(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 March 2017 (09.03.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/041054 A1

(51) **International Patent Classification**:
A61P 25/00 (2006.01) ***A61K 31/4439*** (2006.01)
A61K 31/426 (2006.01)

(21) **International Application Number**:
PCT/US2016/050292

(22) **International Filing Date**:
2 September 2016 (02.09.2016)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
62/214,175 3 September 2015 (03.09.2015) US
62/355,810 28 June 2016 (28.06.2016) US

(71) **Applicant**: **PATHWAYS BIOSCIENCE, LLC** [US/US]; 12635 E. Montview Blvd., Suite 130, Aurora, CO 80045 (US).

(72) **Inventors**: **HYBERTSON, Brooks, Michael**; 4900 Fourmile Canyon Dr., Boulder, CO 80302 (US). **MCCORD, Joe, Milton**; 8156 Woodslanding Trail, West Palm Beach, FL 33411 (US).

(74) **Agents**: **CHEN, Peter** et al.; Lathrop & Gage LLP, 4845 Pearl East Circle, Suite 201, Boulder, CO 80301 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2017/041054 A1

(54) **Title**: COMPOSITIONS FOR IMPROVED NRF2 ACTIVATION AND METHODS OF THEIR USE

(57) **Abstract**: Disclosed here are compositions and methods for preventing or treating certain health conditions associated with inflammation or oxidative stress. These compositions are prepared from ingredients containing phytochemicals that activate the Nrf2 pathways. Synergistic effects of the different phytochemicals are also disclosed.

COMPOSITIONS FOR IMPROVED NRF2 ACTIVATION AND METHODS OF THEIR USE

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Patent application 62/214,175 filed September 3, 2015, and U.S. Patent application 62/355,810 filed June 28, 2016, the entire content of which is hereby incorporated by reference into this application.

BACKGROUND

I. Field of the Invention

[0002] The present disclosure relates to methods and compositions for preventing or treating certain health conditions. More particularly, the present disclosure relates to compositions and methods for preventing or treating certain health conditions associated with inflammation and/or oxidative stress.

II. Description of the Related Art

[0003] Nuclear factor-erythroid 2 related factor 2 (Nrf2) is a transcription factor that is regulated by Kelch-like ECH-Associated Protein 1 (Keap1). Nrf2 regulates gene expression of a wide variety of cytoprotective phase II detoxification enzymes and antioxidant enzymes through an enhancer sequence known as the antioxidant-responsive element (ARE) (Maher and Yamamoto 2010, Satoh, Moriguchi et al. 2010). Relevant to oxidative stress, the ARE is a promoter element found in many antioxidant enzymes, including superoxide dismutase (SOD), peroxiredoxins, thioredoxins, catalase, glutathione peroxidase, and heme oxygenase-1 (HO-1). Nrf2 plays a pivotal role in the ARE-driven cellular defense system against oxidative stress. *See*, Kensler, Wakabayashi et al. 2010; Hybertson and Gao 2014, Bocci and Valacchi 2015, Huang, Li et al. 2015, Johnson and Johnson 2015, Moon and Giaccia 2015, Petiwala and Johnson 2015, Sekhar and Freeman 2015, Suzuki and Yamamoto 2015.

SUMMARY

[0004] The presently disclosed instrumentalities advance the art by providing combinations of agents that activate the Nrf2 cell signaling pathway. In one

embodiment, the combinations of agents may activate the Nrf2 pathway more effectively than individual agents. . In another embodiment, the combinations of agents may activate the Nrf2 pathway synergistically.

[0005] In one embodiment, combinations of more than one ingredients are disclosed here. In one aspect, each ingredient may contain one or more phytochemicals. In another aspect, these phytochemicals may be found in rosemary (Rosmarinus officinalis), ginger (Zingiber officinale), luteolin (from Sophora Japonica), milk thistle (Silybum marianum), and bacopa (Bacopa monnieri). In another aspect, the phytochemicals components are carnosol, shogaol, luteolin, silymarin, and bacosides, which may be found in rosemary, ginger, luteolin, milk thistle, and bacopa, respectively. In another aspect, , the disclosed compositions induce ARE-regulated antioxidant genes by the Nrf2-dependent pathway.

[0006] In another embodiment, specific combinations of rosemary, ashwagandha, and luteolin (referred to herein as PB125), specific combinations of rosemary, ginger, luteolin, and silymarin (referred to herein as PB127), and specific combinations of rosemary, ginger, luteolin, silymarin, and bacopa (referred to herein as PB129) are disclosed. In another embodiment, the combination of these agents may result in a synergistic Nrf2 activation, greater than simply the sum of their individual Nrf2 activation contributions. The active agents or combinations of the agents may be candidates for possible drug development. *See, e.g.*, Koehn and Carter 2005, Lee 2010.

[0007] In another embodiment, the disclosed compositions may contain rosemary (carnosol), ginger (6-shogaol and 6-gingerol), ashwagandha (withaferin A), milk thistle (silymarin), bacopa monnieri (bacosides) and luteolin.

[0008] In one aspect, the compositions may be administered orally, for example in the form of a tablet, capsule, softgel, syrup, aqueous solution or suspension, alcohol-extract, or powder. In another aspect, the synergistic compositions may be administered in the form of aerosol, for example to the lungs in the form of a fine aerosol mist or powder which is inhaled and partially deposited within the lung airways. In another aspect, the disclosed compositions may be administered by local administration, for example, by applying to the skin in the form

of a lotion, gel, ointment, aqueous spray, or within a bandage applied to the skin or to a wound.

[0009] In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), and luteolin (specified at 95-98% luteolin), in the mass ratio of 10:5:1, respectively. This formula is also referred to as PB123 in this disclosure.

[0010] In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ashwagandha extract (specified at 1-3% withaferin A), and luteolin (specified at 95-98% luteolin), in the mass ratio of 30:10:4, respectively. This formula is also referred to as PB125 in this disclosure.

[0011] In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), and milk thistle extract (specified at 50-90% silymarin), in the mass ratio of 10:5:1:30, respectively. This formula is also referred to as PB127 in this disclosure.

[0012] In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), milk thistle extract (specified at 50-90% silymarin), and bacopa monnieri extract (specified at 10-60% bacosides) in the mass ratio of 10:5:1:30:48, respectively. This formula is also referred to as PB129 in this disclosure.

[0013] In another embodiment, the disclosed composition may contain a combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), and bacopa monnieri extract (specified at 10-60% bacosides) in the mass ratio of 10:5:1:48, respectively. This formula is also referred to as PB131 in this disclosure.

[0014] In another embodiment, PB123 may be administered at 10 to 1000 mg per day as an oral administration to a human. For example, it may be administered

as a pill, softgel, or capsule to induce Nrf2 activation, and/or to reduce inflammation and oxidative stress, and/or to improve overall health and wellness.

[0015] In another embodiment, PB123 may be administered at 10 to 1000 mg per day as an oral administration to a human to improve protein homeostasis, and/or to prevent aging-related problems associated with protein homeostasis and/or autophagy in humans.

[0016] In another embodiment, PB125 or PB127 or PB129 or PB131 may be administered at 10 to 1000 mg per day as an oral administration to a human. For example, it may be administered as a pill, softgel, or capsule to induce Nrf2 activation, and/or to reduce inflammation and oxidative stress, and/or to improve overall health and wellness.

[0017] In another embodiment, PB125 or PB127 or PB129 or PB131 may be administered at 10 to 1000 mg per day as an oral administration to a human to improve protein homeostasis, and/or to prevent aging-related problems associated with protein homeostasis and/or autophagy in humans.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1 shows the Nrf2 activation pathways and control points.

[0019] Figure 2 shows the "Shutdown Pathway"-Fyn-dependent deactivation of nuclear Nrf2.

[0020] Figure 3 shows the "Positive Feedback Loop"-Keap1 degradation by Nrf2-induced gene products.

[0021] Figure 4 shows Nrf2 activation induced by PB123, PB125, PB127, PB129, and PB131 in a transfected breast cancer cell line.

[0022] Figure 5 shows Nrf2 activation induced by PB123, PB125, PB127, PB129, and PB131 in a transfected liver cancer cell line.

[0023] Figure 6A-6C shows the synergistic effect of Nrf2 activation induced by PB129 in HepG2 (human liver, Fig. 6A), MCF7 (human breast, Fig. 6B), and A172 (human brain, Fig. 6C) cancer cell lines.

[0024] Figure 7A-7C shows the synergistic effect of Nrf2 activation induced by PB127 in HepG2 (human liver, Fig. 7A), MCF7 (human breast, Fig. 7B), and A172 (human brain, Fig. 7C) cancer cell lines.

[0025] Figure 8 shows increase of Mouse Liver HMOX1 gene expression in vivo.

[0026] Figure 9 shows Liver Catalase Activity Induced by PB125 in diet.

[0027] Figure 10 shows overlay of relative light units (RLU) observed with added luciferin after ARE-driven luciferase gene expression was induced by treatment with PB125 in stably transfected HepG2 (human liver), AREc32 (human breast), MCF7 (human breast), A549 (human lung), 293T (human kidney), and A172 (human brain) cancer cell lines. Strong Nrf2 activation was observed in liver, kidney, and breast cell lines by 5, 10, 15, 20, and 25 micrograms of PB125 per mL of culture solution.

[0028] Figure 11 shows that PB125 decreases LPS-induced expression of inflammatory genes.

[0029] Figure 12 shows that PB125 decreases LPS-induced expression of IL-6.

[0030] Figure 13 shows higher GCLM gene expression as a result of PB125 administration.

DETAILED DESCRIPTION

[0031] The Nrf2/ARE pathway has been implicated in the control of oxidative stress (Eggler, Gay et al. 2008, Cho and Kleeberger 2010, Huang, Li et al. 2015, Johnson and Johnson 2015). Certain agents and combinations of such agents (e.g., PB125) that target the Nrf2/ARE pathway may have beneficial effects on cellular function and survival. In one embodiment, these agents and combinations thereof may alleviate inflammatory responses and oxidative stress, and may have beneficial effects on health and wellness.

[0032] Prior studies have failed to demonstrate the therapeutic potential of direct antioxidant vitamins or supplements such as vitamins C and E, carotenoids, N-acetylcysteine, and other compounds that react stoichiometrically with reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. Here, an improved antioxidant defenses is demonstrated by using Nrf2 activating combinations (Koehn 2006, Eggler, Gay et al. 2008, Boutten, Goven et al. 2010, Cho and Kleeberger 2010).

[0033] In the present disclosure, a multiplicity of agents were combined in a novel way, i.e., by acting at different control points in the Nrf2 activation pathway. Figure 1 shows Nrf2 activation pathways and control points A, B, C, D, and E at which low concentrations of agents that act at those control points work together to effect desired Nrf2-dependent gene expression by combinations such as PB125, PB127, and PB129. In the basal state Nrf2 is sequestered and kept inactive by Kelch-like ECH-associated protein 1 (Keap1), which targets Nrf2 for polyubiquitination and degradation by the proteasome. A. Nrf2 activation involves oxidation of specific thiol residues of Keap1, causing it to Nrf2 to be released from Keap1. B. Nrf2 phosphorylation may play a role in targeting it for nuclear import. C. Nrf2 translocation into the nucleus enables Nrf2 to bind promotors containing the Antioxidant Response Element (ARE), initiating transcription of cytoprotective programming. D. Inactive cytosolic Fyn may be phosphorylated by GSK3β, and this now active p-Fyn translocates to the nucleus, where it can phosphorylate Nrf2 at a second site resulting in nuclear export and degradation. E. A "positive feedback loop" involves SESN2, SQSTM1 and ULK1, gene products induced by Nrf2. SESN2, SQSTM1 and ULK1collaborate to activate autophagy of Keap1, liberating more Nrf2, which induces more of these gene products, tending to maintain Nrf2 activation once this positive feedback loop has been triggered.

[0034] Also in the present disclosure, the combinations of agents gave surprisingly high Nrf2 activation levels compared to what would be predicted based on the prior art and also based on concurrent experiments examining the Nrf2 activating properties of each agent alone and what would be predicted based on adding them together. The Nrf2 activation by the combination of the agents show a synergistic effect. See, e.g., Figures 6 and 7.

[0035] An embodiment of the present disclosure comprises combinations of dietary agents - such as in the PB125, PB127, and PB129 combinations - that act on Nrf2 activation by engagement of different, specific control points so that the combination of agents that synergistically activate the Nrf2 pathway. Thus the new combinations of agents that act on different control points of the Nrf2 signaling pathway to increase expression of Nrf2-dependent genes are novel.

[0036] By way of example, a number of embodiments of the present disclosure are listed below:

[0037] Item 1. A composition comprising two or more phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, said one or more phytochemicals being present in the composition in an amount effective to activate the Nrf2 (Nuclear factor-erythroid 2 related factor 2) pathway.

[0038] Item 2. The composition of Item 1, wherein the two or more phytochemicals exert their effects on at least two different control points of the Nrf2 activation pathway when administered to a mammal, said control points being selected from the group consisting of control points A, B, C, D and E. In one embodiment, at least one of the phytochemicals exerts its effects on one control point, while at least another phytochemical exerts its effects on a different control point of the Nrf2 activation pathway as depicted in Fig. 1.

[0039] Item 3. The composition of any of the preceding Items, wherein the two or more phytochemicals have a synergistic effect on Nrf2 activation when administered to a mammal.

[0040] Item 4. The composition of any of the preceding Items, wherein the composition comprises at least two ingredients selected from the group consisting of rosemary, ginger, luteolin, and ashwagandha.

[0041] Item 5. The composition of any of the preceding Items, wherein the composition also comprises one or more phytochemicals selected from the group consisting of milk thistle and bacopa.

[0042] Item 6. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, and luteolin, said rosemary extract being specified at 5-10% carnosol, said ginger extract being specified at 10-20% 6-shogaol, said luteolin being specified at 95-99% luteolin, wherein the ratio between rosemary extract, ginger extract, and luteolin in the composition is approximately 10:5:1 (w/w).

[0043] Item 7. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, said rosemary extract being specified at 5-10% carnosol, said ashwagandha extract being specified at 1-3% withaferin A, said luteolin being specified at 95-99% luteolin,

wherein the ratio between said rosemary extract, ashwagandha extract, and luteolin in the composition is approximately 30:10:4 (w/w).

[0044] Item 8. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, and luteolin, and wherein the ratio between said rosemary extract, ginger extract, and luteolin is approximately 10:5:1 (w/w).

[0045] Item 9. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, the ratio between said rosemary extract, ashwagandha extract, and luteolin being approximately 30:10:4 (w/w).

[0046] Item 10. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, luteolin and milk thistle extract, the ratio between said rosemary extract, ginger extract, luteolin and milk thistle extract being approximately 10:5:1:30 (w/w).

[0047] Item 11. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, luteolin, milk thistle extract, and bacopa monnieri extract, the ratio between said rosemary extract, ginger extract, luteolin, milk thistle extract and bacopa monnieri extract being approximately 10:5:1:30:48 (w/w).

[0048] Item 12. The composition of any of the preceding Items, wherein the composition comprises rosemary extract, ginger extract, luteolin, and bacopa monnieri extract, the ratio between said rosemary extract, ginger extract, luteolin, and bacopa monnieri extract being approximately 10:5:1:48 (w/w).

[0049] Item 13. The composition of any of the preceding Items, wherein the composition is used to prevent and/or treat a disease or a condition selected from the group consisting of oxidative stress, detoxification, inflammation, cancer, or a related disease or condition.

[0050] Item 14. The composition of any of the preceding Items, wherein the composition is used as a nutritional supplement.

[0051] Item 15. The composition of any of the preceding Items, wherein the composition is in the form of a tablet, a capsule, a soft gel, a liquid, a lotion, a gel, a powder, an ointment, or an aerosol.

[0052] Item 16. A method of treating and/or preventing a disease or condition, comprising the step of administering a composition to a mammal, the composition comprising one or more phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, said one or more phytochemicals being present in the composition in an amount effective to activate the Nrf2 (NF-E2 related factor 2) pathway.

[0053] Item 17. The method of any of the preceding Items, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, wherein the rosemary extract is specified at 5-10% carnosol, the ashwagandha extract is specified at 1-3% withaferin A, and the luteolin is specified at 95-99% luteolin, the ratio between said rosemary extract, ashwagandha extract, and luteolin being approximately 30:10:4 (w/w).

[0054] Item 18. The method of Item 17, wherein the composition comprises rosemary extract, ginger extract, and luteolin, wherein the rosemary extract is specified at 5-10% carnosol, the ginger extract is specified at 10-20% 6-shogaol, and the luteolin is specified at 95-99% luteolin, the ratio between said rosemary extract, ginger extract, and luteolin being approximately 10:5:1 (w/w).

[0055] Item 19. The method of any of Items 17-18, wherein the composition is administered orally to a human at 10-1000 mg per day.

[0056] Item 20. The method of any of Items 17-19, wherein the composition comprises at least two phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, wherein the at least two phytochemicals exert their effects on at least two different control points of the Nrf2 activation pathway, said control points being selected from the group consisting of control points A, B, C, D and E.

[0057] It will be readily apparent to those skilled in the art that the compositions and methods described herein may be modified and substitutions may be made using suitable equivalents without departing from the scope of the embodiments disclosed herein. Having now described certain embodiments in detail,

the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting.

Examples

Example 1 Effects on Nrf2 action pathways

[0058] The different agents, PB123, PB125, PB127, PB129, and PB131, each exhibit strong, potent Nrf2 activation as demonstrated in vitro by using these combinations to treat cell lines that have been stably transfected with a promoter/reporter construct containing a known Nrf2-binding antioxidant response element inserted in to drive production of the readily detectable luciferase gene such that Nrf2 activation results in luciferase production which is detected by luciferin-dependent chemiluminescence. As shown in the Figures 4 and 5, potent Nrf2 activation is induced by the PB123, PB125, PB127, PB129, and PB131 combinations in transfected cancer cell lines independent of tissue type (breast and liver cell data are shown).

[0059] These control points include, but are not limited to, Control point A: release of Nrf2 from binding and inhibition by Keap1; Control point B: action on Nrf2 by enzymes such as kinases that phosphorylate and activate Nrf2; Control point C: activation of other transcription factors that improve the gene expression profile; Control point D: action on mechanisms such as Fyn that control the export of Nrf2 from the nucleus; and Control point E: degradation of Keap1 and mTOR inhibition by SESN2/SQSTM1/ULK1. See Figure 1. For example the PB125 combination that includes rosemary (carnosol), ashwagandha (withaferin A), and luteolin acts at multiple control points in the Nrf2 activation pathway. In HepG2 cells stably transfected with an ARE-driven luciferase reporter gene we inhibited Fyn (with 5 µg/ml saracatinib; AZD0530, a Src family kinase inhibitor (Kaufman, Salazar et al. 2015)) and showed that the inhibition of Fyn increased Nrf2 activation caused by another dietary supplement Nrf2 activator (Protandim) by up to 9-fold. In contrast Fyn inhibition did not further increase PB125-induced Nrf2 activation, confirming that while other dietary Nrf2 activators such as Protandim allow the "shutdown pathway" to remain active, PB125 appears to block the pathway, permitting Nrf2 activation by a smaller amount of the PB125 dietary supplement combination.

[0060] By acting on more than one of the control points, a combination of agents such as PB123 or PB125, along with related combinations based on the core Nrf2 activator triads in PB123 or PB125, such as PB127, PB129, or PB131 give an improved Nrf2 activation and gene regulation response and do so at lower doses than would be predicted based on known properties of the active agents in the combinations and based on what is taught by the prior art. The active ingredients in PB123, 125, PB127, PB129, and PB131 act together in a synergistic fashion, whereby the amount of Nrf2 activation and Nrf2-dependent gene expression is higher for the combined ingredients than would be predicted based on the sum of their individual activities on Nrf2 at the same concentrations, even in different cell types (Figures 6 and 7). One of the surprising findings was that relatively small amounts of luteolin added to the other ingredients gave a larger than expected increase in Nrf2 activation and gene regulation.

[0061] A rosemary (6.7% carnosol), ashwagandha (1% withaferin A), and luteolin (98% luteolin) combination of PB125 (at 30:10:4 rosemary:ashwagandha:luteolin) increased Nrf2-dependent gene expression in mice fed 35 days of PB125 added to mouse chow. See Figures 8 and 9.

[0062] The PB125 phytochemical components are standardized, with rosemary extract (specified at 6% carnosol), ashwagandha extract (specified at 1% withaferin A), and luteolin (specified at 98% purity), so 100 ppm equates to 6.83 x 10-5 mg rosemary extract, 2.27 x 10-5 mg ashwagandha extract, and 9.43 x 10-6 mg luteolin per g of diet. PB125 in mouse diet activates the Nrf2 pathway (e.g., increased hmox1 gene expression in mouse liver) and increases catalase activity. The PB125 dosages were well tolerated by mice as evidenced by no change compared to control diet in weight stability, consistent food intake, and no noticeable GI distress or changes in behavior. The 100 ppm PB125 diet produced significant increases in liver hmox1 gene expression in mice (measured after 35 days of diet consumption)(Figure 8).

[0063] The individual ingredients in PB125, PB127, and PB129 have a long history of human consumption and proven safety in both humans and in animal studies (Saller, Meier et al. 2001, Roodenrys, Booth et al. 2002, Aggarwal, Takada et al. 2004, Boon and Wong 2004, Anadon, Martinez-Larranaga et al. 2008, Zick, Djuric et al. 2008, Johnson 2011, Chandrasekhar, Kapoor et al. 2012, Theoharides, Asadi et

al. 2012, Taliou, Zintzaras et al. 2013, Zhang, Gan et al. 2013, Gonzalez-Vallinas, Reglero et al. 2015, Kumar, Srivastava et al. 2015, Nabavi, Braidy et al. 2015, Petiwala and Johnson 2015). Rosemary, ashwagandha, ginger, milk thistle, bacopa monnieri, and luteolin have been extensively studied in various diseases and have an extensive record of safe use (Mishra, Singh et al. 2000, Roodenrys, Booth et al. 2002, Aggarwal, Takada et al. 2004, Boon and Wong 2004). Rosemary (Rosmarinus officinalis) is a common Mediterranean herb widely consumed in foods as a spice and flavoring agent. Also, rosemary has a long history of use in traditional therapies for the treatment of a variety of disorders [1], with emphasis on anti-inflammatory (Emami, Ali-Beig et al. 2013), antioxidant (Klancnik, Guzej et al. 2009, Raskovic, Milanovic et al. 2014, Ortuno, Serrano et al. 2015), and antimicrobial benefits (Del Campo, Amiot et al. 2000, Bozin, Mimica-Dukic et al. 2007). Ashwagandha (Withania somnifera, also known as Indian winter cherry or Indian ginseng) is a member of the Solanaceae family of flowering plants. It has been utilized for centuries in South Asia in traditional therapies, with historical and current emphasis on immunomodulatory (Khan, Subramaneyaan et al. 2015), anti-tumor (Rai, Jogee et al. 2016), neurological (Raghavan and Shah 2015), anti-inflammatory (Kumar, Srivastava et al. 2015), antioxidant (Priyandoko, Ishii et al. 2011), and other benefits (Wankhede, Langade et al. 2015). Ginger has a long history of safe usage for pain, GI, and aging-related conditions, with evidence of benefit against oxidative stress (Wang, Zhang et al. 2014, Lakhan, Ford et al. 2015, Wilson 2015). Silymarin has a good safety profile (Saller, Meier et al. 2001, Jacobs, Dennehy et al. 2002) even in those with cirrhosis, and even at high doses (up to 900mg a day) that are much higher than used in PB127 or PB129. Bacopa moniera has proven to be safe in human studies of memory loss at doses higher than used in PB129, and animal studies have not demonstrated any adverse toxicities for any of its components (Mishra, Singh et al. 2000, Roodenrys, Booth et al. 2002). Luteolin is a bioflavanoid flavone compound commonly consumed in the human diet from multiple food sources (e.g., onions, tea, apples, broccoli, olives, celery, spinach, oranges, oregano, etc.), resulting in a typical dietary intake of approximate 1 mg/day from normal from food sources (Chun, Chung et al. 2007, Seelinger, Merfort et al. 2008, Jun, Shin et al. 2015, Kim, Park et al. 2015, Nabavi, Braidy et al. 2015). Luteolin is frequently utilized as a dietary supplement with emphasis on its antioxidant (Sun, Sun et al. 2012), neurological (Xu, Wang et al.

2014), and anti-inflammatory benefits (Seelinger, Merfort et al. 2008, Taliou, Zintzaras et al. 2013, Paredes-Gonzalez, Fuentes et al. 2015).

[0064] As an example of properties of PB125, we cultured cell lines that had been stably transfected with constructs of the luciferase gene driven in its promoter region by copies of the ARE Nrf2-binding sequence, known as promoter-reporter constructs (Simmons, Fan et al. 2011, Shukla, Huang et al. 2012). Briefly, the stably transfected cells of types HepG2 (human liver), AREc32 (human breast), MCF7 (human breast), A549 (human lung), 293T (human kidney), and A172 (human brain) were seeded at low density in 24-well plates and incubated at 37°C with 10% CO2. After 24 h various concentrations of PB125 were added to the cells. After an additional 18 h of incubation, the cells were lysed in their wells with 100 µl of a lysing buffer that contains 3.5 mM sodium pyrophosphate to stabilize light output by luciferase. A 20 µl aliquot of cell lysate was added to a small test tube, placed in a BD Monolight 3010 luminometer for background luminescence, and then 50 µl of 1 mM luciferin was injected into the tube. Relative Light Units integrated for 10 sec were measured for each sample. The liver, breast, brain, and kidney cell types tested exhibited Nrf2 gene activation and luciferase expression by treatment with PB100-series combinations with (Figure 10).

[0065] As an example of the cell protective mechanisms induced by PB125 treatment, we examined the gene upregulation in cells treated with PB125. Briefly, cultured HepG2 liver cells were treated with PB125 at 8 micrograms/mL concentration for 18 hours, then total RNA was extracted from the HepG2 cells by using the RNeasy Total RNA Isolation Kit (QIAGEN Inc. Valencia, California, USA). The concentration of each sample was determined based on the absorbance at 260 nm (A260). The purity of each sample was determined based on the ratio of A260 to A280. A range of 1.9-2.1 was considered adequately pure. The integrity of Total RNA samples was verified by Agilent 2200 Tape Station. Total RNA (250ng) was converted to double-stranded cDNA (ds-cDNA) by using the cDNA synthesis kit (Affymetrix). An oligo-dT primer containing a T7 RNA polymerase promoter was utilized. The ds-cDNA was then purified and recovered by using purification beads (Affymetrix). Next, in vitro transcription was performed to generate biotin-labeled cRNA using a RNA Transcript Labeling Kit (Affymetrix). Biotin-labeled cRNA was purified using an RNeasy affinity column (Qiagen). To ensure optimal hybridization

to the oligonucleotide array, the cRNA was fragmented. Fragmentation was performed such that the cRNA fragments are between 50-200 bases in length by incubating the cRNA at 94°C for 35 min in a fragmentation buffer. The sample was then added to a hybridization solution containing 100 mM MES, 1 M Na+, and 20 mM EDTA in the presence of 0.01% Tween 20. The final concentration of the fragmented cRNA was 0.05 µg/µL. Hybridization was performed by incubating 200 uL of the sample to the Affymetrix GeneChip® PrimeView™ human gene expression array (Affymetrix Inc., Santa Clara, California, USA) at 45 °C for 16 hours using a GeneChip® Hybridization Oven 640 (Affymetrix). After hybridization, the hybridization solutions were removed and the arrays were washed and stained with Streptavidin-phycoerythrin using a GeneChip® Fluidics Station 450 (Affymetrix). Arrays were read at a resolution of 2.5 to 3 microns using the GeneChip Scanner 3000 (Affymetrix). Each gene was represented by the use of ~11 probes per transcript and many control probes. The Command Console GeneChip software program was used to determine the intensity of expression for all genes on the array. For this experiment, fold-induction of genes by PB125 treatment of HepG2 cells was calculated compared to the average intensity observed in control HepG2 cells in culture solution without any added stimulus such as PB125. As depicted in Table 1, genes upregulated by PB125 included a variety of Nrf2-regulated antioxidant, anti-inflammatory, cell stress response and other protective genes. These genes include, for example, genes involved in GSH production and regeneration, iron sequestration, GSH utilization, thioredoxin (TXN) production, regeneration and ultilization, etc. Table 1 lists relevant example genes that are upregulated by PB125. In summary, this example supports that the mechanism of cellular protection by PB125 involves activation of the Nrf2 cell signaling pathway.

