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(54) Title: COMBINATION THERAPY FOR THE TREATMENT OF RESISTANT BREAST CANCER

(57) **Abstract:** The present invention relates to a pharmaceutical combination comprising a PI3K/m TOR inhibitor of Formula (I) (as described herein) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agent selected from an estrogen antagonist and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof. The present invention also relates to the use of the said combination in the treatment of anti-estrogen resistant endocrine receptor positive (ER+) breast cancer. The invention also relates to a method of treating anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/m TOR inhibitor of Formula (I) (as described herein) or a pharmaceutically acceptable salt thereof and a therapeutically effective amount of an anti-cancer agent selected from an estrogen antagonist, and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof; or a mixture thereof.



COMBINATION THERAPY FOR THE TREATMENT OF RESISTANT BREAST CANCER

FIELD OF THE INVENTION

The present invention relates to a pharmaceutical combination comprising a PI3K/mTOR inhibitor of Formula (I) (as described herein) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agent selected from an estrogen antagonist and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof. The present invention also relates to the use of the said combination in the treatment of anti-estrogen resistant endocrine receptor positive (ER+) breast cancer. The invention also relates to a method of treating anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor of Formula (I) (as described herein) or a pharmaceutically acceptable salt thereof and a therapeutically effective amount of an anti-cancer agent selected from an estrogen antagonist, and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof; or a mixture thereof.

BACKGROUND OF THE INVENTION

Breast cancer; based on the immunohistochemistry (IHC) expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2) can be classified into four groups; namely ER/PR+, Her2+ or triple positive; ER/PR+, Her2- or endocrine receptor positive; ER/PR-, Her2+ or Her2 positive; and ER/PR-, Her2- or triple negative.

Breast cancer is the most common type of cancer essentially affecting female population and endocrine receptor positive (ER+) breast cancer is the most common subtype. The steroid hormones, particularly estrogen is responsible for the growth and development of this subtype of breast cancer. The estrogen circulating in the bloodstream binds to the estrogen receptors (ER) residing inside the cells of the target tissues such as breast and uterus, thereby stimulating cell division and tumor growth of the target tissue.

About 75% of breast cancers are ER positive and hence, patients with this cancer likely benefit from estrogen antagonist therapy, also referred to as anti-

estrogen therapy. However, it is estimated that approximately 50% of these patients will fail anti-estrogen therapy due to either *de novo* or acquired resistance (Mol. Endocrinol., 2012, 26(3), 360-71).

The anti-estrogen therapy is primarily based on targeting ER signaling pathway and acts by: (i) reducing estrogen levels; (ii) blocking estrogen production; (iii) blocking estrogen receptors with anti-estrogens; or (iv) downregulating estrogen receptors.

Gonadotropin-releasing hormone (GnRH) agonists, such as goserelin act by reducing estrogen levels. GnRH agonists downregulate pituitary GnRH receptors, thereby suppressing the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), thus reducing estrogen production.

In postmenopausal women, the conversion of androgens to estrogens is mediated by the enzyme aromatase in normal tissues (adipose tissue, muscle, liver, or brain) as well as in breast tumors. Aromatase inhibitors such as exemestane (irreversible inhibitor), letrozole or anastrozole (reversible inhibitors) inhibit the activity of aromatase enzyme, thereby blocking the production of estrogens. The aromatase inhibitors are primarily used in postmenopausal women.

Estrogen production can also be blocked by removal of ovaries, termed as oophorectomy.

Anti-estrogen drugs such as tamoxifen act by blocking estrogen receptors. These agents competitively block the ER thereby inhibiting estrogen-dependent growth of the breast tumor.

Drugs such as fulvestrant act as estrogen receptor antagonists by downregulating ER. Fulvestrant is known as pure anti-estrogen because, on binding with ER it induces a conformational change leading to ER degradation thus achieving complete inhibition of estrogen signaling through the estrogen receptor.

Fulvestrant has no estrogen agonist activity and also does not exhibit cross-resistance with other estrogen antagonists, such as tamoxifen (William J. Gradishar, Community Oncology, 2007, 4, 220-232). It has been approved in many countries as a second-line treatment for postmenopausal women having hormone sensitive advanced breast cancer after progression or relapse on anti-estrogen therapy (John Robertson, The Oncologist, 2007, 12, 774-784). However, resistance to fulvestrant has given rise to limitation in its use in the treatment of breast cancer.

Rui Huang et al. (PLOS one, 2014, 9(4), e94226) have reported a role of signal transducer and activator of transcription (STAT) signaling in endocrine resistance. STAT3 and STAT5 are known to have involvement in tumorigenicity, cell-cycle progression, cell survival, transformation and angiogenesis. They seemingly have pronounced role in the oncogenesis of breast cancer and in resistance to endocrine therapy.

Angelo Di Lo et al., (Journal of Clinical Oncology, 2012, 30, 16, 1897-1900), describe an early *in-vivo* study, wherein it was demonstrated that fulvestrant alone can be effective in low-estrogen environment. A xenograft model of MCF-7 was developed by injecting the tumor cells in the mouse and the tumors were allowed to develop in an estrogenic environment which was provided by a subcutaneous estrogen pellet. After tumor development, estrogen pellet was removed and endocrine treatment was initiated. In this model, fulvestrant showed more potent antitumor effects compared to tamoxifen or estrogen deprivation. However, it was further reported that the tumors eventually developed resistance to fulvestrant and did not respond to the treatment of tamoxifen or a combination of tamoxifen and fulvestrant. Angelo Di Lo et al also report another study where a combination of fulvestrant with either tamoxifen or aromatase inhibitors (either letrozole or anastrozole) was tested in *in-vivo* model, which was genetically modified to express high levels of aromatase. Tumor growth inhibition (TGI) achieved with the combination of anastrozole and fulvestrant was only comparable with the TGI achieved using anastrozole and fulvestrant alone. Similarly, no significant difference was seen in TGI with a combination of fulvestrant and tamoxifen or letrozole compared with tamoxifen or letrozole alone.

The above findings and observations necessitate a suitable drug combination to improve the effectiveness of fulvestrant, which constitutes anti-estrogen therapy for the treatment of breast cancer. Additionally, further exploration is required to identify strategies to have an effective management of breast cancer.

In an effort to address the burden of breast cancer and to have an effective treatment strategy particularly for resistant breast cancers, the inventors of the present invention have arrived at a suitable drug combination.

SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a pharmaceutical combination comprising a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agents selected from an estrogen antagonist and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof.

In another aspect, the present invention relates to a pharmaceutical composition comprising a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agents selected from an estrogen antagonist and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof and at least one pharmaceutically acceptable carrier or excipient.

In another further aspect, the pharmaceutical combination of the present invention is provided for use in the treatment of anti-estrogen resistant endocrine receptor positive (ER+) breast cancer.

In yet another aspect, the present invention relates to a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof in combination with a therapeutically effective amount of one or more anti-cancer agents selected from an estrogen antagonist and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof.

In further aspect, the present invention relates to the use of a pharmaceutical combination comprising a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agents selected from estrogen antagonists and a cyclin dependent kinase inhibitor of Formula (II) in the manufacture of a medicament for the treatment of anti-estrogen resistant ER+ breast cancer.

In another further aspect, the present invention relates to a method for inhibiting growth of anti-estrogen resistant ER+ breast cancer cells, comprising contacting the said cells with an effective amount of a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; in combination with an effective amount of one or more anti-cancer agents selected from an

estrogen antagonist and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof.

In still further aspect, the present invention relates to a pharmaceutical kit comprising: (a) a pharmaceutical combination comprising a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agents selected from an estrogen antagonist and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof; and (b) optionally a package insert comprising instructions for using the said 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. 1a is a western blot image showing mTORC1 and mTORC2 inhibitory activity of Compound A (a representative example of the PI3K/mTOR inhibitor of Formula (I)) at different concentrations in anti-estrogen-sensitive and anti-estrogen-resistant endocrine receptor positive (ER+) breast cancer cells.

FIG. 1b is a western blot image showing activin receptor-like kinase (ALK1) inhibitory activity of Compound A in anti-estrogen-sensitive endocrine receptor positive (ER+) breast cancer cells.

FIG. 2 is a western blot image showing cyclin dependent kinase (CDK) inhibitory activity of Compound B (a representative example of the CDK inhibitor of Formula (II)) at different concentrations in anti-estrogen-sensitive and anti-estrogen-resistant ER+ breast cancer cells.

FIG. 3a is a graphical representation showing decrease in proliferation and viability of ER+ breast cancer cells when treated with the Compound A.

FIG. 3b is a graphical representation showing decrease in proliferation and viability of ER+ breast cancer cells when treated with the Compound B.

FIG. 4 is a histogram showing the effects of the Compound A, the Compound B and Fulvestrant when used alone and in combination in anti-estrogen-sensitive and anti-

estrogen-resistant ER+ breast cancer cells.

FIG. 5a and FIG. 5b are the graphical representations of tumor growth profile in anti-estrogen-sensitive ER+ breast cancer (MCF-7) xenograft model after administration of the Compound A, the Compound B and fulvestrant, each alone and in combination.

FIG. 6 is a western blot image showing mTORC1, mTORC2 and PI3K inhibitory activity of Compound A and fulvestrant alone and in combination at different concentrations in anti-estrogen-sensitive (MCF-7) endocrine receptor positive (ER+) breast cancer cells.

FIG. 7 is a graphical representation of tumor growth profile in anti-estrogen-resistant ER+ breast cancer (T47D-fulvestrant resistant) xenograft model after administration of fulvestrant alone and in combination with the Compound A.

FIG. 8 is western blot image showing mTORC1, mTORC2 and PI3K inhibitory activity of the Compound A in combination with fulvestrant in anti-estrogen-resistant (T47D-fulvestrant resistant) endocrine receptor positive (ER+) breast cancer cells.

FIG. 9a is an image showing Ki67 scores of the Compound A and fulvestrant in combination compared with vehicle and fulvestrant in anti-estrogen-resistant ER+ breast cancer (T47D-fulvestrant resistant) xenograft model.

FIG. 9b depicts photomicrographs of anti-estrogen-resistant (T47D-fulvestrant resistant) tumors after treatment with vehicle+fulvestrant and the compound A+ fulvestrant; using hematoxylin-eosin (H&E) staining (i), immunohistochemically stained with antibodies against Ki67 (ii), and terminal deoxynucleotidyl transferase-deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL) analysis (iii).

FIG. 10 is western blot image showing effects of the compound A on PI3K and mTOR signaling in human breast tumors *ex vivo*.

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 fluorine, chlorine, bromine and iodine.

The term “(C₁-C₄)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 4 carbon atoms. Examples of alkyl groups include but are not limited to methyl, ethyl, propyl, butyl, isopropyl, isobutyl, *sec*-butyl, *tert*-butyl, and the like.

Also, use of “(s)” as part of a term, includes reference to the term singly or in plurality, for example the term pharmaceutically acceptable salt(s) indicates a single salt or more than one salt of the PI3K/mTOR inhibitor of Formula (I).

The term “solvate” as used herein refers to a compound formed by the interaction of a solute (in respect of the present invention, PI3K/mTOR inhibitor of Formula (I) or CDK inhibitor Formula (II) 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 alcoholates of the compounds according to the invention.

