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(54) Title: COMPOSITIONS COMPRISING SULFORAPHANE OR A SULFORAPHANE PRECURSOR AND URSOLIC ACID

(57) Abstract: The invention relates to the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and ursolic acid, a salt, ester, amide, or derivative thereof. The invention also relates to the combination of a sulforaphane or a derivative thereof and ursolic acid, a salt, ester, amide, or derivative thereof. The invention also relates to the combination of a broccoli extract or powder and ursolic acid, a salt, ester, amide, or derivative thereof. The invention provides compositions and methods relating to these combinations.

COMPOSITIONS COMPRISING SULFORAPHANE OR A SULFORAPHANE PRECURSOR AND URSOLIC ACID

[0001] This application claims priority to the following applications, each of which is incorporated by reference in its entirety: U.S. Provisional Patent Application No. 61/668,328, filed on July 5, 2012; U.S. Provisional Patent Application No. 61/668,342, filed on July 5, 2012; U.S. Provisional Patent Application No. 61/668,386, filed on July 5, 2012; U.S. Provisional Patent Application No. 61/668,396, filed on July 5, 2012; U.S. Provisional Patent Application No. 61/668,364, filed on July 5, 2012; U.S. Provisional Patent Application No. 61,668,374, filed on July 5, 2012; and U.S. Provisional Patent Application No. 61/794,417, filed on March 15, 2013.

FIELD OF THE INVENTION

[0002] The present invention relates to the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and ursolic acid, a salt, ester, amide, or derivative thereof. The present invention also relates to the combination of a sulforaphane or a derivative thereof and ursolic acid, a salt, ester, amide, or derivative thereof. The present invention also relates to the combination of a broccoli extract or powder and ursolic acid, a salt, ester, amide, or derivative thereof. The present invention provides compositions and methods relating to these combinations.

BACKGROUND OF THE INVENTION

[0003] The use of natural products is becoming increasingly popular with humans and companion animals. Some of these natural products are being incorporated into dietary supplements and medical foods. Many of these products can be useful as chemoprotective and/or antioxidant agents.

[0004] Chemoprotection through the use of natural products is evolving as a safe, effective, inexpensive, easily accessible, and practical means to prevent or reduce the occurrence of many conditions affecting humans and domesticated animals. It is known that carcinogens which can damage cells at the molecular level are often ingested and inhaled as non-toxic precursors. These non-toxic precursors

may then convert into carcinogenic substances in the body. Chemoprotective agents, such as natural substances which can activate detoxifying enzymes or their co-factors, can counteract and allow for the elimination of carcinogens. These same natural substances can potentiate other naturally existing defenses such as the immune system.

[0005] Some natural products have antioxidant activity. Oxidative stress plays a major role in aging, the progression of neurodegenerative diseases, as well as physiological trauma, such as ischemia. Antioxidant agents can reduce or inhibit the oxidation of vital biomolecules and may play a role in treating, preventing, or reducing the occurrence of conditions affected by oxidative stress.

[0006] An example of a natural product thought to have chemoprotective and antioxidant properties is sulforaphane. Sulforaphane is an organosulfur compound which is also known as 1-isothiocyanato-4-methylsulfinylbutane. The sulforaphane precursor, glucoraphanin, can be obtained from vegetables of the *Brassicaceae* family, such as broccoli, Brussels sprouts, and cabbage. However, copious amounts of vegetables must be consumed in order to obtain levels adequate for chemoprevention. Glucoraphanin is converted into sulforaphane by a thioglucosidase enzyme called myrosinase, which occurs in a variety of exogenous sources such as *Brassicaceae* vegetables and endogenously in the gut microflora. However, upon ingestion of glucoraphanin, not all animals are capable of achieving its conversion to sulforaphane, most likely due to variations in microflora populations and overall health. In addition, in acidic environments such as the stomach, glucoraphanin can be converted to inert metabolites. The active metabolite, sulforaphane induces nuclear erythroid-2-related factor (Nrf2) which, in turn, upregulates the production of Phase II detoxification enzymes and cytoprotective enzymes such as glutathione S-transferases, NAD(P)H:quinine oxidoreductase (NQO1) and heme-oxygenase-1 (HO-1). Sulforaphane has been thought to induce the production of these enzymes without significantly changing the synthesis of P-450 cytochrome enzymes. The upregulation of Phase II enzymes is thought to play a role in a variety of biological activities, including the protection of the brain from cytotoxicity, the protection of the liver from the toxic effects of fat accumulation, and the detoxification of a variety of other tissues.

[0007] Sulforaphane and its precursor glucoraphanin have been studied extensively. Shapiro *et al.* (*Nutrition and Cancer,* (2006), Vol. 55(1), pp. 53-62)

discuss a clinical Phase I study determining the safety, tolerability, and metabolism of broccoli sprout glucosinolates and isothiocyanates. Shapiro *et al.* discuss a placebo-controlled, double-blind, randomized clinical study of sprout extracts containing either glucosinolates such as glucoraphanin or isothiocyanates such as sulforaphane in healthy human subjects. The study found that administration of these substances did not result in systematic, clinically significant, adverse effects. Ye et al., (*Clinica Chimica Acta*, 200, 316:43-53) discuss the pharmacokinetics of broccoli sprout isiothiocyanates in humans.

[0008] Ursolic acid, also known as (1S,2R,4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid, is a pentacyclic triterpene acid found in many plants, including cranberries, apples, basil, bilberries, elderflower, peppermint, rosemary, lavender, oregano, thyme, prunes, and apple peels. Ursolic acid, a tripterpenoid compound, has been found to induce apoptosis in prostate cancer cells; it decreases the expression of matrix metalloproteinase 9 (MMP-9), a protein involved in the breakdown of extracellular matrix contributing to metastasis; it also decreases inflammation by decreasing the expression of cyclooxygenase-2 (COX-2) through inhibition of nuclear factor-kappa B (NF-kB), a transcription factor protein complex.

[0009] Quercetin, also known as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, is a flavonoid found in fruits, vegetables, leaves, and grains. Food rich in quercetin include black and green teas, capers, apples, onions, red grapes, citrus fruit, tomatoes, and berries, including cranberry, raspberry, bog whortleberry, lingonberry, chokeberry, and rowanberry. Quercetin has been found to have a number of biological effects, including inhibiting proliferation and epidermal growth factor (EGF) receptor expression, free radical scavenging, and increasing the expression of endogenous tissue inhibitors of matrix metalloproteinases. Quercetin has also been found to possess anti-inflammatory effects, in particular affecting inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).

[00010] Zhang *et al.* (*Proc. Natl. Acad. Sci.*, (1994), Vol. 91, pp. 3147-3150) discuss a study in Sprague-Dawley rats to determine the anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isiothiocyanates. The study determined that administration of sulforaphane was effective in blocking the formation of mammary tumors.

[00011] Cornblatt *et al.* (*Carcinogenesis*, (2007), Vol. 38(7): pp. 1485-1490) discuss a study in Sprague-Dawley rats to determine the effect of sulforaphane in chemoprevention in the breast. The study determined that oral administration of sulforaphane resulted in a 3-fold increase in NAD(P)H:quinine oxidoreductase (NQO1) enzymatic activity and a 4-fold elevated immunostaining of the heme oxygenase-1 (HO-1) enzyme in the mammary epithelium.

[00012] Munday *et al.* (*Cancer Res*, (2008), Vol. 68(5): pp. 1593-1600) discuss a study regarding the effects of a freeze-dried aqueous extract of broccoli sprouts on bladder cancer development in rats. The study found that administration of the broccoli sprout extract resulted in a significant induction of glutathione S-transferase and NAD(P)H:quinine oxidoreductase 1 in the bladder, which are the enzymes having protective activity against oxidants and carcinogens.

[00013] European Patent Application No. 2 213 280 discloses formulations comprising glucosinolates such as glucoraphanin, and myrosinase, wherein the formulation is encapsulated or coated.

[00014] All references cited herein are incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[00015] The present invention provides a composition comprising: (i) a sulforaphane precursor, preferably glucoraphanin; (ii) an enzyme capable of converting the sulforaphane precursor to sulforaphane, preferably a glucosidase enzyme, more preferably a thioglucosidase enzyme, and most preferably myrosinase; (iii) an enzyme potentiator, preferably ascorbic acid; and (iv) ursolic acid, a salt, ester, amide or a derivative thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of, cancer, in particular prostate cancer, liver cancer, colon cancer, brain cancer, and bladder cancer in a subject, comprising administering to the subject: (i) a sulforaphane precursor, (ii) an enzyme capable of converting the sulforaphane precursor to sulforaphane, (iii) an enzyme potentiator, and (iv) ursolic acid, a salt, ester, amide or a derivative thereof. The present invention also provides a method of reducing levels or reducing gene expression of matrix metalloproteinase-9 (MMP-9) in a subject, comprising administering to the subject: (i) a sulforaphane precursor, (ii) an

enzyme capable of converting the sulforaphane precursor to sulforaphane, (iii) an enzyme potentiator, and (iv) ursolic acid, a salt, ester, amide or a derivative thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of matrix metalloproteinase-9 (MMP-9) in a subject, comprising administering to the subject: (i) a sulforaphane precursor, (ii) an enzyme capable of converting the sulforaphane precursor to sulforaphane, (iii) an enzyme potentiator, and (iv) ursolic acid, a salt, ester, amide or a derivative thereof.

[00016] The present invention provides a composition comprising: (i) sulforaphane or a derivative thereof, and (ii) ursolic acid, a salt, ester, amide or a derivative thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of, cancer, in particular prostate cancer, liver cancer, colon cancer, brain cancer, and bladder cancer in a subject, comprising administering to the subject: (i) sulforaphane or a derivative thereof, and (ii) ursolic acid, a salt, ester, amide or a derivative thereof. The present invention also provides a method of reducing levels or reducing gene expression of matrix metalloproteinase-9 (MMP-9) in a subject, comprising administering to the subject: (i) sulforaphane or a derivative thereof, and (ii) ursolic acid, a salt, ester, amide or a derivative thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of matrix metalloproteinase-9 (MMP-9) in a subject, comprising administering to the subject: (i) sulforaphane or a derivative thereof, and (ii) ursolic acid, a salt, ester, amide or a derivative thereof.

[00017] The present invention provides a composition comprising: (i) a broccoli extract or powder, and (ii) ursolic acid, a salt, ester, amide or a derivative thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of, cancer, in particular prostate cancer, liver cancer, colon cancer, brain cancer, and bladder cancer in a subject, comprising administering to the subject: (i) a broccoli extract or powder, and (ii) ursolic acid, a salt, ester, amide or a derivative thereof. The present invention also provides a method of reducing levels or reducing

gene expression of matrix metalloproteinase-9 (MMP-9) in a subject, comprising administering to the subject: (i) a broccoli extract or powder, and (ii) ursolic acid, a salt, ester, amide or a derivative thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of matrix metalloproteinase-9 (MMP-9) in a subject, comprising administering to the subject: (i) a broccoli extract or powder, and (ii) ursolic acid, a salt, ester, amide or a derivative thereof.