Table 1 Gene Microarray analysis revealed that PB125 regulates numerous Nrf2 associated genes and genes associated with antioxidant, anti-inflammatory, and other cell protective effects.

Probe Set ID	HepG2 (Control)	Fold Induction by BP125	Representative Public ID	Gene Title	Gene Symbol
11715650_a_at	45.53	10.10	AF208018.1	thioredoxin reductase 1	TXNRD1
11756634_a_at	414.69	2.81	CR597200.1	glutathione reductase	GSR
11750770_a_at	1005.93	2.37	AK304288.1	glutamate-cysteine ligase, catalytic subunit	GCLC
11759710_at	199.19	2.04	BC024223.2	thioredoxin domain containing 9	TXNDC9
11744680_a_at	231.18	7.72	AB040875.1	solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	SLC7A11
11756634_a_at	414.69	2.81	CR597200.1	glutathione reductase	GSR
11716939_a_at	1217.99	8.63	NM_002133.1	heme oxygenase (decycling) 1	HMOX1
11725496_a_at	488.83	8.87	NM_032717.3	1-acylglycerol-3-phosphate O-acyltransferase 9	AGPAT9
11752577_at	771.67	3.62	AY258285.1	ferritin, heavy polypeptide 1	FTH1
11715649_s_at	3236.76	4.73	NM_003330.2	thioredoxin reductase 1	TXNRD1
11716950_s_at	1908.04	5.45	NM_080725.1	sulfiredoxin 1	SRXN1
11752843_x_at	1202.52	4.54	AK304877.1	sequestosome 1	SQSTM1
11750416_a_at	69.07	9.41	AK293322.1	thioredoxin reductase 1	TXNRD1
11756585_a_at	86.47	6.47	CR614710.1	aquaporin 3 (Gill blood group)	AQP3
11735676_a_at	231.82	3.98	NM_182980.2	oxidative stress induced growth inhibitor 1	OSGIN1
11753445_a_at	244.58	10.37	BT019785.1	heme oxygenase (decycling) 1	HMOX1
11723490_at	1195.87	6.07	BC041809.1	glutamate-cysteine ligase, modifier subunit	GCLM
11756915_a_at	63.77	8.33	AL833940.1	cytochrome P450, family 4, subfamily F, polypeptide 11	CYP4F11
11736655_a_at	499.98	7.20	NM_012212.3	prostaglandin reductase 1	PTGR1
11719171_a_at	2722.97	6.99	NM_001353.5	aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)	AKR1C1
11742378_a_at	1112.08	4.32	NM_001080538.1	aldo-keto reductase family 1, member B10 (aldose reductase) /// aldo-keto reductase family 1, member B15	AKR1B10 /// AKR1B15
11729101_a_at	2435.26	6.95	NM_205845.1	aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III) /// aldo-keto reductase family 1 member C2-like	AKR1C2 /// LOC100653286
11757882_x_at	59.22	2.02	BU784580	glutathione S-transferase alpha 1 /// glutathione S-transferase alpha 2	GSTA1 /// GSTA2

[0066] As an example of the anti-inflammatory mechanisms induced by PB125 treatment, we examined cytokine levels in primary cells treated with PB125 and stimulated with bacterial lipopolysaccharide endotoxin (LPS). Mouse peritoneal macrophages were obtained after treatment with thioglycollate into the peritoneal cavity for 1 week followed by lavage recovery of approximately 7 million macrophages. Aliquots of cells were plated and treated with ethanol control (0.1% to match PB125) or PB125 (5 ug/mL) for 16 h, then stimulated with lipopolysaccharide (100 ng/mL) or vehicle (negative control) for 5 h. Total RNA was isolated from the cells for quantitative PCR analysis to measure TNFα (tumor necrosis factor-alpha) and IL-1β (interleukin-1 beta) gene expression, normalized to 18s levels. Notably, PB125 treatment caused a dramatic decrease in LPS-induced expression of the pro-inflammatory cytokines TNFα and IL-1β. See Figure 11.

[0067] A rosemary (6.7% carnosol), ashwagandha (1% withaferin A), and luteolin (98% luteolin) combination of PB125 (at 30:10:4 rosemary:ashwagandha:luteolin) increased Nrf2-dependent gene expression of the GCLM gene in buccal cell samples from a human subject taking 60 mg of PB125 daily p.o., compare to buccal cell samples two normal control subjects (assayed by

quantitative RT-PCR on purified RNA, using human GCLM specific primers (Forward Primer: TTGCCTCCTGCTGTGTGATG (SEQ ID NO. 1), Reverse Primer: GTGCGCTTGAATGTCAGGAA) (SEQ ID NO. 2), normalized to GAPDH, with relative fold change calculated by the 2^(delta delta Ct) method. See Figure 13.

[0068] As additional data supporting the invention, we found surprising amounts of synergy between the Rosemary, Ginger, Ashwagandha, and Luteolin ingredients. For example, low concentrations of Luteolin synergized with combinations of Rosemary extracts and Ginger extracts to activate Nrf2. In the present invention, other agents can be added to the Nrf2-activating combinations provided they do not interfere with the Nrf2 activating functionality. We found that the silymarin and bacosides ingredients did not antagonize the Nrf2 activation of the Rosemary, Ginger, Ashwagandha, and Luteolin ingredients.

[0069] Following up on this experiment in another way, luciferase RLU measured 17, 24, 41, and 48 hours after treatment of HepG2 cells in which the PB125 treatment at 0-10 ug/mL and 0-50 ug/mL ranges was washed off after 2 hours of exposure time and replaced by fresh cell culture media showed that Nrf2-driven production of luciferase was highest at 17 h, then rapidly decreased to approximately baseline levels by 48 hours after treatment.

[0070] Repeating treatments on cultured HepG2 cells with 2 hour exposures once every 24 hours, then read 24 hours later showed that the Nrf2 activation by PB125 wore off between 24 and 48 hours and the cells could still be activated again if treated again with PB125.

[0071] As an example of the anti-inflammatory mechanisms induced by PB123 or PB125 treatment, we examined gene expression and cytokine levels in primary human pulmonary artery endothelial cells (HPAEC) treated with PB123 or PB125 and stimulated with bacterial lipopolysaccharide endotoxin (LPS). LPS stimulation induced the expression of inflammation-related genes, and this upregulation was attenuated by treatment with PB123 or PB125. Table 2 shows the 40 genes most highly upregulated by LPS treatment, and shows that both PB123 treatment and PB125 treatment attenuated LPS-induced gene expression. LPS stimulation increased the release of pro-inflammatory interleukin-6 (IL6) protein from

the HPAEC cells, and this increase was attenuated by treatment with PB125. See Figure 12.

Table 2 Gene Microarray analysis revealed that PB123 and PB125 exhibited anti-inflammatory effects. Both PB123 and PB125 lowered the LPS-induced expression signals of the 40 genes that were the most highly up-regulated by LPS.

Gene Symbol	Control	LPS	LPS+PB123	LPS+PB125	Gene Title	Gene Symbol	LPS/LPS +PB123	LPS/LPS +PB125
CXCL3	33	1441	492	225	chemokine (C-X-C motif) ligand 3	CXCL3	2.9	6.4
CCL20	196	4776	2055	1034	chemokine (C-C motif) ligand 20	CCL20	2.3	4.6
CXCL2	292	5407	2956	2669	chemokine (C-X-C motif) ligand 2	CXCL2	1.8	2.0
CSF2	41	621	132	133	colony stimulating factor 2 (granulocyte-macrophage)	CSF2	4.7	4.7
TNFAIP6	33	390	91	60	tumor necrosis factor, alpha-induced protein 6	TNFAIP6	4.3	6.5
IL8	590	6750	5571	4257	interleukin 8	IL8	1.2	1.6
TNFAIP2	285	3089	798	512	tumor necrosis factor, alpha-induced protein 2	TNFAIP2	3.9	6.0
CXCL10	67	668	47	31	chemokine (C-X-C motif) ligand 10	CXCL10	14.3	21.3
CXCL1	1195	11398	7858	7819	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	CXCL1	1.5	1.5
CX3CL1	386	3618	444	288	chemokine (C-X3-C motif) ligand 1	CX3CL1	8.2	12.5
BIRC3	86	798	349	190	baculoviral IAP repeat containing 3	BIRC3	2.3	4.2
CD69	36	333	111	45	CD69 molecule	CD69	3.0	7.3
TNFAIP3	94	814	309	190	tumor necrosis factor, alpha-induced protein 3	TNFAIP3	2.6	4.3
SELE	1465	12429	5605	2612	selectin E	SELE	2.2	4.8
CXCL6	245	1683	458	178	chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	CXCL6	3.7	9.5
NKX3-1	60	398	141	125	NK3 homeobox 1	NKX3-1	2.8	3.2
CSF3	92	592	272	290	colony stimulating factor 3 (granulocyte)	CSF3	2.2	2.0
RND1	98	601	224	236	Rho family GTPase 1	RND1	2.7	2.5
LTB	244	1478	374	314	lymphotoxin beta (TNF superfamily, member 3)	LTB	3.9	4.7
FAM101A /// Z	63	329	70	78	family with sequence similarity 101, member A /// protein FAM101A	FAM101A /// ZNF664	4.7	4.2
CXCL5	163	844	127	63	chemokine (C-X-C motif) ligand 5	CXCL5	6.7	13.3
CEBPD	183	947	493	489	CCAAT/enhancer binding protein (C/EBP), delta	CEBPD	1.9	1.9
MAP3K8	26	128	75	45	mitogen-activated protein kinase kinase kinase 8	MAP3K8	1.7	2.9
TRAF1	158	730	421	328	TNF receptor-associated factor 1	TRAF1	1.7	2.2
IL6	429	1967	1166	1105	interleukin 6 (interferon, beta 2)	IL6	1.7	1.8
VCAM1	1315	5963	2065	1116	vascular cell adhesion molecule 1	VCAM1	2.9	5.3
ICAM1	288	1290	543	416	intercellular adhesion molecule 1	ICAM1	2.4	3.1
SLC7A2	356	1592	660	383	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	SLC7A2	2.4	4.2
CXCR7	291	1286	660	521	chemokine (C-X-C motif) receptor 7	CXCR7	1.9	2.5
NCOA7	132	561	212	137	nuclear receptor coactivator 7	NCOA7	2.6	4.1
IRF1	240	1014	579	489	interferon regulatory factor 1	IRF1	1.8	2.1
BCL2A1	31	130	39	18	BCL2-related protein A1	BCL2A1	3.3	7.0
TNFRSF9	32	124	33	30	tumor necrosis factor receptor superfamily, member 9	TNFRSF9	3.7	4.1
IL1A	236	888	589	561	interleukin 1, alpha	IL1A	1.5	1.6
MT1G	36	134	116	163	metallothionein 1G	MT1G	1.2	0.8
TIFA	81	293	175	147	TRAF-interacting protein with forkhead-associated domain	TIFA	1.7	2.0
CCL5	95	330	95	83	chemokine (C-C motif) ligand 5	CCL5	3.5	4.0
CAB39	26	91	48	43	calcium binding protein 39	CAB39	1.9	2.1
SOCS1	29	95	73	76	suppressor of cytokine signaling 1	SOCS1	1.3	1.2
IL1B	52	170	58	66	interleukin 1, beta	IL1B	2.9	2.6

Example 2 PB125

[0072] One embodiment of the present disclosure is a combination of rosemary extract (specified at 5 to 50% carnosol), ashwagandha extract (specified at 0.5-10% withaferin A), and luteolin (specified at 10-100% luteolin), in the mass ratios of 30:10:6, 30:10:5, 30:10:4, or 30:10:1 with a daily human dose of the combination ranging from 42 to 1050 mg as shown in Table 3.

Table 3. Composition with specifications for the ingredients and the daily dose ranges of PB125 for human

Ingredient:	Rosemary	Ashwagandha	Luteolin
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-10% withaferin A	10-100% luteolin
Preferred spec range:	5-10% carnosol	1-3% withaferin A	95-99% luteolin
Daily dose range:	30-750 mg	10-250 mg	2-50 mg
Composition range:	30-90%	10-30%	2-8%
Preferred mass ratio	30	10	6
Preferred mass ratio	30	10	5
Preferred mass ratio	30	10	4
Preferred mass ratio	30	10	1

Example 3 PB127

[0073] Another embodiment of the present disclosure is a PB127 combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), and milk thistle extract (specified at 50-90% silymarin), in the mass ratio of 10:5:1:30, respectively, with a daily human dose of the combination ranging from 46 to 920 mg as shown in Table 4.

Table 4. Composition with specifications for the ingredients and the daily dose ranges of PB127 for human

Ingredient:	Rosemary	Ginger	Luteolin	Milk Thistle
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-20% 6-shogaol or 6-gingerol	10-100% luteolin	10-100% silymarin
Preferred spec range:	5-10% carnosol	10-20% 6-shogaol	95-99% luteolin	75-100% silymarin
Daily dose range:	10-200 mg	5-100 mg	1-20 mg	30-600 mg
Composition range:	10-30%	5-15%	1-3%	25-75%

Preferred mass ratio	10	5	1	30

Example 4 PB129

[0074] Another embodiment of the present disclosure is a PB129 combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin), milk thistle extract (specified at 50-90% silymarin), and bacopa monnieri extract (specified at 10-60% bacosides) in the mass ratio of 10:5:1:30:48, respectively, with a daily human dose of the combination ranging from 94 to 1820 mg as shown in Table 5.

Table 5. Composition with specifications for the ingredients and the daily dose ranges of PB129 for human

Ingredient:	Rosemary	Ginger	Luteolin	Milk Thistle	Bacopa
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-20% 6-shogaol or 6-gingerol	10-100% luteolin	10-100% silymarin	10-80% bacosides
Preferred spec range:	5-10% carnosol	10-20% 6-shogaol	95-99% luteolin	75-100% silymarin	20-60% bacosides
Daily dose range:	10-200 mg	5-100 mg	1-20 mg	30-600 mg	48-900 mg
Composition range:	5-15%	2.5-7.5%	0.5-1.5%	12.5-37.5%	25-75%
Preferred mass ratio	10	5	1	30	48

Example 5 PB123

[0075] Another embodiment of the present disclosure is a PB123 combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin) in the mass ratio of 10:5:1, respectively, with a daily human dose of the combination ranging from 16 to 320 mg as shown in Table 6.

Table 6. Composition with specifications for the ingredients and the daily dose ranges of PB123 for human

Ingredient:	Rosemary	Ginger	Luteolin
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-20% 6-shogaol or 6-gingerol	10-100% luteolin
Preferred spec range:	5-10% carnosol	10-20% 6-shogaol	95-99% luteolin
Daily dose range:	10-200 mg	5-100 mg	1-20 mg
Composition range:	10-30%	5-15%	1-3%
Preferred mass ratio	10	5	1

Example 6 PB131

[0076] Another embodiment of the present invention is a PB131 combination of rosemary extract (specified at 5 to 10% carnosol), ginger extract (specified at 1-10% 6-shogaol and/or 10-25% 6-gingerol), luteolin (specified at 90-100% luteolin) and bacopa monnieri extract (specified at 10-60% bacosides) in the mass ratio of 10:5:1:48, respectively, with a daily human dose of the combination ranging from 64 to 1220 mg as shown in Table 7.

Table 7. Composition with specifications for the ingredients and the daily dose ranges of PB131 for human

Ingredient:	Rosemary	Ginger	Luteolin	Bacopa
Spec range:	5-50% carnosol or 10-100% diterpenes	0.5-20% 6-shogaol or 6-gingerol	10-100% luteolin	10-80% bacosides
Preferred spec range:	5-10% carnosol	10-20% 6-shogaol	95-99% luteolin	20-60% bacosides
Daily dose range:	10-200 mg	5-100 mg	1-20 mg	48-900 mg
Composition range:	5-15%	2.5-7.5%	0.5-1.5%	25-75%
Preferred mass ratio	10	5	1	48

[0077] The contents of all cited references (including literature references, patents, patent applications, and websites) that may be cited throughout this application or listed below are hereby expressly incorporated by reference in their entirety for any purpose into the present disclosure. The disclosure may employ, unless otherwise indicated, conventional techniques of microbiology, molecular biology and cell biology, which are well known in the art.

[0078] The disclosed methods and systems may be modified without departing from the scope hereof. It should be noted that the matter contained in the above description or shown in the accompanying drawings should be interpreted as illustrative and not in a limiting sense.

List of References

[0079] The following references, patents and publication of patent applications are either cited in this disclosure or are of relevance to the present disclosure. All documents listed below, along with other papers, patents and publication of patent applications cited throughout this disclosures, are hereby incorporated by reference as if the full contents are reproduced herein.

Aggarwal, B. B., Y. Takada and O. V. Oommen (2004). "From chemoprevention to chemotherapy: common targets and common goals." Expert Opin Investig Drugs 13(10): 1327-1338.

Anadon, A., M. R. Martinez-Larranaga, M. A. Martinez, I. Ares, M. R. Garcia-Risco, F. J. Senorans and G. Reglero (2008). "Acute oral safety study of rosemary extracts in rats." J Food Prot 71(4): 790-795.

Baitharu, I., V. Jain, S. N. Deep, S. Shroff, J. K. Sahu, P. K. Naik and G. Ilavazhagan (2014). "Withanolide A prevents neurodegeneration by modulating hippocampal glutathione biosynthesis during hypoxia." PLoS One 9(10): e105311.

Bjelakovic, G., D. Nikolova, L. L. Gluud, R. G. Simonetti and C. Gluud (2007). "Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis." JAMA 297(8): 842-857.

Bocci, V. and G. Valacchi (2015). "Nrf2 activation as target to implement therapeutic treatments." Front Chem 3: 4.

Boon, H. and J. Wong (2004). "Botanical medicine and cancer: a review of the safety and efficacy." Expert Opin Pharmacother 5(12): 2485-2501.

Boutten, A., D. Goven, J. Boczkowski and M. Bonay (2010). "Oxidative stress targets in pulmonary emphysema: focus on the Nrf2 pathway." Expert Opin Ther Targets 14(3): 329-346.

Bozin, B., N. Mimica-Dukic, I. Samojlik and E. Jovin (2007). "Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils." J Agric Food Chem 55(19): 7879-7885.

Chandrasekhar, K., J. Kapoor and S. Anishetty (2012). "A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of ashwagandha root in reducing stress and anxiety in adults." Indian J Psychol Med 34(3): 255-262.

Cho, H. Y. and S. R. Kleeberger (2010). "Nrf2 protects against airway disorders." Toxicol Appl Pharmacol 244(1): 43-56.

Chun, O. K., S. J. Chung and W. O. Song (2007). "Estimated dietary flavonoid intake and major food sources of U.S. adults." J Nutr 137(5): 1244-1252.

Del Campo, J., M. J. Amiot and C. Nguyen-The (2000). "Antimicrobial effect of rosemary extracts." J Food Prot 63(10): 1359-1368.

Eggler, A. L., K. A. Gay and A. D. Mesecar (2008). "Molecular mechanisms of natural products in chemoprevention: induction of cytoprotective enzymes by Nrf2." Mol Nutr Food Res 52 Suppl 1: S84-94.

Emami, F., H. Ali-Beig, S. Farahbakhsh, N. Mojabi, B. Rastegar-Moghadam, S. Arbabian, M. Kazemi, E. Tekieh, L. Golmanesh, M. Ranjbaran, C. Jalili, A. Noroozzadeh and H. Sahraei (2013). "Hydroalcoholic extract of Rosemary (Rosmarinus officinalis L.) and its constituent carnosol inhibit formalin-induced pain and inflammation in mice." Pak J Biol Sci 16(7): 309-316.

Gonzalez-Vallinas, M., G. Reglero and A. Ramirez de Molina (2015). "Rosemary (Rosmarinus officinalis L.) Extract as a Potential Complementary Agent in Anticancer Therapy." Nutr Cancer: 1-9.

Heistad, D. D., Y. Wakisaka, J. Miller, Y. Chu and R. Pena-Silva (2009). "Novel aspects of oxidative stress in cardiovascular diseases." Circ J 73(2): 201-207.

Huang, Y., W. Li, Z.-y. Su and A.-N. T. Kong (2015). "The complexity of the Nrf2 pathway: Beyond the antioxidant response." The Journal of Nutritional Biochemistry: in press.

Hybertson, B. M. and B. Gao (2014). "Role of the Nrf2 signaling system in health and disease." Clin Genet 86(5): 447-452.

Jacobs, B. P., C. Dennehy, G. Ramirez, J. Sapp and V. A. Lawrence (2002). "Milk thistle for the treatment of liver disease: a systematic review and meta-analysis." Am J Med 113(6): 506-515.

Johnson, D. A. and J. A. Johnson (2015). "Nrf2-a therapeutic target for the treatment of neurodegenerative diseases." Free Radic Biol Med.

Johnson, J. J. (2011). "Carnosol: A promising anti-cancer and anti-inflammatory agent." Cancer letters 305(1): 1-7.

Jun, S., S. Shin and H. Joung (2015). "Estimation of dietary flavonoid intake and major food sources of Korean adults." Br J Nutr: 1-10.

Kaufman, A. C., S. V. Salazar, L. T. Haas, J. Yang, M. A. Kostylev, A. T. Jeng, S. A. Robinson, E. C. Gunther, C. H. van Dyck, H. B. Nygaard and S. M. Strittmatter (2015). "Fyn inhibition rescues established memory and synapse loss in Alzheimer mice." Ann Neurol 77(6): 953-971.

Kensler, T. W., N. Wakabayashi, S. L. Slocum, J. J. Skoko and S. Shin (2010). "When Nrf2 Talks, Who's Listening?" Antioxid Redox Signal.

Khan, M. A., M. Subramaneyaan, V. K. Arora, B. D. Banerjee and R. S. Ahmed (2015). "Effect of Withania somnifera (Ashwagandha) root extract on amelioration of oxidative stress and autoantibodies production in collagen-induced arthritic rats." J Complement Integr Med 12(2): 117-125.

Kim, Y. J., M. Y. Park, N. Chang and O. Kwon (2015). "Intake and major sources of dietary flavonoid in Korean adults: Korean National Health and Nutrition Examination Survey 2010-2012." Asia Pac J Clin Nutr 24(3): 456-463.

Klancnik, A., B. Guzej, M. H. Kolar, H. Abramovic and S. S. Mozina (2009). "In vitro antimicrobial and antioxidant activity of commercial rosemary extract formulations." J Food Prot 72(8): 1744-1752.

Koehn, F. E. (2006). "Therapeutic potential of natural product signal transduction agents." Curr Opin Biotechnol 17(6): 631-637.

Koehn, F. E. and G. T. Carter (2005). "The evolving role of natural products in drug discovery." Nat Rev Drug Discov 4(3): 206-220.

Kumar, G., A. Srivastava, S. K. Sharma, T. D. Rao and Y. K. Gupta (2015). "Efficacy & safety evaluation of Ayurvedic treatment (Ashwagandha powder & Sidh Makardhwaj) in rheumatoid arthritis patients: a pilot prospective study." Indian J Med Res 141(1): 100-106.

Kyung-Soo, C., K. Juthika, C. In Gyeong and K. and Joydeb Kumar (2014). "Carnosol: A Phenolic Diterpene With Cancer Chemopreventive Potential." Journal of Cancer Prevention 19(2): 103-110.

Lakhan, S. E., C. T. Ford and D. Tepper (2015). "Zingiberaceae extracts for pain: a systematic review and meta-analysis." Nutr J 14: 50.

Lee, K. H. (2010). "Discovery and development of natural product-derived chemotherapeutic agents based on a medicinal chemistry approach." J Nat Prod 73(3): 500-516.

Maher, J. and M. Yamamoto (2010). "The rise of antioxidant signaling--the evolution and hormetic actions of Nrf2." Toxicol Appl Pharmacol 244(1): 4-15.

Martin, D., A. I. Rojo, M. Salinas, R. Diaz, G. Gallardo, J. Alam, C. M. R. de Galarreta and A. Cuadrado (2004). "Regulation of Heme Oxygenase-1 Expression through the Phosphatidylinositol 3-Kinase/Akt Pathway and the Nrf2 Transcription Factor in Response to the Antioxidant Phytochemical Carnosol." Journal of Biological Chemistry 279(10): 8919-8929.

Mishra, L. C., B. B. Singh and S. Dagenais (2000). "Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review." Altern Med Rev 5(4): 334-346.

Moon, E. J. and A. Giaccia (2015). "Dual roles of NRF2 in tumor prevention and progression: possible implications in cancer treatment." Free Radic Biol Med 79: 292-299.

Nabavi, S. F., N. Braidy, O. Gortzi, E. Sobarzo-Sanchez, M. Daglia, K. Skalicka-Woźniak and S. M. Nabavi (2015). "Luteolin as an anti-inflammatory and neuroprotective agent: A brief review." Brain Research Bulletin 119, Part A: 1-11.

Ortuno, J., R. Serrano and S. Banon (2015). "Antioxidant and antimicrobial effects of dietary supplementation with rosemary diterpenes (carnosic acid and carnosol) vs vitamin E on lamb meat packed under protective atmosphere." Meat Sci 110: 62-69.

Paredes-Gonzalez, X., F. Fuentes, S. Jeffery, C. L. Saw, L. Shu, Z. Y. Su and A. T. Kong (2015). "Induction of NRF2-mediated gene expression by dietary phytochemical flavones apigenin and luteolin." Biopharm Drug Dispos.

Pechanova, O. and F. Simko (2009). "Chronic antioxidant therapy fails to ameliorate hypertension: potential mechanisms behind." J Hypertens 27 Suppl 6: S32-36.

Petiwala, S. M. and J. J. Johnson (2015). "Diterpenes from rosemary (Rosmarinus officinalis): Defining their potential for anti-cancer activity." Cancer Lett 367(2): 93-102.

Priyandoko, D., T. Ishii, S. C. Kaul and R. Wadhwa (2011). "Ashwagandha leaf derived withanone protects normal human cells against the toxicity of methoxyacetic acid, a major industrial metabolite." PLoS One 6(5): e19552.

Raghavan, A. and Z. A. Shah (2015). "Withania somnifera: a pre-clinical study on neuroregenerative therapy for stroke." Neural Regen Res 10(2): 183-185.

Rai, M., P. S. Jogee, G. Agarkar and C. A. Santos (2016). "Anticancer activities of Withania somnifera: Current research, formulations, and future perspectives." Pharm Biol 54(2): 189-197.

