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(71) Applicant *(for all designated States except US)*: **MARS, INCORPORATED** [US/US]; 6885 Elm Street, McLean, VA 22101-3883 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **SCHMITZ, Harold, H.** [US/US]; 6109 Rudyard Drive, Bethesda, MD 20814 (US).

(74) Agent: **JAIN, Nada**; NADA JAIN, P.C., 560 WHITE PLAINS ROAD, SUITE 460, Tarrytown, NY 10591 (US).

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(54) **Title:** FLAVANOLS AND B-TYPE PROCYANIDINS AND COX-2 EXPRESSION

(57) **Abstract:** This invention relates to compositions, and methods of use thereof, containing polyphenols such as flavanols, procyanidins and derivatives thereof, for treating and preventing certain tumors/cancers, and more specifically to treating and preventing tumors/cancers overexpressing cycloxygenase-2 (COX-2).

FLAVANOLS AND B-TYPE PROCYANIDINS AND COX-2 EXPRESSION

FIELD OF THE INVENTION

This invention relates to compositions, and methods of use thereof, containing certain polyphenols such as flavanols, procyanidins and derivatives thereof for treating and/or preventing certain tumors, and more specifically it is directed to methods of treating and/or preventing cancers involving the overexpression of cycloxygenase-2 (COX-2) gene.

BACKGROUND OF THE INVENTION

The procyanidins have attracted a great deal of attention in the fields of medicine and nutrition due to the wide range of their biological activities (e.g. U.S. Pat. Nos. 6,297,273; 6,670,390; 6,747,059; 6,524,630 and 6,638,971). Applicants have now discovered specific anti-tumor including anti-cancer properties of procyanidins and derivatives thereof and their effect on cyclooxygenase-2 (COX-2) transcription, a key regulating enzyme in the synthesis of prostaglandins in humans and other mammals.

At least two distinct isoforms of cyclooxygenase are known: COX-1 and COX-2. COX-1 is constitutively expressed in many tissues while COX-2 is not normally expressed by most tissues, but is induced rapidly and transiently by proinflammatory mediators and mitogenic stimuli including cytokines, growth factors and tumor promoters. Up-regulation of COX-2 expression is observed at sites of inflammation, in some tumors and under certain physiologic conditions.

An abnormally elevated level of COX-2 is implicated in the pathogenesis of several types of human/mammalian cancers (e. g. colorectal adenocarcinoma, stomach cancer, esophageal cancer, pancreatic cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, endometrial cancer, thyroid cancer, urinary bladder cancer, prostate cancer, skin cancer, lung cancer, oral leukoplakia- a precursor lesion of oral cancer, liver cancer, liver metastases from colon cancer, bone metastases, cholangiosarcome- a highly malignant epithelial neoplasm arising within the biliary tract, teratocarcinomas, head and neck cancers, lymphomas and leukemias) (Mohan and

Epstein, *Oral. Oncol.*, 39: 537–546, 2003). It should be noted that COX-2 overexpression is not seen in every patient with the above-mentioned cancers. COX-2 positive tumors tend to be larger, higher grade, and more likely to spread/metastasize. (Bundred and Barnes, *Br. J. Cancer*, 93 Suppl 1:S10-5, 2005; Munkarah and Ali-Fehmi, *Curr. Opin. Obstet. Gynecol.*, 17(1):49-532005). COX-2 expression by tumors is associated with poor prognosis. Women with gynecological tumors overexpressing COX-2 have been shown to have a lower response to standard therapy and shorter survival times (Munkarah and Ali-Fehmi, *Curr. Opin. Obstet. Gynecol.*, 17(1):49-532005). Further, mice genetically engineered to overexpress COX-2 are found to be susceptible to tumorigenesis (Muller-Decker et al., *Proc. Natl. Acad. Sci.* U.S.A. 99: 12483–12488, 2002) and conversely, knockout of COX-2 results in altered epidermal differentiation and reduced tumor progression (Tiano et al.,*Cancer Res.* 62: 3395–3401, 2002).

Thus, COX-2 is recognized as a molecular target for tumor therapy (Surh, *Food Chem. Toxicol.*, 40: 1091–1097, 2002; Surh et al, *Mutat. Res.* 480–481: 243–268, 2001). At present, there is a need in the art for compounds that can target COX-2 overexpression, particularly at the transcriptional level, in order to prevent and/or treat proliferative growth. It has now been found that compounds of this invention and compositions thereof are effective for such uses. Most of the previously-known COX-2 inhibitors work primarily by blocking COX-2 enzyme activity either directly or indirectly. A disadvantage of inhibition at the enzyme level is that the loss of COX-2 enzyme activity is compensated for (by the body's natural response) by a bio-feedback loop which leads to an increased production of enzyme. The present inventive compounds offer a clear advantage as COX-2 synthesis is inhibited at the level of gene transcription thereby circumventing the formation of additional, undesirable COX-2 via the biofeedback loop mechanism.

SUMMARY OF THE INVENTION

The invention relates to compositions, products and methods for treatment/prophylaxis of certain tumors, particularly certain cancers, and more specifically treatment/prophylaxis of tumors/cancers overexpressing COX-2.

In one aspect, the invention relates to a composition, such as a pharmaceutical, a food, a food additive, or a dietary supplement comprising the compound of the

invention such as a flavanol, a procyanidin or a derivative thereof. The composition may optionally contain an additional chemotherapeutic agent, or may be administered in combination with an additional chemotherapeutic agent. Packaged products containing the above-mentioned compositions and a label and/or instructions for use as described herein, e.g. to treat/prevent COX-2-overexpressing tumors/cancers are also within the scope of the invention.

In another aspect, the invention relates to a method of treating or preventing a COX-2 overexpressing tumor, including COX-2 overexpressing cancer, by administering to a mammal, such as a human or a veterinary animal, an effective amount of a compound of the invention such as a flavanol, a procyanidin or a derivative thereof. Non-limiting examples of cancers are colorectal adenocarcinoma, stomach cancer, esophageal cancer, pancreatic cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, endometrial cancer, thyroid cancer, urinary bladder cancer, prostate cancer, skin cancer, lung cancer, oral leukoplakia- a precurosor lesion of oral cancer, liver cancer, liver metastases from colon cancer, bone metastases, cholangiosarcoma- a highly malignant epithelial neoplasm arising within the biliary tract, teratocarcinomas, head and neck cancers, lymphomas and leukemias, which overexpress COX-2.

In a further aspect, the invention relates to a method comprising (i) profiling a subject for the overexpression of COX-2 and (ii) treating the subject exhibiting overexpression of COX-2 by administering an effective amount of the compounds of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the inhibitory effect of procyanidin dimer B2 on COX-2 mRNA transcription.

FIGURE 2 shows the inhibitory effect of procyanidin dimer B2 on LPS-induced COX-2 protein expression.

FIGURE 3 A-B show that COX-2 enzyme activities are not inhibited by procyanidin dimer B2 (NS398 is a positive control).

FIGURE 4 A-C show the effects of B1 dimer, B2 dimer, (-)-catechin and (+)-epicatechin in comparison with A1 dimer (each 10μM) on the mRNA expression of COX-2 in LPS and fMLP-pretreated macrophages, and their potency comparison. n=3-

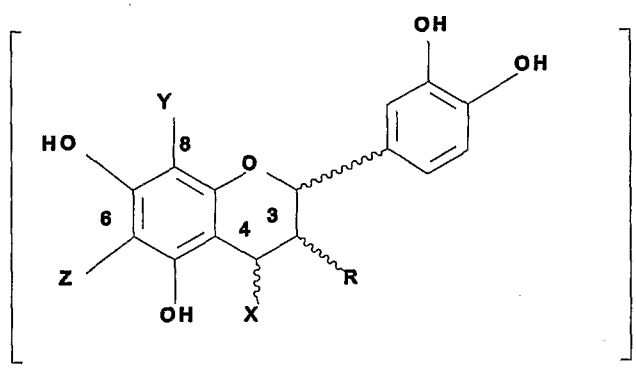
4, Mean ± SD. $^{**}P<0.01$ *versus* control, $^{\#\#}P<0.01$ *versus* LPS, $^{\$\$}P<0.01$ *versus* LPS + fMLP. The normal macrophages without LPS or fMLP treatment were considered as controls.

DETAILED DESCRIPTION

All patents, patent applications and references cited in this application are hereby incorporated herein by reference. In case of any inconsistency, the present disclosure governs.

