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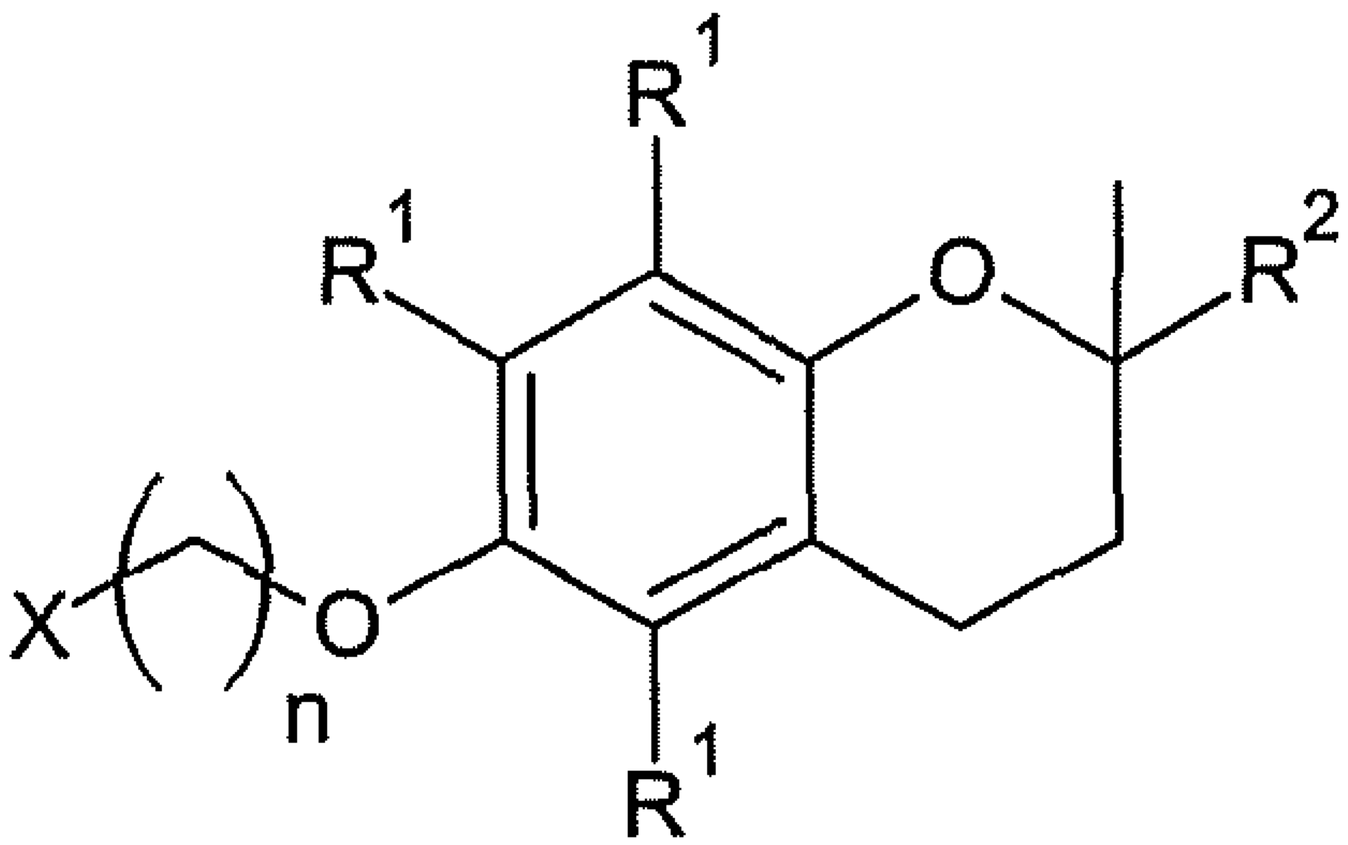


(I)

(57) Abrégé/Abstract:
Tocopheryl succinate derivatives according to formula (I) are described. These compounds increase the activity of protein phosphatase 2A, can be included in pharmaceutical compositions, and can be used for the treatment of androgen receptor-dependent cancers such as prostate cancer.

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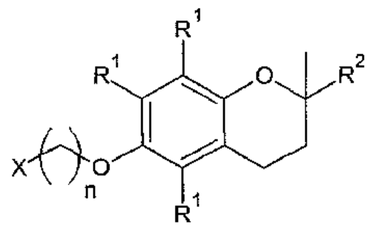


(I)

(57) Abstract: Tocopheryl succinate derivatives according to formula (I) are described. These compounds increase the activity of protein phosphatase 2A, can be included in pharmaceutical compositions, and can be used for the treatment of androgen receptor-dependent cancers such as prostate cancer.

# PROTEIN PHOSPHATASE 2A-ACTIVATING AGENTS

## CONTINUING APPLICATION DATA

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/168,759, filed April 13, 2009, which is incorporated by reference herein.

## GOVERNMENT FUNDING

[0002] The present invention was made with government support under Grant No. CA12250, awarded by the National Cancer Institute (NCI) and Grant No. PC074151, awarded by the Department of Defense Prostate Cancer Research Program. The Government may have certain rights in this invention.

## BACKGROUND

[0003] The translational potential of α-tocopheryl succinate (a.k.a., vitamin E succinate; VES) in cancer therapy has been the focus of many recent investigations in light of its efficacy in suppressing tumor cell proliferation without incurring toxicity to normal cells. See for example Wang *et al.*, Mol. Nutr. Food Res., 50, 675-85 (2006). Substantial evidence indicates that VES exhibits a unique ability to target multiple signaling pathways associated with carcinogenesis, tumor progression, and metastasis, including those mediated by NF-κB, PKCα, sphingolipids, Bcl-2/Bcl-xL, androgen receptor (AR), vascular endothelial growth factor (VEGF), and insulin-like growth factor binding protein-3. Although some of these signaling targets might be cancer type-specific, this broad spectrum of action in conjunction with low toxicity underlies the therapeutic value of developing VES into useful agents for cancer treatment or prevention.

[0004] One of the cancers affected by VES is prostate cancer. A significant challenge in the management of patients with prostate cancer is the treatment of hormone-refractory prostate cancer (HRPC), a hallmark of incurable and lethal prostate cancer progression. To date, chemotherapeutic regimens provide substantive benefits through palliation, but yield no definitive enhancement in survival. A clear need exists for novel strategies that will improve

the treatment of prostate cancer and ultimately increase the survival of prostate cancer patients. Accordingly, significant efforts have been expended to identify small-molecule agents targeting dysregulated pathways associated with HRPC.

[0005] The Ras signaling system provides a potential target for small molecule agents being developed for use against prostate cancer. The proto-oncogenic Ras functions as a molecular switch for signal transduction pathways controlling cell growth and differentiation, including those mediated by Akt, ERKs, RalA GTPase, and the transcription factor c-Myc. As these tumorigenic effectors of Ras regulate various aspects of oncogenesis in different cellular contexts, evidence indicates that Ras signaling represents a major driving force for prostate cancer progression to an androgen-independent state. Weber *et al.*, J. Cell Biochem, 91, p. 13-25 (2004). Moreover, dominant negative inhibition of endogenous Ras activity has been shown to restore androgen sensitivity to hormone-refractory C4-2 prostate cancer cells. Bakin *et al.*, Cancer Res., 63, p. 1975-80 (2003). Together, these finding indicate that Ras signaling represents a therapeutically relevant target for HRPC treatment.

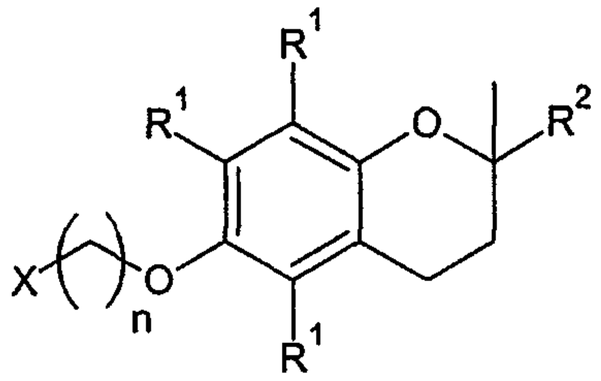
[0006] Farnesyltransferase (FTase) inhibitors were originally developed as anti-Ras compounds and novel target-based drugs for cancer treatment. However, R115777, a potent FTase inhibitor, showed little anti-tumor activity in minimally pretreated patients with androgen-independent prostate cancer. This lack of clinical efficacy underlies uncertainty over whether Ras is a relevant target of FTase inhibitors in humans.

[0007] PP2A is a tumor suppressor that antagonizes Ras signaling. PP2A is a ubiquitously expressed protein serine/threonine phosphatase that accounts for a large fraction of phosphatase activity in human cells. Janssens *et al.*, Biochem J., 353, p. 417-39 (2001). PP2A is composed of a dimeric core enzyme that includes a 65-kDa scaffolding A subunit (Aα or Aβ), a 36-kDa catalytic C subunit, and variable regulatory B subunits. The C subunit of PP2A undergoes reversible methylation on its C terminus, which regulates the binding of B regulatory subunits and PP2A phosphatase activity. Different B subunits confer different properties of PP2A in dephosphorylating downstream substrates, by which PP2A mediates distinct cellular functions. Substantial evidence indicates that PP2A functions as a tumor suppressor through its ability to mediate the dephosphorylation and inactivation of a number of tumorigenic proteins, including Akt, ERKs, and RalA. Mumby M., Cell, 130, p. 21-4 (2007). The fact that all of these tumorigenic PP2A substrates are downstream targets of Ras suggests that a major tumor suppressive activity of PP2A is to antagonize Ras signaling.

Thus, from a therapeutic perspective, developing small-molecule activators of PP2A activity represents a potentially effective strategy to counter Ras signaling and thereby re-sensitize prostate cancer cells to androgen ablation.

## SUMMARY OF THE INVENTION

[0008] One aspect of the invention provides compounds according to formula I:



(I)

wherein $R^1$ is independently selected from hydrogen and methyl; $R^2$ is selected from the group consisting of 4,8-dimethyl-non-1-enyl, 4,8-dimethyl-nonyl, non-1-enyl, and nonanyl groups; X is a carboxyl, phosphonic, or sulfonic moiety, and n is an integer from 1 to 6, or a pharmaceutically acceptable salt thereof. These compounds affect dysregulated pathways such as the RAS signaling system in androgen receptor-dependent cancers such as hormone-refractory prostate cancer.

[0009] Another aspect of the invention provides pharmaceutical compositions including a compound of formula I or a pharmaceutically acceptable salt thereof; as an active ingredient, and a pharmaceutically acceptable liquid or solid carrier or carriers, in combination with the active ingredient. A further aspect of the invention provides a method of treating or preventing the development of androgen receptor-dependent cancer in a subject that includes administering a therapeutically effective amount of a composition including a compound of Formula I or a pharmaceutically acceptable salt thereof. Embodiments of this aspect of the invention may be used for treating prostate cancer, such as hormone-refractory prostate cancer. Yet another aspect of the invention provides a method of increasing protein phosphatase 2A (PP2A) activity by administering an effective amount of a compound of Formula I or a pharmaceutically acceptable salt thereof.

## BRIEF DESCRIPTION OF THE FIGURES

[0010] Figure 1A, top, shows the structures of VES and TS-1, while the bottom provides a bar graph showing that VES and TS-1 increased PP2A phosphatase activity without affecting its expression. Figure 1B shows the effect of VES and TS-1 on facilitating the dephosphorylation of Akt and MAP kinases (ERKs, JNKs, and p38). Except for ERKs in PC-3, these kinases underwent marked dephosphorylation in drug-treated cells.

[0011] Figure 2A provides a schematic model of the factors involved in the antitumor activities of VES and TS-1 through PP2A activation. Figure 2B provides a graph showing the effect of VES and TS-1 on suppressing the cell viability of LNCaP cells versus PrECs.

[0012] Figure 3 provides a table showing the three components with varying structures involved in the combinatorial synthesis of tocopheryl succinate derivatives via coupling.

[0013] Figure 4 provides a synthetic scheme for tocopheryl succinate derivatives.

[0014] Figure 5 shows the effect of VES and TS-1 on the transcriptional regulation of AR expression in LNCaP cells versus PrECs. Section (A) shows the structures of VES and TS-1. Section (B) shows the results of Western blot analysis of the dose- (upper) and time-dependent (lower) effects of VES and TS-1 on the expression of AR and its target gene products PSA and/or EGFR in LNCaP cells in 2.5% FBS-supplemented medium. Percentage values denote the relative intensity of protein bands of drug-treated samples to that of the respective DMSO vehicle-treated control after normalization to the respective internal reference $\beta$-actin. Each value represents the average of two independent experiments. Section (C), left, shows the RT-PCR analysis of the time-dependent suppressive effect of 10 $\mu$M VES or TS-1 on AR mRNA levels in LNCaP cells after 72-h incubation in 2.5% FBS-supplemented medium. Percentage values denote the relative intensity of mRNA bands of drug-treated samples to that of the respective DMSO vehicle-treated control after normalization to the respective internal reference $\beta$-actin. Each value represents the average of two independent experiments. The right side shows the dose-dependent inhibitory effect of VES and TS-1 on luciferase reporter activity in hAR-Luctransfected LNCaP cells after 72-h incubation in 2.5% FBS-supplemented medium. Columns, mean; bars, SD (n = 6). Section (D), upper, shows the differential expression of AR in PrECs versus LNCaP cells, while the lower portion shows a Western blot analysis of the time-dependent effect of 10 $\mu$mol/L VES or TS-1 on the expression of AR and PSA in PrECs in 2.5% FBS-supplemented medium. Cells were exposed to 10 $\mu$mol/L VES or TS-1 for the indicated time intervals, and the

expression levels of AR and PSA were analyzed by Western blot analysis. Section (E) shows the selective dose-dependent suppression of the viability of PrECs versus LNCaP cells by TS-1 after 72-h incubation in 2.5% FBS-supplemented prostate epithelial growth and RPMI 1640 media, respectively, as determined by MTT assays. Each data point represents mean + SD (n = 6).

