

MITOCHONDRIA SPECIFIC TRANSCRIPTION INHIBITORS

FIELD

[0001] The present disclosure relates to mitochondria specific transcription inhibitors, or MSTI compounds, that inhibit the propagation of cancer stem cells (CSCs) and senescent cells through selectively inhibiting CSC mitochondrial transcription.

BACKGROUND

[0002] The biological process of aging continues to receive significant attention in the scientific and medical research communities. Physiologic aging relates, at least in part, to an increase in the rate of oxidative damage to cellular components, including DNA, lipids, proteins, and the like. The increased oxidative damage creates an imbalance that disrupts self-regulating processes at the cellular level. Further, aging correlates to an accumulation of lipofuscin in neuron cytoplasm. Modern research also indicates that aging is a consequence of naturally occurring DNA damage, resulting in abnormal DNA alterations, accumulating over time. Both mitochondrial and nuclear DNA damage can contribute to aging, indirectly through increasing apoptosis and cellular senescence, and directly by increasing cell dysfunction. Accumulated DNA damage can lead to loss of cells and, in surviving cells, loss of gene expression and mutation – effects that, in infrequently dividing cells, produce indicia of aging. Cellular senescence results when aged cells cease cellular division, believed to occur following various environmental damaging events, abnormal cell growth, autophagy, and oxidative stress, among other factors. Senescence Associated Secretory Phenotype (“SASP”) is a characteristic of senescent cells, and lead to a proteotoxic impairment of healthy cell function, including inflammatory or anti-inflammatory and tumor or anti-tumor effects, depending on a host of factors. The impact of SASP-related chronic inflammation impacts the immune system’s normal ability to remove senescent cells, and cells providing an immune function can be conscripted by SASP into senescent cells. Biomarkers of cellular senescence have been found to accumulate as mammals age, and contribute to a wide range of age-related diseases, including Alzheimer’s, lateral sclerosis, and type 2 diabetes. And with respect to frequently dividing cells, accumulated DNA damage can become a prominent cause of cancer.

[0003] Aging thus increases the likelihood of developing cancer, and researchers have struggled to develop new anti-cancer and anti-aging or senolytic treatments. Conventional cancer therapies (e.g. irradiation, alkylating agents such as cyclophosphamide, and anti-metabolites such as 5-Fluorouracil) have attempted to selectively detect and eradicate fast-growing cancer cells by interfering with cellular mechanisms involved in cell growth and DNA

replication. Other cancer therapies have used immunotherapies that selectively bind mutant tumor antigens on fast-growing cancer cells (e.g., monoclonal antibodies). Unfortunately, tumors often recur following these therapies at the same or different site(s), indicating that not all cancer cells have been eradicated. Cancer stem cells, in particular, survive for various reasons, and lead to treatment failure. Relapse may be due to insufficient chemotherapeutic dosage and/or emergence of cancer clones resistant to therapy. Hence, novel cancer treatment strategies are needed to overcome the deficiencies of conventional therapies.

[0004] Advances in mutational analysis have allowed in-depth study of the genetic mutations that occur during cancer development. Despite having knowledge of the genomic landscape, modern oncology has had difficulty with identifying primary driver mutations across cancer subtypes. The harsh reality appears to be that each patient's tumor is unique, and a single tumor may contain multiple divergent clone cells. What is needed, then, is a new approach that emphasizes commonalities between different cancer types. Targeting the metabolic differences between tumor and normal cells holds promise as a novel cancer treatment strategy. An analysis of transcriptional profiling data from human breast cancer samples revealed more than 95 elevated mRNA transcripts associated with mitochondrial biogenesis and/or mitochondrial translation. Sotgia et al., *Cell Cycle*, 11(23):4390-4401 (2012). Additionally, more than 35 of the 95 upregulated mRNAs encode mitochondrial ribosomal proteins (MRPs). Proteomic analysis of human breast cancer stem cells likewise revealed the significant overexpression of several mitoribosomal proteins as well as other proteins associated with mitochondrial biogenesis. Lamb et al., *Oncotarget*, 5(22):11029-11037 (2014).

[0005] Cancer cell mitochondrial metabolism has been the target of recent explorative research, with respect to both searching for anti-cancer therapeutic targets and senolytic therapeutic targets. Mitochondria are extremely dynamic organelles in constant division, elongation and connection to each other to form tubular networks or fragmented granules in order to satisfy the requirements of the cell and adapt to the cellular microenvironment. The balance of mitochondrial fusion and fission dictates the morphology, abundance, function and spatial distribution of mitochondria, therefore influencing a plethora of mitochondrial-dependent vital biological processes such as adenosine triphosphate (ATP) production, mitophagy, apoptosis, and calcium homeostasis. In turn, mitochondrial dynamics can be regulated by mitochondrial metabolism, respiration and oxidative stress.

[0006] ATP is the universal bioenergetic "currency" of all living cells and tissues, including microorganisms, such as prokaryotic bacteria and eukaryotic yeast. In eukaryotes,

mitochondrial organelles function as the “powerhouse” of the cell. Mitochondria generate the vast amount of ATP via the TCA cycle and oxidative phosphorylation (OXPHOS), while glycolysis contributes a minor amount of ATP. Conversely, mitochondrial dysfunction induces ATP-depletion, resulting in mitochondrial-driven apoptosis (programmed cell death) and/or necrosis. Thus, we have proposed that ATP-depletion therapy may be a viable strategy for targeting and eradicating even the “fittest” cancer cells.

[0007] In MCF7 breast cancer cells, mitochondrial-driven OXPHOS contributes to 80-90% of ATP production, while glycolysis only contributes the remaining 10-20%, under normoxic conditions. Therefore, like normal cells, cancer cells are highly dependent on mitochondrial ATP production. However, it still remains largely unknown if ATP levels in cancer cells contribute to undergo 3D anchorage-independent growth and cell migration, two characteristic features of metastatic spread.

[0008] What is needed, then, are novel anti-aging compositions and methods that treat aging at the cellular level, overcoming accumulated oxidative and DNA damage and the numerous undesired effects of aging.

[0009] Further, what is needed are therapeutic agents that target unhealthy senescent cells and SASP, reducing the accumulation of cellular senescence and offsetting chronic senescence.

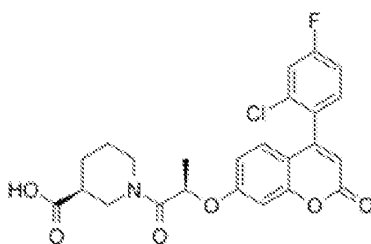
[0010] What is further needed are therapeutic agents that target a broad range of CSCs, through a feature common to CSCs regardless of cancer type, with minimal or negligible impact on normal, healthy cells.

[0011] What is further needed are therapeutic agents that inhibit the propagation of CSCs, including the circulating tumor cells and tumor-initiating cells that have the potential to cause tumor recurrence and/or metastasis.

SUMMARY

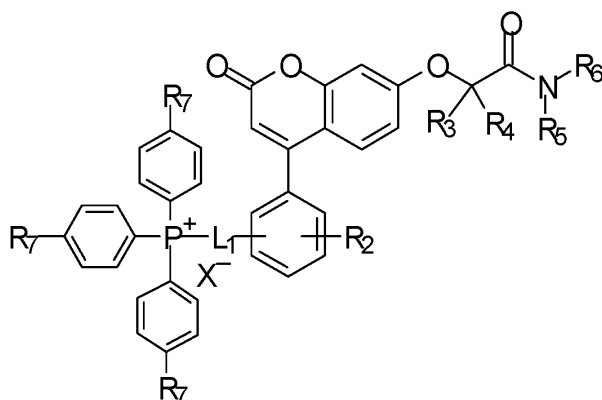
[0012] In view of the foregoing background, it is an object of this disclosure to describe therapeutic agents, or compounds, that may be used to reduce the accumulation of cellular senescence, and inhibit CSC propagation. It is an object of this disclosure to describe therapeutic agents for use in eradicating CSCs and senescent cells. It is an object of this disclosure to describe therapeutic agents for use in preventing and reducing the likelihood of tumor recurrence and metastasis. It is further an object of this disclosure to describe compositions, such as pharmaceutical compositions, and methods for treating and preventing cancer, including tumor recurrence and/or metastasis. It is also an object of this disclosure to describe compositions, such as pharmaceutical compositions, and methods for senolytic therapeutic agents.

[0013] Described herein are mitochondria specific transcription inhibitors (“MSTI”), compounds that may be used as therapeutic agents having anti-cancer activity, pharmaceutical compositions containing such therapeutic agents, methods for synthesizing such compounds, and methods for treating cancer. Embodiments of the MSTI therapeutic agents described herein may be compared to the formula shown below, to demonstrate the unexpectedly advantageous benefits of the present approach:



a compound known to be an orally active, mitochondrial RNA polymerase (POLRMT) inhibitor. The compound was first described in Bonekamp, N.A., Peter, B., Hillen, H.S. et al. Small-molecule inhibitors of human mitochondrial DNA transcription. *Nature* 588, 712–716 (2020), herein incorporated by reference in its entirety. As a POLRMT inhibitor, the compound inhibits mitochondrial DNA expression. The IUPAC name for this compound is 3-piperidinecarboxylic acid, 1-[(2R)-2-[[4-(2-chloro-4-fluorophenyl)-2-oxo-2H-1-benzopyran-7-yl]oxy]-1-oxopropyl]-, (3S)-, and has been assigned CAS Registry No. 2304621-06-3.

[0014] Under the present approach, some embodiments of the MSTI compounds may have the generic chemical structure of Formula [I], shown below:

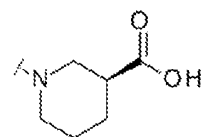
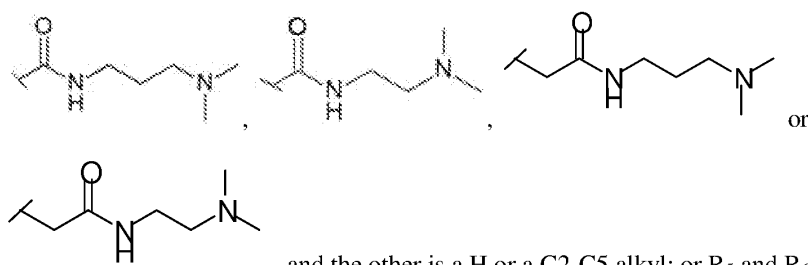


, or pharmaceutically acceptable

salts thereof, in which:

- R₂ is selected from a halogen, CF₂H, -CF₃, -OCF₂H, -OCF₃, substituted or unsubstituted C5-C18 carboxyl, substituted or unsubstituted C5-C18 alkane, substituted or unsubstituted C5-C18 alkene, substituted or unsubstituted C5-C18 cyclic alkene, substituted or unsubstituted C5-C18 alkyne, substituted or unsubstituted C5-C18 ketone, substituted or unsubstituted C5-C18 aldehyde, substituted or unsubstituted C5-C18 ether, substituted or unsubstituted C5-C18 ester, substituted or unsubstituted C5-C18 amine, substituted or unsubstituted C5-C18 amide, substituted or unsubstituted C5-C18 alkyl-amide, monocyclic or polycyclic arene, heteroarene, phenol, or benzoic acid;
- R₃ and R₄ may be the same or different, and are selected from hydrogen, substituted or unsubstituted C1-C10 alkyl, substituted or unsubstituted C3-C8-cycloalkyl, substituted or unsubstituted pyridine, substituted or unsubstituted C2-C10 carboxyl, substituted or unsubstituted C2-C10 alkene, substituted or unsubstituted C2-C10 alkyne, substituted or unsubstituted C2-C10 ketone, substituted or unsubstituted C2-C10 aldehyde, substituted or unsubstituted C2-C10 ether, substituted or unsubstituted C2-C10 ester, substituted or unsubstituted C2-C10 amine, substituted or unsubstituted C2-C10 amide, substituted or unsubstituted C2-C10 alkyl-amide, substituted or unsubstituted phenol, or benzoic acid;
- R₅ and R₆ may be the same or different, and are selected from hydrogen, substituted or unsubstituted C2-C18-alkyl, substituted or unsubstituted C3-C8-cycloalkyl, substituted or unsubstituted pyridine, substituted or unsubstituted C2-C18 carboxyl, substituted or unsubstituted C2-C18 alkene, substituted or unsubstituted C2-C18 alkyne, substituted or unsubstituted C2-C18 ketone, substituted or unsubstituted C2-

C18 aldehyde, substituted or unsubstituted C2-C18 ether, substituted or unsubstituted C2-C18 ester, substituted or unsubstituted C2-C18 amine, substituted or unsubstituted C2-C18 amide, substituted or unsubstituted C2-C18 alkyl-amide, substituted or unsubstituted phenol, or benzoic acid, or one of R₃ and R₄ is the group



; or NR₃R₄ is substituted with OH;

- L₁ is independently selected from: -OR_{1a}⁻, -SR_{1a}⁻, -NR_{1a}⁻, -NR_{1a}C(O)R_{1a}⁻, -OC(O)R_{1a}⁻, -C(O)OR_{1a}⁻, -SO₂R_{1a}, -S(O)R_{1a}⁻, -NR_{1a}C(O)-, -C(O)NR_{1a}⁻, -NR_{1a}S(O)₂⁻, S(O)₂NR_{1a}⁻, -OC(O)NR_{1a}⁻, -NR_{1a}C(O)OR_{1a}⁻, -NR_{1a}C(O)NR_{1a}⁻, -CR_{1a}=CR_{1a}⁻ and -CC-, -CH₂R_{1a}⁻;
 - R_{1a} is independently selected from: H, alkyl, alkenyl, alkynyl, cycloalkyl heterocycloalkyl, heteroaryl or phenyl is optionally substituted where chemically allowed by from 1-4 groups independently selected from oxo, C₁-C₆-alkyl, C₂-C₆-alkynyl, C₂-C₆-alkenyl, C₁-C₆-haloalkyl; OR_a, NR_aR_b, SR_a, C(O)OR_a, C(O)NR_aR_b, halo, cyano, nitro, C(O)R_a, S(O)₂OR_a;
 - wherein R_a is independently at each occurrence selected from: H and C₁-C₆-alkyl; and R_b is independently at each occurrence selected from: H and C₁-C₆-alkyl, C(O)C₁-C₆-alkyl and S(O)₂-C₁-C₆-alkyl;
- and R₇ is selected from -H, -CF₃, -OCF₃, -OCHF₂, -NO₂, -CN;
- and X⁻ selected from a suitable anion to form a pharmaceutically acceptable salt.

[0015] It should be appreciated a wide range of validated counterions are available for forming pharmaceutically acceptable salts of the disclosed compounds. For example, X⁻ may be selected from acetate, benzenesulfonate, benzoate, besylate, bitartrate, bromide, bamsylate, chloride, citrate, decanoate, edetate, esylate, fumarate, gluceptate, hexanoate, iodide,

isethionate, lactate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, octanoate, oleate, pamoate, pantothenate, phosphate, propionate, salicylate, stearate, succinate, sulfate, tartrate, teoclate, and tosylate. In preferred embodiments, X^- is selected from Cl^- , Br^- , I^- , and $MeSO_3^-$. Investigations into the most suitable anions for specific embodiments are ongoing.

[0016] In some embodiments of MSTI compounds, L_1 is one of $-OR_{1a^-}$, $-NR_{1a^-}$, $-NR_{1a}C(O)R_{1a^-}$, $-NR_{1a}C(O)OR_{1a^-}$, $-NR_{1a}C(O)NR_{1a^-}$, or $-CH_2R_{1a^-}$, and then R_{1a} is independently at each occurrence selected from H and C_1 - C_{16} -alkylene attached to $(R_7-(Ph)_3-P^+)-$ at one end, and to N at the other end.

[0017] In some embodiments, L_1 is one of $-OR_{1a^-}$, $-NR_{1a^-}$, $-NR_{1a}C(O)R_{1a^-}$, $-NR_{1a}C(O)OR_{1a^-}$, $-NR_{1a}C(O)NR_{1a^-}$, or $-CH_2R_{1a^-}$, and then R_{1a} is independently at each occurrence selected from H and C_1 - C_{16} -alkylene attached to $(R_7-(Ph)_3-P^+)-$ at one end, and to O at the other end.

[0018] In some embodiments, L_1 is one of $-OR_{1a^-}$, $-NR_{1a^-}$, $-NR_{1a}C(O)R_{1a^-}$, $-NR_{1a}C(O)OR_{1a^-}$, $-NR_{1a}C(O)NR_{1a^-}$, or $-CH_2R_{1a^-}$, and then R_{1a} is independently at each occurrence selected from H and C_1 - C_{16} -alkylene attached to $(R_7-(Ph)_3-P^+)-$ at one end, and to C at the other end.

[0019] When L_1 is $-NR_{1a}C(O)R_{1a}$, R_{1a} is independently at each occurrence selected from H and C_1 - C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)-$ and R_7 is selected from -H and $-CF_3$.

[0020] When L_1 of Formula [I] is $-NR_{1a}C(O)R_{1a}$ attached to para-position in the phenyl ring, R_{1a} is independently at each occurrence selected from H and C_1 - C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)-$ and R_7 is selected from -H and $-CF_3$. Formula [X] and Formula [XI], below, provide examples of such embodiments.

[0021] When L_1 is $-NR_{1a}C(O)R_{1a}$ attached to para-position in the phenyl ring, R_{1a} is independently at each occurrence selected from H and C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)-$ and R_7 is selected from -H and $-CF_3$.

[0022] When R_2 is Cl^- in ortho position in the phenyl ring and L_1 is $-NR_{1a}C(O)R_{1a}$ attached to para-position in the phenyl ring, R_{1a} is independently at each occurrence selected from H and C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)-$ and R_7 is selected from -H and $-CF_3$.

[0023] The present approach may also be used to treat and/or prevent tumor recurrence and/or metastasis. For example, MSTI compounds according to Formula I, above, may be administered as a pharmaceutical agent to treat and/or prevent tumor recurrence and/or metastasis, alone or in connection with other therapies. Anti-cancer treatments often fail

because the tumor recurs or metastasizes, particularly after surgery. CSC mitochondrial activity is understood to be, at least in part, responsible for these causes of treatment failure. Embodiments of the present approach may be used in situations where conventional cancer therapies fail, and/or in conjunction with or prior to anti-cancer treatments, to prevent or reduce the likelihood of treatment failure due to tumor recurrence and/or metastasis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Figs. 1A-1C show mammosphere formation results, SRB assay results on MCF-7 cells, and SRB assay results on BJ1-hTERT cells, respectively, for different concentrations of Compound [I].

[0025] Figs. 2A and 2B show the effect of Compound [I] at different concentrations on MCF-7 colony formation; as representative phase images of 2D colonies and expressed in terms of colony formation relative to the control, respectively.

[0026] Fig. 3A shows mammosphere formation results for Compound [I] at different concentrations on MDA-MB-231 cells, and Fig. 3B shows SRB assay results for Compound [I] on MDA-MB-231 cells.

[0027] Figs. 4A and 4B show the effect of Compound [I] at different concentrations on MDA-MB-231 colony formation; as representative phase images of 2D colonies and expressed in terms of colony formation relative to the control, respectively.

[0028] Figs. 5A and 5B show the results of mammosphere formation assay of Compound [I] on both colonies and spheres, for (A) MCF-7 cells, and (B) MDA-MB-231 cells.

[0029] Figs. 6A-6D show mitochondrial respiration, basal respiration, maximal respiration, and ATP production, respectively, for different concentrations of Compound [I] on MCF-7 cells. The legend of Fig. 6A applies to Figs. 6B-6D.

[0030] Figs. 7A-7D show glycolytic function, glycolysis, glycolytic capacity, and glycolytic reserve, respectively, for different concentrations of Compound [I] on MCF-7 cells. The legend of Fig. 7A applies to Figs. 7B-7D.

[0031] Fig. 8A shows tumor growth results for Compound [I], and Fig. 8B shows metastatic invasion results for Compound [I], both using the CAM assay described herein.

[0032] Fig. 9 shows *in vivo* embryo toxicity results for Compound [I] using the CAM assay described here.

DESCRIPTION

[0033] The following description illustrates embodiments of the present approach in sufficient detail to enable practice of the present approach. Although the present approach is described with reference to these specific embodiments, it should be appreciated that the

present approach can be embodied in different forms, and this description should not be construed as limiting any appended claims to the specific embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the present approach to those skilled in the art.

[0034] This description uses various terms that should be understood by those of an ordinary level of skill in the art. The following clarifications are made for the avoidance of doubt.

[0035] The term “cancer” refers to physiological conditions in mammals that are typically characterized by uncontrolled cell growth. This definition includes benign and malignant cancers. Examples of cancers include cancer types, lymphomas, blastomas (including medullablastomas and retinoblastomas), sarcomas (including liposarcomas and synovial sarcomas), neuroendocrine tumors (carcinoid tumors, gastrin production Includes, but is not limited to, tumors and islet cell carcinomas), sarcomas, Schwannomas (including acoustic neuroma), medullary carcinomas, adenocarcinomas, melanomas, and leukemia or lymphocyte tumors. Specific examples of cancers include bladder cancer, squamous cell carcinoma, small cell lung cancer, non-small cell lung cancer, lung adenocarcinoma, and lung cancer including squamous epithelial cancer of the lung, peritoneal cancer, hepatocellular carcinoma, gastric cancer including gastrointestinal cancer or stomach cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, liver cancer, breast cancer (including metastatic breast cancer), colon cancer, rectal cancer, colon rectal cancer, endometrial cancer or uterine cancer, salivary adenocarcinoma, kidney cancer (kidney cancer) or kidney cancer (renal cancer), prostatic cancer, genital cancer, thyroid cancer, liver cancer, anal cancer, penis cancer, testicular cancer, esophageal cancer, bile duct tumor, and head and neck cancer and multiple myeloma.

[0036] As used herein, the term “tumor” refers to the growth and proliferation of neoplastic cells, whether malignant or benign, including pre-cancerous and cancerous cells and tissues.

[0037] The term “metastasis” refers to the spread of cancer from its primary site to other parts of the body. Cancer cells can escape from the primary tumor, penetrate lymph vessels and blood vessels, circulate through the bloodstream, and grow or “metastasize” in distant lesions in normal tissue elsewhere in the body. Metastases can be local or distant. Metastasis is a sequential process that requires tumor cells to escape from the primary tumor, travel through the bloodstream, and stop at distant sites. At this new site, cells can establish a blood supply and grow to form a life-threatening mass. Both irritating and inhibitory molecular

pathways within tumor cells control this behavior, and the interaction between tumor cells and host cells at distant sites is also important.

