(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
05 December 2019 (05.12.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/232467 A1

(51) International Patent Classification:
A61K 31/4725 (2006.01) *G01N 33/574* (2006.01)
C07D 493/08 (2006.01) *A61P 35/02* (2006.01)

(21) International Application Number:
PCT/US2019/035038

(22) International Filing Date:
31 May 2019 (31.05.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/679,583 01 June 2018 (01.06.2018) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(54) Title: PHARMACODYNAMIC BIOMARKERS FOR THE TREATMENT OF CANCER WITH A CDK8/19 INHIBITOR

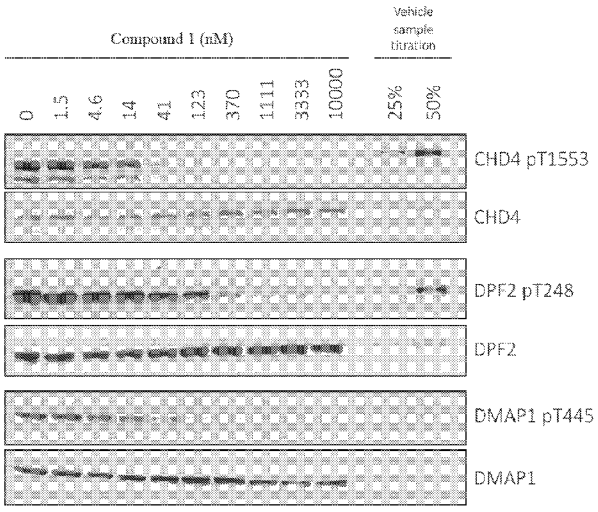

FIG. 6

(57) Abstract: A pharmacodynamic diagnostic is provided that includes the use of a selection of biomarkers to measure the efficiency over time of CDK8 and/or CDK19 inhibitors in medical therapy. This pharmacodynamic approach to assessing CDK8 and/or CDK19 drug efficacy over time is based on the observed loss of phosphorylation of predictive proteins that have been identified for this purpose for the first time using a range of parameters. This advantageous real time diagnostic and kit allows the healthcare provider to monitor the patient during therapy to determine the level of response to treatment with the selected CDK8 and/or CDK19 inhibitor.

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[Continued on next page]

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

PHARMACODYNAMIC BIOMARKERS FOR THE TREATMENT OF CANCER WITH A CDK8/19 INHIBITOR

STATEMENT OF RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 62/679,583 filed June 1, 2018. The entirety of that application is hereby incorporated by reference for all purposes.

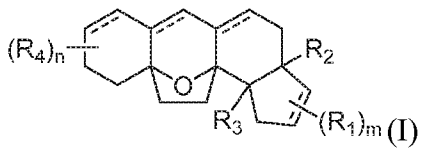
BACKGROUND

CDK8 and CDK19 proteins reversibly associate with the Mediator complex and form its key regulatory unit. CDK8 and CDK19 oncogenes have been implicated in the proliferation of a variety of cancer cell lines via the modulation of various gene expression programs. Currently there is significant interest in inhibiting the CDK8 and CDK19 proteins for the treatment of cancer, particularly acute myeloid leukemia (AML). Super enhancers in AML can be regulated by the use of CDK8 and CDK19 inhibitors such as cortistatin A. This interaction was described in detailed in a 2015 article in Nature titled "Mediator Kinase Inhibition Further Activates Super-Enhancer-Associated Genes in AML" Pelish et al., Nature (2015), 526: 273-276. The various interactions of CDK8/19 mediator kinases are still an active area of research. Recently NFκB has been added to the list of downstream targets (see the 2016 PNAS article titled "CDK8/19 Mediatory Kinases Potentiate Induction of Transcription by NFκB" Chen et al., PNAS (2016), 114, 10208-213). These important discoveries have led to preliminary phosphoproteomic studies such as that described in the 2016 Cell Reports paper titled "Identification of Mediatory Kinase Substrates in Human Cells using Cortistatin A and Quantitative Phosphoproteomics" Poss et al., Cell Reports (2016), 15:436-450.

The mediator complex with CDK8 has a conserved function in transcription as described by Taatjes, D. J., Trends Biochem Sci 35, 315-322 (2010); and Conaway, R. C. and Conaway, J. W., Curr Opin Genet Dev 21, 225-230 (2011). CDK8 has also been reported as an oncogene in both colon cancer (Firestein R. et al., Nature 455:547-51 (2008); Morris E. J. et al., Nature 455:552-6 (2008); Starr T. K. et al., Science 323:1747-50 (2009)) and melanoma (Kapoor A. et al., Nature 468:1105-9 (2010)). CDK8 is upregulated and amplified in a subset of human colon

tumors, is known to transform immortalized cells and is required for colon cancer proliferation in vitro. Similarly, CDK8 has also been found to be overexpressed and essential for proliferation in melanoma. Kapoor, A. et al., Nature 468, 1105-1109 (2010). CDK8 has been shown to regulate several signaling pathways that are key regulators of both ES pluripotency and cancer. CDK8 activates the Wnt pathway by promoting expression of β-Catenin target genes (Firestein, R. et al., Nature 455, 547-551 (2008)) or by inhibiting E2F1, a potent inhibitor of β-Catenin transcriptional activity. Morris, E. J. et al., Nature 455, 552-556 (2008).

Certain selective CDK8/19 inhibitors have been identified that have a cortistatin-like backbone. Harvard University has filed patent applications that describe CDK8/19 inhibitors based on the cortistatin core structure. U.S. Patent 9,127,019 titled "Cortistatin Analogs and Synthesis Thereof" filed by Flyer, et. al., and assigned to the President and Fellows of Harvard College describes compounds of the general Formula I and salts thereof, and the synthesis thereof, wherein R_1, R_2, R_3, R_4, n, and m are as described therein.



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The '019 patent discloses that such compounds are anti-angiogenic and can be used to treat proliferative diseases.

Additional compounds are described in WO 2015/100420 titled "Cortistatin Analogs and Syntheses and Uses Thereof" filed by Shair, et al., also assigned to the President and Fellows of Harvard College, which are useful to treat proliferative disorders such as cancer, and in particular, a hematopoietic cancer such as leukemia, multiple myeloma (MM), acute myelocytic leukemia (AML), a myeloproliferative neoplasm, acute lymphoblastic leukemia (ALL), chronic myelocytic leukemia (CML) and primary myelofibrosis (PMF). WO 2016/182932 titled "Cortistatin Analogues, Syntheses, and Uses Thereof" and WO 2017/004411 titled "Cortistatin Analogues and Syntheses and Uses Thereof" filed by Shair, et al., and also assigned to the President and Fellows of Harvard College describes new compounds with modifications to the A ring of the core skeleton of cortistatin. WO 2017/112815 titled "Cortistatin Analogues and Uses Thereof" and WO

2017/142621 titled "Cortistatin Analogs" filed by Shair, et al., and also assigned to the President and Fellows of Harvard College describes additional compounds that inhibit CDK8 and CDK19.

Harvard has also filed patent applications that describe the use of CDK8/19 biomarkers to select patients for treatment with cortistatin analogues, including WO 2016/182904 titled "Targeted Selection of Patients for Treatment with Cortistatin Derivatives" and WO 2017/112815 titled Targeted Selection of Patients for Treatment with Specific Cortistatin Derivatives."

Synthetic and biological descriptions of Cortistatin A and analogs of Cortistatin A have also been described in other patent applications including: U.S. Patent Application Publication US2013/0217014 and PCT Application WO2013/122609 titled "Methods of Using CDK8 Antagonists" filed by Firestein, et al., and assigned to Genentech, which describes the use of CDK8 antagonists against various cancers.

While a range of CDK8 and CDK19 inhibitors have now been identified, there is a need to know how best to use these compounds to treat patients with a medical disorder such as cancer or a tumor.

SUMMARY OF THE INVENTION

A pharmacodynamic diagnostic is provided that includes the use of a selection of biomarkers to measure the efficiency over time of CDK8 and/or CDK19 inhibitors in medical therapy. This pharmacodynamic approach to assessing CDK8 and/or CDK19 drug efficacy over time is based on the observed loss of phosphorylation of predictive proteins that have been identified for this purpose for the first time using a range of parameters. This advantageous real time diagnostic and kit allows the healthcare provider to monitor the patient during therapy to determine the level of response to treatment with the selected CDK8 and/or CDK19 inhibitor. The CDK8 and/or CDK19 inhibitor may be administered to treat a tumor or cancer, or a non-tumor or cancerous disease that responds to CDK8 and/or CDK19 therapy, as described in more detail below. The ability of these pharmacodynamic biomarkers to offer a real-time evaluation of efficacy is particularly important because cancer cells can develop resistance to treatment. For example, the tumor may acquire resistance by using an alternative kinase (not CDK8 and/or CDK19) to phosphorylate the same target. When this type of resistance arises, the methods described herein may detect the resistance emerging as an increase (or less of a decrease) in the

concentration of the pharmacokinetic biomarker. In the absence of a pharmacodynamic biomarker a care provider may not discover that a patient's cancer either is not sensitive to the drug or has developed resistance to therapy until after the cancer has progressed sufficiently to show (or not show) phenotypic signals.

Most biomarkers are not suitable for use in a pharmacodynamic diagnostic due to multiple requirements. For example, STAT1 and STAT5 are not useful kinase substrates when testing a patient's whole blood, because they are not phosphorylated in sufficient quantities in whole blood and they are phosphorylated by a range of kinases, rendering the assay not meaningful. This is also the case with a number of other kinase substrates.

More accurate biomarkers for use in a pharmacodynamic diagnostic have now been discovered that can conveniently be used in a whole blood assay to monitor over time the effectiveness of the administered CDK8 and/or CDK19 inhibitor drug, such as one of the cortistatin derivatives described further below. These biomarkers are selectively and directly phosphorylated by CDK8 and/or CDK19, and are present in whole blood in sufficient quantities for accurate analysis. Thus, they are superior diagnostic agents for use to determine if CDK8 and/or CDK19 is being effectively inhibited *in vivo* by the drug in the tumor or cancer patient.

These robust pharmacodynamic biomarkers are CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 (which are the phosphorylated forms of the proteins CHD4, DPF2 and DMAP1). They were discovered to be advantageous medical indicators of CDK8/CDK19 pharmacodynamic time course inhibition through a series of complex analyses.

First, phosphoproteomic studies were conducted in two AML cell lines (MOLM-13 and MOLM-14) after CDK8 inhibitor exposure to identify candidate proteins that are not phosphorylated in the presence of the inhibitor. The same analysis was carried out in peripheral blood mononuclear cells from five healthy donors. A list of substrates reported in a previous phosphoproteomics study conducted in colorectal cancer cell line HCT116 using cortistatin A was also evaluated (Poss et al., *Cell Rep* 2016 PMID 27050516). This generated a list of substrate phosphosites that might be phosphorylated by CDK8 and/or CDK19 based on motif, selection of Proline-(Serine/Threonine)-Proline or Proline-X-(Serine/Threonine)-Proline site, as well as the degree of change after short term exposure to the selected CDK8 inhibitor. Thereafter, specific monoclonal mouse antibodies were created against the top phosphorylated protein candidate biomarkers to determine if they are present in sufficient quantities in whole blood. These

antibodies were used to quantify the amount of pharmacodynamic biomarker in the sample which can be indicative of treatment success, as described further herein.

This invention is advantageous in one respect in light of the observation that a CDK8 and/or CDK19 inhibitor may not suppress cancer proliferation in all tissues. It is known that tumors and cancer even within a narrow category can be heterogeneous. Due to the fact that individual tumors can be caused by a range of genetic abnormalities and as a result can express or suppress key proteins, resulting in a range of phenotypes, not all tumors or cancers within the narrow class will respond to the same drug therapy. Even with the most effective oncology drugs, it is expected that there will be responders and non-responders. And, patients who initially respond to a treatment regimen may develop resistance to that treatment. Thus, this invention fills an unmet need to determine in real time over the course of therapy if a patient will or is responding to treatment with the selected CDK8 and/or CDK19 inhibitor. Thus, the present invention also includes a method to determine if a patient's cancer or other disease modulated by CDK8 and/or CDK19, has acquired drug resistance to the administered drug over time. The resulting pharmacodynamic diagnostic and therapeutic methods, materials and kits allow clinicians to monitor the responsiveness of cancer to CDK8 and/or CDK19 treatment in real-time. The diagnostic is sensitive, does not require a biopsy, and allows for rapid feedback regarding treatment efficacy.

In one aspect of the present invention, a method for treating a patient with a disorder that can be treated with a CDK8 and/or CDK19 inhibitor, such as a cancer or tumor is provided comprising the following steps:

i. obtaining a first blood sample from the patient;

ii. administering to the patient a first dose of a CDK8/19 inhibitor;

iii. obtaining a second blood sample from the patient after sufficient time for the CDK 8/19 inhibitor to act;

iv. detecting the concentration of the selected phosphorylated pharmacodynamic biomarker selected from the group consisting of CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in the first and second blood sample;

v. determining if the level of phosphorylated CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 is higher in the first blood sample than the second blood sample;

vi. if the level of phosphorylated CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 is higher in the first blood sample the second blood sample by at least 10, 20 or 30%, then continuing with administration of the CDK 8/19 inhibitor as prescribed by the health care provider; and

vii. optionally repeating steps i)- vi) during the treatment regimen to further confirm that the CDK8 and/or19 inhibitor is continuing to act on the enzyme *in vivo*.

In another aspect of the present invention a diagnostic method or kit is provided to determine if a patient's CDK8 and/or CDK19 mediated disorder is responding to treatment with a CDK8/19 inhibitor. One non-limiting example comprises the following steps:

i. obtaining a first blood sample from the patient before treatment;

ii. administering to the patient a first dose of a CDK8/19 inhibitor;

iii. obtaining a second blood sample from the patient after sufficient time for the CDK 8/19 inhibitor to act;

iv. detecting the concentration of the selected phosphorylated pharmacodynamic biomarker selected from the group consisting of CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in the first and second blood sample;

v. determining if the level of phosphorylated CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 is higher in the first blood sample from the second blood sample, wherein if the decrease in the level of phosphorylated CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 is at least by 10%, then the disorder is responding to treatment.

In one embodiment, a "sufficient time for the CDK8/19 inhibitor to act" as used in the methods herein is at least about or not more than 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, or about 12 hours. Alternatively the sufficient period of time is at least about or not more than 0.2 half lives, 0.5 half lives, 0.7 half lives, 1 half-life, 1.5 half-lives, 2 half-lives, 2.5 half-lives, or 3 half-lives. Or, the sufficient period of time is at least about or not more than the compounds Tmax, 1.5 times its Tmax, 2 times its Tmax, 2.5 times its Tmax, or 3 times its Tmax.

In one embodiment the concentration of pharmacokinetic biomarker must decrease by at least about 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% after treatment with the CDK8/19 inhibitor to indicate that the CDK8 and/or CDK19 mediated disorder is responding to treatment.

In another aspect of any of the methods or kits described herein, the diagnostic includes a reference chart with standardized comparisons of the range of inhibition of phosphorylation in healthy people when given a range of doses of the CDK8 and or CDK19 inhibitor, as well as the range of inhibition of phosphorylation in patients when the drug is considered still effective, and optionally, when it has lost effectiveness.

In another aspect of the present invention the pharmacodynamic biomarkers are used to select patients for treatment with a CDK8/19 inhibitor. As described herein, blood samples bearing a cells from prospective patients can be removed and treated *in vitro* to determine if the patient will likely respond to therapy. One non-limiting example of such a method comprises the following steps:

i. obtaining a blood sample from the patient;

ii. mixing the blood sample with an antibody to pT1553, DPF2 pT248, and/or DMAP1 pT445

iii. detecting the concentration of the selected phosphorylated pharmacodynamic biomarker selected from the group consisting of CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in the first and second blood sample;

iv. adding the CDK8/19 inhibitor to the blood sample to obtain a blood sample treated with the CDK8/19 inhibitor;

v. detecting the concentration of the selected phosphorylated pharmacodynamic biomarker selected from the group consisting of CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in the first and second blood sample; and

vi. determining if the level of phosphorylated CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 is higher in the first blood sample from the second blood sample, wherein if the decrease in the level of phosphorylated CHD4

pT1553, DPF2 pT248, and/or DMAP1 pT445 is at least by 10, 20 or 30% then the patient should be treated with the CDK 8/19 inhibitor.

In one embodiment enough of the CDK8/19 inhibitor is added to the blood sample to make the sample have a concentration of at least 1 nM, 10 nM, 50 nM, 100 nM, 500 nM, 1 μM, 2 μM, or 10 μM of the CDK8/19 inhibitor.

This disclosure thus provides specific, non-limiting examples of methods to treat a patient that includes the administration of a CDK8 and/or CDK19 inhibitor along with a companion diagnostic assessment or kit that includes the use of pharmacodynamic biomarkers. Additionally, the disclosure provides specific, non-limiting methods for determining if a patient's CDK8 and/or CDK19 mediated disorder is responding to treatment with a CDK8/19 inhibitor. The skilled artisan will recognize that other methods are in the alternative and are still encompassed within the scope of this invention. For example, the disclosure describes how to determine the concentration of phosphorylated CHD4 by contacting the sample with an antibody and then quantifying the amount of phosphorylated CHD4 bound to antibody by immunoblot, however the skilled artisan will recognize that other methods could be used, such as other spectrometric techniques or using alternative chemical reactions.

Another non-limiting example of a method for determining if a patient's CDK8 and/or CDK19 mediated disorder is responding to treatment with a CDK8/19 inhibitor is provided below:

i. obtain a first blood sample from the patient;

ii. detect the concentration of one or more pharmacodynamic biomarkers selected from pCHD4, pDPF2, and pDMAP1 in the first blood sample by contacting the blood sample with one or more antibodies to pCHD4, pDPF2, or pDMAP1 and determining the concentration of these proteins by immunoblot;

iii. administer one or more dosages of a CDK8 and/or CDK19 inhibitor drug;

iv. obtain a second blood sample from the patient after one or more treatments with the CDK8/19 inhibitor drug;

v. detect the concentration of the pharmacodynamic biomarker in the second blood sample by contacting the blood sample with one or more antibodies to pCHD4, pDPF2, or pDMAP1 and determining the concentration of these proteins by immunoblot; and

vi. determine if the patient's CDK8 and/or CDK19 mediated disorder is responding to treatment, wherein less than a 10% decrease in the concentration of the one or more pharmacodynamic biomarkers in the second blood sample relative to the first blood sample indicates that the treatment regimen is not efficacious for treating the CDK8 and/or CDK19 mediated disorder in the subject.

In another aspect of the present invention a diagnostic kit is provided for the determination of whether a patient is responding successfully to therapy that includes an antibody that binds to the phosphorylated pharmacodynamic biomarker. In one embodiment, the kit also includes a means to determine the concentration of the phosphorylated pharmacodynamic biomarker antibody complex, for example a colorimetric assay, a western blot assay, or an immunoblot assay. The kit can include multiple antibodies for one or more different pharmacodynamic complexes. In one embodiment, the kit is a combined therapeutic and companion diagnostic that also includes dosage forms of the selected CDK8/19 inhibitor. In another embodiment the kit provides specialized instructions, for example the kit may provide the healthcare provider instructions on how to check patient response *in vitro* as described herein. Some aspects of this disclosure provide kits comprising a drug with a cortistatin framework as described herein or a pharmaceutically acceptable salt, quaternary amine salt, or *N*-oxide thereof, for use as a medicament in the treatment of a CDK8 and/or CDK19 mediated disorder. In one embodiment the kit includes an antibody for pCHD4, pDPF2, or pDMAP1.

In one embodiment the kit is for diagnosing the effectiveness of CDK 8/19 inhibition in the treatment of a hematological cancer. As provided herein, the kit comprises a means for detecting or assaying phosphorylation of CHD4, DPF2, and/or DMAP1, or a combination thereof, both prior to administration of a CDK 8/19 inhibitor to treat a CDK8 and/or CDK19 mediated cancer (for example, a CDK8 and/or CDK19 mediated hematological cancer)and during continued administration of the CDK 8/19 inhibitor. Accordingly, the kit is capable of analyzing the change in phosphorylation of CHD4, DPF2, and/or DMAP1 during treatment using a CDK 8/19 inhibitor, which is indicative of the effectiveness of the CDK 8/19 inhibitor treatment.

Non-limiting examples of hematopoietic lineage tumors or cancers that can be monitored, include acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphoblastic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), childhood B-ALL,

chronic myeloid leukemia, acute monocytic leukemia, acute megakaryoblastic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, AIDS-related lymphoma, chronic myeloproliferative disorder, primary central nervous system lymphoma, T-cell lymphoma, hairy cell leukemia, and multiple myeloma (MM).

The invention also includes a diagnostic for and/or treatment of precursor cells to a hematopoietic tumor or cancer, such as found in myelodysplastic syndrome (MDS).

The tumor or cancer may also be of a non-hematopoietic lineage, such as breast cancer, ovarian cancer, endometrioid carcinoma, squamous cell cancer, angiosarcoma, colon cancer, gastrointestinal tumors, metastasis-prone solid tumors, clear cell carcinoma, renal cell carcinoma, or esophageal cancer.

In one embodiment the CDK8/19 inhibitor is a cortistatin analogue, for example a cortistatin analogue described herein. In one embodiment the pharmacodynamic biomarker is phosphorylated CHD4, DPF2, or DMAP1. In one embodiment the pharmacodynamic biomarker is either CHD4 pT1553, DPF2 pT248, or DMAP1 pT445, wherein the pT# denotes which amino acid is phosphorylated. These particular pharmacodynamic biomarkers are surprisingly effective because they can be used to monitor CDK8 and/or CDK19 mediated disorder progression in blood samples. Several previously identified substrates of CDK8/19 such as STAT1 and STAT5 are not as easily detected in blood samples and thus result in a less sensitive method to follow patient response to treatment.

In one embodiment any of the methods described herein utilize a different patient sample instead of blood. The patient sample can be any sample from which sufficient quantities of cells can be isolated and analyzed because the detected biomarkers are not metabolites or secreted proteins/peptides but rather intracellular proteins. In one embodiment the patient sample is a fluid sample. In another embodiment the patient sample is lymph fluid.

In one aspect of the present invention, a method is provided for determining the pharmacokinetic time course for a specific CDK8/19 inhibitor in a specific patient, comprising the following steps:

i. obtain a first fluid sample from the patient;

ii. detect the concentration of one or more pharmacodynamic biomarkers selected from pCHD4, pDPF2, and pDMAP1 in the first blood sample by contacting the blood sample with one or more antibodies to pCHD4,

pDPF2, or pDMAP1 and determining the concentration of these proteins by immunoblot;

iii. obtain a another fluid sample from the patient after waiting about .5 hours, about 1 hour, about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, or about 4 hours;

iv. repeat step iii 1, 2, 3, 4, 5, or 6 times;

v. detect the concentration of the one or more pharmacodynamic biomarkers selected from pCHD4, pDPF2, and pDMAP1 in the blood samples collected in steps iii and iv wherein the concentration over time of the one or more pharmacodynamic biomarkers is the pharmacokinetic time course for the specific CDK8/19 inhibitor, wherein lower concentration of the one or more pharmacodynamic biomarkers indicates higher inhibition of CDK8 and/or CDK19;

In one embodiment the pharmacokinetic time course determined with the above method is used to establish the dose necessary to achieve full target inhibition, wherein once you see a plateau in pharmacokinetic biomarker concentration the kinase has been inhibited maximally. Combined with tolerated dose data, this data can establish the therapeutic index of a compound. Additionally the data can be used to determine the clearance rate out of tissue and blood.

In one embodiment one or more of the above methods is used to determine when the compound is exerting its effects and when it stops doing so. This data can be compared to pharmacokinetic time courses calculated through means known in the art (such as tracking concentration of the CDK8/19 inhibitor or a metabolite thereof). In an alternative embodiment any of the methods herein are used with samples from xenograph experiments. In another embodiment the methods are used during clinical trials to determine effective dosage regimens.

This summary above is meant to illustrate, in a non-limiting manner, some of the embodiments, advantages, features, and uses of the technology disclosed herein. Other embodiments, advantages, features, and uses of the technology disclosed herein will be apparent from the Detailed Description, the Drawings, the Examples, and the Claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a volcano plot of 5,784 phosphosites identified in TMT mass spectrometry from 5 different donor PBMC samples. The y axis is the t-test value measured as p plotted in logarithmic base 10 units. The x axis is the ratio of DMSO (vehicle) to Cortistatin A plotted in logarithmic base 2 units. DPF2 pT248 and DMAP1 pT445 were top hits. The experimental preparation of these PBMC samples is provided in Example 5.

FIG. 2 is a volcano plot of 5,700 phosphosites identified in TMT mass spectrometry from heavy-labeled MOLM-13 cells treated with Cortistatin A. The y axis is the t-test value measured as p plotted in logarithmic base 10 units. The x axis is the ratio of DMSO (vehicle) to Cortistatin A plotted in logarithmic base 2 units. CHD4 was a top hit.

FIG. 3 is a volcano plot of 5,700 phosphosites identified in TMT mass spectrometry from heavy-labeled MOLM-13 cells treated with vehicle. The y axis is the t-test value measured as p plotted in logarithmic base 10 units. The x axis is the ratio of DMSO (vehicle) to Cortistatin A plotted in logarithmic base 2 units. CHD4 and DMAP1 were top hits.

FIG. 4 is a volcano plot of 5,700 phosphosites identified in TMT mass spectrometry from heavy-labeled MOLM-14 cells treated with Cortistatin A. The y axis is the t-test value measured as p plotted in logarithmic base 10 units. The x axis is the ratio of DMSO (vehicle) to Cortistatin A plotted in logarithmic base 2 units. CHD4 and DPF2 were top hits.

FIG. 5 is a volcano plot of 5,700 phosphosites identified in TMT mass spectrometry from heavy-labeled MOLM-14 cells treated with vehicle. The y axis is the t-test value measured as p plotted in logarithmic base 10 units. The x axis is the ratio of DMSO (vehicle) to Cortistatin A plotted in logarithmic base 2 units. CHD4 and DPF2 were top hits.

FIG. 6 is an immunoblot of freshly drawn whole blood treated with various concentrations of Compound 1 for 3 hours. The PBMCs used were isolated using Lymphoprep and Sepmate tubes, washed, and lysed. The concentration of wild type CHD4, DPF2, DMAP1 and their analogous phosphosites was assessed at various concentrations.

FIG. 7 is a dose response graph of pCHD4 at various concentrations of Compound 1. This graph was generated from the immunoblot in Figure 6. The y-axis is densitometry signal standardized to 100% vehicle. The x-axis is concentration of Compound 1 measured in micromolar units.

FIG. 8 is a dose response graph of pDPF2 at various concentrations of Compound 1. This graph was generated from the immunoblot in Figure 6. The y-axis is densitometry signal standardized to 100% vehicle. The x-axis is concentration of Compound 1 measured in micromolar units.

FIG. 9 is a dose response graph of pDMAP1 at various concentrations of Compound 1. This graph was generated from the immunoblot in Figure 6. The y-axis is densitometry signal standardized to 100% vehicle. The x-axis is concentration of Compound 1 measured in micromolar units.

FIG. 10 is an immunoblot of freshly drawn whole blood treated with various concentrations of Compound 1 for 4 hours. The blood sample used is different from that depicted in Figure 6. The PBMCs were isolated using Lymphoprep and Sepmate tubes, washed, and lysed. The concentration of CHD4 and pCHD4 was assessed at various concentrations.

FIG. 11 is a dose response graph of pCHD4 at various concentrations of Compound 1. This graph was generated from the immunoblot in Figure 10. The y-axis is densitometry signal standardized to 100% vehicle. The x-axis is concentration of Compound 1 measured in micromolar units.

FIG. 12 is an immunoblot of freshly drawn whole blood treated with various concentrations of Compound 1 for 4 hours. The blood sample used is different from that depicted in Figure 6. The PBMCs were isolated using Lymphoprep and Sepmate tubes, washed, and lysed. The concentration of DPF2 and pDPF2 was assessed at various concentrations.

FIG. 13 is a dose response graph of pDPF2 at various concentrations of Compound 1. This graph was generated from the immunoblot in Figure 12. The y-axis is densitometry signal standardized to 100% vehicle. The x-axis is concentration of Compound 1 measured in micromolar units.

FIG. 14 is an immunoblot of freshly drawn whole blood treated with various concentrations of Compound 1 for 4 hours. The blood sample used is the same as the one used in the immunoblots of Figure 10 and Figure 12. The concentration of STAT1, STAT5, pSTAT1, and pSTAT5 was assessed at various concentrations. Despite being canonical CDK8/19 targets neither pSTAT1 or pSTAT5 showed a robust Pharmacodynamic biomarker signal.

FIG. 15 is an immunoblot of freshly drawn whole blood treated with various concentrations of Compound 1 for 3 hours. The PBMC's used for this immunoblot were isolated from freshly

drawn blood using Lymphoprep and Sepmate tubes and then treated in RPMI supplement with 10% serum for 3 hours. The concentration of CHD4 and pCHD4 was assessed at various concentrations.

FIG. 16 is a dose response graph of pCHD4 at various concentrations of Compound 1. This graph was generated from the immunoblot in Figure 15. The y-axis is densitometry signal standardized to 100% vehicle. The x-axis is concentration of Compound 1 measured in nanomolar units. The IC50 determined in Figure 16 (with serum) is different from the IC50 determined in Figure 11 (without serum).

FIG. 17 is an immunoblot of freshly drawn whole blood treated with various concentrations of Compound 1 for 3 hours. The PBMC's used for this immunoblot were isolated from freshly drawn blood using Lymphoprep and Sepmate tubes and then treated in RPMI supplement with 10% serum for 3 hours. The concentration of DPF2 and pDPF2 was assessed at various concentrations.

FIG. 18 is a dose response graph of pDPF2 at various concentrations of Compound 1. This graph was generated from the immunoblot in Figure 17. The y-axis is densitometry signal standardized to 100% vehicle. The x-axis is concentration of Compound 1 measured in nanomolar units. The IC50 determined in Figure 18 (with serum) is different from the IC50 determined in Figure 13 (without serum).

FIG. 19 is an immunoblot of CHD4, pCHD4, DPF2, pDPF2, DMAP1, and pDMAP1 taken from various cells cultured in the absence and presence of 100 nM Cortistatin A. The cells used were EOL-1, HL-60, Kasumi-1, MEG-01, and MOLM-14. All five cell types show biomarker pCHD4, pDPF2, and pDMAP1 as pharmacodynamic biomarkers.

FIG. 20 is an immunoblot of CHD4, pCHD4, DPF2, pDPF2, DMAP1, and pDMAP1 taken from various cells cultured in the absence and presence of 100 nM Cortistatin A. The cells used were NOMO-1, SET-2, SKNO-1, MV4-11, MOLM-16. All five cell types show biomarker pCHD4, pDPF2, and pDMAP1 as pharmacodynamic biomarkers.

FIG. 21 is an immunoblot of CHD4 and pCHD4 taken from mouse xenographs. The mice were either treated with 1 milligram/kg (mpk) of Compound 1 or 2.5 milligram/kg (mpk) of Compound 1 PO. The xenograph model used is MV4-11.

FIG. 22 is an immunoblot of DPF2 and pDPF2 taken from mouse xenographs. The mice were either treated with 1 milligram/kg (mpk) of Compound 1 or 2.5 milligram/kg (mpk) of Compound 1 PO. The xenograph model used is MV4-11.

FIG. 23, FIG. 24, FIG. 25, FIG. 26, FIG. 27, FIG. 28, FIG. 29, FIG. 30, FIG. 31, FIG. 32, FIG. 33, FIG. 34, FIG. 35, FIG. 36, FIG. 37, and FIG. 38 provide non-limiting examples of specific cortistatin analogues that can be used in the present invention.

DETAILED DESCRIPTION OF THE INVENTION

In illustrative embodiments, the present invention includes at least the following features:

A) A method for treating a patient with a CDK8 and/or CDK19 mediated disorder comprising (i) obtaining a first blood sample from the patient; (ii) administering to the patient a first dose of a CDK8/19 inhibitor; (iii) obtaining a second blood sample from the patient after sufficient time for the CDK 8/19 inhibitor to act; (iv) detecting the concentration of the selected phosphorylated pharmacodynamic biomarker selected from the group consisting of CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in the first and second blood sample; (v) determining if the level of phosphorylated CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 is higher in the first blood sample than the second blood sample; (vi) if the decrease in the level of phosphorylated CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 is higher in the first blood sample the second blood sample by at least 10%, then continuing with administration of the CDK 8/19 inhibitor as prescribed by the health care provider; and (vii) optionally repeating steps i)- vi) during the treatment regimen to further confirm that the CDK8/19 inhibitor is continuing to act on the enzyme *in vivo*.

B) The method of embodiment A, wherein the concentration of the cortistatin analogue is administered in a dosage form that provides at least about 10, 25 or 50 ng of the CDK8 and/or CDK19 inhibitor.

C) The method of embodiment A, wherein the concentration of the cortistatin analogue is administered in a dosage form that provides at least about 100, 250 or 500 ng of the CDK8 and/or CDK19 inhibitor.

D) The method of embodiment A, wherein the concentration of the cortistatin analogue is administered in a dosage form that provides at least about 10, 50, 100, 200, 300, 400 or 500 mg of the CDK8 and/or CDK19 inhibitor.

E) The method of embodiment A, that includes a reference chart with standardized comparisons of the range of inhibition of phosphorylation in healthy people when given a range of doses of the CDK8 and or CDK19 inhibitor, and/or the range of inhibition of phosphorylation in patients when the drug is considered still effective, and optionally, when it has lost effectiveness.

F) A method for selecting a patient with a CDK8 and/or CDK19 mediated disorder, for example cancer, for therapy with a cortistatin analogue comprising (i) obtaining a first blood sample from the patient; (ii) detecting the concentration of the selected phosphorylated pharmacodynamic biomarker selected from the group consisting of CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in the first and second blood sample; (iii) adding the CDK8/19 inhibitor to the blood sample to obtain a blood sample treated with the CDK8/19 inhibitor; (iv) detecting the concentration of the selected phosphorylated pharmacodynamic biomarker selected from the group consisting of CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in the first and second blood sample; and (v) determining if the level of phosphorylated CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 is higher in the first blood sample from the second blood sample, wherein if the decrease in the level of phosphorylated CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 is at least by 10% then the patient should be treated.

G) The method of embodiment F, wherein the concentration of the cortistatin analogue is at least about 10 nM.

H) The method of embodiment F, wherein the concentration of the cortistatin analogue is at least about 50 nM.

I) The method of embodiment F, wherein the concentration of the cortistatin analogue is at least about 100 nM, 500nM or 1000 nM.

J) The method of any of embodiments A-I, wherein step (v) is accomplished by comparing the concentration of the pharmacodynamic biomarker in the untreated and treated sample, wherein if the concentration is decreased by at least 10% in the treated sample as compared

to the untreated sample, then the CDK8 and/or CDK19 mediated disorder responds to treatment.

K) The method of embodiment F, wherein the concentration of the pharmacodynamic biomarker is decreased by at least 20%.

L) The method of embodiment F, wherein the concentration of the pharmacodynamic biomarker is decreased by at least 30%.

M) The method of embodiment F, wherein the concentration of the pharmacodynamic biomarker is decreased by at least 40%.

N) The method of embodiment F, that includes a reference chart with standardized comparisons of the range of inhibition of phosphorylation in healthy people when given a range of doses of the CDK8 and or CDK19 inhibitor, and/or the range of inhibition of phosphorylation in patients when the drug is considered still effective, and optionally, when it has lost effectiveness.

O) The method of any of embodiments A-N, wherein the CDK8 and/or CDK19 mediated disorder is a hematological cancer.

P) The method of embodiment O, wherein the hematological cancer is leukemia.

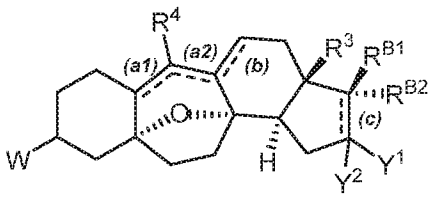
Q) The method of embodiment O, wherein the hematological cancer is lymphoma.

R) The method of embodiment O, wherein the hematological cancer is acute multiple myeloma.

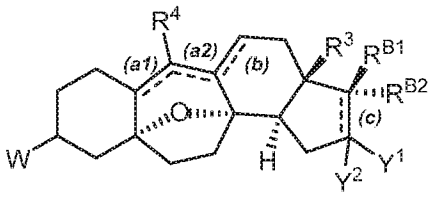
S) The method of any of embodiments A-O, wherein the blood sample is a whole blood sample.

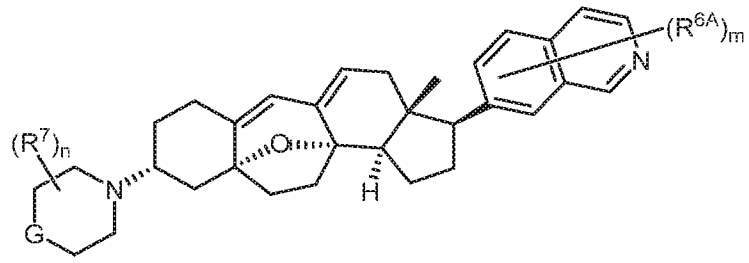
T) The method of any of embodiments A-O, wherein the blood sample is a PBMC blood sample.

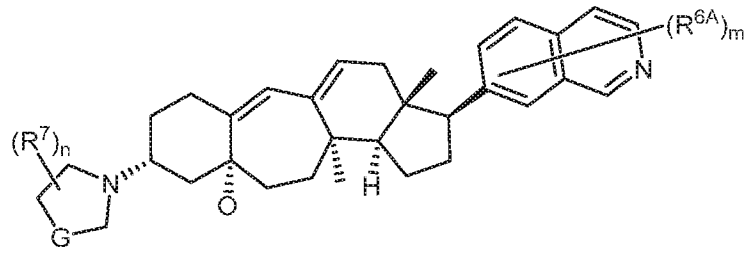
U) The method of any of embodiments A-O, wherein the concentration of the pharmacodynamic biomarker is determined by contacting the sample with an antibody to the phosphorylated pharmacodynamic biomarker.

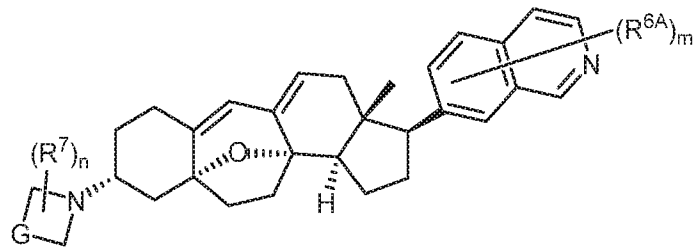
V) The method of any of embodiments A-U, wherein the cortistatin analogue is selected from formula 

, wherein the variables are as defined herein.

W) The method of any of embodiments A-U, wherein the cortistatin analogue is selected from formula  , wherein the variables are as defined herein.

X) The method of any of embodiments A-U, wherein the cortistatin analogue is selected from formula  , wherein the variables are as defined herein.

Y) The method of any of embodiments A-U, wherein the cortistatin analogue is selected from formula  , wherein the variables are as defined herein.

Z) The method of any of embodiments A-U, wherein the cortistatin analogue is selected from formula  , wherein the variables are as defined herein.

AA) The method of any of embodiments A-U, wherein the cortistatin analogue is selected from Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, and Compound 6.

BB) The method of any of embodiments A-U, wherein the pharmacodynamic biomarker is pCHD4.

CC) The method of any of embodiments A-U, wherein the pharmacodynamic biomarker is CHD4 pT1553.

DD) The method of any of embodiments A-U, wherein the pharmacodynamic biomarker is pDPF2.

EE) The method of any of embodiments A-U, wherein the pharmacodynamic biomarker is DPF2 pT248.

FF) The method of any of embodiments A-U, wherein the pharmacodynamic biomarker is pDMAP1.

GG) The method of any of embodiments A-U, wherein the pharmacodynamic biomarker is DMAP1 pT445.

I. PHARMACODYNAMIC BIOMARKERS FOR CDK8 AND CDK19 INHIBITOR TREATMENTS

More accurate biomarkers for use in a pharmacodynamic diagnostic have now been discovered that can conveniently be used in a whole blood assay to monitor over time the effectiveness of the administered CDK8 and/or CDK19 inhibitor drug, such as one of the cortistatin derivatives described further below. These biomarkers are selectively and directly phosphorylated by CDK8 and/or CDK19, and are present in whole blood in sufficient quantities for accurate analysis. Thus, they are superior diagnostic agents for use to determine if CDK8 and/or CDK19 is being effectively inhibited *in vivo* by the drug in the tumor or patient.

These robust pharmacodynamic biomarkers are CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 (which are the phosphorylated forms of the proteins CHD4, DPF2 and DMAP1). They were discovered to be advantageous medical indicators of CDK8/CDK19 pharmacodynamic time course inhibition through a series of complex analyses.

It has been discovered that a patient's CDK8 and/or CDK19 mediated disorder response to treatment with a CDK8/19 inhibitor can be assessed by determining the concentration of a pharmacodynamic biomarker before and after treatment with a CDK8/19 inhibitor. Using methods known in the art in combination with the disclosure herein, a healthcare provider can determine whether the patient is responding to treatment with a CDK8/19 inhibitor.

The term "pharmacodynamic biomarker" as used herein refers to a protein whose concentration changes depending on the effectiveness of therapy with a CDK8/19 inhibitor. The

pharmacodynamic biomarker may serve as an indicator of a particular subtype of a disease or disorder (e.g., cancer) characterized by certain, molecular, pathological, histological, and/or clinical features. In some embodiments the pharmacodynamic biomarker is a phosphorylated protein, for example, pCHD4, pDPF2, or pDMAP1. In some embodiments multiple pharmacodynamic biomarkers are quantified.

The pharmacodynamic biomarker does not have to directly or even indirectly mediate the patient's CDK8 and/or CDK19 mediated disorder. Even if the concentration of the pharmacodynamic biomarker does not predict a cell line's sensitivity to CDK8/19 inhibition the method can still be used. CHD4 pT1553, DPF2 pT248, and DMAP1 pT445 are particularly useful because they are found to be phosphorylated in relatively comparable levels in different AML cell lines and different donors' whole blood unlike phospho-STATs which can often vary widely between different AML cell lines, and between the leukemic blasts and normal PBMCs.

The CDK8/19 inhibitor may act through epigenetic mechanisms. And thus, the inhibitor may cause undifferentiated leukemic blasts to enter a differentiation pathway. Healthy PBMCs are largely differentiated lymphocytes and monocytes, and should be far less sensitive to CDK8/19 inhibition than AML blasts.

As used herein a protein denoted with the letter "p" denotes the protein after one or more phosphorylations. For example, pCHD4 refers to the CHD4 protein with one or more phosphate groups added. CHD4 pT1553 refers to CHD4 that has specifically been phosphorylated at the 1443 amino acid.

In one embodiment the pharmacodynamic biomarker is selected from: pNELFA, pTP53BP1, pAFF4, pOGFR, pMSANTD2, and pBRD4. In an alternative embodiment of the present invention instead of pCHD4, pDPF2, or pDMAP1 being used in a method described herein pNELFA, pTP53BP1, pAFF4, pOGFR, pMSANTD2, or pBRD4 is used. In one embodiment the pharmacodynamic biomarker is selected from: NELFA pTS363, TP53BP1 pTS265, AFF4 pTS814, OGFR pTS349, MSANTD2 pTS27, and BRD4 pTS470. In an alternative embodiment of the present invention instead of pCHD4, pDPF2, or pDMAP1 being used in a method described herein NELFA pTS363, TP53BP1 pTS265, AFF4 pTS814, OGFR pTS349, MSANTD2 pTS27, or BRD4 pTS470 is used, or may be used independently in combination with any of the other biomarkers described herein.

In an alternative embodiment STAT1 pTS727 and/or STAT5 pTS726 is used as the pharmacokinetic biomarker. As described herein STAT1 and STAT5 are not as sensitive to CDK8/19 inhibitors and thus the readouts are not as robust as those achieved for the other pharmacokinetic biomarkers described. However, if the method is sufficiently sensitive for the target method STAT1 and/or STAT5 may be used.

The pharmacodynamic biomarker selected, the particular sample's level of phosphomarks, and the biomarkers dependence on CDK8/19 for phosphorylation will determine what percent reduction is indicative of treatment response. CHD4 pT1553, DPF2 pT248, and DMAP1 pT445 each gave consistent results across multiple donors with CHD4 pT1553 concentration being reduced below 25% of the untreated sample, and DPF2 pT248 and DMAP1 pT445 below 50% of the untreated sample (as quantified by a Western blot set of titrations). Even at maximal CDK8/19 inhibition there is typically not a 100% reduction in the concentration of the pharmacodynamic phosphorylated biomarker used because other kinases may also phosphorylate it.

II. METHODS OF DETECTING PHARMACODYANAMIC BIOMARKER CONCENTRATION

The skilled artisan is aware of numerous methods to detect the concentration of a protein in a sample. Provided herein are non-limiting examples of such techniques such as: capturing the pharmacodynamic biomarker with one or more antibodies, capturing the pharmacodynamic biomarker with a solid support reagent, use of labelled antibodies, use of chemical reagents, or spectroscopic techniques.

In certain embodiments, a monoclonal antibody directed to pCHD4, pDPF2, or pDMAP1 is used to capture the pCHD4, pDPF2, or pDMAP1 from a test sample. The concentration of this antibody protein complex can be determined with immunoassays, mass spectrometry, or any other desired detection technique. In one embodiment the monoclonal antibody is tagged. In one embodiment the monoclonal antibody is a mouse monoclonal antibody. In another embodiment the monoclonal antibody is a rabbit monoclonal antibody.

In one embodiment an additional conjugate antibody is added to detect the presence of pCHD4, pDPF2, or pDMAP1 that has been captured. This additional antibody may possess a tag or utilize radiolabeling. Alternatively the additional antibody just serves as a means to more easily separate the mixture of proteins by SDS page or a related technique.