The present invention relates to a compound, and a composition comprising an effective amount of the compound, having the following formula A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives):

A =



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH, O-sugar or O-gallate;

the substituents of C-4, C-6 and C-8 are X, Z and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

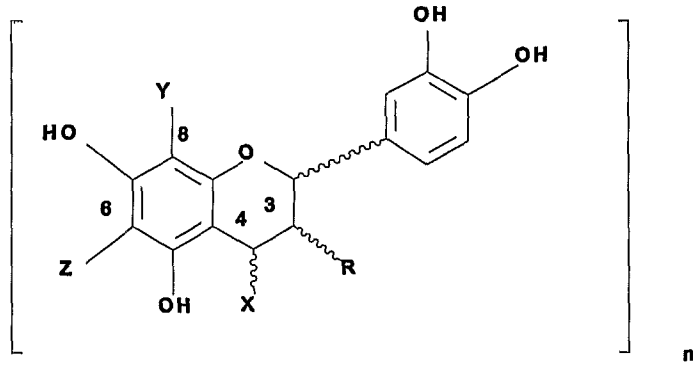
when any C-4, C-6 or C-8 is not bonded to another monomeric unit, X, Y and Z independently are hydrogen or a sugar; and

the sugar is optionally substituted with a phenolic moiety at any position, for instance, via an ester bond.

Monomeric units in the above formula may be bonded via 4→6α; 4 →6β; 4→8α; and/or 4 →8β linkages. The sugar is preferably a monosaccharide or a di-saccharide. The sugar may be selected from the group consisting of glucose, galactose, rhamnose, xylose, and arabinose. The phenolic moiety may be selected from the group consisting of caffeic, cinnamic, coumaric, ferulic, gallic, hydroxybenzoic and sinapic acids. Procyanidin derivatives may include esters such as the gallate esters (e.g. B2 dimer gallate); compounds derivatized with a saccharide moiety such as mono- or di-saccharide moiety (e.g. β-D-glucose), glucuronidated and methylated derivatives, and oxidation products. Oxidation products may be prepared as disclosed in U.S. Pat. No. 5,554,645, the relevant portions of which are incorporated herein by reference. Esters, for example esters with gallic acid, may be prepared using known esterification reactions, and for example as described in US Pat. No. 6,420,572, the disclosure of which is hereby incorporated herein by reference. Methylated derivatives, such as 3'O-methyl-, 4'O-methyl-, and 3'O, 4'O-dimethyl- derivatives may be prepared, for example, as described in Cren-Olive et al., 2002, *J. Chem. Soc. Perkin Trans.* 1, 821-830, and Donovan et al., *Journal of Chromatography* B, 726 (1999) 277-283, the disclosures of which are hereby incorporated herein by reference. Glucuronidated products may be prepared as described in Yu et al, "A novel and effective procedure for the preparation of glucuronides," *Organic Letters*, 2(16) (2000) 2539-41, and as in Spencer et al, ""Contrasting influences of glucuronidation and O-methylation of epicatechin on hydrogen peroxide-induced cell death in neurons and fibroblasts," *Free Radical Biology and Medicine* 31(9) (2001) 1139-46, hereby incorporated herein by reference. It should be noted that this disclosure applies to all formulas recited herein including flavanols.

In certain embodiments, the invention relates to a compound, and the composition comprising an effective amount the compound having the formula A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives):

A =



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH;

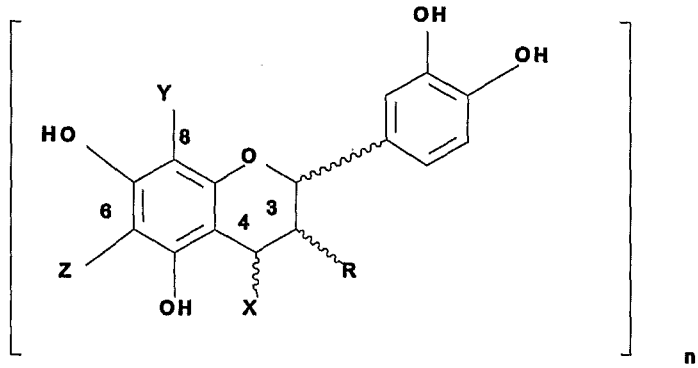
the substituents of C-4, C-6 and C-8 are X, Z and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 and C-8; and

when any C-4, C-6 or C-8 is not bonded to another monomeric unit, X, Y and Z are hydrogen.

Examples of the compounds useful for the products and in the methods of the invention include the compounds described herein wherein the integer n is 3 to 18; 2 to 12; 3 to 12; 2 to 5; 4 to 12; 5 to 12; 4 to 10; or 5 to 10. In some embodiments, the integer n is 2 to 4, for example 2 or 3. This disclosure applies to any compound of formula An herein.

In one embodiment, the invention relates to a compound, and a composition comprising an effective amount of the compound, having the following formula A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives):

A =



wherein

n is 2;

R and X each have either α or β stereochemistry;

R is OH, O-sugar or O-gallate;

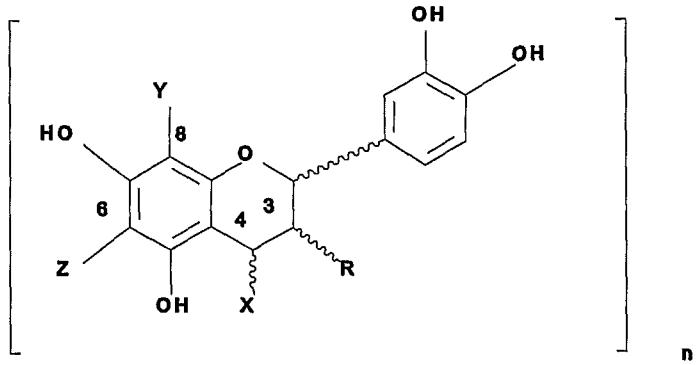
the substituents of C-4, C-6 and C-8 are X, Z and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 and C-8; and

when any C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y and Z independently are hydrogen or sugar; and

the sugar is optionally substituted with a phenolic moiety at any position, for instance, via an ester bond.

In another embodiment, the invention relates to a compound, and a composition comprising an effective amount of the compound, having the following formula A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives):

A =



wherein

n is 2;

R and X each have either α or β stereochemistry;

R is OH;

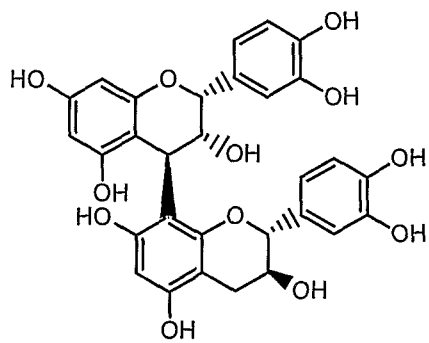
the substituents of C-4, C-6 and C-8 are X, Z and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 and C-8; and

when any C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y and Z are hydrogen.

Examples of dimers within the scope of the invention are dimers B1 [(-)-epicatechin-(4β-8)-(+)-catechin], B2 [(-)-epicatechin-(4β-8)-(-)-epicatechin] and B5 [(-)-epicatechin-(4β-6)-(-)-epicatechin].

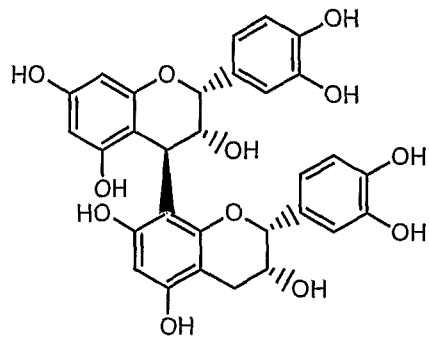
B1 dimer [(-)-epicatechin-(4β-8)-(+)-catechin] has the following formula:

B1 Dimer - epicatechin-(4-β-8)-catechin

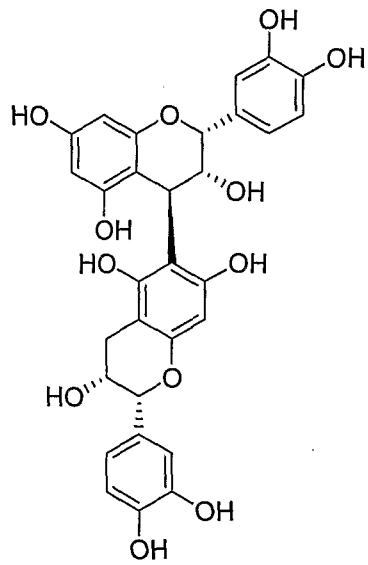


B2 dimer [(-)-epicatechin-(4β-8)-(-)-epicatechin] has the following formula:

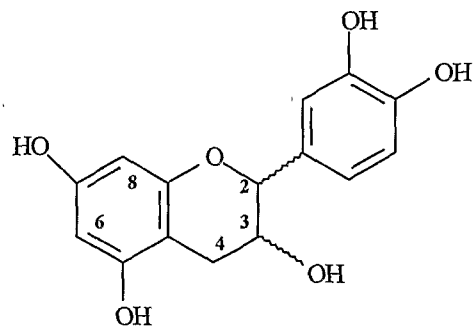
B2 Dimer - epicatechin-(4-β-8)-epicatechin



B5 dimer [(-)-epicatechin-(4β-6)-(-)-epicatechin] has the following formula:

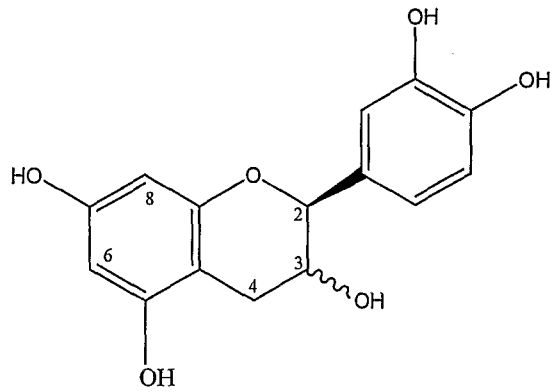


In other embodiments, the present invention relates to a flavanol or a flavan-3-ol. As used herein, the term "flavanol" or "flavan-3-ol" refers to a compound of the following formula:



The invention also relates to a composition comprising an effective amount of the flavanol, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives). Examples of derivatives and their preparation are as described above. In some embodiments, the flavanol derivative is not a gallated derivative.

In certain embodiments, the flavanols of the above formula may have beta stereochemistry at the C2 atom as shown below:



Examples of flavanols are (+)-epicatechin, (-)-epicatechin, (+)-catechin, and (-)-catechin.

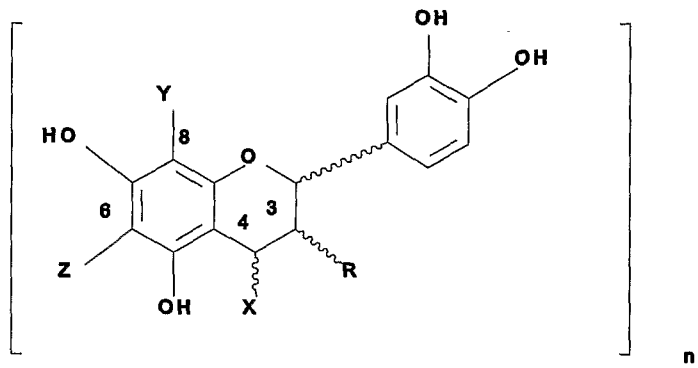
Methods of Use

The invention relates to methods for prevention and/or treatment of certain tumors, particularly certain cancers, and more specifically tumors e.g. cancers that overexpress COX-2 (COX-2 overexpressing tumors/cancers). Non-limiting examples of COX-2 overexpressing cancers to be prevented/treated according to the methods described herein are COX-2 overexpressing colorectal adenocarcinoma, COX-2 overexpressing cancers of the digestive tract (e.g. COX-2 overexpressing stomach cancer, COX-2 overexpressing esophageal cancer and COX-2 overexpressing pancreatic cancer), COX-2 overexpressing breast cancer, COX-2 overexpressing gynecological cancers (e.g. COX-2 overexpressing ovarian cancer, COX-2 overexpressing cervical cancer, COX-2 overexpressing uterine cancer, COX-2 overexpressing endometrial cancer), COX-2 overexpressing thyroid cancer, COX-2 overexpressing urinary bladder cancer, COX-2 overexpressing prostate cancer, COX-2

overexpressing skin cancer; COX-2 overexpressing lung cancer; COX-2 overexpressing oral leukoplakia (a precursor lesion of oral cancer); COX-2 overexpressing liver cancer and liver metastases from colon cancer; COX-2 overexpressing cholangiosarcoma (a highly malignant epithelial neoplasm arising within the biliary tract); COX-2 overexpressing bone metastases; COX-2 overexpressing teratocarcinomas; COX-2 overexpressing head and neck cancers and COX-2 overexpressing leukemias and lymphomas. Any compound described in the application may be used to practice the methods described herein.

In certain embodiments, the invention provides a method of treating or preventing a COX-2 overexpressing tumor/cancer, which comprises administering to a human or a veterinary animal in need thereof an effective amount of a compound having the following formula A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives):

A =



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH, O-sugar or O-gallate;

the substituents of C-4, C-6 and C-8 are X, Z and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

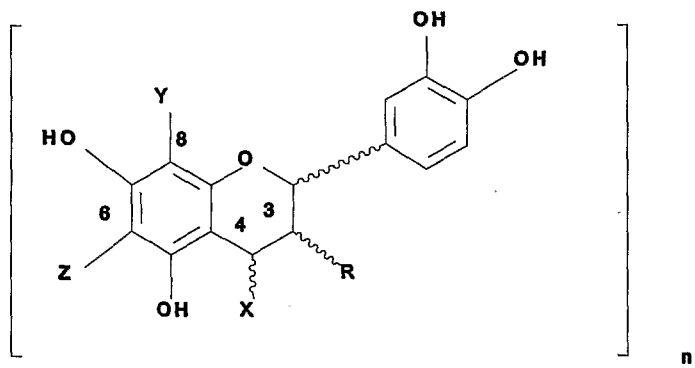
when any C-4, C-6 or C-8 is not bonded to another monomeric unit, X, Y and Z independently are hydrogen or a sugar; and

the sugar is optionally substituted with a phenolic moiety at any position, for instance, via an ester bond.

For example, the above method may involve use of a compound A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives), wherein R is OH, and when any C-4, C-6 or C-8 is not bonded to another monomeric unit, X, Y and Z are hydrogen. Examples of suitable sugars are as described above. Examples of phenolic moieties are as described above. Examples of derivatives are as described above.

In one of the embodiments, the method of treating or preventing a COX-2 overexpressing tumor/cancer comprises administering to a human or a veterinary animal in need thereof an effective amount of a compound having the formula A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives):

A =



wherein

n is 2

R and X each have either α or β stereochemistry;

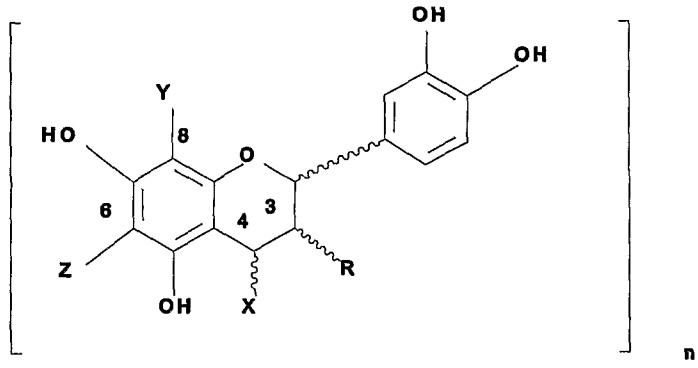
R is OH;

the substituents of C-4, C-6 and C-8 are X, Z and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 and C-8; and

when any C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y and Z are hydrogen.

The invention also relates to a method of treating or preventing a COX-2 overexpressing tumor/cancer, which comprises administering to a human or a veterinary animal suffering from said tumor an effective amount of a compound having the formula A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives):

A =



wherein

n is 2;

R and X each have either α or β stereochemistry;

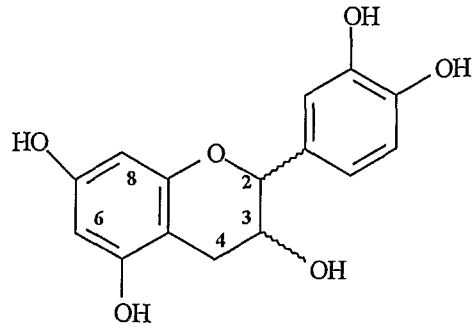
R is OH, O-sugar or O-gallate;

the substituents of C-4, C-6 and C-8 are X, Z and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any C-4, C-6 or C-8 is not bonded to another monomeric unit, X, Y and Z independently are hydrogen or a sugar; and

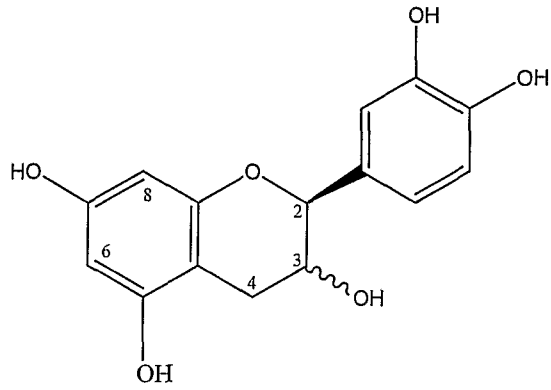
the sugar is optionally substituted with a phenolic moiety at any position, for instance, via an ester bond.

