, a group derived from pyrrole may be pyrrol-1-yl (N-attached) or pyrrol-3-yl (C-attached).

The term "heterocyclyl" or "heterocycle" as used herein refers to a saturated or partially unsaturated monocyclic or polycyclic ring system containing 5 to 20 ring atoms of which 1, 2, 3 or 4 are identical or different heteroatoms selected from N, O and S. The "heterocyclyl" or "heterocycle" may, for example, have 1 to 2 oxygen atoms and/or 1 to 2 sulfur atoms and/or 1 to 4 nitrogen atoms in the ring. The "heterocyclyl" or "heterocycle" preferably is a 5- or 6-membered ring. The ring heteroatoms can be present in any position with respect to each other provided that the resulting "heterocyclyl" or "heterocycle" is stable. Examples of "heterocyclyl" or "heterocycle" include but are not limited to: decahydroquinolinyl, oxadiazolindinyl, imidazolidinyl, indoliny, isobenzofuranyl, morpholiny, octahydroisoquinolinyl, oxazolidinyl, piperidiny, piperaziny, pyrazoliny, pyrazolidinyl, pyrrolidiny, pyrroliny, tetrahydrofuranyl, benzodioxolyl, tetrahydroisoquinolinyl, and tetrahydroquinolinyl.
The term "alkylheterocyclyl" as used herein refers to a heterocyclyl group bonded through an alkyl, wherein the terms "alkyl" and "heterocycle" are as defined herein above. Examples of alkylheterocycle include but are not limited to piperazin-1-ylmethyl, piperidin-1-ylmethyl, pyrrolidin-2-ylmethyl, 2-morpholinoethyl and the like.

The term "stereoisomer" as used herein refers to all isomers of individual compounds of Formula (I) that differ only in the orientation of their atoms in space. The term stereoisomer includes mirror image isomers (enantiomers), mixtures of mirror image isomers (racemates, racemic mixtures), geometric (cis/trans or syn/anti or E/Z) isomers, and isomers of compounds with more than one chiral center that are not mirror images of one another (diastereoisomers). The compounds of Formula (I) may have asymmetric centers and occur as racemates, racemic mixtures, individual diastereoisomers, or enantiomers, or may exist as geometric isomers, with all isomeric forms of said compounds being included in the present invention.

The term "tautomer" as used herein refers to the coexistence of two (or more) compounds that differ from each other only in the position of one (or more) mobile atoms and in electron distribution, for example, keto-enol and imine-enamine tautomers.

The term "solvate" as used herein refers to a compound formed by the interaction of a solute (in respect of the present invention, a compound of Formula (I) or a salt thereof) and a solvent. Such solvents for the purpose of the invention may not interfere with the biological activity of the solute. Examples of suitable solvents include, but are not limited to, water, methanol, ethanol and acetic acid. Preferably the solvent used is a pharmaceutically acceptable solvent. Examples of suitable pharmaceutically acceptable solvents include, without limitation, water, ethanol and acetic acid. Most preferably the solvent used is water. Examples for suitable solvates are the mono- or dihydrates or alcolohates of the compounds according to the invention.

The term "pharmaceutically acceptable salts" as used herein refers to inorganic and organic salts of the compound of Formula (I) contained in the pharmaceutical combination or composition of the invention. The compounds of Formula (I), which contain acidic groups, may be converted into salts with
pharmaceutically acceptable bases. Such salts include, for example, alkali metal salts, like lithium, sodium and potassium salts; alkaline earth metal salts like calcium and magnesium salts; ammonium salts; [tris(hydroxymethyl)aminomethane], trimethylamine salts and diethylamine salts; salts with amino acids such as lysine, arginine, guanidine and the like.

The compounds of Formula (I), which contain one or more basic groups, i.e. groups which can be protonated, can form an addition salt with an inorganic or organic acid. Examples of suitable acid addition salts include: acetates, adipate, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrate, cinnamates, citrates, ethanesulfonates, fumarates, glucuronates, glutamates, glycolates, hydrochlorides, hydrobromides, hydrofluorides, hydroiodides, ketoglutarates, lactates, maleates, malonates, methanesulfonates, nitrates, oxalates, pamoates, palmoates, perchlorates, phosphates, picrates, salicylates, succinates, sulfamate, sulfamates, tartrates, toluenesulfonates and other acid addition salts known to the person skilled in the art.

The term "N-oxide" as used herein in reference to the compounds of Formula (I) refers to the oxide of the nitrogen atom of a nitrogen-containing heteroaryl or heterocycle. N-oxide can be formed in presence of an oxidizing agent for example peroxide such as m-chloro-perbenzoic acid or hydrogen peroxide.

The compounds of Formula (I) contained in the pharmaceutical combination or the composition of the present invention can be used in isotopically labeled form, wherein in the compounds of Formula (I) one or more atoms are replaced by their respective isotopes. All isotopes of any particular atom or element as specified are contemplated within the scope of the compounds of the invention. Examples of isotopes that may be incorporated into the compounds disclosed herein include, but are not limited to, isotopes of hydrogen such as $^2$H and $^3$H, carbon such as $^{11}$C, $^{13}$C and $^{14}$C, nitrogen such as $^{13}$N and $^{15}$N, oxygen such as $^{16}$O, $^{17}$O and $^{18}$O, chlorine such as $^{35}$Cl, fluorine such as $^{18}$F and sulphur such as $^{35}$S. Substitution with heavier isotopes, for example, replacing one or more key carbon-hydrogen bonds with carbon-deuterium bond may show certain therapeutic advantages, resulting from longer metabolism
cycles, (e.g., increased in-vivo half life or reduced dosage requirements), improved safety or greater effectiveness and hence may be preferred in certain circumstances.

The term "alkylating agent" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent which inhibit protein synthesis by alkylating DNA and preventing transcription of DNA into RNA. The non-limiting examples of alkylating agents include cisplatin, mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, altretamine, thiotapec, busulfan, carmustine, lomustine, dacarbazine, procarbazine and temozolomide.

The term "DNA intercalating agent" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent which is capable of inserting themselves between the successive DNA bases, thereby inhibiting protein synthesis. The non-limiting examples of DNA intercalating agent includes doxorubicin, daunorubicin and dactinomycin.

The term "mitotic inhibitor" or antimicrotubule agent refers to an anti-proliferative agent; which is an anti-cancer agent that disrupts mitotic spindle assembly and thereby inhibiting cell division. The non-limiting examples of mitotic inhibitor include paclitaxel, colchicine and vinca alkaloids such as vincristine and vinblastin.

The term "antimetabolite" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that resembles to the metabolite and interferes with the formation or utilization of the metabolite, thus inhibiting essential metabolic routes. Antimetabolites particularly inhibit nucleic acid synthesis or nucleotide synthesis, thus disrupting DNA synthesis resulting in cell death. The non-limiting examples of antimitabolite include methotrexate, 6-thiopurines, mercaptopurine, thioguanine, cladribine, pentostatin, cytarabine, azactidine, fludarabine, 5-fluorouracil, gemcitabine or hydroxyurea.

The term "biguanide" as used herein refers to the compounds comprising two guanidine groups. The non-limiting examples of biguanide include metformin, phenformin or buformin.

The term "insulin-like growth factor-1 receptor (IGF-1 R) kinase inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that disrupts the IGF-1 R signaling pathway. The non-limiting examples of IGF-1 R
kinase inhibitor include figitumumab, AMG-479 (Amgen Inc.), dalotuzumab, PL225B (Merck & Piramal Enterprises Limited) or INSM18 (University of California, San Francisco).

The term "tyrosine kinase inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits tyrosine kinase. The non-limiting examples of tyrosine kinase inhibitor include, Bcr-Abl tyrosine kinase inhibitor such as imatinib, nilotinib; Src family tyrosine kinase inhibitor such as dasatinib; EGFR inhibitors such as gefitinib or erlotinib; ErbB2, Erk-1 and-2 inhibitors such as lapatinib; serine/threonine kinase inhibitors for example mTOR inhibitor such as rapamycin, everolimus or temsirolimus.

The term "epidermal growth factor receptor inhibitor" or "EGFR inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits epidermal growth factor receptor, a receptor tyrosine kinase. Activated EGFR stimulates proliferation, angiogenesis, invasion, metastasis and inhibition of apoptosis. The EGFR inhibitors act, for example by preventing tyrosine kinase activation, inhibition of EGFR signaling pathways, intracellular or extracellular binding. The non-limiting examples of epidermal growth factor receptor inhibitor include gefitinib, erlotinib, lapatinib or cetuximab.

The term "serine-threonine kinase inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits serine-threonine kinase. The non-limiting examples of serine-threonine kinase inhibitor includes Raf kinase inhibitor such as sorafenib or vemurafenib; cyclin dependent kinase inhibitor such as Roscovatine, AT7519M (Astex Pharmaceuticals), dinaciclib, palbociclib or AG-024322 (Pfizer Inc); mitogen activated protein kinase inhibitors such as SB-681323 (GlaxoSmithKline) or PD098059 (Promega Corporation); Akt pathway inhibitor such as MK2206 (Merck Inc.) or SR1 3668 (SRI International).

The term "Ras-Raf-MEK-ERK pathway inhibitor" or "mitogen-activated protein kinase (MAPK) pathway inhibitors" used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits one or more components of MAPK pathway. MAP kinases are major components of the pathway; controlling embryogenesis, cell differentiation, cell proliferation and cell death. The non-limiting examples of MAPK pathway include, p38 MAPK inhibitor such as Selumetinib, MEK inhibitor such as PD-325901 (Pfizer, Inc.) or
Trametinib, B-RAF inhibitor such as Vemurafenib (PLX4032) or Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) inhibitor such as BVD-523 (BioMed Valley Discoveries).

The term "Raf kinase inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits Raf kinase. The Raf kinase includes A-Raf kinase, B-Raf kinase and C-Raf kinase. Raf kinase is responsible for regulation of transcription factors (AP-1, NF-κB, c-Myc), regulation of cell differentiation. Raf kinase inhibitors act, for example by destabilizing Raf proteins, as antisense oligonucleotides, as specific Raf inhibitors, by regulating Raf activity.

The non-limiting examples of B-Raf kinase inhibitors include sorafenib, vemurafenib, PLX3603 (Plexxikon Inc.), dabrafenib, LGX818 (Novartis AG), BMS-908662 or XL281 (Exelixix Inc.), RAF265 or CHIR-265 (Chiron Corp.) or GW-5074 (GlaxoSmithKline). The non-limiting examples of C-Raf inhibitors include sorafenib, or ISIS 5132 (ISIS Pharmaceuticals Inc).

The term "cyclin dependent kinase (CDK) inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits cyclin dependent kinase which is involved in the regulation of cell cycle in mammalian cell. The non-limiting examples of cyclin dependent kinase inhibitor include roscovatine, P276 (Piramal Enterprises Limited), P1446 (Piramal Enterprises Limited), AT7519 (Astex Pharmaceuticals), dinaciclib, palbociclib or AG-024322 (Pfizer Inc).

The term "mitogen activated protein kinase inhibitors" or MAPK inhibitors as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits mitogen activated protein. The non-limiting examples of MAPK inhibitors include p38 MAPK inhibitors such as losmapimod, SB681323 (GlaxoSmithkline) or VX-702 (Vertex Pharmaceuticals Inc / Kissei Pharmaceutical Co Ltd).

The term "Akt pathway inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits Akt pathway. The non-limiting examples of Akt pathway inhibitor include MK2206 (Merck Inc.) or SR13668 (SRI International).

The term "histone deacetylase inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits histone deacetylase.
Histones deacetylase plays a critical role in cell survival and proliferation. Histone deacetylase inhibitors can induce transformed cell cycle arrest and terminal cell differentiation and can also be responsible for cell death. The non-limiting examples of histone deacetylase inhibitor include CHR-3996 (Chroma Therapeutics); CHR-2845 (Chroma Therapeutics), 4SC-202 (4SC AG), Quisinostat or Givinostat.

The term "angiogenesis inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits angiogenesis i.e formation of new blood vessels. Angiogenesis is associated with tumor metastasis. Vascular endothelial growth factor stimulates vasculogenesis and angiogenesis and hence is the primary target for angiogenesis inhibitors. Platelet-derived growth factor is another such target. The non-limiting examples of angiogenesis inhibitor include sunitinib, bevacizumab and sorafenib.

The term "aromatase inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits cytochrome P450 enzyme, aromatase, which catalyses the conversion of androgens to oestrogens. Aromatase inhibitors are primarily used in the treatment of breast and ovarian cancer in postmenopausal women. The non-limiting examples of aromatase inhibitor include testolactone, aminogluthethimide, formestane, fadrozole, exemestane, anastrozole or letrozole.

The term "lipid kinase inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits lipid kinase such as phosphatidylinositol-3-kinase (PI3 kinase), phosphatidylinositol-4-kinase (PI4K) or phosphatidylinositol-5-kinase (PI5K). The non-limiting examples of lipid kinase inhibitor include Wortmannin, BKM-120(Novartis AG); BYL719(Novartis AG); BEZ235(Novartis AG), PF-05212384 (Pfizer Inc), SF1126 (Semafore Pharmaceuticals), MK-2206 (Merck Inc), BAY80-6946 (Bayer Pharma AG) or AZD5363 (Astex Pharmaceuticals).

The term an "apoptosis inducer" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that induces cell death. The apoptosis inducer may cause G2-M arrest, cleavage of essential proapoptotic caspase substrates, or induction of nuclear fragmentation. The non-limiting example of apoptosis inducer includes RO5458640 (Hoffmann-La Roche).
The term an "aurora kinase inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits aurora kinase. Aurora kinase, a serine/threonine kinase comprises of three members, Aurora A, B and C, which are important regulators of mitosis. Aurora kinase inhibitors can delay the entry into mitosis and causes cell to arrest at G2/M checkpoint. The non-limiting examples of aurora kinase inhibitor include PF-03814735 (Pfizer Inc), tozasertib, alisertib or SNS-314 (Sunesis Pharmaceuticals, Inc).

The term "mTOR inhibitors" or mammalian target of rapamycin inhibitor as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits mTOR. mTOR is a protein kinase that controls cell growth by regulating many cellular processes, including protein synthesis and autophagy. mTOR inhibitors can inhibit protein synthesis and angiogenesis. The non-limiting examples of mTOR inhibitors include everolimus, sirolimus or temsirolimus.

The term "progesterone receptor antagonists" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits progesterone receptor. Progesterone receptor antagonists are primarily useful in the treatment of hormone positive breast cancer. The non-limiting examples of progesterone receptor antagonist include Lonaprisan or CDB-2914 (HRA Pharma).

The term "topoisomerase inhibitors" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits the enzymes topoisomerase I or topoisomerase II. Topoisomerases regulate DNA topology and are essential for the integrity of the genetic material during transcription, replication and recombination processes. The topoisomerase inhibitors act by disrupting DNA replication in tumor cells, thus resulting in tumor cell death. The non-limiting examples of topoisomerase inhibitors include irinotecan, topotecan or 9-aminocamptothecin.

The term "DNA dependent protein kinase inhibitor" or "DNA-PK inhibitor" as used herein refers to an anti-proliferative agent; which is an anti-cancer agent that inhibits DNA dependent protein kinase. DNA-PK is essential for repairing double stranded DNA breaks as well as sensing and transmitting a damage signal to downstream targets leading to cell cycle arrest. Abnormal overexpression of DNA-PK may lead to cell proliferation. The non-limiting examples of DNA-PK inhibitor include NU7026 (University of Newcastle) or CC-15 (Celgene Corporation).
The term "therapeutically effective amount", as used herein refers to the amount of the compound of Formula (I) and that of anti-proliferative agent contained in the pharmaceutical combination or composition of the present invention, when administered to a subject in need thereof, is sufficient to (i) prevent or delay one or more symptom of cancer (ii) ameliorate or eliminate of one or more symptom of cancer or (iii) treat the cancer.

The term "treat" and "treatment" as used herein refers to one or more of : (i) inhibition of cancer i.e., arresting the development of the cancer; (ii) reduction in the regression of the cancer or slowing down of the cancer (iii) amelioration of the cancer i.e., reducing the severity of the symptoms associated with the cancer (iv) relief, to some extent, of one or more symptoms associated with cancer (v) achieving a stabilized state of disease; and (vi) prolonging survival of the subject as compared to expected survival.

The term a proliferative disease or disorder as used herein refers to disease or disorder resulting from abnormally increased and/or uncontrolled growth of cell(s) in a mammal.

The term "disease or a disorder mediated by mTOR kinase and/or PI3K" as used herein refers to the disease or disorder which is characterized by abnormal mTOR kinase or PI3 kinase activity or of both, mTOR kinase and PI3K activity.

The term "subject" as used herein, refers to an animal, preferably a mammal, most preferably a human, who is in the need of treatment of cancer. The term subject may be interchangeably used with the term patient in the context of the present invention.

The term "anti-proliferative agent" as used herein refers to a compound that treats a proliferative condition. A proliferative condition is unwanted or uncontrolled abnormal cell growth. An example of a proliferative condition is cancer.

The term "synergistic" or "synergistic effect" or "synergism" as used herein refers to the therapeutic effect of the combination of compounds, which is greater than the additive effect of the compounds used in the pharmaceutical combination. The combination index (CI) method of Chou and Talalay can be used to determine the synergy, additive or antagonism effect of the compounds used in combination. When the CI value is less than 1, there is synergy between
the compounds used in the combination; when the CI value is equal to 1, there is an additive effect between the compounds used in the combination and when CI value is more than 1, there is an antagonistic effect.

The synergistic effect can be attained either by co-formulating the compounds contained in the pharmaceutical combination or the composition of the present invention and administering the said compounds simultaneously through a unit dosage form or as separate formulations administered simultaneously or sequentially.

Thus, in accordance with an aspect, the present invention relates to a pharmaceutical combination comprising a compound of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof having PI3 kinase inhibitory activity; and one or more anti-proliferative agents (as described herein).

**Compounds of Formula (I)**

The compounds of Formula (I) contained in the pharmaceutical combination of the present invention are imidazo[4,5-c]quinoline derivatives, which are disclosed in PCT Application Publication No. WO201 2007926.

In an aspect, the PI3K/mTOR inhibitor contained in the pharmaceutical combination or composition of the present invention is selected from the compounds of Formula (I);

![Formula (I)](image)

wherein,

- $R_1$ is $(C_6-C_{14})$aryl or heteroaryl, wherein each of $(C_6-C_{14})$aryl or heteroaryl is optionally substituted with one or more of $R_3$;
- $R_2$ is -CN;
- $R_3$ is hydrogen or $(CrC_8)$ alkyl, wherein $(CrC_8)$ alkyl is optionally substituted with -CN;
$R_4$ is ($C_6^{-}C_{14}$)aryl or heteroaryl, wherein each of ($C_6^{-}C_{14}$)aryl or heteroaryl is optionally substituted with one or more of $R^a$;

$R^a$ at each occurrence is halogen, -CN, -OR$_x$, -SR$_x$, -NR$_x$R$_y$, (C$_1$-C$_8$) alkyi, (C$_6^{-}C_{14}$)aryl, heterocyclyl or alkyi/heterocyclyl, wherein (CrC$_8$) alkyi and heterocyclyl is optionally substituted with one or more of $R^b$;

$R_x$ and $R_y$ at each occurrence are independently hydrogen, (CrC$_8$) alkyi, (C$_6^{-}C_{14}$)aryl or heteroaryl, wherein each of (C$_1$-C$_8$)alkyl, (C$_6^{-}C_{14}$)aryl or heteroaryl are optionally substituted with $R^b$; and

$R^b$ at each occurrence is halogen, -CN or -NH$_2$; or stereoisomers, tautomers, N-oxides, pharmaceutically acceptable salts or solvates thereof.

In another embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I) wherein, $R_1$ is phenyl or pyridyl, wherein each of phenyl and pyridyl is optionally substituted with one or more of $R^a$;

$R_2$ is -CN;

$R_3$ is (CrC$_8$) alkyi;

$R_4$ is pyridyl, wherein pyridyl is optionally substituted with one or more of $R^b$;

$R^b$ is selected from halogen, -CN, -0-(C$_1$-C$_8$) alkyi, -NR$_x$R$_y$, or (C$_1$-C$_8$) alkyi, wherein (CrC$_8$) alkyi is optionally substituted with one or more of halogen or -CN; and $R_x$ and $R_y$ are independently hydrogen or (CrC$_8$) alkyi; or stereoisomers, tautomers, N-oxides, pharmaceutically acceptable salts or solvates thereof.

In another embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein $R_1$ is pyridyl optionally substituted with one or more of $R^a$; wherein $R^a$ is selected from halogen, -CN, -0-(CrC$_8$) alkyi, or (CrC$_8$) alkyi, wherein (CrC$_8$) alkyi is optionally substituted with one or more of the groups selected from: halogen or -CN.

