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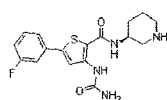
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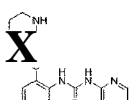
(54) Title: ADMINISTRATION OF HYPOXIA ACTIVATED PRODRUGS IN COMBINATION WITH CHK1 INHIBITORS FOR TREATING CANCER

Figure 1

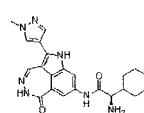
Structure of Chk1 inhibitors



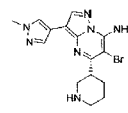
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(57) Abstract: Cancer can be treated by administration of TH-302 in combination with a Chk1 inhibitor.

**ADMINISTRATION OF HYPOXIA ACTIVATED PRODRUGS IN COMBINATION
WITH CHK1 INHIBITORS FOR TREATING CANCER**

FIELD OF THE INVENTION

[0001] The present invention provides methods for treating cancer, and pharmaceutical formulations and unit dose forms useful in those methods. The invention therefore relates to the fields of medicine and pharmacology.

BACKGROUND OF THE INVENTION

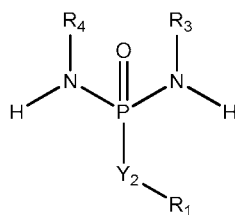
[0002] TH-302 is a hypoxia-activated prodrug in clinical development for the treatment of cancer. See PCT Publication Nos. 2007/002931; 2008/083101; 2010/048330; 2012/006032; 2012/009288; 2012/135757; 2012/142520; and US provisional patent application serial number 61/593,249 filed on 31 January 2012, each of which is incorporated herein by reference. TH-302 releases the DNA cross-linking bromo-isophosphoramidate (sometimes referred to as bromo-isophosphoramidate) mustard (Br-IPM) under hypoxic conditions. TH-302 induces G₂/M arrest at low concentrations and a pan-cell cycle arrest at high concentrations.

[0003] When DNA damage occurs, a signal transduction pathway cascade is activated in response to the damage, which transmits signals to the downstream effectors that connect with the cell cycle machinery. Checkpoint kinase 1 (Chk1) is a vital link between the upstream sensors of the DNA damage checkpoints and the cell cycle effectors. See Cancer Biology & Therapy (2004) 3:3, 305-313, incorporated herein by reference. Generally, cell cycle progression is interrupted at the stage where the cell was when injured to give the cell time to repair the damage by activating DNA damage response and repair pathways. In recent years, Chk1 inhibitors have been studied for use in combination with DNA damaging anticancer agents that cause S and G₂/M arrest in attempts to increase the efficacy of the underlying cancer treatment. However, no Chk1 inhibitor has been approved for cancer treatment alone or in combination with another anti cancer agent.

SUMMARY OF THE INVENTION

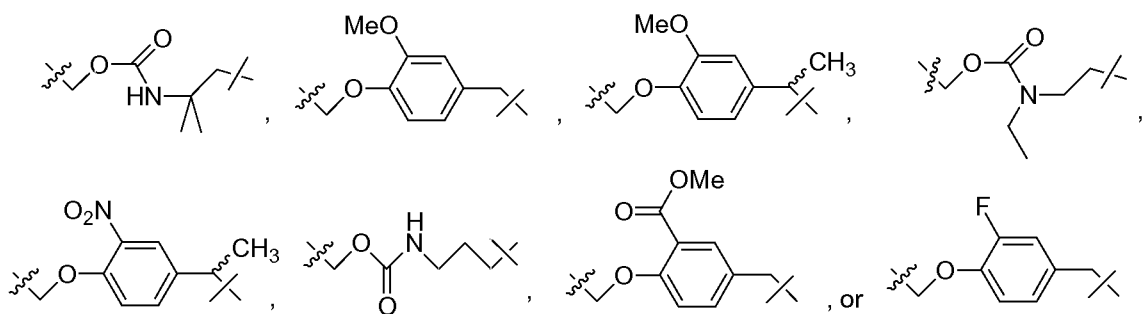
[0004] In a first aspect, the present invention provides a method of treating cancer, said method comprising administering to a patient in need of such treatment a therapeutically effective amount of a hypoxia activated prodrug in combination with a therapeutically effective amount of a Chk1 inhibitor.

[0005] In various embodiments, the hypoxia activated prodrug is a compound of Formula 1:

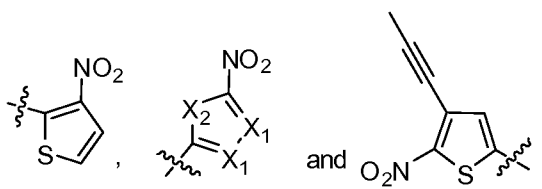


(I)

wherein Y_2 is O, S, NR_6 , $NCOR_6$, or NSO_2R_6 wherein R_6 is $Ci-C_6$ alkyl, $Ci-C_6$ heteroalkyl, aryl, or heteroaryl; R_3 and R_4 are independently selected from the group consisting of 2-haloalkyl, 2-alkylsulfonyloxyalkyl, 2-heteroalkylsulfonyloxyalkyl, 2-arylsulfonyloxyalkyl, and 2-heteroalkylsulfonyloxyalkyl; R_1 has the formula $L-Z_3$; L is $C(Zi)_2$; each Zi independently is hydrogen, halogen, $Ci-C_6$ alkyl, $Ci-C_6$ heteroalkyl, aryl, heteroaryl, C_3-C_8 cycloalkyl, heterocyclyl, $Ci-C_6$ acyl, $Ci-C_6$ heteroacyl, aroyl, or heteroaroyl; or L is:



Z_3 is a bioreductive group having a formula selected from the group consisting of:



wherein each X_i is independently N or CR_8 ; X_2 is NR_7 , S, or O; each R_7 is independently $Ci-C_6$ alkyl, $Ci-C_6$ heteroalkyl, C_3-C_8 cycloalkyl, heterocyclyl, aryl or heteroaryl; and R_8 is independently hydrogen, halogen, cyano, CHF_2 , CF_3 , CO_2H , amino, $Ci-C_6$ alkyl, $Ci-C_6$ heteroalkyl, $Ci-C_6$ cycloalkyl, $Ci-C_6$ alkoxy, $Ci-C_6$ alkylamino, $Ci-C_6$ dialkylamino, aryl, $CON(R_7)_2$, $Ci-C_6$ acyl, $Ci-C_6$ heteroacyl, aroyl or heteroaroyl; or a pharmaceutically acceptable salt thereof. In various embodiments of the invention, the compound utilized in this invention is a compound of Formula I that is TH-281, TH-302, or TH-308 (structures provided below).

[0006] In one embodiment, the hypoxia activated drug is TH-302. In various embodiments, the TH-302 is administered at a dose and frequency described in PCT Publication Nos.

2007/002931; 2008/083101; 2010/048330; 2012/006032; 2012/009288; 2012/135757; and

2012/142520, each of which is incorporated herein by reference. In some embodiments, patients

are selected for treatment and/or the efficacy of treatment is assessed in accordance with the methods described in U.S. Patent Application No. 61/593,249 filed on 31 January 2012, incorporated herein by reference.

[0007] In various embodiments, the Chkl inhibitor is an inhibitor described in Prudhomme, Recent Patents on Anti-Cancer Drug Discovery, 2006, 1, 55-68; Expert Opin. Ther. Patents (2011) 21(8): 1191-1210; and Cell Cycle (2011) 10:13, 2121-2128, each of which is incorporated herein by reference. In various embodiments, the Chkl inhibitor is selected from the group consisting of AZD7762, LY2603618, PF-00477736, and SCH 900776. See Cancer Res. (2010) 70(12): 4972 et seq.; Clin. Cancer Res. (2010) 16(7): 2076-2084; and Shibata et al, Cancer Sci. (2011), each of which is incorporated herein by reference.

[0008] In various embodiments, the patient is identified as having a p53-deficient cancer cell prior to administration of TH-302 and the Chkl inhibitor.

[0009] In one embodiment, the present invention provides a method of increasing antitumor effect of a compound of Formula I on a P53 deficient tumor cell, comprising coadministering to the tumor cell (or to a patient with a tumor) a Chkl inhibitor in combination with a compound of Formula I. As used herein, "increasing the antitumor effect" of a compound of Formula I by coadministration of a Chkl inhibitor refers to one or more of (i) increasing the number of tumor cells killed by the compound of Formula I relative to the number that would be killed in the absence of coadministration of a Chkl inhibitor; (ii) overcoming a tumor cell's resistance to a compound of Formula I; or (iii) abrogating cell-cycle arrest, for example arrest at G₂/M, in a tumor cell. Coadministration, as used herein, contemplates that the two drugs coadministered exert their pharmacological effect in a tumor cell at the same time; such coadministration can be achieved by simultaneous, contemporaneous, or sequential administration of the two drugs. In one embodiment, the Chkl inhibitor is AZD7762, PF477736, or LY603618. In another embodiment, the compound of Formula I is TH-302. In one embodiment, TH-302 is administered once daily, for five consecutive days a week. In other embodiments, TH-302 is administered no more than once a week. Within these embodiments, in some embodiments, TH-302 therapy is continued for multiple weeks.

[0010] In a second aspect, the present invention provides pharmaceutical formulations and unit dose forms suitable for use in the methods of the present invention. In one embodiment, the hypoxia activated prodrug and Chkl inhibitor are formulated separately in distinct unit dose forms. In another embodiment, the hypoxia activated prodrug and Chkl inhibitor are formulated together in an admixture or other combination pharmaceutical formulation and combination unit dose forms. In various embodiments, the hypoxia activated prodrug in the formulation and unit dose forms is TH-302.

BRIEF DESCRIPTION OF THE FIGURES

[0011] Figure 1 shows the structures of illustrative Chkl inhibitors.

[0012] Figure 2 provides a summary table of IC₅₀ values for various cell-based assays described in Examples 1 to 3 below.

[0013] Figure 3 shows the results of cell cycle and immunoblot analyses described in Example 4 below.

[0014] Figures 4A(I)-(III) show the results of daily dosing of TH-302 in the animal model study described in Example 5 below.

[0015] Figures 4B(I)-(III) show the results of intermittent dosing in the animal model study described in Example 5 below.

DETAILED DESCRIPTION OF THE INVENTION

[0016] The practice of the present invention includes the use of conventional techniques of organic chemistry, molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art.

Definitions

[0017] In this specification and in the claims that follow, reference will be made to a number of terms that have the meanings below. All numerical designations, *e.g.*, pH, temperature, time, concentration, and weight, including ranges of each thereof, are approximations that typically may be varied (+) or (-) by increments of 0.1, 1.0, or 10.0, as appropriate. All numerical designations may be understood as preceded by the term "about". Reagents described herein are exemplary and equivalents of such may be known in the art.

[0018] The singular form "a", "an", and "the" includes plural references unless the context clearly dictates otherwise.

[0019] The term "comprising" means any recited elements are necessarily included and other elements may optionally be included. "Consisting essentially of" means any recited elements are necessarily included, elements that would materially affect the basic and novel characteristics of the listed elements are excluded, and other elements may optionally be included. "Consisting of" means that all elements other than those listed are excluded. Embodiments defined by each of these terms are within the scope of this invention.

[0020] Certain terms related to Formula I are defined below.

[0021] "Acyl" refers to -CO- alkyl, wherein alkyl is as defined here.

[0022] "Aroyl" refers to -CO-aryl, wherein aryl is as defined here.

[0023] "Alkoxy" refers to -O-alkyl, wherein alkyl is as defined here.

[0024] "Alkenyl" refers to a linear monovalent hydrocarbon radical or a branched monovalent hydrocarbon radical having the number of carbon atoms indicated in the prefix and containing at least one double bond, but no more than three double bonds. For example, (C₂-C₆)alkenyl includes, ethenyl, propenyl, 1,3-butadienyl and the like. Alkenyl can be optionally substituted with substituents, including for example, deuterium ("D"), hydroxyl, amino, mono or di(Ci-C₆)alkyl amino, halo, C₂-C₆ alkenyl ether, cyano, nitro, ethynyl, Ci-C₆ alkoxy, Ci-C₆ alkylthio, -COOH, -CONH₂, mono- or di(Ci-C₆)alkylcarboxamido, -SO₂NH₂, -OSO₂-(Ci-C₆)alkyl, mono or di(Ci-C₆) alkylsulfonamido, aryl, heteroaryl, alkyl or heteroalkylsulfonyloxy, and aryl or heteroarylsulfonyloxy.

[0025] "Alkyl" refers to a linear saturated monovalent hydrocarbon radical or a branched saturated monovalent hydrocarbon radical having the number of carbon atoms indicated in the prefix. (Ci-C₆)alkyl can be optionally substituted with substituents, including for example, deuterium ("D"), hydroxyl, amino, mono or di(Ci-C₆) alkyl amino, halo, C₂-C₆ alkenyl ether, cyano, nitro, ethenyl, ethynyl, Ci-C₆ alkoxy, Ci-C₆ alkylthio, -COOH, -CONH₂, mono- or di(Ci-C₆)alkylcarboxamido, -SO₂NH₂, -OSO₂-(Ci-C₆)alkyl, mono or di(Ci-C₆) alkylsulfonamido, aryl, heteroaryl, alkylsulfonyloxy, heteroalkylsulfonyloxy, arylsulfonyloxy or heteroarylsulfonyloxy.

[0026] The prefixes (Ci-C_{qq}), Ci-_{qq}, and Ci-C_{qq}, wherein qq is an integer from 2-20, have the same meaning. For example, (Ci-Ce)alkyl, Ci-₆ alkyl, or Ci-C₆ alkyl includes methyl, ethyl, n-propyl, 2-propyl, n-butyl, 2-butyl, tert-butyl, pentyl, and the like. For each of the definitions herein (e.g., alkyl, alkenyl, alkoxy, etc.), when a prefix is not included to indicate the number of main chain carbon atoms in an alkyl portion, the radical or portion thereof will have six or fewer main chain carbon atoms.

[0027] "Alkylamino" or mono-alkylamino refers to -NH-alkyl, wherein alkyl is as defined here.