In certain embodiments, the invention provides a method of treating or preventing a COX-2 overexpressing tumor/cancer, which comprises administering to a human or a veterinary animal in need thereof an effective amount of a flavanol or a flavan-3-ol of the following formula:



or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives). In some embodiments, the flavanol derivative is not a gallated derivative.

In certain embodiments, the above methods may be practiced with a flavanol having beta stereochemistry at the C2 atom as shown below:



Examples of the compounds for use in the methods described herein are (+)-epicatechin, (-)-epicatechin, (+)-catechin, and (-)-catechin, dimers B1, B2, and B5.

As used herein, "treatment" means improving an existing medical condition, for example a COX-2 overexpressing tumor/ cancer, for example by slowing down the disease progression, prolonging survival, reducing the risk of death, and/or inducing a measurable reduction in tumor size. Examples of tumors/cancers to be treated are colorectal adenocarcinoma, stomach cancer, esophageal cancer, pancreatic cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, endometrial cancer, thyroid cancer, urinary bladder cancer, prostate cancer, skin cancer, lung cancer, oral leukoplakia- a precurosor lesion of oral cancer, liver cancer, liver metastases from colon cancer, bone metastases, cholangiosarcoma- a highly malignant epithelial neoplasm arising within the biliary tract, teratocarcinomas, head and neck cancers, lymphomas and leukemias, each overexpressing COX-2. Patients that overexpress

COX-2 can benefit from treatment with the present compounds because COX-2 overexpression is often correlated with more aggressive tumors with poor prognosis.

The present compounds are also suitable for combination therapy with other COX-2 specific inhibitors, most of which primarily target the active COX-2 enzyme. Examples of COX-2 inhibitors include meloxicam, etodolac, nimesulide, flosulide, lumiracoxib, celecoxib, valdecoxib, rofecoxib, deracoxib, parecoxib, etoricoxib, darbufelone, and meclofenamate esters and amides.

The present compounds may be administered, in some embodiments, in combination with and/or to enhance responsiveness to chemotherapeutic agents and/or methods. Examples of such agents include: alkylating agents (e.g. busulfan, cyclophosphamide, melphalan); nitrosureas (e.g. carmustine, lomustine); antimetabolites (e.g. 5-fluorouracil, methotrexate, fludarabine); antitumor antibiotics (e.g. bleomycin, doxorubicin) and mitotic inhibitors (e.g. docetaxel, etoposide, vinorelbine). For that purpose, dosage forms other than pharmaceuticals, e.g. dietary supplements and foods, may also be used.

The invention also encompasses a method of preventing COX-2 overexpressing tumor, particularly COX-2 overexpressing cancer (chemoprevention) comprising administering the compound of the invention to a subject at risk of developing a COX-2 overexpressing tumor/cancer.

The term "preventing" means reducing the risks associated with developing a disease, including reducing the onset of the disease or recurrence of the disease.

The phrase "subject at risk of developing tumor (e.g. cancer)" means a subject with a characteristic(s) which increases the likelihood of tumor (e.g. cancer) in a group of people who have a risk factor for developing the tumor compared to an otherwise healthy group of people who do not. Risk factors may include familial history of COX-2 overexpressing cancers [e.g. colorectal cancers such as Familial Adenomatous Polyposis (FAP) and Hereditary Nonpolyposis Colorectal Cancer (HNPCC) or Familial Juvenile Polyposis (FJP), an inherited cancer of the gastrointestinal tract]. The importance of COX-2 inhibition in these forms of cancers has been underscored by recent clinical studies using specific COX-2 inhibitors (e.g. rofecoxib, celecoxib) which have shown that these compounds can: (i) reduce intestinal polyp burden in patients with FAP; (ii) prevent the occurrence and/or recurrence of colorectal adenomas and cancers. (Peek, Cancer Chemother. Pharmacol., 54 Suppl1:S50-6, 2004).

Chemoprevention is particularly important for such high-risk patients e.g. FAP patients, nearly all whom develop colorectal cancer by age 40, if they hadn't previously undergone polypectomy. (Thompson, Am. J. Health-Syst. Pharm., 62:890-894, 2005).

The methods described herein may be used in a human or a veterinary animal, such as a dog, a cat, and a horse.

Thus, the following uses are within the scope of the invention. Use of a flavanol and/or a compound A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives), as defined above, in the manufacture of a medicament, food, nutraceutical or dietary supplement for inhibiting COX-2 expression. Use of a flavanol and/or a compound of formula A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives), as defined herein, in the manufacture of a medicament, food, nutraceutical or dietary supplement for use in treating or preventing COX-2 overexpressing tumors e.g. cancers.

The above described methods may further comprise determining the effectiveness of the treatment or prophylaxis, for example, by measuring the level of COX-2 expression in a tissue sample using techniques known in the art.

The advantage of the present invention is that can offer a personalized medicine approach to the treatment/prophylaxis of tumors particularly cancers. Each patient can be evaluated for his/her expression of COX-2. Thus, methods of profiling of the patients and their subsequent treatment/prophylaxis are also within the scope of the invention. Such method comprises (i) profiling a subject for the overexpression of COX-2 and (ii) treating the subject exhibiting overexpression of COX-2 by administering an effective amount of the compound of the invention.

It will be understood by a person of skill in the art that overexpression in subjects to be profiled and/or treated can be determined using methods and reagents well known in the art. For example, overexpression at the level of transcription can be assessed using Southern and Northern blotting with appropriate nucleic acid probes or by using Polymerase Chain Reaction (PCR).

The effective amount may be determined by a person of skill in the art using the guidance provided herein and general knowledge in the art. For example, the effective amount may be such as to achieve a physiologically relevant concentration in the body

of a mammal. Such a physiologically relevant concentration may be at least 20 nanomolar (nM), preferably at least about 100 nM, and more preferably at least about 500 nM. In one embodiment, at least about one micromole in the blood of the mammal, such as a human, is achieved. The compounds of formula A_n, as defined herein, may be administered at from about 50 mg/day to about 1000 mg/day, preferably from about 100-150 mg/day to about 900 mg/day, and most preferably from about 300 mg/day to about 500 mg/day. However, amounts higher than stated above may be used. The amounts may be measured as described in Adamson, G.E. et al., *J. Ag. Food Chem.*; 1999; 47 (10) 4184-4188.

The treatments/preventive administration may be continued as a regimen, *i.e.*, for an effective period of time, e.g., daily, monthly, bimonthly, biannually, annually, or in some other regimen, as determined by the skilled medical practitioner for such time as is necessary. The administration may be continued for at least a period of time required to reduce COX-2 expression to therapeutically relevant levels. Preferably, the composition is administered daily, most preferably two or three times a day, for example, morning and evening to maintain the levels of the effective compounds in the body of the mammal. To obtain the most beneficial results, the composition may be administered for at least about 30, or at least about 60 days. These regimens may be repeated periodically.

Compositions and Formulations

The compounds of the invention may be administered as a pharmaceutical, a food, a food additive, or a dietary supplement.

As used herein, a "pharmaceutical" is a medicinal drug. See Merriam-Webster's Collegiate Dictionary, 10th Edition, 1993. A pharmaceutical may also be referred to as a medicament. A "food" is a material containing protein, carbohydrate and/or fat, which is used in the body of an organism to sustain growth, repair and vital processes and to furnish energy. Foods may also contain supplementary substances, for example, minerals, vitamins and condiments. See Merriam-Webster's Collegiate Dictionary, 10th Edition, 1993. The term food includes a beverage adapted for human or animal consumption. As used herein a "food additive" is as defined by the FDA in 21 C.F.R. 170.3(e)(1) and includes direct and indirect additives. As used herein, a "dietary supplement" is a product (other than tobacco) that is intended to supplement

the diet that bears or contains the one or more of the following dietary ingredients: a vitamin, a mineral, an herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract or combination of these ingredients. The above compositions may be prepared as is known in the art.