BRIEF DESCRIPTION OF THE FIGURES

[00018] FIG. 1 is a graph showing the conversion of glucoraphanin at 38°C without ascorbic acid, as described in Example 4.

[00019] FIG. 2 is a graph showing the conversion within about 10 minutes at 38°C as a function of ascorbic acid concentration, as described in Example 4.

[00020] FIG. 3 is a graph showing the conversion to sulforaphane within 30 minutes at 38°C and 1 mM ascorbic acid, as described in Example 4.

[00021] FIG. 4 is a graph showing the conversion of glucoraphanin to sulforaphane in simulated intestinal fluid, as described in Example 5.

[00022] FIG. 5a and 5b and FIG. 6 are graphs showing the results of the experiment described in Example 6.

DETAILED DESCRIPTION OF THE INVENTION

[00023] The present invention relates to the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and ursolic acid, a salt, ester, amide, or derivative thereof. The present invention also relates to the combination of sulforaphane or a derivative thereof and ursolic acid, a salt, ester, amide, or derivative thereof. The present invention also relates to the combination of a broccoli extract or powder and ursolic acid, a salt, ester, amide, or derivative thereof. The present invention also relates to the use of ursolic acid, a salt, ester, amide of derivative thereof, with a mixture of one or more of the following: sulforaphane precursor, sulforaphane or a derivative thereof, and broccoli extract. The present invention provides compositions relating to these combinations.

[00024] The present invention also provides methods comprising administering these combinations. In some embodiments, the combination may be administered for treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of, cancer, in particular prostate cancer, liver cancer, colon cancer, brain cancer, and bladder cancer in a subject. In some embodiments, the combination may be administered for reducing levels or reducing gene expression of matrix metalloproteinase-9 (MMP-9) in a subject, or treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of matrix metalloproteinase-9 (MMP-9) in a subject.

[00025] Sulforaphane is also known as 1-isothiocyanato-4-methylsulfinylbutane. Derivatives of sulforaphane include, but are not limited to sulfoxythiocarbamate analogues of sulforaphane, 6-methylsulfinylhexyl isothiocyanate (6-HITC), and compounds which comprise the structure of sulforaphane with different side chains and/or various lengths of spacers between the isothiocyanato and sulfoxide groups. Examples of derivatives of sulforaphane include those described in the following references, each of which is incorporated herein by reference: Hu et al., *Eur J Med Chem*, 2013, 64:529-539; Ahn et al., *Proc Natl Acad Sci USA*, 2010, 107(21):9590-9595; and Morimistu et al., *J. Biol. Chem.* 2002, 277:3456-3463, and Baird et al., *Arch Toxicol*, 2011, 85(4):241-272.

[00026] In some embodiments, the composition comprises sulforaphane or a derivative thereof, preferably sulforaphane, in an amount of about 1 µg to about 10 g, preferably about 3 µg to about 5 g, preferably about 5 µg to about 1000 mg, preferably about 7 µg to about 750 mg, more preferably about 10 µg to about 500 mg, and most preferably about 100 µg to about 100 mg. In some embodiments, compositions suitable for human use comprise about 1 mg to about 20 mg.

[00027] In some embodiments, the methods of the present invention comprise administration of sulforaphane or a derivative thereof to a subject, preferably sulforaphane, in an amount of about 1 µg to about 10 g, preferably about 3 µg to about 5 g, preferably about 5 µg to about 1000 mg, preferably about 7 µg to about 750 mg, more preferably about 10 µg to about 500 mg, and most preferably about 100 µg to about 100 mg. In some embodiments wherein the subject is a human, the method comprises administration of about 1 mg to about 20 mg. In some

embodiments, the methods of the present invention comprise administration of sulforaphane or a derivative thereof to a subject, preferably sulforaphane, in an amount of about 0.01 µg/kg to about 0.2 g/kg, preferably about 0.05 µg/kg to about 0.07 g/kg, more preferably about 0.07 µg/kg to about 15 mg/kg, more preferably about 0.1 µg/kg to about 11 mg/kg, and most preferably about 0.2 µg/kg to about 7 mg/kg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 2 µg/kg to about 2 mg/kg, and more preferably about 0.01 mg/kg to about 0.3 mg/kg. The above amounts may refer to each dosage administration or a total daily dosage. The total daily dosage refers to the total amount of a compound or ingredient which is administered to a subject in a twenty-four hour period.

[00028] In some embodiments, the method comprises administration of more than one of a sulforaphane or a derivative thereof. In some embodiments, the compositions comprise more than one of a sulforaphane or a derivative thereof. For example, the methods or composition may comprise both sulforaphane and one or more derivatives thereof, or two or more derivatives. In some embodiments wherein the method or composition comprise more than one of a sulforaphane or a derivative thereof, the above amounts may refer to the amount of each sulforaphane or a derivative thereof, or the total amount of the more than one sulforaphane or derivative thereof.

[00029] The term “sulforaphane precursor” refers to any compound, substance or material which can be used to produce sulforaphane. In preferred embodiments, the sulforaphane precursor comprises a compound which can be converted or metabolized to sulforaphane, preferably by an enzyme. In some preferred embodiments, the sulforaphane precursor comprises glucoraphanin. Glucoraphanin is a glucosinolate which is also known as 4-methylsulfinylbutyl glucosinolate and 1-*S*-[(1*E*)-5-(methylsulfinyl)-*N*-(sulfonatooxy) pentanimidoyl]-1-thio-β-D-glucopyranose.

[00030] In some embodiments, the composition comprises about 1 µg to about 10 g, preferably about 250 µg to about 5 g, more preferably about 500 µg to about 2000 mg, even more preferably about 1 mg to about 750 mg, even more preferably about 1.5 mg to about 250 mg, even more preferably about 2 mg to about 100 mg, and most preferably about 3 mg to about 75 mg of the sulforaphane precursor, preferably glucoraphanin. In some embodiments, compositions suitable for human

use comprise about 3.5 mg to about 50 mg of the sulforaphane precursor, preferably glucoraphanin.

[00031] In some embodiments, the method comprises administering the sulforaphane precursor, preferably glucoraphanin to a subject, in an amount of about 1 µg to about 10 g, preferably about 250 µg to about 5 g, more preferably about 500 µg to about 2000 mg, even more preferably about 1 mg to about 750 mg, even more preferably about 1.5 mg to about 250 mg, even more preferably about 2 mg to about 100 mg, and most preferably about 3 mg to about 75 mg. In some embodiments wherein the subject is a human, the method comprises administration of about 3.5 mg to about 50 mg. In some embodiments, the method comprises administering an amount of sulforaphane precursor to a subject in an amount of about 1 µg/kg to about 1000 mg/kg, preferably about 5 µg/kg to about 500 mg/kg, more preferably about 7.5 µg/kg to about 100 mg/kg, even more preferably about 10 µg/kg to about 25 mg/kg, and most preferably about 25 µg/kg to about 10 mg/kg. In some embodiments wherein the subject is a human, the method comprises administration of about 50 µg/kg to about 800 µg/kg. The above amounts may refer to each dosage administration or a total daily dosage.

[00032] In some embodiments, the method comprises administration of more than one sulforaphane precursor. In some embodiments, the composition comprises more than sulforaphane precursor. In some embodiments wherein the method or composition comprises more than one sulforaphane precursor, the above amounts may refer to the amount of each sulforaphane precursor, or the total amount of the sulforaphane precursors.

[00033] The sulforaphane precursor may be converted or metabolized to sulforaphane. In some embodiments, the sulforphane precursor is converted to sulforaphane by an enzyme. In some embodiments, the enzyme capable of converting the sulforaphane precursor to sulforaphane comprises a glucosidase enzyme, preferably a thioglucosidase enzyme, and more preferably myrosinase. Myrosinase is also known as thioglucoside glucohydrolase.

[00034] In some embodiments, the composition comprises the enzyme in an amount of about 1 pg to about 1 ug, preferably about 50 pg to about 500 ng, and most preferably about 1 ng to about 150 ng. In some embodiments, compositions suitable for human use comprise about 5 ng to about 75 ng of the enzyme.

[00035] In some embodiments, the method comprises administering the enzyme, preferably myrosinase, in an amount of about 1 pg to about 1 µg, preferably about 50 pg to about 500 ng, and most preferably about 1 ng to about 150 ng. In some embodiments wherein the subject is a human, the method comprises administration of about 5 ng to about 75 ng of the enzyme. In some embodiments, the method comprises administering the enzyme to a subject in an amount of about 0.02 pg/kg to about 0.02 ug/kg, preferably about 0.7 pg/kg to about 7 ng/kg, and most preferably about .02 ng/kg to about 2 ng/kg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 0.1 ng/kg to about 1 ng/kg. The above amounts may refer to each dosage administration or a total daily dosage.

[00036] In some embodiments, the method comprises administration of more than one enzyme capable of converting the sulforaphane precursor to sulforaphane. In some embodiments, the composition comprises more than one enzyme capable of converting the sulforaphane precursor to sulforaphane. In some embodiments wherein the methods or compositions comprise more than one enzyme, the above amounts may refer to the amount of each enzyme, or the total amount of the enzymes.

[00037] The present invention also provides for the use of a broccoli extract and/or powder, including but not limited to broccoli seed and sprout extracts and powders. The present invention provides methods of administration of broccoli extract and/or powder, and compositions comprising broccoli extract and/or powder. In some embodiments, the broccoli extract or powder is standardized to contain about 1 to about 75% w/w, more preferably about 2.5% to about 50%, even more preferably about 5% to about 25%, and most preferably about 10% to about 20% of a sulforaphane precursor, preferably glucoraphanin. Examples of broccoli extracts and powders include but are not limited to those described in U.S. Patent Nos. 5,411,986; 5,725,895; 5,968,505; 5,968,567; 6,177,122; 6,242,018; 6,521,818; 7,303,770, and 8,124,135, each of which is incorporated by reference in its entirety. Powders of broccoli may be obtained, for example, by air drying, freeze drying, drum drying, spray drying, heat drying and/or partial vacuum drying broccoli, preferably broccoli sprouts. In some embodiments, the compositions and methods comprise use of about 1 µg to about 10 g, more preferably about 250 µg to about 5 g, even more preferably about 500 µg to about 1 g, preferably about 600 µg to about 500

mg, more preferably about 750 µg to about 400 mg, and most preferably about 1 mg to about 300 mg of the broccoli extract. In some embodiments, the broccoli extract or powder are present in a composition or administered to a subject in amounts sufficient to provide a sulforaphane precursor or sulforaphane in the amounts described above. In some embodiments, the composition may further comprise an enzyme potentiator, preferably ascorbic acid. In some embodiments, the method may further comprise administration of an enzyme potentiator, preferably ascorbic acid.

