and hydroxyl radicals, and potentially other reactive species that may be toxic to cells. For example, UV-induced signal transduction and ROS generation have been shown to induce matrix metalloproteinase (MMP) expression in human skin as part of a molecular mechanism underlying photoaging (Kang et al., 2003 *J. Invest. Dermatol.* 120:835).

Clearly there is a need in the art for improved compositions and methods for treating skin conditions that result from ROS generation and oxidative damage, including effective delivery of antioxidants to skin sites of ROS production such as keratinocyte and fibroblast mitochondria. The presently disclosed invention embodiments address this need and offer other related advantages.

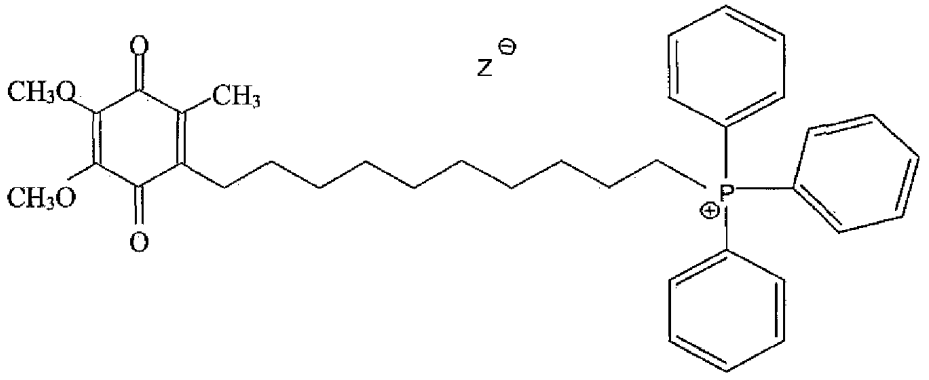
BRIEF SUMMARY

In one aspect, the present invention refers to the use of a topical formulation in the manufacture of a medicament for topically treating a skin condition that results from reactive oxygen species production in skin of a subject and/or for promoting topical wound healing in skin of a subject, the topical formulation comprising

a composition which comprises

(1) an antioxidant compound that is selected from:

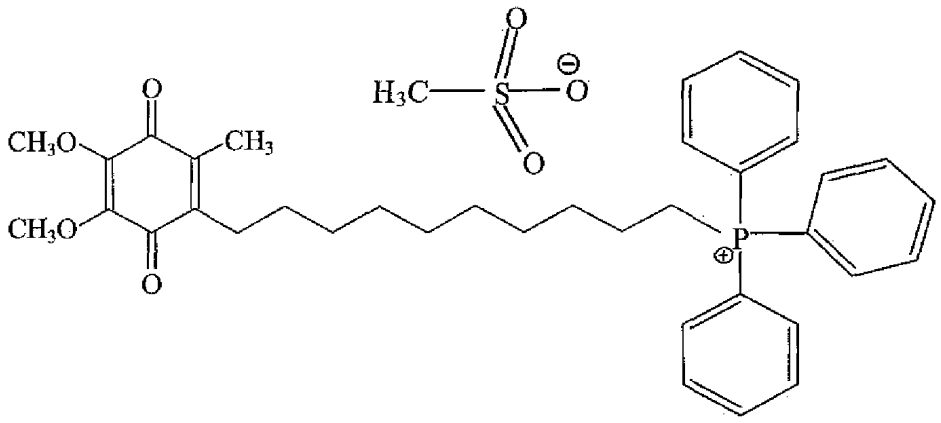
(a) the antioxidant compound that has the formula II:



(II)

or its quinol form, wherein Z is an anionic complement which is an alkyl sulfonate or is selected from the group consisting of methanesulfonate, p-toluenesulfonate, ethanesulfonate, benzenesulfonate or 2-naphthalenesulfonate; and

(b) the antioxidant compound that has the formula III:



(III)

