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N-(2-ALKYL-3-MERCAPTOGLUTARYL)-AMINO-DIAZA CYCLOALKANONE DERIVATIVES AND THEIR USE AS COLLAGENASE INHIBITORS

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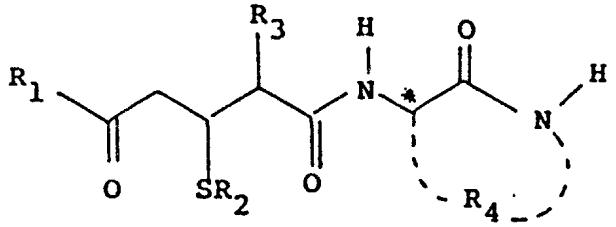
(57) Claim

1. A compound of formula (I) or a salt, solvate or hydrate thereof



(I)

in which,

R_1 is -OH; alkoxy; aryloxy or aralkyloxy in each of which the aryl group is optionally substituted; $-NR_6R_7$, where each of R_6 and R_7 is independently hydrogen or alkyl, or R_6 and R_7 together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring with an

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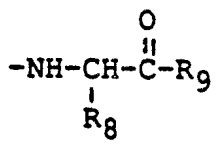
optional oxygen or sulphur atom or an optionally substituted second nitrogen atom in the ring; or a group



where R_8 is hydrogen; alkyl optionally substituted by -OH, alkoxy, $-NR_6R_7$ as defined for R_1, guanidine, $-CO_2H$, $-CONH_2$, -SH, or -S-alkyl; or $-CH_2$-Ar where Ar is optionally substituted aryl; and R_9 is alkoxy; OH; or $-NR_6R_7$ as defined for R_1;

R_2 is hydrogen; C_{2-8} alkanoyl; or optionally substituted aroyl;

R_3 is C_{3-6} alkyl; and

R_4 is $-(CH_2)_p-X-(CH_2)_q-$ where p is an integer from 1 to 9, q is an integer from 2 to 10, and the moiety $-(CH_2)_p-$ is adjacent to the carbon atom marked with an asterisk in formula (I), and X is $-NR_5-$ where R_5 is selected from hydrogen, C_{1-6} alkyl, C_{2-6} alkanoyl, C_{1-6} alkoxycarbonyl and aroyl, aralkyl or aralkyloxycarbonyl in each of which the aryl moiety is optionally substituted.

12. A method of treatment of conditions in which degradation of connective tissue and other proteinaceous compounds of the body occurs which comprises administration to a host in need thereof an effective amount of a compound according to any one of claims 1 to 8 or a pharmaceutically acceptable salt, solvate or hydrate thereof.

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(54) Title: N-(2-ALKYL-3-MERCAPTOGLUTARYL)-AMINO-DIAZA CYCLOALKANONE DERIVATIVES AND THEIR USE AS COLLAGENASE INHIBITORS

(57) Abstract

Thiol carboxylic acid derivatives, processes for their preparation and their use as collagenase inhibitors are described.

N-(2-ALKYL-3-MERCAPTOGLUTARYL)-AMINO-DIAZA CYCLOALKANONE DERIVATIVES AND THEIR USE AS COLLAGENASE INHIBITORS.

The present invention relates to novel thiol carboxylic acid derivatives, processes for their preparation and their use in medicine. In particular, the present invention relates to their use as inhibitors of enzymes of the collagenase family of neutral metalloproteases, for treating arthritic and other diseases.

The mammalian collagenase family of enzymes comprises a number of proteases, exemplified by interstitial (type I) collagenase itself, the stromelysins (also known as proteoglycanases or transins), fibroblast and polymorphonuclear leucocyte gelatinases (also known as collagen-IV-ases), and 'pump-1' (putative metalloprotease 1, uterine metalloprotease). Membership of the mammalian collagenase family of proteases is evident by possession of a number of highly characteristic and experimentally verifiable properties. [Goldberg et al., J. Biol. Chem. 2610, 6600, 1986; Whitham et al., Biochem. J. 240, 913, 1986; Breathnach et al., Nucleic Acids Res., 15, 1139, 1987; Muller et al., Biochem. J., 253, 187, 1988; Collier et al., J. Biol. Chem., 263, 6579, 1988; Murphy et al., Biochem. J., 258, 463, 1989; Quantin et al., Biochem. (N.Y.), 28, 5327, 1989; Birkedal-Hansen, J. Oral Pathol., 17, 445, 1988].

The range of therapeutic applications of the invention described hereinafter reflects the fundamental role of collagen and other proteinaceous substrates of the collagenase family of enzymes in the connective tissue matrix throughout the body. Applications extend to clinical interventions in many diseases and phenomena involving the destruction of collagen and other connective tissue components, and also normal or disordered tissue remodelling.

Inhibitors of the collagenase family of enzymes are considered to provide useful treatments for: arthritic diseases, such as rheumatoid and osteo-arthritis, soft tissue rheumatism, polychondritis and tendonitis; bone resorption diseases, such as osteoporosis, Paget's disease, hyperparathyroidism and cholesteatoma; the enhanced collagen destruction that occurs in association with diabetes; the recessive classes of dystrophic epidermolysis bullosa; periodontal disease and related consequences of gingival production of collagenase, or of PMNL collagenase release following cellular infiltration to inflamed gingiva, including by combating the greater susceptibility of diabetes patients to periodontal disease; corneal ulceration, e.g. that induced by alkali or other burns, by radiation, by vitamin E or retinoid deficiency; ulceration of the skin and gastro-intestinal tract, and abnormal wound healing; post-operative conditions, including colonic anastomosis, in which collagenase levels are raised; cancer, where members of the collagenase family of enzymes have been implicated in the neovascularization required to support tumour growth and survival [P. Basset et al., Nature, 348, 699, 1990] in the tissue remodelling required to accommodate the growing primary and secondary tumours, and in the penetration of tumour cells through the basement membrane of the vascular walls during metastasis; and demyelinating diseases of the central and peripheral nervous systems, including syndromes in which myelin loss is the primary pathological event and those in which demyelination follows axonal atrophy. The degradation of myelin in these diseases, exemplified by multiple sclerosis, is mediated by members of the collagenase family of enzymes.