[0028] "Alkynyl" refers to a linear monovalent hydrocarbon radical or a branched monovalent hydrocarbon radical having the number of carbon atoms indicated in the prefix and containing at least one triple bond, but no more than two triple bonds. For example, (C₂-Ce)alkynyl includes, ethynyl, propynyl, and the like. Alkynyl can be optionally substituted with substituents, including for example, deuterium ("D"), hydroxyl, amino, mono or di(Ci-C₆)alkyl amino, halo, C₂-C₆ alkenyl ether, cyano, nitro, ethenyl, Ci-C₆ alkoxy, Ci-C₆ alkylthio, -COOH, -CONH₂, mono- or di(Ci-C₆)alkylcarboxamido, -SO₂NH₂, -OSO₂-(Ci-Ce)alkyl, mono or di(Ci-C₆)alkylsulfonamido, aryl, heteroaryl, alkyl or heteroalkylsulfonyloxy, and aryl or heteroarylsulfonyloxy.

[0029] "Aryl" refers to a monovalent monocyclic or bicyclic aromatic hydrocarbon radical of 6 to 10 ring atoms which is substituted independently with one to eight substituents, e.g. one, two, three, four or five substituents selected from deuterium ("D"), alkyl, cycloalkyl, cycloalkylalkyl, halo, nitro, cyano, hydroxyl, alkoxy, amino, acylamino, mono-alkylamino, di-alkylamino, haloalkyl, haloalkoxy, heteroalkyl, COR (where R is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, phenyl or phenylalkyl), $-(CR'R'')_n-COOR$ (where n is an integer from 0 to 5, R' and R'' are independently hydrogen or alkyl, and R is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl) or $-(CR'R'')_n-CONR^xR^y$ (where n is an integer from 0 to 5, R' and R'' are independently hydrogen or alkyl, and R^x and R^y are independently selected from hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl). In one embodiment, R^x and R^y together is cycloalkyl or heterocyclyl. More specifically the term aryl includes, but is not limited to, phenyl, biphenyl, 1-naphthyl, and 2-naphthyl, and the substituted forms thereof.

[0030] "Cycloalkyl" refers to a monovalent cyclic hydrocarbon radical of three to seven ring carbons. The cycloalkyl group can have one or more double bonds and can also be optionally substituted independently with one, two, three or four substituents selected from alkyl, optionally substituted phenyl, or $-C(0)R^z$ (where R^z is hydrogen, alkyl, haloalkyl, amino, mono-alkylamino, di-alkylamino, hydroxyl, alkoxy, or optionally substituted phenyl). More specifically, the term cycloalkyl includes, for example, cyclopropyl, cyclohexyl, cyclohexenyl, phenylcyclohexyl, 4-carboxycyclohexyl, 2-carboxamidocyclohexenyl, 2-dimethylaminocarbonyl-cyclohexyl, and the like.

[0031] "Dialkylamino" or di-alkylamino refers to $-N(alkyl)_2$, wherein alkyl is as defined here.

[0032] "Heteroalkyl" refers to an alkyl radical as defined herein with one, two or three substituents independently selected from cyano, $-OR^w$, $-NR^xR^y$, and $-S(0)_pR^z$ (where p is an integer from 0 to 2), with the understanding that the point of attachment of the heteroalkyl radical is through a carbon atom of the heteroalkyl radical. R^w is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, aralkyl, alkoxycarbonyl, aryloxycarbonyl, carboxamido, or mono- or di-alkylcarbonyl. R^x is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, aryl or aralkyl. R^y is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, aralkyl, alkoxycarbonyl, aryloxycarbonyl, carboxamido, mono- or di-alkylcarbonyl or alkylsulfonyl. R^z is hydrogen (provided that p is 0), alkyl, cycloalkyl, cycloalkyl-alkyl, aryl, aralkyl, amino, mono-alkylamino, di-alkylamino, or hydroxyalkyl. Representative examples include, for example, 2-hydroxyethyl, 2,3-dihydroxypropyl, 2-methoxyethyl, benzyloxymethyl, 2-cyanoethyl, and 2-methylsulfonyl-ethyl. For each of the above, R^w, R^x, R^y, and R^z can be further substituted by amino, halo, fluoro, alkylamino, di-alkylamino, OH or alkoxy. Additionally, the prefix indicating the number of carbon atoms (e.g., Ci-Cio) refers to the total number of carbon atoms in the portion of the

heteroalkyl group exclusive of the cyano, $-OR^w$, $-NR^xR^y$, or $-S(0)_pR^z$ portions. In one embodiment, R^x and R^y together is cycloalkyl or heterocyclyl.

[0033] "Heteroaryl" refers to a monovalent monocyclic, bicyclic or tricyclic radical of 5 to 12 ring atoms having at least one aromatic ring containing one, two, or three ring heteroatoms selected from N, O, or S, the remaining ring atoms being C, with the understanding that the attachment point of the heteroaryl radical will be on an aromatic ring. The heteroaryl ring is optionally substituted independently with one to eight substituents, preferably one, two, three or four substituents, selected from alkyl, cycloalkyl, cycloalkyl-alkyl, halo, nitro, cyano, hydroxyl, alkoxy, amino, acylamino, mono-alkylamino, di-alkylamino, haloalkyl, haloalkoxy, heteroalkyl, $-COR$ (where R is hydrogen, alkyl, phenyl or phenylalkyl, $-(CR'R'')_n-COOR$ (where n is an integer from 0 to 5, R' and R'' are independently hydrogen or alkyl, and R is hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, phenyl or phenylalkyl), or $-(CR'R'')_n-CONR^xR^y$ (where n is an integer from 0 to 5, R' and R'' are independently hydrogen or alkyl, and R^x and R^y are, independently of each other, hydrogen, alkyl, cycloalkyl, cycloalkyl-alkyl, phenyl or phenylalkyl). In one embodiment, R^x and R^y together is cycloalkyl or heterocyclyl. More specifically the term heteroaryl includes, but is not limited to, pyridyl, furanyl, thienyl, thiazolyl, isothiazolyl, triazolyl, imidazolyl, isoxazolyl, pyrrolyl, pyrazolyl, pyridazinyl, pyrimidinyl, benzofuranyl, tetrahydrobenzofuranyl, isobenzofuranyl, benzothiazolyl, benzoisothiazolyl, benzotriazolyl, indolyl, isoindolyl, benzoxazolyl, quinolyl, tetrahydroquinolyl, isoquinolyl, benzimidazolyl, benzisoxazolyl, benzothienyl, indazolyl, pyrrolopyrimidinyl, indoliziny, pyrazolopyridinyl, triazolopyridinyl, pyrazolopyrimidinyl, triazolopyrimidinyl, pyrrolotriazinyl, pyrazolotriazinyl, triazolotriazinyl, pyrazolotetrazinyl, hexaaza-indenyl, and heptaaza-indenyl and the derivatives thereof. Unless indicated otherwise, the arrangement of the hetero atoms within the ring can be any arrangement allowed by the bonding characteristics of the constituent ring atoms.

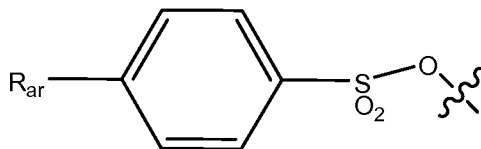
[0034] "Heterocyclyl" or "cycloheteroalkyl" refers to a saturated or unsaturated non-aromatic cyclic radical of 3 to 8 ring atoms in which one to four ring atoms are heteroatoms selected from O, NR (where R is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl), $P(=O)OR^w$, or $S(0)_p$ (where p is an integer from 0 to 2), the remaining ring atoms being C, wherein one or two C atoms can optionally be replaced by a carbonyl group. The heterocyclyl ring can be optionally substituted independently with one, two, three or four substituents selected from alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, cycloalkyl, cycloalkylalkyl, halo, nitro, cyano, hydroxyl, alkoxy, amino, mono-alkylamino, di-alkylamino, haloalkyl, haloalkoxy, $-COR$ (where R is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl), $-(CR'R'')_n-COOR$ (n is an integer from 0 to 5, R' and R'' are independently hydrogen or alkyl, and

R is hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl), or $-(CR'R'')_n-$ CONR^xR^y (where n is an integer from 0 to 5, R' and R'' are independently hydrogen or alkyl, R^x and R^y are, independently of each other, hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, phenyl or phenylalkyl). More specifically the term heterocyclyl includes, but is not limited to, tetrahydropyranyl, N-methylpiperidin-3-yl, N-methylpyrrolidin-3-yl, 2-pyrrolidon-1-yl, pyrrolidinyl, piperidinyl, morpholinyl, tetrahydrofuranyl, tetrahydrothiofuranyl, 1,1-dioxo-hexahydro-1A⁶-thiopyran-4-yl, tetrahydroimidazo[4,5-c]pyridinyl, imidazoliny, piperazinyl, and piperidin-2-yl and the derivatives thereof. The prefix indicating the number of carbon atoms (e.g., C₃-Cio) refers to the total number of carbon atoms in the portion of the cycloheteroalkyl or heterocyclyl group exclusive of the number of heteroatoms.

[0035] "Heteroacyl" refers to -CO-heteroalkyl, wherein heteroalkyl is as defined here.

[0036] "Heteroaroyl" refers to -CO-heteroaryl, wherein heteroaryl is as defined here.

[0037] "R_{sui} sulfonyloxy" refers to R_{sui}-S(=O)₂-O- and includes alkylsulfonyloxy, heteroalkylsulfonyloxy, cycloalkylsulfonyloxy, heterocyclylsulfonyloxy, arylsulfonyloxy and heteroaroylsulfonyloxy wherein R_{sui} is alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl respectively, and wherein alkyl, heteroalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are as defined here. Examples of alkylsulfonyloxy include Me-S(=O)₂-O-, Et-S(=O)₂-O-, C₂F₅-S(=O)₂-O- and the like, and examples of arylsulfonyloxy include:



wherein R_{ar} is H, methyl, or bromo.

[0038] "Substituents" refer to, along with substituents particularly described in the definition of each of the groups above, those selected from: deuterium, -halogen, -OR', -NR'R'', -SR', -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NR'S(O)₂R'', -CN, -NO₂, -R', -N₃, perfluoro(Ci-C₄)alkoxy, and perfluoro(Ci-C₄)alkyl, in a number ranging from zero to the total number of open valences on the radical; and where R', R'' and R''' are independently selected from hydrogen, Ci-g alkyl, C₃₋₆ cycloalkyl, C₂₋₈ alkenyl, C₂₋₈ alkynyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-Ci-₄ alkyl, and unsubstituted aryloxy-Ci-₄ alkyl, aryl substituted with 1-3 halogens, unsubstituted Ci-₈ alkyl, Ci-galkoxy or Ci-gthioalkoxy groups, or unsubstituted aryl-Ci-₄ alkyl groups. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 3-, 4-, 5-, 6-, or 7-membered ring. For example, -NR'R'' is meant to include 1-pyrrolidinyl and 4-morpholinyl. Other suitable substituents include each of the above aryl

substituents attached to a ring atom by an alkylene tether of from 1-4 carbon atoms. Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-T^2-C(O)-(CH_2)_q-U^3-$, wherein T^2 and U^3 are independently $-NH-$, $-O-$, $-CH_2-$ or a single bond, and q is an integer of from 0 to 2. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-A-(CH_2)_r-B-$, wherein A and B are independently $-CH_2-$, $-O-$, $-NH-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2NR'-$ or a single bond, and r is an integer of from 1 to 3. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-(CH_2)_s-X^5-(CH_2)_t-$, wherein s and t are independently integers of from 0 to 3, and X^5 is $-O-$, $-NR'-$, $-S-$, $-S(O)-$, $-S(O)_2-$, or $-S(O)_2NR'-$. The substituent R' in $-NR'-$ and $-S(O)_2NR'-$ is selected from hydrogen or unsubstituted C_{1-6} alkyl.

[0039] Certain compounds utilized in the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers, regioisomers and individual isomers (e.g., separate enantiomers) are all intended to be encompassed within the scope of the present invention. The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example, and without limitation, tritium (3H), iodine-125 (^{125}I), or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

[0040] Other terms related to this invention are defined below.

[0041] "Administering" or "administration of a drug to a patient (and grammatical equivalents of this phrase) refers to direct administration, which may be administration to a patient by a medical professional or may be self-administration, and/or indirect administration, which may be the act of prescribing a drug. For example, a physician who instructs a patient to self-administer a drug and/or provides a patient with a prescription for a drug is administering the drug to the patient.

[0042] "Cancer" refers to malignant solid tumors of potentially unlimited growth, as well as various blood cancers that may originate from cancer stem cells in the bone marrow, which can expand locally by invasion and systemically by metastasis. Examples of cancers include, but are not limited to, cancer of the adrenal gland, bone, brain, breast, bronchi, colon and/or rectum, gallbladder, gastrointestinal tract, head and neck, kidneys, larynx, liver, lung, neural tissue, pancreas, prostate, parathyroid, skin, stomach, and thyroid. Other examples of cancers include,

adenocarcinoma, adenoma, basal cell carcinoma, cervical dysplasia and in situ carcinoma, Ewing's sarcoma, epidermoid carcinomas, giant cell tumor, glioblastoma multiforma, hairy-cell tumor, intestinal ganglioneuroma, hyperplastic corneal nerve tumor, islet cell carcinoma, Kaposi's sarcoma, leiomyoma, leukemias, lymphomas, malignant carcinoid, malignant melanomas, malignant hypercalcemia, marfanoid habitus tumor, medullary carcinoma, metastatic skin carcinoma, mucosal neuroma, myelodysplastic syndrome, myeloma, mycosis fungoides, neuroblastoma, osteosarcoma, osteogenic and other sarcoma, ovarian tumor, pheochromocytoma, polycythemia vera, primary brain tumor, small-cell lung tumor, squamous cell carcinoma of both ulcerating and papillary type, seminoma, soft tissue sarcoma, retinoblastoma, rhabdomyosarcoma, renal cell tumor or renal cell carcinoma, veticulum cell sarcoma, and Wilm's tumor. Examples of cancers also include astrocytoma, a gastrointestinal stromal tumor (GIST), a glioma or glioblastoma, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), and a pancreatic neuroendocrine cancer.

