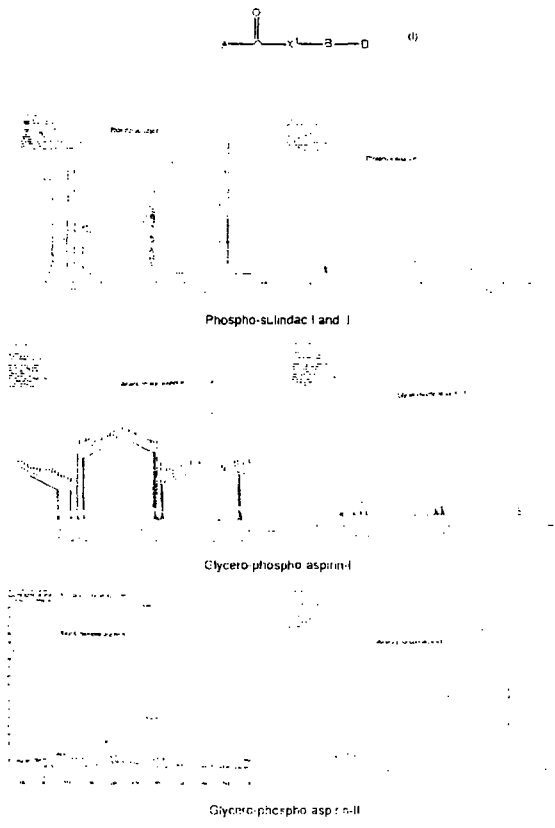


(54) Title: ANTI-INFLAMMATORY COMPOUNDS AND USES THEREOF

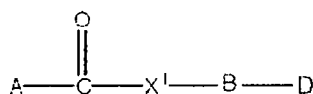


(57) Abstract: Compounds of the general Formula (I) are disclosed with activity towards treating diseases related to inflammation, such as cancer, neurodegenerative and cardiovascular diseases. Pharmaceutical compositions and methods of use are also described.

FIGURE 1A

5 **WHAT IS CLAIMED IS:**

1. A compound of Formula I



Formula (I)

or an enantiomer, a diastereomer, a racemate, a tautomer thereof, or a prodrug, salt,
10 hydrate or ester thereof,

wherein A is an optionally substituted aliphatic, alicyclic, aryl, aralkyl, heteroaliphatic, heterocyclic, or heteroaromatic group,

wherein X¹ is selected from the group consisting of -O-, -NH-, and -S-;

wherein B is an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic,
15 aryl, aralkyl, or heteroaromatic group, wherein

B is optionally substituted with one or more X² which is independently selected from the group consisting of hydrogen, halogen, hydroxyl, alkoxy, -CN; an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl, aralkyl, heteroaromatic moiety; -OR^R, -S(=O)_nR^d, -NR^bR^c, -C(=O)R^a and
20 -C(=O)OR^a; wherein

n is 0-2,

R^a, for each occurrence, is independently selected from the group consisting of hydrogen and an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl, aralkyl, or a heteroaromatic moiety;

25 R^b and R^c, for each occurrence, are independently selected from the group consisting of hydrogen; hydroxy; SO₂R^d; and aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl, aralkyl, heteroaromatic or an acyl moiety;

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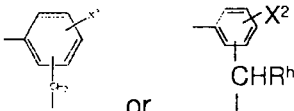
5 R^d , for each occurrence, is independently selected from the group consisting of hydrogen; $-N(R^e)_2$; aliphatic, aryl and heteroaryl;

R^e , for each occurrence, is independently hydrogen or aliphatic; and

R^R is an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl, aralkyl, heteroaromatic or acyl moiety;

10 and

wherein D is hydroxyl; halide; tosylate; phosphate; $-\text{OSO}_2\text{NR}_x\text{R}_y$, where R_x and R_y are independently hydrogen, or an aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl, aralkyl, heteroaromatic or acyl moiety; $-\text{O}-\text{C}_6\text{H}_4\text{OC}(=\text{O})\text{CH}_3$; an alkoxy moiety; or an acyl moiety; provided that if B is

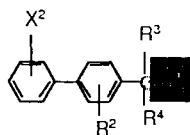
15 , or where R^h is aryl, aralkyl, alkyl, alkenyl or alkynyl, then D is phosphate ester ($-\text{O}-\text{P}(\text{OR}^f)_3$) or a phosphite ester ($-\text{O}-\text{P}(\text{OR}^g)_2$), wherein R^f and R^g are independently each an H, alkyl, alkenyl, an alkynyl group, an aryl, or an aralkyl group, which may in turn be substituted or unsubstituted.

2. The compound of claim 1 wherein A is



where Y is $(-\text{C}-)_n$, wherein n is 0 to 4, optionally containing one or more unsaturated bonds in the $(-\text{C}-)_n$ moiety when n is 2 or greater.

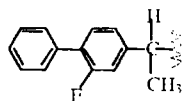
- 5 3. The compound of claim 1 wherein A is



Formula (III)

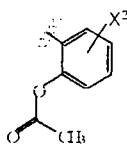
wherein R² is at least one halogen, and R₃ and R₄ are independently hydrogen or an aliphatic group.

- 10 4. The compound of claim 1 wherein A is

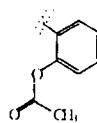


Formula (IV).

5. The compound of claim 1 wherein A is



Formula (V)



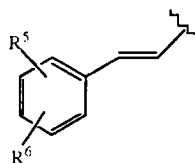
Formula (VI).

15

or

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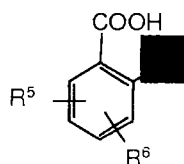
- 5 6. The compound of claim 1 wherein A is



Formula (VII)

- where R⁵ and R⁶ are independently hydrogen, -OH, alkoxy, halide, trifluoroalkyl, alpha-haloalkyl, trifluoroalkoxy, or R^a, wherein R^a is independently hydrogen or an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl or aralkyl, or a heteroaromatic moiety.
- 10

7. The compound of claim 1 wherein A is

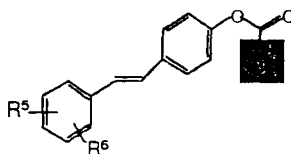


Formula (VIII)

- 15 wherein R⁵ and R⁶ are independently hydrogen, -OH, alkoxy, halide, trifluoroalkyl, alpha-haloalkyl, trifluoroalkoxy, or R^a, wherein R^a is hydrogen or an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, or a heteroaromatic moiety.

8. The compound of claim 1 wherein A is a straight chain or branched aliphatic moiety.
- 20

5 9. The compound of claim 1 wherein X² is

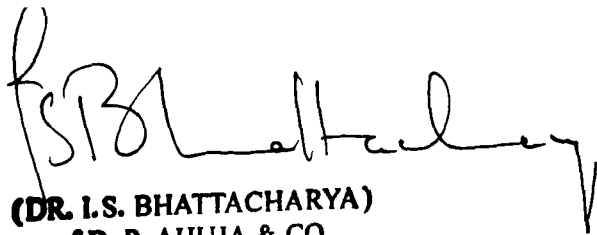


- wherein R⁵ and R⁶ are independently hydrogen, -OH, alkoxy, halide, trifluoroalkyl, alpha-haloalkyl, trifluoroalkoxy, or R^a where R^a, for each occurrence, is independently hydrogen or an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl or aralkyl, or a heteroaromatic moiety.
- 10) A compound of claim 1, wherein A is a non-steroidal anti-inflammatory drug.
11. A compound of claim 10, wherein A is a derivative of acetylsalicylic acid, or a derivative of arylalkanoic acid.
12. A compound according to Claim 11, selected from the group consisting of 2-acetoxy-benzoic acid 4-(diethoxy-phosphoryloxymethyl)-phenyl ester, 2-acetoxy-benzoic acid 3-(diethoxy-phosphoryloxymethyl)-phenyl ester, and phospho-sulindac I, phospho-sulindac II, phosphoflurbiprofen, phosphoibuprofen, phosphoaspirin I and phosphoraspirin II..
13. A compound according to Claim 1, wherein A is valproic acid.
14. A pharmaceutical composition comprising the compound of any one of the preceding Claims and a pharmaceutically acceptable excipient.
15. The compound of any one of Claims 1-13 for use as a medicament.
16. Use of a compound according to any one of Claims 1-13 in the preparation of a medicament for the treatment of inflammation.
17. Use of a compound according to any one of claims 1-13 in the preparation of a medicament for the treatment of an inflammation-related disease.

18. The use according to Claim 17, wherein the inflammation related disease is selected from the group consisting of rheumatologic disease, a cardiovascular disease, a neurodegenerative disease, a cerebrovascular disease, an autoimmune disease, chronic inflammation of an organ, a neoplastic and pre-neoplastic disease.

19. The use according to Claim 18, wherein the disease is selected from the group consisting of rheumatoid arthritis, Sjogren's syndrome, coronary artery disease, peripheral vascular disease, hypertension, Alzheimer's disease and its variants, lupus erythematosus, chronic bronchitis, chronic sinusitis, benign prostatic hypertrophy, prostate cancer, colon adenomas, colon cancer, cancer of the lung, lymphoma and leukemia.

