A MONOETHER OF PROBUCOL AND METHODS FOR THE INHIBITION OF THE EXPRESSION OF VCAM-1

EIN MONOETHER VON PROBUCOL UND METHODEN ZUR HEMMUNG DER VCAM-1 EXPRESSION

UN MONOETHER DU PROBUCOL ET METHODES D’Hemmage DE L’EXPRESSION DE VCAM-1

Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

Designated Extension States:
AL LT LV MK RO SI

Priority: 14.05.1997 US 47020 P

Date of publication of application: 26.04.2000 Bulletin 2000/17

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EP-A- 0 317 165
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US-A- 4 115 590


Remarks:
The file contains technical information submitted after the application was filed and not included in this specification.
Description

This invention is in the area of compounds and compositions for the treatment of diseases mediated by VCAM-1, including cardiovascular and inflammatory diseases.

[0002] Coronary heart disease (CHD) remains the leading cause of death in the industrialized countries. The primary cause of CHD is atherosclerosis, a disease characterized by the deposition of lipids in the arterial vessel wall, resulting in a narrowing of the vessel passages and ultimately hardening the vascular system.

Atherosclerosis as manifested in its major clinical complication, ischemic heart disease, continues to be a major cause of death in industrialized countries. It is now well accepted that atherosclerosis can begin with local injury to the arterial endothelium followed by proliferation of arterial smooth muscle cells from the medial layer to the intimal layer along the deposition of lipid and accumulation of foam cells in the lesion. As the atherosclerotic plaque develops it progressively occludes more and more of the affected blood vessel and can eventually lead to ischaemia or infarction.

Therefore, it is desirable to provide methods of inhibiting the progression of atherosclerosis in patients in need thereof.

[0003] Cardiovascular disease has been linked to several causative factors, which include hypercholesterolemia, hyperlipidemia, and the expression of VCAM-1 in vascular endothelial cells.

Expression of VCAM-1


VCAM-1 is expressed in cultured human vascular endothelial cells after activation by lipopolysaccharide (LPS) and cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF-α). These factors are not selective for activation of cell adhesion molecule expression.

U. S. Patent No. 5,380,747 to Medford, et al., teaches the use of dithiocarbamates such as pyrrolidine dithiocarbamate for the treatment of cardiovascular and other inflammatory diseases.

U. S. Patent No. 5,750,351 to Medford, et al., and WO95/30415 to Emory University describe the discovery
that polyunsaturated fatty acids ("PUFAs") and their hydroperoxides ("ox-PUFAs"), which are important components of oxidatively modified low density lipoprotein (LDL), induce the expression of VCAM-1, but not intracellular adhesion molecule-1 (ICAM-1) or E-selectin in human aortic endothelial cells, through a mechanism that is not mediated by cytokines or other noncytokine signals. This is a fundamental discovery of an important and previously unknown biological pathway in VCAM-1 mediated immune responses.

[0011] As non-limiting examples, linoleic acid, linolenic acid, arachidonic acid, linoleyl hydroperoxide (13-HPODE) and arachidonic hydroperoxide (15-HPETE) induce cell-surface gene expression of VCAM-1 but not ICAM-1 or E-selectin. Saturated fatty acids (such as stearic acid) and monounsaturated fatty acids (such as oleic acid) do not induce the expression of VCAM-1, ICAM-1 or E-selectin.

[0012] The induction of VCAM-1 by PUFAs and their fatty acid hydroperoxides is suppressed by dithiocarbamates, including pyrrolidine dithiocarbamate (PDTC). This indicates that the induction is mediated by an oxidized signal molecule, and that the induction is prevented when the oxidation of the molecule is blocked (i.e., the oxidation does not occur), reversed (i.e., the signal molecule is reduced), or when the redox modified signal is otherwise prevented from interacting with its regulatory target.

[0013] Cells that are chronically exposed to higher than normal levels of polyunsaturated fatty acids or their oxidized counterparts can initiate an immune response that is not normal and which is out of proportion to the threat presented, leading to a diseased state. The oversensitization of vascular endothelial cells to PUFAs and ox-PUFAs can accelerate the formation, for example, of atherosclerotic plaque.

[0014] Based on these discoveries, a method for the treatment of atherosclerosis, post-angioplasty restenosis, coronary artery diseases, angina, small artery disease and other cardiovascular diseases, as well as noncardiovascular inflammatory diseases that are mediated by VCAM-1, was described in WO95/30415 that includes the removal, decrease in the concentration of, or prevention of the formation of oxidized polyunsaturated fatty acids including but not limited to oxidized linoleic (C18Δ9,12), linolenic (C18Δ6,9,12), arachidonic (C20Δ3,5,8,11,14) and eicosatrienoic (C20Δ3,5,8,11,14) acids.

[0015] Nonlimiting examples of noncardiovascular inflammatory diseases that are mediated by VCAM-1 include rheumatoid and osteoarthritis, asthma, dermatitis, and multiple sclerosis.

**Hypercholesterolemia and hyperlipidemia**

[0016] Hypercholesterolemia is an important risk factor associated with cardiovascular disease. Serum lipoproteins are the carriers for lipids in the circulation. Lipoproteins are classified according to their density: chylomicrons, very low-density lipoproteins (VLDL), low density lipoproteins (LDL) and high-density lipoproteins (HDL). Chylomicrons primarily participate in transporting dietary triglycerides and cholesterol from the intestine to adipose tissue and liver. VLDL deliver endogenously synthesized triglycerides from liver to adipose and other tissues. LDL transports cholesterol to peripheral tissues and regulate endogenous cholesterol levels in those tissues. HDL transports cholesterol from peripheral tissues to the liver. Arterial wall cholesterol is derived almost exclusively from LDL. Brown and Goldstein, *Ann. Rev. Biochem.* 52, 223 (1983); Miller, *Ann. Rev. Med.* 31, 97 (1980). In patients with low levels of LDL, the development of atherosclerosis is rare.

