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(54) Title: MITOCHONDRIA-TARGETED LAPACHONE COMPOUNDS AND USES THEREFOR

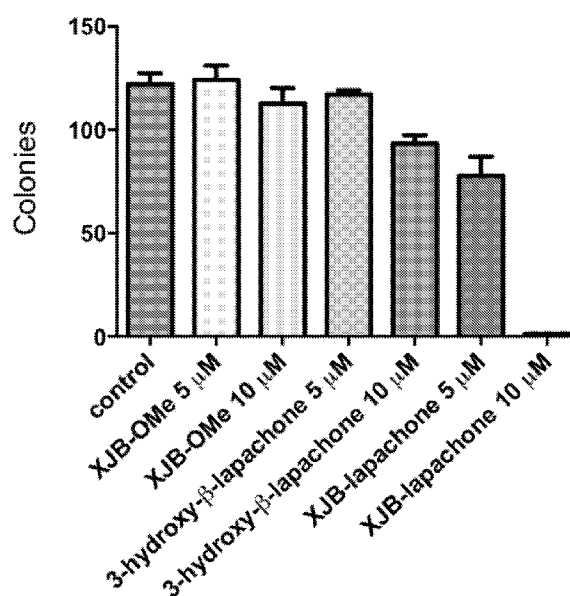


FIG. 5

(57) Abstract: Compounds, compositions and methods useful for treating cancer and neurodegeneration are provided. The compounds comprise a mitochondria-targeting moiety linked to β -lapachone or a β -lapachone derivative.

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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MITOCHONDRIA-TARGETED LAPACHONE COMPOUNDS AND USES THEREFOR

STATEMENT REGARDING FEDERAL FUNDING

This invention was made with government support under GM102989 and GM067082 awarded by the National Institute of Health. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Application No. 62/172,422, filed June 8, 2015, the contents of which are incorporated by reference herein in their entirety.

BACKGROUND

Mitochondria play important roles in tumor cell physiology and survival by providing energy and metabolites for growth and proliferation. As part of their oncogenic status, cancer cells frequently produce increased levels of mitochondrial-generated reactive oxygen species (ROS). However, extensive stimulation of ROS generation in mitochondria can induce cancer cell death, and is one of the major mechanisms of action of many anticancer agents.

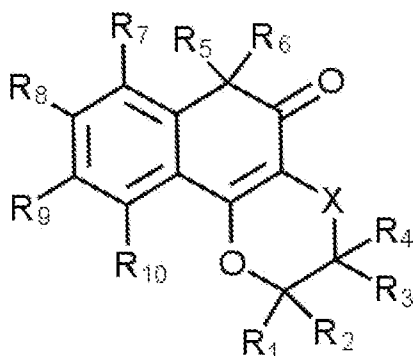
Mitochondria control many growth and survival pathways in eukaryotic cells, not only as the major energy-producing organelle and a regulator of apoptosis and autophagy, but also as a source of steroids, hemes, amino acids, neurotransmitters, and organic acids. One side product of ATP production in the electron transfer chain (ETC) is the generation of up to 90% of total intracellular reactive oxygen species (ROS), including superoxide radical anion, hydroxyl radical, and reactive nitrogen species (RNS). ROS bursts can cause oxidative stress levels associated with acute and chronic damage to cellular components, including mitochondrial membrane lipids such as cardiolipin and mitochondrial DNA (mtDNA). Cumulative oxidative damage will result in functional aberrations of cellular metabolism and signaling pathways and various pathological disorders. However, rather than just representing a chemical nuisance and dangerous progenitor of lipid, protein, and DNA oxidation products, leading to apoptosis, ROS also mediate a diverse range of cellular processes such as signaling cascades, cell cycle control, and autophagy. In fact, controlled ROS release can serve as a modulator of redox-homeostasis and cell signaling pathways. Therefore, rather than complete abolition of ROS, controlled inflection of ROS levels may offer treatment options for a large number of diseases such as cancer, diabetes, cardiovascular and neurodegenerative disorders, where mitochondria have emerged as a key contributing factor.

Cancer cells apparently increase their ROS production relative to normal cells, which is believed to be essential for maintaining oncogenic signaling. Furthermore, disrupting redox homeostasis by either suppressing antioxidant enzymes or enhancing the ROS production in cancer cells has been shown to be able to induce cancer cell death and thus offers an effective strategy for cancer therapy. Directly or indirectly, ROS are also known to play important roles in the anticancer activities of many chemotherapeutic drugs. Unfortunately, these agents often fail to induce cell death in cancer cells due to alterations in their endogenous cell death signaling, such as the p53 pathway. Heat shock proteins compensating for oxidative stress are also frequently upregulated in cancer tissue. Therefore, agents that directly target mitochondria to induce mitochondria-initiated cell death are thought to have greater potential in circumventing tumor cell resistance compared to standard chemotherapeutic drugs. Furthermore, in addition to ROS production, other aspects of mitochondrial metabolism are also required for the function of many types of tumors, including melanoma. Induction of mitochondrial dysfunction is thus considered to be a promising strategy for cancer treatment.

SUMMARY

Provided herein are compounds and compositions comprising a mitochondria-targeting moiety and a β -lapachone or a derivative thereof. In one aspect, the mitochondria-targeting moiety is a membrane-active fragment of gramicidin S or an *E*-alkylene isostere thereof conjugated to a cargo, wherein the fragment of gramicidin S or *E*-alkylene isostere thereof has the sequence of one of: Leu-^DPhe-Pro-Val-Orn, Leu-(*E*)-^DPhe-Pro-Val-Orn, or acylated derivatives thereof, in which pendant amines are acylated or modified with an amine protecting group as indicated herein.

Provided herein is a compound including a mitochondria-targeting group covalently linked to β -lapachone or a β -lapachone derivative, and a salt or ester thereof, including pharmaceutically-acceptable salts or esters thereof. In some aspects, the compound includes a β -lapachone derivative. In further aspects, the β -lapachone derivative has the structure:

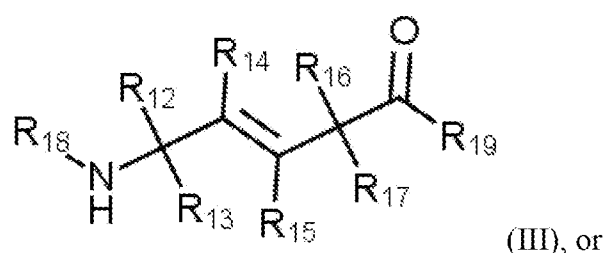


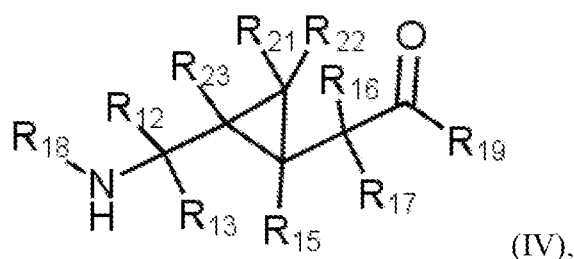
wherein X is $-(CH_2)_n-$ where $n=0-3$, $-S-$, or $-S-CH_2-$; R_1 , is H, OH, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkoxy, C_1-C_6 alkoxy carbonyl, $-(CH_2)_n$ -aryl, $-(CH_2)_n$ -heteroaryl, $-(CH_2)_n$ -phenyl, $-OR_\alpha$, unsubstituted or substituted alkylamino; unsubstituted or substituted dialkylamino, or substituted heterocycle, wherein R_α is H, unsubstituted or substituted amide, unsubstituted or substituted heterocycle, or substituted silyl; R_2 is H, OH, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkoxy, C_1-C_6 alkoxy carbonyl, $-(CH_2)_n$ -aryl, $-(CH_2)_n$ -heteroaryl, $-(CH_2)_n$ -phenyl, a carboxylic acid, or a carboxylic ester; R_3 and R_4 are, independently, H, OH, unsubstituted or substituted C_1-C_6 alkyl, unsubstituted or substituted C_1-C_6 alkenyl, unsubstituted and substituted C_1-C_6 alkoxy, unsubstituted or substituted C_1-C_6 acyl, C_1-C_6 alkoxy carbonyl, amino, alkylamino, dialkylamino, unsubstituted or substituted aryl, unsubstituted or substituted phenyl, unsubstituted or substituted benzyl, unsubstituted or substituted heteroaryl, or unsubstituted or substituted heterocycle, $-(CH_2)_n$ -aryl, $-(CH_2)_n$ -heteroaryl, or $-(CH_2)_n$ -phenyl; R_5 is sulfonate and R_6 is hydroxyl, or R_5 and R_6 , together, are carbonyl ($=O$); R_7 , R_8 , R_9 , and R_{10} are each, independently, H, halogen, hydroxyl, unsubstituted or substituted C_1-C_6 alkyl, unsubstituted or substituted C_1-C_6 alkoxy, C_1-C_6 alkoxy carbonyl, amino, alkylamino, dialkylamino, unsubstituted or substituted aryl, unsubstituted or substituted phenyl, unsubstituted or substituted benzyl, unsubstituted or substituted heteroaryl, or unsubstituted or substituted heterocycle, nitro, cyano, carboxylic acid, or amide; wherein X is not C when R_1 and R_2 are methyl, R_5 and R_6 , together are carbonyl, and R_3 , R_4 , R_7 , R_8 , R_9 , and R_{10} are H (that is, the β -lapachone derivative is not β -lapachone), or an isostere thereof, or a pharmaceutically acceptable salt or ester thereof.

In aspects, the β -lapachone or the β -lapachone derivative is linked to the mitochondria-targeting group at position C_2 or C_3 . In some aspects, the β -lapachone or the β -lapachone derivative is linked to a mitochondria-targeting group at position C_3 .

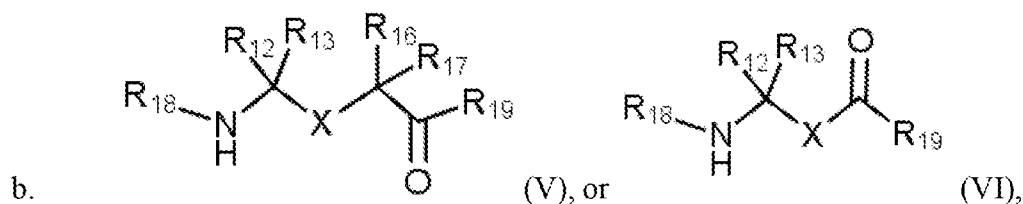
In aspects, the compound has a structure:

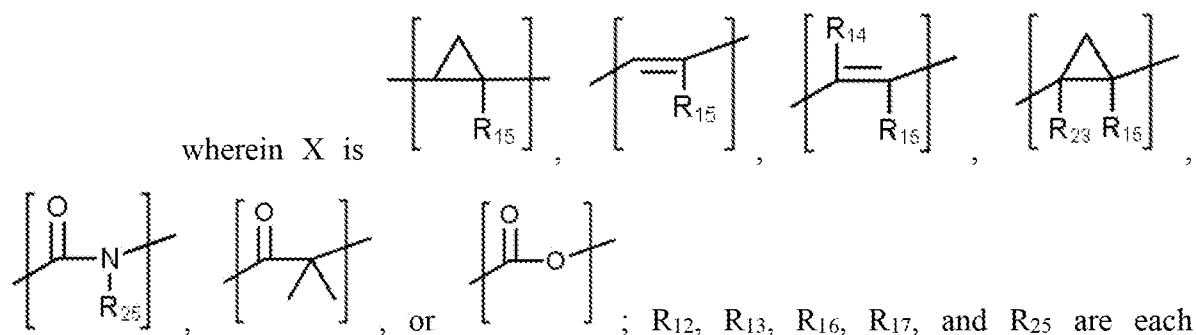
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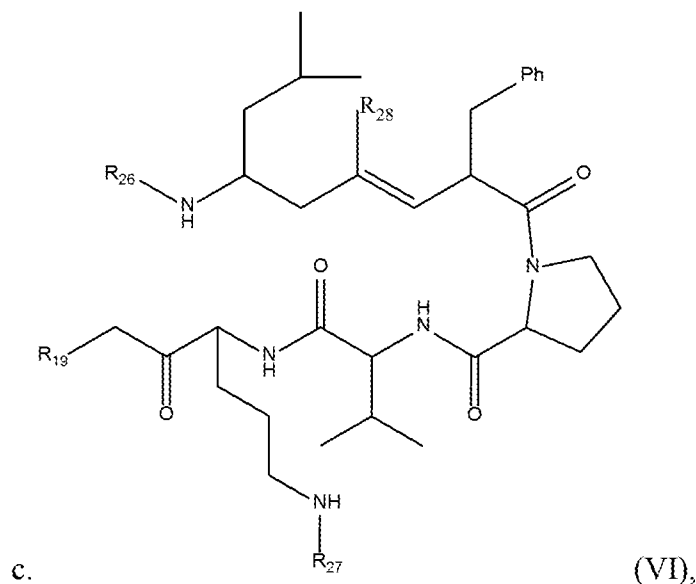


wherein R₁₂, R₁₃, R₁₆, and R₁₇ are independently hydrogen, hydroxyl, halo, a C₁-C₆ straight or branched-chain alkyl, or a C₁-C₆ straight or branched-chain alkyl further comprising a phenyl (C₆H₅) group, wherein the C₁-C₆ straight or branched-chain alkyl group or the C₁-C₆ straight or branched-chain alkyl group comprising a phenyl group is unsubstituted or is methyl-, hydroxyl- or halo-substituted, for example, and without limitation, R₁₂, R₁₃, R₁₆, and R₁₇ are independently methyl-, hydroxyl- or fluoro-substituted, including: methyl, ethyl, propyl, 2-propyl, butyl, t-butyl, pentyl, hexyl, benzyl, hydroxybenzyl (e.g., 4-hydroxybenzyl), phenyl, or hydroxyphenyl; R₁₅ is hydrogen, a halo, a C₁-C₆ straight or branched-chain alkyl, or a C₁-C₆ straight or branched-chain alkyl further comprising a phenyl (C₆H₅) group, wherein the C₁-C₆ straight or branched-chain alkyl group or the C₁-C₆ straight or branched-chain alkyl group comprising a phenyl group is unsubstituted or is methyl-, hydroxyl- or halo-substituted; R₁₈ is -C(O)-R₂₄, -C(O)O-R₂₄, or -P(O)-(R₂₄)₂, wherein R₂₄ is C₁-C₆ straight or branched-chain alkyl or a C₁-C₆ straight or branched-chain alkyl optionally comprising one or more (C₆H₅) groups that are independently unsubstituted, or methyl-, ethyl-, hydroxyl-, halo-substituted or fluoro-substituted, for example and without limitation, R₁₈ is Ac (Acetyl, R = -C(O)-CH₃), Boc (R = -C(O)O-*tert*-butyl), Cbz (R = -C(O)O-benzyl (Bn)), or a diphenylphosphate group; R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β-lapachone or a derivative thereof according to any aspect as described above; R₁₄ is a halo, a C₁-C₆ straight or branched-chain alkyl or a C₁-C₆ straight or branched-chain alkyl further comprising one or more (C₆H₅) groups that are independently unsubstituted, or methyl-, ethyl-, hydroxyl- or halo-substituted; and R₂₁, R₂₂, and R₂₃ are independently H or a halo;





independently hydrogen, halo, a C₁-C₆ straight or branched-chain alkyl, or a C₁-C₆ straight or branched-chain alkyl further comprising a phenyl (C₆H₅) group, wherein the C₁-C₆ straight or branched-chain alkyl group or the C₁-C₆ straight or branched-chain alkyl group comprising a phenyl group is unsubstituted or is methyl-, hydroxyl- or halo-substituted; R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β-lapachone or a derivative thereof according to any aspect as described above; and R₁₈ is -C(O)-R₂₄, -C(O)O-R₂₄, or -P(O)-(R₂₄)₂, wherein R₂₄ is C₁-C₆ straight or branched-chain alkyl or a C₁-C₆ straight or branched-chain alkyl optionally comprising one or more (C₆H₅) groups that are independently unsubstituted, or methyl-, ethyl-, hydroxyl-, halo-substituted or fluoro-substituted, for example and without limitation, R₁₈ is Ac (Acetyl, R = -C(O)-CH₃), Boc (R = -C(O)O-*tert*-butyl), Cbz (R = -C(O)O-benzyl (Bn)), or a diphenylphosphate group; or

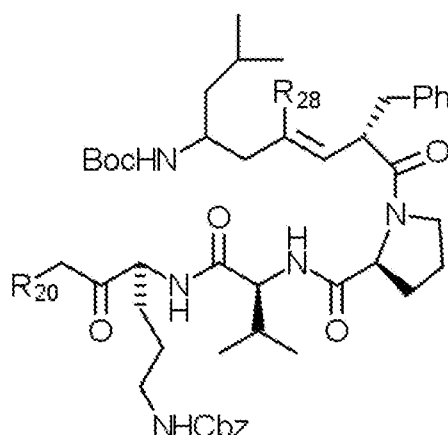


wherein R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β-lapachone, or a β-lapachone derivative according to any aspect described above; R₂₆ and R₂₇, independently are an amine protecting group or acylated. In one aspect, R₂₆ and R₂₇ are protecting groups independently selected from the group consisting of: 9-fluorenylmethyloxy carbonyl (Fmoc), *t*-butyloxycarbonyl (Boc), benzhydryloxycarbonyl (Bhoc), benzyloxycarbonyl (Cbz), O-

nitroveratryloxycarbonyl (Nvoc), benzyl (Bn), allyloxycarbonyl (alloc), trityl (Trt), 1-(4,4-dimethyl-2,6-dioxacyclohexylidene)ethyl (Dde), diathiasuccinoyl (Dts), benzothiazole-2-sulfonyl (Bts), dimethoxytrityl (DMT) and monomethoxytrityl (MMT), and R_{28} is H or methyl. In one aspect, R_{26} is Boc and R_{27} is Cbz. Ph is phenyl.

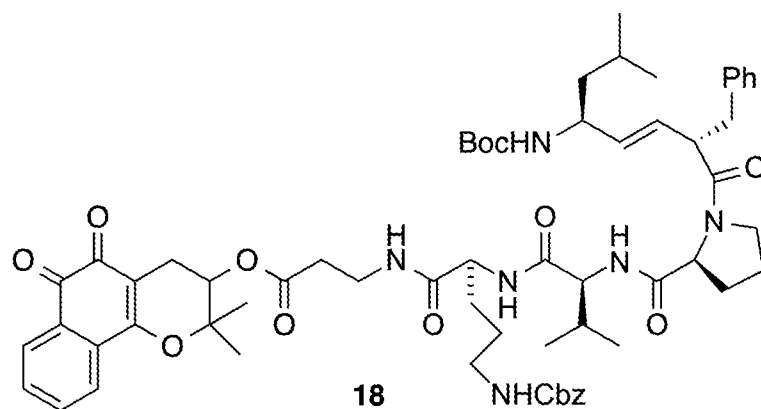
In some aspects, the compound has the structure of (III) or (IV) above. In other aspects, the compound has the structure of (V) or (VI) above. In still further aspects, the compound has the structure of (VII) above.

In aspects, the compound has the structure:



wherein R_{19} is $-\text{NH}-R_{20}$, $-\text{O}-R_{20}$ or $-\text{CH}_2-R_{20}$, where R_{20} is β -lapachone, or a β -lapachone derivative.

In aspects, the compound has the structure:



an isostere thereof, or a pharmaceutically acceptable salt or ester thereof.

Also provided herein is a composition including a first compound as described above and a pharmaceutically-acceptable excipient. In aspects the composition includes a second chemotherapeutic agent that is different from the first compound. In some aspects, the second chemotherapeutic agent is either β -lapachone, a β -lapachone derivative, abiraterone acetate,

altretamine, amsacrine, anhydro vinblastine, auristatin, bafetinib, bexarotene, bicalutamide, BMS 184476, 2,3,4,5,6-pentafluoro-N-(3-fluoro-4-methoxyphenyl)benzene sulfonamide, bleomycin, bosutinib, busulfan, cachectin, cemadotin, chlorambucil, cyclophosphamide, 3',4'-didehydro-4'-deoxy-8'-norvin-calculoblastine, docetaxol, doxorubicin, carboplatin, carmustine (BCNU), chlorambucil, cisplatin, cryptophycin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, daunorubicin, decitabine, dolastatin, doxorubicin (adriamycin), etoposide, etoposide phosphate, 5-fluorouracil, finasteride, flutamide, hydroxyurea, hydroxyureataxanes, ifosfamide, imatinib, irinotecan, liarozole, lonidamine, lomustine (CCNU), MDV3100, mechlorethamine (nitrogen mustard), melphalan, mitoxantrone, mivobulin isethionate, rhizoxin, sertene, streptozocin, mitomycin, methotrexate, nilotinib, nilutamide, onapristone, oxaliplatin, paclitaxel, ponatinib, prednimustine, procarbazine, RPRI 09881, stramustine phosphate, tamoxifen, tasonermin, taxol, teniposide, topotecan, tretinoin, vinblastine, vincristine, vindesine sulfate, vinflunine, or pharmaceutically acceptable salts or esters thereof.

Also provided herein is a method of treating a cancer in a patient, including the step of administering to a patient an amount of a first compound according described above in an amount effective to increase reactive oxygen species (ROS) in mitochondria of cancer cells of a patient. In some aspects, the method further includes administering a radiation therapy to the patient while the first compound is present in the patient. In some aspects the method further includes administering a second chemotherapeutic agent that differs from the first compound. In further aspects, the second chemotherapeutic agent is either β -lapachone, a β -lapachone derivative, abiraterone acetate, altretamine, amsacrine, anhydro vinblastine, auristatin, bafetinib, bexarotene, bicalutamide, BMS 184476, 2,3,4,5,6-pentafluoro-N-(3-fluoro-4-methoxyphenyl)benzene sulfonamide, bleomycin, bosutinib, busulfan, cachectin, cemadotin, chlorambucil, cyclophosphamide, 3',4'-didehydro-4'-deoxy-8'-norvin-calculoblastine, docetaxol, doxorubicin, carboplatin, carmustine (BCNU), chlorambucil, cisplatin, cryptophycin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, daunorubicin, decitabine, dolastatin, doxorubicin (adriamycin), etoposide, etoposide phosphate, 5-fluorouracil, finasteride, flutamide, hydroxyurea, hydroxyureataxanes, ifosfamide, imatinib, irinotecan, liarozole, lonidamine, lomustine (CCNU), MDV3100, mechlorethamine (nitrogen mustard), melphalan, mitoxantrone, mivobulin isethionate, rhizoxin, sertene, streptozocin, mitomycin, methotrexate, nilotinib, nilutamide, onapristone, oxaliplatin, paclitaxel, ponatinib, prednimustine, procarbazine, RPRI 09881, stramustine phosphate, tamoxifen, tasonermin,

taxol, teniposide, topotecan, tretinoin, vinblastine, vincristine, vindesine sulfate, vinflunine, or pharmaceutically acceptable salts or esters thereof.