In a specific assay of the present invention, the immunoassay at least detects CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in the test sample, but does not detect non-phosphorylated CHD4, DPF2, and/or DMAP1.

For purposes of capture, the antibody in the immunodiagnostic reagent is comprised can be coated on a solid support such as for example, a microparticle, (e.g., magnetic particle), bead, test tube, microtiter plate, cuvette, membrane, scaffolding molecule, film, filter paper, disc or chip. Where the immunodiagnostic reagent comprises one or more antibodies that will be used to capture one or more of pCHD4, pDPF2, or pDMAP1 from the test sample, such antibody or antibodies can be co-coated on the same solid support or can be on separate solid supports.

Notably, the immunodiagnostic reagent antibody may be labeled with a detectable label or labeled with a specific partner that allows capture or detection. For example, the labels may be a detectable label, such as a fluorophore, radioactive moiety, enzyme, biotin/avidin label, chromophore, chemiluminescent label, or the like.

In one embodiment a chemiluminescent microparticle immunoassay is used.

In one embodiment the antibody can also incorporate a detectable label, such as a fluorophore, radioactive moiety, enzyme, biotin/avidin label, chromophore, chemiluminescent label, or the like.

In one embodiment the antibody is an anti-IgG antibody or anti-IgM antibody, that can also incorporate a detectable label, such as a fluorophore, radioactive moiety, enzyme, biotin/avidin label, chromophore, chemiluminescent label, or the like.

Any suitable assay known in the art can be used for detecting pCHD4, pDPF2, and/or pDMAP1 in the test sample. Examples of such assays include, but are not limited to, immunoassay, such as sandwich immunoassay (e.g., monoclonal-polyclonal sandwich immunoassays, including radioisotope detection (radioimmunoassay (RIA)) and enzyme detection (enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) (e.g., Quantikine ELISA assays, R&D Systems, Minneapolis, Minn.)), competitive inhibition immunoassay (e.g., forward and reverse), fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), bioluminescence resonance energy transfer (BRET), and homogeneous chemiluminescent assay, etc.

Typically, immunoassays are performed in a 1-step or 2-step format. Solid phase reagents for capture of immune complexes formed in solution in the 1-step assay include anti-biotin

monoclonal antibody, streptavidin, or neutravidin to capture the biotinylated moiety, e.g., the biotinylated antibody for the capture of pCHD4, pDPF2, and/or pDMAP1 in the test sample.

In a SELDI-based immunoassay, a capture reagent that specifically binds pCHD4, pDPF2, and/or pDMAP1 is attached to the surface of a mass spectrometry probe, such as a pre-activated protein chip array. pCHD4, pDPF2, and/or pDMAP1 is then specifically captured on the biochip, and the captured moiety is detected by mass spectrometry. Alternatively, pCHD4, pDPF2, and/or pDMAP1 can be eluted from the capture reagent and detected by traditional MALDI (matrix-assisted laser desorption/ionization) or by SELDI. A chemiluminescent microparticle immunoassay, in particular one employing the ARCHITECT® automated analyzer (Abbott Laboratories, Abbott Park, Ill.), is an example of an immunoassay in which a combination of multiple anti-pCHD4, pDPF2, and/or pDMAP1 antibodies may readily be employed.

Methods well-known in the art for collecting, handling and processing blood, serum and plasma, and other body fluids, can be used in the practice of the present disclosure. The test sample can comprise further moieties in addition to the polypeptide of interest, such as antibodies, antigens, haptens, hormones, drugs, enzymes, receptors, proteins, peptides, polypeptides, oligonucleotides or polynucleotides. For example, the sample can be a whole blood sample obtained from a subject. It can be necessary or desired that a test sample, particularly whole blood, be treated prior to immunoassay as described herein, e.g., with a pretreatment reagent. Even in cases where pretreatment is not necessary, pretreatment optionally can be done for mere convenience (e.g., as part of a regimen on a commercial platform).

The pretreatment reagent can be any reagent appropriate for use with the kit of the invention. The pretreatment optionally comprises: (a) one or more solvents (e.g., methanol and ethylene glycol) and salt, (b) one or more solvents, salt and detergent, (c) detergent, or (d) detergent and salt. Pretreatment reagents are known in the art, and such pretreatment can be employed, e.g., as used for assays on Abbott TDx, AxSYM®, and ARCHITECT® analyzers (Abbott Laboratories, Abbott Park, Ill.), as described in the literature (see, e.g., Yatscoff et al., Abbott TDx Monoclonal Antibody Assay Evaluated for Measuring Cyclosporine in Whole Blood, Clin. Chem. 36: 1969-1973 (1990), and Wallemacq et al., Evaluation of the New AxSYM Cyclosporine Assay: Comparison with TDx Monoclonal Whole Blood and EMIT Cyclosporine Assays, Clin. Chem. 45: 432-435 (1999)), and/or as commercially available. Additionally, pretreatment can be done as described in Abbott's U.S. Pat. No. 5,135,875, European Pat. Pub. No. 0 471 293, U.S. Provisional

Pat. App. 60/878,017, filed Dec. 29, 2006, and U.S. Pat. App. Pub. No. 2008/0020401 (incorporated by reference in its entirety for its teachings regarding pretreatment). The pretreatment reagent can be a heterogeneous agent or a homogeneous agent.

In a heterogeneous format, after the test sample is obtained from a subject, a mixture is prepared. The mixture contains the test sample being assessed for pCHD4, pDPF2, and/or pDMAP1 and an anti-pDMAP1, pDFP2, and/or pDMAP1 antibody, wherein pCHD4, pDPF2, and/or pDMAP1, if present in the sample, and the respective antibody form a complex.

In some embodiments, the anti-pCHD4, pDPF2, and/or pDMAP1 capture antibody are immobilized on a solid phase. In still other embodiments, none of components are immobilized but are instead all added at the same time to the test sample to effect capture onto the solid phase. The solid phase can be any solid phase known in the art, such as, but not limited to, a magnetic particle, a bead, a test tube, a microtiter plate, a cuvette, a membrane, a scaffolding molecule, a film, a filter paper, a disc and a chip.

Detection can be achieved by addition of a specific detection binding partner to the mixture to form a further complex. The detection binding partner can be an anti-IgG antibody. Moreover, the detection binding partner can be labeled with or contains a detectable label.

Any suitable detectable label as is known in the art can be used as any one or more of the detectable labels. For example, the detectable label can be a radioactive label (such as 3H, 125I, 35S, 14C, 32P, and 33P), an enzymatic label (such as horseradish peroxidase, alkaline peroxidase, glucose 6-phosphate dehydrogenase, and the like), a chemiluminescent label (such as acridinium esters, thioesters, or sulfonamides; luminol, isoluminol, phenanthridinium esters, and the like), a fluorescent label (such as fluorescein (e.g., 5-fluorescein, 6-carboxyfluorescein, 3′6-carboxyfluorescein, 5(6)-carboxyfluorescein, 6-hexachloro-fluorescein, 6-tetrachlorofluorescein, fluorescein isothiocyanate, and the like)), rhodamine, phycobiliproteins, R-phycoerythrin, quantum dots (e.g., zinc sulfide-capped cadmium selenide), a thermometric label, or an immuno-polymerase chain reaction label. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden, Introduction to Immunocytochemistry, 2nd ed., Springer Verlag, N.Y. (1997), and in Haugland, Handbook of Fluorescent Probes and Research Chemicals (1996), which is a combined handbook and catalogue published by Molecular Probes, Inc., Eugene, Oreg. A fluorescent label can be used in FPIA (see, e.g., U.S. Pat. Nos. 5,593,896, 5,573,904, 5,496,925, 5,359,093, and 5,352,803, which are hereby incorporated by reference in

their entireties). An acridinium compound can be used as a detectable label in a homogeneous chemiluminescent assay (see, e.g., Adamczyk et al., Bioorg. Med. Chem. Lett. 16: 1324-1328 (2006); Adamczyk et al., Bioorg. Med. Chem. Lett. 4: 2313-2317 (2004); Adamczyk et al., Biorg. Med. Chem. Lett. 14: 3917-3921 (2004); and Adamczyk et al., Org. Lett. 5: 3779-3782 (2003)).

The signal that is generated by the detectable label can be detected using routine techniques known to those skilled in the art. For example, when using a chemiluminescent based-label, based on the intensity of the signal generated, the amount of pCHD4, pDPF2, and/or pDMAP1 in the sample can be quantified. For example, the amount of pCHD4, pDPF2, and/or pDMAP1 in the sample is proportional to the intensity of the signal generated. The amount pCHD4, pDPF2, and/or pDMAP1 can be quantified by comparing the amount of light generated to a standard curve concentration of pCHD4, pDPF2, and/or pDMAP1 or by comparison to a reference standard. The standard curve can be generated using serial dilutions or solutions of known concentrations of pCHD4, pDPF2, and/or pDMAP1 by mass spectroscopy, gravimetric methods, and other techniques known in the art.

pCHD4, pDPF2, and/or pDMAP1 immunoassays can be conducted using any suitable format known in the art. Generally speaking, a sample being tested for pCHD4, pDPF2, and/or pDMAP1 can be contacted with an antibody to pCHD4, pDPF2, or pDMAP1 and a detection antibody, such as a labeled anti-IgG antibody, either simultaneously or sequentially and in any order.

For example, the test sample can be first contacted with at least one capture antibody and then (sequentially) with at least one detection antibody. Alternatively, the test sample can be first contacted with at least one detection antibody and then (sequentially) with at least one capture antibody. In yet another alternative, the test sample can be contacted simultaneously with a capture antibody and a detection antibody.

The detectable label can be bound to the detection antibody either directly or through a coupling agent. An example of a coupling agent that can be used is EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride), which is commercially available from Sigma-Aldrich, St. Louis, Mo. Other coupling agents that can be used are known in the art. Methods for binding a detectable label to an antibody are known in the art. Additionally, many detectable labels can be purchased or synthesized that already contain end groups that facilitate the coupling of the detectable label to the antibody, such as CPSP-Acridinium Ester (i.e., 9-[N-

tosyl-N-(3-carboxypropyl)]-10-(3-sulfopropyl)acridinium carboxamide) or SPSP-Acridinium Ester (i.e., N10-(3-sulfopropyl)-N-(3-sulfopropyl)-acridinium-9-carboxamide).

After formation of the pCHD4, pDPF2, and/or pDMAP1/antibody/detection antibody complex, the amount of label in the complex can be quantified using techniques known in the art. For example, if an enzymatic label is used, the labeled complex is reacted with a substrate for the label that gives a quantifiable reaction such as the development of color. If the label is a radioactive label, the label is quantified using a scintillation counter. If the label is a fluorescent label, the label is quantified by stimulating the label with a light of one color (which is known as the "excitation wavelength") and detecting another color (which is known as the "emission wavelength") that is emitted by the label in response to the stimulation. If the label is a chemiluminescent label, the label is quantified by detecting the light emitted either visually or by using luminometers, x-ray film, high speed photographic film, a CCD camera, etc. Once the amount of the label in the complex has been quantified, the concentration of pCHD4, pDPF2, and/or pDMAP1 in the test sample is determined by use of a standard curve that has been generated using serial dilutions of pCHD4, pDPF2, and/or pDMAP1 or known concentrations of pCHD4, pDPF2, and/or pDMAP1. Other than using serial dilutions, the standard curve can be generated gravimetrically, by mass spectroscopy and by other techniques known in the art.

In one embodiment the detection technique is an adaptation of an existing assay. In particular, with regard to the adaptation of an assay to the I-STAT® system, the following configuration is exemplary. A microfabricated silicon chip is manufactured with a pair of gold amperometric working electrodes and a silver-silver chloride reference electrode. On one of the working electrodes, polystyrene beads (0.2 mm diameter) with immobilized capture antibody are adhered to a polymer coating of patterned polyvinyl alcohol over the electrode. This chip is assembled into an I-STAT® cartridge with a fluidics format suitable for immunoassay. On a portion of the wall of the sample-holding chamber of the cartridge there is a layer comprising the detection antibody labeled with alkaline phosphatase (or other label). Within the fluid pouch of the cartridge is an aqueous reagent that includes p-aminophenol phosphate.

In operation, a sample is added to the holding chamber of the test cartridge and the cartridge is inserted into the I-STAT® reader. After the detection antibody has dissolved into the sample, a pump element within the cartridge forces the sample into a conduit containing the chip. Here it is oscillated to promote formation of the sandwich between the capture antibody, pCHD4, pDPF2,

and/or pDMAP1, and the labeled detection antibody. In the penultimate step of the assay, fluid is forced out of the pouch and into the conduit to wash the sample off the chip and into a waste chamber. In the final step of the assay, the alkaline phosphatase label reacts with p-aminophenol phosphate to cleave the phosphate group and permit the liberated p-aminophenol to be electrochemically oxidized at the working electrode. Based on the measured current, the reader is able to calculate the amount of pCHD4, pDPF2, and/or pDMAP1in the sample by means of an embedded algorithm and factory-determined calibration curve.

The methods provided herein can be used to monitor treatment in a subject receiving one or more CDK 8/19 inhibitors. Specifically, such methods involve obtaining a first test sample from a subject before the subject has been administered one or more CDK 8/19 inhibitors. The concentration or amount of pCHD4, pDPF2, and/or pDMAP1 in a first test sample from a subject is determined (e.g., using the methods described herein or as known in the art). After the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 is determined, the subject is then administered one or more CDK 8/19 inhibitors in a therapeutic protocol for the treatment of a CDK8 and/or CDK19 mediated disorder, such as cancer. Following a pre-determined amount of time of treatment where multiple doses have been administered, for example, at least about or not more than 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 14 days, 17 days, or 21 days, a second test sample is drawn and the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 is determined and compared with the concentration of pCHD4, pDPF2, and/or pDMAP1 in the first test sample. If the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 as determined in the first test sample is the same or lower than the concentration of amount of pCHD4, pDPF2, and/or pDMAP1 in the second sample, then the subject is administered a higher dose of CDK 8/19 inhibitor, or administered an alternative therapeutic agent. However, If the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 as determined in the first test sample is higher than the concentration of amount of pCHD4, pDPF2, and/or pDMAP1 in the second sample, then the subject is maintained on the administered dose of CDK 8/19 inhibitor.

Alternatively samples may be drawn from the patient following a single dose of CDK8/19 inhibitor. For example, the second sample may be drawn at least about or not more than about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, or about 12 hours after treatment. Then the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 is determined and

compared with the concentration of pCHD4, pDPF2, and/or pDMAP1 in the first test sample. If the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 as determined in the first test sample is the same or lower than the concentration of amount of pCHD4, pDPF2, and/or pDMAP1 in the second sample, then the subject is administered a higher dose of CDK 8/19 inhibitor, or administered an alternative therapeutic agent. However, If the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 as determined in the first test sample is higher than the concentration of amount of pCHD4, pDPF2, and/or pDMAP1 in the second sample, then the subject is maintained on the administered dose of CDK 8/19 inhibitor.

In one embodiment multiple samples are collected from the patient after receiving treatment. In one embodiment the concentration of pharmacodynamic biomarker in each sample is determined. These concentrations may be used to determine how that specific patient is metabolizing the CDK 8/19 inhibitor. For example, if the concentration of pharmacokinetic biomarker begins to increase more rapidly after administration of the CDK 8/19 inhibitor the caregiver may increase the frequency of dosing to ensure an effective dose is maintained.

During the course of treatment with the one or more CDK 8/19 inhibitor, subsequent test samples are then obtained from the subject to monitor the concentration or amount of pCHD4, pDPF2, and/or pDMAP1, and the administration of the CDK 8/19 inhibitor adjusted accordingly. The number of test samples and the time in which said test samples are obtained from the subject are not critical. For example, a subsequent sample could be obtained 30 days after the subject's second test sample.

Generally, for assays in which repeat testing may be done (e.g., monitoring disease progression and/or response to treatment), a subsequent test sample is obtained at a period in time after the second test sample has been obtained from the subject. Specifically, a subsequent test sample from the subject can be obtained days, weeks or months after the second test sample has been obtained from the subject. For example, the subsequent test sample can be obtained from the subject at a time period of about about 7 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11 weeks, about 12 weeks, about 13 weeks, about 14 weeks, about 15 weeks, about 16 weeks, about 17 weeks, about 18 weeks, about 19 weeks, about 20 weeks, about 21 weeks, about 22 weeks, about 23 weeks, about 24 weeks, about 25 weeks, about 26 weeks, about 27 weeks, about 28 weeks, about 29 weeks, about 30 weeks, about 31 weeks, about 32 weeks, about 33 weeks, about

34 weeks, about 35 weeks, about 36 weeks, about 37 weeks, about 38 weeks, about 39 weeks, about 40 weeks, about 41 weeks, about 42 weeks, about 43 weeks, about 44 weeks, about 45 weeks, about 46 weeks, about 47 weeks, about 48 weeks, about 49 weeks, about 50 weeks, about 51 weeks, about 52 weeks, or more after the second test sample from the subject is obtained.

III. DIAGNOSTIC KITS

The present invention also provides a diagnostic kit adapted for carrying out any one of the methods of the present invention comprising means for the determination of the amount of a pharmacodynamic biomarker in a sample of a subject and means for comparing the amounts to reference amounts.

In one embodiment, the term "kit" as used herein refers to a collection of the aforementioned means, preferably, provided in separately or within a single container. The container, also preferably, comprises instructions for carrying out the method of the present invention. Kits are described further below, and can be used with any of the methods described herein.

Diagnostic reagents in the field of specific binding assays, like immunoassays, are often best provided in the form of a kit, which comprises the specific binding agent and the auxiliary reagents required to perform the assay. The present invention therefore also relates to an immunological kit comprising at least one specific binding agent for the pharmacodynamic biomarker and auxiliary reagents for measurement of the pharmacodynamic biomarker. Also preferred is an immunological kit comprising at least one specific binding agent for the pharmacodynamic biomarker, at least one specific binding agent for a well characterized standard and auxiliary reagents for measurement of the pharmacodynamic biomarker and well characterized standard.

Also provided herein is a kit for diagnosing the effectiveness of CDK 8/19 inhibition in the treatment of a CDK8 and/or CDK19 mediated disorder. In one embodiment the CDK8 and/or CDK19 mediated disorder is a cancer. In one embodiment the cancer.is a hematological cancer. As provided herein, the kit comprises a means for detecting or assaying phosphorylation of CHD4, DPF2, and/or DMAP1, or a combination thereof, both prior to administration of a CDK 8/19 inhibitor to treat a CDK8 and/or CDK19 mediated cancer (for example, CDK8 and/or CDK19 mediated hematological cancer) and during continued administration of the CDK 8/19 inhibitor.

Accordingly, the kit is capable of analyzing the change in phosphorylation of CHD4, DPF2, and/or DMAP1 during treatment using a CDK 8/19 inhibitor, which is indicative of the effectiveness of the CDK 8/19 inhibitor treatment.

As provided herein, the kit generally incorporates an immunoassay method for determining concentrations of one or more of the phosphorylated targets CHD4, DPF2, and DMAP1. In exemplary embodiments, such methods include methods for isolating pCHD4, pDPF2, and/or pDMAP1 present in the test sample. In certain embodiments, a monoclonal antibody directed to pCHD4, pDPF2, or pDMAP1 is used to capture the pCHD4, pDPF2, or pDMAP1 from a test sample and then further conjugate antibodies are used to detect the presence of pCHD4, pDPF2, or pDMAP1 that has been captured.

Specifically, such antibodies preferably determine the presence of only phosphorylated CHD4, DPF2, and/or DMAP1 in the test sample. Antibodies directed to only phosphorylated CHD4, DPF2, and/or DMAP1 are described herein, and methods of making such antibodies are generally known to those of skill in the art. In specific exemplary embodiments the antibodies used in the immunoassay are antibodies designed to detect CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in a test sample

In a specific kit of the present invention, the immunoassay at least detects CHD4 pT1553, DPF2 pT248, and/or DMAP1 pT445 in the test sample, but does not detect non-phosphorylated CHD4, DPF2, and/or DMAP1.

In particular embodiments, the pCHD4, pDPF2, or pDMAP1 antibodies described above are contemplated for use as immunodiagnostic reagents in combination immunoassays designed for the detection of one of pCHD4, pDPF2, or pDMAP1 found in a test sample from a subject prior to and after receiving a CDK 8/19 inhibitor for the treatment of a CDK8 and/or CDK19 disorder (for example, a CDK8 and/or CDK19 mediated hematological cancer). Immunodiagnostic reagents will be comprised of the above-described antibodies (typically in combination) such that they can be used in an immunoassay designed for the detection of pCHD4, pDPF2, or pDMAP1. For purposes of capture, the kit may include antibodies coated on a solid support such as for example, a microparticle, (e.g., magnetic particle), bead, test tube, microtiter plate, cuvette, membrane, scaffolding molecule, film, filter paper, disc or chip. In this regard, where the immunodiagnostic reagent comprises one or more antibodies that will be used to capture

one or more of pCHD4, pDPF2, or pDMAP1 from the test sample, such antibodies can be co-coated on the same solid support or can be on separate solid supports.

Notably, the immunodiagnostic reagent antibody may be labeled with a detectable label or labeled with a specific partner that allows capture or detection. For example, the labels may be a detectable label, such as a fluorophore, radioactive moiety, enzyme, biotin/avidin label, chromophore, chemiluminescent label, or the like.

Still further the invention contemplates the preparation of a diagnostic kit comprising the immunodiagnostic reagents described herein and instructions for the use of the immunodiagnostic reagents in immunoassays for determining the change in phosphorylation patterns of CHD4, DPF2, or DMAP1 during treatment of a CDK8 and/or CDK19 mediated disorder (for example, a CDK8 and/or CDK19 mediated hematological cancer) before and after administration of a CDK 8/19 inhibitor in a test sample. For example, the kit can comprise instructions for assaying the test sample for pCHD4, pDPF2, or pDMAP1 by immunoassay. It should be understood that the antibodies used in the immunoassays of the present invention may be used in any immunoassay formats known to those of skill in the art for determining the presence of a protein in a test sample, including for example, but not limited to chemiluminescent microparticle immunoassays. The instructions can be in paper form or computer-readable form, such as a disk, CD, DVD, or the like.

Alternatively or additionally, the kit can comprise a calibrator or control, e.g., purified, and optionally lyophilized pCHD4, pDPF2, and/or pDMAP1 and/or at least one container (e.g., tube, microtiter plates or strips), which can be already coated with one or more antibodies for conducting the assay, and/or a buffer, such as an assay buffer or a wash buffer, either one of which can be provided as a concentrated solution, a substrate solution for the detectable label (e.g., an enzymatic label), or a stop solution. Preferably, the kit comprises all components, i.e., reagents, standards, buffers, diluents, etc., which are necessary to perform the assay.

Any antibodies, which are provided in the kit, such as anti-IgG antibodies and anti-IgM antibodies, can also incorporate a detectable label, such as a fluorophore, radioactive moiety, enzyme, biotin/avidin label, chromophore, chemiluminescent label, or the like, or the kit can include reagents for labeling the antibodies or reagents for detecting the antibodies (e.g., detection antibodies) and/or for labeling the analytes or reagents for detecting the analyte. The antibodies, calibrators and/or controls can be provided in separate containers or pre-dispensed into an appropriate assay format, for example, into microtiter plates.

Optionally, the kit includes quality control components (for example, sensitivity panels, calibrators, and positive controls). Preparation of quality control reagents is well-known in the art and is described on insert sheets for a variety of immunodiagnostic products. Sensitivity panel members optionally are used to establish assay performance characteristics, and further optionally are useful indicators of the integrity of the immunoassay kit reagents, and the standardization of assays.

The kit can also optionally include other reagents required to conduct a diagnostic assay or facilitate quality control evaluations, such as buffers, salts, enzymes, enzyme co-factors, substrates, detection reagents, and the like. Other components, such as buffers and solutions for the isolation and/or treatment of a test sample (e.g., pretreatment reagents), also can be included in the kit. The kit can additionally include one or more other controls. One or more of the components of the kit can be lyophilized, in which case the kit can further comprise reagents suitable for the reconstitution of the lyophilized components.

The various components of the kit optionally are provided in suitable containers as necessary, e.g., a microtiter plate. The kit can further include containers for holding or storing a sample (e.g., a container or cartridge for a sample). Where appropriate, the kit optionally also can contain reaction vessels, mixing vessels, and other components that facilitate the preparation of reagents or the test sample. The kit can also include one or more instrument for assisting with obtaining a test sample, such as a syringe, pipette, forceps, measured spoon, or the like.

If desired, the kit can contain a solid support phase, such as a magnetic particle, bead, test tube, microtiter plate, cuvette, membrane, scaffolding molecule, film, filter paper, disc or chip.

Constructions or equipment for conducting any suitable assay known in the art can be included in the kit for detecting pCHD4, pDPF2, and/or pDMAP1 in the test sample. Examples of such assays include, but are not limited to, immunoassay, such as sandwich immunoassay (e.g., monoclonal-polyclonal sandwich immunoassays, including radioisotope detection (radioimmunoassay (RIA)) and enzyme detection (enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) (e.g., Quantikine ELISA assays, R&D Systems, Minneapolis, Minn.)), competitive inhibition immunoassay (e.g., forward and reverse), fluorescence polarization immunoassay (FPIA), enzyme multiplied immunoassay technique (EMIT), bioluminescence resonance energy transfer (BRET), and homogeneous chemiluminescent assay, etc.

Pretreatment reagents may also be provided in this kit. The test sample can comprise further moieties in addition to the polypeptide of interest, such as antibodies, antigens, haptens, hormones, drugs, enzymes, receptors, proteins, peptides, polypeptides, oligonucleotides or polynucleotides. For example, the sample can be a whole blood sample obtained from a subject. It can be necessary or desired that a test sample, particularly whole blood, be treated prior to immunoassay as described herein, e.g., with a pretreatment reagent. Even in cases where pretreatment is not necessary, pretreatment optionally can be done for mere convenience (e.g., as part of a regimen on a commercial platform).

The pretreatment reagent can be any reagent appropriate for use with the kit of the invention. The pretreatment optionally comprises: (a) one or more solvents (e.g., methanol and ethylene glycol) and salt, (b) one or more solvents, salt and detergent, (c) detergent, or (d) detergent and salt. Pretreatment reagents are known in the art, and such pretreatment can be employed, e.g., as used for assays on Abbott TDx, AxSYM®, and ARCHITECT® analyzers (Abbott Laboratories, Abbott Park, Ill.), as described in the literature (see, e.g., Yatscoff et al., Abbott TDx Monoclonal Antibody Assay Evaluated for Measuring Cyclosporine in Whole Blood, Clin. Chem. 36: 1969-1973 (1990), and Wallemacq et al., Evaluation of the New AxSYM Cyclosporine Assay: Comparison with TDx Monoclonal Whole Blood and EMIT Cyclosporine Assays, Clin. Chem. 45: 432-435 (1999)), and/or as commercially available. Additionally, pretreatment can be done as described in Abbott's U.S. Pat. No. 5,135,875, European Pat. Pub. No. 0 471 293, U.S. Provisional Pat. App. 60/878,017, filed Dec. 29, 2006, and U.S. Pat. App. Pub. No. 2008/0020401 (incorporated by reference in its entirety for its teachings regarding pretreatment). The pretreatment reagent can be a heterogeneous agent or a homogeneous agent.

In a heterogeneous format, after the test sample is obtained from a subject, a mixture is prepared. The mixture contains the test sample being assessed for pCHD4, pDPF2, and/or pDMAP1 and an anti-pDMAP1, pDFP2, and/or pDMAP1 antibody, wherein pCHD4, pDPF2, and/or pDMAP1, if present in the sample, and the respective antibody form a complex.

In one embodiment the kit provides specific detection antibodies. The detection binding partner can be an anti-IgG antibody. Moreover, the detection binding partner can be labeled with or contains a detectable label.

The kits provided herein can be used to monitor treatment in a subject receiving treatment with one or more CDK 8/19 inhibitors. Specifically, such methods involve providing a first test

sample from a subject before the subject has been administered one or more CDK 8/19 inhibitors. The concentration or amount of pCHD4, pDPF2, and/or pDMAP1 in a first test sample from a subject is determined (e.g., using the methods described herein or as known in the art). After the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 is determined, the subject is then administered one or more CDK 8/19 inhibitors in a therapeutic protocol for the treatment of CDK8 and/or CDK19 mediated disorder (for example, a CDK8 and/or CDK19 mediated hematological cancer). Following a pre-determined amount of time of treatment, for example, at least about or not more than 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 14 days, 17 days, or 21 days, a second test sample is drawn and the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 is determined and compared with the concentration of pCHD4, pDPF2, and/or pDMAP1 in the first test sample. If the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 as determined in the first test sample is the same or lower than the concentration of amount of pCHD4, pDPF2, and/or pDMAP1 in the second sample, then the subject is administered a higher dose of CDK 8/19 inhibitor, or administered an alternative therapeutic agent. However, If the concentration or amount of pCHD4, pDPF2, and/or pDMAP1 as determined in the first test sample is higher than the concentration of amount of pCHD4, pDPF2, and/or pDMAP1 in the second sample, then the subject is maintained on the administered dose of CDK 8/19 inhibitor.

During the course of treatment with the one or more CDK 8/19 inhibitor, subsequent test samples are then obtained from the subject to monitor the concentration or amount of pCHD4, pDPF2, and/or pDMAP1, and the administration of the CDK 8/19 inhibitor adjusted accordingly. The number of test samples and the time in which said test samples are obtained from the subject are not critical. For example, a subsequent sample could be obtained 30 days after the subject's second test sample.

Generally, for assays in which repeat testing is desired (e.g., monitoring disease progression and/or response to treatment), a subsequent test sample is obtained at a period in time after the second test sample has been obtained from the subject. Specifically, a subsequent test sample from the subject can be obtained days, weeks or months after the second test sample has been obtained from the subject. For example, the subsequent test sample can be obtained from the subject at a time period of about about 7 days, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, about 9 weeks, about 10 weeks, about 11

weeks, about 12 weeks, about 13 weeks, about 14 weeks, about 15 weeks, about 16 weeks, about 17 weeks, about 18 weeks, about 19 weeks, about 20 weeks, about 21 weeks, about 22 weeks, about 23 weeks, about 24 weeks, about 25 weeks, about 26 weeks, about 27 weeks, about 28 weeks, about 29 weeks, about 30 weeks, about 31 weeks, about 32 weeks, about 33 weeks, about 34 weeks, about 35 weeks, about 36 weeks, about 37 weeks, about 38 weeks, about 39 weeks, about 40 weeks, about 41 weeks, about 42 weeks, about 43 weeks, about 44 weeks, about 45 weeks, about 46 weeks, about 47 weeks, about 48 weeks, about 49 weeks, about 50 weeks, about 51 weeks, about 52 weeks, or more after the second test sample from the subject is obtained.

Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test strips or electronic devices which merely require loading with a sample. The results may be given as output of raw data which need interpretation by the clinician. In one embodiment, the output of the device is, however, processed, i.e. evaluated, raw data the interpretation of which does not require a clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the natriuretic peptide, plasmon surface resonance devices, NMR spectrometers, mass-spectrometers etc.) and/or evaluation units/devices referred to above in accordance with the method of the invention.

The kit (or components thereof), as well as the method of determining the concentration of pCHD4, pDPF2, and/or pDMAP1 can be adapted for use in a variety of automated and semi-automated systems (including those wherein the solid phase comprises a microparticle), as described, e.g., in U.S. Pat. Nos. 5,089,424 and 5,006,309, and as commercially marketed, e.g., by Abbott Laboratories (Abbott Park, Ill.) as ARCHITECT®. Other platforms available from Abbott Laboratories include, but are not limited to, AxSYM®, IMx® (see, e.g., U.S. Pat. No. 5,294,404, which is hereby incorporated by reference in its entirety), PRISM®, EIA (bead), and Quantum™ II, as well as other platforms. Additionally, the assays, kits and kit components can be employed in other formats, for example, on electrochemical or other hand-held or point-of-care assay systems. The present disclosure is, for example, applicable to the commercial Abbott Point of Care (i-STAT®, Abbott Laboratories) electrochemical immunoassay system that performs sandwich immunoassays Immunosensors and their methods of manufacture and operation in single-use test devices are described, for example in, U.S. Pat. No. 5,063,081, U.S. Pat. App. Pub. No. 2003/0170881, U.S. Pat. App. Pub. No. 2004/0018577, U.S. Pat. App. Pub. No.

2005/0054078, and U.S. Pat. App. Pub. No. 2006/0160164, which are incorporated in their entireties by reference for their teachings regarding same.

The kit may include an adaptation of an existing assay. For example, with regard to the adaptation of an assay to the I-STAT® system, the following configuration is exemplary. A microfabricated silicon chip is manufactured with a pair of gold amperometric working electrodes and a silver-silver chloride reference electrode. On one of the working electrodes, polystyrene beads (0.2 mm diameter) with immobilized capture antibody are adhered to a polymer coating of patterned polyvinyl alcohol over the electrode. This chip is assembled into an I-STAT® cartridge with a fluidics format suitable for immunoassay. On a portion of the wall of the sample-holding chamber of the cartridge there is a layer comprising the detection antibody labeled with alkaline phosphatase (or other label). Within the fluid pouch of the cartridge is an aqueous reagent that includes p-aminophenol phosphate.

The present invention relates to a diagnostic kit adapted for carrying out any one of the methods of the present invention comprising means for the determination of the amount of a pharmacodynamic biomarker in a sample of a subject and means for comparing the amounts to reference amounts.

In one embodiment, the term "kit" as used herein refers to a collection of the aforementioned means, preferably, provided in separately or within a single container. The container, also preferably, comprises instructions for carrying out the method of the present invention.

Thus, the invention encompasses pharmaceutical packs and/or kits. Pharmaceutical packs and/or kits provided may comprise a provided composition or antibody and a container (e.g., a vial, ampoule, bottle, syringe, and/or dispenser package, or other suitable container). In some embodiments, provided kits may optionally further include a second container comprising a suitable aqueous carrier for dilution or suspension of the provided composition for preparation to administer the compound to the subject. Alternatively, the kit may include a second container comprising a suitable carrier for dilution of suspension of the provided antibody for ease in quantification. In some embodiments, contents of provided formulation container and solvent container combine to form at least one unit dosage form.

Optionally, a single container may comprise one or more compartments for containing a provided composition or antibody, and/or appropriate aqueous carrier for suspension or dilution.

In some embodiments, a single container can be appropriate for modification such that the container may receive a physical modification so as to allow combination of compartments and/or components of individual compartments. For example, a foil or plastic bag may comprise two or more compartments separated by a perforated seal which can be broken so as to allow combination of contents of two individual compartments once the signal to break the seal is generated. In one embodiment the additional compartment is empty and can be filled with the patient's fluid sample. A pharmaceutical pack or kit may thus comprise such multi–compartment containers including a provided composition or antibody and appropriate solvent and/or appropriate aqueous carrier for suspension. Optionally, instructions for use are additionally provided in such kits of the invention. Such instructions may provide, generally, for example, instructions for dosage and administration. In other embodiments, instructions may further provide additional detail relating to specialized instructions for particular containers and/or systems for administration. Still further, instructions may provide specialized instructions for use in conjunction and/or in combination with additional therapy.

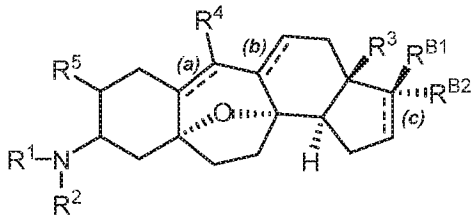
IV. CORTISTATINS

The term "cortistatin" or "cortistatin derivative" or "cortistatin analog" as used herein refers to a compound that has the general cortistatin framework of one of the known naturally occurring cortistatins (Cortistatins A, B, C, D, E, F, G, H, I, J, K or L) or is described in one of the Formulas below. The cortistatin can be used if desired in the form of a pharmaceutically acceptable salt, including a quarternary ammonium salt, an N-oxide and/or in a pharmaceutically acceptable composition.

A. Cortistatin Analogs

In certain embodiments, the CDK8/19 inhibitor for use in the present invention is a cortistatin analogue described in U.S. Patent Publication 2017/029435; PCT Patent Publication WO 2017/112815; PCT Patent Publication WO 2017/142621; each of which is incorporated by reference for all purposes.

Non-limiting examples of cortistatin based CDK8/19 inhibitors include compounds of Formula A, B, C, D, and E: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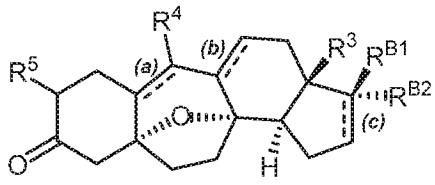
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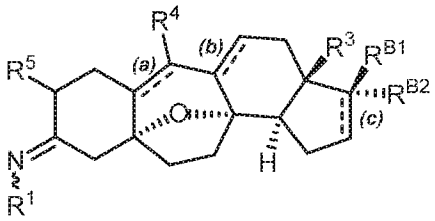
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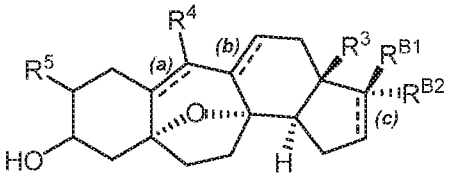
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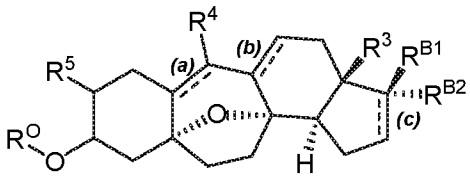

(E)

and pharmaceutically acceptable salts, quaternary amine salts, or N-oxides thereof,

wherein:

R^1 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $-OR^A$, $-SR^A$, $-N(R^A)_2$, $-C(=O)R^A$, $-C(=O)OR^A$, $-C(=O)N(R^A)_2$, $-S(=O)_2R^A$, or a nitrogen protecting group;

R^2 is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $-C(=O)R^A$, $-C(=O)OR^A$, $-C(=O)N(R^A)_2$, $-S(=O)_2R^A$, or a nitrogen protecting group;

or R^1 and R^2 are joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl;

R^3 is hydrogen or optionally substituted alkyl;

R^4 is hydrogen, halogen, optionally substituted alkyl, or $-Si(R^A)_3$;

R^5 is hydrogen, halogen, or optionally substituted alkyl;

each instance of ⎓, designated as (*a*), (*a1*), (*b*), and (*c*), represents a single or double bond, provided that when ⎓ designated as (*c*) represents a double bond, then one of R^{B1} and

R^{B2} is absent, and provided that when ----- designated as (*c*) represents a single bond, then both R^{B1} and R^{B2} are present;

each instance of R^{B1} and R^{B2} is, independently, hydrogen, -L_1-R^{B3}, or –X^AR^A wherein X^A is –O-, -S-, or -N(R^A)-; or R^{B1} and R^{B2} are joined to form an oxo group, provided that at least one of R^{B1} and R^{B2} is not hydrogen;

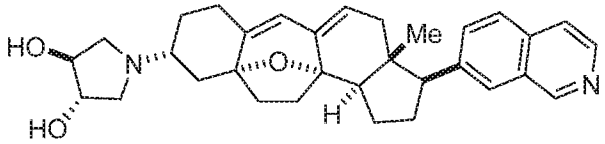
L_1 is a bond, –C(=O)–, –C(=O)O–, –C(=O)S–, –C(=O)N(R^L)–, or -N(R^L)-($C(R^{LL})_2)_p$-, wherein R^L is hydrogen, optionally substituted alkyl, or a nitrogen protecting group, each instance of R^{LL} is independently hydrogen, halogen, or optionally substituted alkyl, and p is 0, 1, or 2;

R^{B3} is hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl, provided that when L_1 is a bond, then R^{B3} is not hydrogen;

each instance of R^A is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, carbonyl, silyl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a nitrogen protecting group when attached to nitrogen, optionally when attached to N the two R^A groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring, and optionally when R^{B1} and R^{B2} are each –X^AR^A then two R^A groups may be joined to form an optionally substituted heterocyclyl ring; and

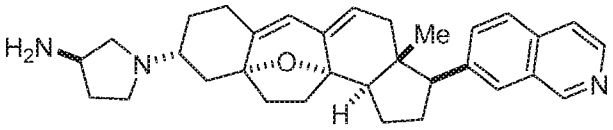
R^O is optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, -C(=O)R^A, -C(=O)OR^A, -C(=O)N(R^A)$_2$, or an oxygen protecting group.

In certain embodiments the compound used is selected from:



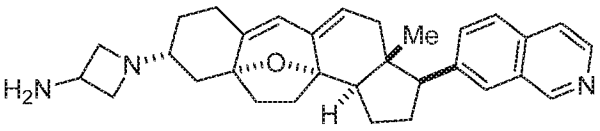
Compound 1

,



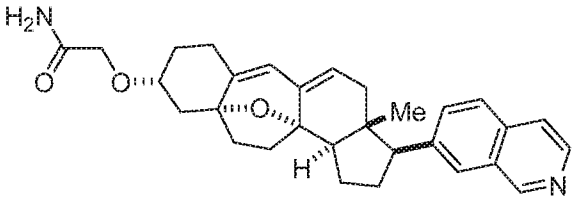
Compound 2

,



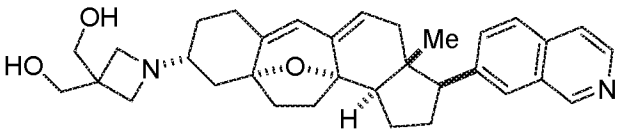
Compound 3

,



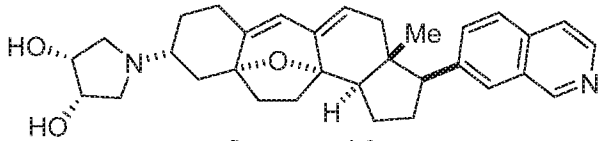
Compound 4

,



Compound 5

, and



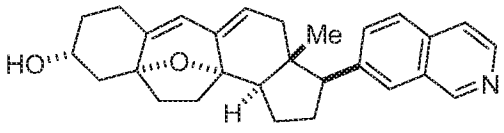
Compound 6

;

or a pharmaceutically acceptable salt thereof.

In one embodiment the cortistatin for use in the present invention is selected from the structures of Figure 23-38.

In another embodiment the compound used is:



Compound 7

;

or a pharmaceutically acceptable salt thereof.

Isotopic Derivatives

In one embodiment, the present invention utilizes compounds of Formula A, B, C, D, or E, and additional active compounds described herein, with at least one desired isotopic substitution of an atom, at an amount above the natural abundance of the isotope, *i.e.*, enriched. Isotopes are atoms having the same atomic number but different mass numbers, *i.e.*, the same number of protons but a different number of neutrons.

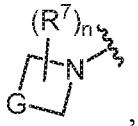
Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, and chlorine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{15}N, ^{18}F ^{31}P, ^{32}P, ^{35}S, ^{36}CI, ^{125}I respectively. The invention includes various isotopically labeled compounds as defined herein, for example those into which radioactive isotopes, such as ^{3}H, ^{13}C, and ^{14}C, are present. Such isotopically labelled compounds are useful in metabolic studies (with ^{14}C), reaction kinetic studies (with, for example ^{2}H or ^{3}H), detection or imaging techniques, such as positron emission tomography (PET) or single-photon emission computed tomography (SPECT) including drug or substrate tissue distribution assays, or in radioactive treatment of patients. In particular, an ^{18}F labeled compound may be particularly desirable for PET or SPECT studies. Isotopically labeled compounds of this invention and prodrugs thereof can generally be prepared by carrying out the procedures disclosed in the schemes or in the examples and preparations described below by substituting a readily available isotopically labeled reagent for a non-isotopically labeled reagent.

Embodiments of R^1 and R^2

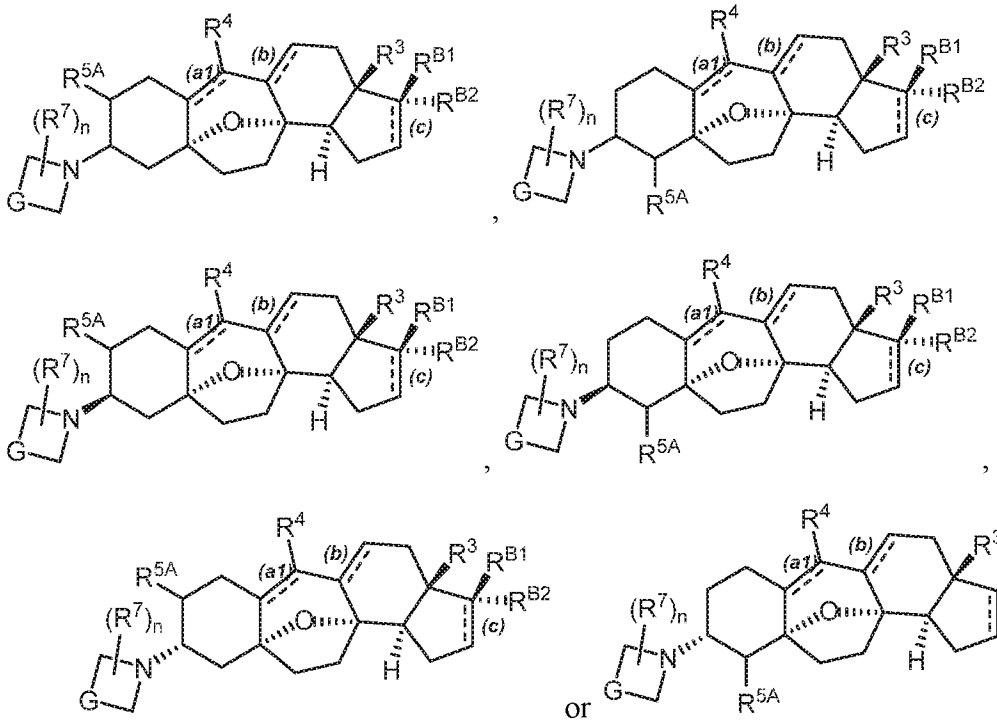
In certain embodiments, R^1 and R^2 are joined to form an optionally substituted heterocyclyl, *e.g.*, an optionally substituted 3-6 membered heterocyclyl. In certain embodiments, R^1 and R^2 are joined to form an optionally substituted 3-membered heterocyclyl, an optionally substituted 4-membered heterocyclyl, optionally substituted 5-membered heterocyclyl, or an optionally substituted 6-membered heterocyclyl. In certain embodiments, R^1 and R^2 are joined to form an optionally substituted 3-membered heterocyclyl, *i.e.*, an optionally substituted aziridinyl. In certain embodiments, R^1 and R^2 are joined to form an optionally substituted 4-membered heterocyclyl, *e.g.*, an optionally substituted azetidinyl. In certain embodiments, R^1 and R^2 are joined to form an optionally substituted 5-membered heterocyclyl, *e.g.*, an optionally substituted pyrrolidinyl or optionally substituted imidazolidine-2,4-dione. In certain embodiments, R^1 and R^2

are joined to form an optionally substituted 6-membered heterocyclyl, *e.g.*, an optionally substituted piperidinyl, optionally substituted tetrahydropyranyl, optionally substituted dihydropyridinyl, optionally substituted thianyl, optionally substituted piperazinyl, optionally substituted morpholinyl, optionally substituted dithianyl, optionally substituted dioxanyl, or optionally substituted triazinanyl.

