FARNESYL TRANSFERASE INHIBITORS

This invention relates to compounds that inhibit farnesylation of gene products through inhibition of the enzyme farnesyl-protein transferase (FPTase). The invention also relates to methods of manufacturing the compounds, pharmaceutical compositions and methods of treating diseases, especially cancer, which are mediated through farnesylation.

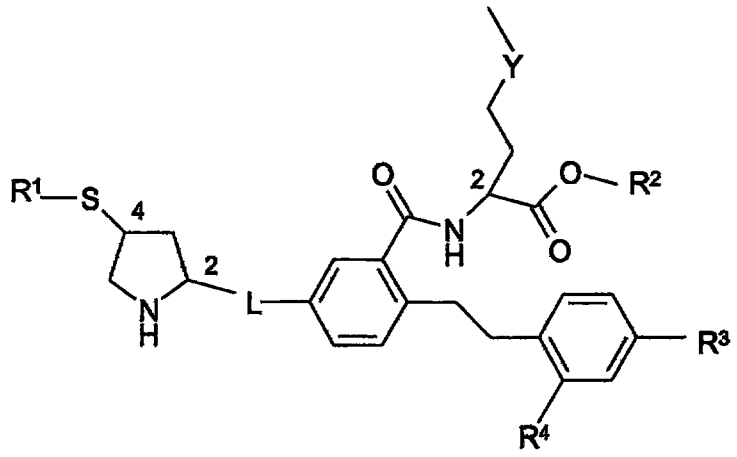
Cancer is believed to involve alteration in expression or function of genes controlling cell growth and differentiation. Whilst not wishing to be bound by theoretical considerations the following text sets out the scientific background to ras in cancer. Ras genes are frequently mutated in tumours. Ras genes encode guanosine triphosphate (GTP) binding proteins which are believed to be involved in signal transduction, proliferation and malignant transformation. H-, K- and N-ras genes have been identified as mutant forms of ras (Barbacid M, Ann. Rev. Biochem. 1987, 56: 779-827). Post translational modification of ras protein is required for biological activity. Farnesylation of ras catalysed by FPTase is believed to be an essential step in ras processing. It occurs by transfer of the farnesyl group of farnesyl pyrophosphate (FPP) to a cysteine at the C-terminal tetrapeptide of ras in a structural motif called the CAAX box. After further post-translational modifications, including proteolytic cleavage at the cysteine residue of the CAAX box and methylation of the cysteine carboxyl, ras is able to attach to the cell membrane for relay of growth signals to the cell interior. In normal cells activated ras is believed to act in conjunction with growth factors to stimulate cell growth. In tumour cells it is believed that mutations in ras cause it to stimulate cell division even in the absence of growth factors (Travis J, Science 1993, 260: 1877-1878), possibly through being permanently in GTP activated form rather than cycled back to GDP inactivated form. Inhibition of farnesylation of mutant ras gene products will stop or reduce activation.

One class of known inhibitors of farnesyl transferase is based on farnesyl pyrophosphate analogues; see for example European patent application EP 534546 from Merck. Inhibitors of farnesyl transferase based on mimicry of the CAAX box have been reported. Reiss (1990) in Cell 62, 81-8 disclosed tetrapeptides such as CVIM (Cys-Val-Ile-Met). James (1993) in Science 260, 1937-1942 disclosed benzodiazepine based peptidomimetic compounds. Lerner (1995) in J. Biol. Chem. 270, 26802 and Eisai in International Patent Application WO 95/25086 disclosed further peptidomimetic

compounds based on Cys as the first residue. EP 696593 and PCT/GB96/01810 disclose further farnesyl transferase inhibitors, including pyrrolidine derivatives. In addition, a range of inhibitors of ras farnesylation are described and claimed in PCT/GB99/00369.

The applicants have found that a particular substitution of the pyrrolidine provides particular advantages in terms of inhibition of farnesyl transferase.

According to one aspect of the present invention there is provided a compound of formula (I)



(I)

wherein:

R^1 and R^2 are independently selected from H or a prodrug moiety;

R^3 is hydrogen or halogen;

R^4 is hydrogen or halogen;

L is -CH=CH- or $-CH_2-Z-$ where Z is NH or O;

Y is S, S(O) or $S(O)_2$;

or a salt thereof.

As used herein, the term "alkyl" refers to straight or branched chain groups, which may, unless otherwise stated have from 1 to 20 and preferably from 1 to 6 carbon atoms. The term"aryl" includes phenyl. The term "halo" includes fluoro, chloro, bromo and iodo.

The term "heterocylyl" or "heterocyclic" include groups having from 4 to 10 ring atoms, up to 5 of which are selected from oxygen, sulphur and nitrogen. The rings may be mono-, or bicyclic and each ring may be aromatic or non-aromatic in character.

Nitrogen atoms may be substituted if the valency of the ring allows it, with either a hydrogen or substituent group, such as an alkyl substituent. Sulphur atoms in a heterocyclic ring may be oxidised to S(O) or $S(O)_2$ groups.

Examples of aromatic 5- or 6-membered heterocyclic ring systems include imidazole, triazole, pyrazine, pyrimidine, pyridazine, pyridine, isoxazole, oxazole, isothiazole, thiazole and thiophene. A 9- or 10-membered bicyclic heteroaryl ring system is an aromatic bicyclic ring system comprising a 6-membered ring fused to either a 5 membered ring or another 6 membered ring. Examples of 5/6 and 6/6 bicyclic ring systems include benzofuran, benzimidazole, benzthiophene, benzthiazole, benzisothiazole, benzoxazole, benzisoxazole, pyridoimidazole, pyrimidoimidazole, quinoline, isoquinoline, quinoxaline, quinazoline, phthalazine, cinnoline and naphthyridine.

Preferably monocyclic heteroaryl rings contain up to 3 heteroatoms and bicyclic heteroaryl rings contain up to 5 heteroatoms. Preferred heteroatoms are N and S, especially N. In general, attachment of heterocyclic rings to other groups is via carbon atoms. Suitable heterocyclic groups containing only N as the heteroatom are pyrrole, pyridine, indole, quinoline, isoquinoline, imidazole, pyrazine, pyrimidine, purine and pteridine.

Hydrogenated or other substituted forms of the above aromatic rings, (which are not aromatic), such as pyrrolidine, piperidine or morpholine rings are examples of non-aromatic heterocyclic groups.

Various forms of prodrugs are known in the art. For examples of such prodrug derivatives, see:

a) Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985) and Methods in Enzymology, Vol. 42, p. 309-396, edited by K. Widder, *et al.* (Academic Press, 1985);

b) A Textbook of Drug Design and Development, edited by Krogsgaard-Larsen;

c) H. Bundgaard, Chapter 5 "Design and Application of Prodrugs", by H. Bundgaard p. 113-191 (1991);

d) H. Bundgaard, Advanced Drug Delivery Reviews, 8, 1-38 (1992);

e) H. Bundgaard, *et al.*, Journal of Pharmaceutical Sciences, 77, 285 (1988); and

f) N. Kakeya, *et al.*, Chem Pharm Bull, 32, 692 (1984).

Suitable examples of groups R^1 are hydrogen or prodrug groups of formula $R^5C(O)$ -where R^5 is an optionally substituted aryl or heterocyclyl group. In particular

R^5 is optionally substituted phenyl, optionally substituted pyridyl, optionally substituted furyl, optionally substituted isoxazole, optionally substituted tetrahydropyridyl or optionally substituted tetrahydrofuryl.

Suitable substituents for R^5 include alkyl groups such as methyl, haloalkyl groups such as trifluoromethyl, hydroxy, alkoxy such as methoxy or cyano.

Preferably R^5 is phenyl, pyridyl or N-methylpiperidine.

Examples of prodrugs groups for R^2 are *in vivo* cleavable ester groups of a pharmaceutically-acceptable ester which is cleaved in the human or animal body to produce the parent acid. Suitably R^2 together with the carboxy group to which it is attached forms a pharmaceutically-acceptable esters such as C_{1-6}alkyl esters or C_{1-6}cycloalkyl esters, for example methyl, ethyl, propyl, iso-propyl, n-butyl or cyclopentyl ; C_{1-6}alkoxymethyl esters, for example methoxymethyl; C_{1-6}alkanoyloxymethyl esters, for example pivaloyloxymethyl; phthalidyl esters; C_{3-8}cycloalkoxycarbonyloxyC_{1-6}alkyl esters, for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolan-2-ylmethyl esters, for example 5-methyl-1,3-dioxolan-2-ylmethyl; C_{1-6}alkoxycarbonyloxyethyl esters, for example 1-methoxycarbonyloxyethyl; aminocarbonylmethyl esters and mono- or di-N-(C_{1-6}alkyl) versions thereof, for example N,N-dimethylaminocarbonylmethyl esters and N-ethylaminocarbonylmethyl esters, and pharmaceutically acceptable esters of optionally substituted heterocyclic groups.

Further examples of such prodrugs for R^2 are *in vivo* cleavable amides of a compound of the invention. Suitably R^2 together with the carboxy group to which it is attached forms a pharmaceutically-acceptable amide, preferably an N-C_{1-6}alkylamide and an N,N-di-(C_{1-6}alkyl)amide, such as N-methyl, N-ethyl, N-propyl, N,N-dimethyl, N-ethyl-N-methyl or N,N-diethylamide.

Thus in particular, R^2 is selected from hydrogen, a C_{1-4}alkyl group such as isopropyl or cyclopentyl, or an optionally substituted heterocyclic group such as N-methyl - tetrahydropyridyl.

R^3 is suitably a halo atom in particular fluorine.

R^4 is preferably a hydrogen or fluorine, and in particular is hydrogen.

The linking group L is preferably a group of formula CH_2-Z- where Z is NH or O.

Group Y is preferably a group S, S(O) or $S(O)_2$.

It is to be understood that, insofar as certain of the compounds of Formula I defined above may exist in optically active or racemic forms by virtue of one or more asymmetric carbon atoms, the invention includes in its definition any such optically active or racemic form which possesses the property of inhibiting FTPase. The synthesis of optically active forms may be carried out by standard techniques of organic chemistry well known in the art, for example by synthesis from optically active starting materials or by resolution of a racemic form. Similarly, inhibitory properties against FTPase may be evaluated using the standard laboratory techniques referred to hereinafter.

Chiral carbon atoms at the 2 and 4 positions of the pyrrolidine ring in Formula I are preferred in the (S) configuration.

The chiral carbon atom at the 2 position between the carbonyl and amine in Formula I is preferred in the (S) configuration.

Compounds of Formula I may form salts which are within the ambit of the invention. Pharmaceutically acceptable salts are preferred although other salts may be useful in, for example, isolating or purifying compounds.

When the compound contains a basic moiety it may form pharmaceutically acceptable salts with a variety of inorganic or organic acids, for example hydrochloric, hydrobromic, sulphuric, phosphoric, trifluoroacetic, citric or maleic acid. A suitable pharmaceutically-acceptable salt of the invention when the compound contains an acidic moiety is an alkali metal salt, for example a sodium or potassium salt, an alkaline earth metal salt, for example a calcium or magnesium salt, an ammonium salt or a salt with an organic base which affords a pharmaceutically-acceptable cation, for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine. Particular salts of compounds of the invention are acetates, alkyl sulphonates such as methyl or ethyl sulphonate, fumarates, formates, succinates and gluconates.