Also provided herein is a method of treating neurodegeneration, including the step of administering to a patient an amount of a compound as described above in an amount effective to decrease reactive oxygen species (ROS) production in mitochondria of a cell of a patient.

Also provided herein is a method of decreasing oxidative damage in a patient including the step of administering to a patient an amount of a compound as described above in an amount effective to decrease reactive oxygen species (ROS) production in mitochondria of a cell of a patient.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a selection of mitocans – cytotoxic agents with mitochondrial targeted mechanisms of action.

FIGURE 2 shows synthesis of a mitochondrial targeted β -lapachone conjugate according to one aspect of the present invention.

FIGURE 3A-3E shows mitochondrial targeted β -lapachone (XJB-lapachone, **18**) shows enhanced efficacy and is tumor cell selective. (3A) A549 non-small cell lung cancer cells were treated with XJB-OMe, 3-hydroxy- β -lapachone (**14**), or XJB-lapachone (**18**) at indicated concentrations for 24 h. Cell viability was determined by a CellTiter-Blue assay. Data represents the mean $\pm$ SEM. (3B) PEO1 ovarian cancer cells, MDA-MB-231 breast cancer cells, and 983B melanoma cells were treated with XJB-lapachone (**18**) at indicated concentrations for 24 h. Cell viability was determined by a CellTiter-Blue assay. Data represents the mean $\pm$ SEM. (3C) Equal numbers of A549 and IMR90 lung fibroblast cells were treated with 10 μ M XJB-lapachone (**18**) for 4 h. XJB-lapachone was then washed away and cells were incubated in drug free media for 3 days. Cell viability was determined by a CellTiter-Blue assay. (3D) A549 cells were treated with XJB-OMe, 3-hydroxy- β -lapachone (**14**), or XJB-lapachone (**18**) at 6 μ M concentrations for 20 h. The formation of vacuoles was examined by phase contrast microscopy. Representative images are shown. (3E) A549 cells were treated as described above. Apoptotic and necrotic cell death were determined by Annexin V and PI staining. Representative images are shown.

FIGURE 4A-4D shows mitochondrial targeted β -lapachone (XJB-lapachone, **18**) induces prominent mitochondrial dysfunction. (4A) A549 cells were treated with XJB-OMe, 3-hydroxy- β -lapachone (**14**), or XJB-lapachone (**18**) at 6 μ M for 16 h. The generation of ROS was determined by staining cells with MitoSox and DCFHDA. The fluorescence intensity was measured by flow cytometry. Data represents the mean $\pm$ S.D. of triplicates. (4B) A549 cells

were treated as described in A, and the oxygen consumption rate was determined by a Seahorse extracellular flux analyzer. These data are the mean $\pm$ S.D. of six wells, and are representative of three experiments. (4C) A549 cells were treated as described in A, and mitochondria morphology was examined by electron microscopy. Representative images are shown. (4D) A549 cells were treated as described above. The expression of the autophagy marker LC3 was examined by western blot. β -actin was used as a loading control. Representative images are shown.

FIGURE 5 shows a graph showing that mitochondrial-targeted β -lapachone (XJB-lapachone) has higher treatment efficacy as compared to unconjugated β -lapachone (3-hydroxy- β -lapachone).

FIGURE 6 shows graphs showing that XJB-lapachone induces mitochondrial ROS generation in a dose and time dependent manner and is independent of NQO1.

FIGURE 7 shows graphs showing that low concentration of XJB-lapachone reduces mitochondrial ROS levels, while high concentration increases mitochondrial ROS.

FIGURE 8A-8B shows graphs showing that XJB-lapachone but not unconjugated beta-lapachone (3-hydroxy- β -lapachone) is effective in inducing cell death in rho0 cells.

FIGURE 9 shows a Western blot showing that XJB-lapachone induces DNA damage.

FIGURE 10 shows a photograph of A549 cell cultures indicating that XJB-lapachone enhances the effect of DNA-damaging anticancer agents.

FIGURE 11 shows synergistic effects of XJB-lapachone and ionizing radiation on colonies of A549 cells.

DETAILED DESCRIPTION

The use of numerical values in the various ranges specified in this application, unless expressly indicated otherwise, are stated as approximations as though the minimum and maximum values within the stated ranges are both preceded by the word "about". In this manner, slight variations above and below the stated ranges can be used to achieve substantially the same results as values within the ranges. Also, unless indicated otherwise, the disclosure of these ranges is intended as a continuous range including every value between the minimum and maximum values. For definitions provided herein, those definitions also refer to word forms, cognates and grammatical variants of those words or phrases.

As used herein, the terms "comprising," "comprise" or "comprised," and variations thereof, in reference to elements of an item, composition, apparatus, method, process, system, claim etc. are intended to be open-ended, meaning that the item, composition, apparatus, method, process, system, claim etc. includes those elements and other elements can be included

and still fall within the scope/definition of the described item, composition, apparatus, method, process, system, claim etc. As used herein, "a" or "an" means one or more. As used herein "another" may mean at least a second or more.

As used herein, the terms "patient" or "subject" refer to members of the animal kingdom including but not limited to human beings.

A "mitochondrial targeting group" is a moiety (that is, a part of a molecule) that partitions specifically to mitochondria. In one aspect, a mitochondria targeting group is a membrane active peptide fragment derived from an antibiotic molecule that acts by targeting a bacterial cell wall. Examples of such antibiotics include: bacitracins, gramicidins, valinomycins, enniatins, alamethicins, beauvericin, serratomolide, sporidesmolide, tyrocidins, polymyxins, monamycins, and lissoclinum peptides. The membrane-active peptide fragment derived from an antibiotic may comprise a complete antibiotic polypeptide, or a portion thereof having mitochondria-targeting abilities, which is readily determined, for example, by cellular partitioning experiments using radiolabeled peptides. Examples of useful gramicidin-derived membrane active peptide fragments are the Leu-^DPhe-Pro-Val-Orn and ^DPhe-Pro-Val-Orn-Leu hemigramicidin fragments. As gramicidin is cyclic, any hemigramicidin 5-mer is expected to be useful as a membrane active peptide fragment, including Leu-^DPhe-Pro-Val-Orn, ^DPhe-Pro-Val-Orn-Leu, Pro-Val-Orn-Leu-^DPhe, Val-Orn-Leu-^DPhe-Pro and Orn-Leu-^DPhe-Pro-Val (from gramicidin S). Any larger or smaller fragment of gramicidin, or even larger fragments containing repeated gramicidin sequences (e.g., Leu-^DPhe-Pro-Val-Orn-Leu-^DPhe-Pro-Val-Orn-Leu-^DPhe-Pro) are expected to be useful for membrane targeting, and can readily tested for such activity. In one aspect, the gramicidin S-derived peptide comprises a β -turn, which appears to confer to the peptide a high affinity for mitochondria. Derivatives of gramicidin, or other antibiotic fragments, include isosteres (molecules or ions with the same number of atoms and the same number of valence electrons - as a result, they can exhibit similar pharmacokinetic and pharmacodynamic properties), such as (E)-alkene isosteres (*see*, United States Patent Publication Nos. 2007/0161573 and 2007/0161544, incorporated herein by reference in their entirety, for exemplary synthesis methods). As with Gramicidin, the structure (amino acid sequence) of bacitracins, other gramicidins, valinomycins, enniatins, alamethicins, beauvericin, serratomolide, sporidesmolide, tyrocidins, polymyxins, monamycins, and lissoclinum peptides are all broadly-known, and fragments of these can be readily prepared and their membrane-targeting abilities can easily be confirmed by a person of ordinary skill in the art.

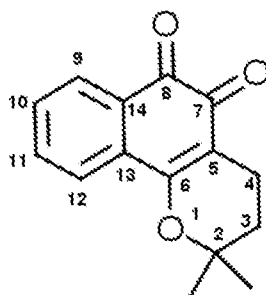
In another aspect, peptide isosteres, e.g., of a portion of gramicidin S are employed as mitochondria-targeting groups. Among the suitable peptide isosteres are trisubstituted (E)-alkene peptide isosteres and cyclopropane peptide isosteres, as well as all imine addition products of hydro- or carbometalated internal and terminal alkynes for the synthesis of d-i and trisubstituted (E)-alkene and cyclopropane peptide isosteres (*See Wipf et al.* Imine additions of internal alkynes for the synthesis of trisubstituted (E)-alkene and cyclopropane isosteres, *Adv Synth Catal.* 2005, 347:1605-1613). These peptide mimetics have been found to act as β -turn promoters (*See Wipf et al.* Convergent Approach to (E)-Alkene and Cyclopropane Peptide Isosteres, *Org Lett.* 2005, 7(1):103-106).

The marked differences between normal and cancer cells in mitochondrial metabolism and function provide support for the hypothesis that selectivity, as well as high therapeutic efficacy can be accomplished by direct targeting of mitochondria. In fact, targeting the electron transfer chain (ETC) in mitochondria with synthetic and natural toxins has attracted significant recent interest. Several classes of mitochondrial targeted anticancer agents, known as 'mitocans', have been reported and categorized into eight classes depending on their sites of action, i.e. 1) hexokinase inhibitors; 2) Bcl-2 family protein ligands; 3) thiol redox system disruptors; 4) mitochondrial membrane transporter/channel inhibitors; 5) electron transfer chain deregulators; 6) inner mitochondrial membrane disruptors; 7) TCA cycle inhibitors; and 8) mtDNA damaging agents (Figure 1). Most of these targets are closely correlated to cancer specific alterations of mitochondrial functions and bioenergetics.

In one aspect, an alkene peptide isostere segment of the antibiotic gramicidin S (GS), i.e. the XJB-peptide, acts as an effective mitochondrial targeting vector. The presence of a type II' β -turn in this pentapeptide sequence facilitates membrane permeability since the polar functionality of the backbone is less solvent exposed. After several structural modifications, the mitochondrial targeting antioxidant nitroxide, XJB-5-131, was developed as a first generation lead compound and a promising therapeutic agent. In parallel to these efforts to generate mitochondrial targeted anti-neurodegenerative compounds, development of a mitochondrial targeted ROS inducer for anticancer therapy has begun. A principal concern with ROS inducers in cancer therapy is whether these compounds have preferential affinities to localize and achieve sufficient concentrations for efficacy within the mitochondrial targets of malignant cells. Due to the distinct features of mitochondrial membranes, major challenges and strategies for a successful treatment rely on the design of effective delivery systems that can penetrate mitochondrial membrane barriers. Toward this goal, various chemistry-based approaches to targeting mitochondria have been reported. The most widely used strategy for

the delivery of organic molecules targeting mitochondria is the use of lipophilic cationic phosphonium ions, which are attracted by the large inner mitochondrial membrane potential and accumulate within the negatively charged mitochondrial matrix. Conjugation of bioactive molecules to peptide-based MT (mitochondria) targeting sequence, such as SS-peptides, mitochondria-penetrating peptides (MPPs), synthetic peptides and amino acid-based transporters are also major strategies for MT delivery. Additionally, the use of targeting systems with nanoparticles and liposomes is expanding. While therapeutic success has been limited, these early results provide support for additional investigations for the design of mitochondrial targeted anticancer drugs. For example, a TPP-tagged dichloroacetate showed three orders of magnitude enhanced potency and cancer cell specificity.

In order to generate a highly effective redox cycling system through direct targeting of mitochondria, an electron-rich *ortho*-quinone, β -lapachone (I, also **1** in Figure 1), was selected for the payload portion of the mitochondrial targeting platform. Numbering of β -lapachone derivatives correspond to the numbering of Formula I. Quinones are frequently involved in redox cycling and glutathione depletion after reductive activation by enzymes, generating bursts of ROS in addition to serving as electrophilic alkylators. β -lapachone (also, 2,2-dimethyl-3,4-dihydro-2H-benzo[h]chromene-5,6-dione, CAS 4707-32-8) is a tricyclic ortho-naphthoquinone compound having the structure depicted in Formula I.



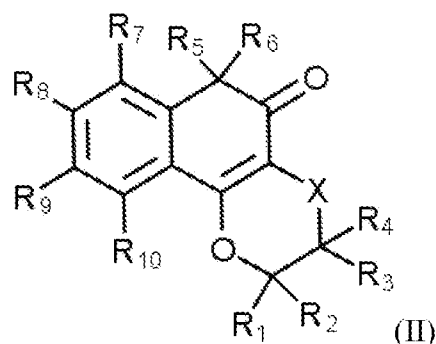
Formula I

β -lapachone is a naturally occurring ortho-naphthoquinone isolated from the lapacho tree (*Tabebuia avellanedae*) that has shown a range of potent biological activities relevant to antibacterial, antifungal, anti-inflammatory, and antitumor pathways. ARQ761, a β -lapachone analog, has entered Phase I/II clinical trials for several types of cancer either as a single agent or in combination with other chemotherapeutics. The cytotoxic effects of β -lapachone are derived from ROS generation dependent on the action of cytosolic NAD(P)H:quinone oxidoreductase 1 (NQO1) which reduces β -lapachone to a reactive hydroquinone and semiquinone redox-cycler. Since cellular levels of NQO1 vary between cell types, especially

between normal and cancer cells, β -lapachone can selectively kill NQO1 overexpressing cancer cells.

Hence, it is hypothesized that a targeted delivery of β -lapachone to mitochondria would 1) provide further insight into the mechanism of action of the active species; 2) trigger a specific activation of a more defined molecular cell death mechanism; 3) provide a novel mitocan causing mitochondrial dysfunction and therefore potentially broadening its therapeutic profile in cancer treatment; and, 4) shed light on the hypothesis that small quantities of ROS generated in mitochondrial might active *protective* cellular signaling pathways.

A very large number of β -lapachone derivatives exhibiting anti-neoplastic, antifungal, and/or antiviral activity, and/or other pharmacologically-relevant activities, have been described, including methods of synthesis of those derivatives. Those β -lapachone derivatives are all expected to be pharmacologically-active (e.g., as an antineoplastic, antifungal, antiviral, etc. therapeutic composition and as a drug product) when incorporated into the compositions, described herein. Relative to the β -lapachone structure of Formula I, above, those derivatives include: modification of one or both of the methyl groups at C₂ and/or addition of one or more groups at C₃ (*See, e.g.*, U.S. Patent Nos. 5,763,625; 5,824,700; and 5,969,163 incorporated herein by reference for their disclosure of β -lapachone derivatives); modification of the size of the hetero ring to have from five to seven members, and/or modification of the composition of the hetero-ring by substituting a carbon for an additional hetero-atom, or adding a hetero-atom, e.g., with S inserted between C₄ and C₅, or with S replacing C₄ (*See, e.g.*, U.S. Patent Nos. 8,068,459 and 5,969,163 incorporated herein by reference for their disclosure of β -lapachone derivatives); Addition of a double bond to the hetero-ring having five members (*See, e.g.*, U.S. Patent No. 5,969,163 incorporated herein by reference for its disclosure of β -lapachone derivatives); and/or replacement of the carbonyl group at C₈ with a sulfonate and a hydroxyl group (8-hydroxyl, 8-sulfonate (*See, e.g.*, U.S. Patent No. 7,790,765 incorporated herein by reference for its disclosure of β -lapachone derivatives). β -lapachone and its derivatives also include salts thereof, including pharmaceutically- or verterinarily-acceptable salts thereof.



wherein

X is $-(CH_2)_n-$ where $n=0-3$, $-S-$, or $-S-CH_2-$;

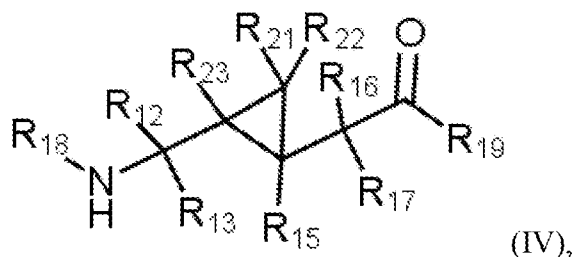
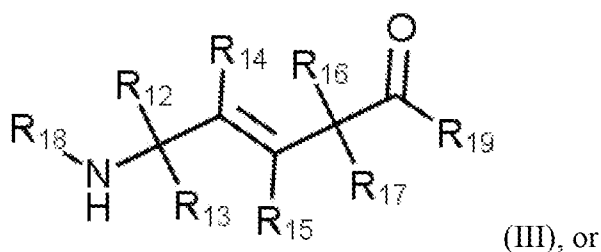
R_1 , R_2 , R_3 and R_4 are, independently, H, C_1 - C_6 alkyl, C_1 - C_6 alkenyl, C_1 - C_6 alkoxy, C_1 - C_6 alkoxy carbonyl, $-(CH_2)_n$ -aryl, $-(CH_2)_n$ -heteroaryl, $-(CH_2)_n$ -phenyl, and in one aspect, R_3 is H and R_4 is OH, and in one aspect, R_1 is $-C-R_{11}$, where R_{11} is H, O; and

R_5 is sulfonate and R_6 is hydroxyl, or R_5 and R_6 , together, are carbonyl ($=O$), and wherein, in one aspect, the β -lapachone derivatives are linked to a mitochondria-targeting group at positions C_2 or C_3 , e.g., via R_1 , R_2 , R_3 , or R_4 , and in one aspect, at position C_3 , that is via R_3 or R_4 .

R_7 , R_8 , R_9 and R_{10} are, independently, H, halogens, hydroxy, C_1 - C_6 alkyl, C_1 - C_6 alkenyl, C_1 - C_6 alkoxy, C_1 - C_6 alkoxy carbonyl, $-(CH_2)_n$ -aryl, $-(CH_2)_n$ -heteroaryl, and $-(CH_2)_n$ -phenyl.

In one aspect, the mitochondria-targeting moiety is a membrane-active fragment of gramicidin S or an *E*-alkylene isostere thereof conjugated to a cargo, wherein the fragment of gramicidin S or *E*-alkylene isostere thereof has the sequence of one of: Leu-^DPhe-Pro-Val-Orn, Leu-(*E*)-^DPhe-Pro-Val-Orn, or acylated derivatives thereof, in which pendant amines are acylated or modified with an amine protecting group as indicated herein.

In another aspect, the compound has the structure:



wherein R_{12} , R_{13} , R_{16} , and R_{17} are independently hydrogen, hydroxyl, halo, a C_1 - C_6 straight or branched-chain alkyl, or a C_1 - C_6 straight or branched-chain alkyl further comprising a phenyl (C_6H_5) group, wherein the C_1 - C_6 straight or branched-chain alkyl group or the C_1 - C_6 straight or branched-chain alkyl group comprising a phenyl group is unsubstituted or is methyl-, hydroxyl- or halo-substituted, for example, and without limitation, R_{12} , R_{13} , R_{16} , and R_{17} are

independently methyl-, hydroxyl- or fluoro-substituted, including: methyl, ethyl, propyl, 2-propyl, butyl, t-butyl, pentyl, hexyl, benzyl, hydroxybenzyl (e.g., 4-hydroxybenzyl), phenyl, or hydroxyphenyl;

R₁₅ is hydrogen, a halo, a C₁-C₆ straight or branched-chain alkyl, or a C₁-C₆ straight or branched-chain alkyl further comprising a phenyl (C₆H₅) group, wherein the C₁-C₆ straight or branched-chain alkyl group or the C₁-C₆ straight or branched-chain alkyl group comprising a phenyl group is unsubstituted or is methyl-, hydroxyl- or halo-substituted;

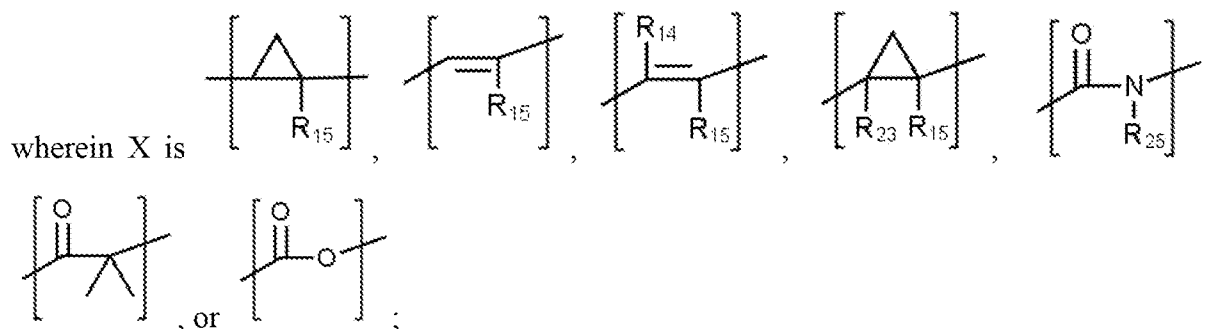
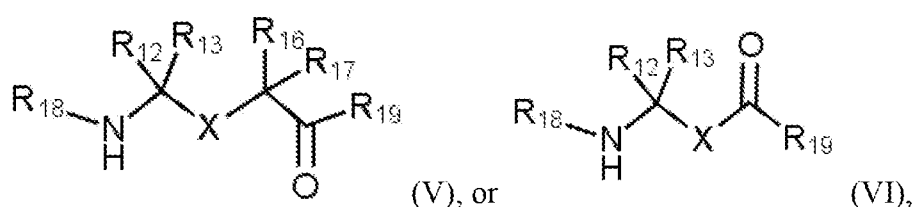
R₁₈ is -C(O)-R₂₄, -C(O)O-R₂₄, or -P(O)-(R₂₄)₂, wherein R₂₄ is C₁-C₆ straight or branched-chain alkyl or a C₁-C₆ straight or branched-chain alkyl optionally comprising one or more (C₆H₅) groups that are independently unsubstituted, or methyl-, ethyl-, hydroxyl-, halo-substituted or fluoro-substituted, for example and without limitation, R₁₈ is Ac (Acetyl, R = -C(O)-CH₃), Boc (R = -C(O)O-*tert*-butyl), Cbz (R = -C(O)O-benzyl (Bn)), or a diphenylphosphate group;

R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β -lapachone or a derivative thereof according to any aspect as described above;

R₁₄ is a halo, a C₁-C₆ straight or branched-chain alkyl or a C₁-C₆ straight or branched-chain alkyl further comprising one or more (C₆H₅) groups that are independently unsubstituted, or methyl-, ethyl-, hydroxyl- or halo-substituted; and

R₂₁, R₂₂, and R₂₃ are independently H or halogens (*See, e.g.*, International Patent Publication Nos. WO 2010/009405 and WO 2012/112851, incorporated herein by reference in their entirety).