The compositions may contain a carrier, a diluent, or an excipient. Depending on the intended use, the carrier, diluent, or excipient may be chosen to be suitable for human or veterinary use, food, additive, dietary supplement or pharmaceutical use. The composition may optionally contain an additional tumor/cancer treating agent. Also depending on use, a person of skill in the art may select the degree of purity of the compound of the invention. For example, when used to prepare pharmaceutical dosage forms, the compound should be as pure as commercially possible, while when preparing food, additive, or supplement, less pure or mixtures of compounds (e.g. plant extracts) may be used.

The compound of the invention may be "isolated and purified," *i.e.,* it may be separated from compounds with which it naturally occurs (e.g. when the compound is of natural origin), or it may be synthetically prepared, in either case such that the level of contaminating compounds and/or impurities does not significantly contribute to, or detract from, the effectiveness of the compound. For example, an "isolated and purified B2 dimer" is separated from B5 dimer, with which it may occur in nature (e.g. in cocoa bean), to the extent achievable by the available commercially viable purification and separation techniques. Such compounds are particularly suitable for pharmaceutical applications.

The compound may also be less pure, *i.e.,* "substantially pure," *i.e.,* it may possess the highest degree of homogeneity achievable by available purification, separation and/or synthesis technology but need not be separated from the like compounds. As used herein, "the like compounds" are the compounds having the same degree of polymerization. For example, a "substantially pure dimer" refers to a mixture of dimers (e.g. B2 and B5, as it would occur in a cocoa extract fraction). While less suitable for pharmaceutical applications, such "substantially pure" compounds may be utilized for food, food additive and dietary supplement applications.

In some embodiments, the compound of the invention is at least 80% pure, at least 85% pure, at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure. Such compounds are particularly suitable for pharmaceutical applications.

Pharmaceuticals containing the inventive compounds, optionally in combination with another cancer treating agent, may be administered in a variety of ways such as orally, sublingually, bucally, nasally, rectally, intravenously, parenterally and topically. A person of skill in the art will be able to determine a suitable mode of administration to maximize the delivery of the compound of formula A_n, and optionally another cancer treating agent, to the site of the tumor. Thus, dosage forms adapted for each type of administration are within the scope of the invention and include solid, liquid and semi-solid dosage forms, such as tablets, capsules, gelatin capsules (gelcaps), bulk or unit dose powders or granules, emulsions, suspensions, pastes, creams, gels, foams or jellies. Sustained-release dosage forms are also within the scope of the invention. Suitable pharmaceutically acceptable carriers, diluents, or excipients are generally known in the art and can be determined readily by a person skilled in the art. The tablet, for example, may comprise an effective amount of the compound of the invention and optionally a carrier, such as sorbitol, lactose, cellulose, or dicalcium phosphate.

The foods comprising the compounds described herein and optionally another tumor/cancer treating/preventing agent may be adapted for human or veterinary use, and include pet foods. The food may be other than a confectionery, for example, a beverage (e.g. cocoa flavored beverage). A confectionery such as a standard of identity (SOI) and non-SOI chocolate, such as milk, sweet and semi-sweet chocolate including dark chocolate, low fat chocolate and a candy which may be a chocolate covered candy are also within the scope of the invention. Other examples include a baked product (e.g. brownie, baked snack, cookie, biscuit) a condiment, a granola bar, a toffee chew, a meal replacement bar, a spread, a syrup, a powder beverage mix, a cocoa or a chocolate flavored beverage, a pudding, a rice cake, a rice mix, a savory sauce and the like. If desired, the foods may be chocolate or cocoa flavored. Food products may be chocolates and candy bars, such as granola bars, containing nuts, for example, peanuts, walnuts, almonds, and hazelnuts.

The compounds for use in the present invention may be of natural origin, for example, derived from a cocoa bean or another natural source known to a person of

skill in the art, or prepared synthetically. A person of skill in the art may select natural or synthetic polyphenol based on the use and/or availability or cost.

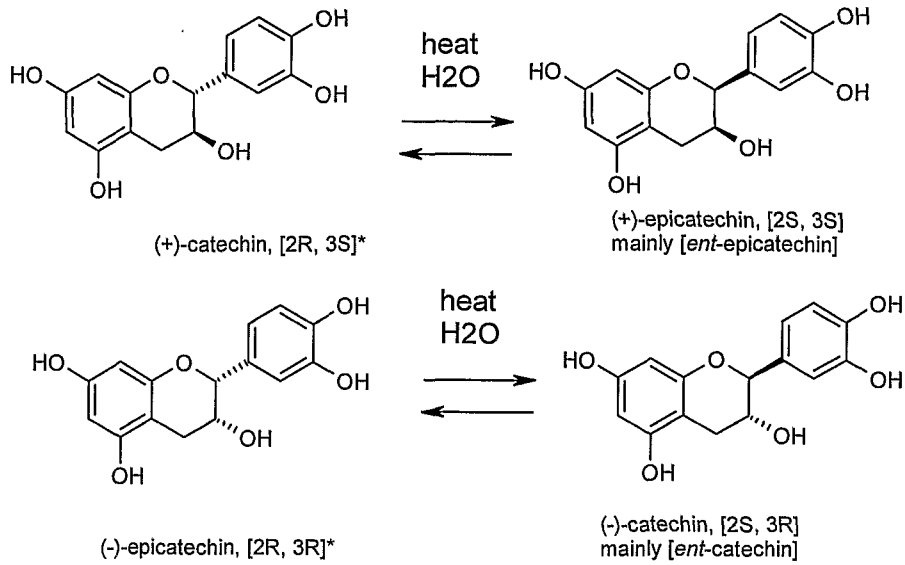
The compounds may be included in the composition in the form of a cocoa ingredient, for example, chocolate liquor included in chocolate, or may be added independently of cocoa ingredients, for example, as an extract, extract fraction, isolated and purified individual compound, pooled extract fractions or a synthetically prepared compound. The term "cocoa ingredient" refers to a cocoa solids-containing material derived from shell-free cocoa nibs such as chocolate liquor and partially or fully-defatted cocoa solids (e.g. cake or powder). The extraction and purification may be conducted as described in U.S. Pat. Nos. 5,554,645 and 6,670,390 to Romanczyk *et al.*, which is hereby incorporated herein by reference.

Synthetic procyanidins may also be used and are prepared by methods known in the art and as described, for example in, U. S. Pat. Nos. 6,420,572; 6,156,912; and 6,864,377, the relevant portions of each of which are hereby incorporated herein by reference.

A flavanol having beta stereochemistry at the C-2 atom may be prepared by thermally treating (in an aqueous solution) a flavanol having alpha stereochemistry at C-2 atom (which are commonly found in nature, for example in cocoa) to cause rotation about the C2 atom resulting in beta stereochemistry at the C-2 atom.

Preparation of a flavanol having beta stereochemistry at the C-2 atom may be conducted according to the following scheme 1 (and as described in Freudenberg, K. and Purrmann, L. (1924). Raumisomere Catechin IV. Liebig's Annalen, 437, 472-85; Fredenberg, K., Bohme, L. and Purrmann, L. (1922). Raumisomere Catechin II. Ber. Dscht. Chem. Ges., 55, 1734-47, the disclosures of which are hereby incorporated herein by reference):

Scheme 1. Epimerization of flavan-3-ols at C-2 in aqueous media (Freudenberg et al., 1922; Freunberg and Purmann,1924).



*known to occur in cocoa

The lower the temperature, the longer the exposure required to cause rotation about the C-2 atom. For examples, such temperatures and times may be at least 40°C, more preferably at least 50°C, for at least 10 hours, more preferably at least 24 hours, or more preferably at least 48 hours; or at least 60°C, at least 70°C, at least 80°C, at least 90°C, at least 100°C, at least 110°C, or at least 120°C, each for at least five, or at least 10, 15 or 20 minutes. For example, the compound may be treated at 120°C for 10 minutes, or 120°C for 20 minutes. Other temperature/time combinations are also effective and the skilled artisan may determine such without undue experimentation using general knowledge in the art and the guidance provided herein. Known techniques, such as HPLC/MS analysis may be used to monitor the success of the reaction.

A daily effective amount of the compound of the invention may be provided in a single serving in case of a food or a single dosage in case of a pharmaceutical or a dietary supplement. For example, a confectionery (e.g. chocolate) may contain at least about 100 mg/serving (e.g. 150-200, 200-400 mg/serving).

The dietary supplement containing the compounds of the invention, and optionally another cancer treating agent, may be prepared using methods known in the

art and may comprise, for example, nutrient such as dicalcium phosphate, magnesium stearate, calcium nitrate, vitamins, and minerals.