## DETAILED DESCRIPTION OF THE INVENTION

[0015] The inventors have demonstrated that VES and a number of tocopheryl derivatives mediate the dephosphorylation of Akt and MAP kinases in LNCaP and PC-3 cells through the activation of PP2A activity, as shown in FIG. 1. Accordingly, the inventors have developed a novel class of protein phosphatase 2A (PP2A)-activating agents based on α-tocopheryl succinate in view of the understanding that activation of PP2A phosphatase activity can delay or block cancer progression by antagonizing Ras-mediated oncogenic signaling pathways.

[0016] Since a major function of PP2A as a tumor suppressor is to antagonize Ras oncogenic signaling by downregulating the phosphorylation/activity of Ras targets, activation of PP2A activity by small-molecule agents represents a therapeutically relevant strategy to block cancer progression, and in particular prostate cancer progression.

Definitions

[0017] The terminology as set forth herein is for description of the embodiments only and should not be construed as limiting of the invention as a whole. Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably. Furthermore, as used in the description of the invention and the appended claims, the singular forms “a”, “an”, and “the” are inclusive of their plural forms, unless contraindicated by the context surrounding such.

[0018] The terms "comprising" and variations thereof do not have a limiting meaning where these terms appear in the description and claims.

[0019] As used herein, the term "organic group" is used for the purpose of this invention to mean a hydrocarbon group that is classified as an aliphatic group, cyclic group, or combination of aliphatic and cyclic groups (*e.g.*, alkaryl and aralkyl groups). In the context of the present invention, suitable organic groups for tocopheryl succinate derivatives are

those that do not interfere with the tocopheryl succinate derivatives' anticancer activity. In the context of the present invention, the term "aliphatic group" means a saturated or unsaturated linear or branched hydrocarbon group. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example.

[0020] As used herein, a carboxyl moiety (COOH) includes a hydroxyl moiety attached to a carbonyl group. A sulfonic moiety ($SO_3H$) is the defining portion of a sulfonic acid, and a phosphonic moiety ($PO_3H_2$) is the defining portion of a phosphonic acid.

[0021] As used herein, the terms "alkyl", "alkenyl", and the prefix "alk-" are inclusive of straight chain groups and branched chain groups and cyclic groups, *e.g.*, cycloalkyl and cycloalkenyl. Unless otherwise specified, these groups contain from 1 to 20 carbon atoms, with alkenyl groups containing from 2 to 20 carbon atoms. In some embodiments, these groups have a total of at most 10 carbon atoms, at most 8 carbon atoms, at most 6 carbon atoms, or at most 4 carbon atoms. Lower alkyl groups are those including at most 6 carbon atoms. Examples of alkyl groups include haloalkyl groups and hydroxyalkyl groups. Cyclic groups can be monocyclic or polycyclic and preferably have from 3 to 10 ring carbon atoms.

[0022] The term "truncated side chain," as used herein, refers to a phytyl side chain of a tocopheryl succinate derivative that has been shortened by the removal of one or more isopranyl units. Such truncated side chains are alkyl groups including from 1 to 11 carbon atoms. Examples of truncated side chains include 4,8-dimethyl-non-1-enyl, 4,8-dimethyl-nonyl, non-1-enyl, and nonanyl groups.

[0023] Unless otherwise specified, "alkylene" and "alkenylene" are the divalent forms of the "alkyl" and "alkenyl" groups defined above. The terms, "alkylenyl" and "alkenylenyl" are used when "alkylene" and "alkenylene", respectively, are substituted. For example, an arylalkylenyl group comprises an alkylene moiety to which an aryl group is attached.

[0024] The term "haloalkyl" is inclusive of groups that are substituted by one or more halogen atoms, including perfluorinated groups. This is also true of other groups that include the prefix "halo-". Examples of suitable haloalkyl groups are chloromethyl, trifluoromethyl, and the like. A halo moiety can be chlorine, bromine, fluorine, or iodine.

[0025] The term "aryl" as used herein includes carbocyclic aromatic rings or ring systems. Examples of aryl groups include phenyl, naphthyl, biphenyl, anthracenyl, phenanthracenyl, fluorenyl and indenyl. Aryl groups may be substituted or unsubstituted.

[0026] Unless otherwise indicated, the term "heteroatom" refers to the atoms O, S, or N.

[0027] The term "heteroaryl" includes aromatic rings or ring systems that contain at least one ring heteroatom (*e.g.*, O, S, N). In some embodiments, the term "heteroaryl" includes a ring or ring system that contains 2 to 12 carbon atoms, 1 to 3 rings, 1 to 4 heteroatoms, and O, S, and/or N as the heteroatoms. Suitable heteroaryl groups include furyl, thienyl, pyridyl, quinolinyl, isoquinolinyl, indolyl, isoindolyl, triazolyl, pyrrolyl, tetrazolyl, imidazolyl, pyrazolyl, oxazolyl, thiazolyl, benzofuranyl, benzothiophenyl, carbazolyl, benzoxazolyl, pyrimidinyl, benzimidazolyl, quinoxalinyl, benzothiazolyl, naphthyridinyl, isoxazolyl, isothiazolyl, purinyl, quinazolinyl, pyrazinyl, 1-oxidopyridyl, pyridazinyl, triazinyl, tetrazinyl, oxadiazolyl, thiadiazolyl, and so on.

[0028] The terms "arylene" and "heteroarylene" are the divalent forms of the "aryl" and "heteroaryl" groups defined above. The terms "arylenyl" and "heteroarylenyl" are used when "arylene" and "heteroarylene", *respectively, are substituted. For example, an alkylarylenyl* group comprises an arylene moiety to which an alkyl group is attached.

[0029] When a group is present more than once in any formula or scheme described herein, each group (or substituent) is independently selected, whether explicitly stated or not. For example, for the formula $-C(O)-NR_2$ each R group is independently selected.

[0030] As a means of simplifying the discussion and the recitation of certain terminology used throughout this application, the terms "group" and "moiety" are used to differentiate between chemical species that allow for substitution or that may be substituted and those that do not so allow for substitution or may not be so substituted. Thus, when the term "group" is used to describe a chemical substituent, the described chemical material includes the unsubstituted group and that group with nonperoxidic O, N, S, Si, or F atoms, for example, in the chain as well as carbonyl groups or other conventional substituents. Where the term "moiety" is used to describe a chemical compound or substituent, only an unsubstituted chemical material is intended to be included. For example, the phrase "alkyl group" is intended to include not only pure open chain saturated hydrocarbon alkyl substituents, such as methyl, ethyl, propyl, *tert*-butyl, and the like, but also alkyl substituents bearing further

substituents known in the art, such as hydroxy, alkoxy, alkylsulfonyl, halogen atoms, cyano, nitro, amino, carboxyl, etc. Thus, "alkyl group" includes ether groups, haloalkyls, nitroalkyls, carboxyalkyls, hydroxyalkyls, sulfoalkyls, etc. On the other hand, the phrase "alkyl moiety" is limited to the inclusion of only pure open chain saturated hydrocarbon alkyl substituents, such as methyl, ethyl, propyl, *tert*-butyl, and the like.

[0031] The invention is inclusive of the compounds described herein (including intermediates) in any of their pharmaceutically acceptable forms, including isomers (*e.g.*, diastereomers and enantiomers), tautomers, salts, solvates, polymorphs, prodrugs, and the like. In particular, if a compound is optically active, the invention specifically includes each of the compound's enantiomers as well as racemic mixtures of the enantiomers. It should be understood that the term "compound" includes any or all of such forms, whether explicitly stated or not (although at times, "salts" are explicitly stated).

[0032] "Treat", "treating", and "treatment", etc., as used herein, refer to any action providing a benefit to a patient at risk for or afflicted with a disease, including improvement in the condition through lessening or suppression of at least one symptom, delay in progression of the disease, prevention or delay in the onset of the disease, etc.

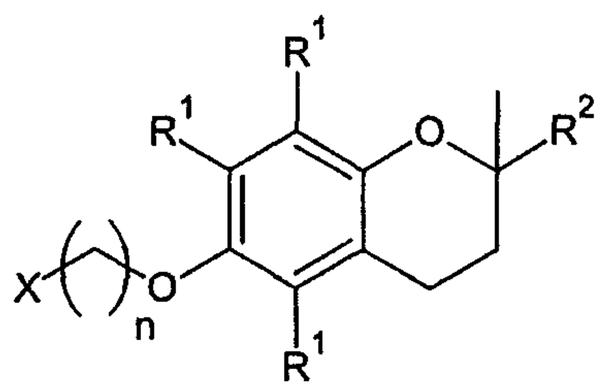
[0033] Androgen receptor-dependent cancers are cancers that are dependent on the presence of androgen receptors on the cancer cells to maintain the ability of the cells to proliferate. For example, hormone refractory prostate cancer is an androgen receptor-dependent cancer in which an increased number of androgen receptors are provided in order to make the cells supersensitive to androgen and thereby able to proliferate even in an environment in which androgen levels have been decreased. See Chen *et al.* Nat. Med. 10, 33-39 (2004), which provides further description of the role of androgen receptors in cancer.

[0034] "Pharmaceutically acceptable" as used herein means that the compound or composition is suitable for administration to a subject to achieve the treatments described herein, without unduly deleterious side effects in light of the severity of the disease and necessity of the treatment.

[0035] "Inhibit" as used herein refers to the partial or complete elimination of a potential effect, while inhibitors are compounds that have the ability to inhibit.

*Tocopheryl succinate derivatives*

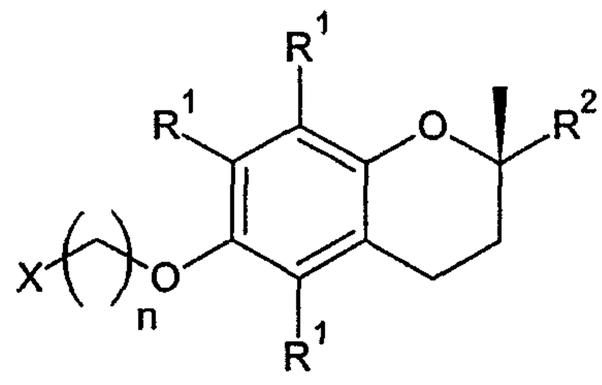
[0036] The compounds of the present invention include a variety of different tocopheryl succinate derivatives. Tocopheryl succinate derivatives, as used herein, refer to compounds that are compounds described herein that are structurally related to tocopheryl succinate. However, the compounds do not have to be synthetically derived from tocopheryl succinate, and do not require a succinate side chain. Tocopheryl succinate derivatives of the invention include compounds according to formula (I):



(I)

wherein $R^1$ is independently selected from hydrogen and methyl; $R^2$ is selected from the group consisting of 4,8-dimethyl-non-1-enyl, 4,8-dimethyl-nonyl, non-1-enyl, and nonanyl groups; X is a carboxyl, phosphonic, or sulfonic moiety, and n is an integer from 1 to 6.

[0037] The tocoperhyl succinate derivatives of the present invention have been shown and named herein without reference to stereochemistry. However, it is understood that vitamin E is [(2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]chroman-6-yl] acetate; *i.e.*, a 2R isomer of the compounds shown, and that the 2R isomers may be preferred in embodiments of the invention. Accordingly, the tocopheryl succinate derivatives of the invention also include compounds according to formula (II):

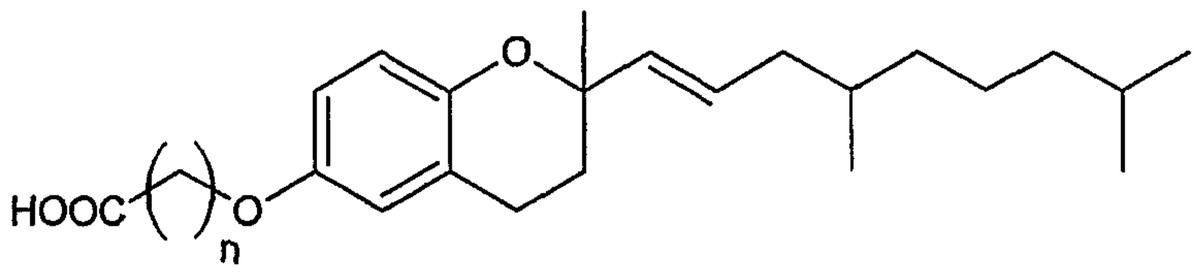


(II)

Wherein the various substituents are defined in the same manner as for formula (I).

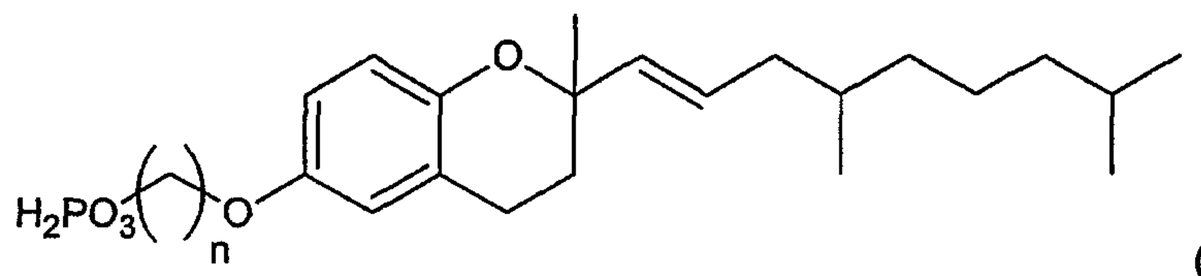
[0038] *In one embodiment, the tocopheryl succinate derivatives of formula (I) are defined* such that $R^1$ is hydrogen, $R^2$ is a 4,8-dimethyl-non-1-enyl group, X is carboxyl moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Ia) and include compounds selected from the group consisting of [2-(4,8-dimethyl-non-1-enyl)-2-methyl-

chroman-6-yloxy]-acitic acid, 3-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-propionic acid, 4-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-butyric acid, 5-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-pentanoic acid, 6-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-hexanoic acid, and 7-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-haptanoic acid.




