COMBINATION THERAPIES WITH CK2 MODULATORS

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 61/228,121, filed July 23, 2009, U.S. Provisional Application No. 61/262,079, filed November 17, 2009, and U.S. Patent Application No. 12/684,053, filed January 7, 2010, the contents of each of which are hereby incorporated in their entirety by reference.

FIELD OF TECHNOLOGY

[0002] The application is in general directed to methods of combination therapy for neoplastic disorders, and combination pharmaceutical compositions. Further, the application is generally directed to methods of treating or ameliorating pain, an inflammatory, autoimmune or infectious disorder.

[0003] Combination therapies can provide improved efficacy of pharmaceutically active agents via additive or synergistic therapeutic effects. Generally synergistic therapy leads to greater therapeutic efficacy.

[0004] Combination therapeutic approaches that permit the use of lower doses of pharmaceutical agents, such as chemotherapeutic agents, than those conventionally used in monotherapy while maintaining therapeutic efficacy are highly desirable. Such combination therapies may lead to a decrease in the frequency and/or severity of adverse side-effects and an improved quality of life for the patient. Further benefits of reducing the incidence of side-effects include improved patient compliance, a reduction in the number of hospitalizations needed for the treatment of adverse effects, and a decrease in the administration of analgesic agents needed to treat pain associated with the adverse effects.

[0005] Combination therapy can also maximize the therapeutic effects of pharmaceutical agents, such as chemotherapeutic agents, administered at normal or even higher doses, in those circumstances where dose limiting toxicity is not an issue. In addition to increased therapeutic efficacy, such combination therapy may reduce the development of resistance, such as in therapy for cancer, pain, inflammation, autoimmune or infectious disorders.

[0006] Current cancer therapy generally involves treatment with surgery, chemotherapy, radiation therapy, or a combination of these approaches. Each of the major treatment approaches has significant limitations. For example, surgery may not completely remove the neoplastic tissue and cannot be used in the treatment of some disseminated neoplastic conditions, such as acute lymphoblastic leukemia, and radiation therapy is effective only when

the irradiated neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue and often causes serious side effects.

[0007] While a variety of chemotherapeutic agents are available, nearly all chemotherapeutic agents are toxic, and chemotherapy frequently causes significant, and often dangerous, side effects. Frequent side-effects include severe nausea and vomiting, bone marrow depression, immunosuppression, cytopenia (including, e.g., anemia, neutropenia, and thrombocytopenia), pain and fatigue. Additional side-effects include cachexia, mucositis, alopecia, cutaneous complications (including hypersensitivity reactions, e.g., pruritis, urticaria, and angioedema), as well as neurological, pulmonary, cardiac, reproductive and endocrine complications.

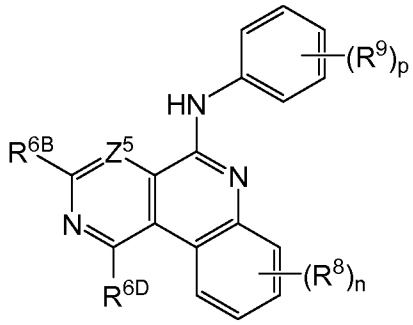
[0008] Side effects associated with chemotherapeutic agents are generally the major factor in defining the agent's dose-limiting toxicity (DLT), and managing the adverse side effects induced by chemotherapy and radiation therapy is of major importance in the clinical management of cancer treatment. In addition, many tumor cells are resistant or develop resistance to chemotherapeutic agents through multi-drug resistance.

[0009] Compounds of Formula I (as shown herein) have been previously reported to be effective in inhibiting tumor progression, reducing pro-inflammatory signaling and blocking infection. See U.S. Serial No. 11/849,230 (filed August 31, 2007).

SUMMARY

[0010] The present application provides compounds, compositions and methods of combination therapy using compounds of Formula I for the treatment of neoplastic disorders. Further, the application is generally directed to methods of treating or ameliorating pain or an inflammatory, autoimmune or infectious disorder. It has been found that contacting proliferating cells with commonly used anticancer agents in combination with a compound of Formula I provides a synergistic effect on inhibiting cell proliferation. Further, the combination of a compound of the application and a therapeutic agent that is effective in the treatment of pain or an inflammatory, autoimmune or infectious disorder provides a synergistic effect.

[0011] In one aspect, the application discloses a method for preventing, treating or ameliorating a neoplastic disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula I:



Formula I,

or a pharmaceutically acceptable salt or ester thereof,

wherein Z^5 is N or CR^{6A};

each R^{6A}, R^{6B}, R^{6D} and R^8 independently is H or an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group,

or each R^{6A}, R^{6B}, R^{6D} and R^8 independently is halo, CF_3, CFN, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, carboxy bioisostere, $CONR_2$, OOCR, COR, or NO_2,

each R^9 is independently an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group, or

each R^9 is independently halo, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, $CONR_2$, OOCR, COR, or NO_2,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, $NR'SO_2R'$, $NR'CONR'_2$, NR′COOR′, NR′COR′, CN, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2,

wherein each R′ is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl,

each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

and wherein two R′ can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S;

n is 0 to 4; and

p is 0 to 4;

and an anticancer agent, or a pharmaceutically acceptable salt or ester thereof; thereby preventing, treating or ameliorating said neoplastic disorder.

[0012] Anticancer agents used in combination with the compounds of the present application may include agents selected from any of the classes known to those of ordinary skill in the art, including, for example, alkylating agents, anti-metabolites, plant alkaloids and terpenoids (e.g., taxanes), topoisomerase inhibitors, anti-tumor antibiotics, hormonal therapies, molecular targeted agents, and the like. Generally such an anticancer agent is an alkylating agent, an anti-metabolite, a vinca alkaloid, a taxane, a topoisomerase inhibitor, an anti-tumor antibiotic, a tyrosine kinase inhibitor, an immunosuppressive macrolide, an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, or a PI3K inhibitor. Commonly, an anticancer agent is selected from the group consisting of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, a PI3K inhibitor, and a monoclonal antibody targeting a tumor/cancer antigen; alternately an anticancer agent is selected from the group consisting of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor and a PI3K inhibitor.

[0013] Another aspect disclosed in the present application is a method for inhibiting cell proliferation in a system comprising administering to the system a compound of Formula I, as disclosed herein, and an anticancer agent or a pharmaceutically acceptable salt or ester thereof, thereby inhibiting cell proliferation. Further, the application is generally directed to methods of treating or ameliorating pain or an inflammatory, autoimmune or infectious disorder comprising administering a compound of Formula I as disclosed herein and a therapeutic agent, e.g., therapeutic compound or antibody useful for treating inflammatory, autoimmune or infectious disorders or targeting CK2 kinase or CK2-regulated pathways.

[0014] A further aspect disclosed in the present application is a pharmaceutical composition comprising a compound of Formula I as disclosed herein, an anticancer agent and at least one pharmaceutically acceptable excipient. In one embodiment, an anticancer agent is

selected from the group consisting of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, a PI3K inhibitor, and a monoclonal antibody targeting a tumor/cancer antigen; alternately an anticancer agent is selected from the group consisting of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor and a PI3K inhibitor. In another aspect, the present application discloses a pharmaceutical composition comprising a compound of Formula I as disclosed herein, a pharmaceutical agent and at least one pharmaceutically acceptable excipient, wherein the pharmaceutical agent is selected from the group consisting of therapeutic compounds or antibodies useful for treating inflammatory, autoimmune or infectious disorders or targeting CK2 kinase or CK2-regulated pathways.

BRIEF DESCRIPTION OF THE FIGURES

[0015] FIG. 1 is a graph of the log drug concentration against the relative fluorescent units (RFU) for Compound A and Compound B for calculation of IC_{50}.

[0016] FIG. 2 is a graph of the log concentration of Compound K and 5-fluorouracil against RFU for calculation of IC_{50} for A375 melanoma cells.

[0017] FIG. 3 is a bar graph showing the percent cell death for Compound K, 5-fluorouracil and the combination thereof for A375 melanoma cells.

[0018] FIG. 4 is a graph of the log concentration of Compound K and fludarabine against RFU for calculation of IC_{50} for A375 melanoma cells.

[0019] FIG. 5 is a bar graph showing the percent cell death for Compound K, fludarabine and the combination thereof for A375 melanoma cells.

[0020] FIG. 6 is a graph of the log concentration of Compound K and gemcitabine against RFU for calculation of IC_{50} for A375 melanoma cells.

[0021] FIG. 7 is a graph of the log concentration of Compound K and paclitaxel against RFU for calculation of IC_{50} for A375 melanoma cells.

[0022] FIG. 8 is a bar graph showing the percent cell death for Compound K, paclitaxel and the combination thereof for A375 melanoma cells.

[0023] FIG. 9 is a graph of the log concentration of Compound K and sunitinib against RFU for calculation of IC_{50} for A375 melanoma cells.

[0024] FIG. 10 is a bar graph showing the percent cell death for Compound K, sunitinib and the combination thereof for A375 melanoma cells.

[0025] FIG. 11 is a graph of the log concentration of Compound K and vinblastine against RFU for calculation of IC_{50} for A375 melanoma cells.

[0026] FIG. 12 is a bar graph showing the percent cell death for Compound K, vinblastine and the combination thereof for A375 melanoma cells.

[0027] FIG. 13 is a graph of the log concentration of Compound K and 5-fluorouracil against RFU for calculation of IC_{50} for MDA-MB-468 breast cancer cells.

[0028] FIG. 14 is a bar graph showing the percent cell death for Compound K, 5-fluorouracil and the combination thereof for MDA-MB-468 breast cancer cells.

[0029] FIG. 15 is a graph of the log concentration of Compound K and 5-fluorouracil against RFU for calculation of IC_{50} for MDA-MB-468 breast cancer cells.

[0030] FIG. 16 is a bar graph showing the percent cell death for Compound K, 5-fluorouracil and the combination thereof for MDA-MB-468 breast cancer cells.

[0031] FIG. 17 is a graph of the log concentration of Compound K and cisplatin against RFU for calculation of IC_{50} for MDA-MB-468 breast cancer cells.

[0032] FIG. 18 is a bar graph showing the percent cell death for Compound K, cisplatin and the combination thereof for MDA-MB-468 breast cancer cells.

[0033] FIG. 19 is a graph of the log concentration of Compound K and cisplatin against RFU for calculation of IC_{50} for MDA-MB-468 breast cancer cells.

[0034] FIG. 20 is a bar graph showing the percent cell death for Compound K, cisplatin and the combination thereof for MDA-MB-468 breast cancer cells.

[0035] FIG. 21 is a graph of the log concentration of Compound K and doxorubicin against RFU for calculation of IC_{50} for MDA-MB-468 breast cancer cells.

[0036] FIG. 22 is a bar graph showing the percent cell death for Compound K, doxorubicin and the combination thereof for MDA-MB-468 breast cancer cells.

[0037] FIG. 23 is a graph of the log concentration of Compound K and doxorubicin against RFU for calculation of IC_{50} for MDA-MB-468 breast cancer cells.

[0038] FIG. 24 is a bar graph showing the percent cell death for Compound K, doxorubicin and the combination thereof for MDA-MB-468 breast cancer cells.

[0039] FIG. 25 is a graph of the log concentration of Compound K and gemcitabine against RFU for calculation of IC_{50} for MDA-MB-468 breast cancer cells.

[0040] FIG. 26 is a bar graph showing the percent cell death for Compound K, gemcitabine and the combination thereof for MDA-MB-468 breast cancer cells.

[0041] FIG. 27 is a graph of the log concentration of Compound K and gemcitabine against RFU for calculation of IC_{50} for MDA-MB-468 breast cancer cells.

[0042] FIG. 28 is a bar graph showing the percent cell death for Compound K, gemcitabine and the combination thereof for MDA-MB-468 breast cancer cells.

[0043] FIG. 29 is a graph of the log concentration of Compound K and vinblastine against RFU for calculation of IC_{50} for MIA PaCa-2 pancreatic cancer cells.

[0044] FIG. 30 is a bar graph showing the percent cell death for Compound K, vinblastine and the combination thereof for MIA PaCa-2 pancreatic cancer cells.

[0045] FIG. 31 is a graph of the log concentration of Compound K and gemcitabine against RFU for calculation of IC_{50} for MIA PaCa-2 pancreatic cancer cells.

[0046] FIG. 32 is a bar graph showing the percent cell death for Compound K, gemcitabine and the combination thereof for MIA PaCa-2 pancreatic cancer cells.

[0047] FIG. 33 is a graph of the log concentration of Compound K and sunitinib against RFU for calculation of IC_{50} for MIA PaCa-2 pancreatic cancer cells.

[0048] FIG. 34 is a bar graph showing the percent cell death for Compound K, sunitinib and the combination thereof for MIA PaCa-2 pancreatic cancer cells.

[0049] FIG. 35 is a graph of the log concentration of Compound K and rapamycin against RFU for calculation of IC_{50} for MIA PaCa-2 pancreatic cancer cells.

[0050] FIG. 36 is a bar graph showing the percent cell death for Compound K, rapamycin and the combination thereof for MIA PaCa-2 pancreatic cancer cells.

[0051] FIG. 37 is a graph of the log concentration of Compound K and 5-fluorouracil against RFU for calculation of IC_{50} for SUM-149PT inflammatory breast carcinoma cells.

[0052] FIG. 38 is a bar graph showing the percent cell death for Compound K, 5-fluorouracil and the combination thereof for SUM-149PT inflammatory breast carcinoma cells.

[0053] FIG. 39 is a graph of the log concentration of Compound K and cisplatin against RFU for calculation of IC_{50} for SUM-149PT inflammatory breast carcinoma cells.

[0054] FIG. 40 is a bar graph showing the percent cell death for Compound K, cisplatin and the combination thereof for SUM-149PT inflammatory breast carcinoma cells.

[0055] FIG. 41 is a graph of the log concentration of Compound K and rapamycin against RFU for calculation of IC_{50} for SUM-149PT inflammatory breast carcinoma cells.

[0056] FIG. 42 is a bar graph showing the percent cell death for Compound K, rapamycin and the combination thereof for SUM-149PT inflammatory breast carcinoma cells.

[0057] FIG. 43 is a graph of the log concentration of Compound K and erlotinib against RFU for calculation of IC_{50} for SUM-149PT inflammatory breast carcinoma cells.

[0058] FIG. 44 is a bar graph showing the percent cell death for Compound K, erlotinib and the combination thereof for SUM-149PT inflammatory breast carcinoma cells.

[0059] FIG. 45 is a graph of the log concentration of Compound K and 5-fluorouracil against RFU for calculation of IC_{50} for SUM-190PT inflammatory breast carcinoma cells.

[0060] FIG. 46 is a bar graph showing the percent cell death for Compound K, 5-fluorouracil and the combination thereof for SUM-190PT inflammatory breast carcinoma cells.

[0061] FIG. 47 is a dose response curve for Compound K, erlotinib and the combination thereof for BT-474 breast carcinoma cells.

[0062] FIG. 48 is a bar graph showing the percent cell death for Compound K, erlotinib and the combination thereof for BT-474 breast carcinoma cells.

[0063] FIG. 49 is a dose response curve of Compound K and Compound K in combination with erlotinib for erlotinib-resistant MDA-MB-453 breast carcinoma cells.

[0064] FIG. 50 is a dose response curve of erlotinib for erlotinib-resistant MDA-MB-453 breast carcinoma cells.

[0065] FIG. 51 is a bar graph showing the percent cell death for Compound K, erlotinib and the combination thereof for erlotinib-resistant MDA-MB-453 breast carcinoma cells.

[0066] FIG. 52 is a dose response curve of Compound K, erlotinib and a combination thereof for erlotinib-resistant T47D breast carcinoma cells.

[0067] FIG. 53 is a bar graph showing the percent cell death for Compound K, erlotinib and the combination thereof for erlotinib-resistant T47D breast carcinoma cells.

[0068] FIG. 54 is a dose response curve of Compound K, erlotinib and a combination thereof for erlotinib-resistant ZR-75-1 breast carcinoma cells.

[0069] FIG. 55 is a bar graph showing the percent cell death for Compound K, erlotinib and the combination thereof for erlotinib-resistant ZR-75-1 breast carcinoma cells.

[0070] FIG. 56 is a dose response curve of Compound K, lapatinib and a combination thereof for T47D breast carcinoma cells.

[0071] FIG. 57 is a dose response curve of Compound K, sorafenib and a combination thereof for T47D breast carcinoma cells.

[0072] FIG. 58 is a bar graph showing the percent cell death for Compound K, sorafenib and the combination thereof for T47D breast carcinoma cells.

[0073] FIG. 59 is a dose response curve of Compound K, sunitinib and a combination thereof for T47D breast carcinoma cells.

[0074] FIG. 60 is a dose response curve of Compound K, Akt1/2 inhibitor and a combination thereof for BT-474 breast carcinoma cells.

[0075] FIG. 61 is a bar graph showing the percent cell death for Compound K, Akt1/2 inhibitor and a combination thereof for BT-474 breast carcinoma cells.

[0076] FIG. 62 is a Western blot analysis using the following in the breast carcinoma cell line MDA-MB-453:

Column 1: Untreated;

Columns 2, 7, 12: 10 uM Compound K;

Columns 3, 8, 13: 100 uM Erlotinib;

Columns 4, 9, 14: 2 uM Lapatinib;

Columns 5, 10, 15: 10 uM Compound K plus 100 uM Erlotinib;

Columns 6, 11, 16: 10 uM Compound K plus 2 uM Lapatinib.

[0077] FIG. 63 is a dose response curve of Compound K, panobinostat and a combination thereof for Hs 578T breast cancer cells.

[0078] FIG. 64 is a bar graph showing the percent cell death for Compound K, panobinostat and a combination thereof for Hs 578T breast cancer cells.

[0079] FIG. 65 is a dose response curve for Compound K, 17-DMAG and a combination thereof for Hs 578T breast cancer cells.

[0080] FIG. 66 is a bar graph showing the percent cell death for Compound K, 17-DMAG and a combination thereof for Hs 578T breast cancer cells.

[0081] FIG. 67 is a dose response curve for Compound K, AKTi VIII and a combination thereof for BT-474 breast cancer cells.

[0082] FIG. 68 is a bar graph showing the percent cell death for Compound K, AKTi VIII and a combination thereof for BT-474 breast cancer cells.

[0083] FIG. 69 is a dose response curve for Compound K, BEZ-235 and a combination thereof for BT-474 breast cancer cells.

[0084] FIG. 70 is a bar graph showing the percent cell death for Compound K, BEZ-235 and a combination thereof for BT-474 breast cancer cells.

[0085] FIG. 71 is a dose response curve for Compound K, LY294002 and a combination thereof for BT-474 breast cancer cells.

[0086] FIG. 72 is a bar graph showing the percent cell death for Compound K, LY294002 and a combination thereof for BT-474 breast cancer cells.

[0087] FIG. 73 is a dose response curve for Compound K, PI-103 and a combination thereof for BT-474 breast cancer cells.

[0088] FIG. 74 is a bar graph showing the percent cell death for Compound K, PI-103 and a combination thereof for BT-474 breast cancer cells.

[0089] FIG. 75 is a dose response curve for Compound K, wortmannin and a combination thereof for BT-474 breast cancer cells.

[0090] FIG. 76 is a bar graph showing the percent cell death for Compound K, wortmannin and a combination thereof for BT-474 breast cancer cells.

[0091] FIG. 77 is a dose response curve for Compound K, PI-103 and a combination thereof for T-47D breast cancer cells.

[0092] FIG. 78 is a bar graph showing the percent cell death for Compound K, PI-103 and a combination thereof for T-47D breast cancer cells.

[0093] FIG. 79 is a Western hybridization analysis in BT-474 breast cancer cells for the following: untreated cells, cells treated with 5 uM Compound K, with 1 uM AKTi VIII and with 5:1 combination thereof.

[0094] FIG. 80 is a graphical representation of the phosphorylation of AKT at S129, at T308, and at S473, as well as of the cleavage of PARP in BT-474 breast cancer cells for the following: untreated cells, cells treated with 5 uM Compound K, with 1 uM AKTi VIII and with 5:1 combination thereof.

[0095] FIG. 81 is a graph of the NCI-H1975 tumor growth in animals treated with vehicle, compound K, cetuximab, or combination of Compound K and cetuximab.

DETAILED DESCRIPTION

[0096] The present application may be understood more readily by reference to the following detailed description of the embodiments and the Examples included herein. It is to be understood that the terminology used herein is for the purpose of describing specific

embodiments only and is not intended to be limiting. It is further to be understood that unless specifically defined herein, the terminology used herein is to be given its traditional meaning as known in the relevant art.

[0097] As used herein, the singular forms "a", "an", and "the" include plural references unless indicated otherwise.

[0098] As used herein, the term "subject" refers to a human or animal subject. Generally, the subject is human.

[0099] The term "neoplastic disorder" as used herein refers to a disorder involving aberrant cell proliferation, such as a cancer, for example. The cancer may result in a tumor in certain instances, and symptoms associated with a tumor sometimes are treated. Neoplastic disorders include, but are not limited to, abnormal cell proliferative conditions (e.g., cancer) of the hemopoietic system (e.g., white blood cell), lung, breast, prostate, kidney, pancreas, liver, heart, skeleton, colon, rectum, skin, brain, eye, lymph node, heart, testes or ovary, for example.

[00100] The term "therapeutically effective amount" or "effective amount" is intended to mean that amount of a drug or pharmaceutical agent that will elicit a biological or medical response of a cell, tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician. When referring to the amount of a compound of the application administered in combination with an additional anticancer agent, the "therapeutically effective amount" of the compound of the application may be an amount sufficient to produce an anticancer effect alone, or may be an amount sufficient to produce an anticancer effect in the presence of the additional anticancer agent. Similarly, the amount of the additional anticancer agent may be sufficient to provide an anticancer effect alone, or may be sufficient to provide an anticancer effect in the presence of the compound of the application. Analogously, a therapeutically effective amount of a compound of the application administered in combination with a therapeutic agent effective in the treatment of pain, inflammation, infection or an autoimmune disorder may be sufficient to produce an analgesic, antiinflammation, antiinfection or anti-autoimmune therapeutic effect or may be an amount sufficient to produce such a therapeutic effect in the presence of the additional therapeutic agent. Similarly, the amount of the therapeutic agent may be sufficient to provide an analgesic, antiinflammation, antiinfection or anti-autoimmune therapeutic effect in the presence of the compound of the application.

[00101] In some embodiments, the combination of a compound of the application and an additional therapeutic agent, e.g., anticancer agent, anti-inflammatory agent, antiinfectious agent, or antiautoimmune agent exhibits an additive effect, such as an additive effect on inhibiting cell proliferation, pain, inflammation, infection, and/or autoimmune disorders. In other embodiments, the combination of a compound of the application and an additional therapeutic agent exhibits a synergistic r effect, such as a synergistic effect on inhibiting cell proliferation, pain, inflammation, infection and/or autoimmune disorders.

[00102] By "inhibiting" or "reducing" cell proliferation, pain, inflammation, infection and/or autoimmune disorders is meant to slow down, to decrease, or, for example, to stop the amount of cell proliferation, pain, inflammation, infection and/or autoimmune disorders, as measured using methods known to those of ordinary skill in the art, by, for example, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, when compared to cells that are not subjected to the methods and compositions of the present application.

[00103] As used herein, the terms "alkyl," "alkenyl" and "alkynyl" include straight-chain, branched-chain and cyclic monovalent hydrocarbyl radicals, and combinations of these, which contain only C and H when they are unsubstituted. Examples include methyl, ethyl, isobutyl, cyclohexyl, cyclopentylethyl, 2-propenyl, 3-butynyl, and the like. The total number of carbon atoms in each such group is sometimes described herein, *e.g.,* when the group can contain up to ten carbon atoms it can be represented as 1-10C or as C1-C10 or C1-10. When heteroatoms (N, O and S typically) are allowed to replace carbon atoms as in heteroalkyl groups, for example, the numbers describing the group, though still written as *e.g.* C1-C6, represent the sum of the number of carbon atoms in the group plus the number of such heteroatoms that are included as replacements for carbon atoms in the backbone of the ring or chain being described.

[00104] Typically, the alkyl, alkenyl and alkynyl substituents contain 1-10C (alkyl) or 2-10C (alkenyl or alkynyl). Generally they contain 1-8C (alkyl) or 2-8C (alkenyl or alkynyl). Sometimes they contain 1-4C (alkyl) or 2-4C (alkenyl or alkynyl). A single group can include more than one type of multiple bond, or more than one multiple bond; such groups are included within the definition of the term "alkenyl" when they contain at least one carbon-carbon double bond, and are included within the term "alkynyl" when they contain at least one carbon-carbon triple bond.

[00105] Alkyl, alkenyl and alkynyl groups are often optionally substituted to the extent that such substitution makes sense chemically. Typical substituents include, but are not limited

to, halo, =O, =N-CN, =N-OR, =NR, OR, NR_2, SR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, C≡CR, COOR, $CONR_2$, OOCR, COR, and NO_2, wherein each R is independently H, C1-C8 alkyl, C2-C8 heteroalkyl, C1-C8 acyl, C2-C8 heteroacyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C6-C10 aryl, or C5-C10 heteroaryl, and each R is optionally substituted with halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, $NR'SO_2R'$, $NR'CONR'_2$, NR′COOR′, NR′COR′, CN, C≡CR′, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2, wherein each R′ is independently H, C1-C8 alkyl, C2-C8 heteroalkyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl or C5-C10 heteroaryl. Alkyl, alkenyl and alkynyl groups can also be substituted by C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl or C5-C10 heteroaryl, each of which can be substituted by the substituents that are appropriate for the particular group.

[00106] "Acetylene" substituents are 2-10C alkynyl groups that are optionally substituted, and are of the formula -C≡C-R^a, wherein R^a is H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl, and each R^a group is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, $NR'SO_2R'$, $NR'CONR'_2$, NR′COOR′, NR′COR′, CN, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2, wherein each R′ is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O; and wherein two R′ can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S. In some embodiments, R^a of -C≡C-R^a is H or Me.

[00107] "Heteroalkyl", "heteroalkenyl", and "heteroalkynyl" and the like are defined similarly to the corresponding hydrocarbyl (alkyl, alkenyl and alkynyl) groups, but the 'hetero' terms refer to groups that contain 1-3 O, S or N heteroatoms or combinations thereof within the backbone residue; thus at least one carbon atom of a corresponding alkyl, alkenyl, or alkynyl group is replaced by one of the specified heteroatoms to form a heteroalkyl, heteroalkenyl, or heteroalkynyl group. The typical sizes for heteroforms of alkyl, alkenyl and alkynyl groups are generally the same as for the corresponding hydrocarbyl groups, and the substituents that may be present on the heteroforms are the same as those described above for the hydrocarbyl

groups. For reasons of chemical stability, it is also understood that, unless otherwise specified, such groups do not include more than two contiguous heteroatoms except where an oxo group is present on N or S as in a nitro or sulfonyl group.

[00108] While "alkyl" as used herein includes cycloalkyl and cycloalkylalkyl groups, the term "cycloalkyl" may be used herein to describe a carbocyclic non-aromatic group that is connected via a ring carbon atom, and "cycloalkylalkyl" may be used to describe a carbocyclic non-aromatic group that is connected to the molecule through an alkyl linker. Similarly, "heterocyclyl" may be used to describe a non-aromatic cyclic group that contains at least one heteroatom as a ring member and that is connected to the molecule via a ring atom, which may be C or N; and "heterocyclylalkyl" may be used to describe such a group that is connected to another molecule through a linker. The sizes and substituents that are suitable for the cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl groups are the same as those described above for alkyl groups. As used herein, these terms also include rings that contain a double bond or two, as long as the ring is not aromatic.

[00109] As used herein, "acyl" encompasses groups comprising an alkyl, alkenyl, alkynyl, aryl or arylalkyl radical attached at one of the two available valence positions of a carbonyl carbon atom, and heteroacyl refers to the corresponding groups wherein at least one carbon other than the carbonyl carbon has been replaced by a heteroatom chosen from N, O and S. Thus heteroacyl includes, for example, -C(=O)OR and $-C(=O)NR_2$ as well as –C(=O)-heteroaryl.

[00110] Acyl and heteroacyl groups are bonded to any group or molecule to which they are attached through the open valence of the carbonyl carbon atom. Typically, they are C1-C8 acyl groups, which include formyl, acetyl, pivaloyl, and benzoyl, and C2-C8 heteroacyl groups, which include methoxyacetyl, ethoxycarbonyl, and 4-pyridinoyl. The hydrocarbyl groups, aryl groups, and heteroforms of such groups that comprise an acyl or heteroacyl group can be substituted with the substituents described herein as generally suitable substituents for each of the corresponding component of the acyl or heteroacyl group.

[00111] "Aromatic" moiety or "aryl" moiety refers to a monocyclic or fused bicyclic moiety having the well-known characteristics of aromaticity; examples include phenyl and naphthyl. Similarly, "heteroaromatic" and "heteroaryl" refer to such monocyclic or fused bicyclic ring systems which contain as ring members one or more heteroatoms selected from O, S and N. The inclusion of a heteroatom permits aromaticity in 5-membered rings as well as

6-membered rings. Typical heteroaromatic systems include monocyclic C5-C6 aromatic groups such as pyridyl, pyrimidyl, pyrazinyl, thienyl, furanyl, pyrrolyl, pyrazolyl, thiazolyl, oxazolyl, and imidazolyl and the fused bicyclic moieties formed by fusing one of these monocyclic groups with a phenyl ring or with any of the heteroaromatic monocyclic groups to form a C8-C10 bicyclic group such as indolyl, benzimidazolyl, indazolyl, benzotriazolyl, isoquinolyl, quinolyl, benzothiazolyl, benzofuranyl, pyrazolopyridyl, quinazolinyl, quinoxalinyl, cinnolinyl, and the like. Any monocyclic or fused ring bicyclic system which has the characteristics of aromaticity in terms of electron distribution throughout the ring system is included in this definition. It also includes bicyclic groups where at least the ring which is directly attached to the remainder of the molecule has the characteristics of aromaticity. Typically, the ring systems contain 5-12 ring member atoms. Often the monocyclic heteroaryls contain 5-6 ring members, and the bicyclic heteroaryls contain 8-10 ring members.