The term “pharmaceutically acceptable salt(s)” as used herein refers to inorganic and organic salts of the PI3K/mTOR inhibitor of Formula (I) and CDK

inhibitor of Formula (II) contained in the pharmaceutical combination or composition of the invention.

The term “therapeutically effective amount”, as used herein refers to the amount of the PI3K/mTOR inhibitor of Formula (I) and that of the anti-cancer agent which is used in combination with the PI3K/mTOR inhibitor of Formula (I), when administered to a subject in need thereof, sufficient to: (i) prevent or delay the progression of anti-estrogen resistant ER+ breast cancer; (ii) prevent or delay the progression of hormone-sensitive breast cancer to hormone-resistant breast cancer; or (iii) treat the anti-estrogen resistant ER+ breast cancer.

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

The term “PI3K/mTOR inhibitor of Formula (I)” used herein refers to PI3K and/or mTOR inhibitor of Formula (I) or pharmaceutically acceptable salts or solvates thereof that inhibits expression or activity of phosphatidylinositol 3 kinase (PI3K) or mammalian target of rapamycin kinase (mTOR) or of both.

The term “anti-cancer agent” as used herein refers to a compound that is capable of suppressing the functions of a cancer cell. The term includes compounds that inhibit cell growth, cell proliferation and/or cell differentiation or cause cell death. Preferably, an anti-cancer agent is selectively toxic against certain cancer cells but is non-toxic or less toxic to the normal cells (non-cancerous cells). In the context of the present invention, the anti-cancer agents are the compounds selected from an estrogen antagonist and a cyclin dependent kinase inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof

The term “CDK inhibitor of Formula (II)” as used herein refers to the compounds of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof,

as disclosed herein which inhibits activity of cyclin dependent kinases (CDK).

The term “estrogen antagonist(s)” or “anti-estrogen(s)” as used herein refers to the compounds that antagonize the effect of estrogen at the estrogen receptor level.

The term “pure anti-estrogen(s)” as used herein refer to the compounds that bind the estrogen receptors and are devoid of any estrogen agonist effect. The term includes a drug such as fulvestrant.

The term “hormone-sensitive breast cancer” as used herein refers to the ER+ breast cancers that depend on the hormones such as estrogen or progesterone for their growth and are sensitive to hormone therapy or endocrine therapy.

The term “hormone-resistant state of breast cancer” or “hormone-resistant breast cancer” as used herein refers to the ER+ breast cancers that are resistant to hormone therapy or endocrine therapy.

The term “subject” as used herein refers to an animal, preferably a mammal, and most preferably a human. The term “mammal” used herein refers to warm-blooded vertebrate animals of the class ‘mammalia’, including humans, characterized by a covering of hair on the skin and, in the female, milk-producing mammary glands for nourishing the young. The term mammal includes animals such as cat, dog, rabbit, bear, fox, wolf, monkey, deer, mouse, pig and human. In the context of the present invention the phrase “a subject in need thereof” means a subject (patient) in need for the treatment of anti-estrogen resistant ER+ breast cancer. Alternatively, the phrase “a subject in need thereof” means a subject (patient) diagnosed having anti-estrogen resistant ER+ breast cancer.

The term “synergistic” or “synergistic effect” or “synergism” as used herein refers to the therapeutic effect of the combination of the compounds (e.g. PI3K/mTOR inhibitor, estrogen antagonists and/or the CDK inhibitor) which is greater than the additive effect of the said compounds used in combination. The synergistic effect can be attained by administering the said compounds simultaneously through a unit dosage form or as separate formulations administered simultaneously or sequentially.

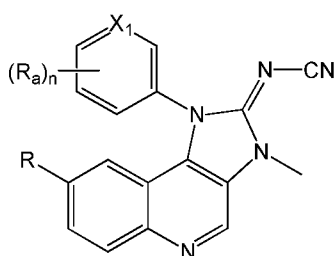
Thus, in accordance with an aspect, the present invention relates to a pharmaceutical combination comprising PI3K/mTOR inhibitor of Formula (I) or a

pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agents (as described herein).

PI3K/mTOR inhibitors of Formula (I)

The PI3K/mTOR inhibitors of Formula (I) used in the pharmaceutical combination as per the present invention are imidazo[4,5-c]quinoline derivatives, which are disclosed in PCT Application Publication No. WO2012007926.

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



Formula (I)

Wherein,

X₁ is C, CH or N;

R is pyridyl, wherein pyridyl is unsubstituted or substituted with one or more of R_b;

R_a and R_b at each occurrence are independently selected from the group consisting of halogen, -CN, -O-(C₁-C₄)alkyl, -NR_cR_d and (C₁-C₄)alkyl, wherein (C₁-C₄)alkyl is unsubstituted or substituted with one or more of halogen or -CN; and R_c and R_d are hydrogen or (C₁-C₄)alkyl; and

n is 0 or an integer from 1 to 5; or

pharmaceutically acceptable salts or solvates thereof.

In another embodiment, the PI3K/mTOR inhibitor is a compound of Formula (I), wherein X₁ is N and R_a at each occurrence is independently selected from the group consisting of Cl, Br, F, -CN, -OCH₃, CH₃, CF₃ or -C(CH₃)₂CN.

In further embodiment, the PI3K/mTOR inhibitor is a compound of Formula (I), wherein R_b is -NR_cR_d or (C₁-C₄)alkyl, wherein (C₁-C₄)alkyl is unsubstituted or substituted with one or more of halogen; and R_c and R_d are hydrogen or (C₁-C₄)alkyl.

In still further embodiment, the PI3K/mTOR inhibitor is a compound of Formula (I), wherein R is 3-pyridyl, which is unsubstituted or substituted with one or more of R_b and R_b at each occurrence is independently selected from $-NR_cR_d$ and $(C_1-C_4)alkyl$, wherein $(C_1-C_4)alkyl$ is unsubstituted or substituted with one or more halogens and R_c and R_d are hydrogen or $(C_1-C_4)alkyl$.

In further embodiment, the PI3K/mTOR inhibitor is a compound of Formula (I), wherein R is pyridyl, which is unsubstituted or substituted with one or more of R_b and R_b at each occurrence is independently selected from the group consisting of $-NH_2$, $-NH(C_1-C_4)alkyl$, $-N(C_1-C_4-alkyl)_2$ and methyl, wherein methyl is unsubstituted or substituted with one to three halogen atoms.

In further embodiment, the PI3K/mTOR inhibitor is a compound of Formula (I), wherein R is pyridyl, which is unsubstituted or substituted with one or more of R_b and R_b at each occurrence is independently selected from the group consisting of $-NH_2$, $-NH-CH_3$, $-N(CH_3)_2$ and $-CF_3$.

In further embodiment, the PI3K/mTOR inhibitor is a compound of Formula (I), wherein R is 3-pyridyl, which is unsubstituted or substituted with one or more of R_b and R_b at each occurrence is independently selected from the group consisting of $-NH_2$, $-NH-CH_3$, $-N(CH_3)_2$ and $-CF_3$.

In still further embodiment, the PI3K/mTOR inhibitor is a compound of Formula (I), wherein n is 1 or 2.

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, and

N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-3-methyl-1-(6-(trifluoromethyl)pyridin-3-yl)-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene) cyanamide, or a pharmaceutically acceptable salt or a solvate thereof.

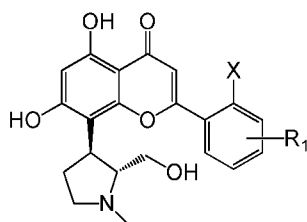
In yet another embodiment the PI3K/mTOR inhibitor of Formula (I) is N-(8-(6-amino-5-(trifluoromethyl)pyridin-3-yl)-1-(6-(2-cyanopropan-2-yl)pyridin-3-yl)-3-methyl-1H-imidazo[4,5-c]quinolin-2(3H)-ylidene) cyanamide, herein after referred to as Compound A (also referred to as panulisib).

Anti-cancer agents

The anti-cancer agents contained and/or used in the combination or composition according to the present invention are selected from cyclin dependent kinase (CDK) inhibitors or estrogen antagonists.

In an embodiment of the present invention, the anti-cancer agent is a cyclin dependent kinase (CDK) inhibitor.

In another embodiment of the present invention, the CDK inhibitor is a compound of Formula (II)



Formula (II)

wherein,

X is chlorine, bromine, fluorine or iodine;

R₁ is hydrogen, (C₁-C₄)alkyl or trifluoromethyl;

or a pharmaceutically acceptable salt or a solvate thereof.

In another embodiment, the CDK inhibitor is a compound of Formula (II), wherein X is chlorine and R₁ is hydrogen, (C₁-C₄)alkyl or trifluoromethyl; or a pharmaceutically acceptable salt or a solvate thereof.

In another embodiment, the CDK inhibitor is a compound of Formula (II), wherein X is chlorine and R₁ is hydrogen; or a pharmaceutically acceptable salt or a solvate thereof.

In another embodiment, the CDK inhibitor is a compound of Formula (II), wherein X is chlorine and R₁ is trifluoromethyl; or a pharmaceutically acceptable salt or a solvate thereof.

In another embodiment, the CDK inhibitor is a compound of Formula (II), wherein X is chlorine and R₁ is 4-trifluoromethyl; or a pharmaceutically acceptable salt or a solvate thereof.

In yet another embodiment, the CDK inhibitor is a compound of Formula (II) selected from:

(+)-*trans*-2-(2-Chlorophenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methylpyrrolidin-3-yl)-chromen-4-one hydrochloride or

(+)-*trans*-2-(2-Chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methylpyrrolidin-3-yl)-chromen-4-one hydrochloride.

In yet another embodiment the CDK inhibitor is (+)-*trans*-2-(2-Chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methylpyrrolidin-3-yl)-chromen-4-one hydrochloride, herein after referred to as Compound B (also referred to as voruciclib).

In further embodiment of the present invention, the anti-cancer agent is an estrogen antagonist.

In further embodiment of the present invention, the anti-cancer agent is a pure anti-estrogen.

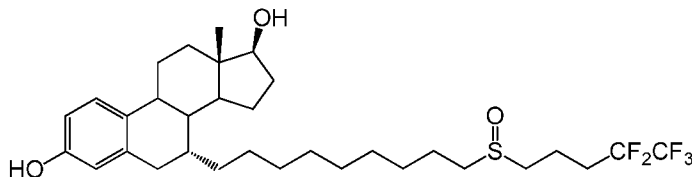
In further embodiment of the present invention, the estrogen antagonist is selected from RU 58668 (Sanofi), EM-652 (Endorecherche) or fulvestrant (AstraZeneca).

In still further embodiment of the present invention, the estrogen antagonist is fulvestrant.

Fulvestrant can be synthesized by methods described in US 4,659,516 or can be obtained through commercial sources.

Fulvestrant (FASLODEX[®], AstraZeneca, CAS Reg. No. 129453-61-8) is used for the treatment of postmenopausal women with hormone receptor-positive metastatic breast cancer after progression or relapse on antiestrogen therapy (The Oncologist, 2007, 12, 774-784). It downregulates estrogen and progesterone receptors and degrades the estrogen receptor. It is a pure anti-estrogen and has no

known agonist activity. Fulvestrant is a compound having the following structure (British Journal of Cancer, 2004, 90 (Suppl 1), S2 – S6).