[0038] The terms “treat,” “treated,” “treating,” and “treatment” include the diminishment or alleviation of at least one symptom associated or caused by the state, disorder or disease being treated, in particular, cancer. In certain embodiments, the treatment comprises diminishing and/or alleviating at least one symptom associated with or caused by the cancer being treated, by the compound of the invention. In some embodiments, the treatment comprises causing the death of a category of cells, such as senescent cells, SASP cells, or CSCs likely to be involved in metastasis or recurrence, of a particular cancer in a host, and may be accomplished through preventing senescent cells and/or cancer cells from further propagation, and/or inhibiting CSC function through, for example, depriving such cells of mechanisms for generating energy. For example, treatment can be diminishment of one or several symptoms of a cancer, or complete eradication of a cancer. As another example, the present approach may be used to inhibit mitochondrial metabolism in the cancer, eradicate (e.g., killing at a rate higher than a rate of propagation) CSCs in the cancer, eradicate TICs in the cancer, eradicate circulating tumor cells in the cancer, inhibit propagation of the cancer, target and inhibit CSCs, target and inhibit TICs, target and inhibit circulating tumor cells, prevent or reduce the likelihood of, metastasis, prevent recurrence, sensitize the cancer to a chemotherapeutic, sensitize the cancer to radiotherapy, sensitize the cancer to phototherapy. As another example, the treatment can reduce the accumulated senescent cells, and/or reduce the rate of senescent cell accumulation.

[0039] In the context of tumor recurrence and/or metastasis, the term “prevent” and “reduce the likelihood of” refer to reducing, in a subject, the presence of CSCs, TICs, and circulating tumor cells, likely to be involved in recurrence or metastasis, to a level at which tumor recurrence and/or metastasis from the primary site is unlikely, relative to a control (i.e., no treatment to prevent or reduce the likelihood of tumor recurrence and/or metastasis). In practice, a treatment to prevent and/or reduce the likelihood of tumor recurrence and/or metastasis as described herein targets and inhibits or eradicates CSCs, TICs, inhibit circulating tumor cells.

[0040] The terms “cancer stem cell” and “CSC” refer to the subpopulation of cancer cells within tumors that have capabilities of self-renewal, differentiation, and tumorigenicity when transplanted into an animal host. Compared to “bulk” cancer cells, CSCs have increased mitochondrial mass, enhanced mitochondrial biogenesis, and higher activation of mitochondrial protein translation. As used herein, a “circulating tumor cell” is a cancer cell that

has shed into the vasculature or lymphatics from a primary tumor and is carried around the body in the blood circulation. The CellSearch Circulating Tumor Cell Test may be used to detect circulating tumor cells.

[0041] The phrase “pharmaceutically effective amount,” as used herein, indicates an amount necessary to administer to a host, or to a cell, tissue, or organ of a host, to achieve a therapeutic result, such as regulating, modulating, or inhibiting protein kinase activity, e.g., inhibition of the activity of a protein kinase, or treatment of cancer. A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required for a given subject, using methods well-known and available in the art. For example, the physician or veterinarian may start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. The determination of a pharmaceutically effective amount is deemed to be within the purview of the person having an ordinary level of skill in the art, having reviewed this disclosure.

[0042] As used herein, the phrase “therapeutic agent” refers to an embodiment of the compound described herein, which may include a pharmaceutically acceptable salt or isotopic analog thereof. It should be appreciated that the therapeutic agent may be administered to the subject through any suitable approach, as would be known to those having an ordinary level of skill in the art. It should also be appreciated that the amount of therapeutic agent and the timing of its administration may be dependent on the individual subject being treated (e.g., the age and body mass, among other factors), on the manner of administration, on the pharmacokinetic properties of the particular therapeutic agent, and on the judgment of the prescribing physician. Thus, because of subject-to-subject variability, any dosages described herein are intended to be initial guidelines, and the physician can titrate doses of the therapeutic agent to achieve the treatment that the physician considers appropriate for the subject. In considering the degree of treatment desired, the physician can balance a variety of factors such as age and weight of the subject, presence of preexisting disease, as well as presence of other diseases. Pharmaceutical formulations can be prepared for any desired route of administration including, but not limited to, oral, intravenous, or aerosol administration, as discussed in greater detail below.

[0043] The phrase “pharmaceutically acceptable carrier” as used herein, means a pharmaceutically acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, solvent, or encapsulating material. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the

patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

[0044] The phrase “pharmaceutically acceptable salt” refers to the relatively non-toxic, inorganic and organic base addition salts of the compounds of the present approach. A pharmaceutically acceptable salt may be formed by, for example, reacting a compound in its free acid form with a base, such as hydroxide or carbonate of a pharmaceutically-acceptable metal cation, with ammonia or with a pharmaceutically-acceptable amine. Representative alkali or alkaline earth salts include sodium, potassium, calcium, magnesium, and aluminum salts, for example. Examples of amines that may be used for base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine. It should be appreciated that other salts may be used, of course, and that the person of ordinary skill in the art may use methods known in the art for identifying suitable salt forms, without departing from the present approach.

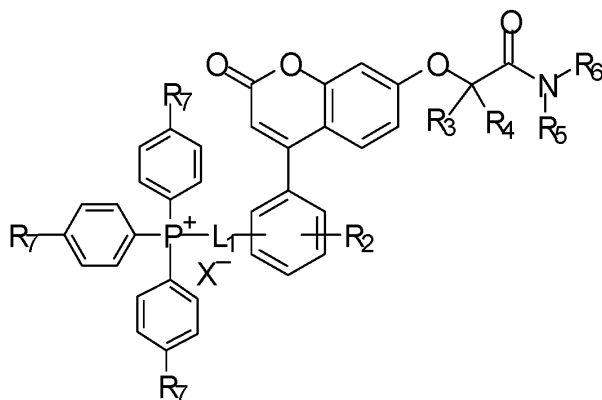
[0045] Suitable salts for MSTI compounds described herein may be selected from acetate, benzenesulfonate, benzoate, besylate, bitartrate, bromide, bamsylate, chloride, citrate, decanoate, edetate, esylate, fumarate, gluceptate, hexanoate, iodide, isethionate, lactate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, octanoate, oleate, pamoate, pantothenate, phosphate, propionate, salicylate, stearate, succinate, sulfate, tartrate, teoate, and tosylate. In preferred embodiments, X^- is selected from Cl^- , Br^- , I^- , and $MeSO_3^-$.

[0046] Recent development identified a first-in-class inhibitor of mitochondrial transcription targeting mitochondrial RNA polymerase (POLRMT), a gene product responsible for mitochondrial gene expression and mitochondrial biogenesis of the OXPHOS system. The inhibitor has been used in a four-week trial in mice. The oral treatment with the compound

showed no evidence of OXPHOS dysfunction or toxicity in the mice, but showed strong anti-tumor effects in human cancer cell xenografts.

[0047] The mitochondria specific transcription inhibitor (MSTI) compounds disclosed herein represent a new class of compounds having exceptional selectivity and activity towards cancer cells. Embodiments of the MSTI compounds disclosed herein may be used as therapeutic agent to selectively eradicate CSCs for treating and/or preventing tumor recurrence and/or metastasis. The data demonstrates that the MSTI compounds disclosed herein have enhanced anti-cancer activity relative to the aforementioned POLRMT inhibitor referenced above, and are suitable for use as therapeutic agents for anti-cancer treatments, including treating and/or preventing tumor recurrence and metastasis. Data described herein demonstrates the anti-cancer activity through inhibition of MCF7 cells via the mammosphere formation assay, and also cell viability using the sulforhodamine B assay (also known in the art and referred to herein as the SRB assay). This assay measures the amount of residual protein that adheres to tissue culture dishes and is a surrogate marker of cell viability.

[0048] Under the present approach, some embodiments of the MSTI compounds may have the generic chemical structure of Formula [I], shown below:



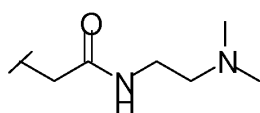
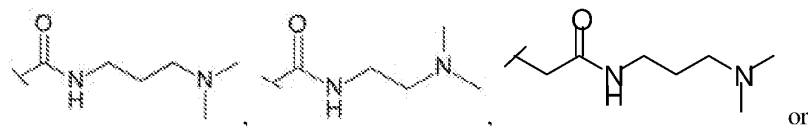
, or pharmaceutically acceptable

salts thereof, in which:

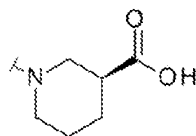
- R₂ is selected from a halogen, CF₂H, -CF₃, -OCF₂H, -OCF₃, substituted or unsubstituted C5-C18 carboxyl, substituted or unsubstituted C5-C18 alkane, substituted or unsubstituted C5-C18 alkene, substituted or unsubstituted C5-C18 cyclic alkene, substituted or unsubstituted C5-C18 alkyne, substituted or unsubstituted C5-C18 ketone, substituted or unsubstituted C5-C18 aldehyde, substituted or unsubstituted C5-C18 ether, substituted or unsubstituted C5-C18 ester, substituted or unsubstituted C5-C18 amine, substituted or unsubstituted C5-C18 amide, substituted

or unsubstituted C5-C18 alkyl-amide, monocyclic or polycyclic arene, heteroarene, phenol, or benzoic acid;

- R₃ and R₄ may be the same or different, and are selected from hydrogen, substituted or unsubstituted C1-C10 alkyl, substituted or unsubstituted C3-C8-cycloalkyl, substituted or unsubstituted pyridine, substituted or unsubstituted C2-C10 carboxyl, substituted or unsubstituted C2-C10 alkene, substituted or unsubstituted C2-C10 alkyne, substituted or unsubstituted C2-C10 ketone, substituted or unsubstituted C2-C10 aldehyde, substituted or unsubstituted C2-C10 ether, substituted or unsubstituted C2-C10 ester, substituted or unsubstituted C2-C10 amine, substituted or unsubstituted C2-C10 amide, substituted or unsubstituted C2-C10 alkyl-amide, substituted or unsubstituted phenol, or benzoic acid;
- R₅ and R₆ may be the same or different, and are selected from hydrogen, substituted or unsubstituted C2-C18-alkyl, substituted or unsubstituted C3-C8-cycloalkyl, substituted or unsubstituted pyridine, substituted or unsubstituted C2-C18 carboxyl, substituted or unsubstituted C2-C18 alkene, substituted or unsubstituted C2-C18 alkyne, substituted or unsubstituted C2-C18 ketone, substituted or unsubstituted C2-C18 aldehyde, substituted or unsubstituted C2-C18 ether, substituted or unsubstituted C2-C18 ester, substituted or unsubstituted C2-C18 amine, substituted or unsubstituted C2-C18 amide, substituted or unsubstituted C2-C18 alkyl-amide, substituted or unsubstituted phenol, or benzoic acid, or one of R₃ and R₄ is the group



, and the other is a H or a C2-C5 alkyl; or R₅ and R₆ form a substituted or unsubstituted C5 or C6 heterocycle; or R₅ and R₆ form the group



; or NR₃R₄ is substituted with OH;

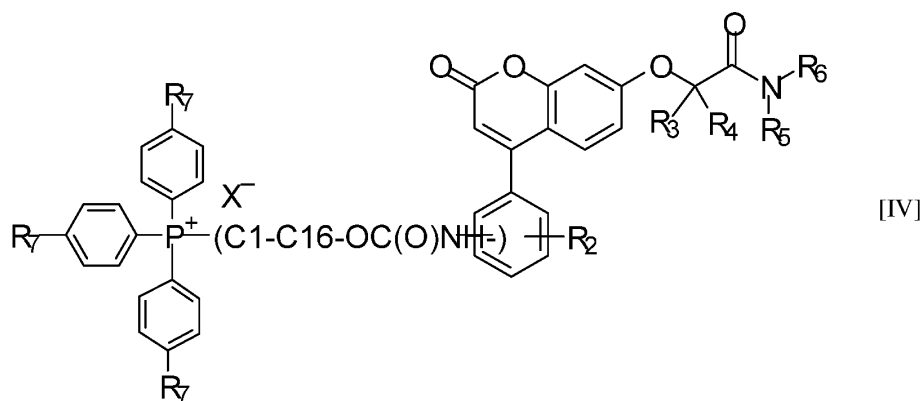
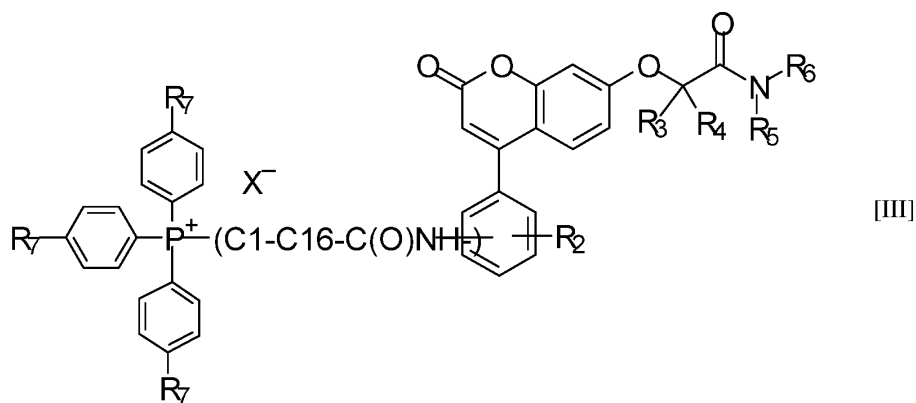
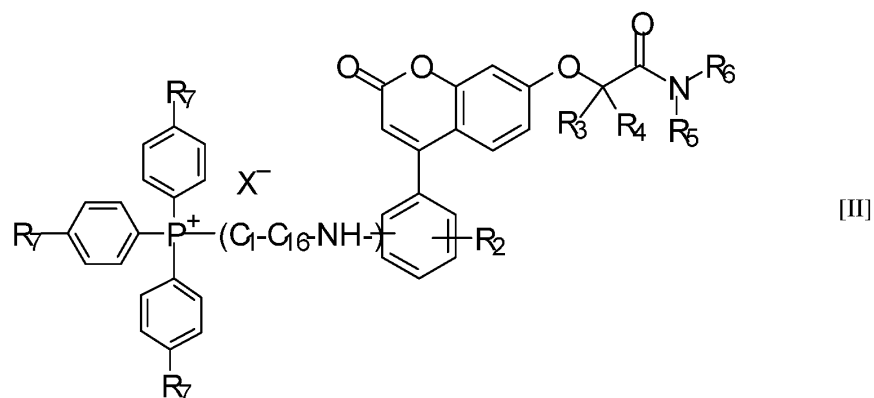
- L₁ is independently selected from: -OR_{1a}-, -SR_{1a}-, -NR_{1a}-, -NR_{1a}C(O)R_{1a}-, -OC(O)R_{1a}-, -C(O)OR_{1a}-, -SO₂R_{1a}-, -S(O)R_{1a}-, -NR_{1a}C(O)-, -C(O)NR_{1a}-, -NR_{1a}S(O)₂-,

$S(O)_2NR_{1a^-}$, $-OC(O)NR_{1a^-}$, $-NR_{1a}C(O)OR_{1a^-}$, $-NR_{1a}C(O)NR_{1a^-}$, $-CR_{1a}=CR_{1a^-}$ and $-CC-$, $-CH_2R_{1a^-}$;

- R_{1a} is independently selected from: H, alkyl, alkenyl, alkynyl, cycloalkyl heterocycloalkyl, heteroaryl or phenyl is optionally substituted where chemically allowed by from 1-4 groups independently selected from oxo, C_1 - C_6 -alkyl, C_2 - C_6 -alkynyl, C_2 - C_6 -alkenyl, C_1 - C_6 -haloalkyl; OR_a , NR_aR_b , SR_a , $C(O)OR_a$, $C(O)NR_aR_b$, halo, cyano, nitro, $C(O)R_a$, $S(O)_2OR_a$;
- wherein R_a is independently at each occurrence selected from: H and C_1 - C_6 -alkyl; and R_b is independently at each occurrence selected from: H and C_1 - C_6 -alkyl, $C(O)C_1$ - C_6 -alkyl and $S(O)_2$ - C_1 - C_6 -alkyl;
- and R_7 is selected from -H, $-CF_3$, $-OCF_3$, $-OCHF_2$, $-NO_2$, $-CN$;
- and X^- selected from a suitable anion to form a pharmaceutically acceptable salt.

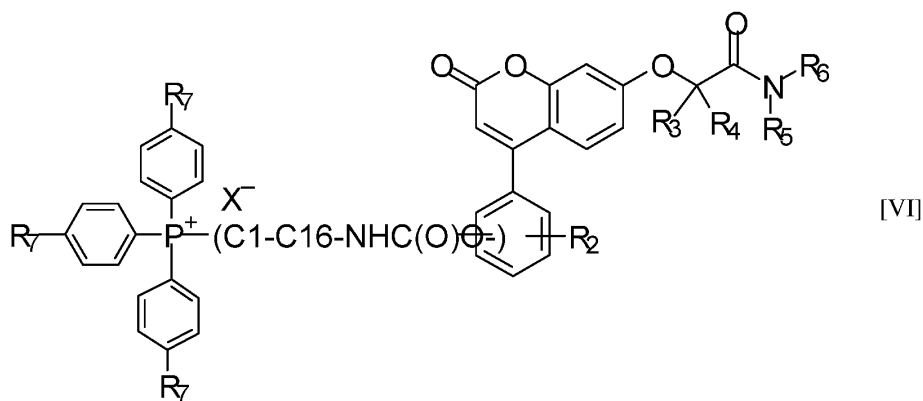
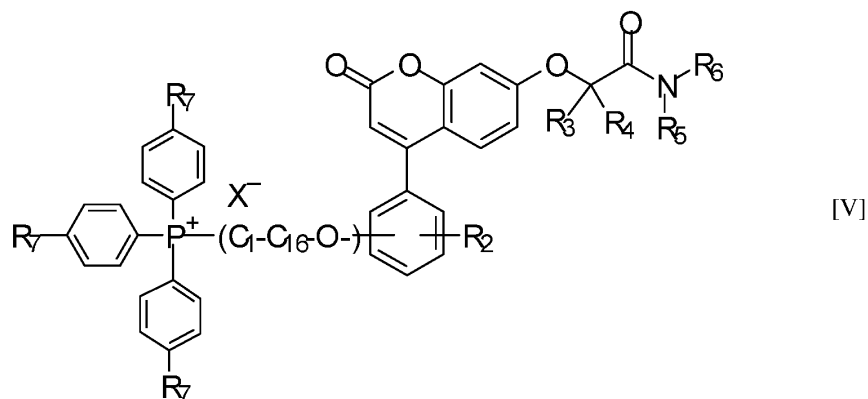
[0049] It should be appreciated a wide range of validated counterions are available for forming pharmaceutically acceptable salts of the disclosed compounds. For example, X^- may be selected from acetate, benzenesulfonate, benzoate, besylate, bitartrate, bromide, bamsylate, chloride, citrate, decanoate, edetate, esylate, fumarate, gluceptate, hexanoate, iodide, isethionate, lactate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, octanoate, oleate, pamoate, pantothenate, phosphate, propionate, salicylate, stearate, succinate, sulfate, tartrate, teoclate, and tosylate. In preferred embodiments, X^- is selected from Cl^- , Br^- , I^- , and $MeSO_3^-$. Investigations into the most suitable anions for specific embodiments are ongoing.

[0050] In some embodiments of MSTI compounds, L_1 is one of $-OR_{1a^-}$, $-NR_{1a^-}$, $-NR_{1a}C(O)R_{1a^-}$, $-NR_{1a}C(O)OR_{1a^-}$, $-NR_{1a}C(O)NR_{1a^-}$, or $-CH_2R_{1a^-}$, and then R_{1a} is independently at each occurrence selected from H and C_1 - C_{16} -alkylene attached to $(R_7-(Ph)_3-P+)$ at one end, and to N at the other end. Example Formulas [II], [III], and [IV] below are illustrative.

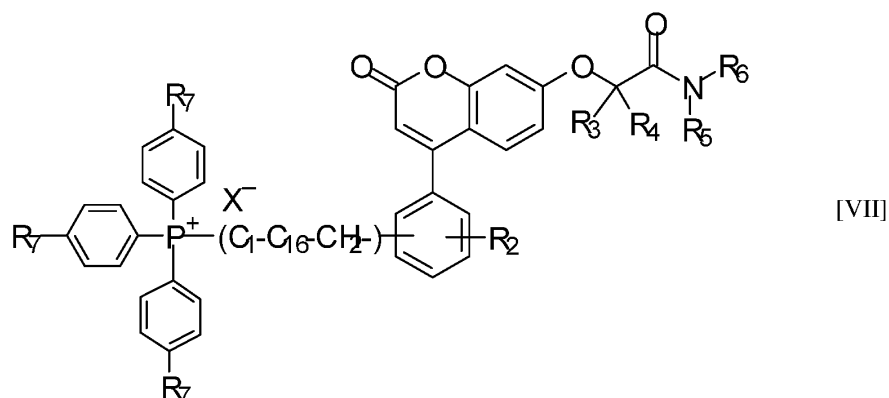


[0051] In the embodiments shown below, Formulas [V] and [VI], L_1 is one of $-OR_{1a-}$, $-NR_{1a-}$, $-NR_{1a}C(O)R_{1a-}$, $-NR_{1a}C(O)OR_{1a-}$, $-NR_{1a}C(O)NR_{1a-}$, or $-CH_2R_{1a-}$, and then R_{1a} is

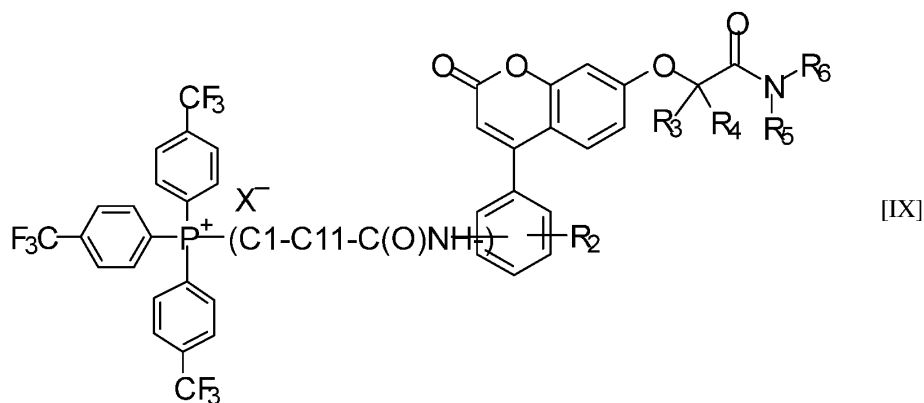
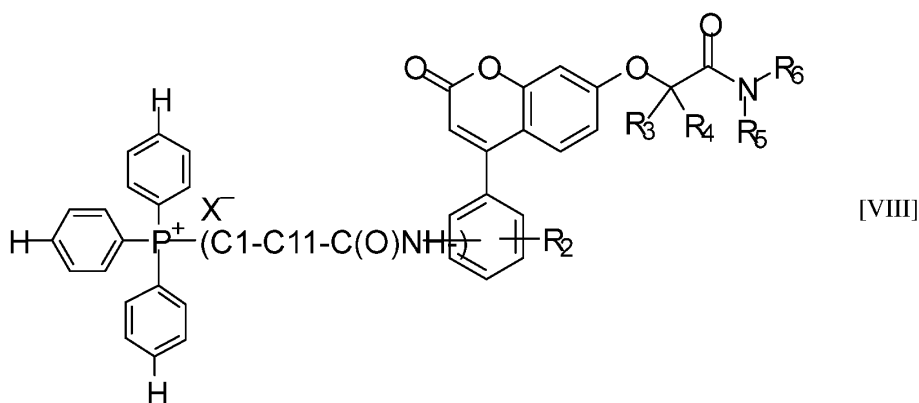
independently at each occurrence selected from H and C₁-C₁₆-alkylene attached to (R⁷-(Ph)₃-P⁺)- at one end, and to O at the other end.