20. The use of any one of claims 16-19, wherein said treatment is treatment of a human.


(DR. I.S. BHATTACHARYA)
of D. P. AHUJA & CO.
APPLICANT'S AGENT

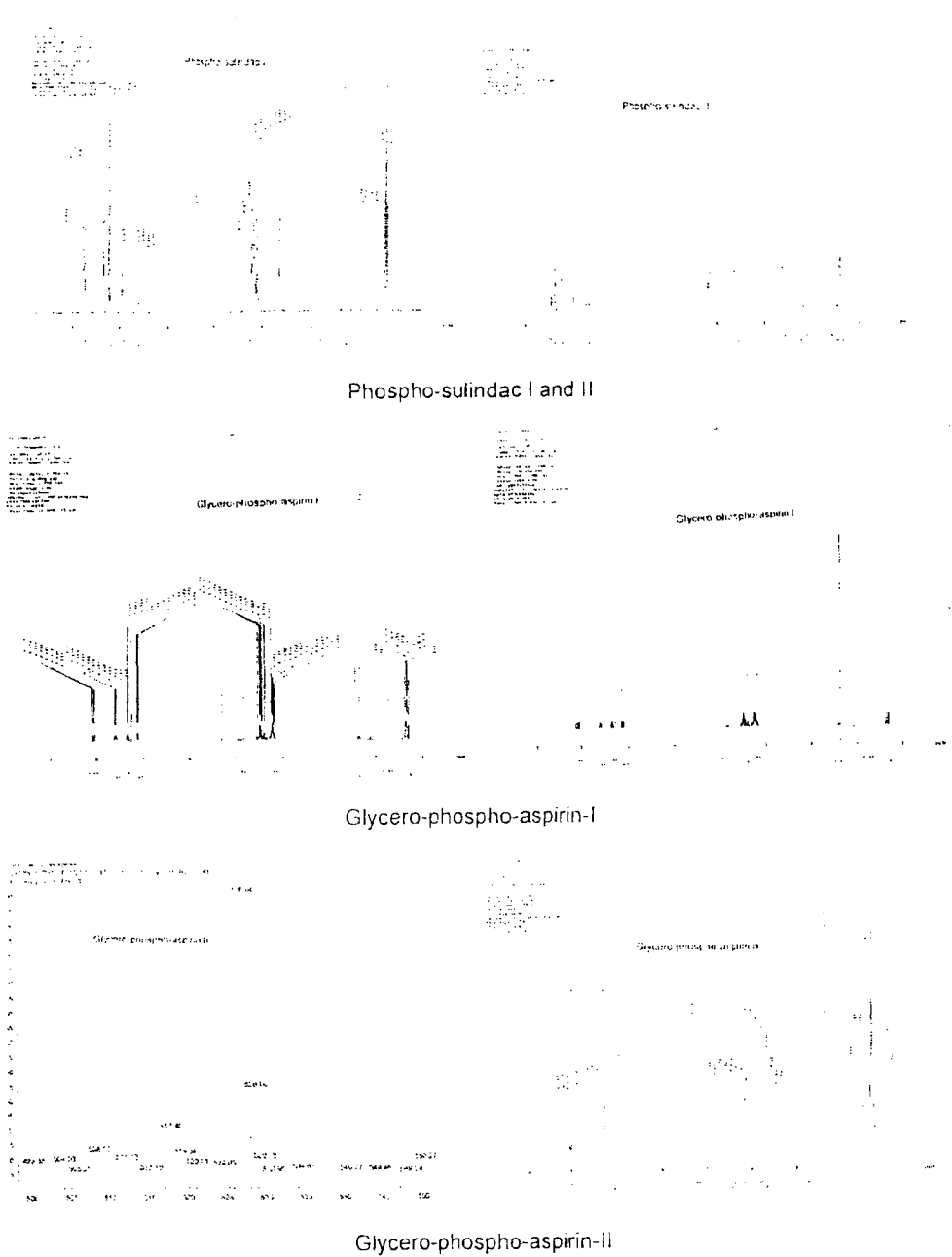


FIGURE 1A

S. D. Ahuja
S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

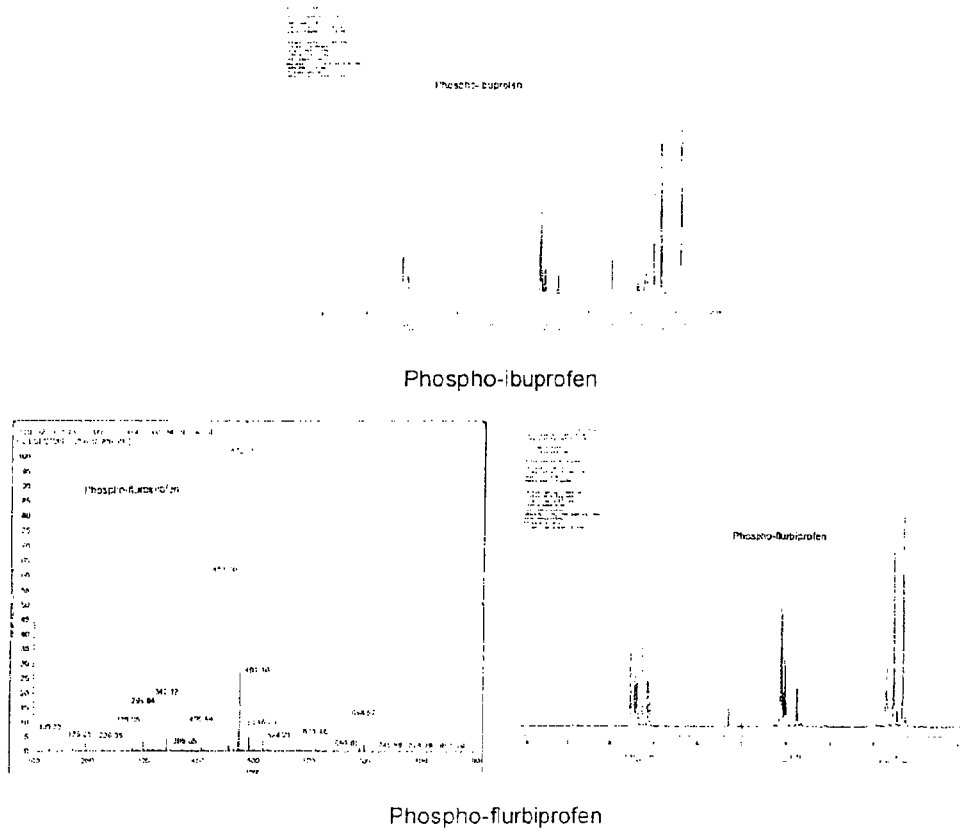


FIGURE 1B

S. D. Ahuja
S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

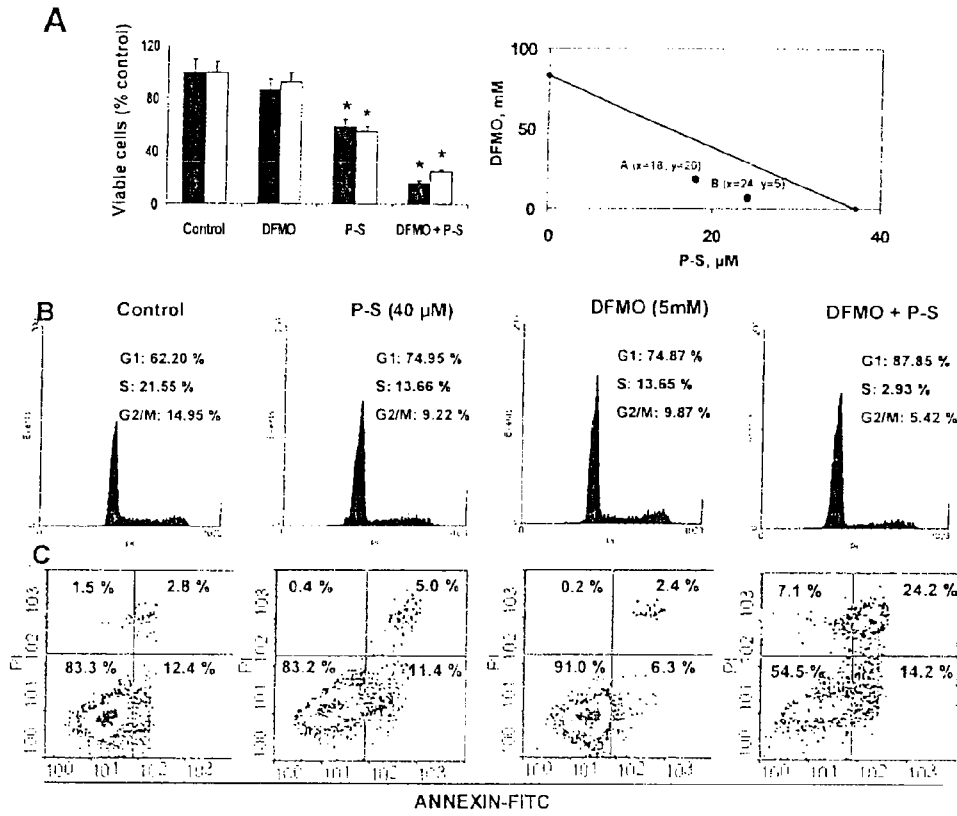


FIGURE 2

S. D. Ahuja
 S. D. AHUJA
 of D. P. AHUJA & CO.
 APPLICANT'S AGENT

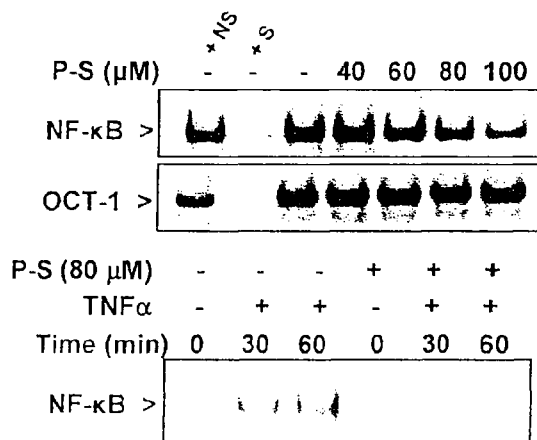


Figure 3

S. D. Ahuja
 S. D. AHUJA
 of D. P. AHUJA & CO.
 APPLICANT'S AGENT

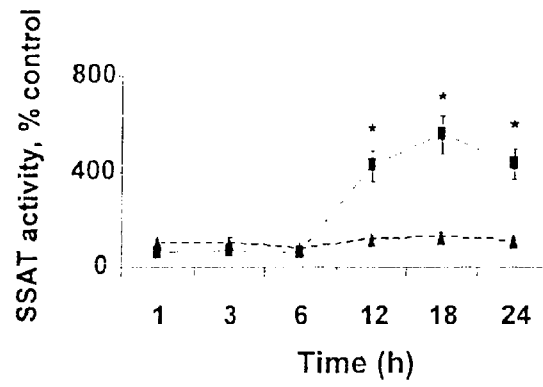
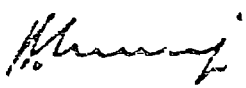


Figure 4

5/5


S. D. AHUJA
of D. P. AHUJA & CO.
APPLICANT'S AGENT

ANTI-INFLAMMATORY COMPOUNDS AND USES THEREOF

CROSS-REFERENCES TO RELATED APPLICATIONS

- 5 [0001] This application claims priority of U.S. Provisional Application Ser. No. 60/955,258, filed August 10, 2007, and U.S. Provisional Application Ser. No. 60/989,584, filed November 21, 2007. The contents of both provisional applications are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

- 10 [0002] The invention is directed to compounds and pharmaceutical compositions for the treatment of inflammation-related diseases, in particular cancer.

BACKGROUND OF THE INVENTION

- [0003] Inflammation, a key component of the immune system, functions in both defense and pathophysiological events to maintain the homeostasis of tissues, organs and individual cells. Inflammation can be classified as either acute or chronic. Acute inflammation is a short-term process characterized by the classic signs of inflammation, i.e. swelling, redness, pain, heat, and loss of function, due to infiltration of tissues by plasma and leukocytes. It occurs as long as the injurious stimulus is present and ceases once the stimulus has been removed. Chronic inflammation is a pathological condition characterized by concurrent active inflammation, tissue destruction, and attempts at repair. Chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes, and plasma cells), tissue destruction, and attempts at healing, which include angiogenesis and fibrosis.

- 25 [0004] Without inflammation, wounds and infections would not be able to heal and progressive destruction of the tissue would threaten the survival of the organism. Unchecked inflammation, on the other hand, can lead to a host of diseases, such as hay fever, atherosclerosis and other cardiovascular diseases, neurodegenerative diseases such as Alzheimer's, cancer and rheumatoid arthritis. For these reasons, 30 inflammation is tightly regulated by the body.

5 [0005] Inflammation is controlled by more than 400 genes. The pro-inflammatory genotype, which appears dominant, increases our vulnerability to, and intensity of, inflammatory reactions, which underlie chronic inflammatory diseases, especially in old age. (Ferencik et al., Inflammation--a lifelong companion. Folia Microbiol (Praha). 2007; 52:159-73). Although joint diseases have long been the
10 prototypical inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, autoimmune diseases, and cancer are now appreciated as having inflammation as a unifying component of their pathogenesis.

[0006] Alzheimer's disease (AD) includes inflammatory processes in the senile plaques and surrounding glia, with increased expression of acute phase
15 proteins such as C-reactive protein (CRP) and IL-6. Increased IL-6 expression during normal brain aging suggests a link of age-related inflammation to the onset of AD during aging. Blood levels of CRP and IL-6 are also associated with higher risk of Alzheimer's disease and cognitive decline during aging (Finch and Morgan, Systemic inflammation, infection, ApoE alleles, and Alzheimer disease: a position paper. Curr
20 Alzheimer Res. 2007; 4:185-9).

[0007] Inflammation plays a crucial role in all steps characterizing the atherosclerotic process. Circulating CRP (C-reactive protein) levels have emerged as a powerful independent determinant of cardiovascular events. Hypertension is closely linked to inflammation. Experimental data and results from cross-sectional
25 studies in humans strongly support this notion (Viridis et al., C-reactive protein and hypertension: is there a causal relationship? Curr Pharm Des. 2007;13:1693-8). In cancer, chronic inflammation often acts as a tumor promoter, resulting in aggressive cancerous growth and spread. Many of the same inflammatory factors that promote tumor growth also are responsible for cancer cachexia/anorexia, pain, debilitation,
30 and shortened survival. A compelling case has been made even for attacking inflammation at initial diagnosis to improving patient quality of life and survival. Serum levels of CRP correlate with poor prognosis in cancer patients (MacDonald N. Cancer cachexia and targeting chronic inflammation: a unified approach to cancer treatment and palliative/supportive care. J Support Oncol. 2007; 5:157-62).

5 **[0008]** Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used anti-inflammatory compounds, with aspirin, the prototypical NSAID, still being one of the oldest and most extensively used medication in the world (Stanley P, Hegedus R. Aspirin--the first hundred years. *Biologist* (London) 2000;47:269-71; Rinsema TJ. One hundred years of aspirin. *Med Hist* 1999;43:502-7). NSAIDs have
10 a significant antineoplastic effect, which should be viewed, at least in part, in the context of the increasingly appreciated role of inflammation in cancer. Aspirin is formally documented to be a chemopreventive agent against colon cancer [3, 4]. For other NSAIDs, the evidence on their antineoplastic properties is quite strong but still it is based mainly on epidemiological studies (Baron JA. What now for aspirin and cancer prevention? *J Natl Cancer Inst* 2004;96:4-5; Jacobs EJ, Rodriguez C, Mondul
15 AM, Connell CJ, Henley SJ, Calle EE, et al. A large cohort study of aspirin and other nonsteroidal anti-inflammatory drugs and prostate cancer incidence. *J Natl Cancer Inst* 2005;97:975-80; Thun MJ, Henley SJ, Gansler T. Inflammation and cancer: an epidemiological perspective. *Novartis Foundation symposium* 2004;256:6-21; discussion 2-8, 49-52, 266-9). For example, a recent meta-analysis of 91
20 epidemiological studies showed a significant exponential decline with increasing intake of NSAIDs in the risk for 7-10 malignancies including the four major types: colon, breast, lung, and prostate cancer (Harris RE, Beebe-Donk J, Doss H, Burr Doss D. Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review). *Oncology reports* 2005;13:559-83; Ratliff TL. Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2
25 blockade (review). *The Journal of urology* 2005;174:787-8).

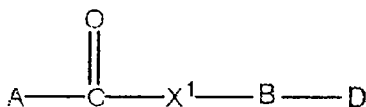
[0009] NSAIDs prevent cancer likely through pleiotropic effects (reviewed in
30 Rayyan Y, Williams J, Rigas B. The role of NSAIDs in the prevention of colon cancer. *Cancer Invest* 2002;20:1002-11; Shiff SJ, Rigas B. Aspirin for cancer. *Nat Med* 1999;5:1348-9.). It is, however, clear that conventional NSAIDs do not meet two important criteria for their wide application as chemopreventive agents against cancer, i.e. safety and high efficacy, as NSAIDs are associated with a considerable
35 number of side effects, and their efficacy is rather limited, not exceeding 50%

- 5 (Rayyan Y, Williams J, Rigas B. The role of NSAIDs in the prevention of colon cancer. *Cancer Invest* 2002;20:1002-11). Thus there is a need to develop compounds with improved efficacy and safety profiles for the treatment of various diseases related to inflammation.

SUMMARY OF THE INVENTION

- 10 **[0010]** The present invention provides such novel therapeutics including a novel group of NSAID derivatives and method of using them in the prevention and treatment of diseases, especially cancers.

[0011] In a first aspect, the present invention provides compounds of general Formula I:



15

Formula (I)

or an enantiomer, racemate, diastereomer, or tautomer thereof, or a prodrug, salt, hydrate or ester thereof;

wherein X^1 is selected from the group consisting of -O-, -S- and -NH-;

- 20 wherein B is an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, or heteroaromatic group,

wherein B is optionally substituted with one or more X^2 which is independently selected from the group consisting of hydrogen, halogen, hydroxyl, alkoxy or -CN; an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic moiety; -OR^R, -S(=O)_nR^d, -NR^bR^c, -C(=O)R^a and -C(=O)OR^a; wherein n is 0-2, R^R is an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl or aralkyl, heteroaromatic group or acyl moiety;

25

5 R^a , for each occurrence, is independently selected from the group consisting of hydrogen and an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, or a heteroaromatic moiety;

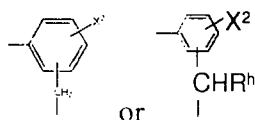
R^b and R^c , for each occurrence, are independently selected from the group consisting of hydrogen; hydroxy; SO_2R^d ; and aliphatic, alicyclic,
10 heteroaliphatic, heterocyclic, aromatic, heteroaromatic or an acyl moiety;

R^d , for each occurrence, is independently selected from the group consisting of hydrogen; $-N(R^e)_2$; aliphatic, aryl and heteroaryl; and

R^e , for each occurrence, is independently hydrogen or an aliphatic group;

15 wherein A is an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, or heteroaromatic group such as but not limited to those moieties described in further detail herein below;

wherein D is hydroxyl; halide; tosylate; phosphate ester ($-O-P(OR^f)_3$) or a phosphite ester ($-O-P(OR^g)_2$), $-OSO_2NR_xR_y$, where R_x and R_y are independently hydrogen, or a
20 substituted or unsubstituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl or aralkyl, heteroaromatic or acyl moiety; $-O-C_6H_4OC(=O)CH_3$; an alkoxy moiety; or an acyl moiety, provided that at least one R^f is not an H, and at least one R^g is not an H, and provided that if B is



where R^h is aryl, aralkyl, alkyl, alkenyl or alkynyl, then D is a
25 phosphate ester ($-O-P(O)(OR^f)_2$) or a phosphite ester ($-O-P(OR^g)_2$), preferably, R^f and R^g is independently each an H, alkyl, alkenyl, alkynyl, aryl or an aralkyl group, which may in turn be substituted or unsubstituted.

[0012] In one embodiment, the present invention relates to mono-, di- or tri-esters
of phosphoric acid or phosphorous acid of aspirin, containing alkyl, alkenyl, aryl,
30 benzyl or cyclic groups and their derivatives, and the use thereof for cancer

5 treatment and prevention. A series of novel aspirin derivatives, in particular 2-
acetoxy-benzoic acid 4-(diethoxy-phosphoryloxymethyl)-phenyl ester, and 2-acetoxy-
benzoic acid 3-(diethoxy-phosphoryloxymethyl)-phenyl ester, provisionally named
phosphoaspirins, and determined these compounds have anticancer activities both
in vitro and *in vivo*. Phosphoaspirin inhibited the growth of HT-29 human colon
10 adenocarcinoma cells ($IC_{50} = 276.6 \pm 12.3 \mu\text{M}$ (mean \pm SEM) through a combined
antiproliferative and mainly proapoptotic effect. While not willing to be bound by any
theory on mechanism, applicant believes that phosphoaspirin achieves this effect by
modulating cell kinetics; the proliferation index of cancer cells was reduced by
18.13% compared to controls ($p < 0.001$) and the apoptosis index was increased by
15 94.6% ($p < 0.003$). No apparent toxicity was shown by phosphoaspirin.