(Ia)

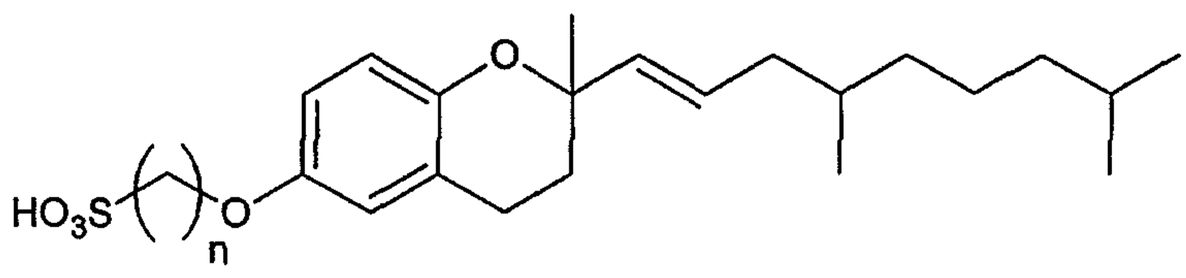
[0039] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a 4,8-dimethyl-non-1-enyl group, X is a phosphonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Ib) and include compounds selected from the group consisting of [2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxymethyl]-phosphonic acid, {2-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-ethyl}-phosphonic acid, {3-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-propyl}-phosphonic acid, {4-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-butyl}-phosphonic acid, {5-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-pentyl}-phosphonic acid, and {6-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-hexyl}-phosphonic acid.




(Ib)

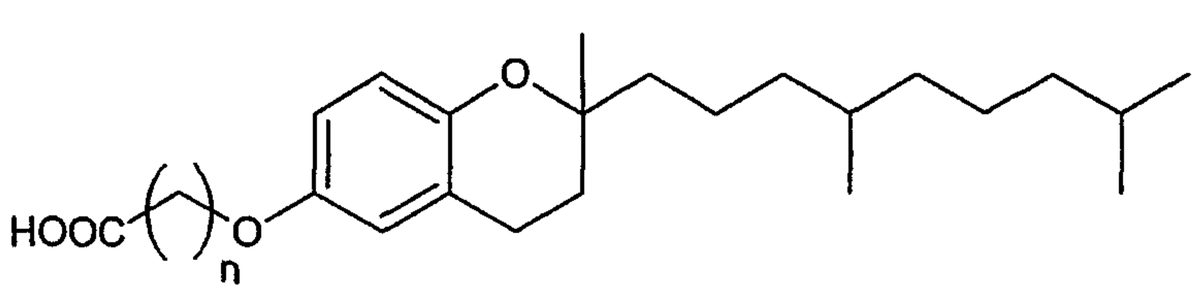
[0040] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a 4,8-dimethyl-non-1-enyl group, X is a sulfonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Ic) and include compounds selected from the group consisting of [2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-methanesulfonic acid; 2-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-ethanesulfonic acid; 3-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-propane-1-sulfonic acid; 4-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-butane-1-sulfonic acid; 5-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-pentane-

1-sulfonic acid; and 6-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-hexane-1-sulfonic acid.



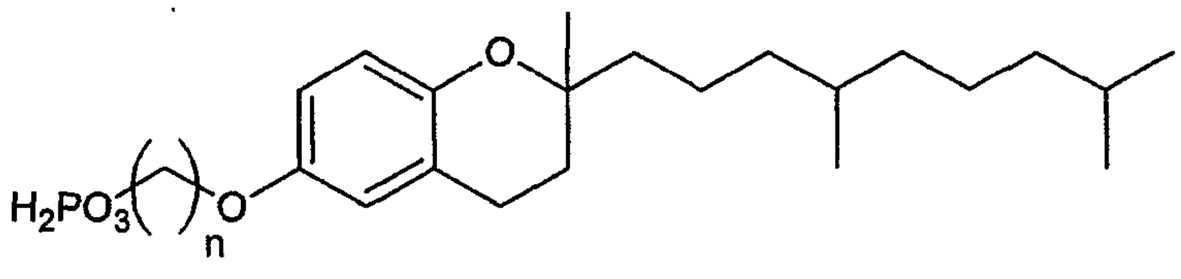

(Ic)

[0041] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a 4,8-dimethyl-nonyl group, X is a carboxyl moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Id) and include compounds selected from the group consisting of [2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-acitic acid, 3-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-propionic acid, 4-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-butyric acid, 5-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-pentanoic acid, 6-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-hexanoic acid, 7-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-haptanoic acid, and 8-[2-(4,8-dimethyl-non-1-enyl)-2-methyl-chroman-6-yloxy]-octanoic acid.



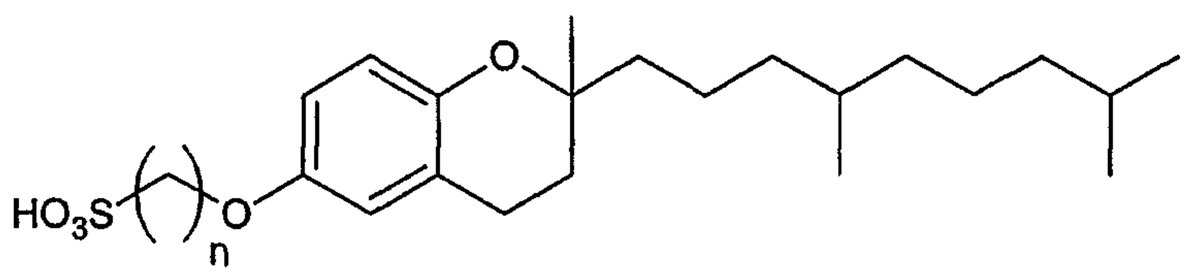

(Id)

[0042] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a 4,8-dimethyl-nonyl group, X is a phosphonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Ie) and include compounds selected from the group consisting of [2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxymethyl]-phosphonic acid, {2-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-ethyl}-phosphonic acid, {3-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-propyl}-phosphonic acid, {4-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-butyl}-phosphonic acid, {5-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-pentyl}-phosphonic acid, and {6-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-hexyl}-phosphonic acid.



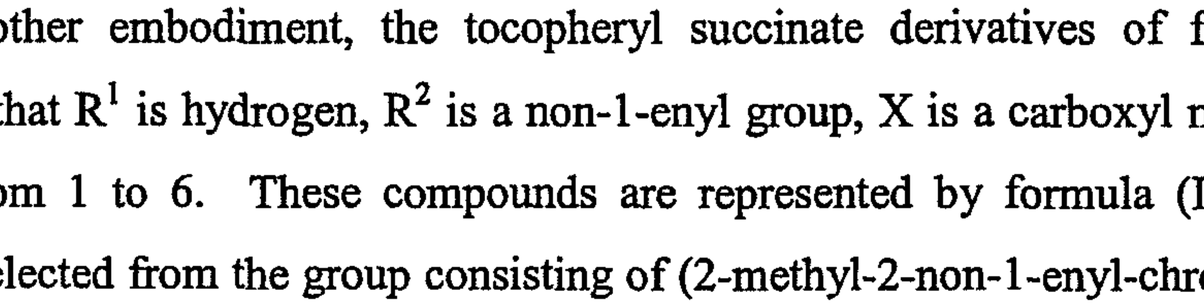

(Ie)

[0043] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a 4,8-dimethyl-nonyl group, X is a sulfonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (If) and include compounds selected from the group consisting of [2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-methanesulfonic acid; 2-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-ethanesulfonic acid; 3-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-propane-1-sulfonic acid; 4-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-butane-1-sulfonic acid; 5-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-pentane-1-sulfonic acid; and 6-[2-(4,8-dimethyl-nonyl)-2-methyl-chroman-6-yloxy]-hexane-1-sulfonic acid.




(If)

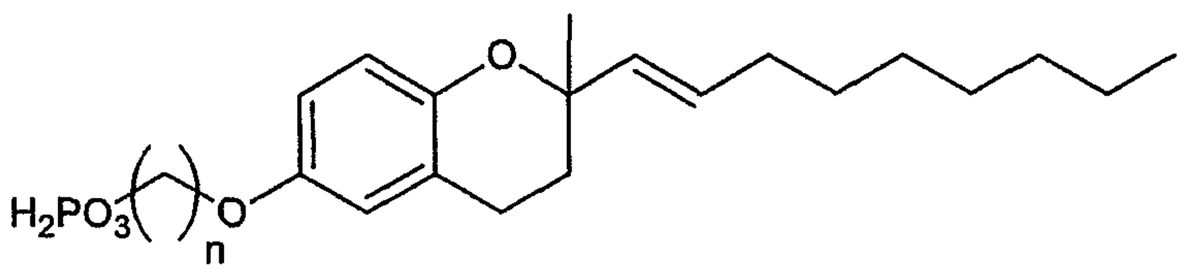
[0044] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a non-1-enyl group, X is a carboxyl moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Ig) and include compounds selected from the group consisting of (2-methyl-2-non-1-enyl-chroman-6-yloxy)-acetic acid; 3-(2-methyl-non-1-enyl-chroman-6-yloxy)-propionic acid, 4-(2-methyl-non-1-enyl-chroman-6-yloxy)-chroman-6-yloxy]-butyric acid, 5-(2-methyl-non-1-enyl-chroman-6-yloxy)-chroman-6-yloxy]-pentanoic acid, 6-(2-methyl-non-1-enyl-chroman-6-yloxy)-chroman-6-yloxy]-hexanoic acid, and 7-(2-methyl-non-1-enyl-chroman-6-yloxy)-chroman-6-yloxy]-heptanoic acid.




(Ig)

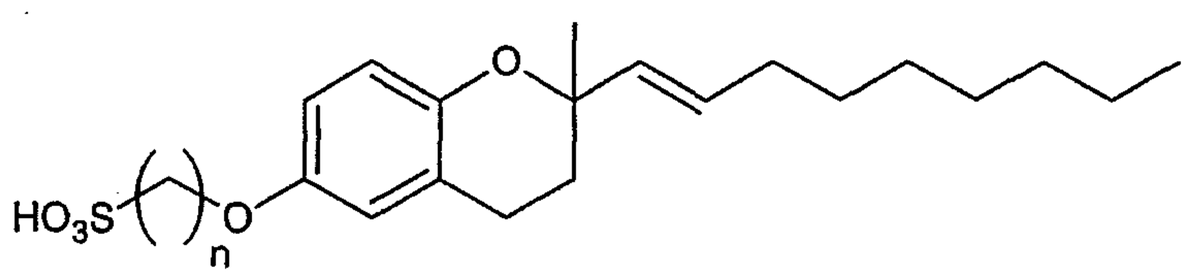
[0045] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a non-1-enyl group, X is a phosphonic moiety, and n

is an integer from 1 to 6. These compounds are represented by formula (Ih) and include compounds selected from the group consisting of (2-methyl-2-non-1-enyl-chroman-6-yloxymethyl)-phosphonic acid; [2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-ethyl]-phosphonic acid; [2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-propyl]-phosphonic acid; [2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-butyl]-phosphonic acid; [2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-pentyl]-phosphonic acid; and [2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-hexyl]-phosphonic acid.




(Ih)

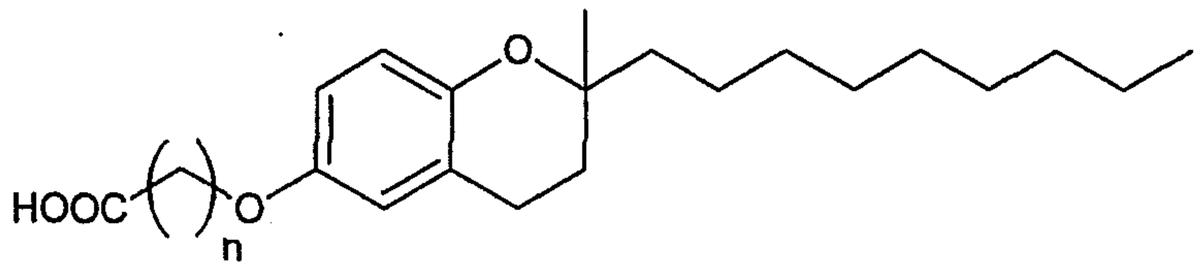
[0046] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a non-1-enyl group, X is a sulfonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Ii) and include compounds selected from the group consisting of 2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-methanesulfonic acid; 2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-ethanesulfonic acid; 2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-propane-1-sulfonic acid; 2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-butane-1-sulfonic acid; 2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-pentane-1-sulfonic acid; and 2-(2-methyl-2-non-1-enyl-chroman-6-yloxy)-hexane-1-sulfonic acid.




