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(19) **United States**(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0049202 A1****Kastelic et al.**(43) **Pub. Date: Mar. 3, 2005**(54) **BENZOIC ACID DERIVATIVES AND USES THEREOF**(76) Inventors: **Tania Kastelic**, Coquitlam (CA);
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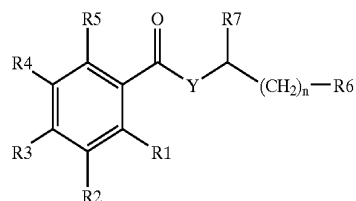
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A61K 31/445; A61K 31/4015(52) **U.S. Cl.** **514/19**; 514/532; 562/450;
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514/473; 546/216; 548/543;
549/321(57) **ABSTRACT**

The present invention provides benzoic acid derivatives and uses thereof. In accordance with one aspect there is provided a compound of formula (I); stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof, or

physiologically acceptable and hydrolysable esters thereof; wherein: R¹ is H, OH, halogen, (C1-C4) alkyl, (C1-C4) alkenyl, (C1-C4) alkoxy, or (C1-C4)alkyl-COO—; R² is H, OH, halogen, (C1-C4) alkyl, (C1-C4) alkenyl, (C1-C4)alkoxy, or (C1-C4) alkyl-COO—; R³ is H, OH, halogen, (C1-C4) alkyl, (C1-C4) alkenyl, (C1-C4)alkoxy, or (C1-C4) alkyl-COO—; R⁴ is H, OH, halogen, (C1-C4) alkyl, (C1-C4) alkenyl, (C1-C4)alkoxy, or (C1-C4) alkyl-COO—; R⁵ is H, OH, halogen, (C1-C4) alkyl, (C1-C4) alkenyl, (C1-C4)alkoxy, or (C1-C4) alkyl-COO—; Y is O or —NR', wherein R' is H or (C1-C4) alkyl; n is 0-8; R⁶ is a 5 to 8 membered lactone or lactam ring; R⁷ is H, (C1-C4) alkyl, (C1-C4) alkenyl, (C1-C4)alkoxy, phenyl or (C1-C4) alkyl-COO—, wherein the lactam or lactone ring optionally comprises one or more C=C double bonds and the lactam or lactone ring is optionally substituted with one or more (C1-C4)alkyl or (C1-C4) alkenyl groups. The compounds of the present invention are useful for the treatment or prevention of disorders with an etiology associated with or comprising excessive cytokine release. The compounds are also used in the treatment of cancer, inflammatory disorders and disorders associated with an increased stability of mRNA which has an mRNA instability sequence. The present invention further provides processes for preparing benzoic acid derivatives of Formula I.



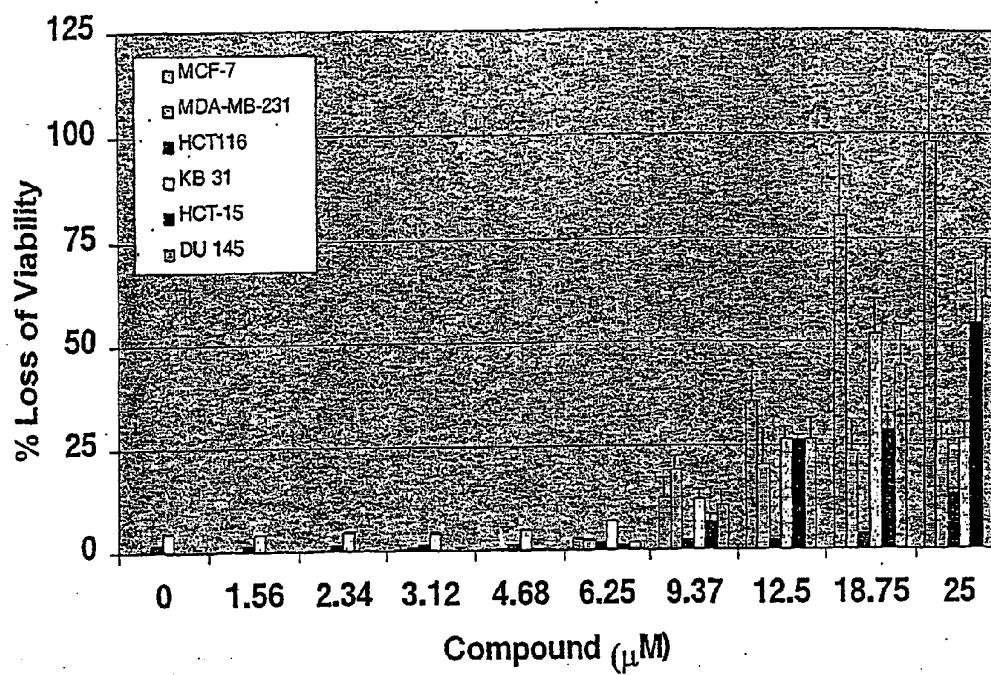


Figure 1

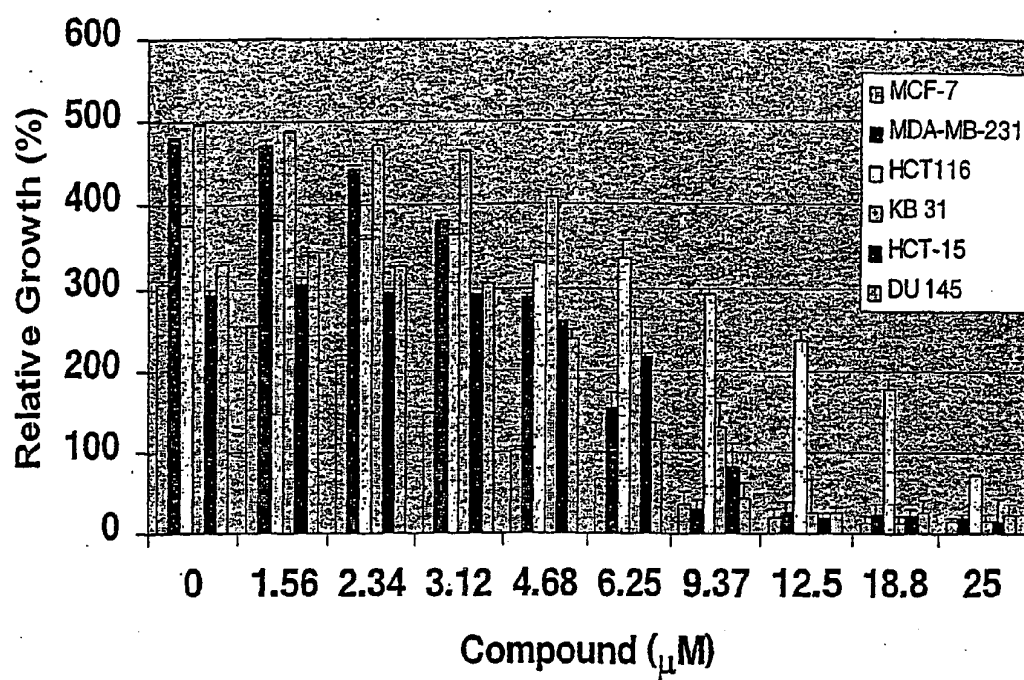


Figure 2

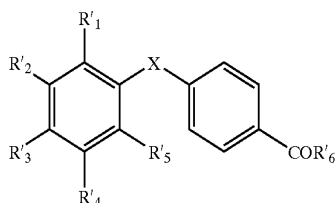
BENZOIC ACID DERIVATIVES AND USES THEREOF

FIELD OF THE INVENTION

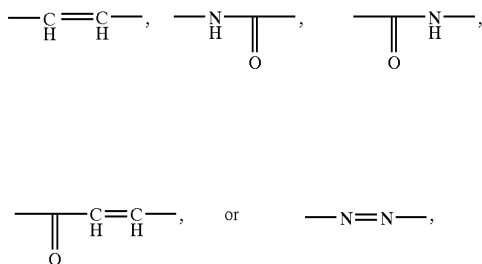
[0001] This invention relates to biologically active benzoic acid derivatives and to their use in the treatment and prophylaxis of disease. In particular the invention relates to a class of compounds which affect the stability of mRNA which contain one or more mRNA instability sequences.

BACKGROUND

[0002] Various benzoic acids have been found to manifest activities of therapeutic value. For example, in Japan Kokai 61-22046, 61-22047 and 61-76440, it was shown that benzoic acid derivatives represented by the following general formula:



[0003] wherein R'1, R'2, R'3, R'4 and R'5 are the same or different, and each represents hydrogen or middle or lower-alkyl, with the proviso that all can not be hydrogen simultaneously, and wherein two neighboring substituents may be combined with each other to form a cyclo-alkyl ring having 5 to 6 carbon atoms, R'6 represents hydroxyl, lower-alkoxy, or lower-alkylamino of the formula $\text{—NR}'7\text{R}'8$, wherein R'7 and R'8 each represents hydrogen or lower-alkyl, and X' represents a group of the formula:



[0004] are capable of inducing the differentiation of malignant cells, especially leukemia cells, to morphologically and functionally mature cells which cannot proliferate further, and are accordingly pharmacologically valuable and useful for treatment of malignant proliferous or immune diseases such as cancer, rheumatism, or psoriasis, and in Japan Kokai 62-215581, there are also shown related compounds.

[0005] The literature also shows the activity and measurement of the activity of those compounds by the differentiation of human acute promyelocytic leukemia cells (HL-60).

[0006] U.S. Pat. No. Re. 36,477 also discloses benzoic acid derivatives which act as differentiation-inducing agents

for neoplastic cells, especially leukemia cells, or as a therapeutic agent for psoriasis or immune and inflammatory diseases

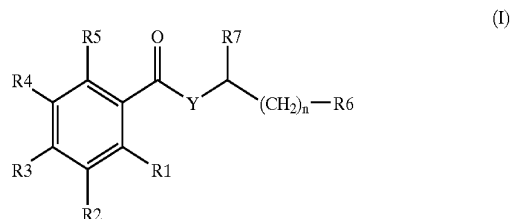
[0007] U.S. Pat. No. 5,849,796 discloses ortho-substituted benzoic acid derivatives that exhibit antiarrhythmic properties and act as inhibitors of the cellular Na^+/H^+ antiporter. In addition, these compounds were shown to be useful as intermediates for the preparation of medicaments, in particular of inhibitors of the cellular Na^+/H^+ antiporter.

[0008] This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present invention. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the present invention. Publications referred to throughout the specification are hereby incorporated by reference in their entireties in this application.

SUMMARY OF THE INVENTION

[0009] An object of the present invention is to provide benzoic acid derivatives and uses thereof.

[0010] In accordance with one aspect of the present invention, there is provided a compound of formula (I):



[0011] stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof, or physiologically acceptable and hydrolysable esters thereof; wherein:

[0012] R1 is H, OH, halogen, $(\text{C}_1\text{—C}_4)$ alkyl, $(\text{C}_1\text{—C}_4)$ alkenyl, $(\text{C}_1\text{—C}_4)$ alkoxy, or $(\text{C}_1\text{—C}_4)$ alkyl-COO—;

[0013] R2 is H, OH, halogen, $(\text{C}_1\text{—C}_4)$ alkyl, $(\text{C}_1\text{—C}_4)$ alkenyl, $(\text{C}_1\text{—C}_4)$ alkoxy, or $(\text{C}_1\text{—C}_4)$ alkyl-COO—;

[0014] R3 is H, OH, halogen, $(\text{C}_1\text{—C}_4)$ alkyl, $(\text{C}_1\text{—C}_4)$ alkenyl, $(\text{C}_1\text{—C}_4)$ alkoxy, or $(\text{C}_1\text{—C}_4)$ alkyl-COO—;

[0015] R4 is H, OH, halogen, $(\text{C}_1\text{—C}_4)$ alkyl, $(\text{C}_1\text{—C}_4)$ alkenyl, $(\text{C}_1\text{—C}_4)$ alkoxy, or $(\text{C}_1\text{—C}_4)$ alkyl-COO—;

[0016] R5 is H, OH, halogen, $(\text{C}_1\text{—C}_4)$ alkyl, $(\text{C}_1\text{—C}_4)$ alkenyl, $(\text{C}_1\text{—C}_4)$ alkoxy, or $(\text{C}_1\text{—C}_4)$ alkyl-COO—;

[0017] Y is O or $\text{—NR}'$, wherein R' is H or $(\text{C}_1\text{—C}_4)$ alkyl;

[0018] n is 0-8;

[0019] R6 is a 5 to 8 membered lactone or lactam ring;

[0020] R7 is H, $(\text{C}_1\text{—C}_4)$ alkyl, $(\text{C}_1\text{—C}_4)$ alkenyl, $(\text{C}_1\text{—C}_4)$ alkoxy, phenyl or $(\text{C}_1\text{—C}_4)$ alkyl-COO—;

[0021] wherein the lactam or lactone ring optionally comprises one or more $\text{C}=\text{C}$ double bonds and the

lactam or lactone ring is optionally substituted with one or more (C₁-C₄)alkyl or (C₁-C₄) alkenyl groups.