For example, in certain embodiments, R^1 and R^2 are joined to form a group of formula:



, *e.g.*, to provide a compound of formula:



or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof, wherein ⋯⋯, R^3, R^4, R^{B1}, and R^{B2} are as defined herein; and

wherein:

G is -O-, -S-, -NH-, -NR^7-, -CH_2-, -$CH(R^7)$-, or -$C(R^7)_2$-;

R^{5A} is selected from hydrogen, halogen, and optionally substituted alkyl;

each instance of R^7 is independently halogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, amino,

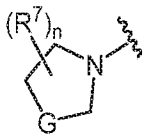
substituted amino, hydroxyl, substituted hydroxyl, thiol, substituted thiol, carbonyl, sulfonyl, sulfinyl, or a nitrogen protecting group when attached to a nitrogen atom;

optionally wherein two R^7 groups are joined to form an optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, an optionally substituted heteroaryl ring, or an oxo (=O) group; and

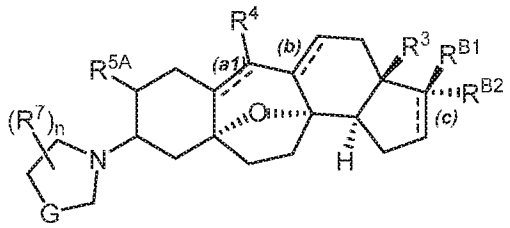
n is 0, 1, 2, 3, or 4.

In an alternative another embodiment R^{5A} is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, $-OR^A$, $-SR^A$, $-N(R^A)_2$, $-C(=O)R^A$, $-C(=O)OR^A$, $-C(=O)N(R^A)_2$, and $-S(=O)_2R^A$.

In certain embodiments, R^1 and R^2 are joined to form a group of formula:



, *e.g.*, to provide a compound of formula:



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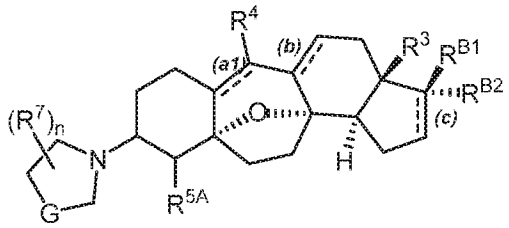

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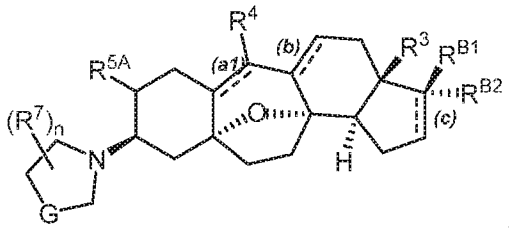

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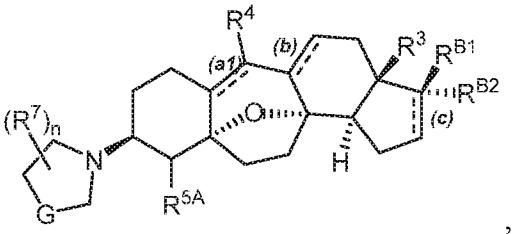

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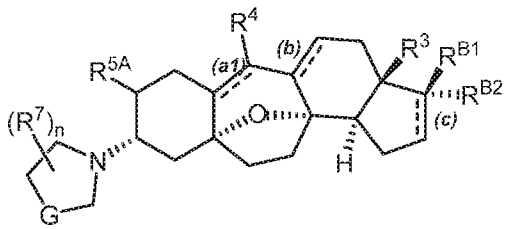
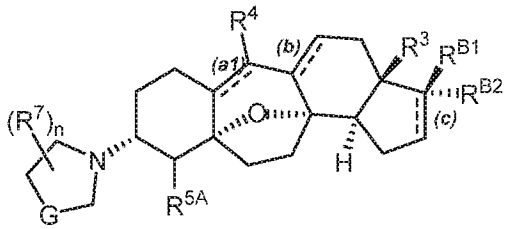


or



or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof, wherein ----, R^3, R^4, R^{5A}, R^{B1}, and R^{B2} are as defined herein; and

wherein:

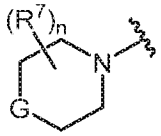
G is -O-, -S-, -NH-, -NR^7-, -CH_2-, -$CH(R^7)$-, or -$C(R^7)_2$-;

each instance of R^7 is independently halogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, amino, substituted amino, hydroxyl, substituted hydroxyl, thiol, substituted thiol, carbonyl, sulfonyl, sulfinyl, or a nitrogen protecting group when attached to a nitrogen atom;

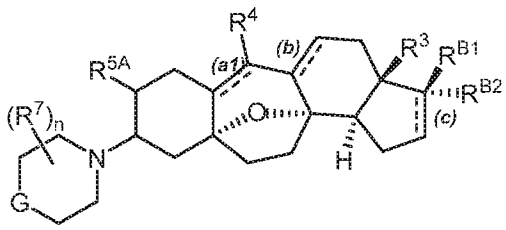
optionally wherein two R^7 groups are joined to form an optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, an optionally substituted heteroaryl ring, or an oxo (=O) group; and

n is 0, 1, 2, 3, or 4.

In certain embodiments, R^1 and R^2 are joined to form a group of formula:



, *e.g.*, to provide a compound of formula:



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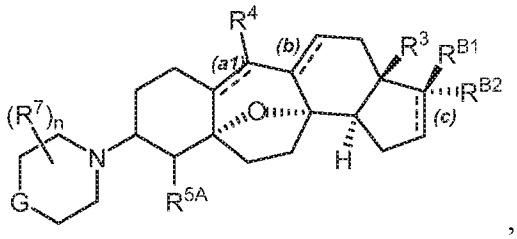

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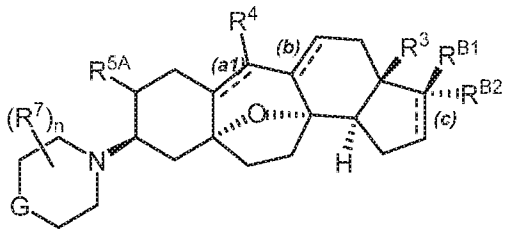

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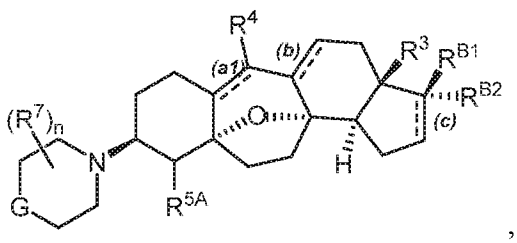

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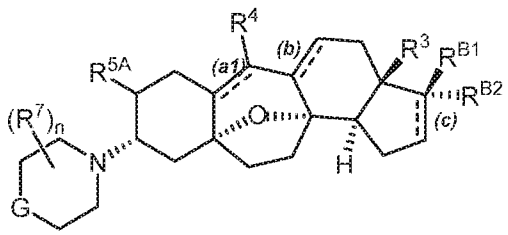
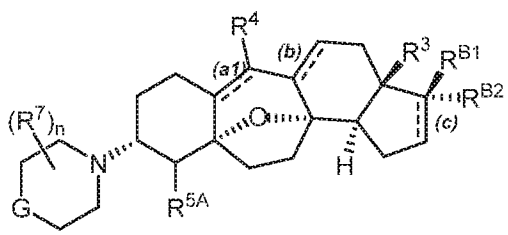


or



or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof, wherein ——, R^3, R^4, R^{5A}, R^{B1}, and R^{B2} are as defined herein; and

wherein:

G is -O-, -S-, -NH-, -NR^7-, -CH_2-, -$CH(R^7)$-, or -$C(R^7)_2$-;

each instance of R^7 is independently halogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, amino, substituted amino, hydroxyl, substituted hydroxyl, thiol, substituted thiol, carbonyl, sulfonyl, sulfinyl, or a nitrogen protecting group when attached to a nitrogen atom;

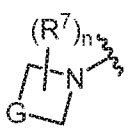
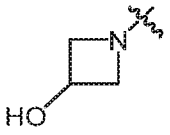
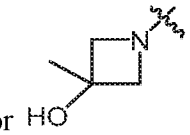
optionally wherein two R^7 groups are joined to form an optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, an optionally substituted heteroaryl ring, or an oxo (=O) group; and

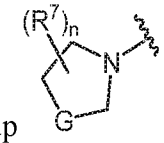
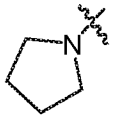
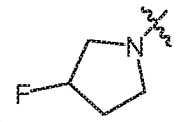
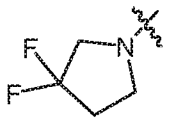
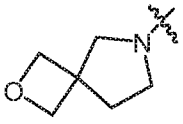
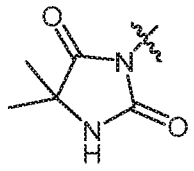
n is 0, 1, 2, 3, or 4.

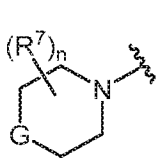
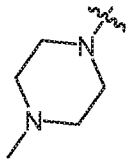
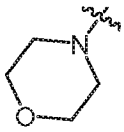
In certain embodiments, n is 0, and the ring system formed by the joining of R^1 and R^2 is not substituted with an R^7 group as defined herein. In certain embodiments, n is 1, 2, 3, or 4, and the ring system is substituted with 1, 2, 3, or 4 R^7 groups as defined herein. In certain embodiments, n is 1. In certain embodiments, n is 2. In certain embodiments, n is 3. In certain embodiments, n is 4.

In certain embodiments, wherein n is not 0 (*i.e.*, n is 1, 2, 3, or 4) and at least one R^7 is attached to a carbon atom, the R^7 is halogen (*e.g.*, fluoro), hydroxyl, substituted hydroxyl, or carbonyl (*e.g.*, -CO_2H). In certain embodiments, wherein n is not 0 (*i.e.*, n is 1, 2, 3, or 4) and two R^7 groups are attached to the same carbon atom, the two R^7 groups are each halogen, *e.g.*, fluoro. In certain embodiments, wherein n is not 0 (*i.e.*, n is 1, 2, 3, or 4) and two R^7 groups are attached to the same carbon atom, the two R^7 groups are joined to form an optionally substituted carbocyclyl ring or optionally substituted heterocyclyl ring (*e.g.*, optionally substituted oxetanyl ring). In certain embodiments, wherein n is not 0 (*i.e.*, n is 1, 2, 3, or 4) and two R^7 groups are attached to a different carbon atom, the two R^7 groups are joined to form an optionally substituted carbocyclyl ring or optionally substituted heterocyclyl ring.

In certain embodiments, G is -O-. In certain embodiments, G is -NR^7-, *e.g.*, wherein R^7 is optionally substituted alkyl (*e.g.*, -CH_3). In certain embodiments, G is -$CH(R^7)$- or -$C(R^7)_2$- wherein at least one R^7 is hydroxyl, substituted hydroxyl, or carbonyl (*e.g.*, -CO_2H).

In certain embodiments, the group  is  ,  , or  .

In certain embodiments, the group  is  ,  ,  ,  ,  , or  .

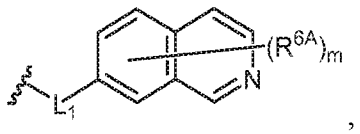
In certain embodiments, the group  is  or  .

In certain embodiments, R^1 is $-S(=O)_2R^A$ and R^2 is optionally substituted alkyl.

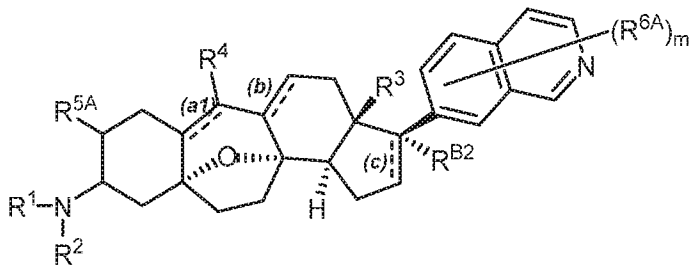
Exemplary compounds

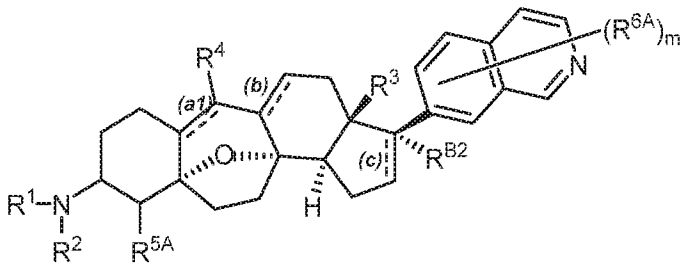
Various combinations of certain embodiments are further contemplated herein.

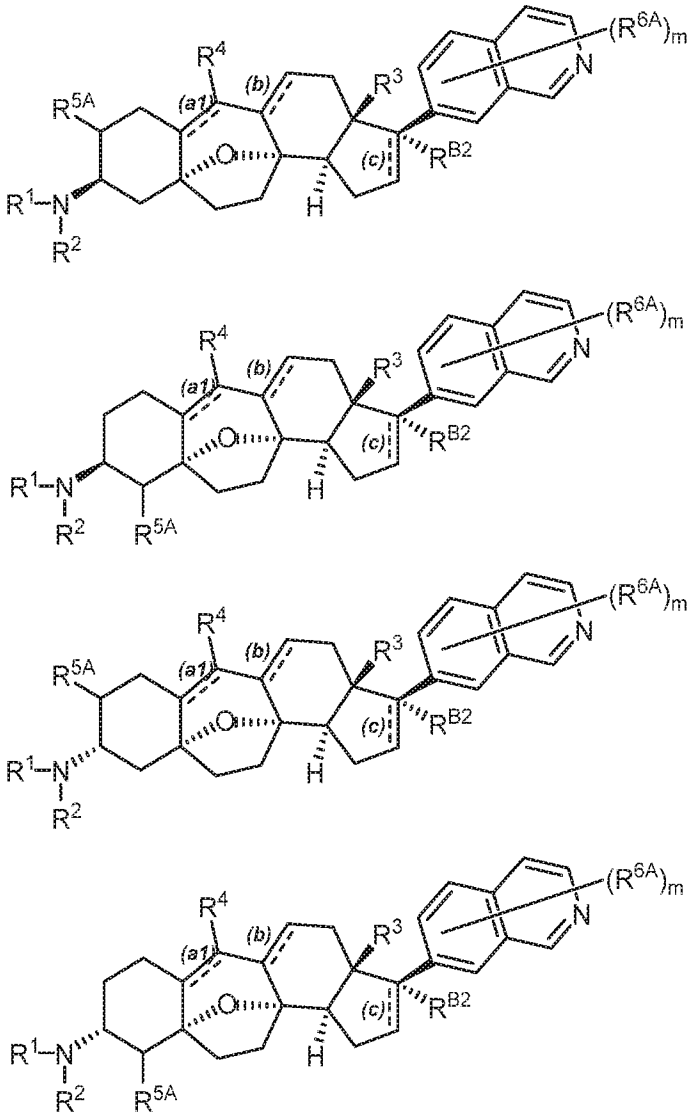
For example, in certain embodiments wherein the group $-L_1-R^{B3}$ is a group of formula:

 ,

and wherein L_1 is a bond, provided are compounds of Formula:







or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof; wherein ....., R^N, R^1, R^2, R^3, R^4, R^{5A}, and m are as defined herein;

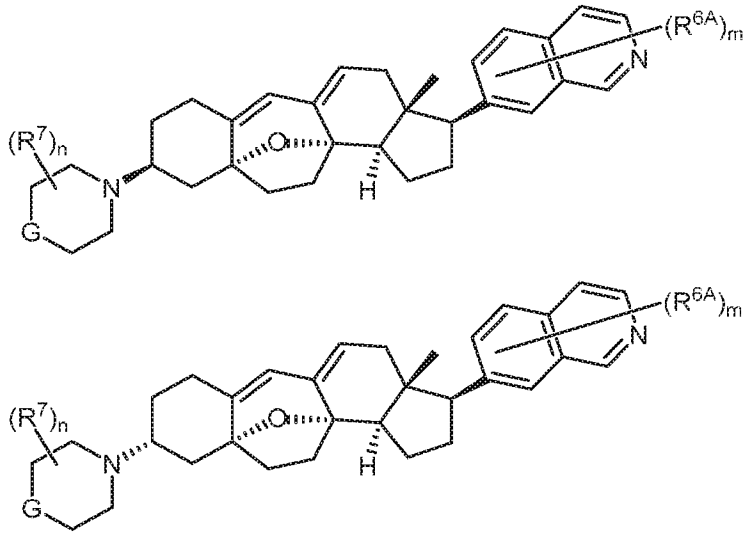
wherein

each instance of R^{6A} is independently halogen, $-NO_2$, -CN, $-OR^{6C}$, $-SR^{6C}$, $-N(R^{6C})_2$, $-C(=O)R^{6C}$, $-C(=O)OR^{6C}$, $-C(=O)N(R^{6C})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and

each instance of R^{6C} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a

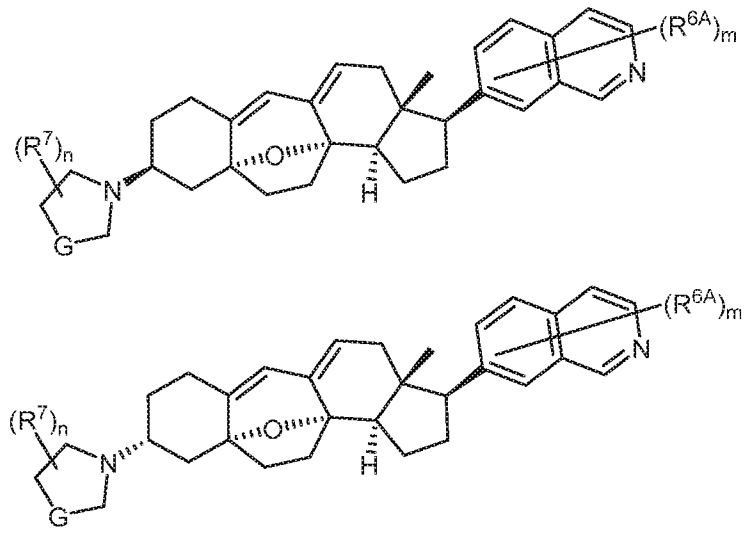
nitrogen protecting group when attached to nitrogen, optionally when attached to N the two R^{6C} groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring.

In certain embodiments, wherein R^1 and R^2 are joined to form an optionally substituted heterocyclyl, provided is a compound of Formula:



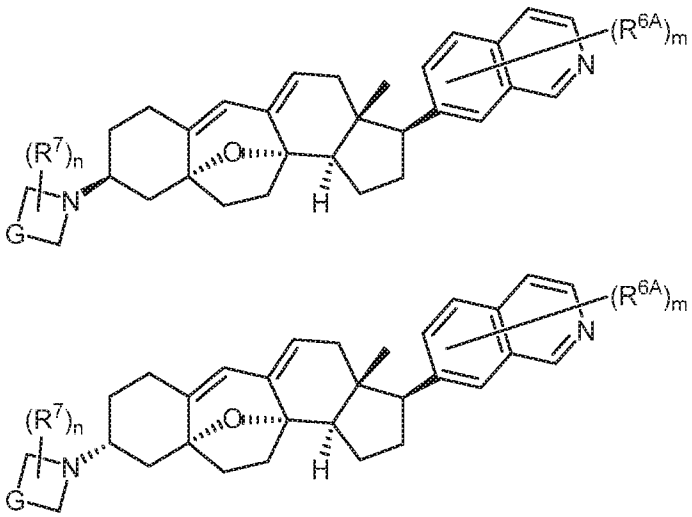
or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof; wherein R^7, R^{6A}, n and m are as defined herein. In certain embodiments, G is O. In certain embodiments, G is N-CH_3. In certain embodiments, m is 0. In certain embodiments, m is 1. In certain embodiments, n is 0. In certain embodiments, n is 1.

In certain embodiments, wherein R^1 and R^2 are joined to form an optionally substituted heterocyclyl, provided is a compound of Formula:



or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof; wherein R^7, R^{6A}, n and m are as defined herein. In certain embodiments, G is not CH_2. In certain embodiments, m is 0. In certain embodiments, m is 1. In certain embodiments, n is 0. In certain embodiments, n is 1.

In certain embodiments, wherein R^1 and R^2 are joined to form an optionally substituted heterocyclyl, provided is a compound of Formula:



or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof; wherein R^7, R^{6A}, n and m are as defined herein. In certain embodiments, G is $-CH_2-$. In certain embodiments, m is 0. In certain embodiments, m is 1. In certain embodiments, n is 0. In certain embodiments, n is 1.

B. Chemical Definitions

Definitions of specific functional groups and chemical terms are described in more detail below. The chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in *Organic Chemistry*, Thomas Sorrell, University Science Books, Sausalito, 1999; Smith and March *March's Advanced Organic Chemistry*, 5th Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; and

Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987.

Compounds described herein can comprise one or more asymmetric centers, and thus can exist in various stereoisomeric forms, *e.g.*, enantiomers and/or diastereomers. Also contemplated are stereoisomers featuring either a Z or E configuration, or mixture thereof, about a double bond. For example, the compounds described herein can be in the form of an individual enantiomer, diastereomer or geometric isomer, or can be in the form of a mixture of stereoisomers, including racemic mixtures and mixtures enriched in one or more stereoisomer. Isomers can be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or preferred isomers can be prepared by asymmetric syntheses. See, for example, Jacques *et al.*, *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen *et al.*, *Tetrahedron* 33:2725 (1977); Eliel, E.L. *Stereochemistry of Carbon Compounds* (McGraw–Hill, NY, 1962); and Wilen, S.H. *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972). The invention additionally encompasses compounds as individual isomers substantially free of other isomers, and alternatively, as mixtures of various isomers.

Isomeric mixtures containing any of a variety of isomer ratios may be utilized in accordance with the present invention. For example, where only two isomers are combined, mixtures containing 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 96:4, 97:3, 98:2, 99:1, or 100:0 isomer ratios are all contemplated by the present invention. Those of ordinary skill in the art will readily appreciate that analogous ratios are contemplated for more complex isomer mixtures. The mixture may contain two enantiomers, two diastereomers, or a mixture of diastereomers and enantiomers.

If, for instance, a particular enantiomer of a compound described herein is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. In some embodiments, a compound described herein is prepared by asymmetric synthesis with an enzyme. Enantiomers and diastereomers may be separated by means of fractional crystallization or chromatography (e.g., HPLC with a chiral column). Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base,

followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium or tritium, replacement of ^{19}F with ^{18}F, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of the disclosure. Such compounds are useful, for example, as analytical tools or probes in biological assays.

When a range of values is listed, it is intended to encompass each value and sub–range within the range. For example, "C_{1-6} alkyl" is intended to encompass, C_1, C_2, C_3, C_4, C_5, C_6, C_{1-6}, C_{1-5}, C_{1-4}, C_{1-3}, C_{1-2}, C_{2-6}, C_{2-5}, C_{2-4}, C_{2-3}, C_{3-6}, C_{3-5}, C_{3-4}, C_{4-6}, C_{4-5}, and C_{5-6} alkyl.

The term "aliphatic," as used herein, refers to alkyl, alkenyl, alkynyl, and carbocyclic groups.

As used herein, "alkyl" refers to a radical of a straight–chain or branched saturated hydrocarbon group having from 1 to 10 carbon atoms ("C_{1-10} alkyl"). In some embodiments, an alkyl group has 1 to 9 carbon atoms ("C_{1-9} alkyl"). In some embodiments, an alkyl group has 1 to 8 carbon atoms ("C_{1-8} alkyl"). In some embodiments, an alkyl group has 1 to 7 carbon atoms ("C_{1-7} alkyl"). In some embodiments, an alkyl group has 1 to 6 carbon atoms ("C_{1-6} alkyl"). In some embodiments, an alkyl group has 1 to 5 carbon atoms ("C_{1-5} alkyl"). In some embodiments, an alkyl group has 1 to 4 carbon atoms ("C_{1-4} alkyl"). In some embodiments, an alkyl group has 1 to 3 carbon atoms ("C_{1-3} alkyl"). In some embodiments, an alkyl group has 1 to 2 carbon atoms ("C_{1-2} alkyl"). In some embodiments, an alkyl group has 1 carbon atom ("C_1 alkyl"). In some embodiments, an alkyl group has 2 to 6 carbon atoms ("C_{2-6} alkyl"). Examples of C_{1-6} alkyl groups include methyl (C_1), ethyl (C_2), n–propyl (C_3), isopropyl (C_3), n–butyl (C_4), tert–butyl (C_4), sec–butyl (C_4), iso–butyl (C_4), n–pentyl (C_5), 3–pentanyl (C_5), amyl (C_5), neopentyl (C_5), 3–methyl–2–butanyl (C_5), tertiary amyl (C_5), and n–hexyl (C_6). Additional examples of alkyl groups include n–heptyl (C_7), n–octyl (C_8) and the like. Unless otherwise specified, each instance of an alkyl group is independently unsubstituted (an "unsubstituted alkyl") or substituted (a "substituted alkyl") with one or more substituents. In certain embodiments, the alkyl group is an unsubstituted C_{1-10} alkyl (*e.g.*, $–CH_3$). In certain embodiments, the alkyl group is a substituted C_{1-10} alkyl.

As used herein, "haloalkyl" is a substituted alkyl group as defined herein wherein one or more of the hydrogen atoms are independently replaced by a halogen, *e.g.,* fluoro, bromo, chloro, or iodo. "Perhaloalkyl" is a subset of haloalkyl, and refers to an alkyl group wherein all of the hydrogen atoms are independently replaced by a halogen, *e.g.,* fluoro, bromo, chloro, or iodo. In some embodiments, the haloalkyl moiety has 1 to 8 carbon atoms ("C_{1-8} haloalkyl"). In some embodiments, the haloalkyl moiety has 1 to 6 carbon atoms ("C_{1-6} haloalkyl"). In some embodiments, the haloalkyl moiety has 1 to 4 carbon atoms ("C_{1-4} haloalkyl"). In some embodiments, the haloalkyl moiety has 1 to 3 carbon atoms ("C_{1-3} haloalkyl"). In some embodiments, the haloalkyl moiety has 1 to 2 carbon atoms ("C_{1-2} haloalkyl"). In some embodiments, all of the haloalkyl hydrogen atoms are replaced with fluoro to provide a perfluoroalkyl group. In some embodiments, all of the haloalkyl hydrogen atoms are replaced with chloro to provide a "perchloroalkyl" group. Examples of haloalkyl groups include $-CF_3$, $-CF_2CF_3$, $-CF_2CF_2CF_3$, $-CCl_3$, $-CFCl_2$, $-CF_2Cl$, and the like.

As used herein, "alkenyl" refers to a radical of a straight–chain or branched hydrocarbon group having from 2 to 10 carbon atoms and one or more carbon-carbon double bonds (*e.g.,* 1, 2, 3, or 4 double bonds). In some embodiments, an alkenyl group has 2 to 9 carbon atoms ("C_{2-9} alkenyl"). In some embodiments, an alkenyl group has 2 to 8 carbon atoms ("C_{2-8} alkenyl"). In some embodiments, an alkenyl group has 2 to 7 carbon atoms ("C_{2-7} alkenyl"). In some embodiments, an alkenyl group has 2 to 6 carbon atoms ("C_{2-6} alkenyl"). In some embodiments, an alkenyl group has 2 to 5 carbon atoms ("C_{2-5} alkenyl"). In some embodiments, an alkenyl group has 2 to 4 carbon atoms ("C_{2-4} alkenyl"). In some embodiments, an alkenyl group has 2 to 3 carbon atoms ("C_{2-3} alkenyl"). In some embodiments, an alkenyl group has 2 carbon atoms ("C_2 alkenyl"). The one or more carbon–carbon double bonds can be internal (such as in 2–butenyl) or terminal (such as in 1–butenyl). Examples of C_{2-4} alkenyl groups include ethenyl (C_2), 1–propenyl (C_3), 2–propenyl (C_3), 1–butenyl (C_4), 2–butenyl (C_4), butadienyl (C_4), and the like. Examples of C_{2-6} alkenyl groups include the aforementioned C_{2-4} alkenyl groups as well as pentenyl (C_5), pentadienyl (C_5), hexenyl (C_6), and the like. Additional examples of alkenyl include heptenyl (C_7), octenyl (C_8), octatrienyl (C_8), and the like. Unless otherwise specified, each instance of an alkenyl group is independently unsubstituted (an "unsubstituted alkenyl") or substituted (a "substituted alkenyl") with one or more substituents. In certain embodiments, the

alkenyl group is an unsubstituted C_{2-10} alkenyl. In certain embodiments, the alkenyl group is a substituted C_{2-10} alkenyl.

As used herein, "alkynyl" refers to a radical of a straight–chain or branched hydrocarbon group having from 2 to 10 carbon atoms and one or more carbon-carbon triple bonds (*e.g.*, 1, 2, 3, or 4 triple bonds) ("C_{2-10} alkynyl"). In some embodiments, an alkynyl group has 2 to 9 carbon atoms ("C_{2-9} alkynyl"). In some embodiments, an alkynyl group has 2 to 8 carbon atoms ("C_{2-8} alkynyl"). In some embodiments, an alkynyl group has 2 to 7 carbon atoms ("C_{2-7} alkynyl"). In some embodiments, an alkynyl group has 2 to 6 carbon atoms ("C_{2-6} alkynyl"). In some embodiments, an alkynyl group has 2 to 5 carbon atoms ("C_{2-5} alkynyl"). In some embodiments, an alkynyl group has 2 to 4 carbon atoms ("C_{2-4} alkynyl"). In some embodiments, an alkynyl group has 2 to 3 carbon atoms ("C_{2-3} alkynyl"). In some embodiments, an alkynyl group has 2 carbon atoms ("C_2 alkynyl"). The one or more carbon–carbon triple bonds can be internal (such as in 2–butynyl) or terminal (such as in 1–butynyl). Examples of C_{2-4} alkynyl groups include, without limitation, ethynyl (C_2), 1–propynyl (C_3), 2–propynyl (C_3), 1–butynyl (C_4), 2–butynyl (C_4), and the like. Examples of C_{2-6} alkenyl groups include the aforementioned C_{2-4} alkynyl groups as well as pentynyl (C_5), hexynyl (C_6), and the like. Additional examples of alkynyl include heptynyl (C_7), octynyl (C_8), and the like. Unless otherwise specified, each instance of an alkynyl group is independently unsubstituted (an "unsubstituted alkynyl") or substituted (a "substituted alkynyl") with one or more substituents. In certain embodiments, the alkynyl group is an unsubstituted C_{2-10} alkynyl. In certain embodiments, the alkynyl group is a substituted C_{2-10} alkynyl.

As used herein, "carbocyclyl" or "carbocyclic" refers to a radical of a non–aromatic cyclic hydrocarbon group having from 3 to 14 ring carbon atoms ("C_{3-14} carbocyclyl") and zero heteroatoms in the non–aromatic ring system. In some embodiments, a carbocyclyl group has 3 to 10 ring carbon atoms ("C_{3-10} carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 9 ring carbon atoms ("C_{3-9} carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 8 ring carbon atoms ("C_{3-8} carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 7 ring carbon atoms ("C_{3-7} carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 6 ring carbon atoms ("C_{3-6} carbocyclyl"). In some embodiments, a carbocyclyl group has 4 to 6 ring carbon atoms ("C_{4-6} carbocyclyl"). In some embodiments, a carbocyclyl group has 5 to 6 ring carbon atoms ("C_{5-6} carbocyclyl"). In some embodiments, a carbocyclyl group has 5 to 10 ring carbon atoms ("C_{5-10} carbocyclyl"). Exemplary C_{3-6} carbocyclyl groups include, without

limitation, cyclopropyl (C_3), cyclopropenyl (C_3), cyclobutyl (C_4), cyclobutenyl (C_4), cyclopentyl (C_5), cyclopentenyl (C_5), cyclohexyl (C_6), cyclohexenyl (C_6), cyclohexadienyl (C_6), and the like. Exemplary C_{3-8} carbocyclyl groups include, without limitation, the aforementioned C_{3-6} carbocyclyl groups as well as cycloheptyl (C_7), cycloheptenyl (C_7), cycloheptadienyl (C_7), cycloheptatrienyl (C_7), cyclooctyl (C_8), cyclooctenyl (C_8), bicyclo[2.2.1]heptanyl (C_7), bicyclo[2.2.2]octanyl (C_8), and the like. Exemplary C_{3-10} carbocyclyl groups include, without limitation, the aforementioned C_{3-8} carbocyclyl groups as well as cyclononyl (C_9), cyclononenyl (C_9), cyclodecyl (C_{10}), cyclodecenyl (C_{10}), octahydro–1*H*–indenyl (C_9), decahydronaphthalenyl (C_{10}), spiro[4.5]decanyl (C_{10}), and the like. As the foregoing examples illustrate, in certain embodiments, the carbocyclyl group is either monocyclic ("monocyclic carbocyclyl") or polycyclic (*e.g.,* containing a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic carbocyclyl") or tricyclic system ("tricyclic carbocyclyl")) and can be saturated or can contain one or more carbon–carbon double or triple bonds. "Carbocyclyl" also includes ring systems wherein the carbocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups wherein the point of attachment is on the carbocyclyl ring, and in such instances, the number of carbons continue to designate the number of carbons in the carbocyclic ring system. Unless otherwise specified, each instance of a carbocyclyl group is independently unsubstituted (an "unsubstituted carbocyclyl") or substituted (a "substituted carbocyclyl") with one or more substituents. In certain embodiments, the carbocyclyl group is an unsubstituted C_{3-14} carbocyclyl. In certain embodiments, the carbocyclyl group is a substituted C_{3-14} carbocyclyl.

As used herein, "heterocyclyl" or "heterocyclic" refers to a radical of a 3– to 14–membered non–aromatic ring system having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("3–14 membered heterocyclyl"). In heterocyclyl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. A heterocyclyl group can either be monocyclic ("monocyclic heterocyclyl") or polycyclic (*e.g.,* a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic heterocyclyl") or tricyclic system ("tricyclic heterocyclyl")), and can be saturated or can contain one or more carbon–carbon double or triple bonds. Heterocyclyl polycyclic ring systems can include one or more heteroatoms in one or both rings. "Heterocyclyl" also includes ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more carbocyclyl groups wherein the point of attachment is either on the

carbocyclyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. Unless otherwise specified, each instance of heterocyclyl is independently unsubstituted (an "unsubstituted heterocyclyl") or substituted (a "substituted heterocyclyl") with one or more substituents. In certain embodiments, the heterocyclyl group is an unsubstituted 3–14 membered heterocyclyl. In certain embodiments, the heterocyclyl group is a substituted 3–14 membered heterocyclyl.

As used herein, "aryl" refers to a radical of a monocyclic or polycyclic (*e.g.*, bicyclic or tricyclic) 4n+2 aromatic ring system (*e.g.*, having 6, 10, or 14 electrons shared in a cyclic array) having 6–14 ring carbon atoms and zero heteroatoms provided in the aromatic ring system ("C_{6-14} aryl"). In some embodiments, an aryl group has 6 ring carbon atoms ("C_6 aryl"; *e.g.*, phenyl). In some embodiments, an aryl group has 10 ring carbon atoms ("C_{10} aryl"; *e.g.*, naphthyl such as 1–naphthyl and 2–naphthyl). In some embodiments, an aryl group has 14 ring carbon atoms ("C_{14} aryl"; *e.g.*, anthracyl). "Aryl" also includes ring systems wherein the aryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the radical or point of attachment is on the aryl ring, and in such instances, the number of carbon atoms continue to designate the number of carbon atoms in the aryl ring system. Unless otherwise specified, each instance of an aryl group is independently unsubstituted (an "unsubstituted aryl") or substituted (a "substituted aryl") with one or more substituents. In certain embodiments, the aryl group is an unsubstituted C_{6-14} aryl. In certain embodiments, the aryl group is a substituted C_{6-14} aryl.

As used herein, "heteroaryl" refers to a radical of a 5–14 membered monocyclic or polycyclic (*e.g.*, bicyclic, tricyclic) 4n+2 aromatic ring system (*e.g.*, having 6, 10, or 14 π electrons shared in a cyclic array) having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–14 membered heteroaryl"). In heteroaryl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. Heteroaryl polycyclic ring systems can include one or more heteroatoms in one or both rings. "Heteroaryl" includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the point of attachment is on the heteroaryl ring, and in such instances, the number of ring members continue to designate the number of ring members

in the heteroaryl ring system. "Heteroaryl" also includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is either on the aryl or heteroaryl ring, and in such instances, the number of ring members designates the number of ring members in the fused polycyclic (aryl/heteroaryl) ring system. Polycyclic heteroaryl groups wherein one ring does not contain a heteroatom (*e.g.,* indolyl, quinolinyl, carbazolyl, and the like) the point of attachment can be on either ring, *i.e.,* either the ring bearing a heteroatom (*e.g.,* 2–indolyl) or the ring that does not contain a heteroatom (*e.g.,* 5–indolyl).

In some embodiments, a heteroaryl group is a 5–10 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–10 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5–8 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–8 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5–6 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–6 membered heteroaryl"). In some embodiments, the 5–6 membered heteroaryl has 1–3 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5–6 membered heteroaryl has 1–2 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5–6 membered heteroaryl has 1 ring heteroatom selected from nitrogen, oxygen, and sulfur. Unless otherwise specified, each instance of a heteroaryl group is independently unsubstituted (an "unsubstituted heteroaryl") or substituted (a "substituted heteroaryl") with one or more substituents. In certain embodiments, the heteroaryl group is an unsubstituted 5–14 membered heteroaryl. In certain embodiments, the heteroaryl group is a substituted 5–14 membered heteroaryl.

Exemplary 5–membered heteroaryl groups containing 1 heteroatom include, without limitation, pyrrolyl, furanyl and thiophenyl. Exemplary 5–membered heteroaryl groups containing 2 heteroatoms include, without limitation, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. Exemplary 5–membered heteroaryl groups containing 3 heteroatoms include, without limitation, triazolyl, oxadiazolyl, and thiadiazolyl. Exemplary 5–membered heteroaryl groups containing 4 heteroatoms include, without limitation, tetrazolyl. Exemplary 6–membered

heteroaryl groups containing 1 heteroatom include, without limitation, pyridinyl. Exemplary 6–membered heteroaryl groups containing 2 heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6–membered heteroaryl groups containing 3 or 4 heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively. Exemplary 7–membered heteroaryl groups containing 1 heteroatom include, without limitation, azepinyl, oxepinyl, and thiepinyl. Exemplary 5,6–bicyclic heteroaryl groups include, without limitation, indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothiophenyl, isobenzothiophenyl, benzofuranyl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzoxadiazolyl, benzthiazolyl, benzisothiazolyl, benzthiadiazolyl, indolizinyl, and purinyl. Exemplary 6,6–bicyclic heteroaryl groups include, without limitation, naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, cinnolinyl, quinoxalinyl, phthalazinyl, and quinazolinyl. Exemplary tricyclic heteroaryl groups include, without limitation, phenanthridinyl, dibenzofuranyl, carbazolyl, acridinyl, phenothiazinyl, phenoxazinyl and phenazinyl.

As used herein, the term "partially unsaturated" refers to a ring moiety that includes at least one double or triple bond. The term "partially unsaturated" is intended to encompass rings having multiple sites of unsaturation, but is not intended to include aromatic groups (*e.g.,* aryl or heteroaryl moieties) as herein defined.

As used herein, the term "saturated" refers to a ring moiety that does not contain a double or triple bond, *i.e.*, the ring contains all single bonds.

As understood from the above, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl groups, as defined herein, are, in certain embodiments, optionally substituted. Optionally substituted refers to a group which may be substituted or unsubstituted (*e.g.,* "substituted" or "unsubstituted" alkyl, "substituted" or "unsubstituted" alkenyl, "substituted" or "unsubstituted" alkynyl, "substituted" or "unsubstituted" heteroalkyl, "substituted" or "unsubstituted" heteroalkenyl, "substituted" or "unsubstituted" "substituted" or "unsubstituted" carbocyclyl, "substituted" or "unsubstituted" heterocyclyl, "substituted" or "unsubstituted" aryl or "substituted" or "unsubstituted" heteroaryl group). In general, the term "substituted" means that at least one hydrogen present on a group is replaced with a permissible substituent, *e.g.,* a substituent which upon substitution results in a stable compound, *e.g.,* a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction. Unless otherwise indicated, a

"substituted" group has a substituent at one or more substitutable positions of the group, and when more than one position in any given structure is substituted, the substituent is either the same or different at each position. The present invention contemplates any and all such combinations in order to arrive at a stable compound. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any suitable substituent as described herein which satisfy the valencies of the heteroatoms and results in the formation of a stable moiety.

Exemplary substituents include, but are not limited to, halogen, $-CN$, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, $-OH$, $-OR^{aa}$, $-ON(R^{bb})_2$, $-N(R^{bb})_2$, $-N(R^{bb})_3^+X^-$, $-N(OR^{cc})R^{bb}$, $-SH$, $-SR^{aa}$, $-SSR^{cc}$, $-C(=O)R^{aa}$, $-CO_2H$, $-CHO$, $-C(OR^{cc})_2$, $-CO_2R^{aa}$, $-OC(=O)R^{aa}$, $-OCO_2R^{aa}$, $-C(=O)N(R^{bb})_2$, $-OC(=O)N(R^{bb})_2$, $-NR^{bb}C(=O)R^{aa}$, $-NR^{bb}CO_2R^{aa}$, $-NR^{bb}C(=O)N(R^{bb})_2$, $-C(=NR^{bb})R^{aa}$, $-C(=NR^{bb})OR^{aa}$, $-OC(=NR^{bb})R^{aa}$, $-OC(=NR^{bb})OR^{aa}$, $-C(=NR^{bb})N(R^{bb})_2$, $-OC(=NR^{bb})N(R^{bb})_2$, $-NR^{bb}C(=NR^{bb})N(R^{bb})_2$, $-C(=O)NR^{bb}SO_2R^{aa}$, $-NR^{bb}SO_2R^{aa}$, $-SO_2N(R^{bb})_2$, $-SO_2R^{aa}$, $-SO_2OR^{aa}$, $-OSO_2R^{aa}$, $-S(=O)R^{aa}$, $-OS(=O)R^{aa}$, $-Si(R^{aa})_3$, $-OSi(R^{aa})_3$ $-C(=S)N(R^{bb})_2$, $-C(=O)SR^{aa}$, $-C(=S)SR^{aa}$, $-SC(=S)SR^{aa}$, $-SC(=O)SR^{aa}$, $-OC(=O)SR^{aa}$, $-SC(=O)OR^{aa}$, $-SC(=O)R^{aa}$, $-P(=O)(R^{aa})_2$, $-OP(=O)(R^{aa})_2$, $-OP(=O)(OR^{cc})_2$, $-NR^{bb}P(=O)(OR^{cc})_2$, $-P(R^{cc})_2$, $-OP(R^{cc})_2$, $-B(R^{aa})_2$, $-B(OR^{cc})_2$, $-BR^{aa}(OR^{cc})$, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, heteroC_{1-10} alkyl, heteroC_{2-10} alkenyl, heteroC_{2-10} alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl;

or two geminal hydrogens on a carbon atom are replaced with the group =O, =S, $=NN(R^{bb})_2$, $=NNR^{bb}C(=O)R^{aa}$, $=NNR^{bb}C(=O)OR^{aa}$, $=NNR^{bb}S(=O)_2R^{aa}$, $=NR^{bb}$, or $=NOR^{cc}$;

each instance of R^{aa} is, independently, selected from C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, heteroC_{1-10} alkyl, heteroC_{2-10}alkenyl, heteroC_{2-10}alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl, or two R^{aa} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring;

each instance of R^{bb} is, independently, selected from hydrogen, $-OH$, $-OR^{aa}$, $-N(R^{cc})_2$, $-CN$, $-C(=O)R^{aa}$, $-C(=O)N(R^{cc})_2$, $-CO_2R^{aa}$, $-SO_2R^{aa}$, $-C(=NR^{cc})OR^{aa}$, $-C(=NR^{cc})N(R^{cc})_2$, $-SO_2N(R^{cc})_2$, $-SO_2R^{cc}$, $-SO_2OR^{cc}$, $-SOR^{aa}$, $-C(=S)N(R^{cc})_2$, $-C(=O)SR^{cc}$, $-C(=S)SR^{cc}$ $-P(=O)(R^{aa})_2$, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, heteroC_{1-10}alkyl, heteroC_{2-10}alkenyl, heteroC_{2-10}alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl, or two R^{bb} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring;

each instance of R^{cc} is, independently, selected from hydrogen, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, heteroC_{1-10} alkyl, heteroC_{2-10} alkenyl, heteroC_{2-10} alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl, or two R^{cc} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring.