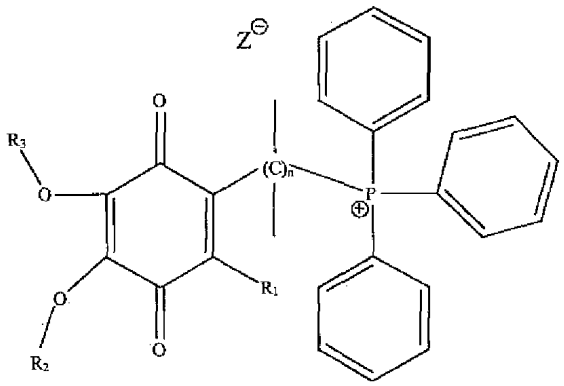
or its quinol form, and

(2) a pharmaceutical excipient or carrier for topical use,

wherein the formulation delivers a therapeutically effective amount of the antioxidant compound to skin fibroblasts and keratinocytes and the cationic moiety is capable of mitochondrially targeting the antioxidant moiety, and

wherein optionally the topical formulation further comprises retinoic acid. According to certain examples, there is provided a method of treating a skin condition that results from reactive oxygen species production in skin of a subject, the method comprising applying to the skin a topical formulation that comprises (a) an antioxidant compound which comprises (i) a lipophilic cationic moiety linked by a linking moiety to an antioxidant moiety, and (ii) an anionic complement for said cationic moiety, and (b) a pharmaceutical excipient or carrier for topical use, wherein the formulation delivers a therapeutically effective amount of the antioxidant compound to skin fibroblasts and keratinocytes and the cationic moiety is capable of mitochondrially targeting the antioxidant moiety, and wherein the anionic complement is a pharmaceutically acceptable anion that is not a bromide ion or a nitrate anion and does not exhibit reactivity against the antioxidant moiety, the cationic moiety or the linking moiety, and thereby treating the skin condition that results from reactive oxygen species production in skin. In certain examples the antioxidant moiety comprises at least one antioxidant moiety that is selected from (i) a quinone or a quinol, (ii) vitamin E or a vitamin E derivative, (iii) ascorbic acid or an ascorbic acid derivative, (iv) alpha-lipoic acid or a derivative thereof, (v) a chain breaking

antioxidant, (vi) a derivatized fullerene, (vii) a spin trap, (viii) an antioxidant moiety that is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, 5,5-dimethylpyrroline-*N*-oxide, *tert*-butylnitrosobenzene, *tert*-nitrosobenzene and α-phenyl-*tert*-butylnitrone, and (ix) N-acetyl cysteine. In certain examples the topical formulation further comprises retinoic acid. In certain examples the antioxidant compound is capable of altering (i) a detectable indicator of reactive oxygen species in a human skin fibroblast, and (ii) a detectable indicator of reactive oxygen species in a human skin keratinocyte. In certain examples the lipophilic cationic moiety is a triphenylphosphonium cation. In certain examples the pharmaceutically acceptable anion is not a halogen ion. In certain examples the pharmaceutically acceptable anion is not nucleophilic. In certain examples the pharmaceutically acceptable anion is an alkyl sulfonate. In certain examples the pharmaceutically acceptable anion is selected from methanesulfonate, p-toluenesulfonate, ethanesulfonate, benzenesulfonate and 2-naphthalenesulfonate. In certain examples the pharmaceutically acceptable anion is methanesulfonate. In certain examples the antioxidant compound has the formula I:

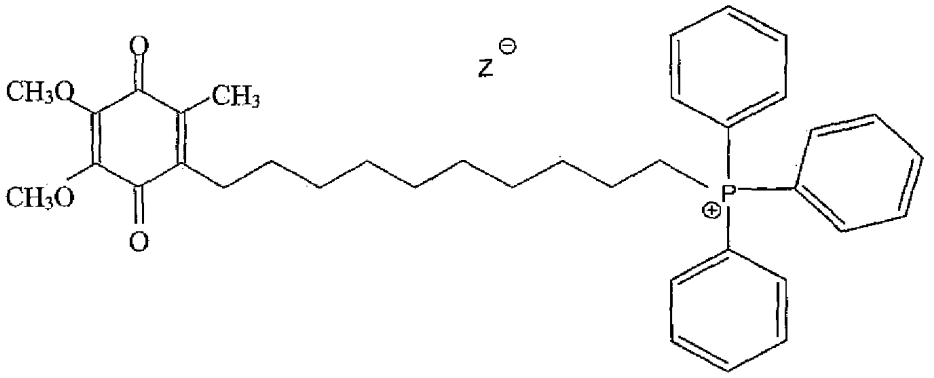


I

or its quinol form, wherein R_1, R_2, and R_3 are the same or different and are selected from C_1 to C_5 alkyl and H, and wherein n is an integer from 2 to 20, and wherein Z is the anionic complement. In certain

further examples Z is selected from an alkyl sulfonate, an aryl sulfonate and nitrate. In certain examples C of $(C)_n$ is saturated.

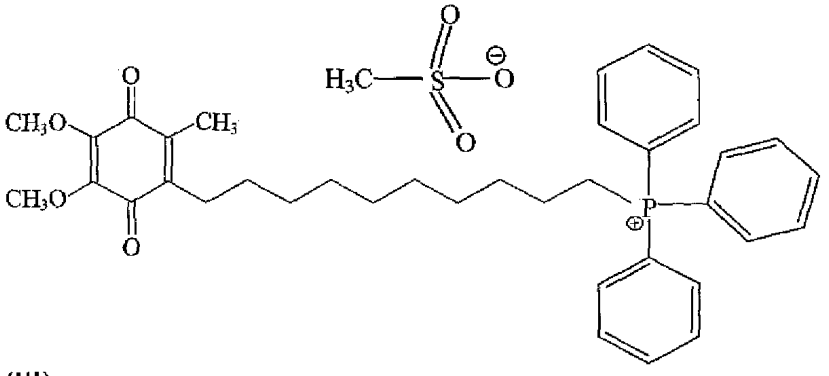
In certain examples of the above described methods the antioxidant compound has the formula:



(II)

or its quinol form, wherein Z is the anionic complement.

In certain examples the antioxidant compound has the formula:



(III)

or its quinol form.

In certain examples the pharmaceutical excipient or carrier comprises cyclodextrin. In certain further examples the antioxidant compound and cyclodextrin are present at a compound-to-cyclodextrin molar ratio that is from about 10:1 to about 1:10. In certain other further examples the antioxidant compound and cyclodextrin are present at a compound-to-cyclodextrin molar ratio that is selected from the group consisting

of (i) from about 5:1 to about 1:5, (ii) from about 4:1 to about 1:4, (iii) from about 2:1 to about 1:2, (iv) about 1:1 and (v) about 1:2. In certain examples the cyclodextrin is β-cyclodextrin. In certain examples the antioxidant compound and cyclodextrin are present at a compound-to-cyclodextrin molar ratio that is about 1:2.

In certain examples of the above described methods, the skin condition that results from reactive oxygen species production is characterized by alteration of at least one of (i) a detectable indicator of reactive oxygen species in a human skin fibroblast, and (ii) a detectable indicator of reactive oxygen species in a human skin keratinocyte. In certain other examples the skin condition that results from reactive oxygen species production is characterized by alteration of (i) a detectable indicator of reactive oxygen species in a human skin fibroblast, and (ii) a detectable indicator of reactive oxygen species in a human skin keratinocyte. In certain examples the skin condition that results from reactive oxygen species production is age-related skin damage. In certain further examples the age-related skin damage comprises skin photoaging. In certain further examples skin photoaging comprises one or more of wrinkling, scar tissue deposition, altered skin elasticity, altered skin color, altered skin texture, altered skin thickness, angioma, telangiectasia, sunburn, dryness, itchiness, neoplasia and precancerous growth. In certain other related examples the skin condition that results from reactive oxygen species production comprises a skin infection. In certain further examples the skin infection comprises at least one of a bacterial infection, a viral infection, a parasitic infection and a fungal infection.