(Ii)

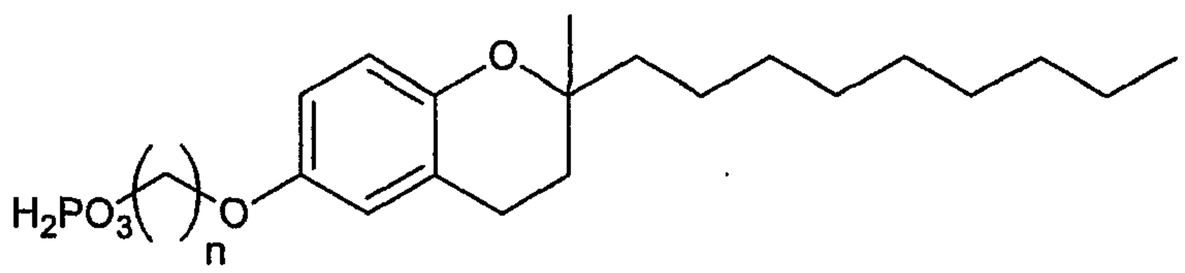
[0047] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a nonanyl group, X is a carboxyl moiety, and n is an integer selected from 1 to 6. These compounds are represented by formula (Ij) and include compounds selected from the group consisting of (2-methyl-2-nonyl-chroman-6-yloxy)-acetic acid, 3-(2-methyl-nonyl-chroman-6-yloxy)-propionic acid, 4-(2-methyl-nonyl-chroman-6-yloxy)-chroman-6-yloxy]-butyric acid, 5-(2-methyl- nonyl-chroman-6-yloxy)-chroman-6-yloxy]-pentanoic acid, 6-(2-methyl-nonyl-chroman-6-yloxy)-chroman-6-yloxy]-

hexanoic acid, 7-(2-methyl-nonyl-chroman-6-yloxy)-chroman-6-yloxy]-haptanoic acid, and 8-(2-methyl-nonyl-chroman-6-yloxy)-chroman-6-yloxy]-octanoic acid.



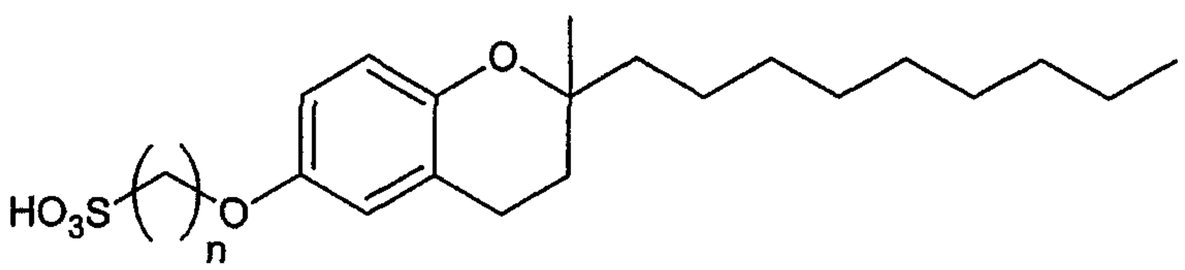

(Ij)

[0048] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a nonanyl group, X is a phosphonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Ik) and include compounds selected from the group consisting of (2-methyl-2-nonyl-chroman-6-yloxymethyl)-phosphonic acid; [2-(2-methyl-2-nonyl-chroman-6-yloxy)-ethyl]-phosphonic acid; [2-(2-methyl-2-nonyl-chroman-6-yloxy)-propyl]-phosphonic acid; [2-(2-methyl-2-nonyl-chroman-6-yloxy)-butyl]-phosphonic acid; [2-(2-methyl-2-nonyl-chroman-6-yloxy)-pentyl]-phosphonic acid; and [2-(2-methyl-2-nonyl-chroman-6-yloxy)-hexyl]-phosphonic acid.



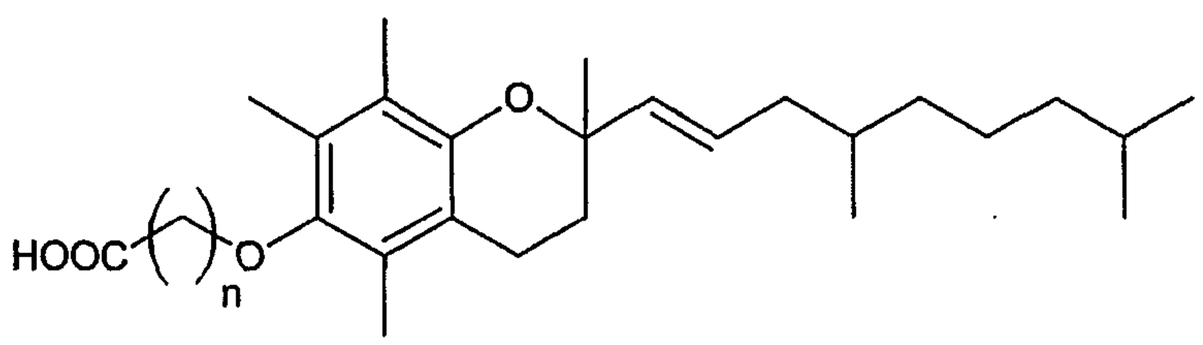

(Ik)

[0049] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is hydrogen, $R^2$ is a nonanyl group, X is a sulfonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Il) and include compounds selected from the group consisting of (2-methyl-2-nonyl-chroman-6-yloxy)-methanesulfonic acid; (2-methyl-2-nonyl-chroman-6-yloxy)-ethanesulfonic acid; (2-methyl-2-nonyl-chroman-6-yloxy)-propane-1-sulfonic acid; (2-methyl-2-nonyl-chroman-6-yloxy)-butane-1-sulfonic acid; (2-methyl-2-nonyl-chroman-6-yloxy)-pentane-1-sulfonic acid; and (2-methyl-2-nonyl-chroman-6-yloxy)-hexane-1-sulfonic acid.



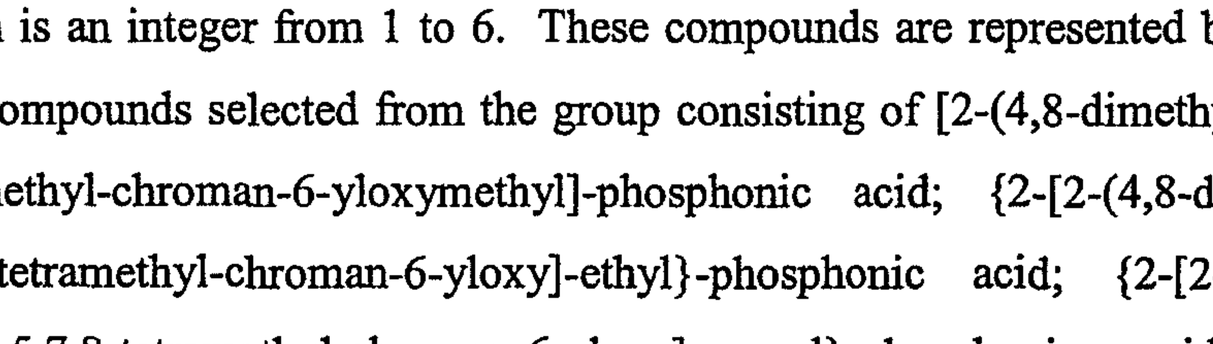

(Il)

[0050] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a 4,8-dimethyl-non-1-enyl group, X is a carboxyl moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Im) and include compounds selected from the group consisting of [2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-acetic acid; 3-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-propionic acid; [2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-butyric acid; [2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-pentanoic acid; [2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-hexanoic acid; and [2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-heptanoic acid.




(Im)

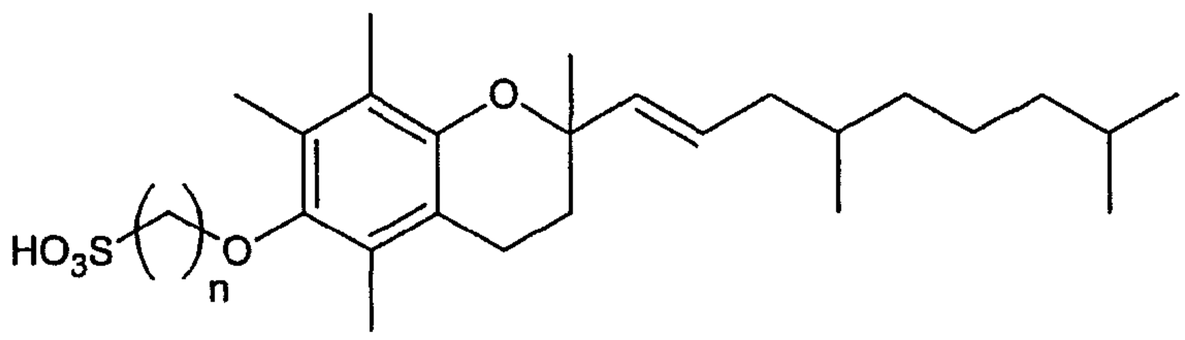
[0051] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a 4,8-dimethyl-non-1-enyl group, X is a phosphonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (In) and include compounds selected from the group consisting of [2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxymethyl]-phosphonic acid; {2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-ethyl}-phosphonic acid; {2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-propyl}-phosphonic acid; {2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-butyl}-phosphonic acid; {2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-pentyl}-phosphonic acid; and {2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-hexyl}-phosphonic acid.




(In)

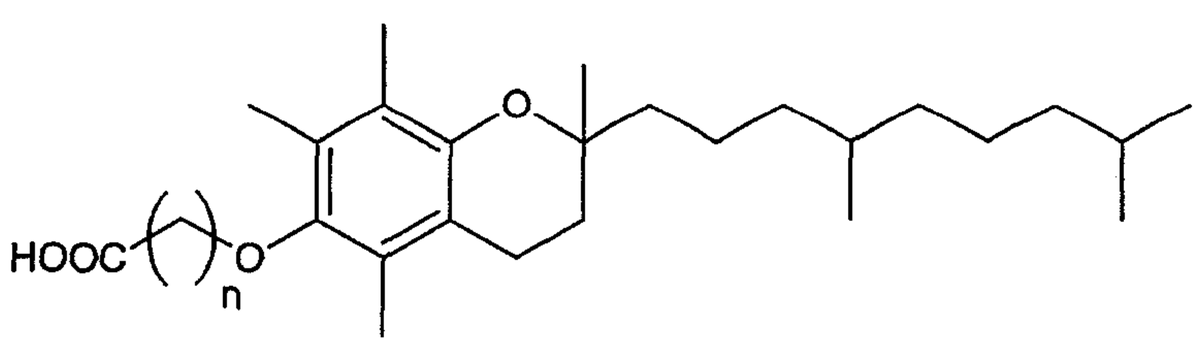
[0052] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a 4,8-dimethyl-non-1-enyl group, X is a sulfonic moiety,

and n is an integer from 1 to 6. These compounds are represented by formula (Io) and include compounds selected from the group consisting of 2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-methanesulfonic acid; 2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-ethanesulfonic acid; 2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-propane-1-sulfonic acid; 2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-butanesulfonic acid; 2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-pentane-1-sulfonic acid; and 2-[2-(4,8-dimethyl-non-1-enyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-hexane-1-sulfonic acid.




(Io)

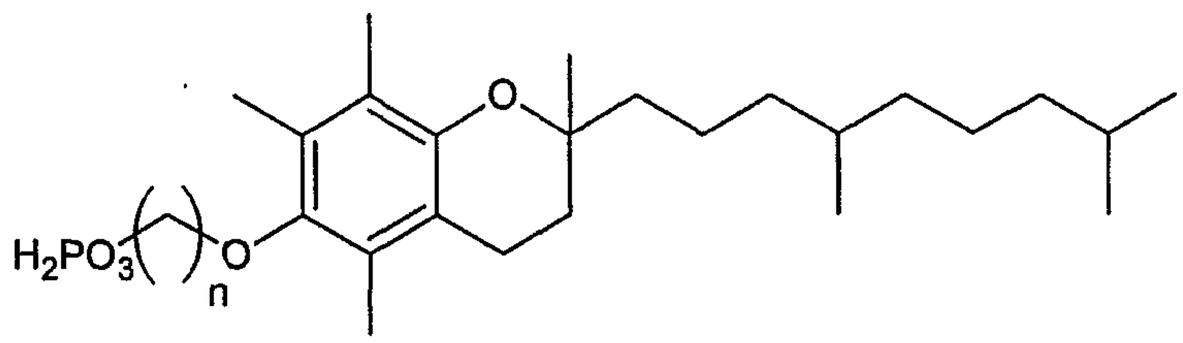
[0053] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a 4,8-dimethyl-nonyl group, X is a carboxyl moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Ip) and include compounds selected from the group consisting of [2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-acetic acid; 3-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-propionic acid; [2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-butyric acid; [2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-pentanoic acid; [2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-hexanoic acid; and [2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-heptanoic acid.




(Ip)

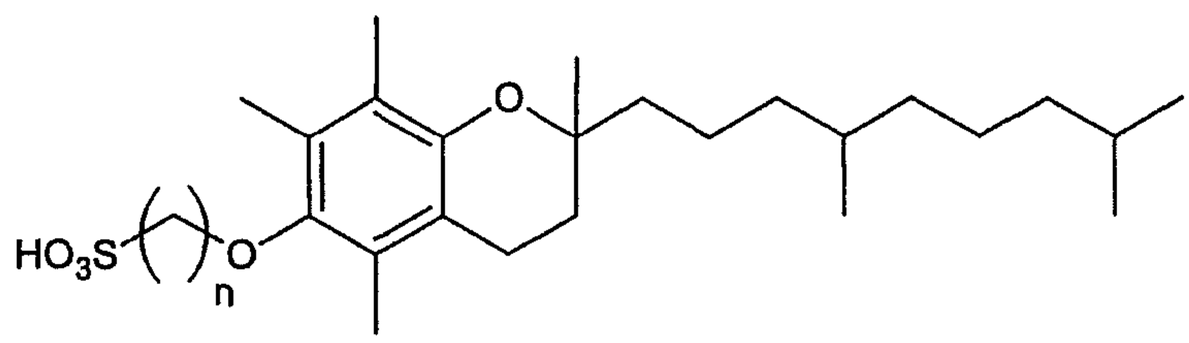
[0054] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a 4,8-dimethyl-nonyl group, X is a phosphonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Iq) and include compounds selected from the group consisting of [2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxymethyl]-phosphonic acid; {2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-ethyl}-phosphonic acid; {2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-

tetramethyl-chroman-6-yloxy]-propyl}-phosphonic acid; {2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-butyl}-phosphonic acid; {2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-pentyl}-phosphonic acid; and {2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-hexyl}-phosphonic acid.