[0043] "Combination therapy" or "combination treatment" refers to the use of two or more drugs in therapy, i.e., use of a hypoxia activated prodrug as described herein together with one or more Chkl inhibitors, and optionally one or more other anti cancer agent(s), to treat cancer. Administration in "combination" refers to the administration of two or more agents (e.g., a hypoxia activated prodrug and a Chkl inhibitor, and optionally one or more anti cancer agents, for treating cancer) in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Thus, administration in combination does not require that a single pharmaceutical composition, the same dosage form, or the same route of administration be used for administration of both agents or that the two agents be administered at precisely the same time. For example, and without limitation, it is contemplated that a Chkl inhibitor can be administered with a hypoxia activated prodrug in accordance with the present invention as a combination therapy.

[0044] "Hyperproliferative disease" refers to a disease characterized by cellular hyperproliferation (e.g., an abnormally increased rate or amount of cellular proliferation). Cancer is a hyperproliferative disease. Examples of hyperproliferative diseases other than cancer include, but are not limited to, allergic angitis and granulomatosis (Churg-Strauss disease), asbestosis, asthma, atrophic gastritis, benign prostatic hyperplasia, bullous pemphigoid, coeliac disease, chronic bronchitis and chronic obstructive airway disease, chronic sinusitis, Crohn's disease, demyelinating neuropathies, dermatomyositis, eczema including atopic dermatitis, eustachean tube diseases, giant cell arteritis, graft rejection, hypersensitivity pneumonitis, hypersensitivity vasculitis (Henoch-Schonlein purpura), irritant dermatitis, inflammatory hemolytic anemia, inflammatory neutropenia, inflammatory bowel disease, Kawasaki's disease,

multiple sclerosis, myocarditis, myositis, nasal polyps, nasolacrimal duct diseases, neoplastic vasculitis, pancreatitis, pemphigus vulgaris, primary glomerulonephritis, psoriasis, periodontal disease, polycystic kidney disease, polyarteritis nodosa, polyangitis overlap syndrome, primary sclerosing cholangitis, rheumatoid arthritis, serum sickness, surgical adhesions, stenosis or restenosis, scleritis, scleroderma, strictures of bile ducts, strictures (of duodenum, small bowel, and colon), silicosis and other forms of pneumoconiosis, type I diabetes, ulcerative colitis, ulcerative proctitis, vasculitis associated with connective tissue disorders, vasculitis associated with congenital deficiencies of the complement system, vasculitis of the central nervous system, and Wegener's granulomatosis.

[0045] "Hypoxia activated prodrug" refers to a drug that is less active or inactive under normoxia than under hypoxia or anoxia. Hypoxia activated prodrugs include drugs that are activated by a variety of reducing agents, including without limitation single electron transferring enzymes (such as cytochrome P450 reductases) and two electron transferring (or hydride transferring) enzymes (see U.S. Pat. App. Pub. Nos. 2005/0256191, 2007/0032455, and 2009/0136521, and PCT Pub. Nos. 2000/064864, 2004/087075, and 2007/002931, each of which is incorporated herein by reference). The hypoxia activated prodrugs useful in the methods of the present invention are compounds of Formula I, including but not limited to compounds where Z_3 , as defined by that formula, is a 2-nitroimidazole moiety. Examples of particular hypoxia activated prodrugs useful in the methods of the invention include without limitation TH-281, TH-302, and TH-308. Methods of synthesizing, formulating, and using TH-302 and other compounds of Formula I are described in the various patent publications and applications referenced in the "Background of the Invention", above, which are incorporated herein by reference.

[0046] "Chkl" or cell cycle checkpoint kinase 1 refers to serine/threonine-protein kinase that in humans is encoded by the CHEK1 gene. Chkl is a kinase that phosphorylates cdc25, an important phosphatase in cell cycle control, particularly for entry into mitosis. Cdc25 is a dual phosphatase responsible for dephosphorylation and activation of Cdc2 kinase, which is a cell cycle kinase responsible for the regulation of G2 progression and G2-M transition, in G2. Cdc25, when phosphorylated by Chkl, becomes bound by an adaptor protein in the cytoplasm. Inhibition of Cdc25 blocks cell cycle progression and, consequently, prevents a cell from entering mitosis.

[0047] "Chkl inhibitor" refers to a compound that inhibits Chkl. Without being bound by theory, Chkl inhibitors can potentiate the anti cancer efficacy of various DNA damaging agents by overriding the last checkpoint defense against DNA damaging agent-induced lethal damage. By binding to and inhibiting Chkl, Chkl inhibitors can cause tumor cells, which might

otherwise bypass Chkl -dependent cell cycle arrest in the S and G2/M phases, to undergo DNA repair prior to entry into mitosis. Tumor cells can thus be sensitized to the DNA-damaging effects of alkylating chemotherapeutic agents. Furthermore, the Chkl inhibitors' action may lead to an accumulation of damaged DNA and promote genomic instability and apoptosis of the cancer cell. Examples of Chkl inhibitors include, without limitation AZD7762, CHIR-124, LY2603618, LY2606368, PF-477736, and SCH 900776. Other examples of Chkl inhibitor are provided in Prudhomme, Recent Patents on Anti-Cancer Drug Discovery, 2006, 1, 55-68; Expert Opin. Ther. Patents (2011) 21(8): 1191-1210; and Cell Cycle (2011) 10:13, 2121-2128, each of which is incorporated herein by reference in its entirety. The structures of illustrative Chkl inhibitors are shown in Figure 1.

[0048] The term "p53 deficient" refers to a cancer cell having mutated p53 or substantially no p53. The p53 molecule (also known as protein 53 or tumor protein 53) is a tumor suppressor protein that in humans is encoded by the TP53 gene.

[0049] "Patient" or "subject" refers to mammals, particularly humans, and so includes animals of veterinary and research interest, such as simians, cattle, horses, dogs, cats, and rodents with cancer or another hyperproliferative disease.

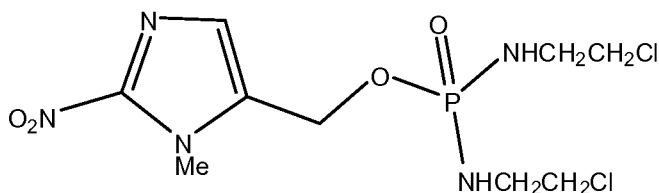
[0050] "Pharmaceutically acceptable salt" refers to pharmaceutically acceptable salts derived from a variety of organic and inorganic counter ions well known in the art that include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, and tetraalkylammonium, and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, and oxalate. Suitable salts include those described in Stahl and Wermuth (Eds.), Handbook of Pharmaceutical Salts Properties, Selection, and Use; 2002.

[0051] QnD or qnd refers to drug administration once every n days. For example, QD (or qd) refers to once every day or once daily dosing, Q2D (or q2d) refers to a dosing once every two days, Q7D refers to a dosing once every 7 days or once a week, Q5D refers to dosing once every 5 days.

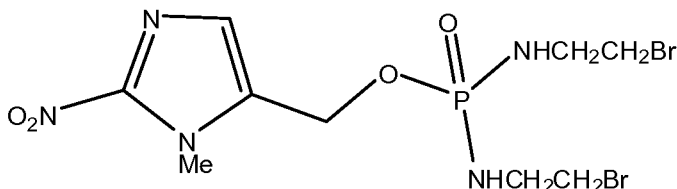
[0052] "Reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) refers to decreasing the severity or frequency of the symptom(s), or elimination of the symptom(s).

[0053] "Relapsed or refractory" refers to a type of cancer that is resistant to treatment with an agent, such a Chkl inhibitor or a hypoxia activated prodrug, or responds to treatment with an agent but recurs with or without being resistant to that agent.

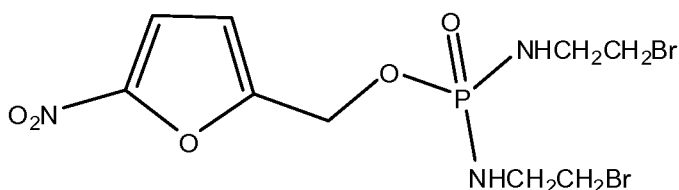
[0054] TH-281 refers to the compound of formula:



[0055] TH-302 refers to the compound of formula:



[0056] TH-308 refers to the compound of formula:



[0057] "Therapeutically effective amount" of a drug or an agent refers to an amount of the drug or the agent that, when administered to a patient with cancer or another hyperproliferative disease, will have the intended therapeutic effect, e.g., alleviation, amelioration, palliation or elimination of one or more manifestations of cancer or another hyperproliferative disease in the patient. A therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations.

[0058] "Treating" or "treatment of" a condition or patient refers to taking steps to obtain beneficial or desired results, including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation or amelioration of one or more symptoms of cancer or another hyperproliferative disease including conditional survival and reduction of tumor load or volume; diminishment of extent of disease; delay or slowing of disease progression; amelioration, palliation, or stabilization of the disease state; or other beneficial results.

[0059] The present invention arises in part from the discovery that the pharmacological inhibition of Chk1 kinase activity can potentiate the efficacy of TH-302 and other drugs of Formula I, when administered in accordance with the methods of the invention. As the examples below demonstrate, three different Chk1 inhibitors (PF477736, AZD7762 and LY2603618) in combination with TH-302 show increased anti cancer efficacy in HeLa cervical, and HT-29 colon, and not in H460 non-small cell lung cell lines. As demonstrated in a 3-day *in vitro* proliferation assay with AlamarBlue as a read-out, TH-302 activity is greatly enhanced (15

to 50 fold) by the presence of any one of these three Chkl inhibitors in the two p53-deficient cell lines (Hela and HT29) tested. In contrast, TH-302 activity is not affected by the presence of the Chkl inhibitors in the p53 wild-type line (H460). These results were confirmed in a clonogenic survival-based assay. In addition, as described in the examples below, an HT29 xenograft animal model was used to demonstrate that AZD7762 enhances the antitumor activity of TH-302.

[0060] Normal cells typically have two mechanisms for repairing DNA damage, one mediated by the p53 pathway and the other mediated by the Chkl pathway. Each of these pathways arrests the cell in its mitotic cycle so that the DNA damage can be repaired prior to cell division, during which the DNA damage, if left unrepaired, could trigger apoptotic pathways, leading to cell death. In many tumor cells, the p53 pathway is inactivated by mutation, making the tumor especially sensitive to the combination therapies of the invention. For example, more than half of all bladder, colon, head/neck, lung, and ovarian cancers have been reported to have p53 mutations (be p53-deficient). Accordingly, in some embodiments, cancer patients to be treated in accordance with the methods of the invention will not be pre-screened to determine p53 status.

[0061] However, in other embodiments of the invention, patients are screened to determine if their cancer is p53-deficient prior to administration of a combination therapy of the invention. Patients most likely to respond favorably to treatment with the combination therapies of the invention are p53-deficient. Whether p53 is present or not, whether p53 is mutated, or whether p53 is over expressed in a cancer cell is readily determined via methods well known to a skilled artisan, such as immunohistochemistry and reverse transcriptase polymerase chain reaction (RT-PCR). For example, suitable methods for assessing p53 status are described in Chiaretti et al., 2011, *Genes, Chrom. & Cancer* 50: 263-274; and Berglind et al., 2008, *Cancer Biol. & Ther.* 7: 5, 699-708, each of which is incorporated herein by reference. Moreover, commercially available test kits, such as the AmpliChip p53 Test (Roche) can be used to assess p53 status of cancer cells from a patient. In some embodiments, patients screened to be p53-deficient are administered the compound of Formula I, e.g., TH-302 in combination with a Chkl inhibitor, in accordance with the present invention.

[0062] While the invention is not to be limited by any theory of mechanism of action, TH-302 is believed to induce cell-cycle arrest at the G₂/M phase mediated by activation of Chkl and prevention of the activation of downstream Cdc2 kinase activity. The results in the examples below show that Chkl inhibitors can abrogate TH-302-induced G₂ checkpoint arrest in Hela and other p53-deficient cells. Chkl affects Cdc2 phosphorylation, and, as reported in the examples below, Cdc2 phosphorylation was evaluated in response to the Chkl inhibitor alone, TH-302

alone, and the combination of a Chkl inhibitor and TH-302. The results demonstrated that TH-302 alone, but not a Chkl inhibitor alone, increases Cdc2-Y15 phosphorylation due to the induced G₂/M arrest. In the combination study, the addition of the Chkl inhibitor blocks the TH-302-induced increase of Cdc2-Y15 phosphorylation. Taken together, the results support that efficacious treatment of cancer can be achieved by administering one or more Chkl inhibitors in combination with tumor-hypoxia targeting hypoxia activated prodrugs of Formula I, such as, TH-302, optionally in combination with other anti-cancer agents.

[0063] In various embodiments of the invention TH-302, or another compound of Formula I, is administered in combination with a Chkl inhibitor to treat cancer. Administration of a number of Chkl inhibitors, for example and without limitation, AZD7762, LY2603618, SCH 900776, PF-00477736 (see below and Figure 1) have been demonstrated for once-a-week administration. In various embodiments of the combination therapies of the invention, a Chkl inhibitor is administered, at a dosing amount reported (for example, and without limitation, see below), no more frequently than once per week, and in many of these embodiments, a patient receives multiple doses, over a period of many weeks to several months or longer, of the Chkl inhibitor. As described more fully below, TH-302 and the other compounds of Formula I can be conveniently administered at a frequency of no more than once per week, and in various embodiments of the invention, TH-302 or another compound of Formula I is administered in combination with the Chkl inhibitor administered as described above, and both compounds are administered no more frequently than once per week. In various of these embodiments, TH-302 or another compound of Formula I is administered in cycles of four weeks, in which TH-302 is administered once per week for three consecutive weeks and is not administered in the fourth week. In various of these embodiments, TH-302 or another compound of Formula I is administered in cycles of three weeks, where TH-302 is administered once per week for two consecutive weeks and is not administered in the third week or where TH-302 is administered only in the first week of the three week cycle. In any of these embodiments, where the compound is TH-302, the dose can be as described below, i.e., 240 - 670 mg/m². When a compound of Formula I and a Chkl inhibitor are coadministered on the same day, the two drugs can be administered simultaneously or one can be given before the other, i.e., a period of 30 minutes to one, two, or four hours can be allowed to lapse after the administration of the first drug before the second drug is administered.