[0022] The compounds of the present invention are useful for the treatment or prevention of disorders with an etiology associated with or comprising excessive cytokine release, particularly IL-1 β release, such as rheumatoid arthritis, osteoarthritis, septic shock, psoriasis, atherosclerosis, inflammatory bowel disease, Crohn's disease and asthma. The disclosure of EP 0606044 A is incorporated by reference in the teaching of the present application.

[0023] The present invention also provides compounds for use in the preparation of a medicament for treatment of a cancer and/or malignant disease.

[0024] Also the invention provides a method for the prophylaxis or treatment of a cancer and/or malignant disease comprising administering to a patient an effective amount of a benzoic acid derivative.

BRIEF DESCRIPTION OF THE FIGURES

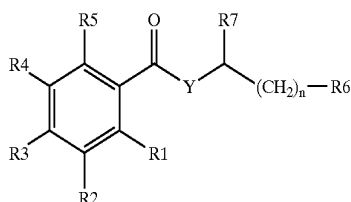
[0025] FIG. 1 graphically depicts the percentage loss of viability of cancer cells following treatment with various concentrations of a benzoic acid derivative, in comparison to viability of control cells.

[0026] FIG. 2 graphically depicts the relative growth of cancer cells following treatment with various concentrations of a benzoic acid derivative, in comparison to growth of control cells.

DETAILED DESCRIPTION OF THE INVENTION

[0027] The terms and abbreviations used in the instant examples have their normal meanings unless otherwise designated. For example "° C." refers to degrees Celsius; "N" refers to normal or normality; "mmol" refers to millimole or millimoles; "g" refers to gram or grams; "ml" means milliliter or milliliters; "M" refers to molar or molarity; "p-" refers to para, "MS" refers to mass spectrometry; "R" refers to infrared spectroscopy; and "NMR" refers to nuclear magnetic resonance spectroscopy.

[0028] The present invention provides a compound of the formula I:



(I)

[0029] stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof or physiologically acceptable and hydrolysable esters thereof, wherein:

[0030] R₁ is H, OH, halogen, (C₁-C₄) allyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

[0031] R₂ is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

[0032] R₃ is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

[0033] R₄ is H, OH, halogen, (C₁-C₄) allyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

[0034] R₅ is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

[0035] Y is O or —NR', wherein R' is H or (C₁-C₄) alkyl;

[0036] n is 0-8;

[0037] R₆ is a 5 to 8 membered lactone or lactam ring;

[0038] R₇ is H, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, phenyl or (C₁-C₄) alkyl-COO—;

[0039] wherein the lactam or lactone ring optionally comprises one or more C=C double bonds and the lactam or lactone ring is optionally substituted with one or more (C₁-C₄)alkyl or (C₁-C₄) alkenyl groups.

[0040] Halogen or halo as used herein refers to F, Cl, Br or I unless otherwise indicated, preferably Cl.

[0041] In one embodiment of the present invention a compound of formula (I) is provided in free form or in the form of a physiologically-hydrolysable and -acceptable ester, wherein:

[0042] R₁=H, OH, MeCOO— or Methoxy;

[0043] R₂=H;

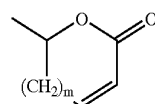
[0044] R₃=H, OH, MeCOO— or Methoxy;

[0045] R₄=H, Methoxy or halogen;

[0046] R₅=H, OH, Methoxy or (C₁-C₄) alkyl;

[0047] Y is O;

[0048] R₆ is



[0049] wherein, m is 0-4;

[0050] R₇=H, Methyl, Isopropyl.

[0051] In another embodiment of the present invention a compound of formula (I) is provided in free form or in the form of a physiologically-hydrolysable and -acceptable ester, wherein:

[0052] R₁ is H, —OH, MeO— or MeCOO—;

[0053] R₂ is H; R₄ is H or halogen;

[0054] R₃ is H, —OH, MeO— or MeCOO—;

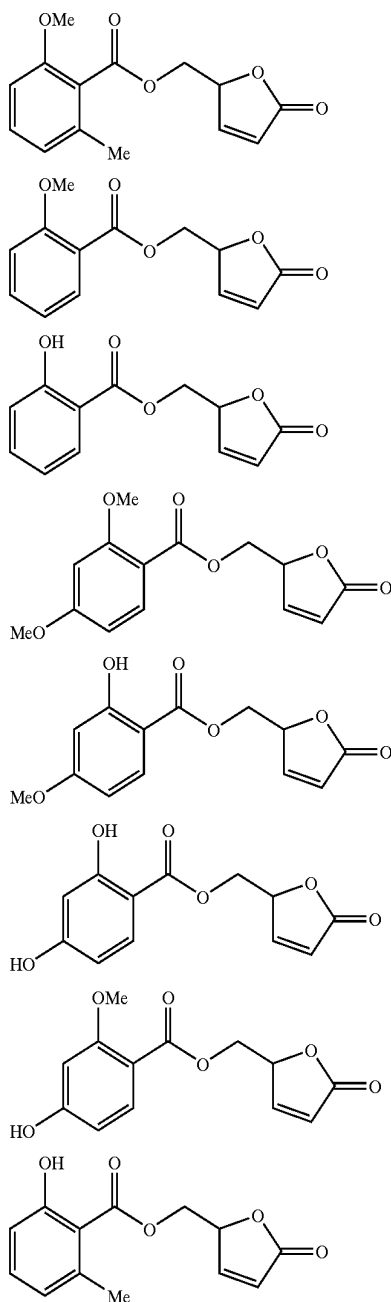
[0055] R₄ is H or halogen;

[0056] R₅ is —OH, MeO—, MeCOO— or Methyl.

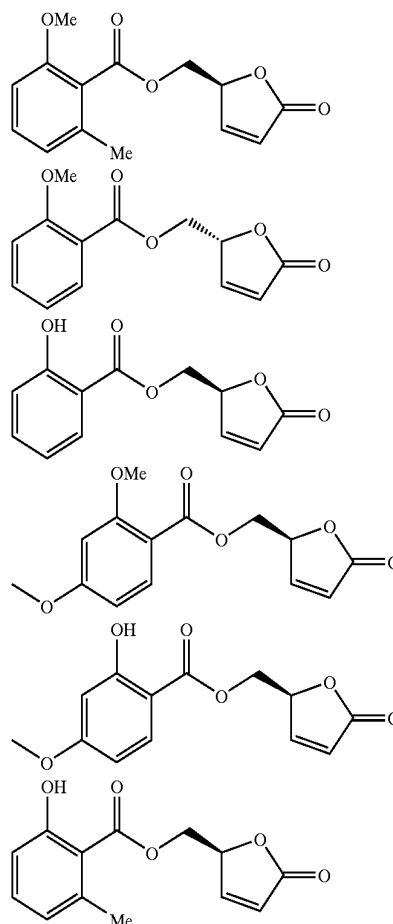
[0057] In another embodiment of the present invention a compound of formula (I) is provided in free form or in the form of a physiologically-hydrolysable and -acceptable ester wherein:

[0058] R₁ is H or MeO, R₃ is H, —OH or MeO—; R₄ is H and R₅ is Methoxy or Methyl.

[0059] Compounds of the present invention include, but are not limited to the following exemplary molecules:



[0060] In another embodiment of the present invention Compounds of the present invention include, but are not limited to the following exemplary molecules:



[0061] As noted supra, this invention includes the pharmaceutically acceptable salts of the compounds defined by Formula I. A compound of this invention can possess a sufficiently acidic, a sufficiently basic, or both functional groups, and accordingly react with any of a number of organic and inorganic bases, and inorganic and organic acids, to form a pharmaceutically acceptable salt.

[0062] This invention further encompasses the pharmaceutically acceptable solvates of the compounds of Formula I. Many of the Formula I compounds can combine with solvents such as water, methanol, ethanol and acetonitrile to form pharmaceutically acceptable solvates such as the corresponding hydrate, methanolate, ethanolate and acetonitrilate.

[0063] The compounds of the present invention may have asymmetric (chiral) centers. As a consequence of these chiral centers, the compounds of the present invention occur as racemates, mixtures of enantiomers and as individual enantiomers, as well as diastereomers and mixtures of diastereomers. All asymmetric forms, individual isomers (which may be prepared via stereospecific synthesis or by separation of a synthesized mixture using conventional methods) and combinations thereof, are within the scope of the present invention.

[0064] The prefixes "R" and "S" are used herein as commonly used in organic chemistry to denote the absolute

configuration of a chiral center, according to the Cahn-Ingold-Prelog system. The stereochemical descriptor R (rectus) refers to that configuration of a chiral center with a clockwise relationship of groups tracing the path from highest to second-lowest priorities when viewed from the side opposite to that of the lowest priority group. The stereochemical descriptor S (sinister) refers to that configuration of a chiral center with a counterclockwise relationship of groups tracing the path from highest to second-lowest priority when viewed from the side opposite to the lowest priority group. The priority of groups is decided using sequence rules as described by Cahn et al., *Angew. Chem.*, 78, 413-447, 1966 and Prelog, V. and Helmchen, G.; *Angew. Chem. Int. Ed. Eng.*, 21, 567-583, 1982).

[0065] In addition to the R,S system used to designate the absolute configuration of a chiral center, the older D-L system is also used in this document to denote relative configuration, especially with reference to amino acids and amino acid derivatives. In this system a Fischer projection of the compound is oriented so that carbon-1 of the parent chain is at the top. The prefix "D" is used to represent the relative configuration of the isomer in which the functional (determining) group is on the right side of the carbon atom at the chiral center and "L", that of the isomer in which it is on the left.

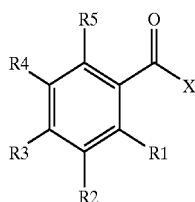
[0066] The compounds of the present invention have carbon-carbon double bonds and can, therefore form cis/trans isomers. The present invention envisages both mixtures of the isomers and the individual isolated/purified isomers.

[0067] The benzoic acid derivatives for use in the invention which comprise —OH substituents may also exist in the form of pharmaceutically acceptable, metabolically labile ester derivatives, and the use of such is included within the scope of the invention. Pharmaceutically acceptable esters are preferably prodrug ester derivatives, such being convertible by solvolysis or under physiological conditions to the free derivatives. Preferred pharmaceutically acceptable prodrug esters are those derived from a carboxylic acid, a carbonic acid monoester or a carbamic acid.

[0068] Preparation of Compounds of Formula (1)

[0069] According to another aspect, the present invention provides a process for the preparation of a compound of Formula I, or a pharmaceutically acceptable metabolically-labile ester or amide thereof, or a pharmaceutically acceptable salt thereof, which comprises:

[0070] a) reacting a compound of formula (I):



(II)

[0071] wherein:

[0072] R1 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

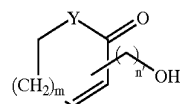
[0073] R2 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

[0074] R3 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

[0075] R4 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—; and

[0076] X is halogen;

[0077] with a compound of formula (III):



(III)

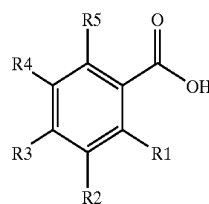
[0078] wherein:

[0079] n' is 1-9; and

[0080] m is 0-4;

[0081] compound (III) optionally contains one or more C=C double bonds and is substituted with one or more (C₁-C₄) alkyl or (C₁-C₄) alkenyl group; in the presence or absence of a base and an appropriate solvent. Non limiting examples of such solvents are dichloromethane and chloroform.

[0082] Compound of formula (II) can be prepared by reacting compound of formula (IV):



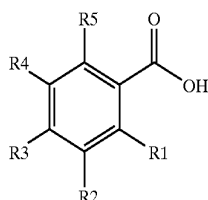
(IV)

[0083] wherein: R1-R5 is as defined above;

[0084] with acid halide of an organic or inorganic acid in the presence of an appropriate solvent known to a worker skilled in the relevant art. Non limiting examples of such acid halides are thionyl chloride, phosphorus trichloride, phosphorus tribromide, phosphorus pentachloride and oxalyl chloride and non limiting examples such solvents are DMF and TBF; or

[0085] b) reacting compound of formula (IV) with compound of formula (II) in the presence of an acid, such as hydrochloric acid, sulfuric acid or aryl sulfonic acid. This reaction can be carried out under Azeotropic distillation conditions using benzene as a solvent; or

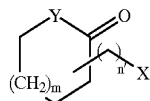
[0086] c) reacting an alkali or alkaline earth metal salt of compound of formula (IV):



(IV)

[0087] wherein R1-R5 are as defined above;

[0088] with a compound of formula (V):



(V)

[0089] wherein;

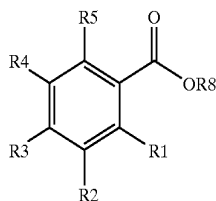
[0090] X is F, Cl, Br or I;

[0091] n' is 1-9; and

[0092] m is 0-4; and compound of formula (V) may optionally contain one or more double bonds and is optionally substituted with one or more (C₁-C₄) alkyl or (C₁-C₄) alkenyl group;

[0093] in the presence of polar aprotic solvents or with crown ether catalysts. Non limiting examples of such polar aprotic solvents are acetone, DMF and DMSO.