In another embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein $R_1$ is 3-pyridyl optionally substituted with one or more of $R^a$; wherein $R^a$ is selected from halogen, -CN, -0-(CrC$_8$) alkyi, or (C$_1$- C$_8$) alkyi, wherein (C$_1$-C$_8$) alkyi is optionally substituted with one or more of the groups selected from: halogen or -CN.
In yet another embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein R₁ is pyridyl optionally substituted with one or more of the groups selected from: Cl, Br, F, -CN, -OCH₃, CH₃, CF₃ or -C(CH₃)₂CN.

In yet another embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein R₁ is 3-pyridyl optionally substituted with one or more of the groups selected from: Cl, Br, F, -CN, -OCH₃, CH₃, CF₃ or -C(CH₃)₂CN.

In further embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein R₃ is CH₃.

In further embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein R₄ is pyridyl optionally substituted with one or more of Rᵃ; wherein Rᵃ is selected from -NRₓRᵧ or (C₁-C₈)alkyl, wherein (C₁-C₈) alkyl is optionally substituted with one or more of halogen; and Rₓ and Rᵧ are independently hydrogen or (C₁-C₈)alkyl.

In still further embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein R₄ is 3-pyridyl optionally substituted with one or more of Rᵃ; wherein Rᵃ is selected from -NRₓRᵧ, or (C₁-C₈) alkyl, wherein (C₁-C₈) alkyl is optionally substituted with one or more halogen and Rₓ and Rᵧ are independently hydrogen or -(C₁-C₈) alkyl.

In further embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein R₆ is pyridyl optionally substituted with one or more groups selected from -NH₂, -NH-(C₁-C₈)alkyl, -N(CrC₈-alkyl)₂ or methyl, wherein methyl is optionally substituted with one to three halogen atoms.

In further embodiment, the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein R₆ is pyridyl optionally substituted with one or more groups selected from -NH₂, -NH-CH₃, -N(CH₃)₂ or -CF₃.

In further embodiment, the PI3K/mTOR inhibitor is selected from the compound of Formula (I), wherein R₆ is 3-pyridyl optionally substituted with one or more groups selected from -NH₂, -NH-CH₃, -N(CH₃)₂ or -CF₃.

In yet another embodiment, the PI3K/mTOR inhibitor is the compound of Formula (I) selected from:
N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-methoxypyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide,
N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(2-chloro-6-methoxypyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide,
N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-(2-cyanopropan-2-yl)pyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide,
N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-methoxy-2-methylpyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide,
N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-cyanopyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide,
N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(2,6-dimethoxypyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide,
N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-cyanopyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide,
N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(2,6-dimethoxypyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide, or
N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide,
and pharmaceutically acceptable salts, solvates, stereoisomers, tautomers or N-oxides thereof.

In yet another embodiment the compound of Formula (I) is N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-(2-cyanopropan-2-yl)pyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide, herein after referred to as Compound A.

Preparation of the compounds of Formula (I):

The compounds of Formula (I) can be prepared by synthetic routes as described in PCT Application Publication No. WO2012007926, which is incorporated herein by reference or by synthetic routes that include similar reaction steps or methods known in the art.

In an embodiment the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), which are in free base form or as pharmaceutically acceptable salts or solvates thereof.

For illustrative purposes, following Scheme 1 depicts the general method for preparing the compounds of Formula (I) including the intermediates thereof.
wherein $R_1$ and $R_3$, are as defined in any one of the embodiments of the invention for the compounds of Formula (I). $R_2$ is -CN and $R_3$ is H, methyl or -CH$_2$CN.

As illustrated in scheme 1, compound of formula (2), can be prepared by reacting nitromethane in the presence of a base such as NaOH at 0-10 °C to RT; then adding the resulting product to concentrated HCl at 0-10 °C and adding the compound of formula (1) in aqueous acid such as water-HCl mixture, and stirring at 0 °C to RT. The resulting compound of formula (2) can be reacted with an acid anhydride such as acetic anhydride in the presence of an alkali metal salt such as potassium acetate or sodium acetate at 80-140 °C to obtain compound of formula (3). The resulting compound of formula (3) can be treated with a halogenating agent, for example with chlorinating agent such as POCl$_3$ at 80-140 °C to obtain compound of formula (4). The resulting compound of formula (4) can be treated with an amine of formula $R_i$-$NH_2$ (wherein $R_i$ is as defined in any one of the embodiments of the invention for the compounds of Formula (I)) at 0-40 °C to obtain compound of formula (5). Catalytic reduction of the nitro group of compound of formula (5) forms the compound of formula (6), a quinoline-diamine compound. The resulting
compound of formula (6) can be coupled with a reagent such as diphenylcyanocarbonimidate or dimethyl cyanocarbonimidodithioate in the presence of a base such as diisopropylethylamine or cesium carbonate and in a solvent such as acetonitrile or dimethylformamide to obtain a compound of formula (7), wherein R₁ is as defined above and R₂ is -CN. The resulting compound of formula (7) can be treated with a methylating agent such as methyl iodide or with bromoacetonitrile in the presence of a base such as sodium hydride to obtain a compound of formula (8), wherein R₁ is as defined above, R₂ is -CN and R₃ is methyl or -CH₂CN. The compound of formula (8) or the compound of formula (7) can be further treated with a compound of formula R₄-B(OH)₃ in the presence of a coupling agent such as palladium dichlorobistriphenylphosphine and a base such as sodium carbonate to form a compound of Formula (I), wherein R₁ and R₄ are as defined in any one of the embodiments of the invention for the compounds of Formula (I), R₂ is -CN and R₃ is H, methyl or -CH₂CN.

The process for the preparation of the compounds of Formula (I) as described herein comprises an optional step of forming a pharmaceutically acceptable salt of the compound of Formula (I). The compound of Formula (I) may be used as a free base or as a pharmaceutically acceptable salt thereof.

The compounds of Formula (I), which contain acidic groups, may be converted into salts with pharmaceutically acceptable bases. Such salts include, for example, alkali metal salts, like lithium, sodium and potassium salts; alkaline earth metal salts like calcium and magnesium salts, ammonium salts, for example, [tris(hydroxymethyl)aminomethane], trimethylamine salts and diethylamine salts; salts with amino acids such as lysine, arginine, guanidine and the like.

The compounds of Formula (I), which contain one or more basic groups, i.e. groups which can be protonated, can form an addition salt with an inorganic or organic acid. Examples of suitable acid addition salts include: acetates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, cinnamates, citrates, ethanesulfonates, fumarates, glucuronates, glutamates, glycolates, hydrochlorides, hydrobromides, hydrofluorides, ketoglutarates, lactates, maleates, malonates, methanesulfonates, nitrates, oxalates, palmoates, perchlorates, phosphates, picrates, salicylates, succinates, sulfamate, sulfates,
tartrates, toluenesulfonates and other acid addition salts known to the person skilled in the art. Salts may be mono salts or bis-salts.

General method for preparation of 4-methylbenzene sulfonic acid salts:

A solution of the compound of Formula (I) in dry dichloromethane is stirred at 0 °C. 4-methylbenzene sulfonic acid dissolved in dry dichloromethane is added drop-wise to the solution of the compound over a period of 0.5 h. Reaction mixture is stirred at the same temperature for 0.5 h, warmed to RT, and is stirred further for 4 h. Solvent is removed and the mesylate salt of the compound of Formula (I) is obtained. The salt so obtained can be characterized by NMR.

General method for preparation of hydrochloride salts:

A solution of the compound of Formula (I) in dry dichloromethane is stirred at 0 °C. Ethereal HCl is added in excess to the solution of the compound. Reaction mixture is stirred at same temperature for 0.5 h, warmed to RT and is further stirred for 4 h. Solvent is removed and the hydrochloride salt of the compound of Formula (I) is obtained. The salt so obtained can be characterized by NMR.

Compounds of Formula (I) may also exist as hydrates or solvates.

The compounds of Formula (I) contained in the pharmaceutical combination of the present invention can be used in their isotopically labeled forms, wherein one or more atoms of the compounds of Formula (I) are replaced with their respective isotopes. All isotopes of any particular atom or element as specified are contemplated within the scope of the compounds disclosed in the invention.

Examples of isotopes of the atoms that can be incorporated into the compounds of Formula (I) disclosed herein include, but are not limited to, isotopes of hydrogen such as ²H and ³H, carbon such as ¹¹C, ¹³C and ¹⁴C, nitrogen such as ¹⁵N and ¹⁵N, oxygen such as ¹⁵O, ¹⁷O and ¹⁸O, chlorine such as ³⁶Cl, fluorine such as ¹⁸F and sulphur such as ³⁵S. Substitution with heavier isotopes, for example, replacing one or more key carbon-hydrogen bonds with carbon-deuterium bond may show certain therapeutic advantages, resulting from longer metabolism cycles, (e.g., increased in-vivo half life or reduced dosage requirements),
improved safety or greater effectiveness and hence may be preferred in certain circumstances.

**Anti-Proliferative Agents**

The antiproliferative agents contained in the pharmaceutical combination of composition of the present invention can be selected from: (i) alkylating agents; (ii) DNA intercalating agents; (iii) mitotic inhibitors; (iv) antimetabolites; (v) biguanides; (vi) insulin-like growth factor-1 receptor inhibitors; (vii) tyrosine kinase inhibitors; (viii) epidermal growth factor receptor (EGFR) inhibitors; (ix) BRAF inhibitors; (x) cRaf kinase inhibitors; (xi) serine-theronine kinase inhibitors including mitogen-activated protein (MAP) kinase inhibitor; (xii) cyclin dependent kinase (CDK) inhibitors; (xiii) aromatase inhibitors; (xiv) histone deacetylase inhibitors; (xv) angiogenesis inhibitors; (xvi) Ras-Raf-MEK-ERK pathway inhibitors; (xvii) lipid kinase inhibitors; (xviii) an apoptosis inducer; (xix) aurora kinase inhibitors; (xx) mTOR inhibitors; (xxi) progesterone receptor antagonists; (xxii) estrogen receptor antagonists; (xxiii) topoisomerase inhibitors; (xxiv) DNA dependent protein kinase inhibitors; (xxv) gamma secretase inhibitors or (xxvi) Notch/Hedgehog pathway inhibitors.

According to an embodiment of the present invention, the antiproliferative agents are selected from: (i) alkylating agents; (ii) DNA intercalating agents; (iii) mitotic inhibitors; (iv) antimetabolites; (v) biguanides; (vi) insulin-like growth factor-1 receptor inhibitors; (vii) tyrosine kinase inhibitors; (viii) epidermal growth factor receptor (EGFR) inhibitors; (ix) cyclin dependent kinase (CDK) inhibitors; (x) aromatase inhibitors; (xi) mTOR inhibitors; (xii) progesterone receptor antagonists; (xiii) estrogen receptor antagonists; (xiv) topoisomerase inhibitors; (xv) BRAF inhibitors (xvi) gamma secretase inhibitors or (xvii) Notch/Hedgehog pathway inhibitors.

According to an embodiment of the present invention, the antiproliferative agents are selected from: (i) alkylating agents; (ii) DNA intercalating agents; (iii) mitotic inhibitors; (iv) antimetabolites; (v) biguanides; (vi) insulin-like growth factor-1 receptor inhibitors; (vii) tyrosine kinase inhibitors; (viii) epidermal growth factor receptor (EGFR) inhibitors; (ix) topoisomerase inhibitors (x) BRAF inhibitors or (xi) gamma secretase inhibitors or (xii) Notch/Hedgehog pathway inhibitors.
According to an embodiment of the present invention, the antiproliferative agent is an alkylating agent.

According to an embodiment of the present invention, the alkylating agent is selected from cisplatin, mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, altretamine, thiotepa, busulfan, carmustine, lomustine, dacarbazine, procarbazine or temozolomide.

According to an embodiment of the present invention, the alkylating agent is cisplatin.

According to an embodiment of the present invention, the antiproliferative agent is DNA intercalating agent.

According to an embodiment of the present invention, the DNA intercalating agent is selected from doxorubicin, daunorubicin or dactinomycin.

According to an embodiment of the present invention, the DNA intercalating agent is doxorubicin.

According to an embodiment of the present invention, the antiproliferative agent is a mitotic inhibitor.

According to an embodiment of the present invention, the mitotic inhibitor is selected from paclitaxel, docetaxel, colchicines, vincristine or vinblastin.

According to an embodiment of the present invention, the mitotic inhibitor is paclitaxel.

According to an embodiment of the present invention, the antiproliferative agent is an antimetabolite.

According to an embodiment of the present invention, the antimetabolite is selected from 5-Fluorouracil (5-FU), methotrexate, 6-thiopurines, mercaptopurine, thioguanine, cladribine, pentostatin, cytarabine, azactidine, fludarabine, gemcitabine or hydroxyurea.

According to an embodiment of the present invention, the antimetabolite is 5-Fluorouracil (5-FU).

According to an embodiment of the present invention, the antiproliferative agent is a biguanide.

According to an embodiment of the present invention, the biguanide is selected from metformin, phenformin or buformin.
According to an embodiment of the present invention, the biguanide is metformin.

According to an embodiment of the present invention, the antiproliferative agent is a tyrosine kinase inhibitor.

According to an embodiment of the present invention, the tyrosine kinase inhibitor is selected from lapatinib, afatinib, or trastuzumab.

According to an embodiment of the present invention, the protein tyrosine kinase inhibitor is lapatinib.

According to an embodiment of the present invention, the antiproliferative agent is an epidermal growth factor receptor (EGFR) inhibitor.

According to an embodiment of the present invention, the EGFR inhibitor is selected from erlotinib, cetuximab, gefitinib or panitumumab.

According to an embodiment of the present invention, the EGFR inhibitor is erlotinib.

According to an embodiment of the present invention, the antiproliferative agent is a topoisomerase inhibitor.

According to an embodiment of the present invention, the topoisomerase inhibitor is selected from irinotecan, topotecan or 9-aminocamptothecin.

According to an embodiment of the present invention, the topoisomerase inhibitor is irinotecan.

According to an embodiment of the present invention, the antiproliferative agent is a BRAF inhibitor.

According to an embodiment of the present invention, the BRAF inhibitor is selected from sorafenib, vemurafenib (PLX4032), PLX3603, dabrafenib, LGX818, BMS-908662, RAF265 or GW-5074.

According to an embodiment of the present invention, the BRAF inhibitor is vemurafenib or sorafenib.

According to an embodiment of the present invention, the notch/hedgehog pathway inhibitor is selected from cyclopamine, vismodegib or RO4929097.

According to an embodiment of the present invention, the notch/hedgehog pathway inhibitor is cyclopamine.
According to an embodiment of the present invention, the insulin-like growth factor-1 receptor inhibitor is selected from figitumumab, AMG-479, dalotuzumab, PL225B or INSM18.

According to an embodiment of the present invention, the insulin-like growth factor-1 receptor inhibitor is PL225B.

According to another embodiment of the present invention, the gamma secretase inhibitor is avagacestat (BMS7081 63).

According to another embodiment of the present invention, the anti-proliferative agent is selected from cisplatin, doxorubicin, paclitaxel, 5-Fluorouracil(5-FU), metformin, lapatinib, erlotinib, irinotecan, vemurafenib, PL225B, sorafenib, avagacestat and cyclopamine.

The compounds of Formula (I) contained in the pharmaceutical combination of the present invention exhibit synergistic effect when used in combination with the anti-proliferative agents. For instance, Compound A, a representative compound of Formula (I) has exhibited synergistic effect when used in combination with anti-proliferative agents such as cisplatin, doxorubicin, paclitaxel, 5-Fluorouracil(5-FU), metformin, lapatinib, erlotinib, irinotecan, vemurafenib, PL225B, sorafenib, avagacestat and cyclopamine.

The anti-proliferative agents used in the present invention can be synthesized by methods known to one skilled in the art or can be obtained through commercial sources. For instance, cisplatin or c/s-diamminedichloroplatinum(II) (CDDP) (CAS Reg. No. 15663-27-1) is a well-known anticancer compound, which is a platinum compound that binds to DNA and forms cisplatin-DNA coordination complexes, or adducts, which affects replication, transcription and repair of DNA, ultimately leading to programmed cell death. Cisplatin is approved to be used alone or in combination with the other drugs for the treatment of various types of cancers, including bladder cancer, cervical cancer, small cell lung cancer and ovarian cancer. Cisplatin has recently been evaluated in a clinical trial that treated 28 women with stage II or stage III TNBC (J. Clin. Oncol, 2010, 28, 7, 1145-1 153).

Another anticancer agent, doxorubicin hydrochloride (CAS REg. No. 25316-40-9) is an anthracycline antibiotic with anticancer activity. Doxorubicin was isolated from the bacterium *Streptomyces peucetius var. caesius*. Doxorubicin intercalates
between DNA base pairs thus inhibiting DNA replication and leading to inhibition of protein synthesis. Doxorubicin is approved to be used alone or in combination with the other drugs for the treatment of various types of cancers, including Hodgkin's lymphoma, Non-Hodgkin's lymphoma, thyroid cancer, small cell lung cancer, gastric cancer, neuroblastoma, and breast cancer.

Paclitaxel (CAS Reg. No.33069-62-4) is a mitotic inhibitor, isolated from the bark of the Pacific yew tree *Taxus brevifolia*. The mechanism of anti-cancer activity of Paclitaxel is unique. Paclitaxel enhances the polymerization of tubulin to stable microtubules and also interacts directly with microtubules, stabilizing them against depolymerization. It has a specific binding site on the microtubule polymer, i.e., B subunit of tubulin. It acts by blocking cells in the G2/M phase of the cell cycle and such cells are unable to form a normal mitotic apparatus. Paclitaxel is approved to be used alone or in combination with the other drugs for the treatment of various types of cancers, including AIDS-related Kaposi sarcoma, non-small cell lung cancer, ovarian cancer and breast cancer.

5-Fluorouracil (5-FU) (CAS Reg no. 51-21-8) is an antimetabolite and a cytotoxic anti-cancer agent. 5-FU is metabolized by the cells to form 5-fluoro-2'-deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). These metabolites interfere with RNA and DNA synthesis. 5-FU is approved to be used alone or in combination with the other drugs for the treatment of various types of cancers, including colorectal cancer, pancreatic cancer, squamous cell carcinoma of head and neck, gastric adenocarcinoma, basal cell carcinoma and breast cancer.

Metformin hydrochloride (CAS Reg no. 1115-70-4) is a biguanide, most widely prescribed anti-diabetic drug. Recently metformin is found to be useful in the treatment of certain cancers, particularly the cancers associated with hyperinsulinemia such as breast cancer and colon cancer. Metformin may also act by directly inhibiting mammalian target of rapamycin (mTOR) signaling and protein synthesis (Biomedcentral Medicine, 2011, 9, 33, 1-6). Metformin is in clinical trial study in combination with other drugs for the treatment of breast cancer, endometrial cancer, kidney cancer, lung cancer, lymphoma and prostate cancer (Cell Cycle, 2010, 9, 6, 1057-1064).
Lapatinib or lapatinib ditosylate (Tykerb®, GlaxoSmithkline) is a 4-anilinoquinazoline kinase inhibitor of the intracellular tyrosine kinase domains of both Epidermal Growth Factor Receptor (HER1/ EGFR/ ErbB1) and of human Epidermal Receptor Type 2 (HER2/ErbB2) receptors. EGFR is seen to be overexpressed in various cancers including head and neck, lung, pancreas, bladder, and breast cancer. Overexpression of ErbB-2 occurs in one third of breast cancers and is also associated with metastasis. The simultaneous inhibition of two receptors involved in tumor proliferation results in more effective treatment of cancer involving ErbB1 and ErbB2. Lapatinib ditosylate is approved to be used in combination with the other drugs for the treatment of breast cancer.

Erlotinib (Tarceva®, Genetech) is a quinazoline derivative and an epidermal growth factor receptor (EGFR) inhibitor. Erlotinib competes with ATP for the ATP-binding site in the cytoplasmic tail of EGFR receptor. This results into inhibition of cellular proliferation, angiogenesis, tumor invasion, and metastasis. Erlotinib is approved to be used alone or in combination with the other drugs for the treatment of non-small-cell-lung cancer and pancreatic cancer.

Irinotecan (Campto®, Yakult Honsha), is a semi-synthetic analog of camptothecin, an alkaloid extract from Camptotheca acuminata. Irinotecan is Topoisomerase-I inhibitor. Topoisomerase-1 is an enzyme that produces reversible single-strand breaks in DNA during DNA replication. These single-strand breaks relieve torsional strain and allow DNA replication to proceed. Irinotecan and its active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin) bind with cellular Topoisomerase 1-DNA complex. Topoisomerase I-irinotecan-DNA cleavable complex leads to the formation of a double-strand DNA break, thus causing irreversible arrest of the replication fork and cell death. The cytotoxic effect occurs in S-phase of cell cycle. Irinotecan is approved to be used alone or in combination with the other drugs for the treatment of colorectal cancer.