Solvates, for example hydrates, are also within the ambit of the invention and may be prepared by generally known methods.

Particular examples of compounds of formula (I) are shown in Table 1

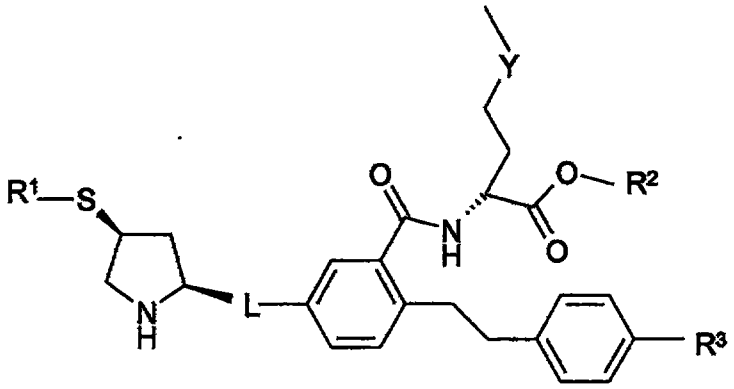
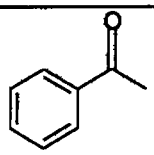
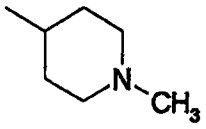
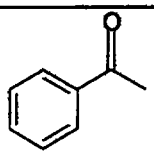
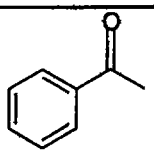
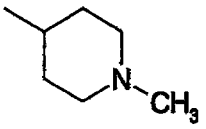
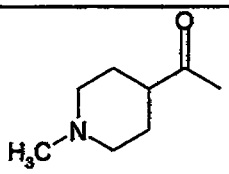
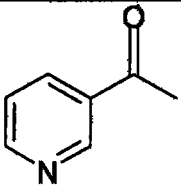
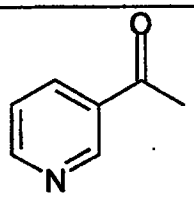
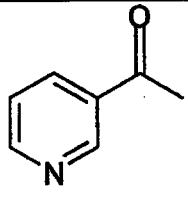


Table 1

Compd. No	R^1	R^2	R^3	L	Y
1	H	H	F	$-CH_2NH-$	S
2	H	$-CH(CH_3)_2$	F	$-CH_2NH-$	S
3			F	$-CH_2O-$	S
4		$-CH(CH_3)_2$	F	$-CH_2NH-$	S
5			F	$-CH_2NH-$	S
6		$-CH(CH_3)_2$	F	$-CH_2NH-$	S
7		$-CH(CH_3)_2$	F	$-CH_2O-$	SO_2

8		$-CH(CH_3)_2$	F	$-CH_2NH-$	S
9		$-CH(CH_3)_2$	F	-CH=CH-	S

According to another aspect of the invention there is provided a compound of Formula I for use as a medicament.

Further according to the invention there is provided a compound of Formula I for use in preparation of a medicament for treatment of a disease mediated through farnesylation of ras, in particular cancer.

The compound is suitably formulated as a pharmaceutical composition for use in this way.

Thus, according to yet another aspect of the invention there is provided a pharmaceutical composition comprising a compound of formula I listed above together with a pharmaceutically acceptable diluent or carrier.

According to another aspect of the present invention there is provided a method of treating ras mediated diseases, especially cancer, by administering an effective amount of a compound of Formula I to a mammal in need of such treatment.

According to a further feature of the invention there is provided a compound of Formula I, or a pharmaceutically-acceptable salt thereof, for use in a method of treatment of the human or animal body by therapy.

The invention also provides the use of a compound of formula (I) in the preparation of a medicament for use in treating farnesylated mediated disease or medical condition such as cancers.

Specific cancers which may be treated by the compound or composition of the invention include:

- carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin;

- hematopoietic tumors of lymphoid lineage, including acute lymphocytic leukemia, B-cell lymphoma and Burketts lymphoma;

- hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia;

- tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; and

- other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma.

The compounds of Formula I are especially useful in treatment of tumors having a high incidence of ras mutation, such as colon, lung, and pancreatic tumors. By the administration of a composition having one (or a combination) of the compounds of this invention, development of tumors in a mammalian host is reduced.

Compounds of Formula I may also be useful in the treatment of diseases other than cancer that may be associated with signal transduction pathways operating through Ras, e.g., neuro-fibromatosis.

Compounds of Formula I may also be useful in the treatment of diseases associated with CAAX-containing proteins other than Ras (e.g., nuclear lamins and transducin) that are also post-translationally modified by the enzyme farnesyl protein transferase.

The compositions of the invention may be in a form suitable for oral use (for example as tablets, lozenges, hard or soft capsules, aqueous or oily suspensions, emulsions, dispersible powders or granules, syrups or elixirs), for topical use (for example as creams, ointments, gels, or aqueous or oily solutions or suspensions), for administration by inhalation (for example as a finely divided powder or a liquid aerosol), for administration by insufflation (for example as a finely divided powder) or for parenteral administration (for example as a sterile aqueous or oily solution for intravenous, subcutaneous, intramuscular or intramuscular dosing or as a suppository for rectal dosing).

The compositions of the invention may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art. Thus, compositions intended for oral use may contain, for example, one or more colouring, sweetening, flavouring and/or preservative agents.

Suitable pharmaceutically acceptable excipients for a tablet formulation include, for example, inert diluents such as lactose, sodium carbonate, calcium phosphate or

calcium carbonate, granulating and disintegrating agents such as corn starch or algenic acid; binding agents such as starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl p-hydroxybenzoate, and anti-oxidants, such as ascorbic acid. Tablet formulations may be uncoated or coated either to modify their disintegration and the subsequent absorption of the active ingredient within the gastrointestinal tract, or to improve their stability and/or appearance, in either case, using conventional coating agents and procedures well known in the art.

Compositions for oral use may be in the form of hard gelatin capsules in which the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions generally contain the active ingredient in finely powdered form together with one or more suspending agents, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as lecithin or condensation products of an alkylene oxide with fatty acids (for example polyoxethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives (such as ethyl or propyl p-hydroxybenzoate, anti-oxidants (such as ascorbic acid), colouring agents, flavouring agents, and/or sweetening agents (such as sucrose, saccharine or aspartame).

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil (such as arachis oil, olive oil, sesame oil or coconut oil) or in a mineral oil (such as liquid paraffin). The oily suspensions may also contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set out above,

and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water generally contain the active ingredient together with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients such as sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, or a mineral oil, such as for example liquid paraffin or a mixture of any of these. Suitable emulsifying agents may be, for example, naturally-occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soya bean, lecithin, an esters or partial esters derived from fatty acids and hexitol anhydrides (for example sorbitan monooleate) and condensation products of the said partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring and preservative agents.

Syrups and elixirs may be formulated with sweetening agents such as glycerol, propylene glycol, sorbitol, aspartame or sucrose, and may also contain a demulcent, preservative, flavouring and/or colouring agent.

The pharmaceutical compositions may also be in the form of a sterile injectable aqueous or oily suspension, which may be formulated according to known procedures using one or more of the appropriate dispersing or wetting agents and suspending agents, which have been mentioned above. A sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example a solution in 1,3-butanediol.

Suppository formulations may be prepared by mixing the active ingredient with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Suitable excipients include, for example, cocoa butter and polyethylene glycols.

Topical formulations, such as creams, ointments, gels and aqueous or oily solutions or suspensions, may generally be obtained by formulating an active ingredient with a

conventional, topically acceptable, vehicle or diluent using conventional procedure well known in the art.

Compositions for administration by insufflation may be in the form of a finely divided powder containing particles of average diameter of, for example, 30μ or much less, the powder itself comprising either active ingredient alone or diluted with one or more physiologically acceptable carriers such as lactose. The powder for insufflation is then conveniently retained in a capsule containing, for example, 1 to 50mg of active ingredient for use with a turbo-inhaler device, such as is used for insufflation of the known agent sodium cromoglycate.

Compositions for administration by inhalation may be in the form of a conventional pressurised aerosol arranged to dispense the active ingredient either as an aerosol containing finely divided solid or liquid droplets. Conventional aerosol propellants such as volatile fluorinated hydrocarbons or hydrocarbons may be used and the aerosol device is conveniently arranged to dispense a metered quantity of active ingredient.

For further information on formulation the reader is referred to Chapter 25.2 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The amount of active ingredient that is combined with one or more excipients to produce a single dosage form will necessarily vary depending upon the host treated and the particular route of administration. For example, a formulation intended for oral administration to humans will generally contain, for example, from 0.5 mg to 2 g of active agent compounded with an appropriate and convenient amount of excipients which may vary from about 5 to about 98 percent by weight of the total composition. Dosage unit forms will generally contain about 1 mg to about 500 mg of an active ingredient. For further information on Routes of Administration and Dosage Regimes the reader is referred to Chapter 25.3 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The size of the dose for therapeutic or prophylactic purposes of a compound of the Formula I will naturally vary according to the nature and severity of the conditions, the age and sex of the animal or patient and the route of administration, according to well known principles of medicine. As mentioned above, compounds of the Formula I are useful in

treating diseases or medical conditions which are due alone or in part to the effects of farnesylation of ras.

In using a compound of the Formula I for therapeutic or prophylactic purposes it will generally be administered so that a daily dose in the range, for example, 0.5 mg to 75 mg per kg body weight is received, given if required in divided doses. In general lower doses will be administered when a parenteral route is employed. Thus, for example, for intravenous administration, a dose in the range, for example, 0.5 mg to 30 mg per kg body weight will generally be used. Similarly, for administration by inhalation, a dose in the range, for example, 0.5 mg to 25 mg per kg body weight will be used. Oral administration is however preferred.

The compounds of formula (I) defined above may be applied as a sole therapy or may involve, in addition to a compound of the invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. In the field of medical oncology it is normal practice to use a combination of different forms of treatment to treat each patient with cancer. In medical oncology the other component(s) of such conjoint treatment in addition to the cell cycle inhibitory treatment defined hereinbefore may be: surgery, radiotherapy or chemotherapy. Such chemotherapy may cover three main categories of therapeutic agent:

(i) other cell cycle inhibitory agents that work by the same or different mechanisms from those defined hereinbefore;

(ii) cytostatic agents such as antioestrogens (for example fulvestrant, tamoxifen, toremifene, raloxifene, droloxifene, iodoxyfene), progestogens (for example megestrol acetate), aromatase inhibitors (for example anastrozole, letrazole, vorazole, exemestane), antiprogestogens, antiandrogens (for example flutamide, nilutamide, bicalutamide, cyproterone acetate), LHRH agonists and antagonists (for example goserelin acetate, luprolide), inhibitors of testosterone 5α-dihydroreductase (for example finasteride), anti-invasion agents (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function) and inhibitors of growth factor function, (such growth factors include for example platelet derived growth factor and hepatocyte growth factor such inhibitors include growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors and serine/threonine kinase inhibitors); and

(iii) antiproliferative/antineoplastic drugs and combinations thereof, as used in medical oncology, such as antimetabolites (for example antifolates like methotrexate, fluoropyrimidines like 5-fluorouracil, purine and adenosine analogues, cytosine arabinoside); antitumour antibiotics (for example anthracyclines like doxorubicin, daunomycin, epirubicin and idarubicin, mitomycin-C, dactinomycin, mithramycin); platinum derivatives (for example cisplatin, carboplatin); alkylating agents (for example nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide, nitrosoureas, thiotepa); antimitotic agents (for example vinca alkaloids like vincristine and taxoids like taxol, taxotere); topoisomerase inhibitors (for example epipodophyllotoxins like etoposide and teniposide, amsacrine, topotecan). According to this aspect of the invention there is provided a pharmaceutical product comprising a compound of the formula (I) as defined above and an additional anti-tumour substance as defined hereinbefore for the conjoint treatment of cancer.

