

BASIC ESTERS OF FATTY ALCOHOLS AND THEIR USE AS ANTI-INFLAMMATORY OR IMMUNOMODULATORY AGENTS

5

FIELD OF THE INVENTION

The present invention relates to basic esters of fatty alcohols and their use as anti-inflammatory and immunomodulatory agents, particularly useful in the treatment of immunologically-mediated inflammation.

Abbreviations: **AA:** adjuvant arthritis; **CFA:** complete Freund's adjuvant; **DTH:** delayed-type hypersensitivity **EAE:** experimental autoimmune encephalomyelitis; **GA:** glatiramer acetate; **IFA:** incomplete Freund's adjuvant; **IV:** intravenously; **MBP:** myelin basic protein; **MS:** multiple sclerosis; **MSCH:** mouse spinal cord homogenate; **OA:** oleyl alcohol; **PBS:** phosphate-buffered saline; **SC:** subcutaneously.

BACKGROUND OF THE INVENTION

20 1. Inflammation

Inflammation is commonly divided into three phases: acute inflammation, the immune response and chronic inflammation. Acute inflammation is the initial response to tissue injury and is mediated by the release of histamine, serotonin, bradykinin, prostaglandins and leukotrienes. The immune response, usually preceded by the acute inflammation phase, occurs when immunologically competent cells are activated in response to foreign organisms or antigenic substances liberated during the acute or chronic inflammatory response. The outcome of the immune response for the host may be beneficial, as it causes invading organisms to be phagocytosed or neutralized. However, the outcome may

be deleterious if it leads to chronic inflammation without resolution of the underlying injurious process as occurs in rheumatoid arthritis.

The treatment of patients with inflammation leads to the slowing or arrest of the tissue-damaging process as well as the relief of pain, which is the presenting
5 symptom and the major continuing complaint of the patient.

Anti-inflammatory agents are usually classified as steroidal or glucocorticoids and nonsteroidal anti-inflammatory agents (NSAIDs). The glucocorticoids are powerful anti-inflammatory agents but the high toxicity associated with chronic corticosteroid therapy inhibits their use except in certain
10 acute inflammatory conditions. Therefore, the nonsteroidal anti-inflammatory drugs have assumed a major role in the treatment of chronic conditions such as rheumatoid arthritis.

Among the non-steroidal anti-inflammatory agents are included derivatives of aminoarylcarboxylic acids, arylacetic acids, arylbutyric acids, arylcarboxylic
15 acids, arylpropionic acids, pyrazole, pyrazolone, salicylic acid and some other derivatives of different chemical structure, including specific anti-arthritic/anti-rheumatic agents.

It would be highly desirable to provide new nonsteroidal anti-inflammatory agents that could serve as alternatives to current anti-inflammatory drugs.

2. References related to the art

Some fatty alcohols and esters of fatty acids have been described as solvents or emulsifiers for use in pharmaceutical compositions. For example, cetyl alcohol may be used in pharmaceutical compositions as emulsifying and stiffening agent (The Merck Index, 2001, 13th edition, pp. 347-8, # 2037), oleyl alcohol may be used as a carrier for medicaments (The Merck Index, 2001, 13th edition, p. 1222, # 6900), and alkyl esters of oleic acid may be used as solvents for medicaments (The Merck Index, 2001, 13th edition, p. 6899, # 6898).

A mixture of higher aliphatic primary alcohols, primarily isolated from beeswax, was described as having moderate anti-inflammatory activity. The composition of such a mixture was not disclosed (Rodriguez et al., 1998).

The mass spectra of nicotines of long chain alcohols, e.g. octadecyl and (Z)-9-octadecen-1-yl nicotines, have been studied to elucidate the structure of long chain alcohols (Vetter and Meister, 1981). No biologic activity was assigned to the compounds.

Esters of 4-aminomethyl-benzoic acid (PAMBA) with C₆-C₁₆ saturated alcohols, e.g. decyl, undecyl, tetradecyl and hexadecyl alcohols, have been tested

for their antifibrinolytic activity and found to be not active (Markwardt et al., 1966). PAMBA esters with short chain alcohols were found to be able to decrease the proliferation of in vitro cultivated endothelial cells, the hexyl ester being the more effective (Beyer and Pilgrim, 1991).

- 5 Alkyl N,N-disubstituted amino acids, e.g. alkyl N,N-dimethylamino acetate wherein the alkyl is octyl, decyl, dodecyl, or tetradecyl, and decyl (4-methyl-1-piperazinyl)acetate, have been described as transdermal penetration enhancers for indomethacin and possibly for other drugs (Wong et al., 1989; US 4,980,378). Crystalline acid addition salts of dodecyl-2-methyl-2-(N,N-dimethyl amino acetate
10 (DDAIP), effective as skin penetration enhancers, are disclosed in US 6,118,020.

Complexes for use in gene therapy comprising a therapeutically active substance and a cationic lipid such as quaternary piperazinium compounds substituted at both the 1 and 4 positions by a methyl and an oleyloxycarbonylmethyl radicals are described in US 6,291,423.

- 15 Esters of N,N-dimethyl-aminoacetic acid with long chain alkanols, e.g. tetradecyl, cetyl and stearyl alcohols, and alkenols, are described in JP 2000-302650 for use in hair cosmetics. The oleyl ester is not specifically disclosed.

Betaine [(carboxymethyl)trimethylammonium hydroxide inner salt] esters with long-chain alcohols such as decyl, lauryl, myristyl, palmityl, stearyl and oleyl
20 alcohol were prepared and their pharmacodynamic properties have been studied (Metayer and Jacob, 1952), or their activity as biocides for cooling water treatment was tested (Rucka et al., 1983).

Quaternary ammonium salts of lauryl, myristyl and cetyl esters of N-carboxymethyl-piperidine, -piperazine and -morpholine compounds were described
25 as germicides (Smith et al., 1951).

Stearyl esters of amino acids, e.g. glycine, phenylglycine, alanine, valine, leucine, lysine, proline, phenylalanine, and tyrosine, and stearyl esters of peptides have been proposed as adjuvants for bacterial and viral human vaccines (Penney et al., 1985, 1993; Nixon-George et al., 1990).

Esters of DL- ω -phenyl-amino acids with C₄-C₁₀ alkanols, such as DL-2-phenylglycine octyl or decyl ester or DL-2-(4-dimethylaminophenyl)glycine octyl ester have been described as antiphlogistic, antihistaminic, spasmolytic, antioxidant

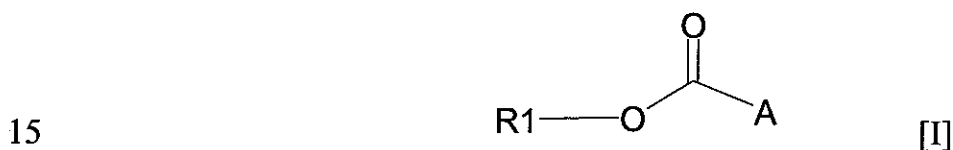
and anti-inflammatory (Schulz et al., 1982; Schewe et al., 1991; Kontogiorgis et al., 2001).

Higher alkyl esters of amino acid, e.g. lauryl, myristyl, cetyl and stearyl esters of glycine, phenylglycine, alanine, valine, norvaline, leucine, isoleucine, lysine, and phenylalanine, and their N-lower alkyl derivatives are described in US 3,821,403 (Misato et al., 1974) as useful for control of plant diseases.

SUMMARY OF THE INVENTION

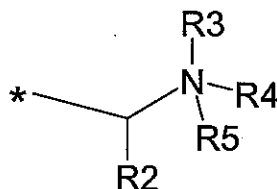
It has now been found, in accordance with the present invention, that certain esters of long-chain fatty alcohols with carboxylic acids containing at least one basic group, act as anti-inflammatory immunomodulators and, therefore, can be used for the treatment of inflammation, particularly immunologically-mediated inflammation.

In one aspect, the present invention relates to the use of a compound of the general formula I:



or of a pharmaceutically acceptable salt thereof, wherein R1 is C₁₂-C₂₄ alkyl or C₁₀-C₂₄ alkenyl, and A is a residue selected from the group consisting of:

(i)



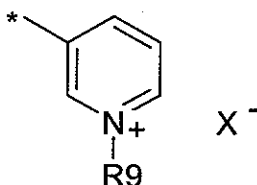
20 wherein R2 is H, C₁-C₆ alkyl, aryl, or aralkyl, wherein any aryl moiety may be unsubstituted or substituted by nitro, cyano, halo, hydroxy, NR₆R₇, or

CR8R8NR6R7; R3 is H, a pair of electrons or C₁-C₆ alkyl; R4 and R5 each independently is H or C₁-C₆ alkyl, or R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and

5 R6, R7 and R8 each independently is H or C₁-C₆ alkyl;

(ii) phenyl substituted by NR6R7 or CR8R8NR6R7, wherein R6, R7 and R8 each independently is H or C₁-C₆ alkyl; and

(iii)



10 wherein R9 is H, lower alkyl or indolyl(C₁-C₄)alkyl, and X⁻ is a counter ion, or R9 is a pair of electrons and X is absent, for the preparation of a pharmaceutical composition for treatment of inflammation.

In another aspect, the present invention relates to pharmaceutical compositions for the treatment of inflammation comprising a compound of the general formula I.

Some of the compounds of the formulas above, as defined hereinafter, are
5 novel and as such constitute a further aspect of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the effect of N,N-dimethyl-aminoacetic acid octadec-(Z)-9-enyl ester (Compound 1) on adjuvant arthritis (AA). Compound 1 was administered
10 intravenously (IV) to Lewis rats on the day of immunization (day 0, triangles) or on day 14, after the onset of AA (day +14, circles).

Fig. 2 shows the effect of Compound 1 on experimental autoimmune encephalomyelitis (EAE). Compound 1 was administered IV to DA rats on the day of immunization (day 0, circles).

15 Fig. 3 shows the effect of Compound 1 on skin allograft survival. Compound 1 was administered IV to mice on the day of grafting (day 0, circles).

Fig. 4 shows inhibition of delayed-type hypersensitivity (DTH) reaction in BALB/c mice after treatment with different concentrations (1, 2 or 4 mg) of (4-methyl-piperazin-1-yl)-acetic acid octadec-(Z)-9-enyl ester tartrate (Compound 3).

Fig 5 shows the effect of different concentrations (0.5, 1, 2 or 4 mg) of Compounds 5, 9, and 11 on DTH in mice. Dexamethazone (2 mg) was used as positive control and (4-methyl-piperazin-1-yl)-acetic acid ethyl ester HCl (0.5, 1, 2 or 4 mg) as negative control.

5 Fig. 6 shows the effect of subcutaneous (SC) administration of different concentrations (10, 50, 100, 200 mg) of Compound 3 on AA in rats. Oleyl alcohol (OA, 100 mg) was used for comparison.

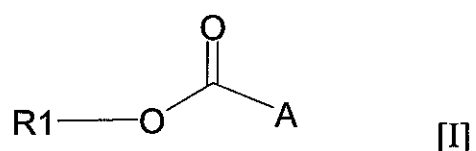
Fig. 7 shows the effect of SC administration of different concentrations (10, 50, 100, 200 mg) of Compound 3 on EAE in rats. Oleyl alcohol (OA, 100 mg) was
10 used for comparison.