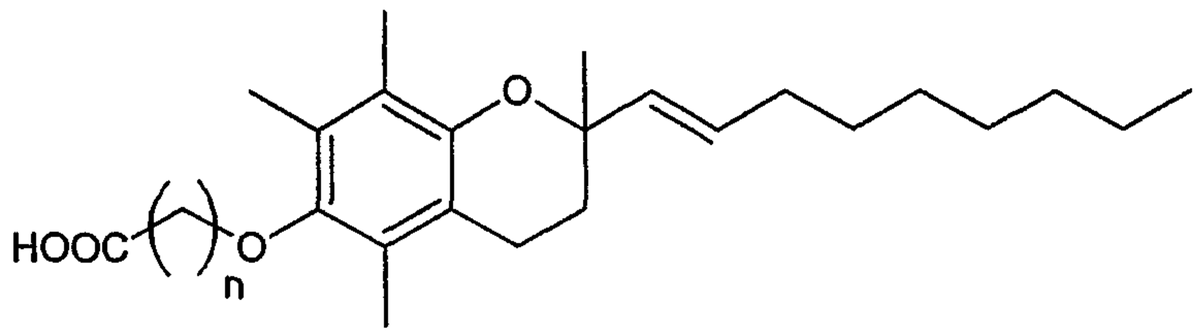
(Iq)

[0055] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a 4,8-dimethyl-nonyl group, X is a sulfonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Ir) and include compounds selected from the group consisting of 2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-methanesulfonic acid; 2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-ethanesulfonic acid; 2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-propane-1-sulfonic acid; 2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-butanesulfonic acid; 2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-pentane-1-sulfonic acid; and 2-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethyl-chroman-6-yloxy]-hexane-1-sulfonic acid.



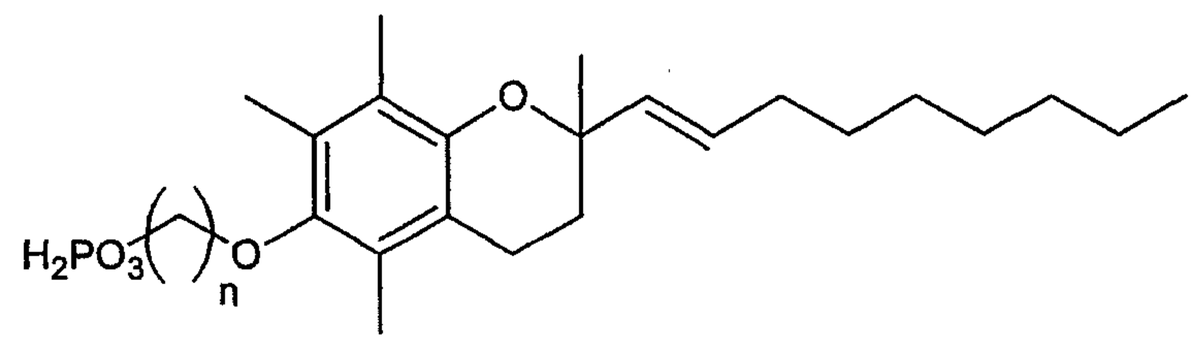
(Ir)

[0056] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a non-1-enyl group, X is a carboxyl moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Is) and include compounds selected from the group consisting of (2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-acetic acid; 3-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-propionic acid; 4-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-butyric acid; 6-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-pentanoic acid; and 7-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-heptanoic acid.



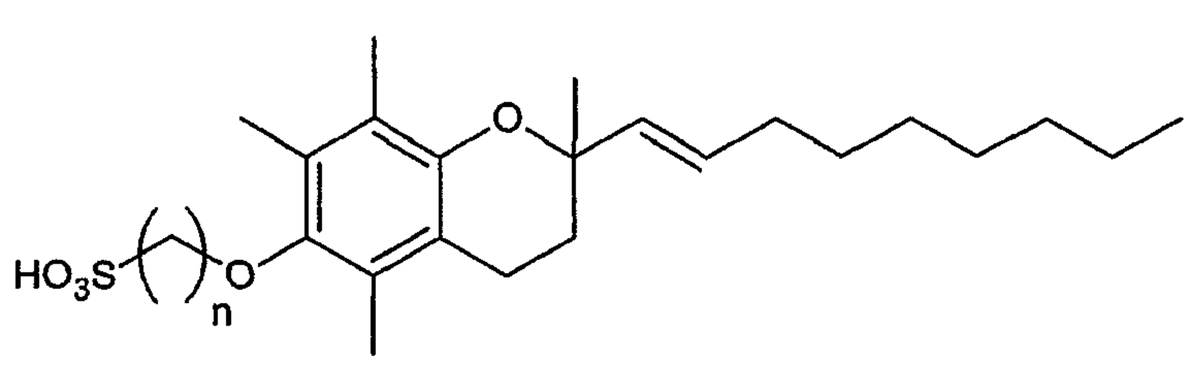

(Is)

[0057] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a non-1-enyl group, X is a phosphonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (It) and include compounds selected from the group consisting of (2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxymethyl)-phosphonic acid; [2-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-ethyl]-phosphonic acid; [2-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-propyl]-phosphonic acid; [2-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-butyl]-phosphonic acid; [2-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-pentyl]-phosphonic acid; and [2-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-hexyl]-phosphonic acid.



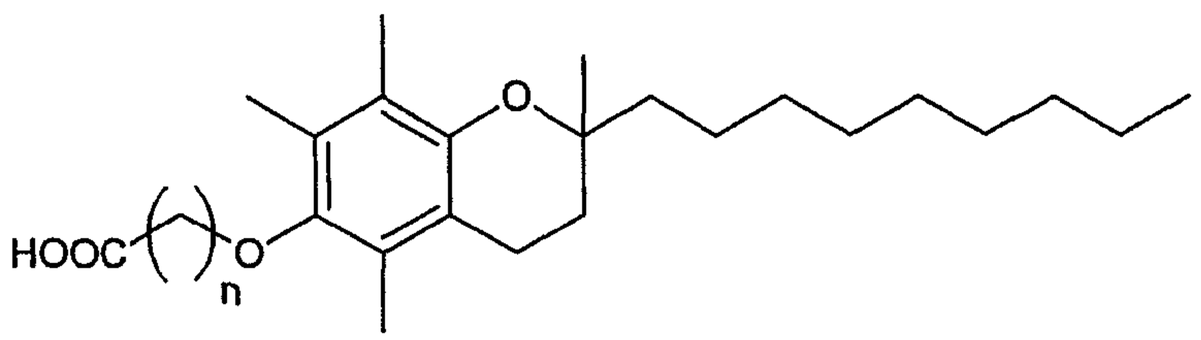

(It)

[0058] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a non-1-enyl group, X is a sulfonic moiety, and n is an integer from 1 to 6, These compounds are represented by formula (Iu) and include compounds selected from the group consisting of (2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-methanesulfonic acid; 2-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-ethanesulfonic acid; 3-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-propanesulfonic acid; 4-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-butanesulfonic acid; 5-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-pentanesulfonic acid; and 6-(2,5,7,8-tetramethyl-2-non-1-enyl-chroman-6-yloxy)-hexanesulfonic acid.



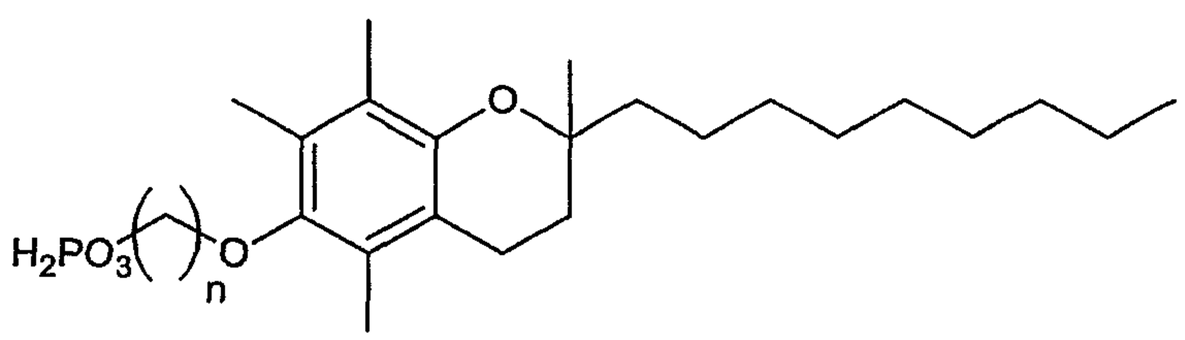

(Iu)

[0059] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a nonyl group, X is a carboxyl moiety, and n is an integer from 1 to 6, These compounds are represented by formula (Iv) and include compounds selected from the group consisting of (2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-acetic acid; 3-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-propionic acid; 4-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-butyric acid; 6-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-pentanoic acid; and 7-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-heptanoic acid.




(Iv)

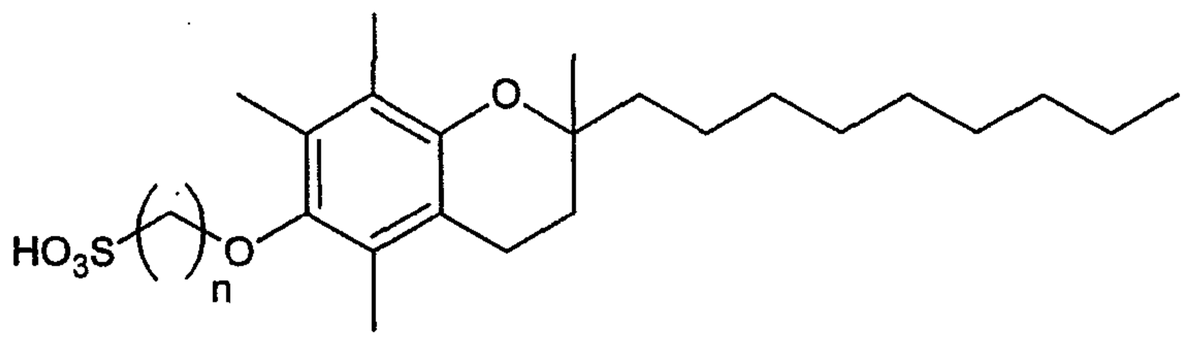
[0060] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a nonyl group, X is a phosphonic moiety, and n is an integer from 1 to 6. These compounds are represented by formula (Iw) and include compounds selected from the group consisting of (2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxymethyl)-phosphonic acid; [2-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-ethyl]-phosphonic acid; [2-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-propyl]-phosphonic acid; [2-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-butyl]-phosphonic acid; [2-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-pentyl]-phosphonic acid; and [2-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-hexyl]-phosphonic acid.




(Iw)

[0061] In another embodiment, the tocopheryl succinate derivatives of formula (I) are defined such that $R^1$ is methyl, $R^2$ is a nonyl group, X is a sulfonic moiety, and n is an integer selected from 1 to 6. These compounds are represented by formula (Ix) and include compounds selected from the group consisting of (2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-methanesulfonic acid; 2-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-ethanesulfonic acid; 3-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-propanesulfonic acid;

4-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-butanesulfonic acid; 5-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-pentanesulfonic acid; and 6-(2,5,7,8-tetramethyl-2-nonyl-chroman-6-yloxy)-hexanesulfonic acid.




(Ix)

[0062] Candidate agents may be tested in animal models. Typically, the animal model is one for the study of cancer. The study of various cancers in animal models (for instance, mice) is a commonly accepted practice for the study of human cancers. For instance, the nude mouse model, where human tumor cells are injected into the animal, is commonly accepted as a general model useful for the study of a wide variety of cancers, including prostate cancer (see, for instance, Polin *et al.*, Investig. New Drugs, 15:99-108 (1997)). Results are typically compared between control animals treated with candidate agents and the control littermates that did not receive treatment. Transgenic animal models are also available and are commonly accepted as models for human disease (see, for instance, Greenberg *et al.*, Proc. Natl. Acad. Sci. USA, 92:3439-3443 (1995)). Candidate agents can be used in these animal models to determine if a candidate agent decreases one or more of the symptoms associated with the cancer, including, for instance, cancer metastasis, cancer cell motility, cancer cell invasiveness, or combinations thereof.

*Treatment of Cancer using Tocopheryl succinate derivatives*

[0063] The present invention provides methods for treating or preventing the development of cancer in a subject using tocopheryl succinate derivatives. Cancer is a disease of abnormal and excessive cell proliferation. Cancer generally is initiated by an environmental insult or error in replication that allows a small fraction of cells to escape the normal controls on proliferation and increase their number. The damage or error generally affects the DNA encoding cell cycle checkpoint controls, or related aspects of cell growth control such as tumor suppressor genes. As this fraction of cells proliferates, additional genetic variants may be generated, and if they provide growth advantages, will be selected in an evolutionary fashion. Cells that have developed growth advantages but have not yet become fully cancerous are referred to as precancerous cells. Cancer results in an increased number of

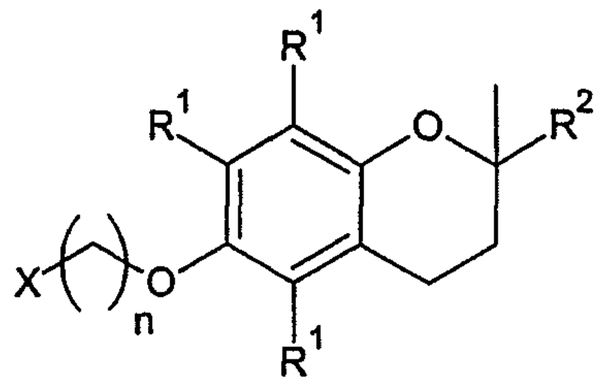
cancer cells in a patient. These cells may form an abnormal mass of cells called a tumor, the cells of which are referred to as tumor cells. The overall amount of tumor cells in the body of a patient is referred to as the tumor load. Tumors can be either benign or malignant. A benign tumor contains cells that are proliferating but remain at a specific site. The cells of a malignant tumor, on the other hand, can invade and destroy nearby tissue and spread to other parts of the body through a process referred to as metastasis.