In another aspect, the compound has the structure:

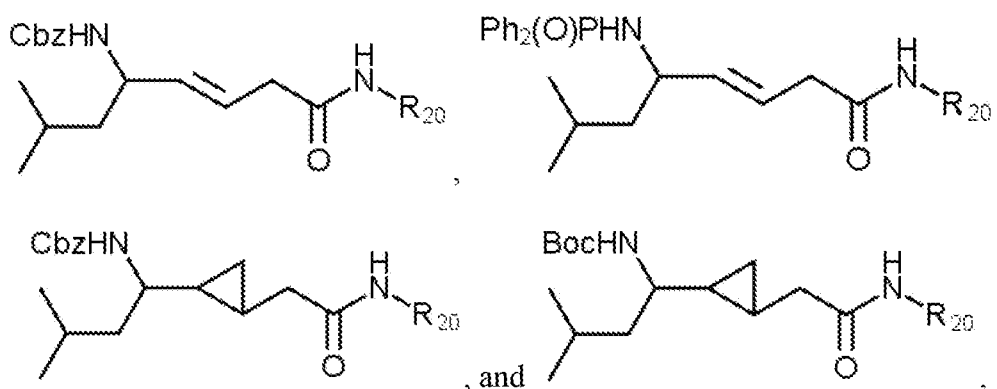


R₁₂, R₁₃, R₁₆, R₁₇, and R₂₅ are each independently hydrogen, halo, a C₁-C₆ straight or branched-chain alkyl, or a C₁-C₆ straight or branched-chain alkyl further comprising a phenyl (C₆H₅) group, wherein the C₁-C₆ straight or branched-chain alkyl group or the C₁-C₆ straight or branched-chain alkyl group comprising a phenyl group is unsubstituted or is methyl-, hydroxyl- or halo-substituted;

R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β -lapachone or a derivative thereof according to any aspect as described above; and

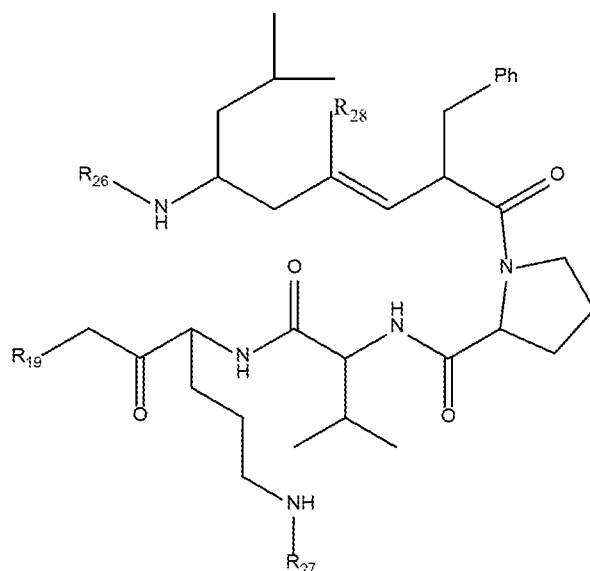
R₁₈ is -C(O)-R₂₄, -C(O)O-R₂₄, or -P(O)-(R₂₄)₂, wherein R₂₄ is C₁-C₆ straight or branched-chain alkyl or a C₁-C₆ straight or branched-chain alkyl optionally comprising one or more (C₆H₅) groups that are independently unsubstituted, or methyl-, ethyl-, hydroxyl-, halo-substituted or fluoro-substituted, for example and without limitation, R₁₈ is Ac (Acetyl, R = -C(O)-CH₃), Boc (R = -C(O)O-*tert*-butyl), Cbz (R = -C(O)O-benzyl (Bn)), or a diphenylphosphate group.

Non-limiting examples of compounds according to (V) include:

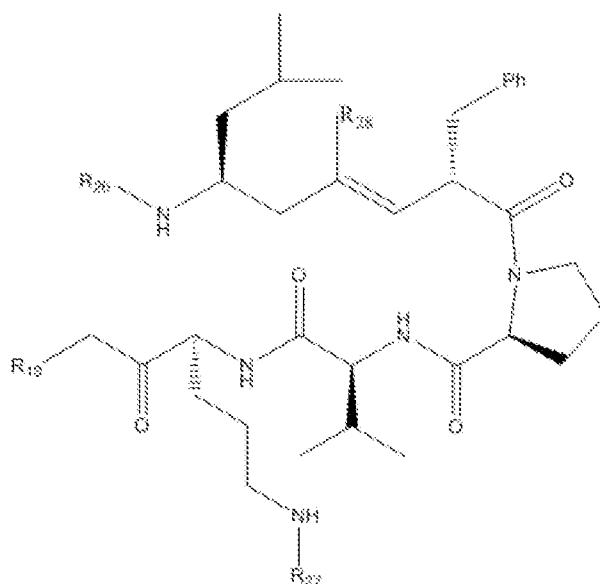


wherein R₂₀ is β -lapachone, or a β -lapachone derivative according to any aspect described above.

In one aspect, the compound has the structure:

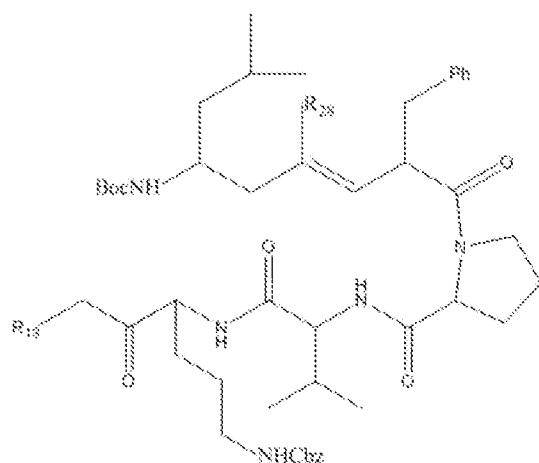


e.g.,

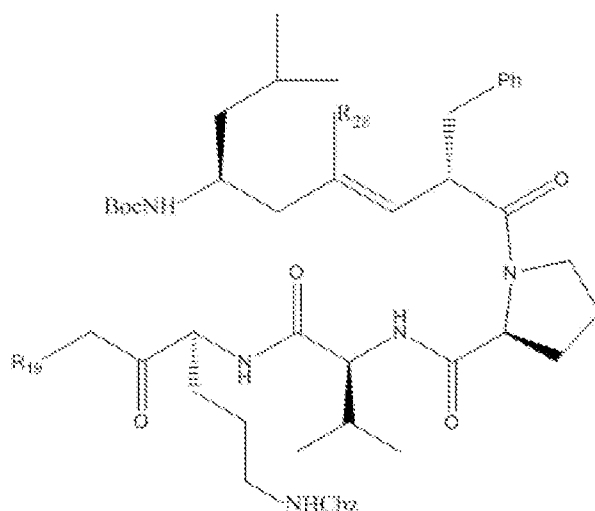


wherein R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β-lapachone or a derivative thereof according to any aspect as described above; R₂₆ and R₂₇, independently are an amine protecting group or acylated. In one aspect, R₂₆ and R₂₇ are protecting groups independently selected from the group consisting of: 9-fluorenylmethyloxy carbonyl (Fmoc), t-butyloxycarbonyl (Boc), benzhydryloxycarbonyl (Bhoc), benzyloxycarbonyl (Cbz), O-nitroveratryloxycarbonyl (Nvoc), benzyl (Bn), allyloxycarbonyl (alloc), trityl (Trt), 1-(4,4-dimethyl-2,6-dioxacyclohexylidene)ethyl (Dde), diathiasuccinoyl (Dts), benzothiazole-2-sulfonyl (Bts), dimethoxytrityl (DMT) and monomethoxytrityl (MMT), and R₂₈ is H or methyl. In one aspect, R₂₆ is Boc and R₂₇ is Cbz. Ph is phenyl.

In another aspect, the compound has the structure:

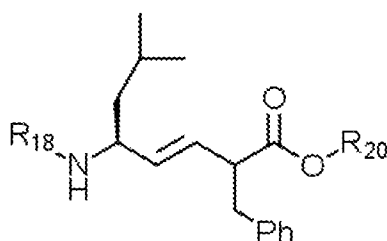


e.g.,



wherein R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β-lapachone, or a β-lapachone derivative according to any aspect described above.

In another aspect, the mitochondria-targeting group has the structure:



wherein R₁₇ is β-lapachone or a β-lapachone derivative, as described herein, and R₂₁ is an amine protecting group or acylated as described above for R₁₈ and R₁₉. In one aspect, R₂₁ is Boc or Cbz and R₂₀ is β-lapachone, and in another R₂₁ is Boc and R₂₀ is β-lapachone.

As used herein, unless indicated otherwise, for instance in a structure, all compounds and/or structures described herein comprise all possible stereoisomers, individually or mixtures thereof.

The following are exemplary definitions of various structural elements described herein. As used herein, "alkyl" refers to straight, branched chain, or cyclic hydrocarbon groups including from 1 to about 20 carbon atoms, for example and without limitation C₁₋₃, C₁₋₆, C₁₋₁₀ groups, for example and without limitation, straight, branched chain alkyl groups such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, and the like. "Substituted alkyl" refers to alkyl substituted at 1 or more, e.g., 1, 2, 3, 4, 5, or even 6 positions, which substituents are attached at any available atom to produce a stable compound, with substitution as described herein. "Optionally substituted alkyl" refers to alkyl or substituted alkyl. "Halogen," "halide," and "halo" refers to -F, -Cl, -Br, and/or -I. "Alkylene" and "substituted alkylene" refer to divalent alkyl and divalent substituted alkyl, respectively, including, without limitation, ethylene (-CH₂-CH₂-). "Optionally substituted alkylene" refers to alkylene or substituted alkylene.

"Alkene or alkenyl" refers to straight, branched chain, or cyclic hydrocarbonyl groups including from 2 to about 20 carbon atoms, such as, without limitation C₁₋₃, C₁₋₆, C₁₋₁₀ groups having one or more, e.g., 1, 2, 3, 4, or 5, carbon-to-carbon double bonds. "Substituted alkene" refers to alkene substituted at 1 or more, e.g., 1, 2, 3, 4, or 5 positions, which substituents are attached at any available atom to produce a stable compound, with substitution as described herein. "Optionally substituted alkene" refers to alkene or substituted alkene. Likewise, "alkenylene" refers to divalent alkene. Examples of alkenylene include without limitation, ethenylene (-CH=CH-) and all stereoisomeric and conformational isomeric forms thereof. "Substituted alkenylene" refers to divalent substituted alkene. "Optionally substituted alkenylene" refers to alkenylene or substituted alkenylene.

"Alkyne or alkynyl" refers to a straight or branched chain unsaturated hydrocarbon having the indicated number of carbon atoms and at least one triple bond. Examples of a (C₂-C₈)alkynyl group include, but are not limited to, acetylene, propyne, 1-butyne, 2-butyne, 1-pentyne, 2-pentyne, 1-hexyne, 2-hexyne, 3-hexyne, 1-heptyne, 2-heptyne, 3-heptyne, 1-octyne, 2-octyne, 3-octyne and 4-octyne. An alkynyl group can be unsubstituted or optionally substituted with one or more substituents as described herein below. The term "alkynylene" refers to divalent alkyne. Examples of alkynylene include without limitation, ethynylene, propynylene. "Substituted alkynylene" refers to divalent substituted alkyne.

The term "alkoxy" refers to an -O-alkyl group having the indicated number of carbon atoms. For example, a (C₁-C₆)alkoxy group includes -O-methyl (methoxy), -O-ethyl (ethoxy), -O-propyl (propoxy), -O-isopropyl (isopropoxy), -O-butyl (butoxy), -O-sec-butyl (sec-butoxy), -O-tert-butyl (tert-butoxy), -O-pentyl (pentoxy), -O-isopentyl (isopentoxy), -O-

neopentyl (neopentoxy), -O-hexyl (hexyloxy), -O-isohexyl (isohexyloxy), and -O-neohexyl (neohexyloxy). "Hydroxyalkyl" refers to a (C₁-C₁₀)alkyl group wherein one or more of the alkyl group's hydrogen atoms is replaced with an -OH group. Examples of hydroxyalkyl groups include, but are not limited to, -CH₂OH, -CH₂CH₂OH, -CH₂CH₂CH₂OH, -CH₂CH₂CH₂CH₂OH, -CH₂CH₂CH₂CH₂CH₂OH, -CH₂CH₂CH₂CH₂CH₂CH₂OH, and branched versions thereof. The term "ether" or "oxygen ether" refers to (C₁-C₁₀)alkyl group wherein one or more of the alkyl group's carbon atoms is replaced with an -O- group. The term ether includes -CH₂-(OCH₂-CH₂)_qOP₁ compounds where P₁ is a protecting group, -H, or a (C₁-C₁₀)alkyl. Exemplary ethers include polyethylene glycol, diethylether, methylhexyl ether and the like.

"Aryl," alone or in combination refers to an aromatic monocyclic or bicyclic ring system such as phenyl or naphthyl. "Aryl" also includes aromatic ring systems that are optionally fused with a cycloalkyl ring. A "substituted aryl" is an aryl that is independently substituted with one or more substituents attached at any available atom to produce a stable compound, wherein the substituents are as described herein. "Optionally substituted aryl" refers to aryl or substituted aryl. "Arylene" denotes divalent aryl, and "substituted arylene" refers to divalent substituted aryl. "Optionally substituted arylene" refers to arylene or substituted arylene.

"Heteroatom" refers to N, O, P and S. Compounds that contain N or S atoms can be optionally oxidized to the corresponding N-oxide, sulfoxide or sulfone compounds. "Hetero-substituted" refers to an organic compound in any embodiment described herein in which one or more carbon atoms are substituted with N, O, P or S.

"Substituted" or "substitution" refer to replacement of a hydrogen atom of a molecule or an R-group with one or more additional R-groups such as halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, nitro, sulfato or other R-groups.

"Cycloalkyl" refer to monocyclic, bicyclic, tricyclic, or polycyclic, 3- to 14-membered ring systems, which are either saturated, unsaturated or aromatic. The cycloalkyl group may be attached via any atom. Cycloalkyl also contemplates fused rings wherein the cycloalkyl is fused to an aryl or hetroaryl ring. Representative examples of cycloalkyl include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. A cycloalkyl group can be unsubstituted or optionally substituted with one or more substituents as described herein below. "Cycloalkylene" refers to divalent cycloalkyl. The term "optionally substituted cycloalkylene"

refers to cycloalkylene that is substituted with 1, 2 or 3 substituents, attached at any available atom to produce a stable compound, wherein the substituents are as described herein.

"Carboxyl" or "carboxylic" refers to group having the indicated number of carbon atoms and terminating in a -C(O)OH group, thus having the structure -R-C(O)OH , where R is a divalent organic group that includes linear, branched, or cyclic hydrocarbons. Non-limiting examples of these include: C_{1-8} carboxylic groups, such as ethanoic, propanoic, 2-methylpropanoic, butanoic, 2,2-dimethylpropanoic, pentanoic, *etc.*

" $(\text{C}_3\text{-C}_8)$ aryl- $(\text{C}_1\text{-C}_6)$ alkylene" refers to a divalent alkylene wherein one or more hydrogen atoms in the $\text{C}_1\text{-C}_6$ alkylene group is replaced by a $(\text{C}_3\text{-C}_8)$ aryl group. Examples of $(\text{C}_3\text{-C}_8)$ aryl- $(\text{C}_1\text{-C}_6)$ alkylene groups include without limitation 1-phenylbutylene, phenyl-2-butylene, 1-phenyl-2-methylpropylene, phenylmethylene, phenylpropylene, and naphthylethylene. The term " $(\text{C}_3\text{-C}_8)$ cycloalkyl- $(\text{C}_1\text{-C}_6)$ alkylene" refers to a divalent alkylene wherein one or more hydrogen atoms in the $\text{C}_1\text{-C}_6$ alkylene group is replaced by a $(\text{C}_3\text{-C}_8)$ cycloalkyl group. Examples of $(\text{C}_3\text{-C}_8)$ cycloalkyl- $(\text{C}_1\text{-C}_6)$ alkylene groups include without limitation 1-cyclopropylbutylene, cyclopropyl-2-butylene, cyclopentyl-1-phenyl-2-methylpropylene, cyclobutylmethylene and cyclohexylpropylene.

"Halo" refers to halogens, including F, Cl, Br, and I.

For therapeutic use, salts of the compounds are those wherein the counter-ion is pharmaceutically acceptable. However, salts of acids and bases which are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

The pharmaceutically acceptable acid and base addition salts as mentioned herein are meant to comprise the therapeutically active non-toxic acid and base addition salt forms which the compounds are able to form. The pharmaceutically acceptable acid addition salts can conveniently be obtained by treating the base form with such appropriate acid. Appropriate acids comprise, for example, inorganic acids such as hydrohalic acids, e.g. hydrochloric or hydrobromic acid, sulfuric, nitric, phosphoric and the like acids; or organic acids such as, for example, acetic, propanoic, hydroxyacetic, lactic, pyruvic, oxalic (i.e. ethanedioic), malonic, succinic (i.e. butanedioic acid), maleic, fumaric, malic (i.e. hydroxybutanedioic acid), tartaric, citric, methanesulfonic, ethanesulfonic, benzenesulfonic, p-toluenesulfonic, cyclamic, salicylic, p-aminosalicylic, pamoic and the like acids. Conversely the salt forms can be converted by treatment with an appropriate base into the free base form.

The compounds containing an acidic proton may also be converted into their non-toxic metal or amine addition salt forms by treatment with appropriate organic and inorganic bases.

Appropriate base salt forms comprise, for example, the ammonium salts, the alkali and earth alkaline metal salts, e.g. the lithium, sodium, potassium, magnesium, calcium salts and the like, salts with organic bases, e.g. the benzathine, N-methyl-D-glucamine, hydrabamine salts, and salts with amino acids such as, for example, arginine, lysine and the like. The term "addition salt" as used hereinabove also comprises the solvates which the compounds described herein are able to form. Such solvates are for example hydrates, alcoholates and the like.

The term "quaternary amine" as used hereinbefore defines the quaternary ammonium salts which the compounds are able to form by reaction between a basic nitrogen of a compound and an appropriate quaternizing agent, such as, for example, an optionally substituted alkylhalide, arylhalide or arylalkylhalide, e.g. methyl iodide or benzyl iodide. Other reactants with good leaving groups may also be used, such as alkyl trifluoromethanesulfonates, alkyl methanesulfonates, and alkyl p-toluenesulfonates. A quaternary amine has a positively charged nitrogen.

Pharmaceutically acceptable counterions include chloro, bromo, iodo, trifluoroacetate and acetate. The counterion of choice can be introduced using ion exchange resins.

"Pharmaceutically acceptable esters" includes those derived from compounds described herein that are modified to include a carboxyl group. An *in vivo* hydrolysable ester is an ester, which is hydrolysed in the human or animal body to produce the parent acid or alcohol. Representative esters thus include carboxylic acid esters in which the non-carbonyl moiety of the carboxylic acid portion of the ester grouping is selected from straight or branched chain alkyl (for example, methyl, n-propyl, t-butyl, or n-butyl), cycloalkyl, alkoxyalkyl (for example, methoxymethyl), aralkyl (for example benzyl), aryloxyalkyl (for example, phenoxymethyl), aryl (for example, phenyl, optionally substituted by, for example, halogen, C₁₋₄ alkyl, or C₁₋₄ alkoxy) or amino); sulphonate esters, such as alkyl- or aralkylsulphonyl (for example, methanesulphonyl); or amino acid esters (for example, L-valyl or L-isoleucyl). A "pharmaceutically acceptable ester" also includes inorganic esters such as mono-, di-, or tri-phosphate esters. In such esters, unless otherwise specified, any alkyl moiety present advantageously contains from 1 to 18 carbon atoms, particularly from 1 to 6 carbon atoms, more particularly from 1 to 4 carbon atoms. Any cycloalkyl moiety present in such esters advantageously contains from 3 to 6 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group, optionally substituted as shown in the definition of carbocyclyl above. Pharmaceutically acceptable esters thus include C₁-C₂₂ fatty acid esters, such as acetyl, t-butyl or long chain straight or branched unsaturated or omega-6 monounsaturated fatty acids such as palmoyl, stearoyl and the like. Alternative aryl or

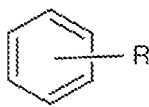
heteroaryl esters include benzoyl, pyridylmethyloyl and the like any of which may be substituted, as defined in carbocyclyl above. Additional pharmaceutically acceptable esters include aliphatic L-amino acid esters such as leucyl, isoleucyl and valyl.

Prodrugs of the disclosed compounds also are contemplated herein. A prodrug is an active or inactive compound that is modified chemically through *in vivo* physiological action, such as hydrolysis, metabolism and the like, into an active compound following administration of the prodrug to a subject. The term "prodrug" as used throughout this text means the pharmacologically acceptable derivatives such as esters, amides and phosphates, such that the resulting *in vivo* biotransformation product of the derivative is the active drug as defined in the compounds described herein. Prodrugs preferably have excellent aqueous solubility, increased bioavailability and are readily metabolized into the active inhibitors *in vivo*. Prodrugs of a compounds described herein may be prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either by routine manipulation or *in vivo*, to the parent compound. The suitability and techniques involved in making and using prodrugs are well known by those skilled in the art.

The term "prodrug" also is intended to include any covalently bonded carriers that release an active parent drug of the present invention *in vivo* when the prodrug is administered to a subject. Since prodrugs often have enhanced properties relative to the active agent pharmaceutical, such as, solubility and bioavailability, the compounds disclosed herein can be delivered in prodrug form. Thus, also contemplated are prodrugs of the presently disclosed compounds, methods of delivering prodrugs and compositions containing such prodrugs. Prodrugs of the disclosed compounds typically are prepared by modifying one or more functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to yield the parent compound. Prodrugs include compounds having a phosphonate and/or amino group functionalized with any group that is cleaved *in vivo* to yield the corresponding amino and/or phosphonate group, respectively. Examples of prodrugs include, without limitation, compounds having an acylated amino group and/or a phosphonate ester or phosphonate amide group. In particular examples, a prodrug is a lower alkyl phosphonate ester, such as an isopropyl phosphonate ester.

As used herein, unless indicated otherwise, for instance in a structure, all compounds and/or structures described herein comprise all possible stereoisomers, individually or mixtures thereof. The compound and/or structure may be an enantiopure preparation consisting essentially of an (-) or (+) enantiomer of the compound, or may be a mixture of enantiomers in either equal (racemic) or unequal proportions.