Fig. 8 shows the effect of per os administration of different concentrations (0.2, 1, 2, 10, 20 mg) of Compound 3 on EAE in rats. Oleyl alcohol (OA, 10 mg) was used for comparison.

Fig. 9 shows the effect of per os administration of piperazin-1-yl-acetic acid
15 octadec-(Z)-9-enyl ester bitartrate (Compound 9, 100mg/kg) on EAE in rats.

DETAILED DESCRIPTION OF THE INVENTION

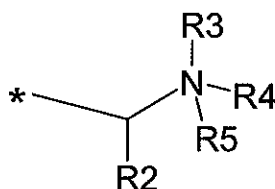
According to one aspect, the present invention provides the use of a
20 compound of the general formula I:



or of a pharmaceutically acceptable salt thereof, wherein R1 is C₁₂-C₂₄ alkyl or C₁₀-C₂₄ alkenyl, and A is a residue selected from the group consisting of:

(i)

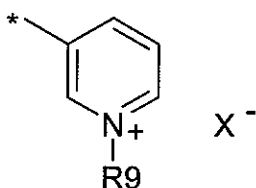
25



wherein R2 is H, C₁-C₆ alkyl, aryl, or aralkyl, wherein any aryl moiety may be unsubstituted or substituted by nitro, cyano, halo, hydroxy, NR₆R₇, or CR₈R₈NR₆R₇; R3 is H, a pair of electrons or C₁-C₆ alkyl; R4 and R5 each independently is H or C₁-C₆ alkyl, or R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and R6, R7 and R8 each independently is H or C₁-C₆ alkyl;

(ii) phenyl substituted by NR₆R₇ or CR₈R₈NR₆R₇, wherein R6, R7 and R8 each independently is H or C₁-C₆ alkyl; and

(iii)



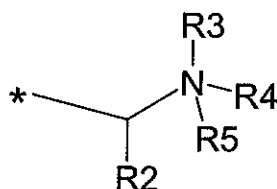
wherein R9 is H, lower alkyl or indolyl(C₁-C₄)alkyl, and X⁻ is a counterion, or R9 is a pair of electrons and X is absent, for the preparation of a pharmaceutical composition for treatment of inflammation.

In a further aspect, the present invention relates to novel compounds of the general formula:



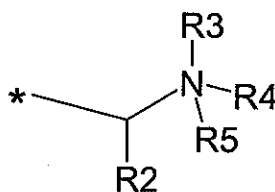
wherein

- 5 (i) R1 is C₂₀-C₂₄ alkyl or C₁₀-C₂₄ alkenyl, and A is a residue of the formula:



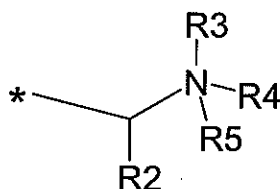
- 10 wherein R2 is H, C₁-C₆ alkyl, aryl, or aralkyl, wherein any aryl moiety may be unsubstituted or substituted by nitro, cyano, halo, hydroxy, NR₆R₇, or CR₈R₈NR₆R₇; R3 is H, a pair of electrons, or C₁-C₆ alkyl; R4 and R5 each independently is H or C₁-C₆ alkyl, or R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and
15 R6, R7 and R8 each independently is H or C₁-C₆ alkyl; or

- (ii) R1 is C₁₈ alkyl and A is a residue of the formula:



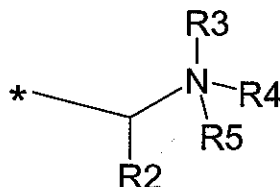
- 20 wherein R2 is H; R3 is a pair of electrons; and R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; or

- (iii) R1 is C₁₂-C₁₆ alkyl and A is a residue of the formula:



wherein R2 is unsubstituted aryl, or aryl or aralkyl wherein the aryl moiety is substituted by nitro, cyano, halo, hydroxy, NR6R7, or CR8R8NR6R7; R3 is H, a pair of electrons, or C₁-C₆ alkyl; R4 and R5 each independently is H or C₁-C₆ alkyl, or R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and R6, R7 and R8 each independently is H or C₁-C₆ alkyl; or

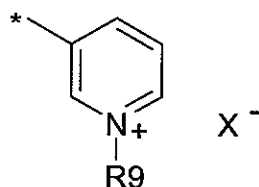
(iv) R1 is C₁₀ alkyl and A is a residue of the formula:



wherein R2 is C₁-C₆ alkyl; R3 is H, a pair of electrons, or C₁-C₆ alkyl; R4 and R5 each independently is H or C₁-C₆ alkyl, or R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and R6, R7 and R8 each independently is H or C₁-C₆ alkyl; or

(v) R1 is C₁₀-C₂₄ alkyl or C₁₀-C₂₄ alkenyl and A is phenyl substituted by NR6R7 or CR8R8NR6R7, wherein R6, R7 and R8 each independently is H or C₁-C₆ alkyl, but excluding the compounds wherein R1 is C₁₀-C₁₆ alkyl and A is phenyl substituted by -CH₂-NH₂; or

(vi) R1 is C₁₀-C₂₄ alkyl or C₁₀-C₂₄ alkenyl and A is a group of the formula:



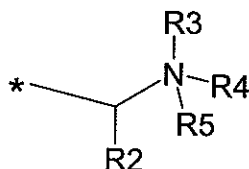
wherein R9 is C₁-C₆ alkyl or indolyl(C₁-C₆)alkyl and X⁻ is a counter ion;
and pharmaceutically acceptable salts thereof.

5 For the sake of clarity, the mark * in all formulas herein in the specification and claims indicates the position linked to the -CO- of the R1-O-CO- group.

According to the invention R1 is a C₁₂-C₂₄ alkyl or C₁₀-C₂₄ alkenyl for the compounds of formula Ia for treatment of inflammation. R1 is preferably C₁₂-C₂₀, more preferably C₁₆-C₁₈, most preferably C₁₈ alkyl or alkenyl. The alkyl groups may
10 be straight or branched and are preferably selected from the group consisting of dodecyl, tetradecyl, hexadecyl and octadecyl such that the radicals R1-O- are derived from the saturated fatty alcohols lauryl alcohol, myristyl alcohol, cetyl alcohol (also known as palmityl alcohol), and stearyl alcohol, respectively. The alkenyl groups may have one or more double bonds and are preferably selected
15 from the group consisting of hexadecenyl, octadecenyl, octadecadienyl and octadecatrienyl such that the radicals R1-O- are derived from the unsaturated fatty alcohols palmitoleyl alcohol or, preferably, from C₁₈-unsaturated fatty alcohols having one or more double bonds in the trans or, preferably, in the cis form, such as, but not being limited to, oleyl alcohol (cis-9-octadecenol), linoleyl alcohol (cis-
20 9,12-octadecadienol), γ-linolenyl alcohol (cis-6,9,12-octadecatrienol) and linolenyl alcohol (cis-9,12,15-octadecatrienol). In a most preferred embodiment, R1 is the cis-9-octadecenyl radical also designated octadec-(Z)-9-enyl or oleyl.

The residue A may comprise a primary, secondary or tertiary amino group or a quaternary ammonium group that may be acyclic or cyclic. When the amino group
25 is acyclic, the resulting compounds are esters of amino acids.

Thus, in one embodiment, in the compounds comprised in the pharmaceutical compositions of the invention or in some of the novel compounds of the invention, A is a radical of the formula:



- 5 wherein R2 is H, C₁-C₆ alkyl, aryl, or aralkyl, wherein any aryl moiety may be unsubstituted or substituted by nitro, cyano, halo, hydroxy, NR₆R₇, or CR₈NR₆R₇; R3 is H, a pair of electrons or C₁-C₆ alkyl; R4 and R5 each independently is C₁-C₆ alkyl, or R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted
 10 by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and R6, R7 and R8 each independently is H or C₁-C₆ alkyl, with the limitations as defined hereinbefore and in the claims. For example, and for the novel compounds, R4 and R5 cannot be H or C₁-C₆ alkyl when R1 is octadecyl (stearyl); for the novel compounds, wherein R1 is C₁₂-C₁₆ alkyl, R2 cannot be H, C₁-C₆ alkyl or
 15 unsubstituted aralkyl, or wherein R1 is C₁₀ alkyl, R2 cannot be H, aryl or aralkyl.

In one preferred embodiment, R2 is H. As used herein, C₁-C₆ alkyl refers to a straight or branched alkyl radical having 1 to 6 carbon atoms such as, but not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, pentyl and hexyl. The term "aryl" as used herein by itself or as part of the "aralkyl" radical
 20 refers to a C₆-C₁₀ aromatic carbocyclic radical such as phenyl and naphthyl. In a preferred embodiment, the aryl radical is phenyl. In the term "aralkyl", the alkyl refers as well to a C₁-C₆ alkyl radical. In a preferred embodiment, the aralkyl group is benzyl. In preferred embodiments, the phenyl and benzyl groups are unsubstituted or are substituted by hydroxy, for example, p-hydroxybenzyl.

- 25 Some compounds of the above formula wherein R3 is a pair of electrons and R4 and R5 are H, are esters of amino acids, for example, esters of glycine (R2 is H), alanine (R2 is methyl), valine (R2 is isopropyl), leucine (R2 is isobutyl), isoleucine

(R2 is sec-butyl), phenylglycine (R2 is phenyl), phenylalanine (R2 is benzyl) , tyrosine (R2 is p-hydroxybenzyl).

According to some embodiments of the invention, R4 and R5 together with the nitrogen atom to which they are attached may form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom such as pyrrolidine, azepine, and, preferably a 6-membered ring, more preferably piperidine, piperazine or morpholine. The piperazine ring may be substituted at the 4-position by a C₁-C₆ alkyl, preferably methyl.

In one preferred embodiment, R2 is H or phenyl, R3 is H, a pair of electrons or methyl, R4 and R5 are each H or methyl, or R4 and R5 together with the N atom to which they are attached form a morpholine or a piperazine ring optionally substituted at the nitrogen atom at position by methyl. Examples of compounds according to this embodiment for use as anti-inflammatory compounds are the novel esters herein identified as Compounds 1, 3, 4, 7, 9, 11:

1. N,N-Dimethylamino-acetic acid octadec-(Z)-9-enyl ester
3. (4-Methyl-piperazin-1-yl)-acetic acid octadec-(Z)-9-enyl ester tartrate
4. (4-Methyl-piperazin-1-yl)-acetic acid octadecyl ester tartrate
7. 4-Methyl-4-octadec-(Z)-9-enyloxycarbonylmethyl-morpholin-4-ium chloride
9. Piperazin-1-yl-acetic acid octadec-(Z)-9-enyl ester bitartrate
11. α -Amino- α -phenyl-acetic acid octadec-(Z)-9-enyl ester HCl salt.