[00112] Aryl and heteroaryl moieties may be substituted with a variety of substituents including C1-C8 alkyl, C2-C8 alkenyl, C2-C8 alkynyl, C5-C12 aryl, C1-C8 acyl, and heteroforms of these, each of which can itself be further substituted; other substituents for aryl and heteroaryl moieties include halo, OR, NR_2, SR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, C≡CR, COOR, $CONR_2$, OOCR, COR, and NO_2, wherein each R is independently H, C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl, and each R is optionally substituted as described above for alkyl groups. The substituent groups on an aryl or heteroaryl group may of course be further substituted with the groups described herein as suitable for each type of such substituents or for each component of the substituent. Thus, for example, an arylalkyl substituent may be substituted on the aryl portion with substituents described herein as typical for aryl groups, and it may be further substituted on the alkyl portion with substituents described herein as typical or suitable for alkyl groups.

[00113] Similarly, "arylalkyl" and "heteroarylalkyl" refer to aromatic and heteroaromatic ring systems which are bonded to their attachment point through a linking group such as an alkylene, including substituted or unsubstituted, saturated or unsaturated, cyclic or acyclic linkers. Typically the linker is C1-C8 alkyl or a hetero form thereof. These linkers may also include a carbonyl group, thus making them able to provide substituents as an acyl or heteroacyl moiety. An aryl or heteroaryl ring in an arylalkyl or heteroarylalkyl group may be

substituted with the same substituents described above for aryl groups. Generally, an arylalkyl group includes a phenyl ring optionally substituted with the groups defined above for aryl groups and a C1-C4 alkylene that is unsubstituted or is substituted with one or two C1-C4 alkyl groups or heteroalkyl groups, where the alkyl or heteroalkyl groups can optionally cyclize to form a ring such as cyclopropane, dioxolane, or oxacyclopentane. Similarly, a heteroarylalkyl group generally includes a C5-C6 monocyclic heteroaryl group that is optionally substituted with the groups described above as substituents typical on aryl groups and a C1-C4 alkylene that is unsubstituted or is substituted with one or two C1-C4 alkyl groups or heteroalkyl groups, or it includes an optionally substituted phenyl ring or C5-C6 monocyclic heteroaryl and a C1-C4 heteroalkylene that is unsubstituted or is substituted with one or two C1-C4 alkyl or heteroalkyl groups, where the alkyl or heteroalkyl groups can optionally cyclize to form a ring such as cyclopropane, dioxolane, or oxacyclopentane.

[00114] Where an arylalkyl or heteroarylalkyl group is described as optionally substituted, the substituents may be on either the alkyl or heteroalkyl portion or on the aryl or heteroaryl portion of the group. The substituents optionally present on the alkyl or heteroalkyl portion are the same as those described above for alkyl groups generally; the substituents optionally present on the aryl or heteroaryl portion are the same as those described above for aryl groups generally.

[00115] "Arylalkyl" groups as used herein are hydrocarbyl groups if they are unsubstituted, and are described by the total number of carbon atoms in the ring and alkylene or similar linker. Thus a benzyl group is a C7-arylalkyl group, and phenylethyl is a C8-arylalkyl.

[00116] "Heteroarylalkyl" as described above refers to a moiety comprising an aryl group that is attached through a linking group, and differs from "arylalkyl" in that at least one ring atom of the aryl moiety or one atom in the linking group is a heteroatom selected from N, O and S. The heteroarylalkyl groups are described herein according to the total number of atoms in the ring and linker combined, and they include aryl groups linked through a heteroalkyl linker; heteroaryl groups linked through a hydrocarbyl linker such as an alkylene; and heteroaryl groups linked through a heteroalkyl linker. Thus, for example, C7-heteroarylalkyl would include pyridylmethyl, phenoxy, and N-pyrrolylmethoxy.

[00117] "Alkylene" as used herein refers to a divalent hydrocarbyl group; because it is divalent, it can link two other groups together. Typically it refers to $-(CH_2)_n-$ where n is 1-8 and often n is 1-4, though where specified, an alkylene can also be substituted by other groups,

and can be of other lengths, and the open valences need not be at opposite ends of a chain. Thus -CH(Me)- and $-C(Me)_2-$ may also be referred to as alkylenes, as can a cyclic group such as cyclopropan-1,1-diyl. Where an alkylene group is substituted, the substituents include those typically present on alkyl groups as described herein.

[00118] In general, any alkyl, alkenyl, alkynyl, acyl, or aryl or arylalkyl group or any heteroform of one of these groups that is contained in a substituent may itself optionally be substituted by additional substituents. The nature of these substituents is similar to those recited with regard to the primary substituents themselves if the substituents are not otherwise described. Thus, where an embodiment of, for example, R^7 is alkyl, this alkyl may optionally be substituted by the remaining substituents listed as embodiments for R^7 where this makes chemical sense, and where this does not undermine the size limit provided for the alkyl *per se*; *e.g.*, alkyl substituted by alkyl or by alkenyl would simply extend the upper limit of carbon atoms for these embodiments, and is not included. However, alkyl substituted by aryl, amino, alkoxy, =O, and the like would be included within the scope of the application, and the atoms of these substituent groups are not counted in the number used to describe the alkyl, alkenyl, etc. group that is being described. Where no number of substituents is specified, each such alkyl, alkenyl, alkynyl, acyl, or aryl group may be substituted with a number of substituents according to its available valences; in particular, any of these groups may be substituted with fluorine atoms at any or all of its available valences, for example.

[00119] "Heteroform" as used herein refers to a derivative of a group such as an alkyl, aryl, or acyl, wherein at least one carbon atom of the designated carbocyclic group has been replaced by a heteroatom selected from N, O and S. Thus the heteroforms of alkyl, alkenyl, alkynyl, acyl, aryl, and arylalkyl are heteroalkyl, heteroalkenyl, heteroalkynyl, heteroacyl, heteroaryl, and heteroarylalkyl, respectively. It is understood that no more than two N, O or S atoms are ordinarily connected sequentially, except where an oxo group is attached to N or S to form a nitro or sulfonyl group.

[00120] "Halo", as used herein includes fluoro, chloro, bromo and iodo. Generally halo refers to fluoro or chloro.

[00121] "Amino" as used herein refers to NH_2, but where an amino is described as "substituted" or "optionally substituted", the term includes NR′R″ wherein each R′ and R″ is independently H, or is an alkyl, alkenyl, alkynyl, acyl, aryl, or arylalkyl group or a heteroform of one of these groups, and each of the alkyl, alkenyl, alkynyl, acyl, aryl, or arylalkyl groups or

heteroforms of one of these groups is optionally substituted with the substituents described herein as suitable for the corresponding group. The term also includes forms wherein R′ and R″ are linked together to form a 3-8 membered ring which may be saturated, unsaturated or aromatic and which contains 1-3 heteroatoms independently selected from N, O and S as ring members, and which is optionally substituted with the substituents described as suitable for alkyl groups or, if NR′R″ is an aromatic group, it is optionally substituted with the substituents described as typical for heteroaryl groups.

[00122] As used herein, the term "carbocycle" refers to a cyclic compound containing only carbon atoms in the ring, whereas a "heterocycle" refers to a cyclic compound comprising a heteroatom. The carbocyclic and heterocyclic structures encompass compounds having monocyclic, bicyclic or multiple ring systems.

[00123] As used herein, the term "heteroatom" refers to any atom that is not carbon or hydrogen, such as nitrogen, oxygen or sulfur.

[00124] Illustrative examples of heterocycles include but are not limited to tetrahydrofuran, 1,3-dioxolane, 2,3-dihydrofuran, pyran, tetrahydropyran, benzofuran, isobenzofuran, 1,3-dihydroisobenzofuran, isoxazole, 4,5-dihydroisoxazole, piperidine, pyrrolidine, pyrrolidin-2-one, pyrrole, pyridine, pyrimidine, octahydropyrrolo[3,4-b]pyridine, piperazine, pyrazine, morpholine, thiomorpholine, imidazole, imidazolidine-2,4-dione, 1,3-dihydrobenzimidazol-2-one, indole, thiazole, benzothiazole, thiadiazole, thiophene, tetrahydro thiophene-1,1-dioxide, diazepine, triazole, guanidine, diazabicyclo[2.2.1]heptane, 2,5-diazabicyclo[2.2.1]heptane, 2,3,4,4a,9,9a hexahydro-1H-β-carboline, oxirane, oxetane, tetrahydropyran, dioxane, lactones, aziridine, azetidine, piperidine, lactams, and may also encompass heteroaryls. Other illustrative examples of heteroaryls include but are not limited to furan, pyrrole, pyridine, pyrimidine, imidazole, benzimidazole and triazole.

[00125] As used herein, the term "inorganic substituent" refers to substituents that do not contain carbon or contain carbon bound to elements other than hydrogen (e.g., elemental carbon, carbon monoxide, carbon dioxide, and carbonate). Examples of inorganic substituents include but are not limited to nitro, halogen, azido, cyano, sulfonyls, sulfinyls, sulfonates, phosphates, etc.

[00126] The terms "treat", "treating" or "treatment" in reference to a particular disease or disorder includes prevention of the disease or disorder, and/or lessening, improving, ameliorating or abrogating the symptoms and/or pathology of the disease or disorder.

Generally the terms as used herein refer to ameliorating, alleviating, lessening, and removing symptoms of a disease or condition. A candidate molecule or compound described herein may be in a therapeutically effective amount in a formulation or medicament, which is an amount that can lead to a biological effect, such as apoptosis of certain cells (e.g., cancer cells), reduction of proliferation of certain cells, or lead to ameliorating, alleviating, lessening, or removing symptoms of a disease or condition, for example. The terms also can refer to reducing or stopping a cell proliferation rate (e.g., slowing or halting tumor growth) or reducing the number of proliferating cancer cells (e.g., removing part or all of a tumor). These terms also are applicable to reducing a titre of a microorganism in a system (i.e., cell, tissue, or subject) infected with a microorganism, reducing the rate of microbial propagation, reducing the number of symptoms or an effect of a symptom associated with the microbial infection, and/or removing detectable amounts of the microbe from the system. Examples of microorganism include but are not limited to virus, bacterium and fungus. Alternatively the terms can refer to reduced or inhibited immune or inflammatory reactions or responses within a subject, e.g., human. For example, these terms are applicable to reduced antibody titers, B cell or T cell reactions, cytotoxocity reactions, any other criteria suitable to measure inflammatory, autoimmune or infectious conditions,

[00127] As used herein, the term “apoptosis” refers to an intrinsic cell self-destruction or suicide program. In response to a triggering stimulus, cells undergo a cascade of events including cell shrinkage, blebbing of cell membranes and chromatic condensation and fragmentation. These events culminate in cell conversion to clusters of membrane-bound particles (apoptotic bodies), which are thereafter engulfed by macrophages.

[00128] As used herein the term “inflammatory disorder” or "inflammation" includes any condition characterized by a localized or a systemic protective response, which may be elicited by physical trauma, infection, chronic diseases, and/or chemical and/or physiological reactions to external stimuli (e.g. as part of an allergic response). Any such response, which may serve to destroy, dilute or sequester both the injurious agent and the injured tissue, may be manifest by, for example, heat, swelling, pain, redness, dilation of blood vessels and/or increased blood flow, invasion of the affected area by white blood cells, loss of function and/or any other symptoms known to be associated with inflammatory conditions. The term "inflammation" or “inflammatory disease” will thus also be understood to include any inflammatory disease, disorder or condition per se, any condition that has an inflammatory component associated with

it, and/or any condition characterized by inflammation as a symptom, including inter alia acute, chronic, ulcerative, specific, allergic and necrotic inflammation. Inflammation can also lead to pain.

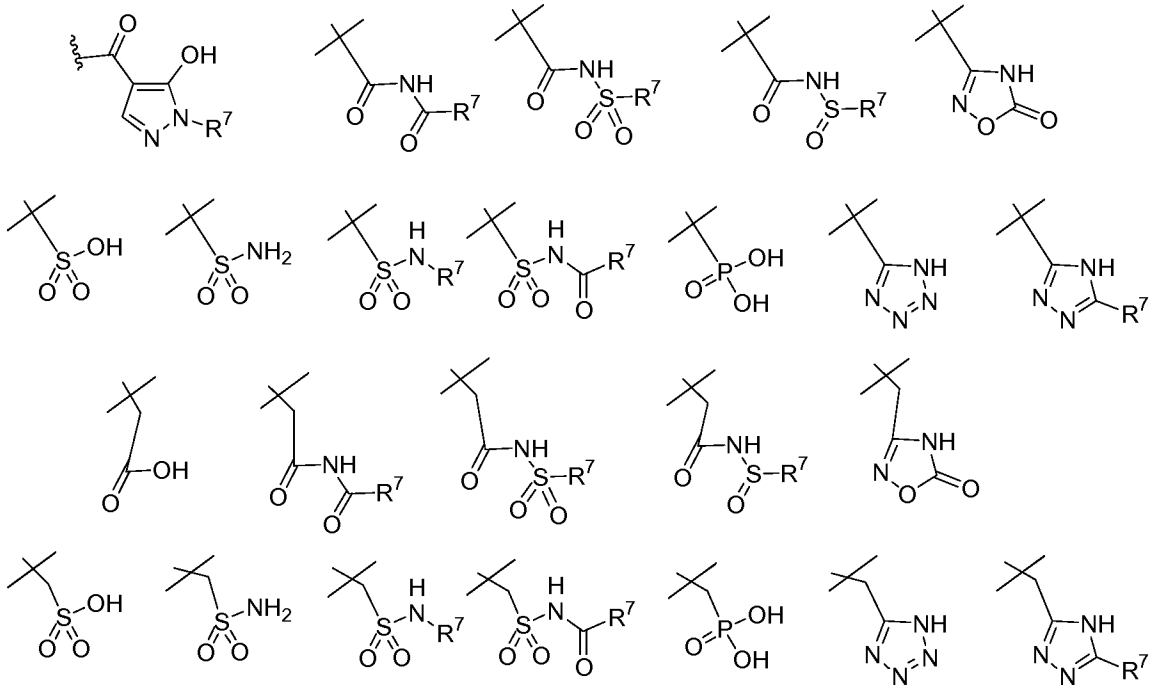
[00129] Conditions associated with inflammation and pain include without limitation acid reflux, heartburn, acne, allergies and sensitivities, Alzheimer's disease, asthma, atherosclerosis, bronchitis, carditis, celiac disease, chronic pain, Crohn's disease, cirrhosis, colitis, dementia, dermatitis, diabetes, dry eyes, edema, emphysema, eczema, fibromyalgia, gastroenteritis, gingivitis, heart disease, hepatitis, high blood pressure, insulin resistance, interstitial cystitis, joint pain/arthritis/rheumatoid arthritis, metabolic syndrome (syndrome X), myositis, nephritis, obesity, osteopenia, osteoporosis, Parkinson's disease, periodontal disease, polyarteritis, polychondritis, psoriasis, scleroderma, sinusitis, Sjogren's syndrome, spastic colon, systemic candidiasis, tendonitis, urinary tract infections, vaginitis, inflammatory cancer (e.g., inflammatory breast cancer) and the like.

[00130] As used herein the term “autoimmune disorder” refers to a condition that occurs when the immune system mistakenly attacks and destroys healthy body tissue. Examples of autoimmune disorders include, but are not limited to Ankylosing Spondylitis, Crohn’s Disease, Dermatomyositis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, Idiopathic thrombocytopenic purpura, Lupus erythematosus, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia gravis, Narcolepsy, Pemphigus vulgaris, Pernicious anaemia, Psoriasis, Psoriatic Arthritis, Polymyositis, Primary biliary cirrhosis, Relapsing polychondritis, Rheumatoid arthritis, Sjögren's syndrome, Temporal arteritis, Ulcerative Colitis, Vasculitis, and Wegener's granulomatosis.

[00131] As used herein, an "infectious disorder" is any disorder characterized by the presence of a microbial infection, such as viral, bacterial or protozoan infections. Such infectious disorders include, for example central nervous system infections, external ear infections, infections of the middle ear, such as acute otitis media, infections of the cranial sinuses, eye infections, infections of the oral cavity, such as infections of the teeth, gums and mucosa, upper respiratory tract infections, lower respiratory tract infections, genitourinary infections, gastrointestinal infections, gynecological infections, septicemia, bone and joint infections, skin and skin structure infections, bacterial endocarditis, burns, antibacterial prophylaxis of surgery, and antibacterial prophylaxis in immunosuppressed patients, such as patients receiving cancer chemotherapy, or organ transplant patients.

[00132] The term "polar substituent" as used herein refers to any substituent having an electric dipole, and optionally a dipole moment (e.g., an asymmetrical polar substituent has a dipole moment and a symmetrical polar substituent does not have a dipole moment). Polar substituents include substituents that accept or donate a hydrogen bond, and groups that would carry at least a partial positive or negative charge in aqueous solution at physiological pH levels. In certain embodiments, a polar substituent is one that can accept or donate electrons in a non-covalent hydrogen bond with another chemical moiety. In certain embodiments, a polar substituent is selected from a carboxy, a carboxy bioisostere or other acid-derived moiety that exists predominately as an anion at a pH of about 7 to 8. Other polar substituents include, but are not limited to, groups containing an OH or NH, an ether oxygen, an amine nitrogen, an oxidized sulfur or nitrogen, a carbonyl, a nitrile, and a nitrogen-containing or oxygen-containing heterocyclic ring whether aromatic or non-aromatic. In some embodiments, the polar substituent represented by R^3 is a carboxylate or a carboxylate bioisostere.

[00133] "Carboxylate bioisostere" or "carboxy bioisostere" as used herein refers to a moiety that is expected to be negatively charged to a substantial degree at physiological pH. In certain embodiments, the carboxylate bioisostere is a moiety selected from the group consisting of:



and salts and prodrugs of the foregoing, wherein each R^7 is independently H or an optionally substituted member selected from the group consisting of C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10}

heteroalkyl, C_{3-8} carbocyclic ring, and C_{3-8} heterocyclic ring optionally fused to an additional optionally substituted carbocyclic or heterocyclic ring; or R^7 is a C_{1-10} alkyl, C_{2-10} alkenyl, or C_{2-10} heteroalkyl substituted with an optionally substituted C_{3-8} carbocyclic ring or C_{3-8} heterocyclic ring.

[00134] In certain embodiments, the polar substituent is selected from the group consisting of carboxylic acid, carboxylic ester, carboxamide, tetrazole, triazole, carboxymethanesulfonamide, oxadiazole, oxothiadiazole, thiazole, aminothiazole and hydroxythiazole.

[00135] In some embodiments, at least one R^8 present is a carboxylic acid or a salt, or ester or a bioisostere thereof. In certain embodiments, at least one R^8 present is a carboxylic acid-containing substituent or a salt, ester or bioisostere thereof. In the latter embodiments, the R^8 substituent may be a C1-C10 alkyl or C1-C10 alkenyl linked to a carboxylic acid (or salt, ester or bioisostere thereof).

Anticancer Agents and other Therapeutic Agents

[00136] Compounds of the application are administered in combination with an additional anticancer agent or other therapeutic agent, as further described herein. Such additional anticancer agents include classic chemotherapeutic agents, as well as molecular targeted therapeutic agents, biologic therapy agents, and radiotherapeutic agents. Other therapeutic agents include those effective for the treatment of pain, infection, inflammation or autoimmune disorders.

[00137] Anticancer agents used in combination with the compounds of the present application may include agents selected from any of the classes known to those of ordinary skill in the art, including, for example, alkylating agents, anti-metabolites, plant alkaloids and terpenoids (e.g., taxanes), topoisomerase inhibitors, anti-tumor antibiotics, hormonal therapies, molecular targeted agents, and the like. Generally such an anticancer agent is an alkylating agent, an anti-metabolite, a vinca alkaloid, a taxane, a topoisomerase inhibitor, an anti-tumor antibiotic, a tyrosine kinase inhibitor, an immunosuppressive macrolide, an Akt inhibitor, an HDAC inhibitor an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, or a PI3K inhibitor.

[00138] Alkylating agents include (a) alkylating-like platinum-based chemotherapeutic agents such as cisplatin, carboplatin, nedaplatin, oxaliplatin, satraplatin, and (SP-4-3)-(cis)-amminedichloro-[2-methylpyridine] platinum(II); (b) alkyl sulfonates such as busulfan;

(c) ethyleneimine and methylmelamine derivatives such as altretamine and thiotepa; (d) nitrogen mustards such as chlorambucil, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, trofosamide, prednimustine, melphalan, and uramustine; (e) nitrosoureas such as carmustine, lomustine, fotemustine, nimustine, ranimustine and streptozocin; (f) triazenes and imidazotetrazines such as dacarbazine, procarbazine, temozolamide, and temozolomide.

[00139] Anti-metabolites include (a) purine analogs such as fludarabine, cladribine, chlorodeoxyadenosine, clofarabine, mercaptopurine, pentostatin, and thioguanine; (b) pyrimidine analogs such as fluorouracil, gemcitabine, capecitabine, cytarabine, azacitidine, edatrexate, floxuridine, and troxacitabine; (c) antifolates, such as methotrexate, pemetrexed, raltitrexed, and trimetrexate. Anti-metabolites also include thymidylate synthase inhibitors, such as fluorouracil, raltitrexed, capecitabine, floxuridine and pemetrexed; and ribonucleotide reductase inhibitors such as claribine, clofarabine and fludarabine.

[00140] Plant alkaloid and terpenoid derived agents include mitotic inhibitors such as the vinca alkaloids vinblastine, vincristine, vindesine, and vinorelbine; and microtubule polymer stabilizers such as the taxanes, including, but not limited to paclitaxel, docetaxel, larotaxel, ortataxel, and tesetaxel.

[00141] Topoisomerase inhibitors include topoisomerase I inhibitors such as camptothecin, topotecan, irinotecan, rubitecan, and belotecan; and topoisomerase II inhibitors such as etoposide, teniposide, and amsacrine.

[00142] Anti-tumor antibiotics include (a) anthracyclines such as daunorubicin (including liposomal daunorubicin), doxorubicin (including liposomal doxorubicin), epirubicin, idarubicin, and valrubicin; (b) streptomyces-related agents such as bleomycin, actinomycin, mithramycin, mitomycin, porfiromycin; and (c) anthracenediones, such as mitoxantrone and pixantrone. Anthracyclines have three mechanisms of action: intercalating between base pairs of the DNA/RNA strand; inhibiting topoiosomerase II enzyme; and creating iron-mediated free oxygen radicals that damage the DNA and cell membranes. Anthracyclines are generally characterized as topoisomerase II inhibitors.

[00143] Hormonal therapies include (a) androgens such as fluoxymesterone and testolactone; (b) antiandrogens such as bicalutamide, cyproterone, flutamide, and nilutamide; (c) aromatase inhibitors such as aminoglutethimide, anastrozole, exemestane, formestane, and letrozole; (d) corticosteroids such as dexamethasone and prednisone; (e) estrogens such as

diethylstilbestrol; (f) antiestrogens such as fulvestrant, raloxifene, tamoxifen, and toremifine; (g) LHRH agonists and antagonists such as buserelin, goserelin, leuprolide, and triptorelin; (h) progestins such as medroxyprogesterone acetate and megestrol acetate; and (i) thyroid hormones such as levothyroxine and liothyronine.

[00144] Molecular targeted agents include (a) receptor tyrosine kinase ('RTK') inhibitors, such as inhibitors of EGFR, including erlotinib, gefitinib, and neratinib; inhibitors of VEGFR including vandetanib, semaxinib, and cediranib; and inhibitors of PDGFR; further included are RTK inhibitors that act at multiple receptor sites such as lapatinib, which inhibits both EGFR and HER2, as well as those inhibitors that act at each of C-kit, PDGFR and VEGFR, including but not limited to axitinib, sunitinib, sorafenib and toceranib; also included are inhibitors of BCR-ABL, c-kit and PDGFR, such as imatinib; (b) FKBP binding agents, such as an immunosuppressive macrolide antibiotic, including bafilomycin, rapamycin (sirolimus) and everolimus; (c) gene therapy agents, antisense therapy agents, and gene expression modulators such as the retinoids and rexinoids, e.g. adapalene, bexarotene, trans-retinoic acid, 9-cis-retinoic acid, and N-(4-hydroxyphenyl)retinamide; (d) phenotype-directed therapy agents, including monoclonal antibodies such as alemtuzumab, bevacizumab, cetuximab, ibritumomab tiuxetan, rituximab, and trastuzumab; (e) immunotoxins such as gemtuzumab ozogamicin; (f) radioimmunoconjugates such as 131I-tositumomab; and (g) cancer vaccines.

[00145] Monoclonal antibodies include, but are not limited to, murine, chimeric, or partial or fully humanized monoclonal antibodies. Such therapeutic antibodies include, but are not limited to antibodies directed to tumor or cancer antigens either on the cell surface or inside the cell. Such therapeutic antibodies also include, but are not limited to antibodies directed to targets or pathways directly or indirectly associated with CK2. Therapeutic antibodies may further include, but are not limited to antibodies directed to targets or pathways that directly interact with targets or pathways associated with the compounds of the present invention. In one variation, therapeutic antibodies include, but are not limited to anticancer agents such as Abagovomab, Adecatumumab, Afutuzumab, Alacizumab pegol, Alemtuzumab, Altumomab pentetate, Anatumomab mafenatox, Apolizumab, Bavituximab, Belimumab, Bevacizumab, Bivatuzumab mertansine, Blinatumomab, Brentuximab vedotin, Cantuzumab mertansine, Catumaxomab, Cetuximab, Citatuzumab bogatox, Cixutumumab, Clivatuzumab tetraxetan, Conatumumab, Dacetuzumab, Detumomab, Ecromeximab, Edrecolomab, Elotuzumab, Epratuzumab, Ertumaxomab, Etaracizumab, Farletuzumab, Figitumumab, Fresolimumab,

Galiximab, Glembatumumab vedotin, Ibritumomab tiuxetan, Intetumumab, Inotuzumab ozogamicin, Ipilimumab, Iratumumab, Labetuzumab, Lexatumumab, Lintuzumab, Lucatumumab, Lumiliximab, Mapatumumab, Matuzumab, Milatuzumab, Mitumomab, Nacolomab tafenatox, Naptumomab estafenatox, Necitumumab, Nimotuzumab, Ofatumumab, Olaratumab, Oportuzumab monatox, Oregovomab, Panitumumab, Pemtumomab, Pertuzumab, Pintumomab, Pritumumab, Ramucirumab, Rilotumumab, Rituximab, Robatumumab, Sibrotuzumab, Tacatuzumab tetraxetan, Taplitumomab paptox, Tenatumomab, Ticilimumab, Tigatuzumab, Tositumomab, Trastuzumab, Tremelimumab, Tucotuzumab celmoleukin, Veltuzumab, Volociximab, Votumumab, Zalutumumab, and Zanolimumab. In some embodiments, such therapeutic antibodies include, alemtuzumab, bevacizumab, cetuximab, daclizumab, gemtuzumab, ibritumomab tiuxetan, pantitumumab, rituximab, tositumomab, and trastuzumab; in other embodiments, such monoclonal antibodies include alemtuzumab, bevacizumab, cetuximab, ibritumomab tiuxetan, rituximab, and trastuzumab; alternately, such antibodies include daclizumab, gemtuzumab, and pantitumumab. In yet another embodiment, therapeutic antibodies useful in the treatment of infections include but are not limited to Afelimomab, Efungumab, Exbivirumab, Felvizumab, Foravirumab, Ibalizumab, Libivirumab, Motavizumab, Nebacumab, Pagibaximab, Palivizumab, Panobacumab, Rafivirumab, Raxibacumab, Regavirumab, Sevirumab, Tefibazumab, Tuvirumab, and Urtoxazumab. In a further embodiment, therapeutic antibodies can be useful in the treatment of inflammation and/or autoimmune disorders, including, but are not limited to, Adalimumab, Atlizumab, Atorolimumab, Aselizumab, Bapineuzumab, Basiliximab, Benralizumab, Bertilimumab, Besilesomab, Briakinumab, Canakinumab, Cedelizumab, Certolizumab pegol, Clenoliximab, Daclizumab, Denosumab, Eculizumab, Edobacomab, Efalizumab, Erlizumab, Fezakinumab, Fontolizumab, Fresolimumab, Gantenerumab, Gavilimomab, Golimumab, Gomiliximab, Infliximab, Inolimomab, Keliximab, Lebrikizumab, Lerdelimumab, Mepolizumab, Metelimumab, Muromonab-CD3, Natalizumab, Ocrelizumab, Odulimomab, Omalizumab, Otelixizumab, Pascolizumab, Priliximab, Reslizumab, Rituximab, Rontalizumab, Rovelizumab, Ruplizumab, Sifalimumab, Siplizumab, Solanezumab, Stamulumab, Talizumab, Tanezumab, Teplizumab, Tocilizumab, Toralizumab, Ustekinumab, Vedolizumab, Vepalimomab, Visilizumab, Zanolimumab, and Zolimomab aritox. In yet another embodiment, such therapeutic antibodies include, but are not limited to adalimumab, basiliximab, certolizumab pegol, eculizumab, efalizumab, infliximab, muromonab-CD3, natalizumab, and omalizumab.

Alternately the therapeutic antibody can include abciximab or ranibizumab. Generally a therapeutic antibody is non-conjugated, or is conjugated with a radionuclide, cytokine, toxin, drug-activating enzyme or a drug-filled liposome.