Further within the scope of the invention is an article of manufacture such as a packaged product comprising the composition of the invention (e.g. a food, a dietary supplement, a pharmaceutical) and a label indicating the presence of, or an enhanced content of the inventive compounds or directing use of the composition to treat a COX-2 expressing tumor (e.g. cancer). The packaged product may contain the composition and the instructions for use to treat a COX-2 overexpressing tumor (e.g. cancer). The label and/or instructions for use may refer to any of the methods of use described in this application. In certain embodiments, the label and/or the instructions for use direct use of the compounds of the invention for treating COX-2 overexpressing tumors.

The invention also relates to a method of manufacturing an article of manufacture comprising any of the compositions described herein, packaging the composition to obtain an article of manufacture and instructing, directing or promoting the use of the composition/article of manufacture for any of the uses described herein. Such instructing, directing or promoting includes advertising.

The invention is further described in the following non-limiting examples.

EXAMPLES

Example 1: Effect of Procyanidin B2 on COX-2 expression

Materials

Procyanidin dimer B2 was prepared from cocoa by solvent extraction, using gel permeation chromatography, followed by further purification/isolation of a dimer enriched fraction using Normal-Phase HPLC (described in detail in Adamson et al., *J. Ag. Food Chem.*, 1999, 47 (10):4184-4188; see also 5,554,645, both hereby incorporated herein by reference). This material was then passed over a C18 column to further enrich B2 dimer (98.3% purity) in the fraction used in the experiments described below.

Phorbol 12-myristate 13-acetate (PMA), Lipopolysaccharides (LPS, from Escherichia coli serotype 0111: B4) and NS398 (a selective COX-2 inhibitor) were purchased from Sigma (St. Louis, MO). RPMI 1640, L-glutamine, HEPES, 2-mercaptoethanol, fetal bovine serum, and penicillin/streptomycin were purchased from

Gibco BRL (Grand Island, NY). Anti-COX-2 was purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA). SuperSignal West Pico chemiluminescent substrate and PGE_2-specific RIA kit was purchased from Beijing East Asia Institute of Immunology (Beijing CN). All other chemicals used were in the purest form available commercially.

Cell culture

Human monocytic THP-1 cells from acute monocytic leukemia (American Type Culture Collection, Manassas, VA) in RPMI 1640 medium (Life Technologies, Rockville, MD), with 4.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, and 50 μM 2-ME supplemented with 10% FBS, were cultured under a humidified 5% CO_2 atmosphere at 37 °C. For differentiation, THP-1 cells were plated at 1×10^6 cells/ml in the medium containing 100 nM PMA and allowed to adhere for 48 h, after which they were fed with PMA-free medium and cultured for 24 h prior to use. LPS was used at concentration of 1μg/ml in the medium. sf9 cells were cultured in monolayer at 28 °C in Grace's supplemented medium with 10 % heat- inactivated fetal bovine serum.

Determination of COX-2 Enzyme Activity

The effect of procyanidin dimer B2 and NS398, a selective COX-2 inhibitor (positive control), on the activity of COX-2 was measured using baculovirus-expressed recombinant human COX-2 enzyme as previously described (Zhang et al., Acta. Pharmacol. Sin. 25(8):1000-1006, 2004). Briefly, 24 h after infecting sf9 cells with hCOX-2 recombinant baculovirus, the cells were collected and washed in HHBS. The assays were performed as follows. One milliliter of Hank's solution containing 1×10^5 COX-2 expressing cells plus 9×10^5 uninfected sf9 cells was dispensed per well of 24-well polypropylene plates. B2, NS398, or DMSO vehicle (10 μL) was added to the appropriate well containing the cell suspension. Following a 15-min drug or DMSO preincubation at 37 °C, the cells were challenged with 10 μmol/L arachidonic acid (Sigma) in ethanol and incubated for 10 min. Reactions were terminated by the addition of 100 μL of 1 mol/L HCl, neutralized with 100 μL of 1 mol/L NaOH. The cells were pelleted for 10 min at 300×g and the levels of PGE_2 in the supernatant were determined by a PGE_2-specific RIA (Beijing East Asia Institute of Immunology). The

concentration of PGE_2 was then determined by interpolation from a standard curve and inhibition calculated by comparison of the PGE_2 production by drug-treated cells (B2 and NS398) with that of DMSO-treated cells.

Real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) to assess COX-2 mRNA expression

Total RNA was extracted from macrophages with TRIzol reagent (Invitrogen Corporation, Carlsbad, CA). Real-time quantitative RT-PCR was performed using the Opticon 2 (MJ Research Inc., Waltham, MA). Sequence specific PCR primers for COX-2 [accession no. NM_000963; forward primer: 5′-GGGCAAAGACTGCGAAGAAG-3′ [SEQ ID NO: 1]; reverse primer: 5′-CCCATGTGACGAAATGACTG-3′ [SEQ ID NO: 2]] and GAPDH [accession no. NM_002046; forward primer: 5′-ACGGATTTGGTCGTATTGGG-3′ [SEQ ID NO: 3]; reverse primer: 5′-CGCTCCTGGAAGATGGTGAT-3′ [SEQ ID NO: 4]] were designed using the Primer Premier software version 5.00. Standard curves were run on the same plate and the relative standard curve method was used to calculate the relative gene expression.

Western Blot analysis

5×10^6 cells were resuspended in modified RIPA lysis buffer (Tris-HCl 50 mM, pH 7.4 NaCl 150 mM, EDTA 1 mM, Na-deoxycholate 0.25%, NP-40 1%, PMSF 1 mM, Na_3VO4 1 mM, NaF 1 mM, Aprotinin 10 μg/ml, leupeptin 5 μg/ml, pepstatin 5 μg/ml), and lysed cells on ice for 45 min. The lysate was centrifuged at 14,000×g for 15 min to sediment the particulate materials. The protein concentration of the supernatant was measured by the method of Lowry (Lowry et al., J. Biol. Chem. 193:265-267, 1951). Samples were electrophoresed in SDS/PAGE gels and separated proteins were transferred onto a PVDF membrane. The blots were blocked with 5% non-fat dry milk in Tris-buffered saline (TBS) for 1 h at room temperature and subsequently incubated overnight at 4 °C with primary antibodies diluted (1 :1000) in TBST [TBS, 1% (v/v) Tween 20 and 5% (w/v) BSA]. Following three washes of 10 min each with TBST, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies in blocking buffer for 1 h at room temperature. After three washes with TBST, the blots

were developed with chemiluminescence reagent and exposed to X-ray film (Kodak XAR5, Eastman Kodak, Rochester, NY, U.S.A.).

Results

Procyanidin dimer B2 inhibits LPS-induced increases in the transcription and expression of COX-2 protein

Treatment of differentiated THP-1 cells with 1μg/ml LPS led to a dramatic increase in COX-2 transcription (Fig. 1). When THP-1 cells were exposed to dimer B2 and LPS for 4 h, the levels of mRNA for COX-2 were reduced in a concentration-dependent manner (Fig. 1). In a separate experiment (30 min cell pretreatment with B2 followed by 4 hour LPS treatment), dimer B2, at a dose of 50 μM, inhibited COX-2 protein expression as illustrated by the Western blot in Fig. 2.

To determine if B2 had a direct effect on COX-2 enzyme activity, a baculovirus-expressed human recombinant COX-2 in a cell-free assay was used. Fig. 3 showed that B2 had no inhibitory effect on PGE2 synthesis, a product of COX-2 enzyme activity, in contrast to the NS398 positive control.

Example 2: Effect of procyanidin dimers (A1, B1 and B2) and flavanols ((-)-catechin and (+)-epicatechin) on COX-2 expression

Materials

Phorbol 12-myristate 13-acetate (PMA), lipopolysaccharide (LPS), and N-formyl-methionyl-leucyl-phenylalanine (fMLP), were obtained from Sigma (St. Louis, MO). Chemicals employed for gel electrophoresis were purchased from Bio-Rad (Hercules, CA). Trypsin sequencing grade was obtained from Promega (Southampton, United Kingdom). EDTA, EGTA, and PMSF were purchased from Amresco (Solon, OH). Flavanols (+)-catechin and (-)-epicatechin were purchased from Sigma, and (-) catechin and (+)-epicatechin were prepared by thermally-treating (+)-catechin and (-)-epicatechin, respectively in an aqueous solution as described above. Procyanidin dimer B2 was prepared from cocoa by solvent extraction, using gel permeation chromatography, followed by further purification/isolation of a dimer enriched fraction using Normal-Phase HPLC (described in detail in Adamson et al., *J. Ag. Food Chem.*, 1999, 47 (10):4184-4188), see also US Pat No. 5,554,645, both of which are hereby

incorporated herein by reference. This material was then passed over a C18 column to further enrich B2 dimer (98.3%) in the fraction which was used in the experiments described below. Procyanidin dimer B1 was prepared synthetically as described in U.S. Patent No. 6,420,572, which is hereby incorporated herein by reference. A1 dimer was prepared from peanut skins as described in U.S. Application Serial No. 11/045,648 (published as. US 2005/0164956), which is hereby incorporated herein by reference.