In another embodiment of the invention, A is phenyl substituted by NR₆R₇ or CR₈R₈NR₆R₇, wherein R₆, R₇ and R₈ each independently is H or C₁-C₆ alkyl. In a preferred embodiment, A is phenyl substituted by CR₈R₈NR₆R₇, wherein R₈ is H and R₆ and R₇ are each H or C₁-C₆ alkyl, preferably methyl. Examples of compounds according to this embodiment are the novel esters of 4-dimethylaminomethylbenzoic acid herein identified as Compounds 5 and 10, for use both as anti-inflammatory agents and as adjuvants.

5. 4-dimethylaminomethyl-benzoic acid octadec-(Z)-9-enyl ester HCl
10. 4-dimethylaminomethyl-benzoic acid octadec-(E)-9-enyl ester HCl.

In yet a further embodiment of the invention, A is the pyridyl group:

X^-

wherein R⁹ is a pair of electrons and X^- is absent, or R⁹ is methyl or indolyethyl and X^- is a counter ion selected from the group consisting of chloride, bromide, iodide and tosylate. These compounds are esters of nicotinic acid. Examples of compounds according to this embodiment are the compounds herein identified as Compounds **2**, **6a**, **6b**, **6c**, and **8**, for use as anti-inflammatory, from which Compounds **6a**, **6b**, **6c**, and **8** are novel compounds:

2. Nicotinic acid octadec-(Z)-9-enyl ester;

6a.1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium iodide;

6b.1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium chloride;

6c.1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium tosylate; and

8.1-[(2-(1H-indol-3-yl)-ethyl)-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium bromide;

The esters of the present invention are in general crystalline, non-hygroscopic and water-soluble and are more easily purified and formulated for oral and parenteral formulation than the starting saturated or cis-unsaturated alcohols.

Also contemplated by the present invention are pharmaceutically acceptable salts of the compounds of Formula I, both acid addition salts and quaternary salts of acyclic or cyclic ammonium groups or pyridinium salts with a counter ion. Pharmaceutically acceptable acid addition salts of the compounds include salts between the base and an inorganic acid such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydriodic, phosphorous, and the like, as well as salts derived from an organic acid such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxyalkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. Such salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate,

dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, p-toluenesulfonate (tosylate),
5 phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like.

The acid addition salts of said basic compounds are prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the conventional manner. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The
10 free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base for purposes of the present invention.

In one embodiment of the invention, compounds of general formula I may be prepared by reacting the long-chain fatty alcohol such as oleyl alcohol with the
15 suitable amino acid. Thus, oleyl alcohol is reacted with N,N-dimethylglycine to afford the compound herein designated Compound 1. In another embodiment, the long-chain fatty alcohol is reacted with an halide of nicotinic acid, for example, oleyl alcohol is reacted with nicotinoyl chloride to afford the compound herein designated Compound 2.

20 In a further embodiment, the long-chain fatty alcohol is reacted with halomethylacetic acid chloride and the resulting ester is then reacted with the suitable amine. For example, oleyl alcohol is reacted with chloroacetyl chloride and the resulting oleyl chloroacetic acid ester is reacted with piperazine or with N-methyl-morpholine, to give the free base that is then converted to the salt, e.g.
25 Compound 3 or Compound 7, respectively. Similarly, stearyl alcohol is reacted with chloroacetyl chloride and the resulting stearyl chloroacetic acid ester is reacted with 4-methyl-piperazine to give the free base that is then converted to the salt, e.g. Compound 4.

Other methods according to the invention are described in the following
30 examples and can be used to prepare similar compounds of Formula I herein.

Reactions between oleyl alcohol and activated amino acids may be performed in a wide range of organic solvents, both polar, e.g. acetonitrile, chloroform, and non-polar, e.g. hexane, under a wide temperature range, from ambient to reflux, and over periods of time ranging from 1-2 hours to 1-2 days.

5 Quaternary pyridinium salts may be prepared either as described above, or by alkylating oleyl nicotinate using alkylating agents known to those skilled in the art such as alkyl halides, alkyl sulfonates and haloalkylindoles. Oleyl haloacetates may be reacted with amines in a wide range of organic solvents, both polar, e.g. acetonitrile, and non-polar, e.g. hexane, under a wide temperature range, from
10 ambient to reflux, and over periods of time ranging from 1-2 hours to 1-2 days. The oily or low-melting free bases, obtained after suitable workup and purification are converted into pharmaceutically acceptable salts. The latter are usually high-melting and water soluble solids.

 It should be noted that for the preparation of the esters of the invention
15 wherein R1 is a cis-alkenyl group, the starting cis-unsaturated alcohol such as oleyl alcohol, may be used in a substantially pure cis-unsaturated form meaning that the reagent contains at least about 80% of the cis-form. For example, the commercial oleyl alcohol is about 85% pure and most of the impurity consists of the trans analog (elaidyl alcohol).

20 The immune system, in both its innate and adaptive arms, is involved in regulating inflammation of every type, and inflammation is a key factor in processes such as wound healing, connective tissue re-modeling, angiogenesis, organ regeneration, neuroprotection, as well as in the adaptive immune responses seen in autoimmunity, allergies, graft rejection, and infection. Therefore, anti-inflammatory
25 agents that modulate the inflammatory response such as those described here will be useful in a variety of conditions.

 Inflammatory diseases, disorders or conditions that can be treated with the immunomodulators of the present invention include, but are not limited to, immunologically-mediated chronic or acute inflammatory diseases, disorders or
30 conditions selected from an autoimmune disease, a severe allergy, asthma, or an

inflammation associated with a disease, disorder or condition selected from graft rejection, a chronic degenerative disease such as Alzheimer's disease, neuroprotection, organ regeneration, chronic ulcers of the skin, or schizophrenia.

Examples of autoimmune diseases that can be treated according to the invention are multiple sclerosis or a human arthritic condition, e.g. rheumatoid arthritis, reactive arthritis with Reiter's syndrome, ankylosing spondylitis and other inflammations of the joints mediated by the immune system. Other autoimmune diseases are contemplated and are presented in the following list in the context of the organ or tissue involved.

Thus, the present invention relates to the treatment of an immunologically-mediated inflammatory disease, disorder or condition selected from myasthenia gravis, Guillain-Barré syndrome, or other inflammatory disease of the nervous system; psoriasis, pemphigus vulgaris or other diseases of the skin; systemic lupus erythematosus, glomerulonephritis or other disease affecting the kidneys; atherosclerosis or other inflammation of the blood vessels; autoimmune hepatitis, inflammatory bowel diseases e.g. Crohn's disease, pancreatitis, or other disorder of the gastrointestinal system; type 1 diabetes mellitus (insulin-dependent diabetes mellitus or IDDM), autoimmune thyroiditis (hashimoto's thyroiditis), or other disease of the endocrine system. In one preferred embodiment, the immunologically-mediated inflammatory disease, disorder or condition is psoriasis.

One of the models used to test the anti-inflammatory activity of the agents according to the invention is adjuvant arthritis (AA), an experimental disease of the joints inducible in some strains of rats by immunizing with *Mycobacterium tuberculosis* in complete Freund's adjuvant (CFA). These animals develop an arthritis whose features are similar to those of rheumatoid arthritis in humans and thus serve as animal models of human arthritic conditions such as rheumatoid arthritis, reactive arthritis in Reiter's syndrome, ankylosing spondylitis and other inflammations of the joints which appear to be mediated by the immune system. Adjuvant arthritis also serves as a model of immune-mediated inflammation in general including cell-mediated autoimmune reactions, graft rejection and allergic

reaction. For example, treatments which can suppress rheumatoid arthritis include immunosuppressive agents such as corticosteroids, cyclosporin A, azathioprine, and other immunosuppressive agents which are broadly used in the treatment of autoimmune diseases. Therefore, suppression of adjuvant arthritis by a therapeutic agent indicates that the agent is potentially useful as a broad anti-inflammatory agent.

The present invention further relates to a pharmaceutical composition for the treatment of inflammation comprising a pharmaceutically acceptable carrier and a compound of the general formula I described hereinabove.

The pharmaceutical composition provided by the present invention may be in solid, semisolid or liquid form and may further include pharmaceutically acceptable fillers, carriers or diluents, and other inert ingredients and excipients. The composition can be administered by any suitable route such as, but not limited to, oral, topical, or parenteral e.g. by injection through subcutaneous, intravenous, intramuscular, or any other suitable route. Since many of the compounds are oily, they are preferably administered parenterally, more preferably subcutaneously. If given continuously, the compounds of the present invention are each typically administered by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. The dosage will depend on the state of the patient and severity of the disease and will be determined as deemed appropriate by the practitioner.

For parenteral administration, the compounds may be formulated by mixing the compound at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. Generally, the formulations are prepared by contacting the compound uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably, the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples

of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils can be also useful, as well as liposomes. These preparations can be made by conventional methods known to those skilled in the art, for example as described in "Remington's Pharmaceutical Science", A.R. Gennaro, ed., 17th edition, 1985, Mack Publishing Company, Easton, PA, USA.

The invention will now be illustrated by the following non-limitative Examples.

5

EXAMPLES

In the Examples herein, the following compounds will be identified by their numerals in bold as follows:

1. N,N-Dimethylamino-acetic acid octadec-(Z)-9-enyl ester;
2. Nicotinic acid octadec-(Z)-9-enyl ester;
- 10 3. (4-Methyl-piperazin-1-yl)-acetic acid octadec-(Z)-9-enyl ester tartrate;
4. (4-Methyl-piperazin-1-yl)-acetic acid octadecyl ester tartrate;
5. 4-dimethylaminomethyl-benzoic acid octadec-(Z)-9-enyl ester HCl

- 6a. 1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium iodide;
- 6b. 1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium chloride;
- 6c. 1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium tosylate; and
- 7. 4-Methyl-4-octadec-(Z)-9-enyloxycarbonylmethyl-morpholin-4-ium chloride;
- 8. 1-[(2-(1H-indol-3-yl)-ethyl]-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium bromide;
- 9. Piperazin-1-yl-acetic acid octadec-(Z)-9-enyl ester bitartrate;
- 10. 4-N,N-Dimethylaminomethyl-benzoic acid octadec-(E)-9-enyl ester HCl
- 11. α -Amino- α -phenyl-acetic acid octadec-(Z)-9-enyl ester HCl salt.

EXAMPLE 1

Synthesis of N,N-dimethylamino-acetic acid octadec-(Z)-9-enyl ester (Compound 1)

N,N-Dimethylglycine (1.60 g, 15.4 mmol) was suspended in dry and alcohol-free chloroform (20 ml), and PCl_5 (3.80 g, 17.3 mmol) was added. The yellow suspension was stirred at 55-60°C for 1 h, cooled to 30°C, and oleyl alcohol (5.70 ml, 4.85 g, 18.0 mmol) was added. The yellow solution was heated to 63-65°C and stirred at this temperature for 3-4 h. The solution was cooled to room temperature and a little amount of insoluble materials was filtered off. Water was added to the filtrate, and the pH of the mixture adjusted to 8. The organic phase was separated, washed with 5% NaHCO_3 and brine, dried and evaporated. The yellow oily residue (5.07 g) was triturated with ether and the resultant suspension was filtered. The filtrate was evaporated to dryness to give 3.18 g of a crude product, which was purified by column chromatography (n-hexane followed by ethyl acetate) to give the title compound, herein designated Compound 1, as a yellowish liquid (2.6 g, 7.36 mmol, 41 %).