[0017] Steinberg, et al., (N. Eng. J. Med. 1989; 320:915-924) hypothesized that modification of low-density lipoprotein (LDL) into oxidatively modified LDL (ox-LDL) by reactive oxygen species is the central event that initiates and propagates atherosclerosis. Oxidized LDL is a complex structure consisting of at least several chemically distinct oxidized materials, each of which, alone or in combination, may modulate cytokine-activated adhesion molecule gene expression. R fatty acid hydroperoxides such as linoleyl hydroperoxide (13-HPODE) are produced from free fatty acids by lipoxigenases and are an important component of oxidized LDL.

[0018] It has been proposed that a generation of oxidized lipids is formed by the action of the cell lipoxigenase system and that the oxidized lipids are subsequently transferred to LDL. There is thereafter a propagation reaction within the LDL in the medium catalyzed by transition metals and/or sulfhydryl compounds. Previous investigations have demonstrated that fatty acid modification of cultured endothelial cells can alter their susceptibility to oxidant injury, whereas supplementation with polyunsaturated fatty acids (PUFA) enhances susceptibility to oxidant injury. Supplementation of saturated or monounsaturated fatty acids to cultured endothelial cells reduces their susceptibility to oxidant injury, whereas supplementation with polyunsaturated fatty acids (PUFA) enhances susceptibility to oxidant injury.

[0019] Using reverse-phase HPLC analysis of native and saponified liquid extracts of LDL, it has been demonstrated that 13-HPODE is the predominant oxidized fatty acid in LDL oxidized by activated human monocytes. Chronic exposure to oxidized LDL provides an oxidative signal to vascular endothelial cells, possibly through a specific fatty acid hydroperoxide, that selectively augments cytokine-induced VCAM-1 gene expression.

[0020] Through a mechanism that is not well defined, areas of vessel wall predisposed to atherosclerosis preferentially sequester circulating LDL. Through a poorly understood pathway, endothelial, smooth muscle, and/or inflammatory cells then convert LDL to ox-LDL. In contrast to LDL, which is taken up through the LDL receptor, monocytes avidly
take up ox-LDL through a “scavenger” receptor whose expression, unlike the LDL receptor, is not inhibited as the content of intracellular lipid rises. Thus, monocytes continue to take up ox-LDL and become lipid-engorged macrophage-foam cells that form the fatty streak.

[0021] There is now a large body of evidence demonstrating that hypercholesterolemia is an important risk factor associated with heart disease. For example, in December 1984, a National Institute of Health Consensus Development Conference Panel concluded that lowering definitely elevated blood cholesterol levels (specifically blood levels of low-density lipoprotein cholesterol) will reduce the risk of heart attacks due to coronary heart disease.

[0022] Typically, cholesterol is carried in the blood of warm-blooded animals in certain lipid-protein complexes such as chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). It is widely accepted that LDL functions in a way that directly results in deposition of the LDL cholesterol in the blood-vessel wall and that HDL functions in a way that results in the HDL picking up cholesterol from the vessel wall and transporting it to the liver where it is metabolized [Brown and Goldstein, Ann. Rev. Biochem. 52, 223 (1983); Miller, Ann. Rev. Med. 31, 97 (1980)]. For example, in various epidemiologic studies the LDL cholesterol levels correlate well with the risk of coronary heart disease whereas the HDL cholesterol levels are inversely associated with coronary heart disease [Patton et al., Clin. Chem. 29, 1980 (1983)]. It is generally accepted by those skilled in the art that reduction of abnormally high LDL cholesterol levels is effective therapy not only in the treatment of hypercholesterolemia but also in the treatment of atherosclerosis.

[0023] Furthermore, there is evidence based on animal and laboratory findings that peroxidation of LDL lipid, such as the unsaturated fatty acid portions of LDL cholesteryl esters and phospholipids, facilitate the accumulation of cholesterol in monocyte/macrophages which eventually are transformed into foam cells and become deposited in the sub-endothelial space of the vessel wall. The accumulation of foam cells in the vessel wall is recognized as an early event in the formation of an atherosclerotic plaque. Thus it is believed that peroxidation of LDL lipid is an important prerequisite to the facilitated accumulation of cholesterol in the vessel wall and the subsequent formation of an atherosclerotic plaque. For example, it has been shown that monocyte/macrophages take up and degrade native LDL at relatively low rates and without marked accumulation of cholesterol. In contrast, oxidized LDL is taken up by these monocyte/macrophages at much higher rates and with marked accumulation of cholesterol [Parthasarathy et al., J. Clin. Invest. 77, 641 (1986)]. It is therefore desirable to provide methods of inhibiting LDL lipid peroxidation in a patient in need thereof.

[0024] Elevated cholesterol levels are associated with a number of disease states, including restenosis, angina, cerebral atherosclerosis, and xanthoma. It is desirable to provide a method for reducing plasma cholesterol in patients with, or at risk of developing, restenosis, angina, cerebral arteriosclerosis, xanthoma, and other disease states associated with elevated cholesterol levels.