In certain other examples the skin condition that results from reactive oxygen species production comprises one or more of acne, amyloidosis, a benign skin tumor, a blister or ulcer, bullous disease, skin cancer, dermatitis, eczema, inflammation, ichthyosis, an insect bite or insect sting, keratosis pilaris, pruritis, psoriasis, a scaling disease, a rash, vitiligo and a sweat gland disorder. In certain examples the antioxidant compound is capable of altering (i) at least one detectable indicator of reactive oxygen species in a human skin fibroblast that is selected from the group consisting of

reactive oxygen species generation, matrix metalloproteinase expression and an extracellular signal-related kinase (ERK) phosphorylation state, and (ii) at least one detectable indicator of reactive oxygen species in a human skin keratinocyte that is selected from the group consisting of reactive oxygen species generation, matrix metalloproteinase expression and an extracellular signal-related kinase (ERK) phosphorylation state. In certain other examples the skin condition that results from reactive oxygen species production comprises one or more condition selected from the group consisting of erythema, skin redness and inflammation caused by laser surgery, radiation therapy, sun burn, rosaceae, a burn or sepsis.

According to certain other examples there is provided a method of promoting topical wound healing in skin of a subject, the method comprising applying to the skin a topical formulation that comprises (a) an antioxidant compound which *comprises (i) a lipophilic cationic moiety linked by a linking moiety to an antioxidant* moiety, and (ii) an anionic complement for said cationic moiety, and (b) a pharmaceutical excipient or carrier for topical use, wherein the formulation delivers a therapeutically effective amount of the antioxidant compound to skin fibroblasts and keratinocytes and the cationic moiety is capable of mitochondrially targeting the antioxidant moiety, and wherein the anionic complement is a pharmaceutically acceptable anion that is not a bromide ion or a nitrate anion and does not exhibit reactivity against the antioxidant moiety, the cationic moiety or the linking moiety, and thereby treating the skin condition that results from reactive oxygen species production in skin. In certain further examples the antioxidant moiety comprises at least one antioxidant moiety that is selected from the group consisting of (i) a quinone or a quinol, (ii) vitamin E or a vitamin E derivative, (iii) ascorbic acid or an ascorbic acid derivative, (iv) alpha-lipoic acid or a derivative thereof, (v) a chain breaking antioxidant, (vi) a derivatized fullerene, (vii) a spin trap, (viii) an antioxidant moiety that is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, 5,5-dimethylpyrroline-*N*-oxide, *tert*-butylnitrosobenzene, *tert*-nitrosobenzene and α-phenyl-*tert*-butylnitrone, and (ix) N-acetyl cysteine. In certain examples the topical formulation further comprises retinoic acid. In certain examples the antioxidant compound is

capable of altering (i) a detectable indicator of reactive oxygen species in a human skin fibroblast, and (ii) a detectable indicator of reactive oxygen species in a human skin keratinocyte. In certain examples the lipophilic cationic moiety is a triphenylphosphonium cation. In certain examples the pharmaceutically acceptable anion is not a halogen ion. In certain examples the pharmaceutically acceptable anion is not nucleophilic. In certain examples the pharmaceutically acceptable anion is an alkyl sulfonate. In certain examples the pharmaceutically acceptable anion is selected from methanesulfonate, p-toluenesulfonate, ethanesulfonate, benzenesulfonate and 2-naphthalenesulfonate. In certain examples the pharmaceutically acceptable anion is methanesulfonate.

These and other aspects of the invention will be evident upon reference to the following detailed description and attached drawings. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference in their entirety, as if each was incorporated individually. Aspects of the invention can be modified, if necessary, to employ concepts of the various patents, applications and publications to provide yet further embodiments of the invention.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 shows induction of ROS production in human skin fibroblasts cultured in three-dimensional collagen lattices, following treatment with collagenase (MMP1).