[00038] The sulforaphane or a derivative thereof, the sulforaphane precursor, and/or the enzyme capable of converting the sulforaphane precursor to sulforaphane may be obtained from any source, including but not limited to one or more plants from the *Brassicaceae* (also known as *Cruciferae*) family. Examples of plants from the *Brassicaceae* family include, but are not limited to, the following: broccoli, Brussels sprouts, cauliflower, cabbage, horseradish, parsnip, radish, wasabi, watercress, and white mustard. In some preferred embodiments, sulforaphane precursor, preferably glucoraphanin, and the enzyme, preferably myrosinase, are obtained from broccoli, broccoli sprouts, or broccoli seeds. The sulforaphane precursor and the enzyme may be obtained from the same source or from different sources. In some embodiments, both the sulforaphane precursor and the enzyme may be obtained from an extract or powder from these plants, preferably a broccoli seed or sprout extract or powder.

[00039] The present invention provides for the use of an enzyme potentiator. Enzyme potentiators may be used to enhance the activity of the enzyme that is capable of converting the sulforaphane precursor to sulforaphane. In some embodiments, the enzyme potentiator comprises an enzyme co-factor, preferably ascorbic acid. Ascorbic acid, also known as ascorbate or vitamin C, can potentiate the activity of myrosinase. In some embodiments, without an enzyme potentiator such as ascorbic acid, the conversion reaction to sulforaphane may be too slow to occur in the location needed for peak absorption. The enzyme potentiator may be obtained from a natural source, or it may be produced synthetically.

[00040] In some embodiments, the compositions may comprise about 1 mg to about 500 mg, preferably about 1 mg to about 250 mg, and most preferably about 1 mg to about 125 mg of the enzyme potentiator. In some preferred embodiments,

compositions suitable for human use comprise about 1 mg to about 50 mg of the enzyme potentiator.

[00041] In some embodiments, the method of the present invention comprises administration of an enzyme potentiator, preferably ascorbic acid, in an amount of about 1 mg to about 500 mg, preferably about 1 mg to about 250 mg, and most preferably about 1 mg to about 125 mg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 1 mg to about 50 mg. In some embodiments, the method of the present invention comprises administration of the enzyme potentiator, preferably ascorbic acid, in an amount of about 0.01 mg/kg to about 3 mg/kg, and most about 0.02 mg/kg to about 2 mg/kg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 0.02 mg/kg to about 0.7 mg/kg of the enzyme potentiator. The above amounts may refer to each dosage administration or a total daily dosage.

[00042] In some embodiments, the method comprises administration of more than one enzyme potentiator. In some embodiments, the composition comprises more than one an enzyme potentiator. In some embodiments wherein the method or composition comprises more than one enzyme potentiator, the above amounts may refer to the amount of each enzyme potentiator, or the total amount of the enzyme potentiators.

[00043] The present invention further comprises the use of ursolic acid, or a salt, ester, amide, or derivative thereof. Ursolic acid is also known as (1S,2R,4aS,6aR,6aS,6bR,8aR,10S,12aR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid. Salts of ursolic acid include, but are not limited to sodium and potassium salts. Esters of ursolic acid include, but are not limited to esters formed with alcohols, such as C_1 to C_{20} esters, preferably C_1 to C_6 esters. Derivatives of ursolic acid include conjugates of ursolic acid, analogues of ursolic acid, and compounds of ursolic acid which include any structural modification. The structural modification can occur at any position in ursolic acid, such as the C-3 and/or C-28 positions. Derivatives of ursolic acid include, but are not limited, to: 3β-(3-carboxypropionyloxy)ursa-9(11),12-dien-28-oic acid; 3β-O-acetoxy-ursan-12-en-28-oic acid N-methyl-piperazinamide or a salt thereof, such as the hydrochloride salt; 3β-O-acetyl-ursolic acid N-carbethoxypiperazinamide; and ursolic acid piperazinamide or a salt thereof, such as the hydrochloride salt. Examples of

derivatives of ursolic acid include, but are not limited to, those described in the following references, each of which is incorporated herein by reference: Zhang et al., *Biochim Biophys Acta*, 2006, 1760:1505-1512; Leal et al. *ChemMedChem*, 2012, 7(9): 1635-46; Bai et al., *Bioorg Med Chem Lett*, 2012, 22(7): 2488-93, WO2011/146768, EP1694320, U.S. Patent No. 4,530,934, U.S. Patent No. 3,903,089; and U.S. Patent Application Publication No. 2011/0190388.

[00044] The ursolic acid, salt, ester, amide, or derivative thereof may be obtained from any source, including but not limited to cranberry, bilberry, Devil's claw, elderflowers, lavender, oregano, thyme, hawthorn, rosemary, and extracts thereof, or it may be produced synthetically. In some embodiments, the composition comprises about 1 µg to about 10 g, preferably about 250 µg to about 7.5 g, more preferably about 500 µg to about 5 g, more preferably about 750 µg to about 2,500 mg, even more preferably about 1 mg to about 1000 mg, even more preferably about 250 mg to about 750 mg, even more preferably about 2 mg to about 500 mg, more preferably about 10 mg to about 250 mg, and most preferably about 25 mg to about 100 mg of ursolic acid, a salt, ester, amide, or a derivative thereof, preferably ursolic acid. In some preferred embodiments wherein the compositions are suitable for human use, the composition comprises about 30 mg to about 90 mg of ursolic acid, a salt, ester, amide, or a derivative thereof, preferably ursolic acid.

[00045] In some embodiments, the method comprises administration of about 1 µg to about 10 g, preferably about 250 µg to about 7.5 g, more preferably about 500 µg to about 5 g, more preferably about 750 µg to about 2,500 mg, even more preferably about 1 mg to about 1000 mg, even more preferably about 1.5 mg to about 750 mg, even more preferably about 2 mg to about 500 mg, more preferably about 10 mg to about 250 mg, and most preferably about 25 mg to about 100 mg. In some preferred embodiments wherein the subject is human, the method comprises administration of about 30 mg to about 90 mg ursolic acid, a salt, ester, amide, or a derivative thereof, preferably ursolic acid. In some embodiments, the method comprises administration of ursolic acid, a salt, ester, amide, or a derivative thereof, preferably ursolic acid, in an amount of about 0.01 µg/kg to about 0.2 g/kg, preferably about 3 µg/kg to about 0.1 g/kg, more preferably about 7 µg/kg to about 10 mg/kg, even more preferably about 10 µg/kg to about 3 mg/kg, even more preferably about 20 µg/kg to about 15 mg/kg, even more preferably about 22 µg/kg to about 10 mg/kg, even more preferably about 30 µg/kg to about 7 mg/kg, even

more preferably about 150 µg/kg to about 3 mg/kg, and most preferably about 350 µg/kg to about 12 mg/kg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 350 µg/kg to about 2 mg/kg. The above amounts may refer to each dosage administration or a total daily dosage.

[00046] In some embodiments, the methods comprise administration of more than one ursolic acid, salt, ester, amide or a derivative thereof. In some embodiments, the composition comprises more than one ursolic acid, salt, ester, amide or a derivative thereof. In some embodiments wherein the method or composition comprises more than one ursolic acid, salt, ester, amide or a derivative thereof, the above amounts may refer to the amount of each ursolic acid, salt, ester, amide or a derivative thereof, or the total amount of the ursolic acid, salt, ester, amide or a derivative thereof.

[00047] The methods of the present invention may further comprise administration of one or more additional components. The compositions of the present invention may further comprise one or more additional components. The additional components may include active pharmaceutical ingredients, nutritional supplement, and nutritional extract. Examples of additional components include, but are not limited to, quercetin or a derivative thereof, an aminosugar such as glucosamine, a glycosaminoglycan such as chondroitin, avocado/soybean unsaponifiables, vitamins such as vitamin K2, coffee fruit, magnesium, silymarin, proanthocyanidins, alpha- and beta-glucans, curcumin, phytosterols, and phytostanols. These additional components may be present in milk thistle (*Silybum marianum*) extract (silymarin), cranberry (*Vaccinium macrocarpon*) extract (proanthocyanidins, quercetin, and ursolic acid), turmeric (*Curcuma longa*), medicinal mushroom extracts, such as shiitake (*Lentinus edodes*), maitake (*Grifola frondosa*) mushroom extracts, and reishi (*Ganoderma lucidum*) mushroom extract.

[00048] Quercetin is also known as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one. The present invention provides for the use of quercetin, or a salt, ester, amide, or derivative thereof. Derivatives of quercetin include but are not limited to conjugates of quercetin and analogues of quercetin. Examples of derivatives of quercetin include, but are not limited to quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-arabinofuranoside, quercetin 3-O-rhamnoside, quercetin 3-methyl ether 4'-O-glucoside, quercetin 3-methyl ether 7-O-glucoside, and quercetin 3-methyl ether. The quercetin or a salt, ester, amide, or

derivative thereof may be obtained from any source, including but not limited to cranberries, onions and extracts thereof, or it may be produced synthetically. In some embodiments, the compositions and methods comprise the use of about 1 µg to about 10 g, preferably about 500 µg to about 5 g, more preferably about 750 µg to about 2,500 mg, and most preferably about 1 mg to about 1000 mg of the quercetin or a salt, ester, amide, or derivative thereof, preferably quercetin. The above amounts may refer to each dosage administration or a total daily dosage. In some embodiments, the compositions of the present invention provide quercetin or a salt, ester, amide, or derivative thereof in the above amounts. In some embodiments, the methods comprise administration of more than one quercetin or a salt, ester, amide, or derivative thereof. In some embodiments, the composition comprises more than one quercetin or a salt, ester, amide, or derivative thereof. In some embodiments wherein the methods or compositions comprise more than one quercetin or a salt, ester, amide, or derivative thereof, the above amounts may refer to the amount of each quercetin or a salt, ester, amide, or derivative thereof, or the total amount of the quercetin or a salt, ester, amide, or derivative thereof.

[00049] In some embodiments, the ratio of ursolic acid or a salt, ester, amide, or derivative thereof to sulforaphane or a derivative of is about 50:1 to about 1:1, preferably about 30:1 to about 2:1, more preferably about 20:1 to about 2.5:1, even more preferably about 15:1 to about 2.5:1, even more preferably about 10:1 to about 3:1. In some embodiments, the ratio of ursolic acid or a salt, ester, amide, or derivative thereof to the sulforaphane precursor is about 0.05:1 to about 25:1, preferably about 0.1:1 to about 20:1, more preferably about 0.2:1 to about 15:1, even more preferably about 0.75:1 to about 10:1, and most preferably about 1:1 to about 9:1.

[00050] In some embodiments, the composition comprises a unit dosage form, including but not limited to pharmaceutical dosage forms suitable for oral, rectal, intravenous, subcutaneous, intramuscular, transdermal, transmucosal, and topical. In some preferred embodiments, the composition comprises an orally administrable dosage form or a rectally administrable dosage form. Examples of orally administrable dosage forms include, but are not limited to a tablet, capsule, powder that can be dispersed in a beverage, a liquid such as a solution, suspension, or emulsion, a soft gel/chew capsule, a chewable bar, or other convenient dosage form known in the art. In preferred embodiments, the composition comprises a tablet,

capsule, or soft chewable treat. The orally administrable dosage forms may be formulated for immediate release, extended release or delayed release.