[0025] Since it has been determined that hypercholesterolemia is due to elevated LDL (hyperlipidemia), the lowering of LDL levels by dietary therapy is attempted. There are several drug classes that are commonly used to lower LDL levels, including bile acid sequestrants, nicotinic acid (niacin), and 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors. Probucol and the fibrate derivatives are sometimes used as adjunctive therapy, usually in combination with other medications. The HMG CoA reductase inhibitors have been termed statins or vastatins. Statins among the most effective agents currently on the market for hypercholesterolemia, and include pravastatin (Pravchol, Bristol Myers Squibb), atorvastatin (Warner Lambert/Pfizer), simvastatin (Zocor, Merck), lovastatin (Mevacor, Merck), and fluvastatin (Lescol).

[0026] Evidence suggests that the atherogenic effects of low density lipoprotein (LDL) may be in part mediated through its oxidative modification. Probucol has shown to possess potent antioxidant properties and to block oxidative modification of LDL. Consistent with these findings, probucol has shown to actually slow the progression of atherosclerosis in LDL receptor-deficient rabbits as discussed in Carew et al. Proc. Natl. Acad. Sci. U.S.A. 84: 7725-7729 (1987). Most likely, probucol is effective because it is highly lipid soluble and is transported by lipoproteins, thus protecting them against oxidative damage.

[0027] Probucol is chemically related to the widely used food additives 2,3-[3-tert-butyl-4-hydroxyanisole (BHA) and 2,6-di-tert-butyl-4-methyl phenol (BHT). Its full chemical name is 4,4’-(isopropylidenedithio) bis(2,6-di-tert-butylphenol).

[0028] Probucol is used primarily to lower serum cholesterol levels in hypercholesterolemic patients. Probucol is commonly administered in the form of tablets available under the trademark Loretco™. Unfortunately, probucol is almost insoluble in water and therefore cannot be injected intravenously. In fact, probucol is difficult for cells to absorb in vitro because of its poor miscibility in buffers and media for cell culture. Solid probucol is poorly absorbed into the blood, and is excreted in substantially unchanged form. Further, the tablet form of probucol is absorbed at significantly different rates and in different amounts by different patients. In one study (Heeg et al., Plasma Levels of Probucol in Man After Single and Repeated Oral Doses, La Nouvelle Presse Medicale, 9:2990-2994 (1980)), peak levels of probucol in sera were found to differ by as much as a factor of 20 from patient to patient. Another study, Kazuya et al. J. Lipid Res. 32; 197-204 (1991) observed an incorporation of less than about 1 µg of probucol/10^6 cells when endothelial cells are incubated for 24 h with 50 µM probucol.

[0029] U.S. Patent No. 5,262,439 to Parthasarathy discloses analogs of probucol with increased water solubility in
which one or both of the hydroxyl groups are replaced with ester groups that increase the water solubility of the compound. In one embodiment, the derivative is selected from the group consisting of a mono- or di- probucol ester of succinic acid, glutaric acid, adipic acid, sebamic acid, sebacic acid, azelaic acid, or maleic acid. In another embodiment, the probucol derivative is a mono- or di- ester in which the ester contains an alkyl or alkenyl group that contains a functionality selected from the group consisting of a carboxylic acid group, amine group, salt of an amine group, amide groups, and aldehyde groups.


[0031] A series of French patents disclose that certain probucol derivatives are hypocholesterolemic and hypolipemic agents: FR 2168137 (bis-4-hydroxyphenylthioalkane esters); FR 2140771 (tetralinyl phenoxy alkanoic esters of probucol); FR 2140769 (benzofuryloxyalkanoic acid derivatives of probucol); FR 2134810 (bis-(3-alkyl-5-1-alkyl-4-thiazole-5-carboxy)phenylthio)alkanes; FR 2133024 (bis-(4-2cinoxyloxyphenylthio)propanes; and FR 2130975 (bis-(4-(phenoxyalkanoyloxy)phenylthio)alkanes).

[0032] U. S. Patent No. 5,155,250 to Parker, et al. discloses that 2,6-dialkyl-4-silylphenols are antiatherosclerotic agents. The same compounds are disclosed as serum cholesterol lowering agents in PCT Publication No. WO 95/15760, published on June 15, 1995. U. S. Patent No. 5,608,095 to Parker, et al. discloses that alkylated-4-silylphenols inhibit the peroxidation of LDL, lower plasma cholesterol, and inhibit the expression of VCAM-1, and thus are useful in the treatment of atherosclerosis.

[0033] A series of European patent applications to Shionogi Seiyaku Kabushiki Kaisha disclose phenolic thioethers for use in treating arteriosclerosis. European Patent Application No. 348 203 discloses phenolic thioethers which inhibit the denaturation of LDL and the incorporation of LDL by macrophages. The compounds are useful as anti-arteriosclerosis agents. Hydroxamic acid derivatives of these compounds are disclosed in European Patent Application No. 405 788 and are useful for the treatment of arteriosclerosis, ulcer, inflammation and allergy. Carbamoyl and cyano derivatives of the phenolic thioethers are disclosed in U. S. Patent No. 4,954,514 to Kita, et al.

[0034] U. S. Patent No. 4,752,616 to Hall, et al., disclose arythioalkylphenylcarboxylic acids for the treatment of thrombotic disease. The compounds disclosed are useful as platelet aggregation inhibitors for the treatment of coronary or cerebral thromboses and the inhibition of bronchoconstriction, among others.


[0037] The Dow Chemical Company is the assignee of patents to hypolipidemic 2-(3,5-di-tert-butyl-4-hydroxyphenyl)thio carboxamides. For example, U. S. Patent Nos. 4,029,812, 4,076,841 and 4,078,084 to Wagner, et al., disclose these compounds for reducing blood serum lipids, especially cholesterol and triglyceride levels.