Figure 2 shows effects of $MitoQ_{10}$ mesylate on ROS production in human skin fibroblasts cultured in three-dimensional collagen lattices, following treatment with collagenase (MMP1).

Figure 3 shows effects of $MitoQ_{10}$ mesylate on MMP1 expression in human skin fibroblasts cultured in three-dimensional collagen lattices, following treatment with collagenase (MMP1).

CLAIMS

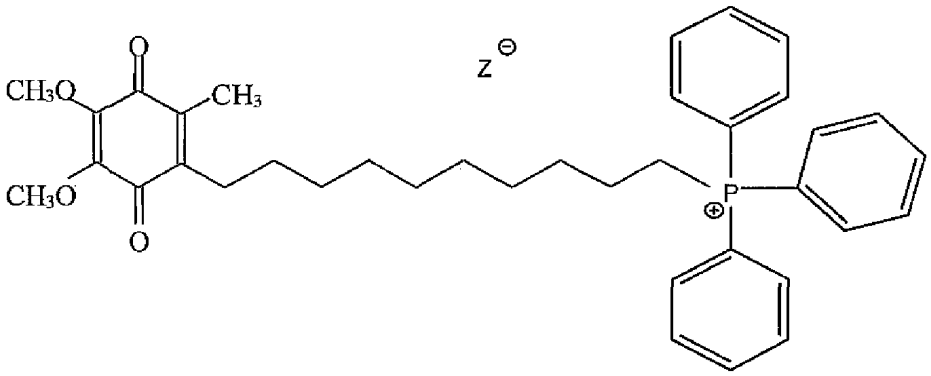
What is claimed is:

1. Use of a topical formulation in the manufacture of a medicament for topically treating a skin condition that results from reactive oxygen species production in skin of a subject and/or for promoting topical wound healing in skin of a subject, the topical formulation comprising

a composition which comprises

(1) an antioxidant compound that is selected from:

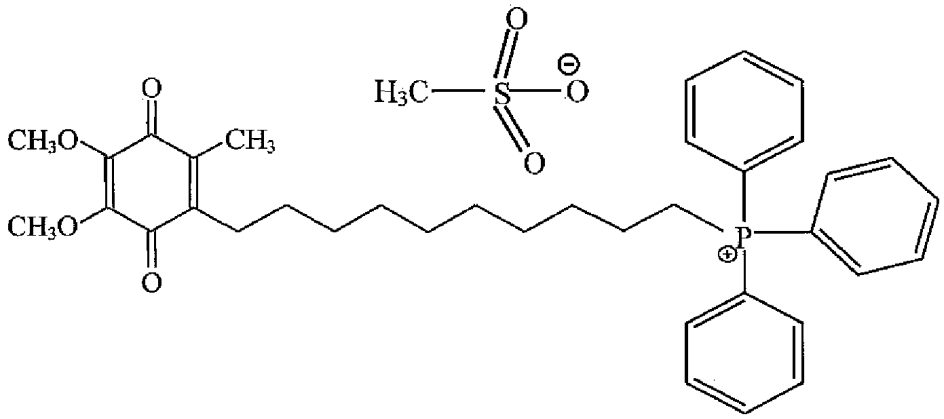
(a) the antioxidant compound that has the formula II:



II

or its quinol form, wherein Z is an anionic complement which is an alkyl sulfonate or is selected from the group consisting of methanesulfonate, p-toluenesulfonate, ethanesulfonate, benzenesulfonate or 2-naphthalenesulfonate; and

(b) the antioxidant compound that has the formula III:



(III)