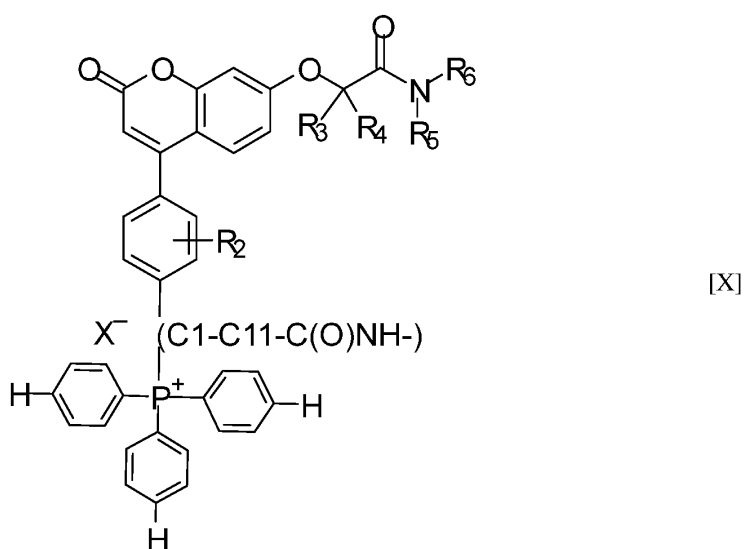
[0052] In some embodiments, L₁ is one of -OR_{1a}-, -NR_{1a}-, -NR_{1a}C(O)R_{1a}-, -NR_{1a}C(O)OR_{1a}-, -NR_{1a}C(O)NR_{1a}-, or -CH₂R_{1a}-, and then R_{1a} is independently at each occurrence selected from H and C₁-C₁₆-alkylene attached to (R⁷-(Ph)₃-P⁺)- at one end, and to C at the other end. Example Formula [VII] below is illustrative.



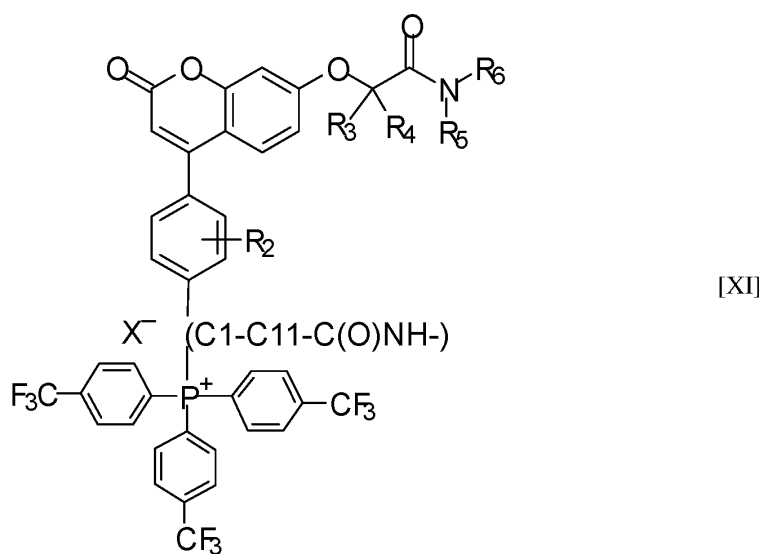
[0053] When L_1 is $-NR_{1a}C(O)R_{1a}$, R_{1a} is independently at each occurrence selected from H and C_1 - C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)$ - and R_7 is selected from -H and $-CF_3$. Formulas [VIII] and [IX], below, are demonstrative.



[0054] When L_1 of Formula [I] is $-NR_{1a}C(O)R_{1a}$ attached to para-position in the phenyl ring, R_{1a} is independently at each occurrence selected from H and C_1 - C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)-$ and R_7 is selected from $-H$ and $-CF_3$. Formula [X] and Formula [XI], below, provide examples of such embodiments.

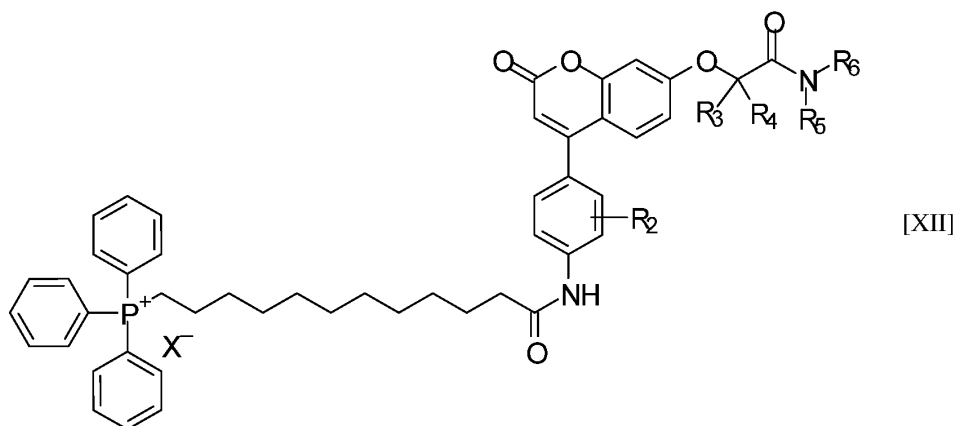


[X]

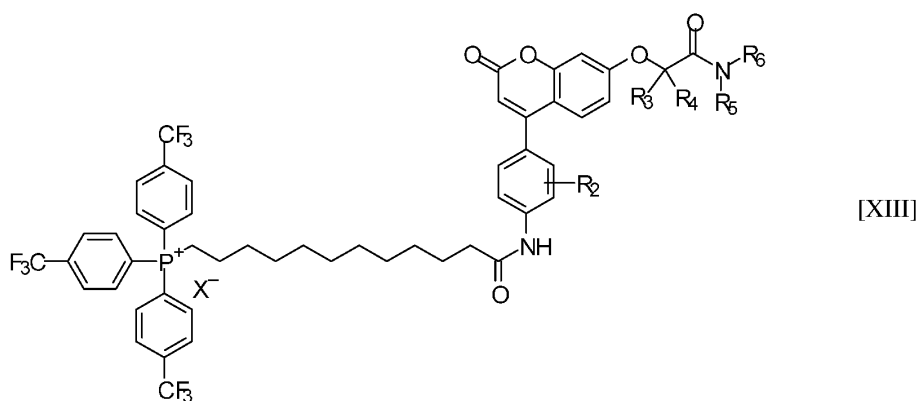


[XI]

[0055] When L_1 is $-NR_{1a}C(O)R_{1a}$ attached to para-position in the phenyl ring, R_{1a} is independently at each occurrence selected from H and C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)-$ and R_7 is selected from $-H$ and $-CF_3$, as demonstrated by Formulas [XII] and [XIII] below.

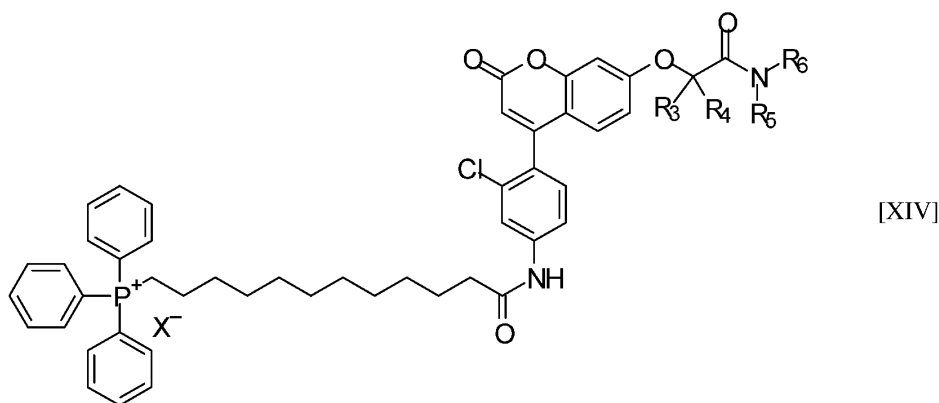


[XII]

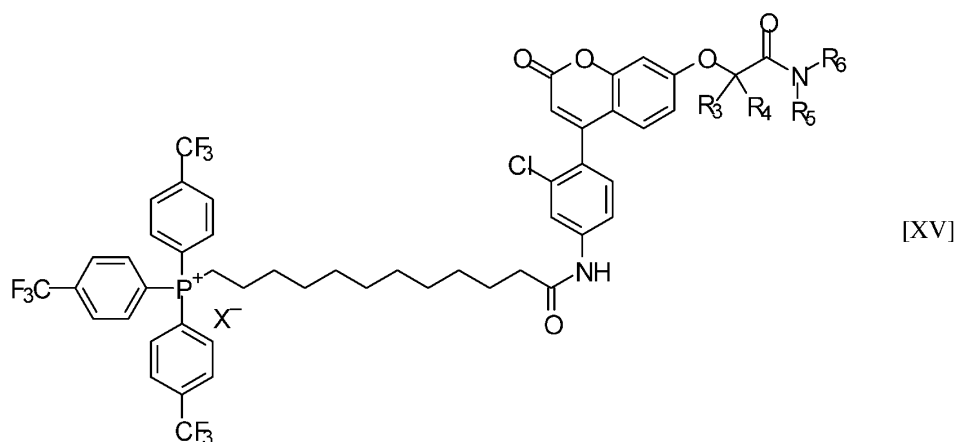


[XIII]

[0056] When R₂ is Cl⁻ in ortho position in the phenyl ring and L₁ is -NR_{1a}C(O)R_{1a} attached to para-position in the phenyl ring, R_{1a} is independently at each occurrence selected from H and C11-alkylene attached to (R₇-(Ph)₃-P⁺)- and R₇ is selected from -H and -CF₃. Formula [XIV] and Formula [XV], below, are demonstrative of such embodiments.

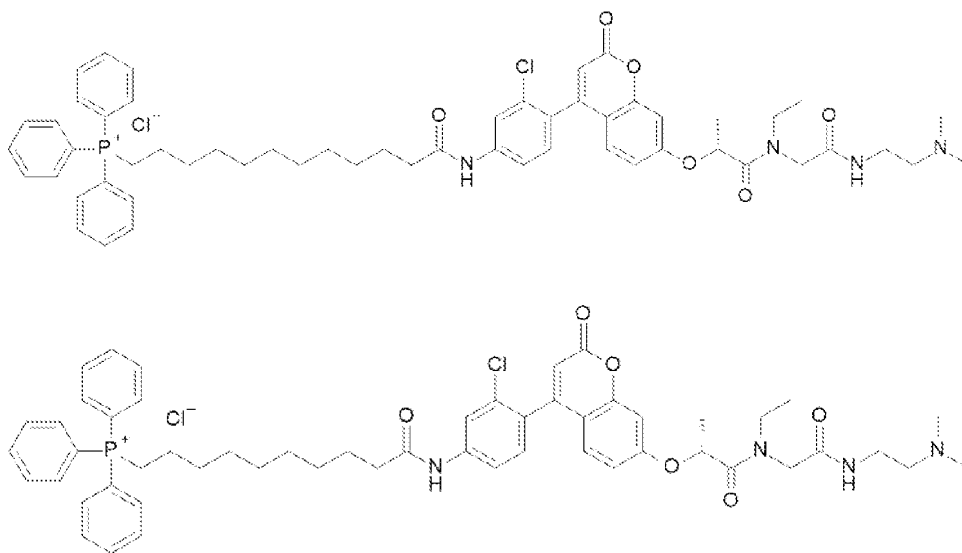


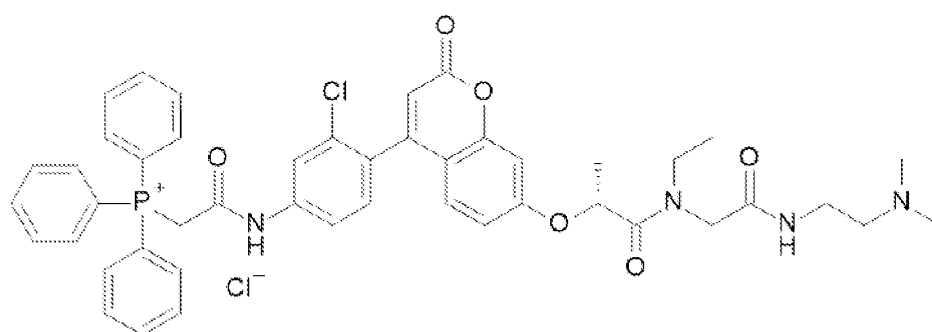
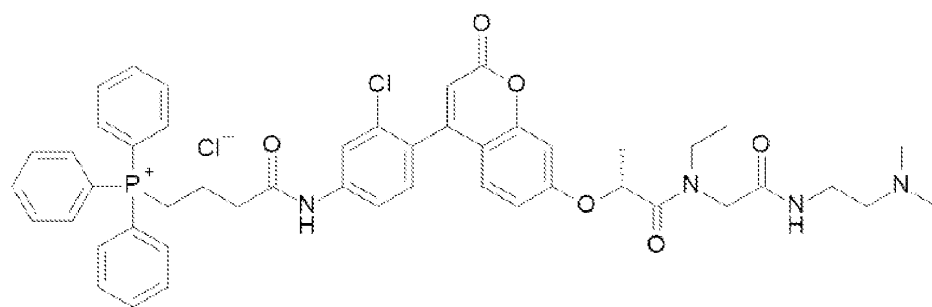
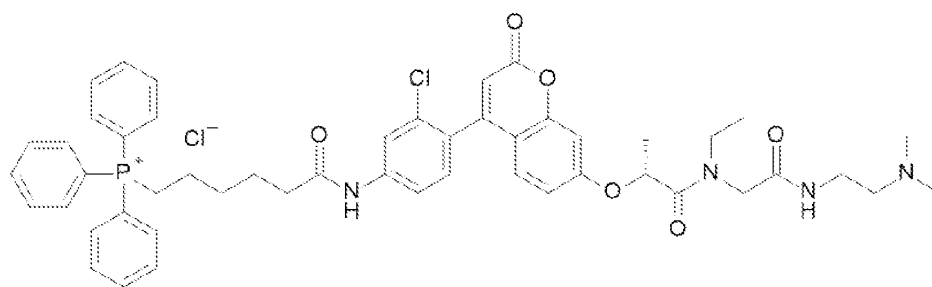
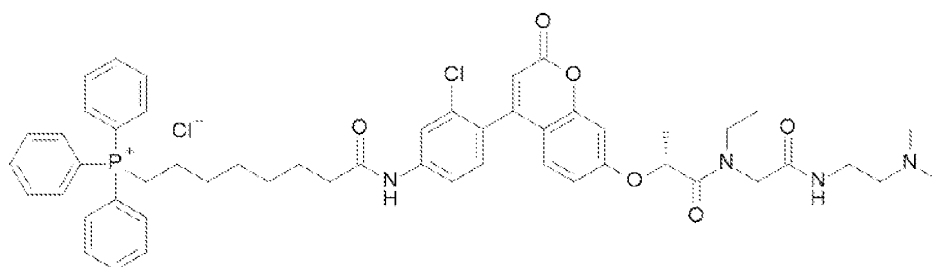
[XIV]

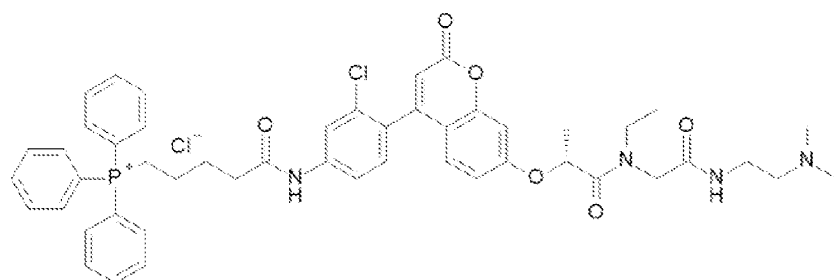
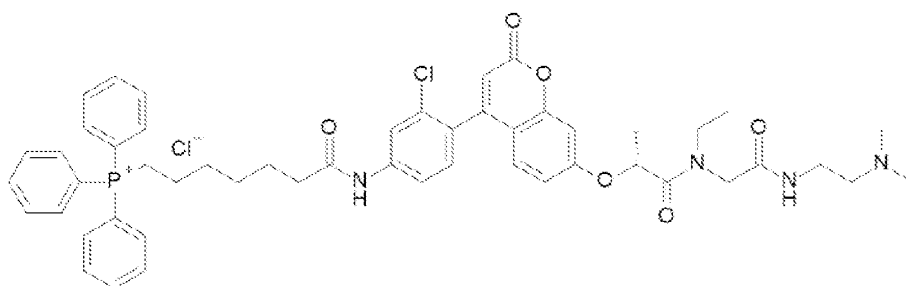
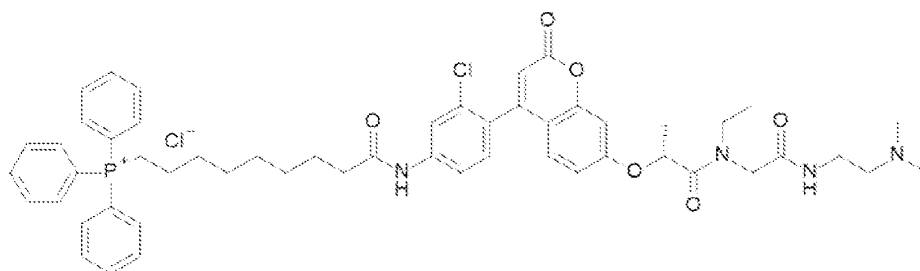
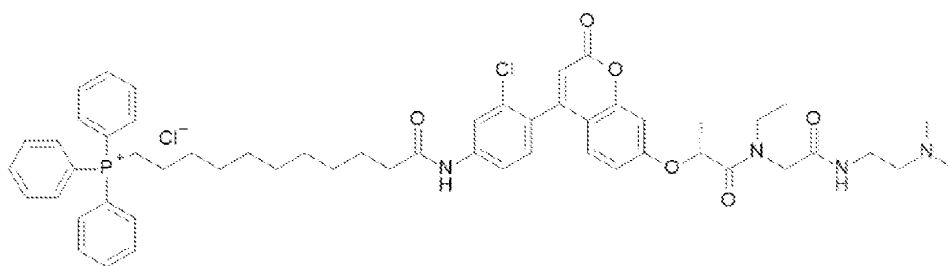


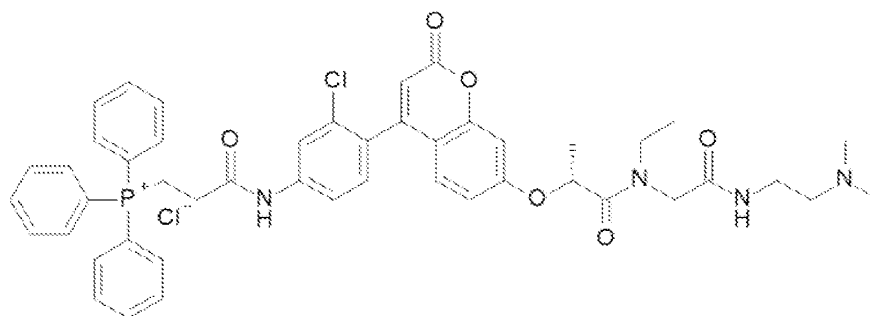
[0057] It should be appreciated that embodiments according to any of Formulas [II]—[XV] would be paired with a pharmaceutically acceptable salt X^- as described above.

[0058] The following compounds are demonstrative preferred embodiments of the present approach. It should be appreciated that several of these embodiments have been synthesized or are in the process of being synthesized. Those embodiments that have been synthesized have demonstrated the pharmaceutical activity described herein, or are in the process of being evaluated to confirm the expected activity based on the common pharmacophore.