Vemurafenib or PLX-4032 (Zelboraf®, Plexxicon) is a potent inhibitor of B-Raf enzyme. PLX4032 inhibits the proliferation of BRAF (V600E) in tumor cells. It is approved for the treatment of melanoma with the BRAF\textsuperscript{V600E} mutation.

PL225B (Merck & Piramal Enterprises Limited) is insulin-like growth factor 1 receptor (IGF-1 R) inhibitor which is disclosed in PCT publication no.
PL225B selectively inhibits IGF-1 R, resulting in inhibition of tumor cell proliferation and the induction of tumor cell apoptosis in IGF-1 R-overexpressing tumor cells. It is being developed for advanced refractory solid tumors.

Sorafenib (Nexavar®, Bayer AG and Onyx Pharmaceuticals) is BRAF kinase inhibitor. It inhibits growth signaling by inhibiting Raf kinase, which controls cell division and proliferation. Sorafenib also inhibits several receptor tyrosine kinase including vascular endothelial growth factor receptor 2 (VEGFR2), platelet-derived growth factor receptor (PDGFR), FLT3, Ret, and c-Kit, thereby inhibiting tumor angiogenesis. Sorafenib is approved to be used for the treatment of hepatocellular carcinoma and renal cell carcinoma.

Avagacestat or BMS708163 (Bristol Myers Squibb) is an orally available gamma secretase inhibitor. It selectively inhibits amyloid β synthesis. There is a cross talk between Notch, hedgehog and PI3K pathways in stem cell maintenance and proliferation.

Cyclopamine (CAS Reg no. 4449-51-8) is a naturally occurring steroidal alkaloid isolated from Veratrum californicum. It inhibits hedgehog pathway by interfering with the initial events of hedgehog signal reception, which involve the multipass transmembrane (TM) proteins Patched (Ptc) and Smoothened. Deregulated hedgehog signaling is implicated in 20-25% of all cancers, including breast cancer.

In an embodiment of the present invention, the pharmaceutical combination comprises a PI3K/mTOR inhibitor selected from the compounds of Formula (I) and one or more anti-proliferative agent selected from the group consisting of: metformin, cisplatin, doxorubicin, paclitaxel, 5-fluorouracil (5-FU), PL225B, cyclopamine, avagacestat or everolimus; wherein the said pharmaceutical combination is provided for use in the treatment of breast cancer. The breast cancer may be estrogen receptor positive (ER+), estrogen receptor negative (ER-), progesterone receptor positive (PR+), progesterone receptor negative (PR-), HER2 positive (HER2+), HER2 negative (HER2-), triple negative breast cancer or herceptin and lapatinib resistance breast cancer.

In an embodiment of the present invention, the pharmaceutical combination comprises a PI3K/mTOR inhibitor selected from the compounds of
Formula (I) and an anti-proliferative agent selected from erlotinib or sorafenib; wherein the said pharmaceutical combination is provided for use in the treatment of lung cancer. The lung cancer may be non-small cell lung cancer (NSCLC) or EGFR mutated NSCLC.

In an embodiment of the present invention, the pharmaceutical combination comprises a PI3K/mTOR inhibitor selected from the compounds of Formula (I) and vemurafenib as the anti-proliferative agent; wherein the said pharmaceutical combination is provided for use in the treatment of melanoma or BRAF mutated melanoma.

In an embodiment of the present invention, the pharmaceutical combination comprises a PI3K/mTOR inhibitor selected from the compounds of Formula (I) and irinotecan as the anti-proliferative agent; wherein the said pharmaceutical combination is provided for use in the treatment of colon cancer.

According to an embodiment of the present invention, the pharmaceutical combination comprising the PI3K/mTOR inhibitor selected from the compounds of Formula I and an antiproliferative agent, is not exclusively limited to those combinations which are obtained by physical association of the said compounds, but also encompass those which permit a separate administration, which can be simultaneous, sequential or spaced out over a period of time so as to obtain maximum efficacy of the combination. Thus, the pharmaceutical combination may be administered simultaneously or sequentially for an effective treatment of a proliferative disease or disorder.

Accordingly, in the combination therapy provided herein, the anti-proliferative agent can be administered simultaneously or sequentially with the compound of Formula (I).

Methods of Treatment

According to one aspect of the present invention there is provided a method of treating a disease or a disorder mediated by mTOR kinase and/or PI3K comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof; in combination with a therapeutically effective amount of one or more anti-proliferative agents.
According to an embodiment of the invention, the disease or a disorder mediated by mTOR kinase and/or PI3K is a proliferative disease or disorder.

Accordingly, there is provided a method for the treatment of a proliferative disease or a disorder comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof; in combination with a therapeutically effective amount of one or more anti-proliferative agents.

According to another aspect of the present invention there is provided use of a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof in combination with one or more anti-proliferative agents for the treatment of a proliferative disease or disorder.

According to another aspect of the present invention there is provided a method for the manufacture of a medicament comprising a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts, or solvates thereof and one or more anti-proliferative agents in association with a pharmaceutically acceptable excipient or carrier; for use in the treatment of a proliferative disease or disorder.

The present invention provides methods for the synergistic treatment of cancers.

According to an embodiment of the present invention, the proliferative disease or disorder is cancer.

According to another embodiment of the present invention, the cancer is solid cancer or hematological cancer.

According to another embodiment of the present invention, the cancer is selected from: leukemia such as acute lymphocytic leukemia, acute myeloid leukemia, adult acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia and hairy cell leukemia; lung cancer such as non-small-cell lung cancer, small-cell lung cancer and mesothelioma; brain tumors such as brain stem glioma and glioblastoma; astrocytoma such as cerebellar astrocytoma and cerebral astrocytoma; and visual pathway and hypothalamic glioma; supratentorial primitive neuroectodermal and
pineal tumors and medulloblastoma; lymphoma such as primary central nervous system lymphoma and non-Hodgkin's lymphoma particularly mantle cell lymphoma; Hodgkin's disease; liver cancer such as hepatocellular carcinoma; kidney cancer such as renal cell carcinoma and Wilms' tumor; sarcoma such as Ewing's sarcoma family of tumors; osteosarcoma; rhabdomyosarcoma and soft tissue sarcomas; bladder cancer; breast cancer; endometrial cancer; head and neck cancer; melanoma; cervical cancer; thyroid cancer; gastric cancer; germ cell tumor; cholangiocarcinoma; extracranial melanoma; resistant multiple myeloma; resistant multiple myeloma; oral cancer; pancreatic cancer; ependymoma; neuroblastoma; skin cancer; ovarian cancer; recurrent ovarian cancer; prostate cancer; testicular cancer; colorectal cancer; lymphoproliferative disease, or myeloproliferative disorder, or a combination of one or more of the said cancers.

According to an embodiment of the present invention, the cancer is selected from: breast cancer, prostate cancer, pancreatic cancer, lung cancer, head and neck cancer, ovarian cancer, colorectal cancer, kidney cancer, gastric cancer, non-Hodgkin's lymphoma, primary central nervous system lymphoma, endometrial cancer, brain tumors, melanoma, liver cancer, thyroid cancer, lymphoid cancer, esophageal cancer, cancer of urinary tract, cervical cancer, bladder cancer, mesothelioma, sarcoma or chronic myeloid leukemia.

According to an embodiment of the present invention, the cancer is selected from: breast cancer, prostate cancer, pancreatic cancer, lung cancer, head and neck cancer, ovarian cancer, colorectal cancer, kidney cancer, gastric cancer, brain tumor, melanoma, liver cancer, thyroid cancer, lymphoid cancer, bladder cancer, mesothelioma, or chronic myeloid leukemia.

According to an embodiment of the present invention, the cancer is selected from: breast cancer, lung cancer, melanoma, liver cancer, ovarian cancer or colorectal cancer.

According to an embodiment of the present invention, the cancer is breast cancer.

According to an embodiment of the present invention, the breast cancer is selected from estrogen receptor positive (ER\(^+\)), estrogen receptor negative (ER\(^-\)).
progesterone receptor positive (PR⁺), progesterone receptor negative (PR⁻), HER2 positive (HER2+), HER2 negative (HER2⁻) or triple negative breast cancer.

According to an embodiment of the present invention, the breast cancer is estrogen receptor positive (ER⁺) breast cancer.

According to another embodiment of the present invention, the breast cancer is estrogen receptor negative (ER⁻) breast cancer.

According to an embodiment of the present invention, the breast cancer is progesterone receptor positive (PR⁺) breast cancer.

According to an embodiment of the present invention, the breast cancer is progesterone receptor negative (PR⁻) breast cancer.

According to an embodiment of the present invention, the breast cancer is HER2 positive (HER2+) breast cancer.

According to an embodiment of the present invention, the breast cancer is HER2 negative (HER2-) breast cancer.

According to an embodiment of the present invention, the breast cancer is triple negative breast cancer (TNBC).

According to an embodiment of the present invention, the cancer is melanoma.

According to an embodiment of the present invention, the liver cancer is hepatocellular carcinoma.

According to an embodiment of the present invention, the cancer is lung cancer.

According to an embodiment of the present invention, the lung cancer is non small cell lung cancer.

According to an embodiment of the present invention, the lung cancer cell has EGFR mutation.

According to an embodiment of the present invention, the EGFR mutation in the lung cancer cell is T790 mutation.

According to another embodiment of the present invention, the cancer is colon cancer.

According to another embodiment of the present invention, the colon cancer cell has k-ras mutation.
In an embodiment of the present invention, in the method for the treatment of a proliferative disease or disorder, the anti-proliferative agent is selected from the group consisting of: metformin, cisplatin, doxorubicin, paclitaxel, 5-fluorouracil (5-FU), PL225B, cyclopamine, avagacestat or everolimus; wherein the said proliferative disease or disorder is breast cancer. The breast cancer may be estrogen receptor positive (ER+) , estrogen receptor negative (ER-) , progesterone receptor positive (PR+) , progesterone receptor negative (PR-) , HER2 positive (HER2+), HER2 negative (HER2-), triple negative breast cancer or herceptin and lapatinib resistance breast cancer.

In an embodiment of the present invention, in the method for the treatment of a proliferative disease or disorder, the anti-proliferative agent is selected from erlotinib or sorafenib; wherein the said proliferative disease or disorder is lung cancer. The lung cancer may be non-small cell lung cancer (NSCLC) or EGFR mutated NSCLC.

In an embodiment of the present invention, in the method for the treatment of a proliferative disease or disorder, the anti-proliferative agent is vemurafenab; wherein the said proliferative disease or disorder is melanoma or BRAF mutated melanoma.

In an embodiment of the present invention, in the method for the treatment of a proliferative disease or disorder, the anti-proliferative agent is irinotecan; wherein the said proliferative disease or disorder is colon cancer.

According to an aspect of the present invention there is provided a method for inhibiting the growth of cancer cells comprising contacting the cancer cell with the pharmaceutical combination or composition of the present invention.

According to an aspect of the present invention, a method is provided for synergistic treatment of cancer.

Administration

The pharmaceutical combination of the present invention, comprising the PI3K/m-TOR inhibitor selected from the compounds of Formula I and an anti-proliferative agent, can be administered simultaneously in a fixed dosage form.
In an embodiment the PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts, stereoisomers, tautomers, N-oxides or solvates thereof and an anti-proliferative agent contained in the pharmaceutical combination or composition can be administered separately wherein the said compounds can be administered simultaneously or the sequentially or spaced out over a period of time.

In an embodiment, the pharmaceutical combination of the present invention comprising a compound of Formula (I) or pharmaceutically acceptable salts, or solvates thereof and one or more anti-proliferative agents can be administered simultaneously in the form of a fixed dosage form.

In another embodiment, the pharmaceutical combination of the present invention comprising a compound of Formula (I) or pharmaceutically acceptable salts or solvates thereof and one or more anti-proliferative agents can be administered in a sequential manner. Sequential administration means administration of one component of the combination before the administration of another component of the combination such that the combination shows a synergistic effect. Accordingly, the compound of Formula (I) can be administered prior to or after the administration of the anti-proliferative agent.

Further, sequential administration of the compound of Formula (I) and the anti-proliferative agents involves administration of the said compounds spaced out over a period of time i.e. after administration of the first component of the combination, the second component is administered after a certain fixed period. According to the present invention, administration of the compounds of Formula (I) and/or anti-proliferative agents can be by any suitable route, including, without limitation, parenteral, oral, sublingual, transdermal, topical, intranasal, aerosol, intraocular, intratracheal or intrarectal.

Pharmaceutical Kit

The present invention provides a pharmaceutical kit comprising a compound of Formula (I) or pharmaceutically acceptable salts or solvates thereof and one or more anti-proliferative agents. The pharmaceutical kit may comprise a container containing a compound of Formula (I) or a pharmaceutically acceptable salt or solvates thereof and one or more anti-proliferative agents as a fixed dose.
formulation; or the kit may comprise two or more separate containers for the compound of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-proliferative agents. The kit may further comprise a package insert, including information about the indication, usage, doses, direction for administration, contraindications, precautions and warnings. The suitable container that can be used, includes a bottle, a vial, an ampoule, a syringe or a blister pack. The pharmaceutical kit may optionally comprise a further container comprising a pharmaceutically acceptable buffer, water for injection, phosphate-buffered saline, Ringer's solution and dextrose solution.

Pharmaceutical Compositions

The pharmaceutical composition of the present invention comprises a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof and one or more anti-proliferative agents and one or more pharmaceutically acceptable carrier, diluent, or excipient. For the production of pills, tablets, coated tablets and hard gelatin capsules, the pharmaceutically active excipients that can be used include, but not limited to, lactose, corn starch or derivatives thereof, gum arabica, magnesia or glucose, etc. For soft gelatin capsules and suppositories, the carriers that can be used include, but not limited to, fats, waxes, natural or hardened oils, etc. Suitable carriers for the production of solutions, are, for example injection solutions, or for emulsions or syrups are, for example, water, physiological sodium chloride solution or alcohols, for example, ethanol, propanol or glycerol, sugar solutions, such as glucose solutions or mannitol solutions, or a mixture of the various solvents which have been mentioned.

The compound of Formula (I) and anti-proliferative agent are formulated into pharmaceutical dosage forms using conventional pharmaceutical techniques familiar to one skilled in the art such as by means of conventional blending, granulating, dissolving or lyophilizing.

The pharmaceutical composition of the present invention includes suitable carriers, diluents or excipients such as, for example, filling agents, binding agents, buffering agents, lubricating agents, antioxidants, dispersants, disintegrants,
emulsifiers, defoamers, flavors, preservatives, surfactants, wetting agents, stabilizing agents, solubilizers, coating agents or colorants.

The pharmaceutical composition may be packaged in a suitable container depending upon the formulation and the method of administration of the composition. Suitable containers known to a person skilled in the art include bottles, vials, ampoules, infusion bag and blister pack.

The pharmaceutical composition can be administered orally, for example in the form of pills, tablets, coated tablets, lozenges, capsules, dispersible powders or granules, suspensions, emulsions, syrups or elixirs. Administration, however, can also be carried out rectally, for example in the form of suppositories, or parenterally, for example intravenously, intramuscularly or subcutaneously, in the form of injectable sterile solutions or suspensions, or topically, for example in the form of solutions or ointments or transdermally, for example in the form of transdermal patches, or in other ways, for example in the form of aerosols, nasal sprays or nasal drops.

The pharmaceutical composition may contain about 1 to 99 %, for example, about 5 to about 70%, or from about 5 to about 30 % by weight of the active ingredients (compound of the Formula (I) or pharmaceutically acceptable salt or solvate thereof and anti-proliferative agent). The amount of the active ingredients in the pharmaceutical composition is from about 1 to about 1000 mg.

Although the effective doses of therapeutic agents (the PI3K/mTOR inhibitor selected from the compounds of Formula (I) and the anti-proliferative agent) for administration vary depending on the type of proliferative disease or disorder, the stage of the disease or disorder, the severity of symptoms, the age, sex, body weight and sensitivity difference of the patient, the mode, time, interval and duration of administration, the nature, formulation and type of the preparation, the type of the active ingredient, etc. In certain embodiments, the therapeutic agents are administered in a time frame where both the agents are still active. One skilled in the art would be able to determine such a time frame by determining the half life of the administered therapeutic agents. As indicated herein before, the active ingredients contained in the pharmaceutical composition can be administered simultaneously or sequentially. Those skilled in
the art will recognize that several variations are possible within the scope and spirit of this invention.

The dose to be administered daily is to be selected to produce the desired effect. A suitable dosage is about 0.001 to 100 mg/kg of the compound of Formula (I) or pharmaceutically acceptable salt thereof, for example, about 0.01 to 50 mg/kg of a compound of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof, about 0.01 to 20 mg/kg of a compound of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof. The anti-proliferative agent can administered at a dose from about 10 mg/day to about 500 mg/day. If required, higher or lower daily doses can also be administered. Actual dosage levels of the active ingredients contained in the pharmaceutical compositions of this invention can be varied so as to obtain an amount of the active ingredient, which is effective to achieve the desired therapeutic response for a particular patient.

In one embodiment, the compound of Formula (I), or pharmaceutically acceptable salts or solvates thereof, is administered at a dose from about 5 mg/kg to about 50 mg/kg; or about 5 mg/kg to about 30 mg/kg; or about 5 mg/kg to about 20 mg/kg.

In an embodiment, the anti-proliferative agent is administered at a suitable dose depending on the type of the proliferative disease or disorder (cancer). Accordingly the dose can vary from about 0.1 mg/kg to about 20 mg/kg. For example metformin can be administered at a dose from about 15 mg/kg to about 20 mg/kg, or about 20 mg/kg; lapatinib can be administered at a dose from about 5 mg/kg to about 50 mg/kg, or about 50 mg/kg; erlotinib can be administered at a dose from about 1 mg/kg to about 25 mg/kg, or about 10 mg/kg; irinotecan can be administered at a dose from about 1 mg/kg to about 50 mg/kg, or about 10 mg/kg, or about 3 mg/kg.

In an embodiment, the therapeutically effective amount of compound A ranges from about 5 mg/kg to about 20 mg/day.

In an embodiment, the therapeutically effective amount of compound A can be about 5 mg/kg, about 10 mg/kg or about 20 mg/kg.

In another embodiment, the therapeutically effective amount of compound A is about 5 mg/kg.
In another embodiment, the therapeutically effective amount of compound A is about 10 mg/kg.

In another embodiment, the therapeutically effective amount of compound A is about 20 mg/kg.

By "pharmaceutically acceptable" it is meant the carrier, diluent, excipients, and/or salt must be compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

According to another aspect of the present invention there is provided a method for the manufacture of a medicament useful for the treatment of a proliferative disease or a disorder comprising a PI3k/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof and one or more anti-proliferative agents in association with a pharmaceutically acceptable excipient or carrier.

It is understood that modifications that do not substantially affect the activity of the various embodiments of this invention are included within the invention disclosed herein.

The one or more pharmaceutical combinations encompassed in this invention have been evaluated in certain assay systems, and in several different administration schedules in vitro. The experimental details are as provided herein below. The data presented herein clearly indicate that the anti-proliferative agent when combined with a compound Formula (I) exhibits synergistic effect.

The representative compound of Formula (I), the compound A used in the pharmacological assays refers to N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-(2-cyanopropan-2-yl)pyridin-3-yl)-3-methyl-1 H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide.

The inventors also established xenograft models to extend in vitro observations to an in vivo system. It is evident from the graphical presentations of Figures 13 to 20d that the pharmaceutical combination of the present invention exhibited therapeutically synergistic activity in various xenograft models.

The synergistic effect of the combination of the present invention is explained in more details with reference to preferred embodiments thereof. It is to
be noted that these are provided only as examples and not intended to limit the invention.

EXAMPLES

In the following examples and elsewhere, abbreviations have the following meanings:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>ATCC</td>
<td>American Type Cell Culture</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>DME</td>
<td>Dulbecco's Modified Eagle Medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>FCS</td>
<td>Fetal Calf serum</td>
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<tr>
<td>g</td>
<td>Gram</td>
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<td>h</td>
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<td>HCl</td>
<td>hydrochloric acid</td>
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<tr>
<td>iv</td>
<td>intravenously</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>μL</td>
<td>microlitre</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium Hydroxide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
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</table>

Activity of pharmaceutical combinations of the present invention can be determined according to any effective *in vitro* or *in vivo* method.

**In vitro** Studies

**Cytotoxicity assay**

**Propidium Iodide Assay (PI assay)**

The assay was designed as in the reference, Anticancer Drugs, 2002, 13, 1-8, the disclosure of which is incorporated by reference for teaching of the assay.