[0094] Compounds of formula (IV) are either commercially available or may be prepared using standard procedures known to a person skilled in the art. For example: by hydrolyzing a compound of formula (VI):



(VI)

[0095] wherein:

[0096] R1-R5 is as defined above;

[0097] R8 is (C₁-C₄) alkyl, aryl or arylalkyl.

[0098] The compounds of Formula (VI) are conveniently hydrolyzed in the presence of an acid, such as hydrochloric acid or sulfuric acid, or a base, such as an alkali metal hydroxide, for example sodium hydroxide. The hydrolysis conveniently performed in an aqueous solvent such as water and at a temperature in the range from 50 to 200° C.

Compounds of formula (VI) are either commercially available or may be prepared using standard procedures known to a person skilled in the art.

[0099] Compounds of formula (III) or (V) are either commercially available or may be prepared using standard procedures known to a person skilled in the art.

[0100] Activity of the Benzoic Acid Derivatives

[0101] The benzoic acid derivatives of the present invention exhibit various biochemical properties including one or more of the following: the ability to inhibit the growth of cancer cells, the ability to increase or restore sensitivity of a cancer cell to one or more anti-neoplastic/cytotoxic drugs; and the ability to induce mRNA degradation. Compounds of the present invention can be assayed to demonstrate the extent of their activities using standard techniques well known to workers skilled in the art. Exemplary testing methods are outlined herein and are not intended to limit the scope of the present invention.

[0102] Anti-Cancer Activity

[0103] As demonstrated herein certain benzoic acid derivatives according to the present invention can inhibit the growth of cancer cells. Compounds of the present invention may be tested for their activity as anti-cancer agents in cell or in vivo assays substantially as described herein or in variants of such assays using appropriate cell lines and conditions as would be readily appreciated by a worker skilled in the art.

[0104] Compounds of this invention may be assayed for anti-cancer activity by conventional methods, including for example, the methods described below.

[0105] 1. In Vitro Methodology

[0106] Cytotoxicity may be measured using a standard methodology for adherent cell lines such as the microculture tetrazolium assay (MTT). Details of this assay have been published (Alley, M C et al, Cancer Research 48:589-601, 1988). Exponentially growing cultures of tumor cells such as the HT-29 colon carcinoma or LX-1 lung tumor are used to make microtiter plate cultures. Cells are seeded at 3000 cells per well in 96-well plates (in 150 μ l or media), and grown overnight at 37° C. Test compounds are added, in 10-fold dilutions varying from 10⁻⁴M to 10⁻¹⁰M. Cells are then incubated for 72 hours. To determine the number of viable cells in each well, the MTT dye is added (50 μ l or 3 mg/ml solution of 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide in saline). This mixture is incubated at 37° C. for 5 hours, and then 50 μ l of 25% SDS, pH2 is added to each well. After an overnight incubation, the absorbance of each well at 550 nm is read using an ELISA reader. The values for the mean \pm SD of data from replicated wells are calculated, using the formula % T/C (% viable cells treated/control). 1 OD of treated cells OD of control cells \times 100 = % T/C. The concentration of test compound which gives a T/C of 50% growth inhibition is designated as the IC₅₀ value.

[0107] An alternative method of evaluating anti-cancer activity of the compounds of the present invention involves determining the effect of a test compound on the viability of cancer cells. Exponentially growing cancer cells are treated with various concentrations of the test compound. Examples of cell lines that can be used in this method are MCF-7,

MDA-MB-231, HCT116, KB 31, HCT-15, and DU 145 which are readily obtainable from ATCC, Bethesda, Md., U.S.A. The cells are further incubated for 24 h, 48 h and 96 h and stained with a molecule that allows a distinction between viable and non-viable cells, for example YOPRO-1 as described in T. Idziorek et al., *J Immunol Meth* 185, 249 (1995). The percent viability is then compared to control cells. A decrease in viability (shown as an increase in % loss of viability in FIG. 1) in the treated cells in comparison to the control cells is indicative of anti-cancer activity.

[0108] Another method of evaluating anti-cancer activity of the compounds of the present invention involves monitoring the effect of the compounds on cell cycle progression. Exponentially growing cancer cells are treated with various concentrations of the test compound. The cells were further incubated for 24 h, 48 h and 96 h. Cells are then lysed and dsDNA is stained with YOPRO-1 as described in T. Idziorek et al., *J Immunol Meth* 185, 249 (1995). Fluorescence measurements are carried out essentially as described in V. L. Singer et al., *Anal Biochem* 249, 228 (1997) and values compared to control cells. A decrease in relative growth of treated cells in comparison to untreated cells is indicative of anti-cancer activity (see FIG. 2).

[0109] In an exemplary method of evaluating the inhibitory effect of the compounds of the present invention on the growth of cancer cells, cells are treated with the test compound and gene expression levels are measured in comparison to untreated cells. For example, human breast cancer cells, MCF-7, are cultured at 5% CO₂ and 37° C. in MEM containing 0.1 mM non-essential amino acids, 2 mM L-glutamine and 10% FBS. Total RNA from cells approximately 50% confluent is isolated using a Qiagen RNeasy Midi™ kit according to manufacturer's instructions. Total RNA is harvested at various times in the presence and absence of the benzoic acid derivative being evaluated. RNase protection analysis is performed using the Ribo-Quant™ Multi-Probe Ribonuclease Protection Assay System from Pharmingen utilizing hAPO-2c and custom human (bclx L/S, p53, GADD45, c-fos, bax, bcl-2, c-myc, L32 and GAPDH) templates, 17 RNA polymerase and [α -³²P]UP (as per manufacturer's instructions). Quantitation of the expression levels is performed using a Storm 860™ Molecular Dynamics Phosphorimager with ImageQuant™ software. Decreased expression of a proto-oncogene, for example bcl-2, in treated cells in comparison to untreated cells is indicative of anti-cancer activity.

[0110] 2. In Vivo Methodology

[0111] Compounds of this invention may be further tested in pre-clinical assay for in vivo activity which is indicative of clinical utility. Such assays are conducted with nude mice into which tumor tissue, preferably of human origin, has been transplanted (xenografted), as is well known in this field. Test compounds are evaluated for their anti-tumor efficacy following administration to the xenograft-bearing mice.

[0112] For example, human breast tumors (MX-1) which have been grown in athymic nude mice are transplanted into new recipient mice, using tumor fragments which are about 50 mg in size. The day of transplantation is designated as day 0. Six to ten days later, mice are treated with the test compounds given as an intravenous injection or orally, in groups of 5-10 mice at each dose. Compounds are given every other day, for 3 weeks.

[0113] Tumor diameters and body weights are measured twice weekly. Tumor volumes were calculated using the diameters measured with Vernier calipers, and the formula

$$(\text{Length} \times \text{width}^2) \div 2 = \text{mm}^3 \text{ of tumor volume}$$

[0114] Mean tumor volumes are calculated for each treatment group, and T/C values determined for each group relative to the untreated control tumors.

[0115] Increasing or Restoring Drug Sensitivity

[0116] Certain benzoic acid derivatives according to the present invention can increase or restore the sensitivity of a cancer cell to one or more anti-neoplastic/cytotoxic drugs. A suitable cell-based assay for assessing the ability of a candidate compound to restore sensitivity of cancer cells to anti-neoplastic/cytotoxic drug substances in vitro is as follows. Cancer cell lines (CCL), e.g. from human small cell carcinoma of the lung, resistant to one or more cancer therapeutic drug substances (CTDS) selected from the group comprising Daunorubicin (DR); Vincristine (VC); Adriamycin (AM); Etoposide (ET); Tenoposide (TE); Colchicine (CC); and Taxol are developed in accordance with the methods described by Twentyman et al., *Br. J. Cancer*, 54, 253 (1986).

[0117] Sensitivity of resistant sub-lines (CCL-R) is compared with parental sensitive lines (CCL-S) by assaying inhibition of cell growth during continuous CTDS exposure, e.g. in the case of a DR-resistant line (CCL-DRR) by comparing growth of CCL-DRS and CCL-DRR lines in the presence of DR contained in the growth medium ab initio. For the purpose, cell proliferation is measured by cell counting using an electronic cell counter, counting being effected close to the termination of the exponential growth phase. CCL-R lines are selected for which the IC₈₀ (drug concentration, e.g. DR concentration, required to reduce final cell number to 20% of that for non-CTDS (e.g. DR) treated controls is >80x, preferably >100x, greater than that of the parental CCL-S lines.

[0118] Sensitivity of selected CCL-R lines to CTDS (e.g. DR) in the presence or absence of test benzoic acid derivatives is then performed, employing cell counting as a measure of proliferation as described above. For this purpose cells are cultured ab initio in the presence of varying concentrations of both CTDS and test benzoic acid derivatives. For screening, concentrations of the latter are chosen which do not themselves cause a significant reduction in proliferation. Appropriate concentrations are established by culturing CCL-S and CCL-R in the presence of varying concentrations of benzoic acid derivatives in the absence of CTDS. Benzoic acid derivatives are routinely tested at concentrations of from 0.01 to 50 $\mu\text{g/ml}$, in particular 0.1 to 10.0 $\mu\text{g/ml}$, e.g. at concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 50 $\mu\text{g/ml}$. The ratio of CTDS (e.g. DR) required to inhibit cell proliferation by 50% in the absence of test benzoic acid derivatives (IC₅₀-CS) compared with that obtained in the presence of test benzoic acid derivatives (IC₅₀+CS) is taken as a measure of increased sensitivity of the CCL-R line to CTDS which has been induced by the benzoic acid derivatives.

[0119] Stability of the CCL-R line used is ensured by cross checking its sensitivity to CTDS with that previously established.

[0120] Additional procedures for assessing utility in restoring sensitivity of cancer cells to anti-neoplastic/cytotoxic, drug substances, including in vivo procedures are described in EP 0296122 B, the relevant disclosures of which are incorporated by reference in the teaching of the present application.

[0121] Induction of mRNA Degradation

[0122] Recently, it has become increasingly apparent that the regulation of RNA half-life plays a critical role in the tight control of gene expression and that mRNA degradation is a highly controlled process. RNA instability allows for rapid up- or down-regulation of mRNA transcript levels upon changes in transcription rates. A number of critical cellular factors, e.g. transcription factors such as c-myc, or gene products which are involved in the host immune response such as cytokines, are required to be present only transiently to perform their normal functions. Transient stabilization of the mRNAs which code for these factors permits accumulation and translation of these messages to express the desired cellular factors when required; whereas, under non-stabilized, normal conditions the rapid turnover rates of these mRNAs effectively limit and "switch off" expression of the cellular factors. However, abnormal regulation of mRNA stabilization can lead to unwanted build up of cellular factors leading to undesirable cell transformation, e.g. tumor formation, or inappropriate and tissue damaging inflammatory responses.

[0123] Although the mechanisms which control mRNA stability are far from understood, sequence regions have been identified in a number of mRNAs, which appear to confer instability on the mRNAs which contain them. These sequence regions are referred to herein as "mRNA instability sequences". For example, typical mRNA instability sequences are the AREs (AU rich elements), which are found in the 3'UTR (untranslated region) of certain genes including a number of immediate early genes and genes coding for inflammatory cytokines, e.g. IL-1 β and TNF α .

[0124] mRNA instability sequences have been identified in the UTRs, in particular the 3'UTRs, of a large number of transiently expressed genes including genes for cytokines, chemokines, nuclear transcription factors, protooncogenes, immediate early genes, cell cycle controlling genes, oxygenases, and genes involved in and controlling of apoptosis. The natural RNA sequences which comprise the mRNA instability sequences are alternatively referred to as adenylate/uridylylate (AU)-rich elements, or AREs. Transiently expressed genes which contain mRNA instability sequences include, for example, the genes coding for GM-CSF, c-fos, c-myc, c-jun, krox-20, nur-77, zif268, IFN- β , uPA, IL-1, IL-3, TNF- α , MCP1, syn1, β_2 -AR, E-selectin, VCAM-1, ICAM-1, P-glycoproteins (MDR), MRPs, P γ 1 (pf mndr), COX II, metalloproteinases (MMPs), bcl-2 and MIP-2 α .