Hypoxia activated drug administration

[0064] In one aspect, the present invention provides a method of treating cancer comprising administering a therapeutically effective amount of a hypoxia activated prodrug of Formula I

and a therapeutically effective amount of a Chkl inhibitor to a patient in need of such treatment thereby treating the cancer. In one embodiment, the combination therapy is administered to a patient that has been previously treated with a Chkl inhibitor or a hypoxia activated prodrug of Formula I, but the cancer is progressing despite the therapy, or the therapy has been discontinued due to cancer progression. In other embodiments, the patient has not been previously treated with any anti-cancer drug. In other embodiments, the patient has been previously treated with an anti-cancer drug other than a Chkl inhibitor or a hypoxia activated prodrug of Formula I.

[0065] In one embodiment, the hypoxia activated prodrug of Formula I is selected from the group consisting of TH-281, TH-302, and TH-308. In one embodiment, the hypoxia activated prodrug administered is TH-302. In various embodiments, the TH-302 or other hypoxia activated prodrug of Formula I is administered once daily, once every 3 days, weekly, or once every 3 weeks. In one embodiment, the TH-302 or other hypoxia activated prodrug of Formula I is administered parenterally. In another embodiment, the TH-302 or other hypoxia activated prodrug is administered orally (see PCT application no. PCT/US2012/033671, filed April 13, 2012, incorporated herein by reference).

[0066] In one embodiment, the hypoxia activated prodrug is TH-302, which is administered in a daily dose of about 240 mg/m² to about 670 mg/m². Suitable administration schedules for doses of TH-302 in this range include the following:

once a week at 575 mg/m²;

once every three weeks at 670 mg/m²;

days one and eight of a twenty-one day cycle at 300, 340, or 480 mg/m²;

days one, eight, and fifteen of a twenty-one day cycle at 240 or 340 mg/m²;

days one, four, eight, and eleven of a twenty-one day cycle at 240 to 480 mg/m²;

days one to five of a twenty-one day cycle at 460 mg/m²;

days one, eight, and fifteen of a twenty-eight day cycle at 240 to 575 mg/m²;

days eight, fifteen, and twenty-two of a twenty-eight day cycle at 240 to 575 mg/m², e.g. 480 mg/m², where the Chkl inhibitor administration is initiated on day one; and

once every two weeks at 240 to 670 mg/m², which may follow surgery.

[0067] Each of the above schedules can be considered a "cycle" of therapy. Patients will generally receive more than one cycle of therapy, although there may be breaks of at least a day, and more generally a week or longer, between each cycle of therapy. Other compounds of Formula I are generally dosed in accordance with the above schedules and amounts, with the amount adjusted to reflect how active the compound is relative to TH-302.

[0068] When a Chkl inhibitor is combined with a hypoxia activated prodrug according to the present invention, the Chkl inhibitor is contemplated to be administered in amounts and dosing frequencies as disclosed herein below, or in amounts and frequencies apparent to the skilled artisan in view of this disclosure, or in amounts and frequencies approved by the FDA or other regulatory authority for use in the treatment of cancer.

[0069] In various embodiments, the patient's cancer treated is a metastatic cancer or a refractory and/or relapsed cancer that is refractory to first, second, or third line treatment. In another embodiment, the treatment is a first, a second, or a third line treatment. As used herein, the phrase "first line" or "second line" or "third line" refers to the order of treatment received by a patient. First line treatment regimens are treatments given first, whereas second or third line treatment are given after the first line therapy or after the second line treatment, respectively. Therefore, first line treatment is the first treatment for a disease or condition. In patients with cancer, primary treatment can be surgery, chemotherapy, radiation therapy, or a combination of these therapies. First line treatment is also referred to those skilled in the art as primary therapy or primary treatment. Typically, a patient is given a subsequent chemotherapy regimen because the patient did not show a positive clinical or only showed a sub-clinical response to the first line therapy, or the first line treatment has stopped. In this context, "chemotherapy" is used in its broadest sense to incorporate not only classic cytotoxic chemotherapy but also molecularly targeted therapies and immunotherapies.

[0070] In another aspect, the treatment methods of the present invention are used for treating hyperproliferative diseases other than cancer.

[0071] Methods of preparation of and pharmaceutical compositions of hypoxia activated prodrugs, and other methods of treating cancer by administering various hypoxia activated prodrugs of Formula I are described in Duan et al, *J. Med. Chem.* 2008, 51, 2412-2420, and PCT Pub. Nos. 2007/002931, 2008/083101, and 2010/048330, each of which is incorporated herein by reference.

[0072] Other methods of treating cancers, which may be used in combination with the methods of the present invention, are known to one of skilled in the art, and are described, for example, in the product descriptions found in the 2010 or more current edition of the Physician's Desk Reference, Medical Economics Company, Inc., Oradell, NJ; Goodman and Gilman's The pharmacological basis of therapeutics., Eds. Hardman *et al.*, McGraw-Hill. New York. (US) 2011, 12th Ed., and in publications of the U.S. Food and Drug Administration and the NCCN Guidelines (National Comprehensive Cancer Network). Such methods can be appropriately modified by one of skill in the art, in view of this disclosure, to practice the treatment methods of the present invention.

[0073] In one embodiment, the TH-302 is provided in 100 mg vials, lyophilized, and dissolved in D5W and administered intravenously (i.v.) over approximately 30 - 60 minutes via an infusion pump. The infusion volume depends on the total dose given (in mg) during the infusion. If less than about 1000 mg is being infused, about 500 mL of D5W are used for infusion. If the total dose is greater than about 1000 mg, about 1000 mL of D5W are used for infusion.

Chkl inhibitors and their administration

[0074] The following compounds are useful for administration in combination with a hypoxia activated prodrug of Formula I according to the present invention.

[0075] AZD7762 (see Figure 1) is a Chkl inhibitor with an IC_{50} of 5 and <10 nM for Chkl and Chk2, respectively. For treating cancer, AZD7762 has previously been administered (6, 9, 14, 21, 32, 48, 64, 96, and 144 mg) by i.v. infusion in combination with irinotecan (100 or 125 mg/m^2 i.v.), and by i.v. infusion (6, 9, 14, 21, 32, 40 mg) in combination with gemcitabine (for example, at 750 mg/m^2 and 1000 mg/m^2), as described below (see, Sausville et al, J. Clin. Oncol, 29: 2011 (suppl; abstr 3058), incorporated herein by reference). Thus, AZD7762 was administered alone on days 1 and 8 (Cycle 0), and after 7 days' observation, AZD7762 was administered after irinotecan on days 1 and 8 of 2 1-day cycles. When combined with irinotecan, the dose limiting toxicity of AZD7762 was 96 mg with irinotecan at 100 mg/m^2 . At 96 mg, AZD7762 showed cardiac side effects. When combined with gemcitabine, the dose limiting toxicity of AZD7762 was 30 mg.

[0076] Accordingly, in one embodiment of the present invention, AZD7762 is administered in a daily amount of up to but not exceeding 144 mg. In other embodiments, the daily amount of AZD7762 administered is up to but not exceeding 96 mg, 64 mg, 48 mg, 32 mg, and 30 mg. Thus, in various embodiments, AZD7762 is administered in a daily amount of 6, 9, 14, 21, 32, 40, 48, 64, 96, or 144 mg. In various embodiments, AZD7762 is administered once weekly. In various embodiments, AZD7762 is administered once weekly for 2 weeks followed by a week's AZD7762 holiday. In some embodiments, AZD7762 is co-administered with irinotecan or gemcitabine in combination with a hypoxia activated prodrug of Formula I, e.g. TH-302), and the irinotecan or gemcitabine is administered at doses and frequencies described above.

[0077] LY2603618 (IC-83; see Figure 1) is a selective Chkl inhibitor. For treating solid advanced or metastatic tumors, LY2603618 (at a dose of 170 mg or 230 mg) has been administered by i.v. infusion on days 2, 9 and 16 of at least one 28-day cycle in combination with gemcitabine (dosed at 1000 mg/m^2) administered intravenously on days 1, 8 and 15 of at least one 28-day cycle. For treating pancreatic cancer, LY2603618 has been administered (at a dose of 70-300 mg/m^2 , by i.v. infusion) once weekly for 3 weeks followed by 1 week of no

treatment, repeated every 28 days for a minimum of 2 cycles in combination with gemcitabine administered 1000 mg/m^2 , i.v., once weekly for 3 weeks followed by 1 week of no treatment, repeated every 28 days for a minimum of 2 cycles. LY2603618 has also been administered to cancer patients at a dose of 40 mg/m^2 , with a possibility of dose escalation, on days 1 and 9 of cycle 1, and day 2 of subsequent cycles of unlimited 21 day cycles in combination with pemetrexed (500 mg/m^2 , i.v.) administered on day 8 of cycle 1 and on day 1 of subsequent cycles of unlimited 21 day cycles. LY2603618 has also been administered to cancer patients, after prior treatment with a 50 mg oral dose of desipramine administration on day 1 of cycle 1, at a dose of 275 mg delivered by intravenous infusion followed by a 50 mg oral dose of desipramine on day 1 of cycle 2. In these studies, additional doses of LY2603618 in combination with other agents were contemplated as follows: 1000 mg/m^2 intravenous dose of gemcitabine on days 1, 8, and 15 and 230 mg intravenous doses of LY2603618 on days 2, 9 and 16 of 28 day cycles; and 500 mg/m^2 intravenous dose of pemetrexed on day 1 and 275 mg intravenous dose of LY2603618 on day 2 of 21 day cycles. In accordance with the methods of the invention, a hypoxia activated prodrug of Formula I, e.g. TH-302, can be administered in combination with LY2603618 administered at any of the foregoing doses and dosing frequencies and with or without co-administration of desipramine, pemetrexed, and gemcitabine.

[0078] Accordingly, in one embodiment of the present invention, LY2603618 is administered in a daily amount of $40\text{-}300 \text{ mg/m}^2$ (about 70-510 mg) in combination with a hypoxia activated prodrug of Formula I. In various embodiments, LY2603618 is administered in a daily amount of 70, 170, 230, or 275 mg. In various embodiments, LY2603618 is administered in a daily amount of $70\text{-}300 \text{ mg/m}^2$ or about 120-510 mg. In various embodiments, LY2603618 is administered once weekly. In various embodiments, LY2603618 is administered for 3 weeks followed by a week of no administration of LY2603618. In other embodiments, LY2603618 is administered once every three weeks.

[0079] CHIR-124, which inhibits Chkl with an IC_{50} of 0.3 nM, is another Chkl inhibitor useful in accordance with the methods of the present invention.

[0080] SCH 900776 inhibits Chkl, cyclin dependent kinase 2 (CDK2), and Chk2 with IC_{50} values of 3 nM, 0.16 μM , and 1.5 μM , respectively, and so is another Chkl inhibitor useful in accordance with the methods of the present invention. For treating advanced solid tumors, SCH 900776 has been administered at 10, 20, 40, 80, and 112 mg/m^2 alone and following gemcitabine (800 mg/m^2) administration on days 1 and 8 every 21 days.

[0081] Accordingly, in one embodiment of the present invention, SCH 900776 is administered in a daily amount of up to 112 mg/m^2 (e.g. up to 190 mg) in combination with a hypoxia activated prodrug of Formula I, e.g. TH-302. In some embodiments, gemcitabine is also co-

administered. In various embodiments, the daily amount of SCH 900776 administered is 10, 20, 40, 80, or 112 mg/m² (e.g. 17, 34, 68, 136, or 190 mg). In various embodiments, SCH 900776 is administered once weekly. In various embodiments, SCH 900776 is administered in a 3 week cycle, once weekly for two weeks, followed by a week of no treatment with SCH 900776.

[0082] PF-00477736 (PF477736) is a Chkl inhibitor useful in accordance with the methods of the present invention. In some embodiments, Chkl and a compound of Formula I, e.g., TH-302, are co-administered to treat cancer, and in other embodiments, additional anti-cancer agents are used. For example, PF-00477736 can be used for treating advanced solid tumors when administered in combination with a compound of Formula I, e.g., TH-302, and gemcitabine. In any of these embodiments escalating doses of PF-00477736 or any dose in the range of 750 to 1250 mg/m² can be administered intravenously on days 2 and 9 and gemcitabine administered intravenously on days 1 and 8 of a 21-day cycle, in combination with a compound of Formula I, e.g., TH-302, administered as described herein. If a patient is administered a dose at Cycle 0, then, in some embodiments, PF-00477736 is administered intravenously on days 1 and 8 of a 21-day cycle for patients who have a 3-hour infusion, and on days 1 and 8 of a 14-day cycle for patients who have a 24-hour infusion, and gemcitabine is administered intravenously on days 1 and 8 of a 21-day cycle.

[0083] Accordingly, in one embodiment of the present invention, PF-00477736 is administered in a daily amount of up to 1250 mg/m² (e.g. up to 2125 mg) in combination with a hypoxia activated prodrug of Formula I, e.g. TH-302. In some embodiments, gemcitabine is also co-administered. In various embodiments, the daily amount of PF-00477736 administered is 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, or 1250 mg/m² (e.g. from 1275 to 2125 mg in increments of 85 mg). In various embodiments, PF-00477736 is administered once weekly. In various embodiments, PF-00477736 is administered in a 3 week cycle, once weekly for two weeks, followed by a week of no treatment with PF-00477736, or is administered in a 2 week cycle, once weekly for two weeks. PF-00477736 can also be used for treating Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL), diffuse large B-cell lymphoma, and such other types of blood cancers in combination with hypoxia activated prodrugs of Formula I.