[00146] Akt inhibitors include 1L6-Hydroxymethyl-chiro-inositol-2-(R)-2-O-methyl-3-O-octadecyl-*sn*-glycerocarbonate, SH-5 (Calbiochem Cat. No. 124008), SH-6 (Calbiochem Cat. No. Cat. No. 124009), Calbiochem Cat. No. 124011, Triciribine (NSC 154020, Calbiochem Cat. No. 124012), 10-(4′-(N-diethylamino)butyl)-2-chlorophenoxazine, Cu(II)Cl_2(3-Formylchromone thiosemicarbazone), 1,3-dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, GSK690693 (4-(2-(4-amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[(3S)-3-piperidinylmethyl]oxy}-1H-imidazo[4,5-c]pyridin-4-yl)-2-methyl-3-butyn-2-ol), SR13668 ((2,10-dicarbethoxy-6-methoxy-5,7-dihydro-indolo[2,3-b] carbazole), GSK2141795, Perifosine, GSK21110183, XL418, XL147, PF-04691502, BEZ-235 [2-Methyl-2-[4-(3-methyl-2-oxo-8-quinolin-3-yl-2,3-dihydro-imidazo[4,5-c]quinolin-1-yl)-phenyl]-propionitrile], PX-866 ((acetic acid (1S,4E,10R,11R,13S,14R)-[4-diallylaminomethylene-6-hydroxy-1-methoxymethyl-10,13-dimethyl-3,7,17-trioxo-1,3,4,7,10,11,12,13,14,15,16,17-dodecahydro-2-oxa-cyclopenta[a]phenanthren-11-yl ester)), D-106669, CAL-101, GDC0941 (2-(1H-indazol-4-yl)-6-(4-methanesulfonyl-piperazin-1-ylmethyl)-4-morpholin-4-yl-thieno[3,2-d]pyrimidine), SF1126, SF1188, SF2523, TG100-115 [3-[2,4-diamino-6-(3-hydroxyphenyl)pteridin-7-yl]phenol]. A number of these inhibitors, such as, for example, BEZ-235, PX-866, D 106669, CAL-101, GDC0941, SF1126, SF2523 are also identified in the art as PI3K/mTOR inhibitors; additional examples, such as PI-103 [3-[4-(4-morpholinylpyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]phenol hydrochloride] are well-known to those of skill in the art. Additional well-known PI3K inhibitors include LY294002 [2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one] and wortmannin. mTOR inhibitors known to those of skill in the art include temsirolimus, deforolimus, sirolimus, everolimus, zotarolimus, and biolimus A9. A representative subset of such inhibitors includes temsirolimus, deforolimus, zotarolimus, and biolimus A9.

[00147] HDAC inhibitors include (i) hydroxamic acids such as Trichostatin A, vorinostat (suberoylanilide hydroxamic acid (SAHA)), panobinostat (LBH589) and belinostat (PXD101) (ii) cyclic peptides, such as trapoxin B, and depsipeptides, such as romidepsin (NSC 630176), (iii) benzamides, such as MS-275 (3-pyridylmethyl-N-{4-[(2-aminophenyl)-carbamoyl]-benzyl}-carbamate), CI994 (4-acetylamino-N-(2aminophenyl)-benzamide) and MGCD0103

(N-(2-aminophenyl)-4-((4-(pyridin-3-yl)pyrimidin-2-ylamino)methyl)benzamide), (iv) electrophilic ketones, (v) the aliphatic acid compounds such as phenylbutyrate and valproic acid.

[00148] Hsp90 inhibitors include benzoquinone ansamycins such as geldanamycin, 17-DMAG (17-Dimethylamino-ethylamino-17-demethoxygeldanamycin), tanespimycin (17-AAG, 17-allylamino-17-demethoxygeldanamycin), EC5, retaspimycin (IPI-504, 18,21-didehydro-17-demethoxy-18,21-dideoxo-18,21-dihydroxy-17-(2-propenylamino)-geldanamycin), and herbimycin; pyrazoles such as CCT 018159 (4-[4-(2,3-dihydro-1,4-benzodioxin-6-yl)-5-methyl-1H-pyrazol-3-yl]-6-ethyl-1,3-benzenediol); macrolides, such as radicocol; as well as BIIB021 (CNF2024), SNX-5422, STA-9090, and AUY922.

[00149] Miscellaneous agents include altretamine, arsenic trioxide, gallium nitrate, hydroxyurea, levamisole, mitotane, octreotide, procarbazine, suramin, thalidomide, lenalidomide, photodynamic compounds such as methoxsalen and sodium porfimer, and proteasome inhibitors such as bortezomib.

[00150] Biologic therapy agents include: interferons such as interferon-α2a and interferon-α2b, and interleukins such as aldesleukin, denileukin diftitox, and oprelvekin.

[00151] In addition to anticancer agents intended to act against cancer cells, combination therapies including the use of protective or adjunctive agents, including: cytoprotective agents such as armifostine, dexrazonxane, and mesna, phosphonates such as pamidronate and zoledronic acid, and stimulating factors such as epoetin, darbeopetin, filgrastim, PEG-filgrastim, and sargramostim, are also envisioned.

[00152] Numerous anti-inflammatory agents are familiar to those of skill in the art, such as, for example glucocorticoids, NSAIDs, coxibs, corticosteroids, analgesics, such as paracetamol, opiates, morphinomimetics, inhibitors of 5-lipoxygenase, inhibitors of 5-lipoxygenase activating protein, and leukotriene receptor antagonists. Examples of nonsteroidal anti-inflammatory agents include, but are not limited to aspirin, ketoprofen, flurbiprofen, ibuprofen, naproxen, fenoprofen, benoxaprofen, indoprofen, pirprofen, carprofen, oxaprozin, pranoprofen, suprofen, alminoprofen, butibufen, diclofenac, ketorolac, aspirin, bextra, celebrex, vioxx and acetominophen. Examples of opiates include but are not limited to morphine, codeine, hydrocodone, oxycodone, penthidine, dihydromorphine, tramadol, and buprenorphine. In one embodiment, anti-inflammatory agents are monoclonal antibodies. In another embodiment, anti-inflammatory agents are monoclonal antibodies targeting at receptors

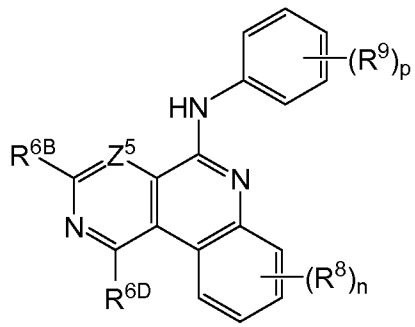
or antigens directly or indirectly associated with inflammation. In another embodiment, anti-inflammatory agents are monoclonal antibodies targeting CK2 kinase or CK2-regulated pathways. In yet another embodiment, anti-inflammatory agents include, but are not limited to Adalimumab, Atlizumab, Atorolimumab, Aselizumab, Bapineuzumab, Basiliximab, Benralizumab, Bertilimumab, Besilesomab, Briakinumab, Canakinumab, Cedelizumab, Certolizumab pegol, Clenoliximab, Daclizumab, Denosumab, Eculizumab, Edobacomab, Efalizumab, Erlizumab, Fezakinumab, Fontolizumab, Fresolimumab, Gantenerumab, Gavilimomab, Golimumab, Gomiliximab, Infliximab, Inolimomab, Keliximab, Lebrikizumab, Lerdelimumab, Mepolizumab, Metelimumab, Muromonab-CD3, Natalizumab, Ocrelizumab, Odulimomab, Omalizumab, Otelixizumab, Pascolizumab, Priliximab, Reslizumab, Rituximab, Rontalizumab, Rovelizumab, Ruplizumab, Sifalimumab, Siplizumab, Solanezumab, Stamulumab, Talizumab, Tanezumab, Teplizumab, Tocilizumab, Toralizumab, Ustekinumab, Vedolizumab, Vepalimomab, Visilizumab, Zanolimumab, and Zolimomab aritox.

[00153] Antiinfection agents include those agents known in the art to treat viral, fungal, parasitic or bacterial infections. The term, "antibiotic," as used herein, refers to a chemical substance that inhibits the growth of, or kills, microorganisms. Encompassed by this term are antibiotic produced by a microorganism, as well as synthetic antibiotics known in the art. Antibiotics include, but are not limited to, clarithromycin, ciprofloxacin, and metronidazole. In one embodiment, antiinfection agents are monoclonal antibodies directed to antigens associated with infectious agents or microorganisms. Non-limiting examples of monoclonal antibodies effective in the treatment of infections include Afelimomab, Efungumab, Exbivirumab, Felvizumab, Foravirumab, Ibalizumab, Libivirumab, Motavizumab, Nebacumab, Pagibaximab, Palivizumab, Panobacumab, Rafivirumab, Raxibacumab, Regavirumab, Sevirumab, Tefibazumab, Tuvirumab, and Urtoxazumab.

[00154] Examples of the immunotherapeutic agents useful for the treatment of pain, inflammation, infection and/or autoimmune disorders include but are not limited to microorganism or bacterial components (e.g., muramyl dipeptide derivative, Picibanil), polysaccharides having immunity potentiating activity (e.g., lentinan, schizophyllan, krestin), cytokines obtained by genetic engineering techniques (e.g., interferon, interleukin (IL)), colony stimulating factors (e.g., G-CSF (Filgrastim/Pegfilgrastim, Lenograstim), GM-CSF (Molgramostim, Sargramostim), SCF (Ancestim), and erythropoietin) and the like. Monoclonal antibodies that have such therapeutic effects include, but are not limited to Adalimumab,

Atlizumab, Atorolimumab, Aselizumab, Bapineuzumab, Basiliximab, Benralizumab, Bertilimumab, Besilesomab, Briakinumab, Canakinumab, Cedelizumab, Certolizumab pegol, Clenoliximab, Daclizumab, Denosumab, Eculizumab, Edobacomab, Efalizumab, Erlizumab, Fezakinumab, Fontolizumab, Fresolimumab, Gantenerumab, Gavilimomab, Golimumab, Gomiliximab, Infliximab, Inolimomab, Keliximab, Lebrikizumab, Lerdelimumab, Mepolizumab, Metelimumab, Muromonab-CD3, Natalizumab, Ocrelizumab, Odulimomab, Omalizumab, Otelixizumab, Pascolizumab, Priliximab, Reslizumab, Rituximab, Rontalizumab, Rovelizumab, Ruplizumab, Sifalimumab, Siplizumab, Solanezumab, Stamulumab, Talizumab, Tanezumab, Teplizumab, Tocilizumab, Toralizumab, Ustekinumab, Vedolizumab, Vepalimomab, Visilizumab, Zanolimumab, and Zolimomab aritox.

[00155] In one aspect, the application discloses a method for treating or ameliorating a neoplastic disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula I:



Formula I,

or a pharmaceutically acceptable salt or ester thereof,

wherein Z^5 is N or CR^{6A};

each R^{6A}, R^{6B}, R^{6D} and R^8 independently is H or an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group,

or each R^{6A}, R^{6B}, R^{6D} and R^8 independently is halo, CF_3, CFN, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, carboxy bioisostere, $CONR_2$, OOCR, COR, or NO_2,

each R^9 is independently an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group, or

each R^9 is independently halo, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, $CONR_2$, OOCR, COR, or NO_2,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, $NR'SO_2R'$, $NR'CONR'_2$, NR′COOR′, NR′COR′, CN, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2,

wherein each R′ is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

and wherein two R′ can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S;

n is 0 to 4; and

p is 0 to 4;

and an anticancer agent, or a pharmaceutically acceptable salt or ester thereof;

thereby treating or ameliorating said neoplastic disorder.

[00156] In one embodiment of any disclosed aspect or alternative described herein, the anticancer agent used in combination with a compound of the present application is selected from an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, a PI3K inhibitor and a monoclonal antibody targeting a tumor/cancer antigen; in another embodiment, the anticancer agent used in combination with a compound of the present application is selected from an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, and a PI3K inhibitor. In one embodiment, the anticancer agent used in combination with a compound of the present application is selected from an inhibitor of Akt1/2, an hydroxamic acid inhibitor of HDAC, and a benzoquinone ansamycin inhibitor of Hsp90. In another embodiment, the anticancer agent used in combination with a compound of the present invention is selected from 1,3-dihydro-1-

(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat and 17-DMAG. In another embodiment, the anticancer agent used in combination with a compound of the present application is selected from an imidazo[4,5-c]quinoline derivative that inhibits PI3K and mTOR kinase activity, a benzopyran derivative that inhibits PI3K, a pyrido[3',2':4,5]furo[3,2-d]pyrimidine derivative that inhibits PI3K and mTOR kinase activity and a furanosteroid derivative that inhibits PI3K. In yet another embodiment, the anticancer agent used in combination with a compound of the present invention is selected from BEZ-235, LY294002, PI-103, and wortmannin. In yet another embodiment, the anticancer agent used in combination with a compound of the present invention is selected from the group consisting of 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat, 17-DMAG, BEZ-235, LY294002, PI-103, wortmannin and cetuximab. In yet a further embodiment, the anticancer agent used in combination with a compound of the present invention is selected from the group consisting of 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat, 17-DMAG, BEZ-235, LY294002, PI-103, and wortmannin.

[00157] In one alternative, the application discloses a method for treating or ameliorating a neoplastic disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula I as described herein and an anticancer agent, or a pharmaceutically acceptable salt or ester thereof, wherein the anticancer agent is not doxorubicin.

[00158] In another alternative, the application discloses a method for treating or ameliorating a neoplastic disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula I as described herein and an anticancer agent, or a pharmaceutically acceptable salt or ester thereof, wherein the anticancer agent is not a topoisomerase II inhibitor.

[00159] In still another alternative, the application discloses a method for treating or ameliorating a neoplastic disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of Formula I as described herein and an anticancer agent, or a pharmaceutically acceptable salt or ester thereof, wherein the anticancer agent is not an anti-tumor antibiotic.

[00160] In one embodiment of any of aspect or alternative described herein, the anticancer agent is not 5-fluorouracil. In another embodiment, the anticancer agent is not a thymidylate synthase inhibitor. In yet another embodiment, the anticancer agent is not an antimetabolite pyrmidine analog. In still another embodiment, the anticancer agent is not an antimetabolite.

[00161] In one embodiment of any of aspect or alternative described herein, the anticancer agent is not rapamycin. In another embodiment, the anticancer agent is not an immunosuppressive macrolide antibiotic. In yet another embodiment, the anticancer agent is not FKBP binding agent.

[00162] In one embodiment of any of aspect or alternative described herein, the anticancer agent is not erlotinib (Tarceva). In another embodiment, the anticancer agent is not a small molecule EGFR inhibitor. In yet another embodiment, the anticancer agent is not a receptor tyrosine kinase inhibitor.

[00163] In one embodiment of any of aspect or alternative described herein, the anticancer agent is not sunitinib (Sutent). In another embodiment, the anticancer agent is not an inhibitor of VEGFR, PDGFR and cKIT. In yet another embodiment, the anticancer agent is not a receptor tyrosine kinase inhibitor.

[00164] In another embodiment of any aspect or alternative described herein, the anticancer agent is not doxorubicin, 5-fluorouracil, rapamycin, erlotinib or sunitinib. In another embodiment, the anticancer agent is not any one of any four of doxorubicin, 5-fluorouracil, rapamycin, erlotinib or sunitinib. For example in one such embodiment, the anticancer agent is not 5-fluorouracil, rapamycin, erlotinib or sunitinib. In another such embodiment the anticancer agent is not doxorubicin, 5-fluorouracil, erlotinib or sunitinib. In another embodiment, the anticancer agent is not any one of any three of doxorubicin, 5-fluorouracil, rapamycin, erlotinib or sunitinib. For example in one such embodiment, the anticancer agent is not 5-fluorouracil, erlotinib or sunitinib. In another such embodiment the anticancer agent is not doxorubicin, erlotinib or sunitinib. In another embodiment, the anticancer agent is not any one of any two of doxorubicin, 5-fluorouracil, rapamycin, erlotinib or sunitinib.

[00165] In another embodiment of any aspect or alternative described herein, the anticancer agent is not a topoisomerase II inhibitor, a thymidylate synthase inhibitor, an immunosuppressive macrolide antibiotic, a small molecule EGFR inhibitor or an inhibitor of VEGFR, PDGFR and cKIT. In another embodiment, the anticancer agent is not any one of any four of a topoisomerase II inhibitor, a thymidylate synthase inhibitor, an immunosuppressive

macrolide antibiotic, a small molecule EGFR inhibitor or an inhibitor of VEGFR, PDGFR and cKIT. For example in one such embodiment, the anticancer agent is not a thymidylate synthase inhibitor, an immunosuppressive macrolide antibiotic, a small molecule EGFR inhibitor or an inhibitor of VEGFR, PDGFR and cKIT. In another such embodiment the anticancer agent is not a topoisomerase II inhibitor, thymidylate synthase inhibitor, a small molecule EGFR inhibitor or an inhibitor of VEGFR, PDGFR and cKIT. In another embodiment, the anticancer agent is not any one of any three of a topoisomerase II inhibitor, thymidylate synthase inhibitor, an immunosuppressive macrolide antibiotic, a small molecule EGFR inhibitor or an inhibitor of VEGFR, PDGFR and cKIT. For example in one such embodiment, the anticancer agent is not a topoisomerase II inhibitor, thymidylate synthase inhibitor, or an inhibitor of VEGFR, PDGFR and cKIT. In another such embodiment the anticancer agent is not a thymidylate synthase inhibitor, a small molecule EGFR inhibitor or an inhibitor of VEGFR, PDGFR and cKIT. In another embodiment, the anticancer agent is not any one of any two of a topoisomerase II inhibitor, thymidylate synthase inhibitor, an immunosuppressive macrolide antibiotic, a small molecule EGFR inhibitor or an inhibitor of VEGFR, PDGFR and cKIT.

[00166] In another embodiment of any aspect or alternative described herein, the anticancer agent is not a topoisomerase II inhibitor, an antimetabolite pyrimidine analog, an FKBP binding agent, or a receptor tyrosine kinase inhibitor. In another embodiment, the anticancer agent is not any one of any three of a topoisomerase II inhibitor, an antimetabolite pyrimidine analog, an FKBP binding agent, or a receptor tyrosine kinase inhibitor. For example in one such embodiment, the anticancer agent is not a topoisomerase II inhibitor, an antimetabolite pyrimidine analog, or a receptor tyrosine kinase inhibitor. In another such embodiment the anticancer agent is not an antimetabolite pyrimidine analog, an FKBP binding agent, or a receptor tyrosine kinase inhibitor. In another embodiment, the anticancer agent is not any one of any two of a topoisomerase II inhibitor, an antimetabolite pyrimidine analog, an FKBP binding agent, or a receptor tyrosine kinase inhibitor.

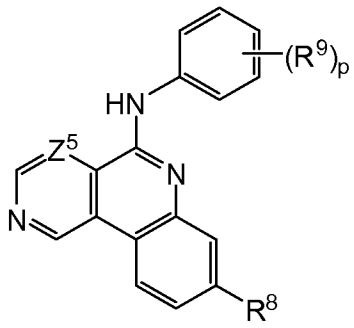
[00167] In one embodiment of any aspect or alternative described herein, the anticancer agent used in combination with a compound of the present application is selected from 5-fluorouracil (5-FU), cisplatin, doxorubicin, fludarabine, gemcitabine, paclitaxel, rapamycin, sunitinib, lapatinib, sorafenib, erlotinib, and vinblastine. In one embodiment of any aspect or alternative described herein, the anticancer agent used in combination with a compound of the present application is selected from 5-fluorouracil (5-FU), cisplatin, doxorubicin, fludarabine,

gemcitabine, paclitaxel, rapamycin, sunitinib, erlotinib, and vinblastine. In another embodiment, the anticancer agent is selected from 5-fluorouracil, cisplatin, fludarabine, gemcitabine, paclitaxel, rapamycin, sunitinib, erlotinib, and vinblastine. In yet another embodiment, the anticancer agent is selected from cisplatin, doxorubicin, fludarabine, gemcitabine, paclitaxel, rapamycin, sunitinib, erlotinib, and vinblastine. In still another embodiment, the anticancer agent is selected from cisplatin, fludarabine, gemcitabine, paclitaxel, rapamycin, sunitinib, erlotinib, and vinblastine. In another embodiment, the anticancer agent is selected from 5-fluorouracil, cisplatin, doxorubicin, fludarabine, gemcitabine, paclitaxel, sunitinib, erlotinib, and vinblastine. In yet another embodiment, the anticancer agent is selected from 5-fluorouracil, cisplatin, fludarabine, gemcitabine, paclitaxel, sunitinib, erlotinib, and vinblastine. In still another embodiment, the anticancer agent is selected from 5-fluorouracil, cisplatin, doxorubicin, fludarabine, gemcitabine, paclitaxel, rapamycin, sunitinib, and vinblastine. In a further embodiment, the anticancer agent is selected from 5-fluorouracil, cisplatin, fludarabine, gemcitabine, paclitaxel, rapamycin, sunitinib, and vinblastine. In an additional embodiment, the anticancer agent is selected from 5-fluorouracil, cisplatin, doxorubicin, fludarabine, gemcitabine, paclitaxel, rapamycin, erlotinib, and vinblastine. In another embodiment, the anticancer agent is selected from 5-fluorouracil, cisplatin, fludarabine, gemcitabine, paclitaxel, rapamycin, erlotinib, and vinblastine. In yet another embodiment, the anticancer agent used in combination with a compound of the present application is selected from doxorubicin, cisplatin, fludarabine, gemcitabine, paclitaxel, and vinblastine. In still another embodiment, the anticancer agent used in combination with a compound of the present application is selected from cisplatin, fludarabine, gemcitabine, paclitaxel, and vinblastine. In yet another embodiment, the anticancer agent used in combination with a compound of the present application is selected from sunitinib, lapatinib, sorafenib and erlotinib.

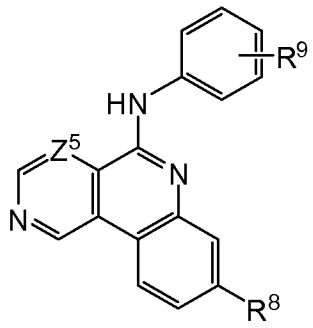
[00168] In still another embodiment, the anticancer agent used in combination with a compound of the present application is selected from a therapeutic antibody such as monoclonal murine antibody; in another embodiment, the therapeutic antibody is a monoclonal chimeric antibody; in yet another embodiment, the therapeutic antibody is a monoclonal humanized antibody. In some embodiments, therapeutic antibodies can include but are not limited to alemtuzumab, bevacizumab, cetuximab, daclizumab, gemtuzumab, ibritumomab tiuxetan, pantitumumab, rituximab, tositumomab, and trastuzumab; in other embodiments, such

monoclonal antibodies include alemtuzumab, bevacizumab, cetuximab, ibritumomab tiuxetan, rituximab, and trastuzumab; alternately, such antibodies include daclizumab, gemtuzumab, and pantitumumab. In yet another embodiment, the therapeutic antibody is cetuximab. In one variation of any of the disclosed embodiments, the therapeutic antibody is non-conjugated. In another variation, the antibody is conjugated with a radionuclide, cytokine, toxin, drug-activating enzyme or a drug-filled liposome.

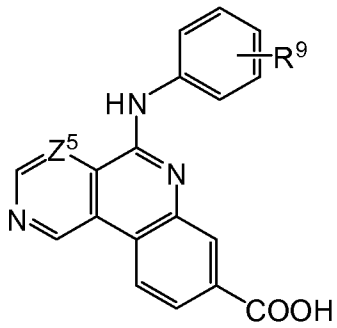
[00169] In one embodiment of any disclosed aspect or alternative, the compound of Formula I has the structure of Formula II, III, IV, V or VI:



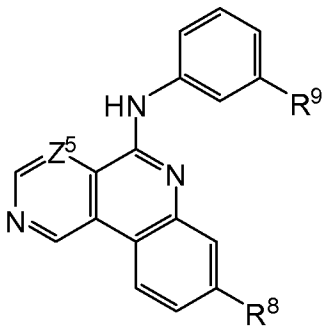
Formula II ;



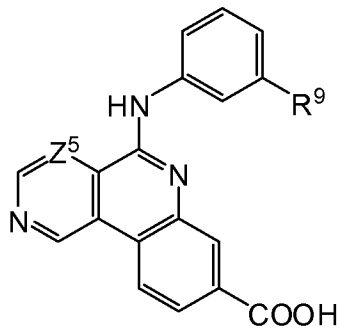
Formula III ;



Formula IV ;



Formula V ; or



Formula VI ;

or a pharmaceutically acceptable salt or ester thereof;

wherein Z^5 is N or CR^{6A};

each R^{6A} and R^8 independently is H or an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group,

or each R^{6A} and R^8 independently is halo, CF_3, CFN, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, carboxy bioisostere, $CONR_2$, OOCR, COR, or NO_2,

each R^9 is independently an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group, or

each R^9 is independently halo, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, $CONR_2$, OOCR, COR, or NO_2,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, $NR'SO_2R'$, $NR'CONR'_2$, NR′COOR′, NR′COR′, CN, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2,

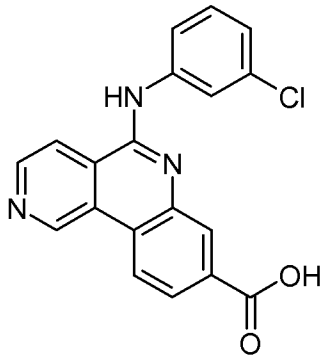
wherein each R′ is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

and wherein two R′ can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S; and

p is 0 to 4.

[00170] In one embodiment of any disclosed aspect or alternative, the compound of Formula I has the structure of Formula II. In another embodiment, the compound of Formula I has the structure of Formula III. In yet another embodiment, the compound of Formula I has the structure of Formula IV. In still a further embodiment, the compound of Formula I has the structure of Formula V. In yet another embodiment of any disclosed aspect or alternative, the compound of Formula I has the structure of Formula VI. In one variation of any disclosed embodiment, Z^5 is CR^{6A}. In one particular variation of any disclosed embodiment, Z^5 is CH.

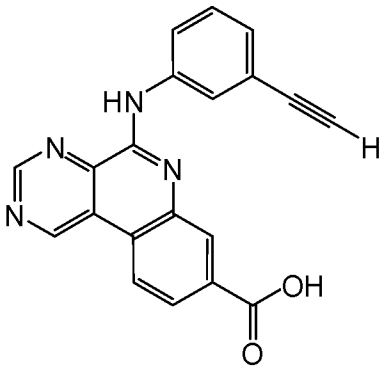
[00171] In a particular embodiment of any disclosed aspect or alternative, the compound of Formula I is a compound (Compound K) having the formula:



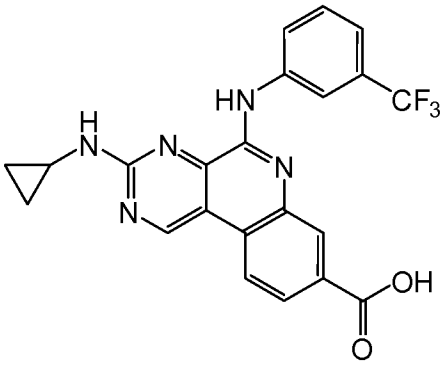
Compound K,

or a pharmaceutically acceptable salt or ester thereof.

[00172] In another embodiment of any disclosed aspect or alternative, the compound of formula I is a compound having formula (1) or (2):



(1), or



(2),

or a pharmaceutically acceptable salt or ester thereof.

[00173] Compounds of Formulae I, II, III, IV, V, and VI can exert biological activities that include, but are not limited to, inhibiting cell proliferation, and modulating protein kinase activity. Compounds of such Formulae can modulate CK2 activity, for example. Such compounds therefore can be utilized in multiple applications by a person of ordinary skill in the art. For example, compounds described herein may find uses that include, but are not limited to, (i) modulation of protein kinase activity (e.g., CK2 activity), (ii) modulation of cell proliferation, (iii) modulation of apoptosis, and (iv) treatment of cell proliferation related disorders, such as neoplastic disorders, when administered alone or in combination with another anticancer agent. Compounds described herein may further find uses that include (a) reduction of pro-inflammatory signaling, (b) treatment of inflammation related disorders, such as inflammatory or autoimmune disorders, and (c) treatment of infectious disorders, such as viral,

bacterial or protozoan infection, when administered alone or in combination with a therapeutic agent.

[00174] Without being bound to any particular theory of mechanism, the compositions described herein provide a therapeutic response as the combination of pharmaceutically active agents synergistically or comprehensively affect multiple pathways associated with neoplastic disorders, pain, inflammatory, autoimmune or infectious disorders.

[00175] Anticancer treatments that use such combination therapies may result in synergistic (e.g., greater than additive) result compared to administration of either therapy alone. Improved results can be explained in part because the compounds of the application act at least in part through a mechanism, e.g., CK2 modulation, that differs from that of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, a PI3K inhibitor or an anti-tumor/anti-cancer monoclonal antibody.

[00176] Similarly, modulation of CK2 activity by the compounds of the application, when administered in combination with compounds effective for the treatment of pain, inflammatory, autoimmune or infectious disorders can lead to synergistic effects. For example, diseases of the immune system can be treated via administration of a CK2 modulator of the current application in combination with immunomodulators. The activity of immunomodulators such as cytokines, including colony-stimulating factors, interferons, and interleukins can be complemented by the activity of a CK2 modulator of the current application. As a further example, inflammation or pain can be treated via administration of a CK2 modulator in combination with an anti-inflammatory, such as COX inhibitors, including NSAIDs, or glucocorticoids.

[00177] In one aspect, the application discloses a method for inhibiting or slowing cell proliferation in a system, comprising administering to said system an effective amount of a compound of Formula I, II, III, IV, V, or VI, as described herein, or a pharmaceutically acceptable salt or ester thereof, and an anticancer agent or a pharmaceutically acceptable salt or ester thereof; thereby inhibiting or slowing cell proliferation. The system may be a cell, tissue or subject.

[00178] In yet another aspect, the present application discloses a method for treating, or ameliorating pain, inflammatory, infection and/or an autoimmune comprising administering to a patient in need thereof an effective amount of a compound of Formula I, II, III, IV, V, or VI, as described herein, or a pharmaceutically acceptable salt or ester thereof, and a therapeutic agent. In one variation, the therapeutic agent is an antiinfection agent. In another variation, the

therapeutic agent is an anti-inflammatory agent. In another variation, the therapeutic agent is an immunotherapeutic agent.

