

PHOSPHOLIPID-FLAVAGLINE CONJUGATES AND METHODS OF USING THE SAME FOR TARGETED CANCER THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/913,571, filed October 10, 2019, the content of which is incorporated herein by reference in its entirety.

FIELD

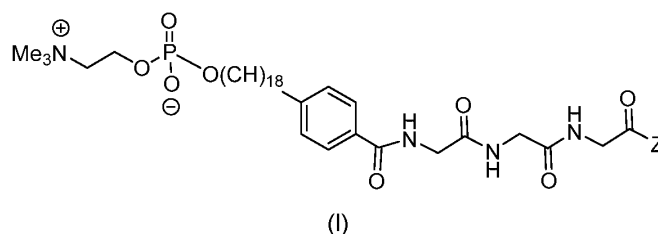
[0002] This disclosure relates to phospholipid-flavagline conjugates and targeted cancer therapies.

INTRODUCTION

[0003] The majority of anticancer drugs in clinical use have their utility limited by their toxicity to all proliferating cells and/or the inability to exert their effect on all of the tumor cells. Novel agents continue to be developed with unique mechanisms of action meant to provide increased targeting, however, many of these compounds still lack absolute tumor selectivity and continue to be limited in their therapeutic utilization due to off-target effects. Antibody drug conjugates (ADCs) have been designed to bind to specific epitopes on the surface of tumor cells and have offered an alternative method to target tumor cells in an effort to reduce associated toxicities. Although highly selective, very few antibody drug conjugates are therapeutically useful since they only achieve modest cellular uptake and limited cell killing activity. More effective tumor targeting platforms are needed.

SUMMARY

[0004] In an aspect, the disclosure provides a compound of formula (I), or a pharmaceutically acceptable salt thereof,



wherein Z is a flavagline analog.

[0005] Another aspect of the disclosure provides a pharmaceutical composition comprising a compound as described herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[0006] Another aspect of the disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject a compound as described herein, or a pharmaceutically acceptable salt thereof.

[0007] Another aspect of the disclosure provides a method of targeting a drug to a tumor or cancer cell in a subject, the method comprising administering to the subject a compound as described herein, or a pharmaceutically acceptable salt thereof.

[0008] In some embodiments, the compounds as described herein localize or travel to the cytoplasm or organelle of the tumor or cancer cell. In some embodiments, the compounds are selective for cancer cells in the subject. In some embodiments, the compounds are incorporated into at least about 2-fold more tumor or cancer cells than healthy cells. In some embodiments, the cancer is melanoma, brain cancer, lung cancer, adrenal cancer, liver cancer, renal or kidney cancer, pancreatic cancer, esophageal cancer, gastric cancer, stomach cancer, colon cancer, colorectal cancer, anal cancer, prostate cancer, ovarian cancer, breast cancer, cervical cancer, lymphoma, leukemia, myeloma, hematologic cancer, hepatocarcinoma, retinoblastoma, glioma, sarcoma, blastoma, squamous cell carcinoma, adenocarcinoma, or a combination thereof. In some embodiments, the subject is human.

[0009] The disclosure provides for other aspects and embodiments that will be apparent in light of the following detailed description and accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[00010] FIG. 1A, FIG. 1B, FIG. 1C, FIG. 1D, FIG. 1E are images of tumor cells with lipid rafts labeled. Tumor cells had a greater concentration of lipid rafts compared to normal cells. FIG. 1F is an image of normal fibroblasts and Caki-2 tumor cells with CLR 1501 (compound (1)). CLR 1501 was highly localized in Caki-2 cells and minimally in normal fibroblasts. FIG. 1G is an image of control A549 cells, and FIG. 1H is an image of A549 cells treated with methyl β -cyclodextrin to disrupt lipid rafts. Cells in both FIG. 1G and FIG. 1H were incubated with CLR 1501 (compound (1)), and disruption of the majority of lipid rafts in A549 cells resulted in 60% reduction in uptake of CLR 1501 (compound (1)). FIG. 1I, FIG. 1J, and FIG. 1K are images of PC3 cells incubated with CLR 1501 (compound (1)) and stained for endoplasmic reticulum (ER). CLR 1501 (compound (1)) co-localized with ER in

malignant cells but not normal cells (not shown). **FIG. 1L, FIG. 1M, and FIG. 1N** are images of PC3 cells incubated with CLR 1501 (compound (1)) and stained for the nucleus and mitochondria. CLR 1501 (compound (1)) co-localized with mitochondria.

[00011] **FIG. 2** is an image of a colorectal (HCT-116) tumor bearing mouse injected with CLR 1502 (compound (2)), showing localization to the tumor.

[00012] **FIG. 3** is a graph of cytotoxicity versus concentration for a cytotoxic compound (FLV1) compared to the cytotoxic compound conjugated to a PLE (CLR 1865, compound (8)) in A549 (human lung adenocarcinoma) cells or normal human dermal fibroblasts (NHDF).

[00013] **FIG. 4** is a graph of fold increase versus time for the uptake of the PLE conjugate CLR 1852 (compound (9)) in A375 (human melanoma) and HEK293 (human embryonic kidney) cells.

[00014] **FIG. 5** are images of a breast cancer model in mice, showing *in vivo* uptake of CLR 1502 (compound (2)).

[00015] **FIG. 6** are images of myeloma cell lines, showing the uptake of CLR 1501 (compound (1)).

[00016] **FIG. 7** are images of cancer stem cells, normal brain tissue, and normal stem cells, showing the specific uptake of CLR 1501 (compound (1)) into the cancer cells.

[00017] **FIG. 8** is a graph of the percent cytotoxicity versus concentration for CLR 1852 (compound (9)) compared to FLV3 in A549 (human lung adenocarcinoma) cells and normal human dermal fibroblasts (NHDF).

[00018] **FIG. 9** is a graph of tumor volume versus time for vehicle compared to CLR 1852 (compound (9)) in a HCT 116 tumor model.

[00019] **FIG. 10** is a graph of body weight versus time for vehicle compared to CLR 1852 (compound (9)) in a HCT 116 tumor model.

[00020] **FIG. 11** is a graph of concentration versus time for FLV3 detected in the cytosol (normalized to the cytosolic volume) in A375 (human melanoma) and A549 (human lung adenocarcinoma) cell lines.

[00021] **FIG. 12** shows representative results for body weight changes in mouse model after administration of CLR 1899 (at 0.5 mg/kg, 1 mg/kg, and 2 mg/kg).

[00022] FIG. 13. shows representative results of in vivo efficacy of CLR 1899 in MCF-7 xenograft model (10 mice per dose group).

DETAILED DESCRIPTION

[00023] Described herein are phospholipid compounds and phospholipid-flavagline conjugates. Based on numerous animal and human tumors containing higher concentrations of naturally occurring ether lipids than normal tissues, phospholipid ether (PLE) molecules were developed. The PLE molecules detailed herein may be used as a tumor targeting platform to selectively deliver drugs to tumors and cancer cells.

[00024] As detailed herein, the tissue distribution of the PLE molecules was examined in over 100 different tumor cells, including fresh human tumor samples. The PLE molecules demonstrated increased uptake in tumor tissue versus normal tissue. The PLE molecules may be conjugated to flavagline molecules and derivatives thereof via linkers to form phospholipid-flavagline conjugates.

1. Definitions

[00025] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[00026] The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “and” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments “comprising,” “consisting of” and “consisting essentially of,” the embodiments or elements presented herein, whether explicitly set forth or not.

[00027] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range

6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[00028] The term “about” as used herein as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain aspects, the term “about” refers to a range of values that fall within 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[00029] Definitions of specific functional groups and chemical terms are described in more detail below. For purposes of this disclosure, 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*, 7th Edition, John Wiley & Sons, Inc., New York, 2013; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; Carruthers, *Some Modern Methods of Organic Synthesis*, 3rd Edition, Cambridge University Press, Cambridge, 1987; the entire contents of each of which are incorporated herein by reference.

[00030] The term “alkoxy” or “alkoxyl” as used herein, refers to an alkyl group, as defined herein, appended to the parent molecular moiety through an oxygen atom. Representative examples of alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, 2-propoxy, butoxy and tert-butoxy.

[00031] The term “alkyl” as used herein, means a straight or branched, saturated hydrocarbon chain containing from 1 to 20 carbon atoms. The term “lower alkyl” or “C₁₋₆ alkyl” means a straight or branched chain hydrocarbon containing from 1 to 6 carbon atoms. The term “C₁₋₄ alkyl” means a straight or branched chain hydrocarbon containing from 1 to 4 carbon atoms. The term “C₁₋₃ alkyl” means a straight or branched chain hydrocarbon containing from 1 to 3 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, *n*-pentyl, isopentyl, neopentyl, *n*-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, *n*-heptyl, *n*-octyl, *n*-nonyl, and *n*-decyl.

[00032] The term “alkenyl” as used herein, means an unsaturated hydrocarbon chain containing from 2 to 20 carbon atoms and at least one carbon-carbon double bond.

[00033] The term “alkynyl” as used herein, means an unsaturated hydrocarbon chain containing from 2 to 20 carbon atoms and at least one carbon-carbon triple bond.

[00034] The term “alkoxyalkyl” as used herein, refers to an alkoxy group, as defined herein, appended to the parent molecular moiety through an alkylene group, as defined herein.

[00035] The term “arylalkyl” as used herein, refers to an aryl group, as defined herein, appended to the parent molecular moiety through an alkylene group, as defined herein.

[00036] The term “alkylamino,” as used herein, means at least one alkyl group, as defined herein, is appended to the parent molecular moiety through an amino group, as defined herein.

[00037] The term “alkylene” as used herein, refers to a divalent group derived from a straight or branched chain hydrocarbon of 1 to 10 carbon atoms, for example, of 2 to 5 carbon atoms. Representative examples of alkylene include, but are not limited to, $-\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$, and $-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$.

[00038] The term “amide,” as used herein, means $-\text{C}(\text{O})\text{NR}-$ or $-\text{NRC}(\text{O})-$, wherein R may be hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, heterocycle, alkenyl, or heteroalkyl.

[00039] The term “aminoalkyl,” as used herein, means at least one amino group, as defined herein, is appended to the parent molecular moiety through an alkylene group, as defined herein.

[00040] The term “amino” as used herein, means $-\text{NR}_x\text{R}_y$, wherein R_x and R_y may be hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, heterocycle, alkenyl, or heteroalkyl. In the case of an aminoalkyl group or any other moiety where amino appends together two other moieties, amino may be $-\text{NR}_x-$, wherein R_x may be hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, heterocycle, alkenyl, or heteroalkyl.

[00041] The term “aryl” as used herein, refers to an aromatic group such as a phenyl group, or a bicyclic fused ring system. Bicyclic fused ring systems are exemplified by a phenyl group appended to the parent molecular moiety and fused to a cycloalkyl group, as defined herein, a phenyl group, a heteroaryl group, as defined herein, or a heterocycle, as defined herein. Representative examples of aryl include, but are not limited to, indolyl,

naphthyl, phenyl, quinolinyl, and tetrahydroquinolinyl. "Arylalkyl" refers to an alkyl as defined herein substituted with an aryl radical.

[00042] "Arylene" refers to an aryl as defined herein having two monovalent radical centers derived by the removal of two hydrogen atoms from two different carbon atoms of a parent aryl. Typical arylene radicals include, but are not limited to, phenylene and naphthylene. "Arylalkylene" refers to an arylalkyl as defined herein having two monovalent radical centers derived by the removal of one hydrogen atom from the aryl radical and the other hydrogen removed from the alkyl radical of the group.

[00043] The term "carboxyl" as used herein, means a carboxylic acid, or -COOH .

[00044] The term "cycloalkyl" means a monovalent saturated hydrocarbon ring or a bicyclic group. Cycloalkyl groups have zero heteroatoms and zero double bonds. Cycloalkyl groups are monocyclic, or are fused, spiro, or bridged bicyclic ring systems. Monocyclic cycloalkyl groups contain 3 to 10 carbon atoms, preferably 4 to 7 carbon atoms, and more preferably 5 to 6 carbon atoms in the ring. Bicyclic cycloalkyl groups contain 8 to 12 carbon atoms, preferably 9 to 10 carbon atoms in the ring. Cycloalkyl groups may be substituted or unsubstituted. Cycloalkyl groups include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

[00045] The term "cycloalkenyl," as used herein, means a non-aromatic monocyclic or multicyclic ring system containing at least one carbon-carbon double bond and preferably having from 5-10 carbon atoms per ring. Exemplary monocyclic cycloalkenyl rings include cyclopentenyl, cyclohexenyl, and cycloheptenyl.

[00046] The term "cycloalkynyl," as used herein, means a monocyclic or multicyclic ring system containing at least one carbon-carbon triple bond and preferably having from 5-10 carbon atoms per ring or more than 10 carbon atoms per ring.

[00047] The term "haloalkyl" as used herein, means an alkyl group, as defined herein, in which one, two, three, four, five, six, seven or eight hydrogen atoms are replaced by a halogen. Representative examples of haloalkyl include, but are not limited to, 2-fluoroethyl, 2,2,2-trifluoroethyl, trifluoromethyl, difluoromethyl, pentafluoroethyl, and trifluoropropyl such as 3,3,3-trifluoropropyl.

[00048] The term "halogen" or "halo" as used herein, means Cl, Br, I, or F.

[00049] The term "heteroalkyl" as used herein, means an alkyl group, as defined herein, in which at least one of the carbons of the alkyl group is replaced with a heteroatom, such as

oxygen, nitrogen, and sulfur. Representative examples of heteroalkyls include, but are not limited to, alkyl ethers, secondary and tertiary alkyl amines, amides, and alkyl sulfides.