[0013] Preferably, compound of formula I is selected from the following: Diethyl 4-
(2-acetoxybenzoyloxy)benzyl phosphate, 2-acetoxy-benzoic acid 4-(diethoxy-
phosphoryloxymethyl)-phenyl ester, 2-acetoxy-benzoic acid 3-(diethoxy-
phosphoryloxymethyl)-phenyl ester, and phospho-sulindac I, phospho-sulindac II,
20 phosphoflurbiprofen, phosphoibuprofen, glycerophosphoaspirin I, and glycerophospho-
aspirin II. The chemical structures of these compounds are provided
hereinbelow.

[0014] In a further aspect, the invention is directed to a pharmaceutical
composition comprising a compound of Formula I, as described generally herein,
25 and a pharmaceutically acceptable excipient. In a specific embodiment, the
composition is useful in the treatment of human and animal inflammation related
diseases, including, but not limited to neoplasms, cancer, rheumatologic diseases
such as rheumatoid arthritis and Sjogren's syndrome; cardiovascular diseases, such
as coronary artery disease, peripheral vascular disease and hypertension;
30 neurodegenerative diseases, such as Alzheimer's disease and its variants or
cerebrovascular diseases; and autoimmune diseases for example lupus
erythematosus. Such compositions can comprise one or more other pharmaceutical
agents in addition to one or more compounds of the invention.

5 [0015] In another embodiment, the invention is directed to a method for inhibiting inflammation, in particular chronic inflammation in a subject in need thereof by administering to the subject an amount of the compound or composition of the present invention effective to inhibit inflammation. The subject may be a human patient or animal.

10 [0016] In yet another aspect, the present invention provides methods for treating any disorder related to undesirable inflammation comprising administering to a subject (e.g., human patient or animal) in need thereof a therapeutically effective amount of a compound of Formula I of the invention or a pharmaceutical composition comprising a compound of the invention. In a preferred embodiment, the disorder
15 includes, but is not limited to rheumatologic diseases such as for example rheumatoid arthritis and Sjogren's syndrome; cardiovascular diseases, such as, for example, coronary artery disease, peripheral vascular disease and hypertension; neurodegenerative diseases, such as, for example, Alzheimer's disease and its variants or cerebrovascular diseases; autoimmune diseases such as for example
20 lupus erythematosus; and other conditions characterized by chronic inflammation of organs such as for example the lung, such as chronic bronchitis or the sinuses, such as chronic sinusitis.

[0017] The compounds of the present invention may be used for the manufacture of a medicament for treatment of a disease listed above.

25 [0018] **BRIEF DESCRIPTION OF THE FIGURES**

[0019] Figure 1 is the NMR profiles of several of the compounds of the present invention.

[0020] Figure 2 shows that DFMO enhances phospho-sulindac-induced inhibition of colon cancer cell proliferation, as well as cell cycle arrest and cell death by
30 apoptosis. **A- Left panel:** Cell viability was determined in HT-29 (full bars) and SW-480 (empty bars) cells after 48 h of incubation with 5 mM DFMO, 40 μ M phospho-sulindac (P-S) or simultaneous combination of DFMO and P-S. Data are expressed as percentage of control cells (cells incubated only with DMSO). Values are mean \pm

5 SEM of 4 independent experiments. *Significantly different from control cells ($p < 0.02$, one way ANOVA test). **Right panel:** In this *isobologram* the additivity line connects the IC_{50} value of each compound used alone. A and B represent two different dose pairs of each compound (their respective concentrations are shown in parentheses). The location of both A and B below the additivity line signifies synergy.

10 **B-** Cell cycle progression in HT-29 cells incubated for 48 h with 5 mM DFMO, 40 μ M phospho-sulindac (P-S) or simultaneous combination of DFMO and P-S. Representative profiles of the distribution of cells in G1, G2/M and S phases, out of 4 independent experiments are shown. DNA content was determined from propidium iodide (PI) fluorescence. **C-** Apoptosis and necrosis were determined by combined

15 staining with annexin V and PI and determining fluorescence intensity. The percentages of apoptotic cells were determined using the dual staining with annexin V and propidium iodide and are indicated in each quadrant: left bottom quadrant, viable cells (annexin V-negative/PI-negative); right bottom quadrant, early apoptotic cells (annexin V-positive/PI-negative); right upper quadrant, late apoptotic cells

20 (annexin V-positive/PI-positive); left upper quadrant, necrotic cells (annexin V-negative, PI-positive). These images are representative of 3 independent experiments.

[0021] Figure 3 shows that phospho-sulindac I inhibits NF- κ B activation in colon cancer cells. Phospho-sulindac inhibits constitutive and TNF α -induced NF- κ B

25 activation. **Upper panel:** Nuclear fractions were isolated from HT-29 cells after 4 h of incubation in the absence or in the presence of 40-100 μ M phospho-sulindac I (P-S). Electrophoretic mobility shift assay (EMSA) for NF- κ B and OCT-1 from cells treated with various concentrations of phospho-sulindac I. To determine the specificity of each transcription factor-DNA complex, the control nuclear fraction (-) was incubated

30 in the presence of 100-fold molar excess of unlabeled oligonucleotide containing the consensus sequence for either the specific (+ S) or a nonspecific (+ NS) transcription factor before the binding assay. **Lower panel:** Nuclear fractions were isolated from HT-29 cells, after 4 h of preincubation in the absence or in the presence of 80 μ M phospho-sulindac I (P-S) and further 0, 30 or 60 min incubation without (-) or with 10

5 ng/ml TNF α . EMSA for NF- κ B incubated with various concentrations of phospho-sulindac I.

[0022] Figure 4 shows that Phospho-sulindac I induces SSAT enzymatic activity in colon cancer cells. SW480 cells were incubated without or with phospho-sulindac I (P-S) for up to 24 h and SSAT activity was determined at each time point by
10 determining the amount of labelled *N*1-acetylspermidine synthesized from [¹⁴C]acetyl-CoA and unlabeled spermidine. Values are shown as means \pm SEM of 4 independent experiments. *Significantly different from control cells ($p < 0.01$, one way ANOVA test).

DETAILED DESCRIPTION OF THE INVENTION

15 [0023] DEFINITIONS

[0024] The term "aliphatic", as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched) or branched aliphatic hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include,
20 but is not limited to, alkyl, alkenyl, and alkynyl moieties. Thus, as used herein, the term "alkyl" includes straight and branched alkyl groups. An analogous convention applies to other generic terms such as "alkenyl", "alkynyl" and the like. Furthermore, as used herein, the terms "alkyl", "alkenyl", "alkynyl" and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein,
25 "lower alkyl" is used to indicate those alkyl groups (substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms.

[0025] In certain embodiments, the alkyl, alkenyl and alkynyl groups employed in the invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic
30 carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and

5 alkynyl groups employed in the invention contain 1-4 carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, n-propyl, isopropyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, n-hexyl, sec-hexyl, moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for
10 example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl and the like.

[0026] The term "alicyclic", as used herein, refers to compounds, which combine the properties of aliphatic and cyclic compounds and include but are not
15 limited to monocyclic, or polycyclic aliphatic hydrocarbons and bridged cycloalkyl compounds, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "alicyclic" is intended herein to include, but is not limited to, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties, which are optionally substituted with one or more functional groups. Illustrative
20 alicyclic groups thus include, but are not limited to, for example, cyclopropyl, -CH₂-cyclopropyl, cyclobutyl, -CH₂-cyclobutyl, cyclopentyl, -CH₂-cyclopentyl, cyclohexyl, -CH₂-cyclohexyl, cyclohexenylethyl, cyclohexanylethyl, norborbyl moieties and the like, which again, may bear one or more substituents.

[0027] The term "alkoxy" or "alkyloxy", as used herein refers to a saturated
25 (*i.e.*, O-alkyl) or unsaturated (*i.e.*, O-alkenyl and O-alkynyl) group attached to the parent molecular moiety through an oxygen atom. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon
30 atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, *i*-butoxy, *sec*-butoxy, *tert*-butoxy, neopentoxy, n-hexoxy and the like.

- 5 [0028] The term "alkylthio" or "thioalkyl" as used herein refers to a saturated (*i.e.*, S-alkyl) or unsaturated (*i.e.*, S-alkenyl and S-alkynyl) group attached to the parent molecular moiety through a sulfur atom. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.
- 10
- 15 [0029] The term "alkylamino" refers to a group having the structure $-NHR'$ wherein R' is alkyl, as defined herein. The term "aminoalkyl" refers to a group having the structure NH_2R' , wherein R' is alkyl, as defined herein. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkylamino include, but are not limited to, methylamino, ethylamino, iso-propylamino and the like.
- 20
- 25 [0030] Some examples of substituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to aliphatic; alicyclic; heteroaliphatic; heterocyclic; aromatic; heteroaromatic; aryl; heteroaryl; alkylaryl; heteroalkylaryl; alkylheteroaryl; heteroalkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; C1; Br; I; -OH; -NO₂; -ONO₂; -CN; -CF₃; -CH₂CF₃; -CHC1₂; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -OC(O)R_x; -OCO₂R_x; -OCON(R_x)₂; -N(R_x)₂; -S(O)₂R_x; -NR_x(CO)R_x wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl, heteroaryl, alkylaryl, alkylheteroaryl, heteroalkylaryl or
- 30

5 heteroalkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic, heterocyclic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, saturated or unsaturated, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally
10 applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0031] In general, the term "aromatic moiety", as used herein, refers to a stable mono- or polycyclic, unsaturated moiety having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. In certain embodiments, the term
15 "aromatic moiety" refers to a planar ring having p-orbitals perpendicular to the plane of the ring at each ring atom and satisfying the Huckel rule where the number of pi electrons in the ring is $(4n+2)$ wherein n is an integer. A mono- or polycyclic, unsaturated moiety that does not satisfy one or all of these criteria for aromaticity is defined herein as "non-aromatic", and is encompassed by the term "alicyclic."

20 **[0032]** In general, the term "heteroaromatic moiety", as used herein, refers to a stable mono- or polycyclic, unsaturated moiety having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted; and comprising at least one heteroatom selected from O, S and N within the ring (*i.e.*, in place of a ring carbon atom). In certain embodiments, the term "heteroaromatic moiety" refers to a
25 planar ring comprising at least one heteroatom, having p-orbitals perpendicular to the plane of the ring at each ring atom, and satisfying the Huckel rule where the number of pi electrons in the ring is $(4n+2)$ wherein n is an integer.

[0033] It will also be appreciated that aromatic and heteroaromatic moieties, as defined herein may be attached via an alkyl or heteroalkyl moiety and thus also
30 include -(alkyl)aromatic, -(heteroalkyl)aromatic, -(heteroalkyl)heteroaromatic, and -(heteroalkyl)heteroaromatic moieties. Thus, as used herein, the phrases "aromatic or heteroaromatic moieties" and "aromatic, heteroaromatic - (alkyl)aromatic, -(heteroalkyl)aromatic, -(heteroalkyl)heteroaromatic, and - (heteroalkyl)heteroaromatic" are interchangeable. Substituents include, but are not

5 limited to, any of the previously mentioned substituents, *i.e.*, the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound.

[0034] The term "aryl", as used herein, does not differ significantly from the common meaning of the term in the art, and refers to an unsaturated cyclic moiety
10 comprising at least one aromatic ring. In certain embodiments, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like.

[0035] The term "heteroaryl", as used herein, does not differ significantly from the common meaning of the term in the art, and refers to a cyclic aromatic radical
15 having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl,
20 thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

[0036] It will be appreciated that aryl and heteroaryl groups (including bicyclic aryl groups) can be unsubstituted or substituted, wherein substitution includes replacement of one or more of the hydrogen atoms thereon independently with any one or more of the following moieties including, but not limited to: aliphatic; alicyclic;
25 heteroaliphatic; heterocyclic; aromatic; heteroaromatic; aryl; heteroaryl; alkylaryl; heteroalkylaryl; alkylheteroaryl; heteroalkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; C1; Br; I; -OH; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHC1₂; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -OC(O)R_x; -OCO₂R_x; -OCON(R_x)₂; -N(R_x)₂; -S(O)R_x; -S(O)₂R_x; -NR_x(CO)R_x wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic, aryl, heteroaryl, alkylaryl, alkylheteroaryl, heteroalkylaryl or heteroalkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic, heterocyclic, alkylaryl, or alkylheteroaryl substituents described above and herein
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5 may be substituted or unsubstituted, branched or unbranched, saturated or
unsaturated, and wherein any of the aromatic, heteroaromatic, aryl, heteroaryl, -
(alkyl)aryl or -(alkyl)heteroaryl substituents described above and herein may be
substituted or unsubstituted. Additionally, it will be appreciated, that any two
adjacent groups taken together may represent a 4, 5, 6, or 7-membered substituted
10 or unsubstituted alicyclic or heterocyclic moiety. Additional examples of generally
applicable substituents are illustrated by the specific embodiments shown in the
Examples that are described herein.

[0037] The term "cycloalkyl", as used herein, refers specifically to groups
having three to seven, preferably three to ten carbon atoms. Suitable cycloalkyls
15 include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl,
cycloheptyl and the like, which, as in the case of aliphatic, alicyclic, heteroaliphatic or
heterocyclic moieties, may optionally be substituted with substituents including, but
not limited to aliphatic; alicyclic; heteroaliphatic; heterocyclic; aromatic;
heteroaromatic; aryl; heteroaryl; alkylaryl; heteroalkylaryl; alkylheteroaryl;
20 heteroalkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio;
heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHCl₂;
-CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -
OC(O)R_x; -OCO₂R_x; -OCON(R_x)₂; -N(R_x)₂; -S(O)₂R_x; -NR_x(CO)R_x wherein each
occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic,
25 heteroaliphatic, heterocyclic, aromatic, heteroaromatic, aryl, heteroaryl, alkylaryl,
alkylheteroaryl, heteroalkylaryl or heteroalkylheteroaryl, wherein any of the aliphatic,
alicyclic, heteroaliphatic, heterocyclic, alkylaryl, or alkylheteroaryl substituents
described above and herein may be substituted or unsubstituted, branched or
unbranched, saturated or unsaturated, and wherein any of the aromatic,
30 heteroaromatic, aryl or heteroaryl substituents described above and herein may be
substituted or unsubstituted. Additional examples of generally applicable
substituents are illustrated by the specific embodiments shown in the Examples that
are described herein.

[0038] The term "heteroaliphatic", as used herein, refers to aliphatic moieties
35 in which one or more carbon atoms in the main chain have been substituted with a

5 heteroatom. Thus, a heteroaliphatic group refers to an aliphatic chain which contains one or more oxygen, sulfur, nitrogen, phosphorus or silicon atoms, *e.g.*, in place of carbon atoms. Heteroaliphatic moieties may be linear or branched, and saturated or unsaturated. In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or
10 more moieties including, but not limited to aliphatic; alicyclic; heteroaliphatic; heterocyclic; aromatic; heteroaromatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHCl₂; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -OC(O)R_x; -
15 OCO₂R_x; -OCON(R_x)₂; -N(R_x)₂; -S(O)₂R_x; -NR_x(CO)R_x wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic, aryl, heteroaryl, alkylaryl, alkylheteroaryl, heteroalkylaryl or heteroalkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic, heterocyclic, alkylaryl, or alkylheteroaryl substituents described
20 above and herein may be substituted or unsubstituted, branched or unbranched, saturated or unsaturated, and wherein any of the aromatic, heteroaromatic, aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described
25 herein.