As used herein, a ring structure showing a bond/group that is not attached to any single

carbon atom, for example and without limitation, depicted as , can be substituted at any position with one or more groups designated "R", and, unless indicated otherwise, each instance of R on the ring can be (independently) the same or different from other R moieties on the ring. Thus, if R is H, the group contains nothing but H groups. If R is "halo", it is a single halo (e.g., F, Cl, Br and I) group. If R is one or more independently of halo and CN, the ring may comprise one, two, three, four, halo or CN groups, such as, for example and without limitation: 2, 3, 4, or 5 chloro; 2, 3, 4, or 5 bromo; 2, 3- or 3,4- or 4,5- or 2,4-dichloro; 3-bromo-4-chloro; 3-bromo-4-cyano, and any other possible permutation of the listed groups.

Protected derivatives of the disclosed compounds also are contemplated. Many suitable protecting groups for use with the disclosed compounds are broadly-known in the art. In general, protecting groups are removed under conditions which will not affect the remaining portion of the molecule. These methods are well known in the art and include acid hydrolysis, hydrogenolysis and the like. One method involves the removal of an ester, such as cleavage of a phosphonate ester using Lewis acidic conditions, such as in TMS-Br mediated ester cleavage to yield the free phosphonate. A second method involves removal of a protecting group, such as removal of a benzyl group by hydrogenolysis utilizing palladium on carbon in a suitable solvent system such as an alcohol, acetic acid, and the like or mixtures thereof. A t-butoxy-based group, including t-butoxy carbonyl protecting groups can be removed utilizing an inorganic or organic acid, such as HCl or trifluoroacetic acid, in a suitable solvent system, such as water, dioxane and/or methylene chloride. Another exemplary protecting group, suitable for protecting amino and hydroxy functions amino is trityl. Other conventional protecting groups are known and suitable protecting groups can be selected by those of skill in the art in consultation with any of the large number of broadly-available publications. When an amine is deprotected, the resulting salt can readily be neutralized to yield the free amine. Similarly, when an acid moiety, such as a phosphonic acid moiety is unveiled, the compound may be isolated as the acid compound or as a salt thereof.

According to one aspect, amine side chains are protected using protective groups, for example and without limitation by acylation (*See, e.g.*, U.S. Patent Nos. 7,528,174; 7,718,603; and 9,006,186, and International Patent Publication Nos. WO 2010/009405 and WO 2012/112851, incorporated herein by reference in their entirety). Protecting groups are known in the art and include, without limitation: 9-fluorenylmethyloxy carbonyl (Fmoc), t-

butyloxycarbonyl (Boc), benzhydryloxycarbonyl (Bhoc), benzyloxycarbonyl (Cbz), O-nitroveratryloxycarbonyl (Nvoc), benzyl (Bn), allyloxycarbonyl (alloc), trityl (Trt), 1-(4,4-dimethyl-2,6-dioxacyclohexylidene)ethyl (Dde), diathiasuccinoyl (Dts), benzothiazole-2-sulfonyl (Bts), dimethoxytrityl (DMT) and monomethoxytrityl (MMT) groups. A protecting group also includes acyl groups, such as acetyl groups, for example, as described.

The compounds typically are administered in an amount and dosage regimen to treat (a) a cancer (e.g., a malignancy), which includes, without limitation, any abnormal cells that divide without control and can invade nearby tissues, or (b) a hyperplasia, which is an increase in the number of cells in an organ or tissue, where the cells appear normal, and are not a cancer, but may become cancer. The compounds also are useful in reducing ROS production and/or the impact of ROS production, e.g., for treating neurodegeneration by virtue of their ability to reduce ROS damage at lower concentrations. For example, at concentrations of 1 μ M or less XJB-lapachone decreased ROS as described in the examples below. Treatment of neurodegeneration includes treatment of neurodegenerative diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD), Multiple Sclerosis (MS) and amyotrophic lateral sclerosis (ALS). The compounds may be administered in any manner that is effective to treat, mitigate or prevent any of the above conditions, including cancer, hyperplasia, neurodegeneration, PD, AD, MS, and ALS. Examples of delivery routes include, without limitation: topical, for example, epicutaneous, inhalational, enema, ocular, otic and intranasal delivery; enteral, for example, orally, by gastric feeding tube and rectally; and parenteral, such as, intravenous, intraarterial, intramuscular, intracardiac, subcutaneous, intraosseous, intradermal, intrathecal, intraperitoneal, transdermal, iontophoretic, transmucosal, epidural and intravitreal, with oral, intravenous, intramuscular and transdermal approaches being preferred in many instances.

As indicated above, and in the examples, depending upon the dosage of the compounds, the compounds can exhibit ROS-inhibiting activities, e.g., at low doses equivalent to 1 μ M or less in a patient (that is, ranging from >0 μ M up to, and including 1 μ M) of the XJB-lapachone compound described in the examples below. At higher concentrations, e.g., the equivalent to >1.0 μ M for XJB-lapachone described in the examples below, the compounds described herein increase ROS production in the mitochondria and due to the altered mitochondrial function and ROS metabolism in cancer cells compared to normal cells, will preferentially attack and kill the cancer cells. In one aspect the concentration of XJB-lapachone effective to increase ROS production in the mitochondria is, >2.0 μ M, for example 2.5 μ M and greater. Therefore, an "effective amount" of the compound or composition described herein is an amount effective in

a dosage regimen (amount of the compound and timing of delivery), to achieve a desired end-point, such as maintaining concentrations at a site of treatment within a range effective to achieve an outcome. Suitable outcomes include killing of cancer cells, improvement or maintenance of neurological function, neuroprotection, shrinking a tumor, or reducing ROS levels.

The compounds may be compounded or otherwise manufactured into a suitable composition for use, such as a pharmaceutical dosage form or drug product in which the compound is an active ingredient. Compositions may comprise a pharmaceutically acceptable carrier, or excipient. An excipient is an inactive substance used as a carrier for the active ingredients of a medication. Although “inactive,” excipients may facilitate and aid in increasing the delivery or bioavailability of an active ingredient in a drug product. Non-limiting examples of useful excipients include: antiadherents, binders, rheology modifiers, coatings, disintegrants, emulsifiers, oils, buffers, salts, acids, bases, fillers, diluents, solvents, flavors, colorants, glidants, lubricants, preservatives, antioxidants, sorbents, vitamins, sweeteners, etc., as are available in the pharmaceutical/compounding arts.

Useful dosage forms include: intravenous, intramuscular, or intraperitoneal solutions, oral tablets or liquids, topical ointments or creams and transdermal devices (e.g., patches). In one embodiment, the compound is a sterile solution comprising the active ingredient (drug, or compound), and a solvent, such as water, saline, lactated Ringer’s solution, or phosphate-buffered saline (PBS). Additional excipients, such as polyethylene glycol, emulsifiers, salts and buffers may be included in the solution.

In one aspect, the dosage form is a transdermal device, or “patch”. The general structure of a transdermal patch is broadly known in the pharmaceutical arts. A typical patch includes, without limitation: a delivery reservoir for containing and delivering a drug product to a subject, an occlusive backing to which the reservoir is attached on a proximal side (toward the intended subject’s skin) of the backing and extending beyond, typically completely surrounding the reservoir, and an adhesive on the proximal side of the backing, surrounding the reservoir, typically completely, for adhering the patch to the skin of a patient. The reservoir typically comprises a matrix formed from a non-woven (e.g., a gauze) or a hydrogel, such as a polyvinylpyrrolidone (PVP) or polyvinyl acetate (PVA), as are broadly known. The reservoir typically comprises the active ingredient absorbed into or adsorbed onto the reservoir matrix, and skin permeation enhancers. The choice of permeation enhancers typically depends on empirical studies. Certain formulations that may be useful as permeation enhancers include, without limitation: DMSO; 95% Propylene Glycol + 5% Linoleic Acid; and 50% EtOH +40%

HSO + 5% Propylene Glycol + 5% Brij30.

Therapeutic/pharmaceutical compositions are prepared in accordance with acceptable pharmaceutical procedures, such as described in Remington: The Science and Practice of Pharmacy, 21st edition, ed. Paul Beringer *et al.*, Lippincott, Williams & Wilkins, Baltimore, MD Easton, Pa. (2005) (see, *e.g.*, Chapters 37, 39, 41, 42 and 45 for examples of powder, liquid, parenteral, intravenous and oral solid formulations and methods of making such formulations).

As shown in the examples below, the compositions as described herein exhibit unexpected synergy when combined with other anticancer therapies, such as chemotherapeutic or radiation therapies as are known in the art. Therefore in one aspect, a method of treatment of a cancer is provided, comprising treating the cancer with a composition comprising a compound comprising a mitochondria-targeting moiety covalently linked to β -lapachone or a β -lapachone derivative according to any aspect described herein and either co-administering a second chemotherapeutic agent, or applying a radiation therapy to the patient while the compound is present in the patient.

As used herein, “chemotherapeutic agents” are compounds or compositions used to treat cancer, including, for example and without limitation: abiraterone acetate, altretamine, amsacrine, anhydro vinblastine, auristatin, bafetinib, bexarotene, bicalutamide, BMS 184476, 2,3,4,5,6-pentafluoro-N-(3-fluoro-4-methoxyphenyl)benzene sulfonamide, bleomycin, bosutinib, busulfan, cachectin, cemadotin, chlorambucil, cyclophosphamide, 3',4'-didehydro-4'-deoxy-8'-norvin-calceculin, docetaxol, doxorubicin, carboplatin, carmustine (BCNU), chlorambucil, cisplatin, cryptophycin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, daunorubicin, decitabine, dolastatin, doxorubicin (adriamycin), etoposide, etoposide phosphate, 5-fluorouracil, finasteride, flutamide, hydroxyurea, hydroxyureataxanes, ifosfamide, imatinib, irinotecan, liarozole, lonidamine, lomustine (CCNU), MDV3100, mechlorethamine (nitrogen mustard), melphalan, mitoxantrone, mivobulin isethionate, rhizoxin, sertenefer, streptozocin, mitomycin, methotrexate, nilotinib, nilutamide, onapristone, oxaliplatin, paclitaxel, ponatinib, prednimustine, procarbazine, RPRI 09881, stramustine phosphate, tamoxifen, tasonermin, taxol, teniposide, topotecan, tretinoin, vinblastine, vincristine, vindesine sulfate, and vinflunine, and pharmaceutically acceptable salts thereof.

EXAMPLES

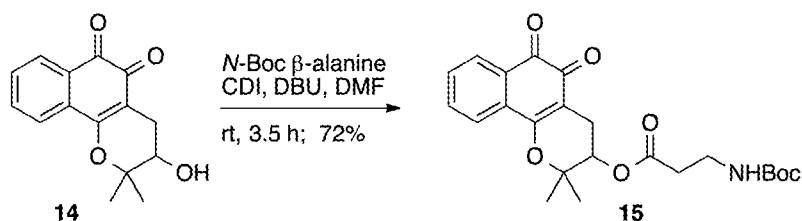
Example 1 - synthesis of XJB-conjugated β -lapachone

For the synthesis of the XJB-conjugated β -lapachone (**Figure 2**, Scheme 1), 3-hydroxy β -lapachone (**14**) was prepared from commercially available lapachol by epoxidation and

intramolecular ring opening using *meta*-chloroperoxybenzoic acid (*m*-CPBA). The secondary alcohol in **14** was acylated in 72% yield with the *N*-Boc β -alanine spacer group in the presence of 1,1'-carbonyldiimidazole (CDI) and DBU in DMF. Cleavage of the Boc group in the resulting ester **15** with TFA in CH₂Cl₂ afford amine **16** in quantitative yield. The XJB-derived Boc-Leu-^DPhe-Pro-Val-Orn(Cbz)-OH targeting sequence **17** was coupled to the amine **16** by EDCI in the presence of HOAt, Hünig's base (DIPEA) and DMAP to afford the desired XJB β -lapachone conjugate **18** in 82% yield.

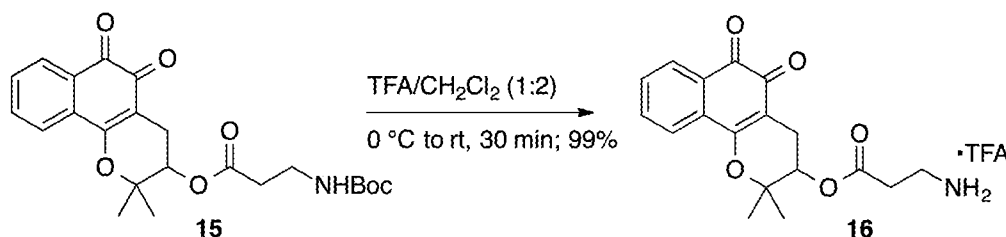
General. All reactions were performed under N₂ atmosphere and all glassware was flame dried in an oven at 150 °C prior to use. CH₂Cl₂ was purified using an alumina column filtration system. All other materials were obtained from commercial sources and used as received. Reactions were monitored by TLC analysis (EM Science pre-coated silica gel 60 F₂₅₄ plates, 250 μ m layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a PMA solution (5 g of phosphomolybdic acid in 100 mL of 95% EtOH), p-anisaldehyde solution (2.5 mL of p-anisaldehyde, 2 mL of AcOH, and 3.5 mL of conc. H₂SO₄ in 100 mL of 95% EtOH) or a KMnO₄ solution (1.5 g of KMnO₄ and 1.5 g of K₂CO₃ in 100 mL of a 0.1% NaOH solution). Flash chromatography on SiO₂ (SiliaFlash[®] F60, Silicycle) was used to purify the crude reaction mixtures. Melting points (uncorrected) were determined using a Mel-Temp instrument with a fiber-optic temperature probe. Infrared spectra were obtained on an Identity IR-ATR spectrometer. ¹H NMR, ¹³C NMR, and variable temperature NMR spectra were recorded on a Bruker Avance 400/100 MHz instruments and the ¹³C NMR spectrum for compound **18** (XJB-lapachone) was obtained using a Bruker Avance III 700 MHz instruments equipped with a 1.7 mm microprobe. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard. ¹H NMR spectra are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, td = triplet of doublet, dt = doublet of triplet, m = multiplet, br = broad, app = apparent), coupling constant(s) and number of protons. All 1D NMR spectra were processed using Bruker Topspin NMR. Final products were >95% purity as analyzed by reverse-phase HPLC (Alltech Prevail C-18, 100 $\times$ 4.6 mm, 1 mL/min, CH₃CN, H₂O and 0.1% TFA) with UV (210, 220 and 254 nm), ELS (nebulizer 45 °C, evaporator 45 °C, N₂ flow 1.25 SLM), and MS detection using a Thermo Scientific Exactive Orbitrap LC-MS (ESI positive).

2,2-dimethyl-5,6-dioxo-3,4,5,6-tetrahydro-2*H*-benzo[*h*]chromen-3-yl 3-((*tert*-butoxy carbonyl)amino)propanoate (**15**) (Sun, J. S.; *et al.* A preparative synthesis of lapachol and related naphthoquinones. Tetrahedron Lett. 1998 39(45):8221-8224).



To a solution of *N*-Boc β -alanine (77.0 mg, 0.40 mmol) in DMF (1.00 mL) was added 1,1'-carbonyldiimidazole (CDI, 67.0 mg, 0.40 mmol) at room temperature. The mixture was stirred for 30 min and treated with alcohol **14** (Salas, C, *et al. Trypanosoma cruzi: Activities of lapachol and α - and β -lapachone derivatives against epimastigote and trypomastigote forms*, Bioorg. Med. Chem. 2008 16(2):668-674) (70.0 mg, 0.27 mmol) and DBU (54.0 μ L, 0.35 mmol). The solution ure was stirred for an additional 3 h, poured into water, and extracted with CH_2Cl_2 . The combined organic layers were dried (MgSO_4) and concentrated. The crude residue was purified by chromatography on SiO_2 (Hexanes/EtOAc, 2:1) to give ester **15** (83.3 mg, 72%) as a yellow oil: ^1H NMR (400 MHz, CDCl_3) δ 8.09 (dd, $J = 7.6, 1.2$ Hz, 1 H), 7.84 (br d, $J = 7.6$ Hz, 1 H), 7.68 (td, $J = 7.7, 1.5$ Hz, 1 H), 7.55 (td, $J = 7.5, 0.9$ Hz, 1 H), 5.15 (t, $J = 4.6$ Hz, 1 H), 4.93 (br s, NH), 3.41–3.35 (m, 2 H), 2.84 (dd, $J = 18.2, 5.0$ Hz, 1 H), 2.68 (dd, $J = 18.4, 4.4$ Hz, 1 H), 2.62–2.48 (m, 2 H), 1.48 (s, 3 H), 1.44 (s, 3 H), 1.42 (s, 9 H); ^{13}C NMR (100 MHz, CDCl_3) δ 179.3, 178.5, 171.5, 161.1, 155.7, 134.9, 131.9, 131.1, 130.1, 128.9, 124.3, 109.9, 79.6, 69.4, 34.6, 28.3, 24.9, 23.2, 22.7; IR (CH_2Cl_2) 3340, 2975, 2932, 1737, 1705, 1605, 1506, 1390, 1247, 1159, 1133, 727 cm^{-1} ; HRMS (ESI) calcd for $\text{C}_{23}\text{H}_{27}\text{O}_7\text{NNa}$ $[\text{M}+\text{Na}]^+$ 452.1680, found 452.1677.

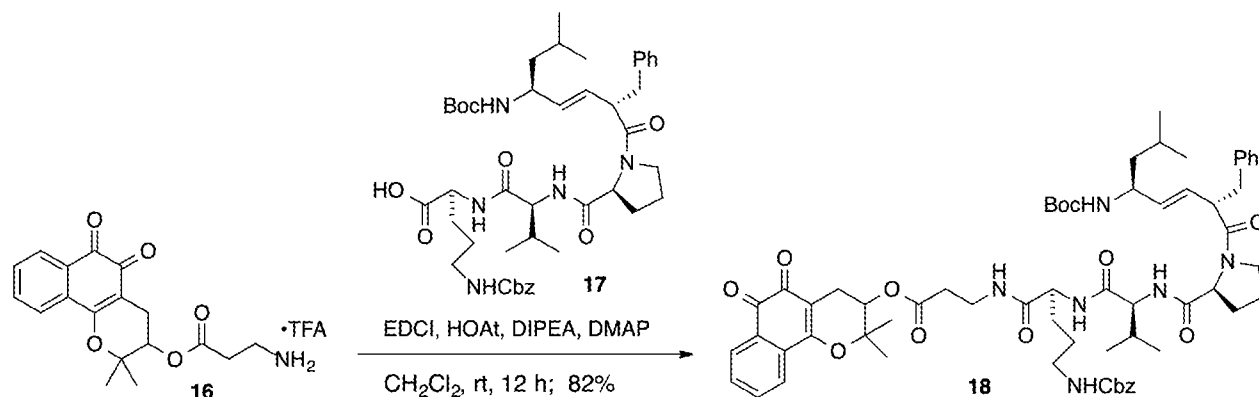
2,2-Dimethyl-5,6-dioxo-3,4,5,6-tetrahydro-2*H*-benzo[*h*]chromen-3-yl 3-aminopropanoate (**16**).



To a solution of *N*-Boc-protected ester **15** (80.0 mg, 0.19 mmol) in CH_2Cl_2 (2.00 mL) was added TFA (1.00 mL) at 0 $^\circ\text{C}$. The reaction mixture was stirred at room temperature for 30 min and concentrated under reduced pressure to provide amine **16** (82.0 mg, 99%) as a brick-red solid: Mp 55–60 $^\circ\text{C}$; ^1H NMR (400 MHz, D_2O) δ 7.99 (dd, $J = 7.8, 1.0$ Hz, 1 H), 7.94 (dd, $J = 7.6, 0.8$ Hz, 1 H), 7.77 (td, $J = 7.7, 1.5$ Hz, 1 H), 7.62 (td, $J = 7.6, 1.2$ Hz, 1 H), 5.29 (dd, $J = 4.6, 4.5$ Hz, 1 H), 3.28 (t, $J = 6.6$ Hz, 2 H), 2.93–2.76 (m, 3 H), 2.69 (dd, $J = 18.0, 3.2$ Hz, 1

H), 1.56 (s, 3 H), 1.47 (s, 3 H); ^{13}C NMR (100 MHz, D_2O) δ 180.7, 179.8, 171.5, 164.0, 135.7, 131.6, 131.5, 129.4, 128.4, 124.7, 117.7, 114.8, 109.2, 80.7, 70.6, 34.7, 30.9, 23.5, 22.5, 21.8; IR (CH_2Cl_2) 3098, 2986, 1735, 1672, 1651, 1601, 1398, 1323, 1197, 1174, 1129, 721; HRMS (ESI) calcd for $\text{C}_{18}\text{H}_{20}\text{O}_5\text{N}$ $[\text{M}+\text{H}]^+$ 330.1336, found 330.1335.

2,2-Dimethyl-5,6-dioxo-3,4,5,6-tetrahydro-2*H*-benzo[*h*]chromen-3-yl 3-(((*S*)-2-(((*S*)-2-(((*S*)-1-(((2*S*,5*S*,*E*)-2-benzyl-5-((*tert*-butoxycarbonyl)amino)-7-methyloct-3-enoyl)pyrrolidine-2-carboxamido)-3-methylbutanamido)-5-(((benzyloxy)carbonyl)amino)pentanamido)propanoate (**18**).