[0038] Given that cardiovascular disease is currently the leading cause of death in the United States, and ninety percent of cardiovascular disease is presently diagnosed as atherosclerosis, there is a strong need to identify new methods and pharmaceutical agents for its treatment. Important to this goal is the identification and manipulation of the specific oxidized biological compounds that act as selective regulators of the expression of mediators of the inflammatory process, and in particular, VCAM-1. A more general goal is to identify selective methods for suppressing the expression of redox sensitive genes or activating redox sensitive genes that are suppressed.

[0039] It is therefore an object of the present invention to provide new compounds, and compositions for the treatment of cardiovascular and inflammatory diseases.

[0040] It is still another object of the present invention to provide new compounds and compositions which are useful as inhibitors of LDL lipid peroxidation.

[0041] It is still another object of the present invention to provide new compounds and compositions which are useful as antiatherosclerotic agents.

[0042] It is still another object of the present invention to provide new compounds and compositions which are useful as LDL lipid lowering agents.

**SUMMARY OF THE INVENTION**

[0043] The present invention provides a compound and composition for inhibiting the expression of VCAM-1, and thus can be used in the treatment of a disease mediated by VCAM-1, which includes administering the compound of the invention or a pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier.

[0044] Accordingly the present invention provides a compound of the following formula:
or a pharmaceutically acceptable salt thereof.

[0045] The invention also provides a pharmaceutical composition comprising an effective treatment amount of the compound of the invention or a pharmaceutically acceptable salt thereof.

[0046] The invention also provides the use of the compound of the invention or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of an inflammatory disorder or a cardiovascular disease or a disorder selected from asthma, dermatitis, multiple sclerosis, psoriasis, rheumatoid arthritis and osteoarthritis or a disorder selected from atherosclerosis, post-angioplasty restenosis, coronary artery disease, angina and small artery disease.

DETAILED DESCRIPTION OF THE INVENTION

[0047] The term alkyl, as used herein, unless otherwise specified, refers to a saturated straight, branched, or cyclic, primary, secondary, or tertiary hydrocarbon of C₁ to C₁₀, and specifically includes methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, isobutyl, t-butyl, pentyl, cyclopentyl, isopentyl, neopentyl, hexyl, 2-hexyl, cyclohexyl, cyclohexylmethyl, 3-methylpentyl, 2,2-dimethylbutyl, and 2,3-dimethylbutyl. The alkyl group can be optionally substituted with one or more moieties selected from the group consisting of alkyl, halo, hydroxyl, carboxyl, acyl, acyloxy, amino, alkyamine, alkoxy, aryl, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference.

[0048] The term lower alkyl, as used herein, and unless otherwise specified, refers to a C₁ to C₅ saturated straight, branched, or if appropriate, a cyclic (for example, cyclopropyl) alkyl group.

[0049] Likewise the term alkylene refers to a saturated hydrocarbyldiyl radical of straight or branched configuration made up of from one to ten carbon atoms. Included within the scope of this term are methylene, 1,2-ethane-diyl, 1,1-ethane-diyl, 1,3-propane-diyl, 1,2-propane-diyl, 1,3-butanediyl, 1,4-butanediyl and the like. The alkylene group can be optionally substituted with one or more moieties selected from the group consisting of alkyl, halo, hydroxyl, carboxyl, acyl, acyloxy, amino, alkyamine, alkoxy, aryl, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference.

[0050] The term “-(CH₂)ₙ-” represents a saturated hydrocarbyldiyl radical of straight chain configuration. The term “n” is defined as 0-10. The moiety “-(CH₂)ₙ-” thus represents a bond (i.e., when n=0), methylene, 1,2-ethanediyl or 1,3-propanediyl, etc.

[0051] The term aryl, as used herein, and unless otherwise specified, refers to phenyl, biphenyl, or naphthyl, and preferably phenyl. The term aralkyl, as used herein, and unless otherwise specified, refers to an aryl group as defined above linked to the molecule through an alkyl group as defined above. In each of these groups, the alkyl group can be optionally substituted as described above and the aryl group can be optionally substituted with one or more moieties selected from the group consisting of alkyl, halo, hydroxyl, carboxyl, acyl, acyloxy, amino, alkyamine, arylamine, alkoxy, aryloxy, nitro, cyano, sulfonic acid, sulfate, phosphonic acid, phosphate, or phosphonate, either unprotected, or protected as necessary, as known to those skilled in the art, for example, as taught in Greene, et al., Protective Groups in Organic Synthesis, John Wiley and Sons, Second Edition, 1991, hereby incorporated by reference.

[0052] The term “protected” as used herein and unless otherwise defined refers to a group that is added to an oxygen, nitrogen, or phosphorus atom to prevent its further reaction or for other purposes. A wide variety of oxygen and nitrogen protecting groups are known to those skilled in the art of organic synthesis.
The term halo, as used herein, includes chloro, bromo, iodo, and fluoro.

The term alkoxy, as used herein, and unless otherwise specified, refers to a moiety of the structure -O-alkyl, wherein alkyl is as defined above.

The term acyl, as used herein, refers to a group of the formula C(O)R', wherein R' is an alkyl, aryl, alkaryl or aralkyl group, or substituted alkyl, aryl, aralkyl or alkaryl, wherein these groups are as defined above.

As used herein, the term polyunsaturated fatty acid (PUFA) refers to a fatty acid (typically C_8 to C_24) that has at least one alkyl bond, and includes but is not limited to linoleic (C_18^Δ6,9,12), linolenic (C_18^Δ6,9,12), arachidonic (C_20^Δ6,9,11,14).