**Protocol**

Cancer cell lines were seeded at a density of 3000 cells/well per 198 μL or 199 μL in culture medium (RPMI 1640 medium or DME) containing 10 % FCS in tissue...
culture grade 96-well white plate (Nuclon Cat. No. 136101) and allowed to recover for 24 h in humidified 5% CO₂ incubator at 37 °C.

Following incubation for a period of 18-24 h, various concentrations of the test compounds (as described herein) were added to the wells and the plates were incubated for 48 h in humidified 5% CO₂ incubator at 37°C. At the end of the treatment, the culture medium was discarded, the cells were washed with 1x PBS and 200 μl of 7 μg/ml propidium iodide was added to each well. The plates were frozen at -70 °C for 16 h. For analysis, the plates were warmed to RT, allowed to thaw and were read in Polar Star fluorimeter with the fluorescence setting (544 nm excitation and 610-620 nm emission wavelength). The percentage of viable cells in the non-treated set of wells was considered to be 100 and the percentage viability following treatment was calculated accordingly.

Percent cytotoxicity was calculated at various drug (the test compounds of Formula (I) and the anti-proliferative agents) concentrations using the formula

\[
100 - \% T/C = \frac{\text{Reading of test sample}}{\text{Reading of Control}}
\]

Combination Index (CI):
Graph for cytotoxicity vs drug concentration was plotted to obtain % Cytotoxicity for the concentrations of the test compounds and for obtaining concentration dependent cytotoxicity curves. These curves were then used for calculation of combination index.

Combination Index is calculated as using the formula

\[
CI = \frac{CA, X}{C_{X,A}} + \frac{CB, X}{C_{X,B}}
\]

wherein,

CA,X and CB,X are the concentrations of the test compounds that are used in combination to achieve x % drug effect.

ICX,A and IC X,B are the concentrations for single test compounds to achieve the same effect.
CI of less than, equal to, and more than 1 indicates synergy, additivity, and antagonism, respectively.

Alternatively, CI can be calculated using COMPUSYN software version 1.0 by Chou and Talale.

Sample preparation

The term test compound used herein above refers to Compound A and the Anti-proliferative agent (combination drug) such as metformin, lapatinib, cisplatin, doxorubicin, paclitaxel, 5-fluoro uracil, PL225B, erlotinib, PLX4032, sorafenib, BMS7081 63, cyclopamine and everolimus used in Examples 1-12.

Compound A was dissolved in DMSO to prepare a stock solution of 10 mM concentration. The solution was further diluted using DMSO to obtain a 200 times concentrated solution of the desired concentration, and 1 µl of this solution was used in the assay.

The efficacy of the combinations of Compound A and the combination drug was studied in vitro by PI assay, in following Examples 1-12.

Example 1:

Effect of compound A on triple negative breast cancer (TNBC) cell lines alone and in combination with metformin

The different combinations of metformin and Compound A were tested by PI assay in a sequential mode or simultaneous mode.

Materials:

Compound A: The desired concentrations of Compound A used were 100, 30, 10, 3, 1, 0.3, 0.1, 0.03 nM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Metformin: A stock solution of 1 M was prepared by dissolving required amount of metformin in RPMI 1640 medium and further dilutions were made in the culture medium to obtain the concentrations of 100, 30, 10, 3, 1, 0.3, 0.1 and 0.03 mM.

Cancer cell line: TNBC cell line MDAMB453 (ATCC, HTB-131).
Assay

(a) Sequential treatment of metformin and Compound A to TNBC cells

The wells seeded and incubated with TNBC cell lines were treated with metformin alone, compound A alone and combination of metformin and compound A.

Treatment of TNBC cells with metformin alone: Metformin was added to the wells by replacing the growth medium with 200 µL of growth medium containing metformin at 100, 30, 10, 3, 1, 0.3, 0.1, 0.03 mM concentrations.

Treatment of TNBC cells with Compound A alone: Compound A was added to the wells by replacing the growth medium with 199 µL of growth medium to which 1 µL of 100, 30, 10, 3, 1, 0.3, 0.1 and 0.03 nM, (200X each), was added to the wells.

Treatment of TNBC cells with combination of Compound A and metformin: Metformin was added to the wells by replacing the growth medium with 200 µL of growth medium containing metformin at 100, 30, 10, 3, 1, 0.3, 0.1, 0.03 mM concentrations. After 48 h of metformin treatment, 1 µL of compound A of concentrations 100, 30, 10, 3, 1, 0.3, 0.1 and 0.03 nM, (200X each), was added to the wells and the well-plate was allowed to stand for 16 h.

Control wells were treated with vehicle (DMSO). The final concentration of DMSO was 0.5% per test well. Percent cytotoxicity was calculated. A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 1aa, FIG. 1ab and FIG. 1ac and concentration dependent cytotoxicity curves were obtained. These curves were used for the calculation of combination index. The combination index obtained from cytotoxicity curves of figures; FIG. 1aa, FIG. 1ab and FIG. 1ac are depicted below in tables viz; Table 1aa, Table 1ab and Table 1ab respectively.

Table 1aa:

<table>
<thead>
<tr>
<th>Test sample</th>
<th>% Cytotoxicity</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound A 1nM</td>
<td>8.37</td>
<td></td>
</tr>
<tr>
<td>Compound A 3nM</td>
<td>18.69</td>
<td></td>
</tr>
<tr>
<td>Compound A 10nM</td>
<td>22.47</td>
<td></td>
</tr>
<tr>
<td>Metformin 3mM</td>
<td>51.37</td>
<td></td>
</tr>
<tr>
<td>Metformin 3 mM + Compound A 1nM</td>
<td>65.86</td>
<td>0.43</td>
</tr>
<tr>
<td>Metformin 3 mM + Compound A 3nM</td>
<td>60.24</td>
<td>0.60</td>
</tr>
<tr>
<td>Metformin 3 mM + Compound A 10nM</td>
<td>62.43</td>
<td>0.50</td>
</tr>
</tbody>
</table>
Table 1ab:

<table>
<thead>
<tr>
<th>Test sample</th>
<th>% Cytotoxicity</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin 10 mM</td>
<td>20.69</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 30 mM</td>
<td>54.83</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 10 nM</td>
<td>22.62</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 30 nM</td>
<td>29.61</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 10 mM + Compound A 10 nM</td>
<td>39.757</td>
<td>0.50</td>
</tr>
<tr>
<td>Metformin 10 mM + Compound A 30 nM</td>
<td>41.013</td>
<td>0.50</td>
</tr>
<tr>
<td>Metformin 30 mM + Compound A 30 nM</td>
<td>63.495</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Table 1ac:

<table>
<thead>
<tr>
<th>Test sample</th>
<th>% Cytotoxicity</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin 3 mM</td>
<td>18.78</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 10 mM</td>
<td>30.52</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 30 mM</td>
<td>86.38</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 10 nM</td>
<td>26.90</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 30 nM</td>
<td>30.11</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 100 nM</td>
<td>34.86</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 3 mM + Compound A 10 nM</td>
<td>42.61</td>
<td>0.29</td>
</tr>
<tr>
<td>Metformin 30 mM + Compound A 10 nM</td>
<td>96.08</td>
<td>0.50</td>
</tr>
<tr>
<td>Metformin 30 mM + Compound A 30 nM</td>
<td>92.546</td>
<td>0.75</td>
</tr>
<tr>
<td>Metformin 30 mM + Compound A 100 nM</td>
<td>96.744</td>
<td>0.50</td>
</tr>
</tbody>
</table>

(b) Simultaneous treatment of metformin and Compound A to TNBC cells

The wells seeded and incubated with TNBC cell lines were treated with metformin alone, compound A alone and combination of metformin and compound A.

Treatment of TNBC cells with metformin alone: Metformin was added to the wells by replacing the growth medium with 200 µL of growth medium containing metformin at 100, 30, 10, 3, 1, 0.3, 0.1, 0.03 mM concentrations.

Treatment of TNBC cells with Compound A alone: Compound A was added to the wells by replacing the growth medium with 199 µL of growth medium to which 1 µL of 100, 30, 10, 3, 1, 0.3, 0.1 and 0.03 nM, (200X each), was added to the wells.

Treatment of TNBC cells with combination of Compound A and metformin: Metformin was added to the wells by replacing the growth medium with 199 µL of growth medium containing metformin at 100, 30, 10, 3, 1, 0.3, 0.1, 0.03 mM.
concentrations. To these wells, 1 µL of Compound A of concentrations 100, 30, 10, 3, 1, 0.3, 0.1 and 0.03 nM (200X each) was added and the well-plate was allowed to stand for 48 h.

Control wells were treated with vehicle (DMSO). The final concentration of DMSO was 0.5% per test well. Percent cytotoxicity was calculated. A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 1ba and FIG. 1bb and concentration dependent cytotoxicity curves were obtained. These curves were used for the calculation of combination index. The combination index obtained from cytotoxicity curves of figures; FIG. 1ba and FIG. 1bb are depicted below in tables viz; Table 1ba and Table 1bb respectively.

### Table 1ba:

<table>
<thead>
<tr>
<th>Test sample</th>
<th>% Cytotoxicity</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin 1 mM</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 3 mM</td>
<td>0.68</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 10 mM</td>
<td>26.83</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 1 nM</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 3 nM</td>
<td>13.72</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 10 nM</td>
<td>35.66</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 1 mM + Compound A 1 nM</td>
<td>25.707</td>
<td>0.28</td>
</tr>
<tr>
<td>Metformin 3 mM + Compound A 1 nM</td>
<td>33.168</td>
<td>0.33</td>
</tr>
<tr>
<td>Metformin 1 mM + Compound A 3 nM</td>
<td>29.800</td>
<td>0.51</td>
</tr>
<tr>
<td>Metformin 3 mM + Compound A 3 nM</td>
<td>40.839</td>
<td>0.39</td>
</tr>
<tr>
<td>Metformin 10 mM + Compound A 3 nM</td>
<td>44.937</td>
<td>0.68</td>
</tr>
<tr>
<td>Metformin 1 mM + Compound A 10 nM</td>
<td>52.643</td>
<td>0.50</td>
</tr>
<tr>
<td>Metformin 3 mM + Compound A 10 nM</td>
<td>49.327</td>
<td>0.24</td>
</tr>
<tr>
<td>Metformin 10 mM + Compound A 10 nM</td>
<td>52.679</td>
<td>0.45</td>
</tr>
</tbody>
</table>

### Table 1bb

<table>
<thead>
<tr>
<th>Test sample</th>
<th>% Cytotoxicity</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin 1 mM</td>
<td>0.000</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 3 mM</td>
<td>17.843</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 10 mM</td>
<td>29.023</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 1 nM</td>
<td>12.626</td>
<td>-</td>
</tr>
<tr>
<td>Compound A 3 nM</td>
<td>38.291</td>
<td>-</td>
</tr>
<tr>
<td>Metformin 1 mM + Compound A 1 nM</td>
<td>34.07</td>
<td>0.46</td>
</tr>
<tr>
<td>Metformin 3 mM + Compound A 1 nM</td>
<td>37.57</td>
<td>0.45</td>
</tr>
<tr>
<td>Metformin 10 mM + Compound A 1 nM</td>
<td>41.35</td>
<td>0.67</td>
</tr>
<tr>
<td>Metformin 1 mM + Compound A 3 nM</td>
<td>49.97</td>
<td>0.60</td>
</tr>
<tr>
<td>Metformin 3 mM + Compound A 3 nM</td>
<td>61.07</td>
<td>0.33</td>
</tr>
<tr>
<td>Metformin 10 mM + Compound A 3 nM</td>
<td>64.17</td>
<td>0.23</td>
</tr>
</tbody>
</table>
Conclusion: The combination of Compound A and metformin at various concentrations showed synergistic effect in triple negative breast cancer cell lines.

Example 2:

Effect of compound A on Her 2 positive breast cancer cell lines alone and in combination with lapatinib

The different combinations of lapatinib and Compound A were tested by PI assay in a simultaneous mode.

Materials:

Compound A: The desired concentrations of Compound A used were 100, 30, 10, 3, 1, 0.3, 0.1, 0.03 nM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Lapatinib: The desired concentrations of lapatinib di-tosylate (LC Laboratories, US) used were 30, 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 µM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Cancer cell line: Her 2 amplified breast cancer cell lines HCC1569 (ATCC CRL-2330), HCC1954 (ATCC) and SKBR3 (ATCC).

Assay:

Simultaneous treatment of lapatinib and Compound A to Her 2 amplified breast cancer cells:

The wells seeded and incubated with Her 2 amplified breast cancer cell lines were treated with lapatinib alone, compound A alone and combination of lapatinib and compound A.
Treatment with lapatinib alone: 1 \( \mu \text{L} \) of lapatinib at concentrations of 30, 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 \( \mu \text{M} \) (200X each), was added to the wells seeded and incubated with Her 2 amplified breast cancer cell lines.

Treatment with Compound A alone: 1 \( \mu \text{L} \) of Compound A at concentrations of 100, 30, 10, 3, 1, 0.3, 0.1 and 0.03 nM (200X each), was added to the wells seeded and incubated with Her 2 amplified breast cancer cell lines.

Treatment with combination of Compound A and lapatinib: 1 \( \mu \text{L} \) of Compound A at concentrations of 100, 30, 10, 3, 1, 0.3, 0.1 and 0.03 nM (200X each), and 1 \( \mu \text{L} \) of lapatinib at concentrations of 30, 10, 3, 1, 0.3, 0.1, 0.03 and 0.01 \( \mu \text{M} \) (200X each), was added to the wells seeded and incubated with Her 2 amplified breast cancer cell lines simultaneously. The well-plate was allowed to stand for 48 h.

Control wells were treated with the vehicle (DMSO). The final concentration of DMSO was 0.5% per test well. Percent cytotoxicity was calculated. A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 2a, FIG. 2b and FIG. 2c and concentration dependent cytotoxicity curves were obtained. These curves were used for the calculation of combination index. The combination index obtained from cytotoxicity curves of figures; FIG. 2a, FIG. 2b and FIG. 2c are depicted below in tables viz; Table 2a, Table 2b and Table 2c respectively.

**Table 2a**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of Compound A (nM)</th>
<th>Concentration of lapatinib (( \mu \text{M} ))</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC1569</td>
<td>30</td>
<td>0.1</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>10</td>
<td>0.77</td>
</tr>
</tbody>
</table>

**Table 2b**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of Compound A (nM)</th>
<th>Concentration of lapatinib (( \mu \text{M} ))</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC1954</td>
<td>3</td>
<td>0.1</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.1</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Conclusion: The combination of Compound A and lapatinib at various concentrations showed synergistic effect in Her 2 amplified breast cancer cell lines.

**Example 3:**

Effect of compound A on triple negative breast cancer (TNBC) cell lines alone and in combination with cisplatin.

The different combinations of cisplatin and compound A were tested by PI assay in a simultaneous mode.

**Materials:**

Compound A: The desired concentrations of compound A used were 10, 3 and 1 nM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Cisplatin: The desired concentrations of cisplatin used were 30, 10, 3, 1, and 0.1 µM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Cancer cell line: TNBC cell lines MDAMB231 (ATCC), MDAMB453 (ATCC), MDAMB468 (ATCC) and BT549 (ATCC).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of Compound A (nM)</th>
<th>Concentration of lapatinib (µM)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKBR3</td>
<td>1</td>
<td>0.01</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.03</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.03</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.03</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.1</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.1</td>
<td>0.43</td>
</tr>
</tbody>
</table>
Assay:
Simultaneous treatment of cisplatin and Compound A to TNBC cells:

The wells seeded and incubated with TNBC cell lines were treated with cisplatin alone, compound A alone and combination of cisplatin and compound A.

Treatment with cisplatin alone: $1 \mu$L of cisplatin at 30, 10, 3, 1, and 0.1 $\mu$M (200X each), was added to the wells seeded and incubated with TNBC cell lines.

Treatment with compound A alone: $1 \mu$L of Compound A at 10, 3, and 1 nM (200X each), was added to the wells seeded and incubated with TNBC cell lines.

Treatment of TNBC cells with combination of Compound A and cisplatin: $1 \mu$L of Compound A of concentrations 10, 3, and 1nM (200X each), and $1 \mu$L of cisplatin of concentrations 30, 10, 3, 1, and 0.1 $\mu$M (200X each) was added to the wells seeded and incubated with TNBC cell lines simultaneously. The well-plates were allowed to stand for 48 h.

Control wells were treated with vehicle (DMSO). The final concentration of DMSO was 0.5% per test well. Percent cytotoxicity was calculated. A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 3a, FIG. 3b, FIG. 3c and FIG. 3d and concentration dependent cytotoxicity curves were obtained. These curves were used for the calculation of combination index. The combination index obtained from cytotoxicity curves of figures viz.; FIG. 3a, FIG. 3b, FIG. 3c and FIG. 3d are depicted below in tables viz; Table 3a, Table 3b, Table 3c and Table 3d respectively.

Table 3a

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of Compound A (nM)</th>
<th>Concentration of cisplatin (μM)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDAMB-231</td>
<td>1</td>
<td>10</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>30</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>30</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>0.202</td>
</tr>
</tbody>
</table>

Table 3b

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of Compound A (nM)</th>
<th>Concentration of cisplatin (μM)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT549</td>
<td>1</td>
<td>1</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>0.53</td>
</tr>
</tbody>
</table>
Table 3c

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of Compound A (nM)</th>
<th>Concentration of cisplatin (µM)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDAMB453</td>
<td>3</td>
<td>10</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>0.307</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>30</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>30</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table 3d

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of Compound A (nM)</th>
<th>Concentration of Cisplatin (µM)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDAMB468</td>
<td>1</td>
<td>0.1</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.1</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Conclusion: The combination of Compound A and cisplatin at various concentrations showed synergistic effect in triple negative breast cancer cell lines.

Example 4:

Effect of compound A on triple negative breast cancer (TNBC) cell lines alone and in combination with doxorubicin

The different combinations of doxorubicin and Compound A were tested by PI assay in a simultaneous mode.

Materials:

Compound A: The desired concentrations of Compound A used were 10, 3 and 1 nM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Doxorubicin: The desired concentrations of doxorubicin (Sigma Aldrich, US) used were 0.3 and 0.1 µM. Each concentration was prepared by following the
procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Cancer cell line: TNBC cell lines BT549 (ATCC), MDMAB468 (ATCC) and MDMAB453 (ATCC).

Assay:
Simultaneous treatment of doxorubicin and Compound A to TNBC cells:

The wells seeded and incubated with TNBC cell lines were treated with doxorubicin alone, compound A alone and combination of doxorubicin and compound A.

Treatment with doxorubicin alone: 1 μL of doxorubicin at concentrations of 0.3 and 0.1 μM (200X each), was added to the wells seeded and incubated with TNBC cell lines.

Treatment with Compound A alone: 1 μL of Compound A at concentrations of 10, 3, and 1 nM (200X each), was added to the wells seeded and incubated with TNBC cell lines.

Treatment with combination of compound A and doxorubicin: 1 μL of Compound A at 10, 3, and 1 nM (200X each), and 1 μL of doxorubicin at 0.3 and 0.1 μM (200X each), was added to the wells seeded and incubated with TNBC cell lines. The well-plates was allowed to stand for 48 h.

Control wells were treated with vehicle (DMSO). The final concentration of DMSO was 0.5% per test well. Percent cytotoxicity was calculated. A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 4a, FIG. 4b and FIG. 4c and concentration dependent cytotoxicity curves were obtained. These curves were used for the calculation of combination index. The combination index obtained from cytotoxicity curves of figures viz.; FIG. 4a, FIG. 4b and FIG. 4c are depicted below in tables viz; Table 4a, Table 4b and Table 4c respectively.

Table 4a

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of compound A (nM)</th>
<th>Concentration of doxorubicin (μM)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT549</td>
<td>1</td>
<td>0.1</td>
<td>0.47</td>
</tr>
</tbody>
</table>
Conclusion: The combination of Compound A and doxorubicin at various concentrations showed synergistic effect in triple negative breast cancer cell lines.

**Example 5:**

Effect of compound A on triple negative breast cancer (TNBC) cell lines alone and in combination with paclitaxel

The different combinations of paclitaxel and Compound A were tested by PI assay in a simultaneous mode.

**Materials:**

Compound A: The desired concentrations of Compound A used were 10, 3 and 1 nM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Paclitaxel: The desired concentrations of paclitaxel used were 3, 1.5 and 1 nM. Each concentration was prepared by following the procedure described above in
PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Cancer cell line: TNBC cell lines BT549 (ATCC), MDAMB453 (ATCC) and MDAMB468 (ATCC).

Assay:
Simultaneous Treatment of paclitaxel and Compound A to TNBC cells:
The wells seeded and incubated with TNBC cell lines were treated with paclitaxel alone, compound A alone and combination of paclitaxel and compound A.