As a particular example of the therapeutic value of inhibitors of the collagenase family of enzymes such as are disclosed in the present invention, chronic arthritic diseases leading to extensive loss of the collagen, proteoglycan and elastin components of the cartilage, bone and tendons within the joints, should be amenable to treatment with inhibitors of the collagenases, proteoglycanases (stromelysins) and gelatinases currently thought to be the major enzymes involved.

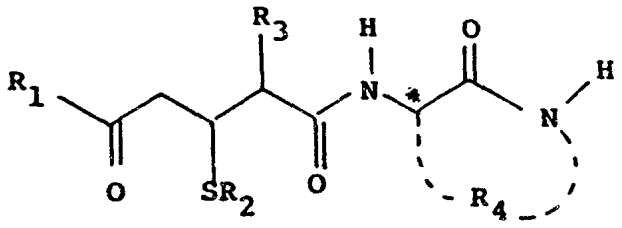
These enzymes have been detected in extracts of synovial and cartilage tissue, and have also been extensively studied in tissue cultures of a wide range of connective tissues. Apart from control of the biosynthesis, secretion and activation of the enzymes, the most important natural regulation of these enzymes in normal and diseased states, is considered to be the endogenous production of inhibitors such as the family of Tissue Inhibitor of Metalloproteases (TIMPS), and alpha-2 macroglobulin. An imbalance between the local levels of the proteolytic enzymes and natural inhibitors will allow destruction of connective tissue components to occur.

The compounds described in the present invention, being synthetic and low molecular weight inhibitors of this family of enzymes, offer a therapeutically useful way in which a more normal or non-pathological balance between inhibition and enzymic activity can be restored: they thus act to complement and supplement the endogenous enzyme inhibitors. Indeed, because these enzymes usually act only within restricted pericellular environments, before being inactivated by inhibitors circulating in the blood and present in most inflammatory exudates, the low molecular weight inhibitors disclosed here may be more effective than endogenous proteinaceous inhibitors that are excluded by their size from the localized regions of connective tissue destruction.

European Patent Application 0273689 (Beecham Group) discloses a class of thiol-carboxylic acid derivatives having activity as inhibitors of collagenase and useful in the treatment of rheumatoid arthritis and related diseases in which collagenolytic activity is a contributing factor.

A novel class of thiol-carboxylic acid derivatives has now been discovered, which are collagenase inhibitors and thus of potential utility in the treatment of diseases in which activity of members of the collagenase family of neutral metalloproteases is implicated.

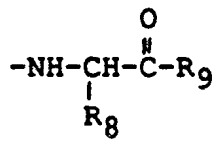
According to the present invention there is provided a compound of general formula (I), or a salt, solvate or hydrate thereof:



(I)

in which,

R_1 is -OH; alkoxy; aryloxy or aralkyloxy in each of which the aryl group is optionally substituted; $-NR_6R_7$, where each of R_6 and R_7 is independently hydrogen or alkyl, or R_6 and R_7 together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring with an optional oxygen or sulphur atom or an optionally substituted second nitrogen atom in the ring; or a group



where R_8 is hydrogen; alkyl optionally substituted by -OH, alkoxy, $-NR_6R_7$ as defined for R_1, guanidine, $-CO_2H$, $-CONH_2$, -SH, or -S-alkyl; or $-CH_2$-Ar where Ar is optionally substituted aryl; and R_9 is alkoxy; OH; or $-NR_6R_7$ as defined for R_1;

R_2 is hydrogen; C_{2-8} alkanoyl; or optionally substituted aroyl;

R_3 is C_{3-6} alkyl; and

R_4 is $-(CH_2)_p-X-(CH_2)_q-$ where p is an integer from 1 to 9, q is an integer from 2 to 10, and the moiety $-(CH_2)_p-$ is adjacent to the carbon atom marked with an asterisk in formula (I), and X is $-NR_5-$ where R_5 is selected from hydrogen, C_{1-6} alkyl, C_{2-6} alkanoyl, C_{1-6} alkoxycarbonyl and aroyl, aralkyl or aralkyloxycarbonyl in each of which the aryl moiety is optionally substituted.

Unless otherwise specified, each alkyl or alkoxy group is a C_{1-8} group, more preferably a C_{1-6} group, and may be straight chain or branched.

Values for aryl groups include naphthyl and phenyl, preferably phenyl.

Optional substituents for aryl groups may be selected from -OH, C_{1-6} alkyl, C_{1-6} alkoxy and halogen.

Values for R_1 include hydroxy; C_{1-6} alkoxy, such as methoxy, ethoxy, iso-propoxy or t-butyloxy; benzyloxy; and $-NR_6R_7$ in which R_6 is hydrogen, and R_7 is hydrogen or C_{1-8} alkyl such as methyl or ethyl, or $-NR_6R_7$ is N'-methyl-N-piperazinyl or N-morpholinyl.

R_1 is preferably hydroxy, C_{1-4} alkoxy or C_{1-6} alkylamino. Most preferably R_1 is hydroxy, methoxy, iso-propoxy or methylamino.