In certain embodiments, an exemplary substituent is selected from the group consisting of halogen, –CN, $–NO_2$, $–N_3$, $–SO_2H$, $–SO_3H$, –OH, $–OR^{aa}$, $–N(R^{bb})_2$, –SH, $–SR^{aa}$, $–SSR^{cc}$, $–C(=O)R^{aa}$, $–CO_2H$, –CHO, $–CO_2R^{aa}$, $–OC(=O)R^{aa}$, $–OCO_2R^{aa}$, $–C(=O)N(R^{bb})_2$, $–OC(=O)N(R^{bb})_2$, $–NR^{bb}C(=O)R^{aa}$, $–NR^{bb}CO_2R^{aa}$, $–NR^{bb}C(=O)N(R^{bb})_2$,$–C(=O)NR^{bb}SO_2R^{aa}$, $–NR^{bb}SO_2R^{aa}$, $–SO_2N(R^{bb})_2$, $–SO_2R^{aa}$, $–S(=O)R^{aa}$, C_{1-10} alkyl, C_{1-10} perhaloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, heteroC_{1-10} alkyl, heteroC_{2-10} alkenyl, heteroC_{2-10} alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl.

As used herein, the term "halo" or "halogen" refers to fluorine (fluoro, –F), chlorine (chloro, –Cl), bromine (bromo, –Br), or iodine (iodo, –I).

As used herein, a "counterion" is a negatively charged group associated with a positively charged quarternary amine in order to maintain electronic neutrality. Exemplary counterions include halide ions (*e.g.,* F^-, Cl^-, Br^-, I^-), NO_3^-, ClO_4^-, OH^-, $H_2PO_4^-$, HSO_4^-, sulfonate ions (*e.g.,* methansulfonate, trifluoromethanesulfonate, p–toluenesulfonate, benzenesulfonate, 10–camphor sulfonate, naphthalene–2–sulfonate, naphthalene–1–sulfonic acid–5–sulfonate, ethan–1–sulfonic acid–2–sulfonate, and the like), and carboxylate ions (*e.g.,* acetate, ethanoate, propanoate, benzoate, glycerate, lactate, tartrate, glycolate, and the like).

As used herein, a "leaving group" is an art–understood term referring to a molecular fragment that departs with a pair of electrons in heterolytic bond cleavage, wherein the molecular fragment is an anion or neutral molecule. See, for example, Smith, March *Advanced Organic Chemistry* 6th ed. (501–502). Exemplary leaving groups include, but are not limited to, halo (*e.g.,* chloro, bromo, iodo) and $–OSO_2R^{aa}$, wherein R^{aa} as defined herein. The group $–OSO_2R^{aa}$ encompasses leaving groups such as tosyl, mesyl, and besyl, wherein R^{aa} is optionally substituted alkyl (*e.g.,* $-CH_3$) or optionally substituted aryl (*e.g.,* phenyl, tolyl).

As used herein, the term "hydroxyl" or "hydroxy" refers to the group –OH. The term "substituted hydroxyl" or "substituted hydroxyl," by extension, refers to a hydroxyl group wherein the oxygen atom directly attached to the parent molecule is substituted with a group other than

hydrogen, and includes groups selected from $-OR^{aa}$, $-ON(R^{bb})_2$, $-OC(=O)SR^{aa}$, $-OC(=O)R^{aa}$, $-OCO_2R^{aa}$, $-OC(=O)N(R^{bb})_2$, $-OC(=NR^{bb})R^{aa}$, $-OC(=NR^{bb})OR^{aa}$, $-OC(=NR^{bb})N(R^{bb})_2$, $-OS(=O)R^{aa}$, $-OSO_2R^{aa}$, $-OSi(R^{aa})_3$, $-OP(R^{cc})_2$, $-OP(=O)(R^{aa})_2$, and $-OP(=O)(OR^{cc})_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein.

As used herein, the term "thiol" or "thio" refers to the group $-SH$. The term "substituted thiol" or "substituted thio," by extension, refers to a thiol group wherein the sulfur atom directly attached to the parent molecule is substituted with a group other than hydrogen, and includes groups selected from $-SR^{aa}$, $-S=SR^{cc}$, $-SC(=S)SR^{aa}$, $-SC(=O)SR^{aa}$, $-SC(=O)OR^{aa}$, and $-SC(=O)R^{aa}$, wherein R^{aa} and R^{cc} are as defined herein.

As used herein, the term, "amino" refers to the group $-NH_2$. The term "substituted amino," by extension, refers to a monosubstituted amino or a disubstituted amino, as defined herein. In certain embodiments, the "substituted amino" is a monosubstituted amino or a disubstituted amino group.

As used herein, the term "monosubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with one hydrogen and one group other than hydrogen, and includes groups selected from $-NH(R^{bb})$, $-NHC(=O)R^{aa}$, $-NHCO_2R^{aa}$, $-NHC(=O)N(R^{bb})_2$, $-NHC(=NR^{bb})N(R^{bb})_2$, $-NHSO_2R^{aa}$, and $-NHP(=O)(OR^{cc})_2$, wherein R^{aa}, R^{bb} and R^{cc} are as defined herein, and wherein R^{bb} of the group $-NH(R^{bb})$ is not hydrogen.

As used herein, the term "disubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with two groups other than hydrogen, and includes groups selected from $-N(R^{bb})_2$, $-NR^{bb}\ C(=O)R^{aa}$, $-NR^{bb}CO_2R^{aa}$, $-NR^{bb}C(=O)N(R^{bb})_2$, $-NR^{bb}C(=NR^{bb})N(R^{bb})_2$, $-NR^{bb}SO_2R^{aa}$, and $-NR^{bb}P(=O)(OR^{cc})_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein, with the proviso that the nitrogen atom directly attached to the parent molecule is not substituted with hydrogen.

As used herein, the term "sulfonyl" refers to a group selected from $-SO_2N(R^{bb})_2$, $-SO_2R^{aa}$, and $-SO_2OR^{aa}$, wherein R^{aa} and R^{bb} are as defined herein.

As used herein, the term "sulfinyl" refers to the group $-S(=O)R^{aa}$, wherein R^{aa} is as defined herein.

As used herein, the term "carbonyl" refers a group wherein the carbon directly attached to the parent molecule is sp^2 hybridized, and is substituted with an oxygen, nitrogen or sulfur atom,

e.g., a group selected from ketones ($-C(=O)R^{aa}$), carboxylic acids ($-CO_2H$), aldehydes (–CHO), esters ($-CO_2R^{aa}$, $-C(=O)SR^{aa}$, $-C(=S)SR^{aa}$), amides ($-C(=O)N(R^{bb})_2$, $-C(=O)NR^{bb}SO_2R^{aa}$, $-C(=S)N(R^{bb})_2$), and imines ($-C(=NR^{bb})R^{aa}$, $-C(=NR^{bb})OR^{aa}$), $-C(=NR^{bb})N(R^{bb})_2$), wherein R^{aa} and R^{bb} are as defined herein.

As used herein, the term "silyl" refers to the group $-Si(R^{aa})_3$, wherein R^{aa} is as defined herein.

As used herein, the term "oxo" refers to the group =O, and the term "thiooxo" refers to the group =S.

Nitrogen atoms can be substituted or unsubstituted as valency permits, and include primary, secondary, tertiary, and quarternary nitrogen atoms.

In certain embodiments, the substituent present on the nitrogen atom is a nitrogen protecting group (also referred to herein as an "amino protecting group"). Nitrogen protecting groups include, but are not limited to, –OH, $-OR^{aa}$, $-N(R^{cc})_2$, $-C(=O)R^{aa}$, $-C(=O)N(R^{cc})_2$, $-CO_2R^{aa}$, $-SO_2R^{aa}$, $-C(=NR^{cc})R^{aa}$, $-C(=NR^{cc})OR^{aa}$, $-C(=NR^{cc})N(R^{cc})_2$, $-SO_2N(R^{cc})_2$, $-SO_2R^{cc}$, $-SO_2OR^{cc}$, $-SOR^{aa}$, $-C(=S)N(R^{cc})_2$, $-C(=O)SR^{cc}$, $-C(=S)SR^{cc}$, C_{1-10} alkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, heteroC_{1-10} alkyl, heteroC_{2-10} alkenyl, heteroC_{2-10} alkynyl, C_{3-10} carbocyclyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl groups. Nitrogen protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

In certain embodiments, the substituent present on an oxygen atom is an oxygen protecting group (also referred to herein as an "hydroxyl protecting group"). Oxygen protecting groups include, but are not limited to, $-R^{aa}$, $-N(R^{bb})_2$, $-C(=O)SR^{aa}$, $-C(=O)R^{aa}$, $-CO_2R^{aa}$, $-C(=O)N(R^{bb})_2$, $-C(=NR^{bb})R^{aa}$, $-C(=NR^{bb})OR^{aa}$, $-C(=NR^{bb})N(R^{bb})_2$, $-S(=O)R^{aa}$, $-SO_2R^{aa}$, $-Si(R^{aa})_3$, $-P(R^{cc})_2$, $-P(=O)(R^{aa})_2$, and $-P(=O)(OR^{cc})_2$, wherein R^{aa}, R^{bb}, and R^{cc} are as defined herein. Oxygen protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

C. Exemplary Syntheses of Cortistatin Analogs

Non-limiting examples of synthetic methods to prepare the cortistatin analogues described

herein for use with the present invention include the procedures listed in: WO 2010/024930; WO 2015/100420; WO 2016/182904; WO 2016/182932; WO 2017/004411; WO 2017/112815; WO 2017/112823; and WO 2017/142621; each of which is incorporated by reference.

V. CDK8/19 Inhibitors

In any of the embodiments described herein, a CDK8/19 inhibitor other than cortistatin can be used in combination with the method of targeted selection of patients for therapy using the biomarkers identified herein. A range of CDK8/19 inhibitors are known in the art, including but not limited to those described in the following publications: Schiemann, K. *et al.* Discovery of potent and selective CDK8 inhibitors from an HSP90 pharmacophore. *Bioorg. Med. Chem. Lett.* **26,** 1443–1451 (2016); Mallinger, A. *et al.* Discovery of Potent, Selective, and Orally Bioavailable Small-Molecule Modulators of the Mediator Complex-Associated Kinases CDK8 and CDK19. *J. Med. Chem.* **59,** 1078–1101 (2016); Koehler, M., Bergeron, P. & Blackwood, E. M. Potent, Specific CDK8 Kinase Inhibitors Lack CDK8-dependent Antiproliferative Activity in HCT-116 Colon Cancer Cell Line. *ACS Med. Chem. Lett.* (2016). doi:10.1021/acsmedchemlett.5b00278; Dale, T. *et al.* A selective chemical probe for exploring the role of CDK8 and CDK19 in human disease. *Nat. Chem. Biol.* **11,** 973–980 (2015).

Additional non-limiting examples of CDK8/19 inhibitors known in the art are provided in the following U.S. Patent Applications: US2013/0217014; US2015/027953; US2004/0180848; US2004/018844; US2014/0038958; US2012/0071477; US2011/0229484; US2005/0009846; US2008/0287439; US2010/0093769; US2005/0256142; US2003/0018058; US2001/0047019; US2002/002178; US2009/0318441; US2005/0192300; US2009/0325983; US2006/0235034; US2010/0215644; US2010/00120781; US2006/0183760; US2009/0270427; US20020165259; US2006/0241297; US2004/0186288; US2006/0241112; US2006/0270687; US2006/0270687; US2004/0254094; US2003/0176699; US2006/0148824; US2003/0114672; US2009/0215805; US2009; 0137572; and US2007/0161635.

In one embodiment the CDK8/19 inhibitor is an analog of Senexin. In another embodiment the CDK8/19 inhibitor is an analog of Selvita.

VI. PHARMACEUTICAL COMPOSITIONS

The cortistatin or other CDK8/19 inhibitor can be administered as the neat chemical, but are more typically administered as a pharmaceutical composition, that includes an effective amount for a host, typically a human, in need of such treatment of the selected cortistatin or the CDK8/19 inhibitor, as described herein. Accordingly, the disclosure provides pharmaceutical compositions comprising an effective amount of cortistatin or its pharmaceutically acceptable salt together with at least one pharmaceutically acceptable carrier for all of the uses described herein. The pharmaceutical composition may contain the cortistatin as the only active agent, or, in an alternative embodiment, the compound and at least one additional active agent. In certain embodiments the pharmaceutical composition is in a dosage form that contains from about 0.1 mg to about 2000 mg, from about 10 mg to about 1000 mg, from about 5 mg to about 200 mg, or from about 5 mg to about 100 mg of the active compound and optionally an appropriate dosage amount of an additional active agent, in a unit dosage form. Examples are dosage forms with at least 5, 10, 15, 25, 50, 75, 100, 200, 250, 300, 400, 500, 600, 700, or 750 mg of active compound. The cortistatin or CDK8/19 inhibitor may be administered orally, topically, parenterally, by inhalation or spray, sublingually, via implant, including ocular implant, transdermally, via buccal administration, rectally, as an ophthalmic solution, injection, intravenous, intra-aortal, intracranial, subdermal, intraperitoneal, subcutaneous, transnasal, sublingual, or rectal or by other means, in dosage unit formulations containing conventional pharmaceutically acceptable carriers.

In some embodiments, the present disclosure provides compositions comprising a cortistatin or CDK8/19 inhibitor, such as a cortistatin described herein or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof, for administration to a subject having a cancer or tumor that exhibits impaired RUNX1 activity. In some embodiments, the composition comprises a CDK8/19 inhibitor. In some embodiments, the composition further comprises a Jak1/2 inhibitor.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally also suitable for administration to animals. If required, modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled person in the art will be able to design and perform such modification with merely ordinary, if any, experimentation. Subjects to which

administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, e.g., cattle, pigs, horses, sheep, cats, dogs, rodents, mice, hamsters, and/or rats; birds, e.g., chickens, ducks, geese, and turkeys.

Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

Salts can be prepared from inorganic acids sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydriodic, phosphorus, and the like. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactobionate, laurylsulphonate and isethionate salts, and the like. Salts can also be prepared from organic acids, such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. and the like. Representative salts include acetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Pharmaceutically acceptable salts can include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium and the like, as well as non-toxic ammonium, quaternary ammonium, and amine cations including, but not limited to, ammonium, tetramethylammonium, tetraethylammonium, methylamine,

dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. Also contemplated are the salts of amino acids such as arginate, gluconate, galacturonate, and the like. See, for example, Berge et al., J. Pharm. Sci., 1977, 66, 1-19, which is incorporated herein by reference.

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) of active ingredient, e.g. a CDK8/19 inhibitor, or a cortistatin or cortistatin analog thereof, such as a compound described herein, or a pharmaceutically acceptable salt, quaternary amine salt, or N-oxide thereof, and, optionally, any additional active ingredients, such as, for example, a JAK1/2 inhibitor. In some embodiments, the composition comprises between 0.1% and 1%, between 1% and 10%, between 10% and 20%, between 20% and 30%, between 30% and 40%, between 40% and 50%, between 50% and 60%, between 60% and 70%, between 70% and 80%, between 80% and 90%, or between 90% and 100% (w/w) of active ingredient, and more generally, between 0.1 and 100% (w/w) of active ingredient.

Pharmaceutical formulations as provided herein may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

In some embodiments, the excipient is one already approved for use in humans and for veterinary use, for example, by United States Food and Drug Administration. In some embodiments, an excipient meets the standards of the United States Pharmacopoeia (USP), the

European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in pharmaceutical formulations. Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, etc., and/or combinations thereof.

Exemplary granulating and/or dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, etc., and/or combinations thereof.

Exemplary surface active agents and/or emulsifiers include, but are not limited to, natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and Veegum® [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g. stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (e.g.

carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate [Tween®20], polyoxyethylene sorbitan [Tween®60], polyoxyethylene sorbitan monooleate [Tween®80], sorbitan monopalmitate [Span®40], sorbitan monostearate [Span®60], sorbitan tristearate [Span®65], glyceryl monooleate, sorbitan monooleate [Span®80]), polyoxyethylene esters (e.g. polyoxyethylene monostearate [Myrj®45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and Solutol®), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. Cremophor®), polyoxyethylene ethers, (e.g. polyoxyethylene lauryl ether [Brij®30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, Pluronic®F 68, Poloxamer®188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof.

Exemplary binding agents include, but are not limited to, starch (e.g. cornstarch and starch paste); gelatin; sugars (e.g. sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol,); natural and synthetic gums (e.g. acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (Veegum®), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; etc.; and combinations thereof.

Exemplary preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and/or other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and/or trisodium edetate. Exemplary antimicrobial preservatives include, but are not limited to,

benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and/or thimerosal. Exemplary antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Exemplary alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Exemplary acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluened (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, Glydant Plus®, Phenonip®, methylparaben, Germall®115, Germaben®II, Neolone™, Kathon™, and/or Euxyl®.

Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, d-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, etc., and/or combinations thereof.

Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behanate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, etc., and combinations thereof.

Exemplary oils include, but are not limited to, almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and/or combinations thereof.

Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents. In certain embodiments for parenteral administration, compositions are mixed with solubilizing agents such as Cremophor®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. 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. Fatty acids such as oleic acid can be used in the preparation of injectables.

Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

In order to prolong the effect of an active ingredient, it is often desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

Compositions for rectal or vaginal administration are typically suppositories which can be prepared by mixing compositions with suitable non-irritating excipients such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active ingredient.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, an active ingredient is mixed with at least one inert, pharmaceutically acceptable excipient such as sodium citrate or dicalcium phosphate and/or fillers or extenders (e.g. starches, lactose, sucrose, glucose, mannitol, and silicic acid), binders (e.g. carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia), humectants (e.g. glycerol), disintegrating agents (e.g. agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate), solution retarding agents (e.g. paraffin), absorption accelerators (e.g. quaternary ammonium compounds), wetting agents (e.g. cetyl alcohol and glycerol monostearate), absorbents (e.g. kaolin and bentonite clay), and

lubricants (e.g. talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. Solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

Dosage forms for topical and/or transdermal administration of a composition may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants and/or patches. Generally, an active ingredient is admixed under sterile conditions with a pharmaceutically acceptable excipient and/or any needed preservatives and/or buffers as may be required. Additionally, the present invention contemplates the use of transdermal patches, which often have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms may be prepared, for example, by dissolving and/or dispensing the compound in the proper medium. Alternatively or additionally, rate may be controlled by either providing a rate controlling membrane and/or by dispersing the compound in a polymer matrix and/or gel.

Suitable devices for use in delivering intradermal pharmaceutical compositions described herein include short needle devices such as those described in U.S. Patents 4,886,499; 5,190,521; 5,328,483; 5,527,288; 4,270,537; 5,015,235; 5,141,496; and 5,417,662. Intradermal compositions may be administered by devices which limit the effective penetration length of a needle into the skin, such as those described in PCT publication WO 99/34850 and functional equivalents thereof. Jet injection devices which deliver liquid compositions to the dermis via a liquid jet injector and/or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis are suitable. Jet injection devices are described, for example, in U.S. Patents 5,480,381; 5,599,302;

5,334,144; 5,993,412; 5,649,912; 5,569,189; 5,704,911; 5,383,851; 5,893,397; 5,466,220; 5,339,163; 5,312,335; 5,503,627; 5,064,413; 5,520,639; 4,596,556; 4,790,824; 4,941,880; 4,940,460; and PCT Publications WO 97/37705 and WO 97/13537. Ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis are suitable. Alternatively or additionally, conventional syringes may be used in the classical mantoux method of intradermal administration.

Formulations suitable for topical administration include, but are not limited to, liquid and/or semi liquid preparations such as liniments, lotions, oil in water and/or water in oil emulsions such as creams, ointments and/or pastes, and/or solutions and/or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 nm to about 7 nm or from about 1 nm to about 6 nm. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder and/or using a self-propelling solvent/powder dispensing container such as a device comprising the active ingredient dissolved and/or suspended in a low-boiling propellant in a sealed container. Such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nm and at least 95% of the particles by number have a diameter less than 7 nm. Alternatively, at least 95% of the particles by weight have a diameter greater than 1 nm and at least 90% of the particles by number have a diameter less than 6 nm. Dry powder compositions may include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally, the propellant may constitute 50% to 99.9% (w/w) of the composition, and active ingredient may constitute 0.1% to 20% (w/w) of the composition. A propellant may further comprise additional ingredients such as a liquid non-ionic

and/or solid anionic surfactant and/or a solid diluent (which may have a particle size of the same order as particles comprising the active ingredient).

Pharmaceutical compositions formulated for pulmonary delivery may provide an active ingredient in the form of droplets of a solution and/or suspension. Such formulations may be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic solutions and/or suspensions, optionally sterile, comprising active ingredient, and may conveniently be administered using any nebulization and/or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, and/or a preservative such as methylhydroxybenzoate. Droplets provided by this route of administration may have an average diameter in the range from about 0.1 nm to about 200 nm.

Formulations described herein as being useful for pulmonary delivery are useful for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 μm to 500 μm. Such a formulation is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of active ingredient, and may comprise one or more of the additional ingredients described herein. In some embodiments, the formulation suitable for nasal administration comprises between 0.1% and 1%, between 1% and 10%, between 10% and 20%, between 20% and 30%, between 30% and 40%, between 40% and 50%, between 50% and 60%, between 60% and 70%, between 70% and 80%, between 80% and 90%, or between 90% and 100% (w/w) of active ingredient. In the absence of a statement to the contrary, the formulation suitable for nasal administration comprises between 0.1 and 100% (w/w) of active ingredient. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, 0.1% to 20% (w/w) active ingredient, the balance comprising an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized

solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or atomized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1/1.0% (w/w) solution and/or suspension of the active ingredient in an aqueous or oily liquid excipient. Such drops may further comprise buffering agents, salts, and/or one or more other of any additional ingredients described herein. Other opthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form and/or in a liposomal preparation. Ear drops and/or eye drops are contemplated as being within the scope of this invention.

General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

VII. METHODS OF TREATMENT

The methods described herein may be used to monitor or predict treatment outcomes for any disorder mediated by CDK8 and/or CDK19. Non-limiting examples of disorders mediated by CDK8 and CDK19, include tumors, cancers, disorders related to abnormal cellular proliferation, inflammatory disorders, immune disorders, and autoimmune disorders.

In certain embodiments, the method is an *in vitro* method. In certain embodiments, the method is an *in vivo* method. In another aspect, a method of treating a condition associated with CDK8 and/or CDK19 kinase activity is provided, comprising administering to a subject in need thereof a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog such as a deuterated derivative, or prodrug thereof, wherein a method utilizing a pharmacodynamics biomarker described herein is utilized during treatment.

In certain embodiments, the condition associated with CDK8 and/or CDK19 kinase activity is a disorder related to abnormal cellular proliferation.

Abnormal cellular proliferation, notably hyperproliferation, can occur as a result of a wide variety of factors, including genetic mutation, infection, exposure to toxins, autoimmune disorders,

and benign or malignant tumor induction.

There are a number of skin disorders associated with cellular hyperproliferation. Psoriasis, for example, is a benign disease of human skin generally characterized by plaques covered by thickened scales. The disease is caused by increased proliferation of epidermal cells of unknown cause. Chronic eczema is also associated with significant hyperproliferation of the epidermis. Other diseases caused by hyperproliferation of skin cells include atopic dermatitis, lichen planus, warts, pemphigus vulgaris, actinic keratosis, basal cell carcinoma and squamous cell carcinoma.

Other hyperproliferative cell disorders include blood vessel proliferation disorders, fibrotic disorders, autoimmune disorders, graft-versus-host rejection, tumors and cancers.

Blood vessel proliferative disorders include angiogenic and vasculogenic disorders. Proliferation of smooth muscle cells in the course of development of plaques in vascular tissue cause, for example, restenosis, retinopathies and atherosclerosis. Both cell migration and cell proliferation play a role in the formation of atherosclerotic lesions.

Fibrotic disorders are often due to the abnormal formation of an extracellular matrix. Examples of fibrotic disorders include hepatic cirrhosis and mesangial proliferative cell disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. Hepatic cirrhosis can cause diseases such as cirrhosis of the liver. An increased extracellular matrix resulting in a hepatic scar can also be caused by viral infection such as hepatitis. Lipocytes appear to play a major role in hepatic cirrhosis.

Mesangial disorders are brought about by abnormal proliferation of mesangial cells. Mesangial hyperproliferative cell disorders include various human renal diseases, such as glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic micro-angiopathy syndromes, transplant rejection, and glomerulopathies.

Another disease with a proliferative component is rheumatoid arthritis. Rheumatoid arthritis is generally considered an autoimmune disease that is thought to be associated with activity of autoreactive T cells, and to be caused by autoantibodies produced against collagen and IgE.

Other disorders that can include an abnormal cellular proliferative component include Bechet's syndrome, acute respiratory distress syndrome (ARDS), ischemic heart disease, post-dialysis syndrome, leukemia, acquired immune deficiency syndrome, vasculitis, lipid histiocytosis, septic shock and inflammation in general.

In certain embodiments, the condition associated with CDK8 and/or CDK19 kinase activity is a diabetic condition.

In certain embodiments, the condition associated with CDK8 and/or CDK19 kinase activity is a viral disease.

Human host proteins, including transcriptional cyclin-dependent kinases (CDKs), are known to contribute to the replication of several viruses, including herpes simplex virus (HSV), human immunodeficiency virus (HIV) and human cytomegalovirus (HCMV). CDK8 activity plays a role in interferon response, which is also important in cancer cell survival. Treatment with Cortistatin A increases expression of genes in MOLM-14 AML cells that have been identified as interferon gamma signalling genes and interferon responsive genes. Viruses such as HIV block interferon induction to allow more effective replication. Further, Cortistatin A has been shown to inhibit the HIV virus as well as the HIV viral protein TAT-1.

In certain embodiments, the condition associated with CDK8 and/or CDK19 kinase activity is an infection. In certain embodiments, the infection is a bacterial infection. In certain embodiments, the infection is a fungal infection. In certain embodiments, the infection is a protozoal infection. In certain embodiments, the infection is a viral infection. In certain embodiments, the viral infection is a retroviral infection, and the virus is a retrovirus, *i.e.*, of the family *Retroviridae*. In certain embodiments, the viral infection is a retroviral infection, and the virus is of the family *Retroviridae* and subfamily *Orthoretrovirinae*, *Alpharetrovirus*, *Betaretrovirus*, *Deltaretrovirus*, *Epsilonretrovirus*, *Gammaretrovirus*, or *Lentivirus*. In certain embodiments, the viral infection is a retroviral infection, and the virus is of the family *Retroviridae* and subfamily *Lentivirus*. Exemplary virus of the subfamily Lentivirus include human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), equine infectious anemia virus (EIAV), and Visna virus are all examples of lentiviruses. In certain embodiments, the viral infection is a human immunodeficiency virus (HIV) infection. Other viral infections contemplated are infections with the herpes simplex virus (HSV), human immunodeficiency virus (HIV) or human cytomegalovirus (HCMV). In certain embodiments, the virus is an oncovirus, *i.e.*, a virus which is associated with oncogenesis and/or causes cancer. In certain embodiments, treatment of the viral infection is associated with inhibition of CDK8 and/or CDK19 kinase activity.

In certain embodiments the methods provided herein are used to predict outcomes related to the treatment of HIV infections and other related conditions such as AIDS-related complex (ARC), persistent generalized lymphadenopathy (PGL), AIDS-related neurological conditions, anti-HIV antibody positive and HIV-positive conditions, Kaposi's sarcoma, thrombocytopenia purpura and opportunistic infections. In addition, these compounds or formulations can be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HIV antibody or HIV-antigen positive or who have been exposed to HIV.

In certain embodiments the methods provided herein are used to predict outcomes related to the treatment of HBV infections and other related conditions such as anti-HBV antibody positive and HBV-positive conditions, chronic liver inflammation caused by HBV, cirrhosis, acute hepatitis, fulminant hepatitis, chronic persistent hepatitis, and fatigue. These compounds or formulations can also be used prophylactically to prevent or retard the progression of clinical illness in individuals who are anti-HBV antibody or HBV-antigen positive or who have been exposed to HBV.

In certain embodiments, the condition is associated with an immune response.

Cutaneous contact hypersensitivity and asthma are just two examples of immune responses that can be associated with significant morbidity. Others include atopic dermatitis, eczema, Sjogren's Syndrome, including keratoconjunctivitis sicca secondary to Sjogren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, and drug eruptions. These conditions may result in any one or more of the following symptoms or signs: itching, swelling, redness, blisters, crusting, ulceration, pain, scaling, cracking, hair loss, scarring, or oozing of fluid involving the skin, eye, or mucosal membranes.

In atopic dermatitis, and eczema in general, immunologically mediated leukocyte infiltration (particularly infiltration of mononuclear cells, lymphocytes, neutrophils, and eosinophils) into the skin importantly contributes to the pathogenesis of these diseases. Chronic eczema also is associated with significant hyperproliferation of the epidermis. Immunologically mediated leukocyte infiltration also occurs at sites other than the skin, such as in the airways in asthma and in the tear producing gland of the eye in keratoconjunctivitis sicca.

In certain embodiments, the condition associated with CDK8 and/or CDK19 kinase activity

is a degenerative disorder, e.g., Alzheimer's disease (AD) or Parkinson's Disease.

In another aspect, the condition is mediated by the β-catenin pathway.

In another aspect, the condition is mediated by the JAK-STAT pathway. In another aspect, the condition is mediated by STAT1 activity in a cell (e.g., the disorder may be treated by inhibiting phosphorylation of STAT1 S727 in the JAK-STAT pathway, leading to up- or down-regulation of specific STAT1-associated genes).

It has been reported that nuclear CDKs, such as CDK8, drive SMAD transcriptional activation and turnover in BMP and TGF-beta. See, e.g., Alarcon et al., Cell (2009), 139: 757–769. Thus, in yet another aspect, the disorder is mediated by the TGF-beta/BMP pathway.

CDK8 has been linked to regulation of hypoxic response, playing a role in induction of HIF-1-A (HIF-1-alpha) target genes. These genes are involved in angiogenesis, glycolysis, metabolic adaption, and cell survival, processes critical to tumor maintenance and growth. See, e.g., Galbraith, et al., Cell 153:1327–1339. Thus, in one aspect, the monitored condition is related to hypoxia comprising. In yet another aspect, the monitored condition is related to modulating HIF-1-A (HIF-1-alpha) activity (e.g., by inhibiting the expression HIF-1-alpha associated genes) in a cell.

In certain embodiments, the method is an *in vitro* method. In certain embodiments, the method is an *in vivo* method.

In yet another aspect, the monitored condition is associated with angiogenesis, such as, for example, a diabetic condition (e.g., diabetic retinopathy), an inflammatory condition (e.g., rheumatoid arthritis), macular degeneration, obesity, atherosclerosis, or a proliferative disorder, comprising administering to a subject in need thereof a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof.

As used herein, a "diabetic condition" refers to diabetes and pre-diabetes. Diabetes refers to a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). There are several types of diabetes. Type I diabetes results from the body's failure to produce insulin, and presently requires the person to inject insulin or wear an insulin pump. Type 2 diabetes results from insulin resistance a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.

Gestational diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. Other forms of diabetes include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes, e.g., mature onset diabetes of the young (e.g., MODY 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10). Pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of diabetes.

In certain embodiments, the condition associated with angiogenesis is macular degeneration. In certain embodiments, provided is a method of treating macular degeneration comprising administering to a subject in need thereof a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof.

In certain embodiments, the condition associated with angiogenesis is obesity. As used herein, "obesity" and "obese" as used herein, refers to class I obesity, class II obesity, class III obesity and pre-obesity (e.g., being "over-weight") as defined by the World Health Organization. In certain embodiments, a method of treating obesity is provided comprising administering to a subject in need thereof a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof.

In certain embodiments, the condition associated with angiogenesis is atherosclerosis. In certain embodiments, provided is a method of treating atherosclerosis comprising administering to a subject in need thereof a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof.

In certain embodiments, the condition associated with angiogenesis is a proliferative disorder. In certain embodiments, provided is a method of treating a proliferative disorder comprising administering to a subject in need thereof a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof.

Exemplary proliferative disorders include, but are not limited to, tumors (e.g., solid tumors), benign neoplasms, pre–malignant neoplasms (carcinoma in situ), and malignant neoplasms (cancers).

Exemplary cancers include, but are not limited to, acoustic neuroma, adenocarcinoma, adrenal gland cancer, anal cancer, angiosarcoma (e.g., lymphangiosarcoma, lymphangioendotheliosarcoma, hemangiosarcoma), appendix cancer, benign monoclonal

gammopathy, biliary cancer (e.g., cholangiocarcinoma), bladder cancer, breast cancer (e.g., adenocarcinoma of the breast, papillary carcinoma of the breast, mammary cancer, medullary carcinoma of the breast), brain cancer (e.g., meningioma; glioma, e.g., astrocytoma, oligodendroglioma; medulloblastoma), bronchus cancer, carcinoid tumor, cervical cancer (e.g., cervical adenocarcinoma), choriocarcinoma, chordoma, craniopharyngioma, colorectal cancer (e.g., colon cancer, rectal cancer, colorectal adenocarcinoma), epithelial carcinoma, ependymoma, endotheliosarcoma (e.g., Kaposi's sarcoma, multiple idiopathic hemorrhagic sarcoma), endometrial cancer (e.g., uterine cancer, uterine sarcoma), esophageal cancer (e.g., adenocarcinoma of the esophagus, Barrett's adenocarinoma), Ewing's sarcoma, eye cancer (e.g., intraocular melanoma, retinoblastoma), familiar hypereosinophilia, gall bladder cancer, gastric cancer (e.g., stomach adenocarcinoma), gastrointestinal stromal tumor (GIST), head and neck cancer (e.g., head and neck squamous cell carcinoma, oral cancer (e.g., oral squamous cell carcinoma (OSCC), throat cancer (e.g., laryngeal cancer, pharyngeal cancer, nasopharyngeal cancer, oropharyngeal cancer)), hematopoietic cancers (e.g., leukemia such as acute lymphocytic leukemia (ALL) – also known as acute lymphoblastic leukemia or acute lymphoid leukemia (e.g., B–cell ALL, T–cell ALL), acute myelocytic leukemia (AML) (e.g., B–cell AML, T–cell AML), chronic myelocytic leukemia (CML) (e.g., B–cell CML, T–cell CML), and chronic lymphocytic leukemia (CLL) (e.g., B–cell CLL, T–cell CLL); lymphoma such as Hodgkin lymphoma (HL) (e.g., B–cell HL, T–cell HL) and non–Hodgkin lymphoma (NHL) (e.g., B–cell NHL such as diffuse large cell lymphoma (DLCL) (e.g., diffuse large B–cell lymphoma (DLBCL)), follicular lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), mantle cell lymphoma (MCL), marginal zone B–cell lymphomas (e.g., mucosa–associated lymphoid tissue (MALT) lymphomas, nodal marginal zone B–cell lymphoma, splenic marginal zone B–cell lymphoma), primary mediastinal B–cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma (i.e., "Waldenström's macroglobulinemia"), hairy cell leukemia (HCL), immunoblastic large cell lymphoma, precursor B–lymphoblastic lymphoma and primary central nervous system (CNS) lymphoma; and T–cell NHL such as precursor T–lymphoblastic lymphoma/leukemia, peripheral T–cell lymphoma (PTCL) (e.g., cutaneous T–cell lymphoma (CTCL) (e.g., mycosis fungiodes, Sezary syndrome), angioimmunoblastic T–cell lymphoma, extranodal natural killer T–cell lymphoma, enteropathy type T–cell lymphoma, subcutaneous panniculitis–like T–cell lymphoma, anaplastic large cell lymphoma); a mixture of one or more leukemia/lymphoma as

described above; and multiple myeloma (MM)), heavy chain disease (e.g., alpha chain disease, gamma chain disease, mu chain disease), hemangioblastoma, inflammatory myofibroblastic tumors, immunocytic amyloidosis, kidney cancer (e.g., nephroblastoma a.k.a. Wilms' tumor, renal cell carcinoma), liver cancer (e.g., hepatocellular cancer (HCC), malignant hepatoma), lung cancer (e.g., bronchogenic carcinoma, small cell lung cancer (SCLC), non–small cell lung cancer (NSCLC), adenocarcinoma of the lung), leiomyosarcoma (LMS), mastocytosis (e.g., systemic mastocytosis), myelodysplastic syndrome (MDS), mesothelioma, myeloproliferative disorder (MPD) (e.g., polycythemia Vera (PV), essential thrombocytosis (ET), agnogenic myeloid metaplasia (AMM) a.k.a. myelofibrosis (MF), chronic idiopathic myelofibrosis, chronic myelocytic leukemia (CML), chronic neutrophilic leukemia (CNL), hypereosinophilic syndrome (HES)), neuroblastoma, neurofibroma (e.g., neurofibromatosis (NF) type 1 or type 2, schwannomatosis), neuroendocrine cancer (e.g., gastroenteropancreatic neuroendoctrine tumor (GEP–NET), carcinoid tumor), osteosarcoma, ovarian cancer (e.g., cystadenocarcinoma, ovarian embryonal carcinoma, ovarian adenocarcinoma), papillary adenocarcinoma, pancreatic cancer (e.g., pancreatic andenocarcinoma, intraductal papillary mucinous neoplasm (IPMN), Islet cell tumors), penile cancer (e.g., Paget's disease of the penis and scrotum), pinealoma, primitive neuroectodermal tumor (PNT), prostate cancer (e.g., prostate adenocarcinoma), rectal cancer, rhabdomyosarcoma, salivary gland cancer, skin cancer (e.g., squamous cell carcinoma (SCC), keratoacanthoma (KA), melanoma, basal cell carcinoma (BCC)), small bowel cancer (e.g., appendix cancer), soft tissue sarcoma (e.g., malignant fibrous histiocytoma (MFH), liposarcoma, malignant peripheral nerve sheath tumor (MPNST), chondrosarcoma, fibrosarcoma, myxosarcoma), sebaceous gland carcinoma, sweat gland carcinoma, synovioma, testicular cancer (e.g., seminoma, testicular embryonal carcinoma), thyroid cancer (e.g., papillary carcinoma of the thyroid, papillary thyroid carcinoma (PTC), medullary thyroid cancer), urethral cancer, vaginal cancer and vulvar cancer (e.g., Paget's disease of the vulva).

In another embodiment, the disorder is myelodysplastic syndrome (MDS).

In certain embodiments, the cancer or tumor is associated with CDK8 and/or CDK19 kinase activity. In certain embodiments, the cancer or tumor is associated with CDK8 kinase activity. In certain embodiments, the cancer or tumor is associated with CDK19 kinase activity. In certain embodiments, the cancer or tumor is associated with aberrant CDK8 kinase activity. In certain embodiments, the cancer or tumor is associated with aberrant CDK19 kinase activity. In

certain embodiments, the cancer or tumor is associated with increased CDK8 kinase activity. In certain embodiments, the cancer is associated with increased CDK19 kinase activity.

In certain embodiments, the cancer is a hematopoietic cancer. In certain embodiments, the hematopoietic cancer is a lymphoma. In certain embodiments, the hematopoietic cancer is a leukemia. In certain embodiments, the leukemia is acute myelocytic leukemia (AML).

In certain embodiments, the proliferative disorder is a myeloproliferative neoplasm. In certain embodiments, the myeloproliferative neoplasm (MPN) is primary myelofibrosis (PMF).

In certain embodiments, the cancer is a solid tumor. A solid tumor, as used herein, refers to an abnormal mass of tissue that usually does not contain cysts or liquid areas. Different types of solid tumors are named for the type of cells that form them. Examples of classes of solid tumors include, but are not limited to, sarcomas, carcinomas, and lymphomas, as described above herein. Additional examples of solid tumors include, but are not limited to, squamous cell carcinoma, colon cancer, breast cancer, prostate cancer, lung cancer, liver cancer, pancreatic cancer, and melanoma.

VIII. COMBINATIONS

In one embodiment the patient is being treated with a CDK8/19 inhibitor and an additional active agent. Non-limiting examples of such combination treatments are provided below. In one embodiment the additional active agent is added after determining if the cancer or tumor is responding to treatment.

Therefore, using the method described herein, a treatment regimen is provided comprising the administration of a compound of this disclosure or a pharmaceutically acceptable composition, salt, isotopic analog (such as a deuterated derivative), or prodrug thereof in combination or in alternation with at least one additional therapeutic agent. The combinations and/or alternations disclosed herein can be administered for beneficial, additive, or synergistic effect in the treatment of abnormal cellular proliferative disorders.

In specific embodiments, the treatment regimen includes the administration of a compound of this disclosure or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof in combination or alternation with at least one additional kinase inhibitor. In one embodiment, the at least one additional kinase inhibitor is selected from a phosphoinositide 3-

kinase (PI3K) inhibitor, a Bruton's tyrosine kinase (BTK) inhibitor, another cyclin-dependent kinase inhibitor, or a spleen tyrosine kinase (Syk) inhibitor, or a combination thereof.

In one embodiment, a compound of this disclosure or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof is combined in a dosage form with the PIk3 inhibitor.

PI3k inhibitors that may be used in the present invention are well known. Examples of PI3 kinase inhibitors include but are not limited to Wortmannin, demethoxyviridin, perifosine, idelalisib, Pictilisib, Palomid 529, ZSTK474, PWT33597, CUDC-907, and AEZS-136, duvelisib, GS-9820, GDC-0032 (2-[4-[2-(2-Isopropyl-5-methyl-1,2,4-triazol-3-yl)-5,6-dihydroimidazo[1,2-d][1,4]benzoxazepin-9-yl]pyrazol-1-yl]-2-methylpropanamide), MLN-1117 ((2R)-1-Phenoxy-2-butanyl hydrogen (S)-methylphosphonate; or Methyl(oxo) {[(2R)-l-phenoxy-2-butanyl]oxy}phosphonium)), BYL-719 ((2S)-N1-[4-Methyl-5-[2-(2,2,2-trifluoro-1,1-dimethylethyl)-4-pyridinyl]-2-thiazolyl]-1,2-pyrrolidinedicarboxamide), GSK2126458 (2,4-Difluoro-N-{2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide), TGX-221 ((±)-7-Methyl-2-(morpholin-4-yl)-9-(l-phenylaminoethyl)-pyrido[l,2-a]-pyrimidin-4-one), GSK2636771 (2-Methyl-1-(2-methyl-3-(trifluoromethyl)benzyl)-6-morpholino-lH-benzo[d]imidazole-4-carboxylic acid dihydrochloride), KIN-193 ((R)-2-((l-(7-methyl-2-morpholino-4-oxo-4H-pyrido[1,2-a]pyrimidin-9-yl)ethyl)amino)benzoic acid), TGR-1202/RP5264, GS-9820 ((S)- l-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-mohydroxypropan- 1 -one), GS-1101 (5-fluoro-3-phenyl-2-([S)]-1-[9H-purin-6-ylamino]-propyl)-3H-quinazolin-4-one), AMG-319, GSK-2269557, SAR245409 (N-(4-(N-(3-((3,5-dimethoxyphenyl)amino)quinoxalin-2-yl)sulfamoyl)phenyl)-3-methoxy-4 methylbenzamide), BAY80-6946 (2-amino-N-(7-methoxy-8-(3-morpholinopropoxy)-2,3-dihydroimidazo[l,2-c]quinaz), AS 252424 (5-[l-[5-(4-Fluoro-2-hydroxy-phenyl)-furan-2-yl]-meth-(Z)-ylidene]-thiazolidine-2,4-dione), CZ 24832 (5-(2-amino-8-fluoro-[l,2,4]triazolo[l,5-a]pyridin-6-yl)-N-tert-butylpyridine-3-sulfonamide), Buparlisib (5-[2,6-Di(4-morpholinyl)-4-pyrimidinyl]-4-(trifluoromethyl)-2-pyridinamine), GDC-0941 (2-(lH-Indazol-4-yl)-6-[[4-(methylsulfonyl)-l-piperazinyl]methyl]-4-(4-morpholinyl)thieno[3,2-d]pyrimidine), GDC-0980 ((S)-1-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-6 yl)methyl)piperazin-l-yl)-2-hydroxypropan-l-one (also known as RG7422)), SF1126 ((8S,14S,17S)-14-(carboxymethyl)-8-(3-guanidinopropyl)-17-(hydroxymethyl)-3,6,9,12,15-

pentaoxo-1-(4-(4-oxo-8-phenyl-4H-chromen-2-yl)morpholino-4-ium)-2-oxa-7,10,13,16-tetraazaoctadecan-18-oate), PF-05212384 (N-[4-[[4-(Dimethylamino)-1-piperidinyl]carbonyl]phenyl]-N'-[4-(4,6-di-4-morpholinyl-l,3,5-triazin-2-yl)phenyl]urea), LY3023414, BEZ235 (2-Methyl-2-{4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydro-lH-imidazo[4,5-c]quinolin-l-yl]phenyl}propanenitrile), XL-765 (N-(3-(N-(3-(3,5-dimethoxyphenylamino)quinoxalin-2-yl)sulfamoyl)phenyl)-3-methoxy-4-methylbenzamide), and GSK1059615 (5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidenedione), PX886 ([(3aR,6E,9S,9aR,10R,11aS)-6-[[bis(prop-2-enyl)amino]methylidene]-5-hydroxy-9-(methoxymethyl)-9a,11a-dimethyl-l,4,7-trioxo-2,3,3a,9,10,ll-hexahydroindeno[4,5h]isochromen-10-yl] acetate (also known as sonolisib)).