[0039] The term "heterocycloalkyl", "heterocycle" or "heterocyclic", as used herein, refers to compounds which combine the properties of heteroaliphatic and cyclic compounds and include, but are not limited to, saturated and unsaturated mono- or polycyclic cyclic ring systems having 5-16 atoms wherein at least one ring
30 atom is a heteroatom selected from O, S and N (wherein the nitrogen and sulfur heteroatoms may be optionally be oxidized), wherein the ring systems are optionally substituted with one or more functional groups, as defined herein. In certain embodiments, the term "heterocycloalkyl", "heterocycle" or "heterocyclic" refers to a non-aromatic 5-, 6- or 7- membered ring or a polycyclic group wherein at least one
35 ring atom is a heteroatom selected from O, S and N (wherein the nitrogen and sulfur

5 heteroatoms may be optionally be oxidized), including, but not limited to, a bi- or tri-
cyclic group, comprising fused six-membered rings having between one and three
heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i)
each 5-membered ring has 0 to 2 double bonds, each 6-membered ring has 0 to 2
10 double bonds and each 7-membered ring has 0 to 3 double bonds, (ii) the nitrogen
and sulfur heteroatoms may be optionally be oxidized, (iii) the nitrogen heteroatom
may optionally be quaternized, and (iv) any of the above heterocyclic rings may be
fused to an aryl or heteroaryl ring. Representative heterocycles include, but are not
limited to, heterocycles such as furanyl, thiofuranyl, pyranlyl, pyrrolyl, thienyl,
pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazoliny, imidazolidinyl, piperidinyl,
15 piperazinyl, oxazolyl, oxazolidinyl, isooxazolyl, isoxazolidinyl, dioxazolyl, thiadiazolyl,
oxadiazolyl, tetrazolyl, triazolyl, thiazotriazolyl, oxatriazolyl, thiadiazolyl, oxadiazolyl,
morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, dithiazolyl,
dithiazolidinyl, tetrahydrofuryl, and benzofused derivatives thereof. In certain
embodiments, a "substituted heterocycle, or heterocycloalkyl or heterocyclic" group
20 is utilized and as used herein, refers to a heterocycle, or heterocycloalkyl or
heterocyclic group, as defined above, substituted by the independent replacement of
one, two or three of the hydrogen atoms thereon with but are not limited to aliphatic;
alicyclic; heteroaliphatic; heterocyclic; aromatic; heteroaromatic; aryl; heteroaryl;
alkylaryl; heteroalkylaryl; alkylheteroaryl; heteroalkylheteroaryl; alkoxy; aryloxy;
25 heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl;
Br; I; -OH; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHCl₂; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -
CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -OC(O)R_x; -OCO₂R_x; -OCON(R_x)₂; -
N(R_x)₂; -S(O)₂R_x; -NR_x(CO)R_x wherein each occurrence of R_x independently
includes, but is not limited to, aliphatic, alicyclic, heteroaliphatic, heterocyclic,
30 aromatic, heteroaromatic, aryl, heteroaryl, alkylaryl, alkylheteroaryl, heteroalkylaryl or
heteroalkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic,
heterocyclic, alkylaryl, or alkylheteroaryl substituents described above and herein
may be substituted or unsubstituted, branched or unbranched, saturated or
unsaturated, and wherein any of the aromatic, heteroaromatic, aryl or heteroaryl
35 substituents described above and herein may be substituted or unsubstituted.

5 Additional examples or generally applicable substituents are illustrated by the specific embodiments shown in the Examples, which are described herein.

[0040] Additionally, it will be appreciated that any of the alicyclic or heterocyclic moieties described above and herein may comprise an aryl or heteroaryl moiety fused thereto. Additional examples of generally applicable substituents are
10 illustrated by the specific embodiments shown in the Examples that are described herein.

[0041] The terms "halo" "halide" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

[0042] The term "haloalkyl" denotes an alkyl group, as defined above, having
15 one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like.

[0043] The term "amino", as used herein, refers to a primary (-NH₂), secondary (-NHR_x), tertiary (-NR_xR_y) or quaternary (-N⁺R_xR_yR_z) amine, where R_x, R_y and R_z are independently an aliphatic, alicyclic, heteroaliphatic, heterocyclic,
20 aromatic or heteroaromatic moiety, as defined herein. Examples of amino groups include, but are not limited to, methylamino, dimethylamino, ethylamino, diethylamino, diethylaminocarbonyl, methylethylamino, iso-propylamino, piperidino, trimethylamino, and propylamino.

[0044] The term "acyl", as used herein, refers to a group having the general
25 formula -C(=O)R, where R is an aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic or heteroaromatic moiety, as defined herein.

[0045] The term "sulfonamido", as used herein, refers to a group of the general formula -SO₂NR_xR_y, where R_x and R_y are independently hydrogen, or an aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic or acyl
30 moiety, as defined herein.

5 [0046] The term "benzamido", as used herein, refers to a group of the general formula PhCONR_x -, where R_x is hydrogen, or an aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic or acyl moiety, as defined herein.

[0047] The term "C₁₋₆alkylidene", as used herein, refers to a substituted or unsubstituted, linear or branched saturated divalent radical consisting solely of
10 carbon and hydrogen atoms, having from one to six carbon atoms, having a free valence "-" at both ends of the chain.

[0048] The term "C₂₋₆alkenylidene", as used herein, refers to a substituted or unsubstituted, linear or branched unsaturated divalent radical consisting solely of carbon and hydrogen atoms, having from two to six carbon atoms, having a free
15 valence "-" at both ends of the radical, and wherein the unsaturation is present only as double bonds and wherein a double bond can exist between the first carbon of the chain and the rest of the molecule.

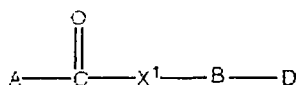
[0049] As used herein, the terms "aliphatic", "heteroaliphatic", "alkyl", "alkenyl", "alkynyl", "heteroalkyl", "heteroalkenyl", "heteroalkynyl", and the like encompass
20 substituted and unsubstituted, saturated and unsaturated, and linear and branched groups. Similarly, the terms "alicyclic", "heterocyclic", "heterocycloalkyl", "heterocycle" and the like encompass substituted and unsubstituted, and saturated and unsaturated groups. Additionally, the terms "cycloalkyl", "cycloalkenyl", "cycloalkynyl", "heterocycloalkyl", "heterocycloalkenyl", "heterocycloalkynyl",
25 "aromatic", "heteroaromatic", "aryl", "heteroaryl" and the like encompass both substituted and unsubstituted groups.

[0050] The phrase, "pharmaceutically acceptable derivative", as used herein, denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of such compound, or any other adduct or derivative which, upon administration to a patient,
30 is capable of providing (directly or indirectly) a compound as otherwise described herein, or a metabolite or residue thereof. Pharmaceutically acceptable derivatives thus include among others pro-drugs. A pro-drug is a derivative of a compound, usually with significantly reduced pharmacological activity, which contains at least

- 5 one additional moiety, which is susceptible to removal *in vivo* yielding the parent molecule as the pharmacologically active species. An example of a pro-drug is an ester, which is cleaved *in vivo* to yield a compound of interest. Pro-drugs of a variety of compounds, and materials and methods for derivatizing the parent compounds to create the pro-drugs, are known and may be adapted to the present invention.
- 10 Certain exemplary pharmaceutical compositions and pharmaceutically acceptable derivatives will be discussed in more detail herein below.

[0051] The present invention discloses compounds and pharmaceutical compositions thereof that possess anti-inflammatory activities.

- [0052]** The compounds of the invention include compounds of the general
 15 formula (I) as defined below:



Formula (I)

or a stereoisomer such as an enantiomer or a diastereomer or a racemate, or a tautomer thereof, or a prodrug, salt, hydrate or ester thereof;

- 20 wherein X^1 is selected from the group consisting of -O-, -S-, and -NH-;

wherein B is an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aryl, aralkyl or a heteroaromatic group, and wherein B is optionally substituted by one or more substituents X^2 which is independently selected from the group consisting of hydrogen, halogen, hydroxyl, -NO₂, -ONO₂, -CN; an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic
 25 moiety; -OR^R, -S(=O)_nR^d, -NR^bR^c, -C(=O)R^a and -C(=O)OR^a; wherein n is 0-2, R^R is an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, heteroaromatic or acyl moiety;

5 R^a , for each occurrence, is independently selected from the group consisting of hydrogen and an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic, aromatic, or a heteroaromatic moiety;

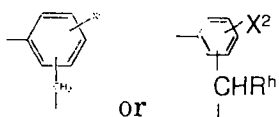
R^b and R^c , for each occurrence, are independently selected from the group consisting of hydrogen; hydroxy; SO_2R^d ; and aliphatic, alicyclic, heteroaliphatic,
10 heterocyclic, aromatic, heteroaromatic or an acyl moiety;

R^d , for each occurrence, is independently selected from the group consisting of hydrogen; $-N(R^e)_2$; aliphatic, aryl and heteroaryl; and

R^e , for each occurrence, is independently hydrogen or aliphatic;

wherein A is an optionally substituted aliphatic, alicyclic, heteroaliphatic, heterocyclic,
15 aromatic, or heteroaromatic group such as but not limited to those moieties described in further detail herein below;

wherein D is hydroxyl; halide; tosylate; phosphate ester ($-O-P(O)(OR^f)_2$) or a phosphite ester ($-O-P(OR^g)_2$), $-OSO_2NR_xR_y$, where R_x and R_y are independently hydrogen, or a substituted or unsubstituted aliphatic, alicyclic, heteroaliphatic,
20 heterocyclic, aromatic, heteroaromatic or acyl moiety; $-O-C_6H_4OC(=O)CH_3$; an alkoxy moiety; or an acyl moiety, provided that at least one R^f is not an H, and at least one R^g is not an H, and provided that if B is

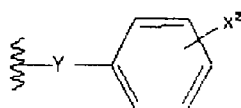


where R^h is aryl, aralkyl, alkyl, alkenyl or alkynyl, then D
is phosphate ester ($-O-P(OR^f)_3$) or a phosphite ester ($-O-P(OR^g)_2$), preferably, R^f and
25 R^g is independently each an H, alkyl, alkenyl or an alkynyl group, which may in turn be substituted or unsubstituted.

[0053] The compounds of Formula I are not limited by the position of the substituents on an aromatic ring. For example, if B is an aromatic ring, the -D moiety may be meta, ortho or para to the $A-C(=O)-X^1$ moiety, in particular when X^2 is H. If
30 one or more X^2 substituents are present, they may be positioned at any unoccupied

5 position(s). Thus, any and all positional isomers of compounds of Formula I are embraced by the invention. As will be apparent from the further discussion below on synthetic methods for the compounds of the invention, the A-C(=O)-X¹- moiety is facilely derived from a carboxylic acid-containing reactant (A-C(=O)-OH) or an amide-containing reactant (A-C(=O)-NH), and thus the A-C(=O)-X¹- moiety may be referred to herein as being derived from a compound with the structure A-C(=O)-OH or A-C(=O)-NH.

[0054] In one embodiment of compounds of Formula (I) of the invention, A is



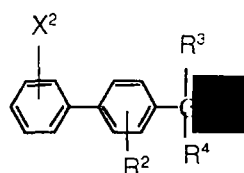
Formula (II)

15 wherein X² is one or more substituents as defined above, and Y is (-C-)_n, wherein n is 0 to 4, and when n is 2 or more, Y optionally contains one or more unsaturated bonds. For example, when n=0, the optionally substituted aromatic ring is bonded to the -C(=O)-X¹- substituent of Formula (I). When n=1, Y is -CH₂-. When n=2, Y may be -CH₂-CH₂-, -CH=CH- or ethynyl radical. When n=3, Y may be -CH₂-CH₂-CH₂-, an allyl radical, -CH=CH-CH₂- or -CH₂-CH=CH-, or a triple bond within the radical. When n=4, the divalent radical may have any combination of saturation and unsaturation.

[0055] Among the preferred but non-limiting selections of substituent A of Formula I, in a first embodiment, A is derived from among non-steroidal anti-inflammatory drugs (NSAIDs) including but not limited to aspirin, sulindac, ibuprofen, flurbiprofen, or formula IV or an analog of either of the foregoing.

[0056] Suitable analogs of formula IV include but are not limited to derivatives with one or more fluorine atoms substituted on one or both of the benzene rings of the formula IV moiety; and compounds with one or more substitutions on the alpha carbon, such as ethyl, dimethyl, diethyl, propyl and other such aliphatic substitutions. Thus, in one embodiment, A may be

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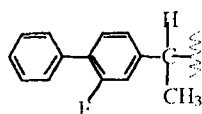


Formula (III)

wherein X^2 is one or more substituents as described above, R^2 is at least one halogen, and R_3 and R_4 are independently hydrogen or an aliphatic group. In a preferred embodiment, R^2 is F. In a more preferred embodiment, X^2 is H, R^2 is F (at position 3 relative to CR^3R^4) and R^3 and R^4 are H and CH_3 , respectively.

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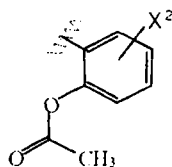
Thus, in one preferred but non-limiting embodiment, A can be



Formula (IV).

[0057] In a second embodiment, A is derived from aspirin, such as shown below:

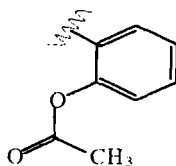
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Formula (V)

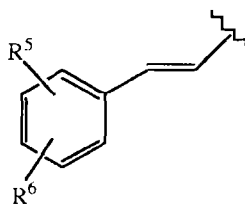
where X^2 is one or more substituents as described above.

5 [0058] In a preferred but non-limiting embodiment, A is



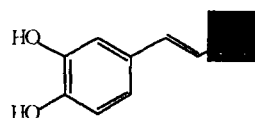
Formula (VI).