[00051] In some embodiments, at least the sulforaphane precursor, the enzyme, and the enzyme potentiator are provided in a dosage form which allows for the release in an area of the gastrointestinal tract having a pH of at least 4 and preferably at least 5, such as the small intestine, preferably the duodenum. In some embodiments, at least the sulforaphane or derivative thereof and/or the broccoli extract or powder are provided in a dosage form which allows for the release in an area of the gastrointestinal tract having a pH of at least 4 and preferably at least 5, such as the small intestine, preferably the duodenum. In some embodiments, the ursolic acid (or a salt, ester, amide or a derivative thereof) and/or any optional additional components are also released in an area of the gastrointestinal tract having a pH of at least 4 and preferably at least 5, such as the small intestine, preferably the duodenum. The small intestine includes the duodenum, jejunum, and ileum.

[00052] In some embodiments, each of these components (i.e, sulforaphane precursor, enzyme, enzyme potentiator, sulforaphane or a derivative thereof, broccoli extract or powder, ursolic acid (or a salt, ester, amide or a derivative thereof), and/or additional components) are released simultaneously or concomitantly (i.e., within a short period of time of each other). This provides benefits over glucoraphanin-containing compositions formulated to release the glucoraphanin in an area of the gastrointestinal tract having a pH below 4, such as the stomach. In low pH environments such as this, the acidic environment may divert conversion of sulforaphane precursor to other, physiologically inactive end products, such as sulforaphane nitrile and epithionitrile.

[00053] In some embodiments, the compositions may comprise orally administrable compositions which comprise enteric coated dosage forms or any dosage form which is resistant to degradation in an area of the gastrointestinal tract having pH below 4, such as the stomach. For example, the orally administrable composition may comprise a tablet or capsule comprising an enteric coating. The enteric coating may comprise materials including, but not limited to cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, polyvinyl acetate phthalate, methacrylic acid copolymer, methacrylic acid:acrylic ester copolymer, hydroxypropyl methylcellulose acetate succinate, hydroxypropyl methylcellulose trimellitate, shellac,

cellulose acetate trimellitate, carboxymethylethylcellulose, and mixtures thereof. The enteric coating may comprise any suitable enteric polymers known in the art. In some embodiments, one or more of the components in the composition may be embedded in a matrix of enteric polymers. In some embodiments, the orally administrable compositions comprise a capsule that dissolves slowly in gastric acid and travels to the small intestine, such as DRCAPS™ acid resistant capsules, which are marketed by CAPSUGEL® or any other acid resistant capsules.

[00054] In the most preferred form, the orally administrable composition is surrounded by a coating that does not dissolve unless the surrounding medium is at a pH of at least 4, and more preferably at least 5. Alternatively, a coating may be employed which controls the release by time, as opposed to pH, with the rate adjusted so that the components are not released until after the pH of the gastrointestinal tract has risen to at least 4, and more preferably at least 5. Thus, a time-release formulation may be used to prevent gastric presence of the sulforaphane precursor, the enzyme capable of converting the sulforaphane precursor to sulforaphane, and the enzyme potentiator, or of the sulforaphane. The coating layer(s) may be applied onto orally administrable composition using standard coating techniques. The enteric coating materials may be dissolved or dispersed in organic or aqueous solvents. The pH at which the enteric coat will dissolve can be controlled by a polymer, or combination of polymers, selected and/or ratio of pendant groups. For example, dissolution characteristics of the polymer film can be altered by the ratio of free carboxyl groups to ester groups. Enteric coating layers also contain pharmaceutically acceptable plasticizers such as triethyl citrate, dibutyl phthalate, triacetin, polyethylene glycols, polysorbates or other plasticizers. Additives such as dispersants, colorants, anti-adhering and anti-foaming agents may also be included.

[00055] The compositions may contain one or more non-active pharmaceutical ingredients (also known generally as “excipients”). Non-active ingredients, for example, serve to solubilize, suspend, thicken, dilute, emulsify, stabilize, preserve, protect, color, flavor, and fashion the active ingredients into an applicable and efficacious preparation that is safe, convenient, and otherwise acceptable for use. The excipients are preferably pharmaceutically acceptable excipients. Examples of classes of pharmaceutically acceptable excipients include lubricants, buffering agents, stabilizers, blowing agents, pigments, coloring agents, flavoring agents,

fillers, bulking agents, fragrances, release modifiers, adjuvants, plasticizers, flow accelerators, mold release agents, polyols, granulating agents, diluents, binders, buffers, absorbents, glidants, adhesives, anti-adherents, acidulants, softeners, resins, demulcents, solvents, surfactants, emulsifiers, elastomers and mixtures thereof.

[00056] In some embodiments, the combination of (i) a sulforaphane precursor, preferably glucoraphanin, (ii) an enzyme capable of converting the sulforaphane precursor to sulforaphane, preferably a glucosidase enzyme, more preferably a thioglucosidase enzyme, and most preferably myrosinase, (iii) an enzyme potentiator, preferably an enzyme co-factor, more preferably ascorbic acid, and (iv) ursolic acid or a salt, ester, amide, or derivative thereof demonstrates a synergistic effect. In some embodiments, the combination of sulforaphane (or a derivative thereof) and ursolic acid (or a salt, ester, amide, or derivative thereof) demonstrates a synergistic effect. Synergy refers to the effect wherein a combination of two or more components provides a result which is greater than the sum of the effects produced by the agents when used alone. In preferred embodiments, the synergistic effect is greater than an additive effect. In some embodiments, the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and ursolic acid or a salt, ester, amide, or derivative thereof has a statistically significant, greater effect compared to: (i) each component alone, (ii) the combination of sulforaphane precursor and the enzyme alone; and/or (iii) the combination of sulforaphane precursor, the enzyme, and the enzyme potentiator alone.

[00057] In preferred embodiments, the combination of the sulforaphane precursor, the enzyme, the enzyme potentiator, and ursolic acid or a salt, ester, amide, or derivative thereof demonstrates synergy by having a statistically significant and/or greater than additive effect compared to the sulforaphane precursor alone and the ursolic acid (or a salt, ester, amide, or derivative thereof) alone. In some embodiments, the combination of glucoraphanin, myrosinase, ascorbic acid, and ursolic acid has a synergistic effect compared to the combination of glucoraphanin, myrosinase, ascorbic acid alone; and compared to ursolic acid alone.

[00058] In some embodiments, the combination of a sulforaphane (or a derivative thereof) and ursolic acid (or a salt, ester, amide, or derivative thereof) has a statistically significant and/or greater than additive effect than: (i) sulforaphane (or

a derivative thereof) alone, and/or (ii) ursolic acid (or a salt, ester, amide, or derivative thereof) alone. In some embodiments, the combination of sulforaphane and ursolic acid has a synergistic effect compared to sulforaphane alone, and ursolic acid alone.

[00059] In some embodiments, the combination of broccoli extract or powder and ursolic acid (or a salt, ester, amide, or derivative thereof) has a statistically significant and/or greater than additive effect than: (i) broccoli extract or powder alone, and/or (ii) ursolic acid (or a salt, ester, amide, or derivative thereof) alone. In some embodiments, the combination of broccoli extract or powder and ursolic acid has a synergistic effect compared to broccoli extract or powder alone, and ursolic acid alone.

[00060] The present invention provides methods of use, including methods of administration to a subject in need thereof. In some embodiments, the method comprises administration of the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and ursolic acid, a salt, ester, amide, or derivative thereof. In some embodiments, the method comprises administration of the combination of a sulforaphane or a derivative thereof and ursolic acid, a salt, ester, amide, or derivative thereof. In some embodiments, the method comprises administration of the combination of a broccoli extract or powder and ursolic acid, a salt, ester, amide, or derivative thereof.

[00061] In some embodiments, the method relates to treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of, cancer, in particular metastatic cancers such as prostate cancer, liver cancer, colon cancer, brain cancer, and bladder cancer, in a subject. The present invention provides methods of treating, preventing, decreasing the symptoms associated with, and/or reducing secondary recurrences of diseases and conditions associated with the hematopoietic system, liver, prostate, breast, colon, kidney, central nervous system, cardiovascular system, pulmonary system, and joints. The present invention provides methods of treating, preventing, decreasing the symptoms associated with, and/or reducing secondary recurrences of lyme disease. The methods may also be useful for decreasing toxicity caused by environmental toxins and toxins from ingestion of plants.

[00062] In some embodiments, the method relates to reducing levels or reducing or downregulating gene expression of matrix metalloproteinase-9 (MMP-9) in a subject, or treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of matrix metalloproteinase-9 (MMP-9) in a subject. The disease or condition may include any disease or condition which would be improved , cured, or ameliorated with a reduction in MMP-9 levels or gene expression. High MMP-9 levels may correlate with poor prognosis in the cancer setting and specifically shorter overall survival times in those patients with high expression. Diseases or conditions associated with elevated or abnormal levels of MMP-9 include, but are not limited to cancer, pulmonary and central nervous system tuberculosis, multiple sclerosis, Crohn's disease, atherosclerosis, osteoarthritis, asthma, stroke, emphysema, diabetic nephropathy, chronic histiocytic intervillositis of the placenta, hypertension, abdominal aortic aneurysm, inflammatory bowel disease, chronic rhinosinusitis, coronary artery disease, and kidney disease.

[00063] In some embodiments, the methods relate to providing a beneficial effect on biomarkers. In some embodiments, the methods relate to reducing levels or reducing gene expression of biomarkers, including matrix metalloproteinases, phase II enzymes, NAD(P)H dehydrogenase , quinone 1 (NQO1), heme oxygenase (HO-1), UDP-glucuronosyltransferase 1-1 (UGT-A1), glutathione S-transferase (GST), cyclooxygenase-1 (COX-1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFk-B). In some embodiments, the method relates to treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with abnormal or elevated levels of these biomarkers in a subject. Diseases or conditions associated with elevated or abnormal levels of these biomarkers include, but are not limited to cancer, pulmonary and central nervous system tuberculosis, multiple sclerosis, Crohn's disease, atherosclerosis, osteoarthritis, asthma, stroke, emphysema, diabetic nephropathy, chronic histiocytic intervillositis of the placenta, hypertension, abdominal aortic aneurysm, inflammatory bowel disease, chronic rhinosinusitis, coronary artery disease, and kidney disease.

[00064] In some embodiments, the method comprises administering to a subject in need thereof a combination of sulforaphane and ursolic acid. In some embodiments the method comprises administering to a subject in need thereof a

combination of broccoli extract or powder and ursolic acid. In some preferred embodiments, the method comprises administering to the subject a combination of glucoraphanin, myrosinase, ascorbic acid, and ursolic acid. In preferred embodiments, the combinations demonstrate a synergistic effect in the methods of the present invention.