To a mixture of acid **17** (Skoda, EM, *et al.*, *Allylic Amines as Key Building Blocks in the Synthesis of (E)-Alkene Peptide Isosteres* Org. Process Res. Dev., 2012, 16 (1), pp 26–34) (48.0 mg, 60.0 μmol) and amine **16** (34.0 mg, 77.0 μmol) in CH_2Cl_2 (2.00 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 15.0 mg, 77.0 μmol), 1-hydroxy-7-azabenzotriazole (HOAt, 10.7 mg, 77.0 μmol), *N,N*-diisopropylethylamine (26.0 μL , 150 μmol), and 4-dimethylaminopyridine (0.7 mg, 6.00 μmol). The reaction mixture was stirred at room temperature for 12 h and poured into saturated NH_4Cl . The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 . The combined organic layers were dried (MgSO_4) and evaporated, and the residue was purified by chromatography on SiO_2 ($\text{CH}_2\text{Cl}_2/\text{EtOAc}$, 2:1, followed by 1–5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to provide amide **5** (56 mg, 82%) as an orange, foamy solid: Mp 93–100 $^\circ\text{C}$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$, mixture of rotamers, room temperature) δ 8.06–7.88 (m, 3 H), 7.81–7.73 (m, 2.5 H), 7.66–7.61 (m, 1.5 H), 7.36–7.09 (m, 10.2 H) 6.98 (d, $J = 7.2$ Hz, 0.8 H), 6.78–6.65 (m, 1 H), 5.45–5.28 (m, 2 H), 5.08–4.92 (m, 3 H), 4.56–4.48 (m, 0.4 H), 4.40–4.33 (m, 0.6 H), 4.25–4.09 (m, 2 H), 3.86–3.77 (m, 1 H), 3.50–3.43 (m, 2 H), 3.26–3.15 (m, 3 H), 2.94–2.81 (m, 4 H), 2.72 (dd, $J = 18.2, 4.6$ Hz, 1 H), 2.67–2.56 (m, 1 H), 2.53–2.41 (m, 3 H), 2.11–1.54 (m, 8 H), 1.42 (s, 3 H), 1.38 (s, 3 H), 1.35 (s, 9 H), 1.23–0.98 (m, 3 H), 0.81–0.71 (m, 12 H); ^1H NMR (400 MHz, $\text{DMSO}-d_6$, 100 $^\circ\text{C}$)

δ 7.95 (d, $J = 7.2$ Hz, 1 H), 7.82 (d, $J = 7.2$ Hz, 1 H), 7.77 (td, $J = 7.6, 0.8$ Hz, 1 H), 7.62 (t, $J = 7.4$ Hz, 1 H), 7.56–7.53 (m, 2 H), 7.36–7.10 (m, 11 H), 6.70 (br s, 1 H), 6.08 (br d, $J = 7.2$ Hz, 1 H), 5.48 (m, 2 H), 5.09 (t, $J = 4.8$ Hz, 1 H), 5.02 (s, 2 H), 4.41 (m, 1 H), 4.27–4.08 (m, 2 H), 3.89–3.87 (m, 1 H), 3.51–3.45 (m, 2 H), 3.39–3.28 (m, 3 H), 3.01 (m, 4 H), 2.79 (dd, $J = 18.0, 4.8$ Hz, 1 H), 2.68 (dd, $J = 13.8, 7.4$ Hz, 1 H), 2.57–2.45 (m, 3 H), 2.09–1.48 (m, 8 H), 1.46 (s, 3 H), 1.44 (s, 3 H), 1.38 (s, 9 H), 1.31–1.10 (m, 3 H), 0.87–0.77 (m, 12 H); ^{13}C NMR (175 MHz, DMSO- d_6 , mixture of rotamers, room temperature) δ 178.8, 178.0, 172.1, 171.6, 171.5, 171.1, 170.7, 159.8, 156.2, 154.9, 154.8, 139.3, 139.2, 137.3, 135.1, 134.6, 134.3, 131.6, 131.1, 130.4, 130.0, 129.2, 128.4, 128.1, 128.0, 127.9, 127.8, 127.3, 126.1, 125.8, 124.8, 123.9, 110.0, 79.5, 77.6, 77.5, 69.1, 65.2, 59.3, 59.0, 58.0, 57.8, 52.3, 50.4, 50.2, 49.0, 48.5, 46.9, 46.7, 44.3, 43.8, 40.0, 38.5, 37.8, 34.7, 33.7, 32.0, 30.5, 30.3, 29.5, 28.3, 26.0, 24.3, 24.1, 23.9, 23.0, 22.6, 22.4, 22.3, 22.1, 19.4, 19.3, 18.4, 18.2, 18.0; IR (CH_2Cl_2) 3329, 3062, 2975, 1696, 1651, 1606, 1573, 1508, 1392, 1260, 1178, 744, 707 cm^{-1} ; HRMS (ESI) calcd for $\text{C}_{62}\text{H}_{81}\text{O}_{13}\text{N}_6$ $[\text{M}+\text{H}]^+$ 1117.5856, found 1117.5872.

Example 2

Cell culture: A549 non-small cell lung cancer cells and MDA-MB-231 breast carcinoma cells were obtained from American Type Culture Collection (ATCC). Ovarian cancer cells PEO1 were kindly provided by Dr. Karyn J. Hansen (Magee-Womens Hospital of UPMC). 983B melanoma cells were kindly provided by Dr. Stergios J. Moschos (University of North Carolina). IMR90 normal lung fibroblast cells were kindly provided by Dr. Christopher Bakkenist (University of Pittsburgh Cancer Institute). Cells were cultured in either RPMI 1640 or DMEM media supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin-streptomycin in 5% CO_2 at 37 °C.

Cell viability and apoptosis assays: Cell viability was determined using a CellTiter-Blue Cell Viability Assay (Promega, Madison, WI). The survival fractions were calculated after setting untreated control cells at 100%. An FITC Annexin V Apoptosis Detection Kit (BD PharMingen, San Diego, CA) was used to quantify apoptotic cells and necrotic cells, according to the manufacturer's instructions.

Determination of ROS generation: To measure intracellular generation of ROS, cells were incubated with 5 μM DCF-DA (Sigma) for 20 min at 37°C after drug exposure. To measure mitochondrial generated ROS, cells were incubated with 5 μM of MitoSox (Invitrogen) for 20 min at 37 °C. After wash with PBS, cells were trypsinized and suspended in HBSS containing

1% BSA. The fluorescence intensity of DCF and MitoSox were analyzed using an Accuri C6 flow cytometer (BD Accuri Cytometers, Ann Arbor, MI).

Extracellular flux (XF) analysis: Oxygen consumption rate (OCR) was measured using a Seahorse XF96 Extracellular Flux Analyzer (Seahorse Bioscience, North Billerica, MA), as previously described (Qian W, *et al.*, Alterations in bioenergetics due to changes in mitochondrial DNA copy number. *Methods* 2010 51:452–457). Briefly, A549 cells were plated in a 6-well plate and treated with compounds for 16 h. After treatment, cells were trypsinized, counted, and plated at 40,000 cells per well in a XF96 microplate using Cell-Tak cell adhesive. OCR measurements were then performed using a XF96 analyzer.

Microscopy: For vacuole imaging, A549 cells were plated in 12-well plate, treated with compounds and the phase contrast images were captured using a Nikon Eclipse TS100 inverted microscope. For transmission electron microscopy analysis, A549 cells cultured in 6-well plate were fixed with 2.5% glutaraldehyde (Sigma-Aldrich) in PBS for 1 h at room temperature, and post-fixed for 1 h at 4 °C in 1% OsO₄ with 1% K₃Fe(CN)₆. After dehydration and embedding, ultrathin (70 nm) sections were cut and mounted onto copper grids. Sections were stained with 2% uranyl acetate followed by 1% lead citrate, and imaged using a JEOL JEM 1011 transmission electron microscope (Peabody, MA) at 80 kV.

Western blot analysis: Western blot was performed. Primary antibody LC3 was obtained from Novus (Littleton, CO) and β -actin was purchased from Sigma-Aldrich (St. Louis, MO).

In order to investigate the cellular toxicity of **18** (XJB-lapachone), A549 nonsmall cell lung cancer cells were treated with various concentrations of XJB-lapachone for 24 h, and compared its effect with XJB-OMe (the mitochondrial targeting moiety) and unconjugated 3-hydroxy- β -lapachone (**14**). A CellTiter-Blue based cell survival assay revealed that XJBLapachone reduced cell viability in a dose dependent manner (Figure 3A). The cytotoxic effect of XJB-Lapachone is superior compared to unconjugated β -lapachone, while XJB-OMe has no effect on cell survival (Figure 3A). Furthermore, XJB-lapachone is also effective against other types of tumor cells, including PEO1 ovarian cancer cells, MDA-MB-231 breast cancer cells, and 983B melanoma cells (Figure 3B). Since MDA-MB-231 breast cancer cells are deficient in NQO1, the high efficacy of XJB-lapachone in this cell type suggests that the effect of the conjugate, mitochondrial targeted XJB-lapachone, is no longer solely dependent on NQO1 activity. β -lapachone is known to have tumor cell-selective toxicity due to higher expression of the enzyme NQO1 in certain tumor cells. Tumor selectivity of β -lapachone, and whether the same is maintained after XJB conjugation, was examined. The cytotoxic effect of XJB-lapachone on A549 lung cancer and non-transformed human lung fibroblast cells

IMR90 was determined by a cell growth/ death assay. It was found that a four-hour exposure to 10 μ M XJB-lapachone significantly reduced the viability of A549 cells, while the toxic effect was less prominent in IMR90 cells, indicating the tumor selectivity of XJB-lapachone (Figure 3C). Remarkably, in A549 cells extensive vacuolization after XJB-lapachone exposure was observed. In contrast, vacuolization was not detected after treatment with XJB-OMe or 3-hydroxy- β -lapachone (Figure 3D). β -lapachone has been shown to induce apoptosis or programmed necrosis depending on cell types, and therefore the mechanism of cell death induced by XJB-lapachone was investigated. Through annexin V and PI staining, it was observed that more annexin V positive cells were induced after treatment with XJB-lapachone, compared to unconjugated 3-hydroxy- β -lapachone (Figure 3E), indicating extensive apoptosis after XJB-lapachone exposure.

Prior studies have shown that conjugation to the XJB sequence enhances the mitochondrial localization of small heterocyclic payloads by >100-fold. Therefore, the focus was on the effect of XJB-lapachone on mitochondrial function. While β -lapachone is known to promote ROS generation after bio-activation by NQO1 and subsequent NQO1-dependent futile cycling in cytoplasm, XJB-lapachone's activity in MDA-MB-231 cells that lack NQO1 suggests other potential mechanisms. After treating A549 cells with 6 μ M XJB-OMe, 3-hydroxy- β -lapachone (**14**), or XJB-lapachone (**18**) for 16 h, a dramatic increase of ROS generation was observed after in the presence of XJB-lapachone, as determined by both DCF and the mitochondria-specific superoxide sensor, MitoSox, through flow cytometry analysis. As expected, the effect of the XJB-conjugated, targeted lapachone **18** on ROS generation is stronger than unconjugated 3-hydroxy- β -lapachone (Figure 4A). Through examining the oxygen consumption of mitochondria, it was further found that following treatment with XJB-lapachone, mitochondrial respiration can no longer be inhibited by oligomycin, which blocks mitochondrial complex V ATP synthase. In contrast, oligomycin was efficient in inhibiting the mitochondrial respiration in cells treated with XJB-OMe or 3-hydroxy- β -lapachone. The absence of response to oligomycin administration indicates a substantial uncoupling effect of the mitochondrial targeted lapachone (Figure 4B). Electron microscopy analysis further revealed that after treatment with XJB-lapachone in A549 cells onion skin-like structures of mitochondria were observed, and cells developed extensive vacuoles (Figure 4C). This finding suggests that XJB-lapachone exposure leads to autophagic degradation of mitochondria (mitophagy). Consistent with the morphologic changes of mitochondria during autophagy, an increase in an LC3-II immunoblot signal was observed after XJB-lapachone exposure, in

contrast to XJB-OMe and 3- hydroxy- β -lapachone exposure (Figure 4D), supporting a potential important role of autophagy in XJB-lapachone induced cell death.

The preferential, dramatic effects of XJB-lapachone on mitochondrial function, as compared to unconjugated **14**, are conceivably the results of its accumulation in mitochondria leading to the generation of ROS and subsequent ROS-mediated mitochondrial damage, and/or covalent modifications of nucleophilic mitochondrial components. Furthermore, the results in Figure 2 indicate that XJB-lapachone induces cell death at least in part independent on NQO1, and therefore the mechanism of action of XJB-lapachone will require further follow-up investigations. Since the XJB-lapachone conjugate **18** contains an *ortho*-quinone/catechol substructure, NQO1-independent mechanisms such as a Cu(II) or Fe(III) chelation may play a significant role in its mitochondrial toxicity. Combined, these data suggest that mitochondrial targeting of β -lapachone results in the activation of unique signaling pathways leading to a yet to be classified type of cell death as highlighted by an extensive formation of vacuoles, in stark contrast to the phenotype of unconjugated β -lapachone.

In summary, a new mitochondrial targeted β -lapachone analog was prepared and its unique effects on cancer cell lines were demonstrated. XJB-lapachone is able to preferentially and efficiently induce mitochondrial ROS generation and subsequent mitochondrial damage, resulting in enhanced cytotoxicity compared to unconjugated β -lapachone. Due to the altered mitochondrial function and ROS metabolism in cancer cells compared to normal cells, mitochondrial targeted lapachone may yield a higher tumor selectivity than unconjugated lapachone. The remarkable cellular vacuolization is a striking result observed upon treatment of A549 cells with XJB-lapachone (**18**), but not with XJB-OMe or the control 3-hydroxy- β -lapachone (**14**).

Taken together, these results support the selection of mitochondria as cancer targets. While many tumors have the capacity to produce a large percentage of their ATP through glycolysis, i.e. the Warburg effect, it is clear that tumor cells are metabolically flexible and can adapt to the harsh tumor environment by altering their carbon sources to provide energy and key metabolic intermediates. In addition, oncogene overexpression, such as Myc, while increasing glycolysis can also increase mitochondrial biogenesis. Thus, mitochondrial function is a vital factor in tumor cells. In addition, cancer cells have a higher ROS load and are likely saturating their scavenging mechanisms. Their ability to respond to additional mitochondrial stress factors is reduced. Furthermore, many apoptosis-avoiding mutations are upstream from mitochondria, thus retaining cellular susceptibility to mitochondria-triggered death signals. Ongoing investigations on the mechanism of XJB-lapachone generation of ROS and its

associated cell death pathway will provide a basis for exploring the therapeutic potential of this novel agent and establish a platform for the design of next generation mitochondrial targeted ROS generators for cancer therapy. Mechanism-based combination designs such as simultaneous inhibition of several antioxidant systems will likely further enhance the efficacy of mitochondrial targeted lapachone.

Further, neurodegenerative diseases are conditions in which nerve cells from the brain and spinal cord are lost. This leads to either functional loss (ataxia), or sensory dysfunction (dementia). Mitochondrial dysfunction, excitotoxicity and apoptosis are considered to be a pathological cause for aging and neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD), Multiple Sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Although neurodegeneration is speculated to involve a number of factors, including environmental and genetic predisposition, but oxidative stress and free radical generation e.g., catalyzed by redox metals, have been shown to play pivotal role in regulating redox reactions *in vivo*, contributing RNS (reactive nitrogen species, such as nitric oxide (NO)) and ROS (reactive oxygen species, such as hydroxyl (OH⁻) and superoxide (O₂⁻)), which are considered to be significant factors in neurodegeneration (*See, e.g.,* Uttara, B, *et al.*, Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options, *Curr Neuropharmacol.* 2009 Mar; 7(1): 65–74 and Chen, X, *et al.*, Oxidative stress in neurodegenerative diseases, *Neural Regen Res.* 2012 Feb 15; 7(5): 376–385; Gruber *et al.*, Mitochondria-targeted antioxidants and metabolic modulators as pharmacological interventions to slow ageing. *Biotechnol. Adv.* 2013, 31, 563-592).

Example 3 – Mitochondrial-targeted beta-lapachone (XJB-lapachone) has higher treatment efficacy as compared to unconjugated beta-lapachone

As shown in Figure 5, A549 non-small cell lung cancer cells were treated with indicated agents for 4 h. Drug efficacy was determined by colony forming capacity using crystal violet 10 days after drug treatment.

Example 4 – XJB-lapachone induces mitochondrial ROS generation in a dose and time dependent manner and is independent of NQO1

A549 non-small cell lung cancer cells (high NQO1) and MDA-MB-231 triple negative breast cancer cells (NQO1 deficient) were treated as indicated in Figure 6. Mitochondrial ROS generation was determined by MitoSox followed by flow cytometry, essentially as described above.

Example 5 – Low concentration of XJB-lapachone reduces mitochondrial ROS levels, while high concentration increases mitochondrial ROS.

MDA-MB-231 triple negative breast cancer cells (NQO1 deficient) were treated as indicated in Figure 3 for 2 h. Mitochondrial ROS generation was determined by MitoSox followed by flow cytometry. Left-shift of peak indicates decrease of ROS and right-shift of peak indicates increase of ROS. Figure 7 indicates that greater concentrations of XJB-lapachone in cancer cells can selectively kill the cancer cells by promoting ROS, while non-enriching cells can be protected through lowering ROS levels.

Example 6 – XJB-lapachone but not unconjugated β -lapachone is effective in inducing cell death in rho0 cells

MDA-MB-231 parental and rho0 cells (established by long-term culture of parental MDA-MB-231 cells in the presence of 50 ng/ml ethidium bromide) were treated with increasing concentrations of XJB-lapachone for 24 h. Cell viability was determined by Celltiter-blue. As shown in Figure 8A-8B, XJB-lapachone but not unconjugated β -lapachone (3-hydroxy- β -lapachone) is effective in inducing cell death in rho0 cells, which are depleted of mtDNA and hence resistant to multiple anticancer agents.

Example 7 – XJB-lapachone induces DNA damage

A549 cells were treated with XJB-lapachone at the concentrations indicated in Figure 5. Western blot was performed 4 h after treatment to examine the activation of DNA damage signaling. Antibodies used in the Western Blot were β -actin (AC-15) and ATM (MAT3-4G10/8) (from Sigma); Chk1 (2G1D5), phospho-Chk2 (Thr68) (C13C1), Chk2 (1C12) (from Cell Signaling Technology); phospho-Chk1 (Ser317) (from R&D systems); and phospho-ATM (S1981) (from Epitomics). Figure 9 shows that XJB-lapachone induces DNA damage.

Example 8 – XJB-lapachone enhances the effect of DNA-damaging anticancer agents

A549 non-small cell lung cancer cells were treated with XJB-lapachone alone or combination with cisplatin or etoposide for 4h at concentrations indicated in Figure 10. Drug efficacy was determined by colony forming capacity 10 days after drug treatment. As seen in Figure 10, XJB-lapachone enhances the effect of DNA-damaging anticancer agents.

Example 9 – Synergistic effect of XJB-lapachone with Ionizing radiation (IR)

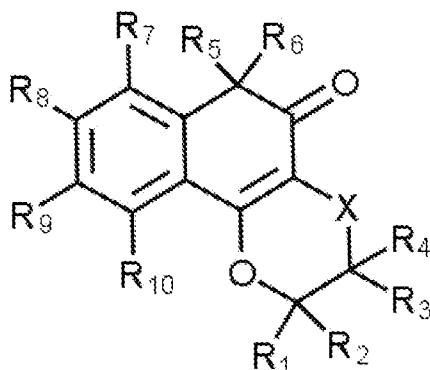
A549 cells were treated with XJB-lapachone for 4h. XJB-lapachone was then washed away before IR exposure (gamma-irradiation with a Shepherd Mark I Model 68 [^{137}Cs] irradiator) at 0, 1, 2, 4, and 6 Gy (grays). The number of colonies was counted 10 days after IR exposure (Figure 11).

Having described this invention above, it will be understood to those of ordinary skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any

embodiment thereof. Any document incorporated herein by reference is only done so to the extent of its technical disclosure and to the extent it is consistent with the present document and the disclosure provided herein.

We claim:

1. A compound comprising a mitochondria-targeting group covalently linked to β -lapachone or a β -lapachone derivative, and a salt or ester thereof, including pharmaceutically-acceptable salts or esters thereof.
2. The compound of claim 1, comprising a β -lapachone derivative.
3. The compound of claim 2, wherein the β -lapachone derivative has the structure:



wherein X is $-(CH_2)_n-$ where $n=0-3$, $-S-$, or $-S-CH_2-$;

R_1 , is H, OH, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, $-(CH_2)_n$ -aryl, $-(CH_2)_n$ -heteroaryl, $-(CH_2)_n$ -phenyl, $-OR_a$, unsubstituted or substituted alkylamino; unsubstituted or substituted dialkylamino, or substituted heterocycle, wherein R_a is H, unsubstituted or substituted amide, unsubstituted or substituted heterocycle, or substituted silyl;

R_2 is H, OH, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl, $-(CH_2)_n$ -aryl, $-(CH_2)_n$ -heteroaryl, $-(CH_2)_n$ -phenyl, a carboxylic acid, or a carboxy ester;

R_3 and R_4 are, independently, H, OH, unsubstituted or substituted C_1-C_6 alkyl, unsubstituted or substituted C_1-C_6 alkenyl, unsubstituted and substituted C_1-C_6 alkoxy, unsubstituted or substituted C_1-C_6 acyl, C_1-C_6 alkoxycarbonyl, amino, alkylamino, dialkylamino, unsubstituted or substituted aryl, unsubstituted or substituted phenyl, unsubstituted or substituted benzyl, unsubstituted or substituted heteroaryl, or unsubstituted or substituted heterocycle, $-(CH_2)_n$ -aryl, $-(CH_2)_n$ -heteroaryl, or $-(CH_2)_n$ -phenyl;

R_5 is sulfonate and R_6 is hydroxyl, or R_5 and R_6 , together, are carbonyl ($=O$);

R_7 , R_8 , R_9 , and R_{10} are each, independently, H, halogen, hydroxyl, unsubstituted or substituted C_1-C_6 alkyl, unsubstituted or substituted C_1-C_6 alkoxy, C_1-C_6 alkoxycarbonyl,

amino, alkylamino, dialkylamino, unsubstituted or substituted aryl, unsubstituted or substituted phenyl, unsubstituted or substituted benzyl, unsubstituted or substituted heteroaryl, or unsubstituted or substituted heterocycle, nitro, cyano, carboxylic acid, or amide;

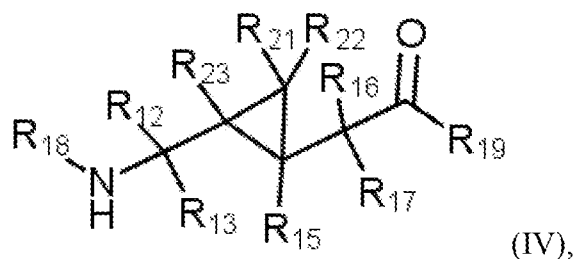
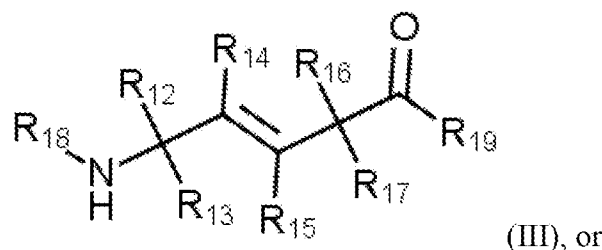
wherein X is not C when R₁ and R₂ are methyl, R₅ and R₆, together are carbonyl, and R₃, R₄, R₇, R₈, R₉, and R₁₀ are H (that is, the β -lapachone derivative is not β -lapachone), or an isostere thereof, or a pharmaceutically acceptable salt or ester thereof.

4. The compound any one of claims 1-3, wherein the β -lapachone or the β -lapachone derivative is linked to the mitochondria-targeting group at position C₂ or C₃.

5. The compound of anyone of claims 1-4, wherein the β -lapachone or the β -lapachone derivative is linked to a mitochondria-targeting group at position C₃.