10 [0059] In a third embodiment, A is derived from cinnamic acid, or an analog of cinnamic acid, such as is shown below:

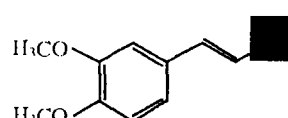


Formula (VII)

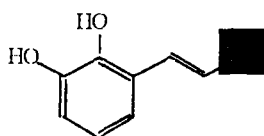
- 5 where R^5 and R^6 are independently hydrogen, -OH, alkoxy, halide, trifluoroalkyl, alpha-haloalkyl, trifluoroalkoxy, or R^a as described above. Non-limiting examples of the foregoing include trifluoromethyl, alpha-fluoromethyl, 4-(anisylideneamino), 2-(hexadecyloxy), and 4-nitro-alpha-(ortho-tolyl). Examples of Formula VI from which group A in Formula I can be selected include but are not limited to 3,4-dihydroxy, o-,
 10 m- and p-hydroxy; 2,3-dihydroxy; 3,5-dihydroxy; 3,4-dimethoxy; 3-hydroxy-4-methoxy and 3,4-dimethoxy. Thus, A can be



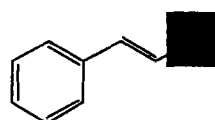
Formula (VIIa)



Formula (VIIb)

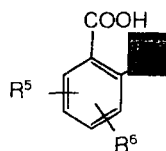


Formula (VIIc)



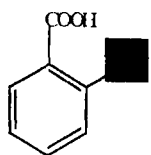
Formula (VII d)

[0060] In another embodiment, A is derived from phthalic acid, or an analog of phthalic acid, shown below:

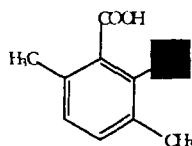


Formula (VIII)

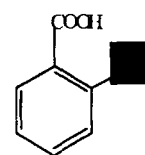
5 wherein R⁵ and R⁶ are as described above. Examples of such A moieties include:



Formula (VIIIa)

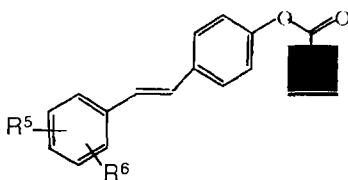


Formula (VIIIb)



Formula (VIIIc)

[0061] In yet a further embodiment, A is a straight chain or branched aliphatic moiety, preferably 1 to 7 carbons. In compounds wherein A is an aliphatic group, X² is preferably a moiety derived from the esterification of resveratrol or an analog thereof to a carboxylic acid on the aromatic ring, i.e. X² is (-C=O)OR^a. Suitable analogs of resveratrol include but are not limited to the compounds described by She Q-B et al. in *Oncogene*, volume 22, pp 2143-2150, 2003, and in the publication by Roberti et al. in *J. Med Chem*, volume 46, pp 3546-3554, 2003. In one embodiment, X² is

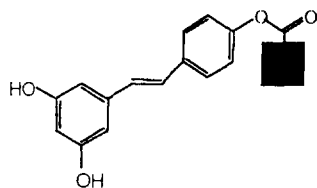


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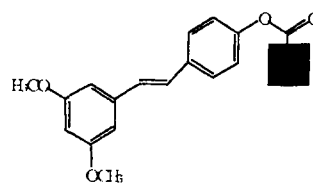
Formula (IX)

wherein R⁵ and R⁶ are as described above.

5 [0062] Non-limiting selections of X^2 are thus, by way of non-limiting examples,



Formula (IXa)



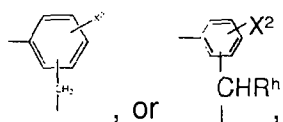
Formula (IXb)

[0063] In a preferred embodiment of the foregoing, A is methyl.

[0064] In addition to the foregoing selections of A, also embraced by the invention are compounds of Formula I wherein A is selected from the group
 10 consisting of an optionally substituted aliphatic, alicyclic, heteroaliphatic, aromatic, heterocyclic or heteroaromatic moiety.

[0065] The D substituent of Formula I is hydroxyl; halide; tosylate; phosphate ester (-O-P(O)(OR^f)₂) or a phosphite ester (-O-P(OR^g)₂), -OSO₂NR_xR_y, where R_x and R_y are independently hydrogen, or a substituted or unsubstituted aliphatic, alicyclic,
 15 heteroaliphatic, heterocyclic, aromatic, heteroaromatic or acyl moiety; -O-C₆H₄OC(=O)CH₃; an alkoxy moiety; or an acyl moiety, provided that at least one R^f is not an H, and at least one R^g is not an H.

[0066] In the compounds of the present invention according to formula I, if B is



20 where R^h is aryl, aralkyl, alkyl, alkenyl or alkynyl then D is phosphate ester (-O-P(O)(OR^f)₂) or a phosphite ester (-O-P(OR^g)₂), wherein R^f and R^g is independently each an H, alkyl, alkenyl or an alkynyl group, which may in turn be substituted or unsubstituted. The D substituent of Formula I is preferably a phosphate ester or a phosphite ester moiety, such as -OPO(alkyloxy)₂, -OPO₂(alkyloxy), -OP(alkyloxy)₂, -OPO(alkyloxy).

5 [0067] As noted above, in case B contains a benzene ring, the substituent containing the aforementioned D moiety, $-\text{CH}_2\text{-D}$, may be at any location on the benzene ring relative to the position of the $-\text{X}_1\text{-C(=O)-A}$ substituent, i.e., meta, ortho or para thereto. The invention embraces all such positional isomers.

[0068] The selections among substituent X^2 are as described above. As
10 mentioned above, in certain cases where A is an aliphatic group such as methyl, X^2 may be a carboxylic acid to which an alcohol or polyphenol is esterified, such as resveratrol or an analog thereof. Suitable analogs of resveratrol include but are not limited to the compounds described by She Q-B et al. in *Oncogene*, volume 22, pp 2143-2150, 2003, and in the publication by Roberti et al. in *J. Med. Chem.*, volume
15 46, pp 3546-3554, 2003. Other preferred examples of X^2 include one or more $-\text{OH}$, $-\text{OCH}_3$, or $-\text{F}$, at one or more positions not occupied by the substituents containing moieties A and D. Other preferred examples of X^2 include $-\text{CH}_3$, and $-\text{C}_2\text{H}_5$.

[0069] Preferably, compound of formula I is selected from the following: 2-
20 acetoxo-benzoic acid 4-(diethoxy-phosphoryloxymethyl)-phenyl ester, 2-acetoxo-benzoic acid 3-(diethoxy-phosphoryloxymethyl)-phenyl ester, and phospho-sulindac I, phospho-sulindac II, phosphoflurbiprofen, phosphoibuprofen, phosphoaspirin I, phosphoraspirin II, and phospho-valproic acid, as described hereinbelow.

[0070] The foregoing compounds are merely illustrative of Formula I and are not intended to be limiting.

25 [0071] In a further aspect, the invention is directed to a pharmaceutical composition comprising a compound of Formula I, as described generally herein, and a pharmaceutically acceptable excipient. In a specific embodiment, the composition is useful in the treatment of human and animal inflammation related diseases including but not limited to neoplasms and cancer, rheumatologic diseases
30 such as rheumatoid arthritis and Sjogren's syndrome; cardiovascular diseases, such as coronary artery disease, peripheral vascular disease and hypertension; neurodegenerative diseases such as Alzheimer's disease and its variants or cerebrovascular diseases; and autoimmune diseases such as lupus erythematosus;

5 and other conditions characterized by chronic inflammation of organs such as the lung, such as chronic bronchitis or the sinuses, such as chronic sinusitis; cardiovascular diseases, for example, coronary artery disease, peripheral vascular disease and hypertension; neurodegenerative diseases, for example, Alzheimer's disease and its variants or cerebrovascular diseases; and autoimmune diseases
10 such as lupus erythematosus; other conditions characterized by chronic inflammation of organs such as the lung, such as chronic bronchitis or the sinuses, such as chronic sinusitis; and various neoplastic and pre-neoplastic diseases, for example, benign prostatic hypertrophy, prostate cancer, colon adenomas and colon cancer, cancer of the lung, lymphomas and leukemias.

15 **[0072]** Such compositions can comprise one or more other pharmaceutical agents in addition to one or more compounds of the invention.

[0073] In another embodiment, the invention is directed to a method for inhibiting inflammation, in particular chronic inflammation in a subject in need thereof by administering to the subject an amount of the compound or composition of the
20 present invention effective to inhibit inflammation. The subject may be a human patient or animal.

[0074] It will be appreciated that for each of the classes and subclasses described above and herein, any one or more occurrences of aliphatic or heteroaliphatic may independently be substituted or unsubstituted, cyclic or acyclic,
25 linear or branched and any one or more occurrences of aryl, heteroaryl, cycloaliphatic, cycloheteroaliphatic may be substituted or unsubstituted.

[0075] Some of the foregoing compounds can comprise one or more asymmetric centers, and thus can exist in various isomeric forms, *e.g.*, stereoisomers and/or diastereomers. Thus, inventive compounds and
30 pharmaceutical compositions thereof may be in the form of an individual enantiomer, diastereomer or geometric isomer, or may be in the form of a mixture of stereoisomers. In certain embodiments, the compounds of the invention are enantiopure compounds. In certain other embodiments, mixtures of stereoisomers

5 or diastereomers are provided. Moreover, when compounds of the invention exist in tautomeric forms, each tautomer is embraced herein.

[0076] Furthermore, certain compounds, as described herein may have one or more double bonds that can exist as either the Z or E isomer, unless otherwise indicated. The invention additionally encompasses the compounds as individual
10 isomers substantially free of other isomers and alternatively, as mixtures of various isomers, *e.g.*, racemic mixtures of stereoisomers. In addition to the above-mentioned compounds *per se*, this invention also encompasses pharmaceutically acceptable derivatives of these compounds and compositions comprising one or more compounds of the invention and one or more pharmaceutically acceptable
15 excipients or additives.

[0077] Thus, the invention is directed to the use of the aforementioned compounds for treating inflammation-related diseases.

[0078] Thus, in a specific embodiment, the invention is directed to a method for obtaining a pharmaceutical composition, comprising formulating the compounds
20 of the present invention into a composition comprising the compound of the present invention and a pharmaceutically acceptable carrier or excipient. The invention is further directed to uses of the compound of the present invention for manufacturing a medicament.

[0079] **Compositions**

25 [0080] As discussed above, this invention provides novel compounds that have biological properties useful for the treatment of any of a number of conditions or diseases generally characterized by abnormal inflammation, or prophylaxis in instances wherein a risk of appearance of such conditions or diseases is present. Moreover, certain compounds known in the art have been newly identified as having
30 activity likewise useful in the prophylaxis or treatment of abnormal inflammation, and the invention is also directed to anti- inflammation compositions comprising such compounds.

5 [0081] Accordingly, in another aspect of the present invention, pharmaceutical compositions are provided, which comprise any one of the compounds described herein (or a prodrug, pharmaceutically acceptable salt or other pharmaceutically acceptable derivative thereof), and optionally comprise a pharmaceutically acceptable carrier. In certain embodiments, these compositions optionally further
10 comprise one or more additional therapeutic agents. Alternatively, a compound of this invention may be administered to a patient in need thereof in combination with the administration of one or more other therapeutic agents. For example, additional therapeutic agents for conjoint administration or inclusion in a pharmaceutical composition with a compound of this invention may be an approved anti-
15 inflammation agent, or it may be any one of a number of agents undergoing approval in the Food and Drug Administration that ultimately obtain approval for the treatment of any disorder related to inflammation. Such additional therapeutic agents may also be provided to promote the targeting of the compounds of the invention to the desired site of treatment, or may increase its stability, increase its half-life, etc. It will
20 also be appreciated that certain of the compounds of present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative thereof. According to the present invention, a pharmaceutically acceptable derivative includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or a pro-drug or other adduct or derivative of a compound of this
25 invention which upon administration to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

[0082] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use
30 in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts of amines, carboxylic acids, and other types of compounds, are well known in the art. For example, S.M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*,
35 66: 1-19 (1977), incorporated herein by reference. The salts can be prepared *in situ*

5 during the final isolation and purification of the compounds of the invention, or
separately by reacting a free base or free acid function with a suitable reagent, as
described generally below. For example, a free base function can be reacted with a
suitable acid. Furthermore, where the compounds of the invention carry an acidic
moiety, suitable pharmaceutically acceptable salts thereof may, include metal salts
10 such as alkali metal salts, *e.g.* sodium or potassium salts; and alkaline earth metal
salts, *e.g.* calcium or magnesium salts. Examples of pharmaceutically acceptable,
nontoxic acid addition salts are salts of an amino group formed with inorganic acids
such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and
perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid,
15 tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used
in the art such as ion exchange. Other pharmaceutically acceptable salts include
adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate,
borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate,
digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate,
20 glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-
hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate,
maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate,
oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate,
phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate,
25 thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like.
Representative alkali or alkaline earth metal salts include sodium, lithium, potassium,
calcium, magnesium, and the like. Further pharmaceutically acceptable salts
include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine
cations formed using counterions such as halide, hydroxide, carboxylate, sulfate,
30 phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

[0083] Additionally, as used herein, the term "pharmaceutically acceptable
ester" refers to esters that hydrolyze *in vivo* and include those that break down
readily in the human body to leave the parent compound or a salt thereof. Suitable
ester groups include, for example, those derived from pharmaceutically acceptable
35 aliphatic carboxylic acids, particularly alkanoic, alkenoic, cycloalkanoic and

5 alkanedioic acids, in which each alkyl or alkenyl moiety advantageously has not more than 6 carbon atoms. Examples of particular esters include formates, acetates, propionates, butyrates, acrylates and ethylsuccinates.

[0084] Furthermore, the term "pharmaceutically acceptable prodrugs" as used herein refers to those prodrugs of the compounds of the present invention which are,
10 within the scope of sound medical judgment, suitable for use in contact with the issues of humans and lower animals with undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" refers to compounds that are
15 rapidly transformed in vivo to yield the parent compound of the above formula, for example by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems, Vol. 14 of the A.C.S. Symposium Series, and in Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and Pergamon Press, 1987, both of which are
20 incorporated herein by reference.

[0085] As described above, the pharmaceutical compositions of the present invention additionally comprise a pharmaceutically acceptable carrier, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying
25 agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is
30 incompatible with the compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, sugars
35 such as lactose, glucose and sucrose; starches such as corn starch and potato

5 starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl
cellulose and cellulose acetate; powdered tragacanth; malt; gelatine; talc; excipients
such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil;
safflower oil, sesame oil; olive oil; corn oil and soybean oil; glycols; such as
propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents
10 such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free
water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer
solutions, as well as other non-toxic compatible lubricants such as sodium lauryl
sulfate and magnesium stearate, as well as coloring agents, releasing agents,
coating agents, sweetening, flavoring and perfuming agents, preservatives and
15 antioxidants can also be present in the composition, according to the judgment of the
formulator.

[0086] Liquid dosage forms for oral administration include, but are not limited
to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions,
syrups and elixirs. In addition to the active compounds, the liquid dosage forms may
20 contain inert diluents commonly used in the art such as, for example, water or other
solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol,
ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol,
1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut
(peanut), corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl
25 alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.
Besides inert diluents, the oral compositions can also include adjuvants such as
wetting agents, emulsifying and suspending agents, sweetening, flavoring, and
perfuming agents.

[0087] Injectable preparations, for example, sterile injectable aqueous or
30 oleaginous suspensions may be formulated according to the known art using
suitable dispersing or wetting agents and suspending agents. The sterile injectable
preparation may also be a sterile injectable solution, suspension or emulsion in a
nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-
butanediol. Among the acceptable vehicles and solvents that may be employed are
35 water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition,

5 sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[0088] The injectable formulations can be sterilized, for example, by filtration
10 through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[0089] In order to prolong the effect of a drug, it is often desirable to slow the
absorption of the drug from subcutaneous or intramuscular injection. This may be
15 accomplished by the use of a liquid suspension or crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable
20 depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also
25 prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

[0090] Compositions to deliver the agent directly to the colon – for example,
pills from which the active agent is released into the colon by a pH-dependent or
other mechanism ensuring exclusive or predominant colonic delivery of said
30 compound, suppositories, enemas and other means for colonic delivery.