Calculated: C,74.73; H,12.26; N,3.96. Found: C,67.88; H,11.69; N,3.13.

¹H-NMR (200MHz, CDCl₃) (ppm) δ: 0.88(t, 3H, Me), 1.27-1.29(m, 22H, 11CH₂), 1.57-1.67 (m, 2H, CH₂), 2.00 (bd, 4H, 2CH₂), 2.37 (s, 6H, NMe₂), 3.18 (s, 2H, CH₂), 4.13 (t, 2H COOCH₂), 5.31-5.39 (m, 2H, CH=CH).

IR: 1755 cm⁻¹ (CO).

5

EXAMPLE 2

Synthesis of nicotinic acid octadec-(Z)-9-enyl ester (Compound 2)

Nicotinoyl chloride hydrochloride (17.8 g, 0.1 mol) and oleyl alcohol (26.85 g, 0.1 mol) were added into hexane (250 ml). Pyridine (15.8 g, 0.2 mol) was added to the stirred mixture over a period of 10 min, and the reaction mixture was stirred at room temperature for 24 h. Pyridine hydrochloride was removed by filtration and washed with hexane. The combined filtrate was extracted with water, dried over MgSO₄ and evaporated to dryness. The residue was digested twice with acetonitrile, and the sticky residue was dried to give a clear water-like liquid which turned soap-like upon cooling (21.1 g, 56.5 mmol, 56.5 %).

¹H-NMR (200MHz, CDCl₃) (ppm) δ: 0.90(t, 3H, Me), 1.30-1.50(m, 22H, 11CH₂), 1.75-1.85 (m, 2H, CH₂), 2.00 (bd, 4H, 2CH₂), 4.35 (t, 2H, COOCH₂), 5.31-5.39 (m, 2H, CH=CH), 7.41(dd, 1H), 8.30(dt, 1H), 8.77(dd, 1H), 9.25(d, 1H).

20

EXAMPLE 3

Synthesis of (4-methyl-piperazin-1-yl)-acetic acid octadec-(Z)-9-enyl ester L-tartrate (Compound 3)

3a) Chloroacetic acid octadec-9-(Z)- enyl ester

Oleyl alcohol (147.9 g, 0.55 mol) was dissolved in n-hexane (300 ml), and chloroacetyl chloride (56.5 g, 0.50 mol) was added dropwise over 10 min. The clear reaction mixture was stirred for 24 h at room temperature. Traces of HCl gas were removed from the reaction mixture by bubbling nitrogen through it. This hexane solution of the crude title product was used in the next step without further purification.

3b) 4-Methyl-piperazin-1-yl-acetic acid octadec-9-(Z)-enyl ester (free base)

n-Hexane was added to the hexane solution obtained in Example 3a above (to a final volume of 500 ml), and 1-methyl-piperazine (103.6 g, 1.03 mol) was added. The reaction mixture was stirred for 36 h at room temperature. 1-Methyl-piperazine HCl was removed by filtration and washed with n-hexane. The combined organic filtrates were extracted with water and brine, dried and evaporated under reduced pressure to give a pale yellow oil. The latter turned into a white solid when cooled to -20°C .

3c) 4-Methyl-piperazin-1-yl-acetic acid octadec-9-(Z)- enyl ester L-tartrate salt

The free base of Example 3b above was dissolved in methanol (150 ml), and a solution of (L)-(+)-tartaric acid (75 g, 0.5 mol) in methanol (400 ml) was added. After stirring for 60 min at room temperature, the solution was filtered through a 20 μ filter and evaporated to dryness. The sticky residue was first digested with acetonitrile, then treated with acetone for 24 h. The solvent was decanted off, and the solid was treated twice with acetone. The resultant suspension was dried under vacuum (filtration was avoided due to hygroscopicity of the wet tartrate salt). The dry product was homogenized and sieved (500 μ) to give 197.3 g of the title Compound 3 as a yellowish powder (35 mmol, 64 %), mp $69-72^{\circ}\text{C}$.

Calculated: C, 62.34; H, 9.74; N, 5.01. Found: C, 60.33; H, 9.71; N, 4.91.

$^1\text{H-NMR}$ (200MHz, DMSO- d_6) ppm δ : 0.85 (t, 3H, Me), 1.18-1.24 (bs, 22H, 11CH₂), 1.54 (bs, 2H, CH₂), 1.96 (bs, 4H, 2CH₂), 2.56 (s, 3H, N-Me), 2.69 (s, 4H, 2CH₂), 2.90 (s, 4H, 2CH₂), 3.26 (s, 2H, N-CH₂COO), 4.0 (t, 2H, CH₂OCO), 4.18, (s, 1.5H, tartrate CH), 5.29 (t, 2H, CH=CH).

IR (cm^{-1}): 1616, 1739 (C=O).

EXAMPLE 4

Synthesis of (4-Methyl-piperazin-1-yl)-acetic acid octadecyl ester L-tartrate (Compound 4)

Stearyl alcohol (27.2 g, 100 mmol) was dissolved in benzene (150 ml) and chloroacetyl chloride (8.0 ml, 100 mmol) was added dropwise over 10 min. The clear reaction mixture was stirred at room temperature for 48 h. Traces of HCl were removed by bubbling nitrogen gas. 1-Methyl-piperazine (28.0 ml, 250 mmol) was added, and the reaction mixture stirred at room temperature for 36 h. 1-Methylpiperazine HCl was removed by filtration and washed with n-hexane. The filtrate and washings were combined and extracted successively with water and brine, dried and evaporated to dryness, to give the free base (38.8 g). The latter was dissolved in ethanol and combined with a solution of (L)-(+)-tartaric acid (14.18 g) in ethanol (150 ml), stirred for 60 min and kept at room temperature for 3 days. The resultant solid was collected by filtration and dried to give 46.5 g (83 mmol, 83 %) of the title Compound 4 as a white solid, mp: 88-9°C.

Calculated: C, 62.11; H, 10.07; N, 5.00. Found: C, 61.02; H, 10.39; N, 5.01.

¹H-NMR (200MHz, DMSO-d₆) ppm δ: 0.85 (t, 3H, Me), 1.18-1.30 (bs, 30H, 15CH₂), 1.54 (m, 2H, CH₂), 1.96 (bs, 4H, 2CH₂), 2.56 (s, 3H, N-Me), 2.69 (s, 4H, 2CH₂), 2.90 (s, 4H, 2CH₂), 3.26 (s, 2H, N-CH₂COO), 4.02 (t, 2H, CH₂OCO), 4.18, (s, 1.5H, tartrate CH), IR: 1742 cm⁻¹ (C=O).

EXAMPLE 5

Synthesis of 4-dimethylaminomethyl-benzoic acid octadec-(Z)-9-enyl ester HCl (Compound 5)

5a) 4-Dimethylaminomethyl-benzoic acid HCl

A suspension of 4-aminomethyl-benzoic acid (92.5 g, 0.61 mol) in 98% formic acid (127 ml, 3.36 mol) was heated under stirring to complete dissolution, and 37% formaldehyde (114 ml, 1.53 mol) was added. The solution was heated and kept at reflux temperature for 10 h. After cooling, 20% HCl (130 ml) was added, and the solution was evaporated to dryness. The white powder (131.5 g, 0.61 mol, ~100 %) thus obtained was used in the next reaction without further purification.

5b) 4-Dimethylaminomethyl-benzoic acid octadec-(Z)-9-enyl ester HCl

Thionyl chloride (28.6 ml, 0.39 mol) was added to a stirred suspension of 4-dimethylaminomethyl-benzoic acid HCl (65.0 g, 0.3 mol) in acetonitrile (300 ml), refluxed for 1 h, and evaporated to dryness. The yellowish solid residue was suspended in acetonitrile (300 ml), oleyl alcohol (99.5 ml, 0.31 mol) was added, and the mixture was refluxed for 2 h. The mixture was allowed to cool, the crude salt was collected by filtration, washed with acetonitrile (500 ml), then acetone (300 ml), and dried to give 105 g (0.225 mol, 75 %) of the title Compound 5 as a slightly yellowish solid, mp: 191-3°C.

Calculated: C, 72.15; H, 10.38; N, 3.00. Found: C, 71.32; H, 10.68; N, 3.15.

¹H-NMR (200MHz, CDCl₃) ppm δ: 0.88 (t, 3H, Me), 1.25-1.33 (bs, 22H, 11CH₂), 1.75 (m, 2H, CH₂), 2.01 (m, 4H, 2CH₂), 2.83 (s, 6H, NMe₂), 4.30(t, 2H, CH₂OCO), 4.39, (s, 2H, PhCH₂), 5.32-5.37 (t, 2H, CH=CH), 7.83 (d, 2H), 8.10 (d, 2H).

EXAMPLE 6

Synthesis of Compounds 6a, 6b and 6c

6a) Synthesis of 1-methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium iodide (Compound 6a)

Nicotinic acid octadec-(Z)-9-enyl ester, prepared by the procedure described in Example 2, (3.74 g, 10 mmol) and iodomethane (3.12 g; 22 mmol) were dissolved in a mixture of nitromethane (5 ml) and methanol (2.5 ml). The reaction mixture was kept at room temperature for 3 days and evaporated to dryness in vacuo. The residue was digested in hexane (20 ml), hexane was decanted off, and the residue dried to give the title compound as a yellow-orange sticky solid (4.95 g, 9.6 mmol, 96%).

¹H-NMR (200MHz, DMSO-d₆) (ppm) δ: 0.85 (t, 3H, Me), 1.20-1.50 (m, 22H, 11CH₂), 1.70-1.80 (m, 2H, CH₂), 1.96 (bd, 4H, 2CH₂), 4.36 (t, 2H, COOCH₂),

4.42 (s, 3H, N-Me), 5.27-5.39 (m, 2H, CH=CH), 8.25 (dd, 1H), 8.95 (dt, 1H), 9.20 (dd, 1H), 9.55 (d, 1H).

6b) Synthesis of 1-methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium chloride (Compound 6b)

1-Methyl-pyridinium-3-carboxylate hydrochloride (1.74 g, 10 mmol) was added to thionyl chloride (7 ml). The reaction mixture was stirred at 68-70°C for 1.5 h, then evaporated to dryness in vacuo. Hexane (25 ml) and oleyl alcohol (2.69 g, 10 mmol) were added to the residue, and the mixture was stirred at 43°C for 3 days and evaporated to dryness under reduced pressure, to give 4.1 g (9.66 mmol, 96 %) of a sticky solid.

¹H-NMR (200MHz, DMSO-d₆) (ppm) δ: 0.85 (t, 3H, Me), 1.20-1.50 (m, 22H, 11CH₂), 1.70-1.80 (m, 2H, CH₂), 1.95 (bd, 4H, 2CH₂), 4.38 (t, 2H, COOCH₂), 4.42 (s, 3H, N-Me), 5.27-5.40 (m, 2H, CH=CH), 8.25 (dd, 1H), 8.95 (dt, 1H), 9.20 (dd, 1H), 9.55 (d, 1H).