The term oxidized polyunsaturated fatty acid (ox-PUFA) refers to an unsaturated fatty acid in which at least one of the alkenyl bonds has been converted to a hydroperoxide. Nonlimiting examples are 13-HPODE and 15-HPETE.

The term pharmaceutically acceptable salts or complexes refers to salts or complexes that retain the desired biological activity of the compounds of the present invention and exhibit minimal undesired toxicological effects. Non-limiting examples of such salts are (a) acid addition salts formed with inorganic acids (for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, and the like), and salts formed with organic acids such as acetic acid, oxalic acid, tartaric acid, succinic acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, naphthalenedisulfonic acid, and polygalcturonic acid; (b) base addition salts formed with metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, sodium, potassium, and the like, or with a cation formed from ammonia, N,N-dibenzylethylenediamine, D-glucosamine, tetraethylammonium, or ethylenediamine; or (c) combinations of (a) and (b); e.g., a zinc tannate salt or the like. Also included in this definition are pharmaceutically acceptable quaternary salts known by those skilled in the art, which specifically include the quaternary ammonium salt of the formula -NR+ A-, wherein R is alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, alkoxy, acyl, alkyl-COOH, alkyl-COOalkyl, alkyl-COOaryl, heteroaryl or substituted heteroaryl; and A is a counterion, including chloride, bromide, iodide, -O-alkyl, toluenesulfonate, methylsulfonate, sulfonate, phosphate, or carboxylate (such as benzoate, succinate, acetate, glycolate, maleate, malate, citrate, tartrate, ascrobate, benzoate, cinnamoate, mandeloate, benzylate, and diphenylacetate).

Diseases mediated by the VCAM-1 include, but are not limited to atherosclerosis, post-angioplasty restenosis, coronary artery disease, angina, small artery disease, and other cardiovascular diseases, as well as noncardiovascular inflammatory diseases such as rheumatoid arthritis, osteoarthritis, asthma, dermatitis, multiple sclerosis and psoriasis.

The compound of the invention can be prepared by known procedures and techniques, or routine modifications thereof. A general synthetic scheme for preparing compounds of the invention is set forth in Scheme A, wherein all substituents, unless otherwise indicated, are previously defined.

Scheme A

A quantity of probucol (commercially available from Sigma Chemicals) in a 0.1 M solution of tetrahydrofuran is treated with 2 equivalents of sodium hydride and stirred at room temperature for 30 minutes. To the reaction mixture is added 3 equivalents of a primary alkyl bromide or iodide and the reaction stirred at room temperature for 16 hours. The reaction is quenched with 1 N aqueous HCl and diluted with ethyl acetate. The aqueous layer is removed and the ethyl acetate layer is washed with water and then with an aqueous saturated sodium chloride solution. The ethyl acetate solution is dried over magnesium sulfate, gravity or vacuum filtered, and then concentrated. The product is purified by silica gel chromatography.

An alternative method for the preparation of the compound of the invention is the treatment of probucol with a primary alcohol according to the method of Mitsunobu (Synthesis, 1981, 1).

A second alternative method for the preparation of the compound of the invention is the treatment of probucol with a primary alkyl bromide or iodide in acetonitrile in the presence of potassium fluoride absorbed on alumina according to the method of Ando et al. (Bull. Chem. Soc. Jpn., 55, 1982, 2504-2507).

Starting materials for use in the general synthetic procedures outlined in the above reaction schemes are readily available or can readily be prepared according to standard techniques and procedures. Probucol is readily available from Sigma Chemicals.

The following example presents a typical syntheses as described in Scheme A. This example is understood to be illustrative only and are not intended to limit the scope of the present invention in any way.
Example 1

Acetic acid, [4-[1-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]thio]-1-methylethyl]thio]2,6-bis (1,1-dimethylethyl)phenoxy]-

Reaction Description:

[0067] To dimethylformamide (1.5 mL) was added probucol (0.5 g, 0.967 mmol) and ethyl-2-iodo acetate (0.31 g, 1.45 mmol) and 40% potassium fluoride on alumina (0.7 g) and the reaction was stirred for 24 hours. The reaction mixture was diluted with ether (25 mL), filtered and washed with water (2X5 mL). The ether layer was dried over MgSO₄, filtered and concentrated. The resulting oil was purified by radial silica gel chromatography by elution with 5:95 ether/hexanes to yield 160 mg of the ethyl ester of the product. The ethyl ester dissolved in THF:H₂O:MeOH(4:1:1)(4 mL) and LiOH·H₂O (50 mg) was added and the reaction stirred for 1 h. The reaction was neutralized with 1N HCl and extracted with ether (2X10 mL), dried over MgSO₄, filtered, and concentrated. Silica gel chromatography and elution with 50:50 ether/hexanes gave 90 mg of the product. 1H NMR (CDCl₃, 400 MHz): δ 7.55 (s, 2 H), 7.40 (s, 2 H), 5.35 (s, 1 H), 4.40 (s, 2 H), 1.43 (s, 6 H), 1.41 (s, 9 H), 1.39 (s, 9 H).

[0068] The following examples illustrate the use of the compound according to the present invention. These examples are illustrative only and are not intended to limit the scope of the invention in any way.

Example 2

LIPID SCREEN & IC₅₀ DETE RMINATION PROTOCOL

Preparation of HEPG2:

[0069] HEPG2 cell was started in 10 ml of MEM, 10% FBS, 1mM Sodium Pyruvate. The cells were incubated in a tissue culture incubator. The cells were split into 4X96-wells plate in MEM, 10% FBS, 1mM Sodium Pyruvate and allowed to grow to about 50% confluency and then removed.