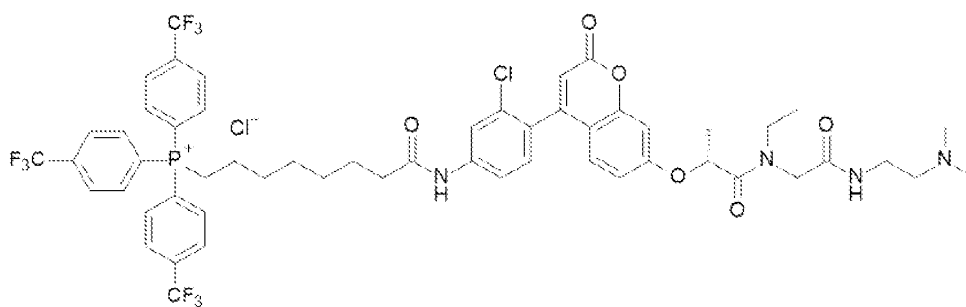
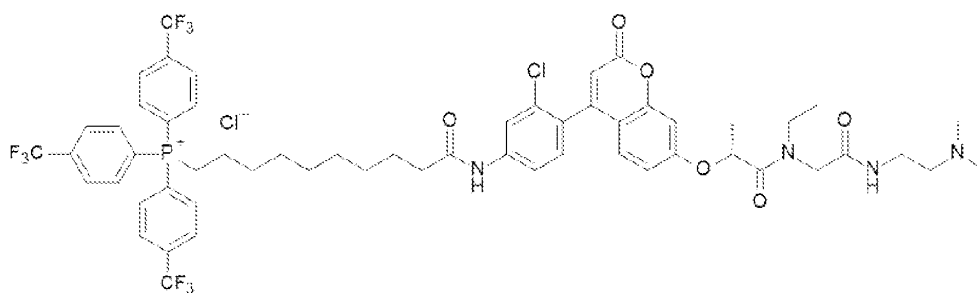
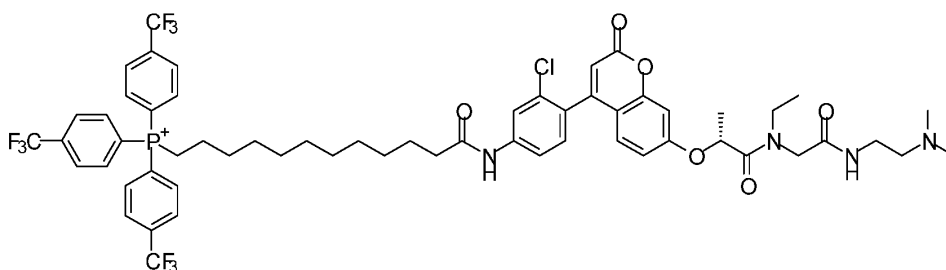


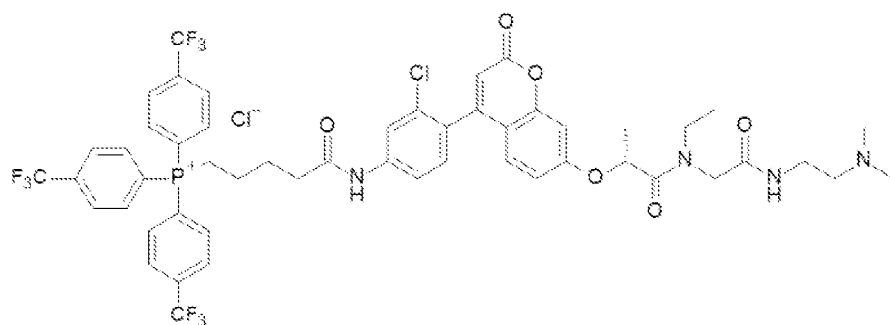
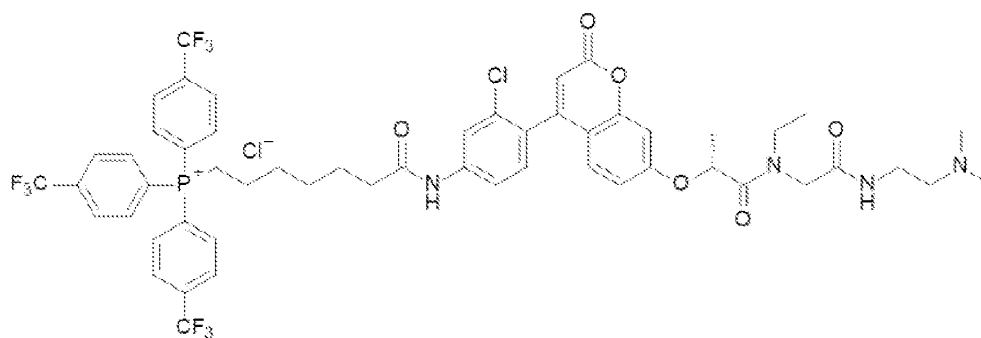
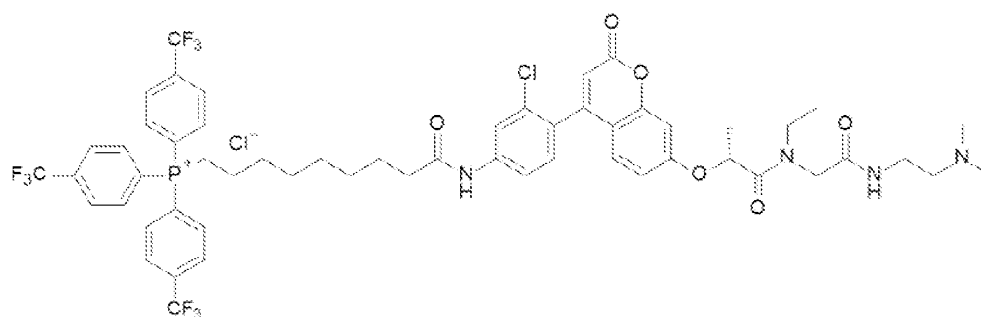


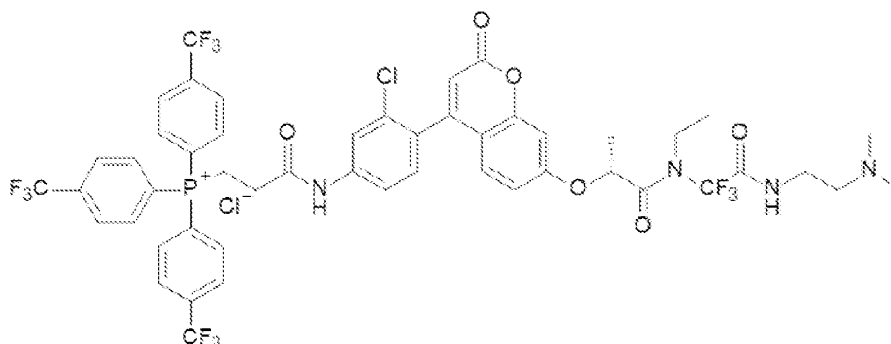




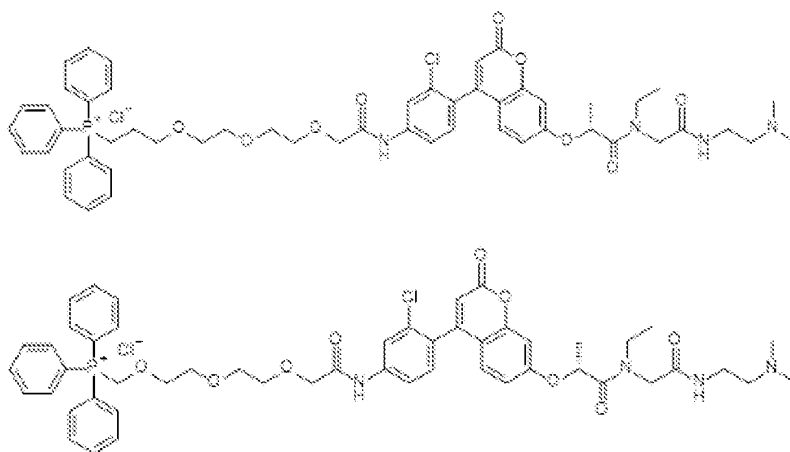
[0059] In the embodiments illustrated above, R₇ is -H. The examples below illustrate selected preferred embodiments in which R₇ is CF₃.

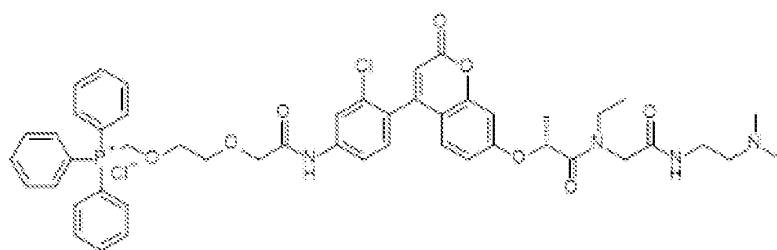
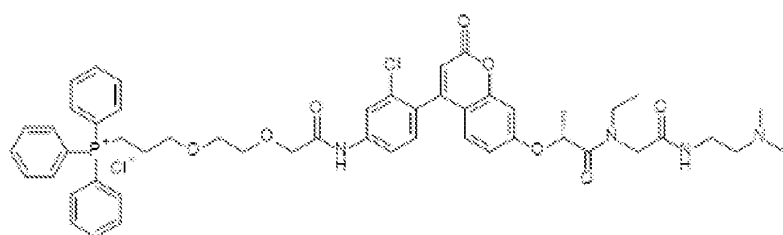
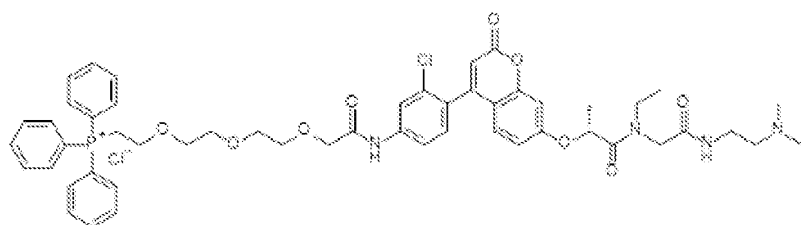
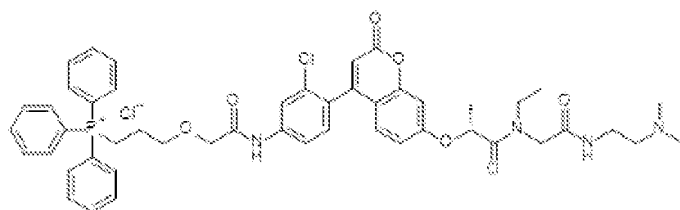
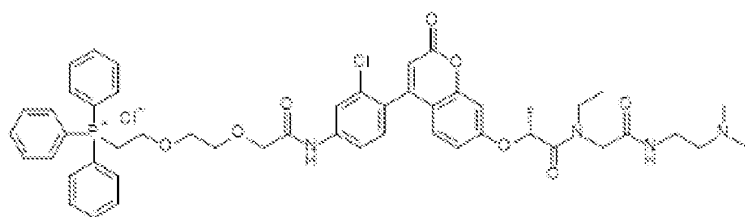


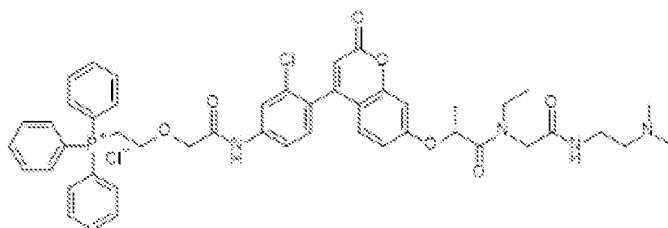




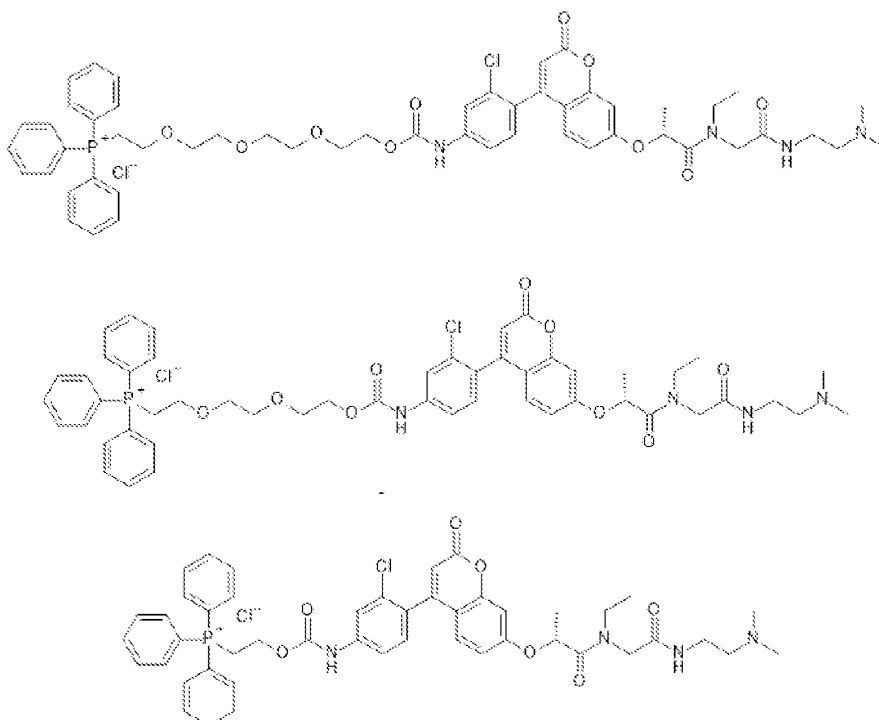
[0060] In some embodiments, linker L_1 may be a polyethylene glycol, carboxylic amide. For example, when L_1 is $-NR_{1a}C(O)R_{1a}$, R_{1a} is independently at each occurrence selected from H and C₂-C₈-polyethylene glycol or $-(CH_2CH_2OH)_x-$, where x is 1-4, attached to $(R_7-(Ph)_3-P^+)-$. The following compounds are demonstrative preferred embodiments of the present approach in which the linker includes polyethylene glycol. As with previous examples, it should be appreciated that several of these embodiments have been synthesized or are in the process of being synthesized. Those embodiments that have been synthesized have demonstrated the pharmaceutical activity described herein, or are in the process of being evaluated to confirm the expected activity based on the common pharmacophore.

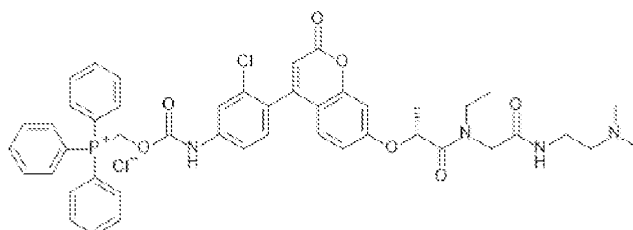
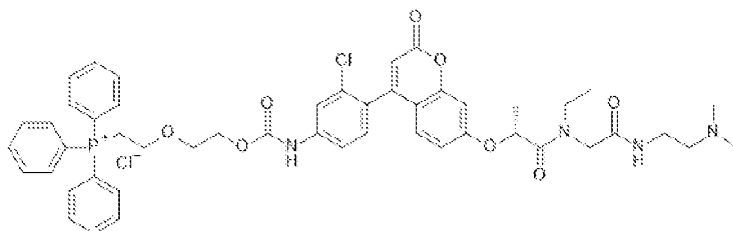
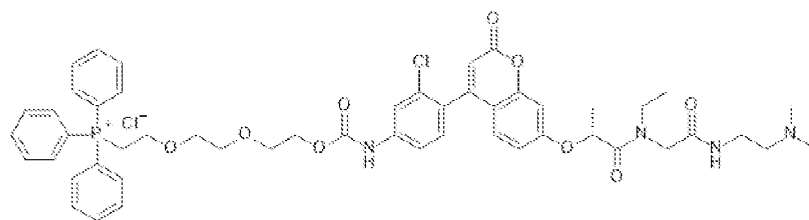






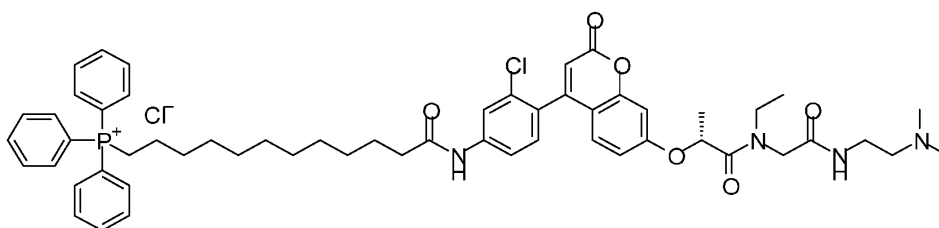
[0061] In some embodiments, linker L_1 may be a polyethylene glycol carbamate. For example, when L_1 is $-NR_{1a}C(O)OR_{1a}$, R_{1a} is independently at each occurrence selected from H and C_2 -C8-polyethylene glycol or $-(CH_2CH_2OH)_x-$, where x is 1-4, attached to $(R_7-(Ph)_3-P^+)-$. The following compounds are demonstrative preferred embodiments of the present approach in which the linker includes polyethylene glycol. As with previous examples, it should be appreciated that several of these embodiments have been synthesized or are in the process of being synthesized. Those embodiments that have been synthesized have demonstrated the pharmaceutical activity described herein, or are in the process of being evaluated to confirm the expected activity based on the common pharmacophore.





[0062] The present approach may also be used to treat and/or prevent tumor recurrence and/or metastasis. For example, MSTI compounds according to Formula I, above, may be administered as a pharmaceutical agent to treat and/or prevent tumor recurrence and/or metastasis, alone or in connection with other therapies.

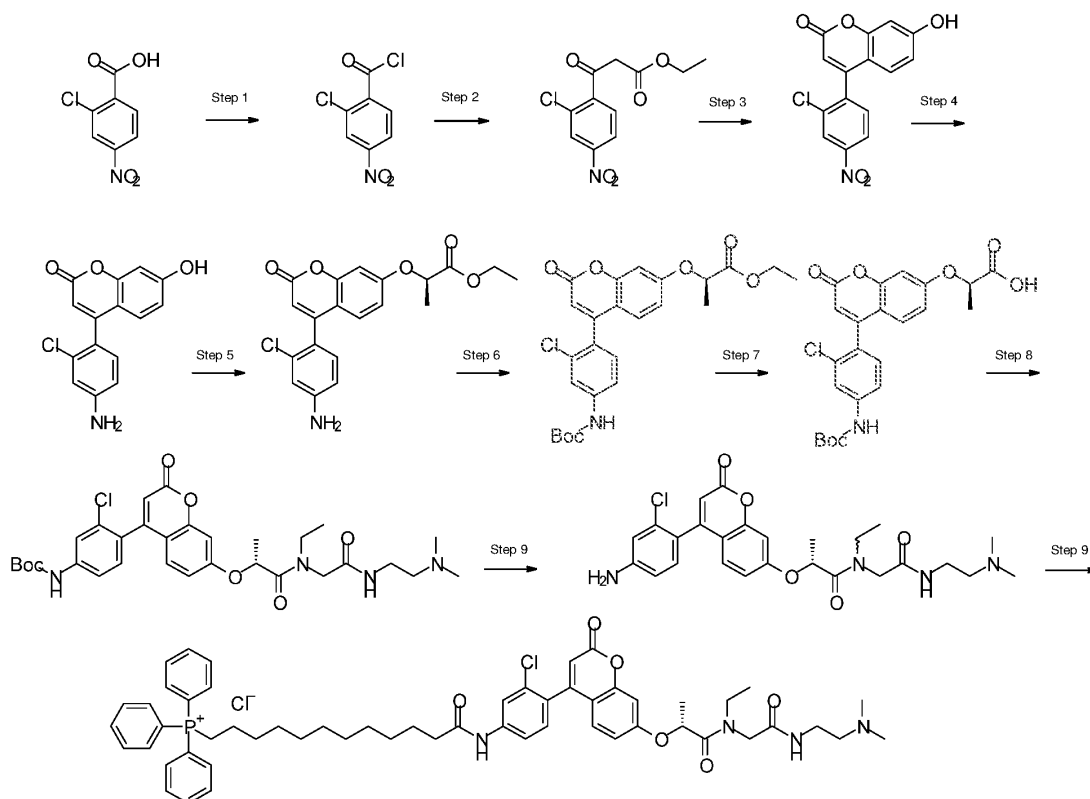
[0063] In one demonstrative embodiment of an MSTI compound of the present approach, based on Formula [I] and Formula [III], is Compound [1] having the formula shown below:



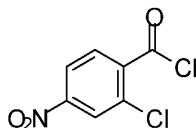
or another pharmaceutically acceptable salt thereof. This MSTI compound has an IUPAC name of [12-[3-chloro-4-[7-[(1R)-2-[[2-[2-(dimethylamino)ethylamino]-2-oxo-ethyl]-ethyl-amino]-

1-methyl-2-oxo-ethoxy]-2-oxo-chromen-4-yl[anilino]-12-oxo-dodecyl]-triphenyl-phosphonium chloride. It should be appreciated that other salts may be used without departing from the present approach.

[0064] Also described herein are methods for manufacturing compounds according to the present approach. For example, the embodiment of Compound [I] was prepared according to the following reaction scheme.

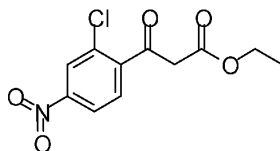


[0065] In Step 1, 2-chloro-4-nitrobenzoic acid is used as the starting material to synthesize 2-chloro-4-nitro-benzoyl chloride, shown below.



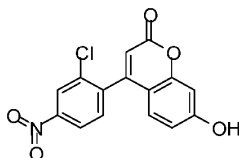
[0066] 2-Chloro-4-nitro-benzoyl chloride was prepared from 2-chloro-4-nitrobenzoic acid following the method in Advance Synthesis and Catalysis, 2014, vol.356, no. 11-12, p2437-2444 yielding crude 2-chloro-4-nitro-benzoyl chloride as a light brown oil (11.78g).

[0067] In Step 2, ethyl 3-(2-chloro-4-nitro-phenyl)-3-oxo-propanoate (shown below) was prepared from 2-chloro-4-nitro-benzoyl chloride.



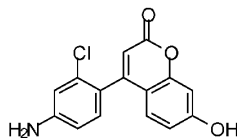
[0068] To a stirred suspension of ethyl potassium malonate (17.0g, 100mmol) in dry MeCN (300ml) under nitrogen atmosphere at +10 °C TEA (14.6ml, 105 mmol), anhydrous magnesium chloride (11.4g, 120mmol) was added, and the mixture was stirred at room temperature for 3 hours. A solution of 2-chloro-4-nitro-benzoyl chloride (11.78g 50mmol) in dry MeCN (100ml) was added over 45 minutes, and the mixture was stirred at room temperature for 16 hours. The reaction mixture was poured into a stirred mixture of saturated NH_4Cl (140ml) and EtOAc (330ml) and most of the solvent was evaporated under reduced pressure. The residue was diluted with EtOAc (200ml) was washed with 4M HCl (2x1000ml), water (100ml), brine (100ml), dried over MgSO_4 , filtered and the filtrate was concentrated under reduced pressure to yield a crude product. Purification on silica gel (10-20% EtOAc in *iso*-hexane) afforded ethyl 3-(2-chloro-4-nitro-phenyl)-3-oxo-propanoate as a light brown solid (11.04g). LC-MS 272.0 $[\text{M}+1]^+$, RT 5.39 min.

[0069] In Step 3, ethyl 3-(2-chloro-4-nitro-phenyl)-3-oxo-propanoate was used to prepare 4-(2-Chloro-4-nitro-phenyl)-7-hydroxy-chromen-2-one (shown below).



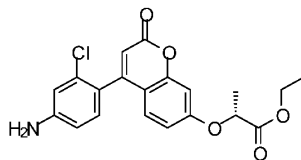
[0070] To a stirred solution of resorcinol (3.53g, 34.0mmol,) in methane sulphonic acid (40ml) at +45 °C under nitrogen atmosphere ethyl 3-(2-chloro-4-nitro-phenyl)-3-oxo-propanoate (10g, 36.8mmol) was added in portions over 10 minutes and the mixture was stirred at +45 °C for 1 hour. The reaction mixture cooled to room temperature, diluted with EtOH (35ml), poured into water (250ml), precipitated purple solid was collected by filtration, washed with EtOAc (100ml), DCM (100ml), water (100ml) and dried under vacuum to yield 4-(2-chloro-4-nitro-phenyl)-7-hydroxy-chromen-2-one as a purple solid (5.85g). LC-MS 318.0 $[\text{M}+1]^+$, RT 5.18 min.

[0071] In Step 4, the product of Step 3 was used to prepare 4-(4-amino-2-chloro-phenyl)-7-hydroxy-chromen-2-one (shown below).



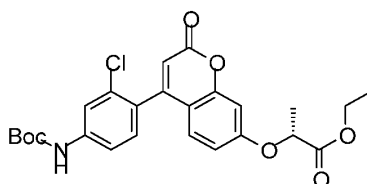
[0072] To a stirred ice cold solution of 4-(2-chloro-4-nitro-phenyl)-7-hydroxy-chromen-2-one (3.18g, 10mmol) and DiPEA (8.60ml, 50mmol) in dry DCM (200ml) under nitrogen atmosphere a solution of trichlorosilane (3.60ml, 35mmol) in dry DCM (20ml) was added over 90 minutes. The mixture was stirred at +0 °C for 1 hour, quenched with water (10ml) and MeOH (10ml), solid NaHCO₃ (10g) was added to adjust pH to 8-9 the suspension that was filtered through a Celite pad, the pad was washed with DCM (2x150ml), organic phase was separated, washed with water (100ml), saturated NaHCO₃ (100ml), brine (100ml), dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure to afford 4-(4-amino-2-chloro-phenyl)-7-hydroxy-chromen-2-one as a dark red-brown solid (3.02g). LC-MS 287.8 [M+1]⁺, RT 4.43 min.