Raskovic, A., I. Milanovic, N. Pavlovic, T. Cebovic, S. Vukmirovic and M. Mikov (2014). "Antioxidant activity of rosemary (Rosmarinus officinalis L.) essential oil and its hepatoprotective potential." BMC Complement Altern Med 14: 225.

Roodenrys, S., D. Booth, S. Bulzomi, A. Phipps, C. Micallef and J. Smoker (2002). "Chronic effects of Brahmi (Bacopa monnieri) on human memory." Neuropsychopharmacology 27(2): 279-281.

Saller, R., R. Meier and R. Brignoli (2001). "The use of silymarin in the treatment of liver diseases." Drugs 61(14): 2035-2063.

Saremi, A. and R. Arora (2009). "Vitamin E and Cardiovascular Disease." Am J Ther.

Satoh, H., T. Moriguchi, K. Taguchi, J. Takai, J. M. Maher, T. Suzuki, P. T. Winnard, Jr., V. Raman, M. Ebina, T. Nukiwa and M. Yamamoto (2010). "Nrf2-deficiency creates a responsive microenvironment for metastasis to the lung." Carcinogenesis 31(10): 1833-1843.

Satoh, T., K. Kosaka, K. Itoh, A. Kobayashi, M. Yamamoto, Y. Shimojo, C. Kitajima, J. Cui, J. Kamins, S. Okamoto, M. Izumi, T. Shirasawa and S. A. Lipton (2008). "Carnosic acid, a catechol-type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1." J Neurochem 104(4): 1116-1131.

Seelinger, G., I. Merfort and C. M. Schempp (2008). "Anti-oxidant, anti-inflammatory and anti-allergic activities of luteolin." Planta Med 74(14): 1667-1677.

Sekhar, K. R. and M. L. Freeman (2015). "NRF2 promotes survival following exposure to ionizing radiation." Free Radic Biol Med.

Shukla, S. J., R. Huang, S. O. Simmons, R. R. Tice, K. L. Witt, D. Vanleer, R. Ramabhadran, C. P. Austin and M. Xia (2012). "Profiling environmental chemicals for activity in the antioxidant response element signaling pathway using a high throughput screening approach." Environ Health Perspect 120(8): 1150-1156.

Simmons, S. O., C. Y. Fan, K. Yeoman, J. Wakefield and R. Ramabhadran (2011). "NRF2 Oxidative Stress Induced by Heavy Metals is Cell Type Dependent." Curr Chem Genomics 5: 1-12.

Sun, G. B., X. Sun, M. Wang, J. X. Ye, J. Y. Si, H. B. Xu, X. B. Meng, M. Qin, J. Sun, H. W. Wang and X. B. Sun (2012). "Oxidative stress suppression by luteolin-induced heme oxygenase-1 expression." Toxicol Appl Pharmacol 265(2): 229-240.

Suzuki, T. and M. Yamamoto (2015). "Molecular basis of the Keap1-Nrf2 system." Free Radic Biol Med.

Taliou, A., E. Zintzaras, L. Lykouras and K. Francis (2013). "An open-label pilot study of a formulation containing the anti-inflammatory flavonoid luteolin and its effects on behavior in children with autism spectrum disorders." Clin Ther 35(5): 592-602.

Theoharides, T. C., S. Asadi and S. Panagiotidou (2012). "A case series of a luteolin formulation (NeuroProtek(R)) in children with autism spectrum disorders." Int J Immunopathol Pharmacol 25(2): 317-323.

Vaishnavi, K., N. Saxena, N. Shah, R. Singh, K. Manjunath, M. Uthayakumar, S. P. Kanaujia, S. C. Kaul, K. Sekar and R. Wadhwa (2012). "Differential activities of the two closely related withanolides, Withaferin A and Withanone: bioinformatics and experimental evidences." PLoS One 7(9): e44419.

Velmurugan, K., J. Alam, J. M. McCord and S. Pugazhenthi (2009). "Synergistic induction of heme oxygenase-1 by the components of the antioxidant supplement Protandim." Free Radic Biol Med 46(3): 430-440.

Wang, S., C. Zhang, G. Yang and Y. Yang (2014). "Biological properties of 6-gingerol: a brief review." Nat Prod Commun 9(7): 1027-1030.

Wankhede, S., D. Langade, K. Joshi, S. R. Sinha and S. Bhattacharyya (2015). "Examining the effect of Withania somnifera supplementation on muscle strength and recovery: a randomized controlled trial." J Int Soc Sports Nutr 12: 43.

Wen, Z., Z. Wang, S. Wang, R. Ravula, L. Yang, J. Xu, C. Wang, Z. Zuo, M. S. Chow, L. Shi and Y. Huang (2011). "Discovery of molecular mechanisms of traditional Chinese medicinal formula Si-Wu-Tang using gene expression microarray and connectivity map." PLoS One 6(3): e18278.

Wilson, P. B. (2015). "Ginger (Zingiber officinale) as an Analgesic and Ergogenic Aid in Sport: A Systemic Review." J Strength Cond Res 29(10): 2980-2995.

Wu, P. S., J. H. Yen, M. C. Kou and M. J. Wu (2015). "Luteolin and Apigenin Attenuate 4-Hydroxy-2-Nonenal-Mediated Cell Death through Modulation of UPR, Nrf2-ARE and MAPK Pathways in PC12 Cells." PLoS One 10(6): e0130599.

Xiang, Q., Z. Liu, Y. Wang, H. Xiao, W. Wu, C. Xiao and X. Liu (2013). "Carnosic acid attenuates lipopolysaccharide-induced liver injury in rats via fortifying cellular antioxidant defense system." Food and Chemical Toxicology 53(0): 1-9.

Xu, J., H. Wang, K. Ding, L. Zhang, C. Wang, T. Li, W. Wei and X. Lu (2014). "Luteolin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE pathway." Free Radic Biol Med 71: 186-195.

Zhang, Y. C., F. F. Gan, S. B. Shelar, K. Y. Ng and E. H. Chew (2013). "Antioxidant and Nrf2 inducing activities of luteolin, a flavonoid constituent in Ixeris sonchifolia Hance, provide neuroprotective effects against ischemia-induced cellular injury." Food Chem Toxicol 59: 272-280.

Zick, S. M., Z. Djuric, M. T. Ruffin, A. J. Litzinger, D. P. Normolle, S. Alrawi, M. R. Feng and D. E. Brenner (2008). "Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects." Cancer Epidemiol Biomarkers Prev 17(8): 1930-1936.

CLAIMS

What is claimed is:

1. A composition comprising two or more phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, said two or more phytochemicals being present in the composition in an amount effective to activate the Nrf2 (Nuclear factor-erythroid 2 related factor 2) pathway.

2. The composition of claim 1, wherein the two or more phytochemicals exert their effects on at least two different control points of the Nrf2 activation pathway when administered to a mammal, said control points being selected from the group consisting of control points A, B, C, D and E.

3 The composition of claim 1, wherein the two or more phytochemicals have a synergistic effect on Nrf2 activation when administered to a mammal.

4. The composition of claim 1, wherein the composition comprises at least two ingredients selected from the group consisting of rosemary, ginger, luteolin, and ashwagandha.

5. The composition of claim 4, wherein the composition further comprises one or more phytochemicals selected from the group consisting of milk thistle and bacopa.

6. The composition of claim 4, wherein the composition comprises rosemary extract, ginger extract, and luteolin, said rosemary extract being specified at 5-10% carnosol, said ginger extract being specified at 10-20% 6-shogaol, said luteolin being specified at 95-99% luteolin, wherein the ratio between rosemary extract, ginger extract, and luteolin in the composition is approximately 10:5:1 (w/w).

7. The composition of claim 4, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, said rosemary extract being specified at 5-10% carnosol, said ashwagandha extract being specified at 1-3% withaferin A, said luteolin being specified at 95-99% luteolin, wherein the ratio between said rosemary extract, ashwagandha extract, and luteolin in the composition is approximately 30:10:4 (w/w).

8. The composition of claim 1, wherein the composition comprises rosemary extract, ginger extract, and luteolin, and wherein the ratio between said rosemary extract, ginger extract, and luteolin is approximately 10:5:1 (w/w).

9. The composition of claim 1, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, the ratio between said rosemary extract, ashwagandha extract, and luteolin being approximately 30:10:4 (w/w).

10. The composition of claim 5, wherein the composition comprises rosemary extract, ginger extract, luteolin and milk thistle extract, the ratio between said rosemary extract, ginger extract, luteolin and milk thistle extract being approximately 10:5:1:30 (w/w).

11. The composition of claim 5, wherein the composition comprises rosemary extract, ginger extract, luteolin, milk thistle extract, and bacopa monnieri extract, the ratio between said rosemary extract, ginger extract, luteolin, milk thistle extract and bacopa monnieri extract being approximately 10:5:1:30:48 (w/w).

12. The composition of claim 5, wherein the composition comprises rosemary extract, ginger extract, luteolin, and bacopa monnieri extract, the ratio between said rosemary extract, ginger extract, luteolin, and bacopa monnieri extract being approximately 10:5:1:48 (w/w).

13. The composition of claim 1, wherein the composition is used to prevent and/or treat a disease or a condition selected from the group consisting of oxidative stress, detoxification, inflammation, cancer, or a related disease or condition.

14. The composition of claim 1, wherein the composition is used as a nutritional supplement.

15. The composition of claim 1, wherein the composition is in the form of a tablet, a capsule, a soft gel, a liquid, a lotion, a gel, a powder, an ointment, or an aerosol.

16. A method of treating and/or preventing a disease or condition, comprising the step of administering a composition to a mammal, the composition comprising one or more phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, said one or more phytochemicals being present in the composition in an amount effective to activate the Nrf2 (NF-E2 related factor 2) pathway.

17. The method of claim 16, wherein the composition comprises rosemary extract, ashwagandha extract, and luteolin, wherein the rosemary extract is specified at 5-10% carnosol, the ashwagandha extract is specified at 1-3% withaferin A, and the luteolin is specified at 95-99% luteolin, the ratio between said rosemary extract, ashwagandha extract, and luteolin being approximately 30:10:4 (w/w).

18. The method of claim 16, wherein the composition comprises rosemary extract, ginger extract, and luteolin, wherein the rosemary extract is specified at 5-10% carnosol, the ginger extract is specified at 10-20% 6-shogaol, and the luteolin is specified at 95-99% luteolin, the ratio between said rosemary extract, ginger extract, and luteolin being approximately 10:5:1 (w/w).

19. The method of claim 18, wherein the composition is administered orally to a human at 10-1000 mg per day.

20. The method of claim 18, wherein the composition comprises at least two phytochemicals selected from the group consisting of carnosol, carnosic acid, shogaol, gingerol, luteolin, and withaferin A, wherein the at least two phytochemicals exert their effects on at least two different control points of the Nrf2 activation pathway, said control points being selected from the group consisting of control points A, B, C, D and E.

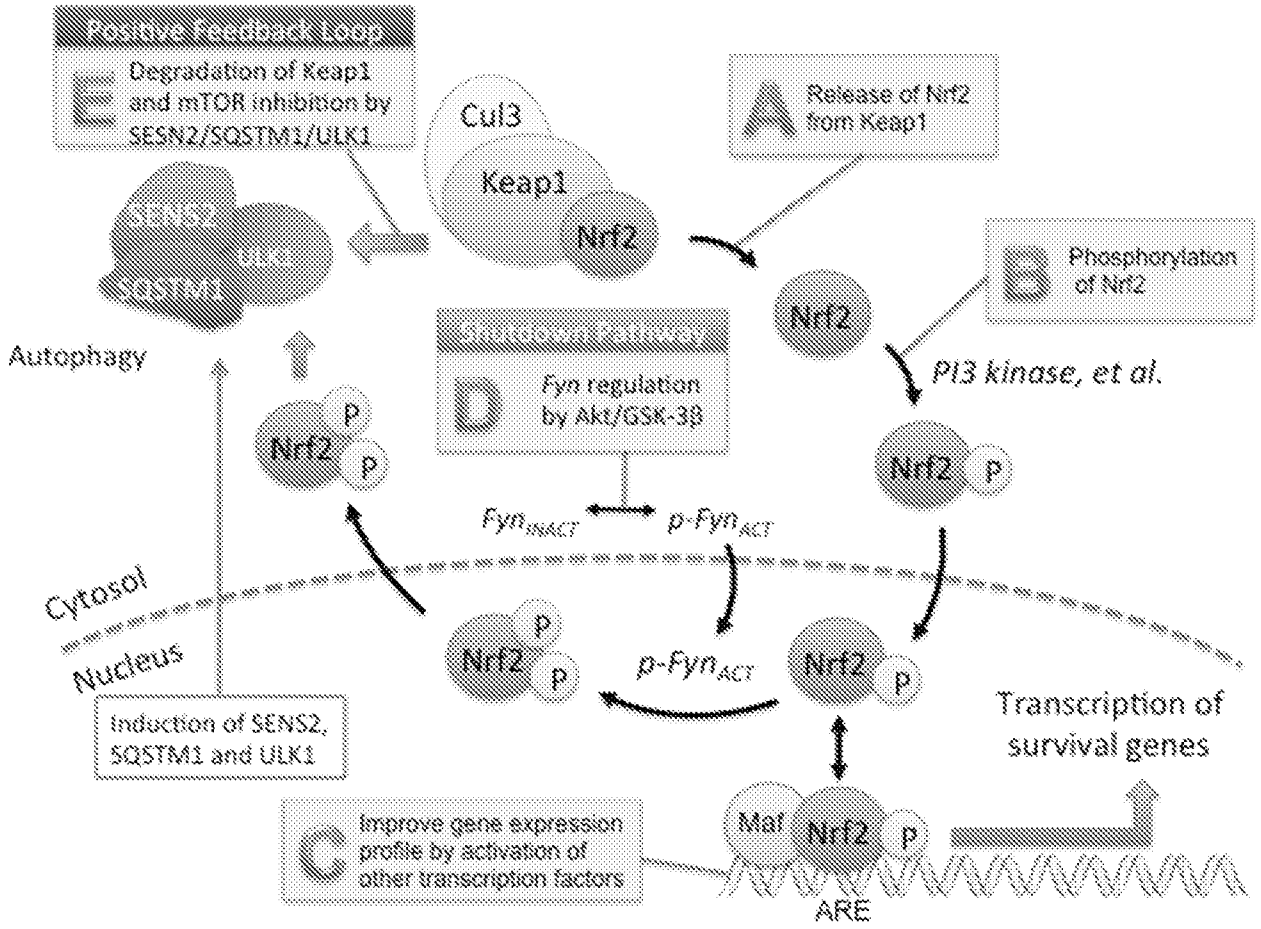
Positive feedback loops
E
Degradation of Keap1 and mTOR inhibition by SESN2/SQSTM1/ULK1
Cul3
Keap1
Nrf2
A
Release of Nrf2 from Keap1
B
Phosphorylation of Nrf2
Nrf2
PI3 kinase, et al.
SENS2
ULK1
SQSTM1
Autophagy
Negative feedback loop
D
Fyn regulation by Akt/GSK-3β
Nrf2
P
P
Fyn_INACT
p-Fyn_ACT
Cytosol
Nucleus
Nrf2
P
P
p-Fyn_ACT
Nrf2
P
Induction of SENS2, SQSTM1 and ULK1
Transcription of survival genes
C
Improve gene expression profile by activation of other transcription factors
Maf
Nrf2
P
ARE

FIG. 1

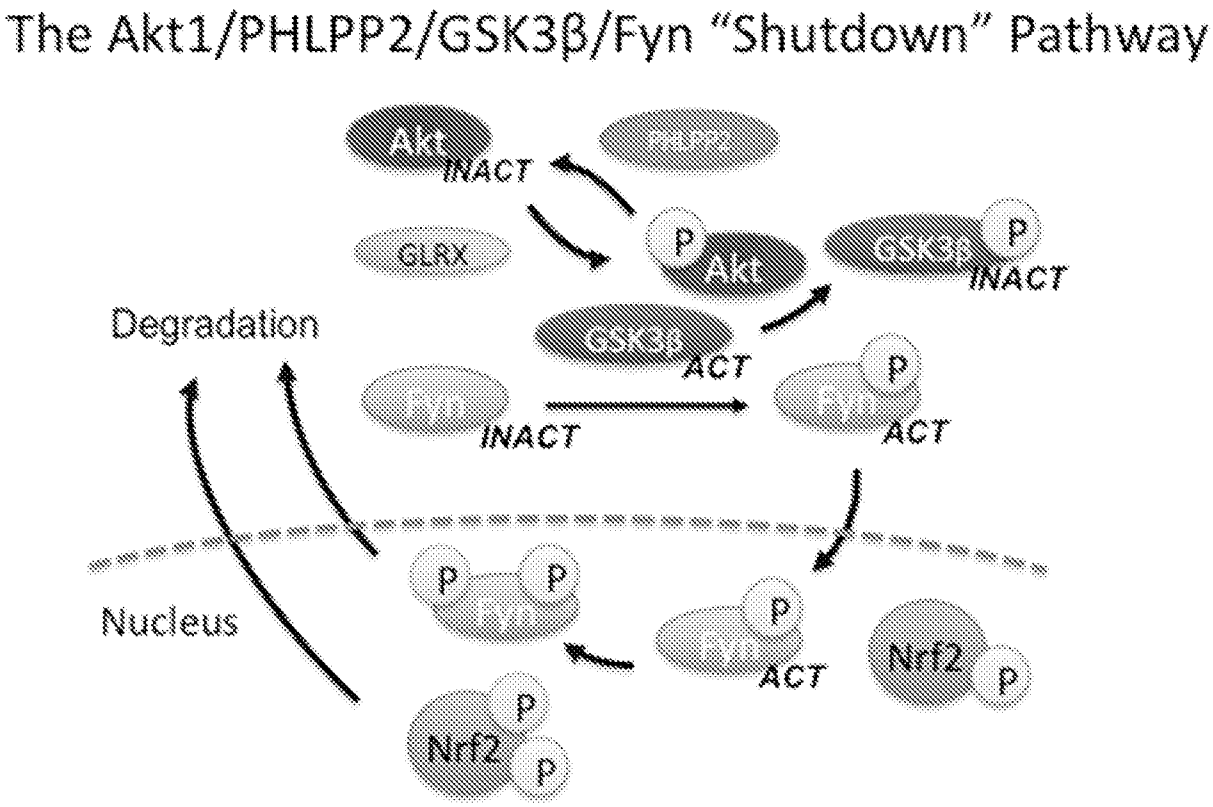
The Akt1/PHLPP2/GSK3β/Fyn "Shutdown" Pathway
Akt
INACT
PHLPP2
GLRX
P
Akt
GSK3β
P
INACT
Degradation
GSK3β
ACT
Fyn
INACT
P
Fyn
ACT
Nucleus
P
P
Fyn
P
Fyn
ACT
Nrf2
P
Nrf2
P
P

FIG. 2

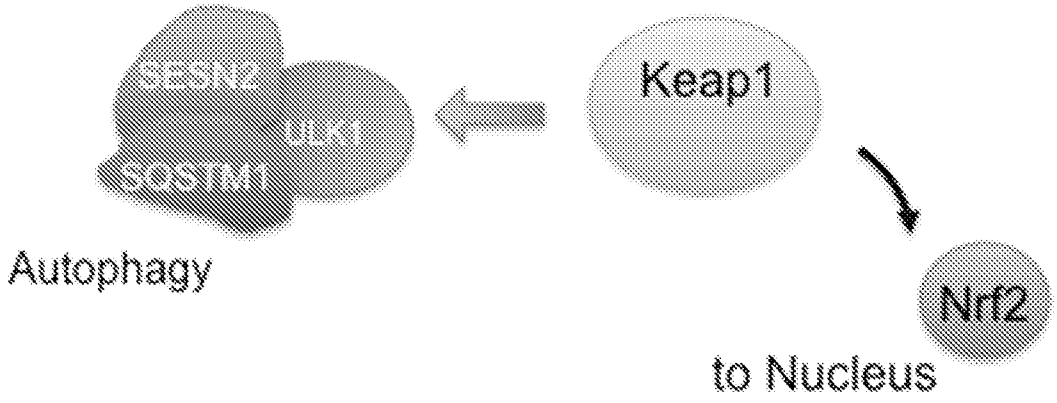
SESN2
ULK1
SQSTM1
Autophagy
Keap1
Nrf2
to Nucleus

FIG. 3

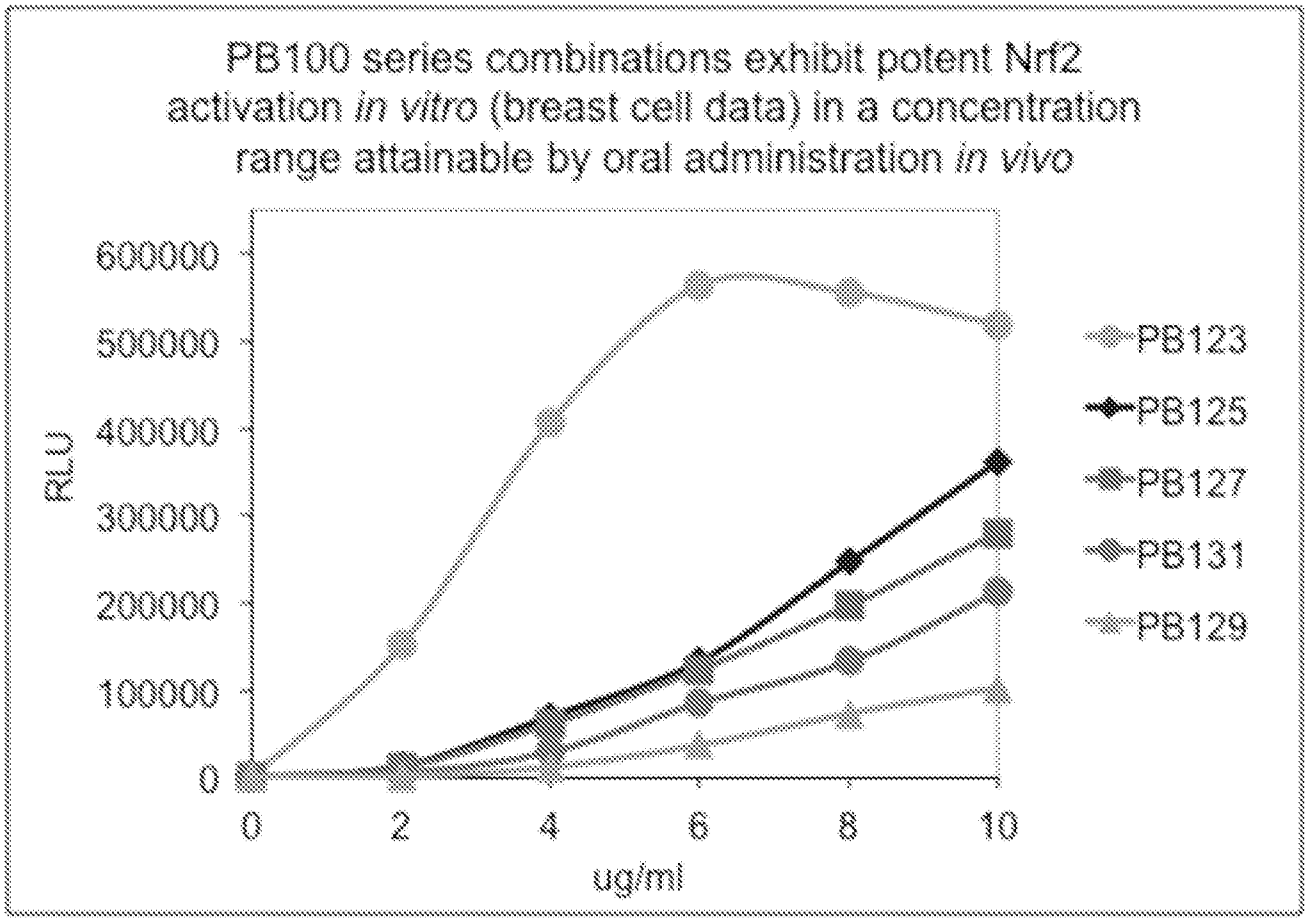
PB100 series combinations exhibit potent Nrf2 activation *in vitro* (breast cell data) in a concentration range attainable by oral administration *in vivo*
RLU
600000
500000
400000
300000
200000
100000
0
0
2
4
6
8
10
ug/ml
PB123
PB125
PB127
PB131
PB129

FIG. 4

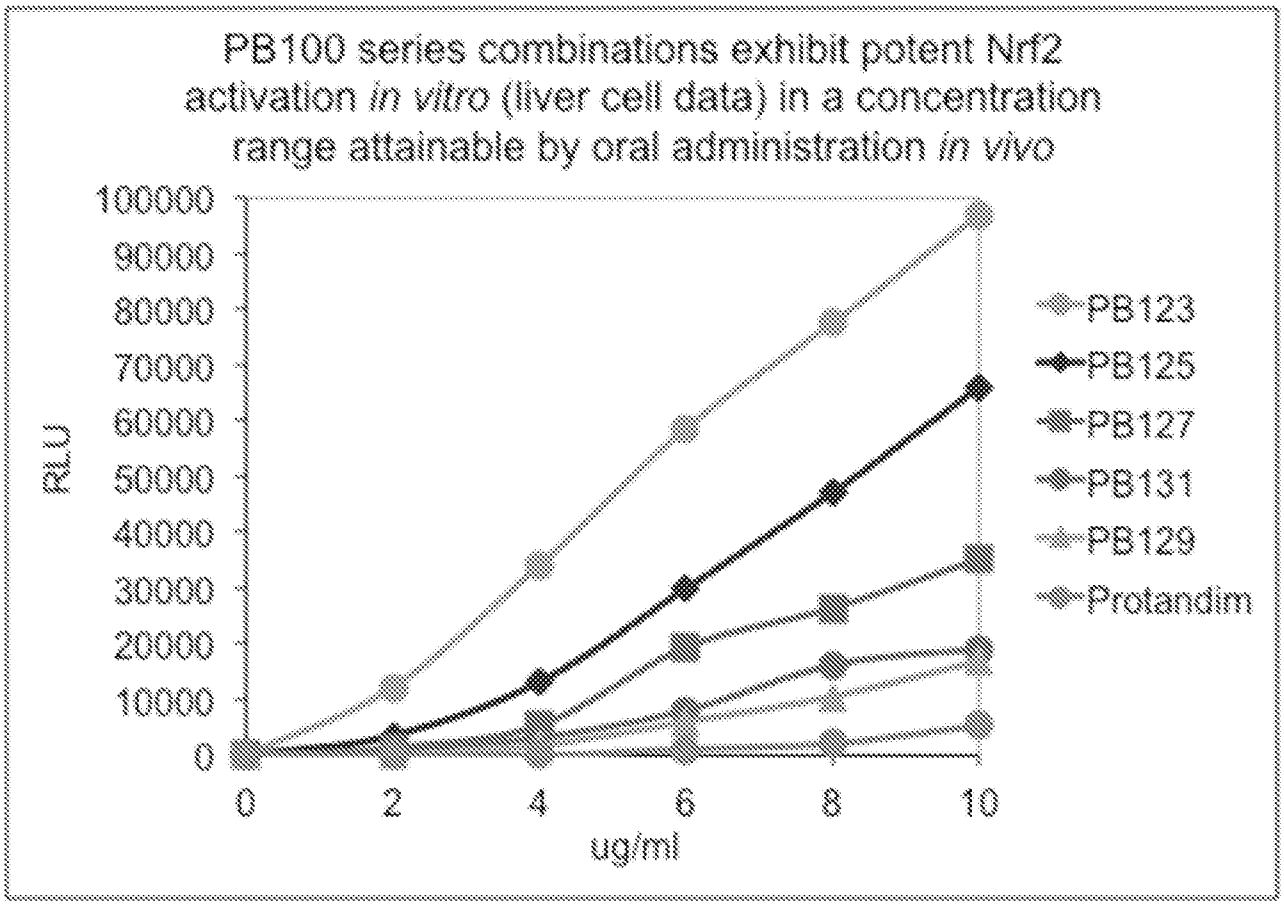
PB100 series combinations exhibit potent Nrf2 activation *in vitro* (liver cell data) in a concentration range attainable by oral administration *in vivo*
RLU
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0
2
4
6
8
10
ug/ml
PB123
PB125
PB127
PB131
PB129
Protandim