[00050] The term “heteroaryl” as used herein, refers to an aromatic monocyclic ring or an aromatic bicyclic ring system containing at least one heteroatom independently selected from the group consisting of N, O, and S. The aromatic monocyclic rings are five or six membered rings containing at least one heteroatom independently selected from the group consisting of N, O, and S. The five membered aromatic monocyclic rings have two double bonds and the six membered aromatic monocyclic rings have three double bonds. The bicyclic heteroaryl groups are exemplified by a monocyclic heteroaryl ring appended to the parent molecular moiety and fused to a monocyclic cycloalkyl group, as defined herein, a monocyclic aryl group, as defined herein, a monocyclic heteroaryl group, as defined herein, or a monocyclic heterocycle, as defined herein. Representative examples of heteroaryl include, but are not limited to, indolyl, pyridinyl (including pyridin-2-yl, pyridin-3-yl, pyridin-4-yl), pyrimidinyl, pyrazinyl, pyridazinyl, pyrazolyl, pyrrolyl, benzopyrazolyl, 1,2,3-triazolyl, 1,3,4-thiadiazolyl, 1,2,4-thiadiazolyl, 1,3,4-oxadiazolyl, 1,2,4-oxadiazolyl, imidazolyl, thiazolyl, isothiazolyl, thienyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, benzoxadiazolyl, benzothienyl, benzofuranyl, isobenzofuranyl, furanyl, oxazolyl, isoxazolyl, purinyl, isoindolyl, quinoxalinyl, indazolyl, quinazolinyl, 1,2,4-triazinyl, 1,3,5-triazinyl, isoquinolyl, quinolyl, 6,7-dihydro-1,3-benzothiazolyl, imidazo[1,2-*a*]pyridinyl, naphthyridinyl, pyridoimidazolyl, thiazolo[5,4-*b*]pyridin-2-yl, thiazolo[5,4-*d*]pyrimidin-2-yl.

[00051] The term “heterocycle” or “heterocyclic” or “heterocyclyl” as used herein means a monocyclic heterocycle, a bicyclic heterocycle (heterobicyclic), or a tricyclic heterocycle. The monocyclic heterocycle is a three-, four-, five-, six-, seven-, or eight-membered ring containing at least one heteroatom independently selected from the group consisting of O, N, and S. The three- or four-membered ring contains zero or one double bond, and one heteroatom selected from the group consisting of O, N, and S. The five-membered ring contains zero or one double bond and one, two, or three heteroatoms selected from the group consisting of O, N, and S. The six-membered ring contains zero, one, or two double bonds and one, two, or three heteroatoms selected from the group consisting of O, N, and S. The seven- and eight-membered rings contains zero, one, two, or three double bonds and one, two, or three heteroatoms selected from the group consisting of O, N, and S. Representative examples of monocyclic heterocycles include, but are not limited to, azetidiny, azepanyl, aziridinyl, diazepanyl, 1,3-dioxanyl, 1,3-dioxolanyl, 1,3-dithiolanyl, 1,3-dithianyl, imidazolinyl, imidazolidinyl, isothiazolinyl, isothiazolidinyl, isoxazolinyl, isoxazolidinyl, morpholinyl, oxadiazolinyl, oxadiazolidinyl, oxazolyl, oxazolidinyl, oxetanyl,

piperazinyl, piperidinyl, pyranyl, pyrazolinyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyridinyl, tetrahydrothienyl, thiadiazolinyl, thiadiazolidinyl, 1,2-thiazinanyl, 1,3-thiazinanyl, thiazolinyl, thiazolidinyl, thiomorpholinyl, 1,1-dioxidothiomorpholinyl (thiomorpholine sulfone), thiopyranyl, and trithianyl. The bicyclic heterocycle is a monocyclic heterocycle fused to a phenyl group, or a monocyclic heterocycle fused to a monocyclic cycloalkyl, or a monocyclic heterocycle fused to a monocyclic cycloalkenyl, or a monocyclic heterocycle fused to a monocyclic heterocycle, or a bridged monocyclic heterocycle ring system in which two non-adjacent atoms of the ring are linked by an alkylene bridge of 1, 2, 3, or 4 carbon atoms, or an alkenylene bridge of two, three, or four carbon atoms. Representative examples of bicyclic heterocycles include, but are not limited to, benzopyranyl, benzothiopyranyl, chromanyl, 2,3-dihydrobenzofuranyl, 2,3-dihydrobenzothienyl, 2,3-dihydroisoquinoline, azabicyclo[2.2.1]heptyl (including 2-azabicyclo[2.2.1]hept-2-yl), 2,3-dihydro-1*H*-indolyl, isoindolyl, octahydrocyclopenta[*c*]pyrrolyl, octahydropyrrolopyridinyl, and tetrahydroisoquinolyl. Tricyclic heterocycles are exemplified by a bicyclic heterocycle fused to a phenyl group, or a bicyclic heterocycle fused to a monocyclic cycloalkyl, or a bicyclic heterocycle fused to a monocyclic cycloalkenyl, or a bicyclic heterocycle fused to a monocyclic heterocycle, or a bicyclic heterocycle in which two non-adjacent atoms of the bicyclic ring are linked by an alkylene bridge of 1, 2, 3, or 4 carbon atoms, or an alkenylene bridge of two, three, or four carbon atoms. Examples of tricyclic heterocycles include, but not limited to, octahydro-2,5-epoxypentalene, hexahydro-2*H*-2,5-methanocyclopenta[*b*]furan, hexahydro-1*H*-1,4-methanocyclopenta[*c*]furan, aza-adamantane (1-azatricyclo[3.3.1.1^{3,7}]decane), and oxa-adamantane (2-oxatricyclo[3.3.1.1^{3,7}]decane). The monocyclic, bicyclic, and tricyclic heterocycles are connected to the parent molecular moiety through any carbon atom or any nitrogen atom contained within the rings, and can be unsubstituted or substituted.

[00052] The term “heteroarylalkyl” as used herein, refers to a heteroaryl group, as defined herein, appended to the parent molecular moiety through an alkylene group, as defined herein.

[00053] The term “heterocyclylalkyl” as used herein, refers to a heterocycle group, as defined herein, appended to the parent molecular moiety through an alkylene group, as defined herein.

[00054] The term “hydroxyl” or “hydroxy” as used herein, means an -OH group.

[00055] The term “hydroxyalkyl” as used herein, means at least one -OH group, is appended to the parent molecular moiety through an alkylene group, as defined herein.

[00056] In some instances, the number of carbon atoms in a hydrocarbyl substituent (e.g., alkyl or cycloalkyl) is indicated by the prefix “C_{x-y}”, wherein x is the minimum and y is the maximum number of carbon atoms in the substituent. Thus, for example, “C₁₋₃ alkyl” refers to an alkyl substituent containing from 1 to 3 carbon atoms.

[00057] The term “substituted” refers to a group that may be further substituted with one or more non-hydrogen substituent groups. Substituent groups include, but are not limited to, halogen, =O (oxo), =S (thioxo), cyano, nitro, fluoroalkyl, alkoxyfluoroalkyl, fluoroalkoxy, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, heteroalkyl, cycloalkyl, cycloalkenyl, aryl, heteroaryl, heterocycle, cycloalkylalkyl, heteroarylalkyl, arylalkyl, hydroxy, hydroxyalkyl, alkoxy, alkoxyalkyl, alkylene, aryloxy, phenoxy, benzyloxy, amino, alkylamino, acylamino, aminoalkyl, arylamino, sulfonylamino, sulfinylamino, sulfonyl, alkylsulfonyl, arylsulfonyl, aminosulfonyl, sulfinyl, -COOH, ketone, amide, carbamate, and acyl.

[00058] The term ““ designates a single bond (—) or a double bond () or a triple bond ().

[00059] For compounds described herein, groups and substituents thereof may be selected in accordance with permitted valence of the atoms and the substituents, such that the selections and substitutions result in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[00060] The term “administration” or “administering,” as used herein, refers to providing, contacting, and/or delivery of a compound or conjugate by any appropriate route to achieve the desired effect. These compounds or conjugates may be administered to a subject in numerous ways including, but not limited to, orally, ocularly, nasally, intravenously, topically, as aerosols, suppository, etc. and may be used in combination.

[00061] As used herein, “cancer” may include any cell or tissue derived from a tumor, neoplasm, cancer, precancer, cell line, malignancy, or any other source of cells that have the potential to expand and grow to an unlimited degree. Cancer cells may be derived from naturally occurring sources or may be artificially created. Cancer cells may also be capable of invasion into other tissues and metastasis. Cancer cells further encompass any malignant cells that have invaded other tissues and/or metastasized. One or more cancer cells in the context of an organism may also be called a cancer, tumor, neoplasm, growth, malignancy, or any other term used in the art to describe cells in a cancerous state. Cancer may include, for example, melanoma, brain cancer, lung cancer, adrenal cancer, liver cancer, renal or

kidney cancer, pancreatic cancer, esophageal cancer, gastric cancer, stomach cancer, colon cancer, colorectal cancer, anal cancer, prostate cancer, ovarian cancer, breast cancer, cervical cancer, lymphoma, leukemia, myeloma, hematologic cancer, hepatocarcinoma, retinoblastoma, glioma, sarcoma, blastoma, squamous cell carcinoma, and adenocarcinoma.

[00062] As used herein the term "cancer stem cell" refers to a cancer cell capable of self-renewing and differentiating into the distinct types of cancer cells found in a malignant tumor.

[00063] In general, reference to "a circulating tumor cell" (CTC) is intended to refer to a single cell, while reference to "circulating tumor cells" or "cluster of circulating tumor cells" is intended to refer to more than one cancer cell. However, one of skill in the art would understand that reference to "circulating tumor cells" is intended to include a population of circulating tumor cells including one or more circulating tumor cells while reference to "a circulating tumor cell" could include more than one circulating tumor cell. The term "circulating tumor cell" or "circulating tumor cells", as used herein, refers to any cancer cell or cluster of cancer cells that are found in a subject's blood or blood serum sample. CTCs may also contain or consist of a cancer stem cell or cluster of cancer stem cells that are found in a subject's blood or blood serum sample.

[00064] The terms "control," "reference level," and "reference" are used herein interchangeably. The reference level may be a predetermined value or range, which is employed as a benchmark against which to assess the measured result. "Control group" as used herein refers to a group of control subjects. The predetermined level may be a cutoff value from a control group. The predetermined level may be an average from a control group. Cutoff values (or predetermined cutoff values) may be determined by Adaptive Index Model (AIM) methodology. Cutoff values (or predetermined cutoff values) may be determined by a receiver operating curve (ROC) analysis from biological samples of the patient group. ROC analysis, as generally known in the biological arts, is a determination of the ability of a test to discriminate one condition from another. A description of ROC analysis is provided in P.J. Heagerty et al. (Biometrics 2000, 56, 337-44), the disclosure of which is hereby incorporated by reference in its entirety. Alternatively, cutoff values may be determined by a quartile analysis of biological samples of a patient group. For example, a cutoff value may be determined by selecting a value that corresponds to any value in the 25th-75th percentile range, preferably a value that corresponds to the 25th percentile, the 50th percentile or the 75th percentile, and more preferably the 75th percentile. Such statistical analyses may be performed using any method known in the art and can be implemented through any number of commercially available software packages (e.g., from

Analyse-it Software Ltd., Leeds, UK; StataCorp LP, College Station, TX; SAS Institute Inc., Cary, NC.). The healthy or normal levels or ranges for a target or for a protein activity may be defined in accordance with standard practice. A control may be a subject, or a sample therefrom, whose disease state is known. The subject, or sample therefrom, may be healthy, diseased, diseased prior to treatment, diseased during treatment, diseased after treatment, or healthy after treatment, or a combination thereof. The term "normal subject" as used herein means a healthy subject, i.e. a subject having no clinical signs or symptoms of disease. The normal subject is clinically evaluated for otherwise undetected signs or symptoms of disease, which evaluation may include routine physical examination and/or laboratory testing. In some embodiments, the control is a healthy control. In some embodiments, the control comprises cancer.

[00065] The term "effective dosage" or "therapeutic dosage" or "therapeutically effective amount" or "effective amount" as used herein means an amount sufficient to elicit a therapeutic effect or a dosage of a drug effective for periods of time necessary, to achieve the desired therapeutic result. An effective dosage may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, the manner of administration, the stage and severity of the disease, the general state of health of the subject, the judgment of the prescribing physician, and the ability of the drug to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of substance are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[00066] The terms "inhibit" or "inhibiting" mean that an activity is decreased or prevented in the presence of an inhibitor as opposed to in the absence of the inhibitor. The term "inhibition" refers to the reduction or down regulation of a process or the elimination of a stimulus for a process, which results in the absence or minimization of the expression or activity of a biomarker or polypeptide. Inhibition may be direct or indirect. Inhibition may be specific, that is, the inhibitor inhibits a biomarker or polypeptide and not others.