BTK inhibitors for use in the present invention are well known. Examples of BTK inhibitors include ibrutinib (also known as PCI-32765)(Imbruvica™)(1-[(3R)-3-[4-amino-3-(4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one), dianilinopyrimidine-based inhibitors such as AVL-101 and AVL-291/292 (N-(3-((5-fluoro-2-((4-(2-methoxyethoxy)phenyl)amino)pyrimidin-4-yl)amino)phenyl)acrylamide) (Avila Therapeutics) (*see* US Patent Publication No 2011/0117073, incorporated herein in its entirety), Dasatinib ([N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide], LFM-A13 (alpha-cyano-beta-hydroxy-beta-methyl-N-(2,5-ibromophenyl) propenamide), GDC-0834 ([R-N-(3-(6-(4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenylamino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide], CGI-560 4-(tert-butyl)-N-(3-(8-(phenylamino)imidazo[1,2-a]pyrazin-6-yl)phenyl)benzamide, CGI-1746 (4-(tert-butyl)-N-(2-methyl-3-(4-methyl-6-((4-(morpholine-4-carbonyl)phenyl)amino)-5-oxo-4,5-dihydropyrazin-2-yl)phenyl)benzamide), CNX-774 (4-(4-((4-((3-acrylamidophenyl)amino)-5-fluoropyrimidin-2-yl)amino)phenoxy)-N-methylpicolinamide), CTA056 (7-benzyl-1-(3-(piperidin-1-yl)propyl)-2-(4-(pyridin-4-yl)phenyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one), GDC-0834 ((R)-N-(3-(6-((4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenyl)amino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide), GDC-0837 ((R)-N-(3-(6-((4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenyl)amino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide), HM-71224, ACP-196, ONO-4059 (Ono Pharmaceuticals), PRT062607 (4-((3-(2H-1,2,3-triazol-2-yl)phenyl)amino)-2-

(((1R,2S)-2-aminocyclohexyl)amino)pyrimidine-5-carboxamide hydrochloride), QL-47 (1-(1-acryloylindolin-6-yl)-9-(1-methyl-1H-pyrazol-4-yl)benzo[h][1,6]naphthyridin-2(1H)-one), and RN486 (6-cyclopropyl-8-fluoro-2-(2-hydroxymethyl-3-{1-methyl-5-[5-(4-methyl-piperazin-1-yl)-pyridin-2-ylamino]-6-oxo-1,6-dihydro-pyridin-3-yl}-phenyl)-2H-isoquinolin-1-one), and other molecules capable of inhibiting BTK activity, for example those BTK inhibitors disclosed in Akinleye et ah, Journal of Hematology & Oncology, 2013, 6:59, the entirety of which is incorporated herein by reference. In one embodiment, a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof is combined in a dosage form with the BTK inhibitor.

In one embodiment the additional cyclin-dependent kinase inhibitor is a CDK7 inhibitor such as THZ1 (N-[3-[[5-chloro-4-(1H-indol-3-yl)pyrimidin-2-yl]amino]phenyl]-4-[[(E)-4-(dimethylamino)but-2-enoyl]amino]benzamide). In an alternative embodiment the additional cyclin-dependent kinase inhibitor is a CDK9 inhibitor such as flavopiridol (alvocidib).

Therefore in one embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with an effective amount of a Syk inhibitor to a host in need thereof. In another embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with an effective amount of a Syk inhibitor to a host in need thereof.

In one embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with imatinib (Gleevec) to a host in need thereof. In another embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with imatinib (Gleevec) to a host in need thereof.

Syk inhibitors for use in the present invention are well known, and include, for example, Cerdulatinib (4-(cyclopropylamino)-2-((4-(4-(ethylsulfonyl)piperazin-1-yl)phenyl)amino)pyrimidine-5-carboxamide), entospletinib (6-(1H-indazol-6-yl)-N-(4-morpholinophenyl)imidazo[1,2-a]pyrazin-8-amine), fostamatinib ([6-({5-Fluoro-2-[(3,4,5-

trimethoxyphenyl)amino]-4-pyrimidinyl}amino)-2,2-dimethyl-3-oxo-2,3-dihydro-4H-pyrido[3,2-b][1,4]oxazin-4-yl]methyl dihydrogen phosphate), fostamatinib disodium salt (sodium (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-3-oxo-2H-pyrido[3,2-b][1,4]oxazin-4(3H)-yl)methyl phosphate), BAY 61-3606 (2-(7-(3,4-Dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino)-nicotinamide HCl), RO9021 (6-[(1R,2S)-2-Amino-cyclohexylamino]-4-(5,6-dimethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide), imatinib (Gleevec; 4-[(4-methylpiperazin-1-yl)methyl]-N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide), staurosporine, GSK143 (2-(((3R,4R)-3-aminotetrahydro-2H-pyran-4-yl)amino)-4-(p-tolylamino)pyrimidine-5-carboxamide), PP2 (1-(tert-butyl)-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine), PRT-060318 (2-(((1R,2S)-2-aminocyclohexyl)amino)-4-(m-tolylamino)pyrimidine-5-carboxamide), PRT-062607 (4-((3-(2H-1,2,3-triazol-2-yl)phenyl)amino)-2-(((1R,2S)-2-aminocyclohexyl)amino)pyrimidine-5-carboxamide hydrochloride), R112 (3,3'-((5-fluoropyrimidine-2,4-diyl)bis(azanediyl))diphenol), R348 (3-Ethyl-4-methylpyridine), R406 (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one), YM193306(see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643), 7-azaindole, piceatannol, ER-27319 (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), PRT060318 (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), luteolin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), apigenin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), quercetin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), fisetin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), myricetin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med. Chem. 2012, 55, 3614-3643 incorporated in its entirety herein), morin (see Singh et al. Discovery and Development of Spleen Tyrosine Kinase (SYK) Inhibitors, J. Med.

Chem. 2012, 55, 3614-3643 incorporated in its entirety herein). In one embodiment a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof is combined in a dosage form with the Syk inhibitor.

In specific embodiments, the method of treatment provided includes the administration of a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof in combination or alternation with at least one additional chemotherapeutic agent.

In one embodiment, the at least one additional chemotherapeutic agent combined or alternated with a compound of the present invention is a protein cell death-1 (PD-1) inhibitor. PD-1 inhibitors are known in the art, and include, for example, nivolumab (BMS), pembrolizumab (Merck), pidilizumab (CureTech/Teva), AMP-244 (Amplimmune/GSK), BMS-936559 (BMS), and MEDI4736 (Roche/Genentech). In one embodiment, a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof is combined in a dosage form with the PD-1 inhibitor. In one embodiment the PD-1 inhibitor is pembrolizumab.

In one embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with an effective amount of a PD-1 inhibitor to a host in need thereof. In another embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with an effective amount of a PD-1 inhibitor to a host in need thereof.

In one embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with pembrolizumab (Keytruda). In another embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with pembrolizumab (Keytruda).

In one embodiment, the at least one additional chemotherapeutic agent combined or alternated with a compound of the present invention is a CTLA-4 inhibitor. CTLA-4 inhibitors are known in the art, and include, for example, ipilimumab (Yervoy) marketed by Bristol-Myers Squibb and tremelimumab marketed by Pfizer.

In one embodiment, the at least one additional chemotherapeutic agent combined or alternated with the compound of the present invention is a BET inhibitor. BET inhibitors are known in the art, and include, for example, JQ1, I-BET 151 (a.k.a. GSK1210151A), I-BET 762 (a.k.a. GSK525762), OTX-015, TEN-010, CPI-203, CPI-0610, RVX-208, and LY294002. In one embodiment the BET inhibitor used in combination or alternation with a compound of the present invention for treatment of a tumor or cancer is JQ1 ((S)-tert-butyl 2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate).

In one embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with an effective amount of a BET inhibitor to a host in need thereof. In another embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with an effective amount of a BET inhibitor to a host in need thereof.

In one embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with JQ1. In another embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with JQ1.

In one embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with I-BET 151. In another embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with I-BET 151.

In one embodiment, the at least one additional chemotherapeutic agent combined or alternated with the compound of the present invention is a MEK inhibitor. MEK inhibitors for use in the present invention are well known, and include, for example, tametinib/GSKl 120212 (N-(3-{3-Cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-d]pyrimidin-l(2H-yl}phenyl)acetamide), selumetinob (6-(4-bromo-2-

chloroanilino)-7-fluoro-N-(2-hydroxyethoxy)-3-methylbenzimidazole-5-carboxamide), pimasertib/AS703026/MSC 1935369 ((S)-N-(2,3-dihydroxypropyl)-3-((2-fluoro-4-iodophenyl)amino)isonicotinamide), XL-518/GDC-0973 (l-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-[(2S)-piperidin-2-yl]azetidin-3-ol), refametinib/BAY869766/RDEAl 19 (N-(3,4-difluoro-2-(2-fluoro-4-iodophenylamino)-6-methoxyphenyl)-1-(2,3-dihydroxypropyl)cyclopropane-1-sulfonamide), PD-0325901 (N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]- benzamide), TAK733 ((R)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3-d]pyrimidine-4,7(3H,8H)-dione), MEK162/ARRY438162 (5-[(4-Bromo-2-fluorophenyl)amino]-4-fluoro-N-(2- hydroxyethoxy)-1-methyl-1H-benzimidazole-6-carboxamide), R05126766 (3-[[3-Fluoro-2- (methylsulfamoylamino)-4-pyridyl]methyl]-4-methyl-7-pyrimidin-2-yloxychromen-2-one), WX-554, R04987655/CH4987655 (3,4-difluoro-2-((2-fluoro-4-iodophenyl)amino)-N-(2-hydroxyethoxy)-5-((3-oxo-l,2-oxazinan-2yl)methyl)benzamide), or AZD8330 (2-((2-fluoro-4-iodophenyl)amino)-N-(2 hydroxyethoxy)-1 , and 5-dimethyl-6-oxo-l,6-dihydropyridine-3-carboxamide). In one embodiment, a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof is combined in a dosage form with the MEK inhibitor.

In one embodiment, the at least one additional chemotherapeutic agent combined or alternated with the compound of the present invention is a Raf inhibitor. Raf inhibitors for use in the present invention are well known, and include, for example, Vemurafinib (N-[3-[[5-(4-Chlorophenyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]carbonyl]-2,4-difluorophenyl]-1-propanesulfonamide), sorafenib tosylate (4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-N-methylpyridine-2-carboxamide;4-methylbenzenesulfonate), AZ628 (3-(2-cyanopropan-2-yl)-N-(4-methyl-3-(3-methyl-4-oxo-3,4-dihydroquinazolin-6-ylamino)phenyl)benzamide), NVP-BHG712 (4-methyl-3-(1-methyl-6-(pyridin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-ylamino)-N-(3-(trifluoromethyl)phenyl)benzamide), RAF-265 (1-methyl-5-[2-[5-(trifluoromethyl)-1H-imidazol-2-yl]pyridin-4-yl]oxy-N-[4-(trifluoromethyl)phenyl]benzimidazol-2-amine), 2-Bromoaldisine (2-Bromo-6,7-dihydro-1H,5H-pyrrolo[2,3-c]azepine-4,8-dione), Raf Kinase Inhibitor IV (2-chloro-5-(2-phenyl-5-(pyridin-4-yl)-1H-imidazol-4-yl)phenol), and Sorafenib N-Oxide (4-[4-[[[[4-Chloro-3(trifluoroMethyl)phenyl]aMino]carbonyl]aMino]phenoxy]-N-Methyl-

2pyridinecarboxaMide 1-Oxide). In one embodiment, a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof is combined in a dosage form with the Raf inhibitor.

In one embodiment, the at least one additional chemotherapeutic agent combined or alternated with the compound of the present invention is a B-cell lymphoma 2 (Bcl-2) protein inhibitor. BCL-2 inhibitors are known in the art, and include, for example, ABT-199 (4-[4-[[2-(4-Chlorophenyl)-4,4-dimethylcyclohex-1-en-1-yl]methyl]piperazin-l-yl]-N-[[3-nitro-4-[[(tetrahydro-2H-pyran-4-yl)methyl]amino]phenyl]sulfonyl]-2-[(lH- pyrrolo[2,3-b]pyridin-5-yl)oxy]benzamide), ABT-737 (4-[4-[[2-(4-chlorophenyl)phenyl]methyl]piperazin-1-yl]-N-[4-[[(2R)-4-(dimethylamino)-1-phenylsulfanylbutan-2-yl] amino]-3-nitrophenyl]sulfonylbenzamide), ABT-263 ((R)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[l, l'-biphenyl]-2-yl)methyl)piperazin-1-yl)-N-((4-((4-morpholino-1-(phenylthio)butan-2-yl)amino)-3((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide), GX15-070 (obatoclax mesylate, (2Z)-2-[(5Z)-5-[(3,5- dimethyl-lH-pyrrol-2-yl)methylidene]-4-methoxypyrrol-2-ylidene]indole; methanesulfonic acid))), 2-methoxy-antimycin A3, YC137 (4-(4,9-dioxo-4,9-dihydronaphtho[2,3-d]thiazol-2-ylamino)-phenyl ester), pogosin, ethyl 2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate, Nilotinib-d3, TW-37 (N-[4-[[2-(1,1-Dimethylethyl)phenyl]sulfonyl]phenyl]-2,3,4-trihydroxy-5-[[2-(1-methylethyl)phenyl]methyl]benzamide), Apogossypolone (ApoG2), or G3139 (Oblimersen). In one embodiment, a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof is combined in a dosage form with the at least one BCL-2 inhibitor. In one embodiment the at least one BCL-2 inhibitor is ABT-199 (Venetoclax).

In one embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with an effective amount of a BCL-2 inhibitor to a host in need thereof. In another embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with an effective amount of a BCL-2 inhibitor to a host in need thereof.

In one embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with ABT-199 to a host in need thereof. In another embodiment, a method of treating a tumor or cancer is provided, comprising administration of an effective amount of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with ABT-199 to a host in need thereof.

In one embodiment, the treatment regimen includes the administration of a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof in combination or alternation with at least one additional chemotherapeutic agent selected from, but are not limited to, Imatinib mesylate (Gleevac), Dasatinib (Sprycel), Nilotinib (Tasigna), Bosutinib (Bosulif), Trastuzumab (Herceptin), Pertuzumab (PerjetaTM), Lapatinib (Tykerb), Gefitinib (Iressa), Erlotinib (Tarceva), Cetuximab (Erbitux), Panitumumab (Vectibix), Vandetanib (Caprelsa), Vemurafenib (Zelboraf), Vorinostat (Zolinza), Romidepsin (Istodax), Bexarotene (Tagretin), Alitretinoin (Panretin), Tretinoin (Vesanoid), Carfilizomib (KyprolisTM), Pralatrexate (Folotyn), Bevacizumab (Avastin), Ziv-aflibercept (Zaltrap), Sorafenib (Nexavar), Sunitinib (Sutent), Pazopanib (Votrient), Regorafenib (Stivarga), and Cabozantinib (CometriqTM).

In some embodiments, the pharmaceutical combination or composition described herein can be administered to the subject in combination or further combination with other chemotherapeutic agents for the treatment of a tumor or cancer. If convenient, the pharmaceutical combination or composition described herein can be administered at the same time as another chemotherapeutic agent, in order to simplify the treatment regimen. In some embodiments, the pharmaceutical combination or composition and the other chemotherapeutic can be provided in a single formulation. In one embodiment, the use of the pharmaceutical combination or composition described herein is combined in a therapeutic regime with other agents. Such agents may include, but are not limited to, tamoxifen, midazolam, letrozole, bortezomib, anastrozole, goserelin, an mTOR inhibitor, a PI3 kinase inhibitor as described above, a dual mTOR-PI3K inhibitor, a MEK inhibitor as described above, a RAS inhibitor, ALK inhibitor, an HSP inhibitor (for example, HSP70 and HSP 90 inhibitor, or a combination thereof), a BCL-2 inhibitor as described above, apopototic inducing compounds, an AKT inhibitor, including but not limited to, Perifosine, (KRX-

0401), GDC-0068, Triciribine, AZD5363, Honokiol, PF-04691502, and Miltefosine, a PD-1 inhibitor as described above including but not limited to, Nivolumab, CT-011, MK-3475, BMS936558, and AMP-514 or a FLT-3 inhibitor, including but not limited to, P406, Dovitinib, Quizartinib (AC220), Amuvatinib (MP-470), Tandutinib (MLN518), ENMD-2076, and KW-2449, or a combination thereof. Examples of mTOR inhibitors include but are not limited to rapamycin and its analogs, everolimus (Afinitor), temsirolimus, ridaforolimus, sirolimus, and deforolimus. Examples of RAS inhibitors include but are not limited to Reolysin and siG12D LODER. Examples of ALK inhibitors include but are not limited to Crizotinib, AP26113, and LDK378. HSP inhibitors include but are not limited to Geldanamycin or 17-N-Allylamino-17-demethoxygeldanamycin (17AAG), and Radicicol. In a particular embodiment, a compound described herein is administered in combination with letrozole and/or tamoxifen. Other chemotherapeutic agents that can be used in combination with the compounds described herein include, but are not limited to, chemotherapeutic agents that do not require cell cycle activity for their anti-neoplastic effect.

In one embodiment, the treatment regimen includes the administration of a compound of the present invention or a pharmaceutically acceptable composition, salt, isotopic analog, or prodrug thereof in combination or alternation with at least one additional therapy, wherein the second therapy is an immunotherapy.

The combination agent can be conjugated to an antibody, radioactive agent, or other targeting agent that directs the active compound as described herein to the diseased or abnormally proliferating cell. In another embodiment, the pharmaceutical combination or composition is used in combination with another pharmaceutical or a biologic agent (for example an antibody) to increase the efficacy of treatment with a combined or a synergistic approach. In an embodiment, the pharmaceutical combination or composition can be used with T-cell vaccination, which typically involves immunization with inactivated autoreactive T cells to eliminate a cancer cell population as described herein. In another embodiment, the pharmaceutical combination or composition is used in combination with a bispecific T-cell Engager (BiTE), which is an antibody designed to simultaneously bind to specific antigens on endogenous T cells and cancer cells as described herein, linking the two types of cells.

In one embodiment, the additional therapy is a monoclonal antibody (MAb). Some MAbs stimulate an immune response that destroys cancer cells. Similar to the antibodies produced

naturally by B cells, these MAbs "coat" the cancer cell surface, triggering its destruction by the immune system. For example, bevacizumab targets vascular endothelial growth factor(VEGF), a protein secreted by tumor cells and other cells in the tumor's microenvironment that promotes the development of tumor blood vessels. When bound to bevacizumab, VEGF cannot interact with its cellular receptor, preventing the signaling that leads to the growth of new blood vessels. Similarly, cetuximab and panitumumab target the epidermal growth factor receptor (EGFR), and trastuzumab targets the human epidermal growth factor receptor 2 (HER-2). MAbs that bind to cell surface growth factor receptors prevent the targeted receptors from sending their normal growth-promoting signals. They may also trigger apoptosis and activate the immune system to destroy tumor cells.

Another group of cancer therapeutic MAbs are the immunoconjugates. These MAbs, which are sometimes called immunotoxins or antibody-drug conjugates, consist of an antibody attached to a cell-killing substance, such as a plant or bacterial toxin, a chemotherapy drug, or a radioactive molecule. The antibody latches onto its specific antigen on the surface of a cancer cell, and the cell-killing substance is taken up by the cell. FDA-approved conjugated MAbs that work this way include ado-trastuzumab emtansine, which targets the HER-2 molecule to deliver the drug DM1, which inhibits cell proliferation, to HER-2 expressing metastatic breast cancer cells.

Immunotherapies with T cells engineered to recognize cancer cells via bispecific antibodies (bsAbs) or chimeric antigen receptors (CARs) are approaches with potential to ablate both dividing and non/slow-dividing subpopulations of cancer cells.

Bispecific antibodies, by simultaneously recognizing target antigen and an activating receptor on the surface of an immune effector cell, offer an opportunity to redirect immune effector cells to kill cancer cells. Another approach is the generation of chimeric antigen receptors by fusing extracellular antibodies to intracellular signaling domains. Chimeric antigen receptor-engineered T cells are able to specifically kill tumor cells in a MHC-independent way.

In certain aspects, the additional therapy is another therapeutic agent, for example, an anti-inflammatory agent, a chemotherapeutic agent, a radiotherapeutic agent, or an immunosuppressive agent.

Suitable chemotherapeutic agents include, but are not limited to, a radioactive molecule, a toxin, also referred to as cytotoxin or cytotoxic agent, which includes any agent that is detrimental to the viability of cells, and liposomes or other vesicles containing chemotherapeutic compounds.

General anticancer pharmaceutical agents for administration as additional agents include: Vincristine (Oncovin) or liposomal vincristine (Marqibo), Daunorubicin (daunomycin or Cerubidine) or doxorubicin (Adriamycin), Cytarabine (cytosine arabinoside, ara-C, or Cytosar), L-asparaginase (Elspar) or PEG-L-asparaginase (pegaspargase or Oncaspar), Etoposide (VP-16), Teniposide (Vumon), 6-mercaptopurine (6-MP or Purinethol), Methotrexate, Cyclophosphamide (Cytoxan), Prednisone, Dexamethasone (Decadron), imatinib (Gleevec marketed by Novartis), dasatinib (Sprycel), nilotinib (Tasigna), bosutinib (Bosulif), and ponatinib (Iclusig™). Examples of additional suitable chemotherapeutic agents include but are not limited to 1-dehydrotestosterone, 5-fluorouracil decarbazine, 6-mercaptopurine, 6-thioguanine, actinomycin D, adriamycin, aldesleukin, an alkylating agent, allopurinol sodium, altretamine, amifostine, anastrozole, anthramycin (AMC)), an anti-mitotic agent, cis-dichlorodiamine platinum (II) (DDP) cisplatin), diamino dichloro platinum, anthracycline, an antibiotic, an antimetabolite, asparaginase, BCG live (intravesical), betamethasone sodium phosphate and betamethasone acetate, bicalutamide, bleomycin sulfate, busulfan, calcium leucouorin, calicheamicin, capecitabine, carboplatin, lomustine (CCNU), carmustine (BSNU), Chlorambucil, Cisplatin, Cladribine, Colchicin, conjugated estrogens, Cyclophosphamide, Cyclothosphamide, Cytarabine, Cytarabine, cytochalasin B, Cytoxan, Dacarbazine, Dactinomycin, dactinomycin (formerly actinomycin), daunirubicin HCL, daunorucbicin citrate, denileukin diftitox, Dexrazoxane, Dibromomannitol, dihydroxy anthracin dione, Docetaxel, dolasetron mesylate, doxorubicin HCL, dronabinol, *E. coli* L-asparaginase, emetine, epoetin-α, *Erwinia* L-asparaginase, esterified estrogens, estradiol, estramustine phosphate sodium, ethidium bromide, ethinyl estradiol, etidronate, etoposide citrororum factor, etoposide phosphate, filgrastim, floxuridine, fluconazole, fludarabine phosphate, fluorouracil, flutamide, folinic acid, gemcitabine HCL, glucocorticoids, goserelin acetate, gramicidin D, granisetron HCL, hydroxyurea, idarubicin HCL, ifosfamide, interferon α-2b, irinotecan HCL, letrozole, leucovorin calcium, leuprolide acetate, levamisole HCL, lidocaine, lomustine, maytansinoid, mechlorethamine HCL, medroxyprogesterone acetate, megestrol acetate, melphalan HCL, mercaptipurine, mesna, methotrexate, methyltestosterone, mithramycin, mitomycin C, mitotane, mitoxantrone, nilutamide, octreotide acetate, ondansetron HCL, paclitaxel, pamidronate disodium, pentostatin, pilocarpine HCL, plimycin, polifeprosan 20 with carmustine implant, porfimer sodium, procaine, procarbazine HCL, propranolol, rituximab, sargramostim, streptozotocin, tamoxifen, taxol, teniposide, tenoposide, testolactone, tetracaine,

thioepa chlorambucil, thioguanine, thiotepa, topotecan HCL, toremifene citrate, trastuzumab, tretinoin, valrubicin, vinblastine sulfate, vincristine sulfate, and vinorelbine tartrate.

Suitable immunosuppressive agents include, but are not limited to: calcineurin inhibitors, e.g. a cyclosporin or an ascomycin, e.g. Cyclosporin A (NEORAL), FK506 (tacrolimus), pimecrolimus, a mTOR inhibitor, e.g. rapamycin or a derivative thereof, e.g. Sirolimus (RAPAMUNE), Everolimus (Certican), temsirolimus, zotarolimus, biolimus-7, biolimus-9, a rapalog, e.g.ridaforolimus, azathioprine, campath 1H, a S1P receptor modulator, e.g. fingolimod or an analog thereof, an anti IL-8 antibody, mycophenolic acid or a salt thereof, e.g. sodium salt, or a prodrug thereof, e.g. Mycophenolate Mofetil (CELLCEPT), OKT3 (ORTHOCLONE OKT3), Prednisone, ATGAM, THYMOGLOBULIN, Brequinar Sodium, OKT4, T10B9.A-3A, 33B3.1, 15-deoxyspergualin, tresperimus, Leflunomide ARAVA, CTLAI-Ig, anti-CD25, anti-IL2R, Basiliximab (SIMULECT), Daclizumab (ZENAPAX), mizorbine, methotrexate, dexamethasone, ISAtx-247, SDZ ASM 981 (pimecrolimus, Elidel), CTLA4lg (Abatacept), belatacept, LFA3lg,, etanercept (sold as Enbrel by Immunex), adalimumab (Humira), infliximab (Remicade), an anti-LFA-1 antibody, natalizumab (Antegren), Enlimomab, gavilimomab, antithymocyte immunoglobulin, siplizumab, Alefacept efalizumab, pentasa, mesalazine, asacol, codeine phosphate, benorylate, fenbufen, naprosyn, diclofenac, etodolac and indomethacin, aspirin and ibuprofen.

In certain embodiments, a pharmaceutical combination or composition described herein is administered to the subject prior to treatment with another chemotherapeutic agent, during treatment with another chemotherapeutic agent, after administration of another chemotherapeutic agent, or a combination thereof.

In some embodiments, the selective pharmaceutical combination or composition can be administered to the subject such that the other chemotherapeutic agent can be administered either at higher doses (increased chemotherapeutic dose intensity) or more frequently (increased chemotherapeutic dose density). Dose-dense chemotherapy is a chemotherapy treatment plan in which drugs are given with less time between treatments than in a standard chemotherapy treatment plan. Chemotherapy dose intensity represents unit dose of chemotherapy administered per unit time. Dose intensity can be increased or decreased through altering dose administered, time interval of administration, or both.

In one embodiment of the invention, the pharmaceutical combination or composition described herein can be administered in a concerted regimen with another agent such as a non-DNA-damaging, targeted anti-neoplastic agent or a hematopoietic growth factor agent. It has recently been reported that the untimely administration of hematopoietic growth factors can have serious side effects. For example, the use of the EPO family of growth factors has been associated with arterial hypertension, cerebral convulsions, hypertensive encephalopathy, thromboembolism, iron deficiency, influenza like syndromes and venous thrombosis. The G-CSF family of growth factors has been associated with spleen enlargement and rupture, respiratory distress syndrome, allergic reactions and sickle cell complications. By combining the administration of the pharmaceutical combination or composition as described herein with the timely administration of hematopoietic growth factors, for example, at the time point wherein the affected cells are no longer under growth arrest, it is possible for the health care practitioner to decrease the amount of the growth factor to minimize the unwanted adverse effects while achieving the desired therapeutic benefit. As such, in one embodiment, the use of the pharmaceutical combination, composition, or methods described herein is combined with the use of hematopoietic growth factors including, but not limited to, granulocyte colony stimulating factor (G-CSF, for example, sold as Neupogen (filgrastin), Neulasta (peg-filgrastin), or lenograstin), granulocyte-macrophage colony stimulating factor (GM-CSF, for example sold as molgramostim and sargramostim (Leukine)), M-CSF (macrophage colony stimulating factor), thrombopoietin (megakaryocyte growth development factor (MGDF), for example sold as Romiplostim and Eltrombopag) interleukin (IL)-12, interleukin-3, interleukin-11 (adipogenesis inhibiting factor or oprelvekin), SCF (stem cell factor, steel factor, kit-ligand, or KL) and erythropoietin (EPO), and their derivatives (sold as for example epoetin-α as Darbopoetin, Epocept, Nanokine, Epofit, Epogin, Eprex and Procrit; epoetin-β sold as for example NeoRecormon, Recormon and Micera), epoetin-delta (sold as for example Dynepo), epoetin- omega (sold as for example Epomax), epoetin zeta (sold as for example Silapo and Reacrit) as well as for example Epocept, EPOTrust, Erypro Safe, Repoeitin, Vintor, Epofit, Erykine, Wepox, Espogen, Relipoeitin, Shanpoietin, Zyrop and EPIAO). In one embodiment, the pharmaceutical combination or composition is administered prior to administration of the hematopoietic growth factor. In one embodiment, the hematopoietic growth factor administration is timed so that the pharmaceutical combination or composition's effect on HSPCs has dissipated.

In one embodiment, the growth factor is administered at least 20 hours after the administration of a pharmaceutical combination or composition described herein.

If desired, multiple doses of a pharmaceutical combination or composition described herein can be administered to the subject. Alternatively, the subject can be given a single dose of a pharmaceutical combination or composition described herein.

In one embodiment, the activity of an active compound for a purpose described herein can be augmented through conjugation to an agent that targets the diseased or abnormally proliferating cell or otherwise enhances activity, delivery, pharmacokinetics or other beneficial property.

A selected compound described herein can be administered in conjugation or combination with a Fv fragment. Fv fragments are the smallest fragment made from enzymatic cleavage of IgG and IgM class antibodies. Fv fragments have the antigen-binding site made of the VH and VC regions, but they lack the CH1 and CL regions. The VH and VL chains are held together in Fv fragments by non-covalent interactions.

In one embodiment, a selected compound as described herein can be administered in combination with an antibody fragment selected from the group consisting of an ScFv, domain antibody, diabody, triabody, tetrabody, Bis-scFv, minibody, Fab2, or Fab3 antibody fragment. In one embodiment, the antibody fragment is a ScFv. Genetic engineering methods allow the production of single chain variable fragments (ScFv), which are Fv type fragments that include the VH and VL domains linked with a flexible peptide When the linker is at least 12 residues long, the ScFv fragments are primarily monomeric. Manipulation of the orientation of the V-domains and the linker length creates different forms of Fv molecules linkers that are 3-11 residues long yield scFv molecules that are unable to fold into a functional Fv domain. These molecules can associate with a second scFv molecule, to create a bivalent diabody. In one embodiment, the antibody fragment administered in combination with a selected compound described herein is a bivalent diabody. If the linker length is less than three residues, scFv molecules associate into triabodies or tetrabodies. In one embodiment, the antibody fragment is a triabody. In one embodiment, the antibody fragment is a tetrabody. Multivalent scFvs possess greater functional binding affinity to their target antigens than their monovalent counterparts by having binding to two more target antigens, which reduces the off-rate of the antibody fragment. In one embodiment, the antibody fragment is a minibody. Minibodies are scFv-CH3 fusion proteins that assemble into bivalent dimers. In one embodiment, the antibody fragment is a Bis-scFv fragment. Bis-scFv

fragments are bispecific. Miniaturized ScFv fragments can be generated that have two different variable domains, allowing these Bis-scFv molecules to concurrently bind to two different epitopes.

In one embodiment, a selected compound described herein is administered in conjugation or combination with a bispecific dimer (Fab2) or trispecific dimer (Fab3). Genetic methods are also used to create bispecific Fab dimers (Fab2) and trispecific Fab trimers (Fab3). These antibody fragments are able to bind 2 (Fab2) or 3 (Fab3) different antigens at once.

In one embodiment, a selected compound described herein is administered in conjugation or combination with an rIgG antibody fragment. rIgG antibody fragments refers to reduced IgG (75,000 daltons) or half-IgG. It is the product of selectively reducing just the hinge-region disulfide bonds. Although several disulfide bonds occur in IgG, those in the hinge-region are most accessible and easiest to reduce, especially with mild reducing agents like 2-mercaptoethylamine (2-MEA). Half-IgG are frequently prepared for the purpose of targeting the exposing hinge-region sulfhydryl groups that can be targeted for conjugation, either antibody immobilization or enzyme labeling.

In other embodiments, a selected active compound described herein can be linked to a radioisotope to increase efficacy, using methods well known in the art. Any radioisotope that is useful against cancer cells can be incorporated into the conjugate, for example, but not limited to, ^{131}I, ^{123}I, ^{192}Ir, ^{32}P, ^{90}Sr, ^{198}Au, ^{226}Ra, ^{90}Y, ^{241}Am, ^{252}Cf, ^{60}Co, or ^{137}Cs.

Of note, the linker chemistry can be important to efficacy and tolerability of the drug conjugates. The thio-ether linked T-DM1 increases the serum stability relative to a disulfide linker version and appears to undergo endosomal degradation, resulting in intra-cellular release of the cytotoxic agent, thereby improving efficacy and tolerability, *See*, Barginear, M.F. and Budman, D.R., Trastuzumab-DM1: A review of the novel immune-conjugate for HER2-overexpressing breast cancer, The Open Breast Cancer Journal, 1: 25-30, (2009).

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In one embodiment the pharmaceutical composition or combination as described herein can be used to treat any disorder described herein.

In one aspect a compound of the present invention is dosed in a combination or composition with an effective amount of a nucleoside or nucleoside analog. Non-limiting examples of nucleosides include: azacitidine, decitabine, didanosine, vidarabine, BCX4430, cytarabine, emtricitabine, lamivudine, zalcitabine, abacavir, aciclovir, entecavir, stavudine, telbivudine, zidovudine, idoxuridine, trifluridine, apricitabine, elvucitabine, amdoxovir, and racivir. In one embodiment the compound of present invention is used in a combination or composition with an effective amount of a nucleoside or nucleoside analog to treat a viral infection. In an alternative

embodiment the compound of present invention is used in a combination or composition with an effective amount of a nucleoside or nucleoside analog to treat a tumor or cancer. In one embodiment the nucleoside analog is azacitidine and the disorder is tumor or cancer.

In one embodiment, provided is a method of treating tumor or cancer in a subject comprising administration of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with an effective amount of a nucleoside analog to a host in need thereof. In another embodiment, provided is a method of treating tumor or cancer in a subject comprising administration of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with an effective amount of a nucleoside analog to a host in need thereof.

In one embodiment, provided is a method of treating tumor or cancer in a subject comprising administration of a compound described herein or a pharmaceutically acceptable salt thereof in combination or alternation with azacitidine to a host in need thereof. In another embodiment, provided is a method of treating tumor or cancer in a subject comprising administration of an analog of a compound described herein or a pharmaceutically acceptable salt thereof as provided herein in combination or alternation with azacitidine to a host in need thereof.

IX. EXAMPLES

Example 1. Exemplary Preparative Technique to Prepare Antigens to form Antibodies to the Pharmacodynamic Biomarkers.

To generate antibodies to a pharmacodynamic biomarker described herein, recombinant expression of the protein is performed to obtain the immunogen. The expression is done by applying a combination of the RTS 100 expression system and bacteria that expresses the pharmacodynamic biomarker. In a first step, the DNA sequence is analyzed and recommendations for high yield cDNA silent mutational variants and respective PCR-primer sequences are obtained using the "ProteoExpert RTS *E. coli* HY" system. This is a commercial web-based service (www.proteoexpert.com). Using the recommended primer pairs, the "RTS 100 *E. coli* Linear Template Generation Set, His-tag" (Roche Diagnostics GmbH, Mannheim, Germany, Cat. No. 3186237) system to generate linear PCR templates from the cDNA for in-vitro transcription and expression of the nucleotide sequence coding for the ASC protein is used. For Western-blot detection and later purification, the expressed protein contains a His-tag. The best expressing

variant is identified. All steps from PCR to expression and detection are carried out according to the instructions of the manufacturer. The respective PCR product, containing all necessary regulatory regions is cloned into the pBAD TOPO® vector (Invitrogen, Karlsruhe, Germany, Cat. No. K 4300/01) following the manufacturer's instructions. For expression using the regulatory sequences, the construct is transformed into *E. coli* BL 21 (DE 3) (Studier, F. W., et al., Methods Enzymol. 185 (1990) 60-89) and the transformed bacteria are cultivated in a 1 l batch for protein expression.

Purification of His-tag fusion protein is done following standard procedures on a Ni-chelate column. Briefly, 1 l of bacteria culture containing the expression vector for the His-tag fusion protein is pelleted by centrifugation. The cell pellet is suspended in lysis buffer, containing phosphate, pH 8.0, 7 M guanidium chloride, imidazole and thioglycerole, followed by homogenization using a Ultra-Turrax®. Insoluble material is pelleted by high speed centrifugation and the supernatant is applied to a Ni-chelate chromatographic column. The column is washed with several bed volumes of lysis buffer followed by washes with buffer, containing phosphate, pH 8.0 and urea. Finally, bound antigen is eluted using a phosphate buffer containing SDS under acid conditions.

Example 2 Exemplary Preparative Technique to form Antibodies to the Pharmacodynamic Biomarkers.

12 week old mice are initially immunized intraperitoneally with 100 µg of antigen (see above). This is followed after 6 weeks by two further intraperitoneal immunizations at monthly intervals. In this process each mouse is administered 100 µg antigen or antigen adsorbed to aluminium hydroxide and 10^9 germs of *Bordetella pertussis*. Subsequently the last two immunizations are carried out intravenously on the 3rd and 2nd day before fusion using 100 µg of antigen for each.

Spleen cells of the mice are fused with myeloma cells according to Galfre, G., and Milstein, C., Methods in Enzymology 73 (1981) 346. In this process ca. 1×10^8 spleen cells of the immunized mouse are mixed with 2×10^7 myeloma cells (P3X63-Ag8-653, ATCC CRL1580) and centrifuged (10 min at 300×g and 4° C.). The cells are then washed once with RPMI 1640 medium and centrifuged again at 400×g in a 50 ml conical tube. The supernatant is discarded, the cell sediment is gently loosened by tapping, 1 ml PEG (molecular weight 4000, Merck, Darmstadt) is added and

mixed by pipetting. After 1 min in a water-bath at 37° C., 5 ml RPMI 1640 is added drop-wise at room temperature within a period of 4-5 min. Afterwards 5 ml RPMI 1640 containing 10% FCS is added drop-wise within ca. 1 min, mixed thoroughly, filled to 50 ml with medium (RPMI 1640+10% FCS) and subsequently centrifuged for 10 min at 400×g and 4° C. The sedimented cells are taken up in RPMI 1640 medium containing 10% FCS and sown in hypoxanthine-azaserine selection medium (100 mmol/l hypoxanthine, 1 μg/ml azaserine in RPMI 1640+10% FCS). Interleukin 6 at 100 U/ml is added to the medium as a growth factor.

After ca. 10 days the primary cultures are tested for specific antibody. Antibody positive primary cultures are cloned in 96-well cell culture plates by means of a fluorescence activated cell sorter. In this process again interleukin 6 at 100 U/ml is added to the medium as a growth additive.

The hybridoma cells obtained are sown at a density of 1×10^5 cells per ml in RPMI 1640 medium containing 10% FCS and proliferated for 7 days in a fermenter (Thermodux Co., Wertheim/Main, Model MCS-104XL, Order No. 144-050). Purification of this antibody from the culture supernatant is carried out by conventional methods in protein chemistry (e.g. according to Bruck, C., et al., Methods in Enzymology 121 (1986) 587-695).

Example 3. Exemplary Western Blot Technique to Detect the Concentration of Pharmacodynamic Biomarker in Human Plasma Samples

SDS-PAGE and Western Blotting are carried out using reagents and equipment of Invitrogen, Karlsruhe, Germany. Human plasma samples are diluted 1:20 in reducing NuPAGE® (Invitrogen) LDS sample buffer and heated for 5 min at 95° C. 10 μl aliquots are run on 4-12% NuPAGE® gels (Bis-Tris) in the MES running buffer system. The gel-separated protein mixture is blotted onto nitrocellulose membranes using the Invitrogen XCell II™ Blot Module (Invitrogen) and the NuPAGE® transfer buffer system. The membranes are washed 3 times in PBS/0.05% Tween-20 and blocked with SuperBlock Blocking Buffer (Pierce Biotechnology, Inc., Rockford, Ill., USA). The biotinylated primary antibody is diluted in SuperBlock Blocking Buffer (0.01-0.2 μg/ml) and incubated with the membrane for 1 h. The membranes are washed 3 times in PBS/0.05% Tween-20. The specifically bound biotinylated primary antibody is labelled with a streptavidin-HRP-conjugate (20 mU_{ABTS}/ml in SuperBlock Blocking Buffer). After incubation for 1 h, the membranes are washed 3 times in PBS/0.05% Tween-20. The bound streptavidin-HRP-conjugate is detected using a chemiluminescent substrate (SuperSignal West Fernto Substrate,

Pierce Biotechnology, Inc., Rockford, Ill., USA) and autoradiographic film. Exposure times varies from 10 min to overnight.

Example 4. Exemplary ELISA Technique to Detect the Concentration of Pharmacodynamic Biomarker in Human Plasma Samples

A 20 µl aliquot of a human serum or plasma sample or a serial dilution of the recombinant standard antigen were incubated with 100 µl biotinylated polyclonal antibody (1 µg/ml) and with digoxygenylated polyclonal antibody (1 µg/ml) in 10 mM phosphate, pH 7.4, 1% BSA, 0.9% NaCl and 0.1% Tween-20. After incubation for 90 min at 30° C., the plates were washed three times with 0.9% NaCl, 0.1% Tween-20. For the detection of antigen-antibody complexes, 150 µl of an monoclonal anti-digoxigenin peroxidase conjugate in 10 mM phosphate, pH 7.4, 1% BSA, 0.9% NaCl and 0.1% Tween-20 were added and incubated for 30 min at 30° C. The excess of conjugate was removed by washing the plates three times with 0.9% NaCl, 0.1% Tween-20. The amount of bound conjugate was detected by incubation with 200 µl TMB solution (Roche Diagnostics GmbH, Penzberg, Germany, Catalog No. 12034425) for 15 min. The enzymatic reaction was stopped by the addition of 50 µl 1 M H_2SO_4. The color development was quantified at 450 nm using an ELISA reader. The concentration of pharmacokinetic biomarker in a serum or plasma sample was calculated from the standard curve using a serial dilution of recombinant biomarker-His-fusion protein.

Example 5. Preparation of PBMCs from Healthy Donors for Tandem Mass Spectrometry Studies

Cryogenically frozen PBMCs from 5 healthy donors (provided by StemCell Technologies) were thawed and maintained in the incubator in RPMI supplemented with 10% heat inactivated FBS in low attachment plates. After 1 hour recovery, 100 nM Cortistatin A or 0.1% DMSO was added to each sample and left in the incubator for 1 hour. PBMCs were collected and flash-frozen. Cells were lysed in 50 mM Tris pH 8.5, 8M Urea, 1% SDS, and protease and phosphatase inhibitors (Roche), reduced with DTT and alkylated with iodoacetamide. Proteins were precipitated by methanol/chloroform, and digested sequentially using LysC (1:50) and trypsin (1:100) protease/protein ratio. Peptides were quantified by micro-BCA (Pierce), and tandem mass tagged for multiplexing. TMT label incorporation was 99.7%. 3278 total peptides, 2254 unique peptides representing 1147 proteins were identified. Phosphopeptide enrichment was 83.9% (2752 total phospho peptides, 1968 unique phosphopeptides). Peptides were separated using a 3 to 25% acetonitrile gradient in 0.125% formic acid over 180 minutes. Peptides were detected (MS1) and quantified (MS3) in the Orbitrap. Peptides were sequenced (MS2) in the ion trap. MS2 spectra were searched using SEQUEST algorithm again a Uniprot composite database. Protein spectral matches were filtered to a 1% FDR using target-decoy strategy combined with linear discriminant analysis. Proteins were filtered to less than 1% FDR and quantified only from peptides with a summed signal/noise threshold of >=200 and MS2 isolation specificity of 0.5. These proteins were used to produce the volcano plot in Figure 1.

Example 6: Generation of Volcano Plots from Heavy Labelled Material.

AML cell lines MOLM-13 and MOLM-14 were split and grown in heavy-labeled and light labeled SILAC medium for weeks. Cells were treated with vehicle or CDK8/19 inhibitor for one hour, and lysed. Lysates were mixed and analyzed by mass spectrometry. Both reciprocal iterations were performed (vehicle or inhibitor-treated cells heavy labeled). CDK8/19 substrates were from phosphopeptides with a high ratio in vehicle: CDK8 inhibitor- treated samples. Confidence was assessed by ztest, calculating number of heavy and light labeled peptides detected in each sample. The resultant volcano plots are shown in Figures 2, 3, 4, and 5 which correspond to MOLM-13 CA treated, MOLM-13 vehicle treated, MOLM-14 CA treated, and MOLM-14 vehicle treated samples respectively.

Example 7. Preparation of Antibodies for Immunoblot Studies.

Peptide synthesis

5 mg of phosphorylated and unphosphorylated version of each peptide was synthesized for immunization, screening, and antibody purification. Phosphorylated peptide was conjugated to keyhole limpet hemocyanin (KLH) and used for immunization. The phosphorylated peptides used in the generation of the antibodies were: CHD4 pT1553: TQPN{pThr}PAPVPPAEDC, DPF2 pT248: DSQPP{pThr}PVSQRSEEC, DMAP1 pT445: VGAPL{pThr}PNSRKRREC. The C-terminal cysteine was used for conjugation to KLH.

Immunization schedule

Three Balb/c and three C57BL/6 mice were injected with the immunogen on the following schedule: Primary immunization: 50 ug/animal with complete Freund's adjuvant (CFA) subcutaneously, 2 boosts of 25 ug/animal on days 14, 28 after primary immunization with incomplete Freund's adjuvant (IFA) subcutaneously. Total sera were screened by Western blotting using lysates of SET-2 cells treated with vehicle or 100 nM Cortistatin A for 3 hours. Two animals mounting the best phosphospecific response were selected for a final intravenous boost at day ~50, using 25 ug/animal and IFA.