[00065] In preferred embodiments, one or more components of the combinations (for example, the sulforaphane precursor, the enzyme capable of converting the sulforaphane precursor to sulforaphane, the enzyme potentiator, ursolic acid (or a salt, ester, amide, or derivative thereof); or the sulforaphane or derivative thereof and the ursolic acid (or a salt, ester, amide, or derivative thereof); or broccoli extract or powder and ursolic acid (or a salt, ester, amide, or derivative thereof)) are administered together in one composition or dosage form, or separately, preferably within a period in which their therapeutic properties overlap. In some embodiments, the components of the combinations may be administered in two or more orally administrable compositions or dosage forms. For example, in some embodiments, the sulforaphane precursor, the enzyme capable of converting the sulforaphane precursor to sulforaphane, and the enzyme potentiator are administered in one orally administrable dosage form, while the ursolic acid (or a salt, ester, amide, or a derivative thereof) are administered in one or more separate or additional orally administrable dosage form(s). In preferred embodiments, the components of the combination are administered in one dosage form.

[00066] In some embodiments, the combination may be administered at a frequency of 1 to 10 times daily, preferably 1 to 5 times daily, more preferably 1 to 3 times daily, and most preferably 1 time daily.

[00067] The dosages disclosed in this application refer generally to dosages suitable for humans. Dosage calculations can be determined by those of skilled in the art by evaluating body weight, surface area, metabolic rate, and species differences.

[00068] The term “subject” refers to any animal, including mammals and birds. Mammals include, but are not limited to, humans, dogs, cats, horses, cows, camels, elephants, lions, tigers, bears, seals, and rabbits. In preferred embodiments, the subjects comprise mammals that are not consumed as food, such as humans, cats, and dogs.

EXAMPLES

[00069] Example 1

[00070] The following is an exemplary orally administrable formulation:

Glucoraphanin-containing broccoli extract (about 12% glucoraphanin, w/w): 50 mg to 5 grams

Myrosinase-containing freeze-dried broccoli sprout powder: 25 mg to 500 mg

Ascorbic acid, 5 mg to 500 mg

Ursolic acid, 1 to 1000 mg

[00071] Example 2

[00072] A Hydrophobic Interaction Chromatographic (HILIC) method was developed, comprising the following conditions:

Column: Waters BEH Amide, 1.7-µm particle size; 2.1 mm x 100 mm

Mobile Phase: 20% 10mM Ammonium Acetate, pH 5.0; 80% Acetonitrile;

Separation mode: isocratic

Column Temperature: 70ºC

Flow Rate: 0.7 mL/min

The above conditions allow separation of five typical *Brassicaceae* glucosinolates, including the sulforaphane precursor, glucoraphanin.

[00073] Example 3.

[00074] Consumption of Glucoraphanin as a Function of the Ascorbic Acid Concentration.

[00075] About 250 mg of broccoli seed extract containing about 12% (w/w) glucoraphanin were subjected to hydrolysis by a fixed concentration of broccoli sprout-derived myrosinase in the presence of variable concentration of ascorbic acid, ranging from 0 to 600 µmoles/Liter. The reaction mixtures were thermostated at 38ºC; aliquots were withdrawn every 15 minutes for 60 minutes, and concentration of glucoraphanin determined chromatographically. The rate of glucoraphanin consumption was interpreted as the rate its conversion to sulforaphane. Graphical representation of glucoraphanin content reduction as a function of increasing ascorbic acid concentration results in a series of linear plots; the slopes of the linear regression lines reflect the rate of glucoraphanin consumption, in µmoles/minute. It is apparent that in the presence of 600 µmoles/Liter concentration of ascorbic acid, the reaction rate increased 13-fold relative to that which proceeded in the absence of modulatory effects of ascorbic acid.

Time, min	Content of Ascorbic Acid							
	0 µM	50 µM	125 µM	250 µM	250 µM Filtered	400 µM	600 µM	
0	93.36	93.36	93.36	93.36	93.36	93.36	93.36	µmoles GR
15	92.24	89.20	84.52	80.95	86.31	78.32	75.02	
30	90.71	84.24	75.92	69.06	79.44	62.78	55.66	
45	89.44	80.30	68.09	57.63	71.94	47.67	37.50	
60	87.79	76.36	59.41	45.76	65.18	33.15	22.09	
Slope	-0.09293	-0.28599	-0.56217	-0.79012	-0.47140	-1.00714	-1.20029	µmol/min
Intercept	93.496	93.271	93.123	93.053	93.386	93.270	92.734	µmol

[00076] Example 4

[00077] Equimolar Conversion of Glucoraphanin to Sulforaphane.

[00078] A two-part experiment was conducted to further elucidate the role of ascorbic acid in modulating myrosinase activity. All solutions were prepared in 20 mM Tris-buffered saline, at pH 7.5, previously identified as an optimal for myrosinase activity; each sample tube had 100 mg of freeze-dried broccoli powder accurately weighed in as a source of myrosinase. Experiment was conducted at 38 ºC for 2

hours, with sample aliquots removed in 30-minute increments, and both glucoraphanin and sulforaphane content assessed by HPLC. A strongly acidic "stop" solution was utilized to instantaneously inhibit further myrosinase activity in the removed aliquots. A control sample contained no ascorbic acid, and the enzymatic conversion proceeded unassisted by a co-factor.

[00079] PART 1. In the presence of the fixed concentration of ascorbic acid, 1 mmol/Liter, an increasing amount of broccoli seed extract (about 12% glucoraphanin, w/w) was added, ranging from 250 mg to 500 mg.

[00080] PART 2. While keeping the amount of broccoli seed extract fixed at 250 mg, the concentration of ascorbic acid was varied from 0.4 mmol/Liter to 3.8 mmol/Liter.

[00081] The table below presents glucoraphanin and sulforaphane expressed in µmoles. It is apparent that within the first 30 minutes in almost all the reaction mixtures, conversion of glucoraphanin to sulforaphane was complete. However, careful examination of the enzymatic conversion occurring in the control sample, without the stimulating effects of ascorbic acid, reveals an equimolar conversion of glucoraphanin to sulforaphane, i.e., the amount of glucoraphanin consumed results in the equivalent amount of sulforaphane produced.

	Glucoraphanin, µmoles					**Sulforaphane, µmoles**				
Time, min	0	30	60	90	120	0	30	60	90	120
GR 250 mg AA 0.0 mM	58.02	48.57	37.52	26.58	15.67	3.42	12.08	22.27	33.17	42.89
GR 250 mg AA 1.0 mM	40.07					21.51	61.95	60.20	60.04	58.25
GR 300 mg AA 1.0 mM	49.31					24.18	74.40	73.04	72.19	70.56
GR 350 mg AA 1.0 mM	61.41					25.00	84.92	84.02	83.19	80.02
GR 400 mg AA 1.0 mM	71.35	1.56				26.71	96.60	95.38	93.39	91.16
GR 500 mg AA 1.0 mM	89.41	1.01				33.52	120.16	118.45	116.45	112.34
GR 250 mg AA 0.4 mM	45.66					15.98	62.06	61.01	60.88	58.90
GR 250 mg AA 1.0 mM	35.24					26.49	62.19	60.62	60.41	59.10
GR 250 mg AA 2.0 mM	24.94					36.05	60.85	59.78	59.65	58.08
GR 250 mg AA 2.9 mM	22.24					38.20	59.95	59.34	58.77	56.99
GR 250 mg AA 3.8 mM	21.70					37.87	58.77	57.79	58.41	56.17

[00082] In the Part 2 of the experiment, the modulatory effect of the increasing concentration of ascorbic acid on the activity of myrosinase was assessed. An initial,

apparently linear, increase in myrosinase-promoted conversion of glucoraphanin to sulforaphane is observed to about 2 mmol/L of ascorbic acid concentration, followed subsequently by a considerable leveling off.

[00083] Finally, examination of sulforaphane yield of after 30 minutes within the PART 1 of the experiment, reveals that in the presence of 1 mmol/Liter of ascorbic acid, the fixed amount of myrosinase contained in 100 mg of freeze-dried broccoli sprout powder is capable of generating at least 200 µmoles of sulforaphane, in a predictably linear fashion. FIG. 1, 2, 3, and 4 demonstrate the results of this study.

[00084] Example 5.

[00085] Conversion of Glucoraphanin to Sulforaphane in the Presence of Simulated Intestinal Fluid.

[00086] Simulated Intestinal Fluid (SIF) powder, a commercially supplied concentrate closely approximating the human intestinal content in terms of composition, pH and ionic strength, was used. The experiment utilized a USP Dissolution Apparatus 2 (paddles), where into six dissolution vessels 500 mL of Simulated Intestinal Fluid was dispensed, along with 150 mg of freeze-dried broccoli sprout powder as a source of myrosinase. In vessels 1-4, the concentration of ascorbic acid was varied from 0.25 to 1.00 mmol/Liter; in vessel 5, in addition to 1 mmol/Liter ascorbic acid, 3.125 g of pancreatin (8x USP) was suspended; in vessel 6, in addition to 1 mmol/Liter ascorbic acid, and 3.125 g of pancreatin (8x USP), a doubled amount of freeze-dried broccoli sprout powder (300 mg) was added. After vessels were brought to 38 ºC, 250 mg of glucoraphanin-rich (8%, w/w) broccoli seed extract was added to each, and the resulting suspensions were stirred at 75 RPM for 2 hours. Aliquots were withdrawn every 15 minutes, and assayed for sulforaphane. FIG. 4 shows direct correlation between larger yield of sulforaphane and higher concentrations of ascorbic acid, especially at the earlier stages of the experiment.

[00087] Example 6

[00088] The following study was conducted to determine the effect of the combination of sulforaphane and ursolic acid on matrix metalloproteinase-9 (MMP-9) gene expression. The MMP-9 gene encodes the protein that is known to degrade the extracellular matrix, which is the support system of cells. In prostate, colon, brain, and other cancers, MMP9 expression is increased, and this increase has been correlated with metastasis. A decrease in MMP-9 is favorable for these cancers.

[00089] In the study, the prostate cancer cell line PC3 was treated with DMSO (vehicle control), sulforaphane (SFN), ursolic acid (UA), or the combination of sulforaphane and ursolic acid, for 24 hours. Gene expression of MMP-9 was analyzed via quantitative RT-PCR. FIG. 5a and 5b and FIG. C show the results of the study.

[00090] In Part 1 of the study, the prostate cancer cell line PC3 was treated with one of the following: (i) DMSO (vehicle control), (ii) 1 μM SFN, (iii) 5 μg/mL UA, or (iv) the combination of 1 μM SFN and 5 μg/mL UA. Two separate treatments were conducted. FIG. 5a shows the results of the first treatment. The following reductions in gene expression of MMP-9 were observed: (i) a 5% decrease with 1 μM SFN alone, (ii) a 31% decrease with 5 μg/mL UA alone, and (iii) a 40% decrease with the combination of 1 μM SFN and 5 μg/mL UA. FIG. 5b shows the results of the second treatment. The following reductions in gene expression of MMP-9 were observed: (i) a 13% decrease with 1 μM SFN alone, (ii) a 28% decrease with 5 μg/mL UA alone, and (iii) a 45% decrease with the combination of 1 μM SFN and 5 μg/mL UA. This demonstrates that the combination of 1 μM SFN and 5 μg/mL UA has a synergistic (greater than the additive) effect on gene expression of MMP-9.