[00067] "Sample" or "test sample" as used herein can mean any sample in which the presence and/or level of compound or target is to be detected or determined. Samples may include liquids, solutions, emulsions, mixtures, or suspensions. Samples may include a medical sample. Samples may include any biological fluid or tissue, such as blood, whole

blood, fractions of blood such as plasma and serum, peripheral blood mononuclear cells (PBMCs), muscle, interstitial fluid, sweat, saliva, urine, tears, synovial fluid, bone marrow, cerebrospinal fluid, nasal secretions, sputum, amniotic fluid, bronchoalveolar lavage fluid, gastric lavage, emesis, fecal matter, lung tissue, peripheral blood mononuclear cells, total white blood cells, lymph node cells, spleen cells, tonsil cells, cancer cells, tumor cells, bile, digestive fluid, skin, or combinations thereof. In some embodiments, the sample comprises an aliquot. In other embodiments, the sample comprises a biological fluid. Samples can be obtained by any means known in the art. The sample can be used directly as obtained from a patient or can be pre-treated, such as by filtration, distillation, extraction, concentration, centrifugation, inactivation of interfering components, addition of reagents, and the like, to modify the character of the sample in some manner as discussed herein or otherwise as is known in the art. Samples may be obtained before diagnosis, before treatment, during treatment, after treatment, or after diagnosis, or a combination thereof.

[00068] The term “specificity” as used herein refers to the number of true negatives divided by the number of true negatives plus the number of false positives, where specificity (“spec”) may be within the range of $0 < \text{spec} < 1$. Hence, a method that has both sensitivity and specificity equaling one, or 100%, is preferred.

[00069] By “specifically binds,” it is generally meant that a compound or conjugate binds to a target when it binds to that target more readily than it would bind to a random, unrelated target.

[00070] “Subject” as used herein can mean a mammal that wants or is in need of the herein described compounds or methods. The subject may be a human or a non-human animal. The subject may be a mammal. The mammal may be a primate or a non-primate. The mammal can be a primate such as a human; a non-primate such as, for example, dog, cat, horse, cow, pig, mouse, rat, camel, llama, goat, rabbit, sheep, hamster, and guinea pig; or non-human primate such as, for example, monkey, chimpanzee, gorilla, orangutan, and gibbon. The subject may be of any age or stage of development, such as, for example, an adult, an adolescent, or an infant. The subject may be male or female. In some embodiments, the subject has a specific genetic marker.

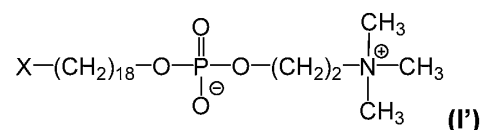
[00071] As used herein, the term “toxic” refers to an amount of a chemical entity, agent, or substance that would be harmful to the subject or cause any adverse effect. The term “non-toxic” refers to a substance that has a relatively low degree to which it can damage a subject. “Cytotoxic” refers to a chemical entity, agent, or substance that is toxic to cells. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, plant, or

other subject as defined herein, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ (organotoxicity), such as the liver (hepatotoxicity). A central concept of toxicology is that effects are dose-dependent; even water can lead to water intoxication when taken in large enough doses, whereas for even a very toxic substance such as snake venom there is a dose below which there is no detectable toxic effect. A composition or compound that is relatively non-toxic may allow a wider range of subjects to be able to safely handle the composition or compound, without serious safety concerns or risks.

[00072] The terms “treat,” “treated,” or “treating” as used herein refers to a therapeutic wherein the object is to slow down (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; stabilization (i.e., not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. “Treatment” or “treating,” when referring to protection of a subject from a disease, means suppressing, repressing, ameliorating, or completely eliminating the disease. Preventing the disease involves administering a composition of the present invention to a subject prior to onset of the disease. Suppressing the disease involves administering a composition of the present invention to a subject after induction of the disease but before its clinical appearance. Repressing or ameliorating the disease involves administering a composition of the present invention to a subject after clinical appearance of the disease. The disease may comprise cancer.

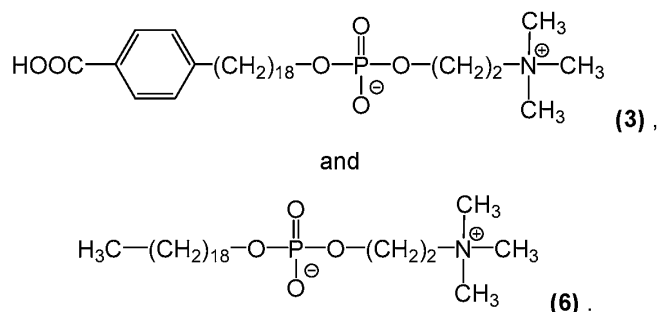
2. Phospholipid Ethers

[00073] Provided herein are phospholipid ether (PLE) molecules. The PLE may be according to Formula (I'), or a salt thereof:



wherein X is hydrogen, methyl, or phenyl substituted with carboxyl.

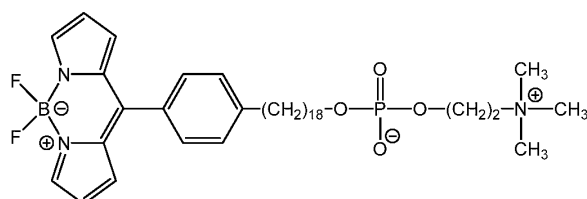
[00074] In some embodiments, the PLE is selected from the following:



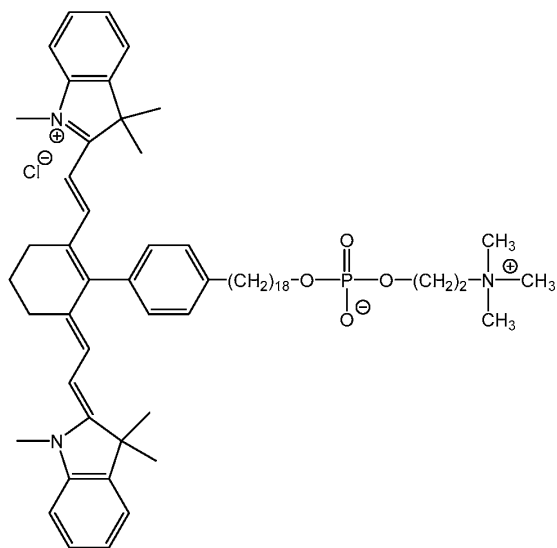
[00075] The PLE may be conjugated to a detectable moiety (also referred to as a reporter or a label), such as, for example, a fluorescent molecule, chemuminescent molecule, radiolabel, magnetic label, infrared molecule, or a combination thereof. Magnetic labels are labeling moieties that, when sufficiently associated with a magnetic proximity sensor, are detectable by the magnetic proximity sensor and cause the magnetic proximity sensor to output a signal. Magnetic labels may include one or more materials selected from paramagnetic, superparamagnetic, ferromagnetic, anti-ferromagnetic materials, combinations thereof, and the like. Fluorescent labels are labeling moieties that are detectable by a fluorescence detector. Suitable fluorescent molecules (fluorophores) include, but are not limited to, fluorescein, fluorescein isothiocyanate, succinimidyl esters of carboxyfluorescein, succinimidyl esters of fluorescein, 5-isomer of fluorescein dichlorotriazine, caged carboxyfluorescein-alanine-carboxamide, Oregon Green 488, Oregon Green 514; Lucifer Yellow, acridine Orange, rhodamine, tetramethylrhodamine, Texas Red, propidium iodide, JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide), tetrabromorhodamine 123, rhodamine 6G, TMRM (tetramethyl rhodamine methyl ester), TMRE (tetramethyl rhodamine ethyl ester), tetramethylrosamine, rhodamine B and 4-dimethylaminotetramethylrosamine, green fluorescent protein, blue-shifted green fluorescent protein, cyan-shifted green fluorescent protein, red-shifted green fluorescent protein, yellow-shifted green fluorescent protein, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid; acridine and derivatives, such as acridine, acridine isothiocyanate; 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS); 4-amino-N-[3-vinylsulfonyl]phenyl]naphthalimide-3,5 disulfonate; N-(4-anilino-1-naphthyl)maleimide; anthranilamide; 4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a diaza-5-indacene-3-propionyl acid BODIPY; cascade blue; Brilliant Yellow; coumarin and derivatives: coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), 7-amino-4-trifluoromethylcoumarin (Coumarin 151); cyanine dyes; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5',5"-dibromopyrogallol-sulfonaphthalein (Bromopyrogallol Red); 7-diethylamino-3-(4'-isothiocyanatophenyl)-4-

methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; 5-(dimethylamino)naphthalene-1-sulfonyl chloride (DNS, dansylchloride); 4-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC); eosin and derivatives: eosin, eosin isothiocyanate, erythrosin and derivatives: erythrosin B, erythrosin, isothiocyanate; ethidium; fluorescein and derivatives: 5-carboxyfluorescein (FAM), 5-(4,6-dichlorotriazin-2-yl)amino-1-fluorescein (DTAF), 2',7' dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE), fluorescein, fluorescein isothiocyanate, QFITC, (XRITC); fluorescamine; IR144; IR1446; Malachite Green isothiocyanate; 4-methylumbelliferone ortho cresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthalaldehyde; pyrene and derivatives: pyrene, pyrene butyrate, succinimidyl 1-pyrene; butyrate quantum dots; Reactive Red 4 (Cibacron™ Brilliant Red 3B-A) rhodamine and derivatives: 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red); N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA); tetramethyl rhodamine; tetramethyl rhodamine isothiocyanate (TRITC); riboflavin; 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS), 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL), rosolic acid; CAL Fluor Orange 560; terbium chelate derivatives; Cy 3; Cy 5; Cy 5.5; Cy 7; IRD 700; IRD 800; La Jolla Blue; phthalocyanine; and naphthalocyanine, coumarins and related dyes, xanthene dyes such as rhodols, resorufins, bmanes, acridines, isoindoles, dansyl dyes, aminophthalic hydrazides such as luminol, and isoluminol derivatives, aminophthalimides, aminonaphthalimides, aminobenzofurans, aminoquinolines, dicyanohydroquinones, fluorescent europium and terbium complexes; combinations thereof, and the like.

[00076] The detectable moiety may be covalently or cleavably linked to the PLE. For example, the labelled PLE may be selected from the following:



CLR 1501 (1), and



CLR 1502 (2).

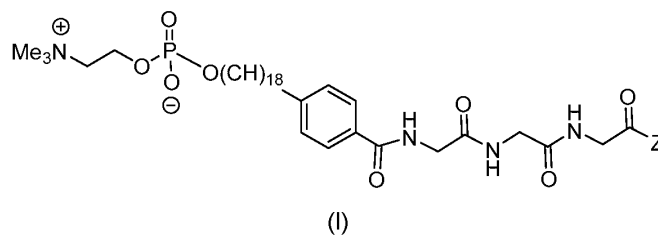
[00077] The above compound (1) is PLE (3) with the fluorescent moiety, BODIPY, stably linked to the PLE, and may also be referred to as CLR 1501. The above compound (2) is PLE (3) with a near infrared molecule, IR-775, stably linked to the PLE, and may also be referred to as CLR 1502. Compounds (1) and (2) may also be referred to as a phospholipid drug conjugate (PDC).

[00078] The PLE, or a conjugate thereof, may be specific for a tumor or cancer cell. Upon administration to a subject, the PLE, or a conjugate thereof, may localize to a tumor or cancer cell. The PLE, or a conjugate thereof, may be incorporated into a tumor or cancer cell more than a healthy cell. The PLE, or a conjugate thereof, may be incorporated into at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold more tumor or cancer cells than healthy cells.

3. Phospholipid-Flavagline Conjugates Compounds

[00079] A PLE may be conjugated via a linker to a flavagline compound to form a phospholipid-flavagline conjugate (also referred to as a PLE-flavagline conjugate).

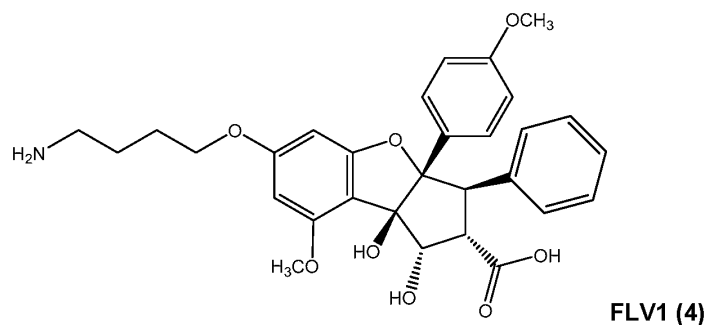
[00080] In one aspect, the present application provides a compound of formula (I), or a pharmaceutically acceptable salt thereof,



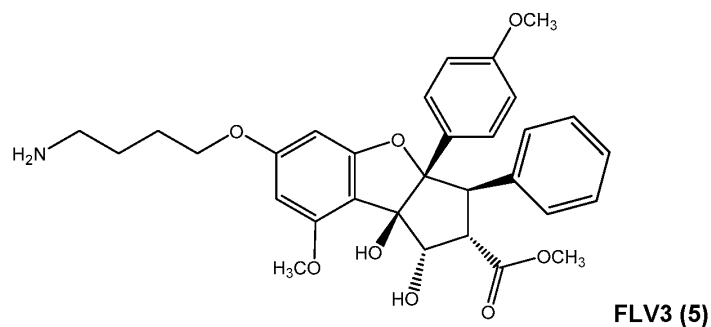
wherein Z is a flavagline analog.

[00081] Flavaglines are a family of natural products that are found in plants of the genus *Aglaia* (Meliaceae). Flavaglines are characterized by a cyclopenta[b]benzofuran skeleton. Flavaglines may have strong insecticidal, antifungal, anti-inflammatory, neuroprotective, cardioprotective, and anticancer activities. Flavaglines may enhance the efficacy of chemotherapies and/or alleviate the cardiac adverse effect of chemotherapies. Suitable flavagline analogs include, for example, the compounds disclosed in U.S. Patent Application Publication US 2018/0086729, the content of which is incorporated herein by reference in its entirety. Flavaglines may include, for example, FLV1, FLV3, a derivative or structural analog thereof, or a combination thereof. As used herein, the term “flavagline analog” or “flavagline anti-cancer drug” includes all natural and synthetic flavagline compounds, derivatives thereof, and structural analogs thereof.