[00179] In one embodiment, the antiinfection agent, the anti-inflammatory agent or the immunotherapeutic agent is an antibody. In one variation the antibody is a murine monoclonal antibody. In another variation, the therapeutic antibody is a chimeric monoclonal antibody; in still another variation, the therapeutic antibody is a humanized monoclonal antibody. In some embodiments, therapeutic antibodies can include but are not limited to Adalimumab, Atlizumab, Atorolimumab, Aselizumab, Bapineuzumab, Basiliximab, Benralizumab, Bertilimumab, Besilesomab, Briakinumab, Canakinumab, Cedelizumab, Certolizumab pegol, Clenoliximab, Daclizumab, Denosumab, Eculizumab, Edobacomab, Efalizumab, Erlizumab, Fezakinumab, Fontolizumab, Fresolimumab, Gantenerumab, Gavilimomab, Golimumab, Gomiliximab, Infliximab, Inolimomab, Keliximab, Lebrikizumab, Lerdelimumab, Mepolizumab, Metelimumab, Muromonab-CD3, Natalizumab, Ocrelizumab, Odulimomab, Omalizumab, Otelixizumab, Pascolizumab, Priliximab, Reslizumab, Rituximab, Rontalizumab, Rovelizumab, Ruplizumab, Sifalimumab, Siplizumab, Solanezumab, Stamulumab, Talizumab, Tanezumab, Teplizumab, Tocilizumab, Toralizumab, Ustekinumab, Vedolizumab, Vepalimomab, Visilizumab, Zanolimumab, and Zolimomab aritox. Alternately, the therapeutic antibody can be selected from the group consisting of adalimumab, basiliximab, certolizumab pegol, eculizumab, efalizumab, infliximab, muromonab-CD3, natalizumab, and omalizumab. The therapeutic antibody may also be abciximab or ranibizumab. In one variation of any disclosed aspect or embodiment, the therapeutic antibody is conjugated with a radionuclide, cytokine, toxin, drug-activating enzyme or a drug-filled liposome.

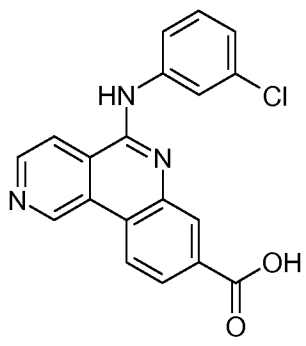
[00180] In yet another variation, the therapeutic agent is an anti-inflammatory agent. In one embodiment, the anti-inflammatory agent is selected from the group consisting of glucocorticoids, NSAIDs, coxibs, corticosteroids, analgesics, inhibitors of 5-lipoxygenase, inhibitors of 5-lipoxygenase activating protein, and leukotriene receptor antagonists. In another embodiment, the anti-inflammatory agent is selected from the group consisting of ketoprofen, flurbiprofen, ibuprofen, naproxen, fenoprofen, benoxaprofen, indoprofen, pirprofen, carprofen, oxaprozin, pranoprofen, suprofen, alminoprofen, butibufen, diclofenac, ketorolac, aspirin, bextra, celebrex, vioxx and acetominophen.

[00181] In yet another variation, the therapeutic agent is an antiinfection agent. In one embodiment, the therapeutic agent is an antiviral agent; alternately the therapeutic agent is an

anti-parasitic agent. In another embodiment, the therapeutic agent is an antifungal agent; alternately, the therapeutic agent is an antibacterial agent. In another variation, the therapeutic agent is an antibiotic. In one variation, the antiinfection agent is selected from the group consisting of penicillin, cephalosporins, aminoglycosides, macrolides, quinolones and tetracyclines. In one variation, the antiinfection agent is selected from the group consisting of Afelimomab, Efungumab, Exbivirumab, Felvizumab, Foravirumab, Ibalizumab, Libivirumab, Motavizumab, Nebacumab, Pagibaximab, Palivizumab, Panobacumab, Rafivirumab, Raxibacumab, Regavirumab, Sevirumab, Tefibazumab, Tuvirumab, and Urtoxazumab.

[00182] In yet another variation, the therapeutic agent is an immunotherapeutic agent. In one embodiment the immunotherapeutic agent is selected from the group consisting of microorganism or bacterial component, such as muramyl dipeptide derivative or Picibanil, a polysaccharide having immunity potentiating activity, such as lentinan, schizophyllan, or krestin, a cytokine, such as interferon or an interleukin, a colony stimulating factor, such as granulocyte colony stimulating factor or erythropoietin. Alternately, the immunotherapeutic agent is selected from the group consisting of Aselizumab, Apolizumab, Benralizumab, Cedelizumab, Certolizumab pegol, Daclizumab, Eculizumab, Efalizumab, Epratuzumab, Erlizumab, Fontolizumab, Mepolizumab, Natalizumab, Ocrelizumab, Omalizumab, Pascolizumab, Pexelizumab, Reslizumab, Rontalizumab, Rovelizumab, Ruplizumab, Siplizumab, Talizumab, Teplizumab, Tocilizumab, Toralizumab, Vedolizumab, and Visilizumab.

[00183] The present application also discloses methods for preventing, treating or ameliorating neoplastic disorders, as well as for inhibiting or slowing cell proliferation, comprising the administration of a therapeutically effective amount of a compound (Compound K) having the formula:



,

or a pharmaceutically acceptable salt or ester thereof, in combination with commonly used anticancer agents, or pharmaceutically acceptable salts or esters thereof.

[00184] In yet another aspect, the present application discloses a method for treating, or ameliorating pain, an inflammatory, autoimmune or infectious disorder comprising administering to a patient in need thereof an effective amount of a compound (Compound K), or a pharmaceutically acceptable salt or ester thereof, and a therapeutic agent, as disclosed herein.

[00185] With regard to the foregoing aspects of the application, the inventors contemplate any combination of the anticancer agents as set forth herein.

[00186] The present application discloses pharmaceutical compositions comprising a compound of Formula I, II, III, IV, V or VI, or a pharmaceutically acceptable salt or ester thereof, and a commonly used anticancer agent, or a pharmaceutically acceptable salt or ester thereof, and at least one pharmaceutically acceptable excipient. A further aspect disclosed in the present application is a pharmaceutical composition comprising a compound of Formula I as disclosed herein, an anticancer agent and at least one pharmaceutically acceptable excipient. In one embodiment, an anticancer agent is selected from the group consisting of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, a PI3K inhibitor, and a monoclonal antibody targeting a tumor/cancer antigen; alternately an anticancer agent is selected from the group consisting of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor and a PI3K inhibitor. In one embodiment, the therapeutic agent used in combination with a compound of the present application is selected from an inhibitor of Akt1/2, an hydroxamic acid inhibitor of HDAC, and a benzoquinone ansamycin inhibitor of Hsp90. In another embodiment, the therapeutic agent used in combination with a compound of the present invention is selected from 1,3-dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat and 17-DMAG. In another embodiment, the therapeutic agent used in combination with a compound of the present application is selected from an imidazo[4,5-c]quinoline derivative that inhibits PI3K and mTOR kinase activity, a benzopyran derivative that inhibits PI3K, a pyrido[3',2':4,5]furo[3,2-d]pyrimidine derivative that inhibits PI3K and mTOR kinase activity and a furanosteroid derivative that inhibits PI3K. In yet another embodiment, the therapeutic agent used in combination with a compound of the present application is selected from BEZ-235, LY294002, PI-103, and wortmannin. In yet another

embodiment, the therapeutic agent used in combination with a compound of the present application is selected from the group consisting of 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat, 17-DMAG, BEZ-235, LY294002, PI-103, wortmannin and cetuximab. In yet a further embodiment, the therapeutic agent used in combination with a compound of the present application is selected from the group consisting of 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat, 17-DMAG, BEZ-235, LY294002, PI-103, and wortmannin. In one variation, the compound is of Formula I; in another variation the compound is of Formula II; in yet another variation, the compound is of Formula III; in yet a further variation, the compound is of Formula IV; in still a further variation, the compound is of Formula V; in another variation, the compound is of Formula VI. In one variation, The combination is administered in an amount effective to inhibit cell proliferation.

[00187] In another aspect, the present application discloses pharmaceutical composition comprising a compound of Formula I as disclosed herein, a therapeutic agent and at least one pharmaceutically acceptable excipient, wherein the therapeutic agent is selected from the group consisting of therapeutic compounds or antibodies useful for treating inflammatory, autoimmune or infectious disorders or targeting CK2 kinase or CK2-regulated pathways as disclosed herein. The combination can be administered in an amount effective to inhibit cell proliferation, reduce inflammation, fight pain or fight infection. In one variation the combination can be administered in an amount effective to reduce inflammation; in another variation the combination can be administered in an amount effective to reduce inflammation fight pain; in yet another variation the combination can be administered in an amount effective to fight infection.

[00188] In one variation, the compound is of Formula I; in another variation the compound is of Formula II; in yet another variation, the compound is of Formula III; in yet a further variation, the compound is of Formula IV; in still a further variation, the compound is of Formula V; in another variation, the compound is of Formula VI. In another embodiment of any of the disclosed aspects or variations, the compound of the application is Compound K, Compound 1, or Compound 2, or a salt or ester thereof.

[00189] Compounds of Formula I, II, III, IV, V and VI, and the pharmaceutically acceptable salts and esters thereof, are sometimes collectively referred to herein as compounds of the application.

[00190] The present application further discloses pharmaceutical compositions comprising a compound of the application or a pharmaceutically acceptable salt or ester thereof, and a commonly used anticancer agent, or a pharmaceutically acceptable salt or ester thereof, and at least one pharmaceutically acceptable excipient. The combination is administered in an amount effective to inhibit cell proliferation. In specific embodiments, the compound of the application is Compound K, Compound 1, or Compound 2, or a salt or ester thereof.

[00191] In one aspect disclosed in the present application, the combination therapy is administered to individuals who have a neoplastic disorder. In another aspect of the present application, the combination therapy is administered to individuals who do not yet show clinical signs of a neoplastic disorder, but who are at risk of developing a neoplastic disorder. Toward this end, the present application discloses methods for preventing or reducing the risk of developing a neoplastic disorder. In another aspect disclosed in the present application, the combination therapy is administered to individuals who have an inflammatory, autoimmune or infectious disorder. In yet another aspect of the present application, the combination therapy is administered to individuals who do not yet show clinical signs of an inflammatory, autoimmune or infectious disorder, but who are at risk of developing an inflammatory, autoimmune or infectious disorder. Toward this end, the present application discloses methods for preventing or reducing the risk of developing an inflammatory, autoimmune or infectious disorder.

[00192] In one embodiment, a single pharmaceutical dosage formulation that contains both a compound of the application, such as Compound K, and the therapeutic agent, such as an anticancer agent, is administered. In another embodiment disclosed in the application, separate dosage formulations are administered; the compound and the therapeutic agent, such as an anticancer agent may be, for example, administered at essentially the same time, for example, concurrently, or at separately staggered times, for example, sequentially. In certain examples, the individual components of the combination may be administered separately, at different times during the course of therapy, or concurrently, in divided or single combination forms.

[00193] The present application discloses, for example, simultaneous, staggered, or alternating treatment. Thus, the compound of the application may be administered at the same time as a therapeutic agent, such as an anticancer agent, in the same pharmaceutical

composition; the compound of the application may be administered at the same time as the therapeutic agent, such as an anticancer agent, in separate pharmaceutical compositions; the compound of the application may be administered before the therapeutic agent, such as an anticancer agent, or the agent may be administered before the compound of the application, for example, with a time difference of seconds, minutes, hours, days, or weeks. In examples of a staggered treatment, a course of therapy with the compound of the application may be administered, followed by a course of therapy with the therapeutic agent, such as an anticancer agent, or the reverse order of treatment may be used, more than one series of treatments with each component may be used. In certain examples of the present application, one component, for example, the compound of the application or the therapeutic agent, is administered to a mammal while the other component, or its derivative products, remains in the bloodstream of the mammal. For example, Compound K may be administered while the therapeutic agent, such as an anticancer agent or its derivative products remains in the bloodstream, or the agent may be administered while Compound K or its derivatives remains in the bloodstream. In other examples, the second component is administered after all, or most of the first component, or its derivatives, have left the bloodstream of the mammal.

Formulation and Administration

[00194] While the compositions and methods of the present application will typically be used in therapy for human patients, they may also be used in veterinary medicine to treat similar or identical diseases. The compositions may, for example, be used to treat mammals, including, but not limited to, primates and domesticated mammals. The compositions may, for example be used to treat herbivores. The compositions of the present application include geometric and optical isomers of one or more of the drugs, wherein each drug is a racemic mixture of isomers or one or more purified isomers.

[00195] Pharmaceutical compositions suitable for use in the present application include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[00196] The compounds of the present application may exist as pharmaceutically acceptable salts. The term "pharmaceutically acceptable salts" is meant to include salts of active compounds which are prepared with relatively nontoxic acids or bases, depending on the

particular substituent moieties found on the compounds described herein. When compounds of the present application contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Included are base addition salts such as sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present application contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids, for example, acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge et al., "Pharmaceutical Salts", Journal of Pharmaceutical Science, 1977, 66, 1-19). Certain specific compounds of the present application contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

[00197] Examples of applicable salt forms include hydrochlorides, hydrobromides, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, tartrates (e.g. (+)-tartrates, (-)-tartrates or mixtures thereof, including racemic mixtures), succinates, benzoates and salts with amino acids such as glutamic acid. These salts may be prepared by methods known to those skilled in art.

[00198] The neutral forms of the compounds are typically regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents.

[00199] The pharmaceutically acceptable esters in the present application refer to non-toxic esters, generally the alkyl esters are methyl, ethyl, propyl, isopropyl, butyl, isobutyl or pentyl esters, more often the alkyl ester is methyl ester. However, other esters such as phenyl-C_{1-5} alkyl may be employed if desired. Ester derivatives of certain compounds may act

as prodrugs which, when absorbed into the bloodstream of a warm-blooded animal, may cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy.

[00200] Certain compounds of the present application can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present application. The term `solvate` is used herein to describe a molecular complex comprising a compound of the application and one or more pharmaceutically acceptable solvent molecules, for example, ethanol; when the solvent is water, the term `hydrate` is commonly employed. Certain compounds of the present application may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present application and are intended to be within the scope of the present application.

[00201] When used as a therapeutic the compounds described herein often are administered with a physiologically acceptable carrier. A physiologically acceptable carrier is a formulation to which the compound can be added to dissolve it or otherwise facilitate its administration. Examples of physiologically acceptable carriers include, but are not limited to, water, saline, physiologically buffered saline.

[00202] Certain compounds of the present application possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisometric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present application. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the application. The compounds of the present application do not include those which are known in art to be too unstable to synthesize and/or isolate. The present application discloses compounds in racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

[00203] The term "tautomer," as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to

another. It will be apparent to one skilled in the art that certain compounds of this application may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the application.

[00204] Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ^{13}C- or ^{14}C-enriched carbon are within the scope of this application. The compounds of the present application may also contain unnatural proportions of atomic isotopes at one or more of atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present application, whether radioactive or not, are encompassed within the scope of the present disclosure.

[00205] In addition to salt forms, the present application provides compounds that are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present application. Additionally, prodrugs can be converted to the compounds of the present application by chemical or biochemical methods in an ex vivo environment. For example, prodrugs can be slowly converted to the compounds of the present application when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

[00206] The descriptions of compounds of the present application are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

[00207] A compound of the present application can be formulated as a pharmaceutical composition. Such a pharmaceutical composition can then be administered orally, parenterally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional

nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administration can also involve the use of transdermal administration such, as transdermal patches or iontophoresis devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques. Formulation of drugs is discussed in, for example, Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa.; 1975. Other examples of drug formulations can be found in Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980.

[00208] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable dilutent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Dimethyl acetamide, surfactants including ionic and non-ionic detergents, polyethylene glycols can be used. Mixtures of solvents and wetting agents such as those discussed above are also useful.

[00209] Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter, synthetic mono- di- or triglycerides, fatty acids and polyethylene glycols that are sold at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

[00210] Solid dosage forms for oral administration can include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the compounds of this application are ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. If administered *per os*, a contemplated aromatic sulfone hydroximate inhibitor compound can be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets can contain a controlled-release formulation

as can be provided in a dispersion of active compound in hydroxypropylmethyl cellulose. In the case of capsules, tablets, and pills, the dosage forms can also comprise buffering agents such as sodium citrate, magnesium or calcium carbonate or bicarbonate. Tablets and pills can additionally be prepared with enteric coatings.

[00211] For therapeutic purposes, formulations for parenteral administration can be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions can be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. A contemplated aromatic sulfone hydroximate inhibitor compound can be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

[00212] Liquid dosage forms for oral administration can include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions can also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

[00213] The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the mammalian host treated and the particular mode of administration.

[00214] The dosage regimen utilizing the compounds of the present application in combination with therapeutic agent is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt or ester thereof employed. A consideration of these factors is well within the purview of the ordinarily skilled clinician for the purpose of determining the therapeutically effective dosage amounts to be given to a person in need of the instant combination therapy.

Dosages

[00215] In one embodiment, a compound of the application or a pharmaceutically acceptable salt or ester thereof is administered at about 0.1 mg/kg to about 500 mg/kg. In another embodiment, a compound of the application a pharmaceutically acceptable salt or ester

thereof is administered in an amount of from about 0.5 mg/kg to about 450 mg/kg; alternately a compound of the application or a pharmaceutically acceptable salt or ester thereof administered in an amount of from about 1 mg/kg to about 250 mg/kg. In another embodiment, a compound of the application or a pharmaceutically acceptable salt or ester thereof is administered in an amount of about 1 mg/kg to about 200 mg/kg; alternately in an amount of 1 mg/kg to 100 mg/kg. Generally, the amount administered of a compound of the application, such as a compound of Formula I, II, III, IV, V, or VI, is 0.01-15 mg/kg, and sometimes 0.1-10 mg/kg.

[00216] The therapeutic agents disclosed herein may, of course, cause multiple desired effects; and the amount of the compound of the application to be used in combination with the therapeutic agent should be an amount that increases one or more of these desired effects. The compound of the application is to be administered in an amount that is effective to enhance a desired effect of the therapeutic agent. An amount is "effective to enhance a desired effect of the therapeutic agent", as used herein, if it increases by at least about 25% at least one of the desired effects of the therapeutic agent alone. Preferably, it is an amount that increases a desired effect of the therapeutic agent by at least 50% or by at least 100% (i.e., it doubles the effective activity of the therapeutic agent.) In some embodiments, it is an amount that increases a desired effect of the therapeutic agent by at least 200%.

Kits

[00217] Further disclosed herein are pharmaceutical kits. In one embodiment, a kit comprises a first dosage form comprising a compound of the present application, e.g., a compound of Formula I, II, III, IV, V or VI or alternately Compound K, Compound 1 or Compound 2 or a salt or ester thereof. In some embodiments the kit comprises a container housing a plurality of dosage forms and instructions for carrying out drug administration therewith. In one embodiment, a kit comprises a first dosage form comprising a compound of the present application in one or more of the forms disclosed herein and at least a second dosage form, in quantities sufficient to carry out the methods of the present invention. The second dosage form, and any additional dosage forms (e.g., a third, fourth of fifth dosage form) can comprise any therapeutic agent disclosed herein for the treatment of cancer, infection, pain, inflammation or autoimmune disorders. All dosage forms together can comprise a therapeutically effective amount of each compound for treatment of a disclosed indication. Alternately, the kit comprises each active ingredient at a dose lower than the therapeutically effective amount. In some embodiments a kit for use by a subject comprises at least one dosage

form, a container housing a plurality of said dosage form and instructions for carrying out drug administration therewith, wherein said at least one dosage form comprises a combination of a therapeutically effective daily dose of a compound of the application, or a pharmaceutically acceptable salt or ester thereof and a dosage form of one or more therapeutic compounds or antibodies useful for treating inflammatory, autoimmune or infectious disorders or targeting CK2 kinase or CK2-regulated pathways. In some embodiments the one or more agents can be in distinct individual dosage forms or combined in a single dosage form or a combination of dosage forms thereof. In some embodiments, a compound of the application or a pharmaceutically acceptable salt or ester thereof is in a distinct individual dosage form or combined in a single dosage form with one or more agents or a combination of dosage forms thereof.

Examples

[00218] The examples set forth below illustrate but do not limit the disclosure.

Example 1: Cell Inhibition Assays

[00219] Three-thousand (3000) cells are plated per well in each well of two 96 well plates (duplicates). Cells are incubated overnight at 37 degrees C. The following day, one or more of the compounds are added to the plates, and concentrations of each of the compounds are systematically varied across the plates. Typically, one compound is varied vertically using two, three or four-fold dilutions and the second compound is varied horizontally using two, three or four-fold dilutions across each plate (shown hereafter). The top concentration for Compound K is 100, 30 or 10 micromolar. The top concentration for other drugs, such as rapamycin or cisplatin, varies between 200 micromolar and 30 nanomolar. In some cases observed synergy is affected by the order of addition of the two compounds. In these cases the first drug was added one day prior to the second. The analysis is performed with Alamar Blue cell viability. In short, twenty microliters of AlamarBlue reagent (Invitrogen, Carlsbad CA) was added per well. The plates were incubated for four hours at 37 degrees Celsius and the resulting fluorescence was measured at Ex 560 nm/ Em 590 nm.

Example 1a: Calculating IC50s for single agents

[00220] To determine IC50s for single agents for each combination, the duplicates of the raw data in Relative Fluorescent Units (RFU) from Alamar Blue Assay were corrected for background and analyzed with Sigmoidal dose-response (variable slope) using GraphPad Prism Software (GraphPad, San Diego CA). The following constrains were applied: Bottom was fixed at equal to zero; in cases where calculated Top was unreasonably high its value was fixed at less or equal to the highest value that was observed in the analyzed data set. See Figure 1.

Example 2: Calculating Synergy From A Plate Of Percent Inhibition Data

[00221] A percent inhibition is calculated for every well in the plate based on the response data gathered as stated in Example 1. The concentration of Compound K increases regularly as the row number increases from 1 to 8 . The high concentration (*e.g.* 100 micromolar) is serially diluted (*e.g.* three-fold). The concentration of drug increases regularly as the column letter increases from A to L (as noted in the table below). The high concentration (*e.g.* 30 micromolar) is serially diluted (*e.g.* three-fold). A representative plate utilized for the studies is shown hereafter.

	Conc. Drug µM	0	0.0005	0.0015	0.0046	0.014	0.041	0.12	0.37	1.1	3.3	10	30
Conc. Compound K µM		A	B	C	D	E	F	G	H	I	J	K	L
0	1												
0.14	2												
0.41	3												
1.2	4												
3.7	5												
11	6												
33	7												
100	8												

[00222] The expected percent inhibition value is derived by assuming exact additivity between the effect of Compound K and the added drug. Hence the expected value for any well of interest is calculated as the percent inhibition observed for Compound K alone at the same concentration present in that well multiplied by the percent inhibition observed for the added drug alone at the same concentration present in that well. In practice this means the percent inhibition observed for Compound K comes from column A as the concentration of the added

drug is 0 here. Similarly, the percent inhibition observed for added drug comes from row 2 (as the concentration of Compound K is 0 here) *e.g.* the expected value for well D8 is obtained by multiplying the percent inhibition observed in well A8 by the percent inhibition observed in well D2.

[00223] Controls for these studies are the dose response curves for each of the two drugs by themselves. Such controls allow one to predict the cytotoxicity for each possible combination for each of the two drugs based simply on adding the cytotoxicity observed for each of the two drugs when used alone.

[00224] Assessment of synergy is completed by comparing the actual percent inhibition to the expected percent inhibition. If the expected value for well D8 is 60% but 80% inhibition is observed, the compounds are enhancing each other's effect and synergy is observed, for example. The number shown in the table will be 20.0. Conversely, a negative number is obtained when the two compounds produce less than the expected inhibitory effect.

[00225] For example, if concentration X of compound A inhibits by 20%, and concentration Y of compound B inhibits by 20%, one could expect a combination of concentration X of compound A and concentration Y of compound B to inhibit by 40%. That leaves another 60% inhibition possible. For example an overall inhibition of 70% corresponds to 50% inhibition of the remaining 60%, showing as a "50" for that particular combination. In practice, a program is written in the PilotScript programming language to calculate the quantities outlined above.

Example 2a: Calculating Synergy Using Combination Index

[00226] Combination index (CI) provides quantitative measure of the extent of drug interactions $CI=[A]/IC50_A+[B]/IC50_B$, where $IC50_A$ and $IC50_B$ concentrations of singe agents to achieve 50% effect alone and [A] and [B] concentrations of these two agents to achieve 50% effect in combination. A CI of less than, equal to, and more than 1 indicates synergy, additivity, and antagonism respectively. To calculate CI for our combinations we used IC50s that were determined with Sigmoidal dose-response (variable slope) using GraphPad Prism Software. The value of 50% effect was calculated as a half of an average between the Top value for compound K and combination compound. CI value is calculated at the lowest drug concentrations at which the 50% effect was achieved.

Example 3: 5-Fluorouracil/Compound K combination testing in A375 melanoma cells

[00227] 5-Fluorouracil, thymidylate synthase inhibitor, was tested in combination with Compound K in the melanoma cell line A375. 5-Fluorouracil was added 24 hours before Compound K in a 5 day assay. Results are shown hereafter; see Figure 2 and Figure 3. Synergy up to 55% is observed at concentrations tested. CI=0.02.

[00228] 5-Fluorouracil was added first, Compound K the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug µM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15.00	30
Conc. K µM												
0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.04	0.0	51.0	62.4	58.7	62.9	56.4	53.8	39.2	17.9	10.2	4.4	2.2
0.12	0.0	30.8	37.6	33.1	41.6	33.5	36.3	22.6	11.3	6.0	2.9	1.1
0.37	0.0	30.3	35.0	34.9	39.8	37.5	33.3	22.9	10.6	6.1	2.8	0.7
1.11	0.0	35.5	39.3	35.6	39.0	36.8	34.9	26.3	11.7	5.6	2.4	0.4
3.33	0.0	26.7	29.0	25.2	29.1	24.4	24.4	15.6	7.4	4.0	1.5	-0.6
10.00	-0.0	8.7	9.4	8.6	9.8	8.9	8.3	5.4	1.7	0.4	-0.4	-1.4
30	0.0	0.5	0.3	0.4	0.4	0.3	0.1	-0.1	-0.4	-0.6	-0.6	-1.5

Conc. Drug µM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15.00	30
Conc. K µM												
0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0
0.04	0.0	48.9	27.4	50.4	30.6	46.3	38.7	33.1	18.3	9.5	3.6	0.7
0.12	0.0	52.6	20.8	44.6	23.1	33.0	30.0	24.4	15.6	7.8	3.0	0.9
0.37	0.0	23.8	6.1	19.7	8.8	11.4	11.1	11.8	6.5	3.0	0.1	-3.3
1.11	-0.0	37.9	17.4	32.7	18.1	25.1	25.8	18.8	10.2	5.5	1.0	-2.0
3.33	0.0	33.3	17.4	29.2	17.2	15.9	19.1	12.6	5.3	2.4	-0.1	-2.5
10.00	-0.0	5.7	1.2	5.4	2.0	3.1	3.5	2.7	1.0	0.1	-0.7	-2.1
30	0.0	0.1	-0.1	0.3	0.1	0.1	-0.4	-0.2	-0.5	-0.5	-0.5	-0.4

[00229] Compound K: IC50=4.6 uM, Top=7711 RFU

[00230] 5-FU: IC50=3.0 uM, Top=9383 RFU

[00231] Value of 50% effect=4274 RFU

[00232] 50% Effect was achieved by combining 40 nM Compound K and 30 nM 5-Fluorouracil.

[00233] CI=[Compound K]/$IC50_{Compound\ K}$+[5-FU]/$IC50_{5\text{-}FU}$= (0.04/4.6)+(0.03/3.0)=0.02

Example 4: Fludarabine/Compound K combination testing in A375 melanoma cells

[00234] Fludarabine, a purine analog, was tested in combination with Compound K in the melanoma cell line A375. Fludarabine was added 24 hours before Compound K in a 4 day assay. Results are shown hereafter; see Figure 4 and Figure 5. Synergy up to 65% is observed at concentrations tested. CI=0.03.

[00235] Fludarabine was added first, Compound K the next day (4 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.20	0.39	0.78	1.56	3.13	6.25	12.50	25.00	50.00	100.00	200
Conc. K μM												
0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	-0.0	0.0
0.04	0.0	22.7	28.4	41.1	30.0	34.9	11.9	11.7	4.6	0.2	-0.1	-0.1
0.12	0.0	32.1	38.7	43.4	37.2	44.3	27.1	22.5	9.1	0.7	-0.2	-0.1
0.37	0.0	60.5	58.0	73.1	61.0	70.8	46.6	36.0	13.9	1.6	-0.0	-0.0
1.11	0.0	52.8	63.8	75.6	68.7	74.2	49.8	35.9	17.1	1.7	-0.1	0.1
3.33	0.0	42.3	43.9	52.1	44.3	47.8	31.2	26.4	10.0	0.8	-0.5	-0.2
10.00	0.0	12.4	12.8	14.5	13.1	14.8	8.9	6.7	2.2	-0.3	-0.7	-0.3
30	0.0	0.3	0.2	0.3	0.2	0.2	-0.1	-0.3	-0.5	-0.6	-0.4	-0.0

Conc. Drug μM	0	0.20	0.39	0.78	1.56	3.13	6.25	12.50	25.00	50.00	100.00	200
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.04	0.0	-0.8	30.7	41.4	24.9	24.9	45.4	43.0	28.8	4.7	0.1	-0.0
0.12	0.0	29.8	53.3	68.3	47.4	45.2	71.1	64.4	41.6	6.2	0.2	0.1
0.37	0.0	37.3	57.7	74.5	56.0	57.0	73.8	65.2	42.3	6.0	0.1	-0.1
1.11	0.0	41.5	64.2	73.2	57.6	57.1	81.3	68.5	44.6	6.4	0.1	-0.0
3.33	0.0	26.1	39.5	50.9	37.9	41.1	50.8	48.5	30.4	4.6	-0.1	-0.1
10.00	0.0	5.1	10.9	11.9	9.1	8.6	12.4	11.9	7.2	0.7	-0.4	-0.2
30	0.0	0.1	0.3	0.3	0.1	-0.1	0.2	-0.0	-0.0	-0.3	-0.2	-0.0

[00236] Compound K: IC50=5.0 uM, Top=8874 RFU

[00237] Fludarabine: IC50=22.9 uM, Top=8227 RFU

[00238] Value of 50% effect=4276 RFU

[00239] 50% Effect was achieved by combining 40 nM Compound K and 390 nM Fludarabine.