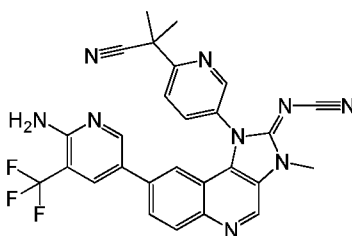


In an embodiment of the present invention, the pharmaceutical combination comprises a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof and a CDK inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof.

In another embodiment of the present invention, the pharmaceutical combination comprises a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or solvate thereof and an estrogen antagonist.

In yet another embodiment of the present invention, the pharmaceutical combination comprises a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof, a CDK inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof and an estrogen antagonist.

In further embodiment of the present invention, there is provided a pharmaceutical combination comprising compound A



Compound A

or pharmaceutically acceptable salts or solvates thereof and one or more anticancer agents selected from CDK inhibitors of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof and an estrogen antagonist.

In an embodiment of the present invention, the pharmaceutical combination comprises Compound A and a CDK inhibitor of Formula (II); or a pharmaceutically acceptable salt or a solvate thereof.

In further embodiment of the present invention, the CDK inhibitor of Formula (II) is Compound B.

In an embodiment of the present invention, the pharmaceutical combination comprises Compound A or pharmaceutically acceptable salts or solvates thereof; and an estrogen antagonist.

In still further embodiment the estrogen antagonist is a pure anti-estrogen.

In yet another embodiment, the estrogen antagonist is fulvestrant.

In an embodiment of the present invention, the pharmaceutical combination comprises Compound A or a pharmaceutically acceptable salt or a solvate thereof; and fulvestrant.

In an embodiment of the present invention, the pharmaceutical combination comprises Compound A or a pharmaceutically acceptable salt or a solvate thereof; Compound B and fulvestrant.

In an embodiment, the pharmaceutical combination as described above in one or more embodiments of the present invention are provided for use in the treatment of anti-estrogen resistant ER+ breast cancer.

Preparation of PI3K/mTOR inhibitors of Formula (I) and CDK inhibitors of Formula (II):

PI3K/mTOR inhibitors of Formula (I) can be prepared by synthetic routes as described in PCT Application Publication No. WO2012007926, which is incorporated herein by reference. The compounds may also be prepared by synthetic routes that include similar reaction steps or methods known in the art.

CDK inhibitors of Formula (II), can be prepared by synthetic routes as described in US Application Publication No. US20070015802, which is incorporated herein by reference.

PI3K/mTOR inhibitors of Formula (I) and CDK inhibitors of Formula (II) can be converted into their pharmaceutically acceptable salts by following procedures known to persons skilled in the art.

PI3K/mTOR inhibitors of Formula (I) and CDK inhibitors of Formula (II) 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.

PI3K/mTOR inhibitors of Formula (I) and CDK inhibitors of Formula (II), 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 can be mono salts or bis-salts.

PI3K/mTOR inhibitors of Formula (I) and CDK inhibitors of Formula (II) can also exist as hydrates or solvates.

PI3K/mTOR inhibitors of Formula (I) and CDK inhibitors of Formula (II) contained and/or used in the combination of the present invention can be used in their isotopically labeled forms, wherein one or more atoms of the compounds 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 disclosed herein include, but are not limited to, isotopes of hydrogen such as ^2H and ^3H , carbon such as ^{11}C , ^{13}C and ^{14}C , nitrogen such as ^{13}N and ^{15}N , oxygen such as ^{15}O , ^{17}O and ^{18}O , chlorine such as ^{36}Cl , fluorine such as ^{18}F and sulfur 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 (+)-*trans*-enantiomers of the CDK inhibitor of Formula (II) can be obtained by methods disclosed in PCT Application Publication Nos. WO2004004632, WO2007148158 and WO2008007169 incorporated herein by reference or the enantiomers of the CDK inhibitor of Formula (II) can also be obtained by methods

well known in the art, such as chiral HPLC and enzymatic resolution. Alternatively, the enantiomers can be synthesized by using optically active starting materials.

Methods of Treatment

According to one aspect of the present invention, there is provided a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or solvate thereof; and a therapeutically effective amount of one or more anti-cancer agents selected from an estrogen antagonist and a CDK inhibitor of Formula (II) or a pharmaceutically acceptable salt or solvate thereof.

According to one embodiment of the present invention there is provided a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or solvate thereof; and a therapeutically effective amount of a CDK inhibitor of Formula (II); or a pharmaceutically acceptable salt or a solvate thereof.

According to another embodiment of the present invention there is provided a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof and a therapeutically effective amount of an estrogen antagonist.

According to another embodiment of the present invention there is provided a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; a therapeutically effective amount of a CDK inhibitor of Formula (II); or a pharmaceutically acceptable salt or a solvate thereof and a therapeutically effective amount of an estrogen antagonist.

According to another embodiment of the present invention there is provided a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of the Compound A or a pharmaceutically acceptable salt or a solvate thereof and a

therapeutically effective amount of a CDK inhibitor of Formula (II); or a pharmaceutically acceptable salt or a solvate thereof.

According to another embodiment of the present invention there is provided a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of the Compound A or a pharmaceutically acceptable salt or a solvate thereof and a therapeutically effective amount of the Compound B.

According to another embodiment of the present invention there is provided a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of the Compound A or a pharmaceutically acceptable salt or a solvate thereof; and a therapeutically effective amount of an anti-estrogen antagonist.

According to another embodiment of the present invention there is provided a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of the Compound A or a pharmaceutically acceptable salt or a solvate thereof; and a therapeutically effective amount of fulvestrant.

According to another embodiment of the present invention there is provided a method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of the Compound A or a pharmaceutically acceptable salt or solvate thereof; a therapeutically effective amount of the Compound B and a therapeutically effective amount of fulvestrant.

According to another aspect of the present invention, there is provided a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or solvate thereof; and a therapeutically effective amount of one or more anti-cancer agents selected from an estrogen antagonist and a CDK inhibitor of Formula (II) or a pharmaceutically acceptable salt or solvate thereof for use in the treatment of anti-estrogen resistant ER+ breast cancer.

According to another embodiment of the present invention there is provided a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof in combination with a CDK inhibitor of Formula (II); or a

pharmaceutically acceptable salt or a solvates thereof; for use in the treatment of anti-estrogen resistant ER+ breast cancer.

According to another embodiment of the present invention there is provided a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof in combination with an estrogen antagonist for use in the treatment of anti-estrogen resistant ER+ breast cancer.

According to another embodiment of the present invention there is provided a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof in combination with a CDK inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof and an estrogen antagonist for use in the treatment of anti-estrogen resistant ER+ breast cancer.

According to another embodiment of the present invention there is provided a pharmaceutical combination for use in the treatment of anti-estrogen resistant ER+ breast cancer, wherein the anticancer agent is a pure anti-estrogen.

According to another embodiment of the present invention there is provided a pharmaceutical combination of the Compound A or a pharmaceutically acceptable salt or a solvate thereof and the Compound B for use in the treatment of anti-estrogen resistant ER+ breast cancer.

According to another embodiment of the present invention there is provided a pharmaceutical combination of the Compound A or a pharmaceutically acceptable salt or a solvate thereof and fulvestrant for use in the treatment of anti-estrogen resistant ER+ breast cancer.

According to another embodiment of the present invention there is provided a pharmaceutical combination of the Compound A or a pharmaceutically acceptable salt or a solvates thereof; the Compound B and fulvestrant for use in the treatment of anti-estrogen resistant ER+ breast cancer.

According to a further aspect of the invention there is provided use of a pharmaceutical combination as described hereinbefore in the manufacture of a medicament for the treatment of anti-estrogen resistant ER+ breast cancer.

The present invention provides methods for the synergistic treatment of anti-estrogen resistant ER+ breast cancer.

According to an embodiment of the present invention, the anti-estrogen resistant ER+ breast cancer is fulvestrant resistant breast cancer.

According to another embodiment of the present invention there is provided a pharmaceutical combination as described hereinbefore, for use in the prevention of the progression of hormone-sensitive breast cancer to hormone-resistant state of the breast cancer.

According to another embodiment of the present invention there is provided a pharmaceutical combination as described hereinbefore, for use in the delay of the progression of hormone-sensitive breast cancer to hormone-resistant state of the breast cancer.

According to another embodiment of the present invention there is provided a pharmaceutical combination as described hereinbefore, for use in the treatment of anti-estrogen resistant ER+ breast cancer synergistically.

According to another aspect of the present invention there is provided a method for inhibiting growth of anti-estrogen resistant ER+ breast cancer cells, comprising contacting the cells with an effective amount of a pharmaceutical combination comprising a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agents selected from an estrogen antagonist and CDK inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof.

According to another embodiment of the present invention there is provided a method for inhibiting growth of anti-estrogen resistant ER+ breast cancer cells, comprising contacting the cells with an effective amount of a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; and an effective amount of one or more anti-cancer agents selected from an estrogen antagonist and a CDK inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof; wherein the anti-estrogen resistant ER+ breast cancer cells are MCF-7/fulvestrant resistant breast cancer cells or T47D/fulvestrant resistant breast cancer cells.

According to another embodiment of the present invention there is provided a method for inhibiting growth of anti-estrogen resistant ER+ breast cancer cells, comprising contacting the cells with an effective amount of the Compound A or a pharmaceutically acceptable salt or a solvate thereof; and an effective amount of fulvestrant; wherein anti-estrogen resistant ER+ breast cancer cells are MCF-

7/fulvestrant resistant breast cancer cells or T47D/fulvestrant resistant breast cancer cells.

According to another embodiment of the present invention there is provided a method for inhibiting growth of anti-estrogen resistant ER+ breast cancer cells, comprising contacting the cells with an effective amount of the Compound A or a pharmaceutically acceptable salt or solvate thereof and an effective amount of the Compound B; wherein anti-estrogen resistant ER+ breast cancer cells are MCF-7/fulvestrant resistant breast cancer cells or T47D/fulvestrant resistant breast cancer cells.

Administration

In another aspect, the PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof and an anti-cancer agent (as described herein) contained and/or used in the pharmaceutical combination according to the present invention; can be administered simultaneously or sequentially or spaced out over a period of time.

In another embodiment, the PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof and the anti-cancer agent (as described herein) contained and/or used in the pharmaceutical combination according to the present invention; can be administered simultaneously.

In another embodiment, the compound A or a pharmaceutically acceptable salt, a solvate thereof and the anti-cancer agent (as described herein) contained and/or used in the pharmaceutical combination according to the present invention; can be administered simultaneously.

In another embodiment, the compound A or a pharmaceutically acceptable salt, or a solvate thereof; and CDK inhibitor of Formula (II) contained and/or used in the pharmaceutical combination according to the present invention; can be administered simultaneously.

In another embodiment, the compound A or a pharmaceutically acceptable salt or a solvate thereof and the Compound B contained and/or used in the pharmaceutical combination according to the present invention; can be administered simultaneously.

In another embodiment, the compound A or a pharmaceutically acceptable salt, or a solvate thereof and fulvestrant contained and/or used in the pharmaceutical combination can be administered simultaneously.