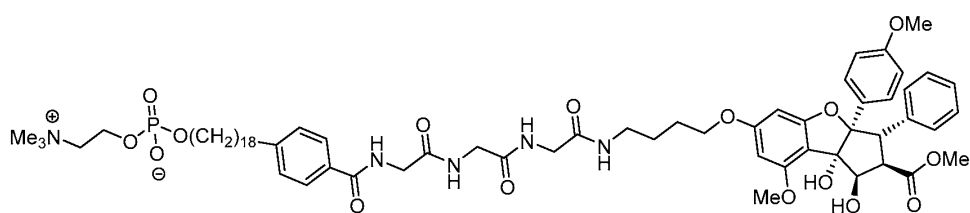
[00082] In some embodiments, the flavagline analog comprises FLV1, or a salt thereof:



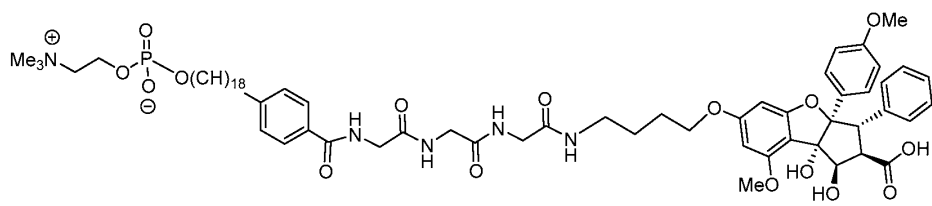
[00083] In some embodiments, the flavagline analog comprises FLV3, or a salt thereof:



[00084] Suitable compounds as disclosed herein include:

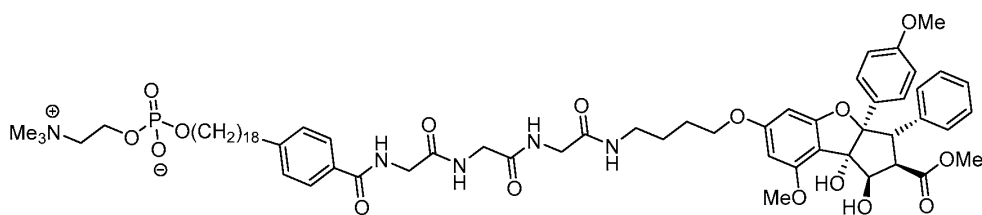


(CLR 1899), and



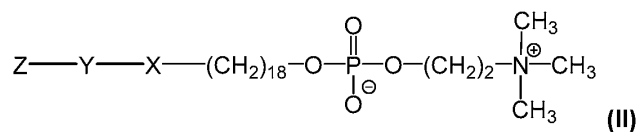
or a pharmaceutically acceptable salt thereof.

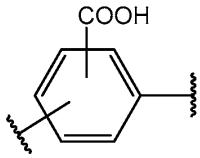
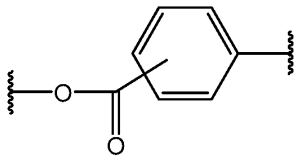
[00085] In some embodiments, the compound is



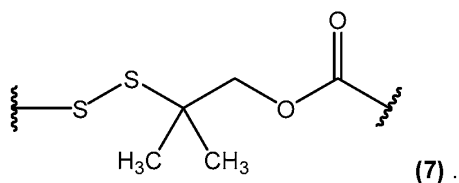
(CLR 1899), or a pharmaceutically acceptable salt thereof.

[00086] Also provided are conjugate compounds of Formula (II), or a salt thereof:

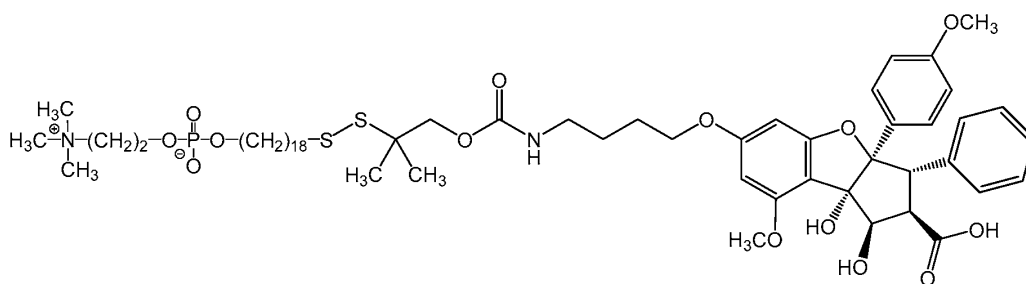


wherein X is , or , or methylene, or bond; Y is a linker comprising a disulfide; and Z is a flavagline anti-cancer drug. A conjugate compound of Formula (II) may also be referred to as a phospholipid drug conjugate (PDC).

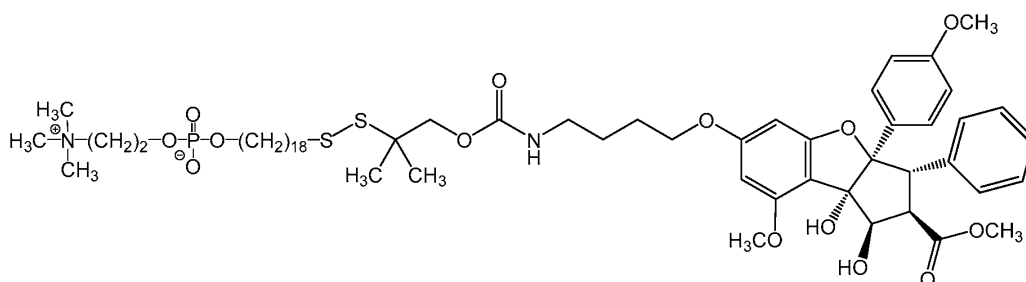
[00087] In formula (II), the linker may be a cleavable linker, such as a disulfide, and is specifically designed to deliver the flavagline to a tumor or cancer cell. In some embodiments, the linker comprises a disulfide. In some embodiments, the linker comprises the following:



[00088] In some embodiments, the phospholipid-flavagline conjugate compound of formula (II) is selected from the following, or a pharmaceutically acceptable salt thereof:



CLR 1865 (with FLV1) (8), and

**CLR 1852 (with FLV3) (9).**

[00089] The compounds as described herein may exist as a stereoisomer wherein asymmetric or chiral centers are present. The stereoisomer is “*R*” or “*S*” depending on the configuration of substituents around the chiral carbon atom. The terms “*R*” and “*S*” used herein are configurations as defined in IUPAC 1974 Recommendations for Section E, Fundamental Stereochemistry, in *Pure Appl. Chem.*, 1976, 45, 13-30. The disclosure contemplates various stereoisomers and mixtures thereof and these are specifically included within the scope of this invention. Stereoisomers include enantiomers and diastereomers, and mixtures of enantiomers or diastereomers. Individual stereoisomers of the compounds may be prepared synthetically from commercially available starting materials, which contain asymmetric or chiral centers or by preparation of racemic mixtures followed by methods of resolution well-known to those of ordinary skill in the art. These methods of resolution are exemplified by (1) attachment of a mixture of enantiomers to a chiral auxiliary, separation of the resulting mixture of diastereomers by recrystallization or chromatography and optional liberation of the optically pure product from the auxiliary as described in Furniss, Hannaford, Smith, and Tatchell, “Vogel's Textbook of Practical Organic Chemistry,” 5th edition (1989), Longman Scientific & Technical, Essex CM20 2JE, England, or (2) direct separation of the mixture of optical enantiomers on chiral chromatographic columns, or (3) fractional recrystallization methods. It should be understood that the compounds as described herein may include tautomeric forms, as well as geometric isomers, and that these isomers also constitute embodiments of the disclosure. The compounds as described herein may exist as a racemic mixture.

[00090] The present disclosure also includes an isotopically-labeled compound, such as an isotopically-labeled PLE, an isotopically-labeled flavagline, an isotopically-labeled linker, or an isotopically-labeled phospholipid-flavagline conjugate. An isotopically-labeled compound is identical to those recited detailed herein but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes suitable for inclusion in

the compounds of the invention are hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, and chlorine, such as, but not limited to ^2H , ^3H , ^{13}C , ^{14}C , ^{15}N , ^{18}O , ^{17}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , and ^{36}Cl , respectively. Substitution with heavier isotopes such as deuterium, i.e. ^2H , can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. The compound may incorporate positron-emitting isotopes for medical imaging and positron-emitting tomography (PET) studies for determining the distribution of receptors. Suitable positron-emitting isotopes that can be incorporated in compounds are ^{11}C , ^{13}N , ^{15}O , and ^{18}F . Isotopically-labeled compounds can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the accompanying Examples using appropriate isotopically-labeled reagent in place of non-isotopically-labeled reagent.

[00091] The disclosed PLE or phospholipid-flavagline conjugate may exist as pharmaceutically acceptable salts. The term "pharmaceutically acceptable salt" refers to salts or zwitterions of the compounds which are water or oil-soluble or dispersible, suitable for treatment of disorders without undue toxicity, irritation, and allergic response, commensurate with a reasonable benefit/risk ratio and effective for their intended use. The salts may be prepared during the final isolation and purification of the compounds or separately by reacting an amino group of the compounds with a suitable acid. For example, a compound may be dissolved in a suitable solvent, such as but not limited to methanol and water and treated with at least one equivalent of an acid, like hydrochloric acid. The resulting salt may precipitate out and be isolated by filtration and dried under reduced pressure. Alternatively, the solvent and excess acid may be removed under reduced pressure to provide a salt. Representative salts include acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, glycerophosphate, hemisulfate, heptanoate, hexanoate, formate, isethionate, fumarate, lactate, maleate, methanesulfonate, naphthylenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, oxalate, maleate, pivalate, propionate, succinate, tartrate, trichloroacetate, trifluoroacetate, glutamate, para-toluenesulfonate, undecanoate, hydrochloric, hydrobromic, sulfuric, phosphoric, and the like. The amino groups of the compounds may also be quaternized with alkyl chlorides, bromides and iodides such as methyl, ethyl, propyl, isopropyl, butyl, lauryl, myristyl, stearyl, and the like.

[00092] Basic addition salts may be prepared during the final isolation and purification of the disclosed compounds by reaction of a carboxyl group with a suitable base such as the

hydroxide, carbonate, or bicarbonate of a metal cation such as lithium, sodium, potassium, calcium, magnesium, or aluminum, or an organic primary, secondary, or tertiary amine. Quaternary amine salts can be prepared, such as those derived from methylamine, dimethylamine, trimethylamine, triethylamine, diethylamine, ethylamine, tributylamine, pyridine, *N,N*-dimethylaniline, *N*-methylpiperidine, *N*-methylmorpholine, dicyclohexylamine, procaine, dibenzylamine, *N,N*-dibenzylphenethylamine, 1-ephedrine and *N,N'*-dibenzylethylenediamine, ethylenediamine, ethanolamine, diethanolamine, piperidine, piperazine, and the like.

[00093] The phospholipid-flavagline conjugate compounds as described herein may be specific for a tumor or cancer cell. Upon administration to a subject, the compounds may localize to a tumor or cancer cell. The compounds may localize or travel to the cytoplasm or organelle of a tumor or cancer cell. The compounds may be incorporated into a tumor or cancer cell more than a healthy cell. The compounds may be incorporated into at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold more tumor or cancer cells than healthy cells.

[00094] The flavagline or flavagline analog may be cleaved from the present compounds, such as cleavage *in vivo* upon administration to a subject. The flavagline or flavagline analog may localize or travel to the cytoplasm or organelle of a tumor or cancer cell. The flavagline or flavagline analog may be incorporated into a tumor or cancer cell more than a healthy cell. The flavagline or flavagline analog may be incorporated into at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold more tumor or cancer cells than healthy cells.

[00095] The compounds as described herein may be synthesized according to the method described in Example 1 or other methods known in the art. The synthesis process may include steps for the preparation of the PLE moiety, the flavagline analog moiety, and the complete compound as shown in Example 1. Suitable flavaglines include commercially available products, for example, those from Haoyuan Chemexpress Co. (Shanghai, China).

4. Pharmaceutical Compositions

[00096] The compounds as described herein, or pharmaceutically acceptable salts thereof, may be formulated into pharmaceutical compositions in accordance with standard techniques well known to those skilled in the pharmaceutical art. In one aspect, the present disclosure provides a pharmaceutical composition comprising a compound as described herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier," as used herein, means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

[00097] The route by which the present compounds are administered and the form of the composition will dictate the type of carrier to be used. The pharmaceutical composition may be in a variety of forms, suitable, for example, for systemic administration (e.g., oral, rectal, sublingual, buccal, implants, intranasal, intravaginal, transdermal, intravenous, intraarterial, intratumoral, intraperitoneal, or parenteral) or topical administration (e.g., dermal, pulmonary, nasal, aural, ocular, liposome delivery systems, or iontophoresis). In some embodiments, the pharmaceutical composition is for administration to a subject's central nervous system. Techniques and formulations may generally be found in "Remington's Pharmaceutical Sciences," (Meade Publishing Co., Easton, Pa.). Pharmaceutical compositions must typically be sterile and stable under the conditions of manufacture and storage. All carriers are optional in the compositions.