[00240] CI=[Compound K]/$IC50_{Compound\ K}$+[Fludarabine]/$IC50_{Fludarabine}$= (0.04/5.0)+(0.39/22.9)=0.03

Example 5: Gemcitabine/Compound K combination testing in A375 melanoma cells

[00241] Gemcitabine, a pyrimidine, was tested in combination with Compound K in the melanoma cell line A375. Gemcitabine was added 24 hours before Compound K in a 4 day assay. Results are shown hereafter; see Figure 6. Synergy up to 45% is observed at concentrations tested. CI=0.04.

[00242] Gemcitabine was added first, Compound K the next day (4 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug µM	0	0.00003	0.00006	0.00012	0.00023	0.00047	0.00094	0.00188	0.00375	0.0075	0.015	0.03
Conc. K µM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0
0.04	0.0	25.9	26.6	39.6	29.6	30.5	36.3	27.4	12.9	2.0	0.1	0.1
0.12	0.0	44.1	37.9	40.9	37.5	36.0	39.7	36.3	13.0	1.6	-0.1	0.1
0.37	0.0	28.0	23.6	30.1	23.7	27.0	25.5	22.4	8.3	0.9	-0.2	0.1
1.11	0.0	26.0	27.3	25.6	27.9	12.4	27.4	26.6	10.5	1.1	-0.1	0.2
3.33	0.0	19.1	15.6	16.7	15.3	17.6	18.2	16.6	5.8	0.5	-0.4	-0.0
10.00	0.0	2.4	2.8	3.6	3.3	0.4	1.2	1.4	0.5	-0.2	-0.4	-0.1
30	0.0	-0.0	-0.2	-0.1	-0.2	-0.3	-0.3	-0.3	-0.4	-0.3	-0.3	-0.2

Conc. Drug µM	0	0.00003	0.00006	0.00012	0.00023	0.00047	0.00094	0.00188	0.00375	0.0075	0.015	0.03
Conc. K µM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.04	0.0	-13.5	14.8	6.8	2.4	1.0	12.1	2.4	4.4	0.8	0.1	0.0
0.12	0.0	39.2	44.4	46.3	42.1	42.5	46.8	44.0	20.8	2.3	0.1	-0.1
0.37	0.0	47.9	52.5	51.7	47.5	51.2	58.8	48.4	26.5	2.6	0.0	0.0
1.11	0.0	46.4	50.7	51.3	47.8	48.8	54.1	48.5	25.2	2.4	-0.0	0.0
3.33	0.0	43.2	49.7	48.5	47.5	46.0	52.8	47.8	24.8	2.3	-0.2	0.0
10.00	0.0	14.8	15.9	17.5	16.8	17.2	19.1	17.3	8.8	0.6	-0.3	0.0
30	0.0	0.3	0.2	0.2	0.2	0.1	0.0	0.0	-0.3	-0.4	-0.2	-0.2

[00243] Compound K: IC50=4.8 uM, Top=8646 RFU

[00244] Fludarabine: IC50=3.5 nM, Top=7461 RFU

[00245] Value of 50% effect=4027 RFU

[00246] 50% Effect was achieved by combining 120 nM Compound K and 30 pM Gemcitabine

[00247] CI=[Compound K]/$IC50_{Compound\ K}$+[Gemcitabine]/$IC50_{Gemcitabine}$= (0.12/4.8)+(0.03/3.5)=0.04

Example 6: Paclitaxel/Compound K combination testing in A375 melanoma cells

[00248] Paclitaxel, a mitotic inhibitor, was tested in combination with Compound K in the melanoma cell line A375. Paclitaxel was added 24 hours before Compound K in a 5 day assay. Results are shown hereafter; see Figure 7 and Figure 8. Synergy up to 30% is observed at concentrations tested. CI=0.17.

[00249] Paclitaxel was added first, Compound K the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.00002	0.00005	0.00015	0.00046	0.0014	0.0041	0.012	0.037	0.11	0.33	1
Conc. K μM												
0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.02	0.0	18.0	31.4	36.1	29.8	14.6	11.3	10.2	1.9	4.0	3.7	-2.9
0.10	0.0	20.1	8.7	14.5	16.5	21.4	11.5	8.1	1.2	1.1	0.2	-6.0
0.39	0.0	0.0	0.0	14.9	31.3	26.5	17.1	13.6	5.9	4.6	5.6	-3.2
1.56	0.0	10.3	19.7	27.2	26.8	22.3	14.0	12.0	5.9	6.4	5.2	-1.5
6.25	0.0	26.4	27.5	29.6	20.6	17.8	11.4	10.6	7.0	8.2	6.9	0.5
25.00	0.0	0.7	4.8	5.8	3.6	2.7	1.2	1.3	0.6	1.0	1.3	0.6
100	0.0	0.5	1.0	1.0	0.8	0.6	0.4	0.6	0.2	0.2	0.1	0.1

Conc. Drug μM	0	0.00002	0.00005	0.00015	0.00046	0.0014	0.0041	0.012	0.037	0.11	0.33	1
Conc. K μM												
0.00	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0	-0.0	0.0	0.0
0.02	0.0	13.8	29.4	28.2	39.7	17.2	8.2	5.6	3.8	3.0	2.7	-0.8
0.10	0.0	16.8	20.1	19.9	21.3	4.0	1.1	-0.7	-0.4	-1.5	-4.5	-7.5
0.39	0.0	16.6	4.9	15.8	19.0	9.3	4.6	2.1	-0.7	0.5	0.4	-4.6
1.56	0.0	22.7	14.8	19.1	17.4	4.7	3.7	1.4	1.3	2.1	1.5	-2.6
6.25	0.0	18.0	15.8	18.2	16.4	5.0	2.7	3.0	4.2	5.9	4.4	0.4
25.00	0.0	1.1	0.8	1.2	1.5	-0.8	-1.1	-0.7	-0.9	0.1	0.7	0.4
100	0.0	0.3	0.4	0.6	0.5	0.2	0.2	0.1	0.3	0.1	0.2	-0.2

[00250] Compound K: IC50=11.5 uM, Top=23452 RFU

[00251] Fludarabine: IC50=2.9 nM, Top=26000 RFU

[00252] Value of 50% effect=12363 RFU

[00253] 50% Effect was achieved by combining 100 nM Compound K and 460 pM Paclitaxel.

[00254] CI=[Compound K]/$IC50_{Compound\ K}$+[Paclitaxel]/$IC50_{Paclitaxel}$= (0.1/11.5)+(0.46/2.9)=0.17

Example 7: Sunitinib/Compound K combination testing in A375 melanoma cells

[00255] Sunitinib, a multi tyrosine-kinase inhibitor, was tested in combination with Compound K in the melanoma cell line A375. Sunitinib was added 24 hours before Compound K in a 4 day assay. Results are shown hereafter; see Figure 9 and Figure 10. Synergy up to 60% is observed at concentrations tested. CI=0.04.

[00256] Sunitinib was added first, Compound K the next day (4 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.003	0.006	0.012	0.023	0.047	0.094	0.188	0.375	0.75	1.5	3
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.04	0.0	30.0	41.1	29.7	39.1	35.4	34.8	18.5	8.8	1.9	-0.1	0.0
0.12	0.0	53.4	58.6	48.9	50.6	52.6	42.2	22.9	11.3	3.1	-0.2	-0.1
0.37	0.0	58.4	64.4	54.2	59.7	61.4	47.0	23.7	10.8	3.3	-0.2	-0.2
1.11	0.0	65.8	63.9	58.3	63.6	63.0	46.8	25.5	11.8	2.7	-0.3	-0.1
3.33	0.0	46.5	46.0	40.0	45.4	42.5	31.8	16.2	6.9	1.7	-0.7	-0.3
10.00	0.0	11.0	11.8	9.9	10.9	10.4	7.4	2.7	0.3	-0.3	-0.6	-0.2
30	0.0	0.3	0.3	0.3	0.2	0.3	0.0	-0.2	-0.3	-0.4	-0.3	-0.2

Conc. Drug μM	0	0.003	0.006	0.012	0.023	0.047	0.094	0.188	0.375	0.750	1.5	3
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.04	0.0	-5.0	21.8	30.4	44.8	32.7	39.2	27.9	13.2	3.7	-0.0	-0.1
0.12	0.0	34.5	42.2	40.0	54.0	33.7	37.1	22.8	10.2	2.9	-0.2	-0.1
0.37	0.0	48.7	55.1	51.4	61.7	42.6	41.9	25.6	11.7	4.0	-0.2	-0.1
1.11	0.0	50.3	58.5	50.5	65.0	45.7	42.8	26.4	11.6	3.5	-0.1	0.1
3.33	0.0	37.5	38.2	34.8	45.0	31.3	28.6	16.1	6.7	1.4	-0.5	-0.1
10.00	0.0	14.9	15.8	14.3	17.2	11.6	10.0	5.0	1.7	0.3	-0.7	-0.3
30	0.0	0.1	-0.1	-0.2	-0.2	-0.2	-0.1	-0.3	-0.4	-0.5	-0.4	-0.1

[00257] Compound K: IC50=5.1 uM, Top=8150 RFU

[00258] Sunitinib: IC50=145 nM, Top=7914 RFU

[00259] Value of 50% effect=4016 RFU

[00260] 50% Effect was achieved by combining 120 nM Compound K and 3 nM Sunitinib.

[00261] CI=[Compound K]/$IC50_{Compound\ K}$+[Sunitinib]/$IC50_{Sunitinib}$= (0.12/5.1)+(0.003/0.145)=0.04

Example 8: Vinblastine/Compound K combination testing in A375 melanoma cells

[00262] Vinblastine, a mitotic inhibitor, was tested in combination with Compound K in the melanoma cell line A375. Vinblastine was added 24 hours before Compound K in a 5 day assay. Results are shown hereafter; see Figure 11 and Figure 12. Synergy up to 35% is observed at concentrations tested. CI=0.39.

[00263] Vinblastine was added first, Compound K the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive

value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.00002	0.00005	0.00015	0.00046	0.0014	0.0041	0.012	0.037	0.11	0.33	1
Conc. K μM												
0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0
0.02	0.0	16.8	0.8	25.4	25.3	11.2	1.7	1.2	1.0	0.3	-1.0	1.8
0.10	0.0	-1.4	-1.4	16.0	23.3	12.1	0.4	-0.2	-0.3	-3.9	-3.7	-5.7
0.39	0.0	-1.4	-0.9	14.8	23.3	11.5	0.1	0.6	0.2	-2.8	-4.1	-2.9
1.56	0.0	7.8	4.0	39.1	27.6	11.4	-0.0	-0.8	0.7	1.0	-1.2	4.9
6.25	0.0	18.5	27.5	51.6	24.0	13.6	4.2	3.7	5.4	4.5	5.8	9.2
25.00	0.0	1.7	5.3	8.8	5.5	3.8	1.2	0.5	0.2	-0.7	-0.3	1.7
100	0.0	1.3	1.7	2.1	1.5	0.9	0.6	0.6	0.6	0.4	0.5	0.5

Conc. Drug μM	0	0.00002	0.00005	0.00015	0.00046	0.0014	0.0041	0.012	0.037	0.11	0.33	1
Conc. K μM												
0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.02	0.0	7.8	5.1	21.2	48.1	11.1	9.6	6.7	6.5	2.6	0.8	-0.8
0.10	0.0	-0.7	3.9	0.1	36.2	11.1	7.8	5.7	7.4	3.7	4.3	-2.9
0.39	0.0	11.2	-0.3	23.5	36.4	7.5	3.0	4.3	2.1	0.2	-0.4	-4.5
1.56	0.0	-4.4	8.6	26.0	49.6	16.7	11.9	7.1	10.1	7.5	6.8	3.0
6.25	0.0	3.2	21.0	30.9	29.9	7.7	3.0	2.0	3.6	3.5	2.5	3.7
25.00	0.0	-3.3	2.8	4.9	6.2	0.8	-0.5	-1.1	-1.0	-1.9	-2.4	-0.9
100	0.0	0.3	0.8	1.6	1.7	0.7	0.6	0.5	0.4	0.3	0.4	0.5

[00264] Compound K: IC50=12 uM, Top=25176 RFU

[00265] Vinblastine: IC50=1.2 nM, Top=28000 RFU

[00266] Value of 50% effect=13294 RFU

[00267] 50% Effect was achieved by combining 20 nM Compound K and 460 pM Vinblastine.

[00268] CI=[Compound K]/$IC50_{Compound\ K}$+[Vinblastine]/$IC50_{Vinblastine}$= (0.02/12)+(0.46/1.2)=0.39

Example 9: 5-Fluorouracil/Compound K combination testing in MDA-MB-468 breast cancer cells

[00269] 5-Fluorouracil, a pyrimidine analog, was tested in combination with Compound K in the breast cancer cell line MDA-MB-468. The effects of order of addition are

examined. Results are shown hereafter; see Figure 13 and Figure 14. Synergy up to 40% is observed at concentrations tested. CI=0.18-0.24. Synergy did not depend on the order of addition.

[00270] 5-Fluorouracil was added first, Compound K the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15	30
Conc. K μM												
0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	5.4	16.5	28.1	21.4	29.7	28.8	18.4	21.7	13.1	9.5	2.4
0.41	0.0	25.1	34.7	43.0	36.1	39.2	39.2	30.7	27.2	18.9	17.1	1.7
1.2	0.0	28.6	38.3	44.6	36.9	39.3	36.2	31.7	29.3	20.4	17.3	2.8
3.7	0.0	20.1	24.3	26.8	24.9	25.4	26.7	22.9	19.7	16.0	11.0	2.5
11	0.0	1.4	1.6	1.9	1.0	0.6	0.2	0.9	-0.8	-1.3	-1.4	-3.9
33	0.0	2.3	0.7	2.9	1.2	2.0	1.6	0.9	-0.8	-0.4	-1.3	-3.0
100	0.0	2.6	2.3	2.4	2.5	1.4	0.6	-0.9	-0.5	-1.8	-1.8	-3.1

Conc. Drug μM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15	30
Conc. K μM												
0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0
0.14	0.0	12.2	14.5	17.8	15.7	25.7	24.1	25.5	28.2	15.5	8.2	0.9
0.41	0.0	21.4	32.9	34.2	36.0	37.0	35.0	36.2	26.8	18.8	16.8	1.2
1.2	0.0	16.2	29.8	33.1	32.7	35.9	32.5	31.3	28.2	18.7	14.3	1.2
3.7	0.0	10.7	18.6	23.9	26.5	25.8	23.8	24.3	20.1	16.1	10.2	3.4
11	0.0	0.7	1.7	2.0	1.2	0.9	0.4	1.1	-0.2	-0.3	-0.6	-3.9
33	0.0	1.5	0.5	2.6	1.4	2.4	2.2	1.3	-0.3	0.1	-0.4	-3.6
100	0.0	2.1	1.8	1.9	2.2	0.9	0.4	-0.6	-0.7	-2.3	-1.7	-3.3

[00271] Compound K: IC50=4.4 uM, Top=10446 RFU

[00272] 5-Fluorouracil: IC50=6.6 uM, Top=10485 RFU

[00273] Value of 50% effect=5233 RFU

[00274] 50% Effect was achieved by combining 410 nM Compound K and 940 nM 5-Fluorouracil.

[00275] CI=[Compound K]/$IC50_{Compound\ K}$+[5-Fluorouracil]/$IC50_{5\text{-}Fluorouracil}$= (0.41/4.4)+(0.94/6.6)=0.24

[00276] Compound K was added first, 5-Fluorouracil the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. See Figure 15 and Figure 16. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15	30
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	10.3	-2.5	0.4	0.1	9.9	15.5	13.4	13.5	13.5	-1.7	3.3
0.41	0.0	8.3	20.0	27.6	22.7	31.4	38.1	35.0	25.1	18.4	14.4	3.3
1.2	0.0	15.8	21.6	28.1	24.6	27.2	27.4	28.3	18.7	15.3	9.1	-1.7
3.7	0.0	20.7	24.3	28.5	26.5	26.6	27.2	24.5	18.5	12.3	13.1	8.1
11	0.0	2.2	3.4	3.3	2.5	2.3	1.8	2.3	0.6	0.2	-0.8	-0.8
33	0.0	1.2	-0.3	2.1	0.1	1.2	0.2	-0.0	-1.6	-0.9	-1.7	-2.0
100	0.0	1.1	1.2	1.4	1.6	0.7	-0.1	-0.7	-0.5	-1.8	-1.7	-2.2

Conc. Drug μM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15	30
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	7.1	0.4	17.4	16.5	10.1	19.0	24.6	22.4	24.4	2.3	4.2
0.41	0.0	12.4	15.9	25.8	18.1	30.0	36.6	30.7	29.2	19.6	22.2	1.6
1.2	-0.0	15.8	19.0	31.3	29.1	29.3	37.8	35.5	27.3	21.8	20.2	2.9
3.7	0.0	8.0	11.8	15.5	16.8	20.6	23.6	23.0	16.8	19.2	13.2	0.1
11	0.0	0.7	1.1	2.1	1.2	0.2	0.2	0.9	-0.7	-0.2	-1.7	-2.4
33	0.0	1.3	-0.4	1.8	0.2	1.3	0.4	-0.2	-1.6	-0.3	-1.5	-2.5
100	0.0	1.5	0.7	1.2	1.4	0.1	-0.0	-0.5	-0.2	-1.5	-1.7	-2.8

[00277] Compound K: IC50=4.6 uM, Top=10630 RFU

[00278] 5-Fluorouracil: IC50=10.6 uM, Top=10384 RFU

[00279] Value of 50% effect=5254 RFU

[00280] 50% Effect was achieved by combining 410 nM Compound K and 940 nM 5-Fluorouracil.

[00281] CI=[Compound K]/$IC50_{Compound\ K}$+[5-Fluorouracil]/$IC50_{5\text{-}Fluorouracil}$= (0.41/4.6)+(0.94/10.6)=0.18

Example 10: Cisplatin/Compound K combination testing in MDA-MB-468 breast cancer cells

[00282] Cisplatin, an alkylating-like agent, was tested in combination with Compound K in the breast cancer cell line MDA-MB-468. The effects of order of addition are examined. Results are shown hereafter; see Figure 17 and Figure 18. Synergy up to 15% is observed at concentrations tested. CI=0.3-0.84. Synergy did not depend on the order of addition.

[00283] Cisplatin was added first, Compound K the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15	30
Conc. K μM												
0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	1.4	12.5	5.9	4.6	7.2	2.9	1.2	0.4	0.5	1.2	0.7
0.41	0.0	5.0	9.6	12.5	9.6	8.5	4.3	2.3	0.8	0.4	0.9	0.9
1.2	0.0	11.9	10.5	13.2	12.7	8.6	3.8	1.9	0.4	1.2	0.9	0.7
3.7	0.0	9.3	14.0	8.8	5.2	4.3	2.3	1.4	0.7	0.2	0.4	0.7
11	0.0	-0.2	0.3	0.3	-0.3	-0.4	-0.8	-0.2	-0.6	-0.6	-0.3	-0.5
33	0.0	1.2	-0.2	1.2	-0.4	0.2	-0.4	-0.3	-1.6	-0.7	-0.5	-0.9
100	0.0	0.8	0.7	0.4	-0.2	-1.5	-1.4	-1.7	-1.6	-2.5	-1.3	-1.8

Conc. Drug μM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15	30
Conc. K μM												
0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0
0.14	0.0	-1.6	0.9	7.0	4.4	7.6	2.0	1.4	0.8	0.4	1.7	0.9
0.41	0.0	3.6	10.1	16.4	11.4	10.4	4.7	2.6	0.5	0.4	1.1	1.3
1.2	0.0	7.7	16.6	23.2	18.9	11.9	5.1	2.4	1.1	1.6	1.2	1.1
3.7	0.0	10.7	19.8	19.5	10.3	7.3	3.2	2.0	0.7	0.4	-0.6	0.2
11	0.0	-0.3	0.4	0.3	-0.5	-0.4	-2.0	0.2	-1.9	-2.3	-2.0	-2.2
33	0.0	1.3	-0.4	0.9	-0.7	-0.5	-1.0	-1.6	-2.2	-2.3	-2.4	-2.3
100	0.0	0.7	0.6	-0.2	-0.6	-2.2	-2.7	-4.1	-3.5	-4.3	-3.2	-3.6

[00284] Compound K: IC50=4.3 uM, Top=10513 RFU

[00285] 5-Fluorouracil: IC50=107 nM, Top=11803 RFU

[00286] Value of 50% effect=5579 RFU

[00287] 50% Effect was achieved by combining 1.2 uM Compound K and 60 nM Cisplatin.

[00288] CI=[Compound K]/$IC50_{Compound\ K}$+[Cisplatin]/$IC50_{Cisplatin}$= (1.2/4.3)+(0.06/0.107)=0.84

[00289] Compound K was added first, Cisplatin the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism; see Figure 19 and Figure 20. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15	30
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	28.5	32.5	35.0	21.1	21.0	19.8	13.3	4.7	2.5	2.2	1.5
0.41	0.0	25.7	30.2	35.5	19.3	19.7	20.2	13.4	5.0	3.2	1.5	1.7
1.2	0.0	21.0	24.0	38.5	25.7	21.8	20.4	14.2	5.7	3.7	1.6	1.7
3.7	-0.0	13.9	22.6	27.0	21.3	21.0	13.2	9.6	3.8	2.2	1.0	0.7
11	0.0	1.7	2.1	2.6	1.2	0.4	-1.0	0.0	-1.5	-0.7	-2.0	-2.0
33	0.0	1.7	-0.1	2.0	0.1	0.7	-0.8	-1.1	-2.6	-1.4	-2.2	-2.9
100	0.0	1.8	1.4	1.4	1.4	-0.3	-1.5	-2.3	-1.9	-3.1	-2.7	-3.5

Conc. Drug μM	0	0.03	0.06	0.12	0.23	0.47	0.94	1.88	3.75	7.50	15	30
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	-6.0	4.0	3.1	6.0	5.8	7.1	3.1	1.4	0.8	1.0	0.6
0.41	0.0	-2.7	-3.4	1.9	4.1	5.8	7.3	3.4	0.9	0.8	0.6	0.6
1.2	0.0	-1.6	3.9	5.9	6.0	6.2	8.0	3.2	1.5	1.4	0.5	0.8
3.7	0.0	5.5	2.3	1.5	6.0	9.3	5.5	2.6	1.2	0.6	-0.1	-0.0
11	0.0	0.2	0.7	0.9	0.4	-0.5	-1.2	-0.4	-1.6	-1.2	-2.2	-1.9
33	0.0	1.0	-0.8	1.5	-0.8	0.1	-0.9	-1.1	-2.6	-1.6	-2.5	-2.8
100	0.0	0.7	0.2	0.2	0.2	-1.2	-2.0	-2.9	-2.6	-3.7	-3.4	-3.8

[00290] Compound K: IC50=4.5 uM, Top=9530 RFU

[00291] Cisplatin: IC50=430 nM, Top=9646 RFU

[00292] Value of 50% effect=4794 RFU

[00293] 50% Effect was achieved by combining 1.2 uM Compound K and 120 nM Cisplatin.

[00294] CI=[Compound K]/$IC50_{Compound\ K}$+[Cisplatin]/$IC50_{Cisplatin}$= (1.2/4.5)+(0.12/0.43)=0.3

Example 11: Doxorubicin/Compound K combination testing in MDA-MB-468 breast cancer cells

[00295] Doxorubicin, an anthracycline, was tested in combination with Compound K in the breast cancer cell line MDA-MB-468. The effects of order of addition are examined. Results are shown hereafter; see Figure 21 and Figure 22. Synergy up to 30% is observed. CI=0.56-0.76. Synergy did not depend on the order of addition.

[00296] Doxorubicin was added first, Compound K the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.001	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	14.2	11.7	10.1	-2.4	7.3	11.5	8.6	3.7	1.1	1.3	1.3
0.41	0.0	10.9	30.4	31.3	29.1	20.0	15.1	11.4	5.5	1.8	1.1	1.7
1.2	-0.0	20.0	36.3	24.8	28.9	25.2	14.3	11.6	6.6	3.8	1.4	1.7
3.7	0.0	17.8	27.6	24.4	22.2	24.4	9.0	7.4	5.4	2.5	0.3	0.5
11	0.0	1.3	1.8	2.4	1.8	1.3	-0.4	0.5	-0.9	-0.9	-1.2	-1.7
33	0.0	2.0	1.1	2.6	1.4	1.2	-0.3	-0.7	-2.2	-1.6	-2.6	-2.1
100	0.0	1.3	1.3	1.6	1.3	0.1	-2.1	-3.0	-2.5	-3.0	-2.9	-2.8

Conc. Drug μM	0	0.001	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1
Conc. K μM												
0	0.0	-0.0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	-1.8	11.2	10.6	8.3	2.8	7.0	7.8	3.6	1.1	1.3	0.6
0.41	0.0	-11.0	22.3	18.3	27.6	19.8	11.5	11.0	5.0	1.6	1.2	1.3
1.2	0.0	0.0	18.5	25.1	20.5	20.3	10.4	10.8	5.7	3.1	1.1	1.0
3.7	0.0	-3.8	20.1	16.6	21.4	19.2	5.7	6.6	3.9	1.3	-0.1	0.1
11	0.0	1.0	1.5	1.9	0.8	0.1	-1.1	-0.5	-1.4	-1.4	-1.2	-2.2
33	-0.0	1.4	0.5	2.2	0.7	0.3	-0.2	-1.0	-2.6	-1.9	-2.2	-2.5
100	0.0	0.9	1.1	0.9	0.6	-1.3	-2.5	-3.7	-3.1	-4.5	-4.2	-3.8

[00297] Compound K: IC50=4.5 uM, Top=10577 RFU

[00298] Doxorubicin: IC50=17 nM, Top=10942 RFU

[00299] Value of 50% effect=5380 RFU

[00300] 50% Effect was achieved by combining 410 nM Compound K and 8 nM Doxorubicin.

[00301] CI=[Compound K]/$IC50_{Compound\ K}$+[Doxorubicin]/$IC50_{Doxorubicin}$= (0.41/4.5)+(0.008/0.017)=0.56

[00302] Compound K was added first, Doxorubicin the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. See Figure 23 and Figure 24. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.001	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1
Conc. K μM												
0	0.0	0.0	-0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	8.9	10.4	15.7	15.7	17.5	10.6	11.1	8.0	3.4	2.7	1.3
0.41	0.0	25.3	10.4	20.6	16.6	19.4	11.1	11.0	5.9	4.5	2.4	2.0
1.2	0.0	10.6	18.5	13.8	12.9	21.0	11.3	12.7	9.0	5.6	2.8	2.6
3.7	0.0	10.8	9.7	7.4	12.5	13.2	9.8	7.3	5.3	3.5	1.7	1.9
11	0.0	2.0	1.9	2.6	1.8	0.2	-1.3	-0.6	-1.6	-1.3	-2.1	-2.2
33	0.0	1.5	-0.0	1.7	0.0	0.7	-0.9	-1.5	-2.8	-1.8	-2.4	-3.3
30	0.0	1.7	0.9	1.0	1.3	-0.6	-1.4	-2.1	-1.7	-2.8	-2.5	-3.5

Conc. Drug μM	0	0.001	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	11.9	17.2	5.2	6.9	3.9	1.9	9.0	3.0	3.3	2.8	1.2
0.41	0.0	12.0	26.3	17.1	13.3	8.9	8.7	10.6	3.6	4.2	2.9	1.2
1.2	0.0	23.0	27.8	18.5	15.4	9.8	12.7	10.5	5.1	6.1	3.2	2.3
3.7	0.0	12.3	17.8	10.5	8.5	7.9	7.5	7.2	3.7	4.6	2.5	2.1
11	0.0	1.1	1.7	1.5	0.6	-0.6	-2.4	-0.8	-1.7	-0.7	-1.8	-1.6
33	-0.0	1.6	-0.1	1.6	-0.3	0.3	-1.1	-1.2	-2.5	-1.4	-2.0	-2.6
100	0.0	1.7	1.2	1.1	1.3	-0.7	-1.6	-2.1	-2.0	-3.1	-2.7	-3.5

[00303] Compound K: IC50=4.6 uM, Top=9652 RFU

[00304] Doxorubicin: IC50=16 nM, Top=11475 RFU

[00305] Value of 50% effect=5282 RFU

[00306] 50% Effect was achieved by combining 1.2 uM Compound K and 8 nM Doxorubicin.

[00307] CI=[Compound K]/$IC50_{Compound\ K}$+[Doxorubicin]/$IC50_{Doxorubicin}$= (1.2/4.6)+(0.008/0.016)=0.76

Example 12: Gemcitabine/Compound K combination testing in MDA-MB-468 breast cancer cells

[00308] Gemcitabine, a pyrimidine analog, was tested in combination with Compound K in the breast cancer cell line MDA-MB-468. The effects of order of addition are examined. Results are shown hereafter; see Figure 25 and Figure 26. Synergy up to 30% is observed at concentrations tested. CI=0.29-0.84. Synergy did not depend on the order of addition.