Treatment with paclitaxel alone: 1 µL of paclitaxel at concentrations of 3, 1.5 and 1 nM (200X each), was added to the wells seeded and incubated with TNBC cell lines.

Treatment with Compound A alone: 1 µL of Compound A at concentrations of 10, 3, and 1 nM (200X each), was added to the wells seeded and incubated with TNBC cell lines.

Treatment with combination of Compound A and paclitaxel: 1 µL of Compound A at concentrations of 10, 3, and 1 nM (200X each), and 1 µL of paclitaxel concentrations of 3, 1.5 and 1 nM (200X each), was added to the wells seeded and incubated with TNBC cell lines simultaneously. The well-plates were allowed to stand for 48 h.

Control wells were treated with vehicle (DMSO). The final concentration of DMSO was 0.5% per test well. Percent cytotoxicity was calculated. A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 5a, FIG. 5b and FIG. 5c and concentration dependent cytotoxicity curves were obtained. These curves were used for the calculation of combination index. The combination index obtained from cytotoxicity curves of figures viz.; FIG. 5a, FIG. 5b and FIG. 5c are depicted below in tables viz; Table 5a, Table 5b and Table 5c respectively.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of compound A (nM)</th>
<th>Concentration of paclitaxel (nM)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>0.008</td>
</tr>
</tbody>
</table>
Conclusion: The combination of Compound A and paclitaxel at various concentrations showed synergistic effect in triple negative breast cancer cell lines.

Example 6:

Effect of compound A on triple negative breast cancer (TNBC) cell lines alone and in combination with 5-fluorouracil (5-FU)

The different combinations of 5-FU and Compound A were tested by PI assay in a simultaneous mode.

Materials:

Compound A: The desired concentrations of Compound A used were 10, 3 and 1 nM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

5-FU: The desired concentrations of 5-FU (Sigma Aldrich, US) used was 10 µM. The concentration was prepared by following the procedure described above in PI
 assay such that the concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Cancer cell line: TNBC cell lines BT549 (ATCC), MDAMB453 (ATCC) and MDAMB468 (ATCC).

Assay:
Simultaneous Treatment of 5-FU and Compound A to TNBC cells:

The wells seeded and incubated with TNBC cell lines were treated with 5-FU alone, compound A alone and combination of 5-FU and compound A.

Treatment with 5-FU alone: 1 µL of 5-FU concentration of 10 µM (200X), was added to the wells seeded and incubated with TNBC cell lines.

Treatment with compound A alone: 1 µL of Compound A at concentrations of 10, 3, and 1 nM (200X each), was added to the wells seeded and incubated with TNBC cell lines.

Treatment with combination of Compound A and 5-FU: 1 µL of Compound A at concentrations of 10, 3, and 1 nM (200X each), and 1 µL of 5-FU at concentration of 10 µM (200X), was added to the wells, seeded and incubated with TNBC cell lines simultaneously. The well-plates was allowed to stand for 48 h.

Control wells were treated with vehicle (DMSO). The final concentration of DMSO was 0.5% per test well. Percent cytotoxicity was calculated. A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 6a, FIG. 6b and FIG. 6c and concentration dependent cytotoxicity curves were obtained. These curves were used for the calculation of combination index. The combination index obtained from cytotoxicity curves of figures viz.; FIG. 6a, FIG. 6b and FIG. 6c are depicted below in tables viz; Table 6a, Table 6b and Table 6c respectively.

Table 6a

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Concentration of Compound A (nM)</th>
<th>Concentration of 5-FU (µM)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT549</td>
<td>1</td>
<td>10</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>0.22</td>
</tr>
</tbody>
</table>
Conclusion: The combination of Compound A and 5-FU at various concentrations showed synergistic effect in triple negative breast cancer cell lines.

**Example 7:**

Effect of compound A on herceptin and lapatinib resistant Her 2 overexpressing breast cancer (HCC 1569) cell lines alone and in combination with PL225B.

The procedure followed was similar to the procedure described in Example 2. The cancer cell line used was HCC 1569.

**Compound A:** The desired concentrations of Compound A used were 10, 3, 1 nM.

Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

**PL225B:** The desired concentration of PL225B used was 1 µM. The concentration was prepared by following the procedure described above in PI assay such that the concentration was 200 times concentrated (200X) than the corresponding desired concentration.

A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figure viz; FIG. 7. The combination index obtained from cytotoxicity curves of FIG. 7, indicate that the CI value for the combination of compound A and PL225B is less than 1.
Conclusion: The combination of Compound A and PL225B at various concentrations showed synergistic effect in Her 2 overexpressing breast cancer (HCC 1569) cell lines.

Example 8:
Effect of compound A on non-small cell lung cancer cell lines alone and in combination with erlotinib

The procedure followed was similar to the procedure described in Example 2.

The cancer cell lines used were A549, H358, H1975 and H1650.

Compound A: The desired concentrations of Compound A used were 6, 5 and 3 nM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Erlotinib: The desired concentration of erlotinib used was 2.5 μM. The concentration was prepared by following the procedure described above in PI assay such that the concentration was 200 times concentrated (200X) than the corresponding desired concentration.

A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 8a, FIG. 8b, FIG. 8c and FIG. 8d.

The combination index obtained from cytotoxicity curves of figures viz; FIG. 8a, FIG. 8b, FIG. 8c and FIG. 8d, indicate that the CI value for the combination of compound A and erlotinib is less than 1.

Conclusion: The combination of Compound A and erlotinib at various concentrations showed synergistic effect in non-small cell lung cancer cell lines.

Example 9:
Effect of compound A on melanoma cell lines alone and in combination with vemurafenib (PLX4032)
The procedure followed was similar to the procedure described for sequential administration in Example 1. The cancer cell lines used were A375, G361, SKMEL-3 and A2058 ( vemurafenib resistant melanoma cancer cell line).

Compound A: The desired concentrations of Compound A used were at the concentrations of 0.01, 0.03 and 0.1 μM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

PLX4032: The desired concentration of PLX4032 used was 0.1, 0.3 and 1 μM. The concentration was prepared by following the procedure described above in PI assay such that the concentration was 200 times concentrated (200X) than the corresponding desired concentration.

A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 9a, FIG. 9b, FIG. 9c and FIG. 9d. The combination index obtained from cytotoxicity curves of figures viz; FIG. 9a, FIG. 9b, FIG. 9c and FIG. 9d, indicate that the CI value for the combination of compound A and PLX4032 is less than 1.

Conclusion: The combination of Compound A and PLX4032 at various concentrations showed synergistic effect in melanoma cell lines.

Example 10:
Effect of compound A on non-small cell lung cancer cell lines alone and in combination with sorafenib

The procedure followed was similar to the procedure described for sequential administration in Example 1. The cancer cell lines used were H460 and A549.

A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 10a and FIG. 10b. The combination index obtained from cytotoxicity curves of figures viz; FIG. 10a, and FIG. 10b, indicate that the CI value for the combination of compound A and sorafenib is less than 1.

Conclusion: The combination of Compound A and sorafenib at various concentrations showed synergistic effect in non-small cell lung cancer cell lines.
Example 11:
Effect of compound A on breast cancer cell lines alone and in combination with BMS7081 63 and cyclopamine

The procedure followed was similar to the procedure described in Example 2. The cancer cell lines used were triple negative breast cancer cell lines viz., MDAMB231, MDAMB453, MDAMB468, BT549 and HCC1937.

Compound A: The desired concentrations of Compound A used were 1, 3 and 10 nM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

BMS7081 63 and cyclopamine: The desired concentration of BMS7081 63 used was 3 and 10 μM and the concentration of cyclopamine used was 3μM. The concentration was prepared by following the procedure described above in PI assay such that the concentration was 200 times concentrated (200X) than the corresponding desired concentration.

A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figures viz; FIG. 11a, FIG. 11b, FIG. 11c, FIG. 11d and FIG. 11e. The combination index obtained from cytotoxicity curves of figures viz; FIG. 11a, FIG. 11b, FIG. 11c, FIG. 11d and FIG. 11e, indicate that the CI value for the compound A in combination with avagacestat (BMS708163) and cyclopamine is less than 1.

Conclusion: The combination of Compound A with BMS708163 and cyclopamine at various concentrations showed synergistic effect in TNBC cell lines.

Example 12:
Effect of compound A on breast cancer cell line alone and in combination with everolimus

The procedure followed was similar to the procedure described in any of the above examples. The cancer cell line used was ER positive, PR positive breast cancer cell line viz., MCF7.
Compound A: The desired concentrations of Compound A used were 3 and 10 nM. Each concentration was prepared by following the procedure described above in PI assay such that each concentration was 200 times concentrated (200X) than the corresponding desired concentration.

Everolimus: The desired concentration of everolimus used was 0.001, 0.01, 0.1 and 1 µM. The concentration was prepared by following the procedure described above in PI assay such that the concentration was 200 times concentrated (200X) than the corresponding desired concentration.

A Graph for percent cytotoxicity vs drug concentration was plotted as shown in the figure viz; FIG. 12a and FIG. 12b. The combination index obtained from cytotoxicity curves of figures viz; FIG. 12a and FIG. 12b indicates that the CI value for the compound A in combination with everolimus is less than 1.

Conclusion: The combination of Compound A with everolimus at various concentrations showed synergistic effect in MCF7 cell line.

**In vivo experiments:**

All experiments were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and with the approval of Institutional Animal Ethics Committee (IAEC) in Piramal Enterprises Limited, Goregaon, Mumbai, India.

The efficacy of the combinations of Compound A and the anti-proliferative agent (combination drug) such as metformin, lapatinib, erlotinib and irinotecan was studied in vivo, in the following Examples 13-24.

**Example 13:**

Effect of compound A on triple negative breast cancer (TNBC) (MDA-MB-231) in nude mice xenograft model alone and in combination with metformin

Animals used

Nude Nu/J male mice, 4 to 6 weeks old, weighing 22 to 25 g (Harlan Laboratories, Inc). Animals were housed in animal isolator (Harlan Inc.) under specified pathogen-free conditions maintained at 22 to 25 °C and 55 to 75% humidity, with a 12-hour light/12-hour dark cycle. The mice were acclimatized for
a period of at least 7 days before experimentation. Animals were handled in a laminar flow hood. All food and water was autoclaved. Mice had access to pelleted rodent diet (National Centre for Laboratory Animal Sciences, Hyderabad, India) and water ad libitum.

Generation of Triple Negative Breast Cancer Xenograft Model

Step 1: Preparation of a single-cell suspension for injection into nude mice

The MDA MB 231 cells were grown in RPMI 1640 medium containing 10% fetal calf serum, in an incubator at 37 ºC containing 5% CO₂, using TNBC cell line MDA MB 231 (ATCC CRL-HBT 26). The cells were washed with cold phosphate-buffered saline, trypsin was added and the suspension further incubated at 37 ºC. RPMI 1640 containing 10% fetal bovine serum was added and the cells were collected in centrifuge tube. The cell suspension was centrifuged at 1000 rpm for 10 minutes. The supernatant was removed and the cell pellet was re-suspended in 1 mL serum-free RPMI 1640 medium. The cell count was obtained using 1:100 dilution. The cells were diluted to obtain 5 x 10⁶ cells per 0.2 mL of suspension.

Step 2: Generation of Triple Negative Breast Cancer Xenograft Model

On the day of tumor cell injection, cell suspension of step 1 was stored on ice in the laminar airflow hood. Each nude mouse was injected with 0.2 mL of the cell suspension subcutaneously in the right flank posterior to the ribcage. Mice were observed every alternate day for palpable tumor mass.

Sample preparation and storage

Compound A: 0.5 mg/mL; vehicle: methyl cellulose (0.25%).

Metformin (used as positive control): 200 mg/kg (1 mg/mL); vehicle: Sterile distilled water.

All the compounds were stored at 2 ºC to 8 ºC.

Dosing

Nude mice were housed in a group of minimum 8 per cage (filter-top cages) with autoclaved husk bedding and free access to food and water. Animals were handled as per the standard guidelines. Treatment was initiated when tumor size volume was about 100 mm³. The tumor-bearing mice were randomized (n= 8) in the following groups of treatment:
i) Group 1: Control group: Tumor-bearing mice administered with vehicle.

ii) Group 2: Tumor-bearing mice administered once daily with 5 mg/kg of compound A perorally

iii) Group 3: Tumor-bearing mice were scheduled to receive 200 mg/kg of metformin, administered in a dose of 1 mg/mL in 100 mL drinking water considering average daily intake of water of each mouse is 5 mL.

iv) Group 4: Tumor-bearing mice were administered with Combination of Compound A (5 mg/kg) and metformin (200 mg/kg) perorally.

Treatment

Compound A was administered per orally to nude mice of Group 2, and metformin was administered to nude mice of Group 3 and combination of compound A (5 mg/kg) and metformin (200 mg/kg) was administered to nude mice of Group 4 everyday for 29 days. On the 29th day animals from all groups were scarified and samples were harvested for further analysis.

The volume of Compound A administered to Group 2 and Group 4 was 10 mL/kg.

Observations and measurement

Following parameters were observed during treatment:

1. Gross animal health was observed everyday
2. Body weight was observed everyday
3. Tumor was measured 2-3 days apart, using vernier caliper.

Tumor volume in mm³ was calculated using the formula for a prolate ellipsoid:

\[ \text{Tumor volume (mm}^3\text{)} = \text{Length (mm)} \times \text{[Breadth (mm)}^2\text{]} \times 0.5 \]

assuming specific gravity of tumor as 1 and \( \pi \) as 3

Treated to control ratio (\( \Delta T/AC \%) \) on a given day was calculated using the formula:

\[
\frac{\text{Tumor size Compound}_x \text{Day } x - \text{Tumor size Compound}_o \text{Day } x}{\text{Tumor size control}_x \text{Day } x - \text{Tumor size control}_o \text{Day } x} \times 100
\]

Growth inhibition (Gl) was calculated using the formula
GI on Day X = 100 - AT/AC% on Day X

Tumor growth inhibition results are given in FIG. 13.

Conclusion: A combination of metformin and compound A showed significant tumor growth inhibition in MDA MB 231 xenograft model.

Example 14:
Effect of compound A on herceptin and lapatinib resistant Her 2 overexpressing breast cancer (HCC 1569) xenograft model alone and in combination with lapatinib

Animals used
NOD/SCID female mice, 4 to 6 weeks old, weighing 22 to 25 g (Harlan Laboratories, Inc). Animals were housed in animal isolator (Harlan Inc.) under specified pathogen-free conditions maintained at 22 to 25 °C and 55 to 75% humidity, with a 12- hour light/12- hour dark cycle. The mice were acclimatized for a period of at least 7 days before experimentation. Animals were handled in a laminar flow hood. All food and water was autoclaved. Mice had access to pelleted rodent diet (National Centre for Laboratory Animal Sciences, Hyderabad, India) and water ad libitum.

Generation of Her 2 overexpressing breast cancer (HCC 1569) xenograft model

Step 1: Preparation of a single-cell suspension for injection into nude mice
Adherent HCC 1569 cells were grown in RPMI 1640 medium containing 10% fetal calf serum, in an incubator at 37 °C containing 5% CO₂, using epithelial breast cancer cell line HCC 1569 (ATCC CRL- 2330). The cells were washed with cold phosphate-buffered saline, trypsin was added and the suspension further incubated at 37 °C. RPMI 1640 containing 10% fetal bovine serum was added and the cells were collected in centrifuge tube. The cell suspension was centrifuged at 1000 rpm for 10 minutes. The supernatant was removed and the cell pellet was re-suspended in 1 mL serum-free RPMI 1640 medium. The cell count was obtained using 1:100 dilution. The cells were diluted to obtain 10 x 10⁶ cells per 0.2 mL of suspension.
Step 2: Generation of Her 2 overexpressing breast cancer (HCC 1569) xenograft model

On the day of tumor cell injection, cell suspension of step 1 was stored on ice in the laminar airflow hood. Each NOD/SCID mouse was injected with 0.2 mL of the cell suspension subcutaneously in the right flank posterior to the ribcage. Mice were observed every alternate day for palpable tumor mass.

Sample preparation and storage

Compound A: 0.5 mg/mL; vehicle: methyl cellulose (0.25%).

Lapatinib (LC Laboratories): (used as positive control) 10 mg/mL; vehicle: methyl cellulose (0.25%).

Compound A was stored at 2 °C to 8 °C and lapatinib was prepared for immediate use.

Dosing

NOD/SCID mice were housed in a group of 8 per cage (filter-top cages) with autoclaved husk bedding and free access to food and water. Animals were handled as per the standard guidelines. Treatment was initiated when tumor size volume was about 100 mm³. The tumor-bearing mice were randomized (n= 8) in the following groups of treatment:

ii) Group 1: Control group: Tumor-bearing mice administered with vehicle.

iii) Group 2: Tumor-bearing mice administered once daily with 5 mg/kg of compound A perorally

iv) Group 3: Tumor-bearing mice administered once daily with 100 mg/kg of lapatinib perorally

v) Group 4: Tumor-bearing mice were administered with a Combination of Compound A (5 mg/kg) and lapatinib (100 mg/kg) perorally.

Treatment

Compound A was administered per orally to NOD/SCID mice of Group 2 and lapatinib was administered to NOD/SCID mice of Group 3 everyday for 15 days. Combination of Compound A (5 mg/kg) and lapatinib (100 mg/kg) was administered to NOD/SCID mice of Group 4 on days 1-5, 12 and 13th day of
treatment. On the 15th day animals from all groups were scarified and samples were harvested for further analysis.

The volume of Compound A administered to Group 2 and Group 4 was 10 mL/kg. Observations and measurement

Following parameters were observed during treatment:
1. Gross animal health was observed everyday
2. Body weight was observed everyday
3. Tumor was measured 2-3 days apart, using vernier caliper.

Tumor volume in mm³ was calculated using the formula for a prolate ellipsoid:

\[ \text{Tumor volume (mm}^3\text{)} = \text{Length (mm)} \times [\text{Breadth (mm)}]^2 \times 0.5 \]

assuming specific gravity of tumor as 1 and \(\pi\) as 3

Treated to control ratio (\(\Delta T/AC\%\)) on a given day was calculated using the formula:

\[ \frac{\text{Tumor size Compound}_{D_{\text{ay}x}} \times - \text{Tumor size Compound}_{D_{\text{ay} \circ}}}{\text{Tumor size control}_{D_{\text{ay}x}} \times - \text{Tumor size control}_{D_{\text{ay} \circ}}} \times 100 \]

Growth inhibition (GI) was calculated using the formula

\[ \text{GI on Day X} = 100 - \frac{\text{AT/AC\% on Day X}}{\Delta T/AC\% on Day X} \]

Tumor growth inhibition results are given in FIG. 14.

Conclusion: A combination of lapatinib and compound A showed significant tumor growth inhibition in herceptin and lapatinib resistant, Her2 overexpressing breast cancer (HCC1569) xenograft model.

**Example 15:**

Effect of compound A on EGFR mutated non-small cell lung cancer (NSCLC) (H1650) in xenograft model alone and in combination with erlotinib

Animals used

Nude Nu/J male mice, 4 to 6 weeks old, weighing 22 to 25 g (Harlan Laboratories, Inc). Animals were housed in animal isolator (Harlan Inc.) under specified pathogen-free conditions maintained at 22 to 25 °C and 55 to 75% humidity, with a 12- hour light/12- hour dark cycle. The mice were acclimatized for
a period of 7 days before experimentation. Animals were handled in a laminar flow hood. All food and water was autoclaved. Mice had access to pelleted rodent diet (National Centre for Laboratory Animal Sciences, Hyderabad, India) and water ad libitum.

Generation of EGFR mutated NSCLC (H1650) xenograft model

Step 1: Preparation of a single-cell suspension for injection into nude mice

The H1650 cells were grown in RPMI 1640 medium containing 10% fetal calf serum, in an incubator at 37 °C containing 5% CO₂, using NSCLC cell line H1650 (ATCC CRL-5883). The cells were rinsed with cold phosphate-buffered saline, trypsin was added and the suspension was further incubated at 37 °C. RPMI 1640 containing 10% fetal bovine serum was added and the cells were collected in a centrifuge tube. The cell suspension was centrifuged at 1000 rpm for 10 minutes. The supernatant was removed and the cell pellet was re-suspended in 1 mL serum-free RPMI 1640 medium. The cell count was obtained using 1:100 dilution. The cells were diluted to obtain 5 x 10⁶ cells per 0.2 mL of suspension.

Step 2: Generation of EGFR mutated NSCLC (H1650) xenograft model

On the day of tumor cell injection, cell suspension of step 1 was stored on ice in the laminar airflow hood. Each nude mouse was injected with 0.2 mL of the cell suspension subcutaneously in the right flank posterior to the ribcage. Mice were observed every alternate day for palpable tumor mass.