R_2 is preferably hydrogen, acetyl or $Ph-\overset{O}{\overset{\|}{C}}-$ in which Ph is an optionally substituted phenyl group. Most preferably R_2 is hydrogen or acetyl.

R_3 is preferably a C_4 alkyl group, such as n-butyl, iso-butyl or sec-butyl. Most preferably R_3 is iso-butyl.

R_4 is preferably $-(CH_2)_p-X-(CH_2)_q-$ where p and q have values such that R_4 forms part of an 11- to 16-membered lactam structure, and X is a group $-NR_5-$ where R_5 is hydrogen, methyl, benzyl, t-butoxycarbonyl or benzyloxycarbonyl.

Most preferably R_4 is $-(CH_2)_p-X-(CH_2)_q-$ where p is 4 and q is 5 or p is 4 and q is 6 and X is a group $-NR_5-$ where R_5 is hydrogen.

The compounds of formula (I) may form salts with bases e.g. sodium hydroxide. When a basic nitrogen atom is present, the compounds of formula (I) may form acid addition salts e.g. with hydrochloric acid. Such compounds form part of the present invention.

The compounds of formula (I) have at least three asymmetric centres and therefore exist in more than one stereoisomeric form. The invention extends to all such forms and to mixtures thereof, including racemates, and diastereoisomeric mixtures.

Where compounds of formula (I), or pharmaceutically acceptable salts thereof, form solvates such as hydrates, these also form an aspect of the invention.

Preferred isomers are those having the (S)-configuration at the chiral centre marked with an asterisk in formula (I).

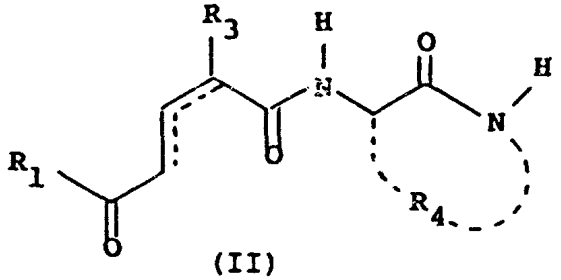
The compounds of formula (I) and their pharmaceutically acceptable salts are preferably in substantially pure form.

A substantially pure form will generally contain at least 50% by weight, preferably 75%, more preferably 90% and still more preferably 95% or 99% or more of the compound of formula (I) or its pharmaceutically acceptable salt.

The present invention provides the compounds of formula (I) or pharmaceutically acceptable salts thereof for use as active therapeutic agents, particularly as agents for treatment of musculo-skeletal disorders resulting from collagenolytic activity, particularly arthritic diseases, and tissue remodelling.

Compounds of formula (I) also have potential utility in the treatment of cancer; for preventing myelin degradation in the central and peripheral nervous system; and in other conditions in which members of the collagenase family of neutral metalloproteases have pathological or other roles.

The present invention also provides a process for the preparation of a compound of formula (I), which process comprises the reaction of a compound of formula (II):

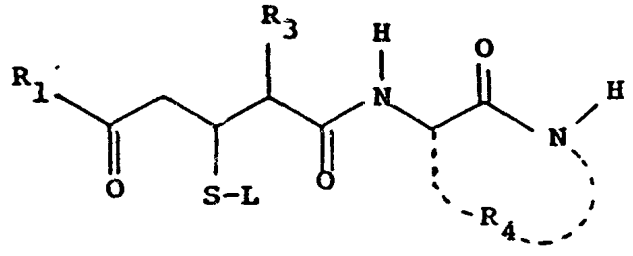


(II)

wherein R_1, R_3 and R_4 are as defined in formula (I), with a thiol of formula (III):

L - SH (III)

wherein L is a conventional sulphur protection group, to give a compound of formula (IV):



(IV)

wherein R_1, R_3 and R_4 are as defined in formula (I) and L is as defined in formula (III); and subsequently as necessary

- cleaving the group L and/or R_5 to give a compound of formula (I) in which R_2 and/or R_5 is hydrogen;

- converting the group R_2 in a compound of formula (I) into another group R_2;

- where appropriate converting the group R_5 in a compound of formula (I) into another group R_5.

Typically a sulphur protection group L is a substituted benzyl group, such as alkoxybenzyl, for example 4-methoxybenzyl, or an aliphatic or aryl acyl group such as acetyl or benzoyl. When L is an acyl group which is C_{2-8} alkanoyl or optionally substituted aroyl it is of course identical to R_2, so that compounds of formula (IV) in which $L=R_2$ are themselves compounds of the invention.

When L is a substituted benzyl sulphur protection group, such as 4-methoxybenzyl, then L may be removed by treatment with mercury acetate in trifluoroacetic acid containing anisole, followed by reaction with hydrogen sulphide in dimethylformamide, in a procedure analogous to that described in Chem. Pharm. Bull 1576, 26, (1978).

When L is an acyl group it may be removed by treatment with a base, for example aqueous ammonia or dilute aqueous sodium hydroxide, or by treatment with an acid, for example methanolic hydrochloric acid.

Other conventional methods for removing sulphur protection groups may also be used.

Compounds of the formula (I) in which R_2 is hydrogen may be converted to compounds of formula (I) in which R_2 is C_{2-8}alkanoyl using standard acylation procedures.

Compounds of formula (IV) can be converted to further compounds of formula (IV) while retaining the same group L, which group can in turn be cleaved to form compounds of the invention in which R_2 is hydrogen.

For example, those compounds of formula (IV) in which R_1 is -OH may be prepared under acid conditions by hydrolysis of compounds in which R_1 is alkoxy, aryloxy or aralkyloxy or by hydrogenolysis of compounds in which R_1 is benzyloxy or substituted benzyloxy in the presence of a catalyst such as palladium black.