[0125] The following publications include extensive discussion of mRNA instability sequences and AREs, the sequences motifs which they contain and (minimum) sequence requirements for mRNA destabilization, as well as identifying a number of mRNA instability sequences and the genes which contain them:

[0126] Shaw & Kamen, Cell, Vol. 46, 659-667, Aug. 29, 1986 (GM-CSF);

[0127] Shyu et al., Genes & Development, 5:221-231 (1991) (c-fos);

[0128] Sachs, Cell, Vol. 74, 413-421, Aug. 13, 1993 (Review. "Messenger RNA Degradation in Eukaryotes");

[0129] Chen et al., Mol. Cell. Biol., January 1994, p. 416-426 (c-fos);

[0130] Akashi et al., Blood, Vol. 83, No. 11, (June 1), 1994: pp 3182-3187 (GM-CSF etc.);

[0131] Nanbu et al., Mol. Cell. Biol., July 1994, p. 4920-4920 (Upa);

[0132] Stoecklin et al., J. Biol. Chem., Vol. 269, No. 46, Nov. 18 1994, pp 28591-28597 (IL-3);

[0133] Lagnado et al., Mol. Cell. Biol., December 1994, p. 7984-7995 (general);

[0134] Zhang et al., Mol. Cell. Biol., April 1995, p. 2231-2244 (yeast);

[0135] Zubiaga et al., Mol. Cell. Biol., April 1995, p. 2219-2230 (general);

[0136] Winstall et al., Mol. Cell. Biol., July 1995, p. 3796-3804 (cfos, GM-CSF);

[0137] Chen et al., Mol. Cell. Biol., October 1995, p. 5777-5788 (cfos, GM-CSF);

[0138] Chen et al., TIBS 20 November 1995, 465-470 (review);

[0139] Levy et al., J. Biol. Chem., Vol. 271, No., Feb. 2, 1996, pp. 2746-2753 (VEGF);

[0140] Kastelic et al., Cytokine, Vol. 8, No. 10 (October), 1996: pp751-761;

[0141] Crawford et al., J. Biol. Chem., Vol. 272, No. 34, August 1997, pp. 21120-21127 (TNF- α);

[0142] Xu et al., Mol. Cell. Biol., August 1997, Vol. 18, No. 8, p. 4611-4621 (general);

[0143] Danner et al., J. Biol. Chem., Vol. 273, No. 6, Feb. 6, 1998, pp. 3223-3229 (human β_2 -adrenergic receptor);

[0144] Lewis et al., J. Biol. Chem., Vol. 273, No. 22, May 29, 1998; pp. 13781-13786 (INF- α).

[0145] As described in the above publications, mRNA instability sequences often contain one or more copies of sequence motifs, e.g. selected from: AUUUA, UAUUUUAU, UUAUUUA(U/A) (U/A), and AUUUUUUA. Such sequence motifs are typically in genes between the stop codon and the poly A signal and may associated with appropriate flanking sequences and may interact in combination with other sequences, e.g. present in the 5'UTR and e.g. with instability motifs present in the coding region.

[0146] As demonstrated herein certain of the benzoic acid derivatives of the present invention can act as inducers of degradation of mRNAs which contain mRNA instability sequences. The activity of compounds for use in the invention as inducers of mRNA degradation may be demonstrated using assays known to workers skilled in the art, for example by means of a reporter gene assay as described herein, or as described in mores detail in International patent application number PCT/CA99/01235.

[0147] One method for identifying a the ability of a compound of the present invention to induce mRNA degradation, comprises i) contacting the compound with a DNA expression system which in the absence of the compound is capable of expressing a protein having a detectable signal, wherein the mRNA which codes for the protein and which is transcribed from the expression system comprises at least one copy of a mRNA instability sequence; ii) measuring the detectable signal in the presence of the test compound and comparing the result obtained with a control. A related method can be used to compare compounds which induce mRNA degradation. This method comprises separately contacting the compounds with a DNA expression system which in the absence of the compounds is capable of expressing a protein having a detectable signal, wherein the mRNA which codes for the protein and which is transcribed from the expression system comprises at least one copy of a mRNA instability sequence, measuring the detectable signal in the presence of each test compound and comparing the signals obtained.

[0148] The DNA expression system used in the above described methods typically comprises a gene coding for expression of the protein having a detectable signal, wherein the gene comprises DNA coding for the amino acid sequence of the protein together with associated 5' and 3'UTR sequences comprising appropriate expression control elements including promoter and/or enhancer regions, and characteristically DNA corresponding to at least one copy of a mRNA instability sequence. An exemplary DNA expression system that can be used in the above methods is a cell based expression system, conveniently in the form of a suitably transformed cell line, optionally a stably transformed cell line. The host cell is typically a eukaryotic host cell, in particular an animal host cell such as a mammalian host cell.

[0149] The host cell may be of the same general cell type as the cells which express the protein which is coded for by the mRNA which it is desired to destabilize. Thus for instance, if the assay is to be used for the characterization of compounds of the present invention to demonstrate their ability to destabilize the mRNA coding for a cytokine, the host cell used is preferably a cell or cell line which is of the same or similar cell type to the cells which normally produce the cytokine in question. For example, monocyte or monocyte-like cell lines may be used as host cells for assaying for compounds which destabilize cytokine, e. g. IL-1 ss, mRNA. Preferred cell lines for oncogene and other cancer related gene mRNA instability assays are, e.g. Colon 205, KB 31, KB 8511, DU145, HCT116, MCF7, MCF7/ADR, MDA-MB-231, MDA-MB-435-and MDA-MB-435/TO.

[0150] The gene coding for expression of the protein having a detectable signal may encode a protein that may itself comprise the detectable signal. For instance, the protein may comprise a fluorescent protein, e.g. green fluorescent protein.

[0151] Alternatively, the protein is such that it is capable of reacting with an appropriate substrate or other substance to give a detectable signal. Conveniently the protein encoded by the mRNA is an enzyme or enzymatically active fragment of an enzyme. Examples of suitable enzymes include horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), alkaline phosphatase (AP),

secreted alkaline phosphatase (SEAP), β -galactosidase, or luciferase. Methods for detecting and quantifying such enzymes are well-known, using appropriate substrates and measurements. It will be appreciated, however, that any suitable detectable protein and measurement procedure may be used the methods described herein.

[0152] Alternative methods for measuring the ability of test molecules to induce mRNA degradation are known to workers skilled in the art and may be used to measure the activity of the benzoic acid derivatives of the present invention.

[0153] Anti-Inflammatory Activity

[0154] As demonstrated herein certain of the benzoic acid derivatives of the present invention exhibit anti-inflammatory activity, for example due to their ability to inhibit release of cytokines or act as antagonists of cytokine activity.

[0155] 1. In Vitro Methodology

[0156] One exemplary method of measuring the ability of the benzoic acid derivatives of the present invention to inhibit cytokine release is performed by monitoring the release of cytokines from cells that have been stimulated to release cytokines either in the presence or the absence of the test compound. Lipopolysaccharide (LPS) is one type of stimulant that can be used to induce cytokine release although a worker skilled in the art would readily appreciate that other stimulants can be used in this assay. Following treatment of the cells in culture by the stimulant, with and without the test compound, the cells are incubated for an appropriate period of time and then the culture medium and cell lysates are collected. Assays are then performed using the isolated media and lysates to determine the amount of cytokine (e.g. IL-1 β (medium and lysate), IL-6 (medium), TNF- α (medium) and PGE₂ (medium and lysate)) present in each sample. In order to ensure that the compounds are also non-toxic to the cells, lactate dehydrogenase (LDH) activity (medium and lysate) and DNA (lysates) are also analyzed. IL-1 β , IL-6 and TNF- α assays can be determined using commercially available ELISA kits (Cistron), PGE₂ can be measured using a standard RIA and DNA fluorimetrically using DAPI (4',6-diamidino-2-phenylindole.2HCl). It should be recognized that this test is not limited by the assay(s) used to quantify the cytokines, LDH release or DNA since multiple assays are well known to workers skilled in the art and the assays listed herein are not intended to limit the scope of the present invention. A reduction in the amount of cytokine in the medium from the treated cells in comparison to the medium from the untreated cells is indicative of the ability of the test compound to inhibit cytokine release. Furthermore, the compounds are considered to be non-toxic if LDH release is unchanged in the treated versus the untreated cells.

[0157] Another exemplary method of measuring the ability of the benzoic acid derivatives of the present invention to inhibit cytokine release is performed by comparing cytokine release from cells stimulated in culture in the presence and absence of the test compound. In an example of such a method mononuclear cells are obtained and cultivated on tissue culture dishes with the test compound at various concentrations [Schnyder et al., Agents & Actions, 30, 350-362 (1990)]. The non-adherent lymphocytes are

removed after 4 hrs by washing several times. Fresh medium, test compound and stimulant, for example lipopolysaccharide (LPS), are added and the monocytes incubated for a further day.

[0158] Purified IL-1, recombinant human IL-1- β (rhIL-1) or conditioned media collected from stimulated human monocytes, mouse macrophages or mouse cell line P388D₁, cause characteristic changes in the secretory pattern of chondrocytes. In particular, a latent metalloproteinase is induced, whilst secretion of plasminogen activator is reduced. The property of the metalloproteinase or stromelysin has been described in detail [Chin et al., J. Biol. Chem. 260, 12367-12376 (1985)], as has that of the plasminogen activator [Schnyder et al., Anal. Biochem. 200, 156-162 (1992)]. In performing the assay the pooled culture media are diluted 1:10 with fresh medium and added to confluent rabbit chondrocytes. Metalloproteinase activity in the chondrocyte culture medium is assayed after a further 2 days using standard techniques known to workers skilled in the art. One example of a technique for measuring metalloproteinase activity is provided in Example 6 of the present disclosure. Compounds having the ability to reduce cytokine release will decrease induction of metalloprotease activity in the chondrocytes that are pooled with the culture media from treated cells in comparison to those pooled with the culture media from untreated cells.

[0159] In order to determine the antagonistic effects of the benzoic acid derivatives of the present invention a cytokine is added to cultured cells that are known to respond to the cytokine and their response is monitored in the presence and the absence of the test compound. Example 7 of the present disclosure demonstrates one example of such a test in which chondrocytes are used. As described herein, chondrocytes will respond to IL-1 by an increase in metalloproteinase activity. A test compound is demonstrated to have an antagonistic effect if the metalloproteinase activity is decreased in the presence of the test compound in comparison to the activity in its absence.

[0160] 2. In Vivo Methodology

[0161] One art recognized animal model of an inflammatory response is LPS-induced fever in rats. An LPS-suspension is injected in rats. At a certain time interval following injection the body temperature of the rat is measured and following the next interval of time the test compound is administered. At the end of the third time interval the body temperature is measured again. The temperature increment shown by the untreated controls can be taken as 100% and that in the treated group can be expressed as a percentage of this value. The ED₅₀ is the dose causing a 50% inhibition of the temperature increase determined in the control rats. Compounds of the present invention typically have an ED₅₀ in this assay of from about 0.1 to about 1 mg/kg.

[0162] An art recognized animal model of inflammation is carrageenan-induced paw edema in the rat. The test compound is administered to the rats one hour prior to the carrageenan injection. Carrageenan is given by subplantar injection into one hind paw. The swelling of the paw is measured. A control reading is taken immediately after the injection, and the swelling is measured after 3 and 5 hrs. The mean value of the 3- and 5-hour reading is taken after deduction of the control reading, and the values obtained from the treated animals are expressed as a percentage of the

value obtained from non-treated controls. The ED₅₀ is the dose causing a 50% inhibition of the carrageenan-induced swelling after 3 hr. Compounds of the invention typically have ED₅₀'s in this assay of from about 0.5 to about 1 mg/kg.

[0163] It should be readily appreciated to a worker skilled in the art that the tests described herein are examples of standard tests that can be used to measure the activity of the compounds of the present invention. A wide variety of tests for measuring anti-cancer or anti-neoplastic activity, ability to increase or restore drug sensitivity, ability to induce mRNA degradation and anti-inflammatory activity are well known and can be used to demonstrate the characteristics of the benzoic acid derivatives of the present invention.

[0164] Use of the Benzoic Acid Derivatives

[0165] In one embodiment of the present invention the benzoic acid derivatives are used in the treatment of patients having pathological conditions associated with abnormal cell proliferation, such as cancer. The pathological conditions include the abnormal cell proliferation of malignant cells of various tissues and/or organs, comprising, with no limitation being implied, muscle, bone or connective tissues, the skin, brain, lungs, sex organs, the lymphatic or renal systems, mammary or blood cells, liver, the digestive tract, pancreas and thyroid or adrenal glands. These pathological conditions can also include solid tumors, cancers of the ovary, breast, brain, prostate, colon, stomach, kidney or testicles, Kaposi's sarcoma, cholangioma, chorioma, neuroblastoma, Wilms' tumor, Hodgkin's disease, melanomas, multiple myelomas, lymphatic leukemias and acute or chronic granulocytic lymphomas.