FIG. 5

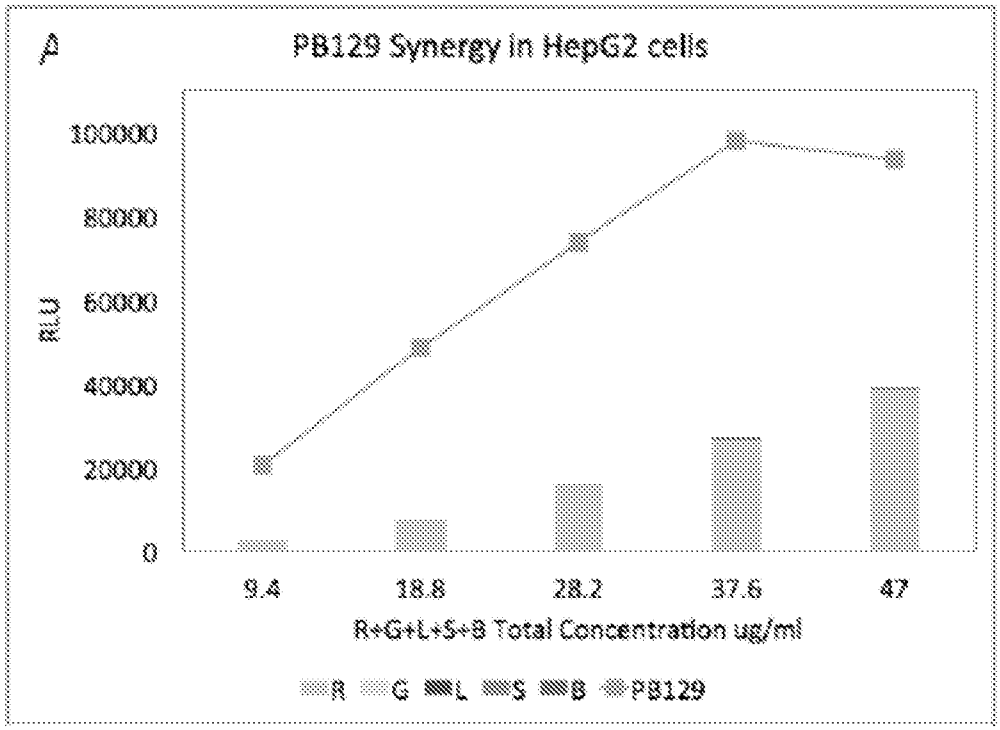
A
PB129 Synergy in HepG2 cells
RLU
100000
80000
60000
40000
20000
0
9.4
18.8
28.2
37.6
47
R+G+L+S+B Total Concentration ug/ml
R G L S B PB129

FIG. 6A

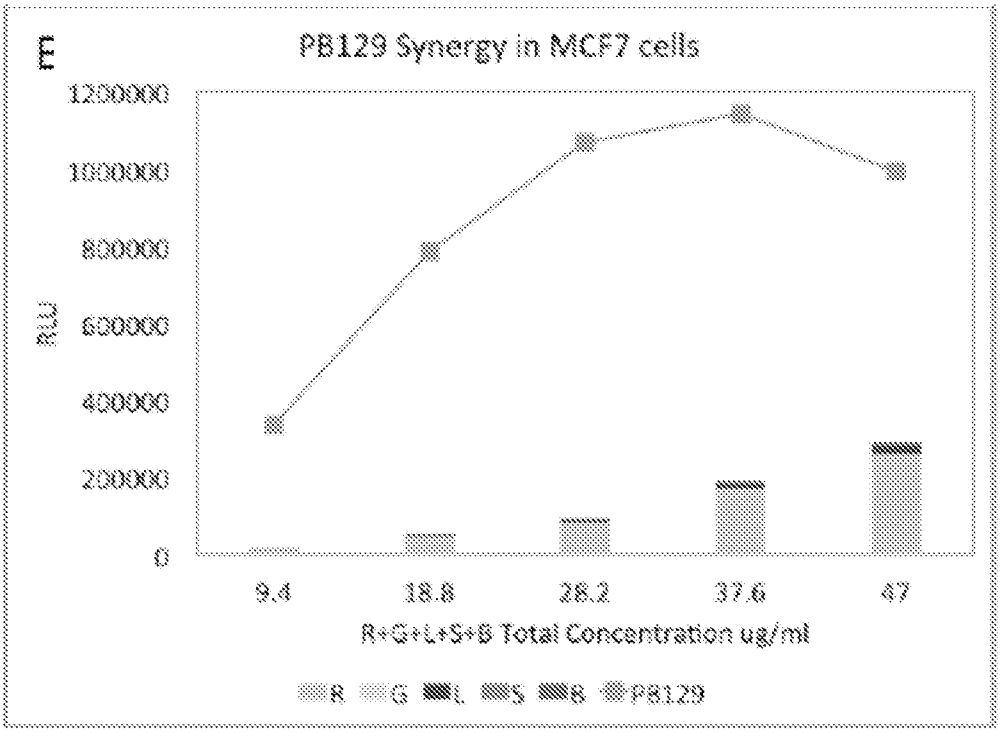
B
PB129 Synergy in MCF7 cells
RLU
1200000
1000000
800000
600000
400000
200000
0
9.4
18.8
28.2
37.6
47
R+G+L+S+B Total Concentration ug/ml
R G L S B PB129

FIG. 6B

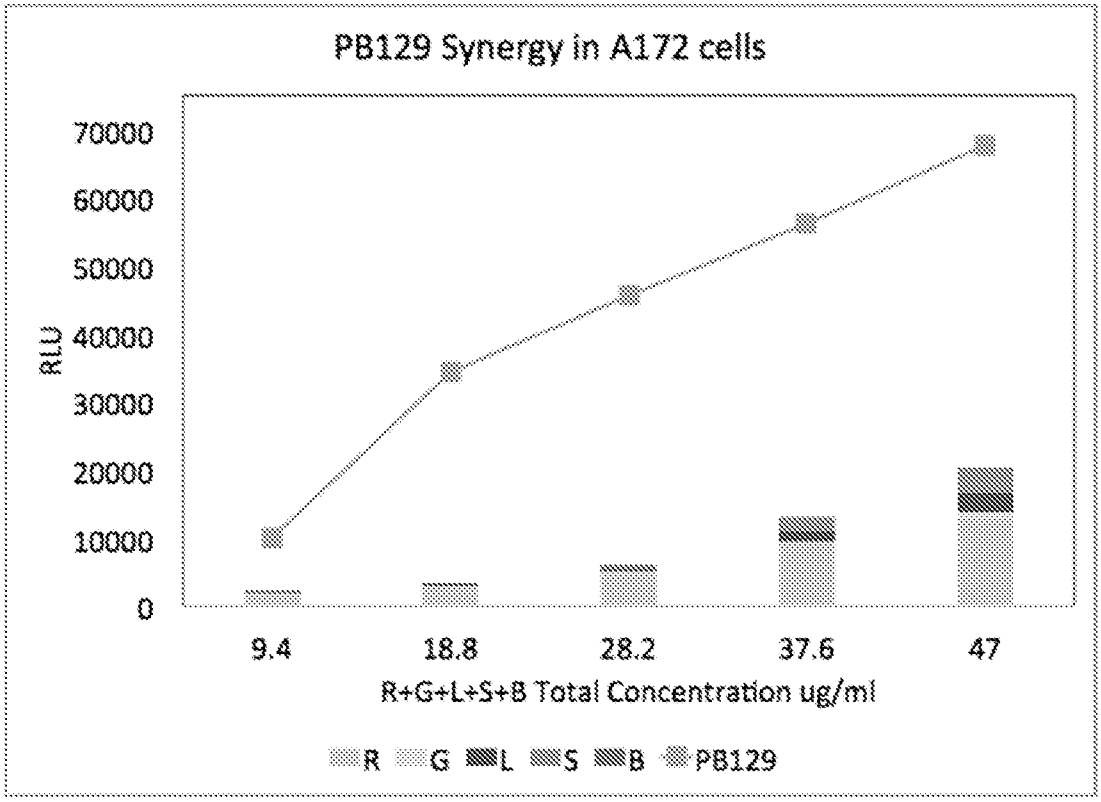
PB129 Synergy in A172 cells
70000
60000
50000
40000
30000
20000
10000
0
RLU
9.4
18.8
28.2
37.6
47
R+G+L+S+B Total Concentration ug/ml
R
G
L
S
B
PB129

FIG. 6C

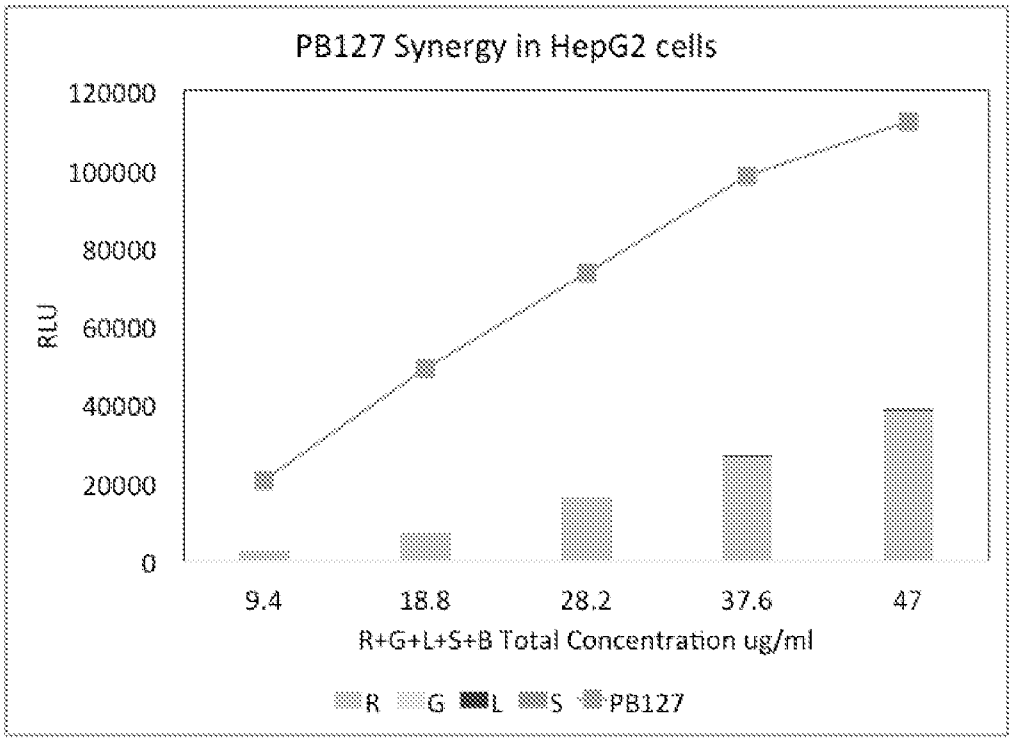
PB127 Synergy in HepG2 cells
120000
100000
80000
60000
40000
20000
0
RLU
9.4
18.8
28.2
37.6
47
R+G+L+S+B Total Concentration ug/ml
R
G
L
S
PB127

FIG. 7A

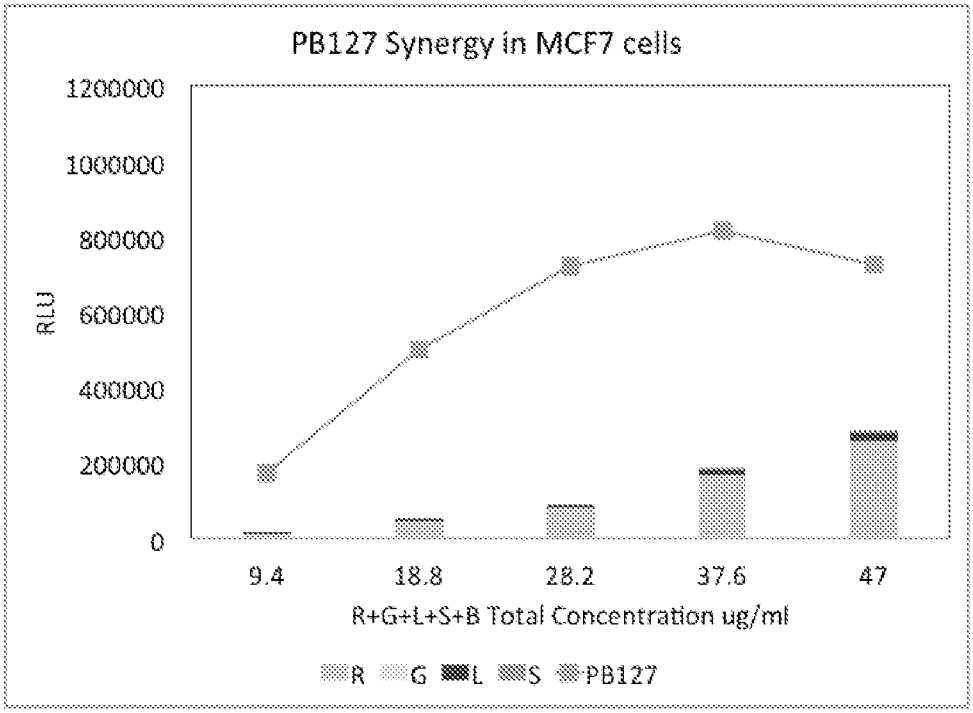
PB127 Synergy in MCF7 cells
1200000
1000000
800000
600000
400000
200000
0
RLU
9.4
18.8
28.2
37.6
47
R+G+L+S+B Total Concentration ug/ml
R G L S PB127

FIG. 7B

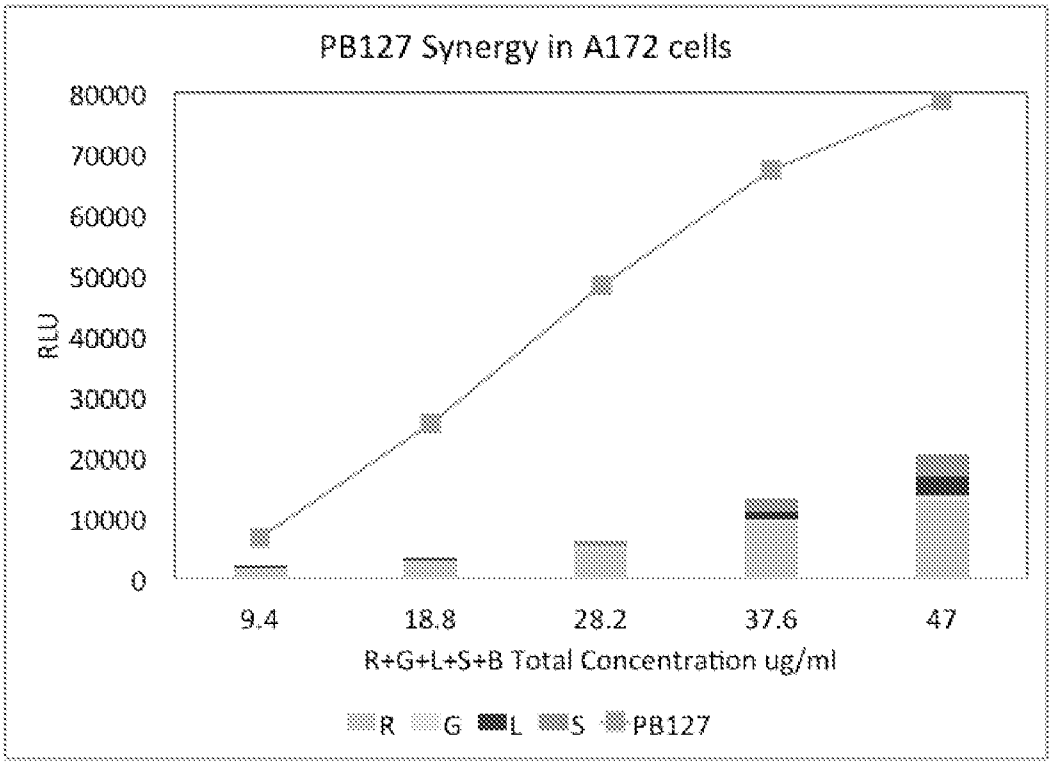
PB127 Synergy in A172 cells
80000
70000
60000
50000
40000
30000
20000
10000
0
RLU
9.4
18.8
28.2
37.6
47
R+G+L+S+B Total Concentration ug/ml
R G L S PB127

FIG. 7C

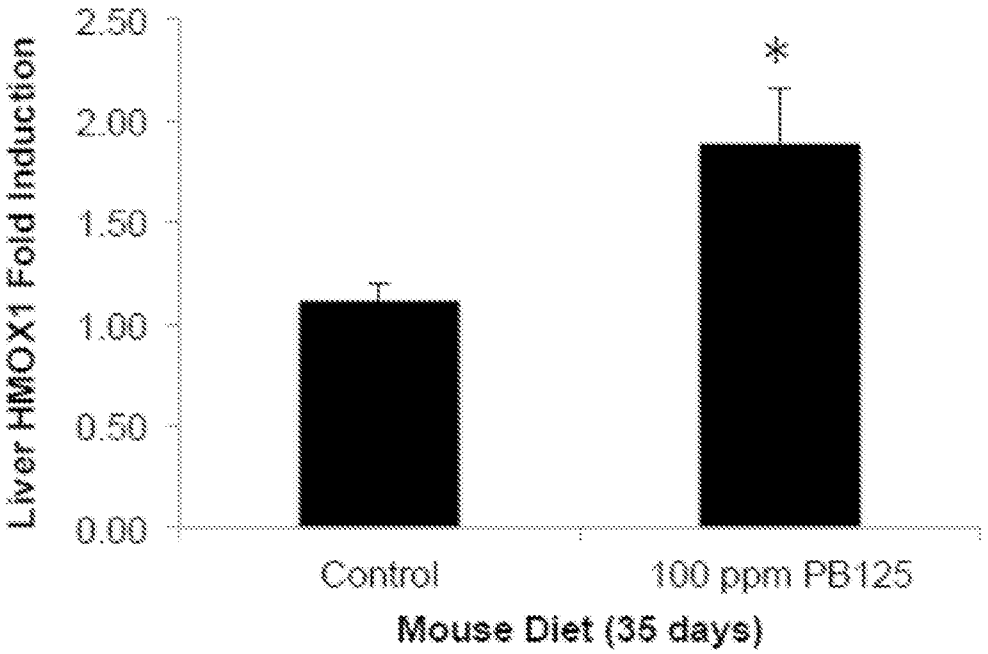
Liver HMOX1 Fold Induction
2.50
2.00
1.50
1.00
0.50
0.00
*
Control
100 ppm PB125
Mouse Diet (35 days)

FIG. 8

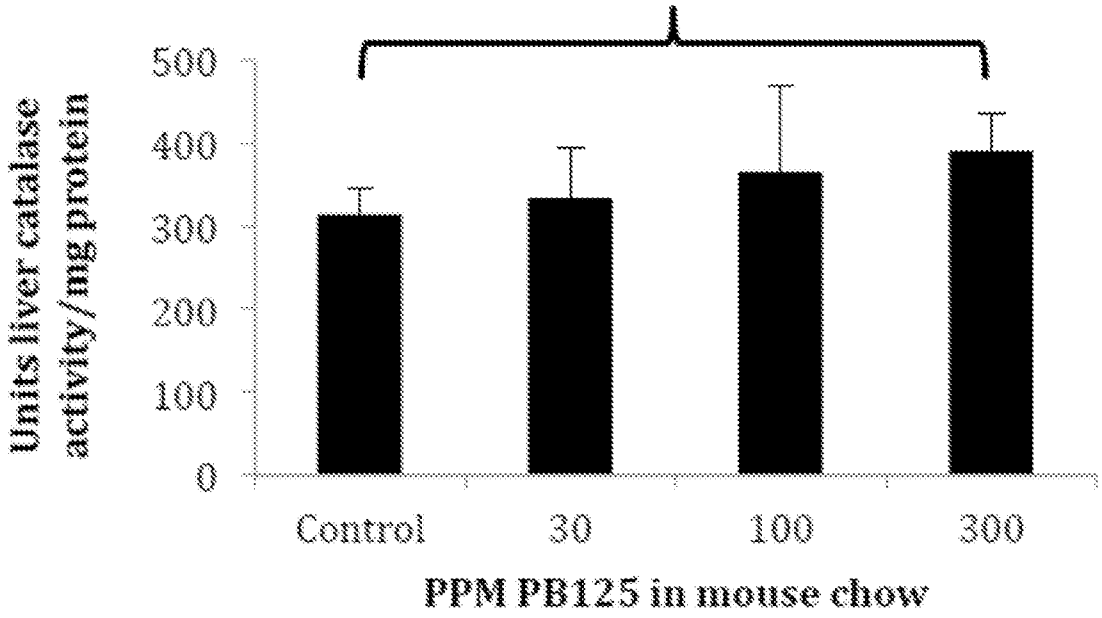
Units liver catalase activity/mg protein
500
400
300
200
100
0
Control
30
100
300
PPM PB125 in mouse chow

FIG. 9

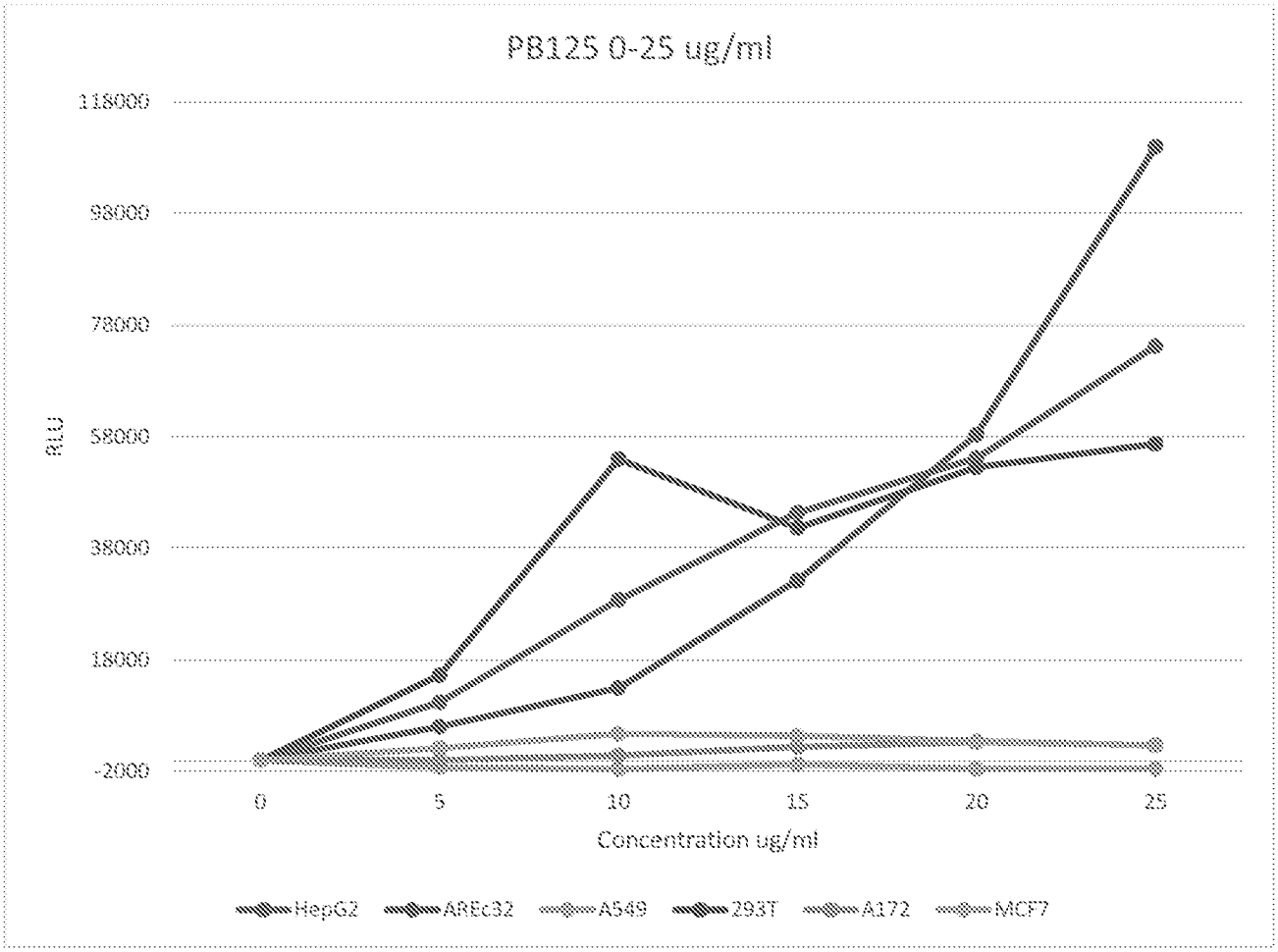
PB125 0-25 ug/ml
118000
98000
78000
58000
38000
18000
-2000
RLU
0
5
10
15
20
25
Concentration ug/ml
HepG2
ARE32
A549
293T
A172
MCF7