Although the compounds of the Formula I are primarily of value as therapeutic agents for use in warm-blooded animals (including man), they are also useful whenever it is required to inhibit farnesylation. Thus, they are useful as pharmacological standards for use in the development of new biological tests and in the search for new pharmacological agents.

According to another aspect of the present invention there is provided individual compounds produced as end products in the Examples set out below and salts thereof.

A compound of the invention, or a salt thereof, may be prepared by any process known to be applicable to the preparation of such compounds or structurally related compounds. Such processes are illustrated by the following representative schemes in which variable groups have any of the meanings defined for Formula I unless stated otherwise. Functional groups may be protected and deprotected using conventional methods. For examples of protecting groups such as amino and carboxylic acid protecting groups (as well as means of formation and eventual deprotection), see T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis", Second Edition, John Wiley & Sons, New York, 1991. Note abbreviations used have been listed immediately before the Examples below.

Protecting groups may be removed by any convenient method as described in the literature or known to the skilled chemist as appropriate for the removal of the protecting

group in question, such methods being chosen so as to effect removal of the protecting group with minimum disturbance of groups elsewhere in the molecule.

Specific examples of protecting groups are given below for the sake of convenience, in which "lower" signifies that the group to which it is applied preferably has 1-4 carbon atoms. It will be understood that these examples are not exhaustive. Where specific examples of methods for the removal of protecting groups are given below these are similarly not exhaustive. The use of protecting groups and methods of deprotection not specifically mentioned is of course within the scope of the invention.

A carboxy protecting group may be the residue of an ester-forming aliphatic or araliphatic alcohol or of an ester-forming silanol (the said alcohol or silanol preferably containing 1-20 carbon atoms).

Examples of carboxy protecting groups include straight or branched chain (1-12C)alkyl groups (e.g. isopropyl, t-butyl); lower alkoxy lower alkyl groups (e.g. methoxymethyl, ethoxymethyl, isobutoxymethyl; lower aliphatic acyloxy lower alkyl groups, (e.g. acetoxymethyl, propionyloxymethyl, butyryloxymethyl, pivaloyloxymethyl); lower alkoxycarbonyloxy lower alkyl groups (e.g. 1-methoxycarbonyloxyethyl, 1-ethoxycarbonyloxyethyl); aryl lower alkyl groups (e.g. p-methoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, benzhydryl and phthalidyl); tri(lower alkyl)silyl groups (e.g. trimethylsilyl and t-butyldimethylsilyl); tri(lower alkyl)silyl lower alkyl groups (e.g. trimethylsilylethyl); and (2-6C)alkenyl groups (e.g. allyl and vinylethyl).

Methods particularly appropriate for the removal of carboxyl protecting groups include for example acid-, metal- or enzymically-catalysed hydrolysis.

Examples of hydroxy protecting groups include lower alkenyl groups (e.g. allyl); lower alkanoyl groups (e.g. acetyl); lower alkoxycarbonyl groups (e.g. t-butoxycarbonyl); lower alkenyloxycarbonyl groups (e.g. allyloxycarbonyl); aryl lower alkoxycarbonyl groups (e.g. benzoyloxycarbonyl, p-methoxybenzyloxycarbonyl, o-nitrobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl); tri lower alkyl/arylsilyl groups (e.g. trimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl); aryl lower alkyl groups (e.g. benzyl) groups; and triaryl lower alkyl groups (e.g. triphenylmethyl).

Examples of amino protecting groups include formyl, aralkyl groups (e.g. benzyl and substituted benzyl, e.g. p-methoxybenzyl, nitrobenzyl and 2,4-dimethoxybenzyl, and triphenylmethyl); di-p-anisylmethyl and furylmethyl groups; lower alkoxycarbonyl (e.g.

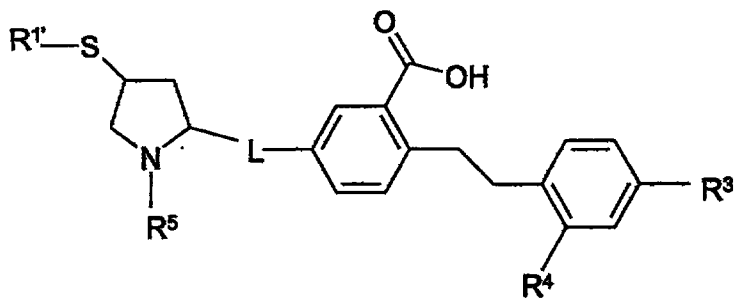
t-butoxycarbonyl); lower alkenyloxycarbonyl (e.g. allyloxycarbonyl); aryl lower alkoxycarbonyl groups (e.g. benzyloxycarbonyl, p-methoxybenzyloxycarbonyl, o-nitrobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl; trialkylsilyl (e.g. trimethylsilyl and t-butyldimethylsilyl); alkylidene (e.g. methylidene); benzylidene and substituted benzylidene groups.

Methods appropriate for removal of hydroxy and amino protecting groups include, for example, acid-, base, metal- or enzymically-catalysed hydrolysis, or photolytically for groups such as o-nitrobenzyloxycarbonyl, or with fluoride ions for silyl groups.

Examples of protecting groups for amide groups include aralkoxymethyl (e.g. benzyloxymethyl and substituted benzyloxymethyl); alkoxymethyl (e.g. methoxymethyl and trimethylsilylethoxymethyl); tri alkyl/arylsilyl (e.g. trimethylsilyl, t-butyldimethylsily, t-butyldiphenylsilyl); tri alkyl/arylsilyloxymethyl (e.g. t-butyldimethylsilyloxymethyl, t-butyldiphenylsilyloxymethyl); 4-alkoxyphenyl (e.g. 4-methoxyphenyl); 2,4-di(alkoxy)phenyl (e.g. 2,4-dimethoxyphenyl); 4-alkoxybenzyl (e.g. 4-methoxybenzyl); 2,4-di(alkoxy)benzyl (e.g. 2,4-di(methoxy)benzyl); and alk-1-enyl (e.g. allyl, but-1-enyl and substituted vinyl e.g. 2-phenylvinyl).

Aralkoxymethyl, groups may be introduced onto the amide group by reacting the latter group with the appropriate aralkoxymethyl chloride, and removed by catalytic hydrogenation. Alkoxymethyl, tri alkyl/arylsilyl and tri alkyl/silyloxymethyl groups may be introduced by reacting the amide with the appropriate chloride and removing with acid; or in the case of the silyl containing groups, fluoride ions. The alkoxyphenyl and alkoxybenzyl groups are conveniently introduced by arylation or alkylation with an appropriate halide and removed by oxidation with ceric ammonium nitrate. Finally alk-1-enyl groups may be introduced by reacting the amide with the appropriate aldehyde and removed with acid.

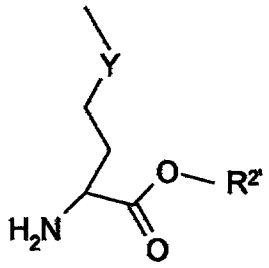
The invention also provides a process for preparing a compound of formula (I) as defined above which process comprises reacting a compound of formula (II)



(II)

where L, R^3 and R^4 are as defined in relation to formula (I), $R^{1'}$ is a group R^1 as defined in relation to formula (I) or a precursor thereof, and R^5 is a protecting group such as BOC,

with a compound of formula (III)



(III)

where Y is as defined in relation to formula (I) and $R^{2'}$ is a group R^2 as defined in relation to formula (I) or a precursor thereof;

and thereafter if desired or necessary, carrying out one or more of the following steps:

a) removing protecting groups R^5;

a) converting any precursor groups $R^{1'}$ and $R^{2'}$ to groups R^1 and R^2; and

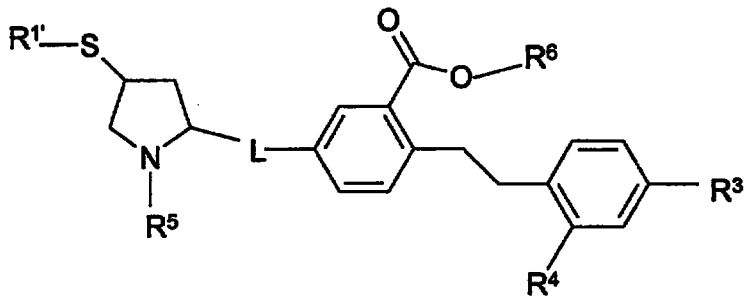
b) changing said groups to different R^1, R^2 groups.

The reaction between compounds of formula (II) and (III) is suitably effected in an organic solvent such as dichloromethane in the presence of a base such as N-methylmorpholine and EDC, and optionally in the presence of a nucleophilic catalyst such as 1-hydroxybenztriazole. Moderate temperatures for example of from 0 to 50°C, conveniently ambient temperature, are employed.

Precursor groups $R^{1'}$ and $R^{2'}$ may include protecting groups such as esters, which are not pharmaceutically acceptable. These may be converted to hydrogen or other prodrug groups using conventional methods as illustrated below.

Removal of protecting groups R^5 can be carried out using conventional methods such as reaction with TFA and/or triethylsilane.

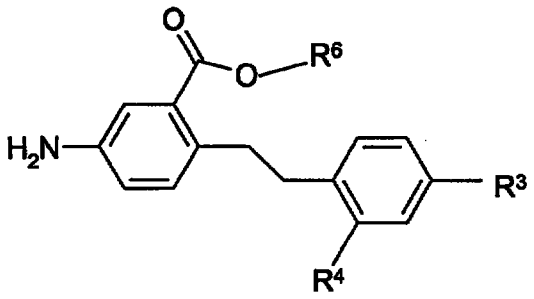
Compounds of formula (II) are suitably prepared by deprotecting a compound of formula (IV)



(IV)

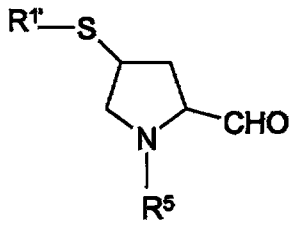
where $R^{1'}$, R^3, R^4, R^5 and L are as defined in relation to formula (II) and R^6 is a protecting group, in particular an alkyl group such as methyl. Deprotection is suitably effected using a strong base such as an alkali metal hydroxide, in particular sodium hydroxide. The reaction is suitably effected in a solvent such as aqueous alcohol and in particular aqueous methanol, at elevated temperatures, conveniently at the reflux temperature of the solvent.