[0166] In an alternative embodiment of the present invention the benzoic acid derivatives are used to inhibit the release of cytokines and/or as functional antagonists of cytokines. In a specific embodiment the benzoic acid derivatives are used to inhibit the release of IL-1, IL-6 and TNF- α and/or as functional antagonists of IL-1.

[0167] A related embodiment of the present invention involves the use of the benzoic acid in the treatment of disorders with an aetiology associated with or comprising excessive cytokine release, particularly IL-1 β release, e.g. in a wide variety of inflammatory states and diseases such as rheumatoid arthritis, osteoarthritis, septic shock, psoriasis, atherosclerosis, inflammatory bowel disease, Crohn's disease and asthma.

[0168] In another embodiment the compounds of the present invention may be used in the prophylaxis or treatment of diseases and medical conditions in general having an etiology associated with the increased or prolonged stability of mRNAs which contain one or more mRNA instability sequences, and which on prolonged or inappropriate expression typically give rise to undesirable effects, e.g. cancer cell growth or an unwanted inflammatory response.

[0169] Compounds of the present invention may be used in connection with diseases and medical conditions associated with any of the genes mentioned above or described in the listed publications, which comprise mRNA instability sequences.

[0170] Examples of diseases and medical conditions which may be treated or prevented by use of the present

invention include: cancers (e.g. of the colon, breast, lung etc.), acute and chronic inflammation, autoimmune diseases, respiratory diseases, infectious diseases and transplant rejection, as well as, neuronal inflammation, Alzheimer's disease and cardiovascular diseases.

[0171] In a related embodiment the compounds of the present invention which induce degradation of mRNA that contains one or more mRNA instability sequences are provided for use as pharmaceuticals, e.g. for use in the prophylaxis or treatment of diseases and medical conditions in general having an etiology associated with the increased or prolonged stability of mRNAs which contain one or more mRNA instability sequences, and which on prolonged or inappropriate expression typically give rise to undesirable effects, e.g. cancer cell growth or an unwanted inflammatory response.

[0172] In view of their activity as inducers of degradation of mRNAs which contain mRNA instability sequences, the compounds of this invention are useful for the prophylaxis and treatment of cancers and malignant diseases which involve inappropriate build-up and expression of mRNAs, which contain mRNA instability sequences, and which code for proteins involved in the initiation, progression or persistence of cancer or malignant disease. Examples of cancer related genes, with mRNAs which contain mRNA destabilising sequences, include various oncogenes and transcription factors, e.g. c-myc, c-fos, Spl, bcl-2 and similar genes. The inappropriate or prolonged expression of such oncogenes is implicated in the initiation of certain forms of cancer, such as colon cancer, breast cancer, lung cancer etc.. Further examples of cancer related genes, with mRNAs which contain mRNA instability sequences are genes for metalloproteinase enzymes, e.g. MBP-1, MMP-2, collagenases etc., involved in tissue remodelling required for tumour growth and metastasis invasion; cell cycle related genes such as p45/SKIP2 etc. and multidrug resistance genes, e.g. mdr-1, MRPs, etc. involved in the intrinsic or acquired multidrug resistance of some cancer cells.

[0173] Treatment with compounds of this invention advantageously leads to degradation of the mRNAs of such genes, resulting in the down-regulation or "switching off" of gene expression. Thus for example, they may be used for treatment and prevention of oncogene mediated cancers and malignant diseases, to treat or prevent tumour growth and metastasis invasion in general, and to prevent or reverse multidrug resistance and thereby facilitate cancer and tumor treatment with conventional, e.g. cytotoxic, anti-cancer agents.

[0174] Characteristically when the benzoic acid derivatives are used to prevent or reverse multidrug resistance of tumor and other malignant cells, they are used in combination with cytostatic or cytotoxic agents.

[0175] The benzoic acid derivatives according to the present invention can be used either alone or in combination with other pharmacologically active compounds, for example together with inhibitors of the enzymes of polyamine synthesis, inhibitors of protein kinase C, inhibitors of other tyrosine kinases, cytokines, negative growth regulators, for example TGF- β or IFN- β , aromatase inhibitors, antioestrogens and/or cytostatic agents.

[0176] Compositions Comprising the Benzoic Acid Derivatives

[0177] In one embodiment of the present invention there is provided compositions comprising one or more benzoic acid derivative of Formula I and one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants and, if desired, other active ingredients. As indicated above such compositions are used in the treatment of various conditions in mammals, including humans.

[0178] The compounds of the invention may be administered by any conventional route, in particular nasally, enterally, preferably orally, e.g. in the form of tablets or capsules, or parenterally e.g. in the form of injectable solutions or suspensions or in a suppository form. Unit dosage forms contain, for example of from about 2 mg to 1 g of the compound of the invention. The invention also provides a pharmaceutical composition comprising an effective amount of the compounds in association with a pharmaceutically acceptable diluent or carrier. Such compositions may be manufactured in conventional manner.

[0179] Suitable pharmaceutical compositions comprising the compounds of the present invention as an active ingredient and that can be used especially in the treatment of the diseases mentioned above include compositions for enteral, such as nasal, buccal, rectal or especially oral, administration and parenteral, such as intravenous, intramuscular or subcutaneous, administration to warm-blooded animals, especially human beings. The compositions comprise the active ingredient on its own or preferably together with a pharmaceutically acceptable carrier. The dosage of the active ingredient depends on the disease to be treated, and on species, age, weight and individual condition, individual pharmacokinetic conditions, and the mode of administration. The dosage can be readily determined by a worker skilled in the art using standard methods in light of the above considerations.

[0180] The pharmaceutical compositions may comprise from approximately 1% to approximately 95% active ingredient, forms of administration in single dose form preferably comprising from approximately 20% to approximately 90% active ingredient and forms of administration that are not in single dose form preferably comprising from approximately 5% to approximately 20% active ingredient. Unit dose forms are, for example, dragees, tablets, ampoules, vials, suppositories or capsules. Other forms of administration are, for example, ointments, creams, pastes, foams, tinctures, lipsticks, drops, sprays, dispersions, etc. Examples are capsules comprising from approximately 0.05 g to approximately 1.0 g of the active ingredient

[0181] The pharmaceutical compositions are prepared in a manner known per se, for example by means of conventional mixing, granulating, confectioning, dissolving or lyophilizing procedures.

[0182] Solutions of the active ingredient, and also suspensions or dispersions, especially isotonic aqueous solutions, dispersions or suspensions, are preferably used, it being possible, for example in the case of lyophilized compositions that contain the active ingredient alone or together with a carrier, for example mannitol, for such solutions, suspensions or dispersions to be made up prior to use. The pharmaceutical compositions may be sterilized and/or may

comprise excipients, for example preservatives, stabilizers, wetting agents and/or emulsifiers, solubilizers, salts for regulating the osmotic pressure and/or buffers, and are prepared in a manner known per se, for example by means of conventional dissolving or lyophilizing procedures. The solutions or suspensions may comprise viscosity-increasing substances, such as sodium carboxymethylcellulose, dextran, polyvinylpyrrolidone or gelatin.

[0183] Suspensions in oil comprise as the oil component the vegetable, synthetic or semi-synthetic oils customary for injection purposes. There may be mentioned as such especially liquid fatty acid esters that contain as acid component a long-chained fatty acid having from 8 to 22, especially from 12 to 22, carbon atoms, for example lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid, or corresponding unsaturated acids, for example oleic acid, elaidic acid, erucic acid, brassidic acid or linoleic acid, if desired with the addition of antioxidants, for example vitamin E, β -carotene or 3,5-di-tert-butyl-4-hydroxytoluene. The alcohol component of those fatty acid esters has a maximum of 6 carbon atoms and is a mono- or poly-hydric, for example a mono-, di- or tri-hydric, alcohol, for example methanol, ethanol, propanol, butanol or pentanol or the isomers thereof, but especially glycol and glycerol. The following examples of fatty acid esters are therefore to be mentioned: ethyl oleate, isopropyl myristate, isopropyl palmitate, "Labrafil M 2375" (polyoxyethylene glycerol trioleate, Gattefosse, Paris), "Labrafil M 1944 CS" (unsaturated polyglycolised glycerides prepared by alcoholysis of apricot kernel oil and consisting of glycerides and polyethylene glycol ester; Gattefosse, France), "Labrasol" (saturated polyglycolised glycerides prepared by alcoholysis of TCM and consisting of glycerides and polyethylene glycol ester; Gattefosse, France) and/or "Miglyol 812" (triglyceride of saturated fatty acids with a chain length of C_8 to C_{12} , Hüls AG, Germany), but especially vegetable oils, such as cottonseed oil, almond oil, olive oil, castor oil, sesame oil, soybean oil and more especially groundnut oil.

[0184] The injection compositions are prepared in customary manner under sterile conditions; the same applies also to introducing the compositions into, for example, ampoules or vials and to sealing the containers.

[0185] Pharmaceutical compositions for oral administration can be obtained, for example, by combining the active ingredient with one or more solid carriers, if desired granulating a resulting mixture, and processing the mixture or granules, if desired, and if necessary by the addition of additional excipients, to form tablets or dragee cores.

[0186] Suitable carriers are especially fillers, such as sugars, for example lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, and also binders, such as starches, for example corn, wheat, rice or potato starch, methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, and/or, if desired, disintegrators, such as the above-mentioned starches, also carboxymethyl starch, crosslinked polyvinylpyrrolidone, or alginic acid or a salt thereof, such as sodium alginate. Additional excipients are especially flow conditioners and lubricants, for example

silicic acid, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol or derivatives thereof.

[0187] Dragee cores can be provided with suitable, optionally enteric, coatings, there being used inter alia concentrated sugar solutions which may contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, or coating solutions in suitable organic solvents or solvent mixtures, or, for the production of enteric coatings, solutions of suitable cellulose preparations, such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate. Colorings or pigments may be added to the tablets or dragee coatings, for example for identification purposes or to indicate different doses of active ingredient.

[0188] Orally administrable pharmaceutical compositions also include dry-filled capsules consisting of gelatin, and also soft, sealed capsules consisting of gelatin and a plasticizer, such as glycerol or sorbitol. The dry-filled capsules may contain the active ingredient in the form of granules, for example in admixture with fillers, such as corn starch, binders and/or glidants, such as talc or magnesium stearate, and optionally stabilisers. In soft capsules, the active ingredient is preferably dissolved or suspended in suitable liquid excipients, such as fatty oils, paraffin oil or liquid polyethylene glycols or fatty acid esters of ethylene or propylene glycol, to which stabilisers and detergents, for example of the polyoxyethylene sorbitan fatty acid ester type, may also be added.

[0189] Other oral forms of administration are, for example, syrups prepared in customary manner which comprise the active ingredient, for example, in suspended form and in a concentration of about 5% to 20%, preferably about 10%, or in a similar concentration that provides a suitable single dose, for example, when administered in measures of 5 or 10 ml. Also suitable are, for example, powdered or liquid concentrates for the preparation of shakes, for example in milk. Such concentrates may also be packaged in single dose quantities.

[0190] Suitable rectally administrable pharmaceutical compositions are, for example, suppositories that consist of a combination of the active ingredient and a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols or higher alkanols.

[0191] For parenteral administration there are suitable especially aqueous solutions of an active ingredient in water-soluble form, for example in the form of a water-soluble salt, or aqueous injection suspensions that contain viscosity-increasing substances, for example sodium carboxymethylcellulose, sorbitol and/or dextran, and, if desired, stabilisers. The active ingredient, optionally together with excipients, can also be in the form of a lyophilisate and can be made into a solution prior to parenteral administration by the addition of suitable solvents.

[0192] The compounds for use in the invention can be administered, prophylactically or therapeutically, as such or in the form of pharmaceutical compositions, preferably in an amount effective against the said diseases, to a warm-blooded animal, for example a human being, requiring such treatment, the compounds being used especially in the form

of pharmaceutical compositions. In such treatment an individual of about 70 kg body weight will be administered a daily dose of from approximately 0.1 g to approximately 5 g, preferably from approximately 0.5 g to approximately 2 g, of a compound.

[0193] The invention also provides a pharmaceutical pack or kit comprising one or more containers fined with one or more of the ingredients of the pharmaceutical -compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the pharmaceutical compositions of the present invention may be employed in conjunction with other therapeutic compounds.