Day 1 Treatment:

[0070] The cells were treated with the desired concentration of compounds in 100 µl DMEM, 1% RSA for 24 hours. The compounds are dissolved in DMSO. For IC₅₀, the range of concentration is 10µM - 40µM, with each concentration being done in triples. On the same day, 4X96-wells NuncImmunoSorb plate is coated with 100 µl of mouse anti-human ApoB monoclonal 1D1 (1:1000 dilution in 1XPBS, pH 7.4). The coating is allowed overnight.

Day 2 ApoB ELISA:

[0071] The coated plate is washed 3 times with 1XPBS, pH 7.4, -0.05% Tween 20. 100 µl of the standards is added to the selected wells. ApoB standards are prepared at 6.25, 3.12, 1.56, 0.78, 0.39 ng, and each concentration is done in triplicates. For samples:

[0072] 90 µl of 1XPBS, pH 7.4, -0.05% Tween 20 is added to each well corresponding to the sample. 10 µl of media is transferred from the treated HEPG2 plates to the ApoB ELISA plate. The plate is incubated at room temperature for 2 hours, rocking gently. Wash the coated plate 3X with 1XPBS, pH 7.4, -0.05% Tween 20. Add 100 µl of sheep anti-human ApoB polyclonal from Boehringer Mannheim. (1:2000 dilution in 1XPBS, pH 7.4, -0.05% Tween 20) from Boehringer Mannheim. Incubate at room temperature for 1 hour, rocking gently. Wash the coated plate 3X with 1XPBS, pH 7.4, -0.05% Tween 20. Add 100 µl of rabbit anti-sheep IgG (1:2000 dilution in 1XPBS, pH 7.4, -0.05% Tween 20). Incubate at room temperature for 1 hour, rocking gently. Wash the coated plate 3X with 1XPBS, pH 7.4, -0.05% Tween 20. Add 100 µl of substrate (10 ml of distilled water, 100 µl of TMB (10mg/ml), and 1 µl of hydrogen peroxide). Allow color to emerge and stop reaction with 25ul of 8N sulfuric acid. Wells are read with MicroPlate Reader @ 450nM. Graph accumulation of ApoB in media as a percentage of control for each sample and their concentration. A determination of IC₅₀ is obtained from the graph.
Example 3

**VCAM-1 Assay**

**Splitting the cells:**

[0073] Two to four confluent P150 plates are trypsinized and the cells transferred to a 50 mL conical centrifuge tube. The cells are pelleted, resuspended, and counted using the trypan blue exclusion method.

[0074] Cells are resuspended at a concentration of 36,000 cells/mL and 1 mL is aliquoted per well.

[0075] Cells are split into 24 well tissue culture plates. The cells in each well should be approximately 90-95% confluent by the following day. Cells should not be older than passage 8.

**Preparation of compounds:**

**Water soluble compounds**

[0076] Compounds are initially screened at 50 µM and 10 µM. A 50 mM stock solution for each compound is prepared in culture medium. The stock solution is diluted to 5 mM and 1 mM. When 10 µL of the 5 mM solution is added to the well (1 mL medium/well), the final concentration will be 50 µM. Adding 10 µL of the 1 mM solution to the well will give a final concentration of 10 µM.

**Water insoluble compounds**

[0077] Compounds which will not go into solution in culture medium are resuspended in DMSO at a concentration of 25 mM. The stock solution is then diluted to the final concentration in culture medium. The old medium is aspirated and 1 mL of the new medium with the compound is added. For example, if the final concentration is 50 µM, the 2 µL of the 25 mM stock is added per mL of culture medium. The 50 mM solution is diluted for lower concentrations.

**Adding the compounds**

[0078] The compounds are added to the plate (each compound is done in duplicate). One plate is done for VCAM expression and one plate is done for ICAM expression.

[0079] Immediately after the compounds are added, TNF is added to each well. 100 units/mL TNF is usually added to each well. Since each lot of TNF varies in the number of units, each new lot is titrated to determine the optimum concentration. Therefore this concentration will change. If 100 units/mL is being used, dilute the TNF to 10 units/µL and add 10 µL to each well.

[0080] The plates are incubated at 37°C, 5% CO₂ overnight (approximately 16 hours). The next day the plates are checked under the microscope to see if there are any visual signs of toxicity. Records are made of any cell death, debris, or morphology changes, as well as insoluble compounds (particulate or turbidity).

Example 4

**ELISA Assay**

[0081] In order to assess MCP-1, the media (500 µL) is saved and frozen at -70°C. Wash cells once with roughly 1 ml/well of Hanks Balance Salt Solution (HBSS) or PBS. Gently empty the wash solution and then tap the plate onto paper towels. Add either 250 µL/well of HBSS +5% FCS to the plank (no primary antibody wells) or 250 µL/well of primary antibody diluted in HBSS +5% FCS. Incubate for 30 minutes at 37°C. Wash the wells twice with .5 mL/well HBSS or PBS and gently tap the plates onto paper towels after the last wash. Add 250µL/well of HRP-conjugated second antibody diluted in HBSS +5% FCS to every well including the blank wells (no primary antibody). Incubate at 37°C for 30 minutes. Wash the wells four times with .5 mL/well HBSS or PBS and gently tap the plates onto paper towels after the last wash. Add 250 µL/well of substrate solution. Incubate at room temperature in the dark until there is adequate color development (blue). Note the length of time incubation was performed (typically 15-30 minutes). Add 75 µL/well stopper solution (8N sulfuric acid), and read A450 nm.
Antibodies and solutions

[0082]

1. Substrate solution is made immediately prior to use and contains:

<table>
<thead>
<tr>
<th>Water</th>
<th>10 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% hydrogen peroxide</td>
<td>1 µL</td>
</tr>
<tr>
<td>TMB (3,3',5,5'-tetramethylbenzidine)</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

TMB stock solution: To 10 mg TMB, add 1 mL acetone. Store at 4°C protected from light.