Sample preparation and storage

Compound A: 0.5 mg/mL; vehicle: methyl cellulose (0.25%)

Erlotinib hydrochloride (used as positive control): 50 mg/kg (5 mg/mL); vehicle: methyl cellulose (0.25%); erlotinib was procured from Taizhou Creating Chemical Company Ltd, China.

All the compounds were stored at 2 °C to 8 °C.

Dosing

Nude mice were housed in a group of minimum 8 per cage (filter-top cages) with autoclaved husk bedding and free access to food and water. Animals were handled as per the standard guidelines. Treatment was initiated when tumor size
volume was about 100 mm$^3$. The tumor-bearing mice were randomized ($n=8$) in
the following groups of treatment:

i) Group 1: Control group: Tumor-bearing mice administered with vehicle.

ii) Group 2: Tumor-bearing mice administered once daily with 5 mg/kg of
compound A perorally

iii) Group 3: Tumor-bearing mice were administered once daily with 50 mg/kg of
eriotinib hydrochloride perorally

iv) Group 4: Tumor-bearing mice were administered with Combination of
Compound A (5 mg/kg) and eriotinib hydrochloride (50 mg/kg) perorally.

Treatment
Compound A was administered per orally to nude mice of Group 2, and eriotinib
hydrochloride was administered to nude mice of Group 3 everyday for 15 days
and Combination of Compound A (5 mg/kg) and eriotinib hydrochloride (50 mg/kg)
was administered to nude mice of Group 4 on days 1 to 6 and stopped further until
termination. On the 15$^{th}$ day animals from all groups were scarificed and samples
were harvested for further analysis.
The volume of Compound A administered was 10 mL/kg and eriotinib
administered was 10 mL/kg.

Observations and measurement
Following parameters were observed during treatment:

1. Gross animal health was observed everyday

2. Body weight was observed everyday

3. Tumor was measured 2-3 days apart, using vernier caliper

Tumor volume in mm$^3$ was calculated using the formula for a prolate
ellipsoid:

\[
\text{Tumor volume (mm}^3\text{)} = \text{Length (mm)} \times \text{[Breadth (mm)}^2\text{]} \times 0.5
\]

assuming specific gravity of tumor as 1 and $\pi$ as 3

Treated to control ratio ($\Delta T/AC$ %) on a given day was calculated using the
formula:
Tumor size Compound Day x - Tumor size Compound Day o
AT/AC% on Day X = \frac{Tumor size control Day x - Tumor size control Day o}{X} \times 100

Growth inhibition (Gl) was calculated using the formula

Gl on Day X = 100 - AT/AC% on Day X

Tumor growth inhibition results are given in FIG. 15.

Conclusion: A combination of erlotinib and compound A showed significant tumor growth inhibition in EGFR & PTEN mutated NSCLC xenograft model (H1 650).

Example 16:

Effect of compound A on EGFR mutated (T790 M) erlotinib resistant non-small cell lung cancer (NSCLC) (H1 975) in xenograft model alone and in combination with erlotinib.

Animals used
Nude Nu/J male mice, 4 to 6 weeks old, weighing 22 to 25 g (Harlan Laboratories, Inc). Animals were housed in animal isolator (Harlan Inc.) under specified pathogen-free conditions maintained at 22 to 25 °C and 55 to 75% humidity, with a 12- hour light/12- hour dark cycle. The mice were acclimatized for a period of 7 days before experimentation. Animals were handled in a laminar flow hood. All food and water was autoclaved. Mice had access to pelleted rodent diet (National Centre for Laboratory Animal Sciences, Hyderabad, India) and water ad libitum.

Generation of EGFR mutated NSCLC (H1975) xenograft model

Step 1: Preparation of a single-cell suspension for injection into nude mice
The H1975 cells were grown in RPMI 1640 medium containing 10% fetal calf serum, in an incubator at 37 °C containing 5% CO₂, using NSCLC cell line H1975 (ATCC CRL-5908). The cells were rinsed with cold phosphate-buffered saline, trypsin was added and the suspension was further incubated at 37 °C. RPMI 1640 containing 10 % fetal bovine serum was added and the cells were collected in a centrifuge tube. The cell suspension was centrifuged at 1000 rpm for 10 minutes. The supernatant was removed and the cell pellet was re-suspended in 1 mL
serum-free RPMI 1640 medium. The cell count was obtained using 1:100 dilution. The cells were diluted to obtain $5 \times 10^6$ cells per 0.2 mL of suspension.

Step 2: Generation of EGFR mutated NSCLC (H1975) xenograft model

On the day of tumor cell injection, cell suspension of step 1 was stored on ice in the laminar airflow hood. Each nude mouse was injected with 0.2 mL of the cell suspension subcutaneously in the right flank posterior to the ribcage. Mice were observed every alternate day for palpable tumor mass.

Sample preparation and storage

Compound A: 0.5 mg/mL; vehicle: methyl cellulose (0.25%)

Erlotinib hydrochloride (used as positive control): 25 mg/kg (2.5 mg/mL); vehicle: methyl cellulose (0.25%); Erlotinib was procured from Taizhou Creating Chemical Company Ltd, China.

All the compounds were stored at 2°C to 8°C.

Dosing

Nude mice were housed in a group of minimum 8 per cage (filter-top cages) with autoclaved husk bedding and free access to food and water. Animals were handled as per the standard guidelines. Treatment was initiated when tumor size volume was about 100 mm$^3$. The tumor-bearing mice were randomized ($n=8$) in the following groups of treatment:

i) Group 1: Control group: Tumor-bearing mice administered with vehicle.

ii) Group 2: Tumor-bearing mice administered once daily with 5 mg/kg of compound A perorally

iii) Group 3: Tumor-bearing mice administered once daily with 15 mg/kg of compound A perorally

iv) Group 4: Tumor-bearing mice were administered once daily with 25 mg/kg of Erlotinib hydrochloride perorally

v) Group 5: Tumor-bearing mice were administered with Combination of Compound A (5 mg/kg) and erlotinib hydrochloride (25 mg/kg) perorally.
Treatment

Compound A was administered perorally to nude mice of Group 2 and Group 3 once daily from day 1 to day 13, and eriotinib hydrochloride was administered to nude mice of Group 4 everyday for 13 days and Combination of Compound A (5 mg/kg) and eriotinib hydrochloride (25 mg/kg) was administered to nude mice of Group 5 on days 1 to 3 and days 10 to 13. On the 13th day animals from all groups were scarified and samples were harvested for further analysis. The volume of Compound A administered was 10 mL/kg and eriotinib administered was 10 mL/kg.

Observations and measurement

Following parameters were observed during treatment:
1. Gross animal health was observed everyday
2. Body weight was observed everyday
3. Tumor was measured 2-3 days apart, using vernier caliper

Tumor volume in mm³ was calculated using the formula for a prolate ellipsoid:

\[
\text{Tumor volume (mm}^3\text{)} = \text{Length (mm)} \times \text{[Breadth (mm)}^2\text{]} \times 0.5
\]

assuming specific gravity of tumor as 1 and \(\pi\) as 3

Treated to control ratio (\(\Delta T/AC\) %) on a given day was calculated using the formula:

\[
\frac{\Delta T/AC\text{% on Day } X}{\text{Tumor size CompoundDay} x - \text{Tumor size CompoundDay} o} = \times
\]

\[
\frac{\text{Tumor size controbay x - Tumor size contrary o}}{100}
\]

Growth inhibition (GI) was calculated using the formula

\[
\text{GI on Day } X = 100 - \frac{\Delta T/AC\text{% on Day } X}{\text{AT/AC% on Day } X}
\]

Tumor growth inhibition results are given in FIG. 16.

Conclusion: A combination of eriotinib and compound A showed significant tumor growth inhibition in EGFR (T790) mutated and eriotinib resistant xenograft model (H1975).
Example 17:
Effect of compound A on K-ras mutated colon cancer (HCT116) in xenograft model alone and in combination with irinotecan

Animals used
Nude Nu/J male mice, 4 to 6 weeks old, weighing 22 to 25 g (Harlan Laboratories, Inc). Animals were housed in animal isolator (Harlan Inc.) under specified pathogen-free conditions maintained at 22 to 25 °C and 55 to 75% humidity, with a 12-hour light/12-hour dark cycle. The mice were acclimatized for a period of 7 days before experimentation. Animals were handled in a laminar flow hood. All food and water was autoclaved. Mice had access to pelleted rodent diet (National Centre for Laboratory Animal Sciences, Hyderabad, India) and water ad libitum.

Generation of K-ras mutated colon cancer (HCT116) xenograft model

Step 1: Preparation of a single-cell suspension for injection into nude mice
The HCT116 cells were grown in McCoy's 5a medium containing 10% fetal calf serum, in an incubator at 37 °C containing 5% CO₂, using colon cancer cell line HCT116 (ATCC CRL-247). The cells were rinsed with cold phosphate-buffered saline, trypsin was added and the suspension was further incubated at 37 °C. McCoy's 5a medium containing 10% fetal bovine serum was added and the cells were collected in a centrifuge tube. The cell suspension was centrifuged at 1000 rpm for 10 minutes. The supernatant was removed and the cell pellet was re-suspended in 1 mL serum-free McCoy's 5a medium. The cell count was obtained using 1:100 dilution. The cells were diluted to obtain 5 x 10⁶ cells per 0.2 mL of suspension.

Step 2: Generation of K-ras mutated colon cancer (HCT116) xenograft model
On the day of tumor cell injection, cell suspension of step 1 was stored on ice in the laminar airflow hood. Each nude mouse was injected with 0.2 mL of the cell suspension subcutaneously in the right flank posterior to the ribcage. Mice were observed every alternate day for palpable tumor mass.
Sample preparation and storage
Compound A: 0.5 mg/mL; vehicle: methyl cellulose (0.25%)  
Irinotecan hydrochloride trihydrate injection (used as positive control): 5 mg/mL;  
Irinotecan hydrochloride trihydrate injection was procured from (Pfizer Products India Private Ltd, Mumbai).

All the compounds were stored at 2 °C to 8 °C.

Dosing
Nude mice were housed in a group of 8 per cage (filter-top cages) with autoclaved husk bedding and free access to food and water. Animals were handled as per the standard guidelines. Treatment was initiated when tumor size volume reached between 100-200 mm³. The treatment was carried out in two arms. Each arm comprised of 8 animals per group.

Treatment Groups of Arm 1:

i) Group 1: Control group: Tumor-bearing mice administered with vehicle.

ii) Group 2: Tumor-bearing mice administered once daily with 15 mg/kg of compound A perorally

iii) Group 3: Tumor-bearing mice were administered once daily with 50 mg/kg of irinotecan hydrochloride intravenously

iv) Group 4: Tumor-bearing mice were administered with combination of compound A (15 mg/kg), perorally and irinotecan hydrochloride (50 mg/kg) intravenously.

Treatment of Arm 1
Compound A was administered perorally to nude mice of Group 2 and Group 4 once daily from day 1 to day 8, and irinotecan hydrochloride was administered to nude mice of Group 3 and Group 4 on day 1 and day 8 via intravenous route and on the 9th day animals from all groups were scarified and samples were harvested for further analysis.

Treatment Groups of Arm 2

i) Group 1: Control group: Tumor-bearing mice administered with vehicle.
ii) Group 2: Tumor-bearing mice administered once daily with 15 mg/kg of compound A perorally

iii) Group 3: Tumor-bearing mice were administered once daily with 50 mg/kg of Irinotecan hydrochloride intarvenously

iv) Group 4: Tumor-bearing mice were administered with Combination of Compound A (15 mg/kg), perorally and Irinotecan hydrochloride (50 mg/kg) intarvenously.

Treatment of Arm 2

Compound A was administered perorally to nude mice of Group 2 daily from day 1 to day 13; Compound A was administered perorally to nude mice of Group 4 scheduled as 3 days ON and 4 days OFF; Irinotecan hydrochloride was administered intravenously to nude mice of Group 3 and Group 4 scheduled as one dose on day 1 only.

On the 14th day animals from all groups were scarified and samples were harvested for further analysis.

The volume of Compound A administered was (10ml/Kg) and irinotecan administered was (10 mL/kg).

Observations and measurement

Following parameters were observed during treatment:

1. Gross animal health was observed everyday
2. Body weight was observed everyday
3. Tumor was measured 2-3 days apart, using vernier caliper

Tumor volume in mm$^3$ was calculated using the formula for a prolate ellipsoid:

\[
\text{Tumor volume (mm}^3\text{)} = \text{Length (mm)} \times [\text{Breadth (mm)}^2] \times 0.5
\]

assuming specific gravity of tumor as 1 and \( \pi \) as 3

Treated to control ratio (\( \Delta \text{T}/\text{AC} \%) \) on a given day was calculated using the formula:

\[
\frac{\Delta \text{T}/\text{AC}\% \text{ on Day } X}{100} = \frac{\text{Tumor size Compound}_{\text{Day } X} - \text{Tumor size Compound}_{\text{Day } 0}}{\text{Tumor size control}_{\text{Day } X} - \text{Tumor size control}_{\text{Day } 0}} \times X
\]
Growth inhibition (Gl) was calculated using the formula

\[ GI_{on\ Day\ X} = 100 - \frac{AT}{AC\%_{on\ Day\ X}} \]

Tumor growth inhibition results for treatment arm 1 are given in FIG. 17a and for treatment arm 2 in FIG. 17b.

Conclusion: A combination of irinotecan and compound A showed significant tumor growth inhibition in Kras mutated colon cancer using HCT116 xenograft model and also reduced toxicity associated with irinotecan.

**Example 18:**

Effect of compound A on triple negative breast cancer (MDAMB231) in xenograft model alone and in combination with PL225B

The procedure followed was similar to the procedure described in Example 13. The dose of PL225B used was 100 mg/kg and was administered perorally. The dose of Compound A alone or in combination was 5 mg/kg.

Tumor growth inhibition results are given in FIG. 18a.

Conclusion: A combination of PL225B and compound A showed significant tumor growth inhibition in MDA MB 231 xenograft model.

**Example 19:**

Effect of compound A on erlotinib resistant (T790M) non-small cell lung cancer (NSCLC) (H1975) in xenograft model alone and in combination with PL225B

The procedure followed was similar to the procedure described in Example 16. The dose of PL225B used was 100 mg/kg and was administered perorally. The dose of Compound A used, alone was 5mg/kg and 15 mg/kg and the dose of compound A when used in combination with PL225B was 5 mg/kg and 15 mg/kg. Tumor growth inhibition results are given in FIG. 18b.

Conclusion: A combination of PL225B and compound A showed significant tumor growth inhibition in EGFR (T790) mutated and erlotinib resistant xenograft model (H1 975).
Example 20:
Effect of compound A on lung cancer (H 460) in xenograft model alone and in combination with Sorafenib

The procedure followed was similar to the procedure described in Example 15. The cancer cell line, H460 was used for the generation of K-ras mutated NSCLC xenograft model. The dose of sorafenib used was 30 mg/kg and was administered perorally and the dose of Compound A used, alone or in combination was 5 mg/kg and 10 mg/kg. Tumor growth inhibition results are given in FIG. 19a and FIG. 19b.

Conclusion: A combination of sorafenib and compound A showed significant tumor growth inhibition in K-ras mutated lung cancer xenograft model (H460).

Example 21: Effect of compound A on K-ras mutated colon cancer (HCT1 16) in xenograft model alone and in combination with Sorafenib

The procedure followed was similar to the procedure described in Example 17. The dose of sorafenib used was 30 mg/kg and was administered perorally. The dose of Compound A used, alone or in combination was 20 mg/kg. Tumor growth inhibition results are given in FIG. 19c.

Conclusion: A combination of sorafenib and compound A showed significant tumor growth inhibition in K-ras mutated colon cancer xenograft model (HCT1 16).

Example 22:
Effect of compound A on hepatocellular carcinoma (Huh-7) in xenograft model alone and in combination with Sorafenib

The procedure followed was similar to the procedure described in Example 15. The hepatocellular carcinoma cell line, Huh-7 was used for the generation of hepatocellular carcinoma in the xenograft model. The dose of sorafenib used was 30 mg/kg and was administered perorally. The dose of Compound A used, alone or in combination was 20 mg/kg. Tumor growth inhibition results are given in FIG. 19d.
Conclusion: A combination of sorafenib and compound A showed significant tumor growth inhibition in hepatocellular carcinoma (Huh-7) xenograft model.

**Example 23:**

Effect of compound A on BRAF mutated melanoma (A375) in xenograft model alone and in combination with PLX4032 (vemurafenib)

The procedure followed was similar to the procedure described in Example 15. The melanoma cell line, A375 was used for the generation of melanoma in the xenograft model. The dose of PLX4032 used was 50 mg/kg and was administered perorally. The dose of Compound A used, alone or in combination was 5 mg/kg and 15 mg/kg. Tumor growth inhibition results are given in figures viz., FIG. 20a and FIG. 20c.

Conclusion: A combination of PLX4032 and compound A showed significant tumor growth inhibition in BRAF mutated melanoma (A375) xenograft model.

**Example 24:**

Effect of compound A on BRAF mutated melanoma (A2058) in xenograft model alone and in combination with PLX4032 (vemurafenib)

The procedure followed was similar to the procedure described in Example 15. The melanoma cell line, A2058, resistant to the treatment of PLX4032, was used for the generation of melanoma in the xenograft model. The dose of PLX4032 used was 50 mg/kg and was administered perorally. The dose of Compound A used, alone or in combination was 5 mg/kg and 15 mg/kg. Tumor growth inhibition results are given in figures viz., FIG. 20b, and FIG. 20d.

Conclusion: A combination of PLX4032 and compound A showed significant tumor growth inhibition in BRAF mutated melanoma (A2058) xenograft model.

It should be noted that, as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.
All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this invention pertains.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.
We Claim

1. A pharmaceutical combination comprising a PI3K/mTOR inhibitor selected from the compounds of Formula (I);

   \[ \text{Formula (I)} \]

   \[ R_1 - \text{N} - \text{N} - R_2 \]

   \[ R_3 \]

   \[ R_4 \]

   wherein,

   \( R_1 \) is \((C_6-C_{14})\)aryl or heteroaryl, wherein each of \((C_6-C_{14})\)aryl or heteroaryl is optionally substituted with one or more of \( R^a \);

   \( R_2 \) is \(-\text{CN}; \)

   \( R_3 \) is hydrogen or \((C_1-C_8)\)alkyl, wherein \((C_1-C_8)\)alkyl is optionally substituted with \(-\text{CN}; \)

   \( R_4 \) is \((C_6-C_{14})\)aryl or heteroaryl, wherein each of \((C_6-C_{14})\)aryl or heteroaryl is optionally substituted with one or more of \( R^a \);

   \( R^a \) at each occurrence is halogen, \(-\text{CN}, -\text{OR}_x, -\text{SR}_x, -\text{NR}_xR_y, (C_1-C_8)\)alkyl, \((C_6-C_{14})\)aryl, heterocyclyl or alkylheterocyclyl, wherein \((C_1-C_8)\)alkyl and heterocyclyl is optionally substituted with one or more of \( R^b \);

   \( R_x \) and \( R_y \) at each occurrence are independently hydrogen, \((C_1-C_8)\)alkyl, \((C_6-C_{14})\)aryl or heteroaryl, wherein each of \((C_1-C_8)\)alkyl, \((C_6-C_{14})\)aryl or heteroaryl are optionally substituted with \( R^b \); and

   \( R^b \) at each occurrence is halogen, \(-\text{CN} \) or \(-\text{NH}_2 \) ; or pharmaceutically acceptable salts or solvates thereof; and one or more anti-proliferative agent.

2. The pharmaceutical combination of claim 1, wherein the PI3K/mTOR inhibitor is selected from the compounds of Formula (I) wherein \( R_1 \) is 3-pyridyl optionally substituted with one or more groups selected from: \( \text{Cl, Br, F}, -\text{CN}, -\text{OCH}_3, \text{CH}_3, \text{CF}_3 \) or \(-\text{C(CH}_3)_2\text{CN}. \)

3. The pharmaceutical combination of claim 1 or claim 2, wherein the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein \( R_3 \) is \( \text{CH}_3. \)
4. The pharmaceutical combination of any one of the preceding claims 1 to 3, wherein the PI3K/mTOR inhibitor is selected from the compounds of Formula (I), wherein R₄ is 3-pyridyl optionally substituted with one or more groups selected from -NH₂, -NH-CH₃, -N(CH₃)₂ or -CF₃.

5. The pharmaceutical combination of any one of the preceding claims 1 to 4, wherein the compound of Formula (I) is N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-(2-cyanopropan-2-yl)pyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene)cyanamide (Compound A).

6. The pharmaceutical combination according to any one of the preceding claims 1 to 5, wherein the anti-proliferative agent is selected from: (i) alkylating agents; (ii) DNA intercalating agents; (iii) mitotic inhibitors; (iv) antimetabolites; (v) biguanides; (vi) insulin-like growth factor-1 receptor inhibitors; (vii) tyrosine kinase inhibitors; (viii) epidermal growth factor receptor (EGFR) inhibitors; (ix) topoisomerase inhibitors; (x) BRAF inhibitors or (xi) gamma secretase inhibitors or (xii) Notch/Hedgehog pathway inhibitors.