In another embodiment, PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof and one or more anti-cancer agents (as described herein) contained and/or used in the pharmaceutical combination can be administered in a sequential manner. Sequential administration means administration of one component of the combination before the administration of the other component of the combination such that the combination shows a synergistic effect. Accordingly, the PI3K/mTOR inhibitor of Formula (I) can be administered prior to or after the administration of the anti-cancer agent.

Further, sequential administration of the PI3K/mTOR inhibitor of Formula (I) and the anti-cancer agents (as described herein) involves administration of the said compounds spaced out over a period of time i.e. after administration of one component of the combination; the other component is administered after a certain fixed period.

In another aspect, the compound A or a pharmaceutically acceptable salt, or a solvate thereof and the Compound B contained and/or used in the pharmaceutical combination or composition according to the present invention; can be administered sequentially.

In another aspect, the compound A or a pharmaceutically acceptable salt or a solvate thereof and fulvestrant contained and/or used in the pharmaceutical combination can be administered sequentially.

According to an embodiment of the present invention, administration of the PI3K/mTOR inhibitor of Formula (I) and/or anti-cancer agents (as described herein) 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 PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof and one or more anti-cancer agents (as described herein). The pharmaceutical kit can

comprise two or more separate containers for the PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agents (as described herein). 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.

In an embodiment of the present invention, the pharmaceutical kit can comprise two or more separate containers for the compound A or a pharmaceutically acceptable salt or a solvate thereof; and one or more anti-cancer agents (as described herein).

In an embodiment of the present invention, the pharmaceutical kit can comprise two or more separate containers for the compound A or a pharmaceutically acceptable salt or a solvate thereof; and a CDK inhibitor of Formula (II).

In an embodiment of the present invention, the pharmaceutical kit can comprise two or more separate containers for the compound A or a pharmaceutically acceptable salt or a solvate thereof; and the compound B.

In an embodiment of the present invention, the pharmaceutical kit can comprise two or more separate containers for the compound A or a pharmaceutically acceptable salt or a solvate thereof; and a pure anti-estrogen.

In an embodiment of the present invention, the pharmaceutical kit can comprise two or more separate containers for the compound A or a pharmaceutically acceptable salt or a solvate thereof; and fulvestrant.

Pharmaceutical Compositions

The pharmaceutical composition of the present invention comprises a PI3K/mTOR inhibitor of Formula (I) or pharmaceutically acceptable salts or solvates thereof; one or more anti-cancer agents selected from an estrogen antagonist and CDK inhibitor of Formula (II) and at least one pharmaceutically acceptable carrier 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 PI3K/mTOR inhibitor of Formula (I) and anti-cancer agents 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 effective doses of the therapeutic agents (the PI3K/mTOR inhibitor of Formula (I) and the anti-cancer agent (as described herein)) for administration will

vary depending on 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, etc. In certain embodiments, the therapeutic agents are administered in a time frame where one or more of the therapeutic 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 therapeutic agents contained and/or used in the pharmaceutical combination 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 of the therapeutic agents (the PI3K/mTOR inhibitors of Formula (I) and the anti-cancer agent (as described herein)) to be administered daily is to be selected to produce the desired effect. A suitable dosage is about 0.01 to 100 mg/kg of the PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof, for example, about 0.01 to 50 mg/kg of the PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof, about 0.01 to 20 mg/kg of a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof. The anti-cancer agent (as described herein) can administered at a dose from about 5 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 an embodiment, the therapeutically effective amount of the compound A or a pharmaceutically acceptable salt or a solvate thereof can be about 5 mg/kg, about 10 mg/kg or about 20 mg/kg.

In another embodiment, the therapeutically effective amount of the compound A or a pharmaceutically acceptable salt or a solvate thereof is about 5 mg/kg.

In another embodiment, the therapeutically effective amount of compound A or a pharmaceutically acceptable salt or a solvate thereof is about 10 mg/kg.

In another embodiment, the therapeutically effective amount of compound A or a pharmaceutically acceptable salt or a solvate thereof 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.

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.

Use of the PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof and one or more anti-cancer agents (as described herein) contained and/or used in the pharmaceutical combination 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-cancer agent when combined with a compound Formula (I) exhibits synergistic effect.

The representative PI3K/mTOR inhibitor 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-1H-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 5 to 9 (FIG. 5a, 5b, 6, 7, 8, 9a and 9b) that the pharmaceutical combination of the present invention exhibited therapeutically synergistic activity in the xenograft models.

The synergistic effect of the PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof and one or more anti-cancer agents (as described herein) contained and/or used in the pharmaceutical combination according to 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:

List of abbreviations			
ANOVA	Analysis of Variance	min	Minutes(s)
ATCC	American Type Cell Culture	μ L	microlitre
BCA assay	Bicinchoninic acid assay	μ M	micromolar
BSA/TBST	Bovine Serum Albumin/ Tris-buffered saline with Tween 20	nM	nanomolar
DMEM	Dulbecco's Modified Eagle Medium	NP-40	Tergitol-type NP-40
EDTA	Ethylenediaminetetraacetic acid	NaF	Sodium fluoride
EGTA	Ethylene Glycol Tetraacetic Acid	NaPPi	sodium pyrophosphate
FBS	Fetal Bovine serum	NaCl	Sodium Chloride
H and E stain	Hematoxylin and eosin stain	Na ₃ VO ₄	Sodium orthovanadate
HRP	Horseradish peroxidase	NOD-Scid	Nonobese diabetic- severe combined immunodeficient
g	Gram(s)	s.c	Subcutaneous(ly)
kg	kilogram	SDS	Sodium dodecyl sulfate
mL	millilitre	RIPA buffer	Radioimmunoprecipitation assay buffer
mmol or mM	millimolar	TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling

Activity of the PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof in combination with one or more anti-cancer agents (as described herein) (the pharmaceutical combination according to the present invention) can be determined according to any effective *in vitro* or *in vivo* method.

***In vitro* experiments:**

Cell Lines:

- (i) All anti-estrogen sensitive parental cell lines (MCF-7, T47-D, HCC-1428, ZR75-1 and BT474) were obtained from ATCC.
- (ii) MCF-7/fulvestrant-resistant and T47D/fulvestrant-resistant cells were provided as

gifts by Matthew Ellis (Washington University). Parental and fulvestrant-resistant cells were maintained in DMEM and 10% FBS (Hyclone) in the absence or presence of 1 μ M fulvestrant (Tocris Bioscience and Abmole BioScience), respectively.

(iii) Long-term estrogen-deprived (LTED) cells were cultured as described in, The Journal of Clinical Investigation, 2010,120, 2406-13 and were maintained in hormone-depleted medium [phenol red-free DMEM with 10% dextran/charcoal-treated FBS (DCC-FBS; Hyclone)].

Example 1:

Effects of the Compound A on mTORC1 and mTORC2 kinase activity in anti-estrogen sensitive and resistant ER positive breast cancer cells

Anti-estrogen sensitive cells (MCF-7, T47-D, HCC-1428, ZR75-1 and BT474), MCF-7/fulvestrant-resistant cells, T47D/ fulvestrant-resistant cells and LTED cells were treated with Compound A (0 to 400 nM) for 16-24 hours. The cell were then lysed in RIPA buffer [50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 1 mM EDTA, 1 mM EGTA, 5 mM NaPPi, 50 mM NaF, 10 mM β -glycerophosphate, 1 mM Na_3VO_4 (New England Biolabs), protease inhibitor cocktail (Pierce)] on ice. Lysates were sonicated for 10 seconds and centrifuged at 18,000 x *g* for 10 min. Protein concentrations of supernatants were determined by Bicinchoninic acid (BCA) assay (Pierce). Samples were reduced and denatured by addition of 1.25% β -mercaptoethanol in NuPage sample buffer (Invitrogen). Samples were heated for 1 min. at 95°C before SDS-PAGE. Proteins were transferred to nitrocellulose membranes, which were blocked with 5% BSA/TBS-T and probed using antibodies against P-AKT_{T308}, P-AKT_{S473}, Actin, P-S6_{S240/244}, PARP, cleaved caspase-3, PR (Cell Signaling), and ER (Santa Cruz).

The cell lysate were also analyzed by immunoblot using antibodies for P-Smad 1/5_{S463/465}.

Antibody binding was detected using HRP-conjugated secondary antibodies against mouse or rabbit Ig (GE Healthcare), and ECL substrate (Pierce).

Result:

- (i) FIG. 1a depicts the mTORC1 and mTORC2 kinase inhibitory activity of Compound A.
- (ii) FIG. 1b depicts the ALK-1 inhibitory activity of Compound A.

Conclusion: Compound A inhibits mTORC1, mTORC2 kinase, and ALK1 activity in anti-estrogen sensitive and resistant ER positive breast cancer cells.

Example 2:

Effects of Compound B on CDK activity in anti-estrogen sensitive and resistant ER positive breast cancer cells

Anti-estrogen sensitive cells (MCF-7, T47-D, HCC-1428, ZR75-1 and BT474), MCF-7/fulvestrant-resistant cells, T47D/ fulvestrant-resistant cells and LTED cells were treated with Compound B (0 to 5 μ M) for 16 to 24 hours. The cells were then lysed in RIPA buffer [50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 1 mM EDTA, 1 mM EGTA, 5 mM NaPPi, 50 mM NaF, 10 mM β -glycerophosphate, 1 mM Na_3VO_4 (New England Biolabs), protease inhibitor cocktail (Pierce)] on ice. Lysates were sonicated for 10 seconds and centrifuged at 18,000 $\times g$ for 10 min. Protein concentrations of supernatants were determined by Bicinchoninic acid (BCA) assay (Pierce). Samples were reduced and denatured by addition of 1.25% β -mercaptoethanol in NuPage sample buffer (Invitrogen). Samples were heated for 1 min. at 95°C before SDS-PAGE. Proteins were transferred to nitrocellulose membranes, which were blocked with 5% BSA/TBS-T and probed using antibodies against P-Rpb1_{S2/5}, P-Rb_{S780}, Mcl-1, PARP, PR, CDK4, CDK9 (Cell Signaling), and ER (Santa Cruz). Antibody binding was detected using HRP-conjugated secondary antibodies against mouse or rabbit Ig (GE Healthcare), and ECL substrate (Pierce).

Result: FIG. 2 depicts the CDK inhibitory activity of Compound B.

Conclusion: Compound B inhibits CDK activity in anti-estrogen sensitive and resistant ER positive breast cancer cells.

Example 3

Effects of Compound A and Compound B on proliferation of anti-estrogen sensitive and resistant ER positive breast cancer cells

Anti-estrogen sensitive cells (MCF-7, T47-D, HCC-1428, ZR75-1 and BT474), MCF-7/fulvestrant-resistant cells, T47D/ fulvestrant-resistant cells and LTED cells were plated in triplicate in respective growth media at 5×10^3 cells/well in 96-well

plates. The next day, cells were treated with Compound A (0 to 30 nM) or Compound B (0 to 5 μ M). Five to eight days later, relative quantities of adherent cells were determined by sulforhodamine B (SRB) assay (as described in Nature Protocols 2006, 1, 1112-1116). Relative cell numbers were used to calculate inhibitory effect.