[00091] In Part 2 of the study, the prostate cancer cell line PC3 was treated with one of the following: (i) DMSO (vehicle control), (ii) 5 μM SFN, (iii) 10 μg/mL UA, or (iv) the combination of 5 μM SFN and 10 μg/mL UA. FIG. 6 shows the results of this study. The following reductions in gene expression of MMP-9 were observed: (i) a 39% decrease with 5 μM SFN alone, (ii) a 15% decrease with 10 μg/mL UA alone, and (iii) a 59% decrease with the combination of 5 μM SFN and 10 μg/mL UA. This demonstrates that the combination of 5 μM SFN and 10 μg/mL UA has a synergistic (greater than the additive) effect on gene expression of MMP-9.

[00092] Example 7

[00093] A subject presents with prostate cancer and is suffering from symptoms including trouble urinating, decreased force in the stream of urine, blood in the urine, and discomfort. He is administered a tablet containing glucoraphanin, myrosinase, ascorbic acid, and ursolic acid. The tablet is an enteric coated formulation which releases the contents in the small intestine. After one month of daily administration of the tablet, the subject experiences improvement in surrogate biomarkers including MMP-9 which correlates with improved symptoms.

WHAT IS CLAIMED:

Claim 1. An orally administrable composition comprising:

a sulforaphane precursor;

an enzyme capable of converting the sulforaphane precursor to sulforaphane;

an enzyme potentiator; and

ursolic acid, a salt, ester, amide or a derivative thereof.

Claim 2. The orally administrable composition of claim 1, wherein the sulforaphane precursor comprises glucoraphanin.

Claim 3. The orally administrable composition of claim 1, wherein the enzyme capable of converting the sulforaphane precursor to sulforaphane comprises myrosinase.

Claim 4. The orally administrable composition of claim 1, wherein the enzyme potentiator comprises ascorbic acid.

Claim 5. The orally administrable composition of claim 1, wherein the composition comprises an enteric-coated dosage form.

Claim 6. The orally administrable composition of claim 1, further comprising one or more additional components selected from the group consisting of: quercetin, an aminosugar, a glycosaminoglycan, avocado/soybean unsaponifiables, a vitamin, coffee fruit, magnesium, silymarin, proanthocyanidins, alpha- and beta-glucans, curcumin, phytosterols, and phytostanols.

Claim 7. The orally administrable composition of claim 1, comprising glucoraphanin, myrosinase, ascorbic acid, and ursolic acid.

Claim 8. The orally administrable composition of claim 1, wherein the composition comprises broccoli extract or powder.

Claim 9. A method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and reducing secondary recurrences of

prostate, colon, or bladder cancer, comprising administering to a subject in need thereof a sulforaphane precursor; an enzyme capable of converting the sulforaphane precursor to sulforaphane; an enzyme potentiator; and ursolic acid, a salt, ester, amide or a derivative thereof.

Claim 10. The method of claim 9, wherein the sulforaphane precursor comprises glucoraphanin.

Claim 11. The method of claim 9, wherein the enzyme capable of converting the sulforaphane precursor to sulforaphane comprises myrosinase.

Claim 12. The method of claim 9, wherein the enzyme potentiator comprises ascorbic acid.

Claim 13. The method of claim 9, comprising administration of glucoraphanin, myrosinase, ascorbic acid, and ursolic acid.

Claim 14. The method of claim 11, comprising administering an enteric-coated dosage form.

FIG. 1

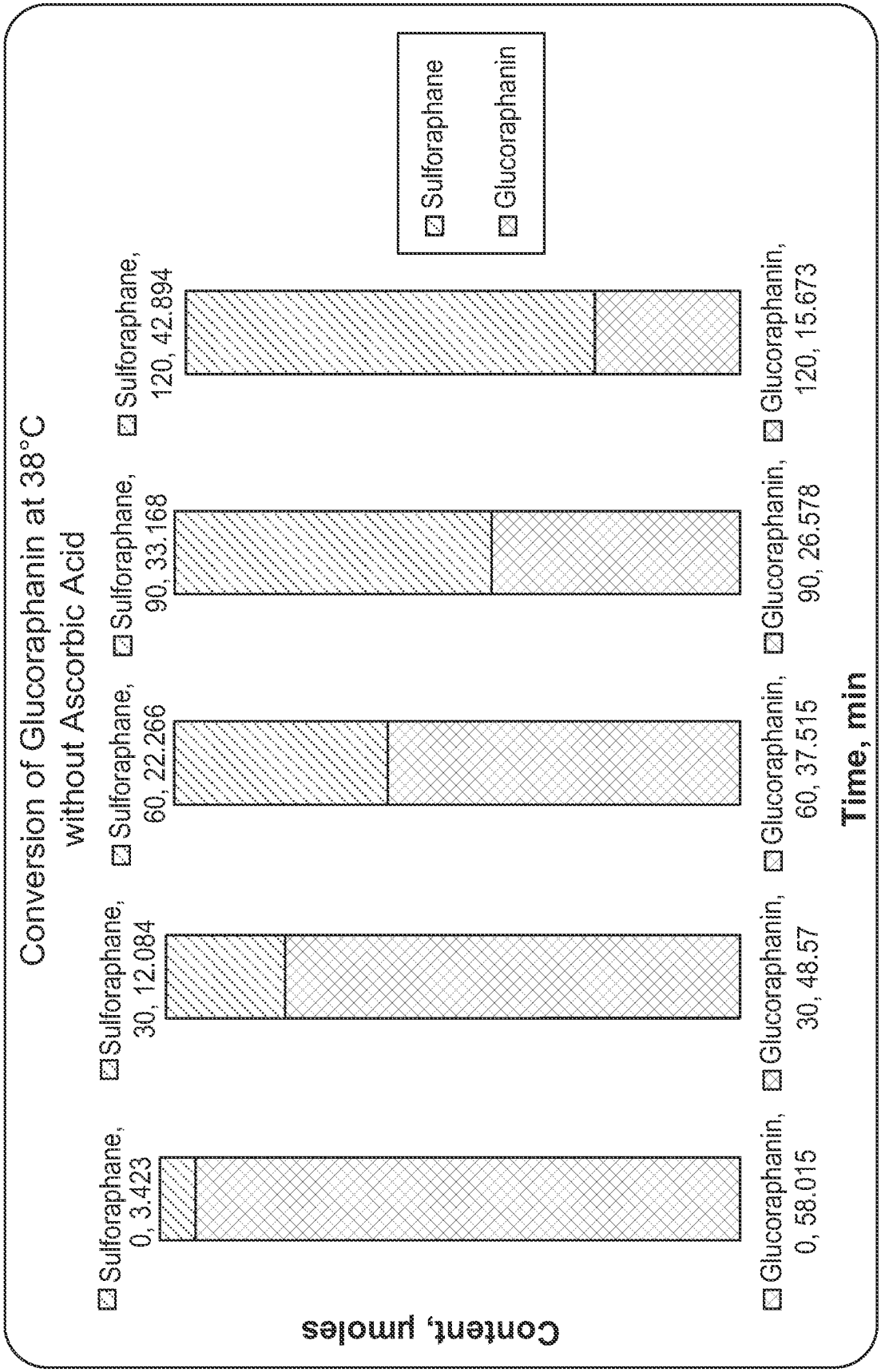

Conversion of Glucoraphanin at 38°C without Ascorbic Acid
Content, μmoles
Time, min
Sulforaphane
Glucoraphanin
Sulforaphane, 0, 3.423
Glucoraphanin, 0, 58.015
Sulforaphane, 30, 12.084
Glucoraphanin, 30, 48.57
Sulforaphane, 60, 22.266
Glucoraphanin, 60, 37.515
Sulforaphane, 90, 33.168
Glucoraphanin, 90, 26.578
Sulforaphane, 120, 42.894
Glucoraphanin, 120, 15.673

FIG. 2

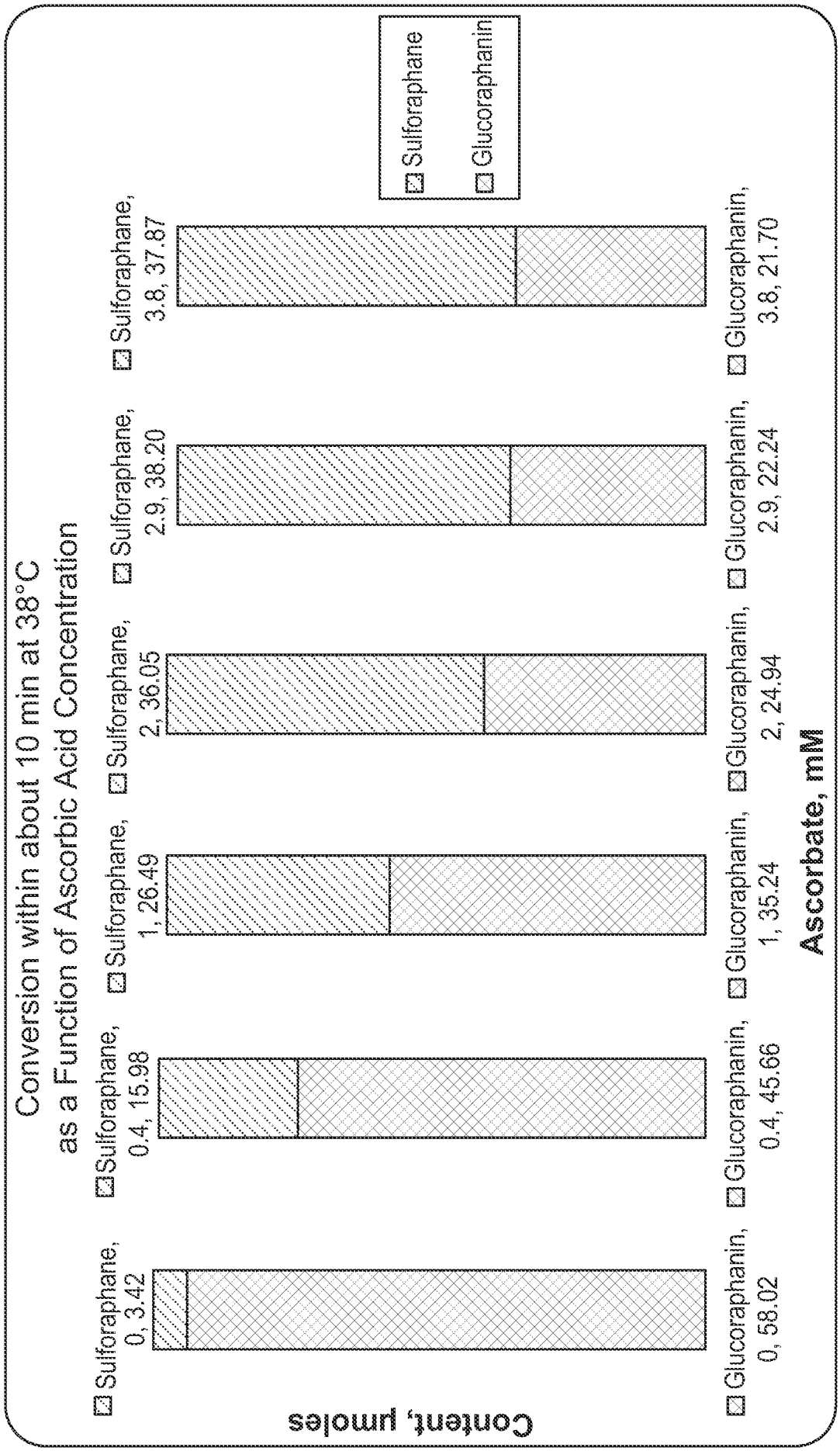

Conversion within about 10 min at 38°C
as a Function of Ascorbic Acid Concentration
Sulforaphane
Glucoraphanin
Content, µmoles
Ascorbate, mM
Sulforaphane, 0, 3.42
Glucoraphanin, 0, 58.02
Sulforaphane, 0.4, 15.98
Glucoraphanin, 0.4, 45.66
Sulforaphane, 1, 26.49
Glucoraphanin, 1, 35.24
Sulforaphane, 2, 36.05
Glucoraphanin, 2, 24.94
Sulforaphane, 2.9, 38.20
Glucoraphanin, 2.9, 22.24
Sulforaphane, 3.8, 37.87
Glucoraphanin, 3.8, 21.70