[0091] Compositions for rectal or vaginal administration are preferably
suppositories which can be prepared by mixing the compounds of this invention with
suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol

5 or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

[0092] Solid dosage forms for oral administration include but are not limited to capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[0093] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

5 [0094] The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms, the active compound
10 may be admixed with at least one inert diluent such as sucrose, lactose and starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may
15 optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include but are not limited to polymeric substances and waxes.

[0095] The present invention encompasses pharmaceutically acceptable
20 topical formulations of inventive compounds. The term "pharmaceutically acceptable topical formulation", as used herein, means any formulation which is pharmaceutically acceptable for intradermal administration of a compound of the invention by application of the formulation to the epidermis. In certain embodiments of the invention, the topical formulation comprises a carrier system. Pharmaceutically
25 effective carriers include, but are not limited to, solvents (*e.g.*, alcohols, poly alcohols, water), creams, lotions, ointments, oils, plasters, liposomes, powders, emulsions, microemulsions, and buffered solutions (*e.g.*, hypotonic or buffered saline) or any other carrier known in the art for topically administering pharmaceuticals. A more complete listing of art-known carriers is provided by
30 reference texts that are standard in the art, for example, Remington's Pharmaceutical Sciences, 16th Edition, 1980 and 17th Edition, 1985, both published by Mack Publishing Company, Easton, Pa., the disclosures of which are incorporated herein by reference in their entireties. In certain other embodiments, the topical formulations of the invention may comprise excipients. Any pharmaceutically
35 acceptable excipient known in the art may be used to prepare the inventive

5 pharmaceutically acceptable topical formulations. Examples of excipients that can be included in the topical formulations of the invention include, but are not limited to, preservatives, antioxidants, moisturizers, emollients, buffering agents, solubilizing agents, other penetration agents, skin protectants, surfactants, and propellants, and/or additional therapeutic agents used in combination to the inventive compound.

10 Suitable preservatives include, but are not limited to, alcohols, quaternary amines, organic acids, parabens, and phenols. Suitable antioxidants include, but are not limited to, ascorbic acid and its esters, sodium bisulfite, butylated hydroxytoluene, butylated hydroxyanisole, tocopherols, and chelating agents like EDTA and citric acid. Suitable moisturizers include, but are not limited to, glycerin, sorbitol,

15 polyethylene glycols, urea, and propylene glycol. Suitable buffering agents for use with the invention include, but are not limited to, citric, hydrochloric, and lactic acid buffers. Suitable solubilizing agents include, but are not limited to, quaternary ammonium chlorides, cyclodextrins, benzyl benzoate, lecithin, and polysorbates. Suitable skin protectants that can be used in the topical formulations of the invention

20 include, but are not limited to, vitamin E oil, allantoin, dimethicone, glycerin, petrolatum, and zinc oxide.

[0096] In certain embodiments, the pharmaceutically acceptable topical formulations of the invention comprise at least a compound of the invention and a penetration enhancing agent. The choice of topical formulation will depend on several

25 factors, including the condition to be treated, the physicochemical characteristics of the inventive compound and other excipients present, their stability in the formulation, available manufacturing equipment, and costs constraints. As used herein the term " penetration enhancing agent " means an agent capable of transporting a pharmacologically active compound through the stratum corneum and

30 into the epidermis or dermis, preferably, with little or no systemic absorption. A wide variety of compounds have been evaluated as to their effectiveness in enhancing the rate of penetration of drugs through the skin. See, for example, *Percutaneous Penetration Enhancers*, Maibach H. I. and Smith H. E. (eds.), CRC Press, Inc., Boca Raton, Fla. (1995), which surveys the use and testing of various skin penetration

35 enhancers, and Buyuktimkin *et al.*, *Chemical Means of Transdermal Drug*

5 Permeation Enhancement in Transdermal and Topical Drug Delivery Systems, Gosh
T. K., Pfister W. R., Yum S. I. (Eds.), Interpharm Press Inc., Buffalo Grove, Ill. (1997).
In certain exemplary embodiments, penetration agents for use with the invention
include, but are not limited to, triglycerides (*e.g.*, soybean oil), aloe compositions
(*e.g.*, aloe-vera gel), ethyl alcohol, isopropyl alcohol, octolyphenylpolyethylene
10 glycol, oleic acid, polyethylene glycol 400, propylene glycol, N-decylmethylsulfoxide,
fatty acid esters (*e.g.*, isopropyl myristate, methyl laurate, glycerol monooleate, and
propylene glycol monooleate) and N-methyl pyrrolidone.

[0097] In certain embodiments, the compositions may be in the form of
ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or
15 patches. In certain exemplary embodiments, formulations of the compositions
according to the invention are creams, which may further contain saturated or
unsaturated fatty acids such as stearic acid, palmitic acid, oleic acid, palmito-oleic
acid, cetyl or oleyl alcohols, stearic acid being particularly preferred. Creams of the
invention may also contain a non-ionic surfactant, for example, polyoxy-40-stearate.
20 In certain embodiments, the active component is admixed under sterile conditions
with a pharmaceutically acceptable carrier and any needed preservatives or buffers
as may be required. Ophthalmic formulation, eardrops, and eye drops are also
contemplated as being within the scope of this invention. Additionally, the present
invention contemplates the use of transdermal patches, which have the added
25 advantage of providing controlled delivery of a compound to the body. Such dosage
forms are made by dissolving or dispensing the compound in the proper medium. As
discussed above, penetration enhancing agents can also be used to increase the
flux of the compound across the skin. The rate can be controlled by either providing
a rate controlling membrane or by dispersing the compound in a polymer matrix or
30 gel.

[0098] It will also be appreciated that the compounds and pharmaceutical
compositions of the present invention can be formulated and employed in
combination therapies, that is, the compounds and pharmaceutical compositions can
be formulated with or administered concurrently with, prior to, or subsequent to, one
35 or more other desired therapeutics or medical procedures. The particular

5 combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an inventive compound may be administered concurrently
10 with another anti-inflammation agent), or they may achieve different effects (e.g., control of any adverse effects).

[0099] In certain embodiments, the pharmaceutical compositions of the present invention further comprise one or more additional therapeutically active ingredients (e.g., anti-inflammatory and/or palliative). For purposes of the invention,
15 the term "*Palliative*" refers to treatment that is focused on the relief of symptoms of a disease and/or side effects of a therapeutic regimen, but is not curative. For example, palliative treatment encompasses painkillers, antinausea medications and anti-sickness drugs.

[00100] In certain embodiments the compounds of the present invention can be
20 covalently or non-covalently bound to for example polyethylene glycol or other similar molecules to make them suitable for administration to the patient either in one of the forms described above or using nanodevices. Alternatively, the compounds of the present invention can be formulated using the principles of nanoscience to optimize their therapeutic application.

25 **[00101] Uses and Methods of Treatment**

[00102] As discussed above, certain of the compounds as described herein exhibit activity generally as inhibitors of inflammation, with inflammation understood as described herein under "BACKGROUND OF THE INVENTION." Thus, in certain
30 embodiments, compounds of the invention are useful for the treatment of any of a number of conditions or diseases in which inflammation, in particular chronic inflammation is the cause of or relates to the onset or continued occurrence of the disease or condition, such as but not limited to rheumatologic diseases such as rheumatoid arthritis and Sjogren's syndrome; cardiovascular diseases, for example,

5 coronary artery disease, peripheral vascular disease and hypertension; neurodegenerative diseases, for example, Alzheimer's disease and its variants or cerebrovascular diseases; and autoimmune diseases such as lupus erythematosus; other conditions characterized by chronic inflammation of organs such as the lung, such as chronic bronchitis or the sinuses, such as chronic sinusitis.

10 **[00103]** Accordingly, in another aspect of the invention, methods for the treatment of inflammation-related disorders are provided comprising administering a therapeutically effective amount of a compound of Formula I to a subject in need thereof. In certain embodiments, a method for the treatment of related disorders is provided comprising administering a therapeutically effective amount of an inventive
15 compound, or a pharmaceutical composition comprising an inventive compound to a subject in need thereof, in such amounts and for such time as is necessary to achieve the desired result.

[00104] The invention is also directed to the use of any compound of Formula (I) for the preparation of a medicament for administration to a human or animal
20 patient in need thereof, to inhibit or block inflammation. Such compounds preferably are administered once an inflammation-related disease or an inflammatory condition that may predispose to disease has been diagnosed in the patient, optionally in combination with other anti- inflammation agents or other agents such as those that maintain therapeutic levels of the compounds within the body. Compounds of the
25 invention also may be administered after other therapies have been tried and failed, and may be administered prophylactically.

[00105] In certain embodiments, the uses and methods of the invention involve the administration of a therapeutically effective amount of the compound or a pharmaceutically acceptable derivative thereof to a subject (including, but not limited
30 to a human or animal, including livestock, domesticated or zoo animals) in need thereof.

[00106] It will be appreciated that the compounds and compositions, according to the method of the present invention, may be administered using any amount and

5 any route of administration effective for the treatment of conditions or diseases in
which anti-inflammation or related activities have a therapeutically useful role. Thus,
the expression "effective amount" as used herein, refers to a sufficient amount of
agent to inhibit inflammation and to exhibit a therapeutic effect. The exact amount
required will vary from subject to subject, depending on the species, age, and
10 general condition of the subject, the severity of the infection, the particular
therapeutic agent, its mode of administration, and the like. The compounds of the
invention are preferably formulated in dosage unit form for ease of administration
and uniformity of dosage. The expression "dosage unit form" as used herein refers
to a physically discrete unit of therapeutic agent appropriate for the patient to be
15 treated. It will be understood, however, that the total daily usage of the compounds
and compositions of the present invention will be decided by the attending physician
within the scope of sound medical judgment. The specific therapeutically effective
dose level for any particular patient or organism will depend upon a variety of factors
including the disorder being treated and the severity of the disorder; the activity of
20 the specific compound employed; the specific composition employed; the age, body
weight, general health, sex and diet of the patient; the time of administration, route of
administration, and rate of excretion of the specific compound employed; the
duration of the treatment; drugs used in combination or coincidental with the specific
compound employed; and like factors well known in the medical arts.

25 **[00107]** Furthermore, after formulation with an appropriate pharmaceutically
acceptable carrier in a desired dosage, the pharmaceutical compositions of this
invention can be administered to humans and other animals orally, rectally,
parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders,
ointments, or drops), buccally, as an oral or nasal spray, or the like, depending on the
30 location and extent of the disease being treated. In certain embodiments, the
compounds of the invention may be parenterally administered at dosage levels of
about 0.001 mg/kg to about 50 mg/kg, from about 0.01 mg/kg to about 25 mg/kg, or
from about 0.1 mg/kg to about 10 mg/kg of subject body weight per day, one or more
times a day, to obtain the desired therapeutic effect. In other embodiments,
35 compounds of the invention may be administered orally or rectally at dosage levels

5 of about 0.01 mg/kg to about 100 mg/kg, from about 0.05 mg/kg to about 50 mg/kg,
or from about 0.1 mg/kg to about 10 mg/kg of subject body weight per day, one or
more times a day, to obtain the desired therapeutic effect. It will also be appreciated
that dosages smaller than 0.001 mg/kg or greater than 50 mg/kg (for example 50-
100 mg/kg) can be administered to a subject. In certain embodiments, compounds
10 are administered orally or parenterally.

[00108] Treatment Kit

[00109] In other embodiments, the present invention relates to a kit for
conveniently and effectively carrying out the methods in accordance with the present
invention. In general, the pharmaceutical pack or kit comprises one or more
15 containers filled with one or more of the ingredients of the pharmaceutical
compositions of the invention. Such kits are especially suited for the delivery of solid
oral forms such as tablets or capsules. Such a kit preferably includes a number of
unit dosages, and may also include a card having the dosages oriented in the order
of their intended use. If desired, a memory aid can be provided, for example in the
20 form of numbers, letters, or other markings or with a calendar insert, designating the
days in the treatment schedule in which the dosages can be administered.
Alternatively, placebo dosages, or calcium dietary supplements, either in a form
similar to or distinct from the dosages of the pharmaceutical compositions, can be
included to provide a kit in which a dosage is taken every day. Optionally associated
25 with such container(s) can be a notice in the form prescribed by a governmental
agency regulating the manufacture, use or sale of pharmaceutical products, which
notice reflects approval by the agency of manufacture, use or sale for human
administration.

[00110] The representative examples that follow are intended to help illustrate
30 the invention, and are not intended to, nor should they be construed to, limit the
scope of the invention. Indeed, various modifications of the invention and many
further embodiments thereof, in addition to those shown and described herein, will
become apparent to those skilled in the art from the full contents of this document,
including the examples which follow and the references to the scientific and patent

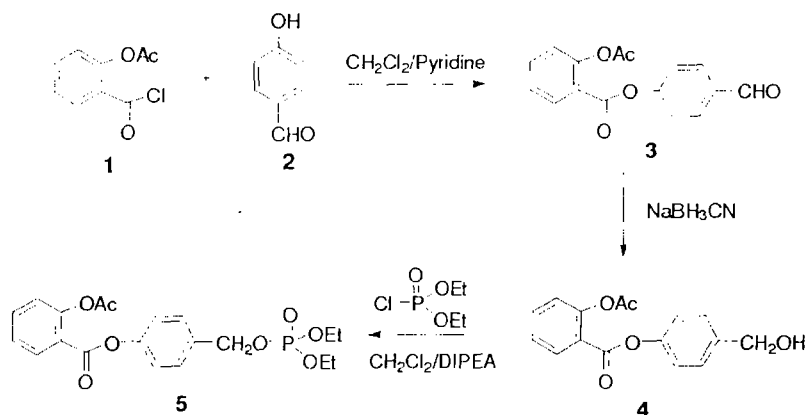
5 literature cited herein. It should further be appreciated that the contents of those cited references are incorporated herein by reference to help illustrate the state of the art.

[00111] The following examples contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in
10 its various embodiments and the equivalents thereof.

EXAMPLES

[00112] Example 1. Method of Synthesis

[00113] The following reaction scheme was followed to obtain Compound 5 (*para*-phosphoaspirin) of this invention shown below. Compound 5 was synthesized
15 starting from *O*-Acetylsalicyloyl chloride (1) and 4-hydroxybenzaldehyde (2) in three steps, as shown below.



[00114] In this scheme -OEt represents $\text{CH}_3\text{CH}_2\text{O}-$.

[00115] **Step 1: Preparation of Compound 3:** To a pre-cooled (0°C) solution
20 of 4-hydroxybenzaldehyde (2, 1.04 g, 8.49 mmole) in dichloromethane (10 mL) and pyridine (4.16 mL, 50 mmole) was added *O*-acetylsalicyloyl chloride (1, 1.98g, 10 mmole) in methylene chloride (10 mL) drop-wise between $0-5^\circ\text{C}$. The temperature of the reaction mixture was slowly raised to room temperature and left over night. At this point TLC of the reaction mixture showed the completion of the reaction. The

5 reaction mixture was washed with water (25 mL), followed by 1N HCl (25 mL) and then finally with aqueous NaHCO₃. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The crude weight of the oil was 2.35 g (97%).