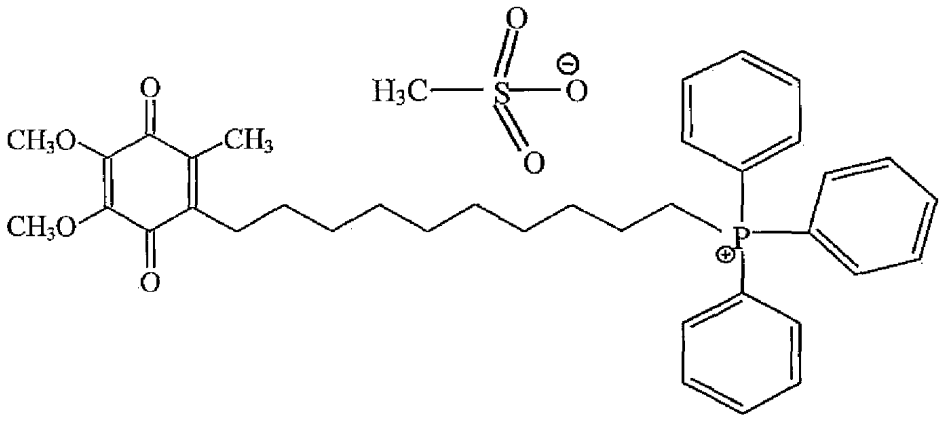
or its quinol form, and

(2) a pharmaceutical excipient or carrier for topical use,

wherein the formulation delivers a therapeutically effective amount of the antioxidant compound to skin fibroblasts and keratinocytes and the cationic moiety is capable of mitochondrially targeting the antioxidant moiety, and

wherein optionally the topical formulation further comprises retinoic acid.

2. The use of the topical formulation according to claim 1, wherein the antioxidant compound is the antioxidant compound that has the formula III:



(III)

or its quinol form.

3. The use of the topical formulation according to claim 1 or claim 2, wherein the antioxidant compound is capable of altering (i) a detectable indicator of reactive oxygen species in a human skin fibroblast, and (ii) a detectable indicator of reactive oxygen species in a human skin keratinocyte.

4. The use of the topical formulation according to claim 1 or claim 2, wherein the pharmaceutical excipient or carrier comprises cyclodextrin, for example, β-cyclodextrin, optionally wherein the antioxidant compound and cyclodextrin are present at a compound-to-cyclodextrin molar ratio that is selected from the group consisting of (i) from about 5:1 to about 1:5, (ii) from about 4:1 to about 1:4, (iii) from about 2:1 to about 1:2, (iv) about 1:1, (v) about 1:2, and (vi) from about 10:1 to about 1:10.

5. The use of the topical formulation according to claim 1 or claim 2, wherein the skin condition that results from reactive oxygen species production is

characterized by alteration of at least one of (i) a detectable indicator of reactive oxygen species in a human skin fibroblast, and (ii) a detectable indicator of reactive oxygen species in a human skin keratinocyte, and is selected from:

(i) age-related skin damage,

(ii) angioma, telangiectasia, sunburn, dryness, itchiness, neoplasia and precancerous growth,

(iii) a skin infection, optionally comprising at least one of a bacterial infection, a viral infection, a parasitic infection and a fungal infection, and

(iv) one or more of acne, amyloidosis, a benign skin tumor, a blister or ulcer, bullous disease, skin cancer, dermatitis, eczema, inflammation, ichthyosis, an insect bite or insect sting, keratosis pilaris, pruritis, psoriasis, a scaling disease, a rash, vitiligo, a sweat gland disorder, erythema, skin redness and inflammation caused by laser surgery, radiation therapy, rosaceae, a burn and sepsis.

6. The use of the topical formulation according to claim 1 or claim 2, wherein the antioxidant compound is capable of altering (i) at least one detectable indicator of reactive oxygen species in a human skin fibroblast that is selected from the group consisting of reactive oxygen species generation, matrix metalloproteinase expression and an extracellular signal-related kinase (ERK) phosphorylation state, and (ii) at least one detectable indicator of reactive oxygen species in a human skin keratinocyte that is selected from the group consisting of reactive oxygen species generation, matrix metalloproteinase expression and an extracellular signal-related kinase (ERK) phosphorylation state.