FIG. 3

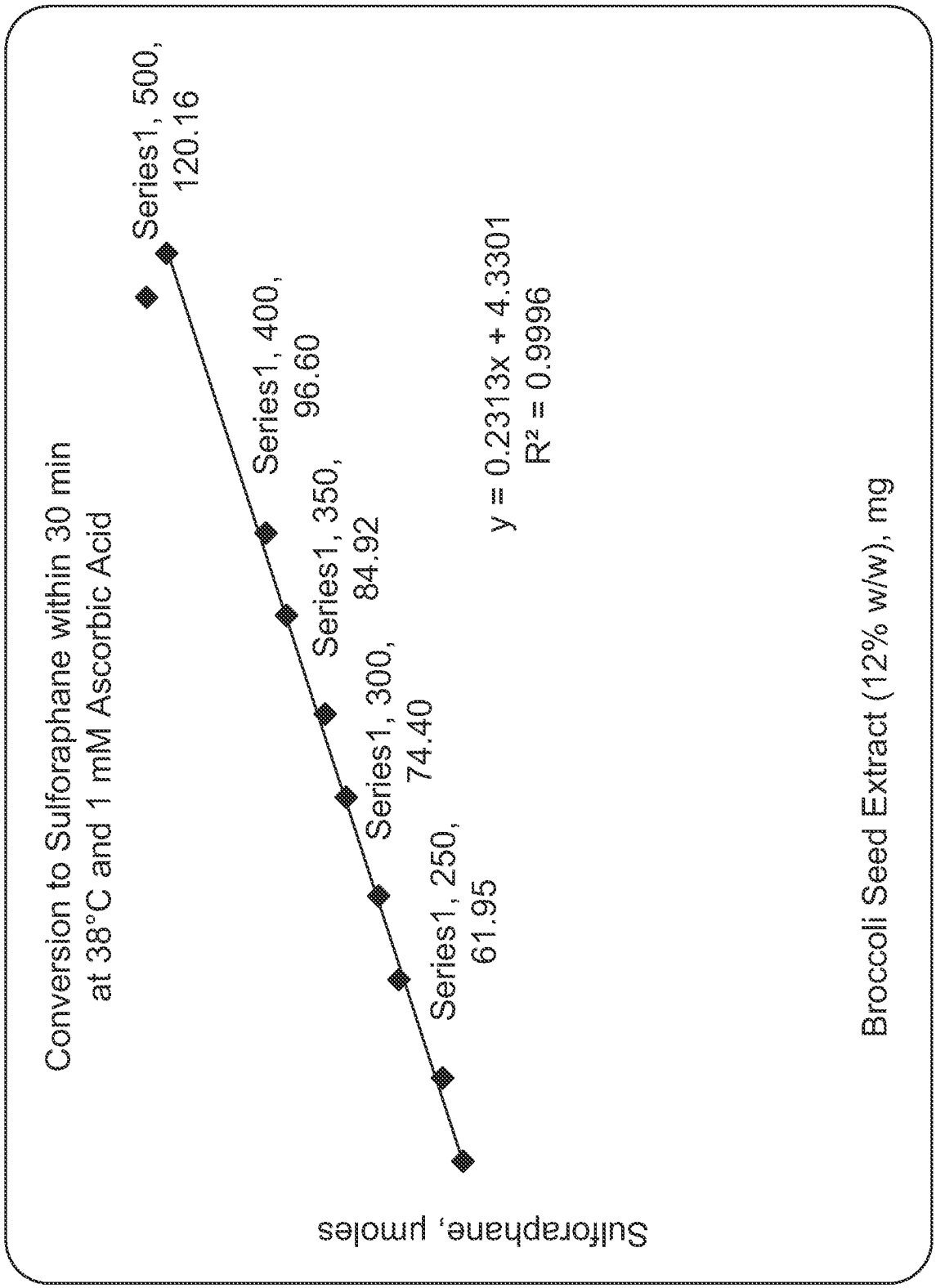

Conversion to Sulforaphane within 30 min
at 38°C and 1 mM Ascorbic Acid
Series1, 500, 120.16
Series1, 400, 96.60
Series1, 350, 84.92
Series1, 300, 74.40
Series1, 250, 61.95
y = 0.2313x + 4.3301
R² = 0.9996
Sulforaphane, µmoles
Broccoli Seed Extract (12% w/w), mg

FIG. 4

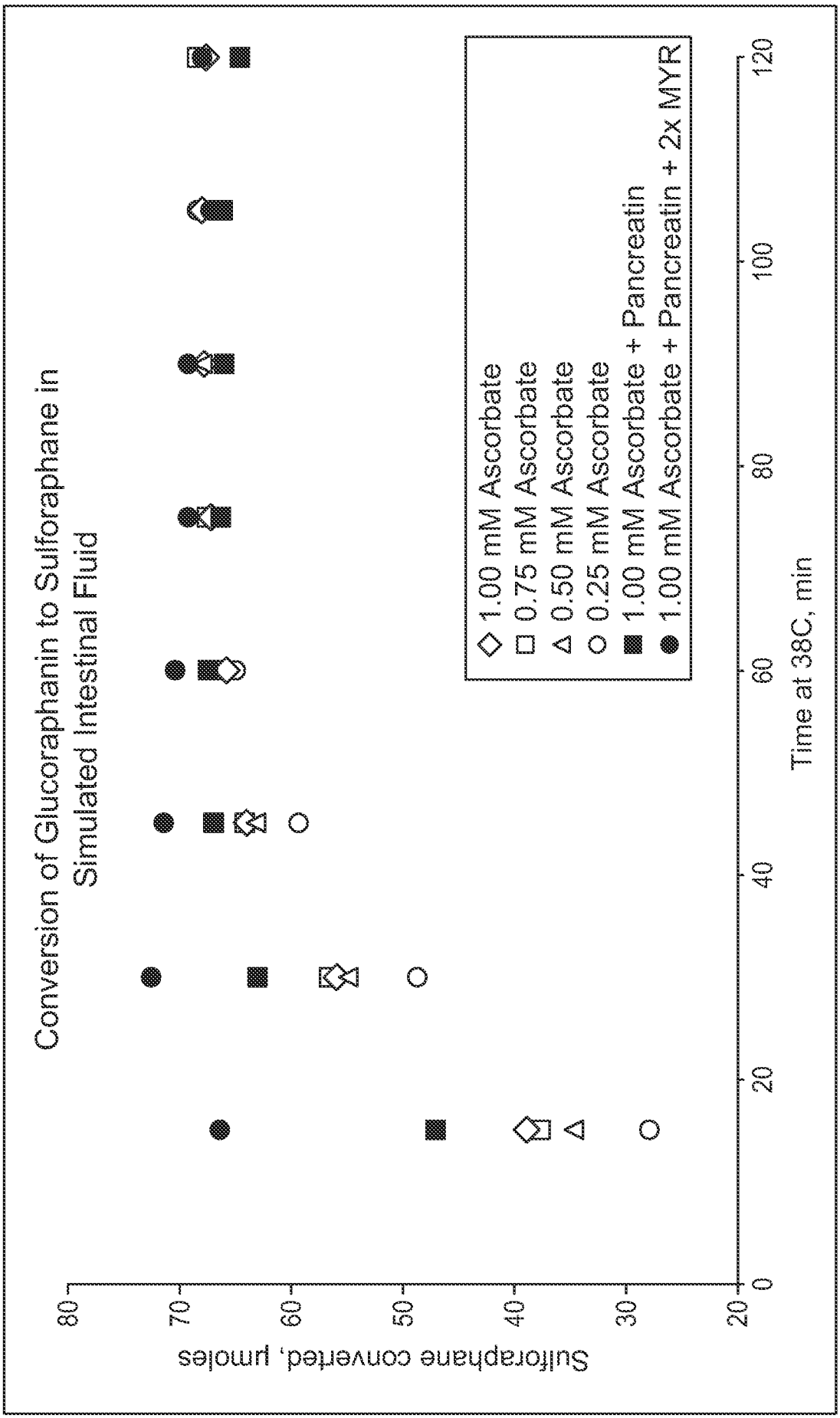

Conversion of Glucoraphanin to Sulforaphane in Simulated Intestinal Fluid
Sulforaphane converted, µmoles
Time at 38C, min
1.00 mM Ascorbate
0.75 mM Ascorbate
0.50 mM Ascorbate
0.25 mM Ascorbate
1.00 mM Ascorbate + Pancreatin
1.00 mM Ascorbate + Pancreatin + 2x MYR
80
70
60
50
40
30
20
0
20
40
60
80
100
120