[00309] Gemcitabine was added first, Compound K the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.00003	0.00006	0.00012	0.00023	0.00047	0.00094	0.0019	0.0038	0.0075	0.015	0.03
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	-4.7	13.2	6.3	16.6	18.5	13.2	9.0	12.3	8.2	3.9	0.1
0.41	0.0	6.1	19.6	9.8	16.1	23.2	25.8	17.1	10.8	-5.5	8.5	1.7
1.2	0.0	-2.0	21.3	20.6	19.7	34.2	25.4	14.8	15.3	10.2	10.3	2.7
3.7	0.0	-0.2	13.8	14.8	16.9	23.4	19.1	11.8	11.5	17.0	8.2	3.2
11	0.0	1.4	1.1	2.0	1.7	1.9	0.8	1.0	1.1	0.9	-0.2	-1.7
33	0.0	1.2	0.5	2.0	1.4	1.3	-0.1	0.3	-0.7	-0.5	-1.5	-2.3
100	0.0	0.5	0.8	0.7	1.5	0.4	-0.1	0.2	-0.7	-1.5	-2.3	-2.9

Conc. Drug μM	0	0.00003	0.00006	0.00012	0.00023	0.00047	0.00094	0.0019	0.0038	0.0075	0.015	0.03
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	5.6	24.7	21.2	26.3	20.2	31.1	30.2	27.6	19.9	11.2	0.8
0.41	0.0	14.9	23.4	24.7	32.2	16.3	23.0	19.3	20.2	20.3	12.4	0.8
1.2	0.0	13.3	19.5	20.9	29.9	23.1	26.2	19.6	19.4	23.7	12.4	1.5
3.7	0.0	11.8	11.5	16.3	24.4	19.9	25.4	22.0	20.6	24.8	11.5	3.1
11	0.0	1.3	0.8	1.8	1.2	1.7	0.9	1.7	2.2	1.6	0.4	-1.4
33	0.0	1.1	0.8	2.2	1.9	1.6	0.6	1.1	0.3	0.5	-1.2	-2.2
100	0.0	0.3	0.6	0.3	1.6	1.1	0.9	1.0	0.0	-1.5	-2.6	-3.3

[00310] Compound K: IC50=4.4 uM, Top=10572 RFU

[00311] Gemcitabine: IC50=8.8 nM, Top=10229 RFU

[00312] Value of 50% effect=5200 RFU

[00313] 50% Effect was achieved by combining 3.7 uM Compound K and 30 pM Gemcitabine.

[00314] CI=[Compound K]/$IC50_{Compound\ K}$+[Gemcitabine]/$IC50_{Gemcitabine}$ = (3.7/4.4)+(0.03/8.8)=0.84

[00315] Compound K was added first, Gemcitabine the next day (5 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. See Figure 27 and Figure 28. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.00003	0.00006	0.00012	0.00023	0.00047	0.00094	0.0019	0.0038	0.0075	0.015	0.03
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	2.3	8.5	17.3	16.3	11.8	10.3	10.9	13.3	8.5	1.1	5.2
0.41	0.0	0.9	17.1	26.1	20.7	20.0	18.9	17.0	15.8	12.3	1.9	4.6
1.2	0.0	8.6	21.1	27.4	20.5	21.4	21.1	20.7	16.7	12.5	8.2	5.4
3.7	0.0	2.0	11.1	15.3	10.7	11.1	13.3	13.9	13.0	10.1	6.9	4.7
11	0.0	-0.4	1.2	1.7	1.2	0.6	0.3	0.9	0.9	0.5	-1.1	-1.6
33	0.0	0.9	-0.2	1.9	0.3	1.2	0.5	0.8	-0.7	-0.2	-1.2	-1.7
100	0.0	0.8	0.8	1.0	1.2	0.5	0.1	-0.5	-0.2	-1.4	-1.4	-2.1

Conc. Drug μM	0	0.00003	0.00006	0.00012	0.00023	0.00047	0.00094	0.0019	0.0038	0.0075	0.015	0.03
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0
0.14	0.0	20.3	18.2	17.1	17.8	17.9	14.8	18.4	7.0	-4.4	-8.0	6.7
0.41	0.0	23.2	33.8	32.9	34.0	28.5	32.3	32.1	22.1	17.3	-7.7	6.6
1.2	0.0	11.5	21.8	27.3	25.4	17.1	27.8	27.2	18.3	18.1	-5.2	5.7
3.7	0.0	5.1	21.0	23.3	20.7	13.9	17.8	16.3	13.7	10.8	4.5	5.5
11	0.0	1.4	2.3	2.4	2.1	1.2	0.8	1.6	0.3	-0.0	-2.7	-2.4
33	0.0	1.5	0.1	2.5	0.6	1.3	0.6	0.4	-1.3	-0.7	-1.9	-2.6
100	0.0	1.5	1.2	1.6	2.0	0.9	0.3	-0.4	-0.4	-1.8	-2.4	-3.0

[00316] Compound K: IC50=4.3 uM, Top=12460 RFU

[00317] Gemcitabine: IC50=8 nM, Top=11772 RFU

[00318] Value of 50% effect=6103 RFU

[00319] 50% Effect was achieved by combining 1.2 uM Compound K and 120 pM Gemcitabine.

[00320] CI=[Compound K]/$IC50_{Compound\ K}$+[Gemcitabine]/$IC50_{Gemcitabine}$ = (1.2/4.3)+(0.12/8)=0.29

Example 13: Vinblastine/Compound K combination testing in MIA PaCa-2 pancreatic cancer cells

[00321] Vinblastine, a mitotic inhibitor, was tested in combination with Compound K in the pancreatic cancer cell line MIA PaCa-2. Vinblastine was added 24 hours before Compound K in a 5 day assay. Results are shown hereafter; see Figure 29 and Figure 30. Synergy up to 45% is observed at concentrations tested. CI=0.07.

[00322] Vinblastine was added first, Compound K the next day (5 day assay in total) . Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug µM	0	1.70E-07	5.10E-07	1.50E-06	4.60E-06	1.40E-05	4.10E-05	1.20E-04	0.00037	0.001	0.003	0.01
Conc. K µM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0
0.01	0.0	-6.0	-25.1	3.3	-2.0	-8.2	1.8	1.3	1.4	0.2	-0.0	-0.2
0.04	0.0	35.2	28.1	58.5	48.6	32.3	3.8	2.7	1.8	1.2	0.0	0.1
0.12	0.0	61.9	26.9	65.6	51.9	34.3	3.2	2.5	1.6	1.4	0.0	-0.1
0.37	0.0	50.1	28.0	43.0	20.4	25.5	2.5	2.0	1.4	1.2	-0.0	-0.3
1.11	0.0	50.6	32.8	30.9	12.0	22.1	2.8	1.7	1.4	1.1	-0.1	-0.3
3.33	0.0	18.5	10.2	12.9	1.5	12.5	0.5	0.9	0.2	0.6	-0.2	-0.4
10	0.0	-20.6	1.6	5.5	0.6	3.9	0.1	-0.3	-0.2	-0.0	-0.1	0.0

Conc. Drug μM	0	1.70E-07	5.10E-07	1.50E-06	4.60E-06	1.40E-05	4.10E-05	1.20E-04	0.00037	0.001	0.003	0.01
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	0.0	35.8	27.2	49.0	23.5	17.0	0.0	0.8	0.0	0.1	-0.2	-0.3
0.04	0.0	41.4	25.6	48.2	38.6	17.8	-1.2	0.5	-0.1	-0.0	-0.1	-0.1
0.12	0.0	15.3	15.4	31.1	32.0	7.7	0.5	0.3	-0.2	-0.1	-0.2	-0.1
0.37	0.0	29.9	23.4	37.2	26.6	9.2	0.4	0.3	-0.1	0.0	-0.1	0.2
1.11	0.0	34.6	17.1	38.1	19.7	8.8	0.4	0.5	-0.4	0.2	-0.3	-0.4
3.33	0.0	31.2	16.5	33.2	28.9	10.8	0.2	0.3	-0.3	-0.3	-0.4	0.1
10	0.0	0.1	-0.5	2.2	2.9	-0.2	-0.9	-0.5	-0.3	-0.2	-0.3	-0.6

[00323] Compound K: IC50=4.1 uM, Top=10022 RFU

[00324] Vinblastine: IC50=14 pM, Top=9697 RFU

[00325] Value of 50% effect=4930 RFU

[00326] 50% Effect was achieved by combining 120 nM Compound K and 0.5 pM Vinblastine.

[00327] CI=[Compound K]/$IC50_{Compound\ K}$+[Vinblastine]/$IC50_{Vinblastine}$ = (0.12/4.1)+(0.5/14)=0.07

Example 14: Gemcitabine/Compound K combination testing in MIA PaCa-2 pancreatic cancer cells

[00328] Gemcitabine, a pyrimidine analog, was tested in combination with Compound K in the pancreatic cancer cell line MIA PaCa-2. Gemcitabine was added 24 hours before Compound K in a 4 day assay. Results are shown hereafter; see Figure 31 and Figure 32. Synergy up to 25% is observed at concentrations tested. CI=0.27.

[00329] Gemcitabine was added first, Compound K the next day (4 day assay in total) . Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.0003	0.0006	0.0012	0.0023	0.0047	0.0094	0.019	0.0375	0.075	0.15	0.3
Conc. K μM												
0	0.0	-0.0	0.0	0.0	-0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.04	0.0	24.6	17.0	27.8	38.8	56.7	26.8	31.7	27.1	35.5	42.8	1.4
0.12	0.0	12.7	18.9	19.4	5.7	17.0	13.4	23.8	23.7	23.3	13.0	-11.4
0.37	0.0	12.0	32.1	32.2	25.2	26.1	8.0	5.9	10.0	18.3	19.6	-3.9
1.1	0.0	19.0	23.7	24.8	4.0	21.3	9.5	9.2	3.9	19.7	15.7	-4.8
3.3	0.0	9.8	1.1	11.7	12.5	13.4	9.1	2.7	5.4	13.9	13.4	-8.3
10	0.0	-0.7	3.5	4.0	4.2	2.7	4.0	4.2	0.9	4.7	1.4	-1.5
30	0.0	0.6	0.4	0.5	0.3	0.2	0.1	-0.3	-0.3	0.1	-0.0	-0.3

Conc. Drug μM	0	0.0003	0.0006	0.0012	0.0023	0.0047	0.0094	0.019	0.0375	0.075	0.15	0.3
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.04	0.0	35.8	27.2	49.0	23.5	17.0	0.0	0.8	0.0	0.1	-0.2	-0.3
0.12	0.0	41.4	25.6	48.2	38.6	17.8	-1.2	0.5	-0.1	-0.0	-0.1	-0.1
0.37	0.0	15.3	15.4	31.1	32.0	7.7	0.5	0.3	-0.2	-0.1	-0.2	-0.1
1.1	0.0	29.9	23.4	37.2	26.6	9.2	0.4	0.3	-0.1	0.0	-0.1	0.2
3.3	0.0	34.6	17.1	38.1	19.7	8.8	0.4	0.5	-0.4	0.2	-0.3	-0.4
10	0.0	31.2	16.5	33.2	28.9	10.8	0.2	0.3	-0.3	-0.3	-0.4	0.1
30	0.0	0.1	-0.5	2.2	2.9	-0.2	-0.9	-0.5	-0.3	-0.2	-0.3	-0.6

[00330] Compound K: IC50=1.5 uM, Top=12202 RFU

[00331] Gemcitabine: IC50=184 nM, Top=13153 RFU

[00332] Value of 50% effect=6339 RFU

[00333] 50% Effect was achieved by combining 370 nM Compound K and 12 nM Gemcitabine.

[00334] CI=[Compound K]/$IC50_{Compound\ K}$+[Gemcitabine]/$IC50_{Gemcitabine}$ = (0.37/1.5)+(12/184)=0.27

Example 15: Sunitinib/Compound K combination testing in MIA PaCa-2 pancreatic cancer cells

[00335] Sunitinib, a multi tyrosine-kinase inhibitor as described herein, was tested in combination with Compound K in the pancreatic cancer cell line MIA PaCa-2. Sunitinib was added 24 hours before Compound K in a 4 day assay. Results are shown hereafter; see Figure 33 and Figure 34. Synergy up to 25% is observed at concentrations tested. CI=0.2.

[00336] Sunitinib was added first, Compound K the next day (4 day assay in total). Results indicate the degree of inhibitory effect found with agent combination, where a positive

value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.0029	0.0059	0.012	0.023	0.047	0.094	0.19	0.38	0.75	1.5	3
Conc. K μM												
0	0.0	0.0	-0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.04	0.0	1.2	-4.3	4.4	17.2	14.7	4.5	-5.9	-7.6	-1.3	-2.7	-0.1
0.12	0.0	23.8	8.6	16.0	4.5	11.5	8.4	-9.1	-5.0	2.8	-1.6	-0.0
0.37	0.0	13.4	-3.9	8.0	2.5	12.2	-8.0	-1.8	-6.5	-6.6	-3.4	-0.1
1.1	0.0	-3.1	-1.2	17.7	6.2	14.4	6.0	4.9	-8.5	-8.3	-3.1	-0.0
3.3	0.0	5.1	3.0	4.6	0.3	9.4	5.3	5.8	-0.8	-6.6	-7.0	-0.3
10	0.0	-4.3	-5.9	-1.3	0.5	-4.4	-0.4	-0.9	-0.6	-3.1	-2.6	-0.1
30	0.0	-0.3	-0.5	-0.1	-0.2	-0.4	-0.2	-0.9	-1.4	-2.0	-1.5	0.0

Conc. Drug μM	0	0.0029	0.0059	0.012	0.023	0.047	0.094	0.19	0.38	0.75	1.5	3
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.04	0.0	10.6	13.8	22.5	28.9	4.2	32.8	39.8	12.4	8.9	4.3	-0.2
0.12	0.0	18.1	18.0	12.7	16.0	10.6	15.0	19.9	17.5	1.7	3.5	-0.3
0.37	0.0	44.2	52.0	37.9	35.2	37.7	41.7	32.2	13.7	14.4	2.3	-0.2
1.1	0.0	19.3	26.1	9.2	19.0	12.2	24.4	21.8	7.3	-5.3	1.6	-0.2
3.3	0.0	-1.2	8.3	19.6	4.7	4.1	-9.8	7.4	0.5	-6.3	-4.3	-0.3
10	0.0	5.2	0.5	2.0	3.3	2.3	2.7	4.3	-0.9	-3.2	-1.0	-0.2
30	0.0	0.0	-0.0	-4.0	-0.4	-0.6	-0.1	-0.7	-1.6	-2.1	-0.9	-0.1

[00337] Compound K: IC50=2.0 uM, Top=10345 RFU

[00338] Sunitinib: IC50=420 nM, Top=12195 RFU

[00339] Value of 50% effect=5635 RFU

[00340] 50% Effect was achieved by combining 370 nM Compound K and 6 nM Sunitinib.

[00341] CI=[Compound K]/$IC50_{Compound\ K}$+[Sunitinib]/$IC50_{Sunitinib}$ = (0.37/1.5)+(12/184)=0.27

Example 16: Rapamycin/Compound K combination testing in MIA PaCa-2 pancreatic cancer cells

[00342] Rapamycin, an immunosuppressive macrolide, was tested in combination with Compound K in the pancreatic cancer cell line MIA PaCa-2. Rapamycin and Compound K

are added simultaneously in a 4 day assay. Results are shown hereafter; see Figure 35 and Figure 36. Synergy up to 30% is observed at concentrations tested. CI=0.25.

[00343] Rapamycin and Compound K are added simultaneously (4 day assay in total) . Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.0005	0.0015	0.0046	0.014	0.041	0.12	0.37	1.1	3.3	10	30
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	1.4	16.7	1.2	9.5	9.4	25.8	10.5	3.9	8.9	4.8	-5.9
0.41	0.0	10.7	35.4	28.4	22.0	24.4	41.8	23.0	20.9	18.6	16.9	-1.3
1.2	0.0	13.9	22.0	15.9	19.4	21.9	29.0	19.4	15.7	14.4	12.7	5.4
3.7	0.0	-1.7	-0.8	-0.2	-2.1	-0.8	-0.2	-1.1	-2.0	-1.3	0.8	0.5
11.1	0.0	-0.4	-0.4	-0.3	-0.5	-0.3	-0.3	-0.5	-0.6	-0.3	-0.8	-0.6
33.3	0.0	-0.4	-0.6	-0.7	-0.9	-0.9	-0.5	-0.7	-1.0	-1.1	-0.7	-0.1
100	0.0	-0.4	-1.0	-1.5	-0.4	-0.6	-1.9	-1.6	-0.7	-0.1	-0.9	-0.3

Conc. Drug μM	0	0.0005	0.0015	0.0046	0.014	0.041	0.12	0.37	1.1	3.3	10	30
Conc. K μM												
0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.14	0.0	7.5	15.0	13.5	3.9	9.0	3.4	16.5	20.8	17.8	3.5	-11.1
0.41	0.0	10.5	16.1	22.3	13.1	4.1	12.7	12.8	19.2	17.6	10.1	0.9
1.2	0.0	29.2	36.0	36.5	29.6	25.9	32.2	32.5	34.1	34.4	24.6	11.0
3.7	0.0	1.5	1.7	0.9	1.0	-0.0	0.4	0.5	0.4	0.1	0.2	0.3
11.1	0.0	-0.2	-0.4	-0.1	-0.3	-0.4	-0.6	-0.4	-0.3	-0.5	-0.6	-0.3
33.3	0.0	-0.1	-0.3	-0.2	-0.6	-0.5	-0.6	-0.6	-0.6	-0.8	-0.5	-0.2
100	0.0	0.4	-2.3	0.5	0.5	-0.0	-0.4	-0.0	0.1	0.6	0.5	0.3

[00344] Compound K: IC50=1.7 uM, Top=13393 RFU

[00345] Rapamycin: IC50=18.1 uM, Top=9864 RFU

[00346] Value of 50% effect=5814 RFU

[00347] 50% Effect was achieved by combining 410 nM Compound K and 120 nM Rapamycin.

[00348] CI=[Compound K]/$IC50_{Compound\ K}$+[Rapamycin]/$IC50_{Rapamycin}$ = (0.41/1.7)+(0.12/18.1)=0.25

Example 17: 5-Fluorouracil/Compound K combination testing in SUM-149PT inflammatory breast carcinoma cells

[00349] 5-Fluorouracil, a pyrimidine analog, was tested in combination with Compound K in the inflammatory breast carcinoma cell line SUM-149PT. 5-Fluorouracil was added 24 hours before Compound K in a 5 day assay. Results are shown hereafter; see Figure 37 and Figure 38. Synergy up to 30% is observed at concentrations tested. CI=0.09.

[00350] 5-Fluorouracil was added first, Compound K the next day (5 day assay in total) . Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug µM	0	0.0098	0.0195	0.039	0.078	0.16	0.31	0.63	1.3	2.5	5	10
Conc. K µM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	0.0	-5.0	-5.8	-8.0	10.9	1.4	-4.9	-9.9	-0.5	-6.3	-2.0	-2.0
0.04	0.0	13.0	4.7	10.3	44.7	25.4	19.1	19.9	10.5	3.4	7.5	5.5
0.12	0.0	-1.7	8.8	-1.5	23.7	15.5	10.9	9.6	0.1	5.5	7.6	3.4
0.37	0.0	0.4	38.3	8.8	29.4	26.3	13.7	14.1	11.6	8.5	8.1	2.7
1.11	0.0	5.2	9.1	5.5	30.4	21.8	21.7	22.5	15.5	14.2	9.1	6.9
3.33	0.0	7.5	8.6	14.3	18.5	18.6	20.6	12.4	9.3	9.3	9.7	7.5
10	0.0	6.9	0.7	7.8	8.5	9.4	8.4	15.3	12.1	10.7	10.8	9.4

Conc. Drug µM	0	0.0098	0.0195	0.039	0.078	0.16	0.31	0.63	1.3	2.5	5	10
Conc. K µM												
0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	0.0	-3.5	-3.0	-0.5	2.9	1.4	-0.6	2.9	-2.6	-18.0	-9.5	-6.8
0.04	0.0	20.5	22.7	30.3	24.7	26.7	11.1	10.5	6.5	-10.1	6.5	-7.2
0.12	0.0	24.3	26.8	41.1	26.5	33.9	18.1	25.7	14.6	1.0	1.6	1.7
0.37	0.0	27.7	18.1	28.1	30.6	25.7	15.4	19.6	13.9	-3.7	5.3	0.4
1.11	0.0	20.9	16.6	21.7	29.0	19.5	12.5	12.7	11.8	6.9	8.1	4.3
3.33	0.0	17.3	17.2	24.5	25.5	22.0	16.3	17.4	11.2	7.2	12.1	10.4
10	-0.0	-1.2	-2.7	8.9	11.1	10.9	5.3	22.5	19.8	13.2	14.9	11.7

[00351] Compound K: IC50=27 uM, Top=17000 RFU

[00352] 5-Fluorouracil: IC50=1.7 uM, Top=19618 RFU

[00353] Value of 50% effect=9154 RFU

[00354] 50% Effect was achieved by combining 1.11 uM Compound K and 78 nM 5-Fluorouracil.

[00355] CI=[Compound K]/$IC50_{Compound\ K}$+[5-Fluorouracil]/$IC50_{5\text{-}Fluorouracil}$ = (1.11/27)+(0.078/1.7)=0.09

Example 18: Cisplatin/Compound K combination testing in SUM-149PT inflammatory breast carcinoma cells

[00356] Cisplatin, an alkylating-like agent, was tested in combination with Compound K in the inflammatory breast carcinoma cell line SUM-149PT. Cisplatin was added 24 hours before Compound K in a 5 day assay. Results are shown hereafter; see Figure 39 and Figure 40. Synergy up to 25% is observed at concentrations tested. CI=0.88.

[00357] Cisplatin was added first, Compound K the next day (5 day assay in total) . Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug µM	0	0.0002	0.0005	0.0015	0.0046	0.014	0.041	0.12	0.37	1.1	3	10
Conc. K µM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	0.0	8.1	14.2	19.3	11.6	20.3	14.5	10.5	7.8	-3.0	9.3	-1.0
0.04	0.0	14.8	17.2	18.8	14.9	8.3	-3.5	4.7	-9.1	-14.4	2.5	-2.2
0.12	0.0	8.5	3.7	12.5	10.3	6.4	-9.6	0.8	-9.4	-13.8	5.3	1.9
0.37	0.0	1.8	16.9	14.5	22.3	11.9	-13.4	3.8	0.1	-10.2	9.3	0.4
1.11	0.0	-11.9	-1.5	-6.7	-5.9	-11.2	-20.6	-11.2	-24.1	-15.5	2.3	1.2
3.33	0.0	3.9	13.0	9.5	10.3	0.2	-14.2	-6.4	-17.0	-3.4	10.1	4.5
10	0.0	26.2	20.1	26.3	28.3	23.0	11.5	10.0	19.6	14.8	25.5	8.8

Conc. Drug µM	0	0.0002	0.0005	0.0015	0.0046	0.014	0.041	0.12	0.37	1.1	3	10
Conc. K µM												
0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	0.0	23.6	24.3	20.0	29.0	16.1	31.2	19.8	-2.7	5.8	1.4	1.4
0.04	0.0	41.9	24.0	30.1	16.4	25.1	34.8	15.4	-1.0	-10.6	-0.5	-2.8
0.12	0.0	13.2	14.1	8.7	6.6	15.1	17.1	9.6	1.9	-10.0	1.8	-2.2
0.37	0.0	29.0	25.9	12.0	29.0	16.7	21.9	20.2	7.3	1.3	8.8	-0.7
1.11	0.0	19.7	12.5	-3.7	8.3	6.9	-3.1	0.8	-10.2	-2.4	4.4	0.7
3.33	0.0	12.8	2.1	5.0	2.3	-10.9	-3.3	-9.2	-7.8	3.8	7.2	2.1
10	0.0	3.3	4.5	3.7	9.7	-2.8	0.9	7.8	6.5	7.7	12.3	4.7

[00358] Compound K: IC50=3.8 uM, Top=16000 RFU

[00359] Cisplatin: IC50=462 nM, Top=14588 RFU

[00360] Value of 50% effect=7547 RFU

[00361] 50% Effect was achieved by combining 3.3 uM Compound K and 46 nM Cisplatin.

[00362] CI=[Compound K]/$IC50_{Compound\ K}$+[Cisplatin]/$IC50_{Cisplatin}$ = (3.3/3.8)+(46/462)=0.88

Example 19: Rapamycin/Compound K combination testing in SUM-149PT inflammatory breast carcinoma cells

[00363] Rapamycin, an immunosuppressive macrolide, was tested in combination with Compound K in the inflammatory breast carcinoma cell line SUM-149PT Rapamycin was added 24 hours before Compound K in a 5 day assay. Results are shown hereafter; see Figure 41 and Figure 42. Synergy up to 40% is observed at concentrations tested. CI=0.03.

[00364] Rapamycin was added first, Compound K the next day (5 day assay in total) . Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug µM	0	0.010	0.020	0.039	0.078	0.16	0.31	0.63	1.3	2.5	5	10
Conc. K µM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	0.0	-3.5	7.9	28.5	15.8	39.1	25.0	23.7	-11.0	-5.0	-11.5	-4.9
0.04	0.0	29.3	22.0	22.2	23.8	25.2	34.3	32.7	-1.0	-19.7	-10.1	-2.2
0.12	0.0	43.5	47.9	54.0	46.1	50.2	51.0	44.7	32.3	15.5	-3.3	0.7
0.37	0.0	42.9	48.0	46.6	42.4	40.8	43.1	39.2	24.2	-4.8	-10.1	4.2
1.11	0.0	33.2	39.7	32.8	42.1	34.3	39.3	36.3	16.3	-7.5	16.2	5.1
3.33	0.0	10.4	36.3	35.9	31.8	34.7	33.0	30.9	27.8	26.5	32.6	2.3
10	0.0	2.9	5.2	5.6	4.4	4.6	2.7	6.0	1.4	-1.4	18.0	3.0

Conc. Drug μM	0	0.010	0.020	0.039	0.078	0.16	0.31	0.63	1.3	2.5	5	10
Conc. K μM												
0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0
0.01	0.0	2.5	1.0	2.1	8.1	-5.6	-14.0	-2.6	-3.6	10.0	-9.8	3.1
0.04	0.0	29.7	28.2	31.6	35.6	28.2	25.2	25.8	19.7	-10.4	-13.8	-3.2
0.12	0.0	25.6	20.6	35.8	38.6	30.7	31.8	22.7	18.4	21.0	-10.1	3.2
0.37	0.0	27.6	25.7	29.1	30.3	28.4	23.3	16.0	5.8	1.9	-15.0	1.6
1.11	0.0	26.8	30.2	27.9	36.4	25.9	20.2	24.2	-2.4	27.1	22.4	4.2
3.33	0.0	16.5	24.2	22.1	30.8	15.2	6.3	16.7	11.0	15.6	25.6	-3.8
10	0.0	-3.0	-9.1	-11.1	-12.0	-10.1	-6.0	-9.4	-10.1	-3.4	13.7	-5.5

[00365] Compound K: IC50=13 uM, Top=18285 RFU

[00366] Rapamycin: IC50=9.7 uM, Top=15915 RFU

[00367] Value of 50% effect=8550 RFU

[00368] 50% Effect was achieved by combining 370 nM Compound K and 39 nM Rapamycin.

[00369] CI=[Compound K]/$IC50_{Compound\ K}$+[Rapamycin]/$IC50_{Rapamycin}$ = (0.37/13)+(0.039/9.7)=0.03

Example 20: Erlotinib/Compound K combination testing in SUM-149PT inflammatory breast carcinoma cells

[00370] Erlotinib, a small molecule EGFR inhibitor, was tested in combination with Compound K in the inflammatory breast carcinoma cell line SUM-149PT. Erlotinib was added 24 hours before Compound K in a 5 day assay. Results are shown hereafter; see Figure 43 and Figure 44. Synergy up to 35% is observed at concentrations tested. CI=0.16.

[00371] Erlotinib was added first, Compound K the next day (5 day assay in total) . Results indicate the degree of inhibitory effect found with agent combination, where a positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.00017	0.00051	0.0015	0.0046	0.014	0.041	0.12	0.37	1.1	3.3	10
Conc. K μM												
0	0.0	-0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	0.0	-7.3	-6.5	-7.6	-9.0	3.3	27.7	5.1	-2.4	-18.1	-10.8	-11.7
0.04	0.0	-7.3	41.6	18.9	28.3	35.1	13.8	32.7	15.3	-2.5	-8.0	-4.5
0.12	0.0	-7.1	28.3	26.0	36.7	41.4	34.4	11.4	5.1	-14.4	-9.4	-11.3
0.37	0.0	-5.3	39.8	19.5	30.0	40.8	43.9	36.0	15.1	-17.3	-7.8	24.4
1.11	0.0	-4.2	23.8	13.7	27.3	31.0	21.8	31.6	6.8	-8.8	-14.7	-15.4
3.33	0.0	6.5	18.1	9.0	11.3	9.5	1.7	6.2	-1.3	-9.9	-20.0	-19.1
10	0.0	-0.0	-2.4	-2.4	-5.1	1.5	2.5	3.0	-2.5	-22.1	-25.6	-29.4

Conc. Drug μM	0	0.00017	0.00051	0.0015	0.0046	0.014	0.041	0.12	0.37	1.1	3.3	10
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0
0.01	0.0	11.5	23.2	29.7	25.2	10.3	17.5	8.4	1.1	5.1	-11.5	6.0
0.04	0.0	-8.3	13.4	18.8	9.5	24.1	9.1	11.1	12.5	17.6	-4.4	13.7
0.12	0.0	-5.0	8.1	17.1	34.3	27.1	16.1	31.0	21.8	31.4	3.3	11.4
0.37	0.0	-3.7	16.7	17.5	13.7	35.9	17.0	25.9	22.5	18.9	3.0	10.3
1.11	0.0	8.7	19.2	10.2	27.4	39.2	25.7	17.1	15.4	20.1	0.4	11.8
3.33	0.0	10.0	9.2	5.9	9.8	9.0	11.7	6.5	-1.9	5.4	-9.1	-4.5
10	0.0	6.5	6.7	8.3	-4.5	19.1	10.1	-1.5	-7.3	-1.7	-11.8	-7.2

[00372] Compound K: IC50=6.9 uM, Top=19848 RFU

[00373] Erlotinib: IC50=2.2 uM, Top=17378 RFU

[00374] Value of 50% effect=9307 RFU

[00375] 50% Effect was achieved by combining 1.1 uM Compound K and 0.5 nM Erlotinib.