[00098] Pharmaceutically acceptable carriers include, for example, diluents, lubricants, binders, disintegrants, colorants, flavors, sweeteners, antioxidants, preservatives, glidants, solvents, suspending agents, wetting agents, surfactants, emollients, propellants, humectants, powders, pH adjusting agents, and combinations thereof.

[00099] Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins (such as human serum albumin), buffer substances (such as phosphates), glycine, sorbic acid, or potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes (such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts), colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, polyacrylates, waxes, polyethylenepolyoxypropylene-block polymers, wool fat, sugars (such as lactose, glucose, and sucrose), starches (such as corn starch and potato starch), cellulose and its derivatives (such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate), powdered tragacanth, malt, gelatin, talc, excipients (such as cocoa butter and suppository waxes), oils (such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil, soybean oil),

glycols (such as propylene glycol or polyethylene glycol), esters (such as ethyl oleate and ethyl laurate), agar, non-toxic compatible lubricants (such as sodium lauryl sulfate and magnesium stearate), coloring agents, releasing agents, coating agents, emulsifying agents, sweetening, flavorant, perfuming agents, preservatives, antioxidants can also be present in the composition, according to the judgment of the formulator.

[000100] Although the amounts of components in the compositions may vary depending on the type of composition prepared, in general, systemic compositions may include 0.01% to 50% of a compound (such as a PLE or phospholipid-flavagline conjugate) and 50% to 99.99% of one or more carriers. Compositions for parenteral administration may typically include 0.1% to 10% of a compound and 90% to 99.9% of one or more carriers. Oral dosage forms may include, for example, at least about 5%, or about 25% to about 50% of a compound. The oral dosage compositions may include about 50% to about 95% of carriers, or from about 50% to about 75% of carriers. The amount of the carrier employed in conjunction with a disclosed compound is sufficient to provide a practical quantity of composition for administration per unit dose of the compound. Techniques and compositions for making dosage forms useful in the methods of this invention are described in the following references: Modern Pharmaceutics, Chapters 9 and 10, Banker & Rhodes, eds. (1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms, 2nd Ed., (1976).

[000101] In some embodiments, the pharmaceutical composition consists essentially of a therapeutically effective amount of a compound as disclosed herein, or a pharmaceutically acceptable salt thereof.

[000102] Liquid dosage forms include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. Solid dosage forms include, but are not limited to, capsules, tablets, pills, powders, cement, putty, and granules. Dosage forms for topical or transdermal administration of the present compounds include, but are not limited to, ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches.

[000103] A liquid carrier or vehicle may be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof.

[000104] The pharmaceutical composition may be in a dosage form suitable for injection or infusion, such as sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient(s) which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions. The ultimate dosage form should be sterile, fluid and stable under manufacture and storage conditions. Sterile injectable solutions may be prepared by incorporating at least a compound as disclosed herein, or a pharmaceutically acceptable salt thereof in the required amount in the appropriate solvent with various other ingredients, as required, optionally followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation may include vacuum drying and freeze-drying techniques, which yield a powder of the active ingredient(s) plus any additional desired ingredient present in the sterile solutions.

[000105] In some embodiments, the composition is a solution, such as a solution suitable for administration by infusion or injection. Solutions may be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. These preparations may contain a preservative to prevent the growth of microorganisms. Prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like.

[000106] Injectable forms may be made by forming microencapsule matrices of the compound(s) as disclosed herein, or pharmaceutically acceptable salt thereof, in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of compound 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). Injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

[000107] In some embodiments, the composition may comprise at least one compound as described herein and at least one additional anti-cancer drug. Anti-cancer drugs that are useful for the present disclosure include, but are not limited to, paclitaxel, irinotecan, topotecan, gemcitabine, cisplatin, geldanamycin, mertansine, abiraterone, afatinib, aminolevulinic acid, aprepitant, axitinib, azacitidine, belinostat, bendamustine, bexarotene, bleomycin, bortezomib, bosutinib, busulfan, cabazitaxel, cabozantinib, capecitabine, carboplatin, carfilzomib, carmustine, ceritinib, cetuximab, chlorambucil, clofarabine, crizotinib, cyclophosphamide, cytarabine, dabrafenib, dacarbazine, dactinomycin, dasatinib, daunorubicin, decitabine, denosumab, dexrazoxane, docetaxel, dolastatins (e.g. monomethyl auristatin E), doxorubicin, enzalutamide, epirubicin, eribulin mesylate, erlotinib,

etoposide, everolimus, floxuridine, fludarabine phosphate, fluorouracil, ganetespib, gefitinib, gemtuzumab ozogamicin, hexamethylmelamine, hydroxyurea, ibritumomab tiuxetan, ibrutinib, idelalisib, ifosfamide, imatinib, ipilimumab, ixabepilone, lapatinib, leucovorin calcium, lomustine, maytansinoids, mechlorethamine, melphalan, mercaptopurine, mesna, methotrexate, mitomycin C, mitotane, mitoxantrone, nelarabine, nelfinavir, nilotinib, obinutuzumab, ofatumumab, omacetaxine mepesuccinate, oxaliplatin, panitumumab, pazopanib, pegaspargase, pembrolizumab, pemetrexed, pentostatin, pertuzumab, plicanycin, pomalidomide, ponatinib hydrochloride, pralatrexate, procarbazine, radium 223 dichloride, ramucirumab, regorafenib, retaspimycin, ruxolitinib, semustine, siltuximab, sorafenib, streptozocin, sunitinib malate, tanespimycin, temozolomide, temsirolimus, teniposide, thalidomide, thioguanine, thiotepa, toremifene, trametinib, trastuzumab, vandetanib, vemurafenib, vinblastine, vincristine, vinorelbine, vismodegib, vorinostat, and ziv-aflibercept. Any compounds that are currently known to or are capable of acting as anti-cancer drugs are also useful for the present disclosure.

5. Administration

[000108] The compounds as described herein, or the pharmaceutically acceptable salts thereof, or the pharmaceutical compositions as described herein, may be administered to a subject. The compounds and compositions may be administered in dosages and by techniques known to those skilled in the medical arts taking into consideration such factors as the age, sex, weight, and condition of the particular subject, and the route of administration.

[000109] The present compounds and compositions may be administered prophylactically or therapeutically. In prophylactic administration, the compounds and compositions may be administered in an effective amount to induce a prophylactic response. In therapeutic applications, the compounds and compositions may be administered to a subject in need thereof in an effective amount to elicit a therapeutic effect.

[000110] For example, a therapeutically effective amount of a compound may be about 1 mg/kg to about 1000 mg/kg, about 5 mg/kg to about 950 mg/kg, about 10 mg/kg to about 900 mg/kg, about 15 mg/kg to about 850 mg/kg, about 20 mg/kg to about 800 mg/kg, about 25 mg/kg to about 750 mg/kg, about 30 mg/kg to about 700 mg/kg, about 35 mg/kg to about 650 mg/kg, about 40 mg/kg to about 600 mg/kg, about 45 mg/kg to about 550 mg/kg, about 50 mg/kg to about 500 mg/kg, about 55 mg/kg to about 450 mg/kg, about 60 mg/kg to about 400 mg/kg, about 65 mg/kg to about 350 mg/kg, about 70 mg/kg to about 300 mg/kg, about

75 mg/kg to about 250 mg/kg, about 80 mg/kg to about 200 mg/kg, about 85 mg/kg to about 150 mg/kg, and about 90 mg/kg to about 100 mg/kg.

[000111] The compound can be administered by methods well known in the art as described in Donnelly et al. (*Ann. Rev. Immunol.* **1997**, *15*, 617-648); Felgner et al. (U.S. Patent No. 5,580,859, issued Dec. 3, 1996); Felgner (U.S. Patent No. 5,703,055, issued Dec. 30, 1997); and Carson et al. (U.S. Patent No. 5,679,647, issued Oct. 21, 1997), the contents of all of which are incorporated herein by reference in their entirety. The compound can be complexed to particles or beads that can be administered to an individual, for example, using a vaccine gun. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration.

[000112] The compound can be delivered via a variety of routes. Typical delivery routes include parenteral administration, e.g., intradermal, intramuscular or subcutaneous delivery. Other routes include oral administration, intranasal, intravaginal, transdermal, intravenous, intraarterial, intratumoral, intraperitoneal, and epidermal routes. In some embodiments, the compound is administered intravenously, intraarterially, or intraperitoneally to the subject. In some embodiments, the compound is administered to the subject intravenously. In some embodiments, the compound is administered to the subject orally.

[000113] In some embodiments, the compound is administered in a controlled release formulation. The compound may be released into the circulation, for example. In some embodiments, the compound may be released over a period of at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 1 week, at least about 1.5 weeks, at least about 2 weeks, at least about 2.5 weeks, at least about 3.5 weeks, at least about 4 weeks, or at least about 1 month.

[000114] The compound can be administered in a single dose or episodically or in repeated doses. For example, the compound may be administered once every hour, 2 hours, 4 hours, 8 hours, 12 hours, 25 hours, 36 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 4 weeks.

6. Methods

[000115] The basis for selective tumor targeting of the compounds described herein lies in differences between the plasma membranes of cancer cells as compared to those of most normal cells. Phospholipid ether (PLE) molecules take advantage of the metabolic shift that

tumors cells undergo in order to generate the energy necessary for the rapid cell division. Tumors enhance the utilization of the beta oxidative pathway to convert long chain fatty acids (LCFA) into energy. In order to increase the uptake of LCFA, tumor cells alter the cell membrane forming specialized microdomains known as "lipid rafts." Lipid rafts form due to metabolic shifts and need for phospholipids. Within tumor cells these regions have become overabundant and stabilized allowing them to be potential tumor specific targets. Specifically, cancer cell membranes are highly enriched in lipid rafts. In normal tissue the presence of lipid rafts is limited and transient (~2 nanoseconds). In tumors, lipid rafts have increased presence and are stabilized (up to 10 days). Cancer cells have five to ten times more lipid rafts than healthy cells. In addition, lipid rafts have been demonstrated to be highly abundant on nearly all tumor types and 100% of individual cancer cells tested. Lipid rafts are highly organized and specialized regions of the membrane phospholipid bilayer, that contain high concentrations of various signaling molecules, sphingolipids, glycosphingolipids and cholesterol, and serve to organize cell surface and intracellular signaling molecules (e.g., growth factor and cytokine receptors, the phosphatidylinositol 3-kinase (PI3K)/Akt survival pathway). Data suggests that lipid rafts serve as portals of entry for phospholipid ethers. The marked selectivity of these compounds for cancer cells versus non-cancer cells is attributed to the high affinity of PLEs for cholesterol and the abundance of cholesterol-rich lipid rafts in cancer cells. The pivotal role played by lipid rafts is underscored by the fact that disruption of lipid raft architecture suppresses uptake of PLEs into cancer cells. It has been shown that the uptake of PLEs is reduced by 60% when lipid rafts are blocked from forming. These features combined with lipid rafts providing rapid internalization of phospholipid drug conjugates, makes them an ideal target.

[000116] The compounds as disclosed herein, such as PLE analogs, may be LCFA mimetics. The molecules as disclosed herein have undergone extensive structure activity relationship (SAR) analysis related to targeting lipid rafts on tumor cells and have been shown to specifically bind to these regions. The molecules as disclosed herein provide entry directly into the cytoplasm and transit to the endoplasmic reticulum and mitochondria along the Golgi-apparatus-network within the cell cytoplasm. In some embodiments, the compounds disclosed herein include a uniquely designed phospholipid ether conjugated to a flavagline (FLV) analogue. FLVs are potent cytotoxins that inhibit translation, cell cycle progression and induce apoptosis.

[000117] The compounds as described herein, or a pharmaceutically acceptable salt thereof, or composition comprising a compound as described herein may be used to treat cancer. In one aspect, the present disclosure provides a method of treating cancer in a

subject in need thereof, comprising administering to the subject an effective amount of a compound as described herein, or a pharmaceutically acceptable salt thereof, or composition comprising a compound as described herein.

[000118] In another aspect, the present disclosure provides compounds, or pharmaceutically acceptable salts thereof, as described herein for use in treating cancer in a subject in need thereof.

[000119] In another aspect, the present disclosure provides use of compounds, or pharmaceutically acceptable salts thereof, as described herein for manufacturing a medicament for treating cancer in a subject in need thereof.

[000120] In one aspect, the present disclosure provides a method of targeting a drug to a tumor or cancer cell in a subject, the method comprising administering to the subject a compound as described herein, or a pharmaceutically acceptable salt thereof, or composition comprising a compound as described herein. The targeting may result in a targeted delivery of the present compounds to a cancer cell and/or a targeted therapy for a cancer.

[000121] In some embodiments, the compound, or the pharmaceutically acceptable salt thereof, localizes or travels to the cytoplasm or organelle of the tumor or cancer cell.

[000122] In some embodiments, the compound, or the pharmaceutically acceptable salt thereof, is selective for cancer cells in the subject.

[000123] In some embodiments, the compound, or the pharmaceutically acceptable salt thereof, is incorporated into at least about 2-fold more tumor or cancer cells than healthy cells. The compound, or the pharmaceutically acceptable salt thereof, may be incorporated into at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold more tumor or cancer cells than healthy cells.