Result: A Graph for inhibitory effect vs drug concentration for Compound A and Compound B was plotted as shown in FIG. 3a and FIG. 3b. IC₅₀ values are indicated by dotted lines.

Conclusion: Compound A and Compound B decrease the proliferation of anti-estrogen sensitive and resistant ER positive breast cancer cells.

Example 4

Apoptotic activity of Compound A and Compound B alone and in combination with fulvestrant in anti-estrogen sensitive and resistant ER positive breast cancer cells

Anti-estrogen sensitive cells (MCF-7), MCF-7/fulvestrant-resistant cells, T47D/fulvestrant-resistant cells and MCF-7/LTED cells were seeded in triplicate in 6-well plates at 0.6×10^6 cells/well and were treated with fulvestrant (1 μ M), Compound A (200 nM) and Compound B (5 μ M), alone and in combination for 3 days. Floating and adherent cells (dislodged by trypsinization) were processed using ApoScreen Annexin Apoptosis kit (Southern Biotech), then analyzed by flow cytometry. Cells staining positively for Annexin-V and/or propidium iodide were considered apoptotic.

Result: FIG. 4 shows the % apoptosis in anti-estrogen sensitive and resistant ER positive breast cancer cells when treated with Compound A and Compound B alone and in combination with fulvestrant.

Conclusion: Compound A and Compound B showed significant increase in apoptosis in all the cell lines, and particularly the combination of Compound A with fulvestrant and Compound B with fulvestrant in MCF-7/fulvestrant resistant and T47D/fulvestrant resistant cells showed significant increase in apoptosis.

***In vivo* experiments:**

All animal studies were approved by the Dartmouth Institutional Animal Care and Use Committee (IACUC).

Animals used

Female NOD-scid IL2R $\gamma^{-/-}$ (NSG; NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice, 5 to 6 weeks old, (obtained from Norris Cotton Cancer Center Transgenics & Genetic Constructs Shared Resource). 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 and water *ad libitum*.

Generation of anti-estrogen sensitive (MCF-7) xenograft model

Each NOD-scid IL2R $\gamma^{-/-}$ (n=59) mice was injected with $5-10 \times 10^6$ MCF-7 cells subcutaneously and a 17 β -estradiol pellet (0.72 mg, 60-day-release, Innovative Research of America). Tumor dimensions were measured twice weekly using calipers, and volumes were calculated using the formula: volume = length x width²/2 (width is the shorter dimension).

Generation of anti-estrogen resistant (T47D/fulvestrant resistant) xenograft model

Each NOD-scid IL2R $\gamma^{-/-}$ mice (n=20) was injected with $5-10 \times 10^6$ T47D/fulvestrant resistant cells and subcutaneously injected weekly with 5mg fulvestrant (Abmole). Tumor dimensions were measured twice weekly using calipers, and volumes were calculated using the formula: volume = length x width²/2 (width is the shorter dimension).

Sample preparation and dosing

Compound A was suspended in (0.5%) methyl cellulose and dosed at 5 or 15 mg/kg/d in 100 μ L.

Fulvestrant was procured as clinical formulation from AstraZeneca or dissolved in ethanol (50 mg/mL), then diluted 10-folds with castor oil to obtain the formulation of 5 mg/mL.

Compound B was dissolved in water and dosed at 75 mg/kg/d in 100 μ L.

Example 5

Effect of compound A on anti-estrogen sensitive tumors (MCF-7) in NOD-scid IL2R γ ^{-/-} mice xenograft model alone and in combination with fulvestrant

Treatment was initiated when tumor size volume of the MCF-7 tumors was about 200mm³. The tumor-bearing mice were randomized (n=59) in the following treatment groups:

- i) Group 1: Control group: Tumor-bearing mice administered with vehicle (n=8).
- ii) Group 2: Tumor-bearing mice administered with fulvestrant (5mg/week s.c. in 100 μ L) (n=8).
- iii) Group 3: Tumor-bearing mice administered with Compound A [5 mg/kg/d p.o. in 100 μ L (n=8) or 15 mg/kg/d p.o. in 100 μ L (n=7)].
- iv) Group 4: Tumor-bearing mice administered with a combination of fulvestrant (5mg/week subcutaneously in 100 μ L) and Compound A (5 mg/kg/d p.o. in 100 μ L) (n=9).
- v) Group 5: Tumor-bearing mice administered with a Compound B (75 mg/kg/d p.o. in 100 μ L) (n=6).
- vi) Group 6: Tumor-bearing mice administered with a combination of Compound A (5 mg/kg/d p.o. in 100 μ L) and Compound B (75 mg/kg/d p.o. in 100 μ L) (n=13).

Tumors were harvested 3 days of treatment or at the end of the study (4 to 6 weeks) and cut in pieces for snap-freezing or formalin fixation followed by paraffin-embedding (FFPE).

Immunoblotting

Tumor cells of mice from treatment groups 1 to 4 were isolated and then lysed in RIPA buffer [50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 1 mM EDTA, 1 mM EGTA, 5 mM NaPPi, 50 mM NaF, 10 mM β -glycerophosphate, 1 mM Na₃VO₄ (New England Biolabs), protease inhibitor cocktail (Pierce)] on ice. Lysates were sonicated for 10 seconds and centrifuged at 18,000 x g for 10 min. Protein concentrations of supernatants were determined by BCA assay (Pierce). Samples were reduced and denatured by addition of 1.25% β -mercaptoethanol in NuPage sample buffer (Invitrogen). Samples were heated for 1 min. at 95°C before SDS-PAGE. Proteins were transferred to nitrocellulose membranes, which were blocked with 5% BSA/TBS-T and probed using antibodies

against P-AKT_{T308}, P-AKT_{S473}, Actin, P-S6_{S240/244}, p70S6K_{T389} and PR. Antibody binding was detected using HRP-conjugated secondary antibodies against mouse or rabbit Ig (GE Healthcare), and ECL substrate (Pierce).

Results:

- (i) FIG. 5a: shows tumor growth inhibition results of Compound A alone and in combination with fulvestrant in anti-estrogen-sensitive ER+ breast cancer (MCF-7) xenograft model.
- (ii) FIG. 5b: shows tumor growth inhibition results of Compound B alone and in combination with Compound A in anti-estrogen-sensitive ER+ breast cancer (MCF-7) xenograft model.
- (iii) FIG. 6 shows mTORC1, mTORC2 and PI3K inhibitory activity of Compound A and fulvestrant in ER+ breast cancer (MCF-7) xenograft model.

Conclusion

Compound A alone, and in combination with fulvestrant shows significant tumor growth inhibition in anti-estrogen-sensitive ER+ breast cancer (MCF-7) xenograft model.

Example 6

Effect of compound A on anti-estrogen resistant tumors (T47D-fulvestrant resistant) in NOD-scid IL2R $\gamma^{-/-}$ mice xenograft model alone and in combination with fulvestrant

Treatment was initiated when tumor size volume of the T47D-fulvestrant resistant tumors were about 200 mm³. The tumor-bearing mice were randomized (n= 20) in the following treatment groups:

- i) Group 1: Control group: Tumor-bearing mice administered with vehicle/fulvestrant (5mg/week subcutaneously in 100 μ L) (n=10).
- ii) Group 2: Tumor-bearing mice administered with Compound A (15 mg/kg/d) and fulvestrant (5mg/week subcutaneously in 100 μ L) (n=10).

Tumors were harvested after 3 days of treatment for immunoblotting, or at the end of 6 weeks for immunohistochemistry. Tumors were cut in pieces for snap-freezing or formalin fixation followed by paraffin-embedding (FFPE).

Immunoblotting:

Tumor cells of mice from treatment groups 1 and 2 were isolated and then lysed in RIPA buffer [50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 1 mM EDTA, 1 mM EGTA, 5 mM NaPPi, 50 mM NaF, 10 mM β -glycerophosphate, 1 mM Na_3VO_4 (New England Biolabs), protease inhibitor cocktail (Pierce)] on ice. Lysates were sonicated for 10 seconds and centrifuged at $18,000 \times g$ for 10 min. Protein concentrations of supernatants were determined by BCA assay (Pierce). Samples were reduced and denatured by addition of 1.25% β -mercaptoethanol in NuPage sample buffer (Invitrogen). Samples were heated for 1 min. at 95°C before SDS-PAGE. Proteins were transferred to nitrocellulose membranes, which were blocked with 5% BSA/TBS-T and probed using antibodies against P-AKT_{T308}, P-AKT_{S473}, P-S6 and Actin. Antibody binding was detected using HRP-conjugated secondary antibodies against mouse or rabbit Ig (GE Healthcare), and ECL substrate (Pierce).

Immunohistochemistry (IHC) and TUNEL

Five-micron sections of FFPE tumor tissue were used for H&E staining, IHC with Ki67 antibody (Biocare Medical), and TUNEL (Promega). For Ki67 IHC and TUNEL, 4-5 high-power (400x magnification) microscopic fields were used to count the numbers of positively-stained and total cells. Percentages of positively stained cells/field were used to calculate a single score for each tumor.

Statistical analyses

Numbers of apoptotic cultured cells, and Ki67- and TUNEL-positive tumor cells were compared between treatment groups by ANOVA with Bonferroni post-hoc test (for MCF-7 tumors), or *t*-test (for T47D/fulv-resistant tumors). Tumor volumes were expressed as percentage relative to baseline for each mouse, and analyzed by mixed modeling using JMP software, with Standard Least Squares personality, Restricted Maximum Likelihood method. Relative tumor volumes at individual time points were compared between groups by *t*-test with Sidak-Bonferroni multiple testing correction using Graphpad Prism software. $p \leq 0.05$ was considered significant.

Results:

- (i) FIG. 7: Compound A and fulvestrant in combination shows significant tumor growth inhibition in anti-estrogen-resistant ER+ breast cancer (T47D- fulvestrant resistant) xenograft model.
- (ii) FIG. 8 shows that Compound A and fulvestrant in combination inhibits mTORC1, mTORC2 and PI3K activity in anti-estrogen-resistant ER+ breast cancer (T47D- fulvestrant resistant) xenograft model.
- (iii) FIG. 9a and FIG. 9b shows significant decrease in Ki67 scores in the treatment group 2 compared with the treatment group 1.
- (iv) FIG. 9b shows significant increase in TUNEL positivity in treatment group 2 compared with treatment group 1.

Conclusion

Compound A and fulvestrant in combination shows significant tumor growth inhibition in anti-estrogen-resistant ER+ breast cancer (T47D- fulvestrant resistant) xenograft model.

Compound A in combination with fulvestrant suppresses T47D-fulvestrant resistant tumor growth by inhibiting proliferation, and increasing apoptosis.

Example 7

Effect of compound A on PI3K and mTOR signaling in human breast tumors *ex vivo*.

Breast tumor samples were obtained under an Institutional Review Board (IRB)-approved protocol, and patients provided written informed consent for study participation. Untreated primary tumors from two patients with early-stage ER+/HER2- breast cancer (confirmed by diagnostic biopsy; no neoadjuvant therapy) were surgically resected. Within 1 hour post-resection, 1-mm punch core biopsies (tool from Miltex) of tumor tissue were taken from the tumor specimen and placed into serum-free DMEM $\pm$ 200 nM the Compound A. After 6 h of *ex vivo* culture, tissue cores were snap-frozen in liquid nitrogen and stored at -80°C.