[0073] In Step 5, 4-(4-amino-2-chloro-phenyl)-7-hydroxy-chromen-2-one was used to prepare ethyl (2R)-2-[4-(4-amino-2-chloro-phenyl)-2-oxo-chromen-7-yl]oxypropanoate (shown below).



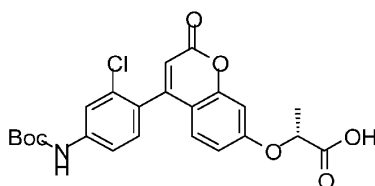
[0074] To a stirred ice cold solution of 4-(4-amino-2-chloro-phenyl)-7-hydroxy-chromen-2-one (0.50g, 1.74mmol), ethyl-S-lactate (0.40ml, 3.48mmol), and triphenylphosphine (0.90g, 3.48mmol) in dry 2-methyltetrahydrofuran (20ml) under nitrogen atmosphere DEAD (0.69ml, 3.48mmol) was added. The mixture was stirred at +0 °C for 0.5 hour, allowed to warm to room temperature and stirred for 64 hours. The solvent was evaporated under reduced pressure to yield a crude product. Purification on silica gel (15-80% EtOAc in *iso*-hexane) afforded ethyl (2R)-2-[4-(4-amino-2-chloro-phenyl)-2-oxo-chromen-7-yl]oxypropanoate as a light brown solid (0.84g). LC-MS 388.1 [M+1]⁺, RT 5.94 min.

[0075] In Step 6, ethyl (2R)-2-[4-[4-(tert-butoxycarbonylamino)-2-chloro-phenyl]-2-oxo-chromen-7-yl]oxypropanoate (shown below) was prepared from the product of Step 7.



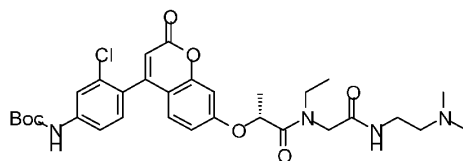
[0076] To a stirred solution of ethyl (2R)-2-[4-(4-amino-2-chloro-phenyl)-2-oxo-chromen-7-yl]oxypropanoate (0.80g, 1.70mmol) and DMAP (0.27g, 2.20mmol) in DCM (20ml) at room temperature under nitrogen atmosphere Boc-anhydride (0.48g, 2.20mmol) was added. The mixture was stirred for 6 hours and the solvent was evaporated under reduced pressure to yield a crude product. The residue was dissolved in EtOAc (50ml), washed with 1M HCl (20ml), saturated NaHCO₃ (20ml), brine (20ml), dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure afforded ethyl (2R)-2-[4-[4-(tert-butoxycarbonylamino)-2-chloro-phenyl]-2-oxo-chromen-7-yl]oxypropanoate (0.54g). LC-MS 488.1 [M+1]⁺, RT 6.35 min.

[0077] The product of Step 6 was used in Step 7 to prepare (2R)-2-[4-[4-(tert-Butoxycarbonylamino)-2-chloro-phenyl]-2-oxo-chromen-7-yl]oxypropanoic acid (shown below).



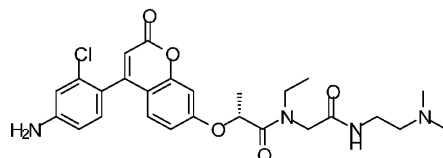
[0078] To a stirred solution of ethyl (2R)-2-[4-[4-(tert-butoxycarbonylamino)-2-chloro-phenyl]-2-oxo-chromen-7-yl]oxypropanoate (0.50g, 1.0mmol) in THF (5ml) and MeOH (5ml) at room temperature under nitrogen atmosphere LiOH hydrate (0.42g, 10.0mmol) in water (5ml) was added. The mixture was stirred for 4 hours, diluted with EtOAc (50ml) washed with 2M HCl (30ml), brine (30ml), dried over MgSO₄, filtered and the solvent was evaporated under reduced pressure affording (2R)-2-[4-[4-(tert-butoxycarbonylamino)-2-chloro-phenyl]-2-oxo-chromen-7-yl]oxypropanoic acid as a light brown solid, (0.45g). LC-MS 460.1 [M+1]⁺, RT 5.92 min.

[0079] Next, tert-Butyl N-[3-chloro-4-[7-[(1R)-2-[[2-[2-(dimethylamino)ethyl amino]-2-oxo-ethyl]-ethyl-amino]-1-methyl-2-oxo-ethoxy]-2-oxo-chromen-4-yl]phenyl] carbamate (shown below) was prepared from the product of the prior step.



[0080] To a stirred solution of (2R)-2-[4-[4-(tert-butoxycarbonylamino)-2-chloro-phenyl]-2-oxo-chromen-7-yl]oxypropanoic acid (0.15g, 0.33mmol) in dry DCM (2ml) at room temperature under nitrogen atmosphere a solution of N-[2-(dimethylamino)ethyl]-2-(ethylamino)acetamide hydrochloride (27mg, 0.11 mmol) was added. To the mixture EDC hydrochloride (82mg, 0.43mmol), HOBt hydrate (58mg, 0.43mmol), Hunig's base (0.265ml, 1.56mmol) was added, the mixture was stirred for 16 hours. The reaction mixture diluted with 2-MeTHF (40ml), washed with water (2x20ml), brine (20ml), dried over MgSO_4 , filtered and the filtrate was evaporated under reduced pressure. Purification on silica gel (5-15% MeOH in DCM) afforded tert-butyl N-[3-chloro-4-[7-[(1R)-2-[[2-[2-(dimethylamino)ethylamino]-2-oxo-ethyl]-ethyl-amino]-1-methyl-2-oxo-ethoxy]-2-oxo-chromen-4-yl]phenyl]carbamate as a colorless solid, (0.102g). LC-MS 615.2 $[\text{M}+1]^+$, RT 5.21 min.

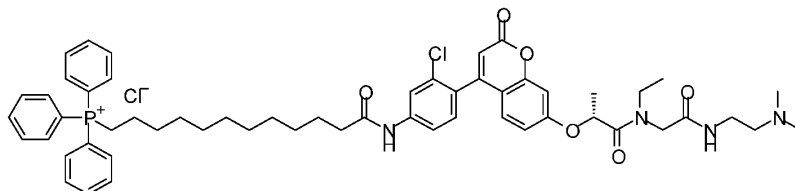
[0081] The product of the prior step was then used to prepare (2R)-2-[4-(4-Amino-2-chloro-phenyl)-2-oxo-chromen-7-yl]oxy-N-[2-[2-(dimethylamino)ethylamino]-2-oxo-ethyl]-N-ethyl-propanamide (shown below).



[0082] To a stirred solution of tert-butyl N-[3-chloro-4-[7-[(1R)-2-[[2-[2-(dimethylamino)ethylamino]-2-oxo-ethyl]-ethyl-amino]-1-methyl-2-oxo-ethoxy]-2-oxo-chromen-4-yl]phenyl]carbamate (0.125g, 0.20mmol) in dry DCM (3ml) at room temperature under nitrogen atmosphere TFA (3ml) was added. The mixture was stirred for 1 hour and the solvent was evaporated under reduced pressure to yield a crude product. The residue was dissolved in EtOAc (30ml), saturated NaHCO_3 (10ml), brine (10ml), dried over MgSO_4 , filtered and the solvent was evaporated under reduced pressure to yield (2R)-2-[4-(4-amino-2-chloro-phenyl)-2-oxo-chromen-7-yl]oxy-N-[2-[2-(dimethylamino)ethylamino]-2-oxo-ethyl]-N-ethyl-propanamide, (0.11g). LC-MS 515.2 $[\text{M}+1]^+$, RT 3.34min.

[0083] Next, Compound [I], having an IUPAC name of [12-[3-chloro-4-[7-[(1R)-2-[[2-[2-(dimethylamino)ethylamino]-2-oxo-ethyl]-ethyl-amino]-1-methyl-2-oxo-ethoxy]-2-oxo-

chromen-4-yl]anilino]-12-oxo-dodecyl]-triphenyl-phosphonium chloride (shown below) was prepared from the product of the prior step.



To a stirred solution of (2R)-2-[4-(4-amino-2-chloro-phenyl)-2-oxo-chromen-7-yl]oxy-N-[2-[2-(dimethylamino)ethylamino]-2-oxo-ethyl]-N-ethyl-propanamide (60mg, 0.12mmol) and 11-carboxyundecyl(triphenyl)phosphonium bromide (57mg, 0.10mmol) in dry DCM (8ml) at room temperature under nitrogen atmosphere DIC (20μl, 0.13mmol) and DMAP (15mg, 0.12mmol)) was added. The mixture was stirred for 18 hour and the solvent was evaporated under reduced pressure to yield a crude product. Purification on silica gel (5-10% MeOH in DCM with 1-2% 7M NH₃ in MeOH) afforded [12-[3-chloro-4-[7-[(1R)-2-[[2-[2-(dimethylamino)ethylamino]-2-oxo-ethyl]-ethyl-amino]-1-methyl-2-oxo-ethoxy]-2-oxo-chromen-4-yl]anilino]-12-oxo-dodecyl]-triphenyl-phosphonium;chloride as a light brown solid, (28.4mg). LC-MS 957.3 [M+1]⁺, RT 5.50 min. ¹H-NMR (MeOD-d₄) δ 8.00 (s, 1H), 7.90-7.85 (m, 3H), 7.82-7.71 (m, 12H), 7.60 (dd, 1H) 7.30 (dd, 1H), 7.30 and 6.96 (m, 1H), 7.08-6.70 (m, 2H), 6.16 (s, 1H), 5.41-5.32 (m, 1H), 4.45-3.96 (m, 2H), 3.79-3.50 (m, 4H), 3.43-3.36 (m, 2H), 3.30-3.25 (m, 2H), 3.00 (s, 3H), 2.90 (s, 3H), 2.41 (t, 2H), 1.76-1.64 (m, 4H), 1.62 (d, 3H), 1.59-1.50 (m, 2H), 1.42-1.18 (m, 12H), 1.10 (t, 3H).

³¹P-NMR (MeOD-d₄) δ 23.7 ppm.

[0084] It should be appreciated that other synthesis methods may be used to arrive at the compounds of the present approach.

[0085] Data generated for embodiments of the present approach show that these compounds have potent anti-cancer activity, in terms of both inhibiting tumor growth and preventing metastasis but are non-toxic towards healthy cells. The following paragraphs summarize data for Compound [I]. It should be appreciated that preliminary results for other embodiments are consistent with the results for Compound [I], but analysis is ongoing. Figs. 1A-1C show mammosphere formation results, SRB assay results on MCF-7 cells, and SRB assay results on BJ1-hTERT cells, for different concentrations of Compound [I]. The asterisks indicate P-value, with ** indicating P < 0.01, *** indicating P < 0.001, and **** indicating P < 0.0001. As can be seen from Fig. 1A, Compound [I] showed inhibition of MCF-7 spheres at

all concentrations and a near complete inhibition at 10 μ M. Compound [I] achieved greater than 50% inhibition at a concentration of 2.5 μ M. Figs. 1B and 1C show results of the SRB assay for (B) MCF-7 cells and (C) hTERT-BJ1 cells. The data show that Compound [I] was cytotoxic starting at 1 μ M towards MCF-7 cells, and cytotoxic starting at about 2.5 μ M towards hTERT-BJ1 cells.

[0086] Figs. 2A and 2B show the effect of Compound [I] at different concentrations on MCF-7 colony formation (A) in petri dishes, and (B) expressed in terms of colony formation relative to the control. As can be seen in the representative phase images of 2D-colonies in Fig. 2A, treatment with Compound [I] significantly decreased MCF-7 colony formation. MCF-7 colony formation was completely inhibited starting at concentrations of 2.5 μ M of Compound [I]. The remarkable inhibition activity against MCF-7 colony formation is clear in graphical form, as Fig. 2B shows that Compound [I] had an IC_{50} of about 0.5 μ M, and complete inhibition at 2.5 μ M.

[0087] Fig. 3A shows mammosphere formation results for Compound [I] at different concentrations on MDA-MB-231 cells, and Fig. 3B shows SRB assay results for Compound [I] on MDA-MB-231 cells. The results show that Compound [I] treatment significantly decreased MDA-MB-231 3D mammosphere formation. Bar graphs are shown as the mean $\pm$ SEM; t-test, two-tailed test: * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.

[0088] Figs. 4A and 4B show the effect of Compound [I] at different concentrations on MDA-MB-231 colony formation (A) representative phase images of 2D-colonies, and (B) expressed in terms of colony formation relative to the control. As can be seen, Compound [I] treatment significantly decreases MDA-MB-231 colony formation. Bar graphs are shown as the mean $\pm$ SEM; t-test, two-tailed test: **** $P < 0.0001$.

[0089] Figs. 5A and 5B show IC_{50} curves for Compound [I] calculated using GraphPad Prism. The results are based on mammosphere formation assay of Compound [I] on both colonies and spheres, for MCF-7 cells (Fig. 5A), and for MDA-MB-231 cells (Fig. 5B). Fig. 5A shows the effect of Compound [I] on MCF-7 spheres and colonies, and Fig. 5B shows the effect of Compound [I] on MDA-MB-231 spheres and colonies. Table 1, below, is a summary of the actual IC_{50} values, demonstrating that Compound [I] is more effective on colony formation, as compared to sphere formation, in both MCF-7 and MDA-MB-231 cell lines.

Cell Line	Colonies IC_{50}	Spheres IC_{50}
MDA-MB-231	1.5 μ M	8.2 μ M

MCF-7	0.7 μ M	3.2 μ M
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[0090] Figs. 6A-6D show mitochondrial respiration, basal respiration, maximal respiration, and ATP production, respectively, for different concentrations of Compound [I] on MCF-7 cells. The metabolic profile MCF-7 was performed using the Seahorse XF96 Analyzer after 6 days of treatment with Compound [I]. The representative tracing of the oxygen consumption rate (OCR) in Fig. 6A shows a reduction in both 0.5 μ M and 1 μ M concentrations, with decreases in basal respiration (Fig. 6B), maximal respiration (Fig. 6C) and ATP production (Fig. 6D). This demonstrates that Compound [I] specifically inhibits mitochondrial respiration.

[0091] Data also shows that Compound [I] does not affect glycolysis. Figs. 7A-7D show glycolytic function, glycolysis, glycolytic capacity, and glycolytic reserve, respectively, for different concentrations of Compound [I] on MCF-7 cells. The representative tracing of the extracellular acidification rate (ECAR) are shown in Fig. 7A. No significant changes are evident in glycolysis (Fig. 7B), glycolytic capacity (Fig. 7C), or glycolytic reserve (Fig. 7D).

[0092] The well-established chorio-allantoic membrane (CAM) assay in chicken eggs were used to quantitatively measure tumor growth in MDA-MB-231 cells. An inoculum of 1×10^6 MDA-MB-231 cells was added onto the Upper CAM of each egg on Day 9 and then eggs were then randomized into groups. On day 10, tumors were detectable and were then treated daily for 8 days with vehicle alone (1% DMSO in PBS), or Compound [I]. After 8 days of drug administration, on day E18, all tumors were weighed. Fig. 8A shows CAM assay tumor growth results for Compound [I]. Bar graphs are shown as the mean $\pm$ SEM; t-test, two tailed test: * $P < 0.05$, **** $P < 0.0001$. These results demonstrate that Compound [I] potently inhibits tumor growth.

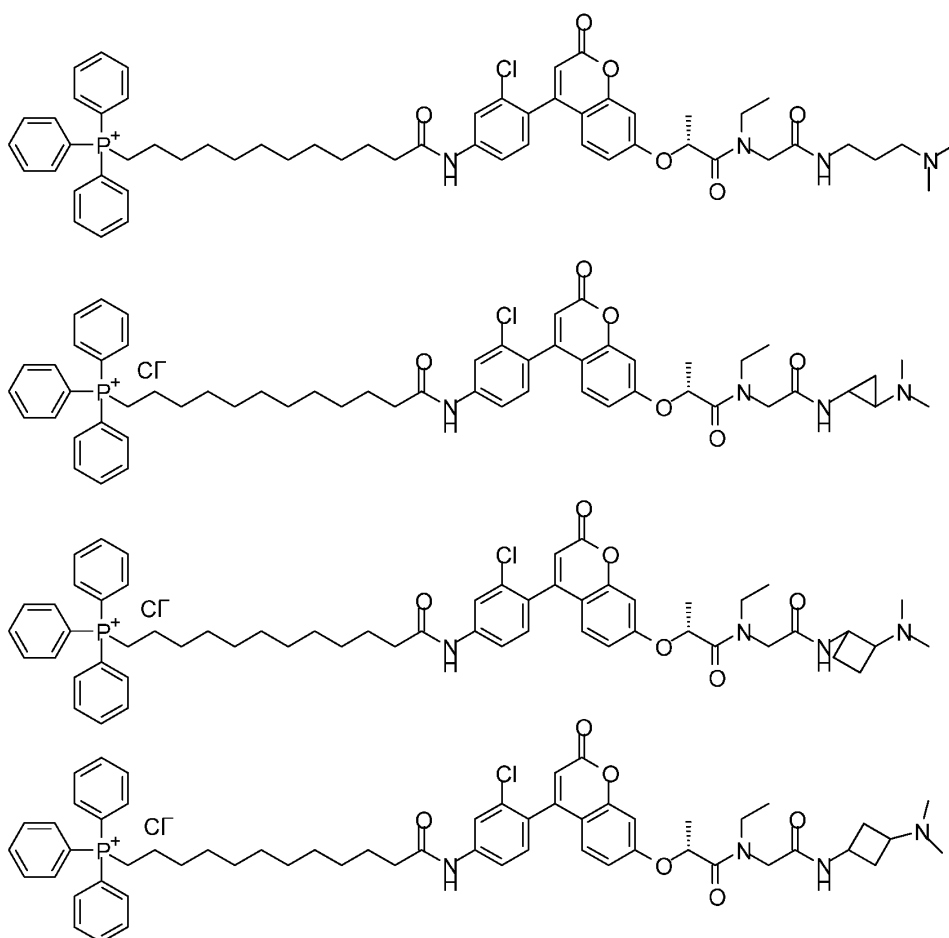
[0093] The data also demonstrates that Compound [I] targets and prevent cancer metastasis. The CAM assay was used in chicken eggs to quantitatively measure spontaneous tumor metastasis in MDA-MB-231 cells. An inoculum of 1×10^6 MDA-MB-231 cells was added onto the Upper CAM of each egg on Day 9 and then eggs were then randomized into groups. On day 10, tumors were detectable and they were then treated daily for 8 days, with vehicle alone (1% DMSO in PBS), or Compound [I]. After 8 days of drug administration, the Lower CAM was collected to evaluate the number of metastatic cells and analyzed by qPCR with specific primers for Human Alu sequences. Fig. 8B shows metastatic invasion results for

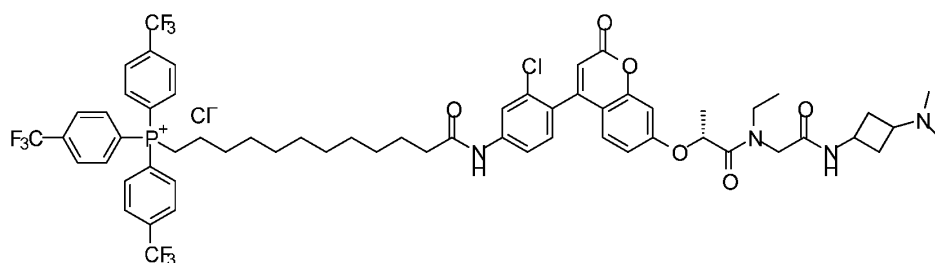
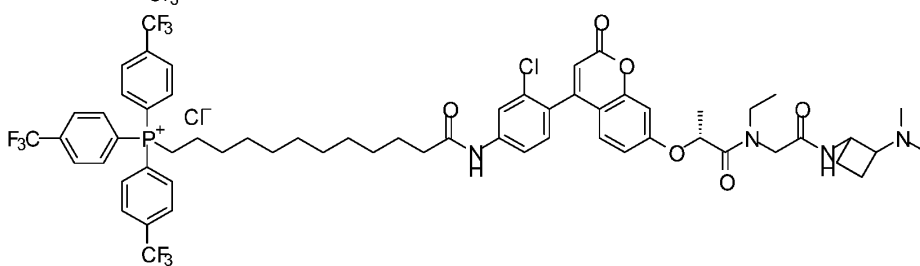
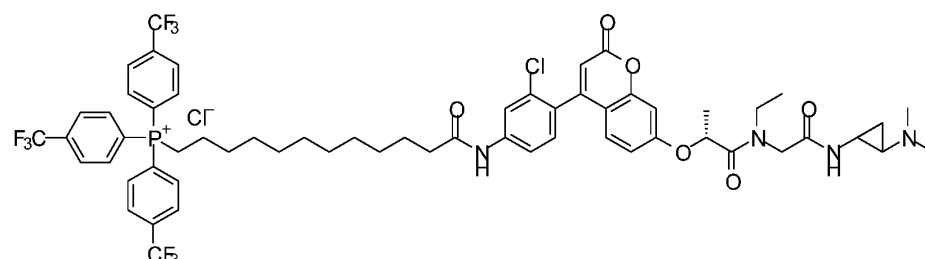
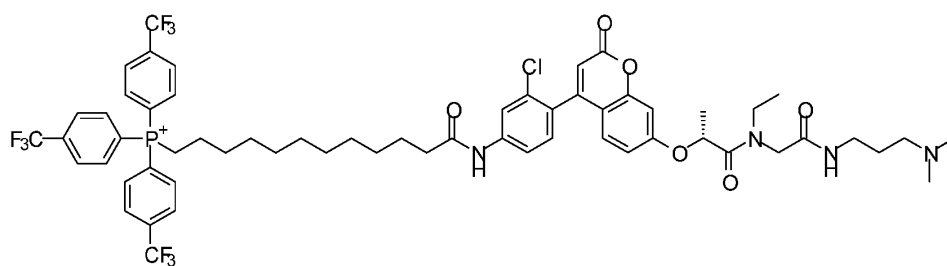
Compound [I]. Bar graph is shown as the mean $\pm$ SEM; t-test, two tailed test: ns $P > 0.05$, ** $P < 0.01$.