[000124] The cancers that may be treated with the compounds as described herein, or a pharmaceutically acceptable salt thereof, or composition comprising a compound as described herein include, but are not limited to: breast cancer including male breast cancer; digestive/gastrointestinal cancers including anal cancer, appendix cancer, extrahepatic bile duct cancer, gastrointestinal carcinoid tumor, colon cancer, esophageal cancer, gallbladder cancer, gastric cancer, gastrointestinal stromal tumors ("gist"), Islet cell tumors, adult primary

liver cancer, childhood liver cancer, pancreatic cancer, rectal cancer, small intestine cancer, and stomach (gastric) cancer; endocrine and neuroendocrine cancers including pancreatic adenocarcinoma, adrenocortical carcinoma, pancreatic neuroendocrine tumors, Merkel cell carcinoma, non-small cell lung neuroendocrine tumor, small cell lung neuroendocrine tumor, parathyroid cancer, pheochromocytoma, pituitary tumor and thyroid cancer; eye cancers including intraocular melanoma and retinoblastoma; genitourinary cancer including bladder cancer, kidney (renal cell) cancer, penile cancer, prostate cancer, transitional cell renal pelvis and ureter cancer, testicular cancer, urethral cancer and Wilms tumor; germ cell cancers including childhood central nervous system cancer, childhood extracranial germ cell tumor, extragonadal germ cell tumor, ovarian germ cell tumor and testicular cancer; gynecologic cancers including cervical cancer, endometrial cancer, gestational trophoblastic tumor, ovarian epithelial cancer, ovarian germ cell tumor, uterine sarcoma, vaginal cancer and vulvar cancer; head and neck cancers including hypopharyngeal cancer, laryngeal cancer, lip and oral cavity cancer, metastatic squamous neck cancer with occult primary, mouth cancer, nasopharyngeal cancer, oropharyngeal cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, pharyngeal cancer, salivary gland cancer and throat cancer; leukemias including adult acute lymphoblastic leukemia, childhood acute lymphoblastic leukemia, adult acute myeloid leukemia, childhood acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia and hairy cell leukemia; lymphomas including AIDS-related lymphoma, cutaneous t-cell lymphoma, adult Hodgkin lymphoma, childhood Hodgkin lymphoma, Hodgkin lymphoma during pregnancy, mycosis fungoides, adult non-Hodgkin lymphoma, childhood non-Hodgkin lymphoma, non-Hodgkin lymphoma during pregnancy, primary central nervous system lymphoma, Sézary syndrome and Waldenström macroglobulinemia; musculoskeletal cancers including Ewing sarcoma, osteosarcoma and malignant fibrous histiocytoma of bone, childhood rhabdomyosarcoma and soft-tissue sarcoma; neurological cancers including adult brain tumor, childhood brain tumor, astrocytomas, brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, craniopharyngioma, ependymoma, neuroblastoma, primary central nervous system (CNS) lymphoma; respiratory/thoracic cancers including non-small cell lung cancer, small cell lung cancer, malignant mesothelioma, thymoma and thymic carcinoma; and skin cancers including Kaposi sarcoma, melanoma and squamous cell carcinoma. In particular embodiments, the cancer may be melanoma, lung cancer, colorectal cancer, breast cancer, or a combination thereof.

[000125] In some embodiments, the cancer is melanoma, brain cancer, lung cancer, adrenal cancer, liver cancer, renal or kidney cancer, pancreatic cancer, esophageal cancer, gastric cancer, stomach cancer, colon cancer, colorectal cancer, anal cancer, prostate

cancer, ovarian cancer, breast cancer, cervical cancer, lymphoma, leukemia, myeloma, hematologic cancer, hepatocarcinoma, retinoblastoma, glioma, sarcoma, blastoma, squamous cell carcinoma, adenocarcinoma, or a combination thereof. In some embodiments, the cancer is melanoma, lung cancer, colorectal cancer, breast cancer, or a combination thereof

[000126] In some embodiments, the cancer may comprise one or more circulating tumor cells. For example, the one or more circulating tumor cells may be selected from the group consisting of a breast cancer, a lung cancer, a thyroid cancer, a cervical cancer, a melanoma, a squamous cell carcinoma, a prostate cancer, a pancreas cancer, a colorectal cancer, and a cancer stem cell, and a malignant plasma cell.

[000127] In some embodiments, the cancer may be metastatic. For example, the metastatic cancer may be selected from the group consisting of a breast cancer, a lung cancer, a melanoma, and a colorectal cancer.

[000128] In some embodiments, the cancer may comprise a cancer stem cell. For example, the cancer stem cell may be selected from the group consisting of a breast cancer, a lung cancer, a melanoma, and a colorectal cancer.

[000129] In some embodiments, the lung cancer may comprise small cell lung cancer, non-small cell lung cancer, or a combination thereof.

[000130] In some embodiments, the melanoma may comprise superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma, amelanotic melanoma, nevoid melanoma, spitzoid melanoma, desmoplastic melanoma, or a combination thereof.

[000131] In some embodiments, the colorectal cancer may comprise adenocarcinoma.

[000132] In some embodiments, the breast cancer may comprise invasive breast ductal carcinoma, metastatic breast cancer, inflammatory breast cancer, triple negative breast cancer, ductal carcinoma in situ, or a combination thereof. In some embodiments, the cancer is breast cancer, the subject may be estrogen receptor positive, both estrogen receptor negative and progesterone receptor negative, expresses HER2 (HER2+), does not express HER2 (HER2-), or a combination thereof.

[000133] In some embodiments, the subject is a human, such as an adult and an infant. In some embodiments, the subject is an animal, such as a mammal.

[000134] The methods may include administering the present compounds, or pharmaceutically acceptable salts thereof, in amounts as described herein. In some embodiments, the methods include administering about 0.0001 to about 1000 mg/kg of a compound as described herein, or a pharmaceutically acceptable salt thereof. Useful dosages of the compound(s) can be determined by comparing their *in vitro* activity and *in vivo* activity in animal models thereof. Methods for the extrapolation of effective dosages in rodents, pigs, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

[000135] In some embodiments, the compounds, or pharmaceutically acceptable salts thereof, or pharmaceutical compositions as disclosed herein may be administered by oral administration or intravenous administration. In general, however, a suitable dose will often be in the range of from about 0.0001 mg/kg to about 1000 mg/kg, such as from about 0.001 mg/kg to about 10.0 mg/kg. For example, a suitable dose may be in the range from about 0.001 mg/kg to about 5.0 mg/kg of body weight per day, such as about 0.01 mg/kg to about 1.0 mg/kg of body weight of the recipient per day, about 0.01 mg/kg to about 3.0 mg/kg of body weight of the recipient per day, about 0.1 mg/kg to about 5.0 mg/kg of body weight of the recipient per day, about 0.2 mg/kg to 4.0 mg/kg of body weight of the recipient per day. The compound may be administered in unit dosage form; for example, containing 1 to 100 mg, 10 to 100 mg, or 5 to 50 mg of active ingredient per unit dosage form.

[000136] The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations.

[000137] Suitable *in vivo* dosages to be administered and the particular mode of administration may vary depending upon the age, weight, the severity of the affliction, and mammalian species treated, the particular compounds employed, and the specific use for which these compounds are employed. The determination of effective dosage levels to achieve the desired result may be accomplished by known methods, for example, human clinical trials, *in vivo* studies and *in vitro* studies. For example, the effective dosages of compounds disclosed herein, or pharmaceutically acceptable salts thereof, may be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Such comparison may be done by comparison against an established drug.

[000138] Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the modulating effects, or minimal

effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vivo* and/or *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, FIPLC assays or bioassays can be used to determine plasma concentrations. Dosage intervals can also be determined using MEC value. Compositions should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

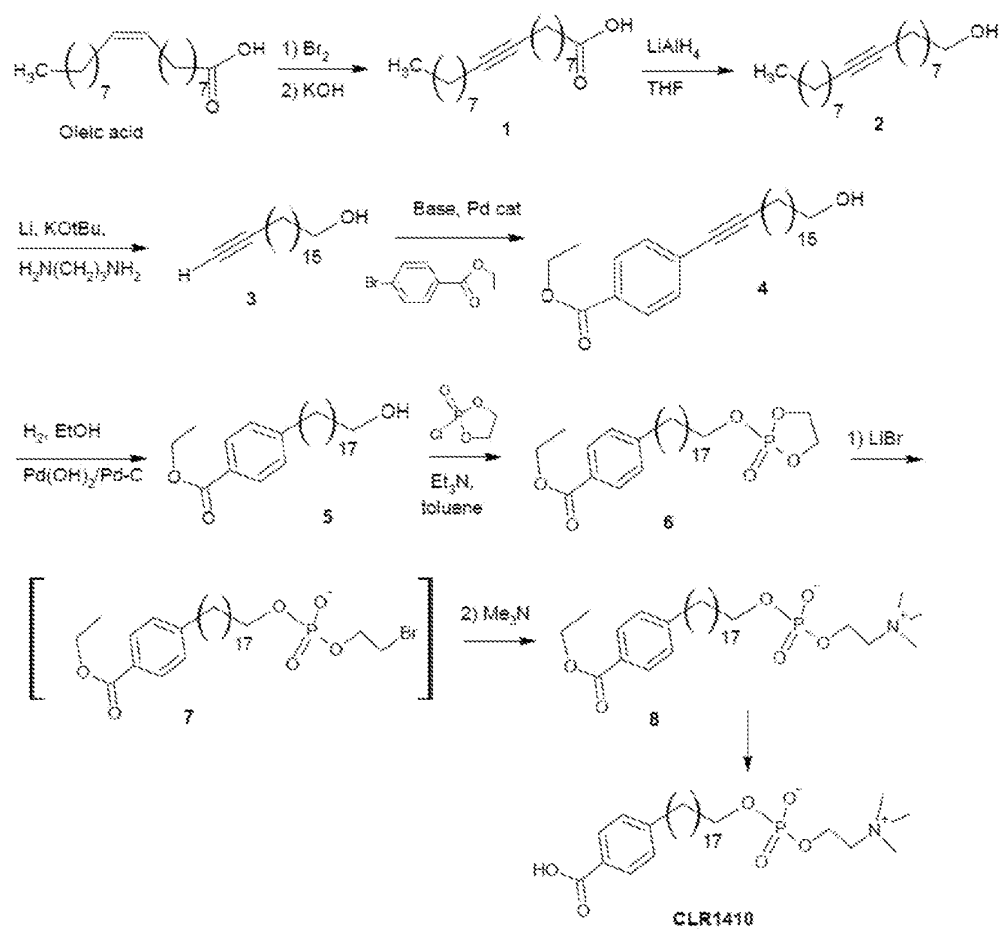
[000139] The compositions described herein may be administered with additional compositions to prolong stability, delivery, and/or activity of the compositions, or combined with additional therapeutic agents, or provided before or after the administration of additional therapeutic agents. Combination therapy includes administration of a single pharmaceutical dosage formulation containing one or more of the compounds described herein and one or more additional pharmaceutical agents, as well as administration of the compounds and each additional pharmaceutical agent, in its own separate pharmaceutical dosage formulation. For example, the compounds as described herein may be administered to a subject with an additional anti-cancer drug as described herein.

7. Examples

Example 1

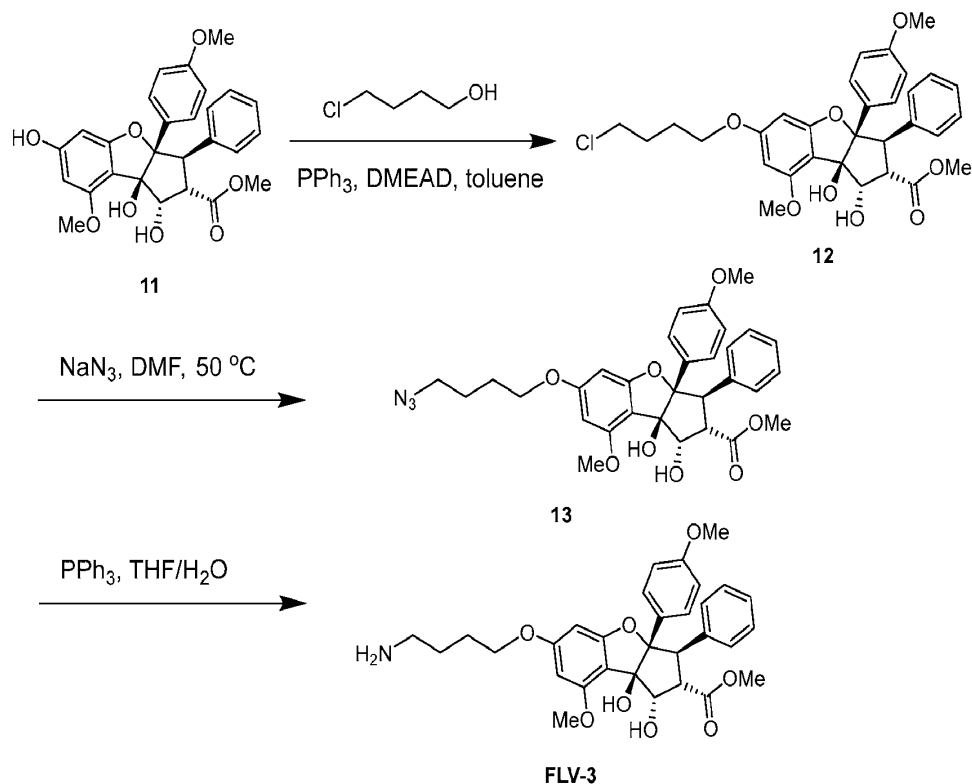
Chemical Synthesis

[000140] *PLEs*. The PLEs were synthetically made according to Scheme 1 below:



Scheme 1

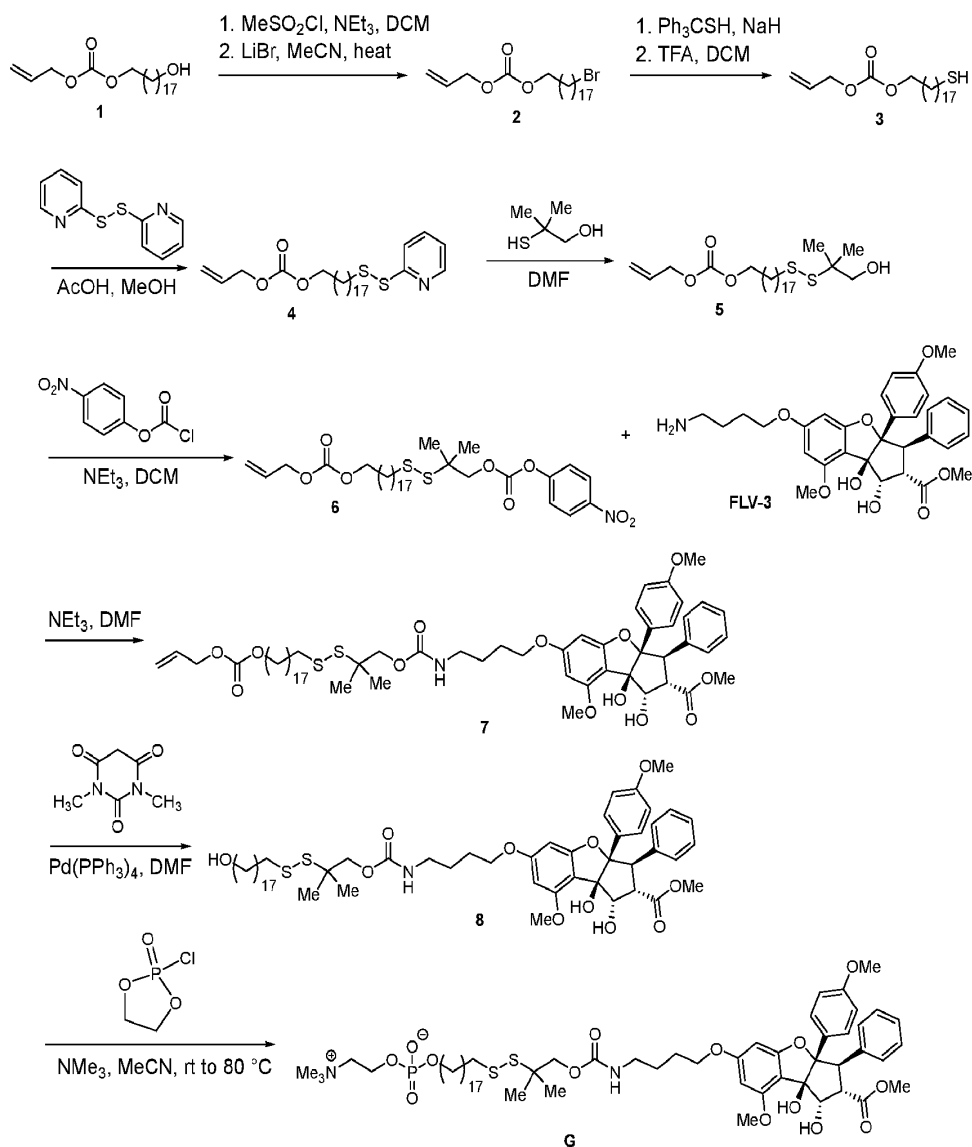
[000141] **Flavaglines.** Flavaglines were commercially available. FLV1 and FLV3 were purchased from Haoyuan Chemexpress Co. (Shanghai, China). FLV1 and FLV3 were alternatively synthetically made according to Scheme 2 below:



Entry	SM	Scale	Product	Conditions	Yield	Comments
1	13	0.317 g	FLV3	50°C , 10 h	0.235 g (78%)	^1H NMR consistent.
2	11	0.170 g	12	0°C to rt, O/N	--	Reaction in progress.

Scheme 2

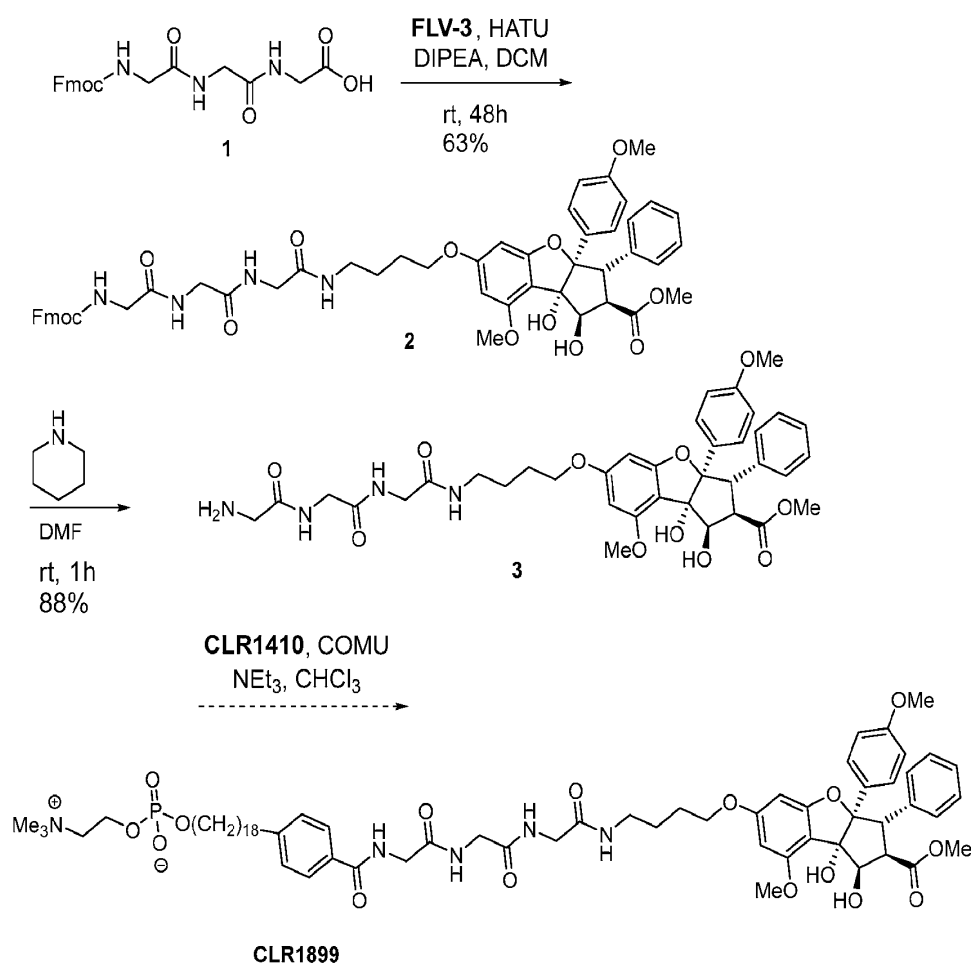
[000142] **PLE-flavagline constructs.** CLR 1852 (compound (9)) and CLR 1865 (compound (8)) were synthetically made according to Scheme 3 below:



Scheme 3

[000143] **Synthesis of CLR 1899.** CLR 1899 was synthesized according to Scheme 4.

Structures were verified by NMR and MS data.



Scheme 4

Example 2

Presence of Lipid Rafts on Tumor Cells

[000144] Over 100 cell lines were stained with cholera toxin subunit B, fixed with 4% formaldehyde, and stained with filipin III for 30 minutes. As shown in **FIG. 1A-FIG. 1D**, almost every tumor type that was tested demonstrated high lipid raft concentration in the cell membrane (over 100 cell lines, fresh patient samples, etc.). As shown in **FIG. 1E**, A549 cells were co-cultured with normal fibroblast cells for 48 hours and then stained with cholera toxin subunit B, fixed with 4% formaldehyde, and stained with filipin III for 30 minutes. These results demonstrated that tumor cells possess a higher concentration of lipid rafts than normal cells.

Example 3

Selective Uptake of PDCs into Tumor Cells

[000145] Normal fibroblast and Caki-2 tumor cells (human clear cell renal cell carcinoma) were plated and co-cultured overnight (**FIG. 1F**). Cells were then incubated with 5 μ M of CLR 1501 (compound (1)) for 24 hours at 37°C in complete media. The next day cells were washed and co-stained with nucleus stain (Hoescht 33342). CLR 1501 was excited and then detected with an Alexa-Fluor 488 filter. CLR 1501 was highly localized in the Caki-2 cells and minimally in the normal fibroblast cells.

Example 4

Disruption of Lipid Rafts Reduced Uptake of PDCs

[000146] A549 cells were plated overnight into separate wells. The following day cells were either not treated (**FIG. 1G**) or treated with methyl β -cyclodextrin (**FIG. 1H**) which has been shown to selectively disrupt lipid rafts. All cells were then incubated for 24 hours with 5 μ M of CLR 1501 (compound (1)). Disruption of the majority of lipid rafts in A549 cells resulted in 60% reduction in uptake of CLR 1501 (**FIG. 1H**) as compared to untreated cells (**FIG. 1G**).

Example 5

PDCs Track to Endoplasmic Reticulum

[000147] Human prostate adenocarcinoma cells (PC3) were plated overnight on the microplate VI (Ibidi, Verona, WI) and then incubated with 5 μ M of CLR 1501 (compound (1)) for 24 hours at 37°C in complete media. After washing, the cells were co-stained with ER-tracker® per protocol and imaged using Nikon A1R confocal light microscope. CLR 1501 and ER were excited and detected using Alexa-Fluor 488 using standard fluorescein filters. CLR 1501 co-localized with ER in malignant (**FIG. 1I-FIG. 1K**) but not with normal cells (not shown).

Example 6

PDCs Track to Endoplasmic Reticulum

[000148] PC3 (grade IV, human prostate adenocarcinoma) cell lines were cultured overnight on micro slide VI (Ibidi, Verona, WI). The next day, the cells were incubated with 5 μ M of CLR 1501 (compound (1)) for 24 hours at 37°C in complete media. The next day after

washing with PBS, the cells were co-stained with nucleus stain (Hoechst 33342) and mitochondria marker (Mitotracker®) (Invitrogen, Carlsbad, CA). The cells were observed using Nikon A1R confocal microscope. CLR 1501 was excited and detected using Alexa-Fluor 488 filter, while nucleus stain and mitochondria stain were excited and detected using DAPI filter and Texas-Red filter, respectively. CLR 1501 was co-localized with mitochondria (FIG. 1L-FIG. 1N).

Example 7

PDCs Provide Targeted Delivery *In vivo*

[000149] Colorectal (HCT-116) tumor bearing nude mouse was injected with 1 mg of CLR 1502 (compound (2)) and imaged on Pearl Infrared Imaging System. Different color reflects the intensity of CLR 1502 over time. Approximately 5.5 hours post injection, the tumor still showed red color (reflecting the highest distribution of CLR 1502)(FIG. 2). Within 24 hours, the maximum distribution of CLR 1502 was obtained. Initial targeting noted within 30 minutes (not shown).

Example 8

Cytotoxic PDCs Provide Targeting and Potentially Improved Therapeutic Index

[000150] A549 (human lung adenocarcinoma) cells and normal human dermal fibroblasts (NHDF) were plated in 96 well dishes overnight. All cells were treated with increasing concentrations of either the parent cytotoxic compound alone (FLV1 or FLV3) or the PDC (parent cytotoxic compound conjugated to PLE moiety with cleavable linker, CLR 1865 (compound (8)) or CLR 1852 (compound (9))). Parent cytotoxic compound showed near equal potency to the A549 cells as it did to the NHDF cells. However, the PDC molecules showed selectivity for the A549 cells (FIG. 3). The PDC molecules showed almost no effect on NHDF cells until the highest concentrations and near similar potency to the parent molecule in A549 cells. The difference between the cytotoxicity of the PDC molecule for tumor cells and that of the normal cells may have indicated a potential to improve the parent molecules therapeutic index.

Example 9

Cytotoxic PDCs Provide Targeting

[000151] The uptake of CLR 1852 (compound (9)) in A375 (human melanoma) and HEK293 (human embryonic kidney) cells was evaluated. The cells were incubated with CLR

1852 (compound (9)) for 24 hours. It was shown that the tumor cells possessed anywhere from a 6 to 28 fold increase in PDCs compared to the normal cells within 24 hours of treatment (FIG. 4).

[000152] Results from Examples 2-9 indicated that phospholipid ether molecules target tumor cells via lipid rafts. PDCs showed significant uptake into tumor cells versus normal cells even in co-culture. Upon entering the tumor cells, PDCs tracked to the mitochondria and endoplasmic reticulum. *In vivo*, the PDCs both targeted and rapidly accumulated within the tumor. Cytotoxic PDCs provided improved targeting and the potential for improved safety.

Example 10

Cellular Uptake of PLE

[000153] Various cancer cell lines were exposed *in vitro* and *in vivo* to a fluorescently labelled PLE (CLR 1501, compound (1)). Tumor cell uptake was measured continuously for 24 hours. Results are shown in TABLE 1 and FIG. 5, FIG. 6, and FIG. 7. The PLE compound was specific for tumors and cancer cells.

[000154] Fluorescently labelled PLE (CLR 1501, compound (1)) was administered to benign tissues, and no uptake was observed (TABLE 2).

TABLE 1. *In vitro* cellular uptake of PLE.