FIG. 5a

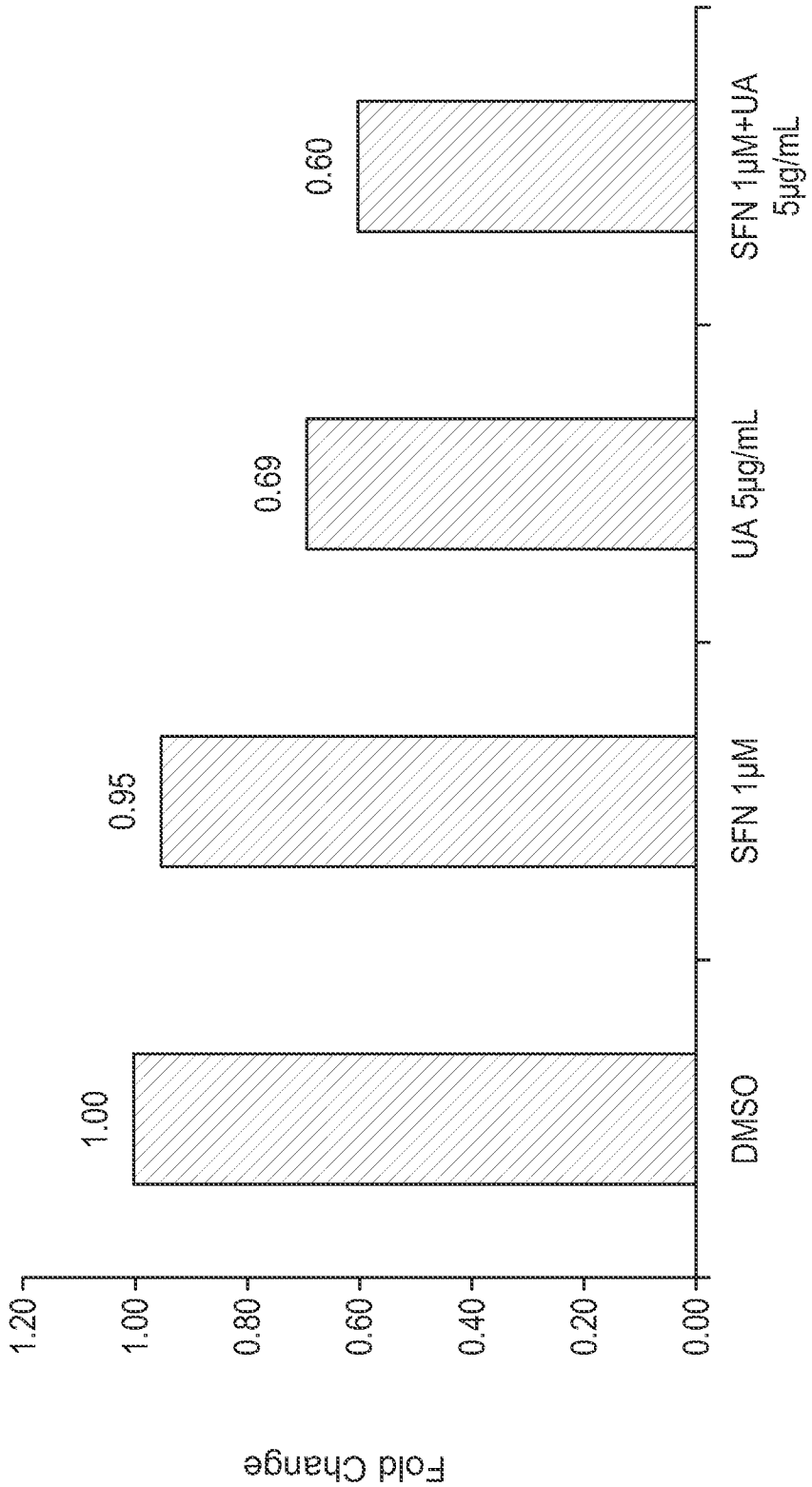

Fold Change
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.00
0.95
0.69
0.60
DMSO
SFN 1μM
UA 5μg/mL
SFN 1μM+UA 5μg/mL

FIG. 5b

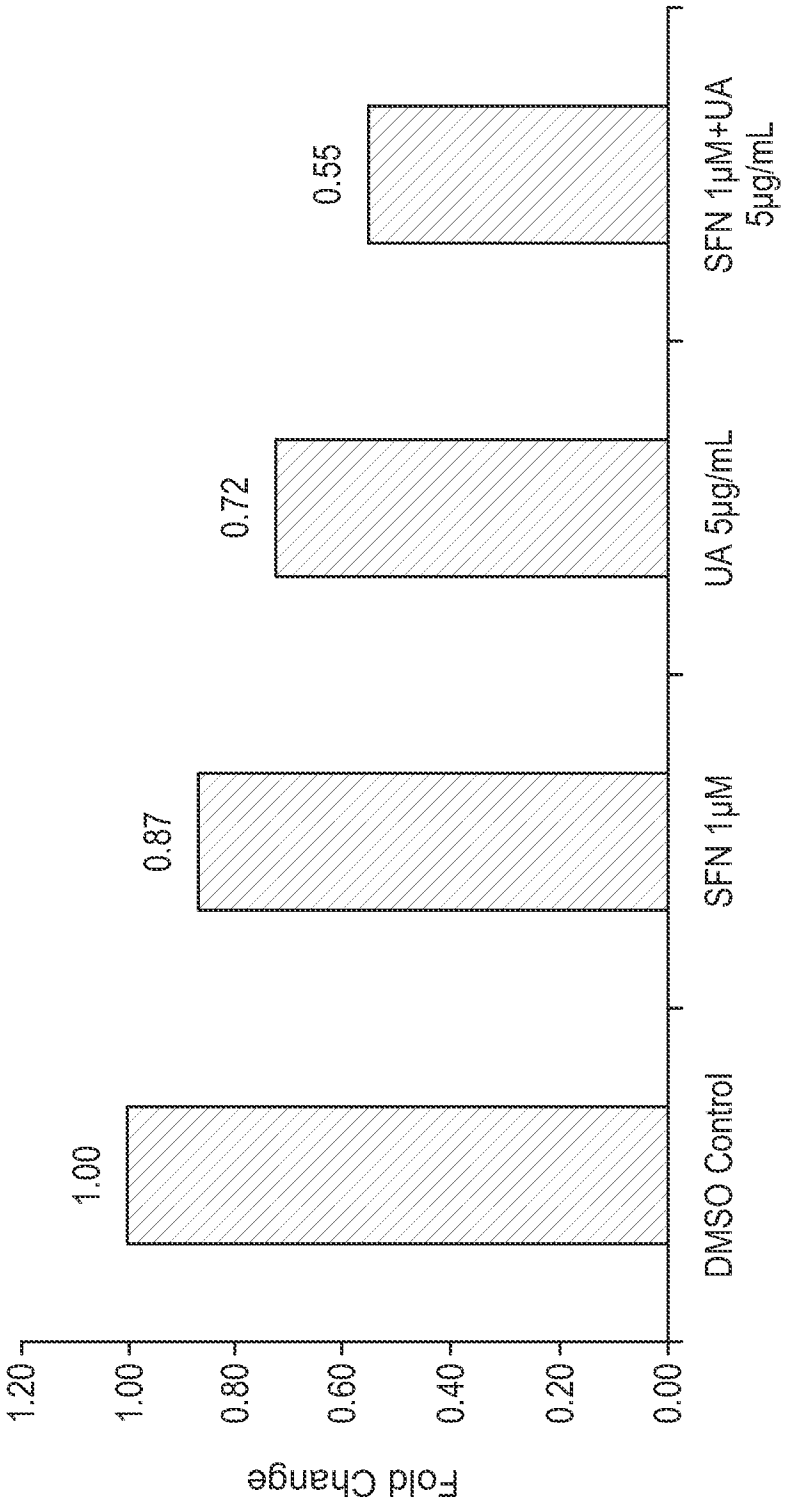
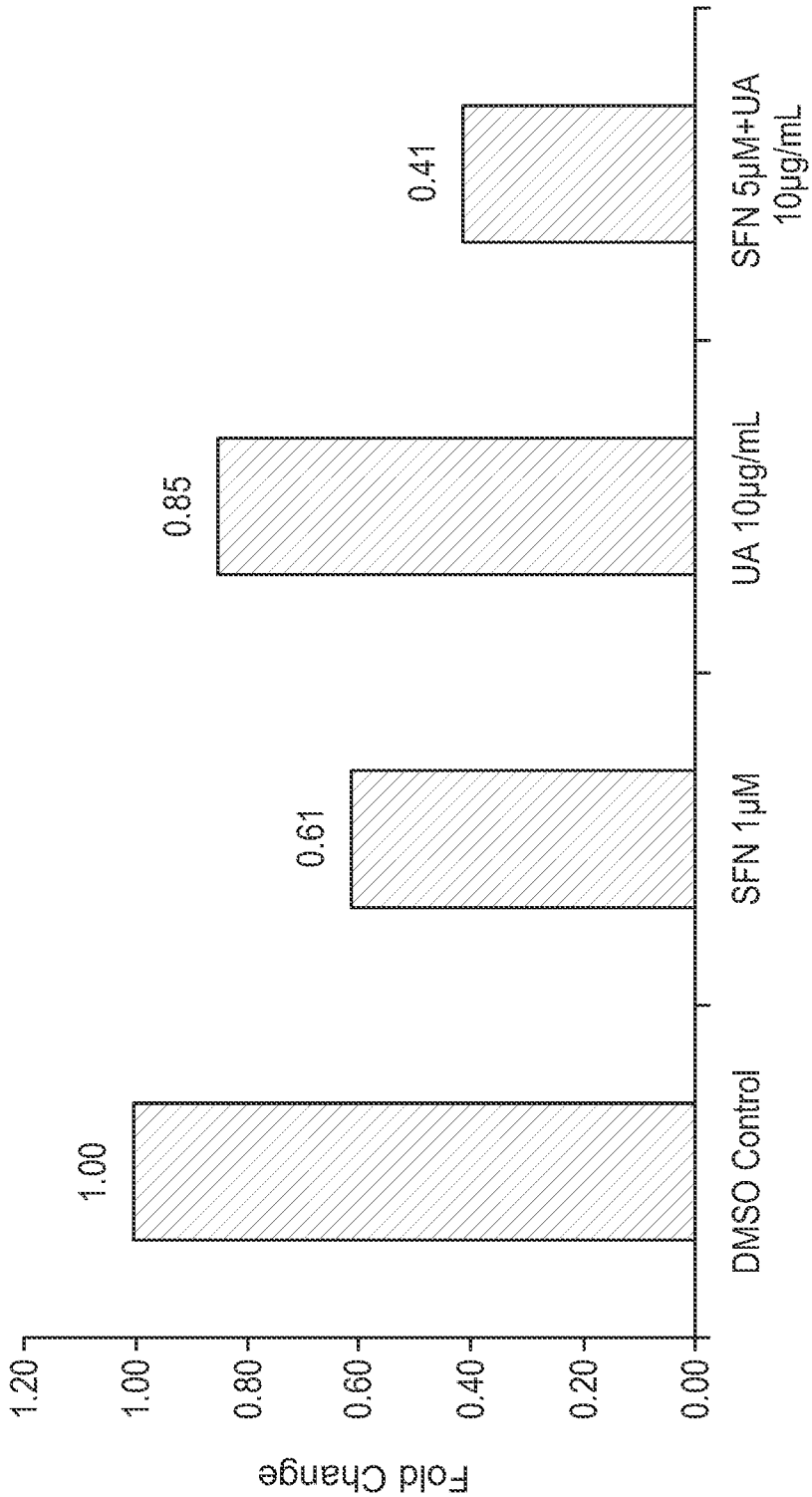

Fold Change
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.00
0.87
0.72
0.55
DMSO Control
SFN 1μM
UA 5μg/mL
SFN 1μM+UA 5μg/mL

FIG. 6


Fold Change
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.00
0.61
0.85
0.41
DMSO Control
SFN 1μM
UA 10μg/mL
SFN 5μM+UA 10μg/mL