2. VCAM-1 Ab: stock .1 µg/µL final concentration 0.25 µg/mL

   mix 25 µL stock VCAM-1 (Southern Biotechnology) and 10 mL HBSS + 5% FCS

3. ICAM-1 Ab: stock .1 µg/µL final concentration 0.25 µg/mL

   mix 25 µL stock ICAM-1 (Southern Biotechnology) and 10 mL HBSS + 5% FCS

4. Secondary Ab: HRP-conjugated goat antimouse IgG diluted 1:500

   mix 20 µL stock (Southern Biotechnology) and 10 mL HBSS + 5% FCS

[0083] The degree of inhibition of the compound of the invention was determined by the assays described in Examples 2-4. The results are provided in Table 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>VCAM-1 IC$_{50}$ or % inhibition at [µM]</th>
<th>LD$_{50}$</th>
<th>ApoB/HepG2 IC$_{50}$ or % inhibition at [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid, [4-[[1-[[3,5-bis</td>
<td>10</td>
<td>50</td>
<td>NE</td>
</tr>
<tr>
<td>(1,1-dimethylethyl)-4-hydroxyphenyl][thio]-1-methylethyl][thio][2,6-bis (1,1-dimethylethyl)[phenoxy]-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pharmaceutical Compositions

[0084] Mammals, and specifically humans, suffering from any of the above-described conditions can be treated by the topical, systemic or transdermal administration of a composition comprising an effective amount of the compound of the invention or a pharmaceutically acceptable salt thereof, optionally in a pharmaceutically acceptable carrier or diluent.

[0085] The composition is administered subcutaneously, intravenously, intraperitoneally, intramuscularly, parenterally, orally, submucosally, by inhalation, transdermally via a slow release patch, or topically, in an effective dosage range to treat the target condition. An effective dose can be readily determined by the use of conventional techniques and by observing results obtained under analogous circumstances. In determining the effective dose, a number of factors are considered including, but not limited to: the species of patient; its size, age, and general health; the specific disease involved; the degree of involvement or the severity of the disease; the response of the individual patient; the particular compound administered; the mode of administration; the bioavailability characteristics of the preparation administered; the dose regimen selected; and the use of concomitant medication. Typical systemic dosages for all of the herein described conditions are those ranging from 0.1 mg/kg to 500 mg/kg of body weight per day as a single daily dose or divided daily doses. Preferred dosages for the described conditions range from 5-1500 mg per day. A more particularly preferred dosage for the desired conditions ranges from 25-750 mg per day. Typical dosages for topical application are those ranging from 0.001 to 100% by weight of the active compound.

[0086] The compound is administered for a sufficient time period to alleviate the undesired symptoms and the clinical signs associated with the condition being treated.

[0087] The active compound is included in the pharmaceutically acceptable carrier or diluent in an amount sufficient to deliver to a patient a therapeutic amount of compound in vivo in the absence of serious toxic effects.

[0088] The concentration of active compound in the drug composition will depend on absorption, inactivation, and excretion rates of the drug as well as other factors known to those of skill in the art. It is to be noted that dosage values will also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional
A preferred mode of administration of the active compound for systemic delivery is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Pharmacologically compatible binding agents, and/or adjuvant materials can be included as part of the composition.

The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

The compound or its salts can be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preserving agents, dyes and colorings and flavors.

The compound can also be mixed with other active materials which do not impair the desired action, or with materials that supplement the desired action. The active compounds can be administered in conjunction with other medications used in the treatment of cardiovascular disease, including lipid lowering agents such as probucol and nicotinic acid; platelet aggregation inhibitors such as aspirin; antithrombotic agents such as coumadin; calcium channel blockers such as varapamil, diltiazem, and nifedipine; angiotensin converting enzyme (ACE) inhibitors such as captopril and enalopril, and β-blockers such as propanalol, terbutalol, and labetolol. The compounds can also be administered in combination with nonsteroidal antinflammatories such as ibuprofen, indomethacin, fenoprofen, mefenamic acid, flufenamic acid, sulindac. The compound can also be administered with corticosteroids.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

If administered intravenously, preferred carriers are physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS).

In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patents No. 4,522,811. For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearyl phosphatidyl ethanolamine, stearyl phosphatidyl choline, arachadyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the compound is then introduced into the container. The container is then swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

Suitable vehicles or carriers for topical application can be prepared by conventional techniques, such as lotions, suspensions, ointments, creams, gels, tinctures, sprays, powders, pastes, slow-release transdermal patches, suppositories for application to rectal, vaginal, nasal or oral mucosa. In addition to the other materials listed above for systemic administration, thickening agents, emollients, and stabilizers can be used to prepare topical compositions. Examples of thickening agents include petrolatum, beeswax, xanthan gum, or polyethylene, humectants such as sorbitol, emollients such as mineral oil, lanolin and its derivatives, or squalene.
Claims

1. A compound of the following formula:

   ![Chemical Structure](image)

   or a pharmaceutically acceptable salt thereof.