Tumor Model		Species	Category	Tumor Uptake
1	Prostate PC-3	SCID Mouse	Adenocarcinoma	Yes
2	Lung A-549 (NSCLC)	SCID Mouse	Adenocarcinoma	Yes
3	Lung NCI H-69 (Oat Cell)	SCID Mouse	Adenocarcinoma	Yes
4	Adrenal H-295	SCID Mouse	Adenocarcinoma	Yes
5	Adrenal RL-251	SCID Mouse	Adenocarcinoma	Yes
6	Colon-51	SCID Mouse	Colorectal adenocarcinoma	Yes
7	ColonLS180	SCID Mouse	Colorectal adenocarcinoma	Yes
8	Colon DLD1	SCID Mouse	Colorectal adenocarcinoma	Yes
9	Colon HT-29	SCID Mouse	Colorectal adenocarcinoma	Yes
10	Colon LS-180	Nude Mouse	Adenocarcinoma	Yes
11	Melanoma A-375	Nude Mouse	Adenocarcinoma	Yes
12	Ovarian HTB-77	Nude Mouse	Adenocarcinoma	Yes
13	Pancreatic BXP3	Nude Mouse	Adenocarcinoma	Yes
14	Pancreatic Capan-1	Nude Mouse	Adenocarcinoma	Yes
15	Renal Cell Caki-2	Nude Mouse (orthotopic)	Adenocarcinoma	Yes

16	Renal Cell ACHN	Nude Mouse (orthotopic)	Adenocarcinoma	Yes
17	SCC1	Nude Mouse	Squamous cell carcinoma	Yes
18	SCC6	Nude Mouse	Adenocarcinoma	Yes
19	Prostate LnCap	Mouse	Adenocarcinoma	Yes
20	Prostate LuCap	Mouse	Adenocarcinoma	Yes
21	Breast MCF-7	Rat	Adenocarcinoma	Yes
22	Breast 4T1	Endogenous Mouse (orthotopic)	Adenocarcinoma	Yes
23	Prostate MatLyLu	Rat	Adenocarcinoma	Yes
24	Walker-256	Rat	Carcinosarcoma	Yes
25	TRAMP prostate	Endogenous Mouse	Adenocarcinoma	Yes
26	Colon CT-26	SCID Mouse	Adenocarcinoma	Yes
27	Min Mouse Intestinal	Endogenous Mouse	Adenocarcinoma	Yes
28	Melanoma	Mouse	Adenocarcinoma	Yes
29	Mammary SCC	Apc ^{Min/+} mouse	Squamous cell carcinoma	Yes
30	Mammary AC	Apc ^{Min/+} mouse	Adenocarcinoma	Yes
31	Hepatocellular Carcinoma	Endogenous Mouse	Adenocarcinoma	Yes
32	Glioma L9	Rat xenograft	Glioma	Yes
33	Glioma C6	Rat xenograft	Glioma	Yes
34	Glioma CNS1	Rat xenograft	Glioma	Yes
35	Glioma RG2	Rat xenograft	Glioma	Yes
36	Retinoblastoma	Endogenous Mouse	Blastoma	Yes
37	Pancreatic c-myc	Endogenous Mouse	Adenocarcinoma	Yes
38	Pancreatic Kras	Endogenous Mouse	Adenocarcinoma	Yes
39	Cervical	Endogenous Mouse	Adenocarcinoma	Yes
40	Sarcoma (Meth-A)	Nude Mouse	Fibrosarcoma	Yes
41	Esophageal	Endogenous Mouse	Adenocarcinoma	Yes

TABLE 2. CLR1404 uptake in benign tissues.

Tumor Model		Species	Category	Tumor Uptake
1	Intestinal Polyp	Endogenous Mouse	Adenoma (benign)	No
2	Mammary alveolar hyperplasia	Endogenous Mouse	Hyperplasia (benign)	No

Example 11

Activity of PLE-Flavagline Conjugates in Cancer Cells

[000155] The PLE-flavagline conjugates CLR 1852 (compound (9)) and CLR 1865 (compound (8)) were administered to cell lines A375 (human malignant melanoma), A549 (human lung adenocarcinoma), HCT 116 (human colon cancer), and NHDF (normal human dermal fibroblasts). The IC₅₀ was calculated. Results are shown in TABLE 3.

TABLE 3. IC₅₀ (μM/mL) in various cancer cell lines.

Compound	A375	A549	HCT 116	NHDF	Therapeutic Index (TI)
FLV3	0.015	0.09	0.009	0.008	0.89
CLR 1852	0.9	0.02	0.06	7.2 (18.3)	360 (915)
CLR 1865	9.75	0.67	1.28	7.8 (25)	11.64 (37.3)

[000156] The cytotoxicity of CLR 1852 was determined over various concentrations. As shown in FIG. 8, CLR 1852 showed modest loss in potency compared to FLV3 alone, perhaps due to incomplete release.

[000157] The stability of CLR 1865 (compound (8)), CLR 1852 (compound (9)), and FLV3 in plasma was examined, with propantheline as a control (TABLE 4). A small amount of molecule was exposed to plasma, and the plasma was then analyzed via HPLC or MS to determine if the molecule was degraded. CLR 1865 and CLR 1852 showed excellent human plasma stability. CLR 1852 outperformed CLR 1865 in mouse plasma. CLR 1865 was only stable in mouse plasma for 3.3 hours. CLR 1852 was stable in plasma for at least 7 hours.

TABLE 4. Plasma stability.

Compound	Human half-life (min)	Mouse half-life (min)
CLR 1852	>400	>400
CLR 1865	>400	199
propantheline	54	85

[000158] The therapeutic index (TI) of CLR 1865 and CLR 1852 was examined in mice (TABLE 5). Listed in Table 5 are the doses administered to the mice, and how many mice were alive after treatment ("3/3" indicates 3 out of 3 mice were alive). Both CLR 1852 and CLR 1865 showed excellent improvement in tolerability compared to FLV3 alone. CLR 1852 did not achieve a maximum tolerated dose (MTD), perhaps because its solubility limited greater dosing. MTD for CLR 1865 was between 5 and 10 mg/kg. The *in vivo* therapeutic index was at least 25 for CLR 1852 and 12.5 for CLR 1865.

TABLE 5. In vivo therapeutic index.

Dose (mg/kg)	FLV3	CLR 1852	CLR 1865
0.1	3/3	3/3	3/3
0.25	3/3	3/3	3/3
0.5	LD	3/3	3/3
1.0	n/a	3/3	3/3
5.0	n/a	3/3	3/3
10.0	n/a	3/3	2/3

[000159] The efficacy for CLR 1852 in HCT 116 cells (human colon cancer) was examined. CLR 1852 was administered to HCT 116 cells in three doses of 1 mg/mg each. Compared to vehicle, CLR 1852 reduced the tumor volume beginning at around 27 days (**FIG. 9**). Compared to vehicle, CLR 1582 caused modest weight loss, presumably due to toxicity (**FIG. 10**). CLR 1582 provided at least a six-fold increase in tolerability compare to FLV3 alone (data not shown).

[000160] CLR 1852 and FLV3 alone were administered to A375 and A549 cells. The levels of FLV3 in cell lysate and growth media were measured using LC/MS. As shown in **FIG. 11**, high levels of FLV3 were present in the cell lysate after 24 hours. Moderate levels of FLV3 were present in the growth media after 24 hours. Levels of intracellular FLV3 appeared to plateau at 24 hours, while extracellular levels of FLV3 continued to increase.

Example 12

Activity of CLR 1899 in Cancer Cells

[000161] CLR 1899 was administered to cell lines A375 (human malignant melanoma), A549 (human lung adenocarcinoma), HCT 116 (human colon cancer), and NHDF (normal human dermal fibroblasts). The in vitro cytotoxicity results are shown in **TABLE 6**.

TABLE 6.

CLR1899	A375	A549	HCT116	MCF7	NHDF
Conc. at half maximal activity(μ M)	0.205	0.656	0.545	1.051	0.186
Minimum % viability	0.671	7.101	13.331	30.253	41.728

[000162] To determine whether CLR 1899 is tolerated in vivo, the compound was administered intravenously to healthy C57BL/6 mice at dose levels of 0.5 mg/kg, 1.0 mg/kg, or 2.0 mg/kg, and body weight changes were observed (**Fig. 12**). CLR 1899 showed no toxicity or adverse events during repeat dosing as measured by changes in weight. The in vivo efficacy of CLR 1899 was studied in MCF-7 xenograft model (**FIG. 13**, 10 mice per dose group). Reduction in tumor volume was recorded. In these studies, CLR 1899 demonstrated equivalent activity as docetaxel in stopping or slowing tumor growth.

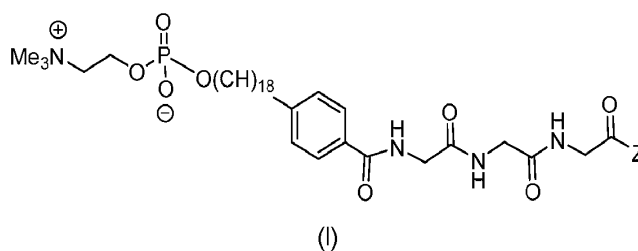
[000163] The foregoing description of the specific aspects will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific aspects, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed aspects, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[000164] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary aspects, but should be defined only in accordance with the following claims and their equivalents.

[000165] All publications, patents, patent applications, and/or other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, and/or other document were individually indicated to be incorporated by reference for all purposes.

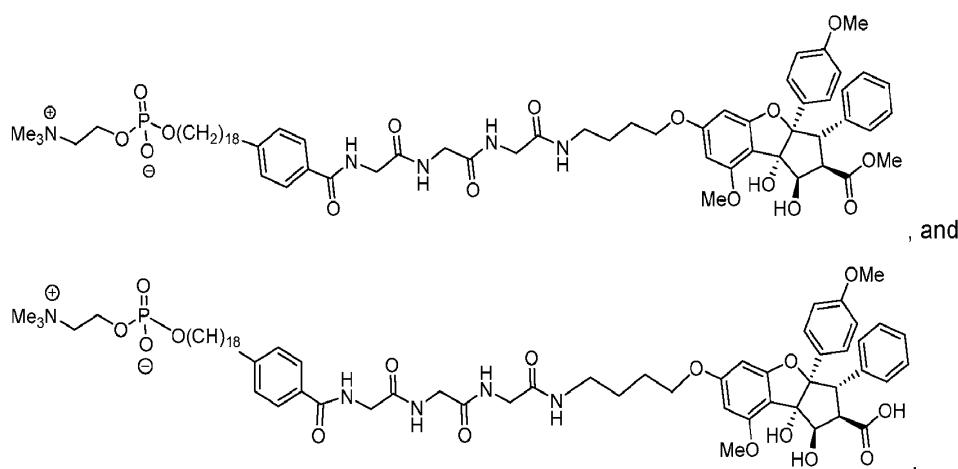
[000166] For reasons of completeness, various aspects of the disclosure are set out in the following numbered clauses:

[000167] Clause 1. A compound of formula (I), or a pharmaceutically acceptable salt thereof,



wherein Z is a flavagline analog.

[000168] Clause 2. The compound of clause 1, which is selected from the group consisting of



or a pharmaceutically acceptable salt thereof.

[000169] Clause 3. A pharmaceutical composition comprising a compound of any one of clauses 1-2, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[000170] Clause 4. A method of treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of a compound of any one of clauses 1-2, or a pharmaceutically acceptable salt thereof.

[000171] Clause 5. A method of targeting a drug to a tumor or cancer cell in a subject, the method comprising administering to the subject a compound of any one of clauses 1-2, or a pharmaceutically acceptable salt thereof.

[000172] Clause 6. The method of any one of clauses 4-5, wherein the compound, or the pharmaceutically acceptable salt thereof, localizes or travels to the cytoplasm or organelle of the tumor or cancer cell.

[000173] Clause 7. The method of any one of clauses 4-6, wherein the compound, or the pharmaceutically acceptable salt thereof, is selective for cancer cells in the subject.

[000174] Clause 8. The method of any one of clauses 4-7, wherein the compound, or the pharmaceutically acceptable salt thereof, is incorporated into at least about 2-fold more tumor or cancer cells than healthy cells.

[000175] Clause 9. The method of any one of clauses 4-8, wherein the cancer is melanoma, brain cancer, lung cancer, adrenal cancer, liver cancer, renal or kidney cancer, pancreatic cancer, esophageal cancer, gastric cancer, stomach cancer, colon cancer, colorectal cancer, anal cancer, prostate cancer, ovarian cancer, breast cancer, cervical cancer, lymphoma, leukemia, myeloma, hematologic cancer, hepatocarcinoma, retinoblastoma, glioma, sarcoma, blastoma, squamous cell carcinoma, adenocarcinoma, or a combination thereof.

[000176] Clause 10. The method of any one of clauses 4-9, wherein the cancer is melanoma, lung cancer, colorectal cancer, breast cancer, or a combination thereof.

[000177] Clause 11. The method of any one of clauses 4-10, wherein
the lung cancer comprises small cell lung cancer, non-small cell lung cancer, or a combination thereof;
the melanoma comprises superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma, amelanotic melanoma, nevoid melanoma, spitzoid melanoma, desmoplastic melanoma, or a combination thereof;
the colorectal cancer comprises adenocarcinoma; or
the breast cancer comprises invasive breast ductal carcinoma, metastatic breast cancer, inflammatory breast cancer, triple negative breast cancer, ductal carcinoma in situ, or a combination thereof.

[000178] Clause 12. The method of any one of clauses 4-11, wherein the cancer comprises cancer stem cells.

[000179] Clause 13. The method of any one of clauses 4-12, wherein the cancer comprises metastatic cancer cells

[000180] Clause 14. The method of any one of clauses 4-13, wherein the cancer comprises circulating tumor cells.

[000181] Clause 15. The method of any one of clauses 4-14, wherein the subject is human.