6. The compound of claim 1, having a structure:

a.



wherein R₁₂, R₁₃, R₁₆, and R₁₇ are independently hydrogen, hydroxyl, halo, a C₁-C₆ straight or branched-chain alkyl, or a C₁-C₆ straight or branched-chain alkyl further comprising a phenyl (C₆H₅) group, wherein the C₁-C₆ straight or branched-chain alkyl group or the C₁-C₆ straight or branched-chain alkyl group comprising a phenyl group is unsubstituted or is methyl-, hydroxyl- or halo-substituted, for example, and without limitation, R₁₂, R₁₃, R₁₆, and R₁₇ are independently methyl-, hydroxyl- or fluoro-substituted, including: methyl, ethyl, propyl, 2-propyl, butyl, t-butyl, pentyl, hexyl, benzyl, hydroxybenzyl (e.g., 4-hydroxybenzyl), phenyl, or hydroxyphenyl;

R₁₅ is hydrogen, a halo, a C₁-C₆ straight or branched-chain alkyl, or a C₁-C₆ straight or branched-chain alkyl further comprising a phenyl (C₆H₅) group, wherein the C₁-C₆

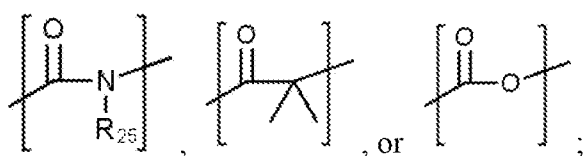
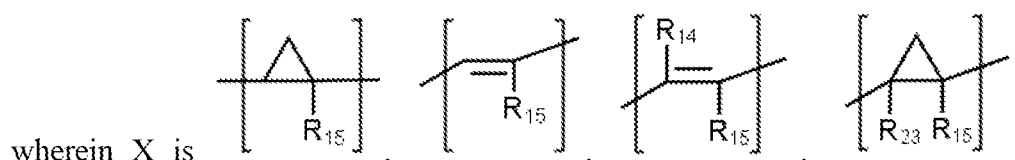
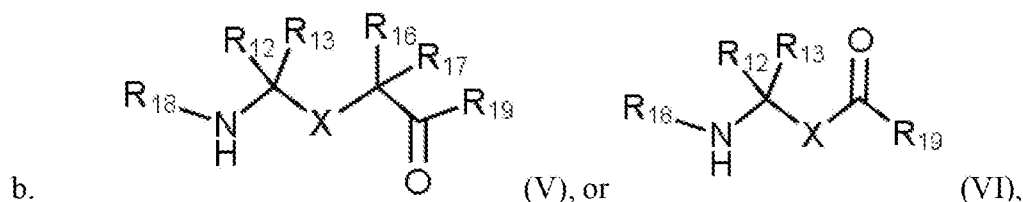
straight or branched-chain alkyl group or the C₁-C₆ straight or branched-chain alkyl group comprising a phenyl group is unsubstituted or is methyl-, hydroxyl- or halo-substituted;

R₁₈ is -C(O)-R₂₄, -C(O)O-R₂₄, or -P(O)-(R₂₄)₂, wherein R₂₄ is C₁-C₆ straight or branched-chain alkyl or a C₁-C₆ straight or branched-chain alkyl optionally comprising one or more (C₆H₅) groups that are independently unsubstituted, or methyl-, ethyl-, hydroxyl-, halo-substituted or fluoro-substituted, for example and without limitation, R₁₈ is Ac (Acetyl, R = -C(O)-CH₃), Boc (R = -C(O)O-*tert*-butyl), Cbz (R = -C(O)O-benzyl (Bn)), or a diphenylphosphate group;

R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β-lapachone or a derivative thereof according to any aspect as described above;

R₁₄ is a halo, a C₁-C₆ straight or branched-chain alkyl or a C₁-C₆ straight or branched-chain alkyl further comprising one or more (C₆H₅) groups that are independently unsubstituted, or methyl-, ethyl-, hydroxyl- or halo-substituted; and

R₂₁, R₂₂, and R₂₃ are independently H or a halo;

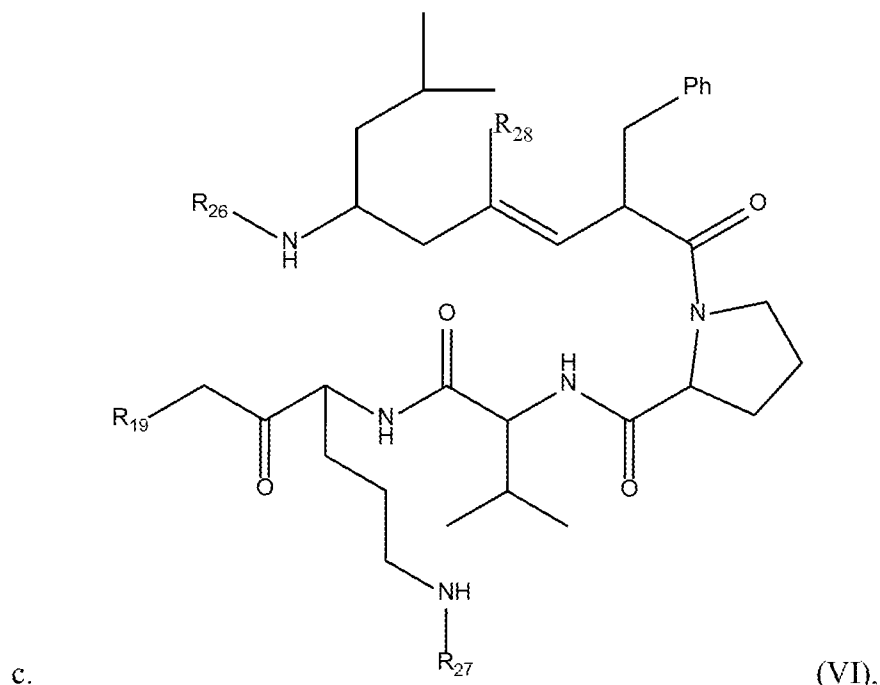


R₁₂, R₁₃, R₁₆, R₁₇, and R₂₅ are each independently hydrogen, halo, a C₁-C₆ straight or branched-chain alkyl, or a C₁-C₆ straight or branched-chain alkyl further comprising a phenyl (C₆H₅) group, wherein the C₁-C₆ straight or branched-chain alkyl group or the C₁-C₆ straight or branched-chain alkyl group comprising a phenyl group is unsubstituted or is methyl-, hydroxyl- or halo-substituted;

R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β-lapachone or a derivative thereof according to any aspect as described above; and

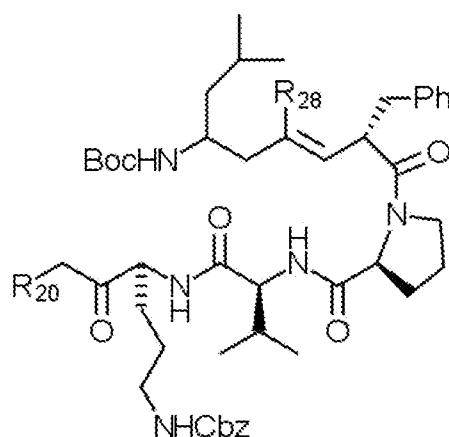
R₁₈ is -C(O)-R₂₄, -C(O)O-R₂₄, or -P(O)-(R₂₄)₂, wherein R₂₄ is C₁-C₆ straight or branched-chain alkyl or a C₁-C₆ straight or branched-chain alkyl optionally comprising one or

more (C₆H₅) groups that are independently unsubstituted, or methyl-, ethyl-, hydroxyl-, halo-substituted or fluoro-substituted, for example and without limitation, R₁₈ is Ac (Acetyl, R = C(O)-CH₃), Boc (R = C(O)O-*tert*-butyl), Cbz (R = C(O)O-benzyl (Bn)), or a diphenylphosphate group; or



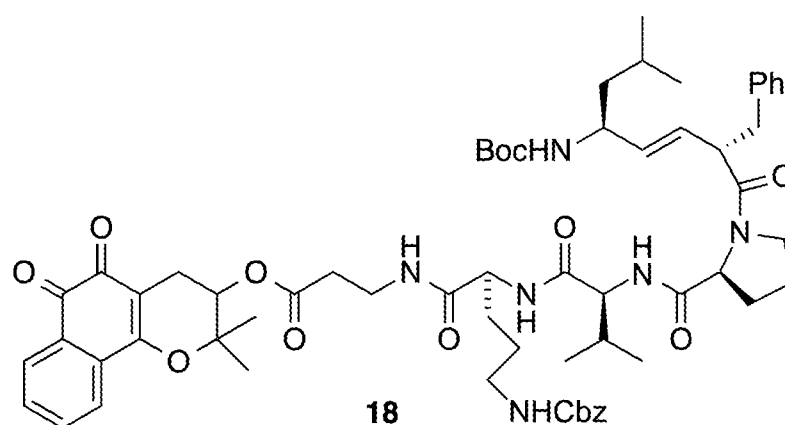
wherein R₁₉ is -NH-R₂₀, -O-R₂₀ or -CH₂-R₂₀, where R₂₀ is β-lapachone, or a β-lapachone derivative according to any aspect described above; R₂₆ and R₂₇, independently are an amine protecting group or acylated. In one aspect, R₂₆ and R₂₇ are protecting groups independently selected from the group consisting of: 9-fluorenylmethyloxy carbonyl (Fmoc), t-butyloxycarbonyl (Boc), benzhydryloxycarbonyl (Bhoc), benzyloxycarbonyl (Cbz), O-nitroveratryloxycarbonyl (Nvoc), benzyl (Bn), allyloxycarbonyl (alloc), trityl (Trt), 1-(4,4-dimethyl-2,6-dioxacyclohexylidene)ethyl (Dde), diathiasuccinoyl (Dts), benzothiazole-2-sulfonyl (Bts), dimethoxytrityl (DMT) and monomethoxytrityl (MMT), and R₂₈ is H or methyl. In one aspect, R₂₆ is Boc and R₂₇ is Cbz. Ph is phenyl.

7. The compound of claim 6, having the structure of (III) or (IV).
8. The compound of claim 6, having the structure of (V) or (VI).
9. The compound of claim 6, having the structure of (VII).
10. The compound of claim 1, having the structure:



wherein R_{19} is $-\text{NH}-R_{20}$, $-\text{O}-R_{20}$ or $-\text{CH}_2-R_{20}$, where R_{20} is β -lapachone, or a β -lapachone derivative.

11. The compound of claim 1, having the structure:



an isostere thereof, or a pharmaceutically acceptable salt or ester thereof.

12. A composition comprising a first compound according to any one of claims 1-11, and a pharmaceutically-acceptable excipient.

13. The composition of claim 12, further comprising a second chemotherapeutic agent that is different from the first compound.

14. The composition of claim 13, wherein the second chemotherapeutic agent is either β -lapachone, a β -lapachone derivative, abiraterone acetate, altretamine, amsacrine, anhydro vinblastine, auristatin, bafetinib, bexarotene, bicalutamide, BMS 184476, 2,3,4,5,6-pentafluoro-N-(3-fluoro-4-methoxyphenyl)benzene sulfonamide, bleomycin, bosutinib, busulfan, cachectin, cemadotin, chlorambucil, cyclophosphamide, 3',4'-didehydro-4'-deoxy-8'-norvin-calculoblastine, docetaxol, doxorubicin, carboplatin, carmustine (BCNU), chlorambucil, cisplatin, cryptophycin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, daunorubicin, decitabine, dolastatin, doxorubicin (adriamycin), etoposide, etoposide phosphate, 5-fluorouracil, finasteride, flutamide, hydroxyurea, hydroxyureataxanes,

ifosfamide, imatinib, irinotecan, liarozole, lonidamine, lomustine (CCNU), MDV3100, mechlorethamine (nitrogen mustard), melphalan, mitoxantrone, mivobulin isethionate, rhizoxin, sertenef, streptozocin, mitomycin, methotrexate, nilotinib, nilutamide, onapristone, oxaliplatin, paclitaxel, ponatinib, prednimustine, procarbazine, RPRI 09881, stramustine phosphate, tamoxifen, tasonermin, taxol, teniposide, topotecan, tretinoin, vinblastine, vincristine, vindesine sulfate, vinflunine, or pharmaceutically acceptable salts or esters thereof.

15. A method of treating a cancer in a patient, comprising administering to a patient an amount of a first compound according to any one of claims 1-11 effective to increase reactive oxygen species (ROS) in mitochondria of cancer cells of a patient.

16. The method of claim 15, further comprising administering a radiation therapy to the patient while the first compound is present in the patient.

17. The method of claim 15 or claim 16, further comprising administering a second chemotherapeutic agent that differs from the first compound.

18. The method of claim 17, wherein the second chemotherapeutic agent is either β -lapachone, a β -lapachone derivative, abiraterone acetate, altretamine, amsacrine, anhydro vinblastine, auristatin, bafetinib, bexarotene, bicalutamide, BMS 184476, 2,3,4,5,6-pentafluoro-N-(3-fluoro-4-methoxyphenyl)benzene sulfonamide, bleomycin, bosutinib, busulfan, cachectin, cemadotin, chlorambucil, cyclophosphamide, 3',4'-didehydro-4'-deoxy-8'-norvin-calceukoblastine, docetaxol, doxetaxel, carboplatin, carmustine (BCNU), chlorambucil, cisplatin, cryptophycin, cyclophosphamide, cytarabine, dacarbazine (DTIC), dactinomycin, daunorubicin, decitabine dolastatin, doxorubicin (adriamycin), etoposide, etoposide phosphate, 5-fluorouracil, finasteride, flutamide, hydroxyurea, hydroxyureataxanes, ifosfamide, imatinib, irinotecan, liarozole, lonidamine, lomustine (CCNU), MDV3100, mechlorethamine (nitrogen mustard), melphalan, mitoxantrone, mivobulin isethionate, rhizoxin, sertenef, streptozocin, mitomycin, methotrexate, nilotinib, nilutamide, onapristone, oxaliplatin, paclitaxel, ponatinib, prednimustine, procarbazine, RPRI 09881, stramustine phosphate, tamoxifen, tasonermin, taxol, teniposide, topotecan, tretinoin, vinblastine, vincristine, vindesine sulfate, vinflunine, or pharmaceutically acceptable salts or esters thereof.

19. A method of treating neurodegeneration, comprising administering to a patient an amount of a compound according to any one of claims 1-11 effective to decrease reactive oxygen species (ROS) production in mitochondria of a cell of a patient.

20. A method of decreasing oxidative damage in a patient comprising administering to a patient an amount of a compound according to any one of claims 1-11

effective to decrease reactive oxygen species (ROS) production in mitochondria of a cell of a patient.

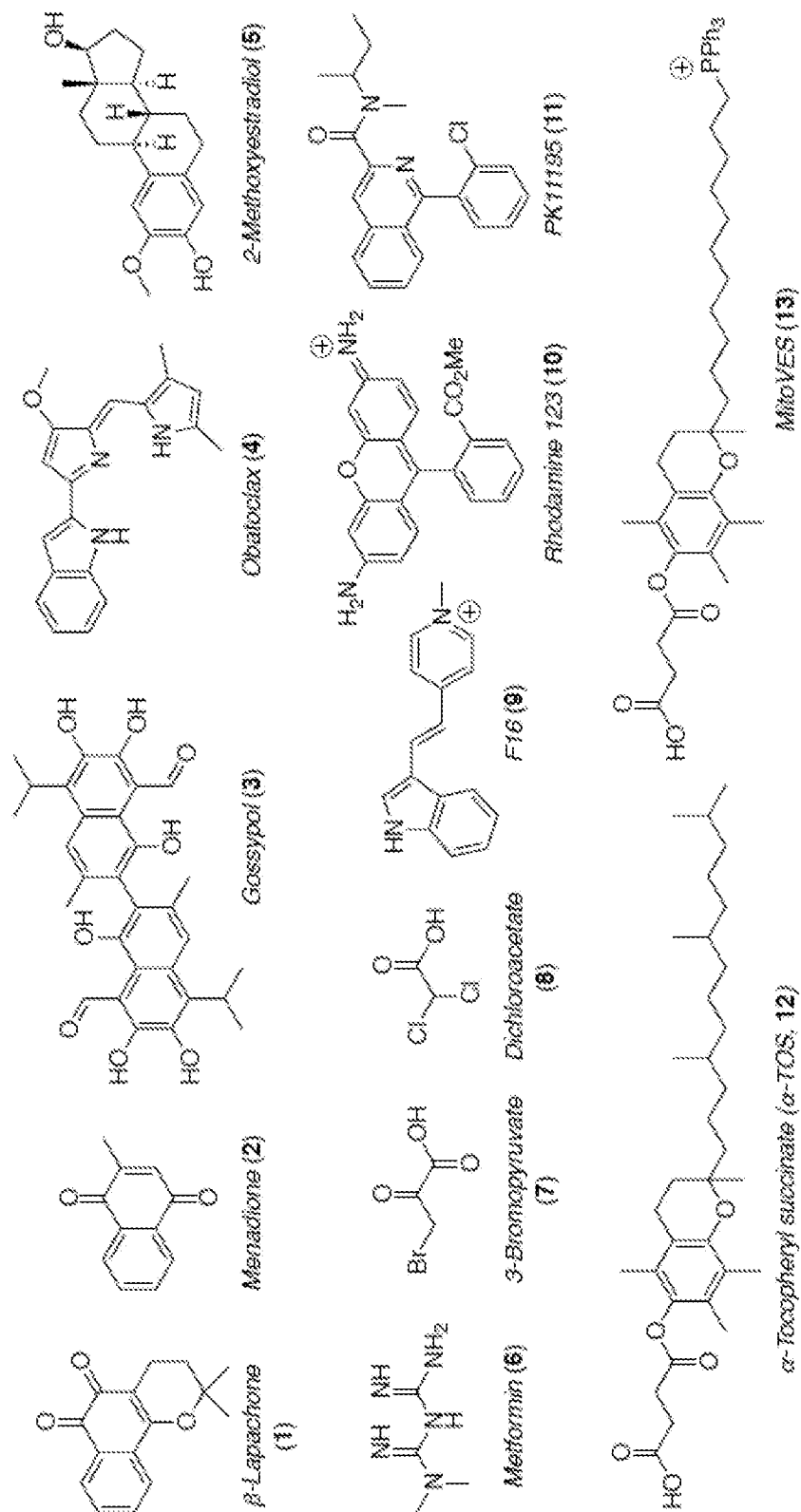


FIG. 1

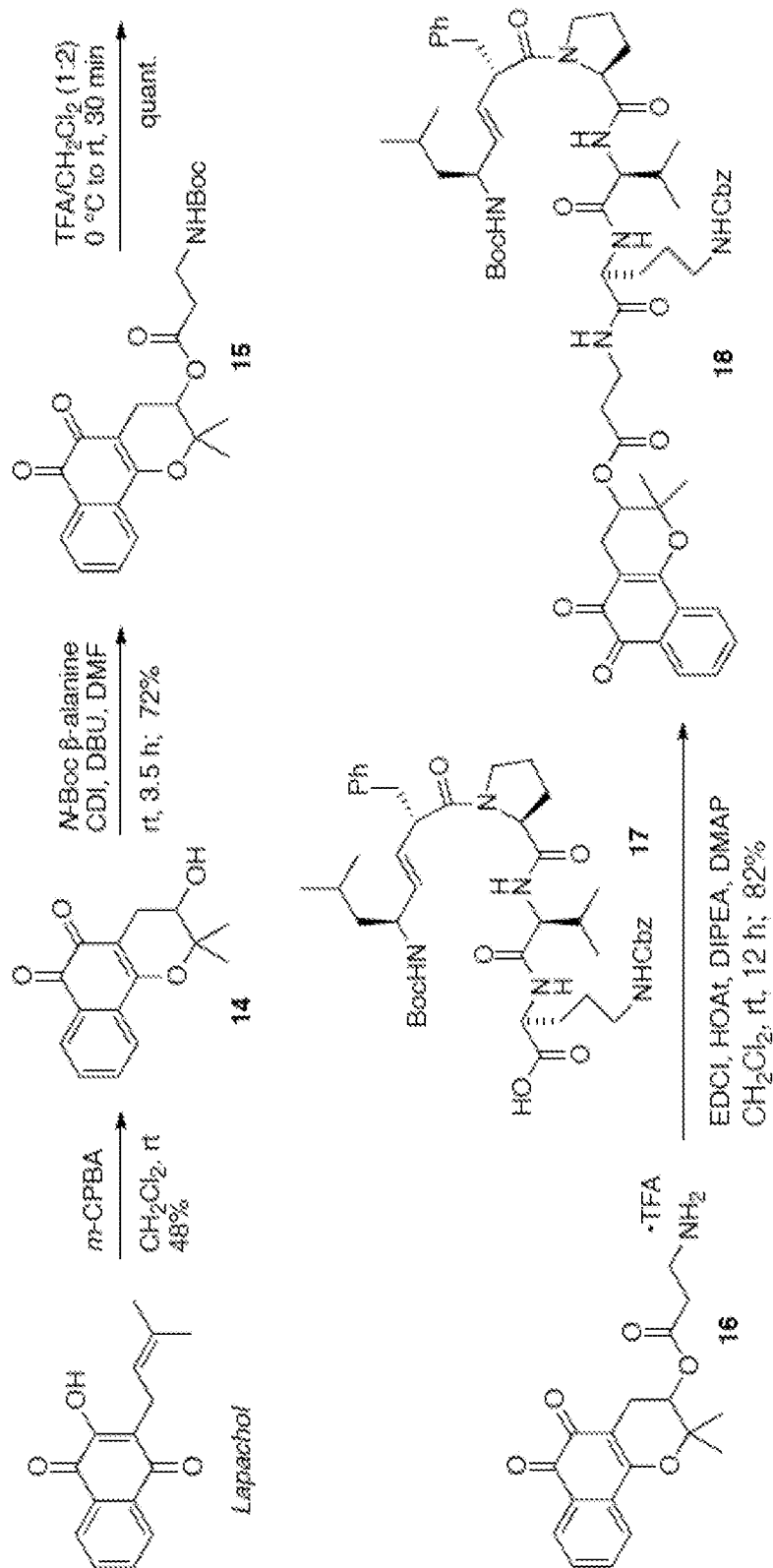
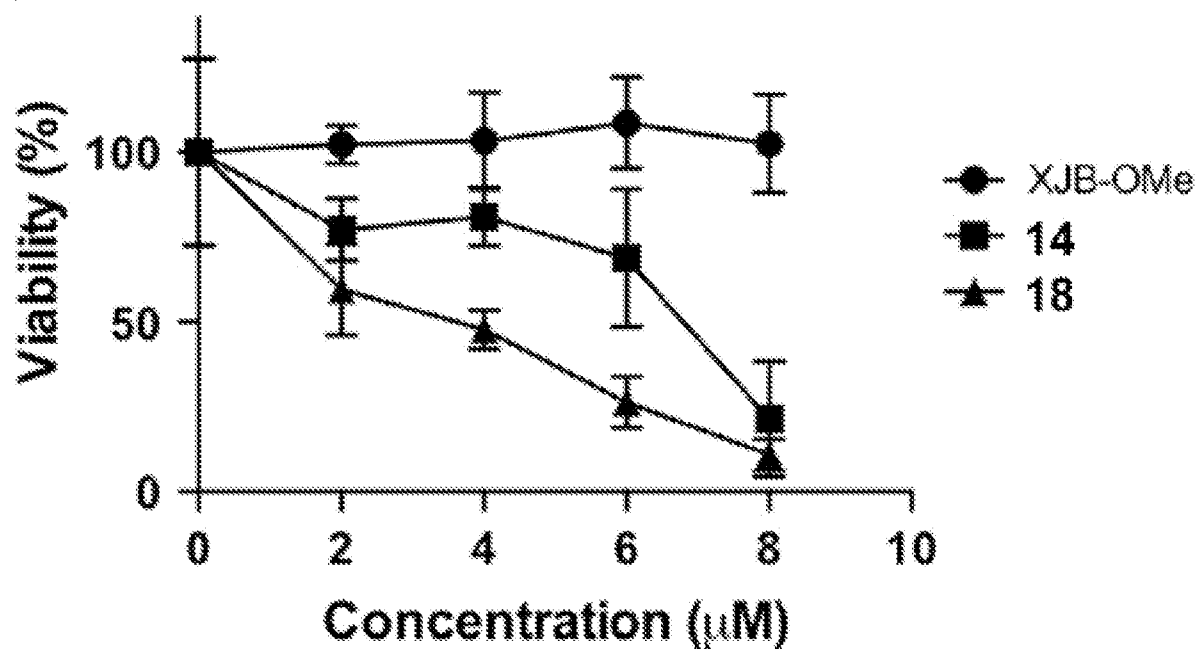