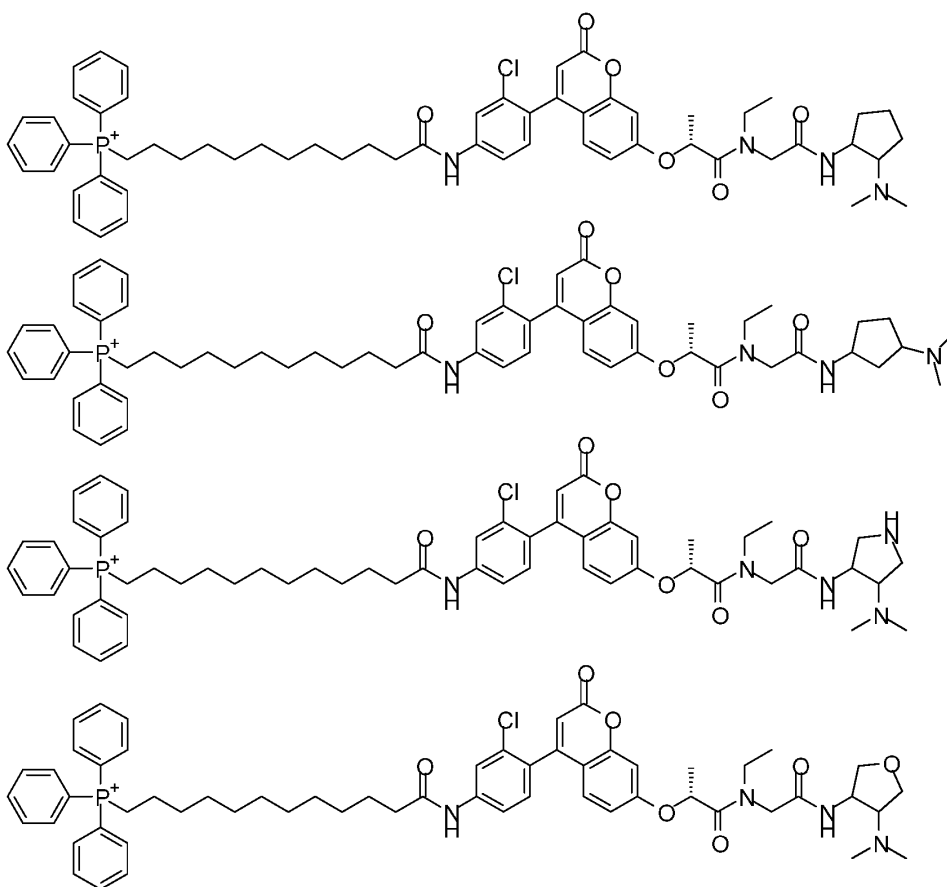
[0094] Fig. 9 shows the results of an *in vivo* chicken embryo assessment for Compound [I]. As can be seen, Compound [I] is not toxic towards normal cells, even at concentrations several orders of magnitude above those found to have potent tumor growth inhibition and anti-metastasis activity.

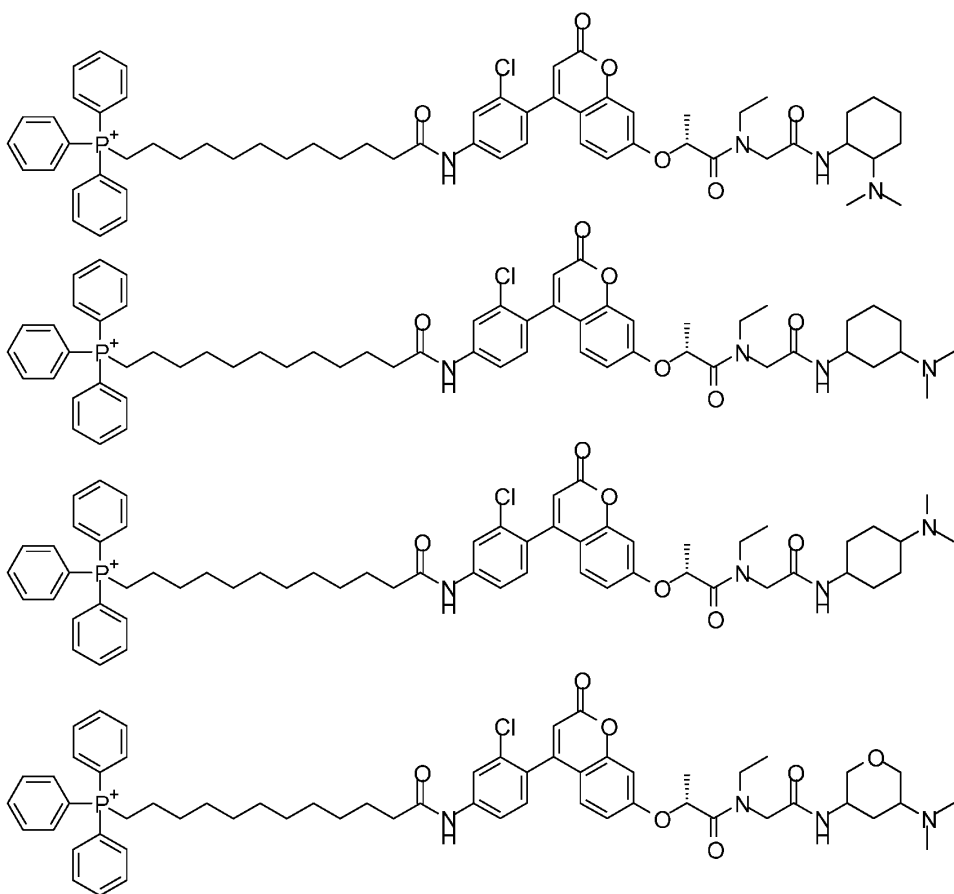
Group	Total	Alive	Dead	%Alive	% Dead
Control	18	16	2	88.89	11.11
Compound [I] 100 μ M	17	17	0	100	0
Compound [I] 250 μ M	16	16	0	100	0
Compound [I] 500 μ M	16	14	2	87.50	12.50

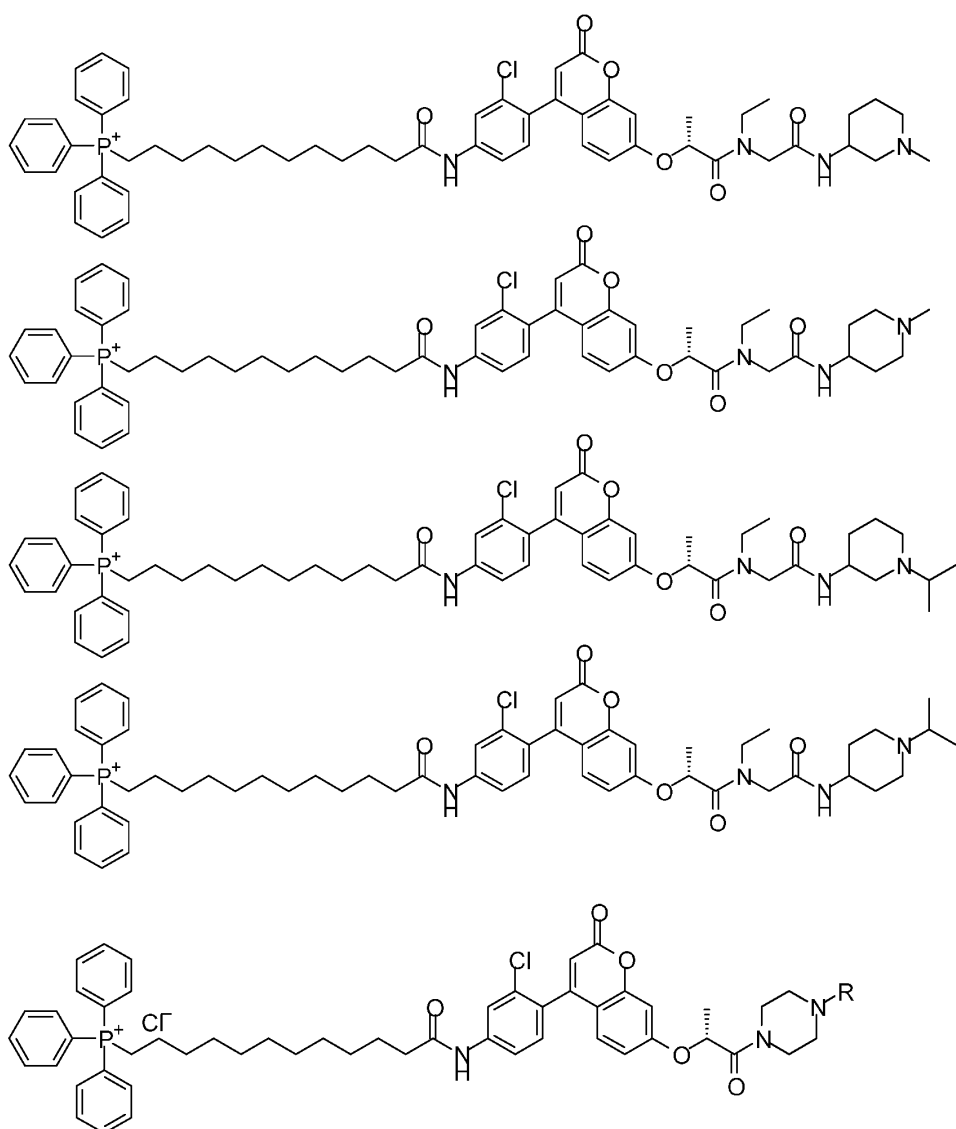
[0095] The following are examples of MSTI compounds according to the present approach. It should be appreciated that while the example compounds are illustrated as chloride salts, other salt forms may be used as described herein.

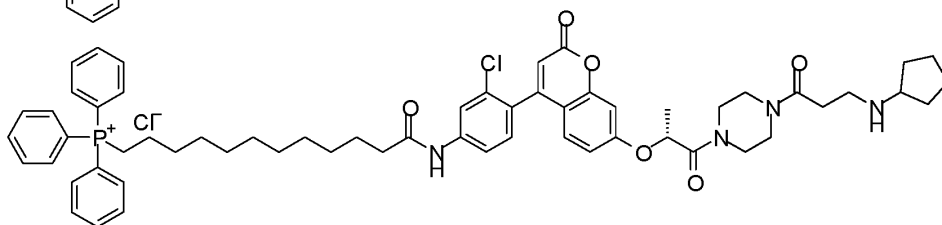
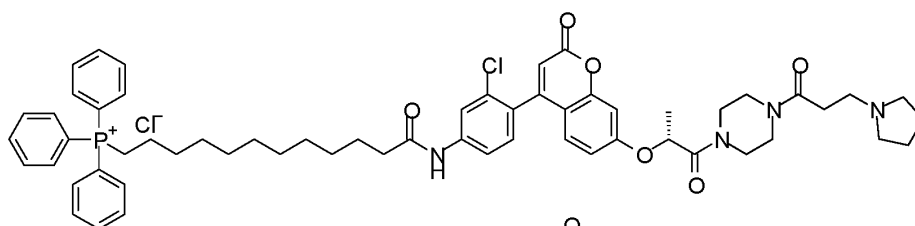
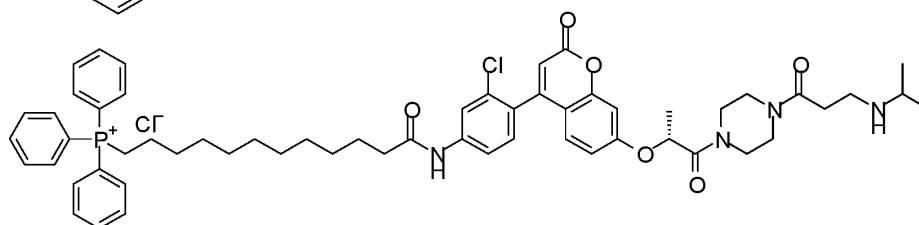
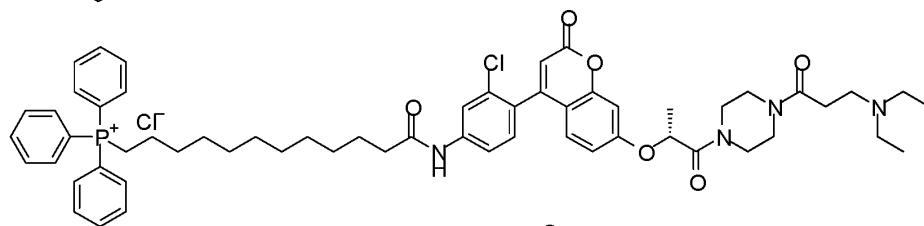
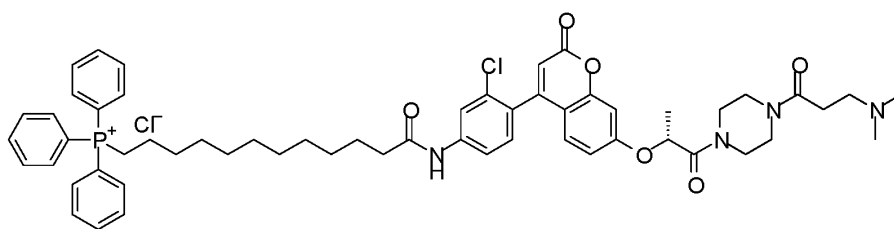


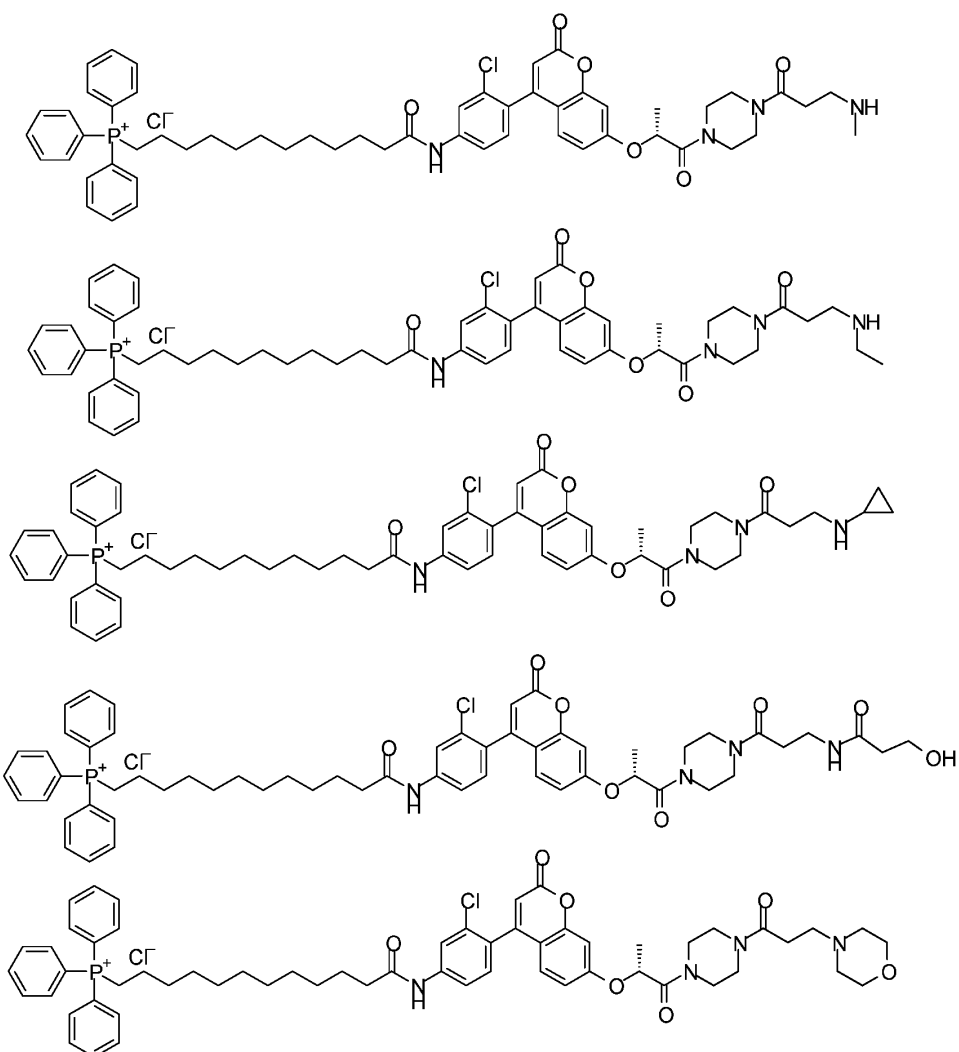


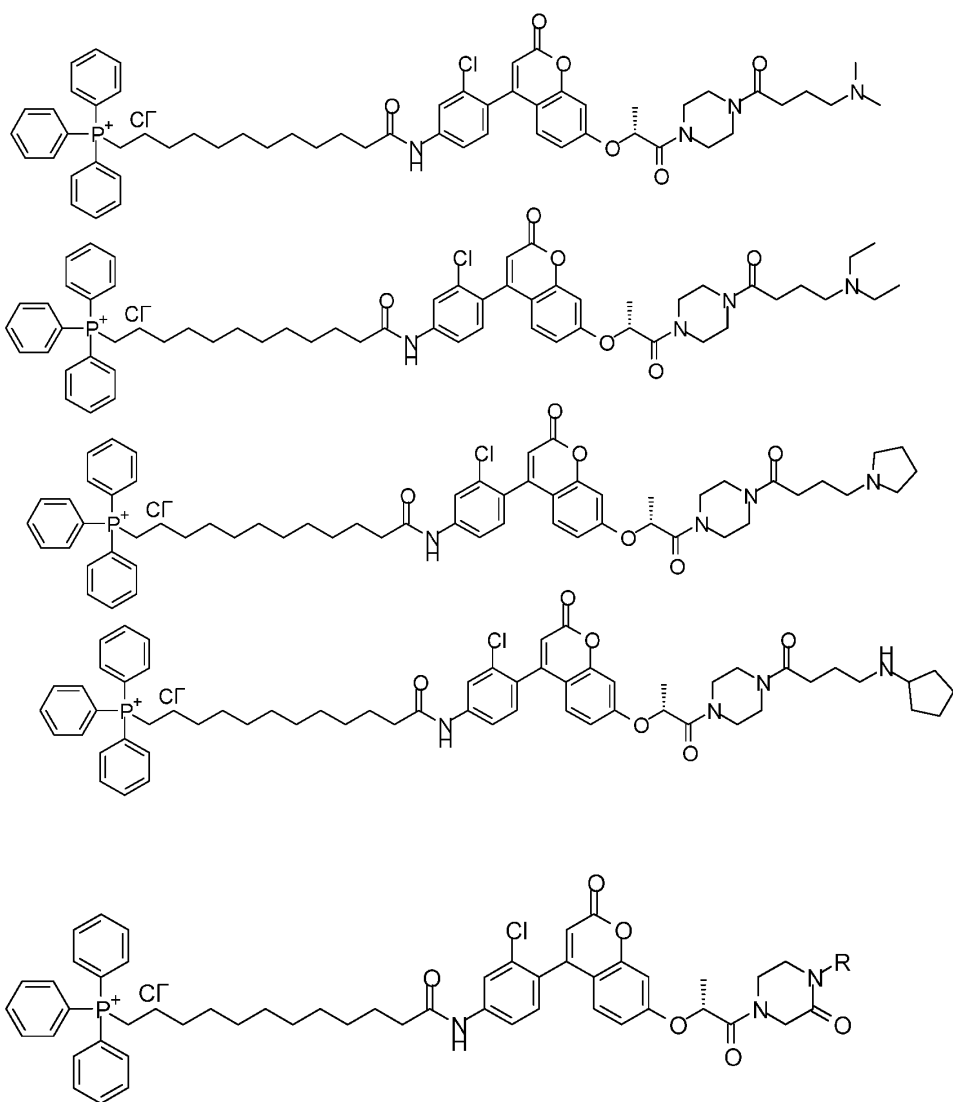


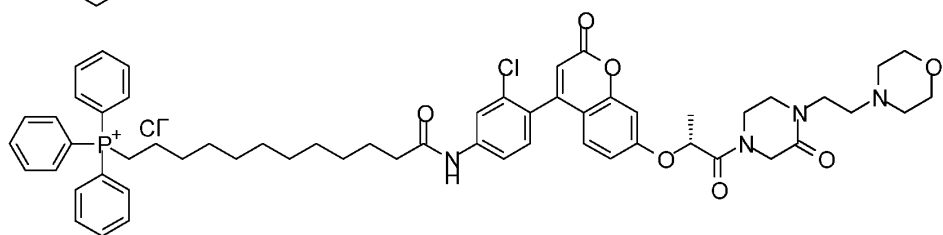
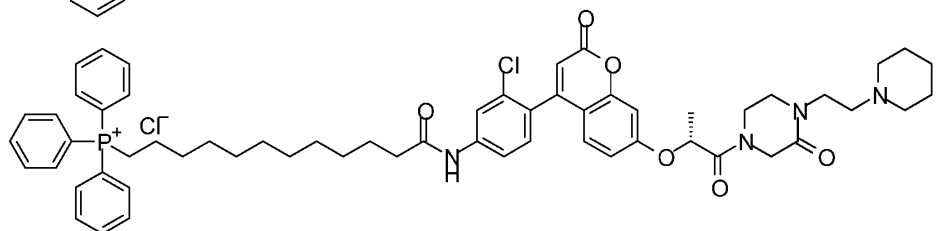
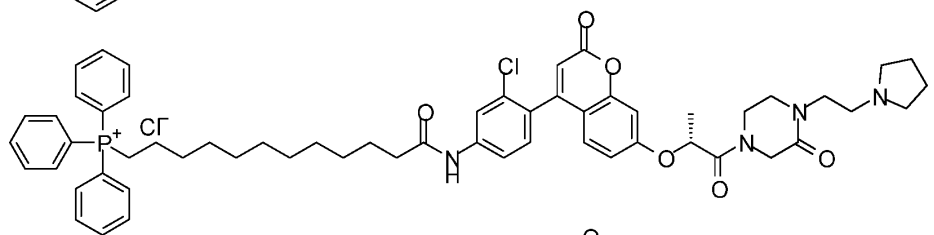
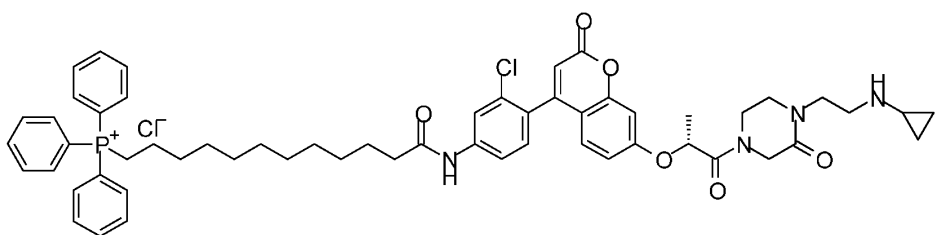


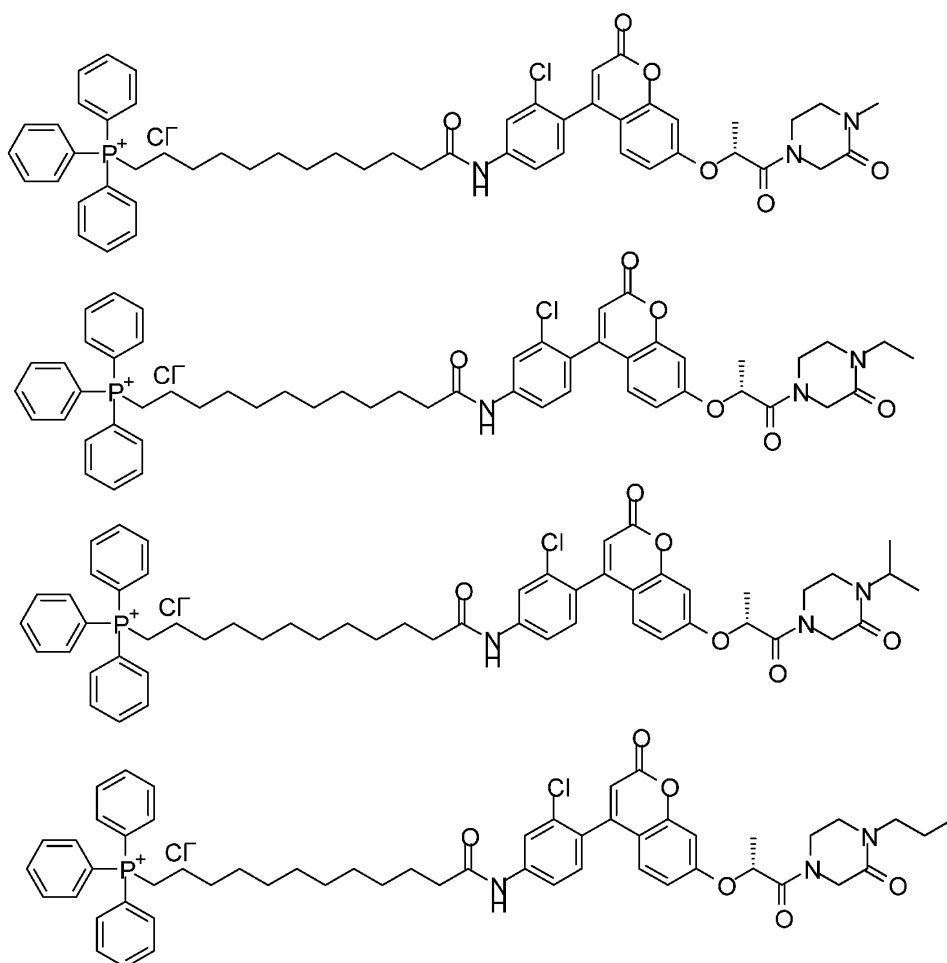












[0096] The description of synthesis methods and reaction schemes use the following abbreviations: acetonitrile (MeCN), ethyl acetate (EtOAc), methanol (MeOH), hydrochloric acid (HCl), magnesium sulphate (MgSO₄), di-isopropyl ethyl amine (DiPEA), dichloromethane (DCM), sodium hydrogen carbonate NaHCO₃, isopropanol (IPA), tetrahydrofuran (THFF), ammonium chloride (NH₄Cl), sodium hydroxide (NaOH), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC HCl), triethylamine (TEA), N-methylmorpholine (NMM), diethylazodicarboxylate (DEAD), dimethylacetamide (DMA), dimethylformamide (DMF), ammonia (NH₃), and 4-dimethylaminopyridine (DMAP), trifluoroacetic acid (TFA), 1-hydroxybenzotriazole (HOBt), ammonia (NH₃).