[00116] **Step 2: Preparation of Compound 4:** To a pre-cooled (0° C) solution
10 of compound **3** (2.3 g, 8.1 mmole) in methylene chloride (10 mL) and acetic acid (2.5 mL) was added sodium cyanoborohydride (253 mg, 4 mmole) in two portions. The temperature of the reaction was slowly raised to room temperature in 30 minutes. At this point TLC showed the completion of the reaction. The reaction mixture was washed with water (2 x 25 mL), followed by saturated aqueous sodium bicarbonate
15 (25 mL) and then finally with brine. It was dried over anhydrous sodium sulfate, filtered and concentrated. The crude weight of the solid was 1.95 g (83%).

[00117] **Step 3: Preparation of 2-Acetoxy-benzoic acid 4-(diethoxy-phosphoryloxymethyl)-phenyl ester (5):** To a solution of alcohol (**4**, 1.9 g, 6.64
20 mmole) in methylene chloride (10 mL) and diisopropylethylamine (2.2 mL, 13.28 mmole) was added diethylchlorophosphate (2.5 mL, 17.26 mmole) drop-wise, followed by DMAP (25 mg) as a solid. The reaction mixture was heated under reflux overnight. At this point the TLC showed the completion of the reaction. The reaction mixture was washed with water (2 x 25 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The crude residue was purified by column
25 chromatography using hexane:ethyl acetate (60:40). The pure fractions were combined and evaporated to give a solid which was triturated with hot hexane several times to give pure title compound 690 mg (25%) as a solid.

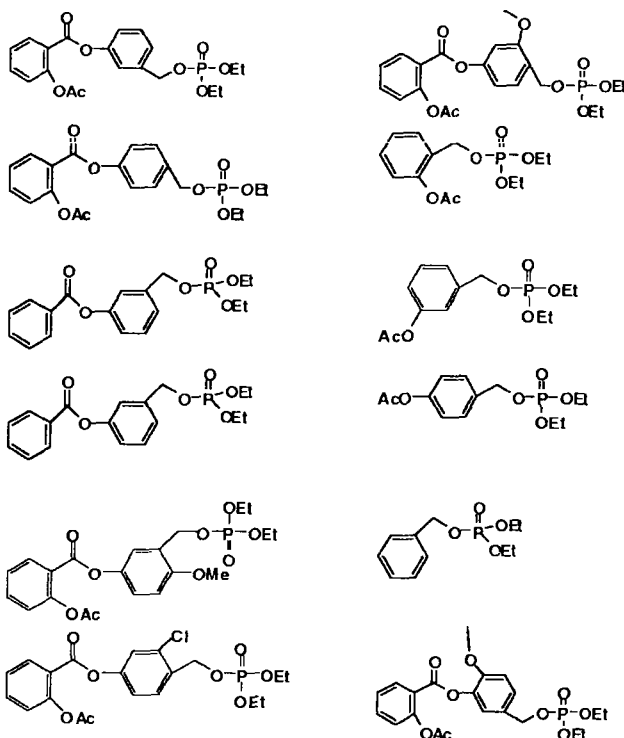
[00118] To confirm purity and identity of compound **5** of this invention, TLC and ¹H NMR was performed. The NMR profile is shown in Figure 1.

30 [00119] **NSAID based compounds** Non-steroidal anti-inflammatory drugs (NSAIDs) comprise a structurally and, to a large extent, functionally diverse group of compounds with nearly 50 individual compounds approved for the treatment of patients with a variety of inflammatory diseases. They all have analgesic,

- 5 antipyretic and anti-inflammatory effects. Some of them, such as acetylsalicylic acid (aspirin), have been demonstrated to have an effect against inflammation-related diseases such as rheumatologic, cardiovascular, neurodegenerative and cancer, be this effect either therapeutic as, for example, in rheumatoid arthritis, or preventive as, for example, in cancer, coronary artery disease or Alzheimer's disease.
- 10 **[00120]** Broadly, NSAIDs can be categorized into the following chemical groups: salicylates, arylalkanoic acids (e.g. sulindac), 2-arylpropionic acids (profens), N-arylanthranilic acids (fenamic acids), pyrazolidine derivatives, oxicams, and sulphonanilides. Most of the available NSAIDs are amenable to derivatization as described herein using methods readily available and known to those ordinarily
- 15 skilled in the art.

[00121] The following are examples of several derivatized NSAIDs according to certain embodiments of the invention.

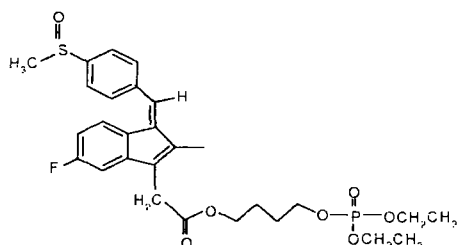
[00122] The following compounds have been similarly synthesized.



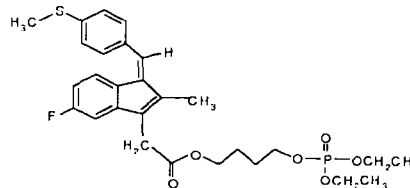
5 Note: -OEt represents $\text{CH}_3\text{CH}_2\text{O}$ -.asdfad

[00123] Two derivatives of sulindac (2-[6-fluoro-2-methyl-3-[(4-methylsulfinylphenyl) methylidene]inden-1-yl]- acetic acid) were synthesized. They are referred to as phospho-sulindac I and phsposulindac II, respectively. Their structures are:

10



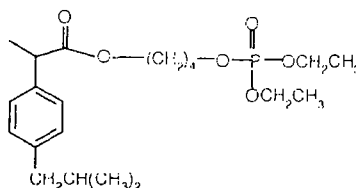
Phospho-sulindac I



Phospho-sulindac II

and their respective $^1\text{H-NMR}$ profiles are shown in Figure 1.

15 [00124] One derivative of ibuprofen has been synthesized and is referred to as phospho-ibuprofen. Its structure is:



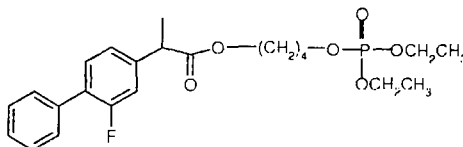
Phospho-ibuprofen

20

and its $^1\text{H-NMR}$ profile is shown in Figure 1.

[00125] One derivative of flurbiprofen has been synthesized and is referred to as phospho-flurbiprofen. Its structure is:

25

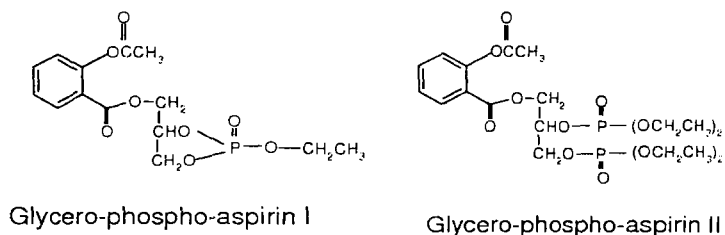


Phospho-flurbiprofen

5 and its $^1\text{H-NMR}$ profile is shown in Figure 1.

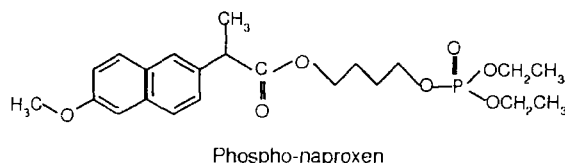
[00126] Two derivatives of aspirin were synthesized. They are referred to as glycerophospho-aspirin I and glycerophospho-aspirin II, respectively. Their structures are shown below, and their respective $^1\text{H-NMR}$ profiles are shown in Figure 1.

10



[00127] A derivative of naproxen is shown below

15



[00128] **EXAMPLE 4 Six novel NSAID-based compounds have significantly enhanced antineoplastic potency compared to their parent compounds**

20 [00129] Six compounds based on four representative NSAIDs, aspirin (2 derivatives differing in their linker), ibuprofen, flurbiprofen and sulindac (2 derivatives, differing in the structure of the sulindac moiety) were synthesized following the methodology of Penning et al (Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, et al. Synthesis and biological evaluation of the 1,5-diarylpirazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, celecoxib). J Med Chem 1997;40:1347-65.) and the methods described herein. Their structures and NMR profiles are shown above.

25

5 [00130] Conventional NSAIDs were purchased from Sigma (St Louis, MO). We examined these six compounds for their antineoplastic properties, determined in cultured human cells derived from colon, breast and pancreatic cancers.

[00131] **Cell culture:** Human breast (MCF-7 and MDA-MB 231), colon (HT-29, and SW-480) and pancreatic (MIA PaCa-2 and BxPC-3) cell lines (American Type
10 Culture Collection, Manassas, VA) were grown as monolayers in the specific medium suggested by American Type Culture Collection and supplemented with 10% fetal calf serum (Mediatech, Herndon, VA), penicillin (50 U/ml) and streptomycin (50 µg/ml; Life Technologies, Grand Island, NY). Cells were incubated at 37°C in 5% CO₂. Cells were seeded at 5.5x10⁴ cells/cm², allowed to attach overnight, and the following
15 morning cells were treated with each of the test compounds. MCF-7 cells are estrogen receptor positive and MDA-MB231 cells are estrogen receptor negative.

[00132] **Cell viability assay:** We used an assay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye (MTT), which was determined according to the manufacture's protocol (Promega, Madison, WI, USA).

20 [00133] **Cell proliferation assay:** To determine cell proliferation, we measured the incorporation of 5-bromo-2'-deoxyuridine (BrdU) into newly synthesized cellular DNA, following the manufacture's instructions (BD Biosciences, San Jose, CA).

[00134] **Annexin V and propidium iodide (PI) staining:** Cells were seeded at a density of 1x10⁵ cells/well and treated for 24 h with various concentrations of each
25 compound or equivalent volumes of DMSO. Briefly, after incubation with the test compounds, cells were trypsinized and stained with Annexin V-FITC (Invitrogen) and PI (0.5 µg/ml). Following incubation at room temperature for 15 min in the dark, annexin V-FITC and PI fluorescence intensities were analyzed by FACScaliber (BD Bioscience). Annexin V (+)/PI (-) cells are in early apoptosis, annexin V (+)/PI (+)
30 cells are in late apoptosis (secondary necrosis), and annexin V (-)/PI (+) cells are necrotic cells.

[00135] **Determination of cell cycle phase distribution (PI incorporation assay):** Cells were seeded in culture plates and treated for 24 h with various

5 concentrations of each compound or equivalent volumes of DMSO. After treatment, cells were trypsinized and fixed in 70% ethanol for 1 h on ice, stained with PI (50 $\mu\text{g}/\text{ml}$) and RNase A (4 U/ml) for 30 min and subjected to flow cytometric analysis for the determination of their distribution in the cell cycle phases.

10 **[00136]** The effect of the six compounds and their parent NSAIDs on six human cancer cell lines is summarized in Table 1, which shows 24-h IC_{50}s (μM) of the NSAID-derivatives in human cancer cell lines. All six compounds showed enhanced potency in inhibiting cell growth compared to their corresponding conventional NSAIDs. The potency enhancement ranged between >6 and >63-fold (we were unable to obtain a precise IC_{50} for conventional aspirin, given its limited solubility).

5

Table 1: 24-h IC₅₀, μM

Compound	BREAST		COLON		PANCREATIC	
	<i>MCF-7</i>	<i>MDA-MB231</i>	<i>HT29</i>	<i>SW480</i>	<i>BxPC-3</i>	<i>MIA-PaCa-2</i>
Sulindac	1128	530	1173	900	489	1036
Phospho-sulindac I	62	17	65	98	62	88
Ratio sulindac/PS I	18	31	18	9	8	12
Phospho-sulindac II	38	18	70	73	32	92
Ratio sulindac/PS II	30	30	17	12	15	11
Flurobiprofen	1433	823	1670	1216	825	1272
Phospho-Flurobiprofen	65	17	80	104	34	135
Ratio flurobiprofen/PF	22	48	21	12	24	9
Ibuprofen	1229	748	1554	1057	1064	1280
Phospho-Ibuprofen	79	28	82	75	53	104
Ratio ibuprofen/PI	16	27	19	14	20	12
Aspirin	>2000	>2000	3996	>2000	>2000	>2000
Glyero-phospho-aspirin I	32	199	54	169	63	303
Ratio aspirin/GPA I	>63	>10	74	>12	>32	>7
Glyero-phospho-aspirin II	248	360	40	170	38	242
Ratio aspirin/GPA II	>8	>6	100	>12	>12	>8

Note: GPA is an abbreviation for glyero-phospho-aspirin

- [00137] To understand the mechanism by which these compounds inhibited cell growth, we evaluated in MDA-MB 231 human breast cancer cells their effects on cell kinetics, namely cell proliferation (i.e., cell renewal), cell death and cell cycle.
- 10 [00138] As summarized in Table 2 below, in MDA-MB231 cells, all six compounds, each used at its IC₅₀ concentration, a) inhibited cell proliferation between 6% and 50% compared to controls; b) induced both early and late apoptosis, as well as necrosis; and c) inhibited the G₁ to S cell cycle phase transition. In this Table, the values for proliferation are the percentage of the corresponding control values.
- 15 Those for apoptosis and necrosis refer to the percentage of cells in each category with respect to the entire cell population, and are to be compared to the control (vehicle only-treated) cells (uppermost row).

5

Table 2: Cell kinetic effect of six compounds

Compound	Proliferation (% control)	Early apoptosis(%)	Late apoptosis(%)	Necrosis (%)
Control	N/A	1.4	2.0	0.6
Phospho- sulindac I	52	1.7	4.7	1.1
Phospho- sulindac II	52	5.0	23.2	2.6
Phospho- flurbiprofen	42	5.3	20.6	1.7
Phospho- ibuprofen	50	7.4	43.2	5.6
Glycero- phospho- aspirin I	94	4.8	3.2	0.3
Glycero- phospho- aspirin II	52	2.4	2.2	0.4

[00139] Following identical methodologies as those described above for MDA-MB231 cells, we determined the effect of phospho-sulindac I on the cell kinetics of SW480 and HT-29 human colon cancer cells by treating them for 24 h with phospho-sulindac I used at its 24-h IC₅₀ concentration. Compared to untreated controls, phospho-sulindac I at a concentration equal to its IC₅₀ for growth at 24 h: inhibited cell proliferation by 72% (from 43% in controls to 12% in phospho-sulindac I treated cells); induced apoptosis (early and late subtypes combined) by 201% (from 6.6% to 20%); induced necrosis by 1350% (from 0.2% to 2.9%) and blocked the transition from the G1 to the S cell cycle phase

5 [00140] Example 5: **Phospho-sulindac I inhibits colon cancer growth in vivo**

[00141] The effect of phospho-sulindac I on tumor growth in vivo was evaluated in two animal models of colon cancer, APC^{Min/+} mice and colon cancer xenografts in nude mice.