Those compounds of formula (IV) in which R_1 is $-NR_6R_7$ may be prepared from compounds in which R_1 is -OH by treating the latter compounds with an amine of formula NHR_6R_7 in the presence of a coupling agent such as N,N-dicyclohexylcarbodiimide or N-ethyl-N'-dimethylaminopropylcarbodiimide.

Compounds of formula (IV) in which R_1 is $-NH-CH(R_8)-COR_9$ may be similarly prepared from compounds in which R_1 is OH by treatment with amine derivatives of formula $NH_2CH(R_8)COR_9$ where R_9 is an alkoxy or amine group, followed by hydrolysis to give an R_9 hydroxy group, if desired.

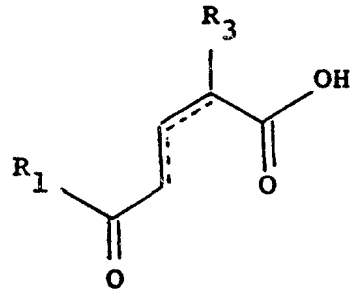
Alternatively, compounds of formula (IV) in which R_1 is $-NR_6R_7$ may be prepared from compounds of formula (IV) in which R_1 is alkoxy by aminolysis of the latter compound with an amine of formula NHR_6R_7 in the presence of a catalytic amount of cyanide. Aminolysis procedures are described by Hogber, T. et al., J. Org. Chem. 1987, 52, 2033-2036; De Ferand, R.J. et al., J. Org. Chem. 1963, 28, 2915-2917.

In addition, compounds of formula (IV) in which L is an acyl group can be converted to compounds of the invention with interconversion of R_1 and concomitant cleavage of the acyl group to give compounds of formula (I) in which R_2 is hydrogen.

It will be appreciated that the above transformations for compounds of formula (IV) will also be applicable for compounds of formula (IV) in which $L=R_2$, i.e. compounds of formula (I).

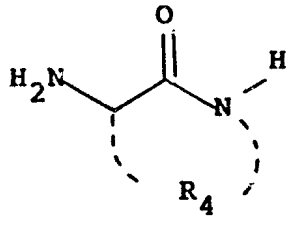
For example, those compounds of formula (I) in which R_1 is -OH and R_2 is hydrogen may be prepared by hydrolysis of compounds of formula (IV) in which R_1 is alkoxy, aryloxy or aralkyloxy and L is acyl, under basic conditions such as treatment with dilute sodium hydroxide.

The intermediate compounds of formula (II) may be prepared by treating a compound of formula (V):



(V)

in which R_1 and R_3 are as defined in formula (I), with a compound of formula (VI):

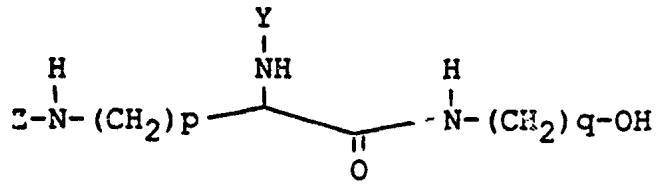


(VI)

wherein R_4 is as defined in formula (I).

The reaction is suitably carried out in the presence of a coupling agent, such as 1,1'-carbonyldiimidazole. Where R_5 in R_4 is hydrogen, the secondary amine is suitably in protected form, for example as a benzyloxycarbonyl derivative.

Compounds of formula (VI) may be prepared by oxidising the primary alcohol function in a compound of formula (VII):



(VII)

wherein p and q are as defined for R_4 in formula (I), Y is a nitrogen protection group, and Z is R_5, to give the corresponding aldehyde, followed by removal of Z when R_5 is an acyl group; cyclisation and reduction; and thereafter, as necessary, removing the nitrogen protection group Y and interconverting R_5.

Suitable values for Y include t-butoxycarbonyl (BOC) and benzyloxycarbonyl groups, preferably t-butoxycarbonyl.

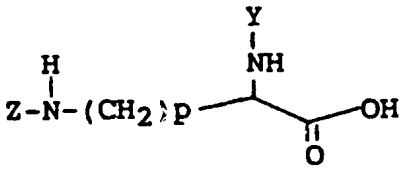
The oxidation may be carried out using pyridinium chlorochromate or under Swern oxidising conditions, for example by treatment with dimethylsulphoxide and an acyl halide followed by triethylamine, as described by D. Swern et al., J. Org. Chem., 43, 2480 (1978). The cyclisation and reductive amination step may be effected by catalytic hydrogenation over a suitable noble metal catalyst, for example palladium on carbon, or by reaction with sodium cyanoborohydride or sodium borohydride.

Nitrogen protection groups may be removed by standard methods. When Y or Z are t-butoxycarbonyl groups these may be removed by treatment with trifluoroacetic acid at reduced temperature. When Y or Z are benzyloxycarbonyl groups these may be removed by conventional hydrogenation over palladium on carbon, or by treatment with either formic acid-methanol and palladium black, or with HBr and glacial acetic acid. The group Z may be selected to undergo concomitant cleavage during the cyclisation reaction to give a compound in which R_5 is hydrogen. For example, when Z is a benzyloxycarbonyl group, it will be readily removed by catalytic hydrogenation.

An R_5 hydrogen in compounds of formulae (I), (II), (IV), (VI) and (VII) may be interconverted to an R_5 C_{1-6} alkyl, aralkyl or acyl group. The nitrogen atom in R_4 may be alkylated, for example methylated to form an R_5 methyl group, or acylated to form an R_5 C_{1-6} alkoxycarbonyl or aralkyoxycarbonyl group.