FIG. 10

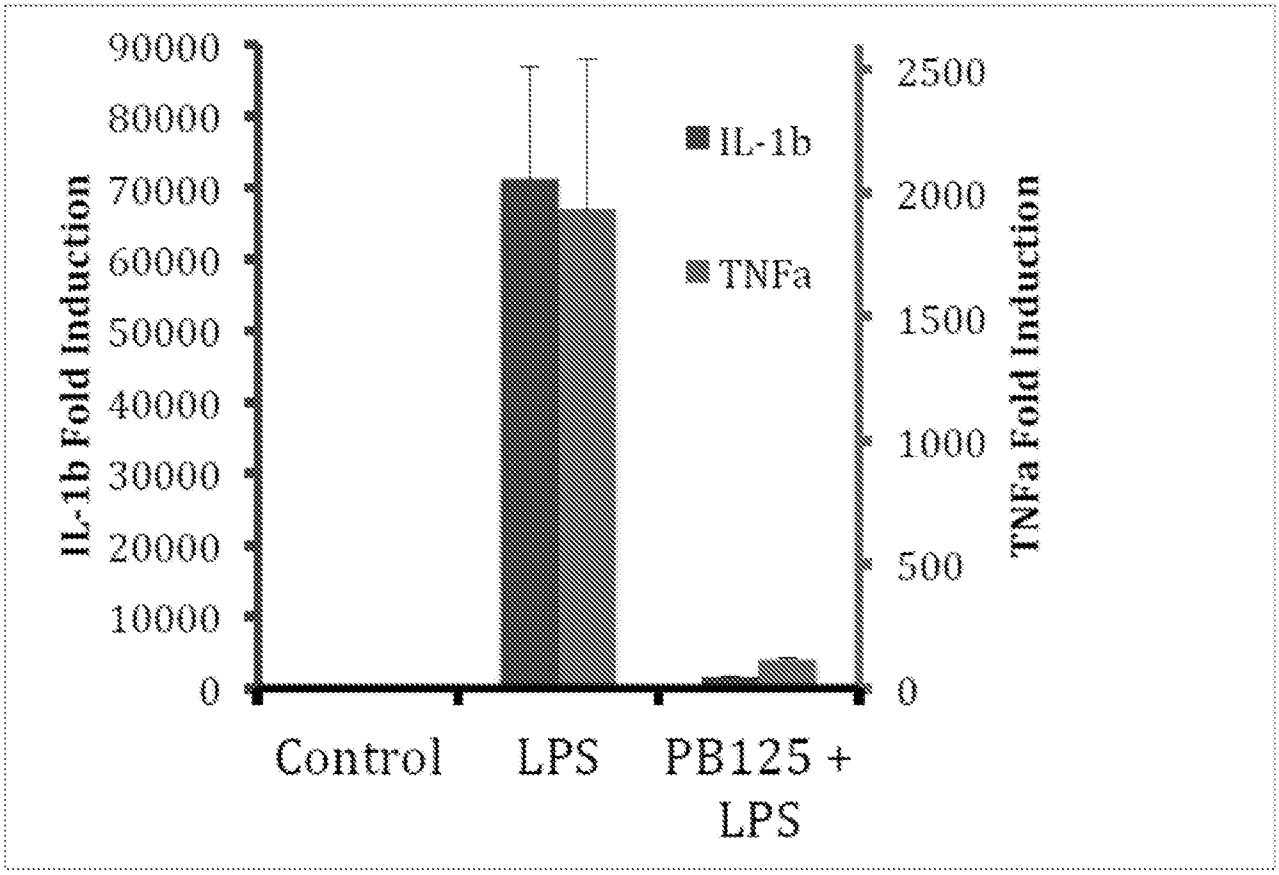
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
IL-1b Fold Induction
2500
2000
1500
1000
500
0
TNFa Fold Induction
IL-1b
TNFa
Control
LPS
PB125 + LPS

FIG. 11

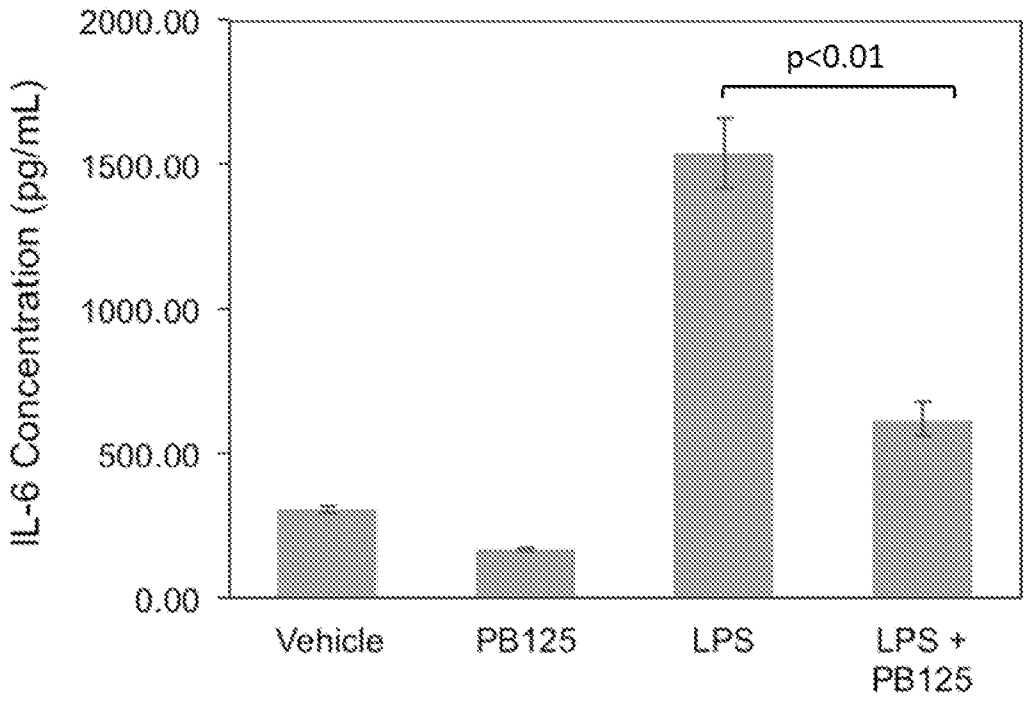
2000.00
1500.00
1000.00
500.00
0.00
IL-6 Concentration (pg/mL)
p<0.01
Vehicle
PB125
LPS
LPS + PB125

FIG. 12

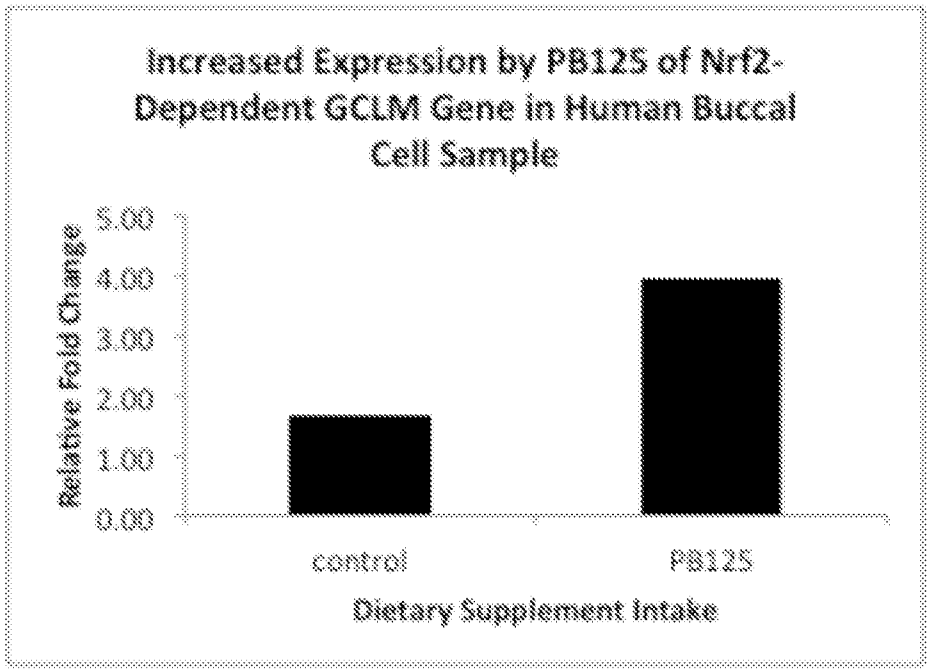
Increased Expression by PB125 of Nrf2-Dependent GCLM Gene in Human Buccal Cell Sample
Relative Fold Change
5.00
4.00
3.00
2.00
1.00
0.00
control
PB125
Dietary Supplement Intake

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/50292

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 25/00; A61K 31/426; A61K 31/4439 (2016.01)
CPC - A61K 31/19; A61K 31/26; A61K 31/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
CPC - A61K 31/19; A61K 31/26; A61K 31/12
IPC (8): A61P 25/00; A61K 31/426; A61K 31/4439 (2016.01)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, PubWest, ProQuest Dialog, Google
Search Terms: Nrf2, Nuclear factor-erythroid 2 related factor 2, pathway, carnosol, carnosic acid, shogaol, gingerol, luteolin, withaforin A, control point, rosemary, ginger, luteolin, ashwagandha, milk thistle, bacopa

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2014/0271944 A1 (McCord et al.) 18 Septmber 2014 (18.09.2014) Title, para [0113], [0116], [0159], [0232], [0281], [0326], [0345], Fig. 1, 34A,	16 ---------- 2-3, 5-13, 17-20
X ---- Y	US 2009/0304823 A1 (Offord Cavin et al.) 10 December 2009 (10.12.2009) para [0047], [0052], [0076], [0114], [0118], Table 1C,	1, 4, 14-15 ---------- 2-3, 5-13, 17-20
X ---- Y	WO 2014/151891 A1 (Walley) 25 Septmber 2014 (25.09.2014) abstract, para [0017], [0076]	16 ---- 1
X ---- Y	US 2014/0287071 A1 (Barnett, III) 25 Septmber 2014 (25.09.2014) abstarct, para [0005]	16 --- 1
X,P	WO 2016/037971 A1 (BIOS LINE S.P.A.) 17 March 2016 (17.03.2016) abstract	1, 16

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2016	02 DEC 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 108136209 A
(43)申请公布日 2018.06.08

(21)申请号 201680050947.0

(22)申请日 2016.09.02

(30)优先权数据
62/214,175 2015.09.03 US
62/355,810 2016.06.28 US

(85)PCT国际申请进入国家阶段日
2018.03.02

(86)PCT国际申请的申请数据
PCT/US2016/050292 2016.09.02

(87)PCT国际申请的公布数据
WO2017/041054 EN 2017.03.09

(71)申请人 路径生物科学有限公司
地址 美国科罗拉多州奥罗拉市

(72)发明人 布鲁克斯·迈克尔·海伯特松
乔·米尔顿·麦考德

(74)专利代理机构 北京天悦专利代理事务所（普通合伙）11311
代理人 田明 任晓航

(51)Int.Cl.
A61P 25/00(2006.01)
A61K 31/426(2006.01)
A61K 31/4439(2006.01)

权利要求书2页 说明书15页 附图10页

(54)发明名称

用于改进NRF2激活的组合物和其使用方法

(57)摘要

本文公开用于预防或处理与炎症或氧化应激相关联的某些健康病况的组合物和方法。这些组合物由含有激活Nrf2路径的植物化学成分的成分制备。还公开不同植物化学成分的协同效应。

CN 108136209 A

1.一种组合物,包含选自鼠尾草酚、鼠尾草酸、姜烯酚、姜醇、毛地黄黄酮和醉茄素A组成的组的两种或更多种植物化学成分,所述两种或更多种植物化学成分以有效激活核因子-红血球2相关因子2(Nrf2)路径的量存在于所述组合物中。

2.根据权利要求1所述的组合物,其中当向哺乳动物投予时,所述两种或更多种植物化学成分对所述Nrf2激活路径的至少两个不同控制点发挥其作用,所述控制点选自控制点A、B、C、D和E组成的组。

3.根据权利要求1所述的组合物,其中当向哺乳动物投予时,所述两种或更多种植物化学成分对Nrf2激活具有协同效应。

4.根据权利要求1所述的组合物,其中所述组合物包含选自迷迭香、姜、毛地黄黄酮和南非醉茄组成的组的至少两种成分。

5.根据权利要求4所述的组合物,其中所述组合物进一步包含选自水飞蓟和假马齿苋属组成的组的一种或多种植物化学成分。

6.根据权利要求4所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物和毛地黄黄酮,所述迷迭香提取物指定在5到10%鼠尾草酚下,所述姜提取物指定在10到20%6-姜烯酚下,所述毛地黄黄酮指定在95到99%毛地黄黄酮下,其中在所述组合物中在迷迭香提取物、姜提取物和毛地黄黄酮之间的比率为大约10:5:1(w/w)。

7.根据权利要求4所述的组合物,其中所述组合物包含迷迭香提取物、南非醉茄提取物和毛地黄黄酮,所述迷迭香提取物指定在5到10%鼠尾草酚下,所述南非醉茄提取物指定在1到3%醉茄素A下,所述毛地黄黄酮指定在95到99%毛地黄黄酮下,其中在所述组合物中在所述迷迭香提取物、南非醉茄提取物和毛地黄黄酮之间的所述比率为大约30:10:4(w/w)。

8.根据权利要求1所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物和毛地黄黄酮,并且其中在所述迷迭香提取物、姜提取物和毛地黄黄酮之间的所述比率为大约10:5:1(w/w)。

9.根据权利要求1所述的组合物,其中所述组合物包含迷迭香提取物、南非醉茄提取物和毛地黄黄酮,在所述迷迭香提取物、南非醉茄提取物和毛地黄黄酮之间的所述比率为大约30:10:4(w/w)。

10.根据权利要求5所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物、毛地黄黄酮和水飞蓟提取物,在所述迷迭香提取物、姜提取物、毛地黄黄酮和水飞蓟提取物之间的所述比率为大约10:5:1:30(w/w)。

11.根据权利要求5所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物、毛地黄黄酮、水飞蓟提取物和假马齿苋提取物,在所述迷迭香提取物、姜提取物、毛地黄黄酮、水飞蓟提取物和假马齿苋提取物之间的所述比率为大约10:5:1:30:48(w/w)。

12.根据权利要求5所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物、毛地黄黄酮和假马齿苋提取物,在所述迷迭香提取物、姜提取物、毛地黄黄酮和假马齿苋提取物之间的所述比率为大约10:5:1:48(w/w)。

13.根据权利要求1所述的组合物,其中所述组合物用于预防和/或处理选自以下组成的组的疾病或病况:氧化应激、解毒、炎症、癌症或相关疾病或病况。

14.根据权利要求1所述的组合物,其中所述组合物用作营养补充剂。

15.根据权利要求1所述的组合物,其中所述组合物呈片剂、胶囊、软凝胶、液体、乳液、

凝胶、粉末、软膏或气溶胶形式。

16.一种处理和/或预防疾病或病况的方法，包含以下步骤：向哺乳动物投予组合物，所述组合物包含选自鼠尾草酚、鼠尾草酸、姜烯酚、姜醇、毛地黄黄酮和醉茄素A组成的组的一种或多种植物化学成分，所述一种或多种植物化学成分以有效激活Nrf2(NF-E2相关因子2)路径的量存在于所述组合物中。

17.根据权利要求16所述的方法，其中所述组合物包含迷迭香提取物、南非醉茄提取物和毛地黄黄酮，其中所述迷迭香提取物指定在5到10％鼠尾草酚下，所述南非醉茄提取物指定在1到3％醉茄素A下，并且所述毛地黄黄酮指定在95到99％毛地黄黄酮下，在所述迷迭香提取物、南非醉茄提取物和毛地黄黄酮之间的比率为大约30:10:4(w/w)。

18.根据权利要求16所述的方法，其中所述组合物包含迷迭香提取物、姜提取物和毛地黄黄酮，所述迷迭香提取物指定在5到10％鼠尾草酚下，所述姜提取物指定在10到20％6-姜烯酚下，并且所述毛地黄黄酮指定在95到99％毛地黄黄酮下，在所述迷迭香提取物、姜提取物和毛地黄黄酮之间的所述比率为大约10:5:1(w/w)。

19.根据权利要求18所述的方法，其中所述组合物以10到1000毫克/每天经口向人类投予。

20.根据权利要求18所述的方法，其中所述组合物包含选自鼠尾草酚、鼠尾草酸、姜烯酚、姜醇、毛地黄黄酮和醉茄素A组成的组的至少两种植物化学成分，其中所述至少两种植物化学成分对Nrf2激活路径的至少两个不同控制点发挥其作用，所述控制点选自控制点A、B、C、D和E组成的组。

用于改进NRF2激活的组合物和其使用方法

[0001] 相关申请

[0002] 本申请要求2015年9月3日提交的美国专利申请62/214,175和2016年6月28日提交的美国专利申请62/355,810的优先权,所述专利的全部内容由此以引用的方式并入到本申请中。

背景技术

[0003] I.技术领域

[0004] 本公开涉及用于预防或处理某些健康病况的方法和组合物。更具体地说,本公开涉及用于预防或处理与炎症和/或氧化应激相关联的某些健康病况的组合物和方法。

[0005] II.现有技术的说明

[0006] 核因子-红血球2相关因子2(Nrf2)为通过Kelch类ECH相关蛋白1(Keap1)调节的转录因子。Nrf2通过被称为抗氧化反应元件(ARE)的强化子序列调节各种细胞保护的II期解毒酶和抗氧化酶的基因表达(Maher和Yamamoto 2010,Satoh,Moriguchi等人2010)。与氧化应激相关,ARE为在包括超氧化歧化酶(SOD)、过氧化物还原酶、硫氧还蛋白、催化酶、麸胱甘肽过氧化酶和血红素加氧酶-1(HO-1)的许多抗氧化酶中发现的启动子元件。Nrf2在针对氧化应激的ARE-驱动细胞防御系统中起关键作用。参见,Kensler,Wakabayashi等人2010;Hybertson和Gao 2014,Bocci和Valacchi 2015,Huang,Li等人2015,Johnson和Johnson 2015,Moon和Giaccia 2015,Petiwala和Johnson 2015,Sekhar和Freeman 2015,Suzuki和Yamamoto 2015。

发明内容

[0007] 本发明所公开的手段通过提供激活Nrf2细胞信号传导路径的试剂的组合推进所属领域。在一个实施例中,试剂的组合可比单一试剂更有效地激活Nrf2路径。。在另一个实施例中,试剂的组合可协同激活Nrf2路径。

[0008] 在一个实施例中,本文公开多于一种成分的组合。在一个方面,每种成分可含有一种或多种植物化学成分。在另一个方面,这些植物化学成分可在迷迭香(rosemary/Rosmarinus officinalis)、姜(生姜)、毛地黄黄酮(来自槐花)、水飞蓟(milk thistle/Silybum marianum)和假马齿苋属(假马齿苋)中发现。在另一个方面,植物化学成分组分为鼠尾草酚、姜烯酚、毛地黄黄酮、水飞蓟素和假马齿苋皂素,其可分别在迷迭香、姜、毛地黄黄酮、水飞蓟和假马齿苋属中发现。在另一个方面,,所公开的组合物通过Nrf2-依赖性路径诱导ARE-调节的抗氧化基因。

[0009] 在另一个实施例中,公开迷迭香、南非醉茄和毛地黄黄酮的特定组合(在本文中被称作PB125)、迷迭香、姜、毛地黄黄酮和水飞蓟素的特定组合(在本文中被称作PB127),和迷迭香、姜、毛地黄黄酮、水飞蓟素和假马齿苋属的特定组合(在本文中被称作PB129)。在另一个实施例中,这些试剂的组合可导致协同Nrf2激活,大于仅其单一Nrf2激活贡献的总和。活性剂或试剂的组合可为可能药物开发的候选者。参见例如Koehn和Carter 2005,Lee 2010。

[0010] 在另一个实施例中，所公开的组合物可含有迷迭香(鼠尾草酚)、姜(6-姜烯酚和6-姜醇)、南非醉茄(醉茄素A)、水飞蓟(水飞蓟素)、假马齿苋(假马齿苋皂素)和毛地黄黄酮。

[0011] 在一个方面，组合物可经口施用，例如呈片剂、胶囊、软凝胶、糖浆、水溶液或悬浮液、醇提取物或粉末形式。在另一个方面，协同组合物可呈气溶胶形式施用，例如呈吸入和部分沉积在肺呼吸道内的细气溶胶雾或粉末形式施用到肺。在另一个方面，所公开的组合物可通过局部施用来施用，例如通过呈乳液、凝胶、软膏、水性喷雾形式向皮肤施用或在绷带内施用到皮肤或伤口。

[0012] 在另一个实施例中，所公开的组合物可含有分别以10:5:1的质量比的迷迭香提取物(指定在5到10％鼠尾草酚下)、姜提取物(指定在1-10％6-姜烯酚和/或10-25％6-姜醇下)，和毛地黄黄酮(指定在95-98％毛地黄黄酮下)的组合。此式在本公开中也被称作PB123。

[0013] 在另一个实施例中，所公开的组合物可含有分别以30:10:4的质量比的迷迭香提取物(指定在5到10％鼠尾草酚下)、南非醉茄提取物(指定在1-3％醉茄素A下)，和毛地黄黄酮(指定在95-98％毛地黄黄酮下)的组合。此式在本公开中也被称作PB125。

[0014] 在另一个实施例中，所公开的组合物可含有分别以10:5:1:30的质量比的迷迭香提取物(指定在5到10％鼠尾草酚下)、姜提取物(指定在1-10％6-姜烯酚和/或10-25％6-姜醇下)、毛地黄黄酮(指定在90-100％毛地黄黄酮下)和水飞蓟提取物(指定在50-90％水飞蓟素下)的组合。此式在本公开中也被称作PB127。

[0015] 在另一个实施例中，所公开的组合物可含有分别以10:5:1:30:48的质量比的迷迭香提取物(指定在5到10％鼠尾草酚下)、姜提取物(指定在1-10％6-姜烯酚和/或10-25％6-姜醇下)、毛地黄黄酮(指定在90-100％毛地黄黄酮下)、水飞蓟提取物(指定在50-90％水飞蓟素下)和假马齿苋提取物(指定在10-60％假马齿苋皂素下)的组合。此式在本公开中也被称作PB129。

[0016] 在另一个实施例中，所公开的组合物可含有分别以10:5:1:48的质量比的迷迭香提取物(指定在5到10％鼠尾草酚下)、姜提取物(指定在1-10％6-姜烯酚和/或10-25％6-姜醇下)、毛地黄黄酮(指定在90-100％毛地黄黄酮下)和假马齿苋提取物(指定在10-60％假马齿苋皂素下)的组合。此式在本公开中也被称作PB131。

[0017] 在另一个实施例中，PB123在向人类口服施用时可以10到1000毫克/每天施用。举例来说，其可作为丸剂、软凝胶或胶囊施用以诱导Nrf2激活和/或降低炎症和氧化应激，和/或以改进整体健康和康乐。

[0018] 在另一个实施例中，PB123在向人类口服施用时可以10到1000毫克/每天施用，以改进蛋白稳态，和/或预防在人体中与蛋白稳态和/或自噬相关联的老化相关问题。

[0019] 在另一个实施例中，PB125或PB127或PB129或PB131在向人类口服施用时可以10到1000毫克/每天施用。举例来说，其可作为丸剂、软凝胶或胶囊施用以诱导Nrf2激活和/或降低炎症和氧化应激，和/或以改进整体健康和康乐。

[0020] 在另一个实施例中，PB125或PB127或PB129或PB131在向人类口服施用时可以10到1000毫克/每天施用，以改进蛋白稳态，和/或预防在人体中与蛋白稳态和/或自噬相关联的老化相关问题。

附图说明

[0021] 图1示出Nrf2激活路径和控制点。

[0022] 图2示出“停工路径”-核Nrf2的Fyn-依赖性失活。

[0023] 图3示出“正反馈回路”-通过Nrf2-诱导的基因产物的Keap1降解。

[0024] 图4示出在经转染的乳癌细胞系中通过PB123、PB125、PB127、PB129和PB131诱导的Nrf2激活。

[0025] 图5示出在经转染的肝癌细胞系中通过PB123、PB125、PB127、PB129和PB131诱导的Nrf2激活。

[0026] 图6A-6C示出在HepG2(人类肝脏,图6A)、MCF7(人类乳房,图6B)和A172(人类大脑,图6C)癌细胞系中通过PB129诱导的Nrf2激活的协同效应。

[0027] 图7A-7C示出在HepG2(人类肝脏,图7A)、MCF7(人类乳房,图7B)和A172(人类大脑,图7C)癌细胞系中通过PB127诱导的Nrf2激活的协同效应。

[0028] 图8示出体内鼠肝脏HMOX1基因表达的提高。

[0029] 图9示出在膳食中通过PB125诱导的肝脏催化酶活性。

[0030] 图10示出在稳定经转染的HepG2(人类肝脏)、AREc32(人类乳房)、MCF7(人类乳房)、A549(人类肺)、293T(人类肾脏)和A172(人类大脑)癌细胞系中在通过用PB125处理诱导ARE-驱动荧光素酶基因表达之后借助添加的荧光素观察到的相对光单位(RLU)的重叠图。通过每mL的培养液5、10、15、20和25微克的PB125,在肝脏、肾脏和乳房细胞系中观察到强Nrf2激活。

[0031] 图11示出PB125降低炎症基因的LPS-诱导表达。

[0032] 图12示出PB125降低IL-6的LPS-诱导表达。

[0033] 图13示出由于施用PB125较高的GCLM基因表达。

具体实施方式

[0034] Nrf2/ARE路径已涉及氧化应激的控制(Eggler,Gay等人2008,Cho和Kleeberger 2010,Huang,Li等人2015,Johnson和Johnson 2015)。靶向Nrf2/ARE路径的某些试剂和这类试剂的组合(例如PB125)可对细胞功能和存活率具有有益作用。在一个实施例中,这些试剂和其组合可缓解炎症反应和氧化应激,并且可对健康和康乐具有有益作用。

[0035] 之前研究尚未证明直接抗氧化维生素或补充剂(如维生素C和E、类胡萝卜素、N-乙酰半胱氨酸)和与反应性氧物质(ROS)(如过氧化物和过氧化氢)化学计量反应的其它化合物的治疗可能性。本文,通过使用Nrf2激活组合证明改进的抗氧化防御(Koehn 2006,Eggler,Gay等人2008,Boutten,Goven等人2010,Cho和Kleeberger 2010)。

[0036] 在本公开中,多种试剂以新颖方式组合,即,通过在Nrf2激活路径中的不同控制点处起作用。图1示出Nrf2激活路径和控制点A、B、C、D和E,在所述控制点处,在那些控制点处起作用的低浓度的试剂一起起作用以通过组合(如PB125、PB127和PB129)影响期望Nrf2-依赖性基因表达。在基础状态下,Nrf2通过靶向Nrf2用于通过蛋白酶体多聚泛素化和降解的Kelch类ECH相关蛋白1(Keap1)隔离并且保持非活性。A.Nrf2激活涉及Keap1的特定硫醇残余物的氧化,使其从Keap1释放Nrf2。B.Nrf2磷酸化可在将其靶向用于核输入中起作用。

C.Nrf2易位到细胞核中使得Nrf2能够结合含有抗氧化反应元件(ARE)的启动子,引发细胞保护的编程的转录。D.非活性胞质Fyn可通过GSK3β磷酸化,并且此现在活性p-Fyn易位到细胞核,其中其可在第二部位处磷酸化Nrf2导致核输出和降解。E.“正反馈回路”涉及通过Nrf2诱导的基因产物SESN2、SQSTM1和ULK1。SESN2、SQSTM1和ULK1协作以激活Keap1的自噬,释放更多Nrf2,这诱导更多这些基因产物,倾向于一旦此正反馈回路已被触发就维持Nrf2激活。

[0037] 另外在本公开中,与基于先前技术预测并且还基于检验每种试剂单独的Nrf2激活特性的同时实验的水平和基于将其一起添加预测的水平相比,试剂的组合产生出人意料高的Nrf2激活水平。通过试剂的组合的Nrf2激活示出协同效应。参见例如图6和7。

[0038] 本公开的一个实施例包含食疗剂的组合-如在PB125、PB127和PB129组合中-其通过不同特定控制点的接合作用于Nrf2激活使得试剂的组合协同激活Nrf2路径。因此作用于Nrf2信号传导路径的不同控制点以提高Nrf2-依赖性基因的表达的试剂的新组合为新颖的。

[0039] 作为实例,下文列出本公开的多个实施例:

[0040] 项目1.一种组合物,包含选自鼠尾草酚、鼠尾草酸、姜烯酚、姜醇、毛地黄黄酮和醉茄素A组成的组的两种或更多种植物化学成分,所述一种或多种植物化学成分以有效激活核因子-红血球2相关因子2(Nrf2)路径的量存在于组合物中。

[0041] 项目2.根据项目1所述的组合物,其中当向哺乳动物施用时,所述两种或更多种植物化学成分对所述Nrf2激活路径的至少两个不同控制点发挥其作用,所述控制点选自控制点A、B、C、D和E组成的组。在一个实施例中,所述植物化学成分中的至少一种对一个控制点发挥其作用,同时至少另一种植物化学成分对所述Nrf2激活路径的不同控制点发挥其作用,如在图1中所描绘。