Cell fusion, clone screening and antibody purification

Cell fusion was performed 4 days after the final boost, using electrofusion and myeloma type SP2/0, generating ~20,000 hybridoma clones per fusion. The clones were plated in multiple 96 well plates. Conditioned media of clones were screened initially for primary binding by ELISA with phosphorylated peptide. Confirmatory screening was then performed by indirect ELISA against unphosphorylated peptide. Positive clones were expanded into 24 well plates and then frozen down. Screening with hybridoma supernatants was performed by Western blot as described earlier for total sera. Clones that performed well by Western blotting were subcloned by limited dilution and rescreened by indirect ELISA. Two stable subclonal cell lines of each primary clone were cryopreserved. Monoclonal antibody was purified by Protein A affinity column chromatography and dialyzed against PBS after elution.

Characterization of antibodies in Western blot analysis

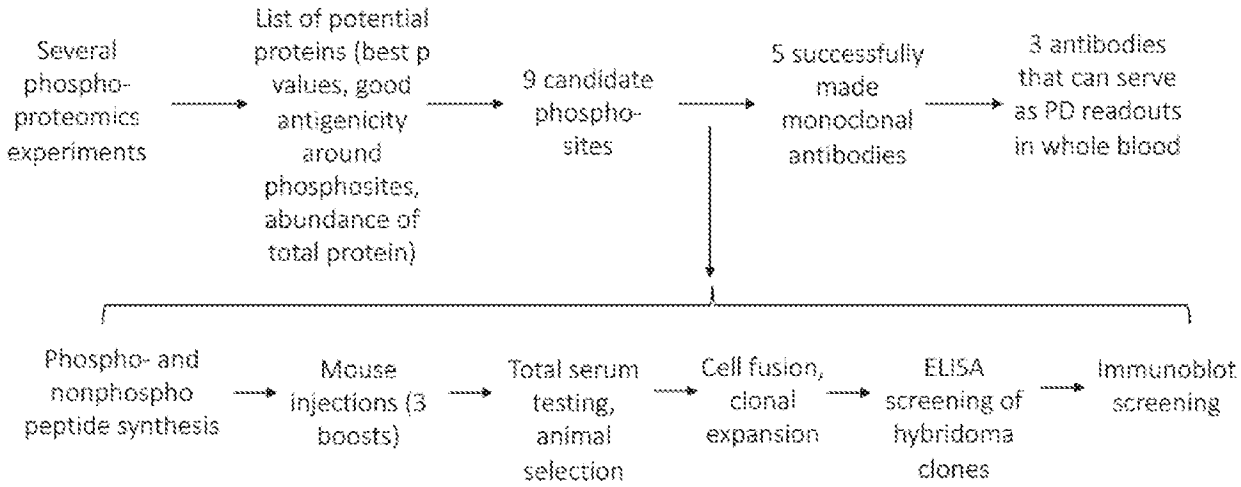
Anti-CHD4 pT1553: clone 7F12 (BALB/c mouse, isotype IgG1), clones 4D8, 7F7, 8B6, and 11B6 (BALB/c mouse, isotype IgG2b); Anti-DPF2 pT248: clones 1D4, 2D1 (BALB/c mouse, isotype IgG2a), clones 11B8, 12C8, 12F1 (BALB/c mouse, isotype IgG2b); Anti-DMAP1 pT445: clone 5G9 (BALB/c mouse, IgG2a), clones 6G4, 14F12 (BALB/c mouse, IgG2b)

The best performing monoclonal antibodies for each target were 11B6 for CHD4, 2D1 for DPF2, and 6G4 for DMAP1 but all successful clones were retained in case other applications (ELISA, flow cytometry) showed a different ranking of performance. Working concentrations for Western blotting by antibodies were 0.2 ug/ml in a blocking solution of 5% fat-free milk in Tris-buffered saline and 0.2% Tween.

Summary

Total serum from animals was screened by immunoblot after the third immunization, and an animal was selected for cell fusion. After one round of cell fusion, hybridoma clones were screened by indirect ELISA, primary screening with phospho peptide, then counter screening with unphosphorylated peptide, and finally by immunoblot, in cell lines and whole blood.

The procedural work flow is provided in Scheme 1 below.



Scheme 1.

Example 8. Preparation of PBMCs for Immunoblot Studies.

Whole blood samples (Research Blood Components) were drawn on the same day as the experiment. SL164 titration stocks were prepared in DMSO and added to blood in T25 flasks that

were incubated at 37 °C 5% CO_2 for 3-4 hours. PBMCs were then collected using Lymphoprep and Sepmate-50 tubes (StemCell Technologies) according to standard protocols. PBMCs were transferred to microtubes, washed with PBS, and lysed in buffer containing 20 mM Tris pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM Na2EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na3VO4, 1 μg/ml, 1X HALT protease/phosphatase inhibitor cocktail, 2 mM PMSF and 1 mM 3,4- dichloroisocoumarin on ice. Samples were quantified by BCA (Thermo Fisher) and run on SDS-PAGE gels at 30 ug total protein per lane. Immunoblotting was performed using the monoclonal antibodies and anti-mouse HRP conjugated antibody.

Example 9. Immunoblot Studies

PBMCs were transferred to microtubes, washed with PBS, and lysed in buffer containing 20 mM Tris pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM Na2EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na3VO4, 1 μg/ml, 1X HALT protease/phosphatase inhibitor cocktail, 2 mM PMSF and 1 mM 3,4-dichloroisocoumarin on ice. Samples were quantified by BCA (Thermo Fisher) and run on SDS-PAGE gels at 30 ug total protein per lane. Immunoblotting was performed using the monoclonal antibodies and anti-mouse HRP conjugated antibody.

The immunoblot studies shown in Figures 6, 7, 8, and 9 all correspond to the same PBMC sample. A different PBMC sample was subjected to the same techniques to result in Figures 10, 11, 12, 13 and 14. Differences in the total amount of the phosphorylated and non phosphoralated proteins can be observed when comparing the immunoblots resulting from these two different PBMC samples. However, this inherent patient variance is reduced by relying on % changes in biomarkers. Similarly, when the blood sample was treated with a 10% solution of serum prior to immunoblotting the maximal concentrations of proteins were also noticeably different. These immunoblots are shown in Figures 15, 16, 17, and 18.

The immunoblot studies were then repeated in various cancer cell lines instead of utilizing patient PBMC samples. The cell lines tested were EOL-1, HL-60, Kasumi-1, MEG-01, MOLM-14, NOMO-1, SET-2, SKNO-1, MV4;11, and MOLM-16. The resultant immunoblots from culturing the cells in the presence and absence of 100 nM Cortistatin A are shown in Figures 19, and 20. Immunoblots resulting from xenograph studies in MV4:11 cells.

This specification has been described with reference to embodiments of the invention. However, one of ordinary skill in the art appreciates that various modifications and changes can be made without departing from the scope of the invention as set forth in the claims below. Accordingly, the specification is to be regarded in an illustrative rather than a restrictive sense, and all such modifications are intended to be included within the scope of invention.

CLAIMS

What is claimed is:

1. A method for treating a patient with a CDK8 and/or CDK19 mediated disorder with a CDK8/19 inhibitor, said method comprising:
    a. obtaining a first blood sample from the patient before treatment;
    b. detecting the concentration of a pharmacodynamic biomarker in the first blood sample by contacting the blood sample with an antibody to the pharmacodynamic biomarker and detecting binding between the antibody and pharmacodynamic biomarker;
    c. administering a first dose of the CDK8/19 inhibitor;
    d. obtaining a second blood sample from the patient after treatment;
    e. detecting the concentration of the pharmacodynamic biomarker in the second blood sample by contacting the blood sample with an antibody to the pharmacodynamic biomarker and detecting binding between the antibody and pharmacodynamic biomarker;
    f. determining if the patient is responding to treatment, wherein less than a 10% decrease in the concentration of the pharmacodynamic biomarker in the second blood sample relative to the first blood sample indicates that the treatment regimen is not efficacious for treating the CDK8 and/or CDK19 mediated disorder in the subject; and
    g. continuing treatment with the CDK8/19 inhibitor if the CDK8 and/or CDK19 mediated disorder is responding to treatment;

wherein the pharmacodynamic biomarker is a phosphorylated protein selected from pCHD4 , pDPF2, and pDMAP1.

2. A method of determining if patient's CDK8 and/or CDK19 mediated disorder is responding to treatment with a CDK8/19 inhibitor analogue, said method comprising:
    a. obtaining a first blood sample from the patient before treatment;
    b. detecting the concentration of a pharmacodynamic biomarker in the first blood sample by contacting the blood sample with an antibody to the pharmacodynamic biomarker and detecting binding between the antibody and pharmacodynamic biomarker;
    c. obtaining a second blood sample from the patient after treatment;
    d. detecting the concentration of the pharmacodynamic biomarker in the second blood sample by contacting the blood sample with an antibody to the pharmacodynamic biomarker and detecting binding between the antibody and pharmacodynamic biomarker; and
    e. determining if the patient is responding to treatment, wherein less than a 10% decrease in the concentration of the pharmacodynamic biomarker in the second blood sample relative to the first blood sample indicates that the treatment regimen is not efficacious for treating the CDK8 and/or CDK19 mediated disorder in the subject;

wherein the pharmacodynamic biomarker is a phosphorylated protein selected from pCHD4, pDPF2, and pDMAP1.

3. The method of claim 1 or 2, wherein a less than 15% decrease in concentration of the pharmacodynamic biomarker indicates that the treatment regimen is not efficacious.

4. The method of claim 1 or 2, wherein a less than 20% decrease in concentration of the pharmacodynamic biomarker indicates that the treatment regimen is not efficacious.

5. The method of claim 1 or 2, wherein a less than 30% decrease in concentration of the pharmacodynamic biomarker indicates that the treatment regimen is not efficacious.

6. A method for determining the dose of a CDK8/19 inhibitor to achieve full target inhibition, said method comprising:

a. obtaining a first blood sample before treatment;
b. detecting the concentration of a pharmacodynamic biomarker in the first blood sample by contacting the blood sample with an antibody to the pharmacodynamic biomarker and detecting the concentration of the pharmacodynamic biomarker;
c. administering a dose of the CDK8/19 inhibitor;
d. obtaining a another blood sample after sufficient time has passed for the CDK8/19 inhibitor to have acted;
e. detecting the concentration of the pharmacodynamic biomarker in the blood sample by contacting the blood sample with an antibody to the pharmacodynamic biomarker and detecting the concentration of the pharmacodynamic biomarker;
f. repeating steps c, d, and e at least 1, 2, 3, or 4 times wherein a higher dose of the CDK8/19 inhibitor is used each time;
g. determining at which dose full target inhibition was achieved, wherein a plateau in the concentration of the pharmacodynamic biomarker is indicative of full target inhibition;

wherein the pharmacodynamic biomarker is a phosphorylated protein selected from pCHD4 , pDPF2, and pDMAP1.

7. The method of claim 6, wherein the blood sample is taken from a patient before and after beginning therapy with a CDK8/19 inhibitor.

8. The method of any of claims 1-7, wherein the pharmacodynamic biomarker is pCHD4.

9. The method of claim 8, wherein the pCHD4 is CHD4 pT1553.

10. The method of claim 8 or 9, wherein the pCHD4 antibody is an antibody to CHD4 pT1553.

11. The method of any of claims 1-7, wherein the pharmacodynamic biomarker is pDPF2.

12. The method of claim 11, wherein the pDPF2 is DPF2 pT248.

13. The method of claim 11 or 12, wherein the pDPF2 antibody is an antibody to DPF2 pT248.

14. The method of any of claims 1-7, wherein the pharmacodynamic biomarker is pDMAP1.

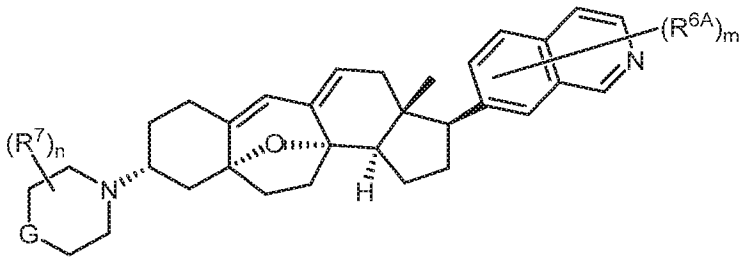
15. The method of claim 14, wherein the pDMAP1 is DMAP1 pT445.

16. The method of claim 14 or 15, wherein the pDMAP1 antibody is an antibody to DMAP1 pT445.

17. The method of any of claims 1-16, wherein the blood sample is a whole blood sample.

18. The method of any of claims 1-16, wherein the blood sample is a PBMC blood sample.

19. The method of any of claims 1-18, wherein the CDK8/19 inhibitor is selected from:



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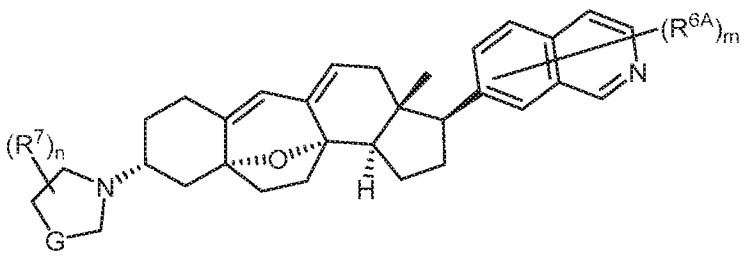
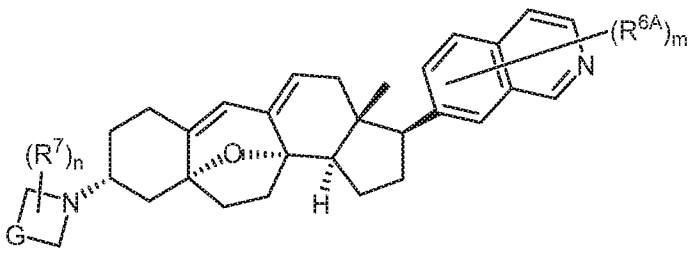


, and



;

or a pharmaceutical acceptable salt thereof;

wherein:

G is -O-, -S-, -NH-, -NR^7-, -CH_2-, -$CH(R^7)$-, or -$C(R^7)_2$-;

each instance of R^{6A} is independently halogen, -NO_2, -CN, -OR^{6C}, -SR^{6C}, -$N(R^{6C})_2$, -$C(=O)R^{6C}$, -$C(=O)OR^{6C}$, -$C(=O)N(R^{6C})_2$, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl;

each instance of R^{6C} is independently hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, an oxygen protecting group when attached to oxygen, a sulfur protecting group when attached to sulfur, or a nitrogen protecting group when attached to nitrogen, optionally when attached to N the two R^{6C} groups may be joined to form an optionally substituted heterocyclyl or optionally substituted heteroaryl ring;

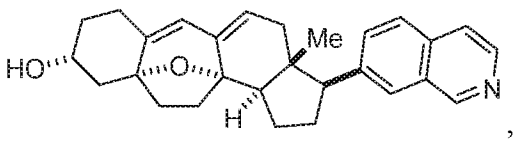
each instance of R^7 is independently halogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, amino, substituted amino, hydroxyl, substituted hydroxyl, thiol, substituted thiol, carbonyl, sulfonyl, sulfinyl, or a nitrogen protecting group when attached to a nitrogen atom;

optionally wherein two R^7 groups are joined to form an optionally substituted carbocyclyl, optionally substituted heterocyclyl, optionally substituted aryl, an optionally substituted heteroaryl ring, or an oxo (=O) group;

n is 0, 1, 2, 3, or 4; and

m is 0, 1, 2, 3, or 4.

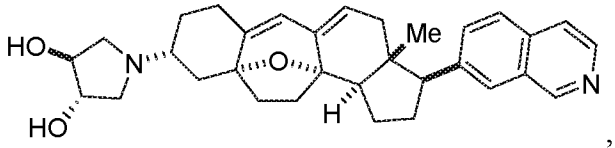
20. The method of any of claims 1-18, wherein the CDK8/19 inhibitor is:



,

or a pharmaceutically acceptable salt thereof.

21. The method of any of claims 1-18, wherein the CDK8/19 inhibitor is selected from:



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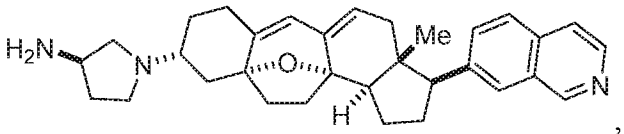
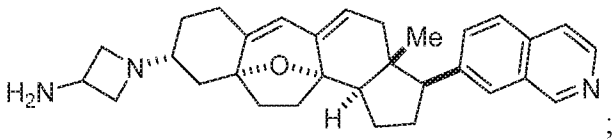


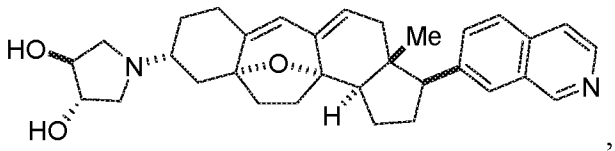
, and



;

or a pharmaceutically acceptable salt thereof.

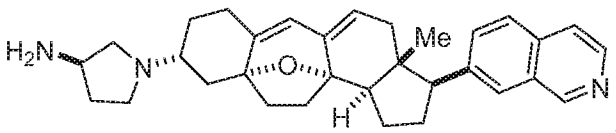
22. The method of claim 21, wherein the CDK8/19 inhibitor is:



,

or a pharmaceutically acceptable salt thereof.

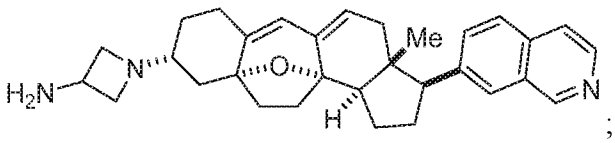
23. The method of claim 21, wherein the CDK8/19 inhibitor is:



, and

or a pharmaceutically acceptable salt thereof.

24. The method of claim 21, wherein the CDK8/19 inhibitor is:



;

or a pharmaceutically acceptable salt thereof.

25. The method of any of claims 1-24, wherein the CDK8 and/or CDK19 mediated disorder is cancer.

26. The method of claim 25, wherein the cancer is acute myeloid leukemia.

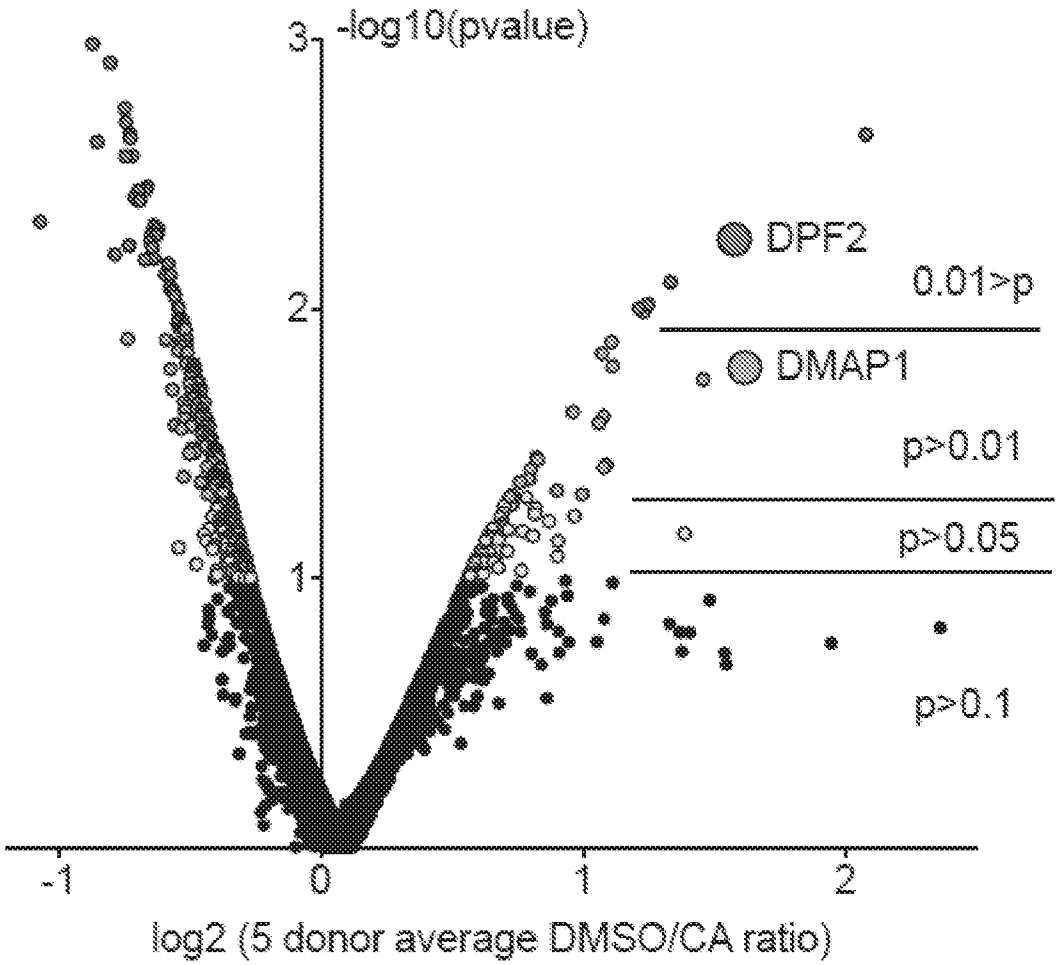

-log10(pvalue)
3
2
1
DPF2
0.01>p
DMAP1
p>0.01
p>0.05
p>0.1
-1
0
1
2
log2 (5 donor average DMSO/CA ratio)

FIG. 1

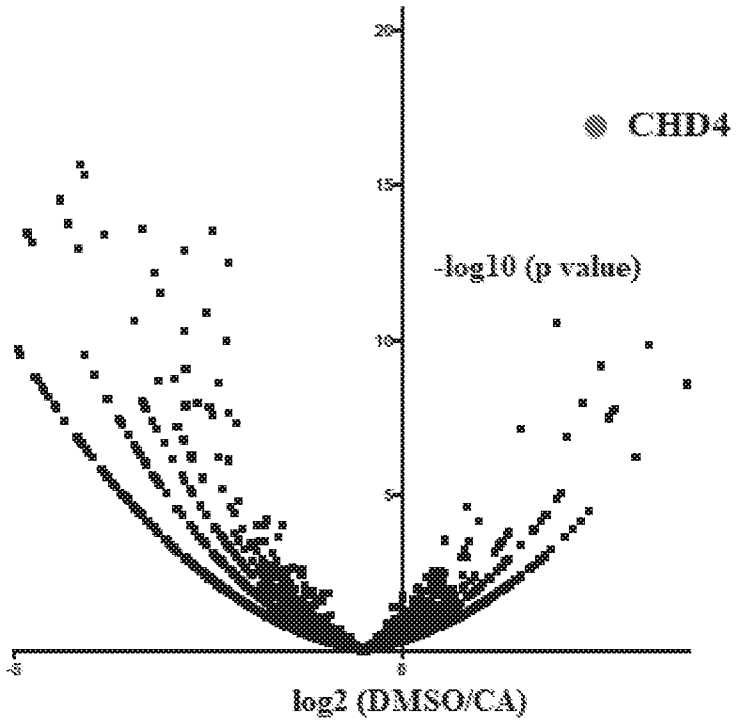


FIG. 2

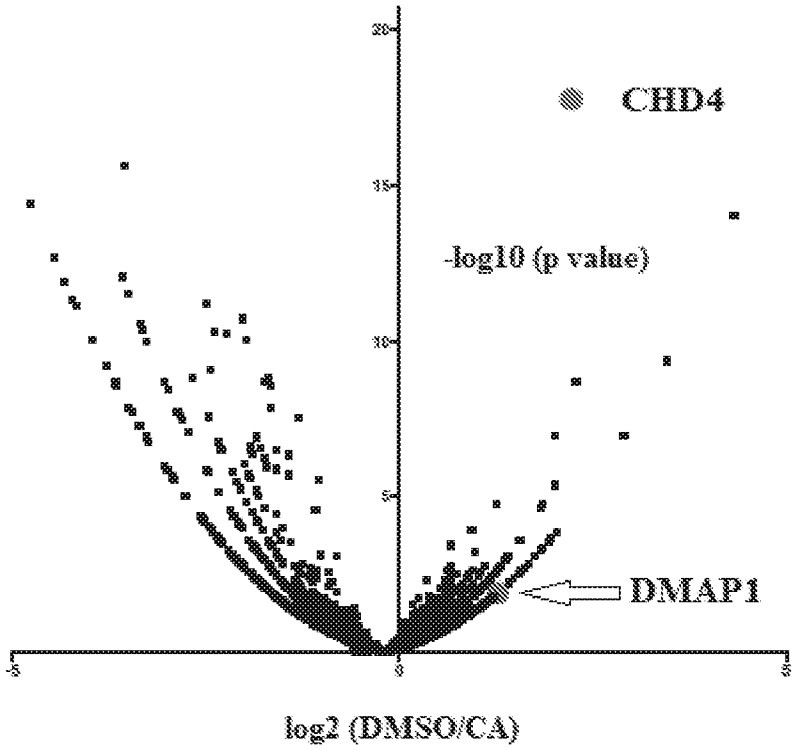


FIG. 3

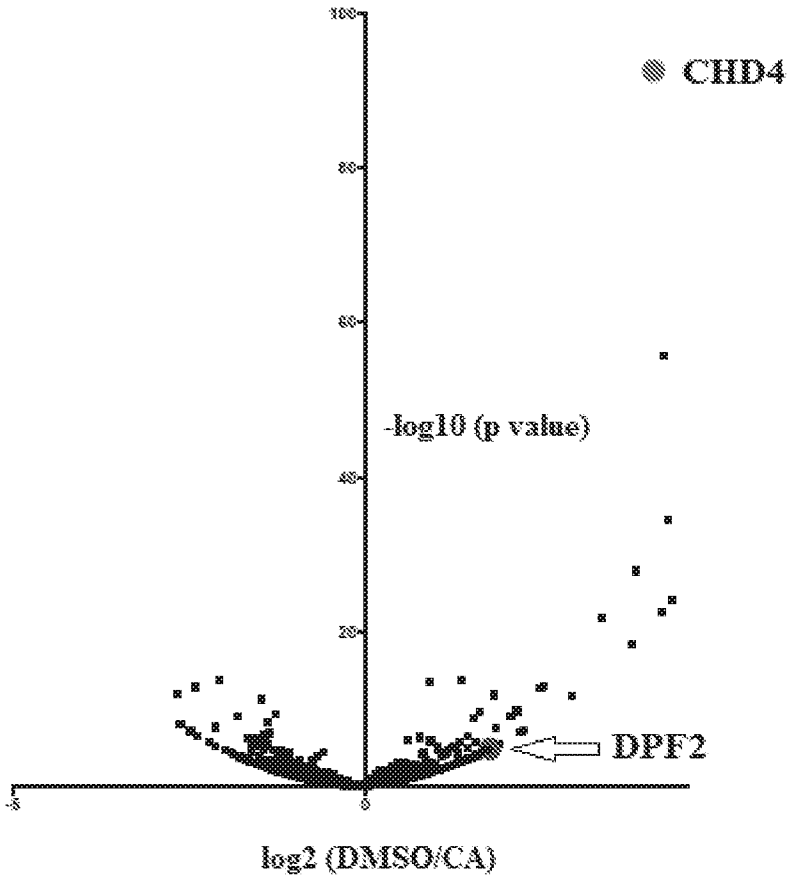


FIG. 4

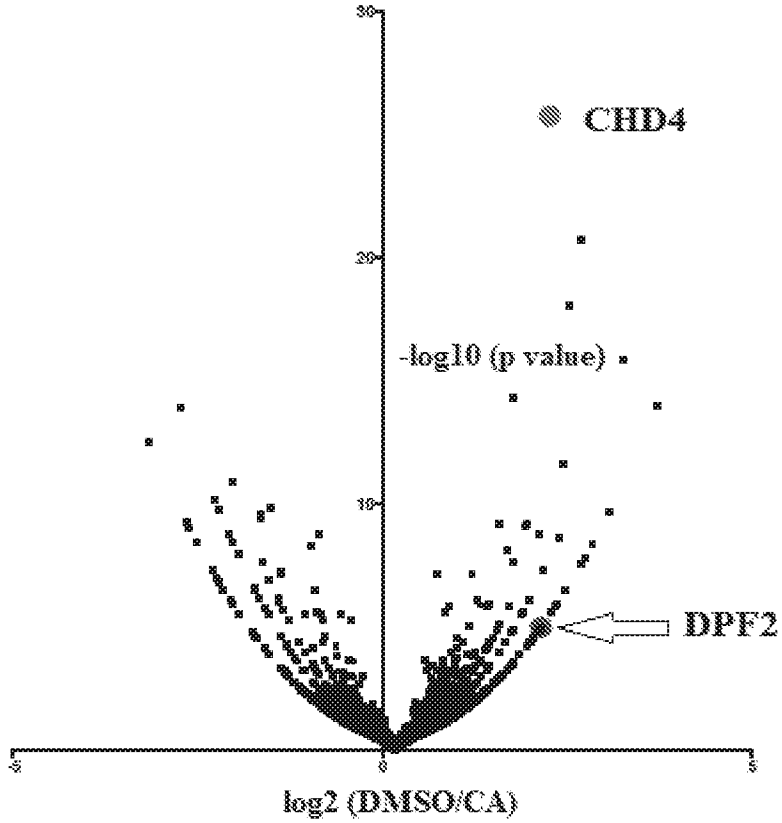


FIG. 5

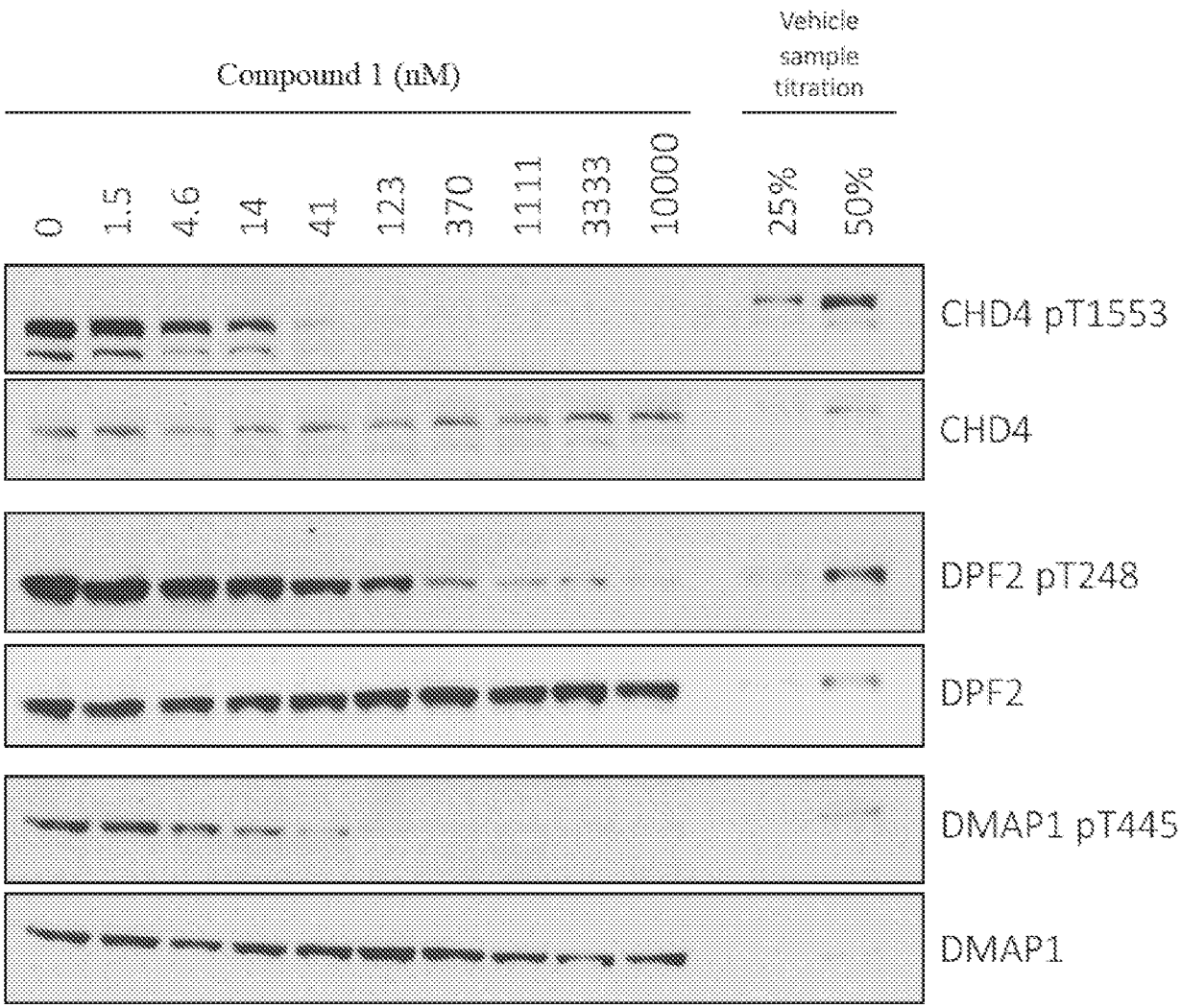

Compound 1 (nM)
Vehicle sample titration
0
1.5
4.6
14
41
123
370
1111
3333
10000
25%
50%
CHD4 pT1553
CHD4
DPF2 pT248
DPF2
DMAP1 pT445
DMAP1

FIG. 6

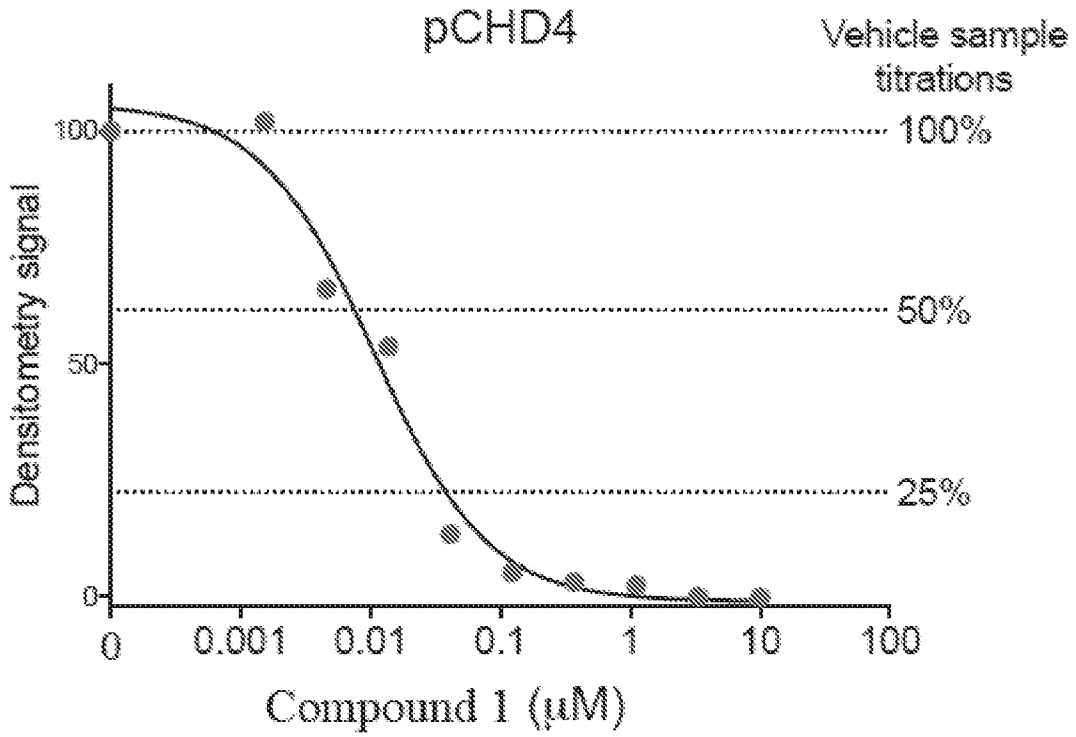

pCHD4
Vehicle sample titrations
100%
50%
25%
Densitometry signal
100
50
0
0
0.001
0.01
0.1
1
10
100
Compound 1 (μM)

FIG. 7

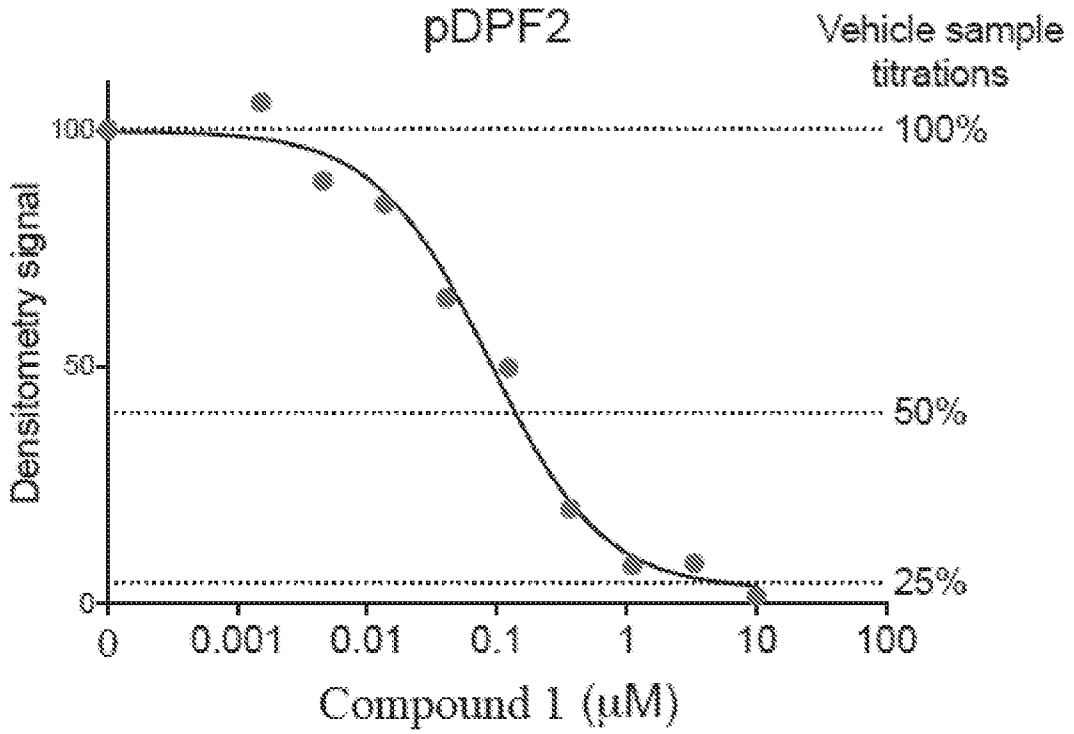

pDPF2
Vehicle sample titrations
100%
50%
25%
100
50
0
Densitometry signal
0
0.001
0.01
0.1
1
10
100
Compound 1 (μM)

FIG. 8

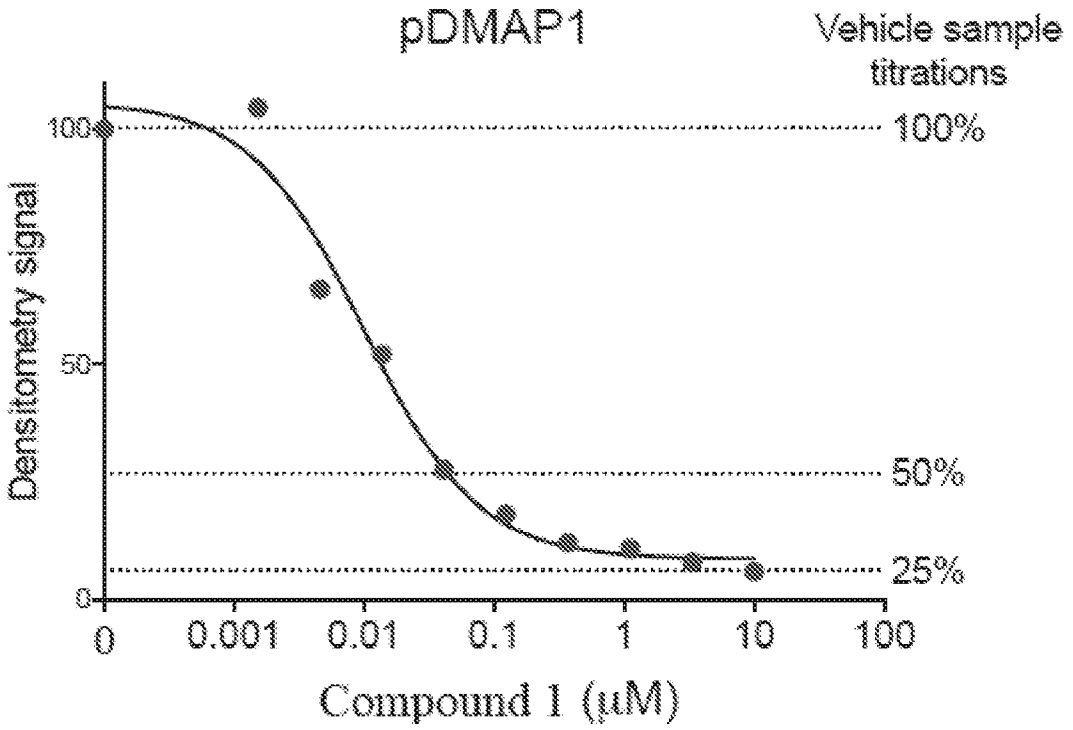

pDMAP1
Vehicle sample titrations
100%
50%
25%
100
50
0
Densitometry signal
0
0.001
0.01
0.1
1
10
100
Compound 1 (μM)

FIG. 9

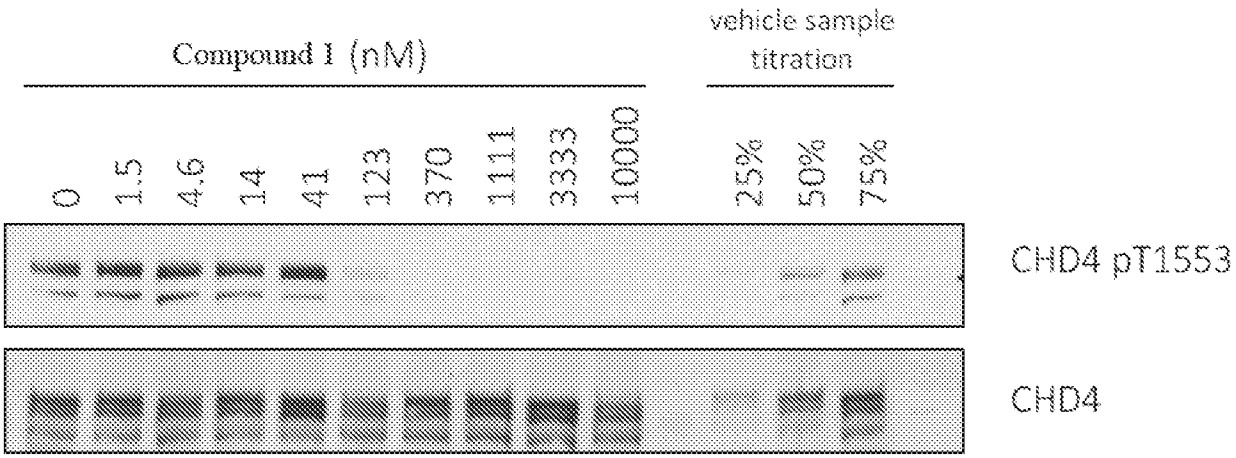

Compound 1 (nM)
vehicle sample titration
0
1.5
4.6
14
41
123
370
1111
3333
10000
25%
50%
75%
CHD4 pT1553
CHD4

FIG. 10

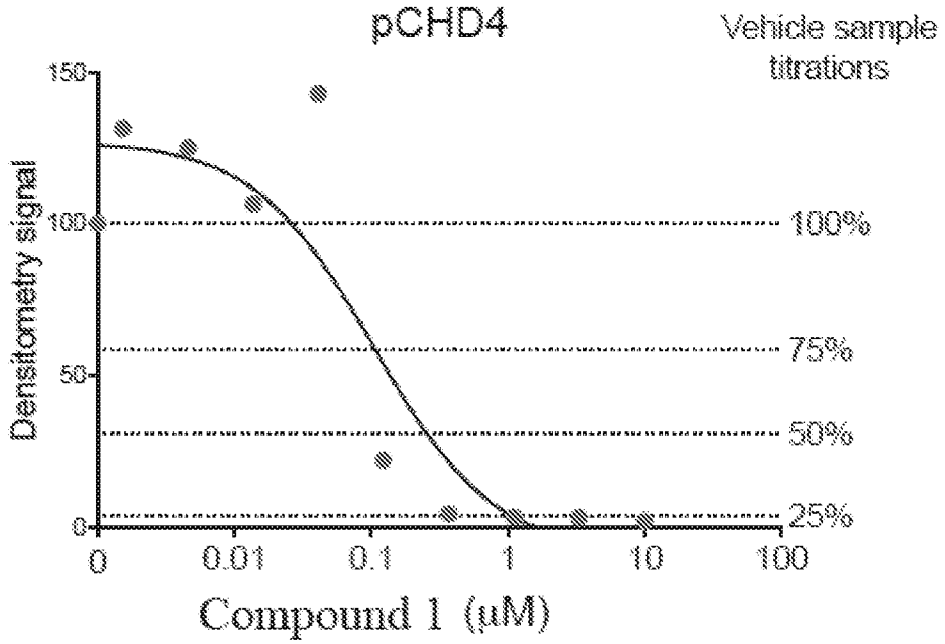

pCHD4
Vehicle sample titrations
Densitometry signal
150
100
50
0
100%
75%
50%
25%
0
0.01
0.1
1
10
100
Compound 1 (μM)