Methylation procedures are described by E. Askitoglu et al., Helv. Chim. Acta., 68, 750, (1985); E. Engler et al., Helv. Chim. Acta., 68, 789, (1985); and M. Lennon et al., J. Chem. Soc. (Perkin I), 622, (1975).

Compounds of formula (VII) may be prepared by reacting a compound of formula (VIII):



(VIII)

wherein p, Y and Z are as defined for formula (VII), with a compound of formula (IX):

$$H_2N-(CH_2)_q-OH \qquad (IX)$$

wherein q is as defined for formula (VII).

The reaction may be carried out using standard procedures for forming an amide from a carboxylic acid and an amine, for example using a coupling agent such as 1,1'-carbonyldiimidazole, 1,3-dicyclohexylcarbodiimide or N-ethyl-N'-dimethylaminopropylcarbodiimide.

Compounds of formula (VIII) are di-aminoalkanoic acid derivatives. These are known compounds or may be prepared from known starting materials by standard methods.

For example the compound of formula (VI) in which R_4 is $-(CH_2)_p-X-(CH_2)_q-$ where p is 3, q is 6 and X is -NH- is prepared from a compound of formula (VIII) derived from ornithine which is commercially available.

The compound of formula (VI) in which R_4 is $-(CH_2)_p-X-(CH_2)_q-$ where p is 4, q is 5 and X is -NH- is prepared from a compound of formula (VIII) derived from the amino acid lysine. The compound of formula (VIII), derived from (S)-lysine, in which Y is t-butoxycarbonyl and Z is benzyloxycarbonyl, is commercially available.

Similarly, the compound of formula (VI) in which R_4 is $-(CH_2)p-X-(CH_2)q-$ where p is 1, q is 8 and X is -NH- may be prepared from 2,3-diaminopropionic acid.

The thiols of formula (III) and the amino alcohols of formula (IX) are known compounds or may be prepared from known compounds by known methods.

Intermediate compounds of formulae (II) and (IV) disclosed herein are novel compounds, ~~and form an aspect of the present invention.~~

The preparation of certain compounds of formula (V) is described in EP-A-0273689.

Where obtainable, pharmaceutically acceptable salts of the compounds of formula (I) may be formed conventionally by reaction with the appropriate acid or base. Solvates may be formed by crystallization from the appropriate solvent.

As mentioned previously, the compounds of formula (I) exist in more than one diastereoisomeric form. Where the processes of the invention produce mixtures thereof, the individual isomers may be separated one from another by chromatography, e.g. column chromatography or HPLC.

Alternatively, separate diastereoisomeric compounds of formula (I) can be obtained by using stereoisomerically pure starting materials or by separating desired isomers of intermediates at any stage in the overall synthetic process, and converting these intermediates to compounds of formula (I).

It will be appreciated that although the absolute configuration at a particular chiral centre may not be known, it is possible to characterise a given diastereoisomer relative to its epimer or to another diastereoisomer using NMR spectroscopy or optical rotation.

The present invention further provides a pharmaceutical composition, which comprises a compound of formula (I), or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

A composition of this invention is useful in the treatment of musculo-skeletal disorders, particularly arthritic diseases and for modulation of tissue remodelling.

A composition of the invention, which may be prepared by admixture, may contain a diluent, binder, filler, disintegrant, flavouring agent, colouring agent, lubricant or preservative in conventional manner. These conventional excipients may be employed in conventional manner, for example as in the preparation of compositions of related peptide enzyme inhibitors, such as the ACE inhibitor captopril.

A composition of the invention may be adapted for oral, topical, rectal or parenteral administration but oral administration is preferred. Parenteral compositions may be administered e.g. intravenously, intramuscularly or intra-articularly. Preferably compositions are in unit dosage form or in a form that a patient can administer to himself in a single dose.

Preferably, a pharmaceutical composition of the invention is in unit dosage form and in a form adapted for use in the medical or veterinarial fields. For example, such preparations may be in a pack form accompanied by written or printed instructions for use as an agent in the treatment or prophylaxis of any of the disorders mentioned above.

Compositions may, for example, be in the form of tablets, capsules, sachets, vials, powders, granules, lozenges, reconstitutable powders, or liquid preparations, for example solutions or suspensions, or suppositories.

The compositions, for example those suitable for oral administration, may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate; disintegrants, for example starch, polyvinylpyrrolidone, sodium starch glycollate or microcrystalline cellulose; or pharmaceutically acceptable wetting agents such as sodium lauryl sulphate.

Solid compositions may be obtained by conventional methods of blending, filling, tabletting or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers. When the composition is in the form of a tablet, powder, or lozenge, any carrier suitable for formulating solid pharmaceutical compositions may be used, examples being magnesium stearate, starch, glucose, lactose, sucrose, rice flour and chalk. Tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating. The composition may also be in the form of an ingestible capsule, for example of gelatin containing the compound, if desired with a carrier or other excipients. For example, a hard gelatin capsule containing the required amount of a compound of the invention in the form of a powder or granulate in intimate mixture with a lubricant, such as magnesium stearate, a filler, such as microcrystalline cellulose, and a disintegrant, such as sodium starch glycollate.

Compositions for oral administration as liquids may be in the form of, for example, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid compositions may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; aqueous or non-aqueous vehicles, which include edible oils, such as almond oil and fractionated coconut oil, oily esters, for example esters of glycerine, propylene glycol, ethyl alcohol, glycerine, water or normal saline; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and if desired conventional flavouring or colouring agents.