[00142] **APC^{Min/+} mice study:** *Min* mice have a truncating mutation in the *Apc* gene that predisposes them to the development of gastrointestinal tumors in the small intestine and colon (Lipkin M, Yang K, Edelmann W, et al. Preclinical mouse models for cancer chemoprevention studies. Ann N Y Acad Sci 1999;889:14-9.). In many important ways, this model system represents a useful (and extensively utilized) experimental system that recapitulates the relevant steps of colon
10
15 carcinogenesis.

[00143] Eleven week-old male C57BL/6J APC^{Min/+} mice divided into four groups of 10 mice/group were treated for 4 weeks via gavage administration as follows: *group 1* was treated with vehicle (corn oil); and *group 2* was treated with phospho-sulindac I 50 mg/kg/day. At the end of treatment, compared with vehicle-treated controls,
20 phospho-sulindac I decreased the number of tumors in the small intestine by 57.2 % (p<0.002) (number of intestinal tumors in vehicle-treated group 33.6±8.7, and in phospho-sulindac I treated mice 19.4±12.0), whereas, specifically, in the colon the reduction by phospho-sulindac I was of 61.8 % (p<0.02) compared to vehicle treated mice (number of colon tumors in vehicle-treated group 1.6±0.8, and in phospho-sulindac I treated mice 0.6±0.5). Of note, as we have shown, conventional sulindac
25 stimulates the formation of tumors in the colon of *Min* mice (Yang K, Fan K, Kurihara N, et al. Regional response leading to tumorigenesis after sulindac in small and large intestine of mice with *Apc* mutations. Carcinogenesis 2003;24(3):605-11). Our results document that P-S exerts a profound inhibitory effect on intestinal carcinogenesis in
30 *Min* mice without any overt signs of toxicity.

[00144] **Nude mice xenograft study:** Female nude mice CByJ.Cg-Foxn1 (5-6 weeks-old) were inoculated subcutaneously in their lower right flank with 1.5x10⁶ SW480 colon cancer cells in a volume of 100 µl (containing 50% matrigel in PBS).

5 Seven days later, animals were randomized into two groups (8 mice/group): *group 1* received vehicle (1% (w/v) carboxy methylcellulose); *group 2* received 50 mg/kg/day phospho-sulindac I. All drugs were administered in a solution of 1% (w/v) carboxy methylcellulose by gavage once daily (phospho-sulindac I and sulindac concentrations are equimolar). Tumors were measured twice a week with a digital
10 microcaliper, and tumor volume (TV) was calculated using the formula: $TV = [L \times W \times (L + W/2) \times 0.56]$, where $L = \text{length}$ and $W = \text{width of tumor}$. After 14 days of treatment, animals were sacrificed, and tumors were removed and weighed. The mean tumor weight for vehicle and phospho-sulindac I were 0.246 ± 0.041 and 0.097 ± 0.018 (*mean \pm SEM*), respectively, indicating a reduction in tumor weight of 60 %
15 ($p < 0.05$) by phospho-sulindac I. Of note, phospho-sulindac I was well tolerated, since no weight loss or other signs of toxicity were observed throughout the treatment period.

[00145] Synergy between difluoromethylornithine (DFMO) and phospho-sulindac

20 **[00146]** We examined the potential synergy between (DFMO) and phospho-sulindac I. A significant development in combination chemoprevention is the use of sulindac plus DFMO to prevent colon cancer (Gerner et al. A comprehensive strategy to combat colon cancer targeting the adenomatous polyposis coli tumor suppressor gene. *Ann N Y Acad Sci* 2005;1059:97-105; Gerner EW, Meyskens FL, Jr.
25 Polyamines and cancer: old molecules, new understanding. *Nat Rev Cancer* 2004;4(10):781-92; Gerner EW, Meyskens FL, Jr., Goldschmid S, Lance P, Pelot D. Rationale for, and design of, a clinical trial targeting polyamine metabolism for colon cancer chemoprevention. *Amino Acids* 2007;33(2):189-95.). The rationale for this combination is simple yet quite powerful: DFMO inhibits the enzyme ornithine
30 decarboxylase, which catalyzes the rate-limiting step in polyamine synthesis, while sulindac stimulates polyamine acetylation and export from the cell by acting on the enzyme spermidine/spermine N^1 -acetyltransferase (SSAT). The end result is reduced polyamine levels leading to suppressed growth of cancer cells. A recently published large clinical trial demonstrated that DFMO plus sulindac reduced the
35 recurrence of all adenomas by 69% and of advanced adenomas by 92% (Meyskens

- 5 FL, McLaren CE, Pelot D, et al. Difluoromethylornithine Plus Sulindac for the Prevention of Sporadic Colorectal Adenomas: A Randomized Placebo-Controlled, Double-Blind Trial. *Cancer Prevention Research* Published Online First on April 14, 2008 as 10.1158/1940-6207.CAPR-08-0042.).

[00147] To evaluate the potential synergy between phospho-sulindac I and DFMO we used HT-29 and SW480 human colon cancer cells and employed the methodologies described above. As shown in Figure 2A, in both HT-29 and SW480 cells DFMO 5 mM and phospho-sulindac I 40 μ M each alone inhibited modestly cell growth at 48 h, but their combination was more effective than the sum of the two: a) in HT-29 cells the reductions in cell number were: DFMO 14%, P-S 41%, both 84%; and b) in SW480 cells: DFMO 8%, P-S 45%, both 75%. Examined by isobologram (Tallarida RJ, Porreca F, Cowan A. Statistical analysis of drug-drug and site-site interactions with isobolograms. *Life Sci* 1989;45(11):947-61), the combined effects of DFMO and P-S on cell growth represent pharmacological synergy.

[00148] We then examined the effect of the synergy between the two compounds on cell kinetic parameters. Cell cycle analysis showed that the combination of DFMO and phospho-sulindac I enhances the magnitude of the effect (S phase: 13.6% for either alone is reduced to 2.9% for both). In addition, the combination of DFMO and phospho-sulindac I presented a G1 population arrest of 88% compared to the 75% of either DFMO or phospho-sulindac I alone (Fig. 2B). Furthermore, we examined whether DFMO increased phospho-sulindac I-induced apoptosis in colon cancer cells. After 48 h of incubation with DFMO and phospho-sulindac I, the percentage of apoptotic cells was 38.4%, compared to 8.7% and 16.5% for DFMO and phospho-sulindac I alone, respectively (Fig. 2C). Of note, the concentrations of both compounds were below their IC_{50} s for cell growth.

[00149] Further evidence of the synergy between the two compounds was provided by their effect on polyamine levels. Conventional sulindac is known to reduce polyamine levels in colon cancer cells (Yerushalmi et al. Role of polyamines in arginine-dependent colon carcinogenesis in Apc(Min) (I+) mice. *Mol Carcinog* 2006;45(10):764-73.; Choi et al. Combination of 5-fluorouracil and N1,N11-

- 5 diethylnorspermine markedly activates spermidine/spermine N1-acetyltransferase expression, depletes polyamines, and synergistically induces apoptosis in colon carcinoma cells. J Biol Chem 2005;280(5):3295-304; and Basuroy and Gerner. Emerging concepts in targeting the polyamine metabolic pathway in epithelial cancer chemoprevention and chemotherapy. J Biochem (Tokyo) 2006;139(1):27-33.
- 10 Phospho-sulindac I markedly diminished the levels of spermidine (34% of control values) and spermine (9% of control values) in SW480 cells, without significantly affecting those of putrescine (91% of control values), the first polyamine in their biosynthetic pathway (ornithine → putrescine → spermidine → spermine).

[00150] The effect of phospho-sulindac I on polyamines is mediated, at least in part, by activation of SSAT by phospho-sulindac I. It is known that conventional sulindac induces SSAT activity (Babbar et al., Cyclooxygenase-independent induction of apoptosis by sulindac sulfone is mediated by polyamines in colon cancer. J Biol Chem 2003;278(48):47762-75.). Incubation of HT-29 and SW480 cells with 85 μ M phospho-sulindac I for 24h leads to a 3- and 4.4-fold increase of SSAT activity (P<0.05 versus control), respectively. On the other hand, incubation with sulindac led to a 1.5 and 2.5-fold increase of SSAT activity (P<0.05 versus control) in HT-29 and SW480 cells, respectively. Examination of the time dependence of the induction of SSAT in SW480 cells exposed to 85 μ M phospho-sulindac I revealed after 12 h of incubation, a 4-fold induction of SSAT compared to vehicle-treated controls, which continued to a maximum of 5.5-fold increase after 18h of incubation (see Fig. 4).

5 **[00151] The anti-inflammatory effect of phospho-sulindac I; Inhibition of NF- κ B activation**

[00152] The anti-inflammatory effect of phospho-sulindac I was examined by evaluating its effect on the activation of nuclear factor-kappa B (NF- κ B), a protein complex that is a transcription factor. NF- κ B is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens (Gilmore TD (1999). "The Rel/NF-kappaB signal transduction pathway: introduction". *Oncogene* **18** (49): 6842-4). NF- κ B plays a key role in regulating the immune response to infection. Consistent with this role, incorrect regulation of NF- κ B has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. NF- κ B has also been implicated in processes of synaptic plasticity and memory (Albensi BC, Mattson MP (2000). "Evidence for the involvement of TNF and NF-kappaB in hippocampal synaptic plasticity". *Synapse* **35** (2): 151-9). In general, NF- κ B represents a major molecular control of inflammation. Of additional interest is the modulating effect of NF- κ B on cell growth and inflammation, especially in the context of cancer (Zhang Z, Rigas B. NF-kappaB, inflammation and pancreatic carcinogenesis: NF-kappaB as a chemoprevention target (review). *Int J Oncol* 2006;29(1):185-92; Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 2005;5(10):749-59).

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[00153] We investigated whether phospho-sulindac I affects the activation of NF- κ B in HT-29 human colon cancer cells, using electrophoretic mobility shift assays. As also demonstrated in Fig. 3, we found that treatment of HT-29 cells with phospho-sulindac I suppressed the constitutively active NF- κ B in a concentration-dependent manner. Furthermore, NF- κ B activation was rapidly induced by exposure to tumor necrosis factor alpha (TNF α) in HT-29 cells; however, 4 h pre-incubation with 80 μ M phospho-sulindac I abrogated this effect).

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5 **[00154] The safety of phospho-sulindac I**

[00155] We assessed the safety of phospho-sulindac I by examining its genotoxicity by the Ames test and its gastrointestinal and other toxicity by performing a toxicological study in mice.

10 [00156] Phospho-sulindac I lacked genotoxicity, as demonstrated by a mutagenicity assay performed by the BioReliance Laboratory (Rockville MD) that conducts such assays under high-quality standardized Good Laboratory Practices conditions. The mutagenic potential of phospho-sulindac I was evaluated by measuring its ability to induce reverse mutations at selected loci of two strains of *Salmonella typhimurium* in the presence and absence of S9 activation. All these studies were negative for
15 genotoxicity.

[00157] Phospho-sulindac I lacked gastrointestinal toxicity in mice; gastrointestinal toxicity is the major side effect of sulindac as well as of the entire class of NSAIDs. We evaluated in mice the potential toxicity of phospho-sulindac I. Three groups, each consisting of 8 female C57BL/6J+/+ mice 6 wks of age, were treated for 5 days by
20 oral gavage with equimolar amounts of phospho-sulindac I (317 mg/kg/d) or conventional sulindac (200 mg/kg/day) or vehicle. Mice were weighed at time 0 and on days 3 and 5. Mice surviving to the end of the study were euthanized and necropsied.

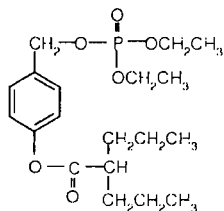
[00158] Phospho-sulindac I- and vehicle-treated mice a) maintained their weight
25 (phospho-sulindac I = $16.3 \pm 1.2 \rightarrow 15.7 \pm 1.2$; vehicle = $16.1 \pm 1.0 \rightarrow 15.7 \pm 1.2$, *mean*±*SD*); b) showed no evidence of gastrointestinal or other toxicity; c) all were alive at the conclusion of the study and appeared healthy; and d) inspection of the heart, lungs, spleen, kidneys and liver showed no abnormalities. In contrast, sulindac-treated mice a) lost 20% of their weight ($16.3 \pm 1.2 \text{ g} \rightarrow 13.0 \pm 0.5 \text{ g}$; *mean*±*SD*); b) showed significant mortality: 75% vs. 0% for phospho-sulindac I and
30 vehicle (5 of the 8 mice died: 1 on day 2; 2 on day 3; 2 on day 4; and 1 on day 5), and c) necropsies revealed upper gastrointestinal toxicity with macroscopically evident gastric ulcers in 3, gastric bleeding in 1, and perforation in 1. The stomachs of

- 5 sulindac-treated animals were larger than those of the other two groups and in some the liver appeared hyperemic.

[00159] Example 6: Phosphovalproic acid inhibits the growth of various human cancer cell lines more potently than conventional valproic acid

- [00160] Valproic acid (VPA) is a in clinical use primarily as an anticonvulsant and mood-stabilizing drug, is now being extensively studied as a potent anticancer agent, especially since it was found to inhibit histone deacetylation (Abend NS, Dlugos DJ. Treatment of refractory status epilepticus: literature review and a proposed protocol. *Pediatr Neurol.* 2008 Jun;38(6):377-90; Oki Y, Issa JP. Review: recent clinical trials in epigenetic therapy. *Rev Recent Clin Trials.* 2006 May;1(2):169-82; Barzman DH, Findling RL. Pharmacological treatment of pathologic aggression in children. *Int Rev Psychiatry.* 2008 Apr;20(2):151-7). VPA has shown potent antitumor effects in several in vitro and in vivo systems, and encouraging results have been reported from early clinical trials (Duenas-Gonzalez A, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. *Cancer Treat Rev.* 2008 May;34(3):206-22.).

- [00161] We synthesized phosphovalproic acid (phosph-VPA), a derivative of VPA, following the methodology described for phospho-sulindac I above and determined its effect on cell growth by determining its 24-h IC_{50} also according to the methods described above. Conventional valproic acid was also studied for comparison purposes. The structure of phosphovalproic acid is:



- 30 [00162] The results summarized in Table 3, demonstrate that phosphovalproic acid a) is very potent in inhibiting the growth of several human cancer cell lines, and b)

5 shows enhanced potency in inhibiting cell growth compared to conventional VPA, with the potency enhancement ranging between 35 and 245-fold.

Table 3. Phospho-valproic acid inhibits the growth of human cancer cells (IC₅₀, μM)

<i>Cell line</i>	<i>VPA</i>	<i>Phospho-VPA</i>	<i>Fold Enhancement</i>
Breast			
MCF-7	1,775	51	35
MDA-MB231	4,049	30	136
Colon			
HT-29	3,210	13	245
SW480	3,639	59	62
Pancreas			
BxPC-3	1,680	36	47
MIA PaCa2	3,082	89	35

These values are representative of two experiments, each performed in quintuplicate; results were within 10%.

[00163] The foregoing description and examples have been set forth merely to
 25 illustrate the invention and are not intended to be limiting. Since modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art, the invention should be construed broadly to include all variations falling within the scope of the appended claims and equivalents thereof. Furthermore, the teachings and disclosures of all references cited herein
 30 are expressly incorporated in their entireties by reference.