2. A pharmaceutical composition comprising an effective treatment amount of a compound of claim 1.

3. The composition of claim 2 suitable for oral administration.

4. The composition of claim 2 suitable for topical or transdermal administration.

5. The composition of claim 2 suitable for intravenous, subcutaneous, intraperitoneal, or intramuscular administration.

6. The composition of claim 2 suitable for submucosal administration.

7. The composition of claim 2 suitable for inhalation administration.

8. A compound according to claim 1 for use in therapy.

9. Use of a compound according to claim 1 in the manufacture of a medicament for the treatment of an inflammatory disorder.

10. Use of a compound according to claim 1 in the manufacture of a medicament for the treatment of a cardiovascular disease.

11. Use of a compound according to claim 1 in the manufacture of a medicament for the treatment of a disorder selected from asthma, dermatitis, multiple sclerosis, psoriasis, rheumatoid arthritis and osteoarthritis.

12. Use of a compound according to claim 1 in the manufacture of a medicament for the treatment of rheumatoid arthritis.

13. Use of a compound according to claim 1 in the manufacture of a medicament for the treatment of a disorder selected from atherosclerosis, post-angioplasty restenosis, coronary artery disease, angina, small artery disease.


15. A compound of claim 1 in conjunction with a non-steroidal anti-inflammatory drug selected from ibuprofen, indomethacin, fenoprofen, mefenamic acid, flufenamic acid and sulindac for use in therapy.

16. A compound of claim 1 in conjunction with a corticosteroid for use in therapy.

17. A compound of claim 1 in conjunction with a cardiovascular drug for use in therapy.

18. A compound of claim 1 in conjunction with a cardiovascular drug selected from lipid lowering agents, platelet aggregation inhibitors, antithrombotic agents, calcium channel blockers, angiotension converting enzyme (ACE) inhibitors and β-blockers for use in therapy.
Patentansprüche

1. Eine Zusammensetzung gemäß folgender Formel:

![Chemical structure diagram]

oder ein pharmazeutisch brauchbares Salz dessen.

2. Eine pharmazeutische Zusammensetzung, die eine wirksame Behandlungsmenge der Zusammensetzung gemäß Anspruch 1 enthält.

3. Eine Zusammensetzung gemäß Anspruch 2, die für eine orale Gabe brauchbar ist.

4. Zusammensetzung nach Anspruch 2, die für topische oder transthermale Gabe brauchbar ist.

5. Zusammensetzung gemäß Anspruch 2, die zur intravenösen, subkutanen, intraperitonealen oder intramuskulären Gabe brauchbar ist.

6. Zusammensetzung gemäß Anspruch 2, die zur submukosalen Gabe brauchbar ist.

7. Zusammensetzung gemäß Anspruch 2, die zur Inhalation brauchbar ist.

8. Zusammensetzung gemäß Anspruch 1 zur therapeutischen Verwendung.


17. Eine Zusammensetzung gemäß Anspruch 1 in Verbindung mit einem kardiovaskulären Medikament zur therapeutischen Verwendung.


Revendications

1. Composé ayant la formule suivante :

![Chemical structure]

ou l'un de ses sels acceptables d'un point de vue pharmaceutique.

2. Composition pharmaceutique comportant une quantité correspondant à un traitement efficace d'un composé suivant la revendication 1.

3. Composition suivant la revendication 2, appropriée pour une administration par voie orale.

4. Composition suivant la revendication 2, appropriée pour une administration par voie topique ou transdermique.

5. Composition suivant la revendication 2, appropriée pour une administration par voie intraveineuse, sous-cutanée, intrapéritonéale ou intramusculaire.

6. Composition suivant la revendication 2, appropriée pour une administration par voie sous-muqueuse.

7. Composition suivant la revendication 2, appropriée pour une administration par inhalation.

8. Composé suivant la revendication 2, destiné à être utilisé en thérapeutique.

9. Utilisation d'un composé suivant la revendication 1 pour la fabrication d'un médicament pour le traitement de troubles inflammatoires.

10. Utilisation d'un composé suivant la revendication 1 pour la fabrication d'un médicament pour le traitement d'une maladie cardiovasculaire.

11. Utilisation d'un composé suivant la revendication 1 pour la fabrication d'un médicament pour le traitement d'un trouble choisi parmi l'asthme, la dermatite, la sclérose multiple, le psoriasis, l'arthrite rhumatoïde et l'ostéoarthrite.

12. Utilisation d'un composé suivant la revendication 1 pour la fabrication d'un médicament pour le traitement du rhumatisme articulaire.

13. Utilisation d'un composé suivant la revendication 1 pour la fabrication d'un médicament pour le traitement d'un trouble sélectionné parmi l'artériosclérose, la resténose post-angioplastie, la maladie des artères coronaires, l'angine et les petites maladies artérielles.
14. Composé suivant la revendication 1, en liaison avec un autre médicament anti-inflammatoire pour l'utilisation en thérapeutique.

15. Composé suivant la revendication 1, en liaison avec un médicament anti-inflammatoire non stéroïde sélectionné parmi l'ibuprofène, l'indométhacine, la fénoprofène, l'acide méfénamique, l'acide flufénamique et le sulindac à utiliser lors d'une thérapeutique.

16. Composé suivant la revendication 1, en liaison avec un corticostéroïde à utiliser dans une thérapeutique.

17. Composé suivant la revendication 1, en liaison avec un médicament cardiovasculaire à utiliser dans une thérapeutique.

18. Composé suivant la revendication 1, en liaison avec un médicament cardiovasculaire sélectionné parmi des agents abaisseurs de lipides, des inhibiteurs d'agrégation de plaquettes, des agents antithrombotiques, des bloqueurs de canal de calcium, des inhibiteurs d'enzymes convertisseurs d'angiotension (ACE) et des β-bloquants à utiliser dans une thérapeutique.