FIG. 2

A



B

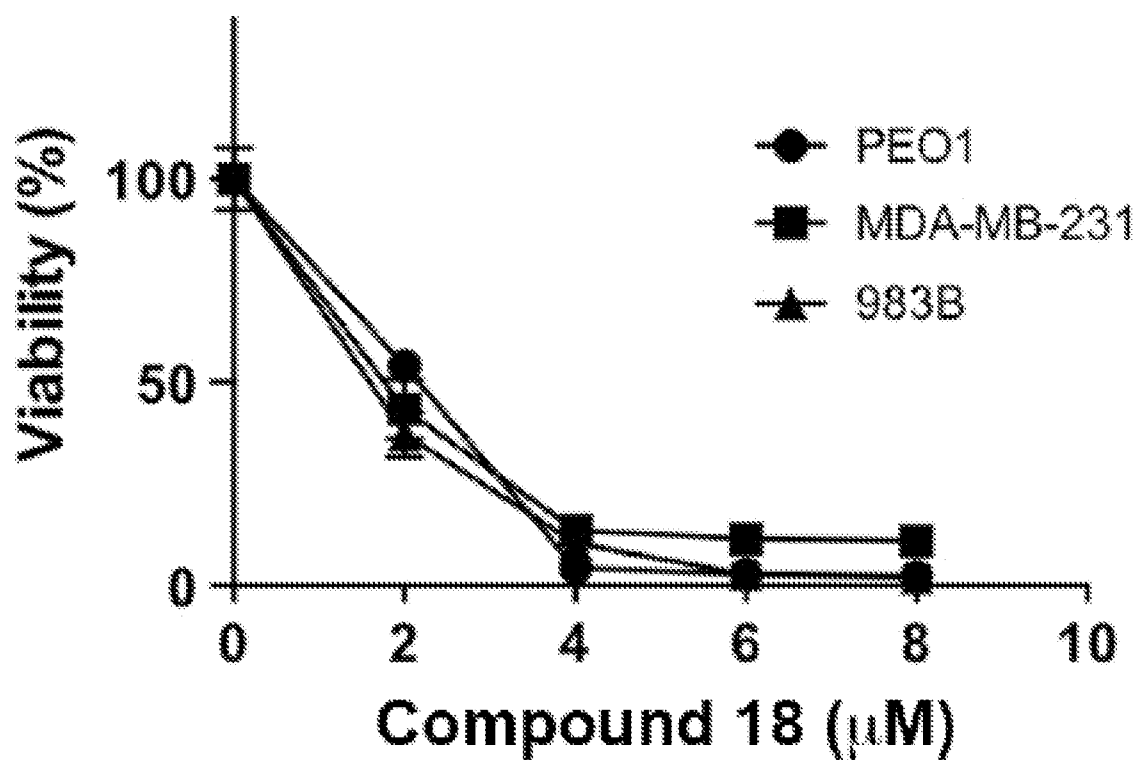


FIG. 3-1

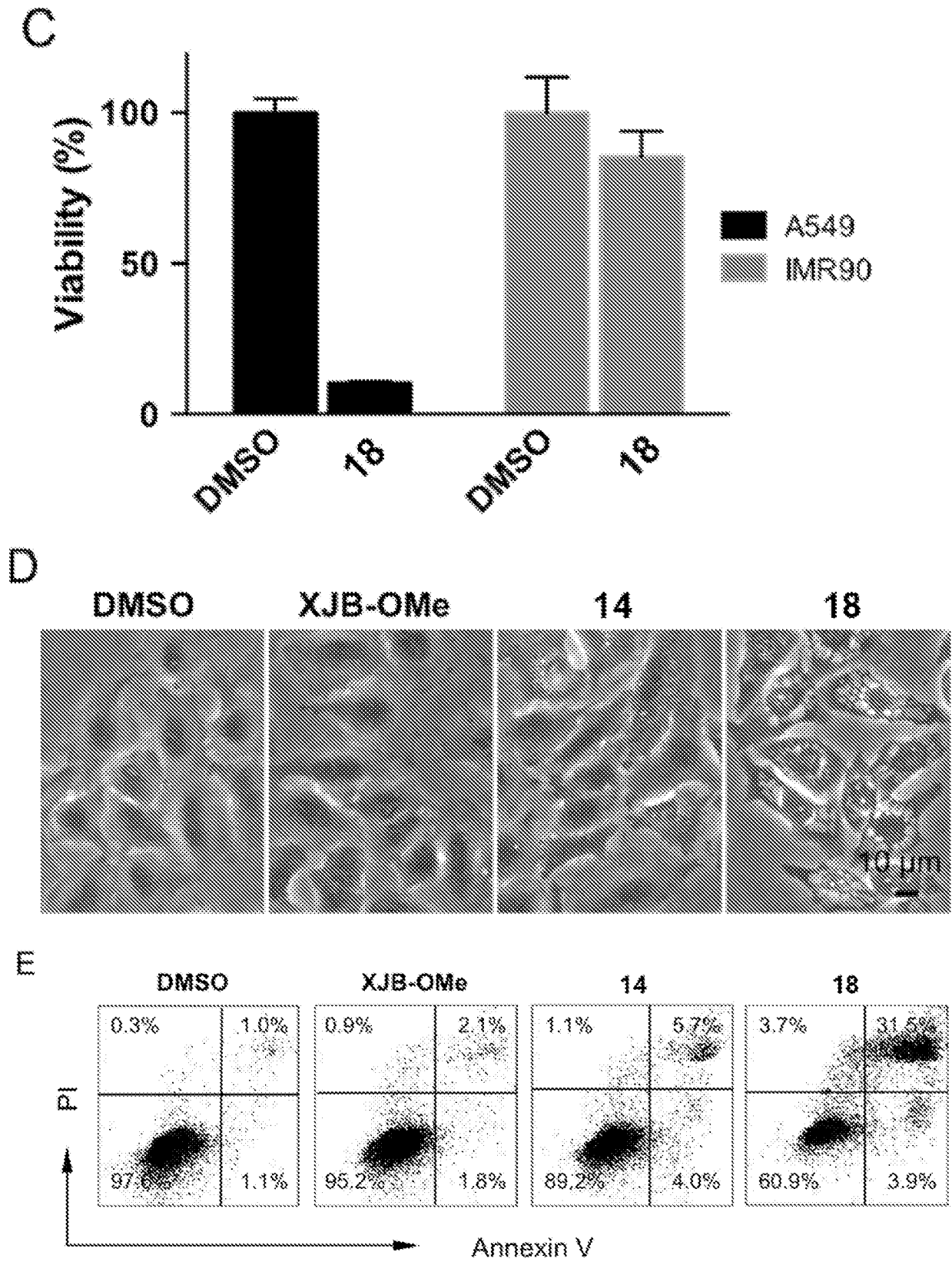


FIG. 3-2

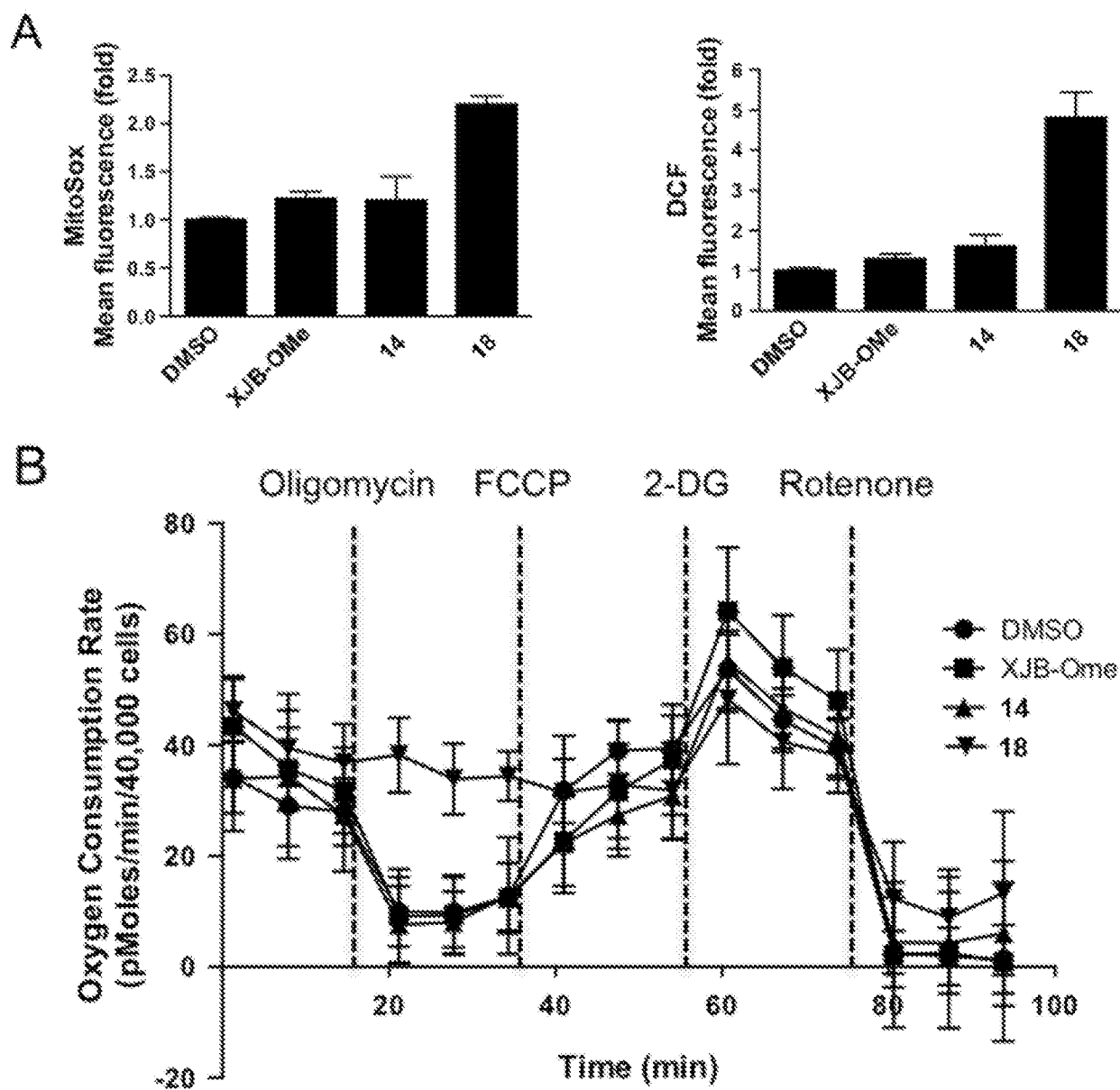


FIG. 4-1

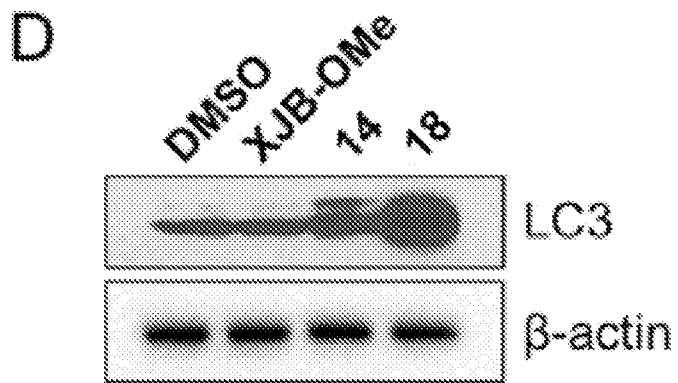
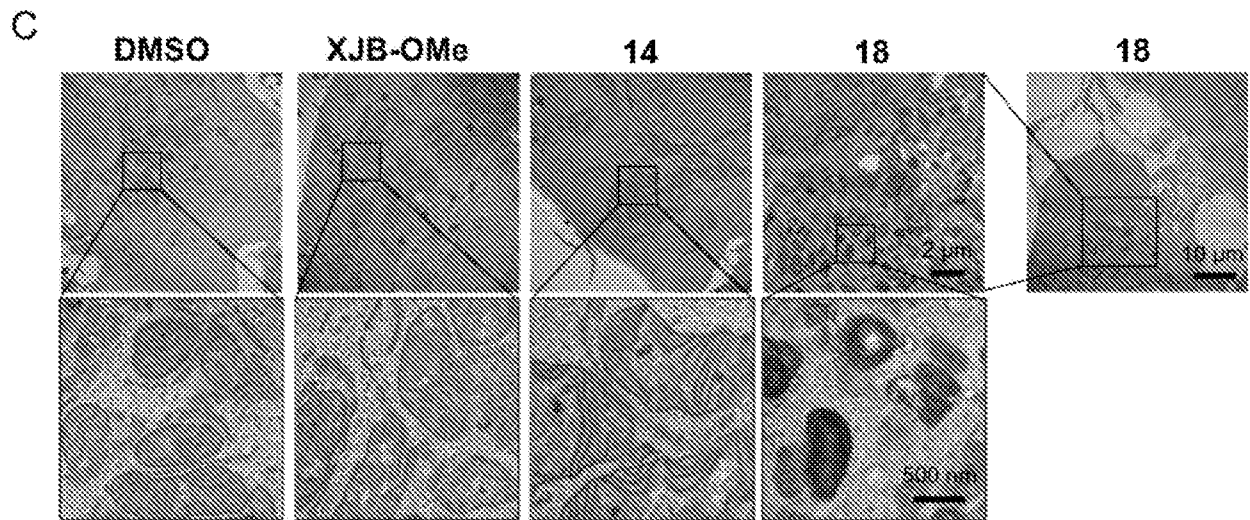