[00376] CI=[Compound K]/$IC50_{Compound\ K}$+[Erlotinib]/$IC50_{Erlotinib}$ = (1.11/6.9)+(0.00051/2.2)=0.16

Example 21: 5-Fluorouracil/Compound K combination testing in SUM-190PT inflammatory breast carcinoma cells

[00377] 5-Fluorouracil, a pyrimidine analog, was tested in combination with Compound K in the inflammatory breast carcinoma cell line SUM-190PT. 5-Fluorouracil was added 24 hours before Compound K in a 5 day assay. Results are shown hereafter; see Figure 45 and Figure 46. Synergy up to 30% is observed at concentrations tested. CI=0.14.

[00378] 5-Fluorouracil was added first, Compound K the next day (5 day assay in total) . Results indicate the degree of inhibitory effect found with agent combination, where a

positive value denotes synergy and a negative value antagonism. The experiment was performed in duplicate. Both data sets are presented.

Conc. Drug μM	0	0.00017	0.00051	0.0015	0.0046	0.014	0.041	0.12	0.37	1.1	3.3	10
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0	0.0	0.0	0.0	-0.0
0.01	0.0	-14.6	-8.8	0.9	-18.9	-27.7	-14.6	9.6	45.6	20.5	9.1	7.2
0.04	0.0	-15.7	41.2	19.5	36.3	24.9	31.6	41.2	35.7	15.4	-21.9	-6.0
0.12	0.0	-12.4	41.4	37.4	29.5	50.7	39.3	35.2	32.9	26.6	-19.0	-4.7
0.37	0.0	-13.1	50.3	33.4	34.9	25.3	34.3	31.4	31.9	21.0	-23.7	-6.4
1.11	0.0	-2.9	-1.5	-9.4	-14.4	-2.7	-10.9	-2.8	-4.2	-9.7	-30.1	-22.8
3.33	0.0	-6.9	-4.9	-4.4	-2.0	-2.6	12.3	5.6	11.3	9.2	-12.1	-7.0
10	0.0	-1.4	-0.8	-2.1	-2.2	-2.0	-2.9	-2.4	-3.1	-4.2	-3.2	0.5

Conc. Drug μM	0	0.00017	0.00051	0.0015	0.0046	0.014	0.041	0.12	0.37	1.1	3.3	10
Conc. K μM												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.0	0.0
0.01	0.0	-41.4	-22.5	-19.5	-34.3	-33.3	-18.5	17.0	19.2	14.5	-0.8	-0.3
0.04	0.0	-29.7	31.3	29.0	10.0	18.9	25.8	25.7	20.1	19.8	-11.7	2.3
0.12	0.0	-9.5	33.5	35.7	26.2	19.5	28.8	32.4	30.6	19.9	11.0	-0.6
0.37	0.0	-43.5	29.0	41.1	23.6	21.9	32.6	27.9	23.0	17.0	11.0	-7.5
1.11	0.0	-13.3	16.0	25.6	11.8	9.9	13.3	21.5	11.1	7.9	0.9	-10.0
3.33	0.0	-8.9	-1.7	0.9	-1.2	-1.1	-0.5	2.6	3.4	3.8	-5.8	-8.3
10	0.0	0.8	-0.1	-0.2	-0.2	-0.6	-1.1	-0.9	-1.0	-0.5	-1.6	-1.9

[00379] Compound K: IC50=852 nM, Top=9958 RFU

[00380] 5-Fluorouracil: IC50=12.2 uM, Top=9141 RFU

[00381] Value of 50% effect=4775 RFU

[00382] 50% Effect was achieved by combining 120 nM Compound K and 46 nM 5-Fluorouracil.

[00383] CI=[Compound K]/$IC50_{Compound\ K}$+[5-Fluorouracil]/$IC50_{5\text{-}Fluorouracil}$ = (120/852)+(0.046/12.2)=0.14

Example 22: Erlotinib/Compound K combination testing in erlotinib-sensitive BT-474 breast carcinoma cells

[00384] Erlotinib, a small molecule EGFR inhibitor, was tested in combination with Compound K in the breast carcinoma cell line BT-474. Erlotinib was added simultaneously with Compound K in a 4 day assay. Results are shown hereafter; see Figure 47 and Figure 48. Synergy was observed with CI=0.55.

[00385] Erlotinib was added simultaneously with Compound K in 1:1 ratio in a 4 day assay. The experiment was performed in triplicate. The dose-response curves for single agents and combination are presented.

[00386] Compound K: IC50=2.7 uM

[00387] Erlotinib: IC50=6.6 uM

[00388] About 60% effect was achieved by combining 1.24 nM Compound K and 1.25 uM erlotinib.

[00389] $CI=[IC50_{Combination}]/IC50_{Compound\ K}+[IC50_{Combination}]/IC50_{Erlotinib}=(1.1/2.7)+(1.1/6.6)=0.57$.

Example 23: Erlotinib/Compound K combination testing in erlotinib-resistant MDA-MB-453 breast carcinoma cells

[00390] Erlotinib, a small molecule EGFR inhibitor, was tested in combination with Compound K in the breast carcinoma cell line MDA-MB453. Erlotinib was added simultaneously with Compound K in a 4 day assay. Results are shown hereafter. Synergy was observed with CI=0.55.

[00391] Compound K alone or in combination with 1 uM erlotinib was added to cells in a 4 day assay. The experiment was performed in triplicate. The dose-response curves for Compound K and combination are presented; see Figure 49 and Figure 50.

[00392] Compound K: IC50=6.15 uM

[00393] Erlotinib: IC50> 100 uM

[00394] Greater than 60% effect was achieved by combining 3.125 uM Compound K and 1 uM erlotinib.

[00395] Combination IC50 Shift = 6.15/1.96 = 3.14.

Example 24: Erlotinib/Compound K combination testing in erlotinib-resistant T47D breast carcinoma cells

[00396] Erlotinib, a small molecule EGFR inhibitor, was tested in combination with Compound K in the breast carcinoma cell line T47D. Erlotinib was added simultaneously with Compound K in 1:2.7 ratio combination a 4 day assay. Results are shown hereafter. Synergy was observed with CI=0.48.

[00397] Erlotinib was added simultaneously with Compound K in 1:1 ratio in a 4 day assay. The experiment was performed in triplicate. The dose-response curves for single agents and combination are presented; see Figure 52.

[00398] Compound K: IC50=5.9 uM; Maximum Concentration = 37.5 uM

[00399] Erlotinib: IC50=47 uM; Maximum Concentration = 100 uM

[00400] Combination: 50% Cell Death at 2.1 uM Compound K plus 5.7 uM Erlotinib; see Figure 53.

[00401] $CI=[IC50_{Combination}]/IC50_{Compound\ K}+[IC50_{Combination}]/IC50_{Erlotinib}=$ (2.1/5.9)+(5.7/47)=0.48.

Example 25: Erlotinib/Compound K combination testing in erlotinib-resistant ZR-75-1 breast carcinoma cells

[00402] Erlotinib, a small molecule EGFR inhibitor, was tested in combination with Compound K in the breast carcinoma cell line ZR-75-1. Compound K was added simultaneously with Erlotinib in 1:2.7 ratio combination a 4 day assay. Results are shown hereafter. Synergy was observed with CI<=0.59.

[00403] Compound K was added simultaneously with Erlotinib in 1:2.7 ratio in a 4 day assay. The experiment was performed in triplicate. The dose-response curves for single agents and combination are presented; see Figure 54.

[00404] Compound K: IC50=4.1 uM; Maximum Concentration = 75 uM

[00405] Erlotinib: IC50>200 uM; Maximum Concentration = 200 uM

[00406] Combination: 50% Cell Death at 2.3 uM Compound K plus 6.2 uM Erlotinib; see Figure 55.

[00407] $CI=[IC50_{Combination}]/IC50_{Compound\ K}+[IC50_{Combination}]/IC50_{Erlotinib}=$ (2.3/4.1)+(6.2/>200)<=0.59.

Example 26: Lapatinib/Compound K combination testing in T47D breast carcinoma cells

[00408] Lapatinib, a small molecule EGFR/Her2 inhibitor, was tested in combination with Compound K in the breast carcinoma cell line T47D. Compound K was added simultaneously with Lapatinib in 1:1.2 ratio combination a 4 day assay. Results are shown hereafter. Synergy was observed with CI=0.49.

[00409] Compound K was added simultaneously with Lapatinib in 1.2:1 ratio in a 4 day assay. The experiment was performed in triplicate. The dose-response curves for single agents and combination are presented; see Figure 56.

[00410] Compound K: IC50=5.87 uM; Maximum Concentration = 75 uM

[00411] Lapatinib: IC50=5.68 uM; Maximum Concentration = 62.5 uM

[00412] Combination: 50% Cell Death at 1.53 uM Compound K plus 1.28 uM lapatinib

[00413] $CI=[IC50_{Combination}]/IC50_{Compound\ K}+[IC50_{Combination}]/IC50_{Lapatinib}=$ (1.53/5.87)+(1.28/5.68)=0.49.

Example 27: Sorafenib/Compound K combination testing in T47D breast carcinoma cells

[00414] Sorafenib, a small molecule Raf/PDGFR/VEGFR2/VEGFR3/cKit inhibitor, was tested in combination with Compound K in the breast carcinoma cell line T47D. Compound K was added simultaneously with Sorafenib in 2:1 ratio combination a 4 day assay. Results are shown hereafter. Synergy was observed with CI=0.80.

[00415] Compound K was added simultaneously with Sorafenib in 2:1 ratio in a 4 day assay. The experiment was performed in triplicate. The dose-response curves for single agents and combination are presented; see Figure 57.

[00416] Compound K: IC50=5.87 uM; Maximum Concentration = 75 uM

[00417] Sorafenib: IC50=3.58 uM; Maximum Concentration = 37.5 uM

[00418] Combination: 50% Cell Death at 2.6 uM Compound K plus 1.3 uM Sorefenib; see Figure 58.

[00419] $CI=[IC50_{Combination}]/IC50_{Compound\ K}+[IC50_{Combination}]/IC50_{Sorafenibb}=$ (2.6/5.87)+(1.3/3.58)=0.80.

Example 28: Sunitinib/Compound K combination testing in T47D breast carcinoma cells

[00420] Sunitinib, a small molecule inhibitor of multiple receptor tyrosine kinases, was tested in combination with Compound K in the breast carcinoma cell line T47D. Compound K was added simultaneously with Sunitinib in 1:1 ratio combination a 4 day assay. Results are shown hereafter. Synergy was observed with CI=0.86.

[00421] Compound K was added simultaneously with Sunitinib in 1:1 ratio in a 4 day assay. The experiment was performed in triplicate. The dose-response curves for single agents and combination are presented; see Figure 59.

[00422] Compound K: IC50=5.87 uM; Maximum Concentration = 75 uM

[00423] Sunitinib: IC50=6.2 uM; Maximum Concentration = 75 uM

[00424] Combination: 50% Cell Death at 2.6 uM Compound K plus 2.6 uM Sunitinib

[00425] $CI=[IC50_{Combination}]/IC50_{Compound\ K}+[IC50_{Combination}]/IC50_{Sunitinib}=(2.6/5.87)+(2.65/6.2)=0.86$.

Example 29: Akt 1/2 Inhibitor/Compound K combination testing in BT-474 breast carcinoma cells

[00426] Isoform specific inhibitor of Akt1/2, 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, was tested in combination with Compound K in the breast carcinoma cell line BT-474. Akt1/2 inhibitor was added simultaneously with Compound K in a 4 day assay. Results are shown hereafter; see Figure 60 and Figure 61. Synergy was observed with CI=0.38.

[00427] Akt1/2 inhibitor was added simultaneously with Compound K in 1:10 ratio in a 4 day assay. The experiment was performed in triplicate. The dose-response curves for single agents and combination are presented in Figure 60.

[00428] Compound K: IC50 = 2.9 uM

[00429] Akt1/2 Inhibitor: IC50 = 0.5 uM

[00430] 50% effect was achieved by combining 700 nM Compound K and 70 nM Akt1/2 Inhibitor.

[00431] $CI=[IC50_{Combination}]/IC50_{Compound\ K}+[IC50_{Combination}]/IC50_{Akt1/2\ Inhibitor}=(0.7/2.9)+(0.07/0.5)=0.38$.

Example 30: Combination of Compound K with Erlotinib and Lapatinib in MDA-MB-453 breast carcinoma cells

[00432] Small molecule inhibitors of EGFR, Erlotinib, and EGFR/Her2, Lapatinib, were tested as single agents or in combination with Compound K in the breast carcinoma cell line MDA-MB-453. 100 uM of Erlotinib or 2 uM Lapatinib were added simultaneously with 10 uM Compound K in a 2, 4 and 8 hour assays. Whole proteomes were isolated from treated

cells and analyzed by Western blot for changes in phosphorylation status of Akt at Ser129 and Ser473 or downstream mediator of Akt activity, PRAS40 at Thr246. Results are shown in Figure 62.

[00433] Treatment with Erlotinib or Lapatinib as single agents decreased phosphorylation of Akt at Ser473 below the detectable levels while having no effect on phosphorylation of Akt at Ser129. There was also a pronounced decrease in phosphorylation of PRAS40 at Thr246 at 2 and 4 hours, that was partially reversed by 8 hours.

[00434] Treatment with compound K as a single agent resulted in significant reduction of phosphorylation of Akt at Ser129 in a time-dependent manner. Phosphorylation of Akt at Ser473 was also affected, but to a lesser degree. The effect on phosphorylation of PRAS40 at Thr246 became evident at 4 and 8 hours, but was significantly less pronounced than for Erlotinib or Lapatinib.

[00435] Treatments with Compound K in combination with either Erlotinib or Lapatinib had similar effects on phosphorylation of Akt at Ser129 and Ser473 to single agents, but had more pronounced and sustained effect on phosphorylation of PRAS40 at Thr246 than any of the drugs alone.

[00436] Combination of Compound K with either Erlotinib or Lapatinib results in enhanced inhibition of Akt signaling.

Example 31: Panobinostat/Compound K combination testing in Hs 578T breast cancer cells

[00437] Panobinostat, an HDAC inhibitor, was tested in combination with Compound K in the breast cancer cell line Hs 578T. Results are shown hereafter; see Figure 63 and Figure 64. Synergy was observed with CI50=0.76.

[00438] Panobinostat was added simultaneously with Compound K in 4 day assay. Drug/Drug molar ratio was 2000:1 (Compound K:Panobinostat). The experiment was performed in triplicate.

[00439] The dose-response curves for Compound K, Panobinostat and (2000:1) combination are presented in Figure 63.

[00440] Compound K: IC50 = 17.63 uM; Maximum Concentration = 200 uM

[00441] Panobinostat: IC50 = 2.76 nM; Maximum Concentration = 100 nM

[00442] Combination: 50% Cell Death at 3.19 uM Compound K plus 1.6 nM Panobinostat.

[00443] $CI50=[IC50_{Combination}]/IC50_{Compound\ K}+[IC50_{Combination}]/IC50_{Panobinostat}=(3.19/17.63)+(1.6/2.76)=0.76$.

Example 32: 17-DMAG/Compound K combination testing in Hs 578T breast cancer cells

[00444] 17-DMAG, an Hsp90 inhibitor, was tested in combination with Compound K in the breast cancer cell line Hs 578T. Results are shown hereafter; see Figure 65 and Figure 66. Synergy was observed with CI50=0.77.

[00445] 17-DMAG was added simultaneously with Compound K in 4 day assay. Drug/Drug molar ratio was 3000:1 (Compound K:17-DMAG). The experiment was performed in triplicate.

[00446] The dose-response curves for Compound K, 17-DMAG and (3000:1) combination are presented in Figure 65.

[00447] Compound K: IC50 = 16.71 uM; Maximum Concentration = 200 uM

[00448] 17-DMAG: IC50 = 6.37 nM; Maximum Concentration = 66 nM

[00449] Combination: 50% Cell Death at 6.84 uM Compound K plus 2.28 nM 17-DMAG;.

[00450] $CI50=[IC50_{Combination}]/IC50_{Compound\ K}+[IC50_{Combination}]/IC50_{17\text{-}DMAG}=(6.84/16.71)+(2.28/6.37)=0.77$.

Example 33: AKTi VIII/Compound K combination testing in BT-474 breast cancer cells

[00451] AKT inhibitor VIII (AKTi VIII, Akt1/2, 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo-[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one (IC_{50} = 58 nM, 210 nM, and 2.12 μM for Akt1, Akt2, and Akt3, respectively) was tested in combination with Compound K in the breast ductal carcinoma cell line BT-474. Results are shown hereafter; see Figures 67 and 68. Synergy was observed with CI50=0.37

[00452] AKTi VIII was added simultaneously with Compound K in 3 day assay. Drug/Drug molar ratios were 20:1 (Compound K/AKTi VIII). The experiment was performed in triplicate.

[00453] The dose-response curves for Compound K, AKTi VIII and (20:1) combination are presented in Figure 67.

[00454] Compound K: IC50=2.68 uM; Maximum Concentration = 10 uM

[00455] AKTi VIII: IC50=550 nM; Maximum Concentration = 500 nM

[00456] Combination: 50% Cell Death at 786 nM Compound K plus 39.3 nM AKTi VIII

[00457] CI50=[IC50Combination]/IC50Compound K+[IC50Combination]/IC50AKYi VIII = (0.786/2.68)+(39.3/550)=0.37.

Example 34: Compound K/BEZ235 combination testing in BT-474 breast cancer cells

[00458] BEZ235 (NVP-BEZ235), a PI3K/mTOR inhibitor, was tested in combination with Compound K in the breast ductal carcinoma cell line BT-474. Results are shown hereafter; see Figures 69 and 70. Synergy was observed with CI50=0.27

[00459] BEZ235 was added simultaneously with Compound K in 3 day assay. Drug/Drug molar ratios were 333:1 (Compound K/BEZ235). The experiment was performed in triplicate.

[00460] The dose-response curves for Compound K, BEZ235 and (333:1) combination are presented in Figure 69.

[00461] Compound K: IC50=2.68 uM; Maximum Concentration = 10 uM

[00462] BEZ235: IC50=10 nM; Maximum Concentration =30 nM

[00463] Combination: 50% Cell Death at 438 nM Compound K plus 1.3 nM BEZ235

[00464] CI50=[IC50Combination]/IC50Compound K+[IC50Combination]/IC50BEZ235 = (0.438/2.68)+(1.3/10)=0.27.

Example 35: Compound K/LY294002 combination testing in BT-474 breast cancer cells

[00465] LY294002, a PI3K inhibitor, was tested in combination with Compound K in the breast ductal carcinoma cell line BT-474. Results are shown hereafter; see Figures 71 and 72. Synergy was observed with CI50=0.61

[00466] LY294002 was added simultaneously with Compound K in 3 day assay. Drug/Drug molar ratios were 1:2 (Compound K/LY294002). The experiment was performed in triplicate.

[00467] The dose-response curves for Compound K, LY294002 and (1:2) combination are presented in Figure 71.

[00468] Compound K: IC50=2.68 uM; Maximum Concentration = 10 uM

[00469] LY294002: IC50=2.26 uM; Maximum Concentration = 20 uM

[00470] Combination: 50% Cell Death at 482 nM Compound K plus 964 nM LY294002

[00471] CI50=[IC50Combination]/IC50Compound K+[IC50Combination]/IC50LY294002 = (0.482/2.68)+(0.964/2.26)=0.61.

Example 36: Compound K/PI-103 combination testing in BT-474 breast cancer cells

[00472] PI-103, a PI3K/mTOR inhibitor, was tested in combination with Compound K in the breast ductal carcinoma cell line BT-474. Results are shown hereafter; see Figures 73 and 74. Synergy was observed with CI50=0.82

[00473] PI-103 was added simultaneously with Compound K in 3 day assay. Drug/Drug molar ratios were 1:1 (Compound K/PI-103). The experiment was performed in triplicate.

[00474] The dose-response curves for Compound K, PI-103 and (1:1) combination are presented in Figure 73.

[00475] Compound K: IC50=2.68 uM; Maximum Concentration = 10 uM

[00476] PI-103: IC50=410 nM; Maximum Concentration = 10 uM

[00477] Combination: 50% Cell Death at 293 nM Compound K plus 293 nM PI-103

[00478] CI50=[IC50Combination]/IC50Compound K+[IC50Combination]/IC50PI-103 = (0.293/2.68)+(293/410)=0.82.

Example 37: Compound K/Wortmannin combination testing in BT-474 breast cancer cells

[00479] Wortmannin, a PI3K inhibitor, was tested in combination with Compound K in the breast ductal carcinoma cell line BT-474. Results are shown hereafter; see Figures 75 and 76. Synergy was observed with CI50=0.59

[00480] Wortmannin was added simultaneously with Compound K in 3 day assay. Drug/Drug molar ratios were 1:2 (Compound K/Wortmannin). The experiment was performed in triplicate.

[00481] The dose-response curves for Compound K, Wortmannin and (1:2) combination are presented in Figure 75.

[00482] Compound K: IC50=2.68 uM; Maximum Concentration = 10 uM

[00483] Wortmannin: IC50=25.92 uM; Maximum Concentration = 20 uM

[00484] Combination: 50% Cell Death at 1.3 uM Compound K plus 1.3 uM Wortmannin

[00485] CI50=[IC50Combination]/IC50Compound K+[IC50Combination]/IC50Wortmannin = (1.3/2.68)+(1.3/25.92)=0.59.

Example 38: Compound K/PI-103 combination testing in T-47D breast cancer cells

[00486] PI-103, a PI3K/mTOR inhibitor, was tested in combination with Compound K in the breast ductal carcinoma cell line T-47D. Results are shown hereafter; see Figures 77 and 78. Synergy was observed with CI50=0.66

[00487] PI-103 was added simultaneously with Compound K in 3 day assay. Drug/Drug molar ratios were 1:1 (Compound K/PI-103). The experiment was performed in triplicate.

[00488] The dose-response curves for Compound K, PI-103 and (1:1) combination are presented in Figure 77.

[00489] Compound K: IC50=3.35 uM; Maximum Concentration = 10 uM

[00490] PI-103: IC50=5.37 uM; Maximum Concentration = 10 uM

[00491] Combination: 50% Cell Death at 1.37 uM Compound K plus 1.37 uM PI-103

[00492] CI50=[IC50Combination]/IC50Compound K+[IC50Combination]/IC50PI-103 = (1.37/3.35)+(1.37/5.37)=0.66.

Example 39: Compound K/AKTi VIII combination testing in BT-474 breast cancer cells

[00493] AKT inhibitor VIII (AKTi VIII, 3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo-[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one (IC_{50} = 58 nM, 210 nM, and 2.12 μM for Akt1, Akt2, and Akt3, respectively) was tested in combination with Compound K in the breast ductal carcinoma cell line BT-474. Results are shown hereafter; see Figures 79 and 80. Synergy induction of apoptosis was observed.

[00494] AKTi VIII was added simultaneously with Compound K in 8 hour assay. Drug/Drug molar ratios were 5:1 (Compound K/AKTi VIII).

[00495] The western hybridization analysis for untreated cells (UTC), Compound K, AKTi VIII and (5:1) combination are presented in Figure 80.

[00496] Compound K: Dramatically reduced phosphorylation of AKT at S129, had moderate effect on phosphorylation of AKT at T308 and S473. Dramatically decreased

phosphorylation of p21 at T145. Had very minor effect on cleavage of PARP (i.e. induction of apoptosis).

[00497] AKTi VIII: Had no effect on phosphorylation of AKT at S129, Dramatically reduced phosphorylation of AKT at T308 and S473. Dramatically decreased phosphorylation of p21 at T145. Had very minor effect on cleavage of PARP (i.e. induction of apoptosis).

[00498] Combination: Dramatically reduced phosphorylation of AKT at S129, T308 and S473. Further decreased phosphorylation of p21 at T145. Had major effect on cleavage of PARP (i.e. induction of apoptosis).

[00499] Combination of Compound K with AKTi VIII inhibits phosphorylation of AKT at S129, T308, S473 and synergistically induces apoptosis (as demonstrated by cleavage of PARP).

Example 40: Tumor Growth Inhibition Assays

[00500] Female immunocompromised mice CrTac:Ncr-Foxn1nu (5-7 weeks old) were obtained from Taconic Farms. Animals were maintained under clean room conditions in sterile filter top cages. Animals received sterile rodent chow and water *ad libitum*. All procedures were conducted in accordance with the Institute for Laboratory Animal Research Guide: The Care and Use of Laboratory Animals. Tumor xenografts were initiated by subcutaneous injection of NCI-H1975 lung adenocarcinoma cells into the right hind flank region of each mouse. When tumors reached a designated volume of 100-150 mm^3 mice were randomized and divided into groups of 10 mice per group. Compound K and therapeutic antibodies were administered according to their schedule. Tumor volumes and body weights were measured every 2-4 days. The length and width of the tumor were measured with calipers and the volume calculated using the following formula:

$$\text{tumor volume} = (\text{length} \times \text{width}^2)/2$$

Example 40a: Determining Tumor Growth Inhibition.

[00501] Percent tumor growth inhibition (TGI) values were calculated on the final day of the study for Compound K-treated, therapeutic antibody-treated or combination-treated compared (Treated) to vehicle-treated (Control) mice and were calculated as 100 x {1-[(Treated on Final day –Treated on Day 1)/(Control on Final day –Control on Day 1)]}. The significance of the differences between the treated versus vehicle groups were determined using one-way ANOVA (Graphpad Prism).

Example 40b: Calculating Synergy From Tumor Growth Inhibition Data

[00502] A Tumor Growth Inhibition is calculated for every treatment as stated in Example 40a.

[00503] The expected percent inhibition value is derived by assuming exact additivity between the effect of Compound K and the combined therapeutic antibody. Hence the expected value for any combined treatment of Compound K with therapeutic antibody is calculated as the percent inhibition observed for Compound K alone at the same dose added to percent inhibition observed for the combined therapeutic antibody alone at the same dose that is multiplied by one hundred percent less the percent inhibition observed for Compound K alone at the same dose divided by a hundred percent, i.e.

Combination TGI = Compound A TGI + Compound B TGI*(100- Compound A TGI)/100

[00504] Controls for these studies are the treatment response curves for each of the two drugs by themselves. Such controls allow one to predict the antitumor effect for each possible combination for each of the two drugs based simply on adding the tumor growth inhibition observed for each of the two drugs when used alone.

[00505] Assessment of synergy is completed by comparing the actual percent inhibition to the expected percent inhibition. If the expected value for combination is 60% but 80% inhibition is observed, the compounds are enhancing each other's effect and synergy is observed, for example.

[00506] For example, if treatment compound A inhibits tumor growth by 20%, and treatment with compound B inhibits tumor growth by 20%, one could expect a combination of treatment with compound A and treatment with compound B to inhibit tumor growth by 36%. That leaves another 64% inhibition possible.

Example 40c: Cetuximab/Compound K combination testing in NCI-H1975 lung adenocarcinoma xenograft bearing mice

[00507] Cetuximab, an EGFR-targeting chimeric antibody, was tested in combination with Compound K in the NCI-H1975 lung adenocarcinoma xenograft bearing mice. Cetuximab was administered intraperitoneally once every three days at 1 mg/kg. Compound K was administered by oral gavage twice daily at 25 or 75 mg/kg. The study was carried out for 27 days. Mice with tumor burden more than 2000 mm^2 were taken of the study and euthanized. On day 18 all animals treated with vehicle or Compound K alone were taken of the study and euthanized tumor burden more than 2000 mm^2.

[00508] Results are shown hereafter; see Figure 81. Synergistic inhibition of tumor growth up to 97% was observed when Compound K was combined with Cetuximab.

[00509] Vehicle: Average Tumor Volume on Day 1 = 131 mm^2, Average Tumor Volume on Day 18 = 2075 mm^2

[00510] Cetuximab: Average Tumor Volume on Day 1 = 130 mm^2, Average Tumor Volume on Day 18 = 665 mm^2

[00511] Compound K (25 mg/kg): Average Tumor Volume on Day 1 = 130 mm^2, Average Tumor Volume on Day 18 = 2000 mm^2

[00512] Compound K (75 mg/kg): Average Tumor Volume on Day 1 = 130 mm^2, Average Tumor Volume on Day 18 = 1899 mm^2

[00513] Combination of Cetuximab with Compound K (25 mg/kg): Average Tumor Volume on Day 1 = 129 mm^2, Average Tumor Volume on Day 18 = 351 mm^2

[00514] Combination of Cetuximab with Compound K (75 mg/kg): Average Tumor Volume on Day 1 = 128 mm2, Average Tumor Volume on Day 18 = 197 mm^2

[00515] Cetuximab:

Tumor Growth Inhibition on Day 18 = 100*(1-(665-130)/(2075-131)) = 73%

[00516] Compound K (25 mg/kg):

Tumor Growth Inhibition on Day 18 = 100*(1-(2000-130)/(2075-131)) = 4%

[00517] Compound K (75 mg/kg):

Tumor Growth Inhibition on Day 18 = 100*(1-(1899-130)/(2075-131)) = 9%

[00518] Combination of Cetuximab with Compound K (25 mg/kg):

Tumor Growth Inhibition on Day 18 = 100*(1-(351-129)/(2075-131)) = 89%

[00519] Combination of Cetuximab with Compound K (75 mg/kg):

Tumor Growth Inhibition on Day 18 = 100*(1-197-129)/(2075-131)) = 97%

[00520] Combination of Cetuximab with Compound K (25 mg/kg):

Additivity = 73 + 4*(100-73)/100 = 74%

[00521] Combination of Cetuximab with Compound K (75 mg/kg):

Additivity = 73 + 9*(100-73)/100 = 75%

[00522] Combination of Cetuximab with Compound K (25 mg/kg):

Additivity = 74%, Observed Tumor Growth Inhibition = 89%.