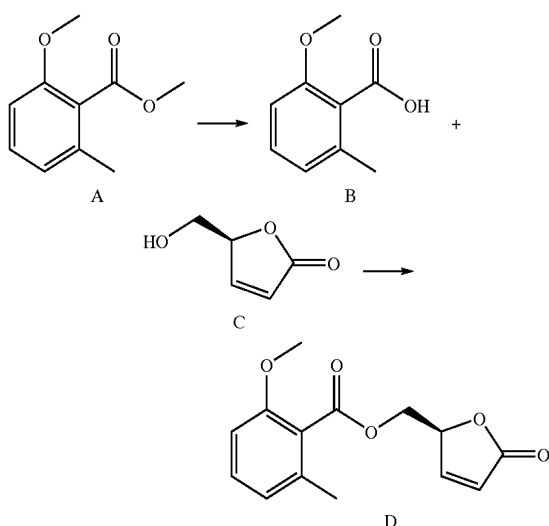
[0194] To gain a better understanding of the invention described herein, the following examples are set forth. It should be understood that these examples are for illustrative purposes only. Therefore, they should not limit the scope of this invention in any way.

EXAMPLES

Example 1

Preparation of the Compound of Formula D and Derivatives

[0195]



[0196] Compound B

[0197] To a solution of 46.7 g compound A (which has been synthesized following the literature procedure in Synthesis 1980, 814) in 800 ml of methanol were added 400 ml of 2N NaOH. This mixture was then refluxed for 24 hrs. Methanol was evaporated off and the remaining aqueous liquor was extracted with diethylether. The aqueous phase was cooled and acidified with conc. HCL. The precipitation was filtered off, washed well with water and dried at 80° C. under vacuum to give 31.8 g of a beige solid. M.p. 138-140° C.

[0198] Compound D

[0199] To a solution of 1.55 g compound B (prepared above) in 42 ml of THF was added 4.1 ml of oxalic acid chloride dropwise at 20° C. The solution was stirred at room temperature (RT) for 2 hrs and then the solvents were removed on the rotary evaporator. The residue was dissolved with 40 ml methylene chloride and 0.8 ml pyridine. A solution of 1 g compound C (which was synthesized following the procedure in J. Chem. Research (3) 1987, 369) in 10 ml methylene chloride was added dropwise. The mixture was stirred under argon at RT for 17 hrs and then extracted with sat. aq. NaHCO₃ solution. The organic phase was dried and evaporated to leave 1.97 g of a brown oil which was purified on a silica gel column with hexane:ethyl acetate=2:1 as solvent giving 0.9 g compound D as colourless crystals. M.p. 74-78° C.

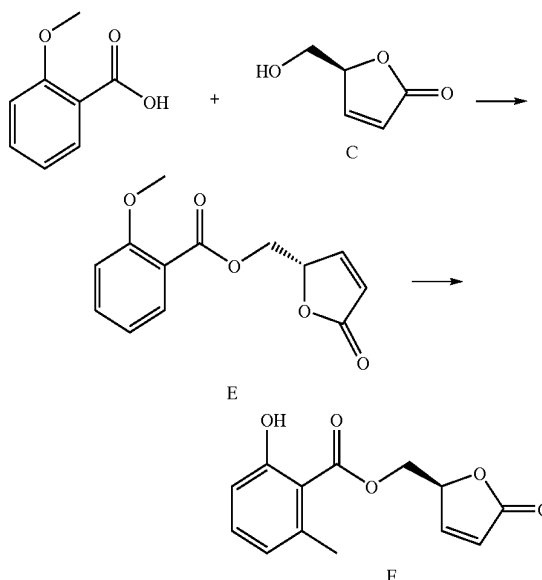
[0200] D: NMR (in CDCl₃): 2.28 ppm (s, 3H); 3.8 ppm (s, 3H); 4.59 ppm (d, 2H); 5.32 ppm (m, 1H); 6.21 ppm (dd, 1H); 6.7-6.85 ppm (m, 2H);

[0201] 7.23 ppm (t, 1H); 7.51 ppm (dd, 1H).

Example 2

Preparation of the Compound of Formula F and Derivatives

[0202]



[0203] Compound E

[0204] To a solution of 3.6 g of 2-Methoxy-benzoic acid were added dropwise 10.1 ml of oxalic acid chloride at 20° C., plus 1 drop of DMF. The solution was stirred at RT for 3 hrs. The pale yellow solution was evaporated down and re-dissolved in 100 ml of dichloromethane. To this solution was added a solution of 2.5 g compound C (prepared above) in 25 ml of dichloromethane. This mixture was stirred at RT for 24 hrs, then washed with water, dried with Na₂SO₄, and evaporated down to give a dark yellow oil. This was

flash-chromatographed on silica gel with toluene: ethyl acetate=4:1 as eluent giving 3.6 g of E as colorless crystals with a m.p. of 53-58° C.

[0205] E: NMR (in CDCl₃): 3.9 ppm (s, 3H); 4.6 ppm (dq, 2H); 5.35 ppm (m, 1 H); 6.21 ppm (dd, 1 H); 6.9-7.05 ppm (m, 2H); 7.4-7.6 ppm (m, 2H);

[0206] 7.78 ppm (dd, 1H).

[0207] Compound F

[0208] To a solution of 3.1 g of E (prepared above) in 100 ml of dichloromethane were added at 0° C. 17.8 ml of a 2.1 molar solution of BCl₃ in ethylene chloride. This mixture was stirred at 0° C. for 30 minutes and then poured on ice-water. The organic phase was washed 2× with water and 1× with sat NaCl solution, dried with Na₂SO₄ and evaporated down. The residue was chromatographed on silica gel with toluene:ethyl acetate=4:1 as eluent giving 2.1 g of F as a colorless oil.

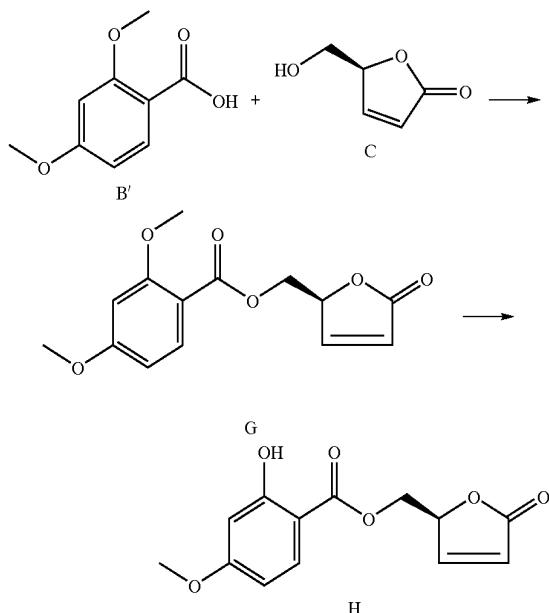
[0209] F: NMR (in CDCl₃): 4.62 ppm (dq, 2H); 5.38 ppm (m, 1H); 6.27 ppm (dd, 1H); 6.8-7.0 ppm (m, 2H); 7.4-7.6 ppm (m, 2H); 7.76 ppm (dd, 1H);

[0210] 10.42 ppm (s, 1H).

Example 3

Preparation of the Compound of Formula H and Derivatives

[0211]



[0212] Compounds G and H were prepared using the above procedures.

[0213] G: NMR (in CDCl₃): 3.85 ppm (s, 3H); 3.88 ppm (s, 3H); 4.57 ppm (dq, 2H); 5.32 ppm (m, 1H); 6.19 ppm (dd, 1H); 6.4-6.55 ppm (m, 2H);

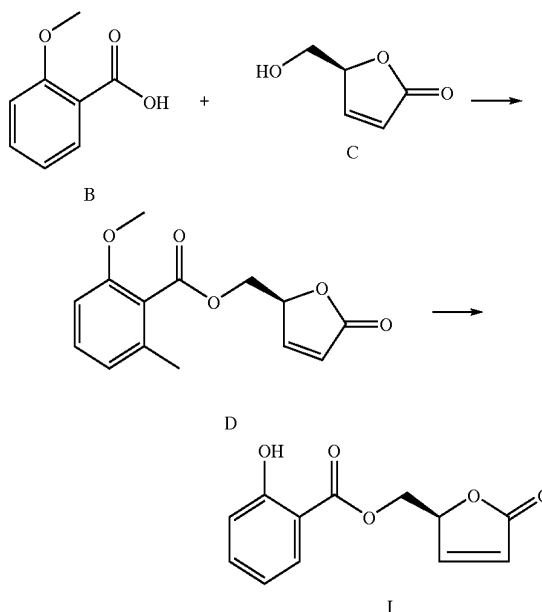
[0214] 7.5 ppm (dd, 1H); 7.8 ppm (d, 1H).

[0215] H: NMR (in CDCl₃): 3.82 ppm (s, 3H); 4.39 ppm (m, 2H); 5.35 ppm (m, 1H); 6.23 ppm (dd, 1H); 6.35-6.5 ppm (m, 2H); 7.5 ppm (dd, 1H); 7.67 ppm (d, 1H); 10.6 ppm (s, 1H).

Example 4

Preparation of the Compound of Formula I and Derivatives

[0216]



[0217] Compound I was prepared using the above procedures.

[0218] I: NMR (in CDCl₃): 2.5 ppm (s, 3H); 4.5-4.8 ppm (m, 2H); 5.38 ppm (m, 1H); 6.27 ppm (dd, 1H); 6.7 ppm (d, 1H); 6.83 ppm (d, 1H); 7.29 ppm (t, 1H); 7.5 ppm (dd, 1H); 11.0 ppm (s, 1H).

[0219] The compounds of the invention have pharmacological properties. In particular, they show cytokine inhibitory effects, acting not only to inhibit the release of IL-1, IL-6 and TNF- α , but also as functional antagonists of IL-1 as indicated in the following in vitro and in vivo test methods described below.

Example 5

Cytokine Release from THP-1 Cells

[0220] The THP-1 cell line is generally available and is described by Tsuchiya et al. [Int. J. Cancer 26 171-176 (1980)]. 900 μ l THP-1 cells (0.5×10^6 cells) together with 100 U γ -interferon/0.9 ml RPMI 1640 medium (containing 2 mM L-glutamine and 5% heat-inactivated fetal calf serum) are pipetted into 24-well culture plates. 100 μ l of the compound to be tested are then added. After 3 hours at 37° C. in 5% CO₂/95% air, 10 μ l lipopolysaccharide (12S) 500 μ g/ml is added and the incubation continued for a further 40 hours. Appropriate controls (with and without stimulus,

solvent) are also included. The media are then removed and clarified by centrifugation at 1000 g for 10 min. 1.0 ml digitonin 0.01% is added to the wells to lyse the cells which are loosened by scraping with a rubber policeman and left at 4° C. for 10 min. Lactate dehydrogenase measurements are then performed immediately and the samples stored at -20° C. until the other determinations can be made. The assays are: IL-1 β (medium and lysate), IL-6 (medium), TNF- α (medium), PGE₂ (medium and lysate), lactate dehydrogenase (LDH) (medium and lysate) and DNA (lysates). IL-1 β , IL-6 and TNF- α assays are determined using commercially available ILISA kits (Cistron), PGE₂ is measured using a standard RIA and DNA fluorimetrically using DAPI (4', 6-diamidino-2-phenylindole.2HCl). In this test, the compounds of the invention inhibit IL-1 β , IL-6, TNF- α and PGE₂ release at a concentration of about 0.1 to 10 μ M. In contrast DNA levels remain substantially unaffected, and the compounds are non-toxic, since LDH release is unchanged.

Example 6

Cytokine Release from Human Monocytes

[0221] Human Monocytes

[0222] Mononuclear cells are obtained from the blood of healthy volunteers via centrifugation and cultivated on tissue culture dishes with the test compound at various concentrations [Schnyder et al., Agents & Actions, 30, 350-362 (1990)]. The non-adherent lymphocytes are removed after 4 hrs by washing several times. Fresh medium, test compound and LPS (10 μ g/ml) as stimulant are added and the monocytes incubated for a further day. The pooled culture media are diluted 1:10 with fresh medium and added to confluent rabbit chondrocytes. Metalloproteinase activity in the chondrocyte culture medium is assayed after a further 2 days as described below. Compounds of the invention are active in suppressing monokine release in this test method at a concentration of the order of from 0.1 to 10 μ M.