Compounds of formula (IV) where L is $-CH_2NH-$ may be prepared by coupling a compound of formula (V)



(V)

where R^3, R^4 and R^6 are as defined above; with an aldehyde of formula (VI)

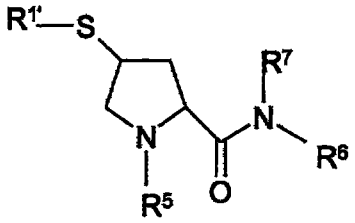


(VI)

where $R^{1'}$ and R^5 are as defined above.

Suitable coupling conditions include the use of a reducing agent (e.g. borane.pyridine with acetic acid, $NaCNBH_3$, BH_3, hydrogen plus catalyst, $LiHBEt_3$, di-isobutyl-aluminiumhydride, lithium aluminium hydride, sodium borohydride) in the presence of a suitable solvent e.g.methanol or ethanol & acetic acid.

Aldehydes of formula (VI) may be prepared by reduction of the compounds of formula (VII)

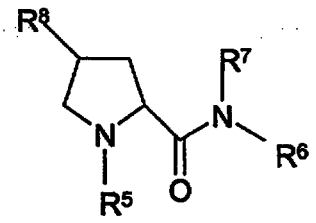


(VII)

where $R^{1'}$ and R^5 are as defined above and R^6 is alkyl such as methyl and R^7 is alkoxy such as methoxy.

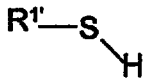
Suitably powerful reducing agents such as lithium aluminium hydride or DIBAL are employed. The reaction is carried out in a solvent such as tetrahydrofuran at low temperatures, for example from -50 to 0°C, in particular at about -40°C.

Compounds of formula (VII) are suitably prepared by reacting a compound of formula (VIII)



(VIII)

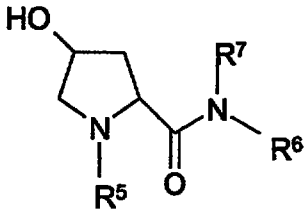
where R^8 is a leaving group such a methansulphonyloxy group, which a compound of formula (IX)



(IX)

where $R^{1'}$ is as defined above and in particular is a triphenylmethyl or trityl group. Reaction conditions would be apparent to the skilled person, but in general, the reaction is effected in an organic solvent such as ethanol at moderate temperatures, for example of from 0 to 60°C and preferably at about 40°C.

Compounds of formula (VIII) may be prepared by reacting compounds of formula (X)



(X)

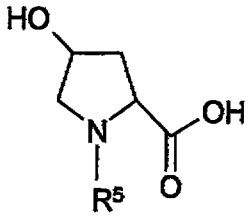
where R^5, R^6 and R^7 are as defined above, with a compound of formula (XI)

R^8-Z

(XI)

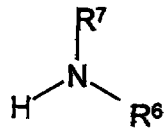
where R^8 is as defined above and Z is a leaving group such as halogen, in particular chlorine. The reaction is suitably effected in an organic solvent such as dichloromethane in the presence of a weak base such as triethylamine. Moderate to low temperatures, for example, from 0-30°C are suitably employed.

Compounds of formula (X) are suitably prepared by reacting a compound of formula (XII)



(XII)

where R^5 is as defined above, with a compound of formula (XIII)

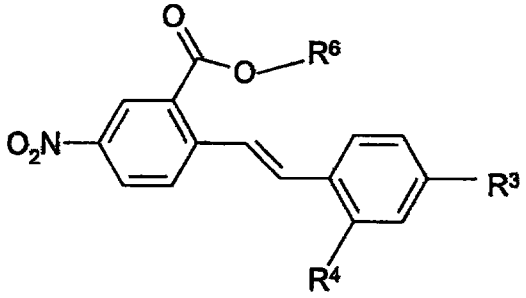


(XIII)

where R^6 and R^7 are as defined above. A particular example of a compound of formula (XIII) is N,O-dimethylhydroxylamine. The reaction is suitably effected in the presence of a base (such as N-methylmorpholine with EDCI) and in an organic solvent such as dichloromethane.

Compounds of formula (XII) may be prepared by N-protection of the corresponding hydroxy proline derivative using known methods.

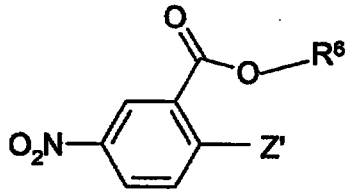
Compounds of formula (V) are suitably prepared by hydrogenation of a compound of formula (XIV)



(XIV)

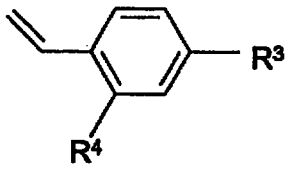
where R^3, R^4 and R^6 are as defined above. Hydrogenation is suitably effected in the presence of a catalyst such as a palladium catalyst.

Compounds of formula (XIV) are suitably prepared by reacting a compound of formula (XV)



(XV)

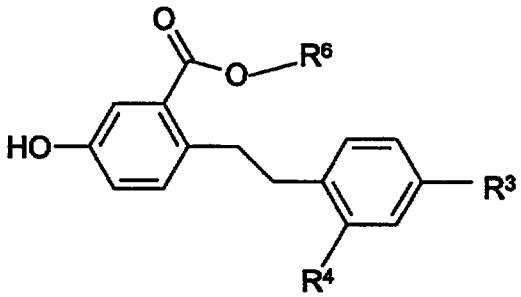
where R^6 is as defined above and Z' is a leaving group such as halogen such as bromine or chlorine, preferably chlorine, with a compound of formula (XVI)



(XVI)

where R^3 and R^4 are as defined above. The reaction is suitably effected in the presence of a base such as tributylamine, preferably sodium carbonate terabutylammonium bromide, and a catalyst such as a palladium catalyst such as. bis-(triphenylphosphine)-palladium(II) chloride in a suitable solvent, preferably dimethylacetamide.

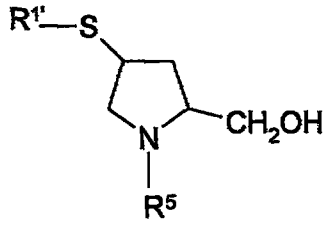
Compounds of formula (IV) where L represents $-CH_2O-$ may be prepared by reacting a compound of formula (XVII)



(XVII)

where R^3, R^4 and R^6 are as defined above,

with a compound of formula (XVIII)



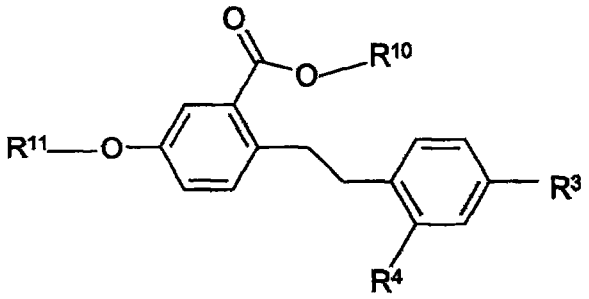
(XVIII)

where $R^{1'}$ and R^5 are as defined above. The reaction is suitably effected under conditions similar to those described above for the reaction between compounds of formulae (V) and (VI).

Compounds of formula (XVIII) are suitably prepared by reduction of a compound of formula (VI), for example using a reducing agent such as lithium aluminium hydride. Reduction is carried out under conventional conditions in a solvent such as tetrahydrofuran.

Compounds of formula (XVII) may be prepared by protection of the corresponding carboxylic acid, for example by esterifying the acid using an alcohol, in particular an alkyl alcohol such as methanol. The reaction is suitably effected in the presence of sulphuryl chloride or the like, at elevated temperatures, conveniently at the reflux temperature of the solvent.

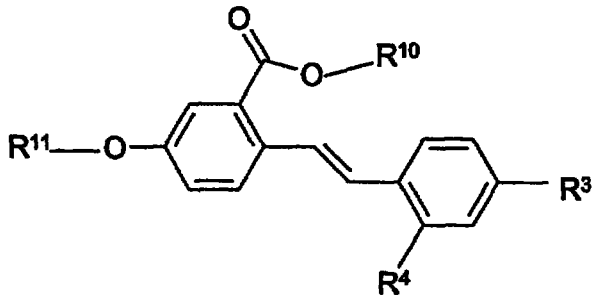
The acid itself may be prepared by deprotection of a compound of formula (XIX)



(XIX)

where R^3 and R^4 are as defined above and R^{10} and R^{11} are protecting groups such as alkyl, and in particular methyl groups. Suitable deprotection conditions include heating the compound with a suitable reagent such as pyridine hydrochloride to high temperatures such, for example from 200 to 250°C, and preferably at about 220°C.

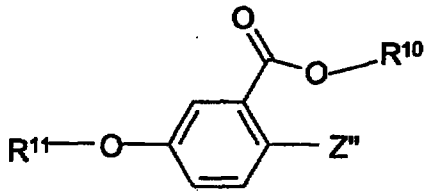
Compounds of formula (XIX) are obtained by hydrogenation of a compound of formula (XX)



(XX)

where R^3, R^4, R^{10} and R^{11} are as described above. Suitable hydrogenation conditions include those described above for the hydrogenation of the compound of formula (XIV).

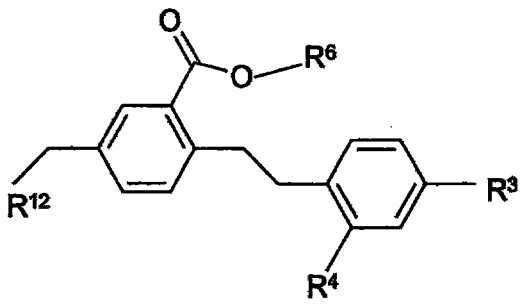
Compounds of formula (XX) may be prepared by reacting a compound of formula (XXI)



(XXI)

where R^{10} and R^{11} are as defined above and Z'' is a leaving group such as halogen and in particular bromine, with a compound of formula (XVI) as defined above using conditions similar to those described above for the reaction between compounds of formula (XV) and (XVI).

Compounds of formula (IV) where L is -CH=CH- are suitably prepared by reacting a compound of formula (VI) as defined above with a compound of formula (XXII)



(XXII)

where R^3, R^4 and R^6 are as defined above and R^{12} is a phosphate ion such as diethylphosphate, or a triphenylphosphine group. The reaction is a Wittig reaction and is suitably carried out under conventional conditions. Suitable reaction conditions include the use of a base (e.g. potassium carbonate, metal hydride, metal alkoxide) in the presence of an organic solvent (e.g. THF, toluene, DMSO) optionally in the presence of an aqueous solvent (2-phase system) and optionally in the presence of a catalyst complexing agent which solubilises alkali metal ions in non-polar solvents such as 1,4,7,10,13-pentaoxacyclopentadecane (also called 15-Crown-5) or 1,4,7,10,13,16-hexaoxacyclooctadecane (also called 18-Crown-6).