FIG. 11

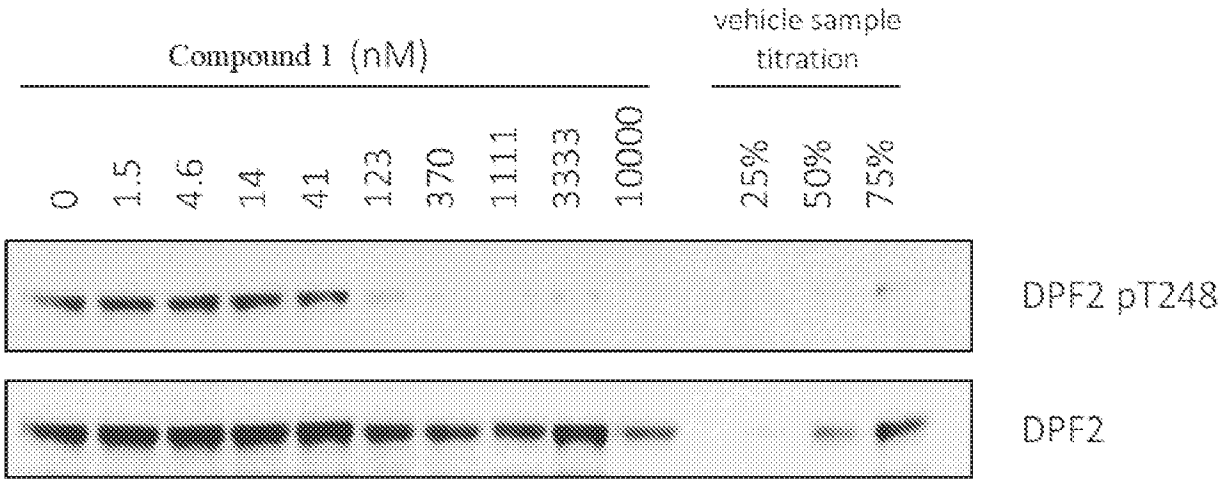

Compound 1 (nM)
vehicle sample titration
0
1.5
4.6
14
41
123
370
1111
3333
10000
25%
50%
75%
DPF2 pT248
DPF2

FIG. 12

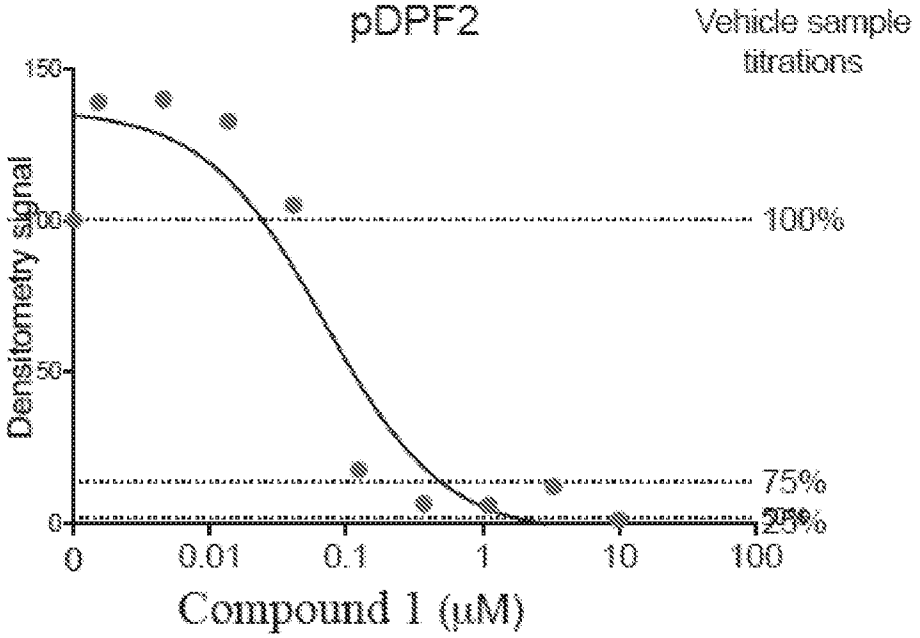

pDPF2
Vehicle sample titrations
Densitometry signal
150
100
50
0
100%
75%
25%
0
0.01
0.1
1
10
100
Compound 1 (μM)

FIG. 13

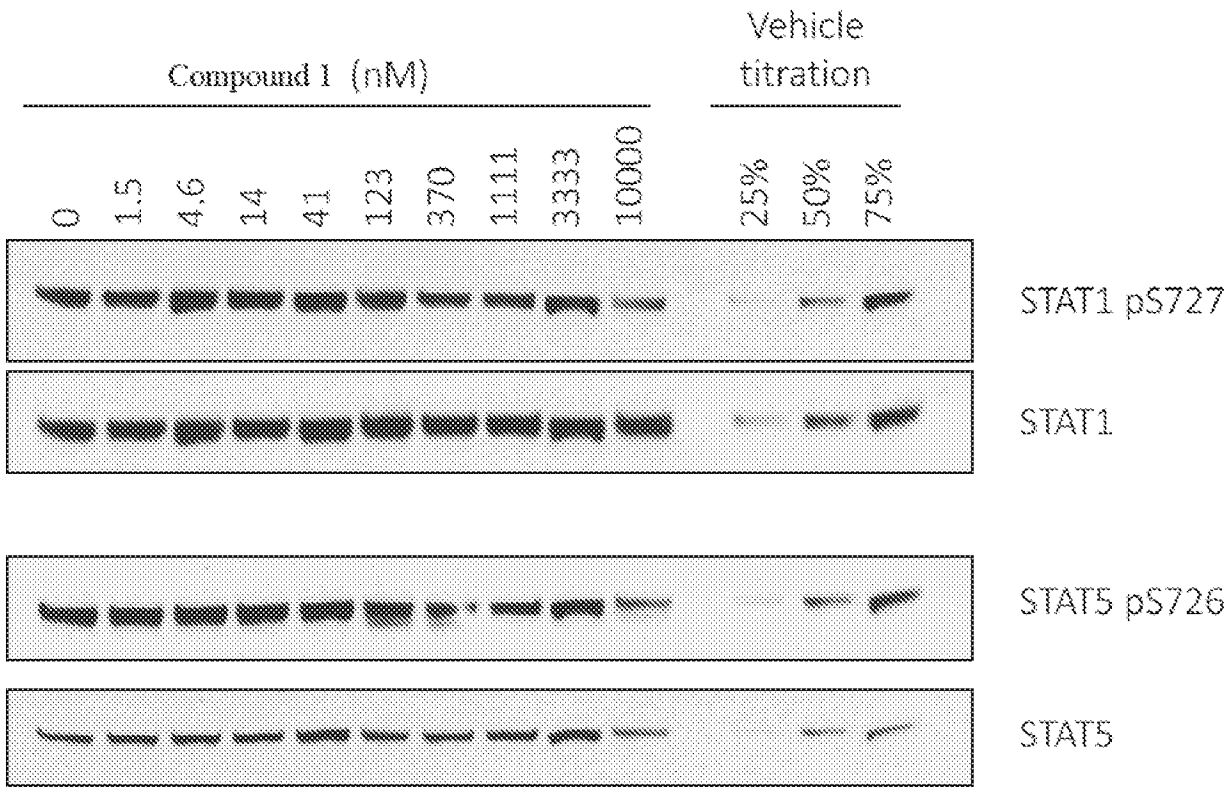

Compound 1 (nM)
Vehicle titration
0
1.5
4.6
14
41
123
370
1111
3333
10000
25%
50%
75%
STAT1 pS727
STAT1
STAT5 pS726
STAT5

FIG. 14

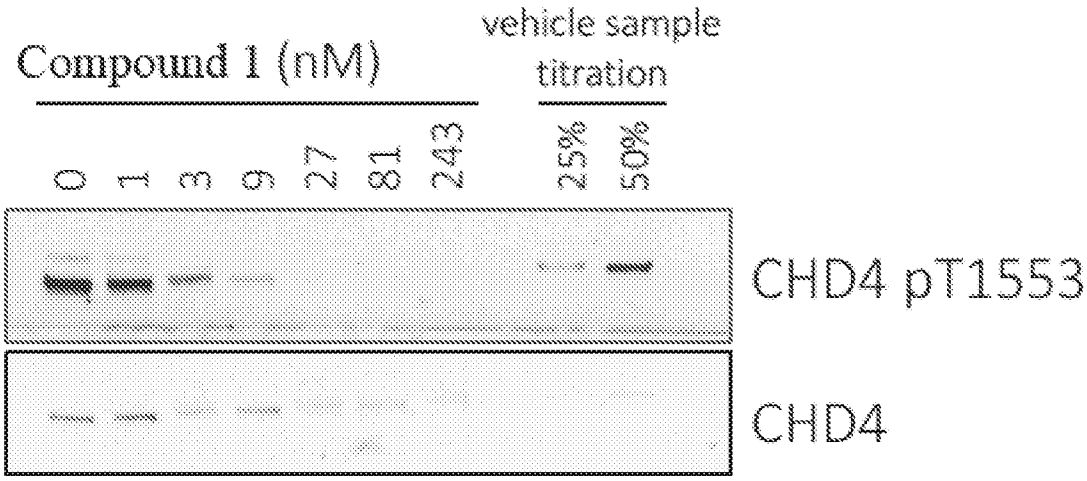

Compound 1 (nM)
vehicle sample titration
0
1
3
9
27
81
243
25%
50%
CHD4 pT1553
CHD4

FIG. 15

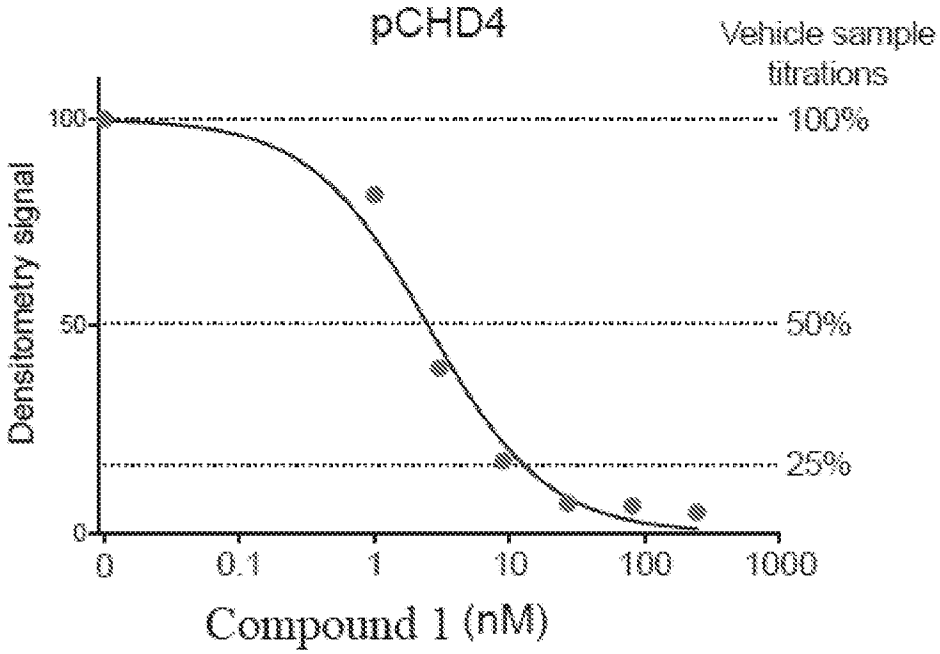

pCHD4
Vehicle sample titrations
100%
50%
25%
Densitometry signal
100
50
0
0
0.1
1
10
100
1000
Compound 1 (nM)

FIG. 16

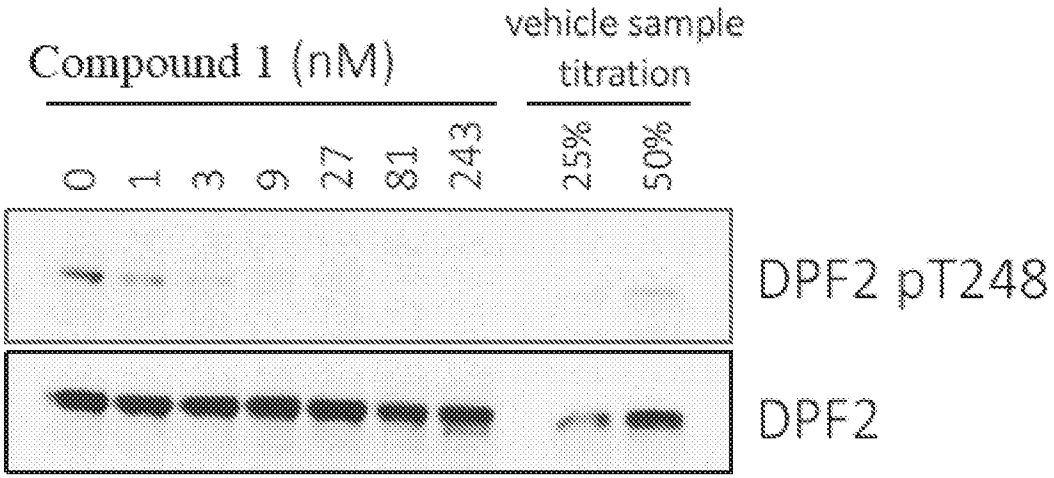

Compound 1 (nM)
vehicle sample titration
0
1
3
9
27
81
243
25%
50%
DPF2 pT248
DPF2

FIG. 17

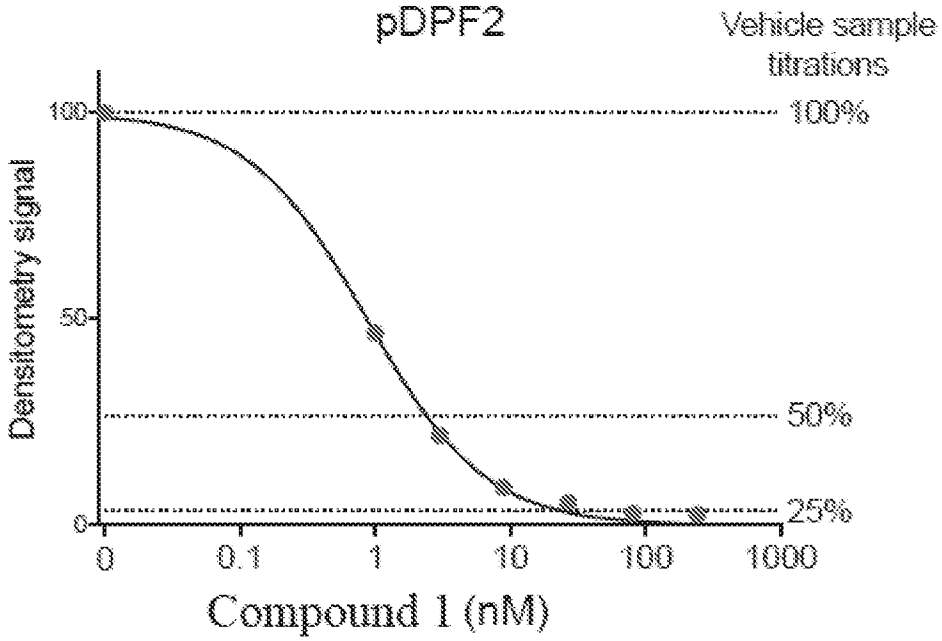

pDPF2
Vehicle sample titrations
100%
50%
25%
Densitometry signal
100
50
0
0
0.1
1
10
100
1000
Compound 1 (nM)

FIG. 18

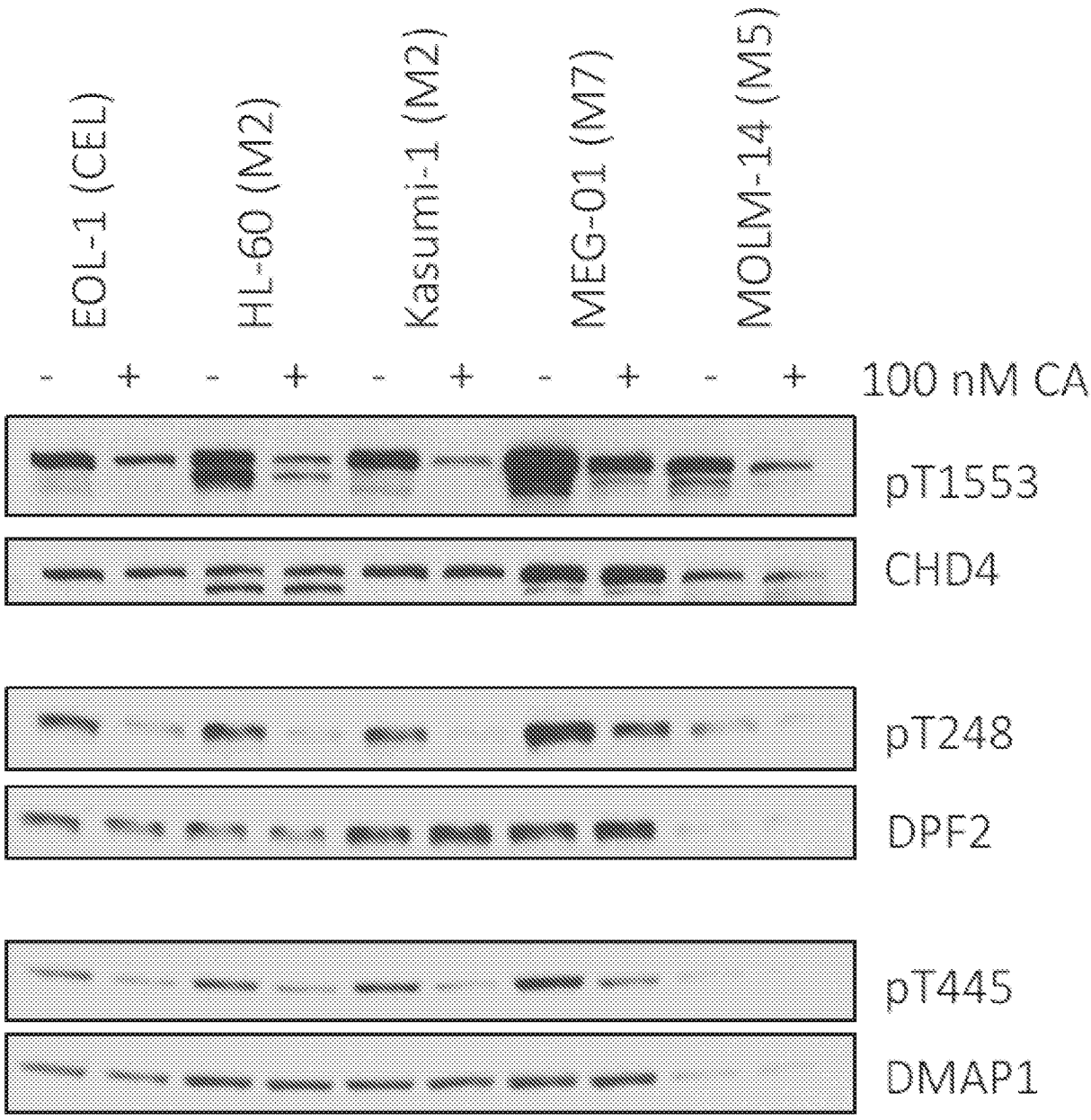

EOL-1 (CEL)
HL-60 (M2)
Kasumi-1 (M2)
MEG-01 (M7)
MOLM-14 (M5)
- + - + - + - + - +
100 nM CA
pT1553
CHD4
pT248
DPF2
pT445
DMAP1

FIG. 19

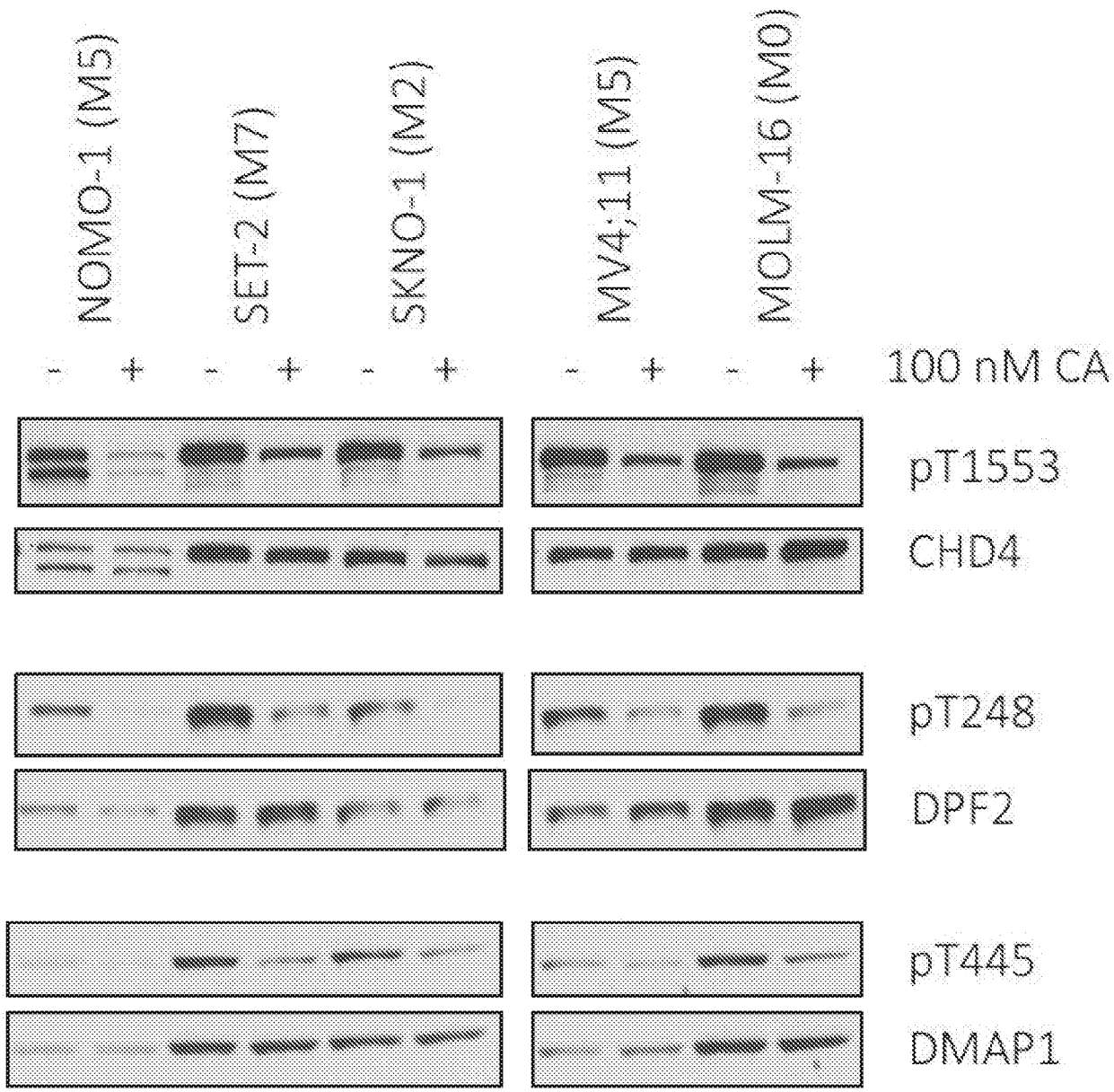

NOMO-1 (M5)
SET-2 (M7)
SKNO-1 (M2)
MV4;11 (M5)
MOLM-16 (M0)
- + - + - + - + - + 100 nM CA
pT1553
CHD4
pT248
DPF2
pT445
DMAP1

FIG. 20

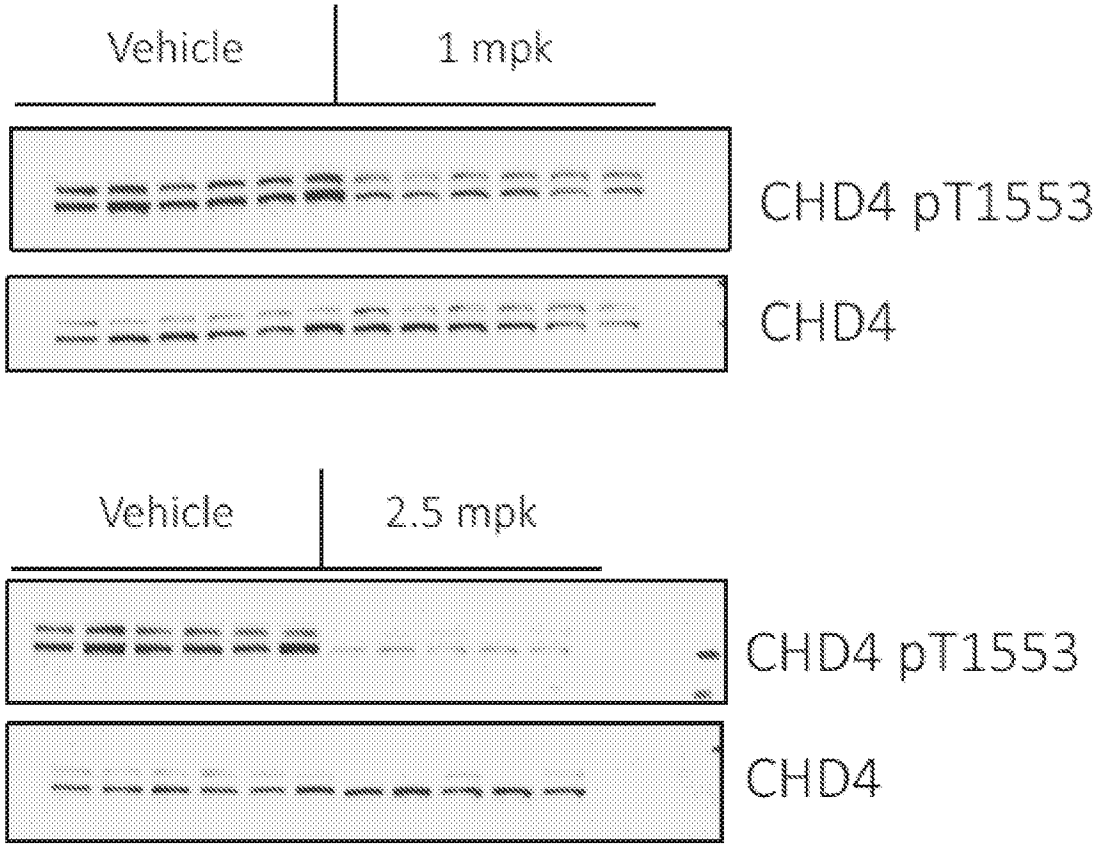

Vehicle
1 mpk
CHD4 pT1553
CHD4
Vehicle
2.5 mpk
CHD4 pT1553
CHD4

FIG. 21

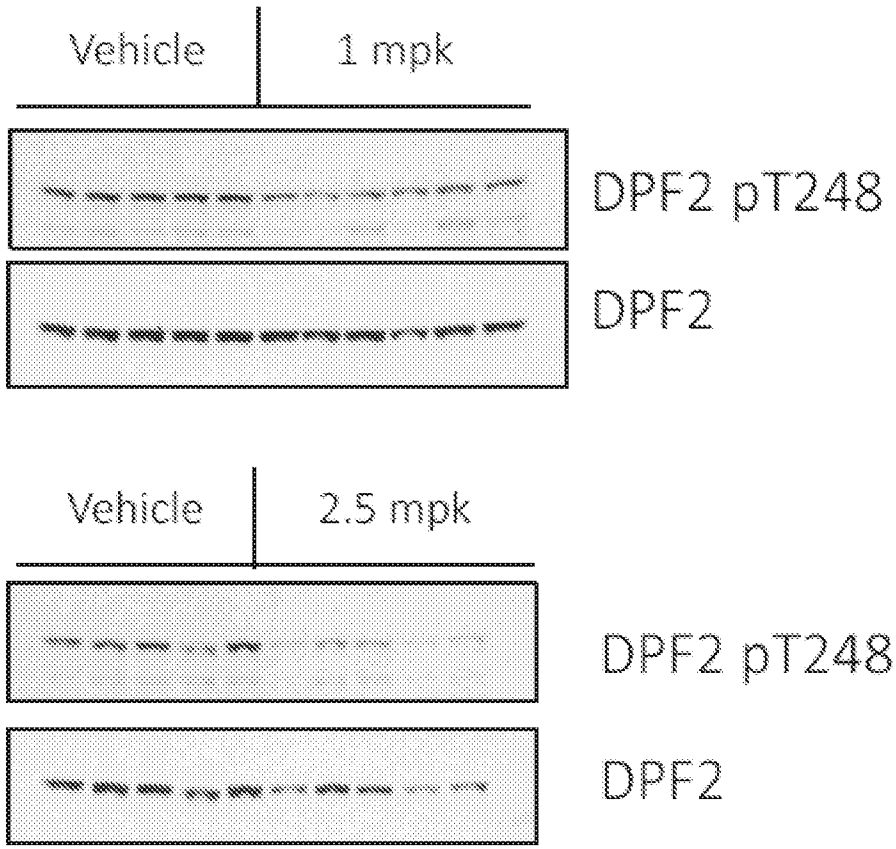


FIG. 22

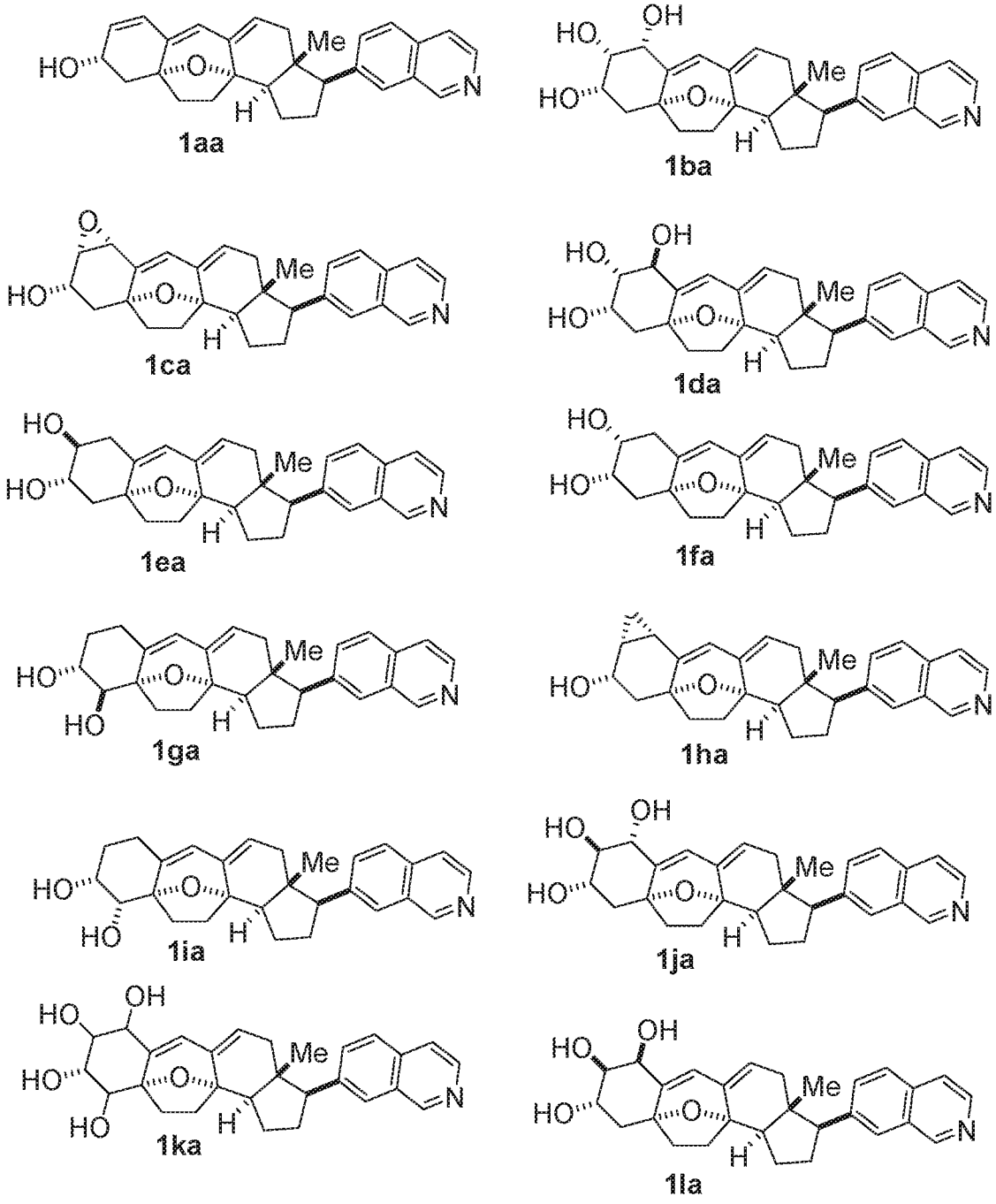

HO
O
Me
H
N
1aa
HO
OH
HO
O
Me
H
N
1ba
O
HO
O
Me
H
N
1ca
HO
OH
HO
O
Me
H
N
1da
HO
HO
O
Me
H
N
1ea
HO
HO
O
Me
H
N
1fa
HO
HO
O
Me
H
N
1ga
HO
O
Me
H
N
1ha
HO
HO
O
Me
H
N
1ia
HO
OH
HO
O
Me
H
N
1ja
HO
OH
HO
HO
O
Me
H
N
1ka
HO
OH
HO
O
Me
H
N
1la

FIG. 23

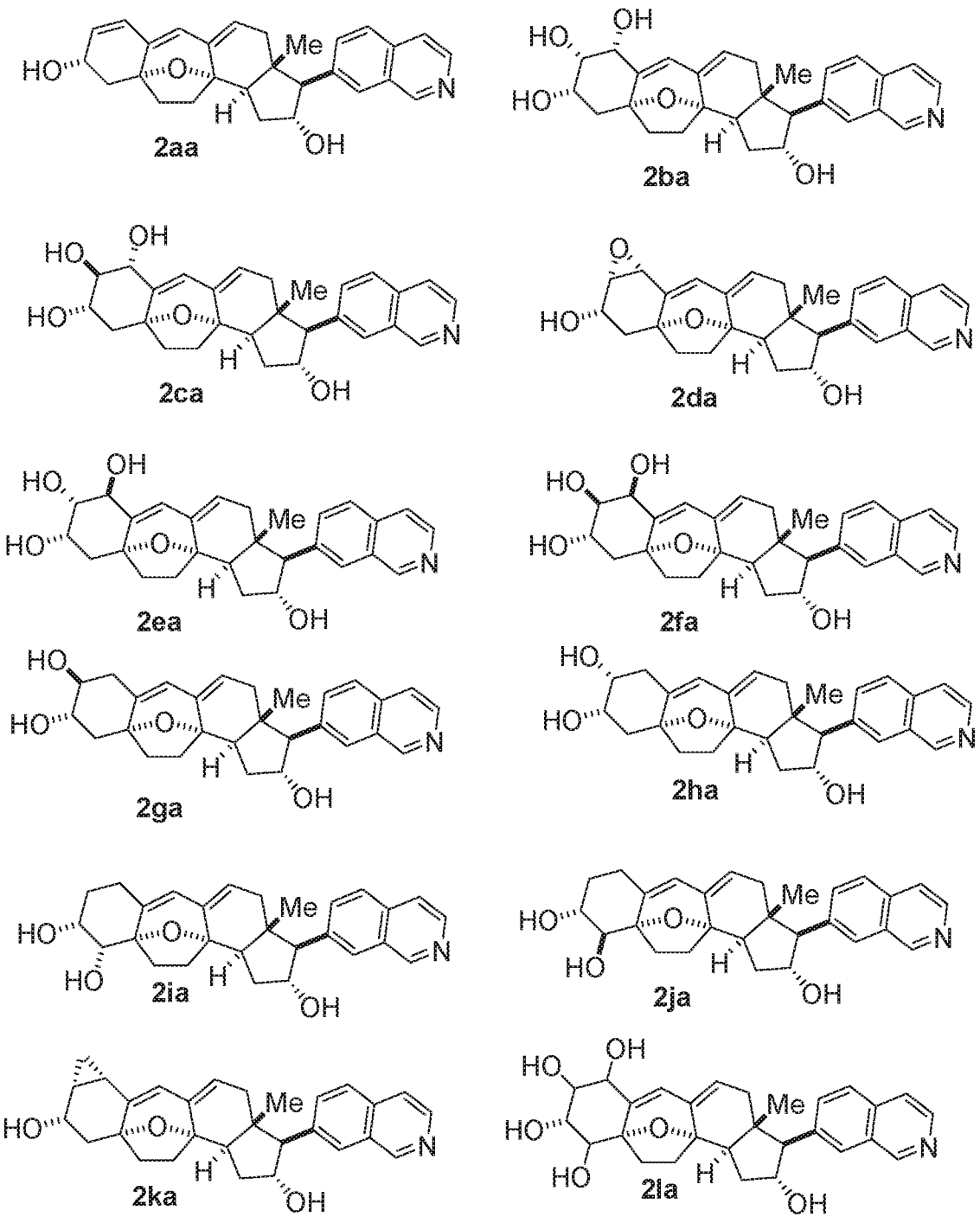

HO
Me
O
N
H
OH
2aa
HO
OH
HO
Me
O
N
H
OH
2ba
HO
OH
HO
Me
O
N
H
OH
2ca
O
HO
Me
O
N
H
OH
2da
HO
OH
HO
Me
O
N
H
OH
2ea
HO
OH
HO
Me
O
N
H
OH
2fa
HO
HO
Me
O
N
H
OH
2ga
HO
HO
Me
O
N
H
OH
2ha
HO
HO
Me
O
N
H
OH
2ia
HO
HO
Me
O
N
H
OH
2ja
HO
Me
O
N
H
OH
2ka
HO
OH
HO
HO
Me
O
N
H
OH
2la

FIG. 24

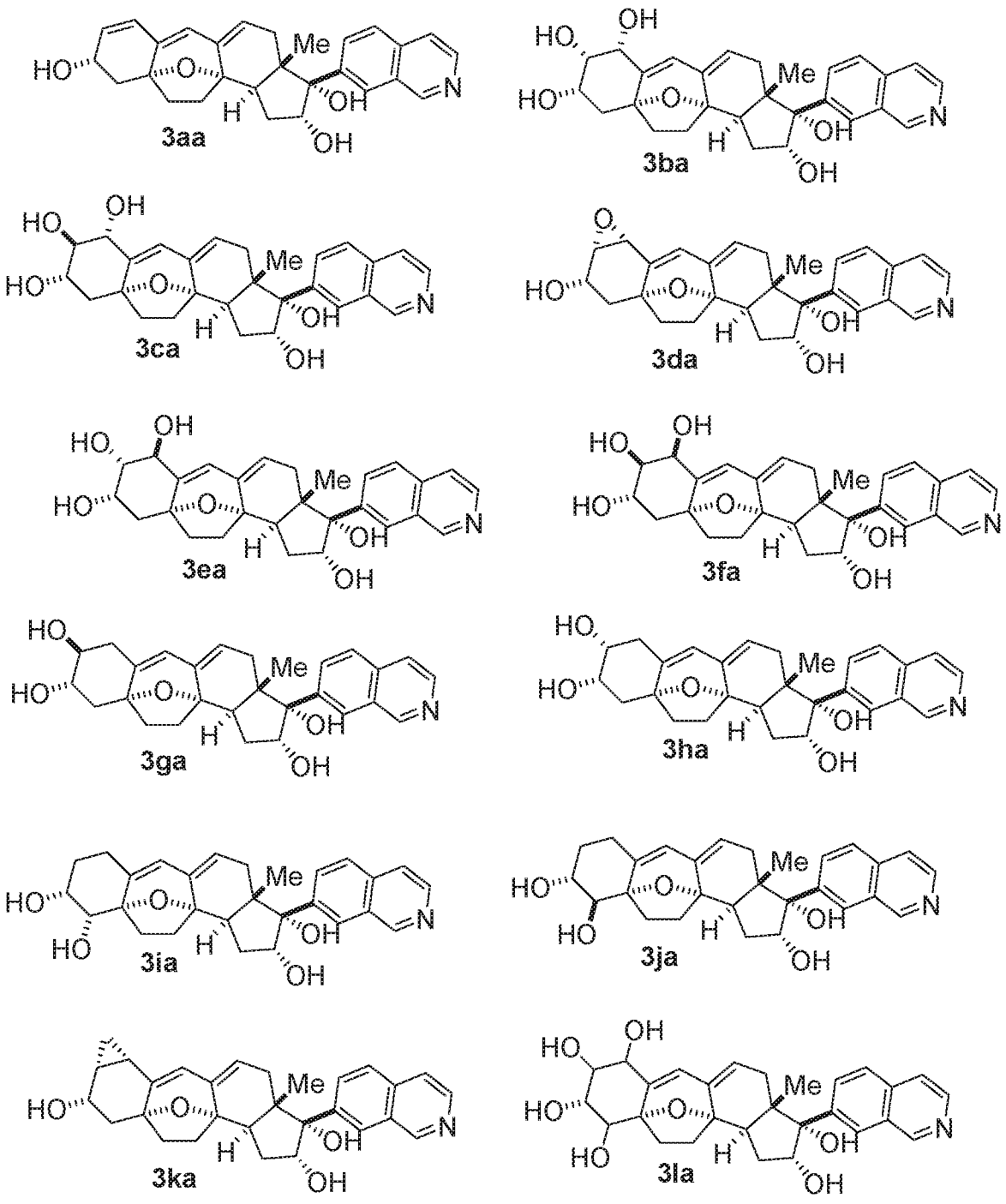


FIG. 25

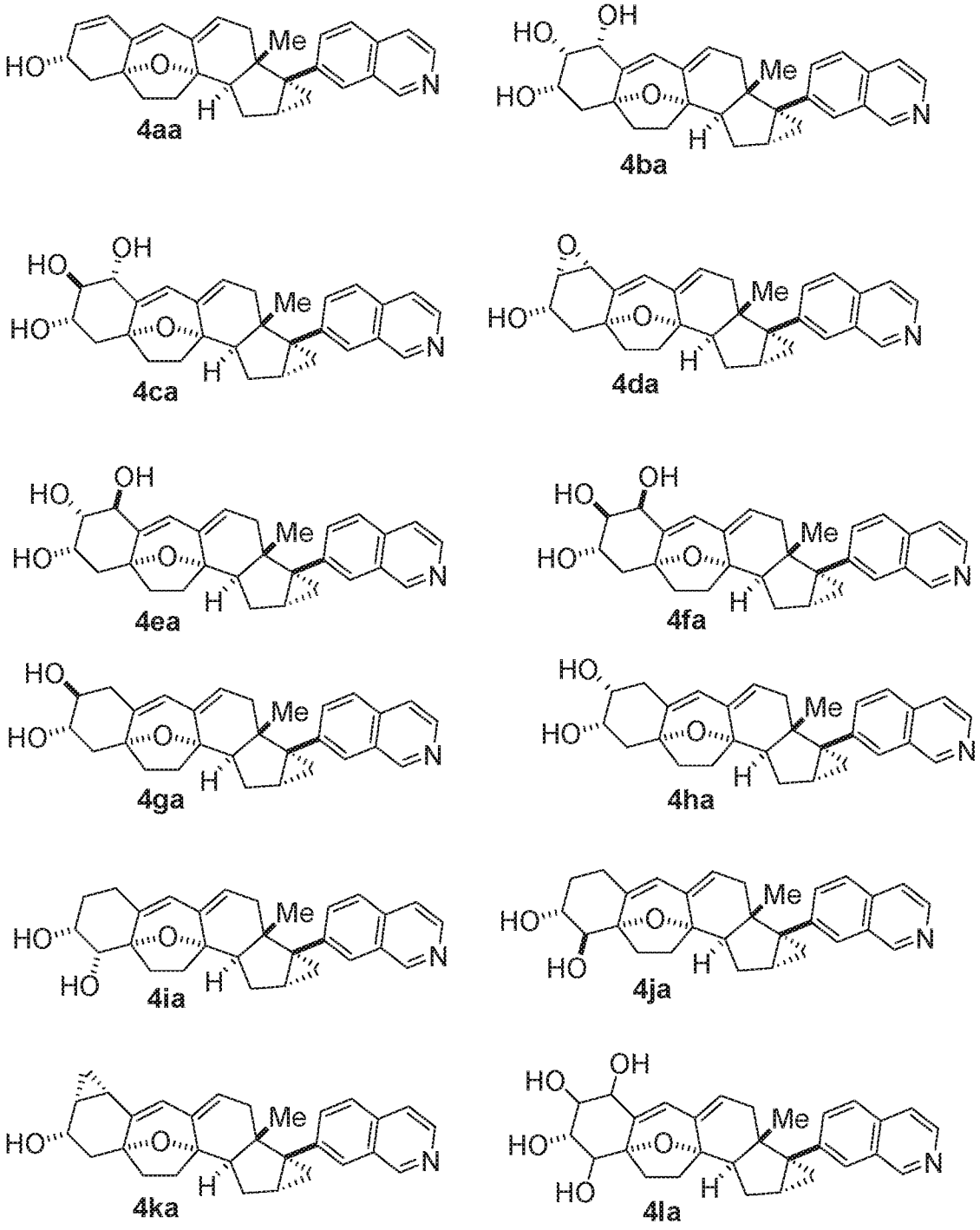

HO
O
Me
H
N
4aa
HO
OH
HO
O
Me
H
N
4ba
HO
OH
HO
O
Me
H
N
4ca
O
HO
O
Me
H
N
4da
HO
OH
HO
O
Me
H
N
4ea
HO
OH
HO
O
Me
H
N
4fa
HO
HO
O
Me
H
N
4ga
HO
HO
O
Me
H
N
4ha
HO
HO
O
Me
H
N
4ia
HO
HO
O
Me
H
N
4ja
HO
O
Me
H
N
4ka
HO
OH
HO
HO
O
Me
H
N
4la