[0064] Cancer is generally named based on its tissue of origin. There are several main types of cancer. Carcinoma is cancer that begins in the skin or in tissues that line or cover internal organs. Sarcoma is cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Leukemia is cancer that starts in blood-forming tissue such as the bone marrow, and causes large numbers of abnormal blood cells to be produced and enter the bloodstream. Lymphoma and multiple myeloma are cancers that begin in the cells of the immune system. Prostate cancer is cancer that initially develops in prostate tissue, but can metastasize to other tissues.

[0065] The tocopheryl succinate derivatives of the present invention can be used to treat various types of cancer and precancers. For example, the tocopheryl succinate derivatives can be used to androgen receptor-dependent cancers. The tocopheryl succinate derivatives can also be used to treat prostate cancer and hormone-refractory prostate cancer.

[0066] Treatment, as used herein, encompasses both prophylactic and therapeutic treatment. Tocopheryl succinate derivatives of the invention can, for example, be administered prophylactically to a subject in advance of the occurrence of cancer. Prophylactic administration is effective to decrease the likelihood of the subsequent occurrence of cancer in the subject, or decrease the severity of cancer that subsequently occurs. Alternatively, tocopheryl succinate derivatives of the invention can, for example, be administered therapeutically to a subject that is already afflicted by cancer (*i.e.*, non-prophylactic treatment). In one embodiment of therapeutic administration, administration of the tocopheryl succinate derivatives is effective to eliminate the cancer; in another embodiment, administration of the tocopheryl succinate derivatives is effective to decrease the severity of the cancer or lengthen the lifespan of the subject so afflicted. The subject is preferably a mammal, such as a domesticated farm animal (*e.g.*, cow, horse, pig) or pet (*e.g.*, dog, cat). More preferably, the subject is a human.

[0067] The present invention also provides a method of increasing protein phosphatase 2A (PP2A) activity, that includes administering an effective amount of a composition including a compound of Formula I:



(I)

wherein $R^1$ is independently selected from hydrogen and methyl; $R^2$ is selected from the group consisting of 4,8-dimethyl-non-1-enyl, 4,8-dimethyl-nonyl, non-1-enyl, and nonanyl groups; X is a carboxyl, phosphonic, or sulfonic moiety, and n is an integer from 1 to 6, or a pharmaceutically acceptable salt thereof. Protein phosphatase 2A activity can be increased in a cell, which can be either in vivo or in vitro. Protein phosphate 2A activity can also be increased in a subject, including a subject with cancer. The ability to evaluate the effect of the compounds to activate PP2A can be evaluated using methods such as the PP2A immunoprecipitation phosphatase assay kit, as further described herein.

[0068] As shown in Fig. 2A, Inactivation of JNK by tocopheryl succinate derivatives facilitates the proteasomal degradation of Sp1, leading to the transcriptional repression of a series of signaling proteins pertaining to prostate carcinogenesis and tumor progression, including VEGF, Mdm2, DNMT1, and AR. From a mechanistic perspective, the ability of tocopheryl succinate derivatives to activate PP2A underscores their broad spectrum of pharmacological activities against many molecular targets. Equally important, relative to malignant cells, normal prostate epithelial cells (PrECs) were resistant to the antiproliferative effects of VES and TS-1, as shown in Fig. 2B.

*Administration and Formulation of Tocopheryl succinate derivatives*

[0069] The present invention also provides pharmaceutical compositions that include tocopheryl succinate derivatives according to formula I as an active ingredient, and a pharmaceutically acceptable liquid or solid carrier or carriers, in combination with the active ingredient. Any of the tocopheryl succinate derivatives described above as being suitable for the treatment of cancer can be included in pharmaceutical compositions of the invention.

[0070] The tocopheryl succinate derivatives can be administered without modification, or can be administered as pharmaceutically acceptable salts. Pharmaceutically acceptable salt refers to the relatively non-toxic, inorganic and organic acid addition salts of the tocopheryl succinate derivatives. These salts can be prepared *in situ* during the final isolation and purification of the tocopheryl succinate derivative, or by separately reacting a purified tocopheryl succinate derivative with a suitable organic or inorganic counterion, and isolating the salt thus formed. Representative cationic counterions suitable for use with tocopheryl succinate derivative anions include ammonium, arginine, diethylamine, ethylenediamine, piperazine, and the like. (See, for example, Handbook of Pharmaceutical Salts: Properties, Selection, and Use, P. H. Stahl and C. G. Wermuth (Eds), Wiley (2008)).

[0071] The pharmaceutical compositions include one or more tocopheryl succinate derivatives together with one or more of a variety of physiological acceptable carriers for delivery to a patient, including a variety of diluents or excipients known to those of ordinary skill in the art. For example, for parenteral administration, isotonic saline is preferred. For topical administration, a cream, including a carrier such as dimethylsulfoxide (DMSO), or other agents typically found in topical creams that do not block or inhibit activity of the peptide, can be used. Other suitable carriers include, but are not limited to, alcohol, phosphate buffered saline, and other balanced salt solutions.

[0072] The formulations may be conveniently presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Preferably, such methods include the step of bringing the active agent into association with a carrier that constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulations. The methods of the invention include administering to a subject, preferably a mammal, and more preferably a human, the composition of the invention in an amount effective to produce the desired effect. The tocopheryl succinate derivatives can be administered as a single dose or in multiple doses. Useful dosages of the active agents can be determined by comparing their *in vitro* activity and the *in vivo* activity in animal models. Methods for extrapolation of effective dosages in mice, and other animals, to humans are known in the art; for example, see U.S. Pat. No. 4,938,949.

[0073] The agents of the present invention are preferably formulated in pharmaceutical compositions and then, in accordance with the methods of the invention, administered to a subject, such as a human patient, in a variety of forms adapted to the chosen route of administration. The formulations include, but are not limited to, those suitable for oral, rectal, vaginal, topical, nasal, ophthalmic, or parental (including subcutaneous, intramuscular, intraperitoneal, intratumoral, and intravenous) administration.

[0074] Formulations of the present invention suitable for oral administration may be presented as discrete units such as tablets, troches, capsules, lozenges, wafers, or cachets, each containing a predetermined amount of the active agent as a powder or granules, as liposomes containing the tocopheryl succinate derivatives, or as a solution or suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion, or a draught. Such compositions and preparations typically contain at least about 0.1 wt-% of the active agent. The amount of tocopheryl succinate derivative (*i.e.*, the active agent) is such that the dosage level will be effective to produce the desired result in the subject.

[0075] Nasal spray formulations include purified aqueous solutions of the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids. Ophthalmic formulations are prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye. Topical formulations include the active agent dissolved or suspended in one or more media such as mineral oil, petroleum, polyhydroxy alcohols, or other bases used for topical pharmaceutical formulations.

[0076] The tablets, troches, pills, capsules, and the like may also contain one or more of the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid, and the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, fructose, lactose, or aspartame; and a natural or artificial flavoring agent. When the unit dosage form is a capsule, it may further contain a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac, sugar, and the like. A syrup or elixir may

contain one or more of a sweetening agent, a preservative such as methyl- or propylparaben, an agent to retard crystallization of the sugar, an agent to increase the solubility of any other ingredient, such as a polyhydric alcohol, for example glycerol or sorbitol, a dye, and flavoring agent. The material used in preparing any unit dosage form is substantially nontoxic in the amounts employed. The active agent may be incorporated into sustained-release preparations and devices.

*Preparation of the Compounds*

[0077] Tocopheryl succinate derivatives of the invention may be synthesized by synthetic routes that include processes similar to those well known in the chemical arts, particularly in light of the description contained herein. The starting materials are generally available from commercial sources such as Aldrich Chemicals (Milwaukee, Wisconsin, USA) or are readily prepared using methods well known to those skilled in the art (*e.g.*, prepared by methods generally described in Louis F. Fieser and Mary Fieser, *Reagents for Organic Synthesis*, v. 1-19, Wiley, New York, (1967-1999 ed.); Alan R. Katritsky, Otto Meth-Cohn, Charles W. Rees, *Comprehensive Organic Functional Group Transformations*, v 1-6, Pergamon Press, Oxford, England, (1995); Barry M. Trost and Ian Fleming, *Comprehensive Organic Synthesis*, v. 1-8, Pergamon Press, Oxford, England, (1991); or *Beilsteins Handbuch der organischen Chemie*, 4, Aufl. Ed. Springer-Verlag, Berlin, Germany, including supplements (also available via the Beilstein online database)).

[0078] Combinatorial synthesis was used to prepare a focused compound library based on the initial TS-1 structure. Structurally, TS-1 can be divided into three sub-structures, *i.e.*, structure A, the acid moiety; structure B, the heterocyclic ring system; and structure C, the aliphatic side chain. These three components can be individually modified and then conjugated to generate new tocopheryl succinate derivatives. The respective substructures prepared are summarized in Figure 3.

[0079] The substructures serve differing functions in the tocopheryl succinate derivatives. For substructure A, the hemisuccinate (an ester linkage) is replaced by an ether-linked acid, *i.e.*, a carboxylic acid (-$CO_2H$), a phosphonic acid (-$P(O)_2OH$), or a sulfonic acid (-$SO_3H$) to provide improved oral bioavailability. The ether linked acids can be attached at the end of an alkyl group with a variety of lengths, such as lengths of 1-6 methylene groups. While the ether linkage improves the bioavailability of the tocopheryl succinate derivatives, it has been

shown to have little effect on the activity of the compounds themselves, outside of their improved bioavailability. For substructure B, structural variants of the chroman ring of TS-1 with different stereochemical properties can be used. For example, the chroman ring can be "clean" (*i.e.*, not have any attached groups other than hydrogen atoms) or it can have one or more attached methyl groups. For substructure C, the chain length can be varied, the methyl branches removed, and an α,β-double bond can be introduced to increase the rigidity of the side chain. Synthesis of these derivatives can be accomplished as illustrated in Fig. 4, which is amenable to scale-up to multi-grams quantities in a laboratory setting.

[0080] Those skilled in the art will appreciate that other synthetic routes may be used to synthesize the compounds of the invention. Although specific starting materials and reagents are depicted in the reaction schemes and discussed below, other starting materials and reagents can be easily substituted to provide a variety of derivatives and/or reaction conditions. In addition, many of the compounds prepared by the methods described below can be further modified in light of this disclosure using conventional methods well known to those skilled in the art.

[0081] The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein.

## EXAMPLES

### Example 1: Vitamin E Succinate derivative mediate the Transcriptional Repression of the Androgen Receptor in Prostate Cancer Cells by targeting the PP2A-JNK-Sp1 Signaling Axis

**Materials and methods**

*Reagents, antibodies, and plasmids.*

[0082] VES and the proteasome inhibitors MG132 and epoxomicin were purchased from EMD Chemicals, Inc (San Diego, CA) and Aldrich-Sigma (St. Louis, MO), respectively. TS1 {succinic acid mono-[2-(4,8-dimethyl-nonyl)-2,5,7,8-tetramethylchroman-6-yl] ester} is a truncated derivative of VES with an improved anti-proliferative potency. Shiau *et al.*, J Biol Chem, 281, 11819-25 (2006). Stock solutions of these agents were made in DMSO and added to medium with a final DMSO concentration of 0.1%. Antibodies against various

proteins were obtained from the following sources. Mouse monoclonal antibodies: AR and prostate specific antigen (PSA), Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit antibodies: Sp1, Santa Cruz; poly (ADP-ribose) polymerase (PARP), p-Ser473-Akt, p-Thr308-Akt, Akt, p-ERK, ERK, p-JNK, JNK, p-p38, and p38, Cell Signaling Technology, Inc. (Beverly, MA). The AR promoter-luciferase reporter vector (hAR-Luc) was constructed as previously described. Yang *et al.*, Cancer Res, 67, 3229-38 (2007). The dominant-negative JNK1 plasmid pCDNA3-Flag-JNK1a1 was obtained from Addgene Inc. (Cambridge, MA). Hemagglutinin (HA)-ubiquitin plasmid and the constitutively active JNK plasmid Flag-MKK7-JNK1 encoding MKK7-JNK1 fusion protein with constitutive JNK activity (Lei *et al.*, Mol Cell Biol., 22, 4929-42 (2002)) were kind gifts from Dr. Hung-Wen Chen (Institute of Biological Sciences, Academia Sinica, Taipei, Taiwan) and Dr. Roger Davis (University of Massachusetts Medical School, Worcester, Massachusetts), respectively. The pCMVSp1 plasmid was purchased from OriGene Technologies, Inc. (Rockville, MD).