[0223] Determination of IL-1 by the Chondrocyte Test

[0224] Purified IL-1, recombinant human IL-1 β (rhIL-1) or conditioned media collected from stimulated human monocytes, mouse macrophages or mouse cell line P388D₁, cause characteristic changes in the secretory pattern of chondrocytes. In particular, a latent metalloproteinase is induced, whilst secretion of plasminogen activator is reduced. The property of the metalloproteinase or stromelysin has been described in detail [Chin et al., J. Biol. Chem. 260, 12367-12376 (1985)], as has that of the plasminogen activator [Schnyder et al., Anal. Biochem. 200, 156-162 (1992)]. Dose-response curves using purified or recombinant IL-1 and neutralisation with an antibody to human monocyte IL-1 have shown that this system can be used as a specific and sensitive bioassay for IL-1. Stimulation of the secretion of a latent metalloproteinase by rabbit articular chondrocytes is relatively IL-1-specific, IL-2, TNF- α , recombinant human interferons- α , and - γ , phorbol myristate acetate, Concanavalin A, E-type prostaglandin and indomethacin having no influence [Schnyder and Payne, Brit. J. Rheumatol. 24 (suppl. 1), 128-132 (1985); Schnyder et al., J. Immunol. 138, 496-503 (1987)].

[0225] Chondrocytes are harvested and cultured as described [Evequoz et al., Biochem. J. 219, 667-677 (1984)]. Briefly, chondrocytes are released from slices of

distal femur articular cartilage from ca. 1.2 kg female New Zealand White rabbits by treatment with proteinases. The washed cells are cultured on 48-well culture plates in DMEM, enriched with 1% antibiotics, 2 mM glutamine and 10% heat-inactivated fetal calf serum. After reaching confluency the cells are incubated with 20 μ l samples of the test culture media for IL-1 bioassay and made up to a volume of 300 μ l Iscove's modified Dulbecco's medium. The supernatant media are collected after 48 h, centrifuged and processed for biochemical analysis.

[0226] Biochemical Assays

[0227] Metalloproteinase is measured kinetically by using a 96-well plate Twinreader (Flow Laboratories AG) linked to a personal computer.

[0228] Ac-Pro-Leu-Gly-S-Leu-Leu-Gly-OC₂H₅, a synthetic substrate for vertebrate collagenase is used for the determination of metalloproteinase [Weingarten and Feder, Anal. Biochem. 147,437440 (1985)]. 50 μ l of the latent metalloproteinase is activated with 50 μ l trypsin (120 μ g/ml in 50 mM PIPES pH 6.8, containing 20 mM CaCl₂) for 30 min at 37° C., after which time the activities of all serine proteinases are stopped by adding 150 μ l Soybean trypsin inhibitor (SBTI; 166 μ g/ml in the above buffer). A 50 μ l aliquot of the activated metalloproteinase is then mixed with 100 μ l reagent solution (2.5 mM 5, 5'-dithio-bis-2-nitrobenzoic acid, 100 μ g/ml SBTI, 20 mM CaCl₂ in the above buffer), and kept for 10 min at room temperature in order to react with all free SH-groups. The reaction is then started by adding 100 μ l substrate solution (1.25 mM in buffer containing 100 μ g/ml SBTI and the changes in absorbance at 414 nm is measured 11 times at 1 min intervals.

Example 7

Functional IL-1 Antagonistic Effects

[0229] The test method described in Example 6 above is repeated, but instead of adding human monocytes to the chondrocyte culture, IL-1 itself (recombinant human IL-1, Sandoz) is added at a concentration of 1 ng/ml. Metalloproteinase activity in the chondrocyte culture medium is assayed after 2 days. Compounds of the invention are active as functional IL-1 antagonists at concentrations of 1-10 μ M.

Example 8

LPS-Fever

[0230] An LPS-suspension (Sigma, No. L-5886; 100 μ g/5 ml glucose solution/kg s.c.) is injected in male Tüttlingen SD rats (150-160 g). Two hours later the body temperature is measured using a thermistor rectal probe connected to an ELLAB telethermometer. After 4 hrs the test compound is administered. Two hours later (6 hrs after LPS administration) the temperature is measured again. The temperature increment shown by the untreated controls is taken as 100% and that in the treated group is expressed as a percentage of this value. The ED₅₀ is the dose causing a 50% inhibition of the temperature increase determined in the control rats. Compounds of the invention typically have an ED₅₀ in this assay of from about 0.1 to about 1 mg/kg.

Example 9

Carrageenan-Induced Paw Edema in the Rat

[0231] Five OFA male rats, 150-170 g body weight, are used for each group. The test compound is administered

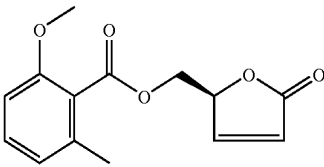
orally as a suspension in physiological saline/0.5% tragacanth one hour prior to the carrageenan injection. Carrageenan (0.1 ml of a 1% suspension in physiological saline) is given by subplantar injection into one hind paw. The swelling of the paw is measured by means of an antiphlogometer according to Kemper & Amelm. A control reading is taken immediately after the injection and the swelling is measured after 3 and 5 hrs. The mean value of the 3- and 5-hour reading is taken after deduction of the control reading, and the values obtained from the treated animals are expressed as a percentage of the value obtained from non-treated controls. The ED₅₀ is the dose causing a 50% inhibition of the carrageenan-induced swelling after 3 hr. Compounds of the invention typically have ED₅₀s in this assay of from about 0.5 to about 1 mg/kg.

Example 10

Reporter Gene Assay for Compounds which Destabilize mRNA

[0232] The THP-1 cell lines, clone No. 63 (containing PGL2_Neo30) and clone No. 53 (containing pGL2-Control) are grown, differentiated and stimulated with IFN- γ and LPS identical to normal THP-1 cells (for details see WO 00/39314). Compound is added 16 hrs after the addition of LPS and cell extracts are then taken 8 hrs later or as indicated. Luciferase activity was inhibited by 1 μ M benzoic acid derivative on average by 40%, whereas up to 5 μ M of benzoic acid derivative had no effects on the control clone No. 53.

TABLE 1

COMPOUND	Luciferase reporter gene assay (% control)			
	clone	0.5 μ M	1 μ M	5 μ M
	63	85	79	29
	53	89	93	99

[0233] The compounds of the invention are therefore indicated for the treatment of disorders with an etiology associated with or comprising excessive cytokine release, particularly IL-1 release, e.g. in a wide variety of inflammatory states and diseases such as rheumatoid arthritis, osteoarthritis, septic shock, psoriasis, atherosclerosis, inflammatory bowel disease, Crohn's disease and asthma.

[0234] For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general however, satisfactory results are achieved at dosage rates of from about 0.1 to 20 mg/kg animal body weight, preferably 0.5 to 5 mg/kg.

Example 11

Preparation of a Pharmaceutical Composition

[0235] Tablets, each comprising e.g. 50 mg of compound or a pharmaceutically acceptable salt, are prepared as follows:

Composition (10000 tablets)

active ingredient	500.0 g
lactose	500.0 g
potato starch	352.0 g
gelatin	8.0 g
talc	60.0 g
magnesium stearate	10.0 g
silicon dioxide (highly dispersed)	20.0 g
ethanol	q.s.

[0236] The active ingredient is mixed with the lactose and 292 g of potato starch and the mixture is moistened with an ethanolic solution of the gelatin and granulated through a sieve. After drying, the remainder of the potato starch, the magnesium stearate, the talc and the silicon dioxide are mixed in and the mixture is compressed to form tablets, each weighing 145.0 mg and comprising 50.0 mg of active ingredient; the tablets may, if desired, be provided with breaking notches for finer adaptation of the dose.

Example 12

Preparation of a Pharmaceutical Composition

[0237] Film-coated tablet, each comprising 100 mg of benzoic acid derivatives or a pharmaceutically acceptable salt are prepared as follows:

Composition (for 1000 film-coated tablets)

active ingredient	100.0 g
lactose	100.0 g
corn starch	70.0 g
talc	60.0 g
calcium stearate	1.5 g
hydroxypropylmethylcellulose	2.36 g
shellac	0.64 g
water	q.s.
methylene chloride	q.s.

[0238] The active ingredient, the lactose and 40 g of the corn starch are mixed and moistened with a paste prepared from 15 g of corn starch and water (with heating) and granulated. The granules are dried, the remainder of the corn starch, the talcum and the calcium stearate are added and mixed with the granules. The mixture is compressed to form tablets (weight: 280 mg) which are then film-coated with a solution of the hydroxypropylmethylcellulose and the shellac in methylene chloride; final weight of the film-coated tablet: 283 mg.

Example 13

Preparation of a Pharmaceutical Composition

[0239] Hard gelatin capsules, comprising 100 mg of active ingredient, for example benzoic acid derivatives or a pharmaceutically acceptable salt are prepared, for example, as follows:

Composition (for 1000 capsules)	
active ingredient	100.0 g
lactose	250.0 g
microcrystalline cellulose	30.0 g
sodium lauryl sulfate	2.0 g
magnesium stearate	8.0 g

[0240] The sodium lauryl sulfate is added to the lyophilized active ingredient through a sieve of 0.2 mm mesh size. The two components are intimately mixed. Then first the lactose is added through a sieve of 0.6 mm mesh size and then the microcrystalline cellulose is added through a sieve of 0.9 mm mesh size. The mixture is then intimately mixed again for 10 minutes. Finally the magnesium stearate is added through a sieve of 0.8 mm mesh size. After mixing for a further 3 minutes, size 0 hard gelatin capsules are each filled with 390 mg of the resulting formulation. Soft gelatin capsules may be prepared using similar ingredients and procedures.

Example 14

Effect on Cell Viability of Cancer Cells

[0241] The cell lines used in this experiment (ACF-7, MDA-MB-231, HCT116, KB 31, HCT-15, and DU 145) were obtained from ATCC, Bethesda, Md., U.S.A. Exponentially growing cells were treated with various concentrations of the test compound. The cells were further incubated for 24 h, 48 h and 96 h. Cells were then stained with YOPRO-1 as described in T. Idziorek et al., J Immunol Meth 185, 249 (1995) and the percentage of dying or dead cells was determined.

Example 15

Effect on Cell Cycle Progression of Cancer Cells

[0242] The cell lines used in this experiment (MCF-7, MDA-MB-231, HCT116, KB 31, HCT-15, and DU 145) were obtained from ATCC, Bethesda, Md., U.S.A. Exponentially growing cells were treated with various concentrations of the test compound. The cells were further incu-

bated for 24 h, 48 h and 96 h. Cells were then lysed and dsDNA was stained with YOPRO-1 as described in T. Idziorek et al., J Immunol Meth 185, 249 (1995). Fluorescence measurements were carried out essentially as described in V. L. Singer et al., Anal Biochem 249, 228 (1997) and values compared to control cells.

Example 16

Effect on Interleukin-1 β mRNA Levels

[0243] Total cellular RNA was isolated from THP-1 cells at different times after the initiation of differentiation using the guanidine isothiocyanate method of Chirgwin et al. (44). 20 μ g of each RNA sample was denatured and analyzed on a 1% agarose gel containing 2.2 M formaldehyde. The gel was transferred to Hybond-N (Amersham), UV-crosslinked by the Stratagene Stratalinker (1600 μ Joules \times 100) and judged for equal loading of RNA by methylene blue staining. Blots were hybridized to a digoxigenin (DIG)-labeled DNA probe. Labeling and detection was carried out by the DIG kit (Boehringer Mannheim) according to the manufacturer's specifications. DNA probes were obtained by sub-cloning RT-PCR derived fragments of the genes of interest, into pGemT-vector (Promega). For the RT-PCR analysis, 1 μ g of total RNA was reverse transcribed using AMV reverse transcriptase (Life Sciences Inc., St. Petersburg, Fla.), then amplified by the PCR method. Each PCR reaction also contained a set of β -actin primers as internal control. The levels of the expected PCR product were compared to the constant levels of β -actin. The size of the PCR products was compared to MspI digested pBR322 standards (New England Biolabs). PCR products were analyzed on a 4% agarose gel. Nucleotide sequences for the oligonucleotide 5' and 3' primers, respectively, were:

5'

3'

β -actin: ATGGGTCAGAAGGATTCTTA AGAGGCGTACAGGGATAGCAC

IL-1 β : GACACATGGGATAACGAGGCT ACGCAGGACAGGTACAGATTC

[0244] Compounds of the invention are reducing mRNA levels of interleukin-1 β up to 70% at concentrations of 10 μ M.