FIG. 26

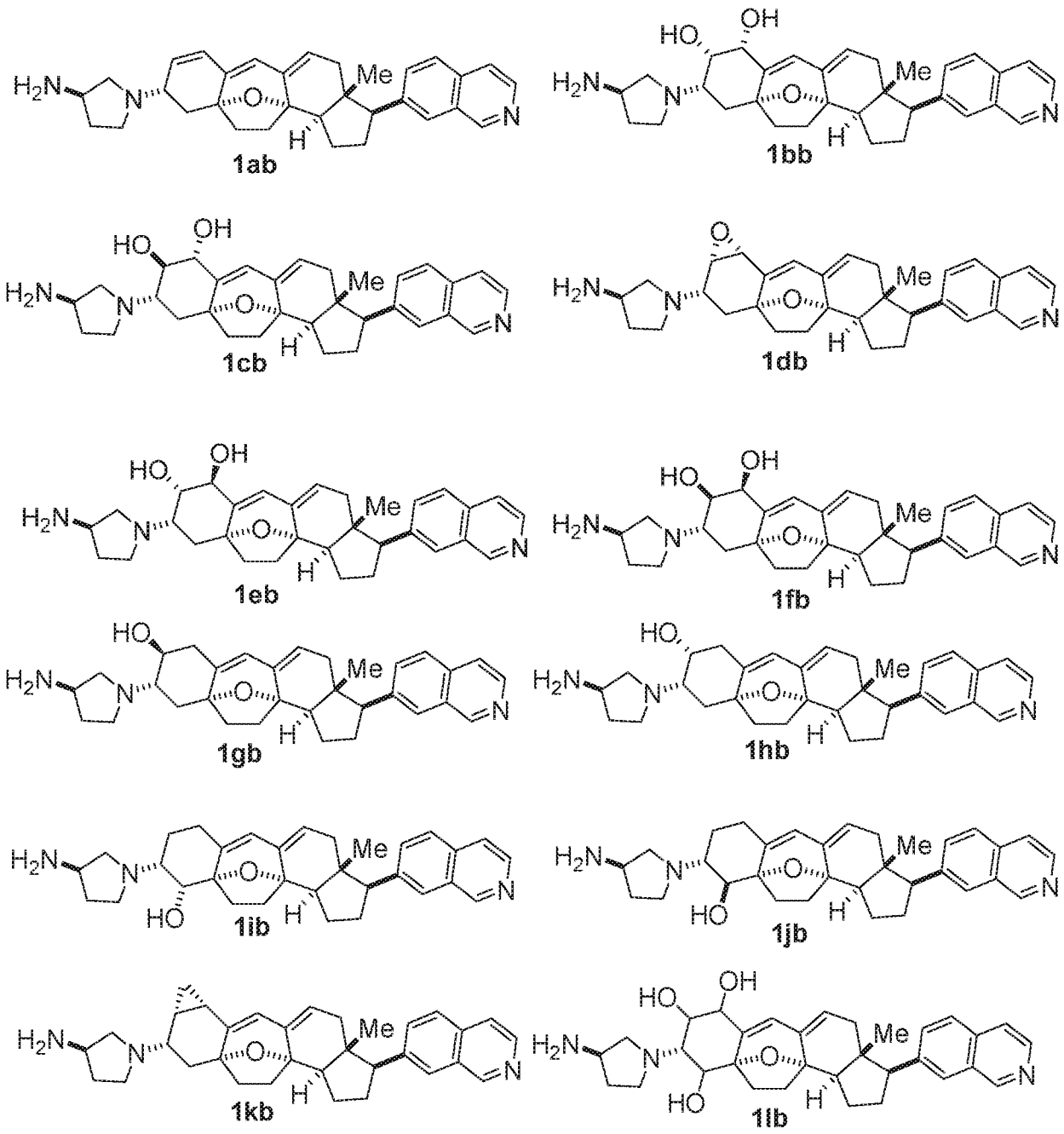


FIG. 27

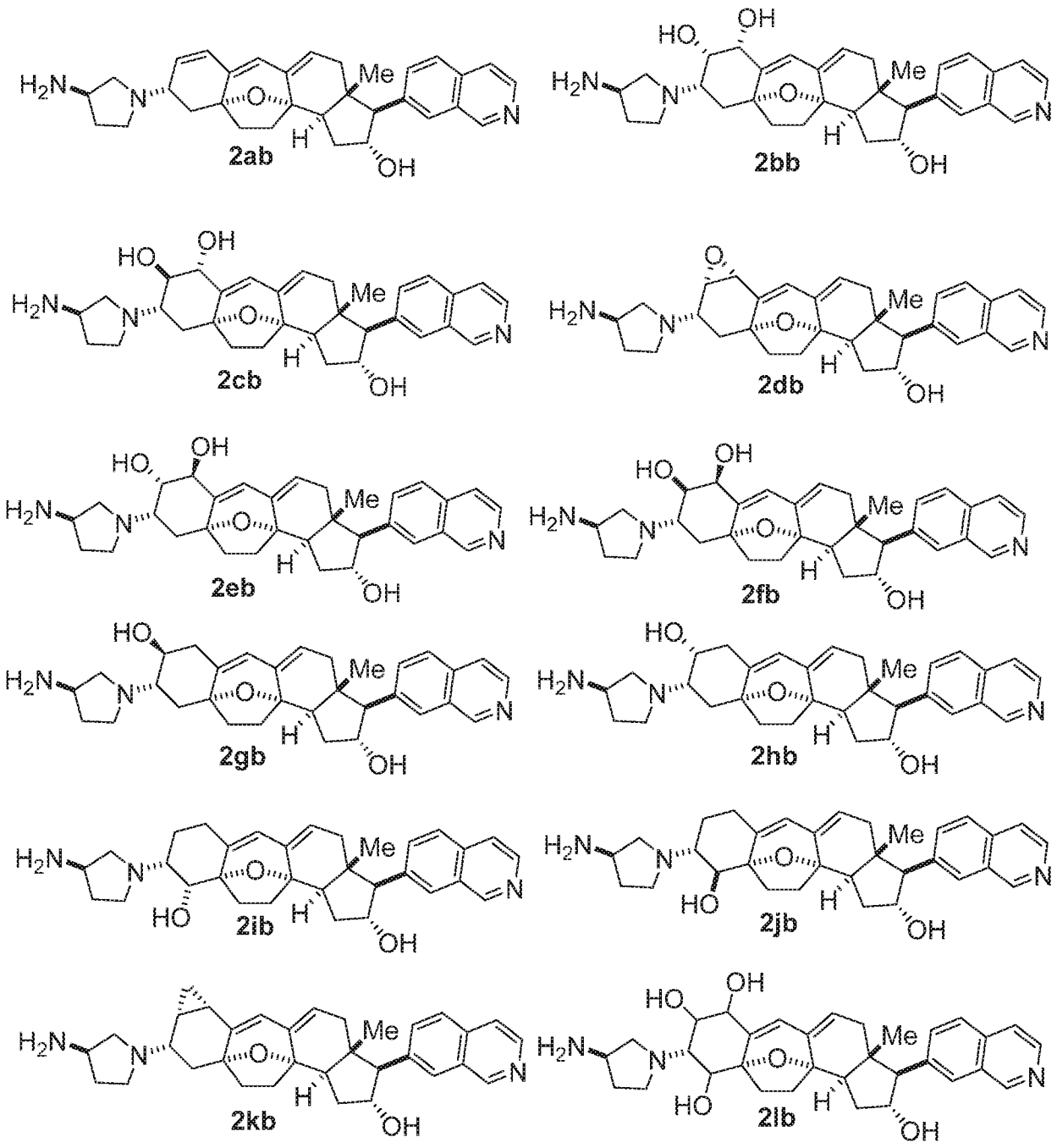


FIG. 28

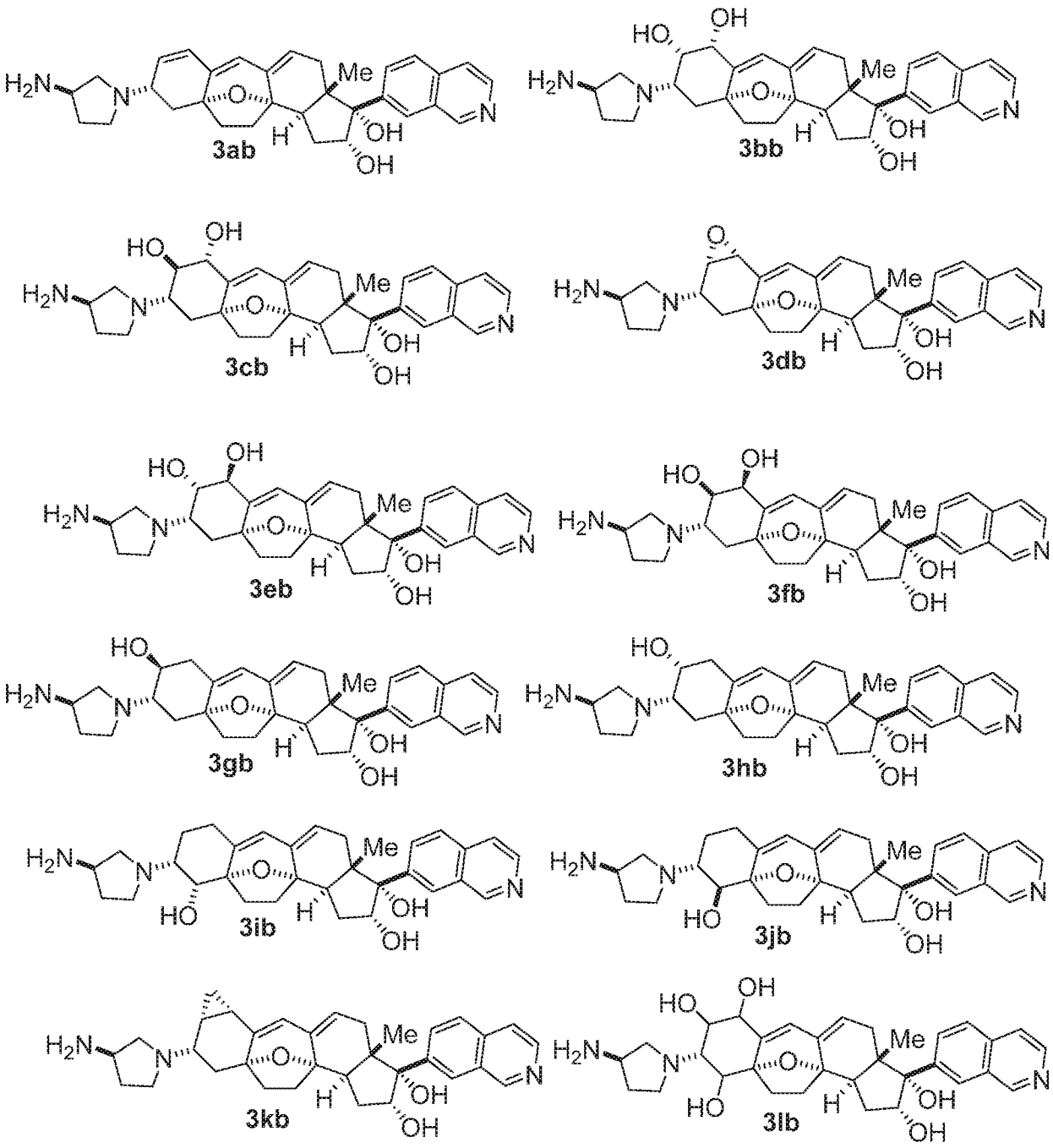


FIG. 29

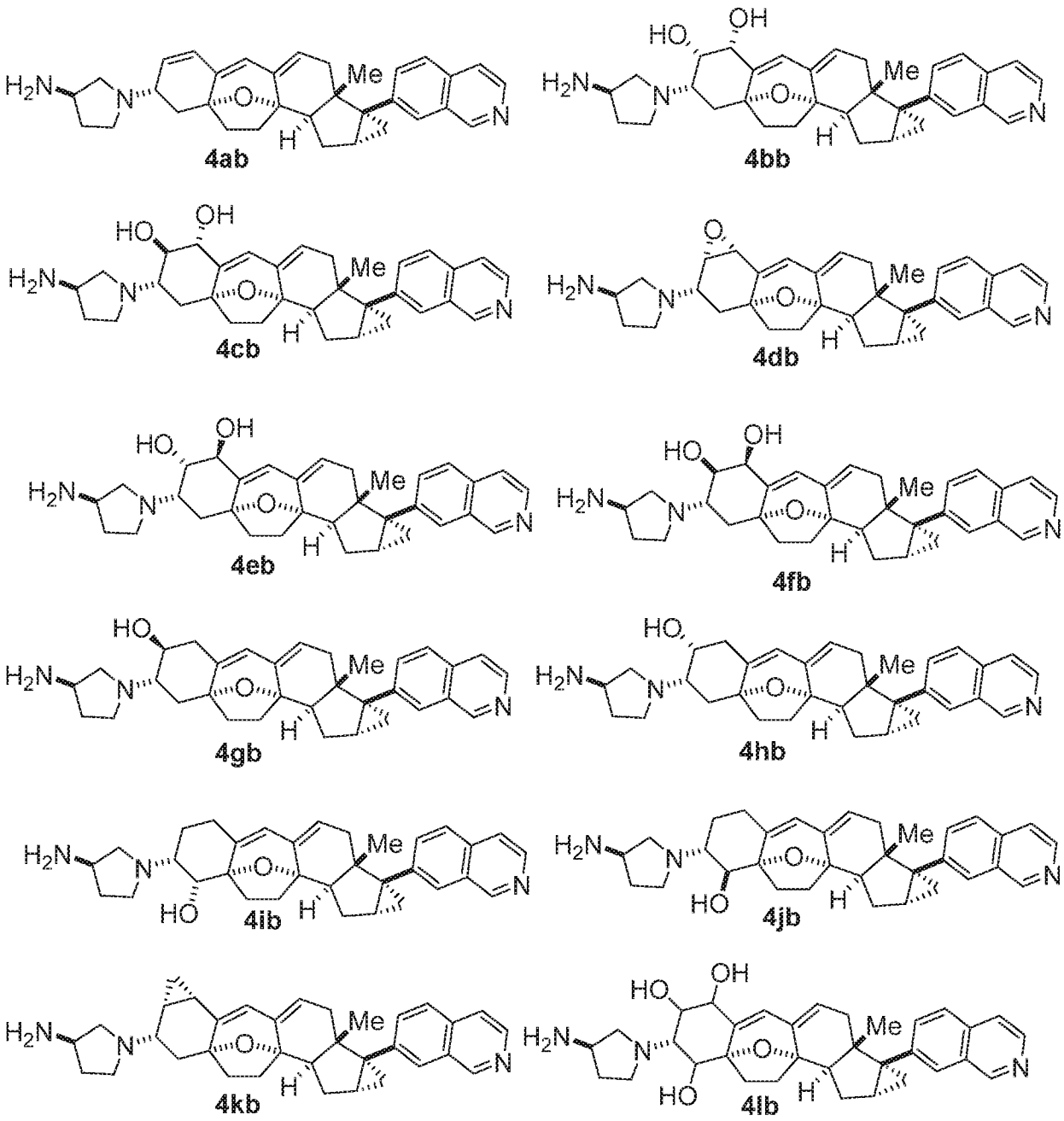


FIG. 30

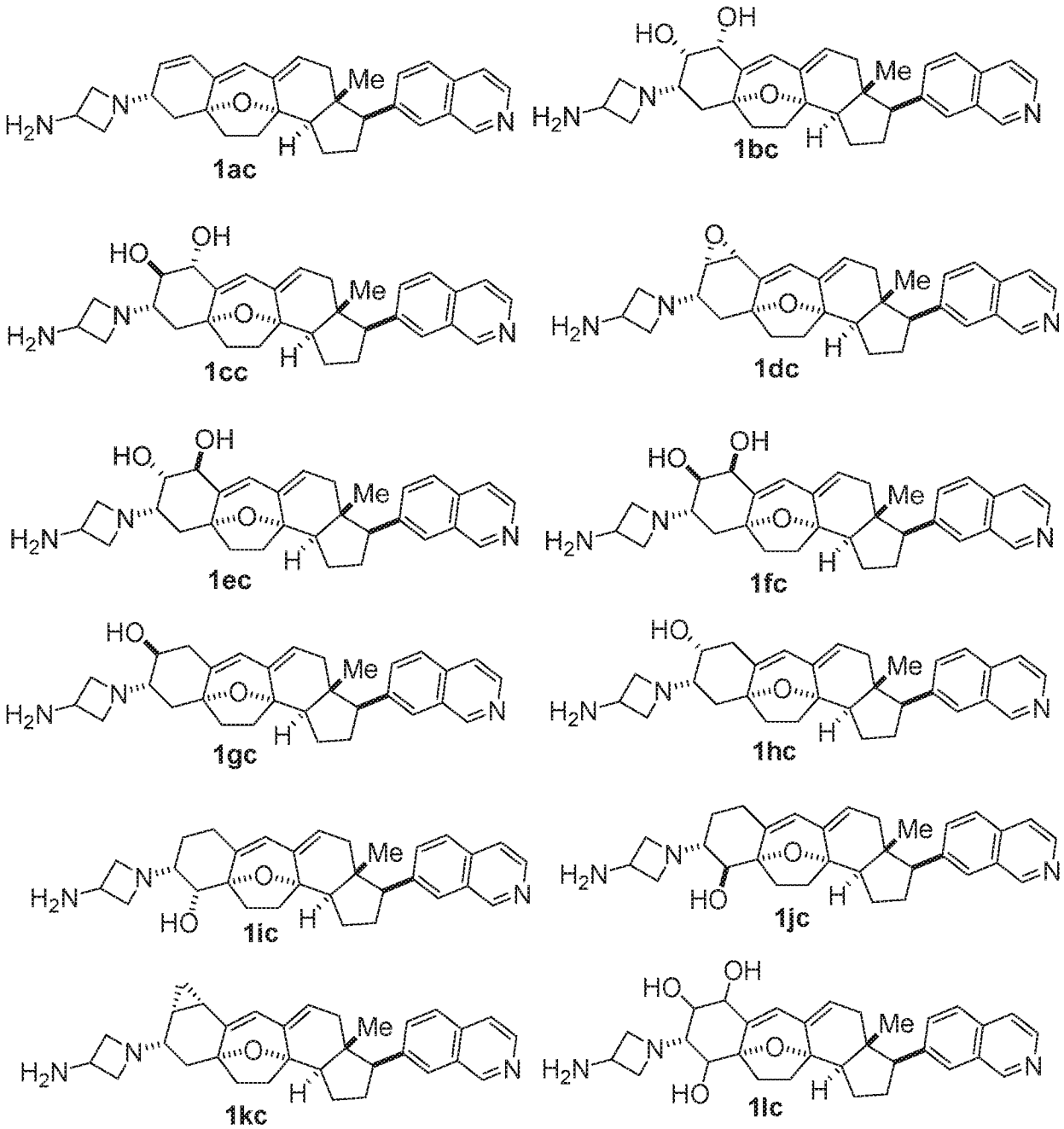


FIG. 31

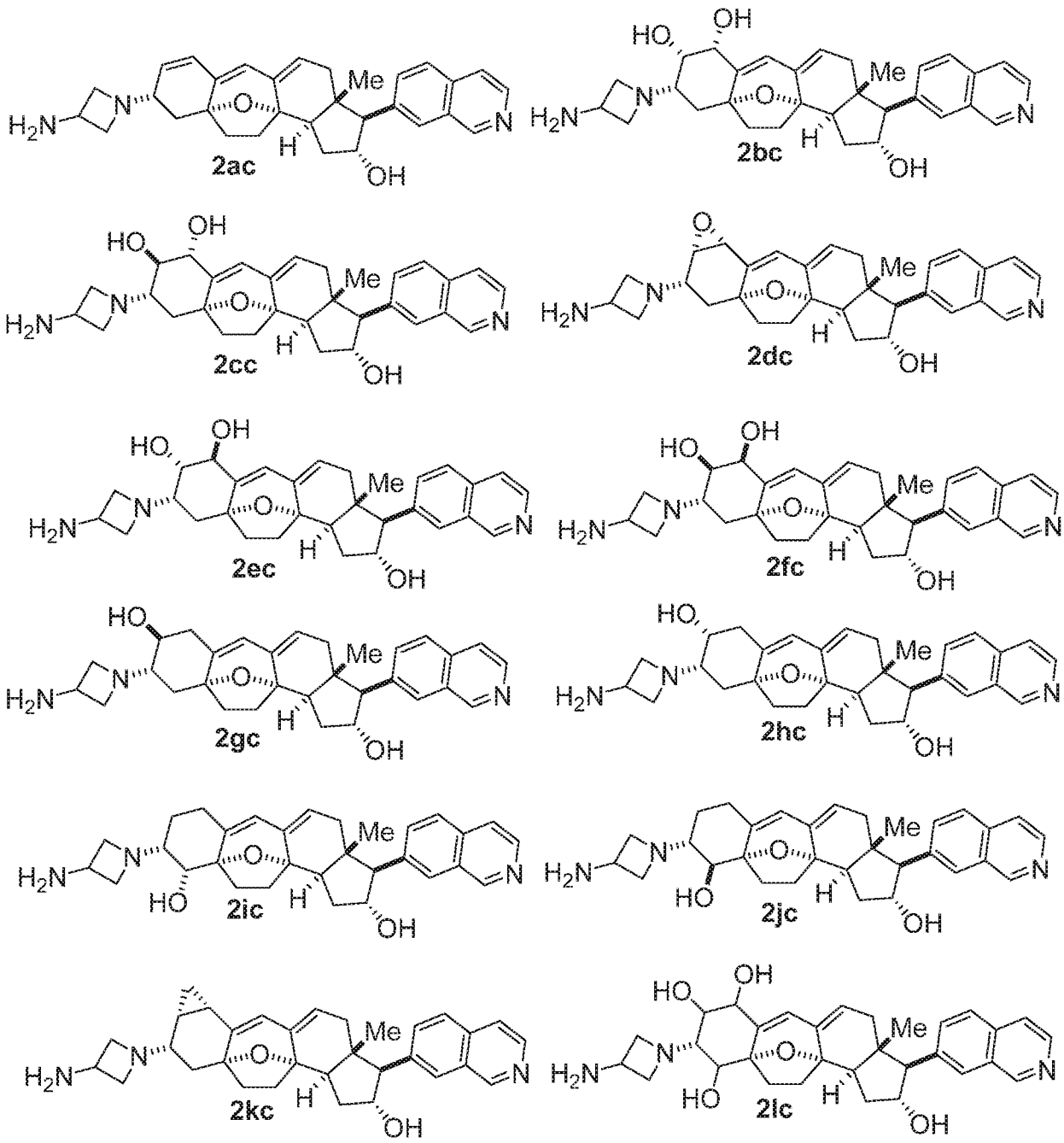


FIG. 32

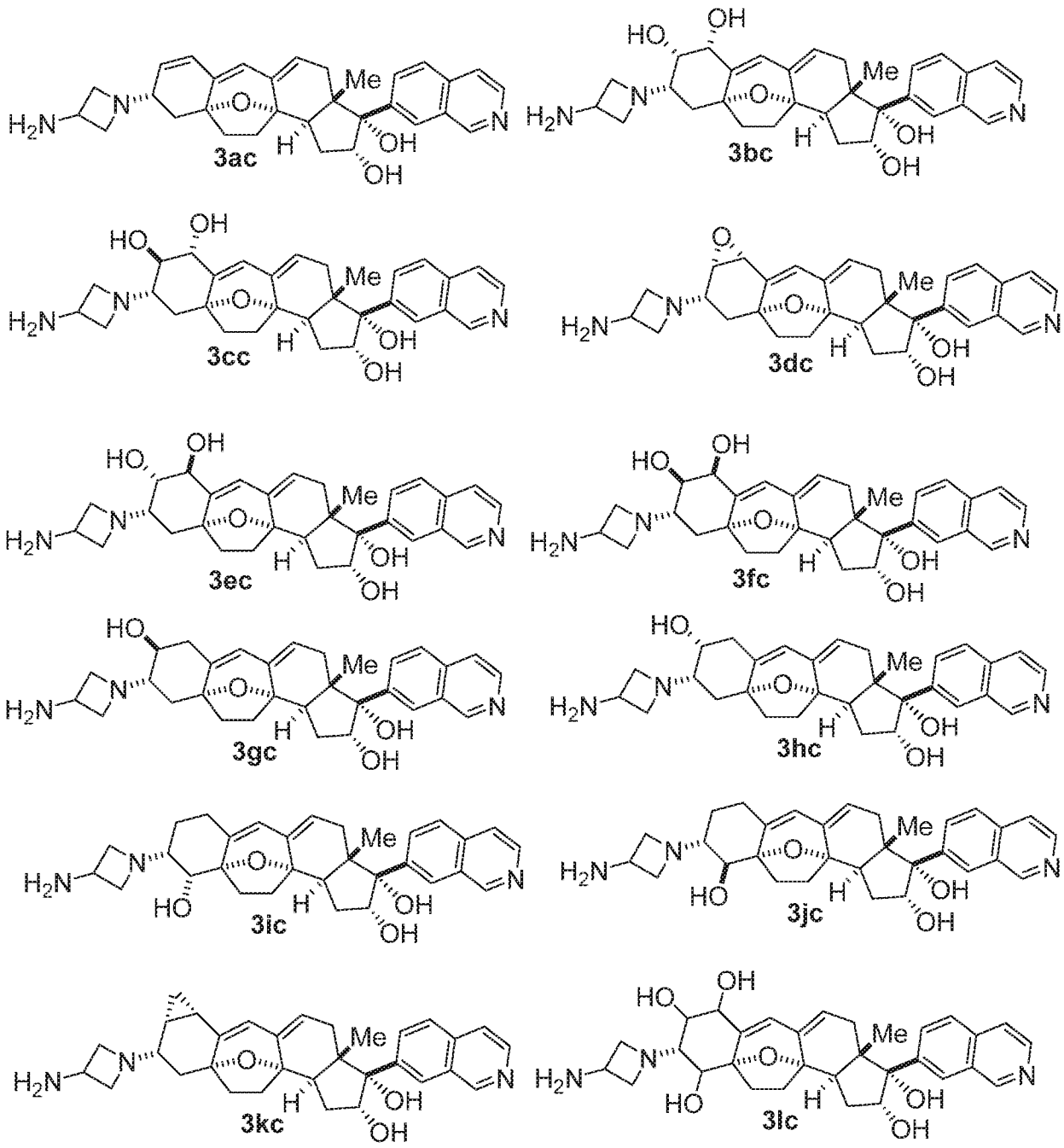


FIG. 33

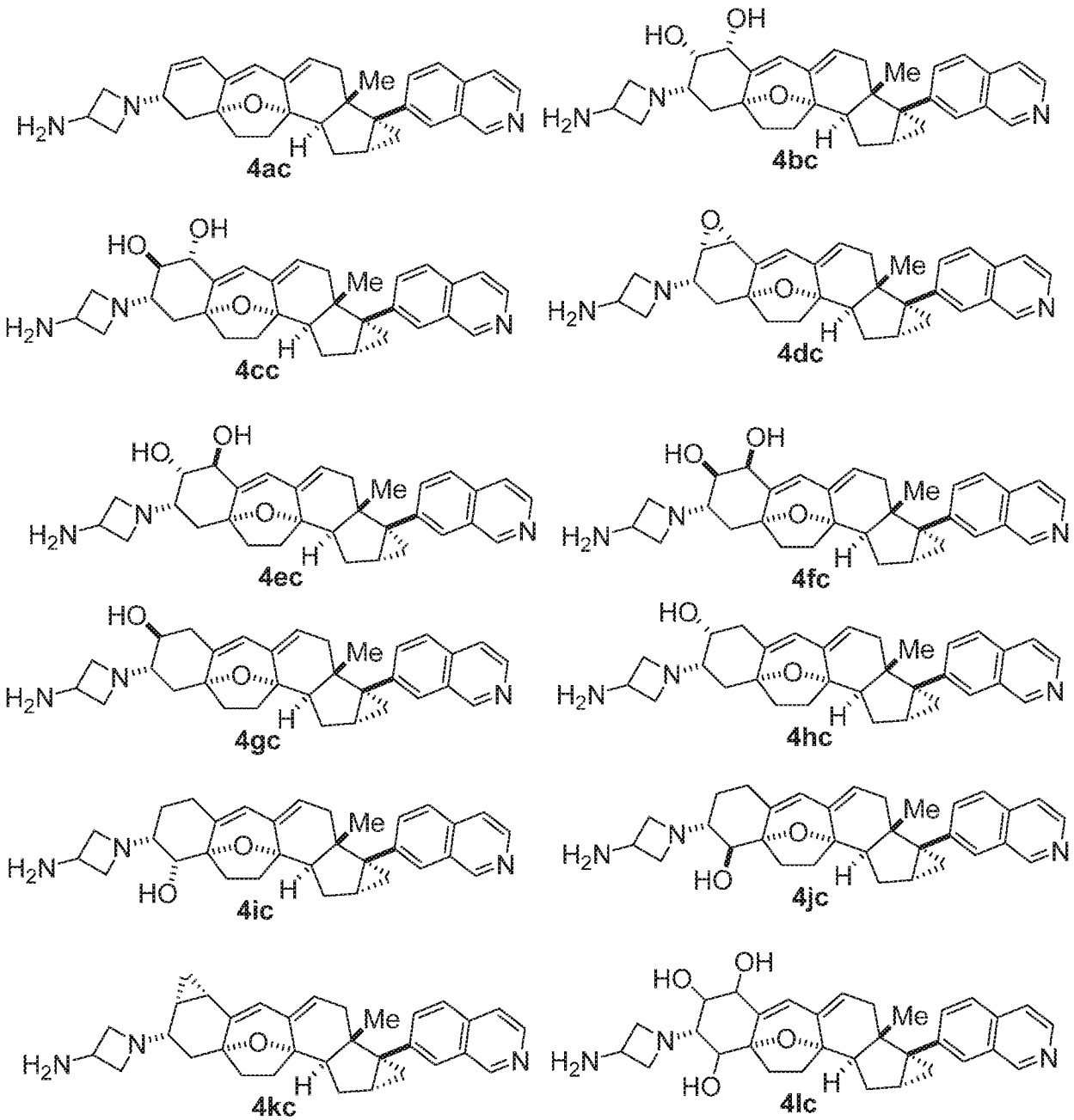


FIG. 34

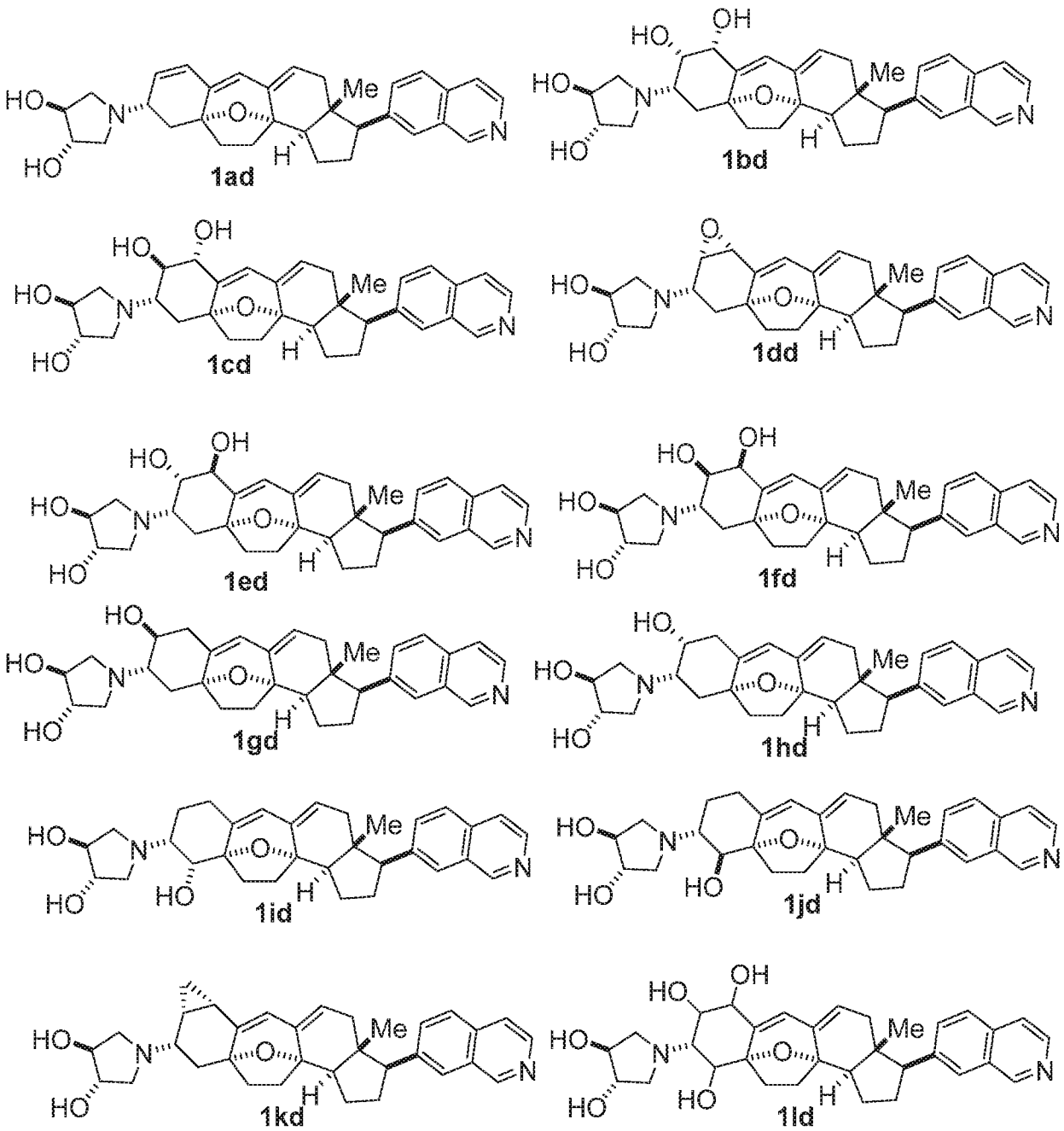


FIG. 35

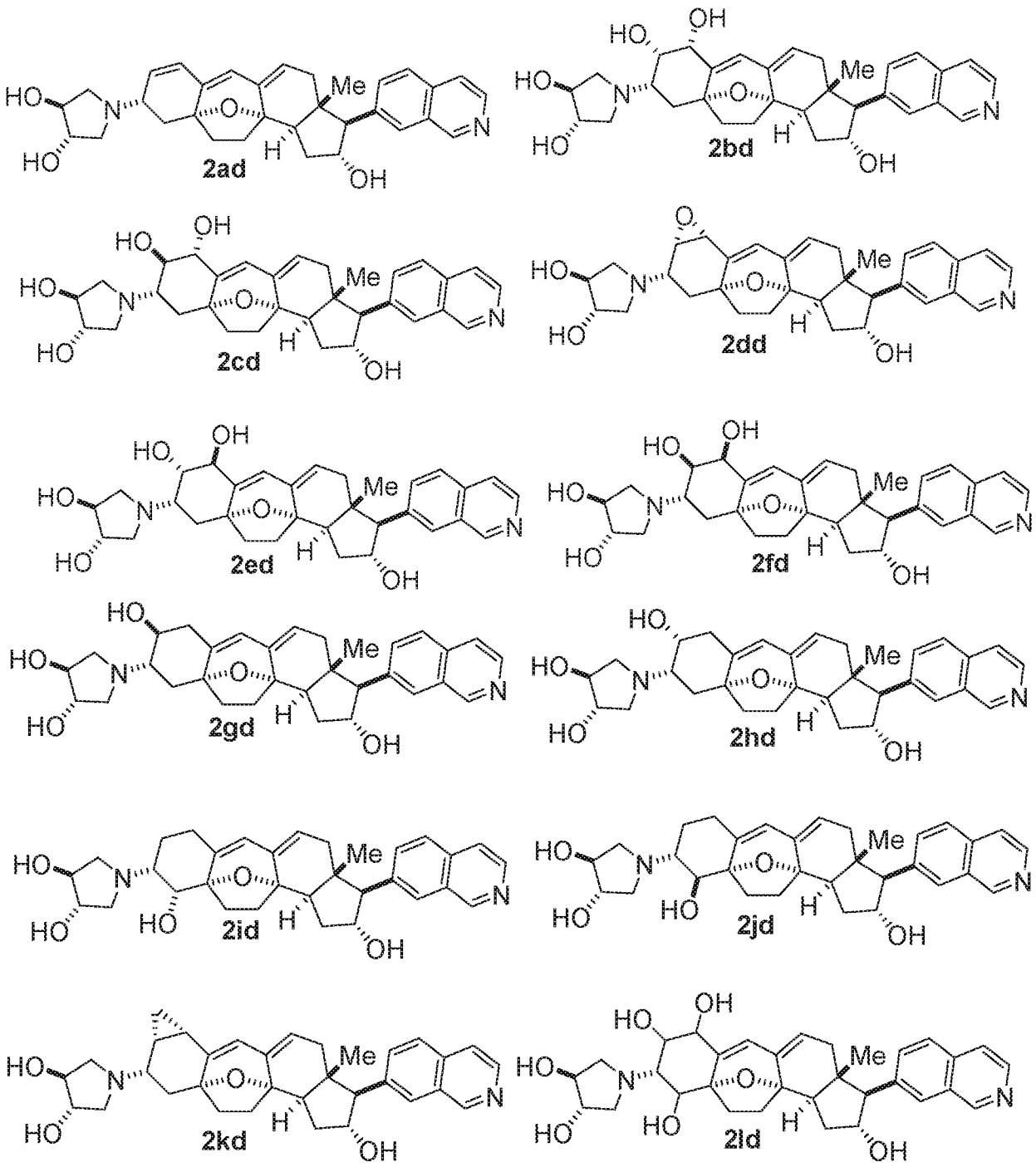


FIG. 36

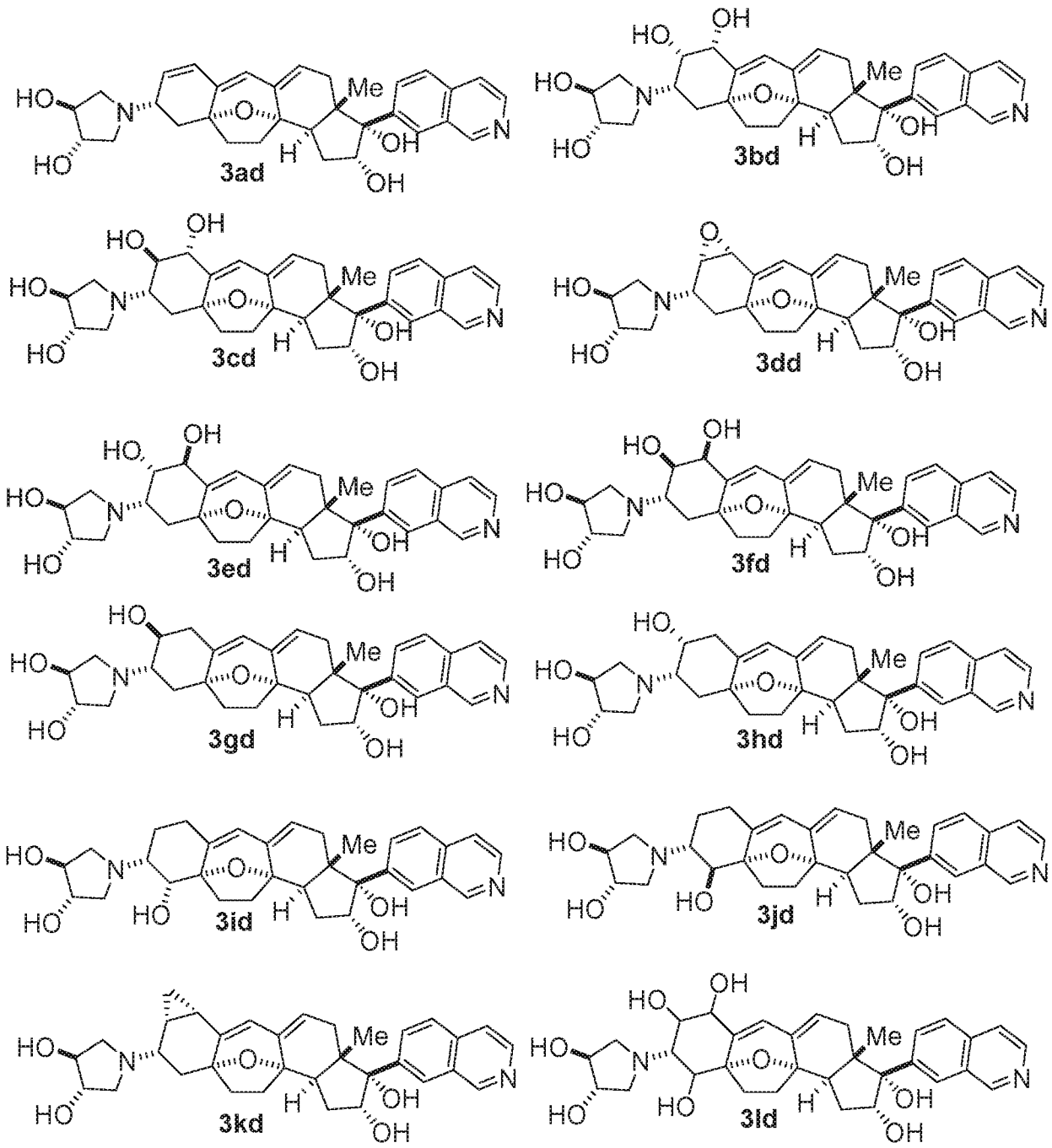


FIG. 37

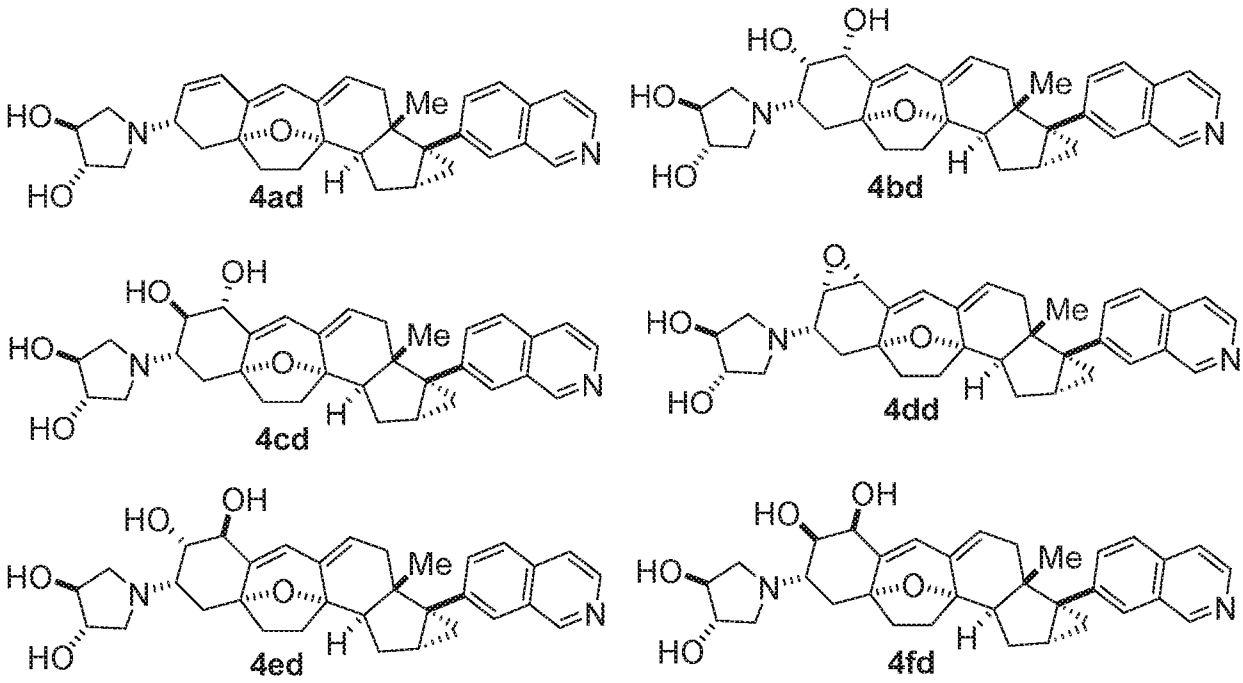

HO
N
HO
O
Me
H
N
4ad
HO
OH
HO
N
HO
O
Me
H
N
4bd
HO
OH
HO
N
HO
O
Me
H
N
4cd
O
HO
N
HO
O
Me
H
N
4dd
HO
OH
HO
N
HO
O
Me
H
N
4ed
HO
OH
HO
N
HO
O
Me
H
N
4fd

FIG. 38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/35038

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/4725; C07D 493/08; G01N 33/574; A61P 35/02 (2019.01)
CPC - A61K 31/4725; A61P 35/02; C07D 493/08; G01N 33/57484

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/182904 A1 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 17 November 2016 (17.11.2016); pg 4/285 ln 17-pg 5/285 ln 4; pg 5/285 ln 9-15; pg 5/285 ln 25-pg 6/285 ln 2; pg 6/285 ln 20-26; pg 9/285 ln 6; pg 27/285 ln 20; pg 215/285 ln 28; pg 221/285 ln 26-29; pg 222/285 ln 5-6	1-7
A	US 2018/0087114 A1 (TROVAGENE, INC) 29 March 2018 (29.03.2018); para [0006]-[0008], [0051], [0058], [0060], [0074], [0096], [0119]-[0120]	1-7
A	WO 2013/148775 A1 (MERCK SHARP & DOHME CORP.) 03 October 2013 (03.10.2013); pg 1 ln 20; pg 3 ln 1-6; pg 7 ln 7, 35; pg 8 ln 21; pg 9 ln 1-4, 36-37; pg 12 ln 29, 33-35; pg 13 ln 33; pg 16 ln 5, 16-18; pg 31 ln 24-29, 31-32; pg 32 ln 29-32; pg 35 ln 29-30	1-7
A	US 2012/0270233 A1 (WALDRON et al.) 25 October 2012 (25.10.2012); see entire document	1-7
A	US 2009/0304594 A1 (FANTIN et al.) 10 December 2009 (10.12.2009); see entire document	1-7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2019	22 AUG 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/35038

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

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

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

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

3. ☒ Claims Nos.: 8-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.