[0042] 项目3.根据前述项目中任一项所述的组合物,其中当向哺乳动物施用时,所述两种或更多种植物化学成分对Nrf2激活具有协同效应。

[0043] 项目4.根据前述项目中任一项所述的组合物,其中所述组合物包含选自迷迭香、姜、毛地黄黄酮和南非醉茄组成的组的至少两种成分。

[0044] 项目5.根据前述项目中任一项所述的组合物,其中所述组合物另外包含选自水飞蓟和假马齿苋属组成的组的一种或多种植物化学成分。

[0045] 项目6.根据前述项目中任一项所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物和毛地黄黄酮,所述迷迭香提取物指定在5-10%鼠尾草酚下,所述姜提取物指定在10-20%6-姜烯酚下,所述毛地黄黄酮指定在95-99%毛地黄黄酮下,其中在所述组合物中在迷迭香提取物、姜提取物和毛地黄黄酮之间的比率为大约10:5:1(w/w)。

[0046] 项目7.根据前述项目中任一项所述的组合物,其中所述组合物包含迷迭香提取物、南非醉茄提取物和毛地黄黄酮,所述迷迭香提取物指定在5-10%鼠尾草酚下,所述南非醉茄提取物指定在1-3%醉茄素A下,所述毛地黄黄酮指定在95-99%毛地黄黄酮下,其中在所述组合物中在所述迷迭香提取物、南非醉茄提取物和毛地黄黄酮之间的所述比率为大约30:10:4(w/w)。

[0047] 项目8.根据前述项目中任一项所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物和毛地黄黄酮,并且其中在所述迷迭香提取物、姜提取物和毛地黄黄酮之间的

所述比率为大约10:5:1(w/w)。

[0048] 项目9.根据前述项目中任一项所述的组合物,其中所述组合物包含迷迭香提取物、南非醉茄提取物和毛地黄黄酮,在所述迷迭香提取物、南非醉茄提取物和毛地黄黄酮之间的所述比率为大约30:10:4(w/w)。

[0049] 项目10.根据前述项目中任一项所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物、毛地黄黄酮和水飞蓟提取物,在所述迷迭香提取物、姜提取物、毛地黄黄酮和水飞蓟提取物之间的所述比率为大约10:5:1:30(w/w)。

[0050] 项目11.根据前述项目中任一项所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物、毛地黄黄酮、水飞蓟提取物和假马齿苋提取物,在所述迷迭香提取物、姜提取物、毛地黄黄酮、水飞蓟提取物和假马齿苋提取物之间的所述比率为大约10:5:1:30:48(w/w)。

[0051] 项目12.根据前述项目中任一项所述的组合物,其中所述组合物包含迷迭香提取物、姜提取物、毛地黄黄酮和假马齿苋提取物,在所述迷迭香提取物、姜提取物、毛地黄黄酮和假马齿苋提取物之间的所述比率为大约10:5:1:48(w/w)。

[0052] 项目13.根据前述项目中任一项所述的组合物,其中所述组合物用于预防和/或处理选自以下组成的组的疾病或病况:氧化应激、解毒、炎症、癌症或相关疾病或病况。

[0053] 项目14.根据前述项目中任一项所述的组合物,其中所述组合物用作营养补充剂。

[0054] 项目15.根据前述项目中任一项所述的组合物,其中所述组合物呈片剂、胶囊、软凝胶、液体、乳液、凝胶、粉末、软膏或气溶胶形式。

[0055] 项目16.一种处理和/或预防疾病或病况的方法,包含以下步骤:向哺乳动物施用组合物,所述组合物包含选自鼠尾草酚、鼠尾草酸、姜烯酚、姜醇、毛地黄黄酮和醉茄素A组成的组的一种或多种植物化学成分,所述一种或多种植物化学成分以有效激活Nrf2(NF-E2相关因子2)路径的量存在于所述组合物中。

[0056] 项目17.根据前述项目中任一项所述的方法,其中所述组合物包含迷迭香提取物、南非醉茄提取物和毛地黄黄酮,其中所述迷迭香提取物指定在5-10%鼠尾草酚下,所述南非醉茄提取物指定在1-3%醉茄素A下,并且所述毛地黄黄酮指定在95-99%毛地黄黄酮下,在所述迷迭香提取物、南非醉茄提取物和毛地黄黄酮之间的所述比率为大约30:10:4(w/w)。

[0057] 项目18.根据项目17所述的方法,其中所述组合物包含迷迭香提取物、姜提取物和毛地黄黄酮,所述迷迭香提取物指定在5-10%鼠尾草酚下,所述姜提取物指定在10-20%6-姜烯酚下,并且所述毛地黄黄酮指定在95-99%毛地黄黄酮下,在所述迷迭香提取物、姜提取物和毛地黄黄酮之间的所述比率为大约10:5:1(w/w)。

[0058] 项目19.根据项目17-18中任一项所述的方法,其中所述组合物以10-1000毫克/每天经口向人类施用。

[0059] 项目20.根据项目17-19中任一项所述的方法,其中所述组合物包含选自鼠尾草酚、鼠尾草酸、姜烯酚、姜醇、毛地黄黄酮和醉茄素A组成的组的至少两种植物化学成分,其中所述至少两种植物化学成分对所述Nrf2激活路径的至少两个不同控制点发挥其作用,所述控制点选自控制点A、B、C、D和E组成的组。

[0060] 所属领域的技术人员将容易了解本文所描述的组合物和方法可在不脱离本文公

开的实施例的范围的情况下修改并且可使用合适的等效物进行替代。现在已详细描述某些实施例，参照以下实例将更清楚地理解本发明，所述实例仅出于说明的目的包括并且并不旨在为限制性的。

[0061] 实例

[0062] 实例1对Nrf2作用路径的效应

[0063] 不同试剂，PB123、PB125、PB127、PB129和PB131，各自显示强强效Nrf2激活，如通过使用这些组合以处理已经用含有插入的已知Nrf2-结合抗氧化反应元件的启动子/报导构建体稳定转染以驱动生产容易地可检测荧光素酶基因使得Nrf2激活导致通过荧光素-依赖性化学发光检测的荧光素酶产生的细胞系体外来证明。如在图4和5中所示，通过在与组织类型无关的经转染的癌细胞系中的PB123、PB125、PB127、PB129和PB131组合诱导强效Nrf2激活(示出乳房和肝脏细胞数据)。

[0064] 这些控制点包括(但不限于)控制点A：从由Keap1的结合和抑制释放Nrf2；控制点B：通过酶(如磷酸化和激活Nrf2的激酶)作用于Nrf2；控制点C：激活改进基因表达曲线的其它转录因子；控制点D：作用于如控制Nrf2从细胞核输出的Fyn的机理；和控制点E：通过SESN2/SQSTM1/ULK1降解Keap1和mTOR抑制。参见图1。举例来说，包括迷迭香(鼠尾草酚)、南非醉茄(醉茄素A)和毛地黄黄酮的PB125组合在Nrf2激活路径中的多个控制点处起作用。在用ARE-驱动荧光素酶报导基因稳定转染的HepG2细胞中，我们抑制Fyn(具有5μg/ml塞卡替尼；AZD0530，其为Src家族激酶抑制因子(Kaufman，Salazar等人2015))并且示出Fyn的抑制将由另一种膳食补充剂Nrf2活化剂(普天登(Protandim))引起的Nrf2激活提高至多9倍。相比之下，Fyn抑制不另外提高PB125-诱导的Nrf2激活，确定虽然其它膳食Nrf2活化剂(如普天登)允许“停工路径”保持活性，但是PB125似乎阻断路径，准许通过较小量的PB125膳食补充剂组合的Nrf2激活。

[0065] 通过作用于多于一个控制点，试剂(如PB123或PB125)的组合连同基于在PB123或PB125中核心Nrf2活化剂三元组的相关组合(如PB127、PB129或PB131)产生改进的Nrf2激活和基因调节响应，并且以比基于在组合中的活性剂的已知特性预测和基于通过先前技术教导的较低剂量进行。在PB123、125、PB127、PB129和PB131中的活性成分以协同方式一起起作用，由此对于组合的成分Nrf2激活和Nrf2-依赖性基因表达的量比基于在相同浓度下甚至在不同细胞类型中其对Nrf2单一活性的总和预测较高(图6和7)。出人意料的发现中的一个为添加到其它成分的相对少量的毛地黄黄酮产生大于Nrf2激活和基因调节的预期提高。

[0066] PB125(在30:10:4迷迭香:南非醉茄:毛地黄黄酮下)的迷迭香(6.7％鼠尾草酚)、南非醉茄(1％醉茄素A)和毛地黄黄酮(98％毛地黄黄酮)的组合提高在进食35天添加到鼠食物中的PB125的小鼠中Nrf2-依赖性基因表达。参见图8和9。

[0067] PB125植物化学成分组分用迷迭香提取物(指定在6％鼠尾草酚下)、南非醉茄提取物(指定在1％醉茄素A下)和毛地黄黄酮(指定在98％纯度下)标准化，因此100ppm等于每g的膳食6.83×10-5mg迷迭香提取物、2.27×10-5mg南非醉茄提取物，和9.43×10-6mg毛地黄黄酮。在鼠膳食中PB125激活Nrf2路径(例如提高在鼠肝脏中hmox1基因表达)并且提高催化酶活性。鼠很好地承受PB125剂量，如通过与对照膳食相比在重量稳定性、恒定食品摄入上没有改变并且没有显著的GI痛苦或在行为上改变来证明。100ppm PB125膳食在鼠中产生肝脏hmox1基因表达的显著提高(在35天膳食消耗之后测量)(图8)。

[0068] 在PB125、PB127和PB129中的单一成分具有很长的人类消耗历史并且证实在人类和动物研究中的安全性(Saller,Meier等人2001,Roodenrys,Booth等人2002,Aggarwal,Takada等人2004,Boon和Wong 2004,Anadon,Martinez-Larranaga等人2008,Zick,Djuric等人2008,Johnson 2011,Chandrasekhar,Kapoor等人2012,Theoharides,Asadi等人2012,Taliou,Zintzaras等人2013,Zhang,Gan等人2013,Gonzalez-Vallinas,Reglero等人2015,Kumar,Srivastava等人2015,Nabavi,Braidy等人2015,Petiwala和Johnson 2015)。迷迭香、南非醉茄、姜、水飞蓟、假马齿苋和毛地黄黄酮已经在多种疾病中广泛地研究并且具有广泛的安全使用记录(Mishra,Singh等人2000,Roodenrys,Booth等人2002,Aggarwal,Takada等人2004,Boon和Wong 2004)。迷迭香(Rosemary/Rosmarinus officinalis)为在食品中作为香辛料和调味剂广泛消耗的常见中海草本植物。另外,迷迭香在用于处理多种疾病[1]的传统治疗中具有很长的使用历史,其中着重于抗炎(Emami,Ali-Beig等人2013)、抗氧化(Klancnik,Guzej等人2009,Raskovic,Milanovic等人2014,Ortuno,Serrano等人2015)和抗微生物益处(Del Campo,Amiot等人2000,Bozin,Mimica-Dukic等人2007)。南非醉茄(催眠睡茄,也被称作印度冬樱桃或印度人参)为开花植物的茄科家族的成员。在南亚的传统治疗中已利用数世纪,其中历史和当前着重于免疫调节(Khan,Subramaneyaan等人2015)、抗肿瘤(Rai,Jogee等人2016)、神经(Raghavan和Shah 2015)、抗炎(Kumar,Srivastava等人2015)、抗氧化(Priyandoko,Ishii等人2011)和其它益处(Wankhede,Langade等人2015)。姜具有用于疼痛、GI和老化相关病况的很长的安全使用历史,具有针对氧化应激的益处的证据(Wang,Zhang等人2014,Lakhan,Ford等人2015,Wilson 2015)。水飞蓟素甚至在具有肝硬化的那些中并且甚至以大大高于在PB127或PB129中使用的高剂量(至多900mg一天)具有良好安全特性(Saller,Meier等人2001,Jacobs,Dennehy等人2002)。假马齿苋已被证实以高于在PB129中使用的剂量在人类记忆损失的研究中安全,并且动物研究尚未证明其任一种组分的任何不良毒性(Mishra,Singh等人2000,Roodenrys,Booth等人2002)。毛地黄黄酮为在来自多种食品来源(例如洋葱、茶、苹果、椰菜、橄榄、芹菜、菠菜、橙子、茉沃刺等)的人类膳食中通常消耗的生物类黄酮黄酮化合物,导致大约1毫克/天来自食品来源的正常典型膳食摄入(Chun,Chung等人2007,Seelinger,Merfort等人2008,Jun,Shin等人2015,Kim,Park等人2015,Nabavi,Braidy等人2015)。毛地黄黄酮经常用作膳食补充剂,其中着重于其抗氧化(Sun,Sun等人2012)、神经(Xu,Wang等人2014)和抗炎益处(Seelinger,Merfort等人2008,Taliou,Zintzaras等人2013,Paredes-Gonzalez,Fuentes等人2015)。

[0069] 作为PB125的特性的一个实例,我们培养已用在其启动子区中通过拷贝ARE Nrf2-结合序列的荧光素酶基因驱动的构建体(被称为启动子-报导构建体)稳定转染的细胞系(Simmons,Fan等人2011,Shukla,Huang等人2012)。简单来说,稳定转染的细胞类型HepG2(人类肝脏)、AREc32(人类乳房)、MCF7(人类乳房)、A549(人类肺)、293T(人类肾脏)和A172(人类大脑)在24孔板中在低密度下接种并且在37℃下用10％CO2培育。在24小时之后,将多种浓度的PB125添加到细胞。在额外培育18小时之后,细胞在其具有含有3.5mM焦磷酸钠的100μl的溶解缓冲液的孔中裂解以通过荧光素酶使光输出稳定。将细胞裂解物的20μl等分试样添加到小试管,放置在BD Monolight 3010光度计中用于背景发光,并且随后将50μl的1mM荧光素注入到管中。对于每个样品测量积分10秒的相对光单位。测试的肝脏、乳房、大脑

和肾脏细胞类型显示通过借助(图10)用PB100-系列组合处理的Nrf2基因激活和荧光素酶表达。

[0070] 作为通过PB125处理诱导的细胞保护机理的一个实例,我们检验在用PB125处理的细胞中基因上调。简单来说,培养的HepG2肝细胞用在8微克/毫升浓度下的PB125处理18小时,随后总RNA通过使用RNeasy总RNA分离试剂盒(美国加利福尼亚州巴伦西亚凯杰公司(QIAGEN Inc.Valencia,California,USA))从HepG2细胞提取。基于在260nm下的吸光度(A260)确定每个样品的浓度。基于A260与A280的比率确定每个样品的纯度。1.9-2.1的范围被视为足够纯。通过Agilent 2200Tape Station证实总RNA样品的完整性。通过使用cDNA合成试剂盒(昂飞(Affymetrix))将总RNA(250ng)转化成双链cDNA(ds-cDNA)。利用含有T7RNA聚合酶启动子的寡聚-dT引物。随后通过使用纯化珠粒(昂飞)纯化并且回收ds-cDNA。接下来,使用RNA转录标记试剂盒(昂飞)执行体外转录以生成生物素标识的cRNA。使用RNeasy亲和柱(凯杰(Qiagen))纯化生物素标识的cRNA。为了确保与寡核苷酸阵列的理想杂交,cRNA分断。执行分断使得通过在分断缓冲液中将cRNA在94℃下培育35分钟cRNA片段在长度上在50-200个碱基之间。随后在存在0.01%TWEEN 20的情况下将样品添加到含有100mM MES、1M Na+和20mM EDTA的杂交溶液中。分断的cRNA的最终浓度为0.05μg/μL。通过使用GeneChip®杂交炉640(昂飞)在45℃下将200uL的样品培育到Affymetrix GeneChip®PrimeView™人类基因表达阵列(美国加利福尼亚州圣克拉拉的昂飞公司(Affymetrix Inc.,Santa Clara,California,USA))持续16小时来执行杂交。在杂交之后,去除杂交溶液并且洗涤阵列并且使用GeneChip®流体站450(昂飞)用抗生蛋白链菌素-藻红蛋白染色。使用GeneChip扫描仪3000(昂飞)在2.5到3微米的分辨率下读取阵列。通过每个转录使用约11个探针和许多对照探针表示每个基因。指控台GeneChip软件程序用于测定用于在阵列上的所有基因的表达的强度。对此实验,与在没有任何添加的刺激物(如PB125)的培养液中的对照HepG2细胞中观察到的平均强度相比,计算通过HepG2细胞的PB125处理的基因诱导倍数。如在表1中所描绘,通过PB125上调的基因包括多种Nrf2-调节的抗氧化、抗炎、细胞应激反应和其它保护基因。这些基因包括例如参与GSH产生和再生、铁螯合作用、GSH利用率、硫氧还蛋白(TXN)产生、再生和利用等的基因。表1列出通过PB125上调的相关实例基因。综上所述,本实例支持通过PB125的细胞保护机构涉及Nrf2细胞信号传导路径的激活。

[0071] 表1基因微阵列分析揭示PB125调节多种Nrf2相关基因和与抗氧化、抗炎和其它细胞保护效应相关联的基因。

[0072]

探针组 ID	HepG2(对照)	通过BP125的诱导倍数	代表性公开 ID	基因名称	基因符号
11715650_a_at	45.53	10.10	AF208018.1	硫氧还蛋白还原酶 1	TXNRD1

[0073]

11756634_a_at	414.69	2.81	CR597200.1	谷胱甘肽还原酶	GSR
11750770_a_at	1005.93	2.37	AK304288.1	谷氨酸-半胱氨酸接合酶，催化亚基	GCLC
11759710_at	199.19	2.04	BC024223.2	含有硫氧还蛋白结构域的9	TXNDC9
11744680_a_at	231.18	7.72	AB040875.1	溶质载体家族7（阴离子氨基酸转运蛋白轻链，xc-体系），成员11	SLC7A11
11756634_a_at	414.69	2.81	CR597200.1	谷胱甘肽还原酶	GSR
11716939_a_at	1217.99	8.63	NM_002133.1	血红素加氧酶（解环）1	HMOX1
11725496_a_at	488.83	8.87	NM_032717.3	1-酰化甘油-3-磷酸酯O-酰基转移酶9	AGPAT9
11752577_at	771.67	3.62	AY258285.1	铁蛋白，重多肽1	FTH1
11715649_s_at	3236.76	4.73	NM_003330.2	硫氧还蛋白还原酶1	TXNRD1
11716950_s_at	1908.04	5.45	NM_080725.1	硫氧还蛋白1	SRXN1
11752843_x_at	1202.52	4.54	AK304877.1	隔离体1	SQSTM1
11750416_a_at	69.07	9.41	AK293322.1	硫氧还蛋白还原酶1	TXNRD1
11756585_a_at	86.47	6.47	CR614710.1	水通道蛋白3（吉尔血型）	AQP3
11735676_a_at	231.82	3.98	NM_182980.2	氧化应激诱导的生长抑制因子1	OSGIN1
11753445_a_at	244.58	10.37	BT019785.1	血红素加氧酶（解环）1	HMOX1
11723490_at	1195.87	6.07	BC041809.1	谷氨酸-半胱氨酸接合酶，改性剂亚基	GCLM
11756915_a_at	63.77	8.33	AL833940.1	细胞色素P450，家族4，F子族，多肽11	CYP4F11
11736655_a_at	499.98	7.20	NM_012212.3	前列腺素还原酶1	PTGR1
11719171_a_at	2722.97	6.99	NM_001353.5	醛-酮还原酶家族1，成员C1（二氢二醇去氢酶1；20-α(3-α)-羟基类固醇去氢酶）	AKR1C1
11742378_a_at	1112.08	4.32	NM_001080538.1	醛-酮还原酶家族1，成员B10（醛糖还原酶）///醛-酮还原酶家族1,成员B15	AKR1B10///AKR1B15
11729101_a_at	2435.26	6.95	NM_205845.1	醛-酮还原酶家族1，成员C2（二氢二醇去氢酶2；胆酸结合蛋白；3-α羟基类固醇去氢酶，类型III）///醛-酮还原酶家族1成员C2类	AKR1C2///LOC100653286
11757882_x_at	59.22	2.02	BU784580	麸胱甘肽S-转移酶α1///麸胱甘肽S-转移酶α2	GSTA1///GSTA2

[0074] 作为通过PB125处理诱导的抗炎机理的一个实例，我们在用PB125处理并且用细菌性脂多糖内毒素(LPS)刺激的原代细胞中检验细胞因子水平。在用巯基醋酸酯处理到腹膜腔中持续1周随后灌洗回收大约7百万巨噬细胞之后获得鼠腹膜巨噬细胞。涂布细胞的等分试样并且用乙醇对照(0.1％匹配PB125)或PB125(5ug/mL)处理16小时，随后用脂多糖(100ng/mL)或媒剂(阴性对照)刺激5小时。从细胞分离总RNA用于定量PCR分析以测量肿瘤坏死因子-α(TNFα)和介白素-1β(IL-1β)基因表达，归一化到18s水平。值得注意地，PB125处理引起促炎性细胞因子TNFα和IL-1β的LPS-诱导的表达的严重降低。参见图11。

[0075] PB125(在30:10:4迷迭香:南非醉茄:毛地黄黄酮下)的迷迭香(6.7％鼠尾草酚)、南非醉茄(1％醉茄素A)和毛地黄黄酮(98％毛地黄黄酮)的组合提高在来自每天经口服用60mg的PB125的人类主体的口腔细胞样品中的GCLM基因的Nrf2-依赖性基因表达,与两个正常对照主体的口腔细胞样品相比(通过对纯化的RNA定量RT-PCR测定,使用人类GCLM特异性引物(正向引物:TTGCCTCCTGCTGTGTGATG(SEQ ID NO.1),反向引物:GTGCGCTTGAATGTCAGGAA)(SEQ ID No.2),归一化到GAPDH,其中通过2^(△△Ct)方法计算相对倍数改变。参见图13。

[0076] 如支撑本发明的额外数据,我们发现在迷迭香、姜、南非醉茄和毛地黄黄酮成分之间出人意料的协同作用的量。举例来说,低浓度的毛地黄黄酮与迷迭香提取物和姜提取物的组合协同激活Nrf2。在本发明中,其它试剂可添加到Nrf2-激活组合,其条件是其不干扰Nrf2激活功能性。我们发现水飞蓟素和假马齿苋皂素成分不拮抗迷迭香、姜、南非醉茄和毛地黄黄酮成分的Nrf2激活。

[0077] 以另一方式跟踪此实验,在2小时的暴露时间之后洗掉在HepG2细胞处理(其中PB125处理在0-10ug/mL和0-50ug/mL范围下)之后17、24、41和48小时测量的荧光素酶RLU并且被示出荧光素酶的Nrf2-驱动产生在17小时处为最高的新鲜细胞培养基代替,随后在处理之后48小时快速降低到大约基线水平。

[0078] 重复对培养的HepG2细胞以每24小时一次2小时暴露的处理,随后24小时后的读数示出在24和48小时之间通过PB125逐渐消逝的Nrf2激活和如果再次用PB125处理那么细胞仍然可被再次激活。

[0079] 作为通过PB123或PB125处理诱导的抗炎机理的一个实例,我们检验在用PB123或PB125处理并且用细菌性脂多糖内毒素(LPS)刺激的原代人类肺动脉内皮细胞(HPAEC)中的基因表达和细胞因子水平。LPS刺激诱导炎症相关基因的表达,并且通过用PB123或PB125处理减弱此上调。表2示出通过LPS处理最高度上调的40种基因,并且示出PB123处理和PB125处理均减弱LPS-诱导的基因表达。LPS刺激提高促炎性白细胞介素-6(IL6)蛋白从HPAEC细胞释放,并且通过用PB125处理减弱此提高。参见图12。

[0080] 表2基因微阵列分析揭示PB123和PB125显示抗炎效应。PB123和PB125均降低通过LPS最高度上调的40种基因的LPS-诱导表达信号。

[0081]

基因符号	对照	LPS	LPS+PB123	LPS+PB125	基因名称	基因符号	LPS/LPS+PB123	LPS/LPS+PB125
CXCL3	33	1441	492	225	趋化因子（C-X-C 基序）配体 3	CXCL3	2.9	6.4
CCL20	196	4776	2055	1034	趋化因子（C-C 基序）配体 20	CCL20	2.3	4.6
CXCL2	292	5407	2956	2669	趋化因子（C-X-C 基序）配体 2	CXCL2	1.8	2.0
CSF2	41	621	132	133	群落刺激因子 2（粒细胞-巨噬细胞）	CSF2	4.7	4.7
TNFAIP6	33	390	91	60	肿瘤坏死因子，α-诱导蛋白 6	TNFAIP6	4.3	6.5
IL8	590	6750	5571	4257	介白素 8	IL8	1.2	1.6
TNFAIP2	285	3089	798	512	肿瘤坏死因子，α-诱导蛋白 2	TNFAIP2	3.9	6.0
CXCL10	67	668	47	31	趋化因子（C-X-C 基序）配体 10	CXCL10	14.3	21.3
CXCL1	1195	11398	7858	7819	趋化因子（C-X-C 基序）配体 1（黑素瘤生长刺激活性，α）	CXCL1	1.5	1.5
CX3CL1	386	3618	444	288	趋化因子（C-X3-C 基序）配体 1	CX3CL1	8.2	12.5
BIRC3	86	798	349	190	含有杆状病毒（baculoviral）IAP 重复的 3	BIRC3	2.3	4.2
CD69	36	333	111	45	CD69 分子	CD69	3.0	7.3
TNFAIP3	94	814	309	190	肿瘤坏死因子，α-诱导蛋白 3	TNFAIP3	2.6	4.3
5ELE	1465	12429	5605	2612	选择蛋白 E	SELE	2.2	4.8
CXCL6	246	1683	458	178	趋化因子（C-X-C 基序）配体 6（粒细胞趋化性蛋白	CXCL6	3.7	9.5

[0082]

					2)			
NKX3-1	60	398	141	125	NK3 同源盒 1	NKX3-1	2.8	3.2
CSF3	92	592	272	290	群落刺激因子 3（粒细胞）	CSF3	2.2	2.0
RND1	98	601	224	236	Rho 家族 GTP 酶 1	RND1	2.7	2.5
LTB	244	1478	374	314	淋巴毒素 β（TNF 超家族，成员 3）	LTB	3.9	4.7
FAM101A///2	63	329	70	78	序列相似性家族 101，成员 A///蛋白 FAM101A	FAM101A///ZNF664	4.7	4.2
CXCL5	163	844	127	63	趋化因子（C-X-C 基序）配体 5	CXCL5	6.7	13.3
CEBPD	183	947	493	489	CCAAT/强化子结合蛋白（C/EBP），△	CEBPD	1.9	1.9
MAP3K8	26	128	75	45	丝裂原活化蛋白激酶激酶激酶 8	MAP3K8	1.7	2.9
TRAF1	158	730	421	328	TNF 受体相关因子 1	TRAF1	1.7	2.2
IL6	429	1967	1166	1105	介白素 6（干扰素，β2）	IL6	1.7	1.8
VCAM1	1315	5963	2065	1116	血管细胞粘附分子 1	VCAM1	2.9	5.3
ICAM1	288	1290	543	416	胞间粘着分子 1	ICAM1	2.4	3.1
SLC7A2	356	1592	660	383	溶质载体家族 7（阳离子氨基酸转运蛋白，y+体系），成员 2	SLC7A2	2.4	4.2
CXCR7	291	1286	660	521	趋化因子（C-X-C 基序）受体 7	CXCR7	1.9	2.5
NCOA7	132	561	212	137	核受体辅活化子 7	NCOA7	2.6	4.1
IRF1	240	1014	579	489	干扰素调节因子 1	IRF1	1.8	2.1
BCL2A1	31	130	39	18	BCL2 相关蛋白 A1	BCL2A1	3.3	7.0
TNFRSF9	32	124	33	30	肿瘤坏死因子受体超家	TNFRSF9	3.7	4.1