The compounds of this invention may also be administered by a non-oral route. In accordance with routine pharmaceutical procedure, the compositions may be formulated, for example for rectal administration as a suppository or for parenteral administration in an injectable form. For injection, for example by intra-articular injection or by injection into the cerebro-spinal fluid or via other routes which will gain access to sites of demyelination, as freely soluble solutions or as poorly dispersed depot stores, the compounds of the invention may be presented in an aqueous or non-aqueous solution, suspension or emulsion in a pharmaceutically acceptable liquid, e.g. sterile pyrogen-free water or a parenterally acceptable oil or a mixture of liquids, which may contain bacteriostatic agents, anti-oxidants or other preservatives, buffers or solutes to render the solution isotonic with the blood,

thickening agents, suspending agents or other pharmaceutically acceptable additives. Such forms will be presented in sterile unit dose form such as ampoules or disposable injection devices or in multi-dose forms such as a bottle from which the appropriate dose may be withdrawn or a solid form or concentrate which can be used to prepare an injectable formulation.

For topical and percutaneous administration, the preparations may also be presented as an ointment, cream, lotion, gel, spray, aerosol, wash, skin paint or patch.

The suitable dosage range for the compounds of the invention may vary from compound to compound and may depend on the condition to be treated. It will also depend, inter alia, upon the relation of potency to absorbability and the mode of administration chosen.

A unit dose for treating diseases and physiological phenomena in which enzymes from the collagenase family are involved will generally contain from 10 to 1000 mg and preferably will contain from 10 to 500 mg, in particular 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 mg. The composition may be administered once or more times a day, for example 2, 3 or 4 times daily, so that the total daily dose for a 70 kg adult will normally be in the range 10 to 3000 mg. Such a dose corresponds to approximately 0.15 to 50 mg/kg per day. Alternatively, in particular for injection, the unit dose will suitably contain from 2 to 20 mg of a compound of the invention and be administered in multiples, if desired, to give the desired daily dose.

The present invention additionally provides a method of treating conditions in which degradation of connective tissue and other proteinaceous components of the body

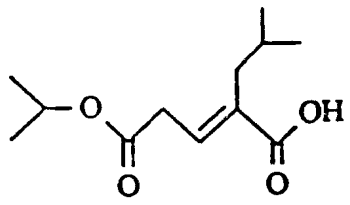
occurs, such as rheumatism and/or arthritic conditions in mammals, such as humans, which comprises administering to the mammal in need of such treatment an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

The present invention also provides the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for use in the treatment of conditions in which degradation of connective tissue and other proteinaceous components of the body occurs such as rheumatism and/or arthritic conditions.

The following Description and Examples illustrate the preparation of compounds of the invention.

Description 1

4-Isopropoxycarbonyl-2-(2-methylpropyl)but-2-enoic acid (D1)

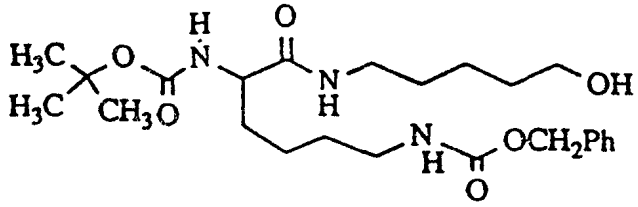


A solution of 2-(2-methylpropyl)pent-2-enedicarboxylic anhydride (prepared as in EP-A-273689) in 2-propanol (100 ml) was heated under reflux for 8h. The solvent was evaporated in vacuo to give the title compound as a brown oil (31.7g, 98%). $\delta(CDCl_3)$:

0.88 (6H,d, J=7Hz), 1.25 (6H,d,J=7Hz), 1.80 (1H, septuplet, J=7Hz), 2.18 (2H,d,J=7Hz), 3.60 (2H, d, J=7Hz), 5.05 (1H, septuplet, J=7Hz) and 6.30 (1H,t,J=7Hz).

Description 2

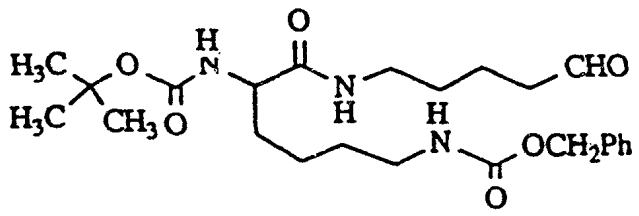
N^{ε}-Benzyloxycarbonyl-N^{α}-tert-butoxycarbonyl-(S)-lysine-(5-hydroxy)pentylamide (D2)



To a solution of N^{ε}-benzyloxycarbonyl-N^{α}-tert-butoxycarbonyl-(S)-lysine (7.8g, 21 mmol) in anhydrous dichloromethane (150ml) maintained at 0°C was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.3g, 22.5 mmol) and 1-hydroxybenzotriazole (3.6g, 26.5 mmol). The mixture was stirred for 0.5h at 0°C, 5-aminopentan-1-ol (2.3g, 22.5 mmol) added and stirring continued at room temperature. After 3h the mixture was washed with saturated aqueous $NaHCO_3$ (60 ml), dried over anhydrous magnesium sulphate and evaporated in vacuo to afford a viscous oil. Purification by flash chromatography [($CHCl_3$:MeOH) (20:1) v/v] gave the title compound (D2) as a clear oil (8.01g).
(Found: C, 61.54; H, 8.52; N, 9.18. $C_{24}H_{39}O_6N_3$ requires C, 61.91; H, 8.44; N, 9.02%). Observed $(M+H)^+$466. $C_{24}H_{39}O_6N_3$ requires M 465.