[0084] Thus, in accordance with the present invention, TH-302 or another compound of Formula I is co-administered with a Chkl inhibitor, optionally in combination with other treatments. A synergistic effect may be achieved by using more than one compound in a pharmaceutical composition of the invention, i.e. a compound of Formula I is combined with at least another agent as active ingredient, which is either another compound of Formula I, or a Chkl inhibitor, or both, or another anti-cancer agent. The active ingredients useful in the methods of the invention can be used either simultaneously (as in an admixed formulation) or

sequentially. Thus, the invention also relates to a compound or pharmaceutical composition for inhibiting abnormal cell growth or cancer in a mammal which comprises an amount of a compound of Formula I, or a pharmaceutically acceptable salt or solvate or prodrug thereof, in combination with an amount of another Chkl inhibitor (and optionally another anti-cancer therapeutic), wherein the amounts of the compound, salt, solvate, or prodrug, and of the Chkl inhibitor (and of the another anti-cancer therapeutic) are together effective in inhibiting abnormal cell growth or cancer in a patient. The combination therapies described herein are thus suitable for use in combination with known anti-cancer agents.

[0085] The invention also relates to a set of items, which may be packaged into a kit, consisting of separate packs of an effective amount of a compound of Formula I and a Chkl inhibitor (or pharmaceutically acceptable salts, derivatives, solvates, and stereoisomers thereof, including mixtures thereof in all ratios, and optionally an effective amount of a further medicament active ingredient). The set or kit comprises suitable containers, such as boxes, individual bottles, bags, or ampoules. The set may, for example, comprise separate ampoules, each containing an effective amount of a compound of Formula I and a Chkl inhibitor (or pharmaceutically acceptable salts, derivatives, solvates, and stereoisomers thereof, including mixtures thereof in all ratios, and optionally an effective amount of a further medicament active ingredient), each in dissolved or lyophilized form. The set or kit of the invention may also contain an article that contains written instructions or directs the user to written instructions that explain the how the compounds are administered in accordance with the invention to treat a disease, such as cancer.

[0086] The invention also relates to the use of compounds of Formula I and Chkl inhibitor compounds and/or physiologically acceptable salts thereof for the prophylactic or therapeutic treatment and/or monitoring of diseases, such as cancer, that are caused, mediated, and/or propagated by abnormal cellular proliferative activity.

[0087] Furthermore, the invention relates to the use of compounds of Formula I and Chkl inhibitors and/or physiologically acceptable salts thereof for the production of a medicament for the prophylactic or therapeutic treatment and/or monitoring of diseases, such as cancer, that are caused, mediated, and/or propagated by abnormal cellular proliferative activity.

[0088] Compounds of Formula I and Chkl inhibitors and/or a physiologically acceptable salt thereof can also be employed as intermediates for the preparation of further medicament active ingredients. The medicament is preferably prepared in a non-chemical manner, e.g. by combining the active ingredient with at least one solid, fluid and/or semi-fluid carrier or excipient, and optionally in conjunction with a single or more other active substances in an appropriate dosage form.

[0089] Another object of the present invention are compounds of Formula I and Chkl inhibitors according to the invention and/or physiologically acceptable salts thereof for use in the prophylactic or therapeutic treatment and/or monitoring of diseases, such as cancer, that are caused, mediated, and/or propagated by abnormal cellular proliferative activity. Another preferred object of the invention concerns compounds of Formula I and Chkl inhibitors according to the invention and/or physiologically acceptable salts thereof for use in the prophylactic or therapeutic treatment and/or monitoring of hyperproliferative disorders, including cancer.

[0090] The prior teaching of the present specification concerning the compounds of Formula I and Chkl inhibitors, including any preferred embodiment thereof, is valid and applicable without restrictions to the compounds Formula I and Chkl inhibitors and their salts for use in the prophylactic or therapeutic treatment and/or monitoring of hyperproliferative disorders.

[0091] The following examples illustrate various aspects and embodiment of the invention.

EXAMPLES

Example 1. Enhanced TH-302 activity in both p53 deficient Hela cells and HT29 cells by CHK1 inhibitors.

[0092] Cells were treated with TH-302 and a Chkl inhibitor (0.4 μ M PF477736, 0.1 μ M AZD7762, or 0.5 μ M LY2603618) for 2 hours under either normoxia or hypoxia. After a wash to remove drugs, cells were incubated with fresh media at 37°C for an additional 3 days in the presence of a Chkl inhibitor. Cell viability was determined using AlamarBlue. For a doxorubicin group (doxorubicin also induces cell cycle arrest in the G2/M phase and was used as a non-hypoxia activated prodrug control), cells were co-treated with doxorubicin and Chkl inhibitors for 3 days. The results demonstrated that TH-302 activity in both Hela cells and HT29 cells was significantly enhanced by the presence of Chkl inhibitors. A similar enhancement of doxorubicin activity was also observed in the presence of a Chkl inhibitor. The IC₅₀ values in μ M obtained are provided in Figures 2.

Example 2. TH-302 activity is not altered by Chkl inhibitor in p53 heterozygous DU145 and p53 wild type H460 cells.

[0093] Appropriate DU145 and H460 cells were treated with TH-302 and Chkl inhibitors as described in Example 1. The results demonstrated that TH-302 activity in both DU145 cells and H460 cells was not enhanced by the presence of Chkl inhibitors. A similar lack of enhanced activity of doxorubicin activity was also observed in the presence of a Chkl inhibitor. These results are consistent with the presence in these cells of a functional p53 pathway for mediating DNA repair. The IC₅₀ values in μ M obtained are provided in Figure 2.

Example 3. Enhanced TH-302 activity by Chkl inhibitors in p53 deficient cells but not in p53 proficient cells.

[0094] Cells (provided by Horizon Discovery Ltd. and generated using Horizon's adeno-associated virus (AAV) technology Genesis™) were treated as described in Example 1. The results demonstrated that TH-302 activity was significantly enhanced by co-treatment with a Chkl inhibitor in p53 deficient cells but not p53 proficient cells. Similar results were obtained for doxorubicin. The IC₅₀ values in μ M obtained are provided in Figures 2.

Example 4. Chkl inhibitor abrogates TH-302-mediated cell-cycle arrest and Cdc2-Y15 phosphorylation.

[0095] HeLa cells were treated with TH-302 and a Chkl inhibitor as described in Example 1. Cells were harvested and analyzed for cell cycle distribution by flow cytometry and for protein expression by immunoblot. The results demonstrated that TH-302 exhibited concentration-dependent cell cycle arrest at G₂/M, and the G₂/M arrest-mediated by TH-302 was abrogated by the Chkl inhibitor, supporting Chkl kinase's role in TH-302-mediated G₂/M arrest.

Immunoblot data demonstrated DNA-damaging agents TH-302 and doxorubicin upregulate phosphorylation of Cdc2-Y15, and the Chkl inhibitor completely eliminated the DNA damaging agent-induced increase of Cdc2-Y15 phosphorylation. The Chkl inhibitor alone did not affect Cdc2-Y15 phosphorylation.

Example 5. Enhancement of TH-302's antitumor activity by AZD7762 in HT29 xenografts.

[0096] HT-29 tumor-bearing mice were treated with TH-302 alone, AZD7762 alone, and TH-302 in combination with AZD7762. TH-302 was dosed intraperitoneally (i.p.) at either 50 mg/kg, QD x 5/wk x 2 wks (daily dosing), or 100 mg/kg, twice/wk x 2 wks (intermittent dosing). AZD7762 was dosed i.p. at either 12.5 mg/kg, QD x 5/wk x 2 wks, or 12.5 mg/kg, four times/wk x 2 wks. For the combination therapy, two dose regimens were used: TH-302 at QD x 5/wk x 2 wks and AZD7762 at 12.5 mg/kg, QD x 5/wk x 2 wks, with AZD7762 dosed 4 hours after TH-302 administration; and TH-302 at 100 mg/kg, twice/wk x 2 wks and AZD7762 at 12.5 mg/kg, four times/wk x 2 wks, with AZD7762 dosed 4 hours and 16 hours after TH-302. The data (shown in Figures 4A-B) demonstrated that TH-302 alone or AZD7762 alone produced a growth delay, and that the combination of AZD7762 with TH-302 significantly prolonged the time required for tumor volume doubling relative to either drug alone.

[0097] These examples demonstrate that TH-302 activity is significantly enhanced by co-administration with a Chkl inhibitor in p53 deficient tumor cell lines but not in p53 proficient cell lines; that Chkl inhibitors can abrogate TH-302-mediated cell-cycle arrest; that TH-302 can increase phosphorylation of Cdc2-Y15 but does not affect the expression of Cdc-2, Mytl and Chkl; that a Chkl inhibitor can completely eliminate TH-302-induced increase of Cdc2-Y15

phosphorylation; and that co-administration of a Chkl inhibitor significantly enhances TH-302-mediated anti-tumor activity in HT29 xenografts.

Example 6. Effect of Combination Therapy on DNA Damage in Cells.

[0098] The preceding examples show that Chkl inhibitors are TH-302 sensitizers both *in vitro* and *in vivo* in the context of p53 checkpoint deficiency. To assess the impact of the Chkl inhibitor AZD7762 on DNA damage in TH-302 treated cells directly, the single-cell electrophoresis-based 'Comet' assay was employed. After seeding HT29 cells at 1×10^6 cells/3 ml/60 mm dish for 24 hr, TH-302 (at the indicated concentrations below) and 0.1 μM of AZD7622 were added and incubated for 24 hr either under air or 0.1% O_2 . Cells were washed twice to remove the compounds. The Comet assay was performed with Trevigen's single-cell electrophoresis system (Gaithersburg, MD). The olive tail moment was calculated using Comet Assay IV software from Perceptive Instruments (Suffolk, UK). The olive tail moment is the distance of DNA migration from the body of the nuclear core and provides an evaluation of the extent of DNA damage. HT29 cells treated (for 24 hr) with vehicle, TH-302 (10 μM with air; 2.5 μM with 0.1% O_2), or AZD7762 (0.1 μM with air) exhibited no visible tail moment (observed tail moment values of from 0.1 to 0.3). In contrast, cells treated with both AZD7762 and TH-302 exhibited an olive tail moment, indicating the induction of double-strand breaks: olive tail moments ranged from 3 (2.5 μM TH-302 and 0.1 μM AZD7762 in air) to 25 (10 μM TH-302 and 0.1 μM AZD7762 in 0.1% O_2).

[0099] To determine the effect of AZD7762 on TH-302-induced DNA damage response, γH2AX was evaluated. HT29 cells were treated with vehicle or TH-302 for 2 hr under either exemplary normoxic (95% O_2 /5% CO_2 , TH-302 at 0-50 μM) or exemplary hypoxic (90% N_2 /5% CO_2 /5% H_2 ; TH-302 at 0-5 μM) conditions with or without 0.1 μM of AZD7762. After washing, cells were continuously incubated for additional 4 hr in the presence of 0.1 μM AZD7762. HT29 cells were permeabilized with 1% Triton X-100 and incubated with γH2AX monoclonal antibody for 2 hr and goat anti-mouse-FITC for 1 hr. Cells were imaged using a fluorescent microscope (Nikon TS-100). The results showed that, in $\text{p53}^{-/-}$ HT29 cells, TH-302 alone and AZD7762 alone could induce γH2AX . However, treatment with both AZD7762 and TH-302 greatly increased γH2AX staining, demonstrating a greater DNA damage response with the combination therapy.

[0100] Induction of apoptosis was also assessed. HT29 cells were seeded at a density of 40,000 cells per well of a 24 well plate. After 24 hr incubation for cell attachment, cells were exposed to TH-302 (0-10 μM under air and 0-1 μM under N_2) and 0.1 μM of AZD7762 for 2 hr. After washing, cells were continuously cultured for an additional 46 hr in the presence of 0.1 μM of AZD7762. A luminescence-based caspase activity assay was performed. TH-302 did not

induce apoptosis in HT29, while AZD7762 caused a slight increase in apoptosis (Caspase Glo3/7 Assay System, Promega). However, treatment with both TH-302 and AZD7762 induced a 3-fold increase in apoptosis, indicating that the Chk1 inhibitor AZD7762 sensitizes HT29 cells, for example, to apoptosis in response to the DNA damage induced by TH-302. To validate the specificity of apoptotic signal, the pan-caspase inhibitor ZVAD was included in the testing. ZVAD reduced the apoptotic signal generated by the combination therapy, consistent with the apoptosis induced being a caspase-dependent process.

[0101] To explore involved signaling pathways, an immunoblot analysis was conducted. HT29 cells were seeded in p60 dishes at a density of 2×10^6 cells per dish and incubated overnight for cell attachment. The following day, cells were exposed to TH-302 (50 μM in air and $1 \mu\text{M}$ in N_2), 0.1 μM AZD7762 or the combination of TH-302 and AZD7762 for 2 hr under either normoxia (air) or anoxia (N_2). After TH-302 was removed by washing, cells were continuously incubated with AZD7762 for 22 hours and then lysed with a protease inhibitor cocktail and brief sonication. Insoluble debris was pelleted and the protein concentration of the resulting supernatant determined using a Sigma BCA kit (St. Louis, MO). About 10 μg of protein from each sample were loaded onto and SDS gel and electrophoresed and then electrotransferred to a nitrocellulose membrane. Proteins were detected with an electrochemiluminescent detection system (ChemiGlow; Protein Simple, Santa Clara, CA) using antibodies recognizing Histone H3. An increase in phospho-histone H3 (at serine 10) was observed in the combination treatment group but not in the TH-302 treated or AZD7762-treated samples, consistent with DNA damaged cells undergoing mitotic catastrophe (TH-302 causes a G2 cell cycle arrest, and AZD7762 unblocks the G2 arrest). Equal loading was confirmed by actin blot, which showed even intensities across the samples.

[0102] To investigate which DNA repair system is affected by Chk-1 inhibitor-mediated enhancement of TH-302 cytotoxicity, CHO cell-based DNA repair mutant cell line pairs were utilized. The experimental details were as described in Example 1. The data showed that potentiation of TH-302 cytotoxicity by AZD7762 (0.1 μM) was observed only in cell lines proficient in HDR (AA8 CHO cells; TH-302 IC_{50} was 260 μM in air and 1.2 μM in N_2) and not cell lines deficient in HDR (cell lines irs1SF and UV41 : TH-302 IC_{50} was 63 μM (irs1SF) and 26 μM (UV41) in air and 0.3 μM (irs1SF) and 0.06 μM (UV41) in N_2). HDR (homology-dependent repair) is the specific type of DNA repair capable of repairing TH-302-induced DNA damage. The IC_{50} of TH-302 monotherapy in AA8, irs1SF, and UV41 cell lines in air is 490 μM , 54 μM , and 29 μM , respectively, and in N_2 is 3.2 μM , 0.4 μM , and 0.07 μM , respectively.