[0083]

					族，成员 9			
IL1A	236	888	589	561	介白素 1，α	IL1A	1.5	1.6
MT1G	36	134	116	163	金属硫蛋白 1G	MT1G	1.2	0.8
TIFA	81	293	175	147	TRAF-与叉头相关结构域互作蛋白	TIFA	1.7	2.0
CCL5	95	330	95	83	趋化因子（C-C 基序）配体 5	CCL5	3.5	4.0
CAB39	26	91	48	43	钙结合蛋白 39	CAB39	1.9	2.1
SOCS1	29	95	73	76	细胞因子信号传递的抑制因子 1	SOCS1	1.3	1.2
IL1B	52	170	58	66	介白素 1，β	IL1B	2.9	2.6

[0084] 实例2 PB125

[0085] 本公开的一个实施例为以30:10:6、30:10:5、30:10:4或30:10:1的质量比的迷迭香提取物(指定在5到50％鼠尾草酚下)、南非醉茄提取物(指定在0.5到10％醉茄素A下)和毛地黄黄酮(指定在10-100％毛地黄黄酮下)的组合，其中组合的每天人类剂量在42到1050mg的范围内，如在表3中所示。

[0086] 表3.具有用于人类的PB125的成分和日剂量范围的规格的组合物

[0087]

成分：	迷迭香	南非醉茄	毛地黄黄酮
规格范围：	5-50%鼠尾草酚或 10-100%二萜烯	0.5-10%醉茄素 A	10-100%毛地黄黄酮
优选的规格范围：	5-10%鼠尾草酚	1-3%醉茄素 A	95-99%毛地黄黄酮
日剂量范围：	30-750 mg	10-250 mg	2-50 mg
组合物范围：	30-90%	10-30%	2-8%
优选的质量比	30	10	6
优选的质量比	30	10	5
优选的质量比	30	10	4
优选的质量比	30	10	1

[0088] 实例3 PB127

[0089] 本公开的另一个实施例为分别以10:5:1:30的质量比的迷迭香提取物(指定在5到10％鼠尾草酚下)、姜提取物(指定在1-10％6-姜烯酚和/或10-25％6-姜醇下)、毛地黄黄酮(指定在90-100％毛地黄黄酮下)和水飞蓟提取物(指定在50-90％水飞蓟素下)的PB127组合，其中组合的每天人类剂量在46到920mg的范围内，如在表4中所示。

[0090] 表4.具有用于人类的PB127的成分和日剂量范围的规格的组合物

[0091]

成分：	迷迭香	姜	毛地黄黄酮	水飞蓟
规格范围：	5-50%鼠尾草酚或10-100%二萜烯	0.5-20% 6-姜烯酚或6-姜醇	10-100%毛地黄黄酮	10-100%水飞蓟素
优选的规格范围：	5-10%鼠尾草酚	10-20% 6-姜烯酚	95-99%毛地黄黄酮	75-100%水飞蓟素
日剂量范围：	10-200 mg	5-100 mg	1-20 mg	30-600 mg
组合物范围：	10-30%	5-15%	1-3%	25-75%
优选的质量比	10	5	1	30

[0092] 实例4 PB129

[0093] 本公开的另一个实施例为分别以10:5:1:30:48的质量比的迷迭香提取物(指定在5到10％鼠尾草酚下)、姜提取物(指定在1-10％6-姜烯酚和/或10-25％6-姜醇下)、毛地黄黄酮(指定在90-100％毛地黄黄酮下)、水飞蓟提取物(指定在50-90％水飞蓟素下)和假马齿苋提取物(指定在10-60％假马齿苋皂素下)的PB129组合，其中组合的每天人类剂量在94到1820mg的范围内，如在表5中所示。

[0094] 表5.具有用于人类的PB129的成分和日剂量范围的规格的组合物

[0095]

成分：	迷迭香	姜	毛地黄黄酮	水飞蓟	假马齿苋属
规格范围：	5-50%鼠尾草酚或10-100%二萜烯	0.5-20% 6-姜烯酚或6-姜醇	10-100%毛地黄黄酮	10-100%水飞蓟素	10-80%假马齿苋皂素
优选的规格范围：	5-10%鼠尾草酚	10-20% 6-姜烯酚	95-99%毛地黄黄酮	75-100%水飞蓟素	20-60%假马齿苋皂素
日剂量范围：	10-200 mg	5-100 mg	1-20 mg	30-600 mg	48-900 mg
组合物范围：	5-15%	2.5-7.5%	0.5-1.5%	12.5-37.5%	25-75%
优选的质量比	10	5	1	30	48

[0096] 实例5 PB123

[0097] 本公开的另一个实施例为分别以10:5:1的质量比的迷迭香提取物(指定在5到10％鼠尾草酚下)、姜提取物(指定在1-10％6-姜烯酚和/或10-25％6-姜醇下)、毛地黄黄酮(指定在90-100％毛地黄黄酮下)的PB123组合，其中组合的每天人类剂量在16到320mg的范围内，如在表6中所示。

[0098] 表6.具有用于人类的PB123的成分和日剂量范围的规格的组合物

[0099]

成分：	迷迭香	姜	毛地黄黄酮
规格范围：	5-50%鼠尾草酚或10-100%二萜烯	0.5-20% 6-姜烯酚或6-姜醇	10-100%毛地黄黄酮
优选的规格范围：	5-10%鼠尾草酚	10-20% 6-姜烯酚	95-99%毛地黄黄酮
日剂量范围：	10-200 mg	5-100 mg	1-20 mg

[0100]

组合物范围：	10-30%	5-15%	1-3%
优选的质量比	10	5	1

[0101] 实例6 PB131

[0102] 本发明的另一个实施例为分别以10:5:1:48的质量比的迷迭香提取物(指定在5到10％鼠尾草酚下)、姜提取物(指定在1-10％6-姜烯酚和/或10-25％6-姜醇下)、毛地黄黄酮(指定在90-100％毛地黄黄酮下)和假马齿苋提取物(指定在10-60％假马齿苋皂素下)的PB131组合,其中组合的每天人类剂量在64到1220mg的范围内,如在表7中所示。

[0103] 表7.具有用于人类的PB131的成分和日剂量范围的规格的组合物

[0104]

成分:	迷迭香	姜	毛地黄黄酮	假马齿苋属
规格范围:	5-50%鼠尾草酚或10-100%二萜烯	0.5-20% 6-姜烯酚或 6-姜醇	10-100%毛地黄黄酮	10-80%假马齿苋皂素
优选的规格范围:	5-10%鼠尾草酚	10-20% 6-姜烯酚	95-99%毛地黄黄酮	20-60%假马齿苋皂素
日剂量范围:	10-200 mg	5-100 mg	1-20 mg	48-900 mg
组合物范围:	5-15%	2.5-7.5%	0.5-1.5%	25-75%
优选的质量比	10	5	1	48

[0105] 可在整个本申请中引用或下文列出的所有引用参考文献(包括文献参考、专利、专利申请和网站)的内容出于任何目的在此明确地全文以引用的方式并入到本公开中。除非另外指示,否则公开内容可采用在所属领域中众所周知的微生物学、分子生物学和细胞生物学的常规技术。

[0106] 可在不脱离本文的范围的情况下修改所公开的方法和系统。应注意,在以上描述所含有或在附图中所示的主题应以说明性而非限制性含义进行解释。

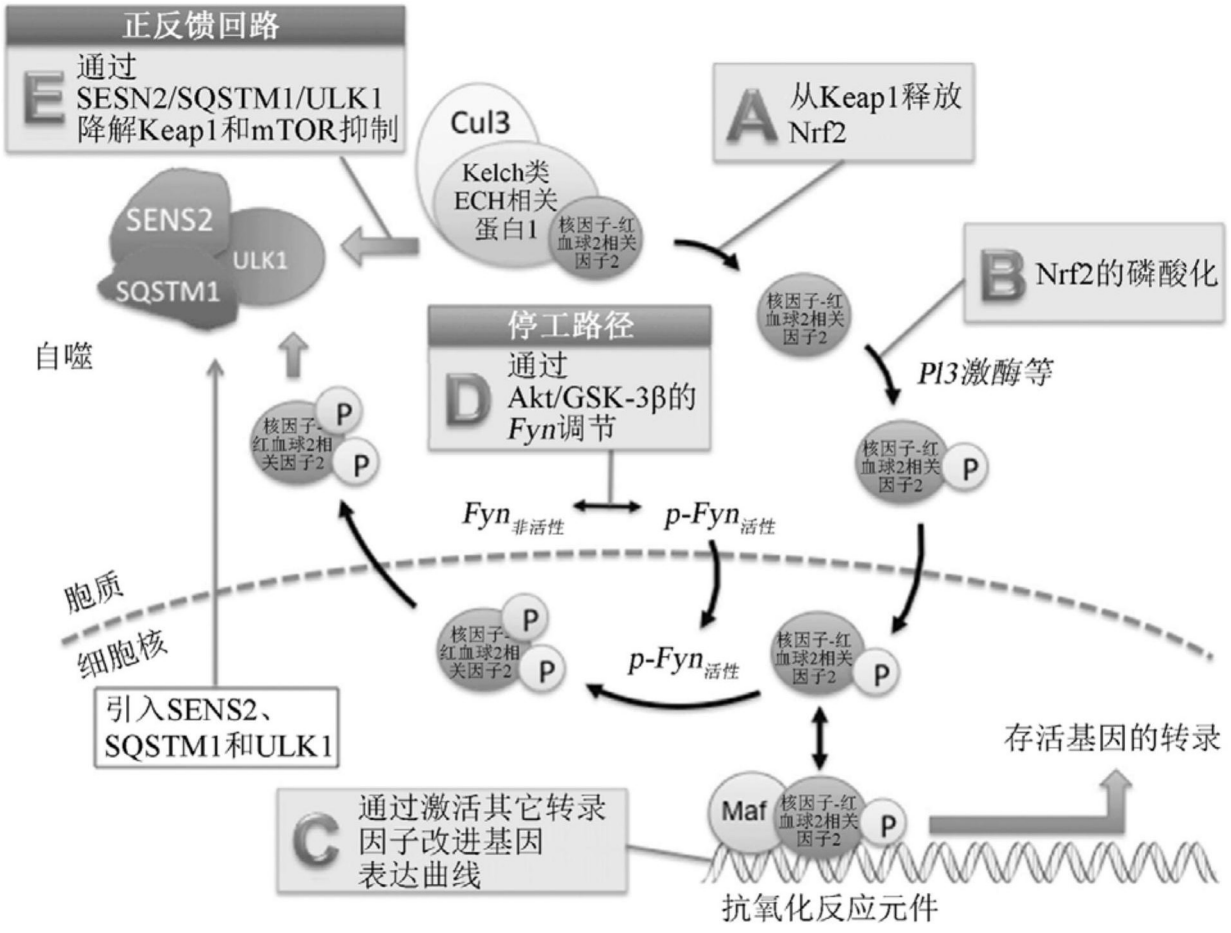
正反馈回路
E 通过SESN2/SQSTM1/ULK1降解Keap1和mTOR抑制
Cul3
Kelch类ECH相关蛋白1
核因子-红血球2相关因子2
A 从Keap1释放Nrf2
B Nrf2的磷酸化
SENS2
SQSTM1
ULK1
自噬
停工路径
D 通过Akt/GSK-3β的Fyn调节
PI3激酶等
P
Fyn非活性
p-Fyn活性
胞质
细胞核
引入SENS2、SQSTM1和ULK1
C 通过激活其它转录因子改进基因表达曲线
Maf
存活基因的转录
抗氧化反应元件

图1

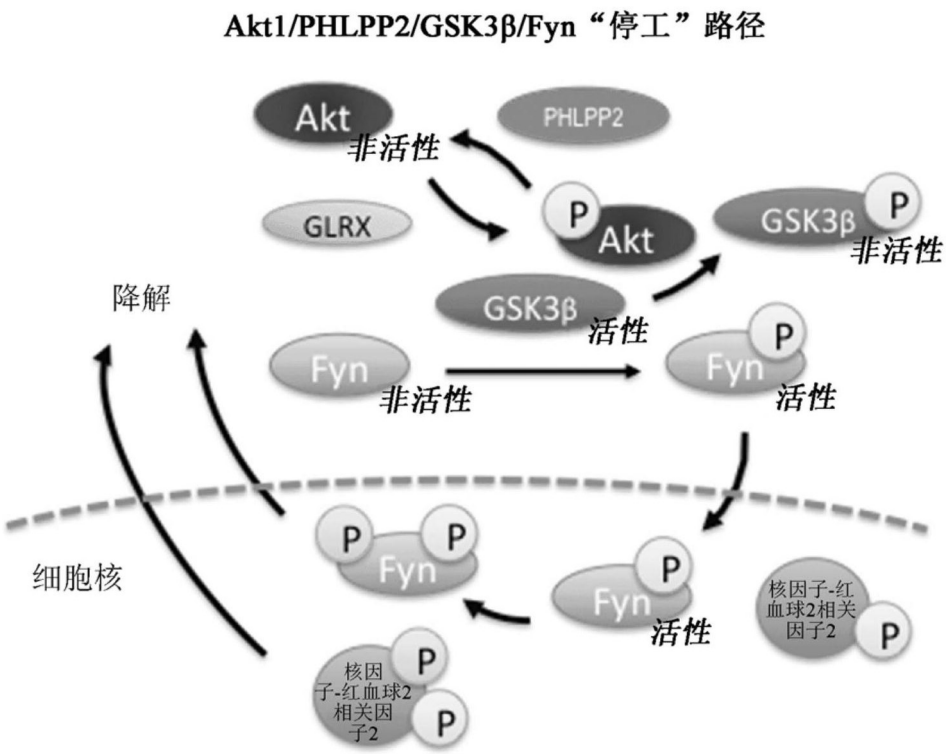
Akt1/PHLPP2/GSK3β/Fyn“停工”路径
Akt
非活性
PHLPP2
GLRX
P
Akt
GSK3β
P
非活性
降解
GSK3β
活性
Fyn
非活性
Fyn
P
活性
细胞核
P
P
Fyn
Fyn
P
活性
核因子-红血球2相关因子2
P
核因子-红血球2相关因子2
P
P

图2

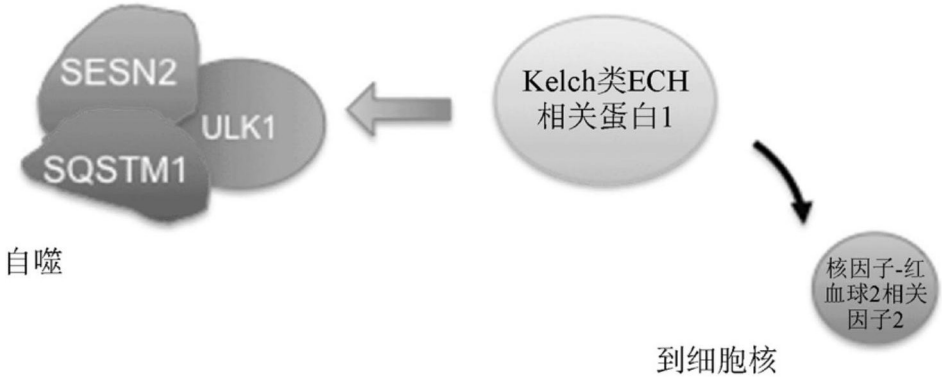
SESN2
ULK1
SQSTM1
自噬
Kelch类ECH相关蛋白1
核因子-红血球2相关因子2
到细胞核

图3

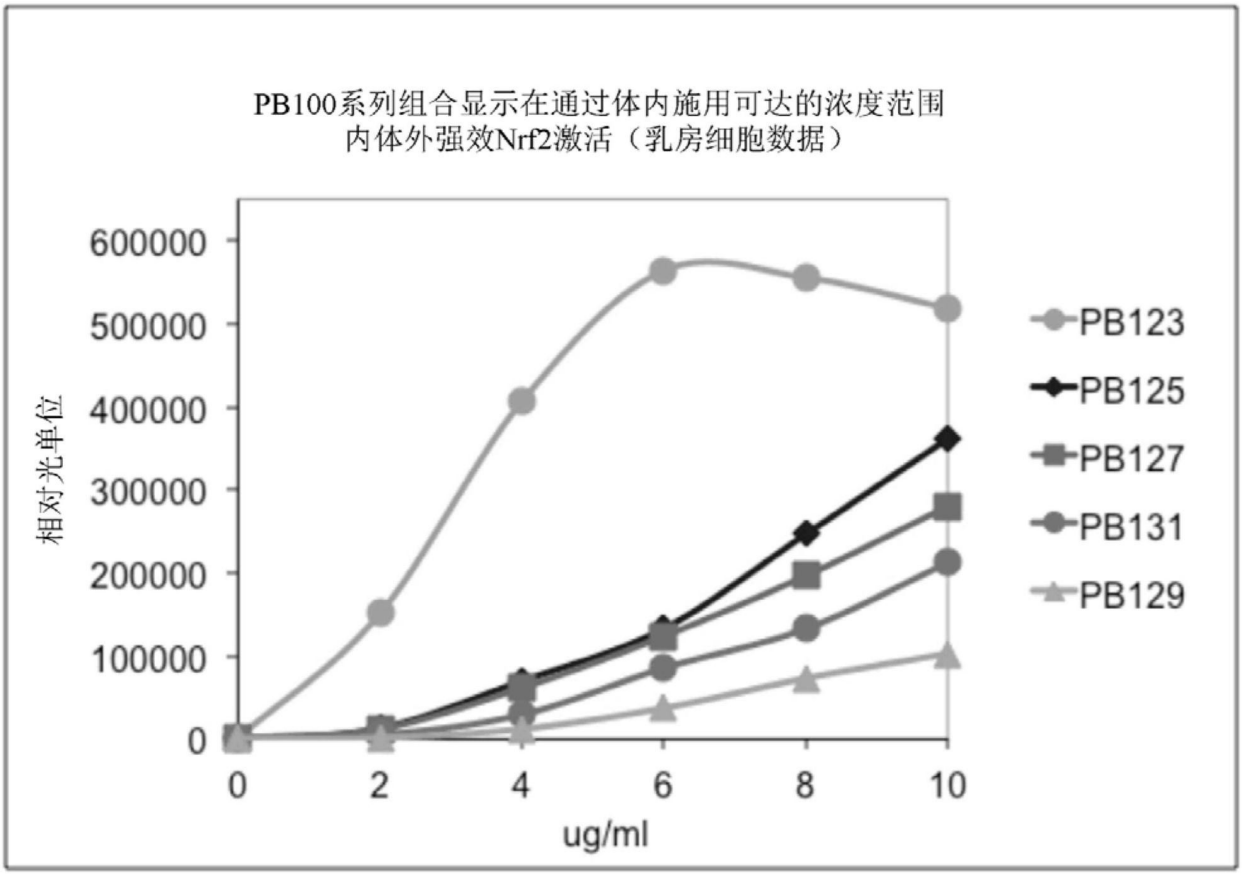
PB100系列组合显示在通过体内施用可达的浓度范围
内体外强效Nrf2激活（乳房细胞数据）
相对光单位
600000
500000
400000
300000
200000
100000
0
0
2
4
6
8
10
ug/ml
PB123
PB125
PB127
PB131
PB129

图4

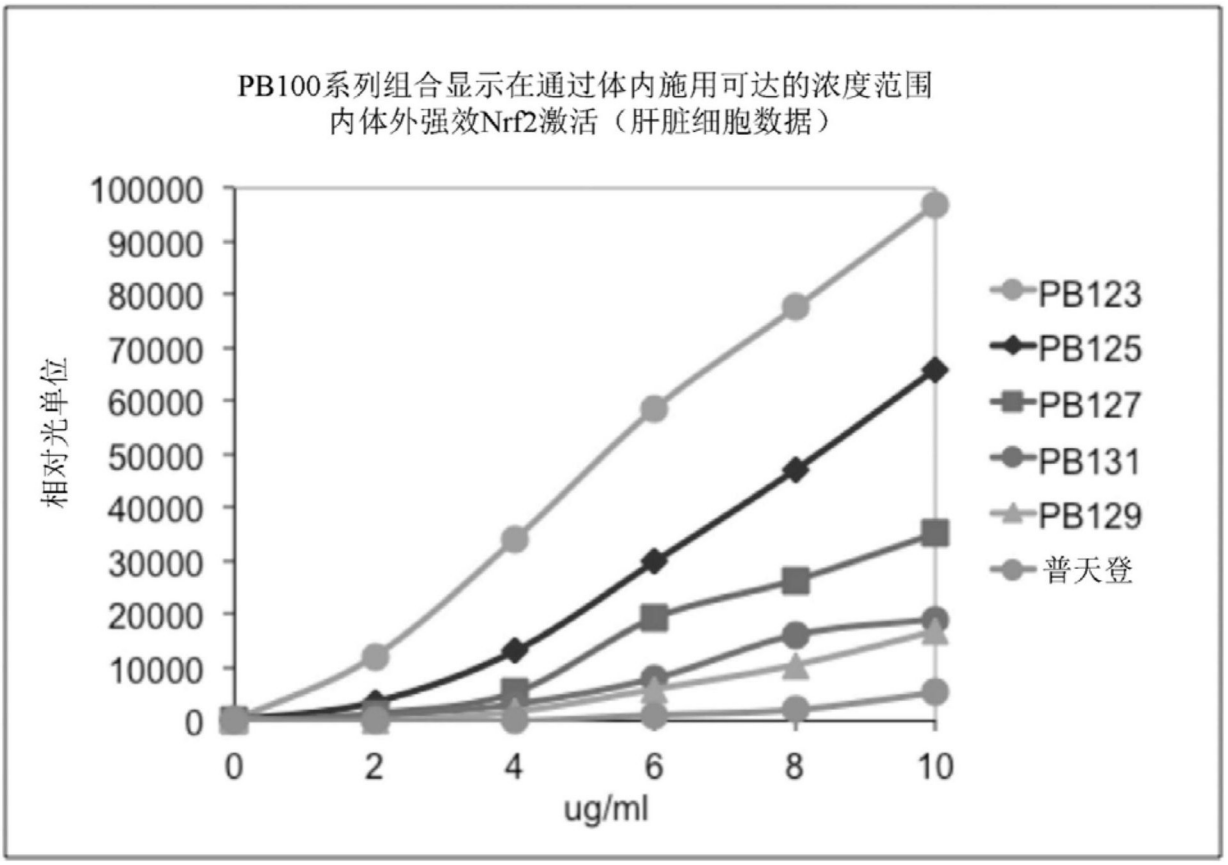
PB100系列组合显示在通过体内施用可达的浓度范围
内体外强效Nrf2激活（肝脏细胞数据）
相对光单位
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0
2
4
6
8
10
ug/ml
PB123
PB125
PB127
PB131
PB129
普天登

图5

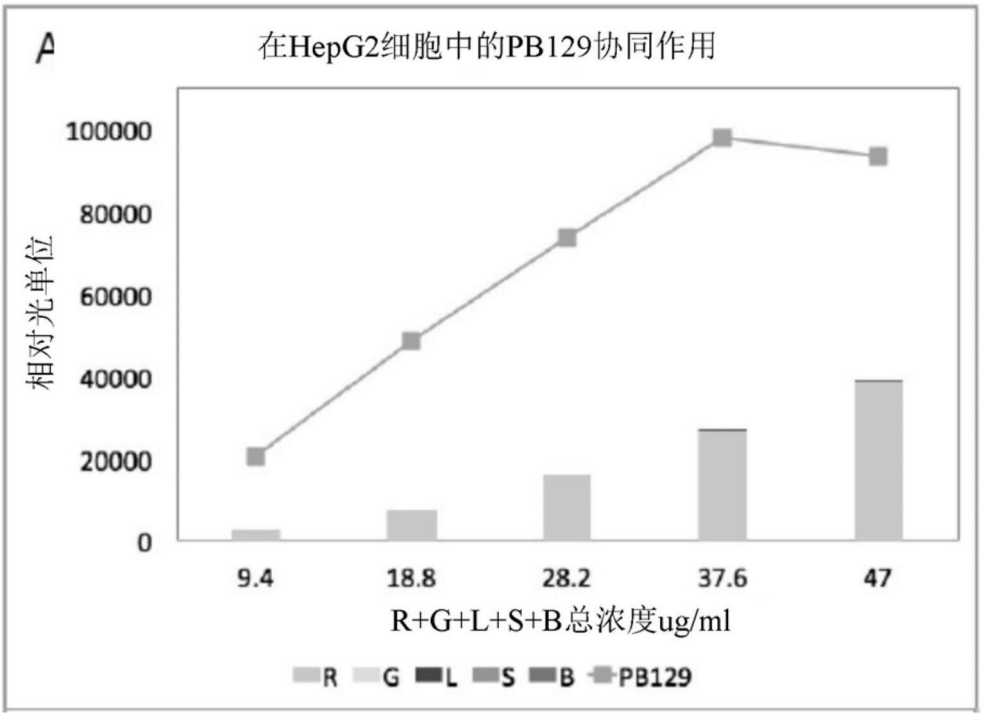
A
在HepG2细胞中的PB129协同作用
相对光单位
100000
80000
60000
40000
20000
0
9.4
18.8
28.2
37.6
47
R+G+L+S+B总浓度ug/ml
R
G
L
S
B
PB129

图6A

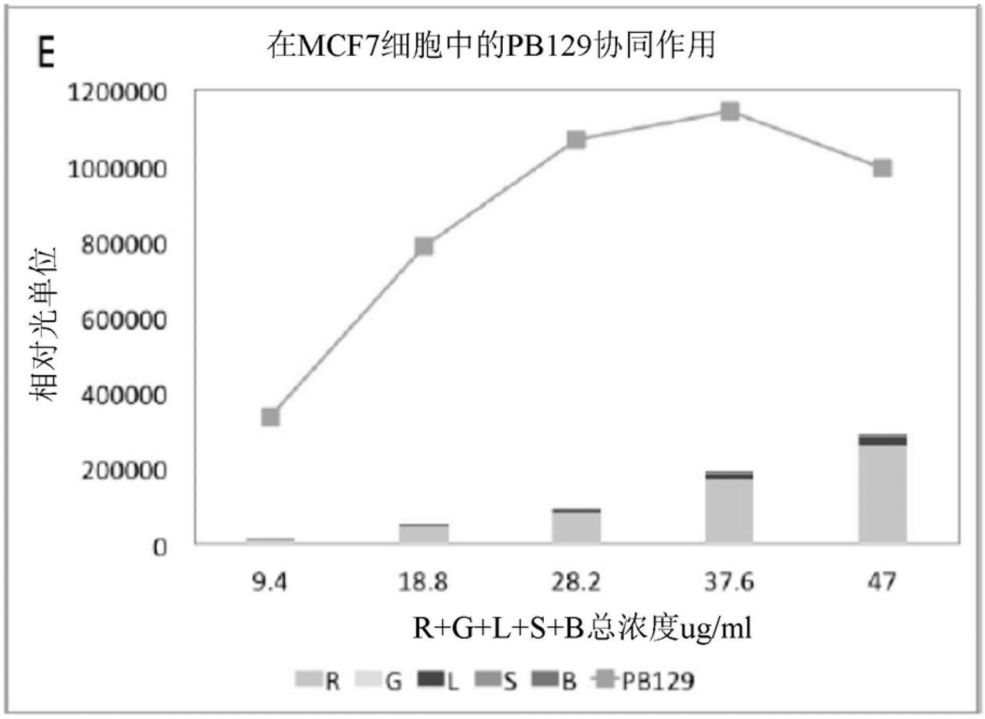
E
在MCF7细胞中的PB129协同作用
1200000
1000000
800000
600000
400000
200000
0
相对光单位
9.4
18.8
28.2
37.6
47
R+G+L+S+B总浓度ug/ml
R G L S B PB129