Description 3

N^{ε}-Benzyloxycarbonyl-N^{α}-tert-butoxycarbonyl-(S)-lysine-(4-formyl)butylamide (D3)

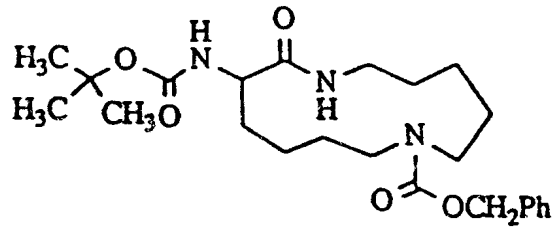


To a stirred solution of oxalyl chloride (1.47g, 12 mmol) in anhydrous dichloromethane (40 ml) maintained under an atmosphere of nitrogen at -60°C was added

dimethylsulphoxide (1.21g, 15 mmol) dropwise, such that the temperature remained below -50°C. The mixture was left stirring at -60°C for 15 minutes, alcohol (D2) (3.6g, 7.7 mmol) diluted in anhydrous dichloromethane (10 ml) was added, and allowed to warm up to -25°C over 1h. The mixture was then cooled down to -60°C, triethylamine (4.7g, 46 mmol) added slowly such that the internal temperature remained below -50°C. On completion of addition, the mixture was gradually warmed up to room temperature, washed with water (30 ml) and sat. aq. NaCl (30 ml). The aqueous washes were back extracted with dichloromethane (2x30 ml) and the combined organic fractions were dried over anhydrous magnesium sulphate and evaporated in vacuo to yield a viscous clear oil. Purification by flash chromatography [(EtOAc:MeOH) (20:1)v/v] afforded the title compound (D3) as an oil (2.8g).Observed $(M+H)^+$ 464. $C_{24}H_{37}O_6N_3$ requires M 463.

Description 4

(S)-3-(N-tert-Butoxycarbonyl)amino-8-(N-benzyloxycarbonyl)-1,8-diazacyclotridecan-2-one (D4)



Method A

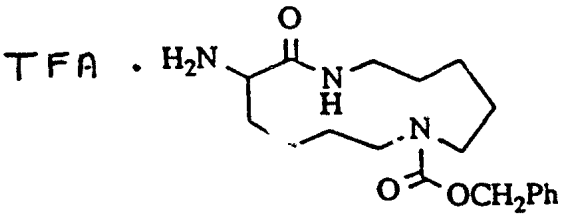
The aldehyde (D3) (1.8g, 3.88 mmol) was dissolved in ethanol (180 ml) and hydrogenated over 10% palladium on charcoal (200 mg) at atmospheric pressure and 35°C for 72h. The suspension was filtered through Keiselguhr and evaporated in vacuo to give crude (S)-3-(N-tert-butoxy-carbonyl)amino-1,8-diazacyclotridecan-2-one. The crude amine was dissolved in a mixed solvent system of tetrahydrofuran/water, (6:20 ml) v/v, cooled to 0°C and treated with benzyl chloroformate (0.66g, 3.88 mmol) and excess sodium carbonate to maintain a pH between 10 and 11. The mixture was left stirring at room temperature overnight, washed with ethyl acetate (3x25 ml), and the combined organic fractions dried over anhydrous magnesium sulphate and evaporated in vacuo to afford a clear oil. Purification by flash chromatography[(EtOAc:MeOH) (20:1)v/v] yielded the title compound (D4) as a white solid (0.2g).Observed M^+ 447. $C_{24}H_{37}O_5N_3$ requires M 447.

Method B

The aldehyde (D3) was hydrogenated at about 100psi of pressure over 10% palladium on charcoal in methanol, and then in acidic methanol to afford crude (S)-3-(N-tert-butoxycarbonyl)amino-1,8-diazacyclotridecan-2-one. The amine was treated with benzyl chloroformate and purified as described in Method A, to yield the identical title compound.

Description 5

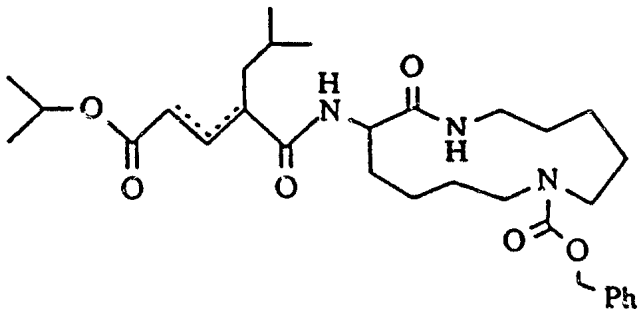
(S)-3-Amino-8-(N-benzyloxycarbonyl)-1,8-diazacyclotridecan-2-one, trifluoroacetate salt (D5)



A cooled (0°C) solution of the lactam (D4) (0.59g, 1.28 mmol) in dichloromethane (10 ml) was treated with trifluoroacetic acid (5ml). The mixture was stirred for 1h at 0°C, warmed up to room temperature and left stirring overnight. The solvent was evaporated under reduced pressure, to afford crude title compound (D5) as the trifluoroacetate salt. This was used as such without further purification.