Frozen patient-derived tumor samples were homogenized in RIPA buffer and were analyzed by immunoblotting as described in Example 1.

Result: FIG. 10 depicts mTORC1, mTORC2 and PI3 kinase inhibitory activity of the Compound A.

Conclusion: Compound A inhibits mTORC1, mTORC2 and PI3 kinase activity in ER+/HER2- human breast tumors.

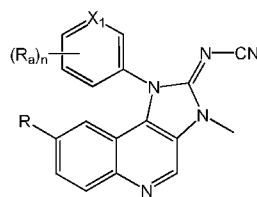
It should be noted that, as used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the content clearly dictates otherwise. It should also be noted that the term “or” is generally employed in its sense including “and/or” unless the content 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 of Formula (I)



Formula (I)

wherein,

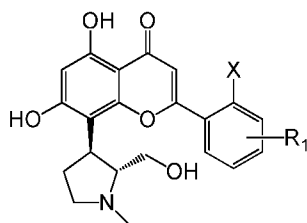
X₁ is C, CH or N;

R is pyridyl, wherein pyridyl is unsubstituted or substituted with one or more of R_b;

R_a and R_b at each occurrence are independently selected from the group consisting of halogen, -CN, -O-(C₁-C₄)alkyl, -NR_cR_d and (C₁-C₄)alkyl, wherein (C₁-C₄)alkyl is unsubstituted or substituted with one or more of halogen or -CN; and R_c and R_d are hydrogen or (C₁-C₄)alkyl;

n is 0 or an integer from 1 to 5; or a pharmaceutically acceptable salt or a solvate thereof; and

one or more anti-cancer agents selected from an estrogen antagonist and a cyclin dependent kinase inhibitor of Formula (II)



Formula (II)

wherein,

X is chlorine, bromine, fluorine or iodine;

R₁ is hydrogen, (C₁-C₄)alkyl or trifluoromethyl;

or a pharmaceutically acceptable salt or a solvate thereof.

2. A pharmaceutical combination according to claim 1, wherein the PI3K/mTOR inhibitor 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 (referred to as Compound A).

3. The pharmaceutical combination according to claims 1 or 2, wherein the anti-cancer agent is a cyclin dependent kinase inhibitor of Formula (II).
4. The pharmaceutical combination according to any one of the claims 1 or 3, wherein the cyclin dependent kinase inhibitor of Formula (II) is selected from (+)-*trans*-2-(2-Chlorophenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methylpyrrolidin-3-yl)-chromen-4-one hydrochloride or (+)-*trans*-2-(2-Chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methylpyrrolidin-3-yl)-chromen-4-one hydrochloride.
5. The pharmaceutical combination according to any one of the claims 1 to 4, wherein the compound of Formula (II) is (+)-*trans*-2-(2-Chloro-4-trifluoromethyl-phenyl)-5,7-dihydroxy-8-(2-hydroxymethyl-1-methylpyrrolidin-3-yl)-chromen-4-one hydrochloride (referred to as Compound B).
6. The pharmaceutical combination according to claim 1 or 2, wherein the anti-cancer agent is an estrogen antagonist.
7. The pharmaceutical combination according to any one of the claims 1, 2 or 6, wherein estrogen antagonist is a pure anti-estrogen.
8. The pharmaceutical combination according to any one of the claims 1, 2, 6 or 7, wherein estrogen antagonist is fulvestrant.
9. A pharmaceutical composition comprising a pharmaceutical combination according to any one of the claims 1 to 8 and at least one pharmaceutically acceptable carrier or excipient.
10. A pharmaceutical combination according to any one of the claims 1 to 8, for use in the treatment of anti-estrogen resistant ER+ breast cancer.
11. The pharmaceutical combination according to claim 10, wherein anti-estrogen resistant ER+ breast cancer is fulvestrant resistant breast cancer.

12. Use of a pharmaceutical combination according to any one of the claims 1 to 8 in the manufacture of a medicament for the treatment of anti-estrogen resistant ER+ breast cancer.

13. A method for the treatment of anti-estrogen resistant ER+ breast cancer comprising administering to a subject in need thereof a therapeutically effective amount of a PI3K/mTOR inhibitor of Formula (I) or a pharmaceutically acceptable salt or a solvate thereof; and a therapeutically effective amount of one or more anti-cancer agents selected from an estrogen antagonist and a CDK inhibitor of Formula (II) or a pharmaceutically acceptable salt or a solvate thereof.