Compounds of formula (XXII) are suitably prepared by routes such as those described in PCT/GB98/000230, and in particular Example 19 of that application. Preparation details are summarised further in Scheme 3 hereinafter.

If necessary or required, groups R^1 and R^2 may be changed for different such groups after any of the above preparation methods using conventional chemistry and examples of this are provided hereinafter.

Biological activity was tested as follows. Farnesyl protein transferase (FPT) was partially purified from human placenta by ammonium sulphate fractionation followed by a single Q-Sepharose® (Pharmacia, Inc) anion exchange chromatography essentially as described by Ray and Lopez-Belmonte (Ray K P and Lopez-Belmonte J (1992) Biochemical Society Transations 20 494-497). The substrate for FPT was Kras (CVIM C-terminal sequence). The cDNA for oncogenic val12 variant of human c-Ki-ras-2 4B was obtained from the plasmid pSW11-1 (ATCC). This was then subcloned into the polylinker of a suitable expression vector e.g. pIC147. The Kras was obtained after expression in the E. coli strain, BL21. The expression and purification of c-KI-ras-2 4B

and the val12 variant in E. coli has also been reported by Lowe et al (Lowe P N et al. J. Biol. Chem. (1991) 266 1672-1678).

Incubations with enzyme contained 300nM tritiated farnesyl pyrophosphate (DuPont/New England Nuclear), 120nM ras-CVIM, 50mM Tris HCl pH 8.0, 5mM $MgCl_2$, 10μM $ZnCl_2$, 5mM dithiotheitol and compounds were added at appropriate concentrations in DMSO (3% final concentration in test and vehicle control). Incubations were for 20 minutes at 37°C and were stopped with acid ethanol as described by Pompliano et al. (Pompliano D L et al (1992) 31 3800-3807). Precipitated protein was then collected onto glass fibre filter mats (B) using a Tomtec® cell harvester and tritiated label was measured in a Wallac®1204 Betaplate scintillation counter.

Although the pharmacological properties of the compounds of the Formula I vary with structural change as expected, in general compounds of the Formula I possess an IC_{50} in the above test in the range, for example, 0.01 to 200μM. Thus by way of example, the compound 5{[(2S,4S),4-acetylsulfanyl-1-(4-nitro-benzyloxycarbonyl)-pyrrolidine-2-carbonyl]-amino} -3(N-methyl-methoxycarbamoyl)-benzoic acid allyl ester (see Example 7) has an IC_{50} of approximately 0.5μM. No physiologically unacceptable toxicity was observed at the effective dose for compounds tested of the present invention.

The invention will now be illustrated in the following non-limiting Examples in which, unless otherwise stated:-

(i) evaporations were carried out by rotary evaporation in vacuo and work-up procedures were carried out after removal of residual solids by filtration;

(ii) operations were carried out at room temperature, that is in the range 18-25°C and under an atmosphere of an inert gas such as argon;

(iii) column chromatography (by the flash procedure) and medium pressure liquid chromatography (MPLC) were performed on Merck Kieselgel silica (Art. 9385) or Merck Lichroprep RP-18 (Art. 9303) reversed-phase silica obtained from E. Merck, Darmstadt, Germany;

(iv) yields are given for illustration only and are not necessarily the maximum attainable;

(v) the end-products of the Formula I have satisfactory microanalyses and their structures were confirmed by nuclear magnetic resonance (NMR) and mass spectral

techniques; chemical shift values were measured on the delta scale; the following abbreviations have been used: s, singlet; d, doublet; t or tr, triplet; m, multiplet; br, broad;

(vi) intermediates were not generally fully characterised and purity was assessed by thin layer chromatographic, infra-red (IR) or NMR analysis;

(vii) melting points are uncorrected and were determined using a Mettler SP62 automatic melting point apparatus or an oil-bath apparatus; melting points for the end-products of the Formula I were determined after crystallisation from a conventional organic solvent such as ethanol, methanol, acetone, ether or hexane, alone or in admixture; and

(viii) the following abbreviations have been used throughout the application:-

BOC	tert-butoxycarbonyl
DCCI	1,3-dicyclohexylcarbodiimide
DMA	N,N-dimethylacetamide
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
EDC	1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
HOBT	1-hydroxybenzotriazole
NMM	N-methylmorpholine
NMM-O	4-methylmorpholine-N-oxide
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMSI	trimethylsilyliodide
TPAP	tetrapropylammonium perruthenate

Example 1

Preparation of Compound No. 6 in Table 1

The procedure followed is summarised in Scheme 1 hereinafter. In particular, a mixture of compound (xv) where R was N-methylpiperidine (4.8 g.), triethylsilane(10 ml.) and TFA(200 ml.) was stirred at ambient temperature for 1 hour under a nitrogen atmosphere. The TFA was evaporated away and the residue dissolved in ethyl acetate(50 ml.). HCl in

ether(1M.,100 ml.) was added followed by more ether(100 ml.). The resulting white solid was isolated by centrifuging, further washing with ether and re-centrifuging(4 times in all). The solid was dried under high vac. to give Compound 6 in Table 1 as the hydrochloride salt(4.5 g.).

Compound 6

^{1}H NMR data (DMSO d_6) δ 1.14 (6H, m), 1.7 (1H, m), 1.8-2.11 (7H, m), 2.02 (3H, s), 2.29-3.0 (10H, m), 2.66 (3H, d), 3.16 (1H, m), 3.2-3.52 (3H, m), 3.62 (1H, m), 3.78 (1H, m), 4.0 (1H, m), 4.46 (1H, q), 4.9 (1H, m), 6.60 (2H, m), 6.9-7.10 (3H, m), 7.19 (2H, m), 8.59 (1H, d).

MS (ES+) m/z 673.7 $(M+H)^+$

The starting material, Compound (xv) in Scheme 1, where R was N-methylpiperidine was prepared as follows:

Compound (xii) is a known compound and was prepared as described in Example 6 of PCT/GB99/00369. A mixture of Compound (xii) (50 g.), L-methionine iso-propyl ester hydrochloride(19.0 g.), DMAP(42.6 g.) and EDC(20.0 g.) in dichloromethane(500 ml.) was stirred at ambient temperature for 18 hours. The solution was washed with aqueous citric acid(1M.), brine and dried. It was then diluted with the same amount of iso-hexane and applied directly to a silica flash column eluting with ethyl acetate/iso-hexane(20:80,30:70) to give Compound (xiii) in Scheme 1 (58.6 g) as a solid white foam.

Compound(xiii):

^{1}H NMR data ($CDCl_3$) δ 1.26 (6H, m), 1.38 (9H, s), 1.41 (1H, m), 1.58 (1H, m), 2.02 (1H, m), 2.05 (3H, s), 2.15-2.4 (2H, m), 2.47-2.7 (2H, m), 2.7-2.98 (6H, m), 3.06-3.34 (2H, m), 3.9 (1H, m), 4.79 (2H, m), 5.08 (1H, m), 6.34 (1H, d), 6.38 (2H, m), 6.91 (3H, m), 7.09 (2H, m), 7.18-7.35 (9H, m), 7.43 (6H, m).

MS (ES+) m/z 890.5 $(M+H)^+$

TFA(80 ml.) was added to a rapidly stirring solution of Compound (xiii)(58.6 g.) and triethylsilane(80 ml.) in dichloromethane(4 l.) under a nitrogen atmosphere. The solution was then stirred at ambient temperature for 4.5 hours, basified with sat. sodium bicarbonate solution and the dichloromethane layer separated. After drying and evaporation to a smaller volume(500 ml.) it was applied directly to a silica flash column and eluted with ethyl acetate/iso-hexane(30:70,50:50) to give Compound (xiv) in Scheme 1 (35.5 g.) as a white solid.

Compound(xiv):

^{1}H NMR data ($CDCl_3$) δ 1.25 (6H, m), 1.46 (9H, s), 1.5 (1H, m), 1.68 (1H, m), 1.70 (1H, d), 2.00 (1H, m), 2.08 (3H, s), 2.21 (1H, m), 2.56 (2H, m), 2.75-2.97 (4H, m), 3.05 (1H, m), 3.26 (2H, m), 3.4 (1H, m), 3.85-4.3 (2H, m), 4.8 (2H, q), 5.05 (1H, m), 6.35 (1H, d), 6.6 (2H, m), 6.92 (3H, m), 7.1 (2H, m).

MS (ES+) m/z 648.7 $(M+H)^+$

A mixture of Compound (xiv) (4.5 g.), N-methylpiperidine-4-carboxylic acid(1.5 g.), N-methylpiperidine(3.5 g.), EDC(1.6 g.) and HOBT(0.94 g.) in dichloromethane(100 ml.) was stirred under a nitrogen atmosphere for 18 hours. It was then applied directly to a silica flash column and eluted with ethyl acetate/iso-hexane(1:1), ethyl acetate, methanol/ethyl acetate(10:90,20:80) to give Compound xv in Scheme 1 where R is N-methylpiperidine (4.8 g.) as a white foam.

^{1}H NMR data (DMSO d_6) δ 1.15 (6H, m), 1.4 (9H, s), 1.69 (1H, m), 1.7-2.0 (7H, m), 2.01 (3H, s), 2.11 (3H, s), 2.4-2.6 (4H, m), 2.63-2.82 (6H, m), 3.04 (2H, m), 3.28 (1H, m), 3.42 (1H, m), 3.7-4.0 (3H, m), 4.44 (1H, m), 4.9 (1H, m), 5.82 (1H, t), 6.6 (2H, m), 6.9 (1H, m), 7.03 (2H, t), 7.38 (2H, m), 8.55 (1H, d).

MS (ES+) m/z 773.6 $(M+H)^+$

Example 2

Using a method analogous to that described in Example 1, Compounds 4 and 8 in Table 1 were prepared from Compound (xiv) in Scheme 1 using the appropriate intermediates.

Compound (xv) in Scheme 1 where R is phenyl:

^{1}H NMR data ($CDCl_3$) δ 1.28 (6H, m), 1.50 (9H, s), 1.50 (1H, m), 1.90 (1H, m), 2.05 (1H, m), 2.07 (3H, s), 2.23 (1H, m), 2.57 (2H, m), 2.69 (1H, m), 2.82 (2H, m), 2.90 (2H, m), 3.27 (2H, m), 3.46 (1H, m), 4.1 (1H, m), 4.23 (1H, m), 4.80 (2H, m), 5.07 (1H, m), 6.40 (1H, d), 6.68(2H, m), 6.93(3H, m), 7.1(2H, m), 7.46(2H, m), 7.59(1H, m), 7.94(2H, d).

MS (ES+) m/z 752.71 $(M+H)^+$

Compound 4 in Table 1:

^{1}H NMR data (DMSO d_6) δ 1.16 (7H, m), 1.82 (1H, m), 1.98 (2H, m), 2.01 (3H, s), 3.4-3.9 (6H, m), 3.22 (1H, m), 3.42 (2H, m), 3.80 (2H, m), 4.20 (1H, m), 4.47 (1H, q), 4.90 (1H, m), 6.62 (2H, m), 6.93-7.10 (3H, m), 7.19 (2H, m), 7.57 (2H, t), 7.70 (1H, t), 7.89 (2H, d), 8.60 (1H, d).