6c) Synthesis of 1-methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium tosylate (Compound 6c)

Nicotinic acid octadec-(Z)-9-enyl ester, prepared by the procedure described in Example 2, (3.74 g, 10 mmol) was dissolved in a mixture of nitromethane (5 ml) and methanol (2.5 ml). A solution of methyl 4-methylbenzenesulfonate (1.86 g, 10 mmol) in a mixture of nitromethane (2 ml) and methanol (1 ml) was added, and the reaction mixture stirred at room temperature for 4 days. The clear solution was evaporated to dryness under reduced pressure to afford 5.2 g (9.3 mmol, 93 %) of a clear sticky solid.

¹H-NMR (200MHz, DMSO-d₆) (ppm) δ: 0.85 (t, 3H, Me), 1.20-1.50 (m, 22H, 11CH₂), 1.70-1.80 (m, 2H, CH₂), 1.96 (bd, 4H, 2CH₂), 2.25 (s, 3H, tolyl Me), 4.36 (t, 2H, COOCH₂), 4.42 (s, 3H, N-Me), 5.27-5.39 (m, 2H, CH=CH), 7.10 (d, 2H, Ph), 7.45 (d, 2H, Ph), 8.25 (dd, 1H), 8.95 (dt, 1H), 9.20 (dd, 1H), 9.55 (d, 1H).

EXAMPLE 7

Synthesis of 4-methyl-4-octadec-(Z)-9-enyloxycarbonylmethyl-morpholin-4-ium chloride (Compound 7)

Chloroacetic acid octadec-9-(Z)-enyl ester (17.2 g, 50 mmol) and N-methyl morpholine (6.6 ml, 60 mmol) were refluxed in acetonitrile (100 ml) for 18 h. The reaction mixture was cooled to room temperature and evaporated to dryness. Acetone was added, and the sticky residue which precipitated was collected by filtration, washed with acetone, and dried, to give the title Compound 7 as a slightly sticky and hygroscopic yellowish solid, mp 129-131 °C (4.11 g, 9.2 mmol, 18 %).

Calculated: C, 67.31; H, 10.84; N, 3.14. Found: C, 65.58; H, 11.35; N, 3.48.

¹H-NMR (200MHz, CDCl₃) (ppm) δ: 0.88 (t, 3H, Me), 1.15-1.50 (m, 22H, 11CH₂), 1.65-1.80 (m, 2H, CH₂), 2.0 (bd, 4H, 2CH₂), 3.78 (s, 3H, N-Me), 4.08-4.36 (m, 10H, COOCH₂, 4CH₂ morpholine), 5.28 (s, 2H, COCH₂N), 5.27-5.39 (m, 2H, CH=CH).

15

EXAMPLE 8

Synthesis of 1-[(2-(1H-indol-3-yl)-ethyl)-3-octadec-(Z)-9-enyloxy-carbonyl-pyridinium bromide (Compound 8)

A solution of 3-(2-bromoethyl)indole (1.12 g, 5 mmol) in methanol (10 ml) was added to a solution of nicotinic acid octadec-(Z)-9-enyl ester (1.87 g, 5 mmol) in methanol (5 ml). The reaction mixture was stirred at room temperature for 24 h and evaporated to dryness under reduced pressure to afford the title Compound 8 as a sticky solid (2.7 g, 4.5 mmol, 90.3 %).

¹H-NMR (200MHz, DMSO-d₆) (ppm) δ: 0.85 (t, 3H, Me), 1.20-1.50 (m, 22H, 11CH₂), 1.70-1.80 (m, 2H, CH₂), 1.90-2.05 (bd, 4H, 2CH₂), 3.4 (2H, CH₂), 4.31 (t, 2H, CH₂OCO), 4.95 (t, 2H, CH₂), 5.30-5.39 (m, 2H, CH=CH), 6.30-7.60 (m, 5H, indole aryl), 8.10-9.50 (4H, pyr), 11.0 (br s, 1H, NH).

EXAMPLE 9

Synthesis of piperazin-1-yl-acetic acid octadec-(Z)-9-enyl ester bitartrate

5

(Compound 9)

To a solution of piperazine (10.77 g, 125 mmol) in acetonitrile (200 ml) was added a solution of crude oleyl chloroacetate (prepared from 25 mmol chloroacetyl chloride) in acetonitrile (30 ml). The mixture was refluxed for 1.5 h, cooled to room temperature and evaporated to dryness. Water and ethyl acetate were added to the residue, and the phases were separated. The organic phase was washed with an equal volume of water, dried on sodium sulfate, and evaporated to dryness. The oily residue was dissolved in a small volume of methanol, and L-tartaric acid (7.50 g, 2 eqs.) in MeOH was added. The solution of the crude salt was evaporated to dryness, and the solid residue treated with acetonitrile, filtered, washed with acetonitrile, and then with acetone. The solid was dissolved in MeOH (400 ml), filtered and evaporated to dryness. The residue was treated with EtOAc, filtered, washed with EtOAc, then with acetone and dried to give the title Compound 9 as a white, non-hygroscopic powder, mp 130-132 °C (10.45 g, 15 mmol, 60 %).

20 Calculated: C, 55.32; H, 8.42; N, 4.03. Found: C, 53.67; H, 8.66; N, 4.14.

¹H-NMR (200MHz, DMSO-d₆) ppm δ: 0.85 (t, 3H, Me), 1.18-1.34 (bs, 22H, 11CH₂), 1.54 (bs, 2H, CH₂), 1.97 (bs, 4H, 2CH₂), 2.73 (s, 4H, 2CH₂), 3.06 (s, 4H, 2CH₂), 3.30(s, 2H, N-CH₂COO), 4.03 (t, 2H, CH₂OCO), 4.22, (s, 1.5H, tartrate CH), 5.33 (t, 2H, CH=CH).

EXAMPLE 10

Synthesis of 4-dimethylaminomethyl-benzoic acid octadec-(E)-9-enyl ester HCl (Compound 10)

The title Compound 10 (1.0 g, 2.1 mmol, 46 %), mp: 194-6°C, was prepared from 4-dimethylaminomethyl-benzoic acid HCl (1.0 g, 4.6 mmol) by the procedure described in Example 5b, except that elaidyl alcohol was used instead of oleyl alcohol.

Calculated: C, 72.14; H, 10.37; N, 3.00. Found: C, 71.36; H, 11.01; N, 3.19.

¹H-NMR (200MHz, CDCl₃) ppm δ: 0.88 (t, 3H, Me), 1.25-1.33 (bs, 22H, 11CH₂), 1.75 (m, 2H, CH₂), 1.96 (m, 4H, 2CH₂), 2.83 (s, 6H, NMe₂), 4.30 (t, 2H, CH₂OCO), 4.39, (s, 2H, PhCH₂), 5.38 (t, 2H, CH=CH), 7.83 (d, 2H), 8.10 (d, 2H).

EXAMPLE 11

Synthesis of aminophenylacetic acid octadec-(Z)-9-enyl ester HCl (Compound 11)

To a solution of N-Boc phenylglycine (2.51 g, 10 mmol) in acetonitrile (50 ml), was added portionwise 1,1'-carbonyldiimidazole (3.24 g, 20 mmol). The solution was stirred at room temperature for 1h, oleyl alcohol (3.15 ml, 2.68 g, 10 mmol) was added, and the reaction mixture further stirred for 2 h at room temperature. The solvent was evaporated, the residue was dissolved in ethyl acetate (150 ml), washed successively with 5% NaHCO₃, 5% citric acid and water, dried on MgSO₄ and evaporated to dryness. The residue was dissolved in 1% HCl in ethyl acetate (100 ml), and the solution set aside for 6 h at room temperature, and evaporated to dryness. The residue thus obtained was crystallized from ether/n-hexane to give 1.6 g 3.65 mmol, 36.5 %) of an off-white solid, mp 101-103°C.

Calculated: C, 71.28; H, 10.12; N, 3.10. Found: C, 70.87; H, 10.33; N, 3.47.

¹H-NMR (200MHz, DMSO-d₆) (ppm) δ: 0.85 (t, 3H, Me), 1.20-1.50 (m, 22H, 11CH₂), 1.70-1.80 (m, 2H, CH₂), 1.96 (bd, 4H, 2CH₂), 4.15 (m, 2H, COOCH₂), 5.25 (s, 1H, PhCHNH₂), 5.27-5.39 (m, 2H, CH=CH), 7.40-7.60 (m, 5H, Ph), 9.20 (br s, 3H, N⁺H₃).

EXAMPLE 12

Effect of dimethylamino-acetic acid octadec-(Z)-9-enyl ester (Compound 1) on adjuvant arthritis (AA)

AA was induced by immunizing inbred female Lewis strain rats (groups of 8 rats obtained from Harlan-Olac), 8-9 weeks old, at the base of the tail with 0.1 ml of incomplete Freund's adjuvant (IFA, mineral oil containing an emulsifier; Sigma) containing 1 mg killed *Mycobacterium tuberculosis* (Difco). Arthritis of the limbs was noted to develop 12-14 days later and was scored on a scale of 0-16 summing the severity of the inflammation of each of the 4 limbs on a scale of 0-4, as described (Holoshitz et al., 1983). The peak of the arthritis usually was observed around day 26 after immunization.

One group of rats was treated intravenously (IV) on the day of immunization with 40 mg of Compound 1 in 0.5 ml of saline (triangles); another group was treated IV with the same amount of Compound 1 on day 14 after the onset of AA (circles), and a third group was treated with saline (squares). AA was scored on a scale of 0-16 by the degree of redness and swelling of each limb, on a scale of 0-4. The results shown in Fig. 1 are the mean + SE. As shown in Fig. 1, Compound 1 could protect the rats against the inflammation caused by AA.

EXAMPLE 13

Anti-inflammatory effect of dimethylamino-acetic acid octadec-(Z)-9-enyl ester (Compound 1) - protection against EAE

EAE is an experimental autoimmune disease inducible in some strains of rats by immunization with myelin basic protein (MBP) in complete Freund's adjuvant (CFA) (in Lewis rats) or with an emulsion of the rat's spinal cord in incomplete Freund's adjuvant (IFA) (in DA rats). The animal experimental disease serves as a model for the human autoimmune disease multiple sclerosis. The disease develops in the animal about 12 days after immunization and is characterized by paralysis of various degrees due to inflammation of the central nervous system. In some strains,

like the Lewis rat, the paralysis can last up to 6-7 days and the rats usually recover unless they die during the peak of their acute paralysis. In other strains of rats like the DA rat, the paralysis can be chronic and remitting.