[0097] The liquid chromatography-mass spectroscopy data was prepared using a Waters Sunfire C18 30x4.6mm column. Gradient eluent: 20-100% acetonitrile/water containing 0.05% formic acid. Time: 0-10min.

[0098] NMR spectra was recorded using Bruker 500MHz Avance III HD spectrometer equipped with broadband prodigy cryoProbe (1H, 13C, 15N, 19F, 31P) and with TopSpin version 3.6, patch level 4, running under a Windows 10 environment.

[0099] The following paragraphs describe the materials and assays used to generate the data described herein. It should be appreciated that the person having an ordinary level of skill in the art may perform the same assays as described herein, and/or may utilize other assays generally known in the art to assess the physical, chemical, and pharmaceutical properties of a compound as described herein.

[00100] Reagents and Model Cell Lines: It should be apparent that other cell lines may be used without departing from the present approach. The human breast adenocarcinoma cell line MCF-7 was from the American Type Culture Collection (ATCC). hTERT-BJ1 cells were from Clontech, Inc. MCF-7 and hTERT-BJ 1 cells were grown in DMEM supplemented with 10% fetal bovine serum, GlutaMAX and 1% penicillin-streptomycin and incubated at 37C in a humidified 5% CO₂ incubator. The medium was changed 2-3 times/week.

[00101] 3D Anchorage Independent Growth Assay: This assay is also referred to as the mammosphere formation assay. A single-cell suspension was prepared using enzymatic, and manual disaggregation (25-g needle). Then, cells were plated at a density of 500 cells/cm² in mammosphere medium (DMEM-F12 + 1X B-27 Plus Supplement + 20 ng/ml EGF + Pen/Strep) under non-adherent conditions, in culture dishes pre-coated with (2-hydroxyethylmethacrylate) (poly-HEMA, Sigma Aldrich Inc.), called "mammosphere plates." Cells were grown for 5 days and maintained in a humidified incubator at 37°C. After 5 days of culture, 3D-mammospheres >50 µm were counted using an eye piece ("graticule"), and the percentage of cells plated which formed spheres was calculated and is referred to as percent mammosphere formation, and was normalized to one (1 = 100% MFE). 3D mammosphere formation efficiency (MFE) was analyzed in both the ATP-low and ATP-high sub-populations of cells. All 3D mammosphere experiments were performed in triplicate, at least 3 times independently.

[00102] Statistical Analysis: All analyses were performed with GraphPad Prism 6. Data were represented as mean ± SD (or ± SEM where indicated). All experiments were conducted at least 3 times independently, with >3 technical replicates for each experimental condition tested (unless stated otherwise, e.g., when representative data is shown). Statistically

significant differences were determined using the Student's t-test or the analysis of variance (ANOVA) test. For the comparison among multiple groups, one-way ANOVA was used to determine statistical significance. $p < 0.05$ was considered significant.

[00103] The present approach includes methods of confirming cell viability. Persons of skill in the art may select one or more methods for confirming cell viability suitable for the particular embodiment. The inventors initially used the sulphorhodamine (SRB) assay, which is based on the measurement of cellular protein content. After treatment for 72 hours in 96-well plates, cells were fixed with 10% trichloroacetic acid (TCA) for 1 hour in the cold room, and were dried overnight at room temperature. Then, cells were incubated with SRB for 15 min, washed twice with 1% acetic acid, and air dried for at least 1 hour. Finally, the protein-bound dye was dissolved in a 10 mM Tris, pH 8.8 solution and read using the plate reader at 540-nm. Using the SRB assay, the inventors selected only the compounds depleting ATP levels without prominent cytotoxicity for further analysis. Prominent cytotoxicity was defined as fewer than 30% of cells still on the plate. Of course, embodiments employing other cell viability confirmation methodology may select compounds for further analysis based on other considerations as may be known in the art.

[00104] The therapeutic agents may be used in the form of pharmaceutical compositions which may be prepared using one or more known methods. For example, a pharmaceutical composition may be prepared by using diluents or excipients such as, for example, one or more fillers, bulking agents, binders, wetting agents, disintegrating agents, surface active agents, lubricants, and the like as are known in the art. Various types of administration unit forms can be selected depending on the therapeutic purpose(s). Examples of forms for pharmaceutical compositions include, but are not limited to, tablets, pills, powders, liquids, suspensions, emulsions, granules, capsules, suppositories, injection preparations (solutions and suspensions), topical creams, and other forms as may be known in the art. For the purpose of shaping a pharmaceutical composition in the form of tablets, any excipients which are known may be used, for example carriers such as lactose, white sugar, sodium chloride, glucose, urea, starch, calcium carbonate, kaolin, cyclodextrins, crystalline cellulose, silicic acid and the like; binders such as water, ethanol, propanol, simple syrup, glucose solutions, starch solutions, gelatin solutions, carboxymethyl cellulose, shalac, methyl cellulose, potassium phosphate, polyvinylpyrrolidone, etc. Additionally, disintegrating agents such as dried starch, sodium alginate, agar powder, laminaria powder, sodium hydrogen carbonate, calcium carbonate, fatty acid esters of polyoxyethylene sorbitan, sodium laurylsulfate, monoglyceride of stearic acid, starch, lactose, etc., may be used. Disintegration inhibitors such as white sugar, stearin, coconut

butter, hydrogenated oils; absorption accelerators such as quaternary ammonium base, sodium laurylsulfate, etc., may be used. Wetting agents such as glycerin, starch, and others known in the art may be used. Adsorbing agents such as, for example, starch, lactose, kaolin, bentonite, colloidal silicic acid, etc., may be used. Lubricants such as purified talc, stearates, boric acid powder, polyethylene glycol, etc., may be used. If tablets are desired, they can be further coated with the usual coating materials to make the tablets as sugar coated tablets, gelatin film coated tablets, tablets coated with enteric coatings, tablets coated with films, double layered tablets, and multi-layered tablets. Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, foams, sprays, aerosols, or oils. Such pharmaceutical compositions may include conventional additives which include, but are not limited to, preservatives, solvents to assist drug penetration, co-solvents, emollients, propellants, viscosity modifying agents (gelling agents), surfactants, and carriers.

[00105] The present approach may be used to prevent and/or reduce the likelihood of tumor recurrence, metastasis. Anti-cancer treatments often fail because the tumor recurs or metastasizes, particularly after surgery. CSC mitochondrial activity is, at least in part, responsible for these causes of treatment failure. Embodiments of the present approach may be used in situations where conventional cancer therapies fail, and/or in conjunction with anti-cancer treatments to prevent or reduce the likelihood of failure due to tumor recurrence and/or metastasis.

[00106] The present approach provides for methods of selectively targeting cancer cells. The target cancer cell may be at least one of a CSC, an energetic cancer stem cell (e-CSC), a circulating tumor cell (CTC, a seed cell leading to the subsequent growth of additional tumors in distant organs, a mechanism responsible for a large fraction of cancer-related deaths), and a therapy-resistant cancer cell (TRCC, a cell that has developed a resistance to one or more of chemotherapies, radiotherapies, and other common cancer treatments).

[00107] As described in Applicant's co-pending U.S. Provisional Patent Application Nos. 62/686,881, filed June 19, 2018, and 62/731,561, filed September 14, 2018, and incorporated by reference in their entirety, e-CSCs represent a CSC phenotype associated with proliferation. In addition to bulk cancer cells and CSCs, it should be appreciated that the present approach may be used to target a hyper-proliferative cell sub-population that the inventors refer to as e-CSCs, which show progressive increases in stemness markers (ALDH activity and mammosphere-forming activity), highly elevated mitochondrial mass, and increased glycolytic and mitochondrial activity.

[00108] In view of the foregoing, it should be appreciated that the present approach may take a wide variety of forms, depending on the embodiment. For example, embodiments of the present approach may take the form of a composition, and in particular a pharmaceutical composition. The therapeutic compound may be the active ingredient, and may be present in a pharmaceutically-effective amount.

[00109] Embodiments of the present approach may also take the form of methods for preventing or reducing the likelihood of at least one of tumor recurrence and metastasis. In some embodiments, an effective amount of a composition having, as a therapeutic agent, a compound of the present approach, may be administered. In some embodiments, an effective amount of a composition having, as its therapeutic agent, an embodiment of a compound as described herein may be administered.

[00110] The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms “a,” “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. The invention includes numerous alternatives, modifications, and equivalents as will become apparent from consideration of the following detailed description.

[00111] It will be understood that although the terms “first,” “second,” “third,” “a),” “b),” and “c),” etc. may be used herein to describe various elements of the invention, and the claims should not be limited by these terms. These terms are only used to distinguish one element of the invention from another. Thus, a first element discussed below could be termed an element aspect, and similarly, a third without departing from the teachings of the present invention. Thus, the terms “first,” “second,” “third,” “a),” “b),” and “c),” etc. are not intended to necessarily convey a sequence or other hierarchy to the associated elements but are used for identification purposes only. The sequence of operations (or steps) is not limited to the order presented in the claims.

[00112] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the present application and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. All

publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. In case of a conflict in terminology, the present specification is controlling.

[00113] Also, as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[00114] The terms “decrease,” “lower,” “lessen,” and “reduce” generally refer to the ability of compositions according to the present approach to produce and/or cause a lesser physiological response (i.e., a measurable downstream effect), as compared to the response caused by either vehicle or a control molecule/composition, e.g., decreased tumor volume. A “decrease” or “reduced” response is typically a “statistically significant” response, and may include an decrease that is 1.1, 1.2, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7, 1.8, etc.) the response produced by normal, untreated, or control-treated subject.

[00115] The phrase “treatment cycle” refers to a course of treatment, such as a dosing schedule that is repeated on a regular or pre-defined basis. A treatment cycle can comprise several days of treatment followed by several days of rest. For example only, an agent may be administered daily for two weeks, followed by two weeks of no treatment, over a 4-week treatment cycle. It should be appreciated that a treatment cycle may depend on a number of factors, such as the disease state, age, sex, and weight of the individual, as well as the particular agent(s) and/or methodologies, to elicit a desired response in the individual.

[00116] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the present invention also contemplates that in some embodiments of the invention, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed.

[00117] The term “about,” as used herein when referring to a measurable value, such as, for example, an amount or concentration and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$, or even $\pm 0.1\%$ of the specified amount. A range provided herein for a measurable value may include any other range and/or individual value therein.

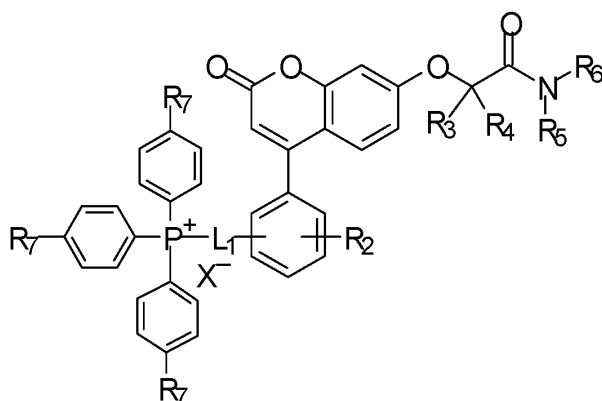
[00118] Having thus described certain embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited by particular

details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope thereof as hereinafter claimed.

CLAIMS

What is claimed is:

1. A compound having the chemical structure:



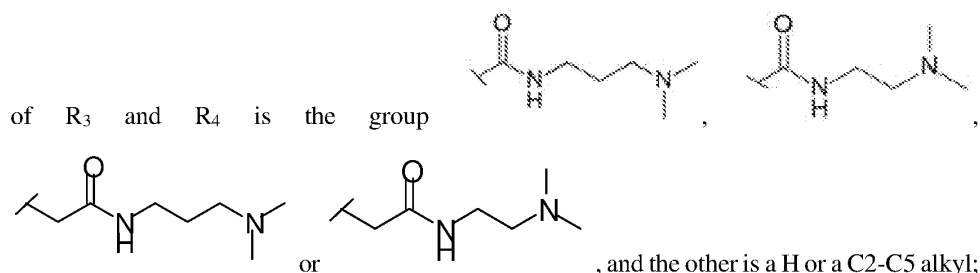
, in which:

R_2 is selected from the group consisting of a halogen, CF_2H , $-CF_3$, $-OCF_2H$, $-OCF_3$, substituted or unsubstituted C5-C18 carboxyl, substituted or unsubstituted C5-C18 alkane, substituted or unsubstituted C5-C18 alkene, substituted or unsubstituted C5-C18 cyclic alkene, substituted or unsubstituted C5-C18 alkyne, substituted or unsubstituted C5-C18 ketone, substituted or unsubstituted C5-C18 aldehyde, substituted or unsubstituted C5-C18 ether, substituted or unsubstituted C5-C18 ester, substituted or unsubstituted C5-C18 amine, substituted or unsubstituted C5-C18 amide, substituted or unsubstituted C5-C18 alkyl-amide, monocyclic or polycyclic arene, heteroarene, phenol, or benzoic acid;

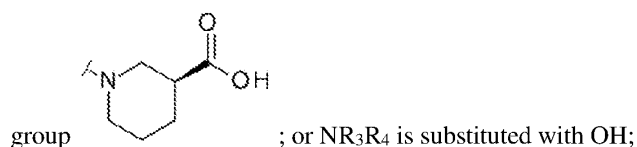
R_3 and R_4 may be the same or different, and are selected from the group consisting of from hydrogen, substituted or unsubstituted C1-C10 alkyl, substituted or unsubstituted C3-C8-cycloalkyl, substituted or unsubstituted pyridine, substituted or unsubstituted C2-C10 carboxyl, substituted or unsubstituted C2-C10 alkene, substituted or unsubstituted C2-C10 alkyne, substituted or unsubstituted C2-C10 ketone, substituted or unsubstituted C2-C10 aldehyde, substituted or unsubstituted C2-C10 ether, substituted or unsubstituted C2-C10 ester, substituted or unsubstituted C2-C10 amine, substituted or unsubstituted C2-C10 amide, substituted or unsubstituted C2-C10 alkyl-amide, substituted or unsubstituted phenol, or benzoic acid;

R_5 and R_6 may be the same or different, and are selected from hydrogen, substituted or unsubstituted C2-C18-alkyl, substituted or unsubstituted C3-C8-cycloalkyl, substituted or unsubstituted pyridine, substituted or unsubstituted C2-C18 carboxyl, substituted or

unsubstituted C2-C18 alkene, substituted or unsubstituted C2-C18 alkyne, substituted or unsubstituted C2-C18 ketone, substituted or unsubstituted C2-C18 aldehyde, substituted or unsubstituted C2-C18 ether, substituted or unsubstituted C2-C18 ester, substituted or unsubstituted C2-C18 amine, substituted or unsubstituted C2-C18 amide, substituted or unsubstituted C2-C18 alkyl-amide, substituted or unsubstituted phenol, or benzoic acid, or one



or R_5 and R_6 form a substituted or unsubstituted C5 or C6 heterocycle; or R_5 and R_6 form the



L_1 is independently selected from the group consisting of $-OR_{1a}$ -, $-SR_{1a}$ -, $-NR_{1a}$ -, $-NR_{1a}C(O)R_{1a}$ -, $-OC(O)R_{1a}$ -, $-C(O)OR_{1a}$ -, $-SO_2R_{1a}$ -, $-S(O)R_{1a}$ -, $-NR_{1a}C(O)-$ -, $-C(O)NR_{1a}$ -, $-NR_{1a}S(O)_2$ -, $S(O)_2NR_{1a}$ -, $-OC(O)NR_{1a}$ -, $-NR_{1a}C(O)OR_{1a}$ -, $-NR_{1a}C(O)NR_{1a}$ -, $-CR_{1a}=CR_{1a}$ - and $-CC-$ -, $-CH_2R_{1a}$ -, wherein

R_{1a} is independently selected from: H, alkyl, alkenyl, alkynyl, cycloalkyl, heterocycloalkyl, heteroaryl or phenyl is optionally substituted where chemically allowed by from 1-4 groups independently selected from oxo, C₁-C₆-alkyl, C₂-C₆-alkynyl, C₂-C₆-alkenyl, C₁-C₆-haloalkyl; OR_a , NR_aR_b , SR_a , $C(O)OR_a$, $C(O)NR_aR_b$, halo, cyano, nitro, $C(O)R_a$, $S(O)_2OR_a$;

R_a is independently at each occurrence selected from: H and C₁-C₆-alkyl; and R_b is independently at each occurrence selected from: H and C₁-C₆-alkyl, $C(O)C_1$ -C₆-alkyl and $S(O)_2$ -C₁-C₆-alkyl;

R_7 is selected from -H, -CF₃, -OCF₃, -OCHF₂, -NO₂, -CN; and

X^- selected from a suitable anion to form a pharmaceutically acceptable salt.

2. The compound of claim 1, wherein X^- is selected from the group consisting of acetate, benzenesulfonate, benzoate, besylate, bitartrate, bromide, bamsylate, chloride, citrate, decanoate, edetate, esylate, fumarate, gluceptate, hexanoate, iodide, isethionate, lactate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, octanoate,

oleate, pamoate, pantothenate, phosphate, propionate, salicylate, stearate, succinate, sulfate, tartrate, teoclate, and tosylate.

3. The compound of any preceding claim, wherein X^- is selected from the group consisting of Cl^- , Br^- , I^- , and $MeSO_3^-$.

4. The compound of any preceding claim, wherein L_1 is selected from the group consisting of $-OR_{1a}$ -, $-NR_{1a}$ -, $-NR_{1a}C(O)R_{1a}$ -, $-NR_{1a}C(O)OR_{1a}$ -, $-NR_{1a}C(O)NR_{1a}$ -, or $-CH_2R_{1a}$ -, and R_{1a} is independently at each occurrence selected from the group consisting of H and C_1 - C_{16} -alkylene attached to $(R_7-(Ph)_3-P^+)$ - at one end, and to N at the other end.

5. The compound of any one of claims 1-3, wherein, L_1 is selected from the group consisting of $-OR_{1a}$ -, $-NR_{1a}$ -, $-NR_{1a}C(O)R_{1a}$ -, $-NR_{1a}C(O)OR_{1a}$ -, $-NR_{1a}C(O)NR_{1a}$ -, or $-CH_2R_{1a}$ -, and R_{1a} is independently at each occurrence selected from the group consisting of H and C_1 - C_{16} -alkylene attached to $(R_7-(Ph)_3-P^+)$ - at one end, and to O at the other end.

6. The compound of any one of claims 1-3, wherein L_1 is selected from the group consisting of $-OR_{1a}$ -, $-NR_{1a}$ -, $-NR_{1a}C(O)R_{1a}$ -, $-NR_{1a}C(O)OR_{1a}$ -, $-NR_{1a}C(O)NR_{1a}$ -, or $-CH_2R_{1a}$ -, and R_{1a} is independently at each occurrence selected from the group consisting of H and C_1 - C_{16} -alkylene attached to $(R_7-(Ph)_3-P^+)$ - at one end, and to C at the other end.

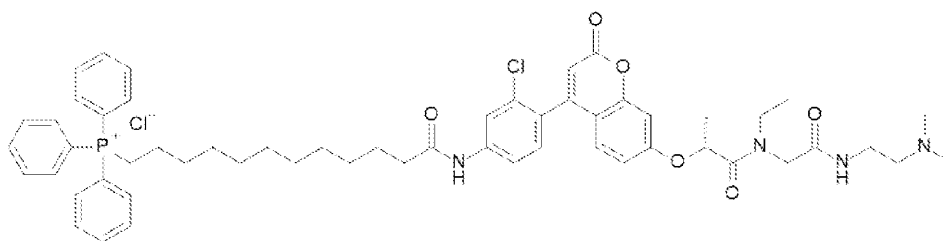
7. The compound of any one of claims 1-3, wherein L_1 is selected from the group consisting of $-NR_{1a}C(O)R_{1a}$ -, R_{1a} is independently at each occurrence selected from the group consisting of H and C_1 - C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)$ -, and R_7 is one of -H and $-CF_3$.

8. The compound of any one of claims 1-3, wherein L_1 is $-NR_{1a}C(O)R_{1a}$ attached to para-position in the phenyl ring, R_{1a} is independently at each occurrence selected from the group consisting of H and C_1 - C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)$ - and R_7 is one of -H and $-CF_3$.