[0103] To explore the role of HDR in AZD7762-mediated potentiation of TH-302 cytotoxicity further, the effects of AZD7762 on Rad51, an enzyme that coordinates recombination and that is

important to the process of error-free DNA repair by recombination, levels were examined. The samples were collected as described above for the Histone H3 blot. In response to TH-302, Rad51 expression levels were increased. However, the combination treatment with AZD7762 and TH-302 led to Rad51 expression levels lower than those observed in control cells. The results are consistent with down-regulation of Rad51-dependent HDR by AZD7762 resulting in the persistence of unrepaired DNA damage caused by TH-302, providing a mechanism for potentiation of TH-302 cytotoxicity by AZD7762.

[0104] The data presented in this example support a new approach for the treatment of cancer in which a Chk1 inhibitor is administered in combination with a tumor-hypoxia targeted prodrug such as TH-302 or another compound of Formula I to provide more efficacious therapy.

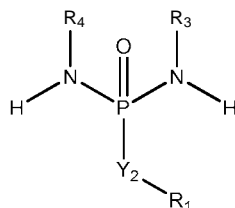
[0105] It should be understood that although the present invention has been specifically disclosed by certain aspects, embodiments, and optional features, modification, improvement and variation of such aspects, embodiments, and optional features can be resorted to by those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this disclosure.

[0106] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

CLAIMS

1. A method of treating cancer, said method comprising administering to a patient in need of such treatment a therapeutically effective amount of a hypoxia activated prodrug in combination with a therapeutically effective amount of a Chkl inhibitor.

2. The method of claim 1, wherein the hypoxia activated prodrug is a compound of Formula I:



(I)

wherein

Y_2 is O, S, NR_6 , $NCOR_6$, or NSO_2R_6

R_6 is $Ci-C_6$ alkyl, $Ci-C_6$ heteroalkyl, aryl, or heteroaryl;

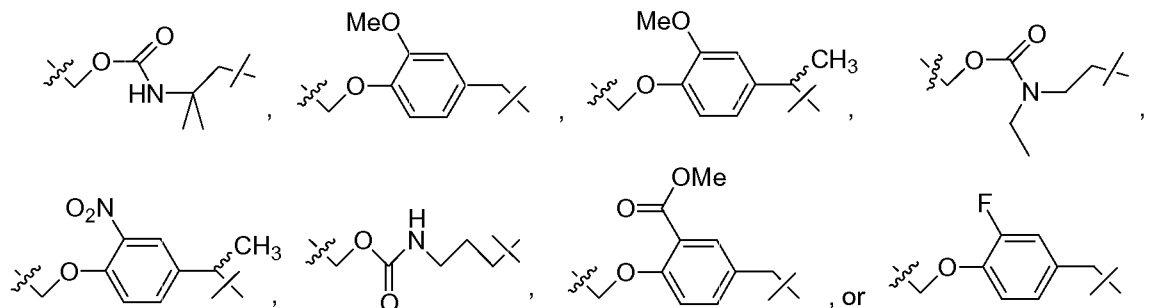
R_3 and R_4 are independently selected from the group consisting of 2-haloalkyl, 2-alkylsulfonyloxyalkyl, 2-heteroalkylsulfonyloxyalkyl, 2-arylsulfonyloxyalkyl, and 2-heteroalkylsulfonyloxyalkyl;

R_1 has the formula $L-Z_3$;

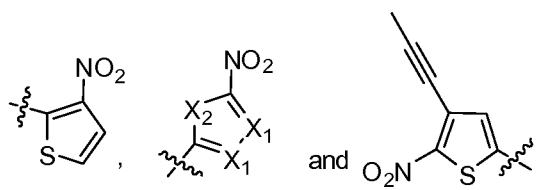
L is $C(Z_i)_2$;

each Z_i independently is hydrogen, halogen, $Ci-C_6$ alkyl, $Ci-C_6$ heteroalkyl, aryl, heteroaryl, C_3-C_6 cycloalkyl, heterocyclyl, $Ci-C_6$ acyl, $Ci-C_6$ heteroacyl, aroyl, or heteroaroyl;

or L is:



Z_3 is a bioreductive group having a formula selected from the group consisting of:



each X_i is independently N or CR₈;

X₂ is NR₇, S, or O;

each R₇ is independently Ci-C₆ alkyl, Ci-C₆ heteroalkyl, C₃-C₈ cycloalkyl, heterocyclyl, aryl or heteroaryl;

and R₈ is independently hydrogen, halogen, cyano, CHF₂, CF₃, CO₂H, amino,

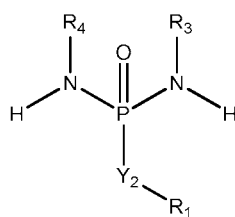
Ci-C₆ alkyl, Ci-C₆ heteroalkyl, Ci-C₆ cycloalkyl, Ci-C₆ alkoxy, Ci-C₆

alkylamino, Ci-C₆ dialkylamino, aryl, CON(R₇)₂, Ci-C₆ acyl, Ci-C₆ heteroacyl,

aroyle or heteroaroyle;

or a pharmaceutically acceptable salt thereof.

3. The method of claim 1 or 2, wherein said compound of Formula I is TH-302.
4. The method of any one of claims 1-3, wherein said Chkl inhibitor is selected from the group consisting of AZD7762, LY2603618, PF-00477736, and SCH 900776.
5. The method of any one of claims 1-4, wherein said patient is identified as having a p53-deficient cancer cell prior to administration of TH-302 and the Chkl inhibitor.
6. A pharmaceutical formulation comprising a compound of Formula I:



(I)

wherein

Y₂ is O, S, NR₆, NCOR₆, or NSO₂R₆

R₆ is Ci-C₆ alkyl, Ci-C₆ heteroalkyl, aryl, or heteroaryl;

R₃ and R₄ are independently selected from the group consisting of 2-haloalkyl, 2-

alkylsulfonyloxyalkyl, 2-heteroalkylsulfonyloxyalkyl, 2-arylsulfonyloxyalkyl,

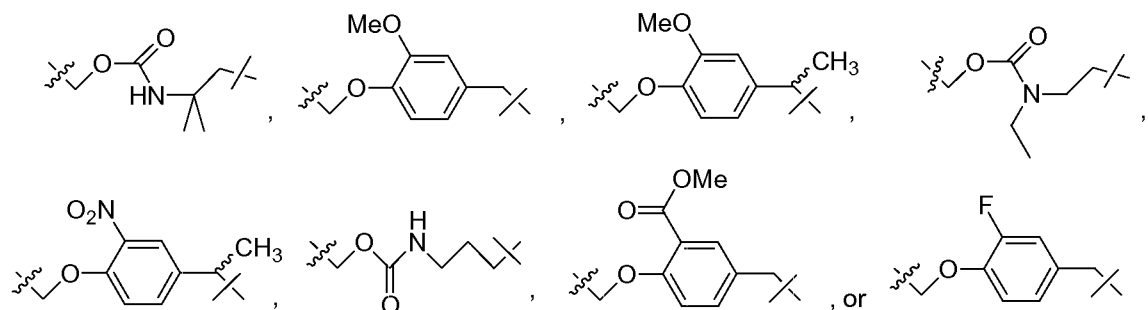
and 2-heteroalkylsulfonyloxyalkyl;

R₁ has the formula L-Z₃;

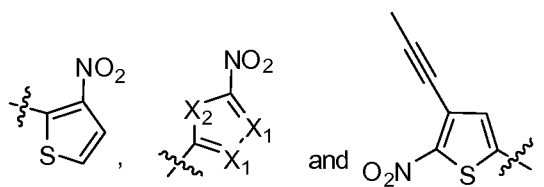
L is C(Zi)₂;

each Zi independently is hydrogen, halogen, Ci-C₆ alkyl, Ci-C₆ heteroalkyl, aryl, heteroaryl, C₃-C₈ cycloalkyl, heterocyclyl, Ci-C₆ acyl, Ci-C₆ heteroacyl, aroyl, or heteroaroyl;

or L is:



Z₃ is a bioreductive group having a formula selected from the group consisting of:



each Xi is independently N or CR₈;

X₂ is NR₇, S, or O;

each R₇ is independently Ci-C₆ alkyl, Ci-C₆ heteroalkyl, C₃-C₈ cycloalkyl, heterocyclyl, aryl or heteroaryl;

and R₈ is independently hydrogen, halogen, cyano, CHF₂, CF₃, CO₂H, amino, Ci-C₆ alkyl, Ci-C₆ heteroalkyl, Ci-C₆ cycloalkyl, Ci-C₆ alkoxy, Ci-C₆ alkylamino, Ci-C₆ dialkylamino, aryl, CON(R₇)₂, Ci-C₆ acyl, Ci-C₆ heteroacyl, aroyl or heteroaroyl;

or a pharmaceutically acceptable salt thereof;

a Chkl inhibitor, and at least a pharmaceutically acceptable excipient.

7. The pharmaceutical formulation of claim 6, wherein said compound of Formula I is TH-302.

8. The pharmaceutical formulation of claim 6 or 7 wherein said Chkl inhibitor is selected from the group consisting of AZD7762, LY2603618, PF-00477736, and SCH 900776.

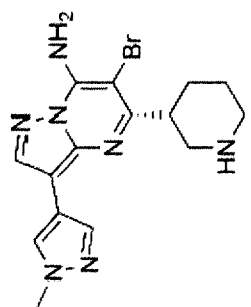
9. A method of increasing antitumor effect of a compound of Formula I on a P53 deficient tumor cell, comprising coadministering to the tumor cell a Chkl inhibitor.

10. The method of claim 9, wherein the Chkl inhibitor is AZD7762, PF477736, or LY603618.
11. The method of claim 9 or 10, wherein the compound of Formula I is TH-302.

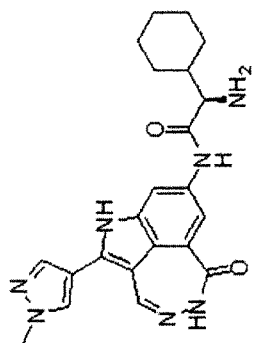
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Figure 1

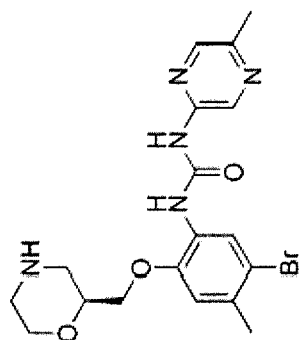
Structure of Chk1 inhibitors



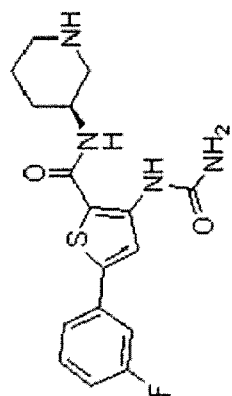
SCH 900776



PF-0477736



LY2603618



AZD7762

Figure 2

Cell line	Tumor type	p53 status	Doxorubicin			TH-302 (air)			TH-302 (N ₂)					
			-	+AZD7762	+LY2603618	+PF477736	-	+AZD7762	+LY2603618	+PF477736	-	+AZD7762	+LY2603618	+PF477736
HeLa	Cervical	p53 ^{-/-}	0.2	0.03	0.03	0.05	>1000	95	90	200	100	0.5	0.4	2.1
H460	NSLCC	p53 ^{+/+}	0.05	0.05	0.05	0.05	25	25	22	19	0.4	0.2	0.2	0.2
HT29	Colon	p53 ^{-/-}					1000	39	46	57	10	0.2	0.4	0.4
DU145	Prostate	p53 ^{+/+}	0.14	0.05	0.05	0.05	210	80	110	340	2.7	1.1	0.9	1.9
MCF10A p53 ^{+/+}	Breast	p53 ^{+/+}	0.05	0.02	0.04	0.03	110	44	61	75	0.8	0.4	0.5	0.6
MCF10A p53 ^{-/-}	Breast	p53 ^{-/-}	0.07	0.02	0.02	0.04	88	24	22	34	0.7	0.1	0.2	0.2
SW48 p53 ^{+/+}	Colon	p53 ^{+/+}	0.01	0.02	0.01	0.02	39	16	35	39	0.09	0.04	0.07	0.2
SW48 p53 ^{-/-}	Colon	p53 ^{-/-}	0.02	0.02	0.02	0.04	110	19	22	27	1.5	0.2	0.2	1