*Cell culture.*

[0083] LNCaP androgen-dependent ($p53^{+/+}$) and PC-3 androgen-nonresponsive ($p53^{-/-}$) prostate cancer cells were purchased from the American Type Culture Collection (Manassas, VA), and cultured in RPMI 1640 medium containing 10% heat-inactivated FBS. Normal prostate epithelial cells (PrECs) were obtained from Lonza, Inc. (Allendale, NJ), and maintained in Prostate Epithelial Growth Media supplemented with a growth factor kit suggested by the vendor. All cell types were cultured at 37 °C in a humidified incubator containing 5% $CO_2$. Cells in log phase growth were harvested by trypsinization for use in the MTT viability assay. LNCaP cells were plated in poly-D-lysine coated culture flasks in order to assist cell adherence to the surface. Prior to drug treatment, cells were plated in a density of 12,000 cells/$cm^2$ surface area in the respective culture medium for 24-48 h, followed by individual test agents in 2.5% FBS-supplemented RPMI medium.

*Immunoblotting.*

[0084] Cells cultured in T25 flasks were collected by scraping, and cell pellets were washed once with PBS. Cells were lysed in a lysis buffer consisting of 1% SDS, 10 mM EDTA and 50 mM Tris-HCl, pH 8.1, in the presence of a commercial protease inhibitor cocktail from Aldrich-Sigma (2 mM AEBSF, 1 mM EDTA, 130 μM bestatin, 14 μM E-64, 1 μM leupeptin, and 0.3 μM aprotinin). Following a 10-sec sonication using 20% output in a Virsonic 300

sonicator (Virtis, Gardiner NY) to disrupt cellular organelles and genomic DNA, cell lysates were centrifuged at 15,200 x g for 15 minutes. One $\mu$L of the suspension was used for protein determination using a colorimetric BCA assay (Pierce, Rockford, IL), and to the remaining solution was added an equivalent volume of 2x SDS-polyacrylamide gel electrophoresis sample loading buffer (62.5 mM Tris-HCl, pH 6.8, 4% SDS, 5% $\beta$-mercaptoethanol, 20% glycerol, and 0.1% bromophenol blue), and boiled for 5 min. Equal amounts of proteins were resolved in 8% SDS-polyacrylamide gels, and transferred to nitrocellulose membranes using a semidry transfer cell. The transblotted membrane was washed twice with Tris-buffered saline containing 0.1% Tween 20 (TBST). After blocking with TBST containing 5% nonfat milk for 40 min, the membrane was incubated with the appropriate primary antibody in TBST-1% nonfat milk at 4 °C overnight. All primary antibodies were diluted 1:1000 in 1% nonfat milk-containing TBST. After treatment with the primary antibody, the membrane was washed three times with TBST for a total of 15 min, followed by incubation with goat anti-rabbit or anti-mouse Immunoglobulin G (IgG)-horseradish peroxidase conjugates (diluted 1:2000) for 1 h at room temperature and four washes with TBST for a total of 1 h. The immunoblots were visualized by enhanced chemiluminescence.

*RNA isolation and reverse transcription (RT)-PCR.*

[0085] LNCaP cells were subject to total RNA isolation by using a Trizol reagent (Invitrogen Corporation, CA). RNA concentrations were determined by measuring absorption at 260 nm in a spectrophotometer. Aliquots of 2 $\mu$g of total RNA from each sample were reverse transcribed to cDNAs using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's instructions. PCR products were resolved electrophoretically in 1.2% agarose gels and visualized by ethidium bromide staining.

*Transfection and luciferase assay.*

[0086] Cells were transfected with 5 $\mu$g of the AR-linked luciferase reporter (hAR-Luc) plasmid in an Amaxa Nucleofector using a cell line–specific nucleofector kit according to the manufacturer's protocol (Amaxa Inc. Gaithersburg, MD) and then seeded in T25 flasks at 3 x $10^5$ cells per flask for 48 h. The transfection efficiency was determined by transfecting cells with 3 $\mu$g of pmaxGFP plasmid followed by fluorescence microscopy to detect green fluorescent protein expression. For each transfection, herpes simplex virus (HSV) thymidine kinase promoter-driven *Renilla reniformis* luciferase was used as an internal control for

normalization. For the luciferase reporter gene assay, after transfection, cells were cultured in 24-well plates in 10% FBS-supplemented RPMI 1640 for 48 h, subjected to different treatments in 2.5 % FBS-supplemented medium for the indicated times, collected, and lysed with passive lysis buffer (Promega). Aliquots of lysates (50 $\mu$L) were mixed with 75 $\mu$L of luciferase substrate (Promega) in 96-well plates, and luciferase activities were monitored in a MicroLumaPlus LB96V luminometer (Berthold Technologies, Oak Ridge, TN) with the WinGlow software package. All transfection experiments were carried out in six replicates.

*Immunoprecipitation.*

[0087] LNCaP cells were co-transfected with 5 $\mu$g each of Flag-Sp1 and HA-ubiquitin plasmids in an Amaxa Nucleofector using a LNCaP-specific nucleofector kit. These transiently transfected cells were seeded in 6-well plates at $2 \times 10^5$ per well. After 48-h incubation, cells were exposed to VES or TS-1 at the indicated concentration for 48 h, and lysed by a radioimmunoprecipitation assay lysis buffer (Santa Cruz Biotechnology) in the presence of a freshly prepared cocktail of phosphatase and protease inhibitors (2 mmol/L AEBSF, 1 mM EDTA, 130 μM bestatin, 14 μM E-64, 1 μM leupeptin, and 0.3 μM aprotinin, 2 mM imidazole, 1 mM sodium fluoride, 1.15 mM sodium molybdate, 1 mM sodium orthovanadate, and 4 mmol/L sodium tartrate dihydrate). After centrifugation at 13,000 x g for 15 min, the supernatants were collected, preincubated with protein A/G agarose (Santa Cruz Biotechnology) for 15 min, and centrifuged at 1,000 x g for 5 min. Supernatant (20 $\mu$L) were stored at 4 °C to be used as input, whereas the remaining supernatant was exposed to 4 $\mu$g of anti-Sp1 antibodies at 4 °C for 12 h, followed by protein A/G agarose beads at 4 °C for another 2 h. After brief centrifugation, immunoprecipitates were collected, washed with the aforementioned lysis buffer four times, suspended in 2x SDS sample buffer, and subjected to Western blot analysis with antibodies against HA and Flag.

*Chromatin immunoprecipitation (ChIP).*

[0088] After drug treatment, LNCaP cells ($2 \times 10^7$) in 50 mL of PBS were cross-linked with 1.35 mL of 37% formaldehyde (final concentration 1%) for 15 min at room temperature. Glycine solution (1 mol/L) was added to a final concentration of 125 mmol/L to stop the cross-linking reaction. Cells were harvested and washed twice with 5 mL of PBS, and the cell pellets were lysed in a ChIP lysis buffer containing (50 mM HEPES-KOH at pH 7.5, 140 mM NaC1, 1% Triton X- 100, 0.1% sodium deoxycholate, 2 mM AEBSF, 1 mM EDTA, 130 μM

bestatin, 14 μM E-64, 1 μM leupeptin, and 0.3 μM aprotinin). The suspension was sonicated at 20 % output in a Virsonic 300 sonicator with 6 sets of 10-sec pulses (resulting in an average fragment size of 0.8-0.2 kb) and centrifuged for 10 min at 15,000 x g at 4 °C. One-μL aliquots of the transparent supernatants were taken for determining protein concentrations by BCA assays. Immunoprecipitation was carried out as described above. Aliquots of 1 mg proteins were used for immunoprecipitation using 4 μg of anti-Sp1 antibody followed by protein A/G agarose beads. The immunoprecipitates were successively washed twice with 1 mL of CHIP lysis buffer, twice with 1 mL of a high salt CHIP lysis buffer (50 mmol/L HEPES-KOH at pH 7.5, 500 mmol/L NaC1, 1% Triton X-100, 0.1% sodium deoxycholate, 2 mmol/L AEBSF, 1 mmol/L EDTA 130 μmol/L bestatin, 14 μmol/L E-64, 1 μmol/L leupeptin, and 0.3 μmol/L aprotinin), twice with 1 ml of CHIP wash buffer (10 mmol/L Tris pH 8.0; 250 mmol/L LiCl; 0.5 % NP-40; 0.5 % sodium deoxycholate; 1 mmol/L EDTA), and twice with 1 mL of TE buffer (10 mmol/L Tris, pH 7.5, 1 mmol/L EDTA). The immunocomplex was eluted by addition of 75 μL of elution buffer (50 mmol/L Tris, pH 8.0, 1 % SDS, 10 mmol/L EDTA), and were incubated at 65 °C for 10 min. The resulting supernatant was collected after brief centrifugation, and the pellets were eluted again with another 75 μl of elution buffer. The combined supernatant was incubated at 65 °C overnight. Ten-μg aliquots (1%) of the original total proteins were added to 150 μl of elution buffer, and were incubated at 65 °C overnight as the input control. Finally, samples were processed for DNA purification using a PCR purification kit (Qiagen, Valencia CA), and the recovered DNA was eluted with 50 μL of 10 mM Tris-HCl, pH 8.5. One-μl aliquots were used for PCR with primers spanning two adjacent Sp1 binding sites on the AR promoter, located at 429–442 of 5'-UTR of the AR gene. Wang *et al.*, Carcinogenesis, 27, 2124-32 (2006). E2TAK taq polymerase (Takara Bio, Inc.) and the corresponding buffer system were used for amplification of PCR products.

*PP2A activity assay.*

[0089] PP2A activity in drug-treated cells was determined by using a PP2A Immunoprecipitation Phosphatase Assay Kit (Millipore) according to the manufacturer's instructions. LNCaP cells were exposed to DMSO, VES, or TS-1 at the indicated concentrations in 2.5 % FBS supplemented medium for 12 h, and subjected to cell lysis in a phosphate-free lysis buffer containing 20 mmol/L imidazole-HCl, pH 7.0, 2 mM EDTA, 2 mM EGTA, 2 mM AEBSF, 1 mM EDTA, 130 μM bestatin, 14 μM E-64, 1 μM leupeptin, and 0.3 μM aprotinin. The suspension was sonicated (Virtis) at 20 % output for 10 sec,

followed by centrifugation at 2000 x g for 5 min. One-$\mu$L aliquots of the supernatants were taken for protein determination by BCA assays, and the remaining supernatants were used for phosphatase activity assays. Aliquots of cell lysates containing 400 $\mu$g of proteins were combined with 4 $\mu$g of anti- PP2Ac antibody (Millipore), to which was added PP2A assay buffer (20 mmol/L Hepes pH 7.0, 100 mM NaCl) to a final volume of 500 $\mu$L followed by 40 $\mu$L of Protein A–agarose. Mixtures were incubated at 4 °C for 2 h, and briefly centrifuged. The immunocomplexes were washed and used for the phosphatase activity assay. The amounts of PP2A in the immunocomplexes were determined semi-quantitatively by Western blotting.

**Results**

*Differential effect of VES and TS1 on suppressing AR expression in LNCaP cells versus normal prostate epithelial cells (PrECs).*

[0090] In the course of investigation of the inhibitory effect of VES on Bcl-xL/Bcl-2 function, a structurally optimized derivative, TS-1 (Figure 5A), was developed in which the phytyl side chain was shortened by one isopranyl unit relative to that of VES. Shiau *et al.*, J Biol Chem, 281, 11819-25 (2006) In this study, Western blot analysis indicates that this side chain truncation also led to higher potency in suppressing the expression of AR and its target gene product PSA in LNCaP cells (Figure 5B). For example, TS-1 at 5 $\mu$mol/L was effectively reduced the expression of these biomarkers by 50% after 72 h of incubation, while VES required at least 10 $\mu$mol/L to achieve the same extent of suppression (Figure 5B). The abilities of VES and TS-1 to repress AR correlated with the respective potencies in inducing apoptosis, as manifest by the extents of PARP cleavage. Furthermore, two lines of evidence reveal that this decrease in AR protein expression was attributed to the transcriptional inhibition of AR gene expression. First, RT-PCR analysis of the mRNA transcript of the AR gene in LNCaP cells showed a time-dependent reduction paralleling that of AR protein in response to 10 $\mu$M VES or TS-1 (Figure 5C, left panel). Second, the AR promoter-luciferase reporter assay confirmed that these agents were able to inhibit AR gene transcription in a dose-dependent manner after 72 h of exposure (Figure 5C, right panel). Together, these data indicate that VES and TS-1 mediated the inhibition of AR mRNA expression by targeting the transcriptional regulation of the AR promoter.

[0091] As compared to LNCaP cells, normal prostate epithelial cells (PrECs), which exhibited low abundance of AR, were resistant to the repressive effect of drug on AR expression (Figure 5D). This selectivity might, in part, account for the differential sensitivity of normal versus malignant cells to the ability of TS-1 to suppress cell viability (Figure 5E).

*VES and TS-1 target Sp1 to downregulate AR gene transcription.*

[0092] In a previous study of the effect of thiazolidinediones on modulating AR expression in LNCaP cells, the inventors demonstrated a mechanistic link between drug-mediated AR ablation and the downregulation of Sp-1 expression. Yang *et al.*, Cancer Res, 67, 3229-38 (2007). To investigate this putative link in VES- and TS-1- induced AR repression, ChIP assays were performed to detect the binding of Sp1 to AR promoter in LNCaP cells treated with various doses of VES or TS-1 for 72 h. After formaldehyde treatment of cells, antibodies against Sp1 or IgG were used to immunoprecipitate Sp1-bound genomic DNA fragments, followed by PCR analysis with a pair of primers spanning the AR promoter. The results demonstrated that VES and TS-1 diminished the Sp1 binding to AR promoter in a dose-dependent manner. Based on Western blot analysis, this reduced binding was attributed to decreases in Sp1 expression in drug-treated cells. Moreover, this repression occurred at the posttranslational level since Sp1 mRNA expression remained unaltered even after treatment of LNCaP cells with high doses of VES and TS-1. The ability of VES and TS-1 to reduce Sp1 levels was confirmed by the dose-dependent transcriptional repression of a series of Sp1 downstream target genes, including those encoding vascular endothelial growth factor (VEGF), the negative p53 regulator Mdm2, and DNA methyltransferase 1 (DNMT1), all of which play important roles in prostate tumorigenesis and cancer progression.