FIG. 4-2

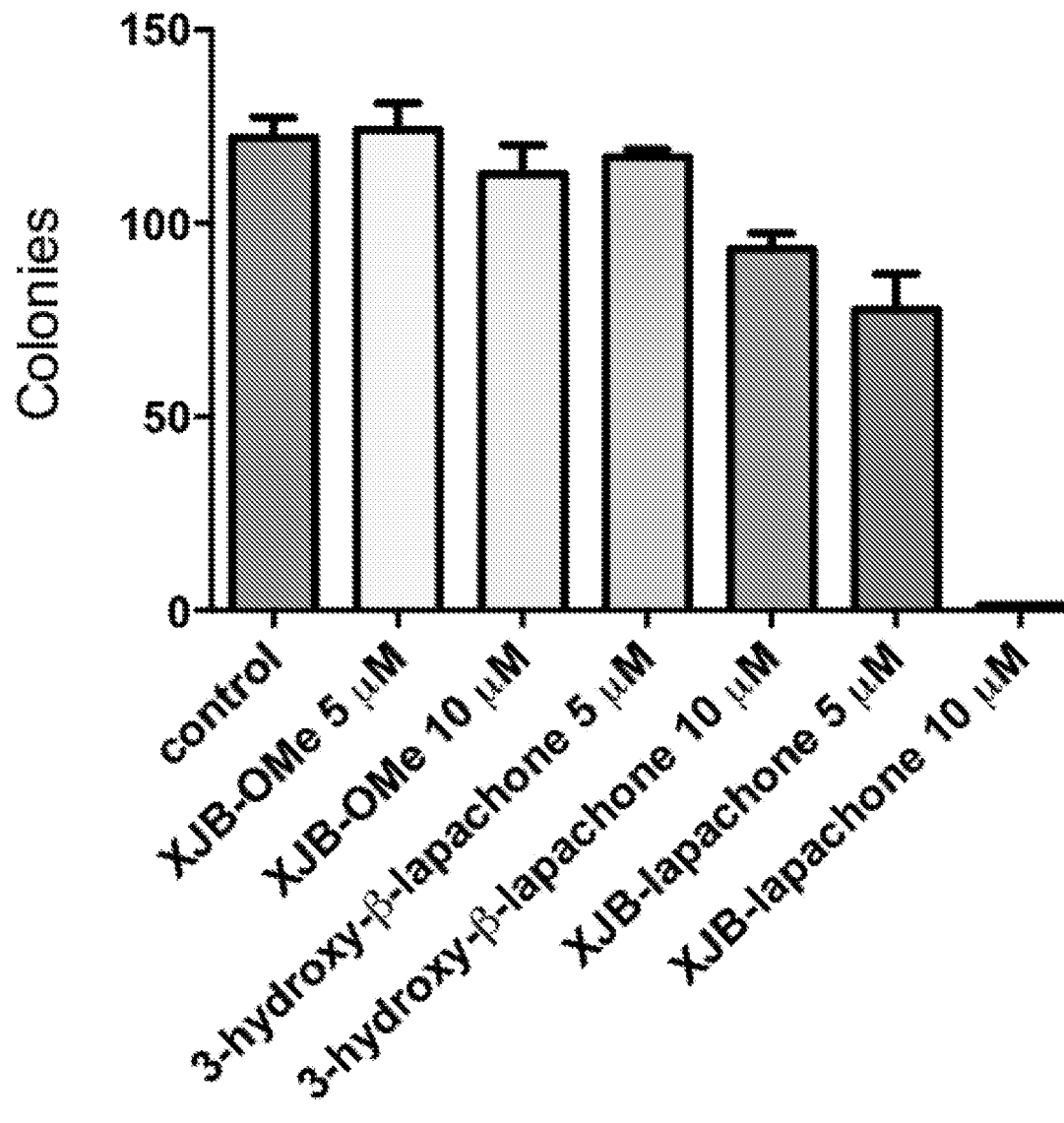


FIG. 5

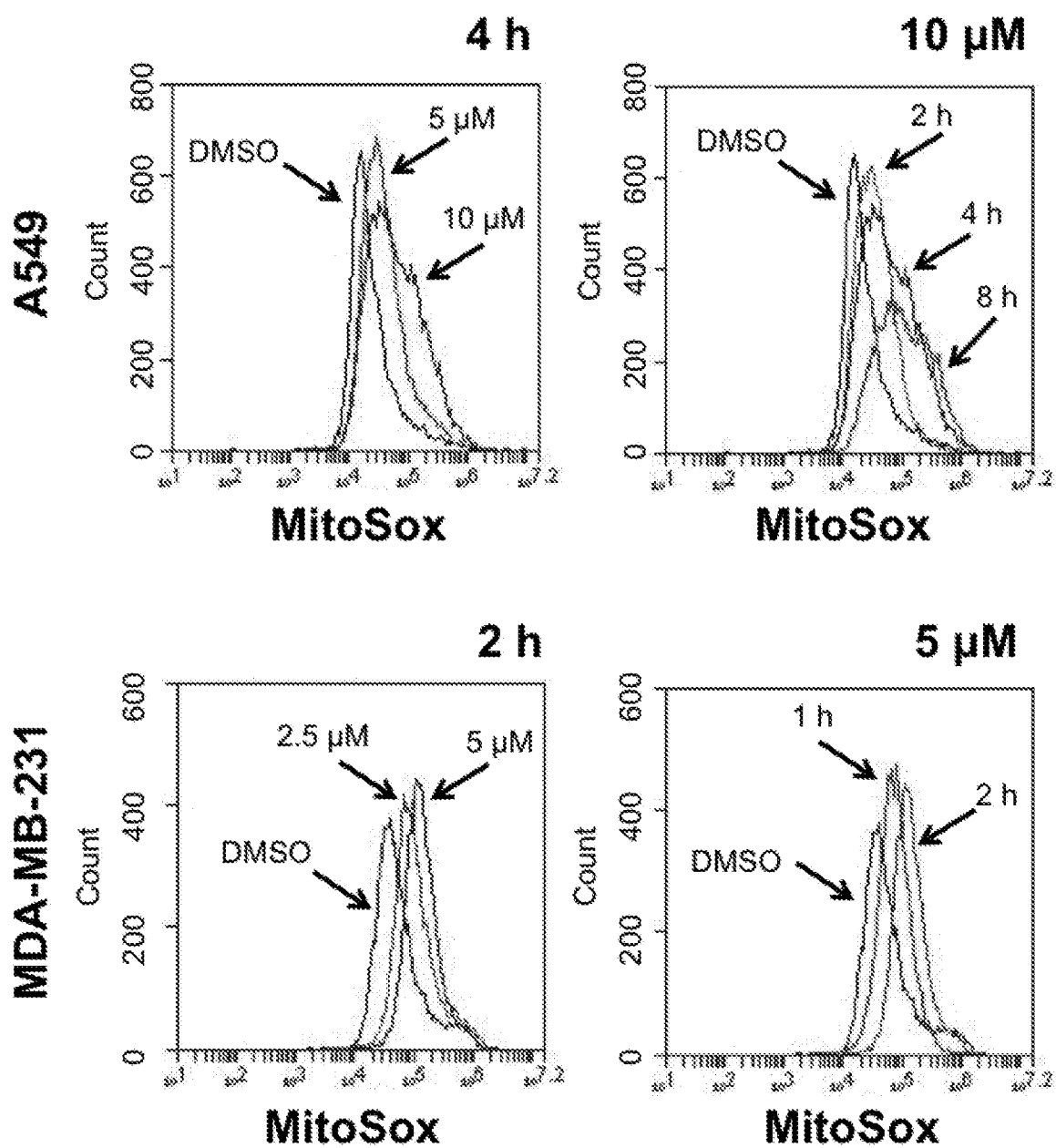


FIG. 6

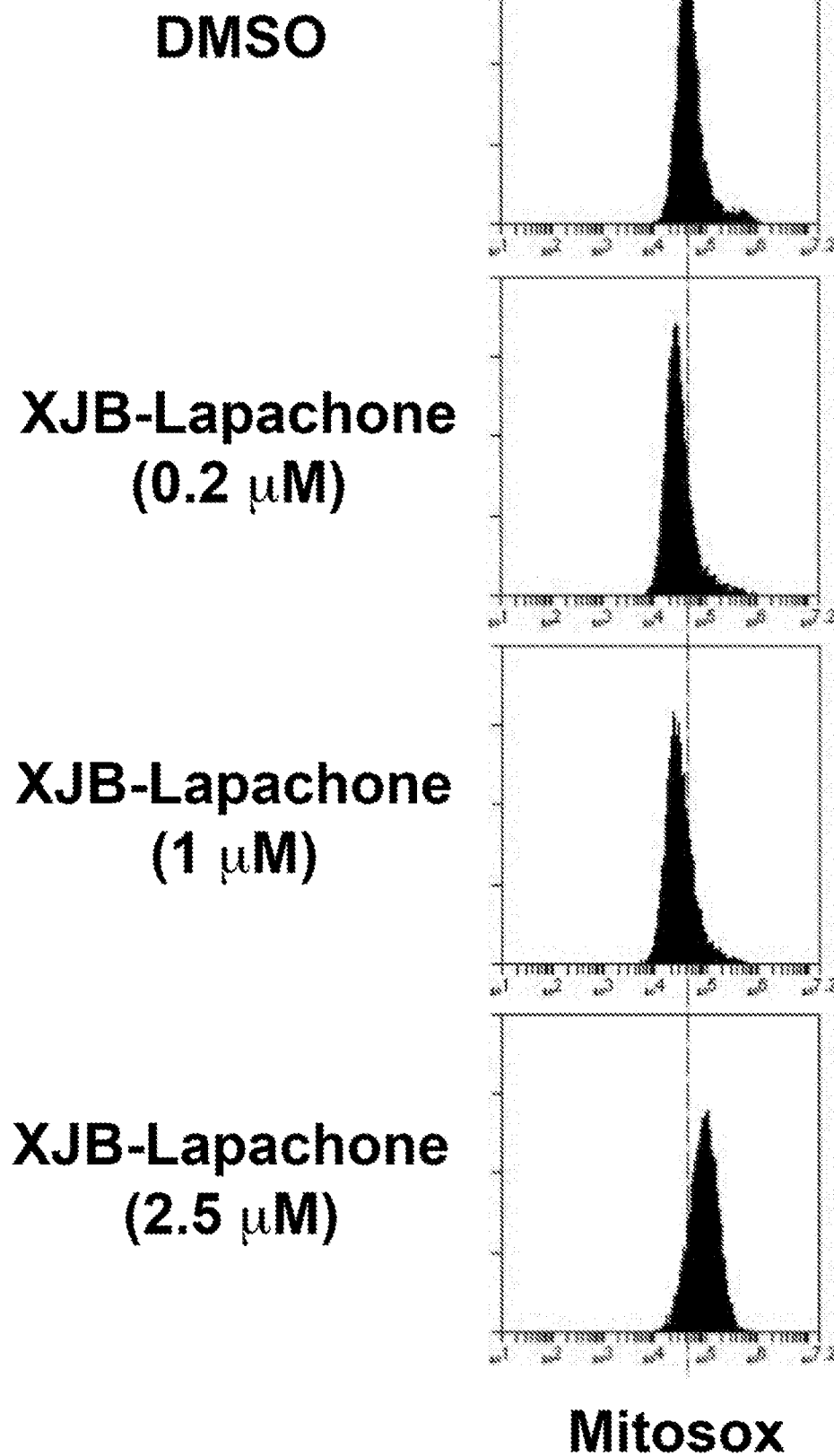
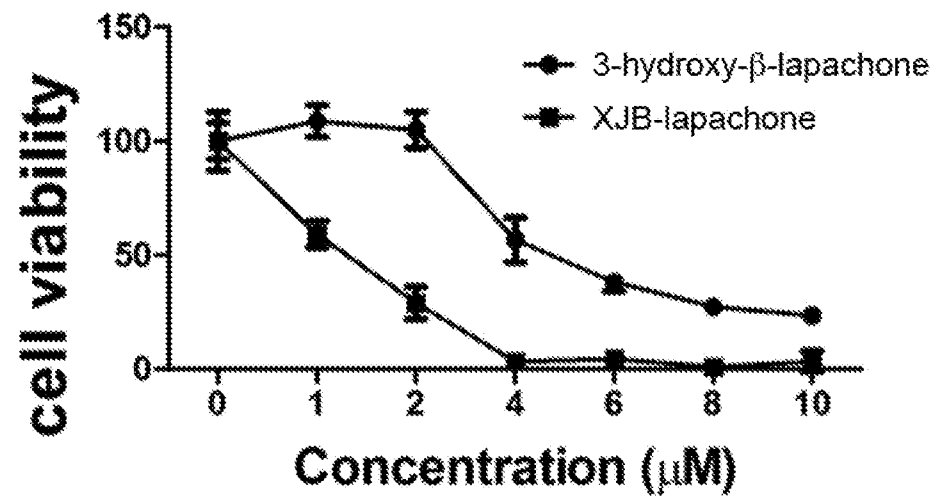
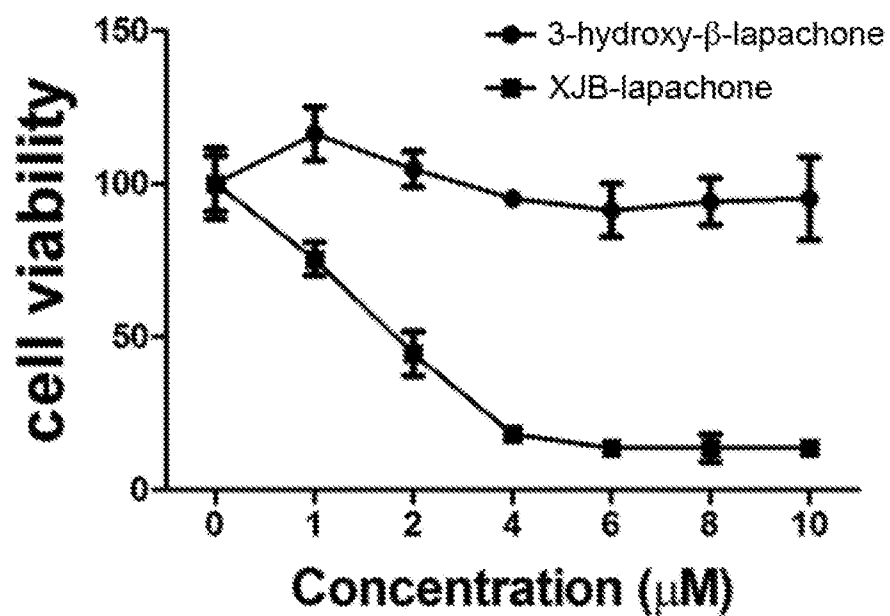


FIG. 7

A. MDA-MB-231 parental**B. MDA-MB-231 rho0****FIG. 8**

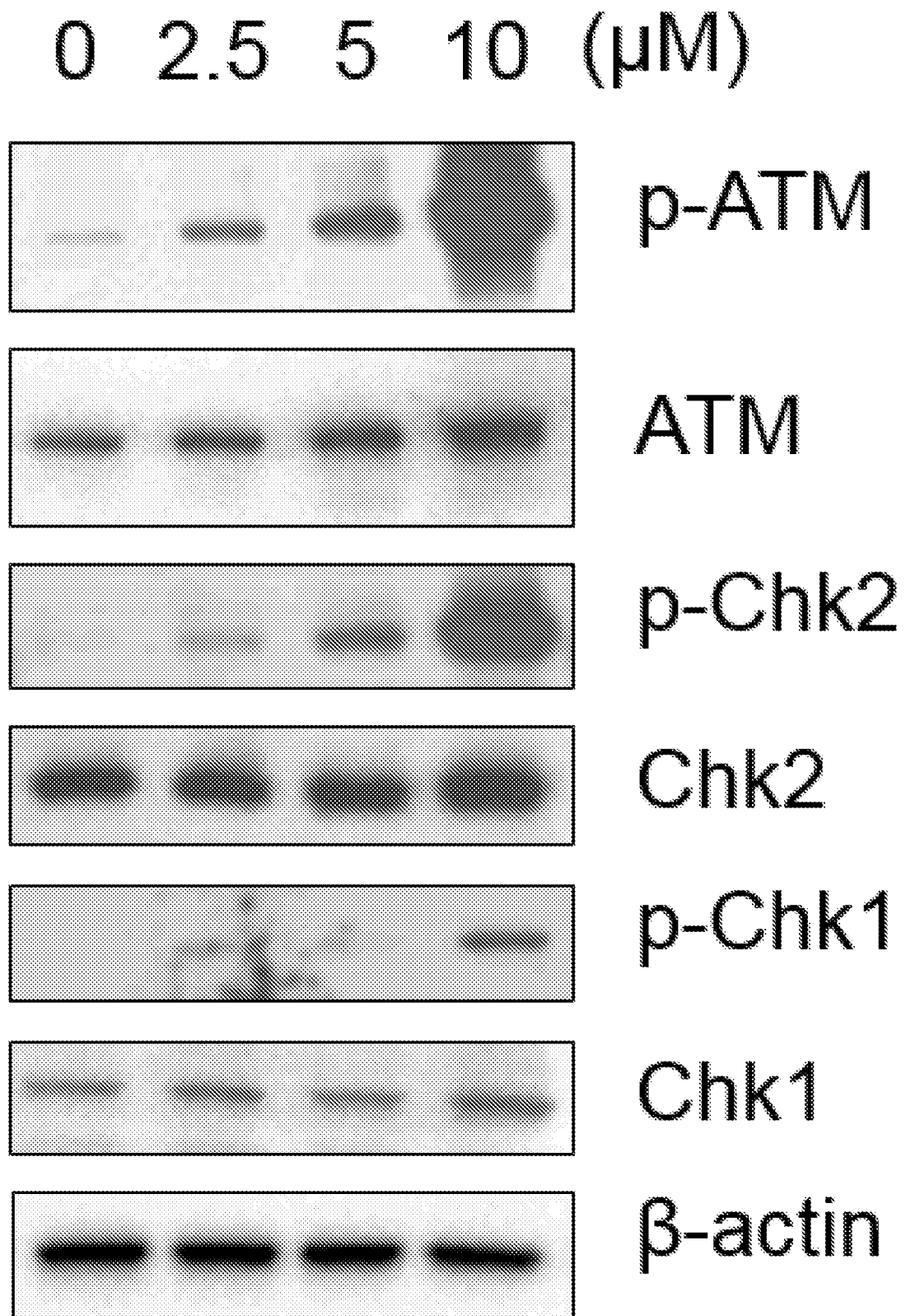


FIG. 9

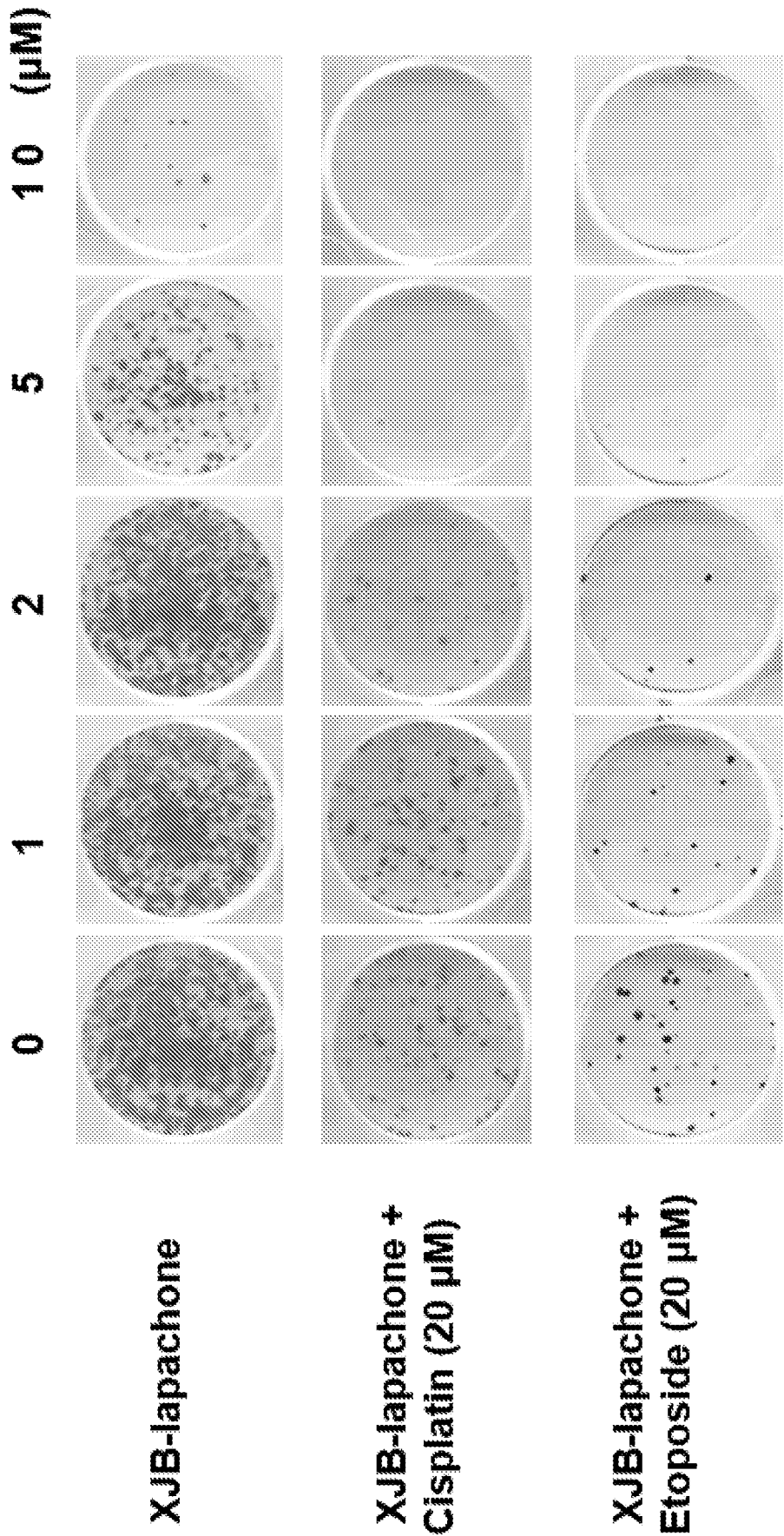


FIG. 10

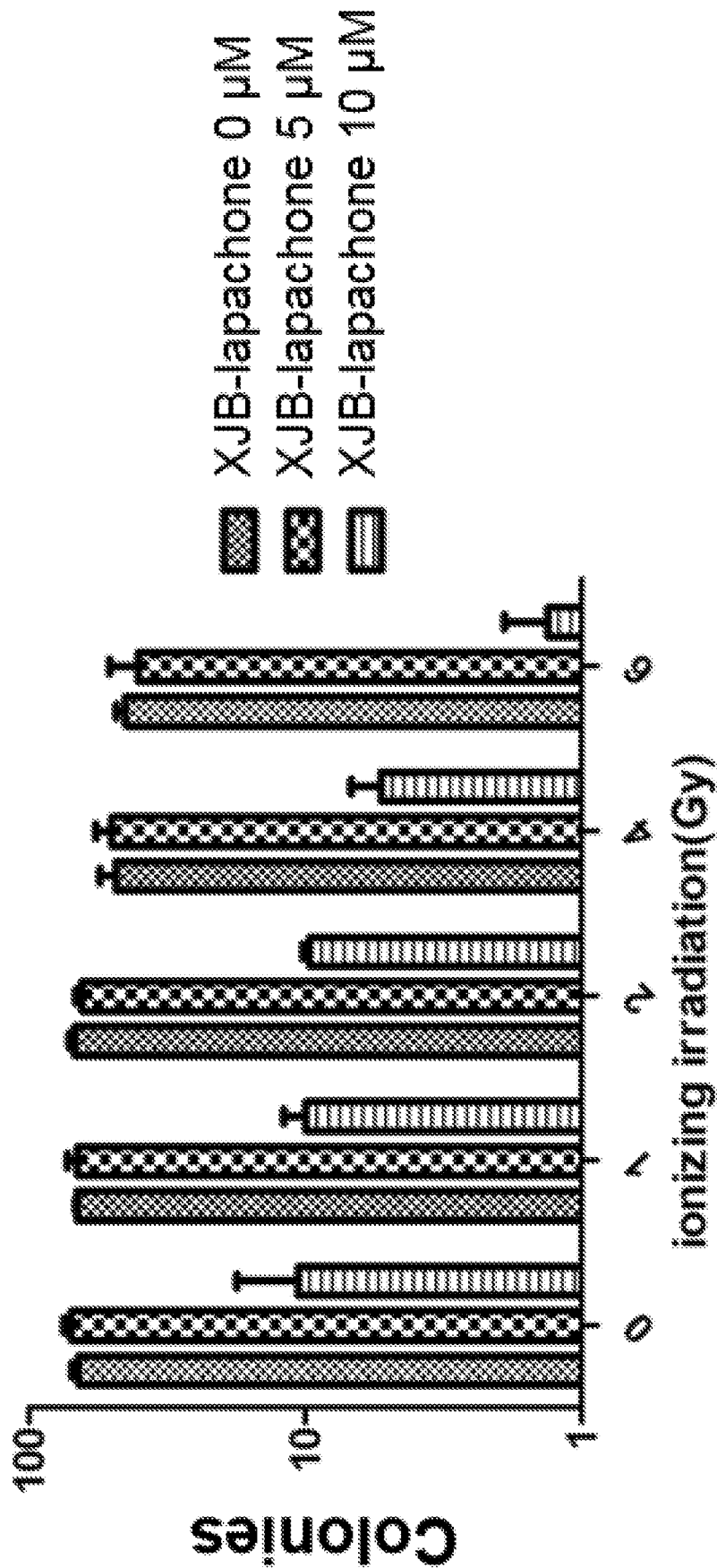


FIG. 11

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US2016/036440**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

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

1. ☒ Claims Nos.: 15-20
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 15-20 pertain to methods for treatment of the human body by therapy and thus relate to a subject matter which this International Searching Authority is not required to search under PCT Article 17(2)(a)(i) and Rule 39.1(iv).
2. ☒ Claims Nos.: 13-14,16,18
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Each of claims 13-14,16 and 18 refers to a claim which is not drafted in accordance with the third sentence of Rule 6.4(a).
3. ☒ Claims Nos.: 5,12,15,17,19-20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/036440**A. CLASSIFICATION OF SUBJECT MATTER****C07D 311/86(2006.01)I, C07D 335/08(2006.01)I, C07D 405/12(2006.01)I, A61K 31/35(2006.01)I, A61K 31/382(2006.01)I**

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D 311/86; C07F 5/02; C07D 211/94; A61K 9/14; A61K 31/352; A61K 31/66; C07D 335/08; C07D 405/12; A61K 31/382

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal), STN(Registry, CAplus), Google & Keywords: mitochondria, targeting, β -lapachone, cancer, reactive oxygen species (ROS)**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KUNG, HSIU-NI et al., 'The Chemotherapeutic Effects of Lapacho Tree Extract: β -Lapachone', Chemotherapy, 19 June 2014, Vol. 3, pp. 1-5 See abstract; pages 1-2; and figures 1-2.	1-4
A		6-11
Y	US 2014-0018317 A1 (WIPF, PETER et al.) 16 January 2014 See paragraphs [0003], [0058], [0081].	1-4
A	WO 2013-123298 A1 (UNIVERSITY OF GEORGIA RESEARCH FOUNDATION, INC.) 22 August 2013 See the whole document.	1-4, 6-11
A	LI, JASON Z. et al., 'Mechanistic studies of cancer cell mitochondria- and NQO1-mediated redox activation of beta-lapachone, a potentially novel anticancer agent', Toxicology and Applied Pharmacology, 29 October 2014, Vol. 281, pp. 285-293 See the whole document.	1-4, 6-11
A	US 2009-0028952 A1 (BARTIS, JUDIT et al.) 29 January 2009 See the whole document.	1-4, 6-11

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

20 September 2016 (20.09.2016)

Date of mailing of the international search report

20 September 2016 (20.09.2016)

Name and mailing address of the ISA/KR

International Application Division

Korean Intellectual Property Office

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/036440

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	MA, JING et al., 'Mitochondrial targeted β -lapachone induces mitochondrial dysfunction and catastrophic vacuolization in cancer cells', Bioorganic & Medicinal Chemistry Letters, 26 June 2015, Vol. 25, pp. 4828-4833 See the whole document.	1-4, 6-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2016/036440

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2014-0018317 A1	16/01/2014	US 2016-0068556 A1 US 9217000 B2 WO 2012-112851 A2 WO 2012-112851 A3	10/03/2016 22/12/2015 23/08/2012 15/11/2012
WO 2013-123298 A1	22/08/2013	EP 2814496 A1 EP 2814496 A4 JP 2015-513533 A US 2016-0022825 A1	24/12/2014 21/10/2015 14/05/2015 28/01/2016
US 2009-0028952 A1	29/01/2009	AU 2008-246067 A1 AU 2008-246067 B2 BR PI0810717 A2 CA 2685739 A1 CA 2685739 C CN 101687835 A CN 101687835 B DK 2152686 T3 EP 2152686 A1 EP 2152686 B1 ES 2532656 T3 HK 1139411 A1 JP 2010-526072 A KR 10-2010-0017483 A MX 2009011822 A TW 200913986 A TW I401081 B US 7790765 B2 WO 2008-134088 A1	06/11/2008 20/10/2011 24/05/2016 06/11/2008 14/06/2016 31/03/2010 19/08/2015 12/01/2015 17/02/2010 17/12/2014 30/03/2015 21/08/2015 29/07/2010 16/02/2010 14/01/2010 01/04/2009 11/07/2013 07/09/2010 06/11/2008