For the induction and clinical assessment of EAE, spinal cord obtained from DA rats was frozen, thawed and minced thoroughly with a spatula before immunization. Rats were immunized by one subcutaneous injection (just under the skin) into the dorsal base of the tail with 200 µl emulsion prepared from 1:1 IFA (Difco, Detroit, MI, USA) and antigen (volume/weight, i.e. 100 µl IFA/100 mg of whole spinal cord). IFA was complemented with 20 mg/ml of *Mycobacterium tuberculosis* strain 37RA (Difco) (= complete Freund's adjuvant, CFA). The emulsion was prepared by trituration with a gas-tight glass syringe and a needle, 1.2 mm diameter. Rats were regularly weighed and examined for clinical signs of EAE. A four-graded scale was used to assess clinical severity: 0, no paralysis; 1, tail weakness (hanging); 2, hind limb paralysis; 3, hind and fore limb paralysis; 4, severe total paralysis (Lorentzen et al., 1995).

Groups of 5 or 7 DA strain female rats, 8-9 weeks old, were immunized in the hind footpads with 0.1 ml per footpad of IFA containing 100 mg of whole, homogenized DA spinal cord, for a total of 200 mg per rat. On the day of immunization, the rats were treated by intravenous (IV) injection with 40 mg Compound 1 in 0.5 ml of saline or saline (control). The rats were scored for EAE on a severity scale of 0 - 4 as described above. The results are shown in Fig. 2 as the mean score + SE. The results in Fig. 2 show that Compound 1 (circles) could prevent the paralysis to a significant degree in comparison with the untreated control (squares) rats.

EXAMPLE 14

Effect of dimethylamino-acetic acid octadec-(Z)-9-enyl ester (Compound 1) on skin allograft survival

Skin graft transplantation was carried out essentially as described (Birk et al., 1999). Thus, mice were shaved and 1cm² sections of skin were cut from the dorsal side of sacrificed donors and cleaned in phosphate-buffered saline (PBS). Two patches of dorsal skin, 1 cm² each, were cut from the anesthetized recipients (Nembutal 6 mg/ml, 0.25 ml/mouse) in preparation for the allograft. Two donor allografts per recipient were grafted onto the dorsal lesioned patches. Histoacryl (B. Braun Melsungen AG, Melsungen, Germany) was applied around the graft. Nobecutan (ASTR, Astra Tech, Glos G15, UK) was sprayed over the grafts.

In the experiment, groups of 6 BALB/c female mice, 8 weeks old, were grafted with 1 cm², full thickness skin grafts from C57BL/6 female mice, 8 weeks old. On the day of grafting, a group of recipient mice was treated either with saline or IV with 100 µl saline containing 10 mg Compound 1. The day of rejection was scored. As shown in Fig. 3, the transplanted skin in the mice treated with Compound 1 (circles) survived longer in comparison with the untreated control (squares) mice.

EXAMPLE 15

(4-Methyl-piperazin-1-yl)-acetic acid octadec-(Z)-9-enyl ester tartrate

(Compound 3) inhibits DTH skin reaction

Delayed-type hypersensitivity (DTH), a localized inflammatory reaction induced by cytokines secreted by certain T_H cells when they encounter certain types of antigens, is an established experimental model for skin inflammation.

As a first step, DTH reactions were induced in the skin of 8-week-old female BALB/c mice. The mice were sensitized by topical application of 200 µl of a 2% oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; Sigma, St. Louis, MO) solution in acetone/olive oil (4:1, vol/vol) to the shaved abdomen of the mice. Six days after sensitization, one ear of each mouse was challenged by topical application of 20 µl of a 0.5% oxazolone solution. Treatment was given 30 min after the challenge by applying 20 µl of Compound 3 at various doses (1, 2 and 4 mg) on the challenged ear of each mouse (control: non-treated ear or treated with PBS). After 24 hours, the extent of inflammation was measured using the mouse ear-swelling test and the percent inhibition induced by the treatment with Compound 3 was quantitated. The results are shown in Fig. 4. Treatment with 1 mg and 2 mg Compound 3 inhibited the DTH reaction (12.5 % and 8 %, respectively).

EXAMPLE 16

Compounds 5, 9, and 11 inhibit DTH skin reaction

Mice were induced with DTH as in Example 15. Treatment was given 30 min after the challenge by applying compounds 5, 9, and 11 in doses of 0.5, 1, 2, and 4 mg in 40 µl. In addition, a positive control of dexamethazone (2mg/40µl) was administered. A group of DTH induced mice was also treated with (4-Methyl-piperazin-1-yl)-acetic acid ethyl ester HCl (negative control.)

The results of test compounds as well as dexamethazone showed positive results in reducing inflammation in the DTH model. The negative control, an ethyl ester, did not show any positive results. The results are shown in Fig. 5.

EXAMPLE 17

Effect of Compound 3 on adjuvant arthritis (AA)

AA was induced in 8-week-old female Lewis rats as described in Example 12 above. Groups of rats were treated with different amounts of Compound 3 (10, 50, 100 or 200 mg) SC at day -7 before induction of the disease. As shown in Fig. 6, Compound 3 could protect the rats against AA.

EXAMPLE 18

Effect of Compound 3 SC and per os on EAE

EAE was induced in 8-week-old female Lewis rats by injecting each foot pad with 25 μ g of guinea MBP (50 μ g total) in CFA (Sigma). The disease develops about 12 days after immunization and is characterized by paralysis of various degrees due to inflammation of the central nervous system. The rats are scored for paralysis on a scale of 0-4, as described above in Example 13.

In one experiment, groups of rats (7 animals per group) were treated with different amounts of Compound 3 (10, 50, 100 or 200 mg) SC in the back on day 7 before induction of the disease. The rats were scored for EAE on a severity scale of 0-4 as described above. The results are shown in Fig. 7.

In another experiment, groups of rats (7 animals per group) were treated with different amounts of Compound 3 (0.2, 1, 2, 10 or 20 mg) per os, every day, starting from the day of EAE induction. The rats were scored for EAE on a severity scale of 0-4 as described above. The results are shown in Table 1 and in Fig. 8.

In a further experiment, Compound 3 was administered per os every other day, starting from the day of EAE induction, and was found to be similarly effective (not shown).

The results of the experiments above show that Compound 3 could protect the rats against EAE when administered subcutaneously or orally.

Table 1

Compound 3 Dose (mg)	Mean Clinic Score	% inhibition
0	2.9	0
0.2	2.1	28
1	2.1	28
2	2.5	14
10	2.1	28
20	1.7	41

EXAMPLE 19**Effect of Compound 9 per os on EAE**

- 5 EAE was induced in 8-week-old female Lewis rats as described in Example 18 above with guinea pig MBP in CFA.

Rats were treated with Compound 9 (100 mg/Kg) per os. The rats were scored for EAE on a severity scale of 0-4 as described above. The results in Table 2 and in Fig. 9 show that Compound 9 could protect the rats against EAE when
10 administered orally.

Table 2

DOSE	Mg/Kg	Mean Clinic Score	% Inhibition	% Sick rats	Mean onset
PBS	0	2.81±0.46	0	100	12.3
Comp. 9	100	1.93±0.45	31.4	100	11.5

REFERENCES

Beyer S. and Pilgrim H. 1991, Die Hemmung der Endothelzellproliferation durch Ester der 4-Aminomethylbenzoesäure (PAMBA), *Pharmazie* 46:597-599.

- 5 Birk et al., 1999. The 60kDa heat shock protein modulates allograft rejection. *Proc. Nat. Acad. Science USA*. 96: 5159-63.

Holoshitz, Y. et al., 1983. Lines of T lymphocytes mediate or vaccinate against autoimmune arthritis, *Science* 219: 56.

- 10 Kontogiorgis C. A., Hadjipavlou-Litina D.J., and Schulz E., 2001, Antioxidant activity of DL ω -phenyl-amino acid octyl esters with anti-inflammatory activity. *Arzneim-Forsch./Drug Res.*, 51(I):485-488.

Lorentzen J.C. et al., 1995. Protracted, relapsing and demyelinating experimental autoimmune encephalomyelitis in DA rats immunized with syngeneic spinal cord and incomplete Freund's adjuvant, *J. Neuroimmunology*. 63: 193-205.

- 15 Markwardt F., Neuland P., and Klocking H.-P. 1966, Über die antifibrinolytische Wirkung von Estern der 4-Aminomethylbenzoesäure (PAMBA) *Pharmazie* 21(6):345-348.

Metayer MM. M. et Jacob J., 1952, Preparation et propriétés pharmacodynamiques de quelques esters de la bétaine, *Ann. Pharm. Française*, 10:435-440.

- 20 Nixon-George A., Moran T., Dionne G., Penney C.L., et al. 1990, The adjuvant effect of stearyl tyrosine on a recombinant subunit hepatitis B surface antigen. *The Journal of Immunology*, 144(12): 4798-4802.

Penney C. L., Shah P., and Landi S., 1985, A simple method for the synthesis of long-chain alkyl esters of amino acids, *J. Org. Chem.* 50:1457-1459.

Penney C. L., Ethier D., Dionne G., Nixon-George A., Zaghoulani H., et al., 1993, Further studies on the adjuvanticity of stearyl tyrosine and ester analogues, *Vaccine* 11(11):1129-1134.

5 Rodriguez, M.D., Gomez, R., Sanchez, M and H. Garcia. 1998, *J. Appl. Toxicol.* 18: 313-6.

Rucka M., Oswiecimska M., Witek S. 1983, New biocides for cooling water treatment: Quaternary ammonium salts derivatives of glycine esters. *Environment Protection Engineering*, 9(3):25-31.

10 Schewe Ch., Schulz E., Vietinghoff G., Sprung W. D., Kobow M., Loose S., and Schewe T., 1991, On the mode of action of antiphlogistically active DL- ω -phenyl amino acid esters, *Biomed. Biochim. Acta*, 50(2):189-198.

Schulz E., Sprung WD, Kroning G. and Lauge, P. 1982, Pharmacological properties of D,L-2-phenylglycine alkyl esters with special consideration of their anti-inflammatory activity, *Agents Actions Suppl.*, AAS 10:119-128.

15 Smith D.R., Maienthal M. and Eifert R.L., 1951, Quaternary ammonium salts of heterocyclic bases, *JACS*, 73:2964-2965.

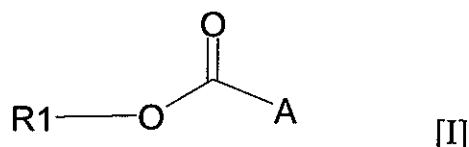
The Merck Index, 13th Edition, 2001, Merck & Co. Inc., Rahway, N.J., U.S.A.

20 Vetter W. and Meister W., 1981, Nicotines as derivatives for the mass spectrometric investigation of long chain alcohols, *Organic Mass Spectrometry*, 16(3): 118-122.

Wong O., Huntington J., Nishihata T., and Rytting J. H., 1989, New alkyl N,N-dialkyl-substituted amino acetates as transdermal penetration enhancers, *Pharmaceutical Research*, 6(4):286-295.