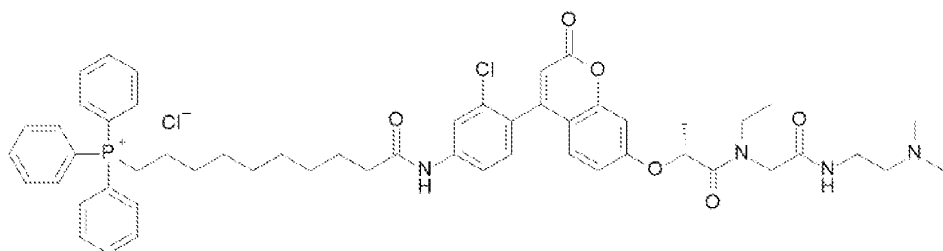
9. The compound of any one of claims 1-3, wherein L_1 is $-NR_{1a}C(O)R_{1a}$ attached to para-position in the phenyl ring, R_{1a} is independently at each occurrence selected from the group consisting of H and C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)$ -, and R_7 is one of -H and $-CF_3$.

10. The compound of any one of claims 1-3, wherein R_2 is Cl^- in ortho position in the phenyl ring and L_1 is $-NR_{1a}C(O)R_{1a}$ attached to para-position in the phenyl ring, R_{1a} is independently at each occurrence selected from the group consisting of H and C_{11} -alkylene attached to $(R_7-(Ph)_3-P^+)$ -, and R_7 is one of -H and $-CF_3$.

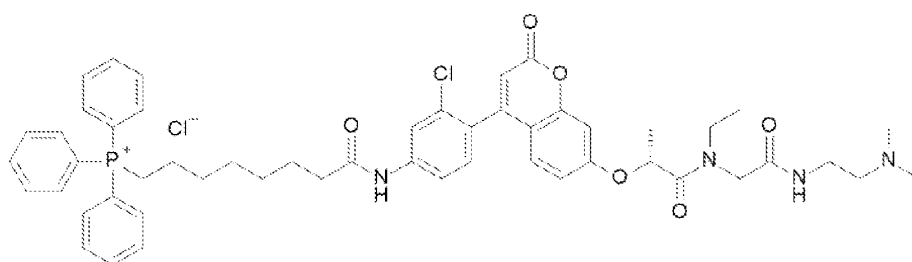
11. The compound of claim 1, having a structure selected from the group consisting of:



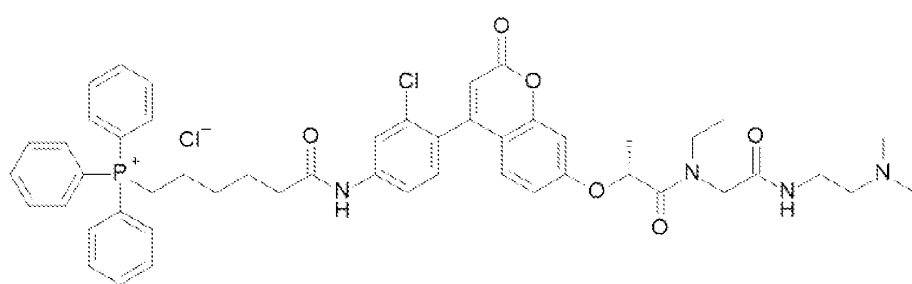
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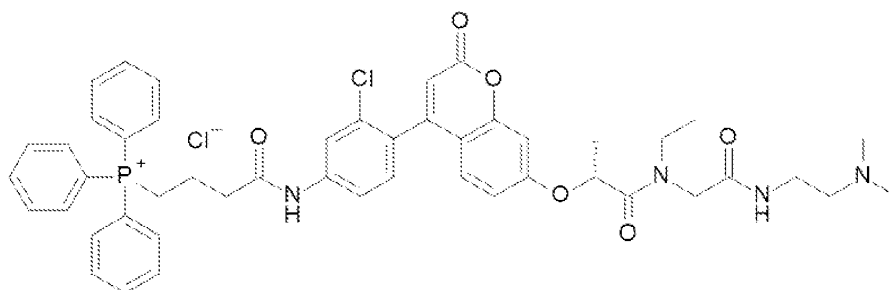
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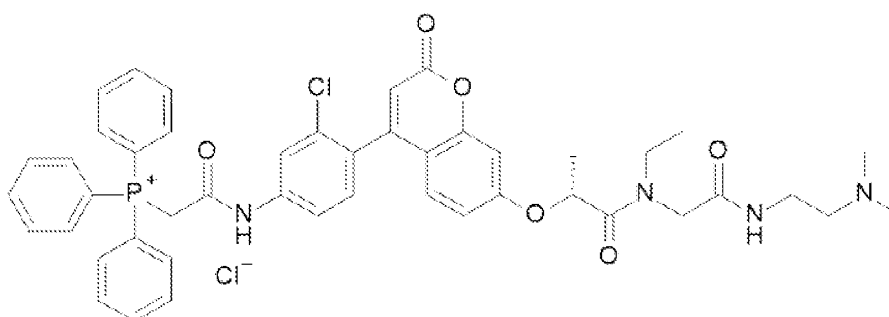
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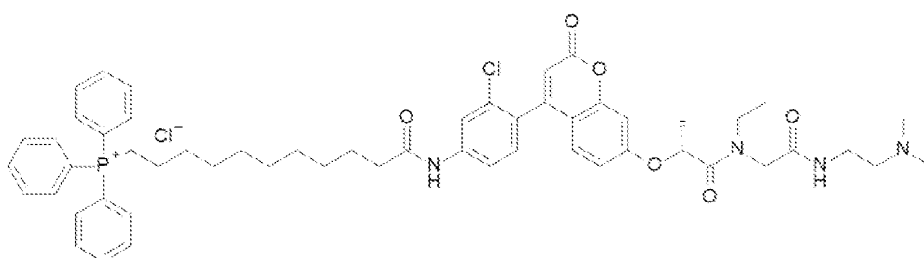
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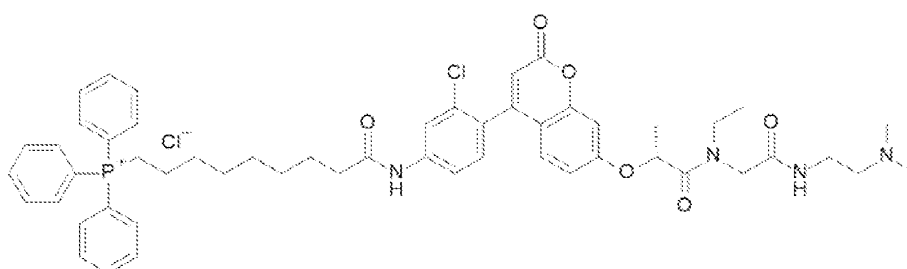
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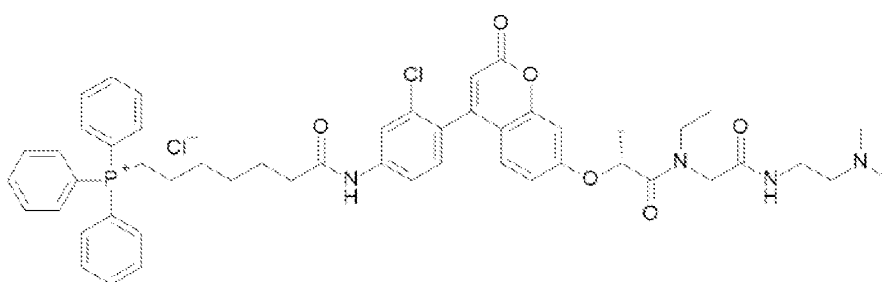
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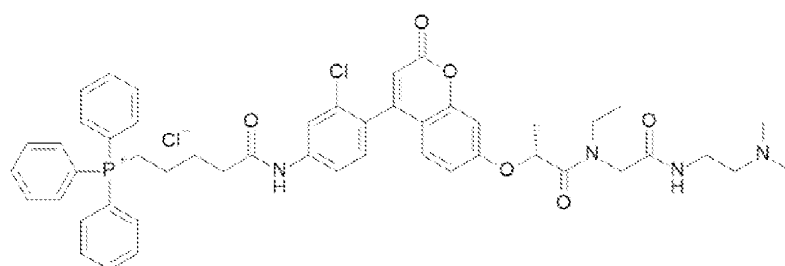
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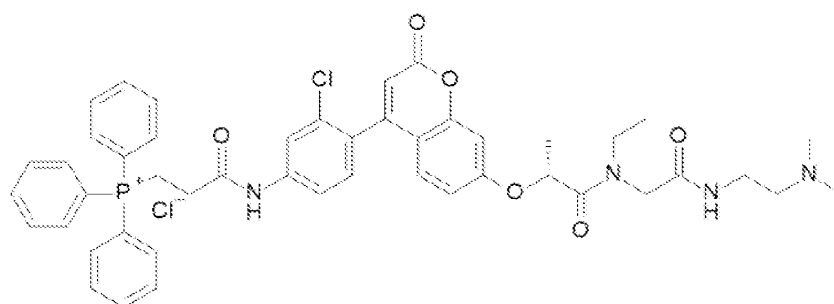


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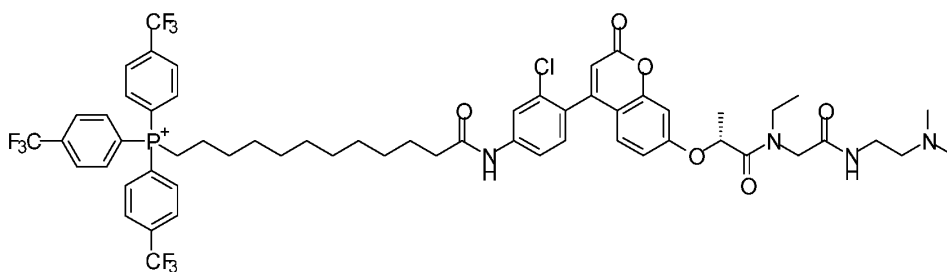


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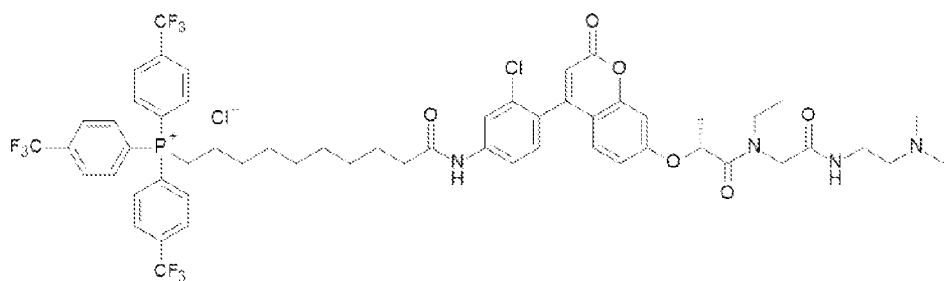
and



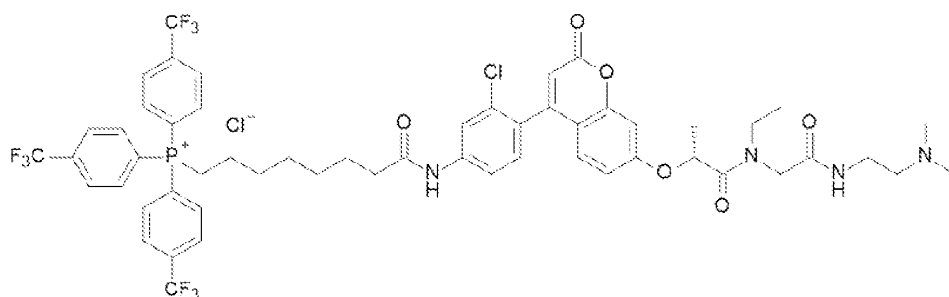
12. The compound of claim 1, having a structure selected from the group consisting of:



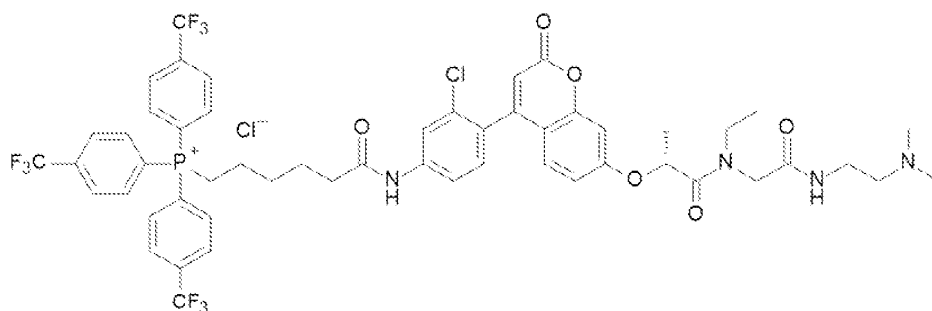
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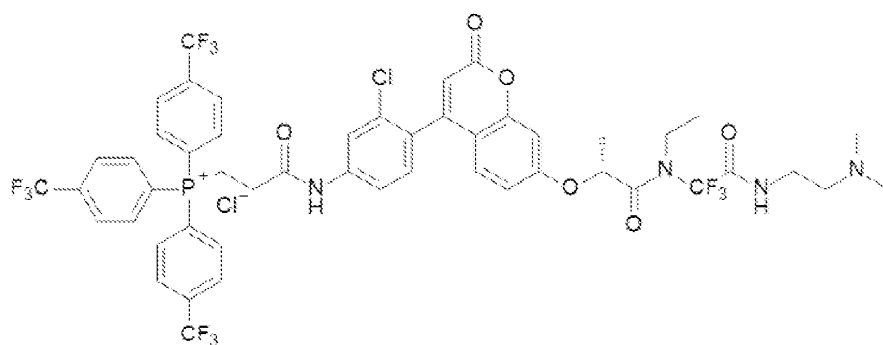
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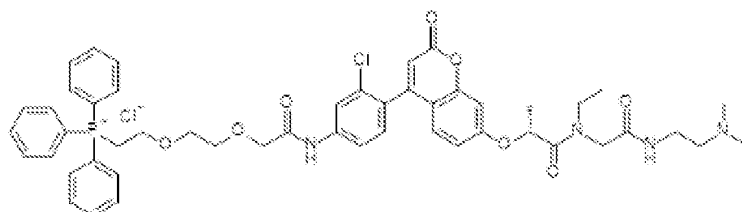
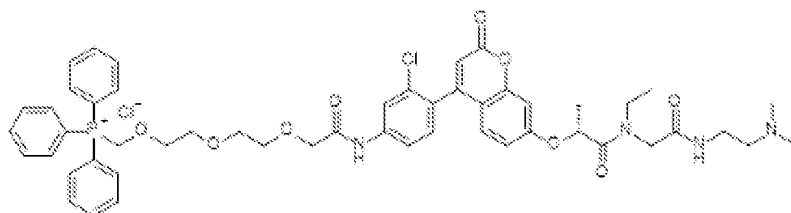
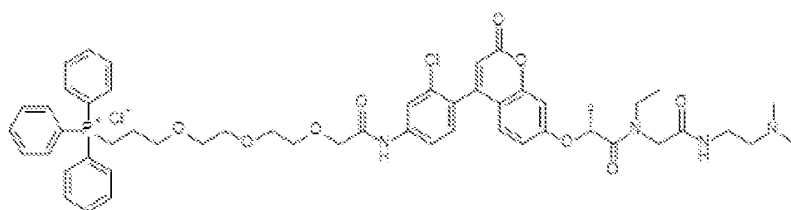
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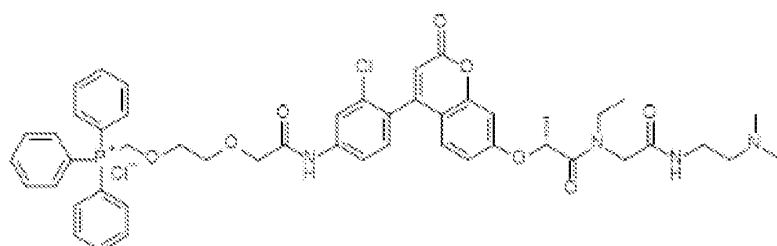
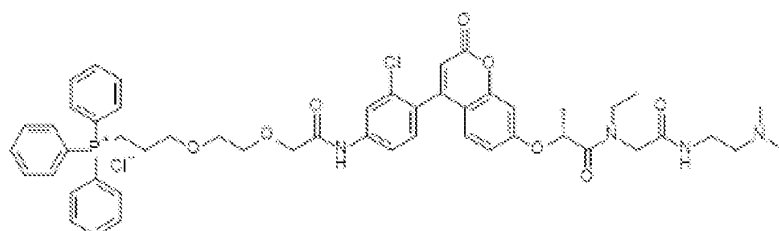
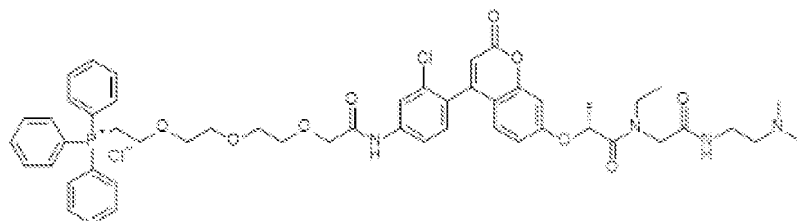
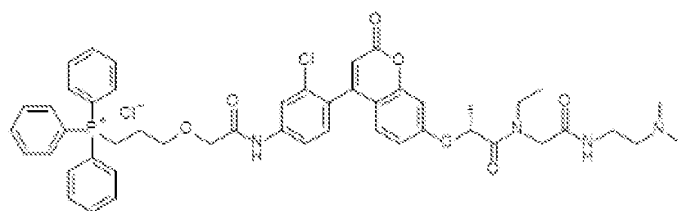


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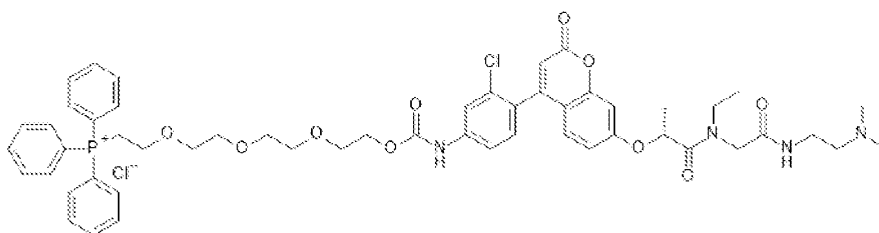


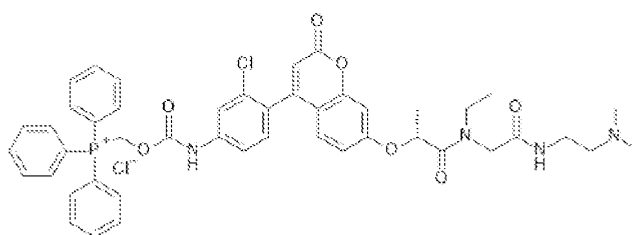
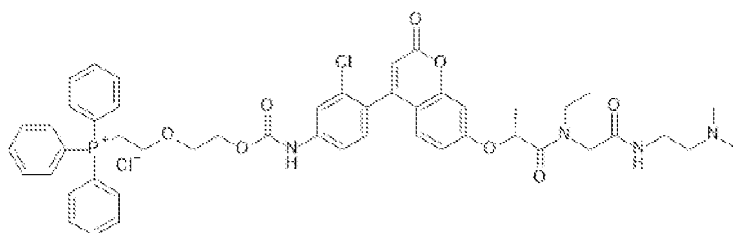
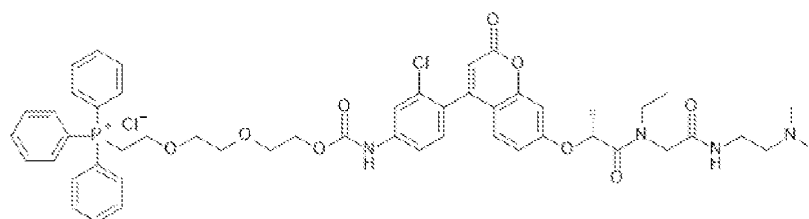
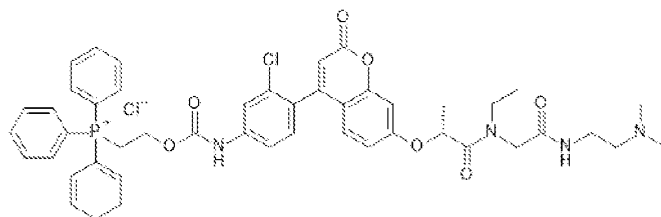
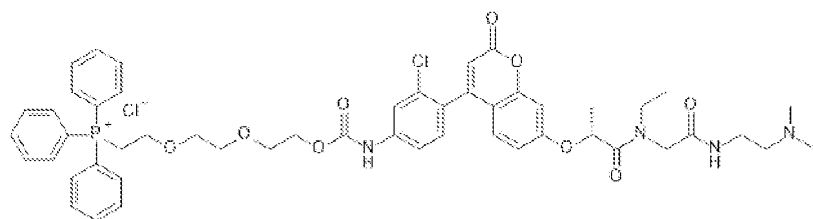
13. The compound of claim 1, having a structure selected from the group consisting of:





14. The compound of claim 1, having a structure selected from the group consisting of:





15. The compound of any one of claims 1-10, wherein R₇ is CF₃ at each occurrence.
16. The compound of any one of claims 1-10, wherein R₇ is H at each occurrence.
17. A pharmaceutical composition comprising the compound of any one of claims 1-16, and a pharmaceutically acceptable carrier.

18. The pharmaceutical composition of claim 17, wherein the carrier is selected from the group consisting of a sugar, lactose, glucose, sucrose, a starch, corn starch, potato starch, cellulose, sodium carboxymethyl cellulose, ethyl cellulose, cellulose acetate, tragacanth, malt, gelatin, talc, cocoa butter, a glycol, propylene glycol, a polyols, glycerin, sorbitol, mannitol, polyethylene glycol, an ester, ethyl oleate, ethyl laurate, agar, a buffering agent, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, a phosphate buffer solution.

19. The pharmaceutical composition of claim 17, wherein the excipient is selected from the group consisting of lactose, white sugar, sodium chloride, glucose, urea, starch, calcium carbonate, kaolin, cyclodextrins, crystalline cellulose, silicic acid.

20. A method for treating or preventing tumor recurrence and/or metastasis, the method comprising administering to a patient at risk of tumor recurrence and/or metastasis a pharmaceutically effective amount of a pharmaceutical composition comprising the compound of any one of claims 1-16.

21. The method of claim 20, wherein the administering is performed at least one of prior to a cancer treatment, with a cancer treatment, and following a cancer treatment.

22. A method for inhibiting the propagation of cancer stem cells in a patient, the method comprising administering to the patient a pharmaceutically effective amount of a pharmaceutical composition comprising the compound of any one of claims 1-16.

23. The method of claim 22, wherein the administering is performed at least one of prior to a cancer treatment, with a cancer treatment, and following a cancer treatment.

24. A method for treating cancer, the method comprising: administering to a person having cancer a pharmaceutically effective amount of a pharmaceutical composition comprising the compound of any one of claims 1-16.

25. The method of claim 24, wherein the administering is performed at least one of prior to a cancer treatment, with a cancer treatment, and following a cancer treatment.