Cell Culture, RNA Extraction and Polymerase Chain Reaction (RT-PCR)

The human monocyte line U937 was obtained from the cell bank in Shanghai Institute of Biological Sciences, Chinese Academy of Sciences. Monocytic cells of 3-4 passages were grown to confluence in RPMI 1640 (GIBCO-BRL, Glasgow, Scotland) containing 10 % fetal bovine serum at 37^0C in a 5 % CO_2 humidified incubator during all experimental procedures. Macrophages were pretreated with procyanidins A1 dimer, B1 dimer, B2 dimer, and flavanols (-)-catechin and (+)-epicatechin (10μM) for 2 h before LPS (1μg.ml^{-1}) priming or fMLP (1μM) stimulation for 2 h. The normal macrophages without LPS or fMLP treatment were considered as controls. They were then differentiated to macrophages in the medium containing 100 nM PMA and allowed to adhere for 48 h, after which they were fed with PMA-free medium and cultured for 24 h prior to use. Cells from different groups were collected and total RNA was prepared with TRI-REAGENT-LS extraction kit. The expression of RNAs was determined by RT-PCR. Complementary DNA was created from RNA using TrueScript MMLV reverse transcriptase and oligo d (T) 18 primers. 5 μg RNA was included in each reaction. The primers of COX-2 and GAPDH are shown in the following table.

Gene Name	*Primer sequnce*	**Products (bp)**
COX-2	sense: 5'-TATACTAGAGCCCTTCCTCCTGTGCC-3' [SEQ ID NO: 5] antisense: 5'-ACATCGCATACTCTGTTGTGTTCCC-3' [SEQ ID NO: 6]	503
GAPDH	sense: 5'-AAGAAGGTGGTGAAGCAGGC-3' [SEQ ID NO: 7]	200

antisense: 5'-CCACCACCCTGTTGCTGTAG-3' [SEQ ID NO. 8]

Statistical analysis of data.

The data are presented as means ± SD and compared with ANOVA and least significant difference test using SPSS statistical program. The level of the statistical significance was set at $P < 0.05$.

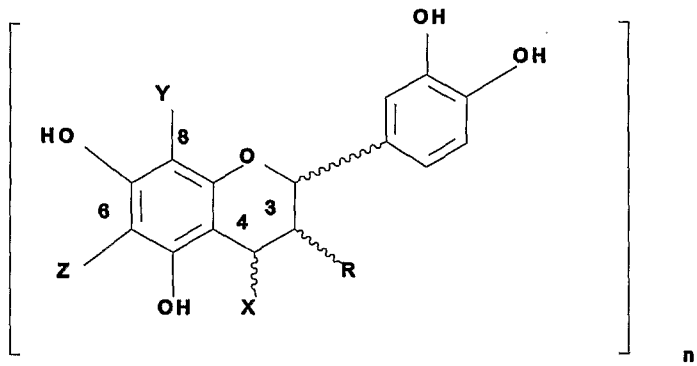
Results

The anti-inflammatory effects of B1 dimer, B2 dimer, (-)-catechin and (+)-epicatechin in comparison with A1 dimer were investigated. As shown in Fig. 4 A-C, (-)-catechin, (+)-epicatechin, A1, B1 and B2 showed significant inhibition of the mRNA expression of COX-2. Further, the order of suppression potency of the COX-2 mRNA expression induced by LPS and fMLP was: (-)-catechin, (+)-epicatechin > B1, B2 >A1 (Fig. 4C).

What is claimed is:

1. A method of treating or preventing a COX-2 overexpressing tumor/cancer, which comprises administering, to a subject in need thereof, a composition comprising an effective amount of a compound having the formula A_n, or a pharmaceutically acceptable salt or derivative thereof (including oxidation products, methylated derivatives and glucuronidated derivatives):

A =



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH, O-sugar or O-gallate;

the substituents of C-4, C-6 and C-8 are X, Z and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y and Z independently are hydrogen or a sugar;

the sugar is optionally substituted with a phenolic moiety at any position, for instance, via an ester bond; and

wherein the subject is a human or a veterinary animal.

2. The method of claim 1, wherein the subject is a human.

3. The method of claim 2, wherein the composition is a pharmaceutical composition.

4. The method of claim 3, wherein n = 2-12.

5. The method of claim 3, wherein n = 2-5.

6. The method of claim 3, wherein n = 2.

7. The method of claim 1, wherein R is OH.

8. The method of claim 7, wherein the subject is a human.

9. The method of claim 8, wherein n = 2-12.

10. The method of claim 8, wherein n = 2-5.

11. The method of claim 8, wherein n = 2.

12. The method of claim 11, wherein the compound is dimer B2.

13. The method of claim 3, wherein the cancer is COX-2-overexpressing colorectal cancer.

14. The method of claim 3, wherein the cancer is COX-2-overexpressing breast cancer.

15. The method of claim 3, wherein the cancer is COX-2-overexpressing gynecological cancer selected from a group consisting of ovarian, uterine, cervical or endometrial cancer.

16. The method of claim 3 wherein the cancer is COX-2-overexpressing cancer of the digestive tract selected from a group consisting of esophagus, stomach or pancreatic cancer.

17. The method of claim 3, wherein the tumor/cancer is selected from a group consisting of COX-2-overexpressing thyroid cancer, urinary bladder cancer, skin cancer, lung cancer, prostate cancer, oral leukoplakia, liver cancer, liver metastases from colon cancer, cholongiosarcoma, bone metastases, teratocarcinoma, head and neck cancers, leukemias and lymphomas.

18. The method of claim 13, wherein the compound is dimer B1 or B2.

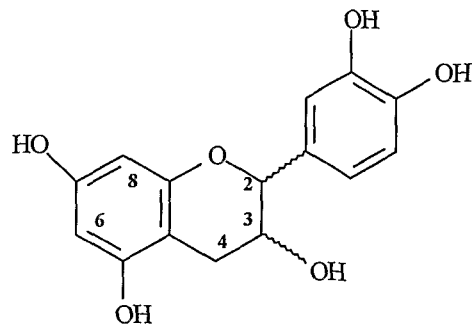
19. The method of claim 14, wherein the compound is dimer B1 or B2.

20. The method of claim 15, wherein the compound is dimer B1 or B2.

21. The method of claim 16, wherein the compound is dimer B1 or B2.

22. The method of claim 17, wherein the compound is dimer B1 or B2.

23. A method of treating or preventing a COX-2 overexpressing tumor/cancer comprising administering, to a subject in need thereof, a composition comprising an effective amount of a compound having the formula:



or a pharmaceutically acceptable salt thereof, wherein the subject is a human or a veterinary animal.