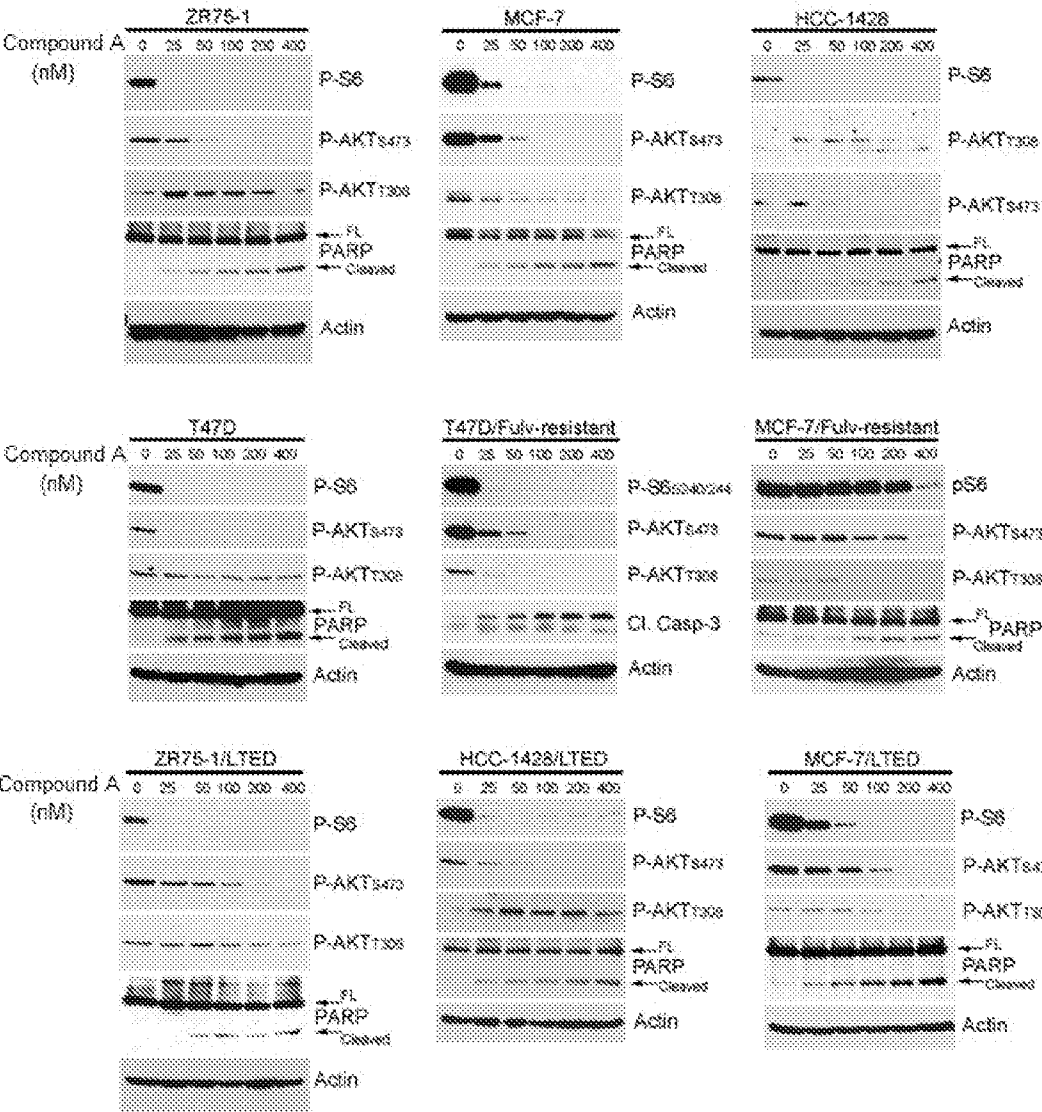


FIG. 1a

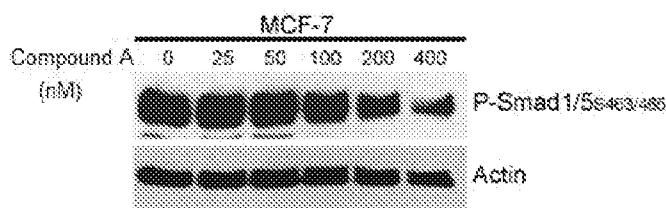


FIG. 1b

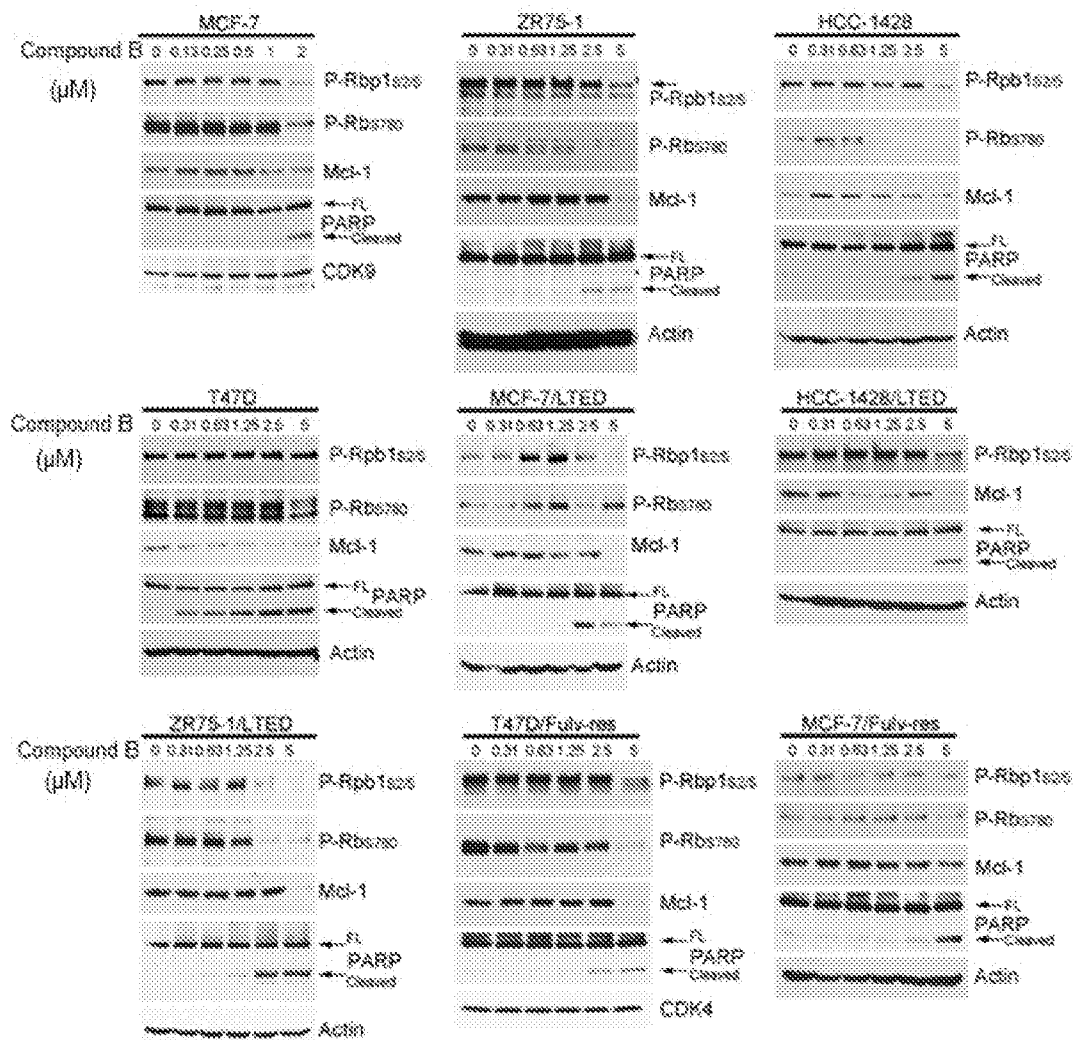


FIG. 2

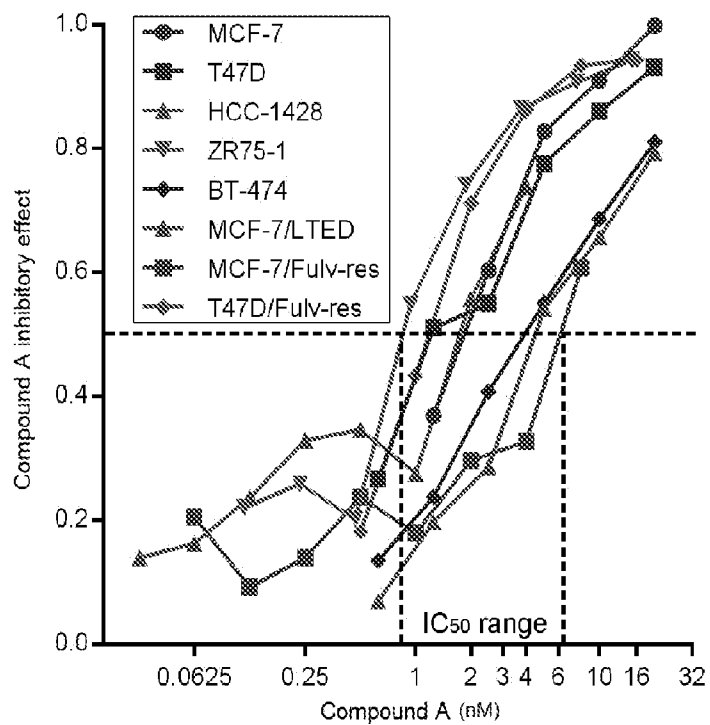


FIG. 3a

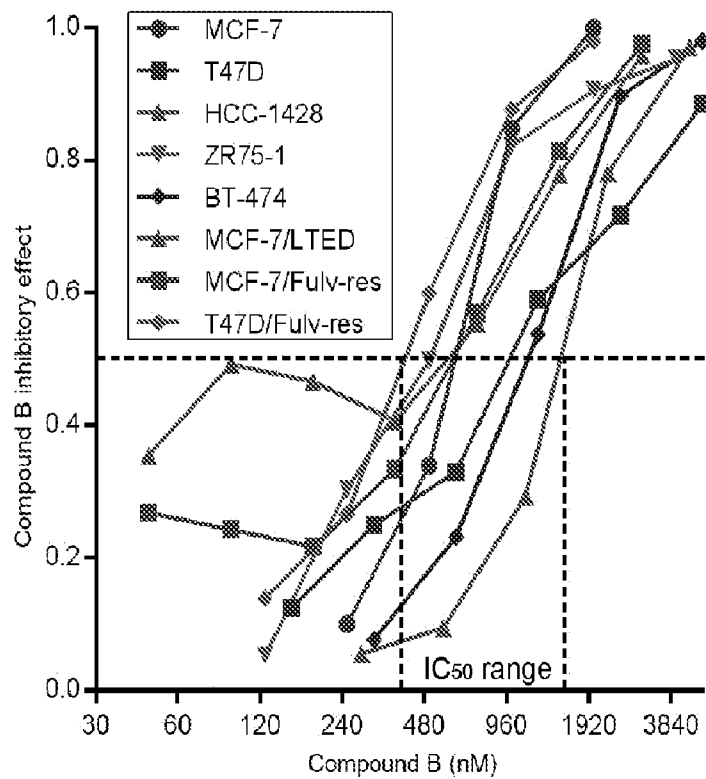


FIG. 3b

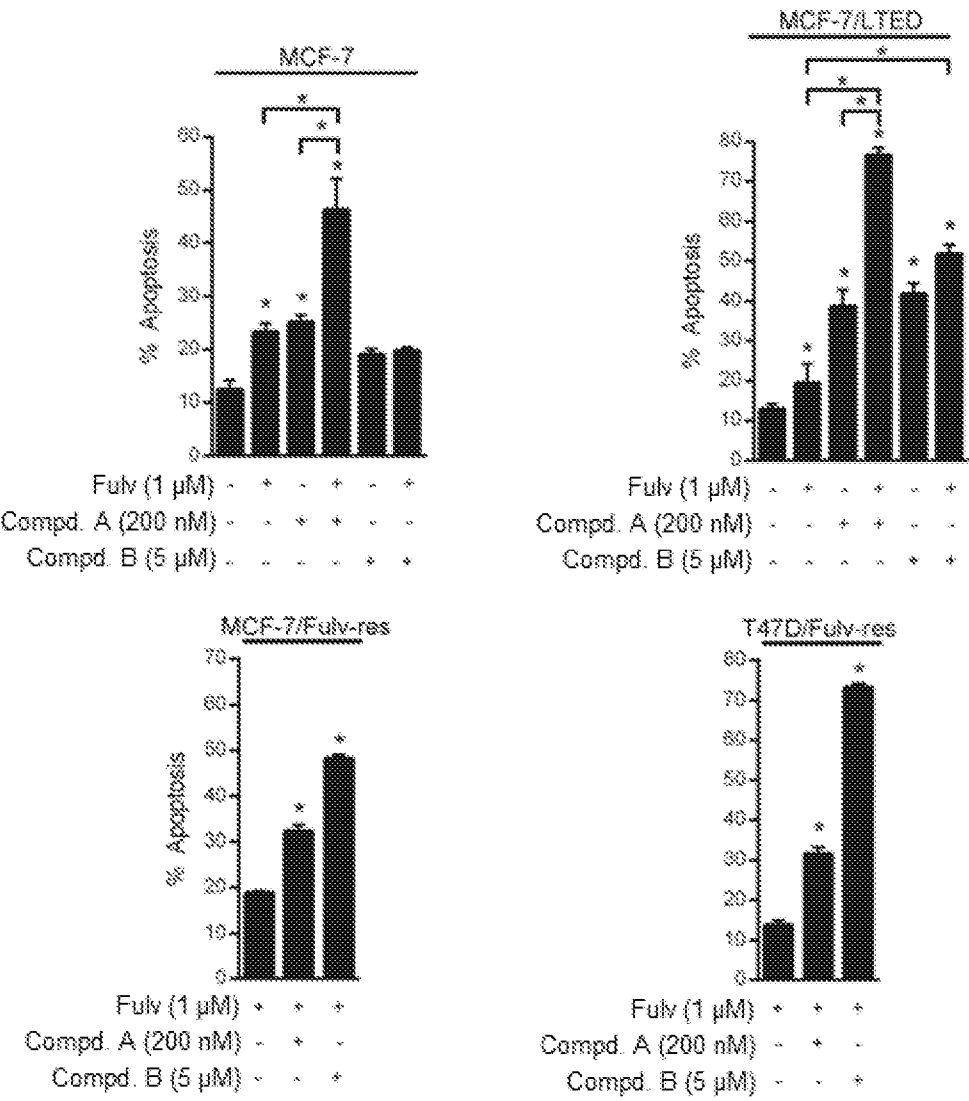


FIG. 4

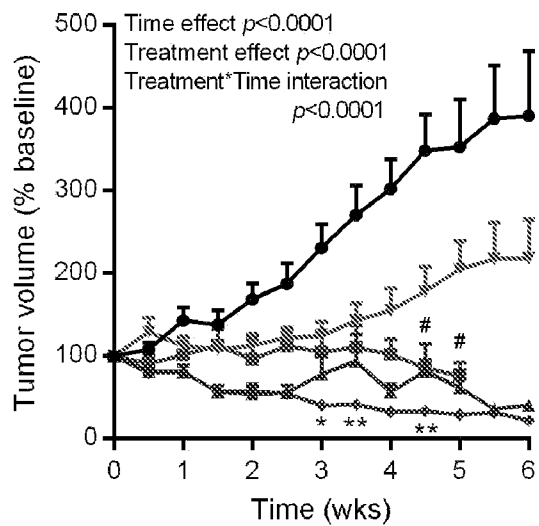


FIG. 5a

- Vehicle ($n=8$)
- Fulvestrant ($n=8$)
- ▲ 5 mg/kg/d Compd. A ($n=8$)
- ◆ Fulv + 5 mg/kg/d Compd. A ($n=9$)
- 15 mg/kg/d Compd. A ($n=7$)
- Compd. B ($n=6$)
- Compd. B + 5 mg/kg/d Compd. A ($n=13$)