图6B

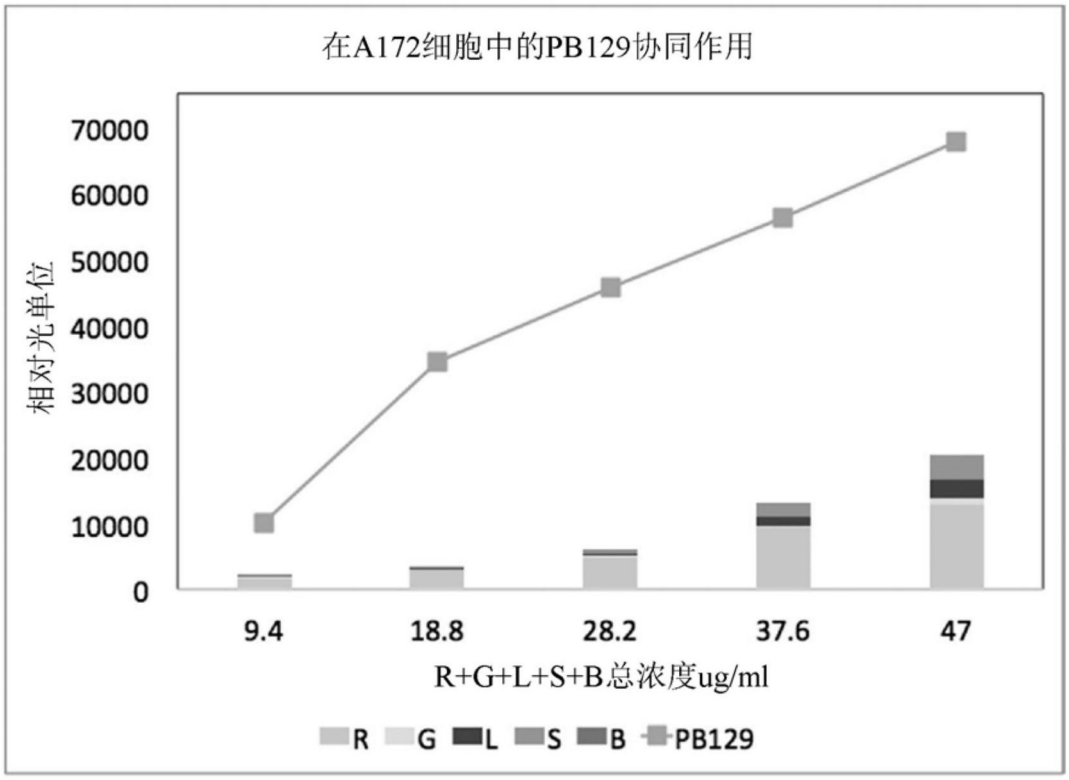
在A172细胞中的PB129协同作用
70000
60000
50000
40000
30000
20000
10000
0
相对光单位
9.4
18.8
28.2
37.6
47
R+G+L+S+B总浓度ug/ml
R G L S B PB129

图6C

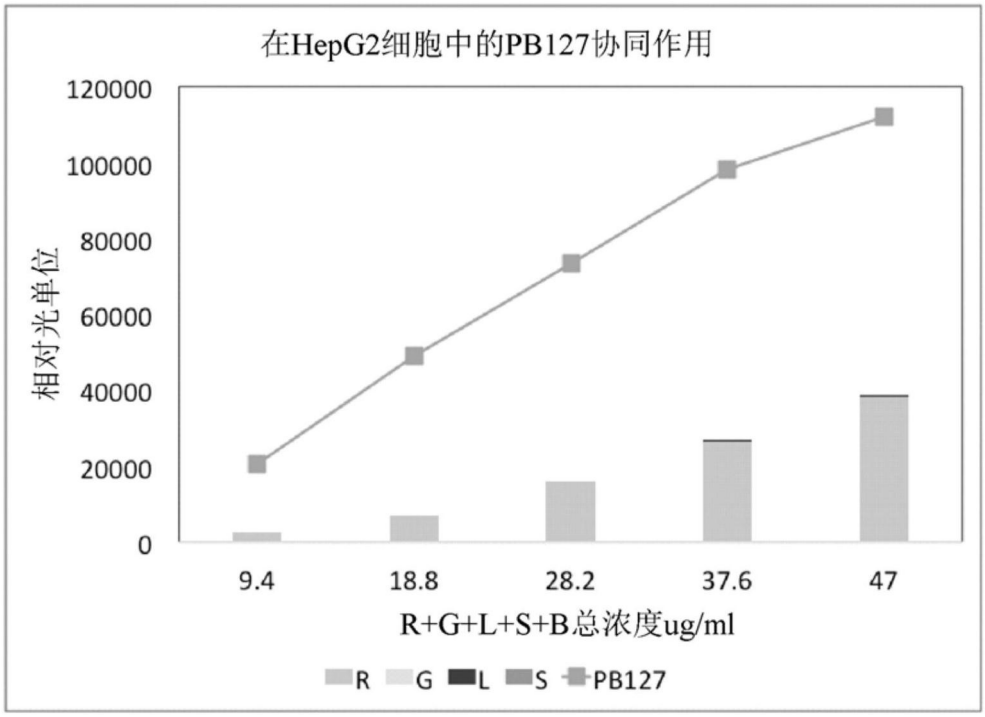
在HepG2细胞中的PB127协同作用
120000
100000
80000
60000
40000
20000
0
相对光单位
9.4
18.8
28.2
37.6
47
R+G+L+S+B总浓度ug/ml
R G L S PB127

图7A

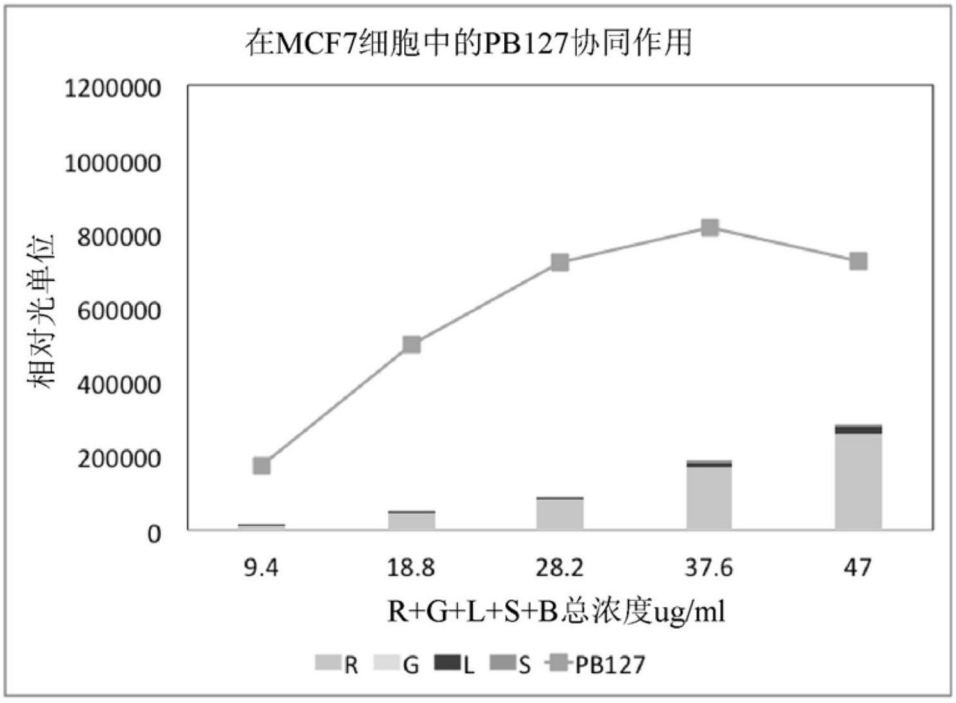
在MCF7细胞中的PB127协同作用
1200000
1000000
800000
600000
400000
200000
0
相对光单位
9.4
18.8
28.2
37.6
47
R+G+L+S+B总浓度ug/ml
R G L S PB127

图7B

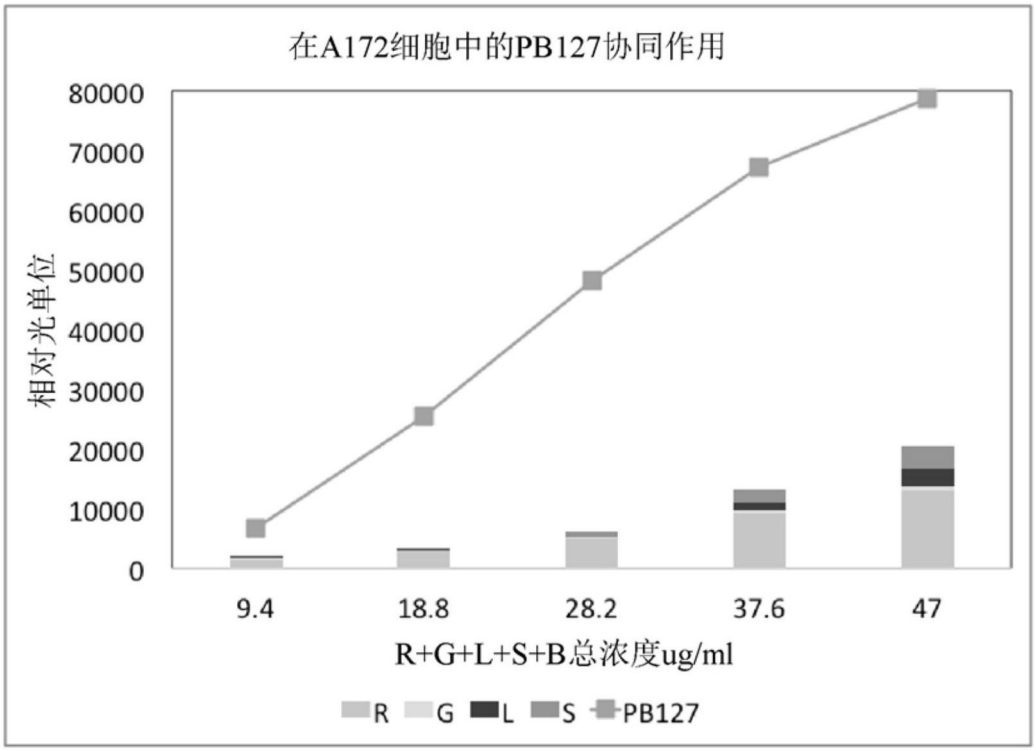
在A172细胞中的PB127协同作用
80000
70000
60000
50000
40000
30000
20000
10000
0
相对光单位
9.4
18.8
28.2
37.6
47
R+G+L+S+B总浓度ug/ml
R
G
L
S
PB127

图7C

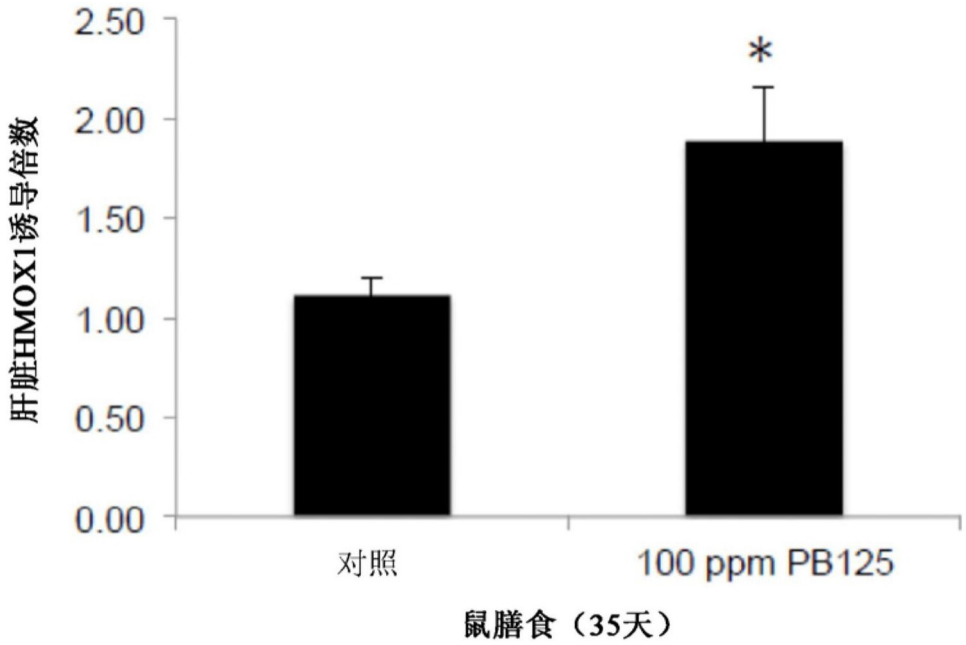
2.50
2.00
1.50
1.00
0.50
0.00
肝脏HMOX1诱导倍数
*
对照
100 ppm PB125
鼠膳食（35天）

图8

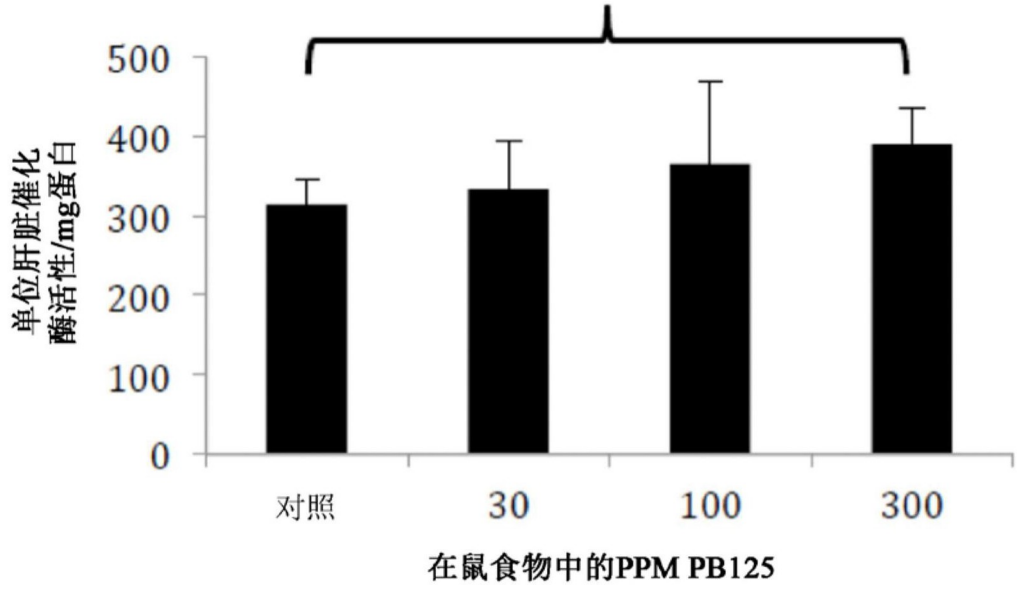
500
400
300
200
100
0
单位肝脏催化
酶活性/mg蛋白
对照
30
100
300
在鼠食物中的PPM PB125

图9

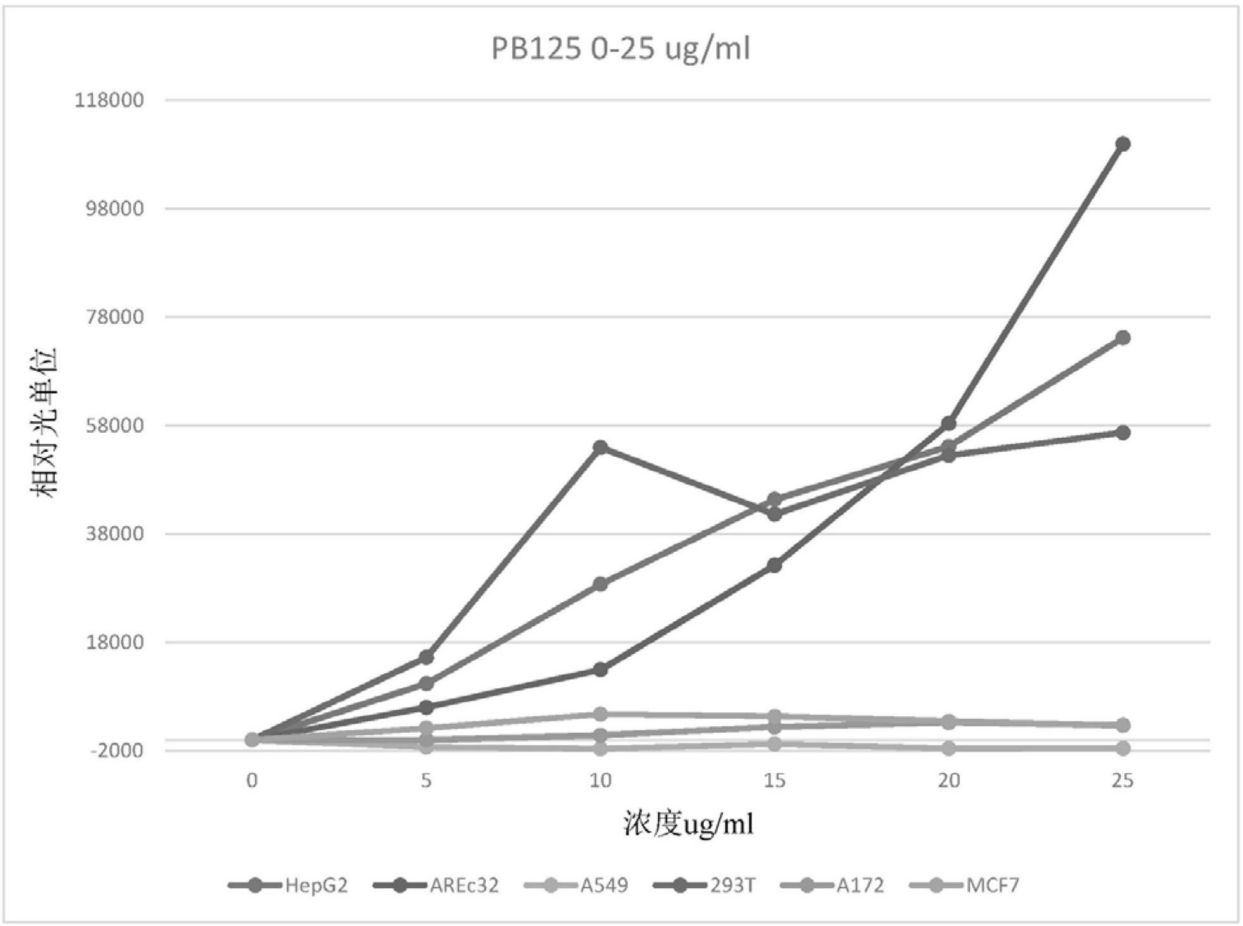
PB125 0-25 ug/ml
118000
98000
78000
58000
38000
18000
-2000
相对光单位
0
5
10
15
20
25
浓度ug/ml
HepG2
AREc32
A549
293T
A172
MCF7

图10

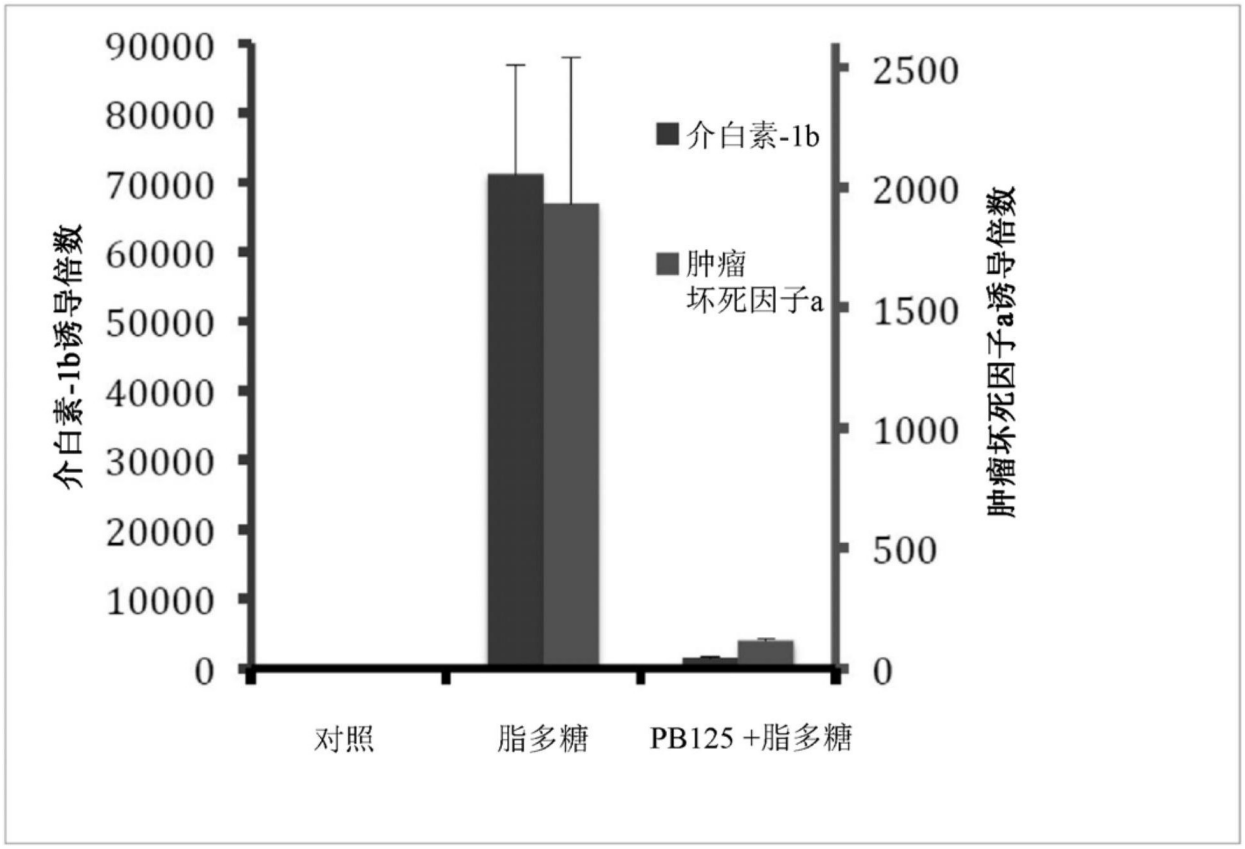
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
介白素-1b诱导倍数
2500
2000
1500
1000
500
0
肿瘤坏死因子a诱导倍数
介白素-1b
肿瘤
坏死因子a
对照
脂多糖
PB125 +脂多糖

图11

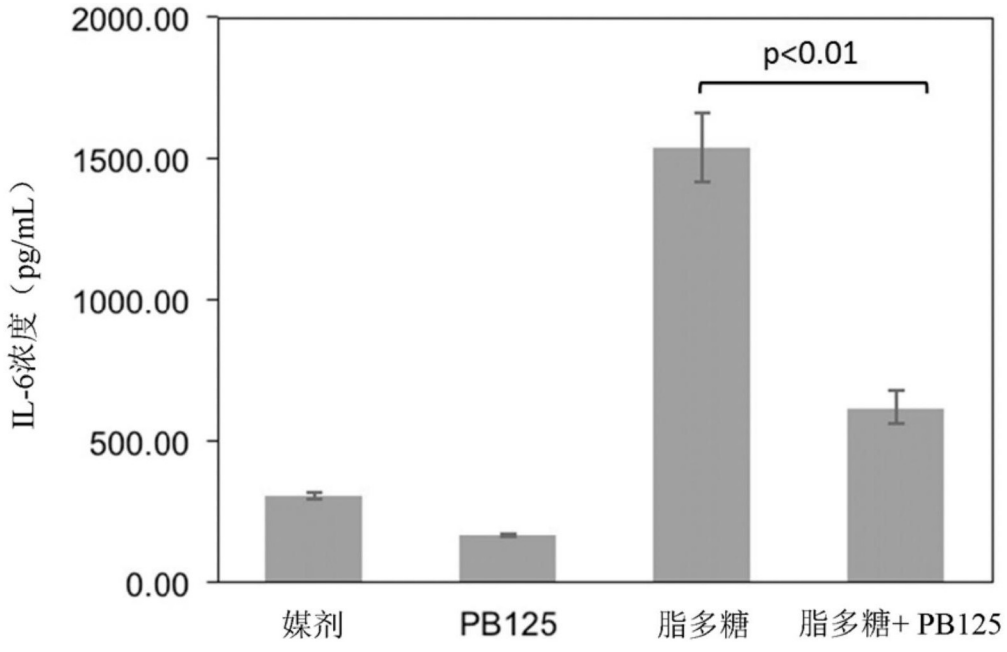
2000.00
1500.00
1000.00
500.00
0.00
IL-6浓度（pg/mL）
p<0.01
媒剂
PB125
脂多糖
脂多糖+ PB125

图12

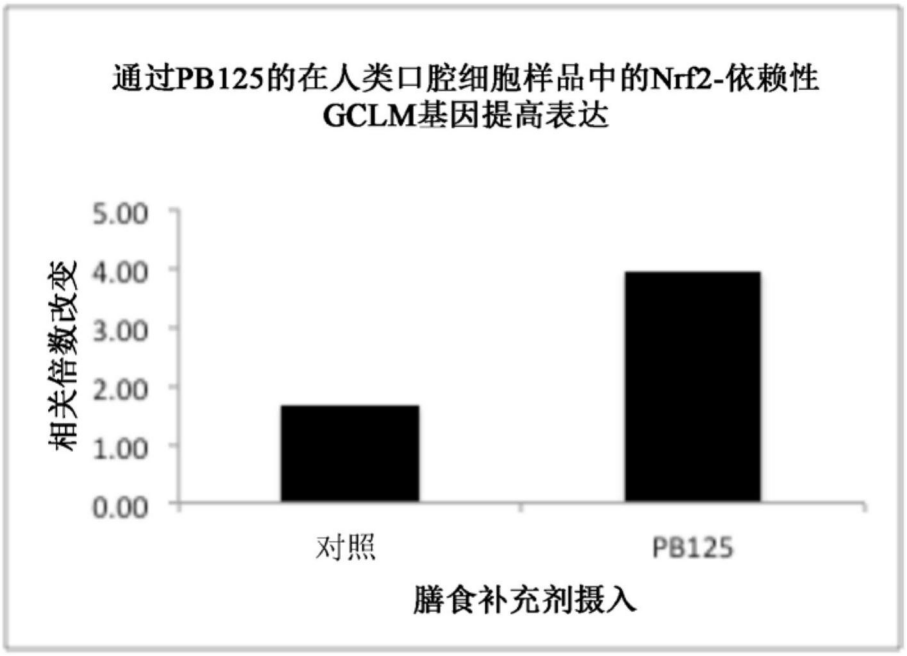
通过PB125的在人类口腔细胞样品中的Nrf2-依赖性GCLM基因提高表达
相关倍数改变
5.00
4.00
3.00
2.00
1.00
0.00
对照
PB125
膳食补充剂摄入

图13