SEQUENCE LISTING

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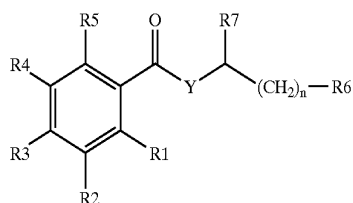
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21

1. A compound having structural formula (I):



(I)

or stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof or physiologically acceptable and hydrolysable esters thereof, wherein:

R1 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R2 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R3 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R4 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R5 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

Y is O or —NR', wherein R' is H or (C₁-C₄) alkyl;

n is 0-8;

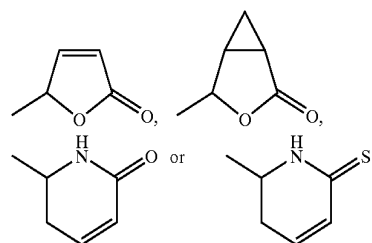
R6 is a 5 to 8 membered lactone or lactam ring;

R7 is H, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, phenyl or (C₁-C₄) alkyl-COO—,

wherein the lactam or lactone ring optionally comprises one or more C=C double bonds and the lactam or

lactone ring is optionally substituted with one or more (C₁-C₄)alkyl or (C₁-C₄) alkenyl groups;

with the proviso that when R1=R2=R3=R4=R5=R7=H, and Y is O, and n is 0, then R6 is other than



2. The compound according to claim 1, wherein:

R1=H, OH, MeCOO— or Methoxy;

R2=H;

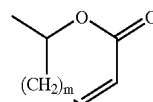
R3=H, OH, MeCOO— or Methoxy;

R4=H, Methoxy or halogen;

R5=H, OH, Methoxy or (C₁-C₄) alkyl;

Y is O;

R6 is



wherein, m is 0-4;

R7=H, Methyl, Isopropyl.

3. The compound according to claim 1, wherein:

R1 is H, —OH, MeO— or MeCOO—;

R2 is H;

R3 is H, —OH, MeO— or MeCOO—;

R4 is H or halogen;

R5 is —OH, MeO—, MeCOO— or Methyl.

4. The compound according to claim 2, wherein:

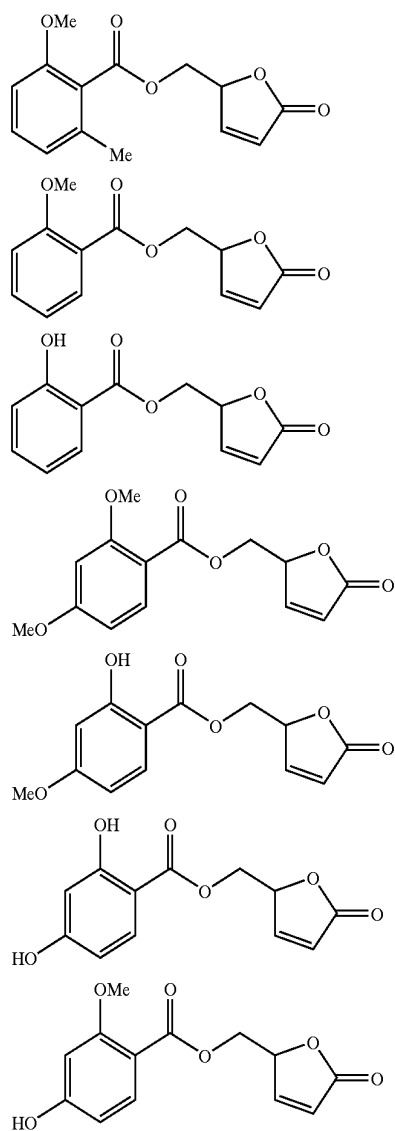
R1 is H or MeO,

R₃ is H, —OH or MeO—;

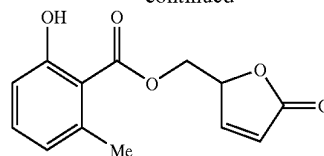
R4 is H and

R₅ is Methoxy or Methyl.

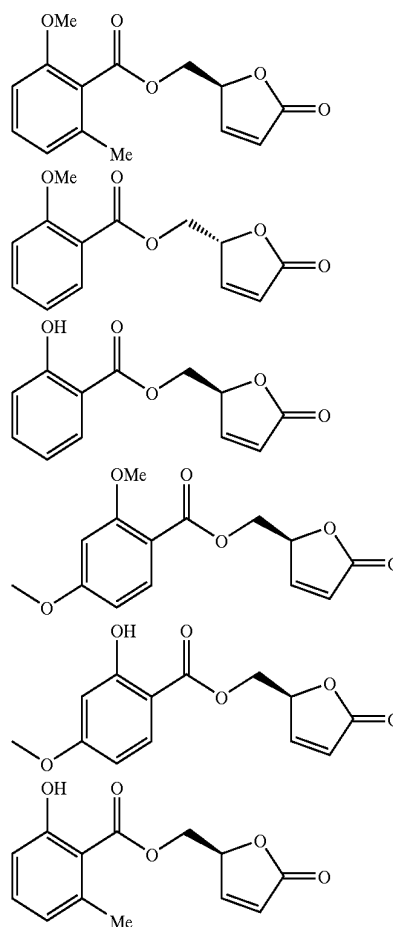
5. The compound according to claim 1, wherein said compound is selected from the group consisting of:



-continued

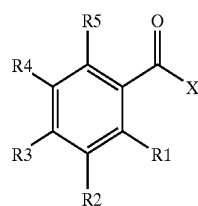


6. The compound according to claim 1, wherein said compound is selected from the group consisting of:



7. A process for the preparation of a compound of claim 1, a pharmaceutically acceptable metabolically labile ester or amide thereof, or a pharmaceutically acceptable salt thereof, which comprises:

a) reacting a compound of formula (II):



(II)

wherein:

R1 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

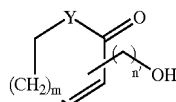
R2 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R3 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R4 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—; and

X is halogen;

with a compound of formula (III):



(III)

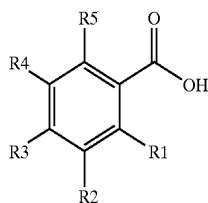
wherein:

n' is 1-9; and

m is 0-4;

compound (III) optionally contains one or more C=C double bonds and is substituted with one or more (C₁-C₄) alkyl or (C₁-C₄) alkenyl group; or

b) reacting a compound of formula (IV):

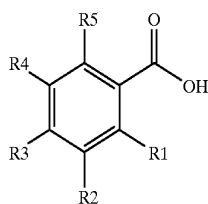


(IV)

wherein R1-R5 are as defined above;

with a compound of formula (II) in the presence of an acid under Azeotropic distillation conditions; or

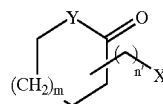
c) reacting an alkali or alkaline earth metal salt of compound of formula (IV):



(IV)

wherein R1-R5 are as defined above;

with a compound of formula (V):



(V)

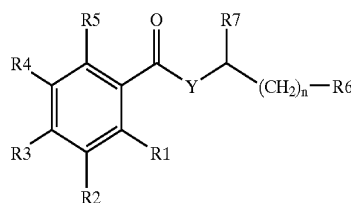
wherein;

X is F, Cl, Br or I;

n' is 1-9; and

m is 0-4; and compound of formula (V) may optionally contain one or more double bonds and is optionally substituted with one or more (C₁-C₄) alkyl or (C₁-C₄) alkenyl group.

8. A pharmaceutical composition comprising a compound having structural formula (I):



(I)

a stereoisomer thereof, or a pharmaceutically acceptable salt or hydrate thereof or a physiologically acceptable and a hydrolysable ester thereof and a pharmaceutically acceptable carrier, diluent or excipient; wherein:

R1 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R2 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R3 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R4 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R5 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

Y is O or —NR', wherein R' is H or (C₁-C₄) alkyl;

n is 0-8;

R6 is a 5 to 8 membered lactone or lactam ring;

R7 is H, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, phenyl or (C₁-C₄) alkyl-COO—,

wherein the lactam or lactone ring optionally comprises one or more C=C double bonds and the lactam or lactone ring is optionally substituted with one or more (C₁-C₄) alkyl or (C₁-C₄) alkenyl groups.

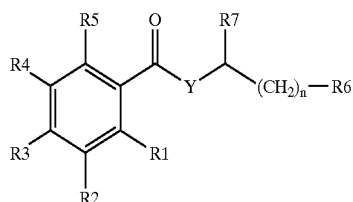
9. (cancelled).

10. The composition according to claim 8 additionally comprising a cytostatic or a cytotoxic agent.

11. The composition according to claim 8 additionally comprising a chemotherapeutic agent.

12-22. (cancelled).

23. A method for the prophylaxis or treatment of a cancer and/or malignant disease comprising administering to a mammal an effective amount of the compound a compound having structural formula (I):



(I)

or stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof or physiologically acceptable and hydrolysable esters thereof, wherein:

R1 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R2 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R3 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R4 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R5 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

Y is O or —NR', wherein R' is H or (C₁-C₄) alkyl;

n is 0-8;

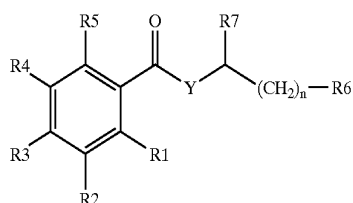
R6 is a 5 to 8 membered lactone or lactam ring;

R7 is H, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, phenyl or (C₁-C₄) alkyl-COO—,

wherein the lactam or lactone ring optionally comprises one or more C=C double bonds and the lactam or lactone ring is optionally substituted with one or more (C₁-C₄)alkyl or (C₁-C₄) alkenyl groups.

24. (cancelled).

25. A method for the prophylaxis or treatment of a disease or medical condition having an etiology associated with the increased stability of mRNA which contains one or more mRNA instability sequences, comprising administering to a mammal an effective amount of a compound having structural formula (I):



(I)

stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof or physiologically acceptable and hydrolysable esters thereof, wherein:

R1 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R2 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R3 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R4 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R5 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

Y is O or —NR', wherein R' is H or (C₁-C₄) alkyl;

n is 0-8;

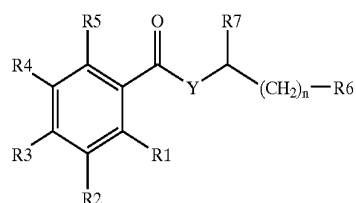
R6 is a 5 to 8 membered lactone or lactam ring;

R7 is H, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, phenyl or (C₁-C₄) alkyl-COO—,

wherein the lactam or lactone ring optionally comprises one or more C=C double bonds and the lactam or lactone ring is optionally substituted with one or more (C₁-C₄)alkyl or (C₁-C₄) alkenyl groups.

26. (cancelled).

27. A method for inducing degradation of mRNA in a mammal, which comprises administering an effective amount of a compound having structural formula (I):



(I)

stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof or physiologically acceptable and hydrolysable esters thereof, wherein:

R1 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R2 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R3 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R4 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R5 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

Y is O or —NR', wherein R' is H or (C₁-C₄) alkyl;

n is 0-8;

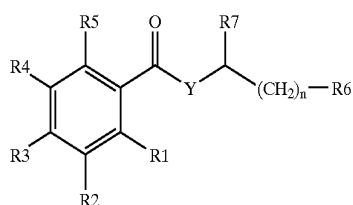
R6 is a 5 to 8 membered lactone or lactam ring;

R7 is H, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, phenyl or (C₁-C₄) alkyl-COO—,

wherein the lactam or lactone ring optionally comprises one or more C=C double bonds and the lactam or lactone ring is optionally substituted with one or more (C₁-C₄)alkyl or (C₁-C₄) alkenyl groups.

28. (cancelled).

29. A method of treating or preventing an immunopathological disorder having an etiology associated with the production of proinflammatory factors, comprising administering to a mammal with the disorder or a predisposition for the disorder with a therapeutically effective amount of a compound having structural formula (I):



stereoisomers thereof, or pharmaceutically acceptable salts or hydrates thereof or physiologically acceptable and hydrolysable esters thereof, wherein:

R1 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R2 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R3 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R4 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

R5 is H, OH, halogen, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, or (C₁-C₄) alkyl-COO—;

Y is O or —NR', wherein R' is H or (C₁-C₄) alkyl;

n is 0-8;

R6 is a 5 to 8 membered lactone or lactam ring;

R7 is H, (C₁-C₄) alkyl, (C₁-C₄) alkenyl, (C₁-C₄) alkoxy, phenyl or (C₁-C₄) alkyl-COO—,

wherein the lactam or lactone ring optionally comprises one or more C=C double bonds and the lactam or lactone ring is optionally substituted with one or more (C₁-C₄)alkyl or (C₁-C₄) alkenyl groups.

30. (cancelled).

31. The method according to claim 29 wherein the immunopathological disorder is selected from the group consisting of microbial infection, malignancy and metastasis, asthma, coronary restinosis, autoimmune disease, cirrhosis, entotoxemia, reperfusion injury and septic shock.

32. The method according to claim 27, wherein said mRNA encodes IL-1 β , IL-6, TNF- α , COX II, or bcl-2.

33. The method according to claim 23, wherein said mammal is a human.

34. The method according to claim 25, wherein said mammal is a human.

35. The method according to claim 27, wherein said mammal is a human.

36. The method according to claim 29, wherein said mammal is a human.

37. The method of claim 29, wherein said proinflammatory factor is selected from the group consisting of cytokines, chemokines, kinase phosphorylation factors and cyclooxygenases.

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