*Proteasomal degradation of Sp1.*

[0093] The ability of VES and TS-1 to modulate the stability of Sp1 protein was confirmed by its shortened half-life in drug-treated LNCaP cells relative to the DMSO control, which was more prominent after TS-1 treatment. Moreover, pharmacological inhibition of proteasomal degradation by epoxomicin and MG-132 protected Sp1 from TS-1-facilitated ablation. Because proteasome-facilitated proteolysis is preceded by ubiquitination, the formation of ubiquitinated Sp1 in response to different doses of VES and TS-1 in LNCaP cells expressing ectopic HA-ubiquitin and Flag-Sp1 was examined. After drug treatment for 24 h, cell lysates were immunoblotted with Sp1 antibodies or immunoprecipitated by anti-

Flag antibody-agarose conjugates. Equivalent amounts of the immunoprecipitated proteins were subjected to immunoblotting with Flag or HA antibodies. Both TS-1 and VES increased the extent of Sp1 ubiquitination as indicated by a complex ladder of ubiquitinated Sp1 bands.

*Ectopic Sp1 expression confers resistance to the effect of VES and TS-1 on AR transcriptional repression.*

[0094] To validate the link between the drug-induced AR repression and Sp1 down-regulation, the ability of ectopic Sp1 expression to protect AR from VES- and TS-1-induced repression was assessed. Transient transfection of LNCaP cells with the pCMVSp1 plasmid resulted in a higher expression level of Sp1 than that of the pcDNA-transfected cells. Although treatment of pCMVSp1-transfected cells with 10 $\mu$M TS-1 or VES caused differential reduction in Sp1 expression, the respective Sp1 levels were still higher than that of untreated pcDNA-transfected cells. As a consequence, the expression level of AR remained virtually unchanged after drug treatment, indicating the protective effect of ectopic Sp1.

*VES and TS-1 mediate Sp1 degradation through a JNK-dependent pathway.*

[0095] Despite recent advances in understanding Sp1's biological functions, the mechanism controlling the turnover of this transcription factor remains unclear. Data obtained by the inventors indicates that VES- and TS-1- facilitated Sp1 degradation was accompanied by concomitant reduction in its phosphorylation level. In light of a recent report that Jun $NH_2$-terminal kinases (JNKs) were involved in maintaining the stability of Sp1 (Chuang *et al.*, Mol Biol Cell, 19, 1139-51 (2008)), this finding suggests a putative role of JNK in mediating the drug-induced Sp1 proteolysis. To corroborate this premise, the effect of VES and TS-1 on the phosphorylation status of JNKs and other kinases including Akt, ERK and p38 in LNCaP cells was examined. The results showed that treatment of LNCaP cells with VES and, to a greater extent, TS-1 led to a dose-dependent reduction in the phosphorylation levels of all four kinases examined, which was also noted in PC-3 cells. As these kinases are known PP2A substrates, their concomitant dephosphorylation raised a possible link with PP2A activation in drug-treated cells. This causal relationship was supported by the ability of VES and TS-1 to increase PP2A phosphatase activity. This enhancement in PP2A activity, however, was not due to increases in PP2A protein levels after drug treatment.

[0096] Furthermore, the mechanistic link between JNK inactivation and Sp1 degradation was borne out by two lines of evidence. First, stable transfection of LNCaP cells with a dominant negative mutant of JNK1 (DN-JNK) mimicked the effect of VES and TS-1 on attenuating Sp1 expression. Second, PC-3 cells were used as a model to demonstrate that the constitutively active fusion protein MKK7-JNK1 conferred protection against VES- and TS-1-induced Sp1 degradation. Relative to PC-3 cells, LNCaP cells were vulnerable to the upregulation of this stress kinase as transient transfection of LNCaP cells with MKK7-JNK1 plasmids resulted in apoptotic death in nearly all transfected cells. Equally important, the PP2A inhibitor okadaic acid could protect cells from the suppressive effect of VES and TS-1 on the phosphorylation or expression of JNK, Sp1, and AR, confirming that VES and TS-1 facilitated the transcriptional repression of AR by targeting the PP2A-JNK-Sp1 signaling axis.

**Discussion**

[0097] In light of the therapeutic relevance of targeting AR in prostate cancer, the mechanism by which VES and its truncated derivative TS-1 suppress AR gene transcription was investigated. The data demonstrated that the effect of VES- and TS-1 on facilitating AR transcriptional repression was attributable to their ability to promote Sp1 degradation, which, in turn, was mediated through PP2A-mediated JNK inactivation. Equally important, relative to malignant cells, PrECs were resistant to the antiproliferative effects of VES and TS-1. From a mechanistic perspective, the function of VES and TS-1 to activate PP2A activity underscores their pleiotropic effects on targeting multiple signaling pathways. This study indicates that these mechanisms included, but were not limited to, those mediated by Akt, ERKs, JNKs, p38, Sp1, AR, and the respective downstream targets, all of which are clinically relevant to prostate carcinogenesis and tumor progression. Based on the ubiquitous action of PP2A in a growing list of phosphoproteins and signaling pathways, PP2A has been recognized as a tumor suppressor protein. Mumby, M., Cell, 130, 21-4 (2007). A recent study demonstrated that suppression of PP2A activity cooperates with other oncogenic changes to cause neoplastic transformation of multiple cell types. Junttila *et al.*, 130, 51-62 (2007). Thus, the effect of VES and TS-1 to activate PP2A phosphatase activity is of translational value to develop novel PP2A-activating agents for prostate cancer therapy and prevention.

[0098] The PP2A-mediated downregulation of MAP kinases in VES/TS-1-treated prostate cancer cells, however, contrasts with recent reports that VES induced differentiation and apoptosis in breast and gastric cancer cells by activating ERKs and JNK. This discrepancy might be caused by differences in the regulation of the respective signaling networks in *different cancer types. At present, the mechanism underlying the effect of VES* and TS-1 on activating PP2A phosphatase activity remains unclear. It may be that PP2A activation is attributable to increased intracellular levels of ceramide, a known PP2A activator, in drug-treated cells since VES has been reported to stimulate ceramide production. The ability of VES and TS-1 to mediate ceramide-induced PP2A activation is currently under investigation.

[0099] In summary, in the course of investigating the mechanism underlying VES- and TS-1-mediated suppression of AR gene transcription, the ability of these small molecule agents to modulate the PP2A-JNK-Sp1 signaling axis was demonstrated, of which the significance is multifold. First, this signaling axis provides a molecular basis to account for the broad spectrum of activities of VES on multiple signaling targets. This pleiotropic effect in conjunction with low toxicity is of clinical relevance to cancer therapy/prevention. Second, the higher potency of TS-1 relative to VES in modulating the PP2A-Sp1-AR signaling pathway demonstrates that these agents could be structurally optimized to develop potent PP2A-targeted agents for prostate cancer therapy.

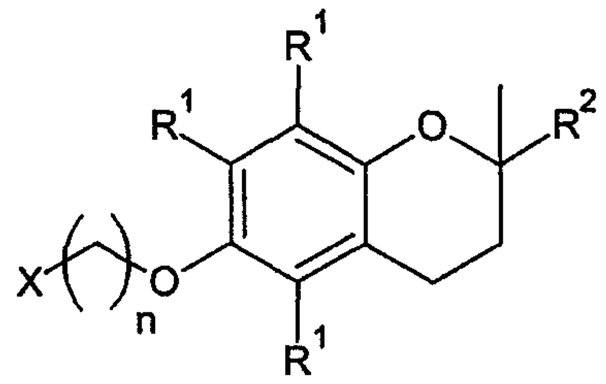
[00100] The complete disclosure of all patents, patent applications, and publications, and electronically available material cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

[00101] All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

# CLAIMS

What is claimed is:

1. A compound according to formula I:



(I)

wherein $R^1$ is independently selected from hydrogen and methyl; $R^2$ is selected from the group consisting of 4,8-dimethyl-non-1-enyl, 4,8-dimethyl-nonyl, non-1-enyl, and nonanyl groups; X is a carboxyl, phosphonic, or sulfonic moiety, and n is an integer from 1 to 6, or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein $R^2$ is a 4,8-dimethyl-non-1-enyl group.

3. The compound of claim 1, wherein $R^2$ is a 4,8-dimethyl-nonyl group.

4. The compound of claim 1, wherein $R^2$ is a non-1-enyl group.

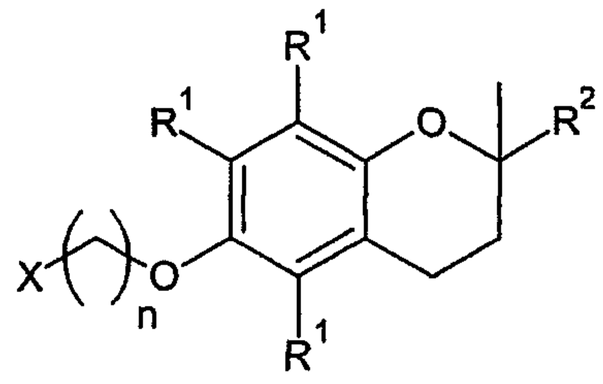
5. The compound of claim 1, wherein $R^2$ is a nonanyl group.

6. The compound of any one of claims 1-5, wherein X is a carboxyl moiety.

7. The compound of any one of claims 1-5, wherein X is a phosphonic moiety.

8. The compound of any one of claims 1-5, wherein X is a sulfonic moiety.

9. A method of treating or preventing the development of androgen receptor-dependent cancer in a subject, comprising administering a therapeutically effective amount of a composition including a compound of Formula I:



(I)

wherein $R^1$ is independently selected from hydrogen and methyl; $R^2$ is selected from the group consisting of 4,8-dimethyl-non-1-enyl, 4,8-dimethyl-nonyl, non-1-enyl, and nonanyl groups; X is a carboxyl, phosphonic, or sulfonic moiety, and n is an integer from 1 to 6, or a pharmaceutically acceptable salt thereof.

10. The method of claim 9, wherein the androgen receptor-dependent cancer is prostate cancer.

11. The method of claim 9, wherein $R^2$ is a 4,8-dimethyl-non-1-enyl group.

12. The method of claim 9, wherein $R^2$ is a 4,8-dimethyl-nonyl group.

13. The method of claim 9, wherein $R^2$ is a non-1-enyl group.

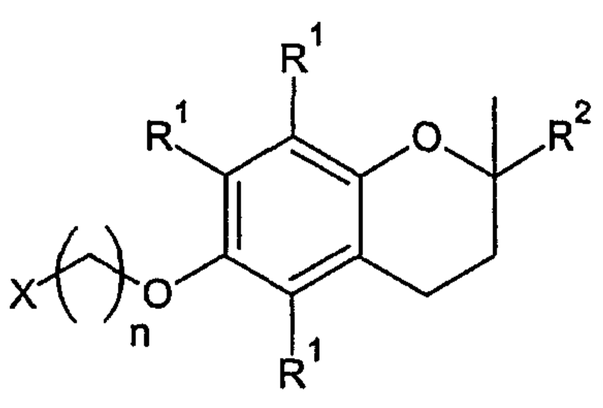
14. The method of claim 9, wherein $R^2$ is a nonanyl group.

15. The method of any one of claims 9-14, wherein X is a carboxyl moiety.

16. The method of any one of claims 9-14, wherein X is a phosphonic moiety.

17. The method of any one of claims 9-14, wherein X is a sulfonic moiety.

18. A method of increasing protein phosphatase 2A (PP2A) activity, comprising administering an effective amount of a composition including a compound of Formula I:



(I)

wherein $R^1$ is independently selected from hydrogen and methyl; $R^2$ is selected from the group consisting of 4,8-dimethyl-non-1-enyl, 4,8-dimethyl-nonyl, non-1-enyl, and nonanyl groups; X is a carboxyl, phosphonic, or sulfonic moiety, and n is an integer from 1 to 6, or a pharmaceutically acceptable salt thereof.

19. The method of claim 18, wherein $R^2$ is a 4,8-dimethyl-non-1-enyl group.

20. The method of claim 18, wherein $R^2$ is a 4,8-dimethyl-nonyl group.

21. The method of claim 18, wherein $R^2$ is a non-1-enyl group.

22. The method of claim 18, wherein $R^2$ is a nonanyl group.

23. The method of any one of claims 18-22, wherein X is a carboxyl moiety.

24. The method of any one of claims 18-22, wherein X is a phosphonic moiety.

25. The method of any one of claims 18-22, wherein X is a sulfonic moiety.

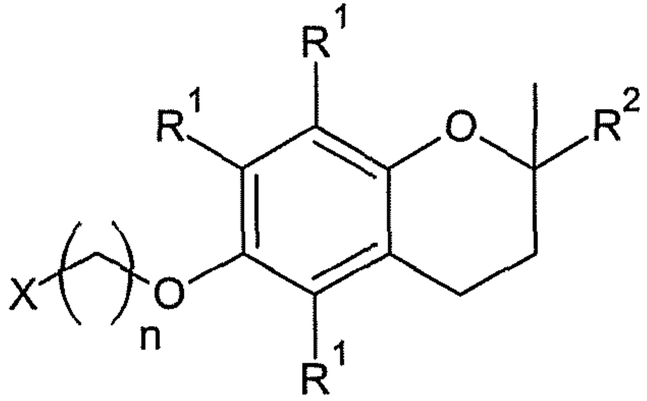
Application number / Numéro de demande: US20100307988

Figures: 1, 2, 3, 4, 5

Pages: 1/2, 2/2

Unscannable items received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de la préparation des dossiers au 10ième étage)




(I)