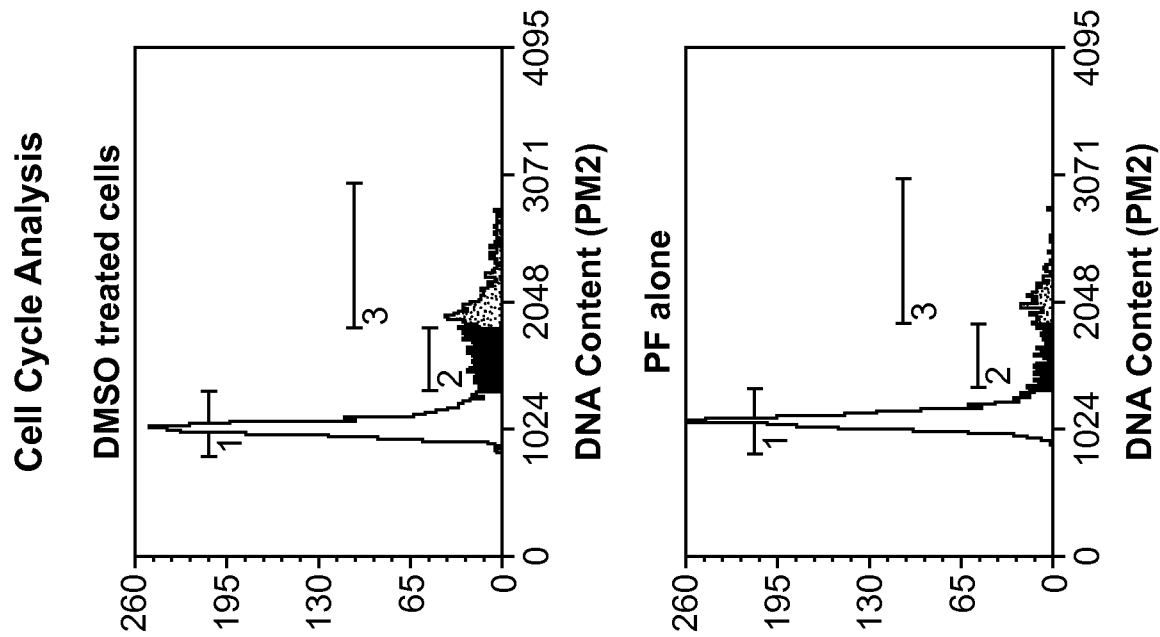


FIG. 3

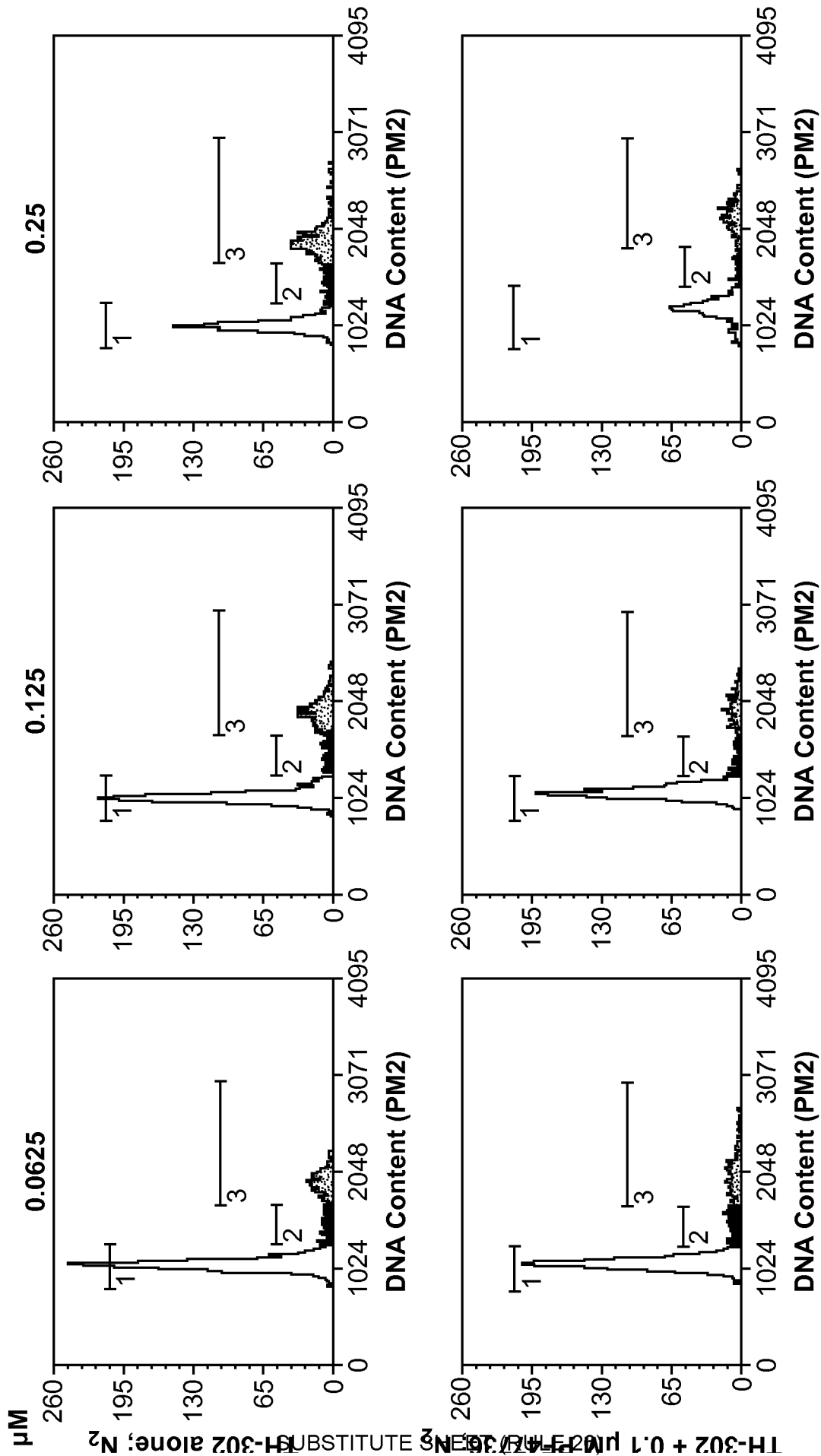


FIG. 3 (Cont. 1)

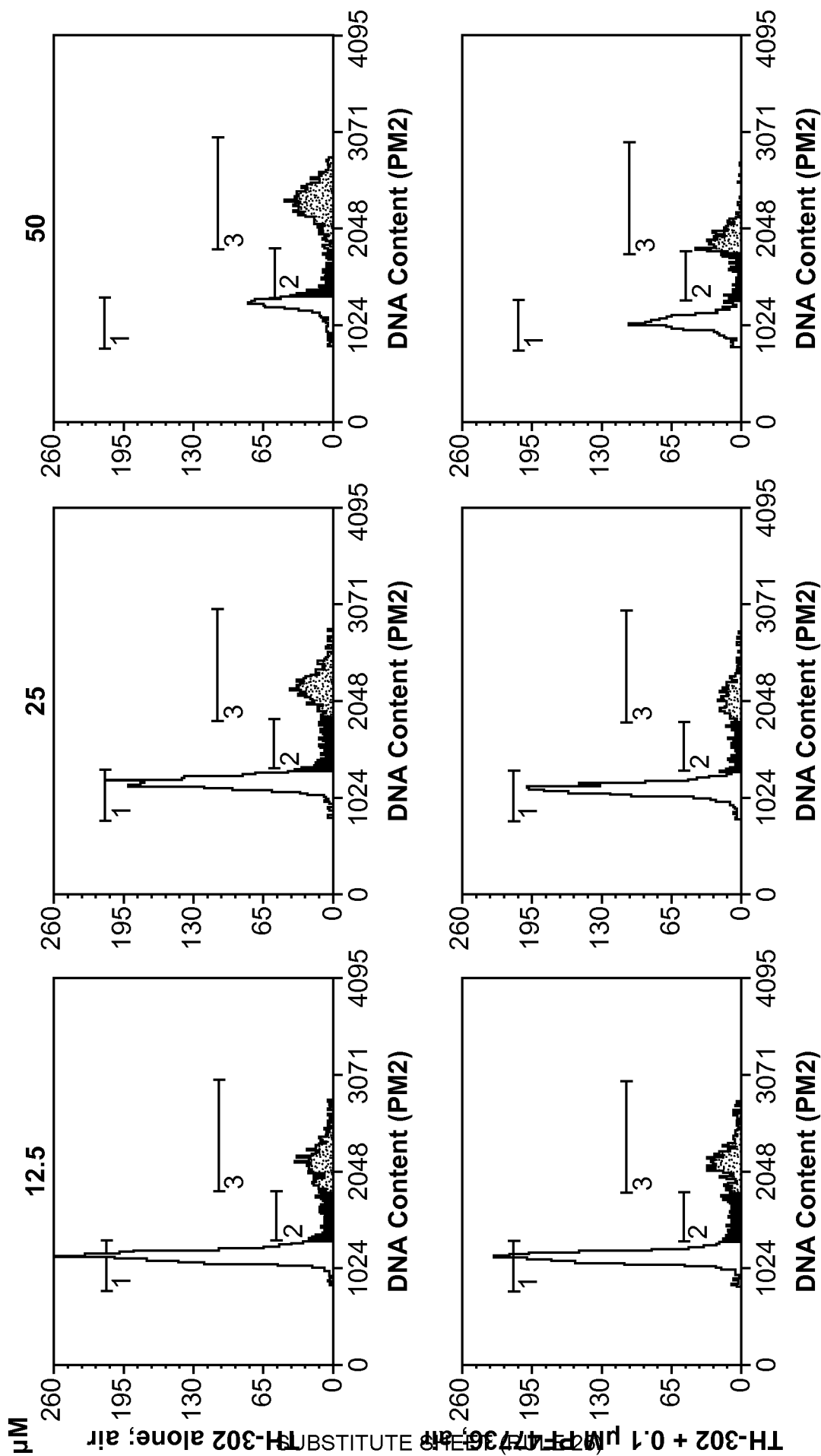


FIG. 3 (Cont. 2)

	TH-302 μ M	TH-302 alone				TH-302+PF			
		%G ₀ /G ₁	%S	%G ₂ /M	%G ₀ /G ₁	%S	%G ₂ /M	%G ₀ /G ₁	%S
N ₂	0	64	18	19					
	0.0625	75	8	17	71	12	18		
	0.125	68	8	24	76	8	16		
	0.25	54	10	36	60	11	29		
Air	12.5	65	11	24	65	13	23		
	25	61	12	27	71	10	19		
	50	31	17	52	57	14	28		

FIG. 3 (Cont. 3)