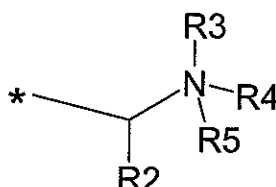
CLAIMS:

1. Use of a compound of the general formula I:



- 5 or of a pharmaceutically acceptable salt thereof, wherein R1 is C₁₂-C₂₄ alkyl or C₁₀-C₂₄ alkenyl, and A is selected from the group consisting of:

(i)

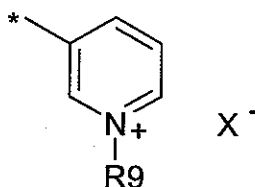


10

wherein R2 is H, C₁-C₆ alkyl, aryl or aralkyl, wherein any aryl moiety may be unsubstituted or substituted by nitro, cyano, halo, hydroxy, NR₆R₇, or CR₈R₈NR₆R₇; R3 is H, a pair of electrons or C₁-C₆ alkyl; R4 and R5 each independently is H or C₁-C₆ alkyl, or R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and R6, R7 and R8 each independently is H or C₁-C₆ alkyl;

(ii) phenyl substituted by NR₆R₇ or CR₈R₈NR₆R₇, wherein R6, R7 and R8 each is independently H or C₁-C₆ alkyl; and

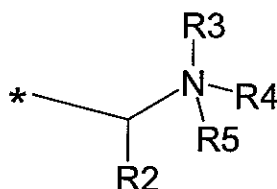
(iii)



wherein R₉ is H, C₁-C₆ alkyl or indolyl(C₁-C₄)alkyl, and X⁻ is a counter ion, or R₉ is a pair of electrons and X is absent, for the preparation of a pharmaceutical composition for treatment of inflammation.

- 5 2. The use according to claim 1 wherein R₁ is a C₁₂-C₂₀ alkyl or alkenyl.
3. The use according to claim 2 wherein R₁ is a C₁₆-C₁₈ alkyl or alkenyl.
4. The use according to claim 3 wherein R₁ is hexadecyl, octadecyl,
10 hexadecenyl, octadecenyl, cis-9-octadecenyl, trans-9-octadecenyl, cis-9,12-octadecadienyl, cis-6,9,12-octadecatrienyl, or cis-9,12,15-octadecatrienyl.
5. The use according to claim 4 wherein R₁ is cis-9-octadecenyl.

6. The use according to claim 5, wherein the residue A is of the formula:



5

wherein R₂ is H; a straight or branched C₁-C₆ alkyl selected from the group consisting of methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, pentyl and hexyl; phenyl, benzyl or p-hydroxybenzyl; R₃ is H, a pair of electrons or C₁-C₆ alkyl; R₄ and R₅ is each independently H or C₁-C₆ alkyl, or R₄ and R₅ together

10

with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl, said ring being selected from the group consisting of pyrrolidine, piperidine, morpholine, piperazine, and 4-methylpiperazine.

5

7. The use according to claim 6, wherein R₂ is H or phenyl, R₃ is H, methyl or a pair of electrons, R₄ and R₅ are each H or C₁-C₆ alkyl, or R₄ and R₅ together with the N atom to which they are attached form a morpholine ring or a piperazine ring optionally substituted at the nitrogen atom at position 4 by methyl.

10

8. The use according to claim 7, wherein said compound is selected from the group consisting of:

N,N-Dimethylamino-acetic acid octadec-(Z)-9-enyl ester;

(4-Methyl-piperazin-1-yl)-acetic acid octadec-(Z)-9-enyl ester tartrate;

15

(4-Methyl-piperazin-1-yl)-acetic acid octadecyl ester tartrate;

4-Methyl-4-octadec-(Z)-9-enyloxycarbonylmethyl-morpholin-4-ium chloride;

α-Amino-α-phenyl-acetic acid octadec-(Z)-9-enyl ester HCl salt; and

Piperazin-1-yl-acetic acid octadec-(Z)-9-enyl ester bitartrate.

20

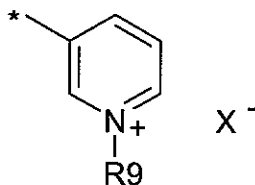
9. The use according to claim 1, wherein the residue A is phenyl substituted by NR₆R₇ or CR₈R₈NR₆R₇, wherein R₆, R₇ and R₈ is each independently H or 1 C₁-C₆ alkyl.

25

10. The use according to claim 9, wherein A is phenyl substituted by CR₈R₈NR₆R₇, wherein R₈ is H and R₆ and R₇ is each methyl.

11. The use according to claim 10, wherein said compound is 4-dimethylaminomethyl-benzoic acid octadec-(Z)-9-enyl ester HCl or 4-dimethylaminomethyl-benzoic acid octadec-(E)-9-enyl ester HCl.

12. The use according to claim 1, wherein A is the group:



wherein R9 is H, C₁-C₆ alkyl or indolyl(C₁-C₄)alkyl and X⁻ is a counter ion,
or R9 is a pair of electrons and X⁻ is absent.

13. The use according to claim 12, wherein R9 is a pair of electrons and X⁻ is absent, or R9 is methyl or indolylethyl and X⁻ is a counter ion selected from the group consisting of chloride, bromide, iodide and tosylate.

14. The use according to claim 13, wherein said compound is selected from the group consisting of:

Nicotinic acid octadec-(Z)-9-enyl ester;

1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium iodide;

1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium chloride;

1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium tosylate; and

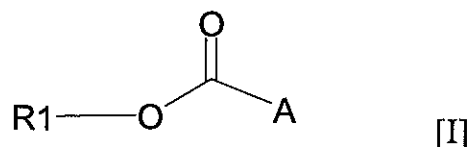
1-[(2-(1H-indol-3-yl)-ethyl)-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium bromide.

15. The use according to any one of claims 1 to 14, wherein said pharmaceutical composition is for treatment of immunologically-mediated inflammation.

16. The use according to claim 15, wherein said pharmaceutical composition is for the treatment of an immunologically-mediated chronic or acute inflammatory disease, disorder or condition.

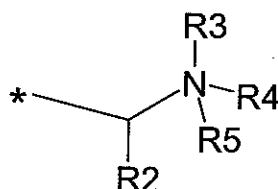
17. The use according to claim 16, wherein said pharmaceutical composition is for the treatment of an autoimmune disease, a severe allergy, asthma, or an inflammation associated with a disease, disorder or condition selected from graft rejection, a chronic degenerative disease such as Alzheimer's disease, neuroprotection, organ regeneration, chronic ulcers of the skin, or schizophrenia.
18. The use according to claim 17, wherein said autoimmune disease is multiple sclerosis or a human arthritic condition.
19. The use according to claim 18, wherein said human arthritic condition is rheumatoid arthritis, reactive arthritis with Reiter's syndrome, ankylosing spondylitis or other inflammation of the joints mediated by the immune system.
20. The use according to claim 16, wherein said immunologically-mediated inflammatory disease, disorder or condition is myasthenia gravis, Guillain-Barré syndrome, or other inflammatory disease of the nervous system; psoriasis, pemphigus vulgaris or other disease of the skin; systemic lupus erythematosus, glomerulonephritis or other disease affecting the kidneys; atherosclerosis or other inflammation of the blood vessels; autoimmune hepatitis, inflammatory bowel diseases, pancreatitis, or other disorder of the gastrointestinal system; type 1 diabetes mellitus, autoimmune thyroiditis, or other disease of the endocrine system.
21. The use according to claim 20, wherein said immunologically-mediated inflammatory disease or disorder is psoriasis.
22. The use according to any one of claims 1 to 21, wherein said pharmaceutical composition is for oral, topical, intradermal or parenteral administration.
23. The use according to claim 22, wherein said pharmaceutical composition is for subcutaneous, intravenous, or intramuscular administration.

24. A pharmaceutical composition for the treatment of inflammation comprising a pharmaceutically acceptable carrier and a compound of the general formula I:



5 or a pharmaceutically acceptable salt thereof, wherein R1 is C₁₂-C₂₄ alkyl or C₁₀-C₂₄ alkenyl, and A is selected from the group consisting of:

(i)

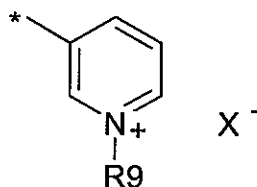


10 wherein R2 is H, C₁-C₆ alkyl, aryl or aralkyl, wherein any aryl moiety may be unsubstituted or substituted by nitro, cyano, halo, hydroxy, NR₆R₇, or CR₈R₈NR₆R₇; R3 is H, a pair of electrons or C₁-C₆ alkyl; R4 and R5 each independently is H or C₁-C₆ alkyl, or R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted
15 by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and R6, R7 and R8 each independently is H or C₁-C₆ alkyl;

(ii) phenyl substituted by NR₆R₇ or CR₈R₈NR₆R₇, wherein R6, R7 and R8 each is independently H or C₁-C₆ alkyl; and

(iii)

20



wherein R⁹ is H, C₁-C₆ alkyl or indolyl(C₁-C₄)alkyl, and X⁻ is a counter ion, or R⁹ is a pair of electrons and X is absent

5 25. The pharmaceutical composition according to claim 24, wherein R¹ is a C₁₂-C₂₀ alkyl or alkenyl.

26. The pharmaceutical composition according to claim 25, wherein R¹ is a C₁₆-C₁₈ alkyl or alkenyl.

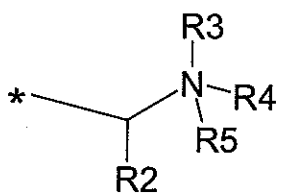
10

27. The pharmaceutical composition according to claim 26, wherein R¹ is hexadecyl, octadecyl, hexadecenyl, octadecenyl, cis-9-octadecenyl, trans-9-octadecenyl, cis-9,12-octadecadienyl, cis-6,9,12-octadecatrienyl, or cis-9,12,15-octadecatrienyl,

15

28. The pharmaceutical composition according to claim 27, wherein R¹ is cis-9-octadecenyl or trans-9-octadecenyl.

29. The pharmaceutical composition according to claim 24, wherein the residue A has the formula:



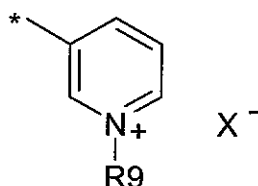
5

- wherein R₂ is H; a straight or branched C₁-C₆ alkyl selected from the group consisting of methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, pentyl and hexyl; phenyl, benzyl or p-hydroxybenzyl; R₃ is H, a pair of electrons or C₁-C₆ alkyl; R₄ and R₅ each is independently H or C₁-C₆ alkyl, or R₄ and R₅ together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl, said ring being selected from the group consisting of pyrrolidine, piperidine, morpholine, piperazine, and 4-methylpiperazine.
30. The pharmaceutical composition according to claim 29, wherein R₂ is H or phenyl, R₃ is H or a pair of electrons, R₄ and R₅ are each H or C₁-C₆ alkyl, or R₄ and R₅ together with the N atom to which they are attached form a morpholine ring or a piperazine ring optionally substituted at the nitrogen atom at position 1 or 4 by methyl.
31. The pharmaceutical composition according to claim 30, wherein said compound is selected from the group consisting of:
- N,N-Dimethylamino-acetic acid octadec-(Z)-9-enyl ester;
 - (4-Methyl-piperazin-1-yl)-acetic acid octadec-(Z)-9-enyl ester tartrate;
 - (4-Methyl-piperazin-1-yl)-acetic acid octadecyl ester tartrate;
 - 4-Methyl-4-octadec-(Z)-9-enyloxycarbonylmethyl-morpholin-4-ium chloride;
 - α-Amino-α-phenyl-acetic acid octadec-(Z)-9-enyl ester HCl salt; and
 - Piperazin-1-yl-acetic acid octadec-(Z)-9-enyl ester bitartrate.
32. The pharmaceutical composition according to claim 24, wherein the residue A is phenyl substituted by NR₆R₇ or CR₈R₈NR₆R₇, wherein R₆, R₇ and R₈ each is independently H or C₁-C₆ alkyl.