MS (ES+) m/z 652 $(M+H)^+$

Compound (xv) in Scheme 1 where R is 3-pyridyl:

^{1}H NMR data ($CDCl_3$) δ 1.27 (6H, m), 1.49 (9H, s), 1.50 (1H, m), 1.92 (1H, m), 2.04 (1H, m), 2.10 (3H, s), 2.23 (1H, m), 2.56 (2H, m), 2.70 (1H, m), 2.83 (2H, m), 2.92 (2H, m), 3.28 (2H, m), 3.45 (1H, m), 4.14 (1H, m), 4.25 (1H, m), 4.80 (2H, m), 5.08 (1H, m), 6.40 (1H, d), 6.65 (2H, m), 6.93(3H, m), 7.10(2H, m), 7.42(1H, m), 8.18(1H, m), 8.80(1H, m), 9.13(1H, m).

MS (ES+) m/z 753.71 $(M+H)^+$

Compound 8 in Table 1:

^{1}H NMR data (DMSO d_6) δ 1.15 (6H, m), 1.20 (1H, m), 1.86 (1H, m), 1.97 (2H, m), 2.02 (3H, s), 2.40-2.92 (5H, m), 3.29 (1H, m), 3.37-3.60 (2H, m), 3.68-3.94 (2H, m), 4.28 (1H,

m), 4.48 (1H, m), 4.89 (1H, m), 6.67 (2H, m), 7.00 (4H, m), 7.19 (2H, t), 7.68 (1H, m), 8.33 (1H, m), 8.61 (1H, d), 8.88(1H, m), 9.06(1H, m).

MS (ES+) m/z 653.56 $(M+H)^+$

Example 3

Preparation of Compound 7 in Table 1

Compound 7 in Table 1 was synthesised from Compound (xxviii) in Scheme 2 using a method analogous to that described in Example 1 for the conversion of Compound (xv) in Scheme 1 to Compound 6.

Compound 7 in Table 1

^{1}H NMR data (DMSO-D_6 +CD_3COOD) δ1.1-1.25 (6H, m), 1.8-2.05 (1H, m), 2.05-2.35 (2H, m), 2.6-3.05 (5H, m), 2.95 (3H, s), 3.05-3.4 (3H, m), 3.82 (1H, dd), 4.0-4.2 (1H, m), 4.2-4.4 (3H, m), 4.4-4.6 (1H, m), 4.85-5.0 (1H, m), 6.9-7.1 (4H, m), 7.1-7.3 (3H, m), 7.65 (1H, dd), 8.3 (1H, dd), 8.87 (1H, dd), 9.07 (1H, d).

MS (ES+) m/z 686.5 $(M+H)^+$

Analysis Calculated for $C_{34}H_{40}N_3S_2O_7F,2HCl,2H_2O$

C,51.4;H,5.8;N,5.3;S,8.0;Cl,8.9

Found

C,51.2;H,5.39;N,5.2;S,7.84;Cl,9.2

The starting material, Compound (xxviii) was synthesised from Compound(xxv) in Scheme 2 in a similar manner to that described in Example 1 for the preparation of Compound(xv) in Scheme 1 using the appropriate intermediates. Compound (xxv) was obtained using the method described in Example 12 of PCT/GB99/00369, and summarised in Scheme 2.

Compound (xxvi) in Scheme 2:

^{1}H NMR data ($CDCl_3$) δ1.2-1.35 (6H, m), 1.35 (9H, s), 1.8-2.05 (1H, m), 2.15-2.4 (1H, m), 2.45-3.3 (10H, m), 2.85 (3H, s), 3.7-4.3 (4H, m), 4.7-4.85 (1H, m), 5.0-5.2 (1H, m), 6.5 (1H, d), 6.8-7.0 (4H, m), 7.0-7.15 (3H, m), 7.15-7.4 (9H, m), 7.4-7.6 (6H, m).

MS (ES+) m/z 945.1 $(M+Na)^+$

Compound (xxvii) in Scheme 2:

^{1}H NMR data ($CDCl_3$) δ1.2-1.35 (6H, m), 1.45 (9H, m), 1.75 (1H, d), 1.9-2.1 (1H, m), 2.2-2.4 (1H, m), 2.45-2.7 (2H, m), 2.8-3.4 (8H, m), 2.85 (3H, s), 3.8-4.4 (4H, m), 4.7-4.85 (1H, m), 5.0-5.2 (1H, m), 6.55 (1H, d), 6.8-7.2 (7H, m).

MS (ES+) m/z 681.2 $(M+H)^+$

MS (ES-) m/z 679.2 $(M-H)^-$

Compound (xxviii) in Scheme 2:

^{1}H NMR data ($CDCl_3$) δ1.2-1.35 (6H, m), 1.45 (9H, s), 2.1-2.4 (2H,m), 2.45-2.65 (1H, m), 2.65-2.8 (1H, m), 2.8-3.45 (8H, m), 2.85 (3H, s), 4.0-4.4 (4H, m), 4.7-4.85 (1H, m), 5.0-5.2 (1H, m), 6.55 (1H, d), 6.8-7.2 (7H, m), 7.42 (1H, dd), 8.18 (1H, d), 8.8 (1H, d), 9.15 (1H, s).

MS (ES+) m/z 786.7 $(M+H)^+$

MS (ES-) m/z 784.6 $(M-H)^-$

Example 4

Preparation of Compound 9 in Table 1

Compound 9 in Table 1 was synthesised from Compound (xxxxi) in Scheme 3 using a method analogous to that described in Example 1 for the conversion of Compound (xv) in Scheme 1 to Compound 6 in Table 1.

Compound 9 in Table 1

^{1}H NMR data (DMSO d_6) δ 1.13 - 1.22 (6H, m), 1.92 - 2.06 (6H, m), 2.39 - 2.46 (1H, m), 2.54 - 2.62 (2H, m), 2.66 - 2.83 (3H, m), 2.92 - 3.00 (2H, m), 3.31 (1H, m), 3.81 (1H, m), 4.30 - 4.39 (1H, m), 4.47 - 4.57 (1H, m), 4.90 (1H, m), 6.37 (1H, dd), 6.81 (1H, d), 7.04 (2H, t), 7.15 - 7.25 (3H, m), 7.39 - 7.46 (2H, m), 7.61 (1H, dd), 8.27 (1H, m), 8.79 (1H, d), 8.87 (1H, dd), 9.05 (1H, d).

MS (ES+) m/z 650.1 $(M+H)^+$

The starting material (Compound (xxxxi) in Scheme 3) was synthesised from Compound(xxxv) as shown in Scheme 3 using the following method. Compound xxxv itself was obtained as described in Example 21 of PCT/GB98/00230 and summarised in Scheme 3.

Compound (xxxv) in Scheme 3 (20g) was dissolved in triethyl phosphite (100 ml) and heated to 160°C under a nitrogen atmosphere for 18 hours. The solution was evaporated to dryness and the residue was dissolved in dichloromethane and applied directly to a silica flash column and eluted with ethyl acetate/iso-hexane(50:50,100:0) to give Compound (xxxvi) as a colourless crystalline solid(19.4g).

Compound (xxxvi) in Scheme 3:

^{1}H NMR data (DMSO d_6) δ : 1.15 (6H, t), 2.79 (2H, t), 3.10 (2H, t), 3.24 (2H, d), 3.81 (3H, s), 3.88 - 3.98 (4H, m), 7.02 - 7.10 (2H, m), 7.16 - 7.27 (3H, m), 7.35 - 7.40 (1H, m), 7.72 (1H, s).

MS (ES+) m/z 409.3 $(M+H)^+$

Compound (xxxvi) in Scheme 3 (17.5g) was dissolved in tetrahydrofuran (500 ml) and cooled to -50°C under a nitrogen atmosphere. n-Butyl lithium (1.6M)(26.9 ml) was slowly added at -40°C followed by Compound (x)(18.5 g) as a solution in tetrahydrofuran (100 ml). The mixture was then allowed to warm to 0°C. Aqueous saturated ammonium chloride solution (300 ml) was then added and the organics were separated and dried over magnesium sulphate. The solution was evaporated to dryness and the residue dissolved in dichloromethane and purified by flash column chromatography eluting with ethyl acetate/iso-hexane (20:80,30:70) to give Compound (xxxvii) in Scheme 3 as a colourless solid foam(10.4g).

Compound (xxxvii) in Scheme 3:

^{1}H NMR data (DMSO d_6) δ : 1.16 - 1.30 (10H, m), 1.52 - 1.65 (1H, m), 2.68 - 2.80 (5H, m), 3.04 - 3.14 (2H, m), 3.80 (3H, s), 4.04 (1H, m), 6.12 (1H, dd), 6.34 (1H, d), 7.06 (2H, t), 7.15 - 7.40 (18H, m), 7.48 (1H, dd), 7.74 (1H, d).

MS (ES+) m/z 728.8 $(M+H)^+$

Compound (xxxvii) in Scheme 3 was converted to Compound (xxxxi) in Scheme 3 using a method analogous to that described in Example 1 for the preparation of Compound (xv) in Scheme 1 using the appropriate intermediates.

Compound (xxxviii) in Scheme 3:

^{1}H NMR data (DMSO d_6) δ : 1.14 - 1.35 (10H, m), 1.53 - 1.66 (1H, m), 2.68 - 2.81 (5H, m), 3.10 (2H, t), 4.04 (1H, m), 6.10 (1H, dd), 6.34 (1H, d), 7.05 (2H, t), 7.14 - 7.39 (18H, m), 7.43 (1H, d), 7.76 (1H, s), 12.90 (1H, br s).