89% > 74%, hence combination is synergistic.

[00523] Combination of Cetuximab with Compound K (75 mg/kg):

Additivity = 75%,

Observed Tumor Growth Inhibition = 97%.

97% > 75%, hence combination is synergistic.

Example 41: Evaluation of Pain management

[00524] ***Study Setup:*** The efficacy of Compound K, an analgesic of choice, and a combination of both is examined following a single dose given 1 hour prior to injection of 5% formalin into the hind paw. Animals are observed for flinching behaviors from 0-60 minutes following the formalin injection. The level of pain is assumed to be directly proportional to the number of flinches of formalin-injected paw.

[00525] ***Animals:*** Male Sprague Dawley rats (Hsd: Sprague Dawley®™, Harlan, Indianapolis, Indiana, U.S.A.) weighing 210-238 g are housed three per cage. Animals have free access to food and water and are maintained on a 12:12h light/dark schedule for the duration of the study. The animal colony is maintained at approximately 21°C and 60% relative humidity. All experiments are conducted in accordance with the International Association for the Study of Pain guidelines.

[00526] ***Induction of persistent pain:*** Rats are allowed to acclimate to round glass observation chambers for at least 30 minutes prior to formalin injection. 50µl of a 5% formalin solution in 0.9% saline is injected subcutaneously into the dorsal surface of the left hind-paw. The number of flinches is recorded continuously from 0 - 60 minutes in 5 minute intervals by direct observation.

[00527] ***Experimental compounds:*** Compound K and other CK2 inhibitors, as well as analgesics: paracetamol, non-steroidal anti-inflammatory drugs (i.e. aspirin, ibuprofen, naproxen), opiates and morphinomimetics (i.e. morphine, codeine, hydrocodone, oxycodone, penthidine, dihydromorphine, tramadol, buprenorphine)

[00528] ***Experimental timeline for formalin testing:***

(1) -60 min = compound administration (vehicle, Compound K, other CK2 inhibitors, analgesics and combination of CK2 inhibitor with analgesics)

(2) 0 min = formalin injection, observation begins

(3) 60 min = observation ends

(4) 70 min = cardiac puncture

[00529] ***Data analysis:*** The total flinches are counted over 60 minutes broken down into the following phases. The phases summarized in this study are:

Phase 1: 0-9 minutes

Phase 2: 10-60 minutes

Phase 2A: 10-40 minutes

Phase 2B: 41-60 minutes

Statistical analyses are conducted using Prism™ 5.0 (GraphPad, San Diego, CA, USA). Compound effect is analyzed by carrying out a one-way analysis of variance (ANOVA) for each test compound versus vehicle. The level of significance is set at $P < 0.05$. Post-hoc analysis is performed using Dunnett's multiple comparison between vehicle and compound treated groups. The level of significance is set at $P < 0.05$.

[00530] Reduction in paw flinching in mice treated with drug combination compared to vehicle or single agent treatments indicates that the combination of Compound K with analgesics provides favorable therapeutic outcome in pain management.

Example 42: Modulation of pro-inflammatory molecules: Combination of Compound K with Bortezomib in Inflammatory Breast Cancer Model SUM-149PT.

[00531] The effect of Bortezomib in combination with Compound K on the production of pro-inflammatory cytokine IL-6 is tested in the inflammatory breast carcinoma cell line SUM-149PT.

[00532] Bortezomib is added simultaneously with Compound K in 6 hour assay. Drug/Drug molar ratios are varied (Compound K/ Bortezomib). The experiment is performed in triplicate.

[00533] The effect of Bortezomib, Compound K and combination thereof is assessed with ELISA kit measuring the release of IL-6 into growth media.

[00534] The dose-response curves for Compound K, Bortezomib and the combination thereof are calculated, as are IC50 for maximum concentrations, as well as the percent inhibition of IL-6 production for each of Compound K, Bortezomib and the combination thereof, and CI50.

Example 42: Treatment of infectious disorder: Compound K/Antiviral drug Combination Efficacy evaluation in Human Peripheral Blood Mononuclear Cells (PBMCs) and U1 Latent/Induced HIV-1 assays

[00535] Fresh human PBMCs, seronegative for HIV and HBV, are isolated from screened donors (Biological Specialty Corporation, Colmar, PA). Cells are pelleted/washed 2-3 times by low speed centrifugation and re-suspension in PBS to remove contaminating platelets. The Leukophoresed blood is then diluted 1:1 with Dulbecco's Phosphate Buffered Saline (DPBS) and layered over 14 mL of Lymphocyte Separation Medium (LSM; Cellgro® by Mediatech, Inc.; density 1.078+/-0.002 g/ml; Cat.# 85-072-CL) in a 50 mL centrifuge tube and then centrifuged for 30 minutes at 600 X g. Banded PBMCs are gently aspirated from the resulting interface and subsequently washed 2X with PBS by low speed centrifugation. After the final wash, cells are enumerated by trypan blue exclusion and re-suspended at 1 x 10^7 cells/mL in RPMI 1640 supplemented with 15 % Fetal Bovine Serum (FBS), and 2 mM L-glutamine, 4 μg/mL Phytohemagglutinin (PHA, Sigma). The cells are allowed to incubate for 48-72 hours at 37°C.

[00536] After incubation, PBMCs are centrifuged and re-suspended in RPMI 1640 with 15% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 20 U/mL recombinant human IL-2 (R&D Systems, Inc). IL-2 is included in the culture medium to maintain the cell division initiated by the PHA mitogenic stimulation. PBMCs are maintained in this medium at a concentration of 1-2 x 10^6 cells/mL with biweekly medium changes until used in the assay protocol. Cells are kept in culture for a maximum of two weeks before being deemed too old for use in assays and discarded. MDMs are depleted from the culture as the result of adherence to the tissue culture flask.

[00537] For the standard PBMC assay, PHA stimulated cells from at least two normal donors are pooled (mixed together), diluted in fresh medium to a final concentration of 1 x 10^6 cells/mL, and plated in the interior wells of a 96 well round bottom microplate at 50 μL/well (5 x 10^4 cells/well). Pooling (mixing) of mononuclear cells from more than one donor is used to minimize the variability observed between individual donors, which results from quantitative and qualitative differences in HIV infection and overall response to the PHA and IL-2 of primary lymphocyte populations. Each plate contains virus/cell control wells (cells plus virus), experimental wells (drug plus cells plus virus) and compound control wells (drug plus media without cells, necessary for MTS monitoring of cytotoxicity). In this *in vitro* assay, PBMC

viability remains high throughout the duration of the incubation period. Therefore, infected wells are used in the assessment of both antiviral activity and cytotoxicity. The dilutions of Compound K, antiviral compounds (i.e. temacrazine, AZT and other constituents of HAART) and combination of thereof are prepared at a 2X concentration in microtiter tubes and 100 μL of each concentration (nine total concentrations) are placed in appropriate wells using the standard format. 50 μL of a predetermined dilution of virus stock is placed in each test well (final MOI ≅ 0.1). The PBMC cultures are maintained for seven days following infection at 37°C, 5% CO_2. After this period, cell-free supernatant samples are collected for analysis of reverse transcriptase activity and/or p24 antigen content. Following removal of supernatant samples, compound cytotoxicity is measured by addition of MTS to the plates for determination of cell viability. Wells are also examined microscopically and any abnormalities are noted.

[00538] Reverse transcriptase activity assay: A microtiter plate-based reverse transcriptase (RT) reaction is utilized (Buckheit et al., *AIDS Research and Human Retroviruses* 7:295-302, (1991)). Tritiated thymidine triphosphate (^{3}H-TTP, 80 Ci/mmol, NEN) is received in 1:1 dH_2O:Ethanol at 1 mCi/mL. Poly rA:oligo dT template:primer (Pharmacia) is prepared as a stock solution by combining 150 μL poly rA (20 mg/mL) with 0.5 mL oligo dT (20 units/mL) and 5.35 mL sterile dH_2O followed by aliquoting (1.0 mL) and storage at -20°C. The RT reaction buffer is prepared fresh on a daily basis and consists of 125 μL 1.0 M EGTA, 125 μL dH_2O, 125 μL 20% Triton X100, 50 μL 1.0 M Tris (pH 7.4), 50 μL 1.0 M DTT, and 40 μL 1.0 M $MgCl_2$. The final reaction mixture is prepared by combining 1 part ^{3}H-TTP, 4 parts dH_2O, 2.5 parts poly rA:oligo dT stock and 2.5 parts reaction buffer. Ten microliters of this reaction mixture is placed in a round bottom microtiter plate and 15 μL of virus containing supernatant is added and mixed. The plate is incubated at 37°C for 60 minutes. Following incubation, the reaction volume is spotted onto DE81 filter-mats (Wallac), washed 5 times for 5 minutes each in a 5% sodium phosphate buffer or 2X SSC (Life Technologies). Next they are washed 2 times for 1 minute each in distilled water, 2 times for 1 minute each in 70% ethanol, and then dried. Incorporated radioactivity (counts per minute, CPM) is quantified using standard liquid scintillation techniques.

[00539] MTS staining for PBMC viability to measure cytotoxicity: At assay termination, assay plates are stained with the soluble tetrazolium-based dye MTS (CellTiter 96 Reagent, Promega) to determine cell viability and quantify compound toxicity. The mitochondrial enzymes of metabolically active cells metabolize MTS to yield a soluble formazan product. This

allows the rapid quantitative analysis of cell viability and compound cytotoxicity. The MTS is a stable solution that does not require preparation before use. At termination of the assay, 20 μL of MTS reagent is added per well. The microtiter plates are then incubated 4-6 hrs at 37°C. The incubation intervals were chosen based on empirically determined times for optimal dye reduction. Adhesive plate sealers are used in place of the lids, the sealed plate is inverted several times to mix the soluble formazan product and the plate is read spectrophotometrically at 490/650 nm with a Molecular Devices Vmax or SpectraMaxPlus plate reader.

[00540] Data Analysis: Using GraphPad Prism, IC_{50} (50% inhibition of virus replication), IC_{90} (90% inhibition of virus replication), IC_{95} (95% inhibition of virus replication), TC_{50} (50% cytotoxicity), TC_{90} (90% cytotoxicity), TC_{95} (95% cytotoxicity) and therapeutic index values (TI = TC/IC; also referred to as Antiviral Index or AI) are measured. The effect of combination versus single agents are assessed.

[00541] The increase in inhibition of virus replication coupled with the increase in therapeutic index with drug combination compared to single agent treatments indicates that combination of Compound K with antivirals provides favorable therapeutic outcome in preventing the HIV infection.

[00542] Description of the U1 Latent/Induced HIV-1 Assay: U1 cells are derived from the histocytic leukemia cell line U937 and contain a single integrated provirus (HIV-1_{IIIB}) for which gene expression is inducible. The cells were obtained from the AIDS Research and Reference Reagent Program and are maintained under standard culture conditions in RPMI 1640 supplemented with 15% fetal bovine serum (heat inactivated), 2 mM L-glutamate, 100 U/mL penicillin and 100 μg/mL streptomycin. The cultures are maintained in such a way as to ensure exponential growth of the populations. Prior to initiating the assay, cells are collected by centrifugation and counted using a hemacytometer. If cell viability, as assessed by Trypan Blue dye exclusion, is less than 70% the assay is terminated. The cells are adjusted to 5×10^5 cells/mL and 100 μL is placed in the cell control wells (5×10^4 cells/mL) of 96 well plates. The remaining cells are treated with Phorbol 12-myristate 13-acetate (PMA, Sigma; final concentration of 250 ng/ml) and incubated for 10 minutes. The treated cells are then added to the appropriate wells of the plate in a volume of 100 μL (5×10^4 cells/mL). Compound K, antiviral compounds (i.e. temacrazine, AZT and other constituents of HAART) and combination of thereof are serially diluted and added to the plates in a volume of 100 μL (200 μL final volume/well). Cultures are incubated for 3 days and supernatants harvested. The level of virus released from

the cells is determined by measuring virion-associated RT activity (described above). Compound cytotoxicity is determined by MTS dye reduction to assess cell viability (described above). The effect of combination versus single agents are assessed.

[00543] The decrease in virus release with drug combination compared to single agent treatments indicates that combination of Compound K with antivirals provides favorable therapeutic outcome in preventing the reactivation of latent HIV infection.

Example 42: Treatment of autoimmune disorder: Combination of Compound K with cyclophosphamides, corticosteroids and immunosuppressants in models of Systematic Lupus Erythematosus.

[00544] Experiments are carried out on MRL/Lpr mice, which is a commonly used model of SLE. Autoimmune-prone MRL mice carrying the Lpr mutation of the Fas gene spontaneously develop a systemic disease which resembles human SLE. Lymphoadenopathy and autoantibody (autoAb) titers in sera (the most common being the anti-nuclear (ANA) ones) are already evident around 12 weeks, and by week 20 almost all animals show glomerular/tubular inflammation evident at histopathology. Death due to kidney failure usually occurs at approximately 6 months of age. Disease severity can be quantified by: measuring autoAb titers; quantifying the severity and age at onset of kidney inflammation at histopathology; and monitoring overall survival.

[00545] 4 Groups of 12 female MRL/Lpr mice are subjected to treatment with inert vehicle, Compound K at 25 mg/kg or 50 mg/kg or 75 mg/kg PO BID, SLE standard of care (i.e. cyclophosphamaides, corticosteroids and immunosuppressants) or combination of thereof beginning at week 8. ANA titers are measured in all mice at 12 and 16 weeks. 4 mice per groups are sacrificed at week 20. Spleens and lymph nodes are weighed and kidneys sectioned and histopathology analysis of kidney sections is carried out at the histopathology lab. Paraffin-embedded sections are stained by hematoxylin and eosin, and immunofluorescence staining for immuno-complexes and complement are performed on additional kidney sections. The remaining 8 mice are followed up and monitored for overall survival. The study is terminated when all mice die or at least one mouse reaches 10 months of age. All deceased mice or sacrificed long-term survivors are dissected and kidney histopathology carried out as described above.

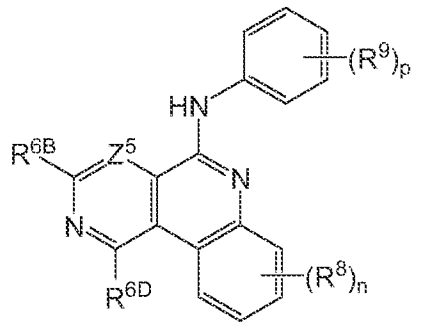
[00546] The reduction in autoAb titers, and/or decrease in kidney inflammation plus increased overall survival in mice treated with drug combination compared to vehicle or single

agent treatments indicates that combination of Compound K with standard of care (i.e. cyclophosphamaides, corticosteroids and immunosuppressants) provides favorable therapeutic outcome in treating SLE.

[00547] The patents and publications listed herein describe the general skill in the art and are hereby incorporated by reference in their entireties for all purposes and to the same extent as if each was specifically and individually indicated to be incorporated by reference. In the case of any conflict between a cited reference and this specification, the specification shall control. In describing embodiments of the present application, specific terminology is employed for the sake of clarity. However, the invention is not intended to be limited to the specific terminology so selected. Nothing in this specification should be considered as limiting the scope of the present invention. All examples presented are representative and non-limiting. The above-described embodiments may be modified or varied, without departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is therefore to be understood that, within the scope of the claims and their equivalents, the invention may be practiced otherwise than as specifically described.

Claims

1. A method for treating or ameliorating a neoplastic disorder, comprising administering to a subject in need thereof a therapeutically effective amount of a compound of formula I:



(I),

or a pharmaceutically acceptable salt or ester thereof,

wherein Z^5 is N or CR^{6A};

each R^{6A}, R^{6B}, R^{6D} and R^8 independently is H or an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group,

or each R^{6A}, R^{6B}, R^{6D} and R^8 independently is halo, CF_3, CFN, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, carboxy bioisostere, $CONR_2$, OOCR, COR, or NO_2,

each R^9 is independently an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group, or

each R^9 is independently halo, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, $CONR_2$, OOCR, COR, or NO_2,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, NR′SO_2R′, NR′CONR′$_2$, NR′COOR′, NR′COR′, CN, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2,

wherein each R′ is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

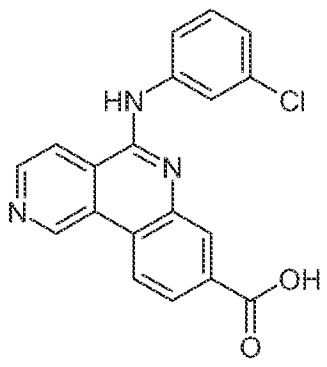
and wherein two R′ can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S;

n is 0 to 4; and

p is 0 to 4;

and an anticancer agent, selected from the group consisting of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, a PI3K inhibitor, and a monoclonal antibody targeting a tumor/cancer antigen, thereby treating or ameliorating said neoplastic disorder.

2. The method of claim 1, wherein the compound of formula I has the structure:



Compound K,

or a pharmaceutically acceptable salt or ester thereof.

3. The method of claim 1, wherein the anticancer agent is selected from the group consisting of 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat, 17-DMAG, BEZ-235, LY294002, PI-103, wortmannin, and cetuximab.

4. The method of claim 1, wherein the neoplastic disorder is cancer.

5. The method of claim 4, wherein the cancer is cancer of the hemopoietic system, lung, breast, prostate, kidney, pancreas, liver, heart, skeleton, colon, rectum, skin, brain, eye, lymph node, heart, testes or ovary.

6. The method of claim 1, wherein the compound of formula I and the anticancer agent are administered simultaneously.

7. The method of claim 1, wherein the compound of formula I and the anticancer agent are administered simultaneously and separately.

8. The method of claim 1, wherein the compound of formula I and the anticancer agent are administered sequentially.

9. The method of claim 8, wherein the compound of formula I is administered prior to the anticancer agent.

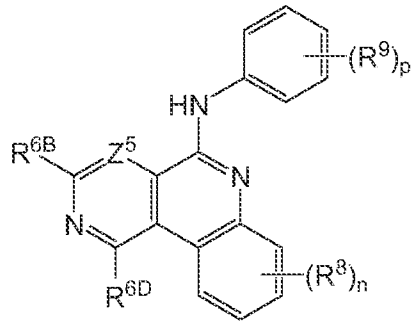
10. The method of claim 8, wherein the compound of formula I is administered after the anticancer agent.

11. The method of claim 1, wherein the subject is human.

12. The method of claim 1, wherein the compound of formula I and the anticancer agent provide at least an additive anticancer effect.

13. The method of claim 1, wherein the compound of formula I and the anticancer agent provide a synergistic anticancer effect.

14. A method for inhibiting cell proliferation in a system, comprising administering to the system an effective amount of a compound of Formula I:



(I),

or a pharmaceutically acceptable salt or ester thereof,

wherein Z^5 is N or CR^{6A};

each R^{6A}, R^{6B}, R^{6D} and R^8 independently is H or an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group,

or each R^{6A}, R^{6B}, R^{6D} and R^8 independently is halo, CF_3, CFN, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, carboxy bioisostere, $CONR_2$, OOCR, COR, or NO_2,

each R^9 is independently an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group, or

each R^9 is independently halo, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, $CONR_2$, OOCR, COR, or NO_2,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, NR′SO_2R′, NR′CONR′$_2$, NR′COOR′, NR′COR′, CN, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2,

wherein each R′ is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

and wherein two R′ can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S;

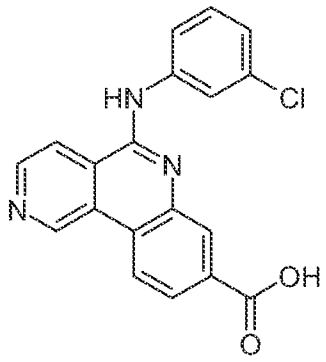
n is 0 to 4; and

p is 0 to 4;

and an anticancer agent selected from the group consisting of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, a PI3K inhibitor, and a monoclonal antibody targeting a tumor/cancer antigen; thereby inhibiting cell proliferation.

15. The method of claim 14, wherein the system is a cell, tissue or subject.

16. The method of claim 14, wherein the compound of formula I has the structure:

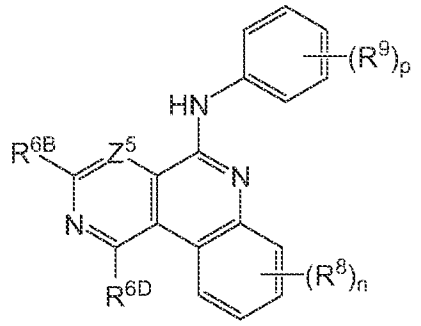


Compound K,

or a pharmaceutically acceptable salt or ester thereof.

17 The method of claim 14, wherein the anticancer agent is selected from the group consisting of 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)-methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat, 17-DMAG, BEZ-235, LY294002, PI-103, wortmannin, and cetuximab.

18. A pharmaceutical composition comprising a compound of formula I:



(I),

or a pharmaceutically acceptable salt or ester thereof,

wherein Z^5 is N or CR^{6A};

each R^{6A}, R^{6B}, R^{6D} and R^8 independently is H or an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group,

or each R^{6A}, R^{6B}, R^{6D} and R^8 independently is halo, CF_3, CFN, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, carboxy bioisostere, $CONR_2$, OOCR, COR, or NO_2,

each R^9 is independently an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group, or

each R^9 is independently halo, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, $CONR_2$, OOCR, COR, or NO_2,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, NR′SO_2R′, NR′CONR′$_2$, NR′COOR′, NR′COR′, CN, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2,

wherein each R′ is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

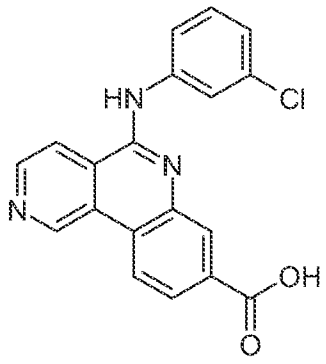
and wherein two R′ can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S;

n is 0 to 4; and

p is 0 to 4;

a pharmaceutical agent selected from the group consisting of an Akt inhibitor, an HDAC inhibitor, an Hsp90 inhibitor, an mTOR inhibitor, a PI3K/mTOR inhibitor, a PI3K inhibitor, and a monoclonal antibody targeting a tumor/cancer antigen, and at least one pharmaceutically acceptable excipient.

19. The composition of claim 18, wherein the compound of Formula I has the structure:



Compound K,

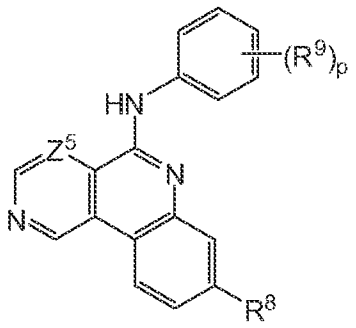
or a pharmaceutically acceptable salt or ester thereof.

20. The composition of claim 18, wherein the pharmaceutical agent is selected from the group consisting of 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat, 17-DMAG, BEZ-235, LY294002, PI-103, wortmannin, and cetuximab.

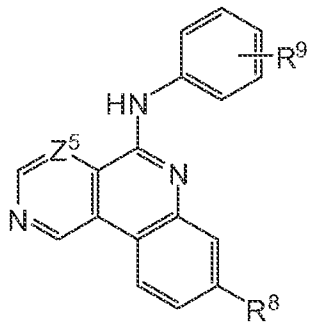
21. The composition of claim 19, wherein pharmaceutical agent is selected from the group consisting of 1,3-Dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinoxalin-7-

yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one, panobinostat, 17-DMAG, BEZ-235, LY294002, PI-103, wortmannin, and cetuximab.

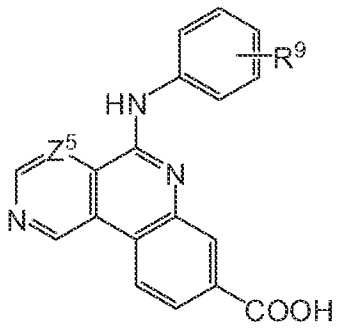
22. The method of claim 1, wherein the compound of Formula I has the structure of Formula II, III, IV, V or VI:



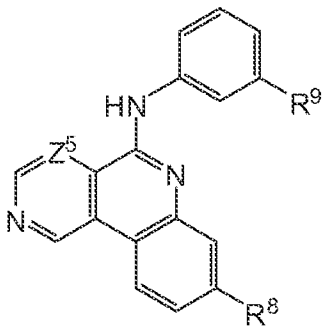
Formula II ;



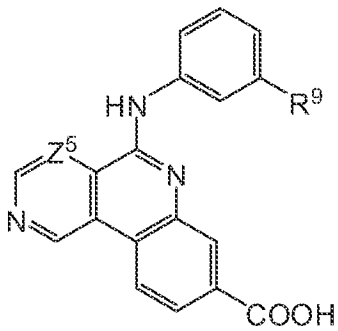
Formula III ;



Formula IV ;



Formula V ; or



Formula VI ;

or a pharmaceutically acceptable salt or ester thereof;

wherein Z^5 is N or CR^{6A};

each R^{6A} and R^8 independently is H or an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group,

or each R^{6A} and R^8 independently is halo, CF_3, CFN, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, carboxy bioisostere, $CONR_2$, OOCR, COR, or NO_2,

each R^9 is independently an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group, or

each R^9 is independently halo, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, $CONR_2$, OOCR, COR, or NO_2,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

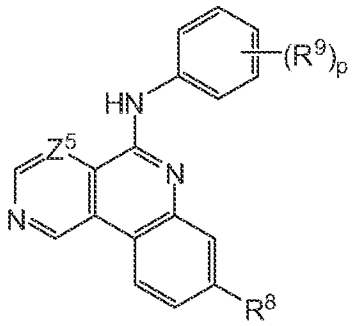
and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, $NR'SO_2R'$, $NR'CONR'_2$, NR′COOR′, NR′COR′, CN, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2,

wherein each R′ is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

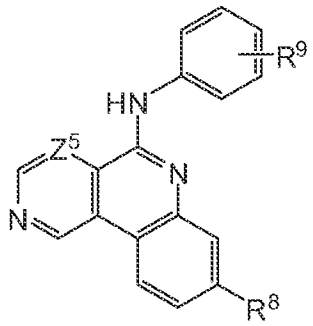
and wherein two R′ can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S; and

p is 0 to 4.

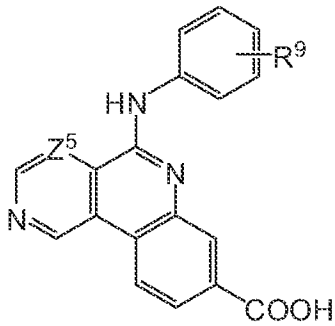
23. The method of claim 14, wherein the compound of Formula I has the structure of Formula II, III, IV, V or VI:



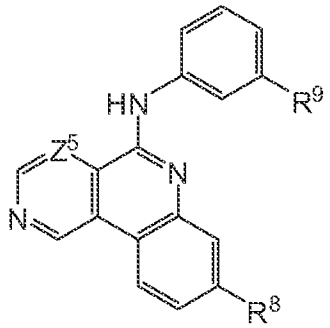
Formula II ;

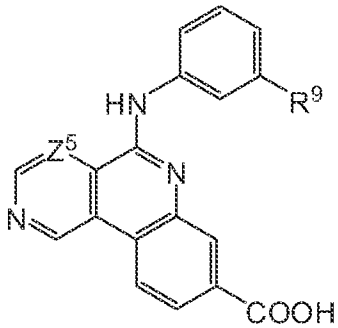


Formula III ;



Formula IV ;



Formula V ; or  Formula VI ;

or a pharmaceutically acceptable salt or ester thereof;

wherein Z^5 is N or CR^{6A};

each R^{6A} and R^8 independently is H or an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group,

or each R^{6A} and R^8 independently is halo, CF_3, CFN, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, carboxy bioisostere, $CONR_2$, OOCR, COR, or NO_2,

each R^9 is independently an optionally substituted C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C12 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl group, or

each R^9 is independently halo, OR, NR_2, NROR, $NRNR_2$, SR, SOR, SO_2R, SO_2NR_2, $NRSO_2R$, $NRCONR_2$, NRCOOR, NRCOR, CN, COOR, $CONR_2$, OOCR, COR, or NO_2,

wherein each R is independently H or C1-C8 alkyl, C2-C8 heteroalkyl, C2-C8 alkenyl, C2-C8 heteroalkenyl, C2-C8 alkynyl, C2-C8 heteroalkynyl, C1-C8 acyl, C2-C8 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-C12 arylalkyl, or C6-C12 heteroarylalkyl,

and wherein two R on the same atom or on adjacent atoms can be linked to form a 3-8 membered ring, optionally containing one or more N, O or S;

and each R group, and each ring formed by linking two R groups together, is optionally substituted with one or more substituents selected from halo, =O, =N-CN, =N-OR′, =NR′, OR′, NR'_2, SR′, SO_2R', $SO_2NR'_2$, NR′SO_2R′, NR′$CONR'_2$, NR′COOR′, NR′COR′, CN, COOR′, $CONR'_2$, OOCR′, COR′, and NO_2,

wherein each R′ is independently H, C1-C6 alkyl, C2-C6 heteroalkyl, C1-C6 acyl, C2-C6 heteroacyl, C6-C10 aryl, C5-C10 heteroaryl, C7-12 arylalkyl, or C6-12 heteroarylalkyl, each of which is optionally substituted with one or more groups selected from halo, C1-C4 alkyl, C1-C4 heteroalkyl, C1-C6 acyl, C1-C6 heteroacyl, hydroxy, amino, and =O;

and wherein two R′ can be linked to form a 3-7 membered ring optionally containing up to three heteroatoms selected from N, O and S; and

p is 0 to 4.