7. The pharmaceutical combination according to claim 6, wherein the anti-proliferative agent is an alkylating agent selected from: cisplatin, mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, altretamine, thiotepa, busulfan, carmustine, lomustine, dacarbazine, procarbazine or temozolomide.

8. The pharmaceutical combination according to claim 7, wherein the anti-proliferative agent is cisplatin.

9. The pharmaceutical combination according to claim 6, wherein the antiproliferative agent is a DNA intercalating agent selected from doxorubicin, daunorubicin or dactinomycin.

10. The pharmaceutical combination according to claim 9, wherein the anti-proliferative agent is doxorubicin.

11. The pharmaceutical combination according to claim 6, wherein the anti-proliferative agent is a mitotic inhibitor selected from paclitaxel, docetaxel, colchicines, vincristine or vinblastin.

12. The pharmaceutical combination according to claim 11, wherein the anti-proliferative agent is paclitaxel.
13. The pharmaceutical combination according to claim 6, wherein the antiproliferative agent is an antimetabolite selected from 5-Fluorouracil (5-FU), methotrexate, 6-thiopurines, mercaptopurine, thioguanine, cladribine, pentostatin, cytarabine, azactidine, fludarabine, gemcitabine or hydroxyurea.

14. The pharmaceutical combination according to claim 13, wherein the antiproliferative agent is 5-Fluorouracil (5-FU).

15. The pharmaceutical combination according to claim 6, wherein the antiproliferative agent is a biguanide selected from metformin, phenformin or buformin.

16. The pharmaceutical combination according to claim 15, wherein the antiproliferative agent is metformin.

17. The pharmaceutical combination according to claim 6, wherein the antiproliferative agent is a tyrosine kinase inhibitor selected from lapatinib, afatinib, or trastuzumab.

18. The pharmaceutical combination according to claim 17, wherein the antiproliferative agent is lapatinib.

19. The pharmaceutical combination according to claim 6, wherein the antiproliferative agent is an EGFR inhibitor selected from erlotinib, cetuximab, gefitinib or panitumumab.

20. The pharmaceutical combination according to claim 19, wherein the antiproliferative agent is erlotinib.

21. The pharmaceutical combination according to claim 6, wherein the antiproliferative agent is a topoisomerase inhibitor selected from irinotecan, topotecan or 9-aminocamptothecin.

22. The pharmaceutical combination according to claim 21, wherein the antiproliferative agent is irinotecan.

23. The pharmaceutical combination according to claim 6, wherein the antiproliferative agent is BRAF inhibitor selected from sorafenib, vemurafenib, PLX3603, dabrafenib, LGX818, BMS-908662, RAF265 or GW-5074.
24. The pharmaceutical combination according to claim 23, wherein the anti-proliferative agent is vemurafenib or sorafenib.

25. The pharmaceutical combination according to claim 6, wherein the anti-proliferative agent is a notch/hedgehog pathway inhibitor selected from cyclopamine, vismodegib or RO4929097.

26. The pharmaceutical combination according to claim 25, wherein the anti-proliferative agent is cyclopamine.

27. The pharmaceutical combination according to claim 6, wherein the anti-proliferative agent is insulin-like growth factor-1 (IGFR-1) receptor inhibitor is selected from figitumumab, AMG-479, dalotuzumab, PL225B or INSM18.

28. The pharmaceutical combination according to claim 27, wherein the IGFR-1 receptor inhibitor is PL225B.

29. The pharmaceutical combination according to claim 6, wherein the anti-proliferative agent is gamma secretase inhibitor, avagacesta.

30. The pharmaceutical combination according to any one of the preceding claims 1 to 29 for use in the treatment of a proliferative disease or disorder.

31. The pharmaceutical combination for the use according to claim 30, wherein the proliferative disease or disorder is cancer.

32. The pharmaceutical combination for the use according to claim 31, wherein the cancer is selected from breast cancer, prostate cancer, pancreatic cancer, lung cancer, head and neck cancer, ovarian cancer, colorectal cancer, kidney cancer, gastric cancer, non-Hodgkin's lymphoma, primary central nervous system lymphoma, endometrial cancer, brain tumor, melanoma, liver cancer, thyroid cancer, lymphoid cancer, esophageal cancer, cancer of urinary tract, cervical cancer, bladder cancer, mesothelioma, sarcoma or chronic myeloid leukemia.

33. The pharmaceutical combination for the use according to claim 32, wherein the cancer is selected from breast cancer, lung cancer, ovarian cancer, melanoma, liver cancer or colorectal cancer.

34. The pharmaceutical combination for the use according to any one of the claims 29 to 32, wherein the compound of Formula (I) and the anti-proliferative agent
contained in the said pharmaceutical combination are administered simultaneously or sequentially.

35. The pharmaceutical combination for the use according to claim 34, wherein the compound of Formula (I) and the anti-proliferative agent contained in the said pharmaceutical combination are administered simultaneously.

36. The pharmaceutical combination for the use according to claim 34, wherein the compound of Formula (I) and the anti-proliferative agent contained in the said pharmaceutical combination are administered sequentially.

37. A pharmaceutical composition comprising a therapeutically effective amount of a PI3K/mTOR inhibitor selected from the compounds of Formula (I); and a therapeutically effective amount of one or more anti-proliferative agents; in association with one or more pharmaceutically acceptable carrier, diluent or excipient.

38. The pharmaceutical composition according to claim 37 for use in the treatment of a proliferative disease or disorder.

39. The pharmaceutical composition for the use according to claim 38, wherein the proliferative disease or disorder is cancer.

40. The pharmaceutical composition for the use according to claim 39, wherein the cancer is selected from breast cancer, prostate cancer, pancreatic cancer, lung cancer, head and neck cancer, ovarian cancer, colorectal cancer, kidney cancer, gastric cancer, non-Hodgkin's lymphoma, primary central nervous system lymphoma, endometrial cancer, brain tumor, melanoma, liver cancer, thyroid cancer, lymphoid cancer, esophageal cancer, cancer of urinary tract, cervical cancer, bladder cancer, mesothelioma, sarcoma or chronic myeloid leukemia.

41. A method for the treatment of a proliferative disease or a disorder comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor selected from the compounds of Formula (I) or pharmaceutically acceptable salts or solvates thereof; in combination with a therapeutically effective amount of one or more anti-proliferative agents.

42. A method according to claim 41, wherein the PI3K/mTOR inhibitor is selected from the compounds of Formula (I) as defined in any one of the claims 1 to 5.
43. A method according to claim 41, wherein the anti-proliferative agent is selected from: (i) alkylating agents; (ii) DNA intercalating agents; (iii) mitotic inhibitors; (iv) antimetabolites; (v) biguanides; (vi) insulin-like growth factor-1 receptor inhibitors; (vii) tyrosine kinase inhibitors; (viii) epidermal growth factor receptor (EGFR) inhibitors; (ix) topoisomerase inhibitors; (x) BRAF inhibitors or (xi) gamma secretase inhibitors or (xii) Notch/Hedgehog pathway inhibitors.

44. A method according to claim 43, wherein the anti-proliferative agent is an alkylating agent selected from: cisplatin, mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, altretamine, thiotepa, busulfan, carmustine, lomustine, dacarbazine, procarbazine or temozolomide.

45. A method according to claim 44, wherein the anti-proliferative agent is cisplatin.

46. A method according to claim 43, wherein the antiproliferative agent is a DNA intercalating agent selected from doxorubicin, daunorubicin or dactinomycin.

47. A method according to claim 46, wherein the anti-proliferative agent is doxorubicin.

48. A method according to claim 43, wherein the anti-proliferative agent is a mitotic inhibitor selected from paclitaxel, docetaxel, colchicines, vincristine or vinblastin.

49. A method according to claim 48, wherein the anti-proliferative agent is paclitaxel.

50. A method according to claim 43, wherein the anti-proliferative agent is an antimetabolite selected from 5-Fluorouracil (5-FU), methotrexate, 6-thiopurines, mercaptopurine, thioguanine, cladribine, pentostatin, cytarabine, azactidine, fludarabine, gemcitabine or hydroxyurea.

51. A method according to claim 50, wherein the anti-proliferative agent is 5-Fluorouracil(5-FU).

52. A method according to claim 43, wherein the antiproliferative agent is a biguanide selected from metformin, phenformin or buformin.

53. A method according to claim 52, wherein the anti-proliferative agent is metformin.
54. A method according to claim 42, wherein the antiproliferative agent is a tyrosine kinase inhibitor selected from lapatinib, afatinib, or tratuzumab.
55. A method according to claim 53, wherein the anti-proliferative agent is Lapatinib.
56. A method according to claim 43, wherein the anti-proliferative agent is an EGFR inhibitor selected from erlotinib, cetuximab, gefitinib or panitumumab.
57. A method according to claim 56, wherein the anti-proliferative agent is erlotinib.
58. A method according to claim 43, wherein the anti-proliferative agent is a topoisomerase inhibitor selected from irinotecan, topotecan or 9-aminocamptothecin.
59. A method according to claim 58, wherein the anti-proliferative agent is irinotecan.
60. A method according to claim 43, wherein the anti-proliferative agent is BRAF inhibitor selected from sorafenib, vemurafenib, PLX3603, dabrafenib, LGX818, BMS-908662, RAF265 or GW-5074.
61. A method according to claim 60, wherein the anti-proliferative agent is vemurafenib or sorafenib.
62. A method according to claim 43, wherein the anti-proliferative agent is a notch/hedgehog pathway inhibitor selected from cyclopamine, vismodegib or RO4929097.
63. A method according to claim 62, wherein the anti-proliferative agent is cyclopamine.
64. A method according to claim 43, wherein the anti-proliferative agent is insulin-like growth factor-1 (IGFR-1) receptor inhibitor is selected from figitumumab, AMG-479, dalotuzumab, PL225B or INSM18.
65. A method according to claim 64, wherein the anti-proliferative agent is PL225B.
66. A method according to claim 43, wherein the anti-proliferative agent is gamma secretase inhibitor, avagacesta.
67. A method according to any one of the preceding claims 42 to 66, wherein the cancer is selected from breast cancer, prostate cancer, pancreatic cancer, lung cancer, head and neck cancer, ovarian cancer, colorectal cancer, kidney cancer, gastric cancer, non-Hodgkin's lymphoma, primary central nervous system lymphoma, endometrial cancer, brain tumor, melanoma, liver cancer, thyroid cancer, lymphoid cancer, esophageal cancer, cancer of urinary tract, cervical cancer, bladder cancer, mesothelioma, sarcoma or chronic myeloid leukemia.

68. A method according to any one of the preceding claims 42 to 67, wherein the compound of Formula (I) and the anti-proliferative agent are administered simultaneously.

69. A method according to any one of the preceding claims 42 to 65, wherein the compound of Formula (I) and the anti-proliferative agent contained in the said pharmaceutical combination are administered sequentially.
Combination of Metformin and Compound A in MDAMB453 cell line - Sequential

![Graph showing cytotoxicity and combination index for different drug concentrations.]

FIG. 1aa

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
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<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>Metformin 3 mM</td>
</tr>
<tr>
<td>D5</td>
<td>Metformin 3 mM + Compound A 1 nM</td>
</tr>
<tr>
<td>D6</td>
<td>Metformin 3 mM + Compound A 3 nM</td>
</tr>
<tr>
<td>D7</td>
<td>Metformin 3 mM + Compound A 10 nM</td>
</tr>
</tbody>
</table>

Combination of Metformin and Compound A in MDAMB231 cell line - Sequential

![Graph showing cytotoxicity and combination index for different drug concentrations.]

FIG. 1ab

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
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<tr>
<td>D1</td>
<td>Metformin 10 mM</td>
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<tr>
<td>D2</td>
<td>Metformin 30 mM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>Compound A 30 nM</td>
</tr>
<tr>
<td>D5</td>
<td>Metformin 10 mM + Compound A 10 nM</td>
</tr>
</tbody>
</table>
D6  Metformin 10 mM + Compound A 30 nM
D7  Metformin 30 mM + Compound A 30 nM

Combination of Metformin and Compound A in BT549 cell line - Sequential

% Cytotoxicity

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
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<tbody>
<tr>
<td>D1</td>
<td>Metformin 3 mM</td>
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<td>D2</td>
<td>Metformin 10 mM</td>
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<tr>
<td>D3</td>
<td>Metformin 30 mM</td>
</tr>
<tr>
<td>D4</td>
<td>Compound A 10 nM</td>
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<tr>
<td>D5</td>
<td>Compound A 30 nM</td>
</tr>
<tr>
<td>D6</td>
<td>Compound A 100 nM</td>
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<tr>
<td>D7</td>
<td>Metformin 3 mM + Compound A 10 nM</td>
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<tr>
<td>D8</td>
<td>Metformin 30 mM + Compound A 10 nM</td>
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<tr>
<td>D9</td>
<td>Metformin 30 mM + Compound A 30 nM</td>
</tr>
<tr>
<td>D10</td>
<td>Metformin 30 mM + Compound A 100 nM</td>
</tr>
</tbody>
</table>

Combination of Metformin and Compound A in BT549 cell line - Simultaneous

% Cytotoxicity

Diagram showing the combination index for different drug concentrations.

FIG. 1ac

FIG. 1ba
Abbreviation | Drug Concentration
--- | ---
D1 | Metformin 1 mM
D2 | Metformin 3 mM
D3 | Metformin 10 mM
D4 | Compound A 1 nM
D5 | Compound A 3 nM
D6 | Compound A 10 nM
D7 | Metformin 1 mM + Compound A 1 nM
D8 | Metformin 3 mM + Compound A 1 nM
D9 | Metformin 1 mM + Compound A 3 nM
D10 | Metformin 3 mM + Compound A 3 nM
D11 | Metformin 10 mM + Compound A 3 nM
D12 | Metformin 1 mM + Compound A 10 nM
D13 | Metformin 3 mM + Compound A 10 nM
D14 | Metformin 10 mM + Compound A 10 nM

**Combination of Metformin and Compound A in MDAMB231 cell line - Simultaneous**

![Graph showing cytotoxicity and combination index](image)

**FIG. 1bb**

Abbreviation | Drug Concentration
--- | ---
D1 | Metformin 1 mM
D2 | Metformin 3 mM
D3 | Metformin 10 mM
D4 | Compound A 1 nM
D5 | Compound A 3 nM
D6 | Metformin 1 mM + Compound A 1 nM
D7 | Metformin 3 mM + Compound A 1 nM
D8 | Metformin 10 mM + Compound A 1 nM
D9 | Metformin 1 mM + Compound A 3 nM
D10 | Metformin 3 mM + Compound A 3 nM
D11 | Metformin 10 mM + Compound A 3 nM
Combination of Lapatinib and Compound A in HCC 1569 cell line - Simultaneous

![Graph showing % Cytotoxicity and Combination Index for different drug concentrations.]

**FIG. 2a**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
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<tbody>
<tr>
<td>D1</td>
<td>Compound A 10 nM</td>
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<tr>
<td>D2</td>
<td>Compound A 30 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Lapatinib 0.1 μM</td>
</tr>
<tr>
<td>D4</td>
<td>Lapatinib 1 μM</td>
</tr>
<tr>
<td>D5</td>
<td>Lapatinib 3 μM</td>
</tr>
<tr>
<td>D6</td>
<td>Lapatinib 10 μM</td>
</tr>
<tr>
<td>D7</td>
<td>Lapatinib 0.1 μM + Compound A 30 nM</td>
</tr>
<tr>
<td>D8</td>
<td>Lapatinib 1 μM + Compound A 10 nM</td>
</tr>
<tr>
<td>D9</td>
<td>Lapatinib 1 μM + Compound A 30 nM</td>
</tr>
<tr>
<td>D10</td>
<td>Lapatinib 3 μM + Compound A 30 nM</td>
</tr>
<tr>
<td>D11</td>
<td>Lapatinib 10 μM + Compound A 30 nM</td>
</tr>
</tbody>
</table>

Combination of Compound A and Lapatinib in HCC 1954 cell line - Simultaneous

![Graph showing % Cytotoxicity and Combination Index for different drug concentrations.]

**FIG. 2b**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 3 nM</td>
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<tr>
<td>D2</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 30 nM</td>
</tr>
<tr>
<td>D4</td>
<td>Lapatinib 0.1 μM</td>
</tr>
<tr>
<td></td>
<td>Drug Concentration</td>
</tr>
<tr>
<td>----</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>D5</td>
<td>Lapatinib 1 μM</td>
</tr>
<tr>
<td>D6</td>
<td>Lapatinib 3 μM</td>
</tr>
<tr>
<td>D7</td>
<td>Lapatinib 10 μM</td>
</tr>
<tr>
<td>D8</td>
<td>Lapatinib 0.1 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D9</td>
<td>Lapatinib 0.1 μM + Compound A 10 nM</td>
</tr>
<tr>
<td>D10</td>
<td>Lapatinib 0.1 μM + Compound A 30 nM</td>
</tr>
<tr>
<td>D11</td>
<td>Lapatinib 1 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D12</td>
<td>Lapatinib 1 μM + Compound A 10 nM</td>
</tr>
<tr>
<td>D13</td>
<td>Lapatinib 1 μM + Compound A 30 nM</td>
</tr>
<tr>
<td>D14</td>
<td>Lapatinib 3 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D15</td>
<td>Lapatinib 3 μM + Compound A 10 nM</td>
</tr>
<tr>
<td>D16</td>
<td>Lapatinib 3 μM + Compound A 30 nM</td>
</tr>
</tbody>
</table>

**Combination of Compound A and Lapatinib in SKBR3 cell line. Simultaneous**

![Graph](image)

**FIG. 2c**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>Lapatinib 0.01 μM</td>
</tr>
<tr>
<td>D5</td>
<td>Lapatinib 0.03 μM</td>
</tr>
<tr>
<td>D6</td>
<td>Lapatinib 0.1 μM</td>
</tr>
<tr>
<td>D7</td>
<td>Lapatinib 0.01 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D8</td>
<td>Lapatinib 0.03 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D9</td>
<td>Lapatinib 0.03 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D10</td>
<td>Lapatinib 0.03 μM + Compound A 10 nM</td>
</tr>
<tr>
<td>D11</td>
<td>Lapatinib 0.1 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D12</td>
<td>Lapatinib 0.1 μM + Compound A 10 nM</td>
</tr>
</tbody>
</table>
Combination of Compound A and Cisplatin in MDAMB231 Cell - Simultaneously

![Graph showing % Cytotoxicity and Combination Index for different drug concentrations.]

**FIG. 3a**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>Cisplatin 10 μM</td>
</tr>
<tr>
<td>D5</td>
<td>Cisplatin 30 μM</td>
</tr>
<tr>
<td>D6</td>
<td>Cisplatin 10 μM + Compound A 10 nM</td>
</tr>
<tr>
<td>D7</td>
<td>Cisplatin 30 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D8</td>
<td>Cisplatin 30 μM + Compound A 10 nM</td>
</tr>
</tbody>
</table>

Combination of Compound A and Cisplatin in BT549 cell line- Simultaneous

![Graph showing % Cytotoxicity and Combination Index for different drug concentrations.]