33. The pharmaceutical composition according to claim 32, wherein A is phenyl substituted by CR₈R₈NR₆R₇, wherein R₈ is H and R₆ and R₇ each is methyl.

34. The pharmaceutical composition according to claim 33, wherein said compound is 4-dimethylaminomethyl-benzoic acid octadec-(Z)-9-enyl ester HCl or 4-dimethylaminomethyl-benzoic acid octadec-(E)-9-enyl ester HCl.

5 35. The pharmaceutical composition according to claim 24, wherein A is the group:



10 wherein R9 is H, C₁-C₆ alkyl or indolyl(C₁-C₄)alkyl and X⁻ is a counter ion, or R9 is a pair of electrons and X⁻ is absent.

36. The pharmaceutical composition according to claim 35, wherein R9 is a pair of electrons and X⁻ is absent, or R9 is methyl or indolyethyl and X⁻ is a counter ion selected from the group consisting of chloride, bromide, iodide and tosylate.

15

37. The pharmaceutical composition according to claim 36, wherein said compound is selected from the group consisting of:

Nicotinic acid octadec-(Z)-9-enyl ester;

1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium iodide;

20

1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium chloride;

1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium tosylate; and

1-[(2-(1H-indol-3-yl)-ethyl)-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium bromide.

25 38. The pharmaceutical composition according to any one of claims 24 to 37, for treatment of immunologically-mediated inflammation.

39. The pharmaceutical composition according to claim 38, for the treatment of an immunologically-mediated chronic or acute inflammatory disease, disorder or condition.

5

40. The pharmaceutical composition according to claim 39, for the treatment of an autoimmune disease, a severe allergy, asthma, or an inflammation associated with a disease, disorder or condition selected from graft rejection, a chronic degenerative disease such as Alzheimer's disease, neuroprotection, organ
10 regeneration, chronic ulcers of the skin, or schizophrenia.

41. The pharmaceutical composition according to claim 40, wherein said autoimmune disease is multiple sclerosis or a human arthritic condition.

15 42. The pharmaceutical composition according to claim 41, wherein said human arthritic condition is rheumatoid arthritis, reactive arthritis with Reiter's syndrome, ankylosing spondylitis or other inflammation of the joints mediated by the immune system.

20 43. The pharmaceutical composition according to claim 39, wherein said immunologically-mediated inflammatory disease, disorder or condition is myasthenia gravis, Guillain-Barré syndrome, or other inflammatory disease of the nervous system; psoriasis, pemphigus vulgaris or other disease of the skin; systemic lupus erythematosus, glomerulonephritis or other disease affecting the kidneys;
25 atherosclerosis or other inflammation of the blood vessels; autoimmune hepatitis, inflammatory bowel diseases, pancreatitis, or other disorder of the gastrointestinal system; type 1 diabetes mellitus, autoimmune thyroiditis, or other disease of the endocrine system.

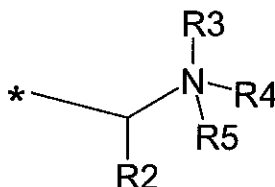
44. The pharmaceutical composition according to claim 43, wherein said immunologically-mediated inflammatory disease or disorder is psoriasis.
- 5 45. The pharmaceutical composition according to any one of claims 24 to 44 for oral, topical, intradermal or parenteral administration.
46. The pharmaceutical composition according to claim 45, for subcutaneous, intravenous, or intramuscular administration.

47. A compound of the general formula:



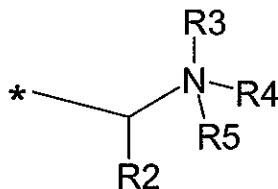
wherein

5 (i) R1 is C₂₀-C₂₄ alkyl or C₁₀-C₂₄ alkenyl and A is a residue of the formula:



wherein R2 is H, C₁-C₆ alkyl, aryl, or aralkyl, wherein any aryl moiety may be unsubstituted or substituted by nitro, cyano, halo, hydroxy, NR₆R₇, or CR₈R₈NR₆R₇; R3 is H, a pair of electrons, or C₁-C₆ alkyl; R4 and R5 each independently is H or C₁-C₆ alkyl, or R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and R6, R7 and R8 each independently is H or C₁-C₆ alkyl; or

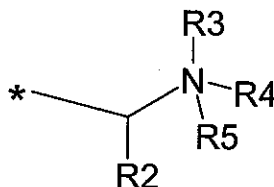
15 (ii) R1 is C₁₈ alkyl and A is a residue of the formula:



wherein R2 is H; R3 is a pair of electrons; and R4 and R5 together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring

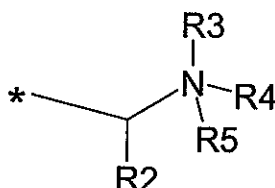
optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; or

(iii) R₁ is C₁₂-C₁₆ alkyl and A is a residue of the formula:



- 5 wherein R₂ is unsubstituted aryl, or aryl or aralkyl wherein the aryl moiety is substituted by nitro, cyano, halo, hydroxy, NR₆R₇, or CR₈R₈NR₆R₇; R₃ is H, a pair of electrons, or C₁-C₆ alkyl; R₄ and R₅ each independently is H or C₁-C₆ alkyl, or R₄ and R₅ together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and R₆, R₇ and R₈ each independently is H or C₁-C₆ alkyl; or

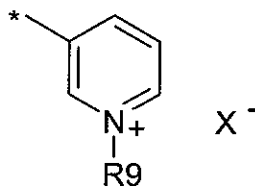
(iv) R₁ is C₁₀ alkyl and A is a residue of the formula:



- 15 wherein R₂ is C₁-C₆ alkyl; R₃ is H, a pair of electrons, or C₁-C₆ alkyl; R₄ and R₅ each independently is H or C₁-C₆ alkyl, or R₄ and R₅ together with the nitrogen atom to which they are attached form a 5-7 membered saturated ring optionally interrupted by an oxygen atom or by a nitrogen atom optionally substituted by C₁-C₆ alkyl; and R₆, R₇ and R₈ each independently is H or C₁-C₆ alkyl; or

- 20 (v) R₁ is C₁₀-C₂₄ alkyl or C₁₀-C₂₄ alkenyl and A is phenyl substituted by NR₆R₇ or CR₈R₈NR₆R₇, wherein R₆, R₇ and R₈ each independently is H or C₁-C₆ alkyl, but excluding the compounds wherein R₁ is C₁₀-C₁₆ alkyl and A is phenyl substituted by -CH₂-NH₂; or

(vi) R₁ is C₁₀-C₂₄ alkyl or C₁₀-C₂₄ alkenyl and A is a group of the formula:



wherein R9 is C₁-C₆ alkyl or indolyl(C₁-C₆)alkyl and X⁻ is a counter ion;

5 and pharmaceutically acceptable salts thereof.

48. A compound according to claim 47(i), (v) or (vi) wherein R1 is a C₁₂-C₁₈ alkenyl.

10 49. A compound according to claim 48, wherein R1 is a C₁₆-C₁₈ alkenyl.

50. A compound according to claim 49, wherein R1 is cis-9-octadecenyl or trans-9-octadecenyl.

15 51. A compound according to claim 50, wherein R2 is H or phenyl, R3 is H or a pair of electrons, and R4 and R5 are methyl or together with the N atom to which they are attached form a morpholino or a piperazine ring optionally substituted at the nitrogen atom at position 4 by methyl.

20 52. A compound according to claim 51, selected from the group consisting of:
 N,N-Dimethylamino-acetic acid octadec-(Z)-9-enyl ester;
 (4-Methyl-piperazin-1-yl)-acetic acid octadec-(Z)-9-enyl ester tartrate;
 4-Methyl-4-octadec-(Z)-9-enyloxycarbonylmethyl-morpholin-4-ium chloride;
 Piperazin-1-yl-acetic acid octadec-(Z)-9-enyl ester bitartrate.

53. A compound according to claim 47(ii), wherein R1 is octadecyl and R4 and R5 together with the N atom to which they are attached form a morpholino or a piperazine ring optionally substituted at the nitrogen atom at position 4 by methyl.

54. A compound according to claim 53, which is (4-methyl-piperazin-1-yl)-acetic acid octadecyl ester tartrate.
55. A compound according to claim 47(vi), wherein R1 is C₁₂-C₂₀ alkyl or C₁₂-C₂₀ alkenyl and A is phenyl substituted by CR₈R₈NR₆R₇, wherein R₈ is H and R₆ and R₇ is each H or C₁-C₆ alkyl.
56. A compound according to claim 55, wherein R1 is C₁₆-C₁₈ alkenyl.
57. A compound according to claim 56, wherein R1 is cis-9-octadecenyl or trans-9-octadecenyl.
58. A compound according to claim 57, which is 4-dimethylaminomethyl-benzoic acid octadec-(Z)-9-enyl ester HCl or 4-dimethylaminomethyl-benzoic acid octadec-(E)-9-enyl ester HCl.
59. A compound according to claim 47(vi), wherein R1 is C₁₂-C₂₀ alkyl or C₁₂-C₂₀ alkenyl.
60. A compound according to claim 59, wherein R1 is C₁₆-C₁₈ alkyl or alkenyl.
61. A compound according to claim 60, wherein R1 is cis-9-octadecenyl or trans-9-octadecenyl.
62. A compound according to claim 61, selected from the group consisting of:
1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium iodide;
1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium chloride;

1-Methyl-3-octadec-(Z)-9-enyloxycarbonyl-pyridinium tosylate; and
1-[(2-(1H-indol-3-yl)-ethyl]-3-octadec-(Z)-9-enyloxycarbonyl-
pyridinium bromide.

63. A compound according to claim 47(i), wherein R1 is cis-9-octadecenyl, R2 is phenyl, R3 is a pair of electrons and R4 and R is each H.

5 64. The compound of claim 63, which is α -amino- α -phenyl-acetic acid octadec-(Z)-9-enyl ester HCl salt.

65. A pharmaceutical composition comprising a compound according to any one of claims 47 to 64 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

10

66. A pharmaceutical composition according to claim 65, for the treatment of inflammation.

ABSTRACT

Basic esters of fatty alcohols of the general formula:



or pharmaceutically acceptable salt thereof, wherein R1 is C₁₂-C₂₄ alkyl or C₁₀-C₂₄ alkenyl, and A is a residue containing at least one acyclic or cyclic amino group and/or at least one heteroaromatic ring containing a tertiary or quaternary nitrogen atom, are anti-inflammatory and immunomodulatory agents, useful in the treatment
10 of immunologically-mediated inflammation.

15