Figure 4A(I)
Daily dosing

Tumor Growth

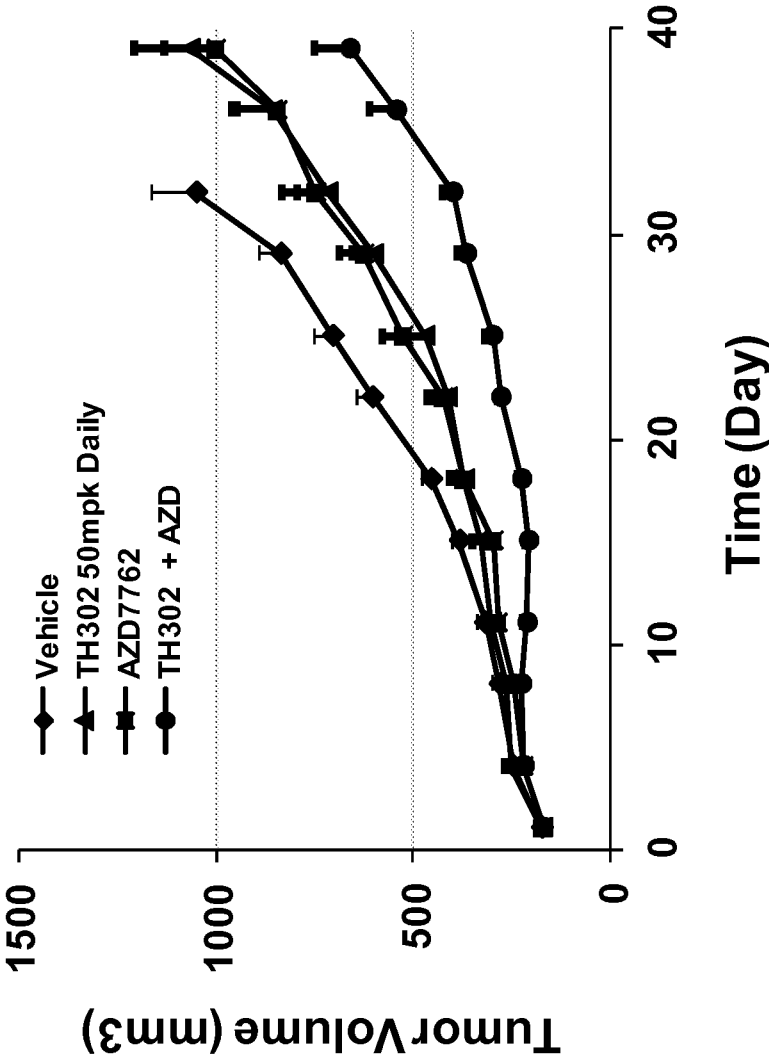


Figure 4A(II)
Daily dosing

Body Weight Change

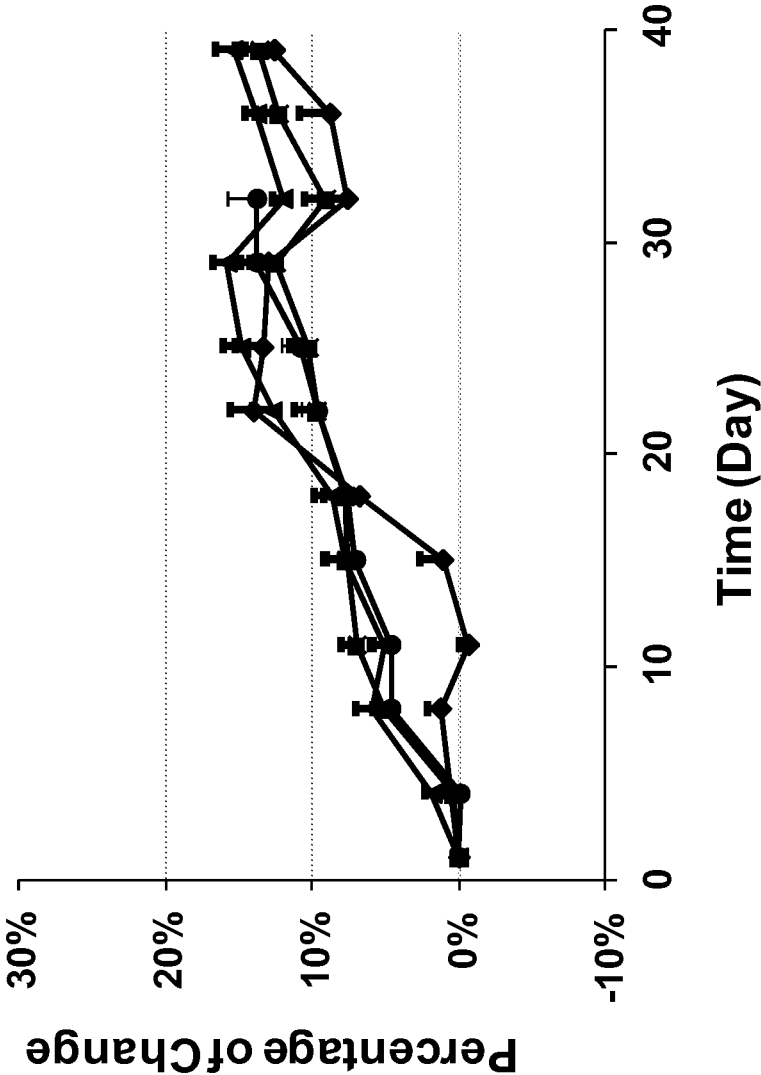


Figure 4A(III)
Daily dosing

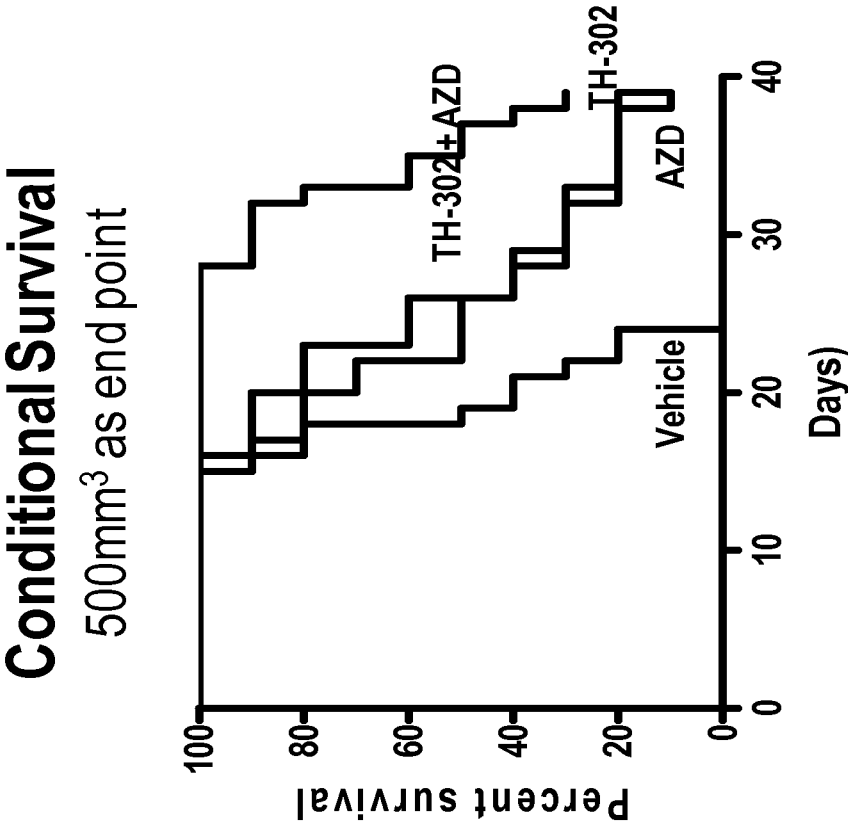


Figure 4B(I)
Intermittent dosing

Tumor Growth

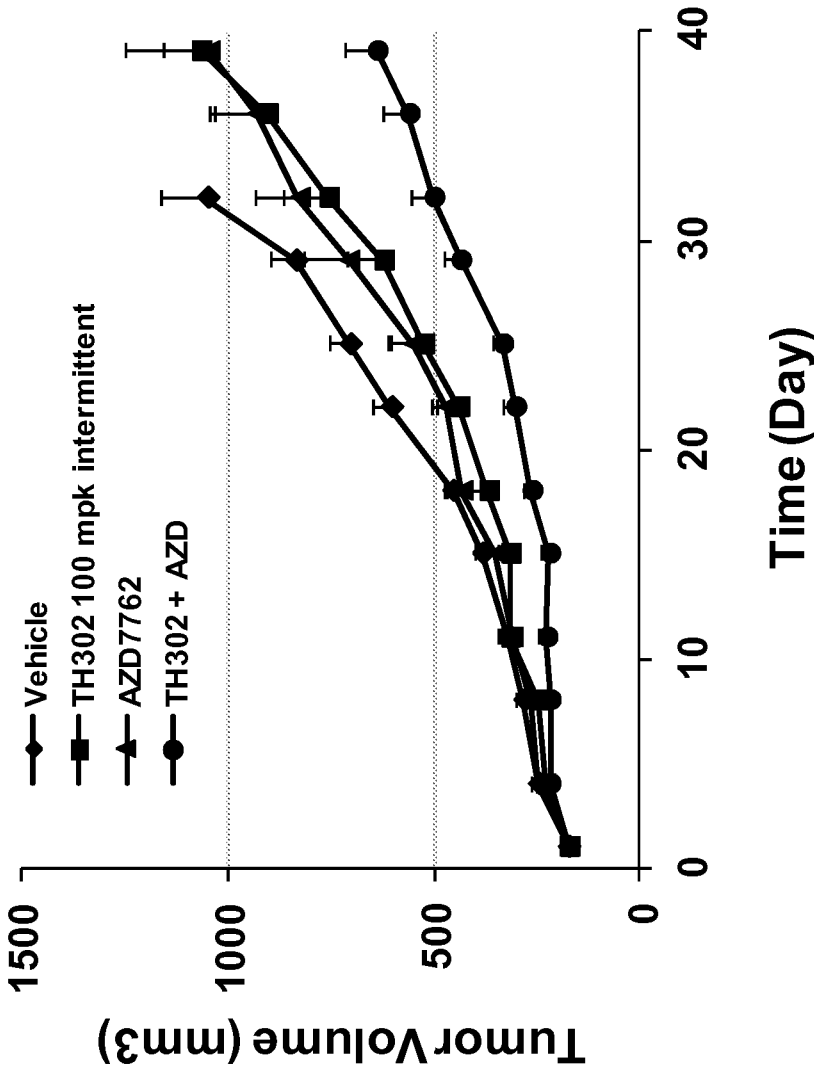


Figure 4B(II)
Intermittent dosing

Body Weight Change

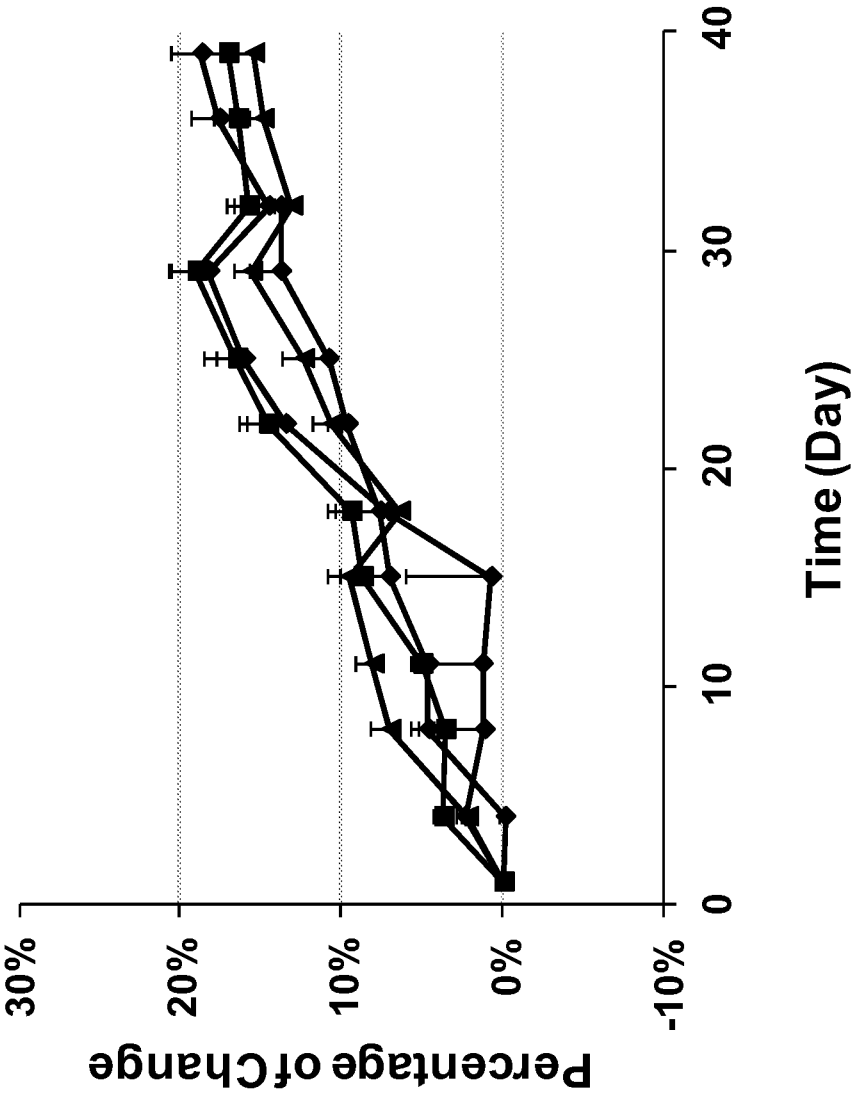
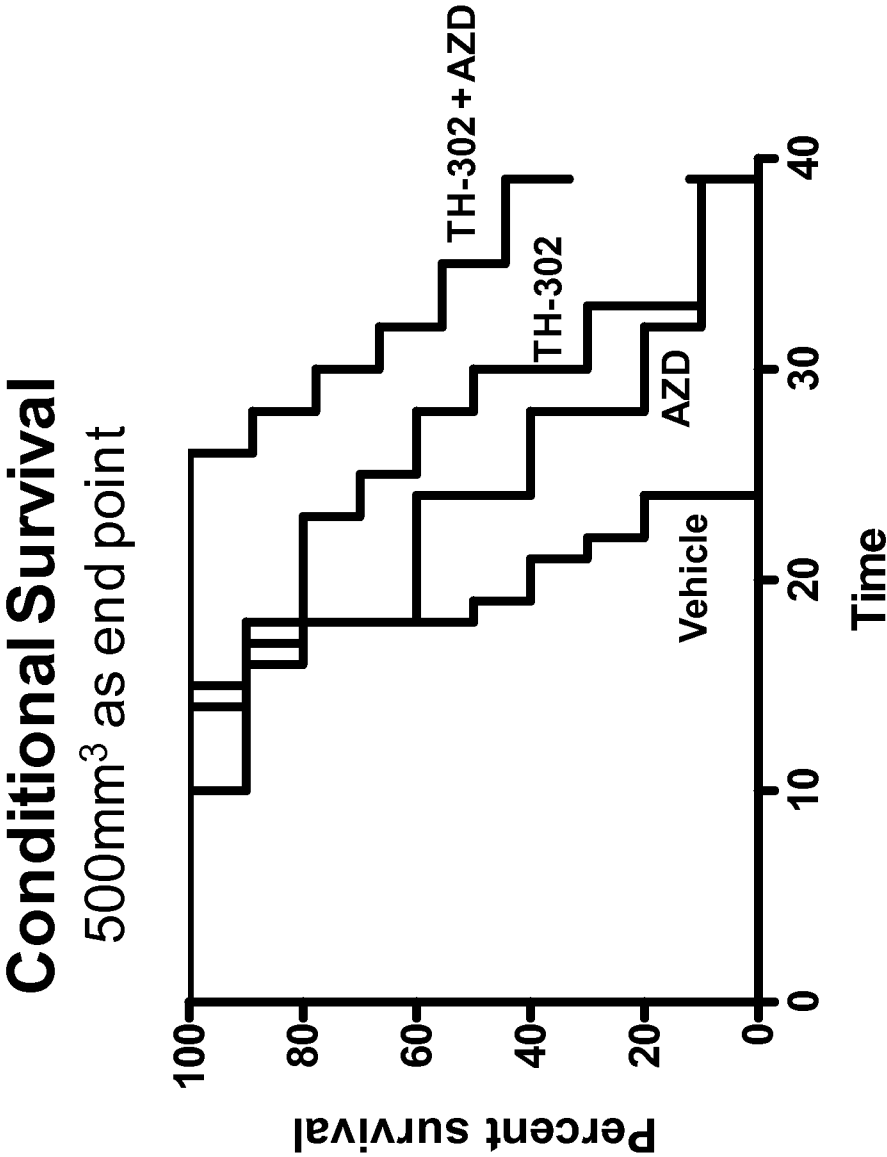


Figure 4B(III)
Intermittent dosing



A. CLASSIFICATION OF SUBJECT MATTER**A61K 31/4164(2006.01)i, A61K 31/34(2006.01)1, A61K 31/66(2006.01)1, A61P 35/00(2006.01)1**

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)

A61K 31/4164; A61K 31/4375; C12N 5/02; C07D 489/02; A61K 9/127; C07D 471/14; GOIN 33/574

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: hypoxia activated prodrug, TH-302, Chkl inhibitor, combination

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MENG, F. et al. 'Molecular and cellular pharmacology of the hypoxia-activated prodrug TH-302' Molecular Cancer Therapeutics, 06 December 2011 (E-pub.), Vol. 11, No. 3, pages 740-751. See pages 741 and 749; and figure 1.	6-8
A	US 2009-0246271 A1 (WANEBO, H. J.) 01 October 2009 See claim 1 and paragraph [0123].	6-8
A	TSE, A. N. et al. 'Targeting checkpoint kinase 1 in cancer therapeutics' Clinical Cancer Research, 2007, Vol. 13, No. 7, pages 1955-1960. See the whole document.	6-8
A	US 2011-0118230 A1 (CHEN, H. et al.) 19 May 2011 See abstract; claims 1-24, 26, and 27; and paragraphs [0053] - [0057].	6-8
A	US 7462713 B2 (BENEDICT, S. et al.) 09 December 2008 See abstract and claims 1-6.	6-8



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 April 2013 (17.04.2013)

Date of mailing of the international search report

19 April 2013 (19.04.2013)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
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City, 302-70 1, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

CHOI, Sung Hee

Telephone No. 82-42-481-8740



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/071074

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

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

1. ☒ Claims Nos.: **1-5 and 9-11**
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-5 and 9-11 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **4, 5**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2012/071074

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

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