**FIG. 3b**
### Abbreviation | Drug Concentration
---|---
D1 | Compound A 1 nM
D2 | Compound A 3 nM
D3 | Compound A 10 nM
D4 | Cisplatin 1 μM
D5 | Cisplatin 3 μM
D6 | Cisplatin 1 μM + Compound A 1 nM
D7 | Cisplatin 1 μM + Compound A 3 nM
D8 | Cisplatin 1 μM + Compound A 10 nM
D9 | Cisplatin 3 μM + Compound A 1 nM
D10 | Cisplatin 3 μM + Compound A 3 nM
D11 | Cisplatin 3 μM + Compound A 10 nM

**Combination of Compound A and Cisplatin in MDAMB453 Cell line: Simultaneous**

**FIG. 3c**

### Abbreviation | Drug Concentration
---|---
D1 | Compound A 1 nM
D2 | Compound A 3 nM
D3 | Compound A 10 nM
D4 | Cisplatin 10 μM
D5 | Cisplatin 30 μM
D6 | Cisplatin 10 μM + Compound A 3 nM
D7 | Cisplatin 10 μM + Compound A 10 nM
D8 | Cisplatin 30 μM + Compound A 1 nM
D9 | Cisplatin 30 μM + Compound A 3 nM
D10 | Cisplatin 30 μM + Compound A 10 nM
Combination of Compound A and Cisplatin in MDAMB468 Cell line- Simultaneous

% Cytotoxicity

Combination Index

% Cytotoxicity

Combination Index

D1  D2  D3  D4  D5  D6  D7

Abbreviation | Drug Concentration
---|---
D1 | Compound A 1 nM
D2 | Compound A 3 nM
D3 | Compound A 10 nM
D4 | Cisplatin 0.1 μM
D5 | Cisplatin 0.1 μM + Compound A 1 nM
D6 | Cisplatin 0.1 μM + Compound A 3 nM
D7 | Cisplatin 0.1 μM + Compound A 10 nM

Combination of Compound A and Doxorubicin in BT549 cell line-Simultaneous

% Cytotoxicity

Combination Index

% Cytotoxicity

Combination Index

D1  D2  D3  D4  D5  D6  D7  D8  D9  D10  D11

Abbreviation | Drug Concentration
---|---
D1 | Compound A 1 nM
D2 | Compound A 3 nM
D3 | Compound A 10 nM
D4 | Doxorubicin 0.1 μM
D5 | Doxorubicin 0.3 μM
D6 | Doxorubicin 0.1 μM + Compound A 1 nM
D7 | Doxorubicin 0.1 μM + Compound A 3 nM
D8 | Doxorubicin 0.1 μM + Compound A 10 nM
D9 | Doxorubicin 0.3 μM + Compound A 1 nM
D10 | Doxorubicin 0.3 μM + Compound A 3 nM
Combination of Compound A and Doxorubicin in MDAMB468 cell line-Simultaneous

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Doxorubicin 0.1 µM</td>
</tr>
<tr>
<td>D4</td>
<td>Doxorubicin 0.3 µM</td>
</tr>
<tr>
<td>D5</td>
<td>Doxorubicin 0.1 µM + Compound A 1 nM</td>
</tr>
<tr>
<td>D6</td>
<td>Doxorubicin 0.1 µM + Compound A 3 nM</td>
</tr>
<tr>
<td>D7</td>
<td>Doxorubicin 0.3 µM + Compound A 1 nM</td>
</tr>
<tr>
<td>D8</td>
<td>Doxorubicin 0.3 µM + Compound A 3 nM</td>
</tr>
</tbody>
</table>

Combination of Compound A with Doxorubicin in MDAMB453 cell line- Simultaneous

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>Doxorubicin 0.3 µM</td>
</tr>
<tr>
<td>D5</td>
<td>Doxorubicin 0.3 µM + Compound A 1 nM</td>
</tr>
<tr>
<td>D6</td>
<td>Doxorubicin 0.3 µM + Compound A 3 nM</td>
</tr>
<tr>
<td>D7</td>
<td>Doxorubicin 0.3 µM + Compound A 10 nM</td>
</tr>
</tbody>
</table>
Combination of Compound A and Paclitaxel in BT459 cell line - Simultaneous

![Graph 1]

Abbreviation | Drug Concentration
--- | ---
D1 | Compound A 1 nM
D2 | Compound A 3 nM
D3 | Compound A 10 nM
D4 | Paclitaxel 3 nM
D5 | Paclitaxel 3 nM + Compound A 1 nM
D6 | Paclitaxel 3 nM + Compound A 3 nM
D7 | Paclitaxel 3 nM + Compound A 10 nM

Combination of Compound A and Paclitaxel in MDA MB453 cell line - Simultaneous

![Graph 2]

Abbreviation | Drug Concentration
--- | ---
D1 | Compound A 1 nM
D2 | Compound A 3 nM
D3 | Compound A 10 nM
D4  Paclitaxel 3 nM  
D5  Paclitaxel 3 nM + Compound A 1 nM  
D6  Paclitaxel 3 nM + Compound A 3 nM  
D7  Paclitaxel 3 nM + Compound A 10 nM  

Combination of Compound A and Paclitaxel in MDAMB468 cell line-Simultaneous

Abbreviation  Drug Concentration  
D1  Compound A 1 nM  
D2  Compound A 3 nM  
D3  Compound A 10 nM  
D4  Paclitaxel 1 nM  
D5  Paclitaxel 1.5 nM  
D6  Paclitaxel 1 nM + Compound A 1 nM  
D7  Paclitaxel 1 nM + Compound A 3 nM  
D8  Paclitaxel 1 nM + Compound A 10 nM  
D9  Paclitaxel 1.5 nM + Compound A 1 nM  
D10  Paclitaxel 1.5 nM + Compound A 3 nM  
D11  Paclitaxel 1.5 nM + Compound A 10 nM  

Combination of Compound A and 5-FU in BT549 cell line- Simultaneous

FIG. 5c

FIG. 6a
Combination of Compound A and 5-FU in MDA MB453 cell line-Simultaneous

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
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</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>5-FU 10 μM</td>
</tr>
<tr>
<td>D5</td>
<td>5-FU 10 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D6</td>
<td>5-FU 10 μM + Compound A 3 nM</td>
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<tr>
<td>D7</td>
<td>5-FU 10 μM + Compound A 10 nM</td>
</tr>
</tbody>
</table>

FIG. 6b

<table>
<thead>
<tr>
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<th>Drug Concentration</th>
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</thead>
<tbody>
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</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>5-FU 10 μM</td>
</tr>
<tr>
<td>D5</td>
<td>5-FU 10 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D6</td>
<td>5-FU 10 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D7</td>
<td>5-FU 10 μM + Compound A 10 nM</td>
</tr>
</tbody>
</table>
Combination of Compound A and 5-FU in MDAMB468 cell line-Simultaneous

![Graph showing % cytotoxicity and combination index for different drug concentrations.]

**FIG. 6c**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>5-FU 10 μM</td>
</tr>
<tr>
<td>D5</td>
<td>5-FU 10 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D6</td>
<td>5-FU 10 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D7</td>
<td>5-FU 10 μM + Compound A 10 nM</td>
</tr>
</tbody>
</table>

Combination of PL225B and Compound A in HCC1959 cell line - Simultaneous

![Graph showing % cytotoxicity and combination index for different drug concentrations.]

**FIG. 7**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
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</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>PL225B 1μM</td>
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</table>
Combination of erlotinib with Compound A in A549 cell lines

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
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</thead>
<tbody>
<tr>
<td>D1</td>
<td>Erlotinib 2.5 μM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 5 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Erlotinib 2.5 μM + Compound A 5 nM</td>
</tr>
<tr>
<td>D4</td>
<td>Erlotinib 2.5 μM</td>
</tr>
<tr>
<td>D5</td>
<td>Compound A 5 nM</td>
</tr>
<tr>
<td>D6</td>
<td>Erlotinib 2.5 μM + Compound A 5 nM</td>
</tr>
</tbody>
</table>
Combination of Erlotinib with Compound A in H358 cell lines

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
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</thead>
<tbody>
<tr>
<td>D1</td>
<td>Erlotinib 2.5 µM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Erlotinib 2.5 µM + Compound A 3 nM</td>
</tr>
<tr>
<td>D4</td>
<td>Erlotinib 5 µM</td>
</tr>
<tr>
<td>D5</td>
<td>Compound A 6 nM</td>
</tr>
<tr>
<td>D6</td>
<td>Erlotinib 5 µM + Compound A 6 nM</td>
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</tbody>
</table>

Combination of erlotinib with Compound A in H1975 cell lines

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<th>Abbreviation</th>
<th>Drug Concentration</th>
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</thead>
<tbody>
<tr>
<td>D1</td>
<td>Erlotinib 2.5 µM</td>
</tr>
</tbody>
</table>
Combination of Compound A with erlotinib in H1650 cell line

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
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<tbody>
<tr>
<td>D1</td>
<td>Erlotinib 2.5 μM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 5 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Erlotinib 2.5 μM + Compound A 5 nM</td>
</tr>
<tr>
<td>D4</td>
<td>Erlotinib 2.5 μM</td>
</tr>
<tr>
<td>D5</td>
<td>Compound A 5 nM</td>
</tr>
<tr>
<td>D6</td>
<td>Erlotinib 2.5 μM + Compound A 5 nM</td>
</tr>
</tbody>
</table>
Combination of Compound A with PLX4032 in A375 cell lines - Sequential

Abbreviation | Drug Concentration
---|---
D1 | PLX4032 0.1 μM
D2 | PLX4032 0.3 μM
D3 | PLX4032 1 μM
D4 | Compound A 0.01 μM
D5 | Compound A 0.03 μM
D6 | Compound A 0.1 μM
D7 | PLX4032 0.1 μM + Compound A 0.01 μM
D8 | PLX4032 0.1 μM + Compound A 0.03 μM
D9 | PLX4032 0.1 μM + Compound A 0.1 μM

Combination of Compound A with PLX4032 in G361 cell line - Sequential
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>PLX4032 0.1 μM</td>
</tr>
<tr>
<td>D2</td>
<td>PLX4032 0.3 μM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 0.003 μM</td>
</tr>
<tr>
<td>D4</td>
<td>Compound A 0.01 μM</td>
</tr>
<tr>
<td>D5</td>
<td>Compound A 0.03 μM</td>
</tr>
<tr>
<td>D6</td>
<td>PLX4032 0.3 μM + Compound A 0.003 μM</td>
</tr>
<tr>
<td>D7</td>
<td>PLX4032 0.3 μM + Compound A 0.01 μM</td>
</tr>
<tr>
<td>D8</td>
<td>PLX4032 0.3 μM + Compound A 0.03 μM</td>
</tr>
</tbody>
</table>

Combination of Compound A with PLX4032 in SKMEL-3 cell line - Sequential

% Cytotoxicity

Combination Index

Abbreviation | Drug Concentration |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>PLX4032 0.1 μM</td>
</tr>
<tr>
<td>D2</td>
<td>PLX4032 0.3 μM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 0.003 μM</td>
</tr>
<tr>
<td>D4</td>
<td>Compound A 0.01 μM</td>
</tr>
<tr>
<td>D5</td>
<td>Compound A 0.03 μM</td>
</tr>
<tr>
<td>D6</td>
<td>PLX4032 0.1 μM + Compound A 0.003 μM</td>
</tr>
<tr>
<td>D7</td>
<td>PLX4032 0.1 μM + Compound A 0.01 μM</td>
</tr>
<tr>
<td>D8</td>
<td>PLX4032 0.1 μM + Compound A 0.03 μM</td>
</tr>
</tbody>
</table>
### Abbreviation and Drug Concentration

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>PLX4032 0.1 μM</td>
</tr>
<tr>
<td>D4</td>
<td>PLX4032 1 μM</td>
</tr>
<tr>
<td>D5</td>
<td>PLX4032 3 μM</td>
</tr>
<tr>
<td>D6</td>
<td>PLX4032 10 μM</td>
</tr>
<tr>
<td>D7</td>
<td>PLX4032 0.1 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D8</td>
<td>PLX4032 1 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D9</td>
<td>PLX4032 3 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D10</td>
<td>PLX4032 10 μM + Compound A 1 nM</td>
</tr>
<tr>
<td>D11</td>
<td>PLX4032 0.1 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D12</td>
<td>PLX4032 1 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D13</td>
<td>PLX4032 3 μM + Compound A 3 nM</td>
</tr>
<tr>
<td>D14</td>
<td>PLX4032 10 μM + Compound A 3 nM</td>
</tr>
</tbody>
</table>
Combination of Compound A with sorafenib in H460 cell lines

![Graph showing % Cytotoxicity and Combination Index for different drug concentrations.]

**FIG. 10a**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Sorafenib IC30</td>
</tr>
<tr>
<td>D2</td>
<td>Sorafenib IC50</td>
</tr>
<tr>
<td>D3</td>
<td>Sorafenib IC70</td>
</tr>
<tr>
<td>D4</td>
<td>Compound A IC30</td>
</tr>
<tr>
<td>D5</td>
<td>Compound A IC50</td>
</tr>
<tr>
<td>D6</td>
<td>Compound A IC70</td>
</tr>
<tr>
<td>D7</td>
<td>Sorafenib IC30 + Compound A IC30</td>
</tr>
<tr>
<td>D8</td>
<td>Sorafenib IC30 + Compound A IC50</td>
</tr>
<tr>
<td>D9</td>
<td>Sorafenib IC30 + Compound A IC70</td>
</tr>
</tbody>
</table>

Combination of Compound A with sorafenib in A549 cell line

![Graph showing % Cytotoxicity and Combination index for different drug concentrations.]

**FIG. 10b**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Sorafenib IC30</td>
</tr>
<tr>
<td>D2</td>
<td>Sorafenib IC50</td>
</tr>
</tbody>
</table>
Combination of Compound A with BMS708163 and Cyclopaamine in MDA MB231 cell line

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Compound A 1 nM</td>
</tr>
<tr>
<td>D2</td>
<td>Compound A 3 nM</td>
</tr>
<tr>
<td>D3</td>
<td>Compound A 10 nM</td>
</tr>
<tr>
<td>D4</td>
<td>BMS708163 3 μM</td>
</tr>
<tr>
<td>D5</td>
<td>Cyclopaamine 3 μM</td>
</tr>
<tr>
<td>D6</td>
<td>BMS708163 3 μM + Cyclopaamine 3 μM</td>
</tr>
<tr>
<td>D7</td>
<td>Compound A 1 nM + BMS708163 3 μM + Cyclopaamine 3 μM</td>
</tr>
<tr>
<td>D8</td>
<td>Compound A 3 nM + BMS708163 3 μM + Cyclopaamine 3 μM</td>
</tr>
<tr>
<td>D9</td>
<td>Compound A 10 nM + BMS708163 3 μM + Cyclopaamine 3 μM</td>
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</tbody>
</table>
Combination of Compound A with BMS708163 and cyclopa mine in MDA MB453 cell line

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Drug Concentration</th>
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<tbody>
<tr>
<td>D1</td>
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Combination of Compound A with BMS708163 and cyclopa mine in MDA MB468 cell line

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Combination of Compound A with BMS708163 and cycloamine in BT 649 cell line

![Graph showing cytotoxicity and combination index for different drug concentrations.]

FIG. 11d

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Combination of Compound A with BMS708163 and cycloamine in HCC1937 cell line

**Figure 11e**

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Combination of Everolimus and Compound A in MCF7 cell line

Abbreviation | Drug Concentration
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D1 | Compound A 3 nM
D2 | Everolimus 0.001 μM
D3 | Everolimus 0.01 μM
D4 | Everolimus 0.1 μM
D5 | Everolimus 1 μM
D6 | Compound A 3 nM + Everolimus 0.001 μM
D7 | Compound A 3 nM + Everolimus 0.01 μM
D8 | Compound A 3 nM + Everolimus 0.1 μM
D9 | Compound A 3 nM + Everolimus 1 μM

Combination of Everolimus and Compound A in Panc-1 cells

FIG. 12a
FIG. 12b
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**FIG. 13**

- **Vehicle control**
- **Metformin- 200 mg/kg, po**
- **Compound A-6 mg/kg, po**
- **Combination of Compound A (5mg/kg) and Metformin (200 mg/kg)**

Tumor Volume (mm$^3$) vs. Treatment Days
**FIG. 17b**

- **Vehicle Control**
- **Compound A: 15 mg/kg, po, QD, Day 1 to 13**
- **Irinotecan: 50 mg/kg, iv, Day 1 (single dose)**
- **Combination of Compound A (15 mg/kg), Day 1-Day 3, Day 10-Day 12 and Irinotecan, iv, D1, single dose**

**FIG. 18a**

- **Vehicle control**
- **Compound A, po**
- **PL225B - 100 mg/kg, po**
- **Combination of Compound A (6 mg/kg) and PL225B (100 mg/kg)**

Tumor Volume [mm³]

Treatment Days
**Fig. 18b**

- Vehicle control
- PL225B - 100 mg/kg, po
- Compound A - 5 mg/kg, po
- Combination of Compound A (5 mg/kg) and PL225B (100 mg/kg), po
- Compound A - 15 mg/kg, po

**Fig. 19a**

- Vehicle Control 1 (CMC+ Tween 80)
- Vehicle Control 2 (cremophore EL+ ethanol)
- Compound A 10 mg/kg, po
- Sorafenib - 30 mg/kg, po
- Combination of Compound A (10 mg/kg) and sorafenib (30 mg/kg)
INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/053433

A. CLASSIFICATION OF SUBJECT MATTER

<table>
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<td>Y the whole document page 4, line 22 - line 26 page 21 - page 30 claim 1 page 43, line 14 - line 15</td>
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Further documents are listed in the continuation of Box C.

See patent family annex.

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

Date of the actual completion of the international search 17 March 2014

Date of mailing of the international search report G3/04/2014

Name and mailing address of the ISA/European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer Langer, Oliver
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Form PCT/I/SA/210 (continuation of second sheet) (April 2008)
## INTERNATIONAL SEARCH REPORT

**Observations where certain claims were found unsearchable**

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

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

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

### 3. 
- **Claims Nos.:**
- because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Observations where unity of invention is lacking**

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

- see additional sheet

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

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

### 3. 
- As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  - 7, 8, 19, 20, 23, 24, 27, 28, 44, 45, 56, 57, 6θ, 61, 64
  - 65 (completely); 1-6, 30-43, 67-69 (partially)

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

**Remark on Protest**

- The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

Form PCT/ISA/21 0 (continuation of first sheet (2)) (April 2005)
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 7, 8, 44, 45 (completely); 1-6, 30-43, 67-69 (partially)
   
   Pharmaceutical combinations and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-proliferative agents, wherein the anti-proliferative agent is an alkylating agent, in particular according to claims 7, 8, 44 and 45.

---

2. claims: 9, 10, 46, 47 (completely); 1-6, 30-43, 67-69 (partially)

   Pharmaceutical combinations and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-proliferative agents, wherein the anti-proliferative agent is a DNA intercalating agent, in particular according to claims 9, 10, 46 and 47.

---

3. claims: 11, 12, 48, 49 (completely); 1-6, 30-43, 67-69 (partially)

   Pharmaceutical combinations and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-proliferative agents, wherein the anti-proliferative agent is a mitotic inhibitor, in particular according to claims 11, 12, 48 and 49.

---

4. claims: 13, 14, 50, 51 (completely); 1-6, 30-43, 67-69 (partially)

   Pharmaceutical combinations and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-proliferative agents, wherein the anti-proliferative agent is an antimetabolite, in particular according to claims 13, 14, 50 and 51.

---

5. claims: 15, 16, 52, 53 (completely); 1-6, 30-43, 67-69 (partially)

   Pharmaceutical combinations and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-proliferative agents, wherein the anti-proliferative agent is a biguanide, in particular according to claims 15, 16, 52 and 53.

---

6. claims: 27, 28, 64, 65 (completely); 1-6, 30-43, 67-69 (partially)
Pharmaceutical compositions and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-PI3K and/or mTOR agents, wherein the anti-PI3K and/or mTOR agent is an insulin-like growth factor-1 receptor inhibitor, in particular one according to claims 27, 28, 64 and 65.

---

7. claims: 17, 18, 54, 55 (completely) ; 1-6, 30-43, 67-69 (partially)

Pharmaceutical compositions and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-PI3K and/or mTOR agents, wherein the anti-PI3K and/or mTOR agent is a tyrosine kinase inhibitor, in particular one according to claims 17, 18, 54 and 55.

---

8. claims: 19, 20, 56, 57 (completely) ; 1-6, 30-43, 67-69 (partially)

Pharmaceutical compositions and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-PI3K and/or mTOR agents, wherein the anti-PI3K and/or mTOR agent is an epidermal growth factor receptor (EGFR) inhibitor, in particular one according to claims 19, 20, 56 and 57.

---

9. claims: 21, 22, 58, 59 (completely) ; 1-6, 30-43, 67-69 (partially)

Pharmaceutical compositions and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-PI3K and/or mTOR agents, wherein the anti-PI3K and/or mTOR agent is a topoisomerase inhibitor, in particular one according to claims 21, 22, 58 and 59.

---

10. claims: 23, 24, 60, 61 (completely) ; 1-6, 30-43, 67-69 (partially)

Pharmaceutical compositions and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-PI3K and/or mTOR agents, wherein the anti-PI3K and/or mTOR agent is a BRAF inhibitor, in particular one according to claims 23, 24, 60 and 61.

---

11. claims: 29, 66 (completely) ; 1-6, 30-43, 67-69 (partially)

Pharmaceutical compositions and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more
anti-proliferative agents, wherein the anti-proliferative agent is a gamma secretase inhibitor, in particular one according to claims 29 and 66.

---

12. claims: 25, 26, 62, 63 (completely); 1-6, 30-43, 67-69 (partially)

Pharmaceutical combinations, compositions and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-proliferative agents, wherein the anti-proliferative agent is a Notch/Hedgehog pathway inhibitor, in particular according to claims 25, 26, 62 and 63.

---

13. claims: 1-5, 30-43, 67-69 (partially)

Pharmaceutical combinations, compositions and methods involving a PI3K/mTOR inhibitor according to the general formula (I) of claim 1 in combination with one or more anti-proliferative agents, as far as not covered by inventions 1 to 12.

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