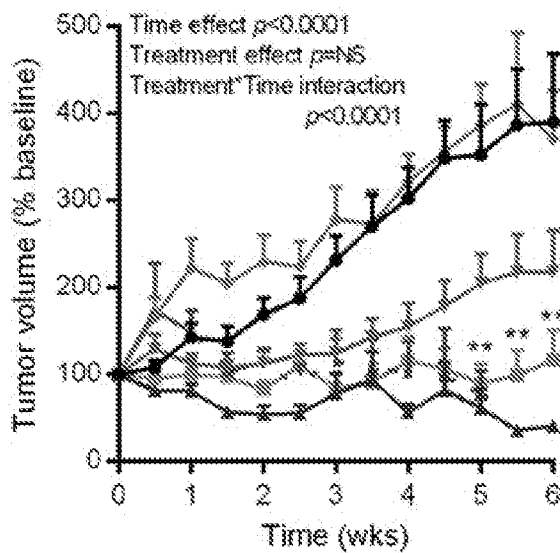


FIG. 5b

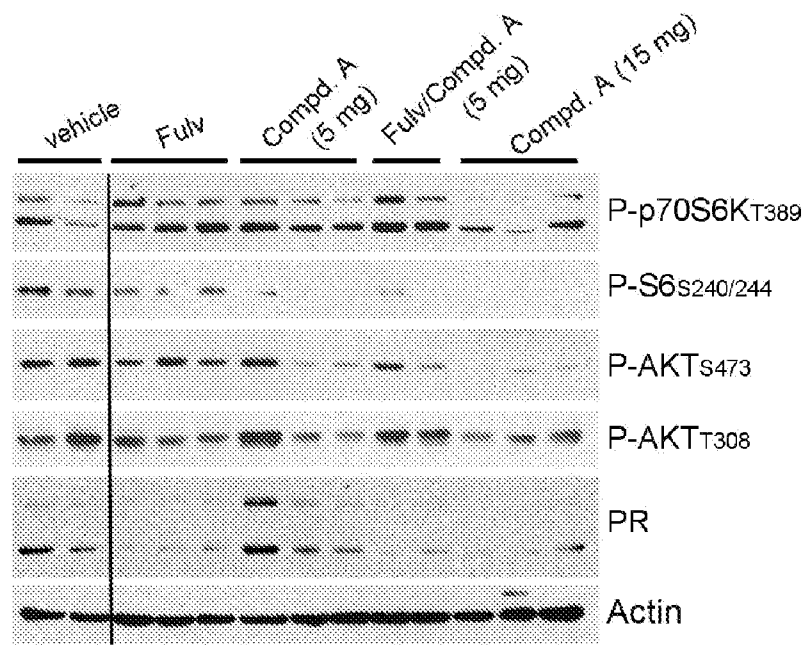


FIG. 6

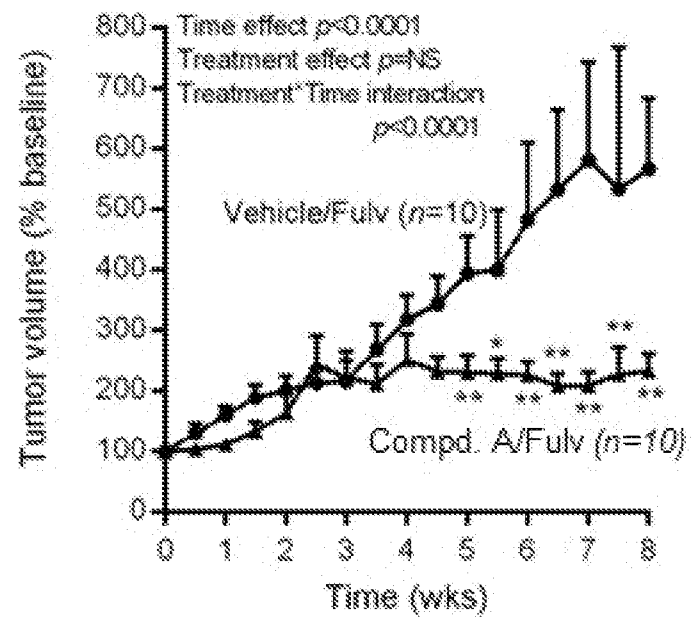


FIG. 7

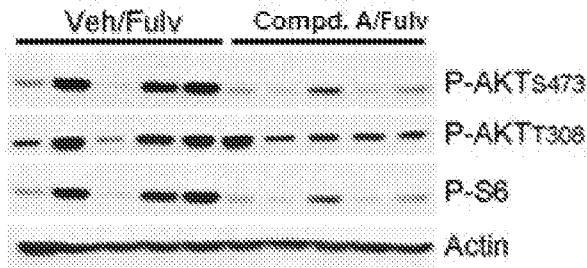


FIG. 8

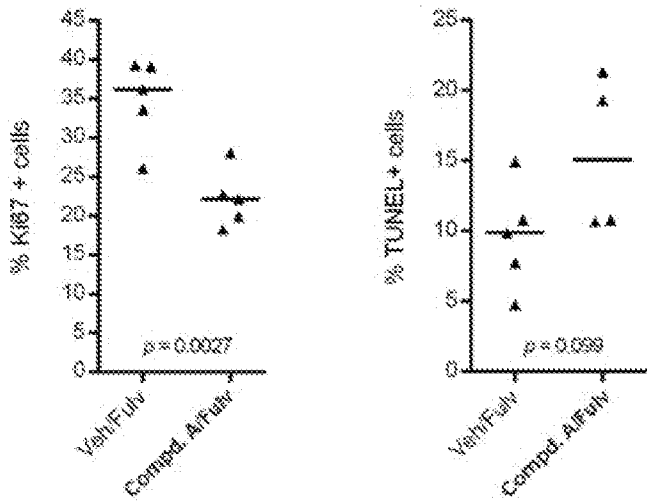


FIG. 9a

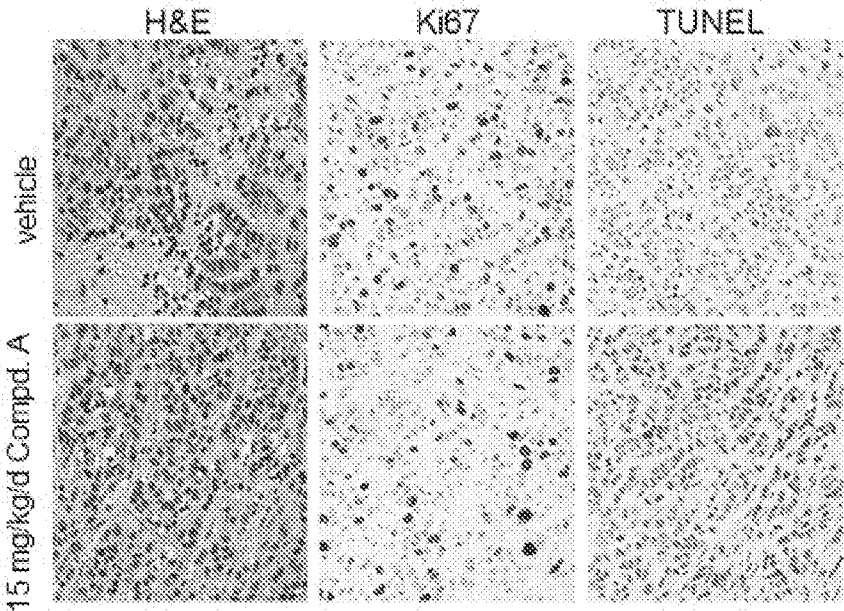


FIG. 9b

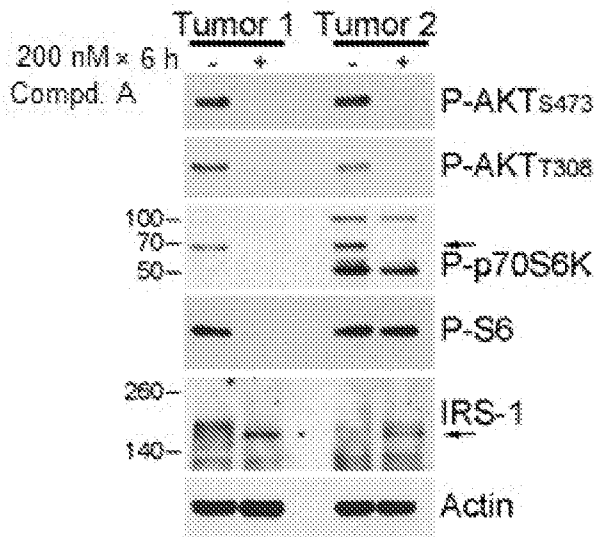


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2015/055616

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/4745 (2006.01)

A61K 31/4025 (2006.01)

A61K 31/565 (2006.01)

A61K 31/453 (2006.01)

C07D 471/02 (2006.01)

C07D 405/04 (2006.01)

C07J 31/00 (2006.01)

C07D 405/12 (2006.01)

A61P 35/00 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

ClinicalTrials.org: Keywords: panulisib, AK-151761, voruciclib, P1446A-05, fulvestrant, faslodex, RU-58668, acolbifene, EM-652.
REGISTRY, HCAPLUS: structure search based on Formula (I) and on Formula (II), 1356033-60-7/rn, 1000023-04-0/rn, 1000023-05-1/rn, panulisib, AK-151761, voruciclib, P1446A-05 & related terms and compound salts. **EPODOC, WPIAP, MEDLINE, TXTE cluster:** panulisib, AK-151761, mTOR, FRAP-1, PI3K, ER antagonist, anti-estrogen, CDK inhibitor, cancer, proliferation, carcinogenic, breast, treatment, therapy, fulvestrant, faslodex, ICI-182780, ZM-182780, RU-58668, EM-652, acolbifene & related terms. **PATENTSCOPE, ESPACENET, GOOGLE PATENTS, PUBMED, AUSPAT, IP AUSTRALIA internal databases:** Name applicant: Piramal; Inventors: AGARWAL, Veena, and MILLER, Todd; Keywords: panulisib, AK-151761, fulvestrant, faslodex, RU-58668, EM652, acolbifene, voruciclib, P1446A-05 & related terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent but published on or after the international filing date	"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
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"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
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 18 December 2015		Date of mailing of the international search report 18 December 2015	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au		Authorised officer Ross Heisey AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833185	

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/IB2015/055616
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2012/007926 A1 (PIRAMAL LIFE SCIENCES LIMITED) 19 January 2012 Abstract; description page 43, lines 4-10; Example 140, last Table; claims 13-16, 18-20	1-2, 6-7, 9-13
Y	WO 2008/076447 A2 (ORDWAY RESEARCH INSTITUTE) 26 June 2008 Abstract; page 5, lines 14-18; page 70, lines 20-34; claims 10, 14	1-2, 6-7, 9-13
Y	FOX, E.M., et al., "Abrogating endocrine resistance by targeting ER α and PI3K in breast cancer", Frontiers in Oncology, 2012, vol. 2, Article 145, pages 1-6 (http://dx.doi.org/10.3389/fonc.2012.00145) Whole document, in particular Abstract; page 2, RH col and page 4. RH col.	1-2, 6-7, 9-13
A	WO 2012/066508 A1 (PIRAMAL LIFE SCIENCES LIMITED) 24 May 2012 Abstract; page 11, lines 24-29; Examples 3-4, 6-7, 16-18; claims 7, 10	
A	WO 2012/069972 A1 (PIRAMAL LIFE SCIENCES LIMITED) 31 May 2012 Abstract; page 12, lines 9-14; Examples 3-4, 6-7, 16-17; claims 7, 10	

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/IB2015/055616	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2012/007926 A1	19 January 2012	WO 2012007926 A1	19 Jan 2012
		AR 084706 A1	05 Jun 2013
		AU 2011277935 A1	07 Mar 2013
		AU 2011277935 B2	22 Jan 2015
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		US 9062046 B2	23 Jun 2015
WO 2008/076447 A2	26 June 2008	WO 2008076447 A2	26 Jun 2008
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		US 2009170715 A1	02 Jul 2009
		US 7890267 B2	15 Feb 2011
		US 2008312199 A1	18 Dec 2008
		US 2009098538 A1	16 Apr 2009
		US 2011251087 A1	13 Oct 2011
WO 2007114896 A2	11 Oct 2007		
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		CA 2818229 A1	24 May 2012
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.
Form PCT/ISA/210 (Family Annex)(July 2009)

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/IB2015/055616	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
		CN 104220066 A	17 Dec 2014
		EP 2640380 A1	25 Sep 2013
		JP 2013542979 A	28 Nov 2013
		KR 20140014086 A	05 Feb 2014
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		TW 201304771 A	01 Feb 2013
		US 2013237582 A1	12 Sep 2013
WO 2012/069972 A1	31 May 2012	WO 2012069972 A1	31 May 2012
		TW 201307330 A	16 Feb 2013
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			