MS (ES-) m/z 712.6 $(M-H)^-$

Compound (xxxx) in Scheme 3:

MS (ES+) m/z 645.6 $(M+H)^+$

Compound (xxxxi):

MS (ES+) m/z 750.6 $(M+H)^+$

Example 5

Pharmaceutical compositions

The following illustrate representative pharmaceutical dosage forms of the invention as defined herein (the active ingredient being termed "Compound X"), for therapeutic or prophylactic use in humans:

(a)	Tablet I	mg/tablet
	Compound X	100
	Lactose Ph.Eur	182.75
	Croscarmellose sodium	12.0
	Maize starch paste (5% w/v paste)	2.25
	Magnesium stearate	3.0

(b)	Tablet II	mg/tablet
	Compound X	50
	Lactose Ph.Eur	223.75
	Croscarmellose sodium	6.0
	Maize starch	15.0
	Polyvinylpyrrolidone (5% w/v paste)	2.25
	Magnesium stearate	3.0

(c)	Tablet III	mg/tablet
	Compound X	1.0
	Lactose Ph.Eur	93.25
	Croscarmellose sodium	4.0
	Maize starch paste (5% w/v paste)	0.75
	Magnesium stearate	1.0

(d)	Capsule	mg/capsule
	Compound X	10
	Lactose Ph.Eur	488.5
	Magnesium	1.5

(e)	Injection I	(50 mg/ml)
	Compound X	5.0% w/v
	1M Sodium hydroxide solution	15.0% v/v

0.1M Hydrochloric acid (to adjust pH to 7.6)

Polyethylene glycol 400..... 4.5% w/v

Water for injection to 100%

(f) Injection II (10 mg/ml)

Compound X..... 1.0% w/v

Sodium phosphate BP..... 3.6% w/v

0.1M Sodium hydroxide solution..... 15.0% v/v

Water for injection to 100%

(g) Injection III (1mg/ml, buffered to pH6)

Compound X..... 0.1% w/v

Sodium phosphate BP..... 2.26% w/v

Citric acid..... 0.38% w/v

Polyethylene glycol 400..... 3.5% w/v

Water for injection to 100%

(h) Aerosol I mg/ml

Compound X..... 10.0

Sorbitan trioleate..... 13.5

Trichlorofluoromethane..... 910.0

Dichlorodifluoromethane..... 490.0

(i) Aerosol II mg/ml

Compound X..... 0.2

Sorbitan trioleate..... 0.27

Trichlorofluoromethane..... 70.0

Dichlorodifluoromethane..... 280.0

Dichlorotetrafluoroethane..... 1094.0

(j)

Aerosol III	mg/ml
Compound X	2.5
Sorbitan trioleate	3.38
Trichlorofluoromethane	67.5
Dichlorodifluoromethane	1086.0
Dichlorotetrafluoroethane	191.6

(k)

Aerosol IV	mg/ml
Compound X	2.5
Soya lecithin	2.7
Trichlorofluoromethane	67.5
Dichlorodifluoromethane	1086.0
Dichlorotetrafluoroethane	191.6

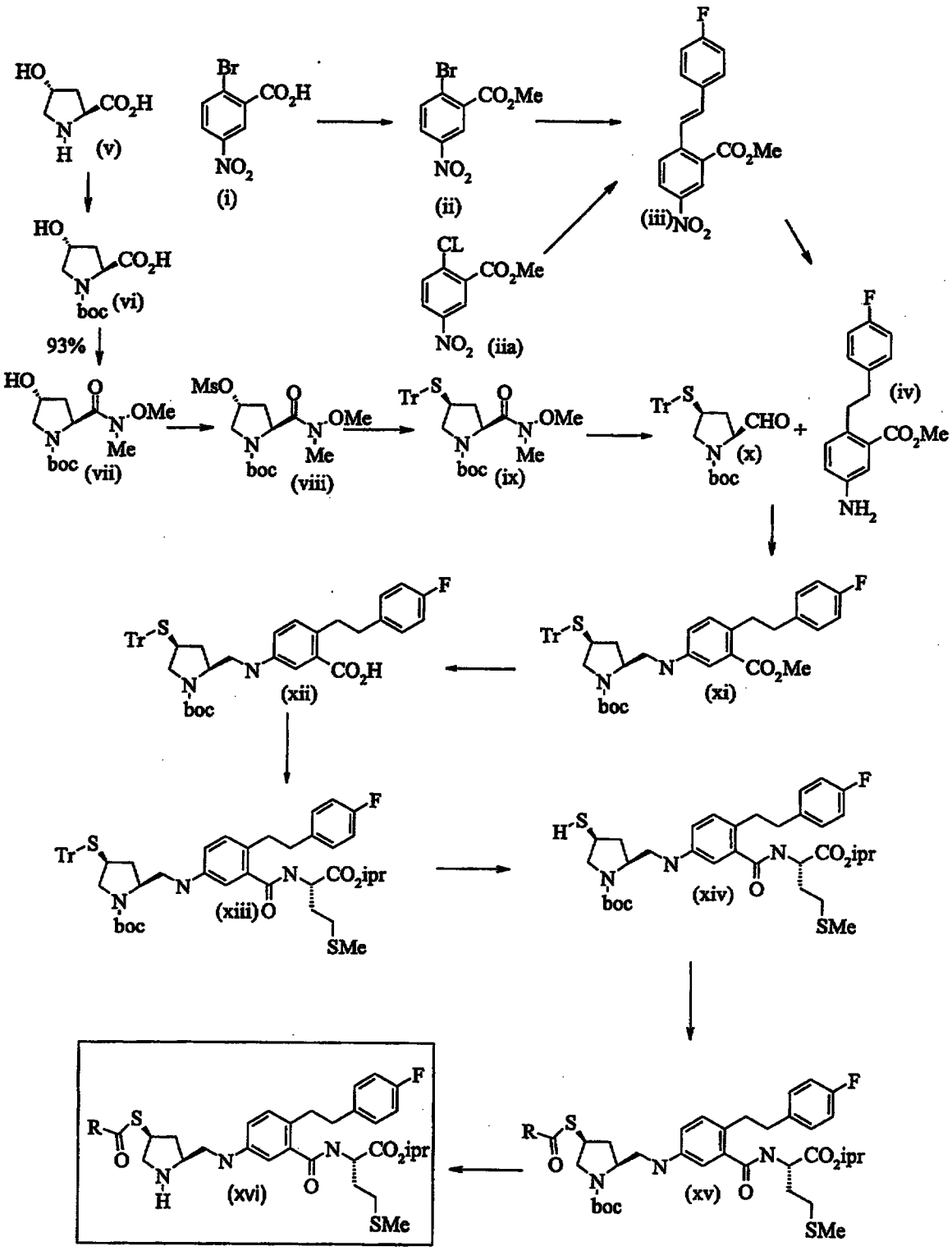
(l)

Ointment	ml
Compound X	40 mg
Ethanol	300 µl
Water	300 µl
1-Dodecylazacycloheptan-2-one	50 µl
Propylene glycol	to 1 ml

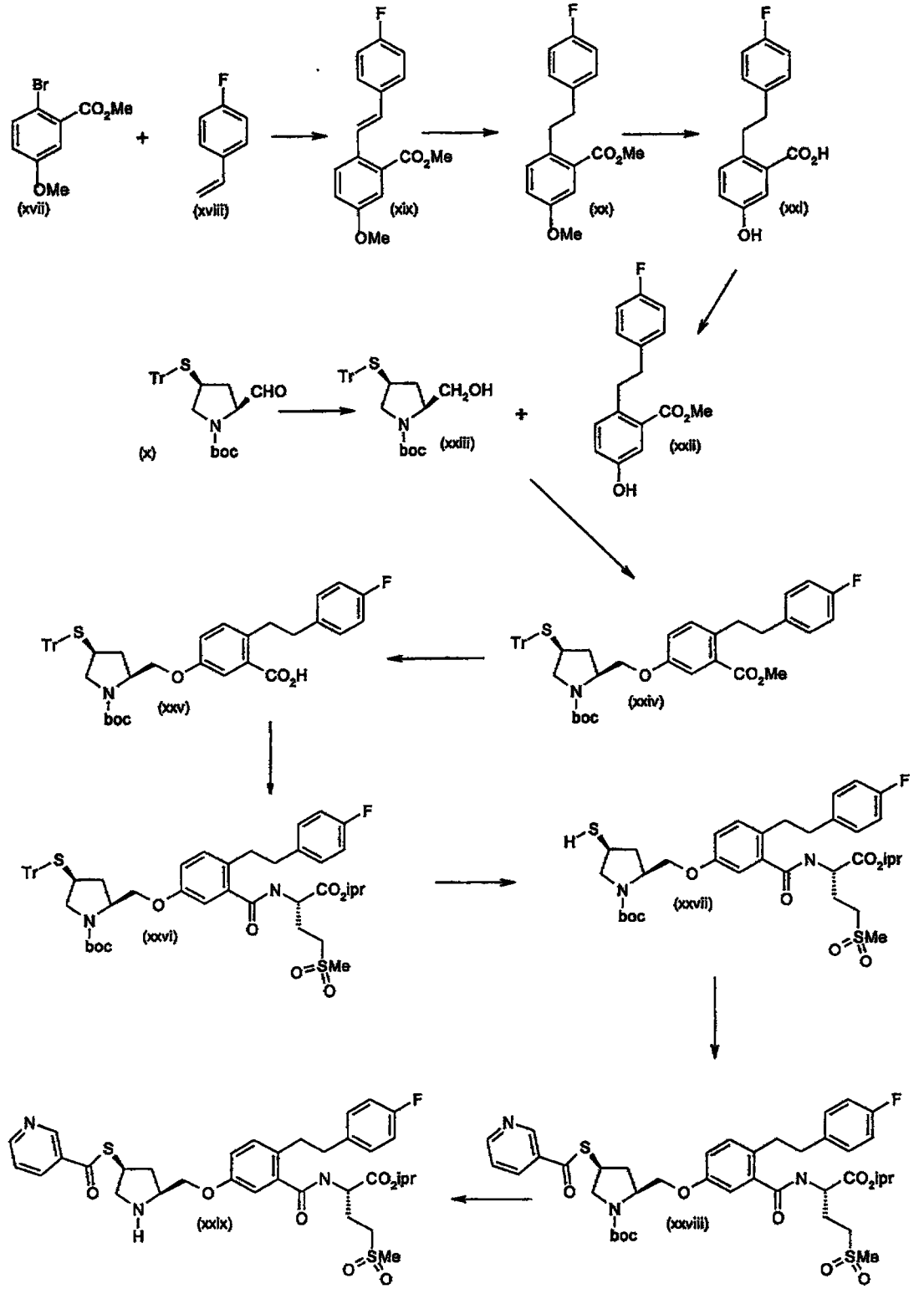
Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate. The aerosol formulations (h)-(k) may be used in conjunction with standard, metered dose aerosol dispensers, and the suspending agents sorbitan trioleate and soya lecithin may be replaced by an alternative suspending agent such as sorbitan monooleate, sorbitan sesquioleate, polysorbate 80, polyglycerol oleate or oleic acid.