24. The method of claim 23, wherein the subject is a human.

25. The method of claim 24, wherein the compound is (+)-epicatechin.

26. The method of claim 24, wherein the compound is (-)-catechin.

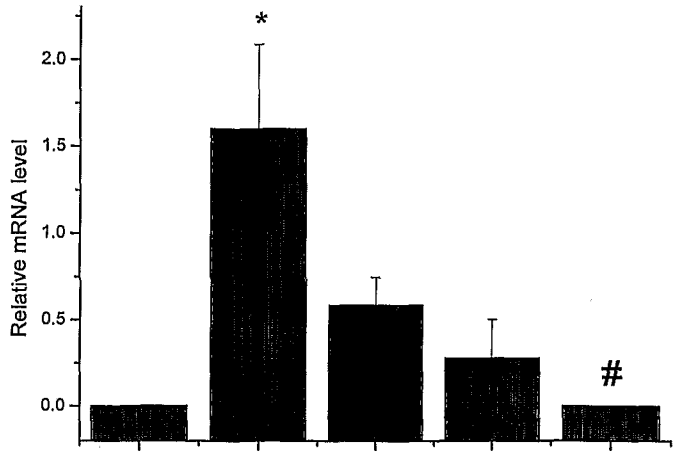


FIG. 1

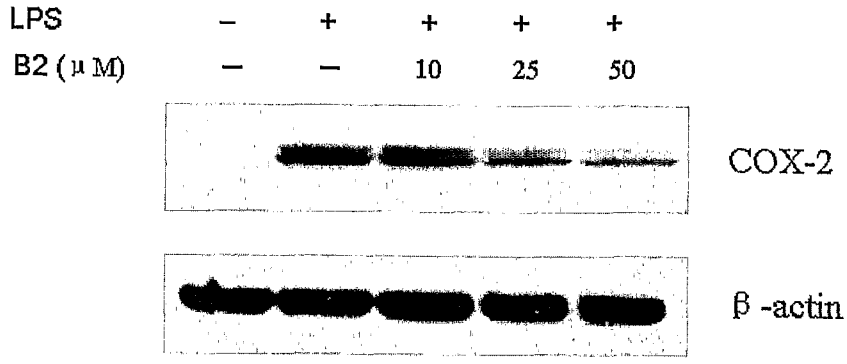
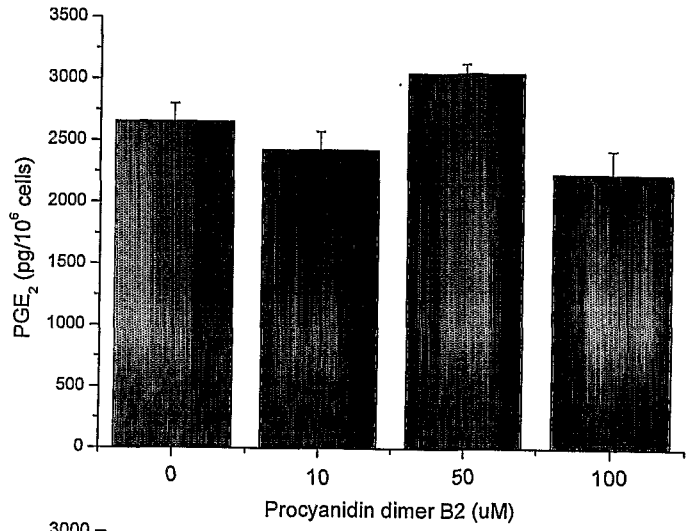
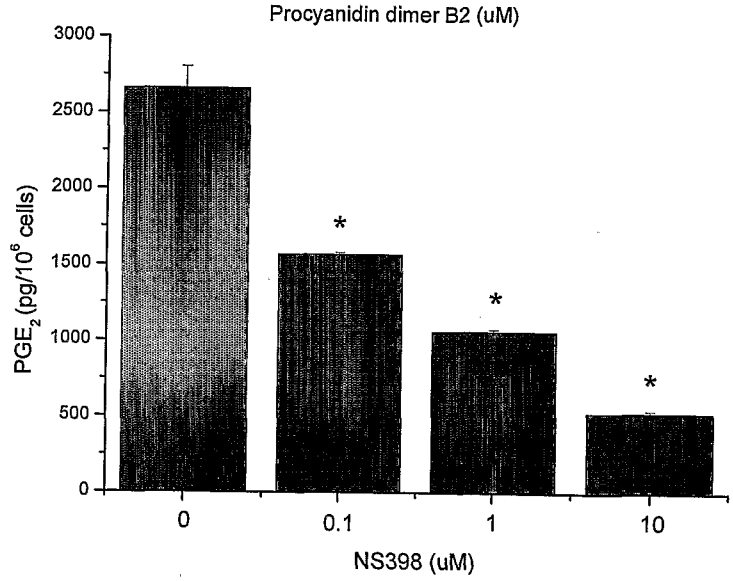


FIG. 2

A.


3500
3000
2500
2000
1500
1000
500
0
PGE2 (pg/10^6 cells)
0
10
50
100
Procyanidin dimer B2 (uM)

B.


3000
2500
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500
0
PGE2 (pg/10^6 cells)
*
*
*
0
0.1
1
10
NS398 (uM)

FIG. 3

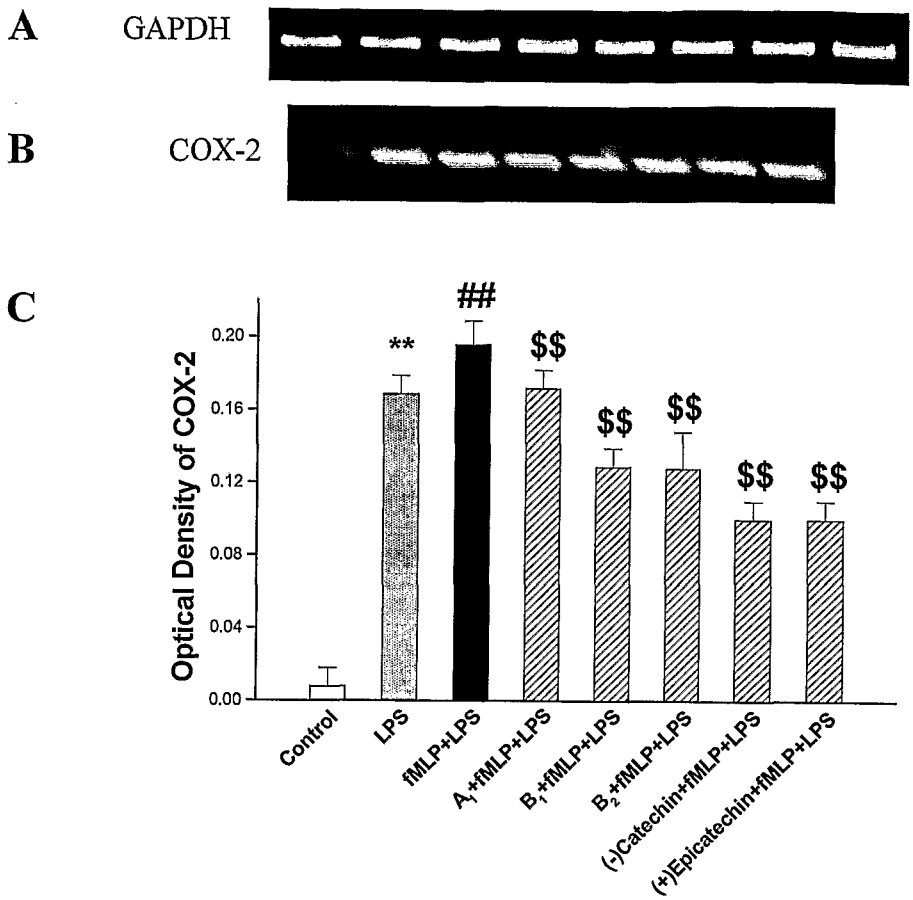
A
GAPDH
B
COX-2
C
Optical Density of COX-2
0.00
0.04
0.08
0.12
0.16
0.20
**
##
$$
$$
$$
$$
Control
LPS
fMLP+LPS
A1+fMLP+LPS
B1+fMLP+LPS
B2+fMLP+LPS
(-)Catechin+fMLP+LPS
(+)Epicatechin+fMLP+LPS

FIG. 4A-C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US06/42547

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **A61K 31/70**(2006.01).**31/7042**(2006.01);**C07H 17/04**(2006.01)

USPC: 514/27,25;536/4.1,8
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)
U.S. : 514/27, 25; 536/4.1, 8

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)
STN Electronic database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,420,572 B1 (ROMANCZYK, JR et al) 16 July 2002(16.07.2002), see example 25, col. 45 line 28 to col. 46, line 13.	1-22
Y	STN ABSTRACT: ACCESSION NO.: 2003204932 MEDLINE; DOCUMENT NO.: PubMed ID: 12724337; TITLE: Does the release of archidonic acid from cells play a role in cancer chemotherapy? AUHOR: LEVINE, LAWRENCE. The FASEB Journal: official publication of the Federation of American Societies for Experimental Biology, (2003 May) Vol. 17, No. 8, pp. 800-2.	1-26
Y	STN ABSTRACT: ACCESSION NO.: 2002:317572 CAPLUS; DOCUMENT NO.: 137:284083 ; TITLE: Potential cancer-chemopreventive activities of wine stilbenoids and flavans extracted from grape (Vitis vinifera) cell cultures; AUTHOR: WAFFO-TEGUO et al., Nutrition and cancer (2001), Vol. 40, No. 2, pages 173-179.	23-26
A	US 6,297,273 B1 (ROMANCZYK, JR) 02 October 2001 (02.10.2001), see entire patent.	1-26

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

* Special categories of cited documents:

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16 March 2007 (16.03.2007)

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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) 273-3201

Authorized officer
Michael C. Henry Felicia D. Roberts for
Telephone No. 703 308-1235

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