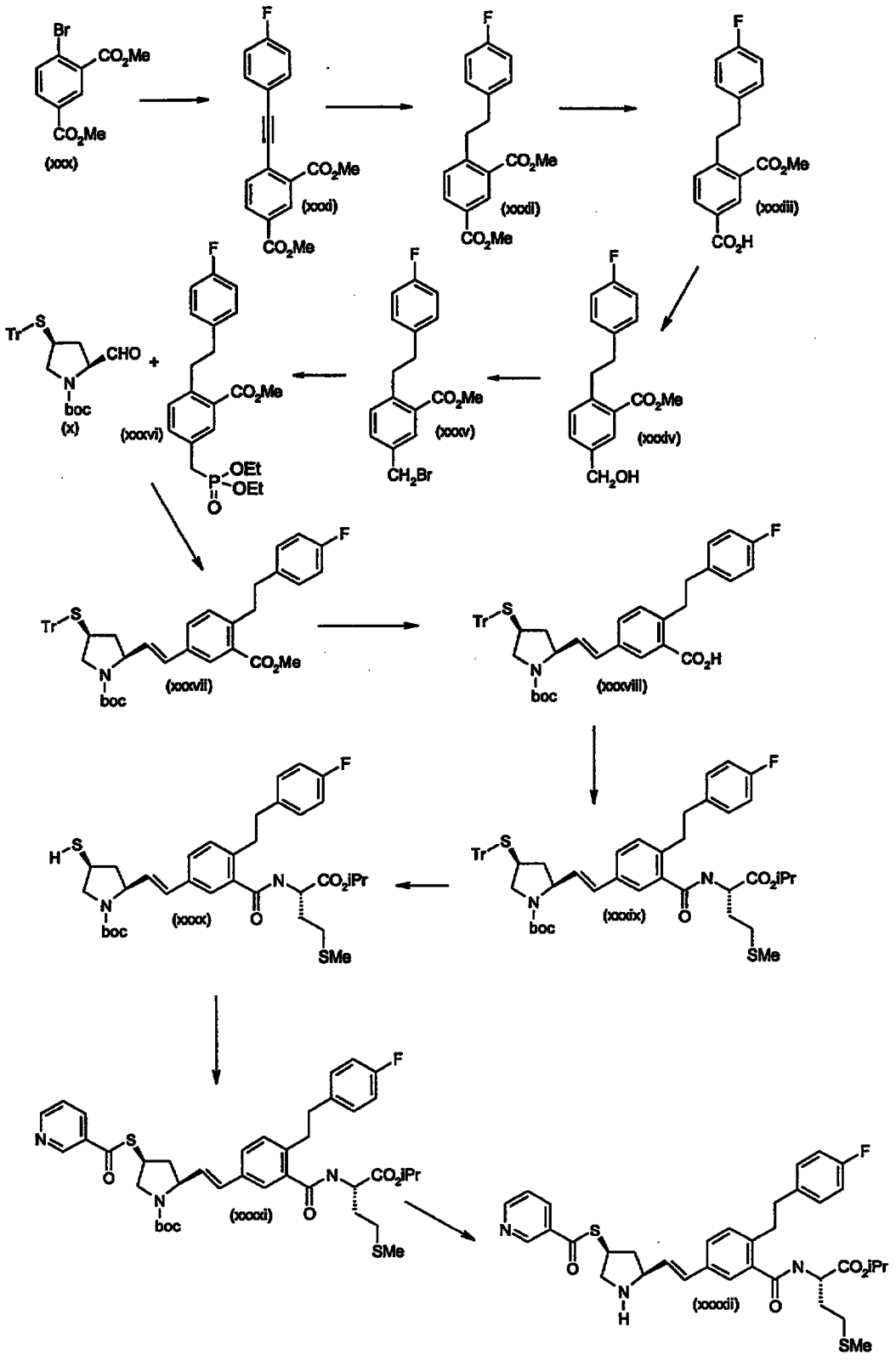
Scheme 1


HO
CO2H
N
H
(v)
Br
CO2H
NO2
(i)
Br
CO2Me
NO2
(ii)
F
CO2Me
(iii) NO2
CL
CO2Me
NO2
(iia)
HO
CO2H
N
boc
(vi)
93%
HO
O
N
OMe
N
Me
boc
(vii)
MsO
O
N
OMe
N
Me
boc
(viii)
Tr-S
O
N
OMe
N
Me
boc
(ix)
Tr-S
CHO
N
boc
(x)
+
F
(iv)
CO2Me
NH2
Tr-S
F
N
CO2Me
N
boc
(xi)
Tr-S
F
N
CO2H
N
boc
(xii)
Tr-S
F
N
N
CO2ipr
N
boc
(xiii)
O
SMe
H-S
F
N
N
CO2ipr
N
boc
(xiv)
O
SMe
R
S
F
O
N
N
CO2ipr
N
H
(xvi)
O
SMe
R
S
F
O
N
N
CO2ipr
N
boc
(xv)
O
SMe

Scheme 2

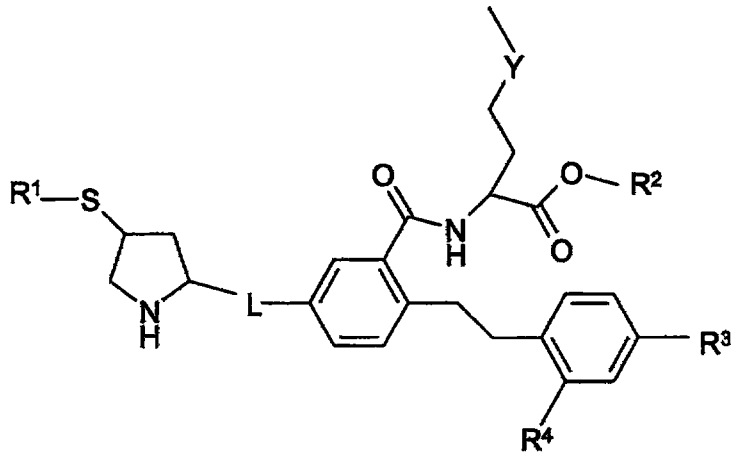

Br
CO2Me
OMe
(xvii)
F
(xviii)
(xix)
(xx)
CO2H
(xxi)
OH
Tr
S
CHO
N
boc
(x)
CH2OH
(xxii)
(xxiii)
(xxiv)
(xxv)
CO2ipr
SMe
O
(xxvi)
(xxvii)
H
(xxviii)
(xxix)

Scheme 3


Br
CO2Me
CO2Me
(xxx)
F
CO2Me
CO2Me
(xxxi)
CO2Me
(xxxii)
CO2H
CO2Me
(xxxiii)
CH2OH
CO2Me
(xxxiv)
CH2Br
CO2Me
(xxxv)
Tr
S
CHO
N
boc
(x)
+
CO2Me
(xxxvi)
P
OEt
OEt
O
Tr
S
N
boc
CO2Me
(xxxvii)
CO2H
(xxxviii)
H
S
CO2iPr
SMe
(xxxx)
(xxxix)
(xxxxi)
(xxxxii)
H

Claims

1. A compound of formula (I)



(I)

wherein:

R^1 and R^2 are independently selected from H or a prodrug moiety;

R^3 is hydrogen or halogen;

R^4 is hydrogen or halogen;

L is -CH=CH- or -CH_2-Z- where Z is NH or O;

Y is S, S(O) or $S(O)_2$;

or a salt thereof.

2. A compound of formula (I) as claimed in claim 1 wherein the group R^1 is hydrogen or prodrug group of formula $R^5C(O)$ -where R^5 is an optionally substituted aryl or heterocyclyl group.

3. A compound of formula (I) as claimed in claim 2 wherein R^5 is optionally substituted phenyl, optionally substituted pyridyl, optionally substituted furyl, optionally substituted isoxazole, optionally substituted tetrahydropyridyl or optionally substituted tetrahydrofuryl.

4. A compound of formula (I) as claimed in claim 3 wherein R^5 is phenyl, pyridyl or N-methylpiperidine.

5. A compound of formula (I) as claimed in claim 2, 3 or 4 wherein R^5 is optionally substituted by alkyl, haloalkyl, hydroxy, alkoxy or cyano.

6. A compound of formula (I) as claimed in any claim from 1 to 5 wherein R^2 together with the carboxy group to which it is attached forms a pharmaceutically-acceptable ester or amide.

7. A compound of formula (I) as claimed in 6 wherein R^2 together with the carboxy group to which it is attached forms a C_{1-6}alkyl ester or C_{1-6}cycloalkyl ester; C_{1-6}alkoxymethyl ester; C_{1-6}alkanoyloxymethyl ester; phthalidyl ester; C_{3-8}cycloalkoxycarbonyloxyC_{1-6}alkyl ester; 1,3-dioxolan-2-ylmethyl ester; C_{1-6}alkoxycarbonyloxyethyl ester; aminocarbonylmethyl ester and mono- or di-N-(C_{1-6}alkyl) versions thereof; or a pharmaceutically acceptable ester of an optionally substituted heterocyclic group.

8. A compound of formula (I) as claimed in claim 6 wherein R^2 together with the carboxy group to which it is attached forms N-C_{1-6}alkylamide or N,N-di-(C_{1-6}alkyl)amide.

9. A compound of formula (I) as claimed in any claim from 1 to 5 wherein R^2 is selected from hydrogen, a C_{1-4}alkyl group, or an optionally substituted heterocyclic group.

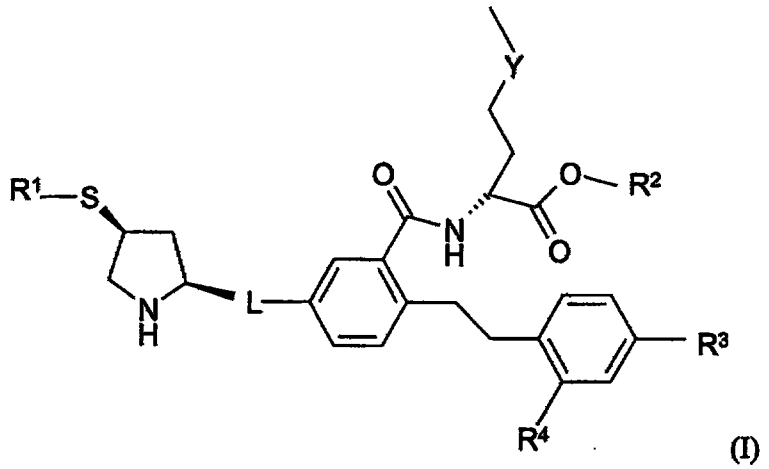
10. A compound of formula (I) as claimed in any claim from 5 to 9 wherein R^3 is a halo atom.

11. A compound of formula (I) as claimed in in any claim from 5 to 9 wherein R^4 is hydrogen or fluorine.

12. A compound of formula (I) as claimed in in any claim from 5 to 9 wherein the linking group L is CH_2-Z- where Z is NH or O.

13. A compound of formula (I) as claimed in in any claim from 5 to 9 wherein the Y is S or $S(O)_2$.

14. A compound of formula (I)



(I)

wherein:

R^1 and R^2 are independently selected from H or a prodrug moiety;

R^3 is hydrogen or halogen;

R^4 is hydrogen or halogen;

L is -CH=CH- or $-CH_2-Z-$ where Z is NH or O;

Y is S, S(O) or $S(O)_2$;

or a salt thereof.

15. Isopropyl (2*S*)-2-({2-(4-fluorophenethyl)-5-[({(2*S*,4*S*)-4-[(3-pyridinylcarbonyl)sulfanyl]tetrahydro-1*H*-pyrrol-2-yl}methyl)amino]benzoyl}amino)-4-(methylsulfanyl)butanoate or a salt thereof.

16. (2*S*)-2-({2-(4-fluorophenethyl)-5-[({(2*S*,4*S*)-4-(mercapto)tetrahydro-1*H*-pyrrol-2-yl}methyl)amino]benzoyl}amino)-4-(methylsulfanyl)butanoic acid or a salt thereof.

17. A compound as defined in any claim from 1 to 16 for use as a medicament.

18. A pharmaceutical composition comprising a compound as defined in any claim from 1 to 16 together with a pharmaceutically acceptable diluent or carrier.

19. Isopropyl (2*S*)-2-({2-(4-fluorophenethyl)-5-[({(2*S*,4*S*)-4-(mercapto)tetrahydro-1*H*-pyrrol-2-yl}methyl)amino]benzoyl}amino)-4-(methylsulfanyl)butanoate or a salt thereof.

20. 2-Amino-3-(4-fluorophenethyl) benzoic acid or a salt thereof.