Description 6

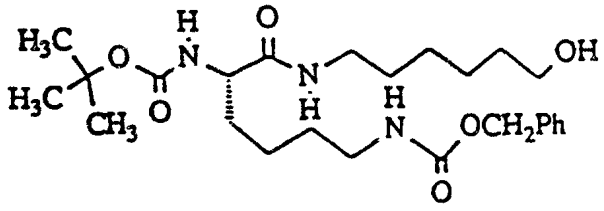
6-Methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotridecan-2-one-3-yl]aminocarbonyl]hept-2(and 3)-enoic acids, isopropyl esters (D6)



A solution of 4-isopropoxycarbonyl-2-(2-methylpropyl) but-2-enoic acid (D1) (3.7g, 16.1 mmol) in anhydrous dichloromethane (10 ml) under nitrogen was cooled to 0°C in an ice bath and then treated with 1,1,-carbonyldi-imidazole (3.03g, 18.7mmol) in one portion. After 1h at 0°C the mixture was sequentially treated with N,N-diisopropylethylamine (2.59g, 20.1 mmol) and the crude diazalactam (D5). The solution was stirred at 0°C for 1h, warmed up to room temperature, and stirring continued overnight. The solution was washed successively with water, 10% citric acid, 10% $NaHCO_3$, brine, dried over anhydrous magnesium sulphate and finally evaporated in vacuo to remove the solvent. The product was subjected to flash-column chromatography on silica gel, eluting with ethyl acetate-pentane (1:1) v/v to afford the title compound (D6) as a white solid (3.04g) m.p. 112-117°C. (Found: C, 66.56; H, 8.32; N, 7.52. $C_{31}H_{47}O_6N_3$ requires C, 66.76; H, 8.49; N, 7.53%).

Description 7

N^{α}-tert-Butoxycarbonyl-N^{ε}-benzyloxycarbonyl-(S)-lysine-(6-hydroxy)hexylamide (D7)



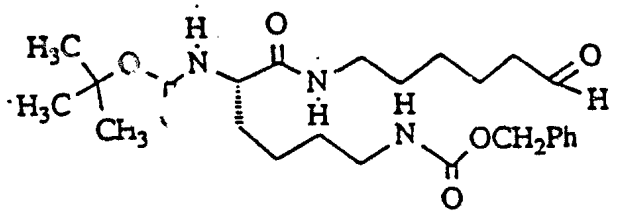
A solution of N^{α}-tert-butoxycarbonyl-N^{ε}-benzyloxycarbonyl-(S)-lysine (15.8g, 0.042 mol) in anhydrous dichloromethane (200 ml) maintained at 0°C, was treated sequentially with

1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (9.96g, 0.051 mol) and 1-hydroxy-benzotriazole (7.0g, 0.051 mol). The solution was stirred at 0°C for 1h, treated with 6-aminohexan-1-ol (4.7g, 0.046 mol), and left stirring overnight at room temperature. The mixture was then washed with saturated aqueous $NaHCO_3$, dried over anhydrous magnesium sulphate, and evaporated in vacuo to afford a viscous oil. Purification by flash chromatography [($CHCl_3$:MeOH) (20:1) v/v] gave the title compound (D7) as a clear oil (16g), which on standing solidified to a white solid.

Observed $(M+H)^+$ 480. $C_{25}H_{41}N_3O_6$ requires M 479.

Description 8

N^{α}-tert-Butoxycarbonyl-N^{ε}-benzyloxycarbonyl-(S)-lysine-(5-formyl)pentylamide (D8)



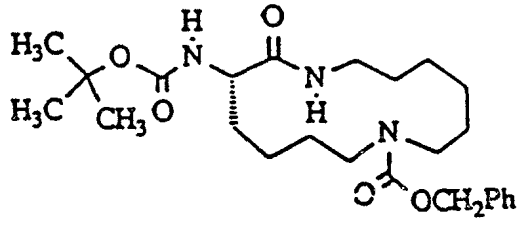
A stirred solution of dimethyl sulphoxide (3.63g, 0.046 mol) in anhydrous dichloromethane (100 ml) maintained at -60°C, was treated with oxalyl chloride (2.58g, 0.0198 mol) diluted in dichloromethane (10 ml) at such a rate, so as to ensure temperature remained below -50°C. After stirring for 20 minutes, the alcohol (D7) (6.35g, 0.013 mol) dissolved in dichloromethane (50 ml) was added

dropwise over 5 mins. The mixture was stirred at -60°C for 15 mins, warmed up to -35°C, stirred for a further 10 mins then cooled down to -60°C. The solution was treated with triethylamine (8g, 0.08 mol), warmed up to room temperature, washed with water (2x100 ml), dried over anhydrous magnesium sulphate and solvent evaporated under reduced pressure to afford a viscous oil. Purification by flash chromatography [(EtOAc:MeOH) (30:1) v/v] gave the title compound (D8) as an oil (5g), which on standing solidified to a white solid.

Observed $(M+H)^+$ 478. $C_{25}H_{39}N_3O_6$ requires M 477.

Description 9

(S)-3-(N-tert-Butoxycarbonyl)amino-8-(N-benzyloxycarbonyl)-1,8-diazacyclotetradecan-2-one (D9)



Method A

The aldehyde (D8) (5.0g) in methanol (450 ml) was treated with 5% palladium on charcoal (5.5g). The suspension was hydrogenated at 140 psi and ambient temperature for 48h, treated with 2.5M aqueous hydrochloric acid (3 ml) and hydrogenation continued at the said pressure for a further

24h. The suspension was filtered through Kieselguhr and evaporated in vacuo to give crude (S)-3-(N-tert-butoxy-carbonyl)amino-1,8-diazacyclotetradecan-2-one. The crude amine was dissolved in a mixed solvent system of tetrahydrofuran/water, (10:40 ml) v/v cooled to 0°C and treated with benzyl chloroformate (2.85g) and excess sodium carbonate to maintain a pH between 10 and 11. The mixture was left stirring at room temperature for 4h, solvent partially evaporated in vacuo and the residue extracted with dichloromethane (3x100 ml). The organic fraction was dried over anhydrous magnesium sulphate and evaporated in vacuo to afford a clear oil. Purification by flash chromatography [(EtOAc:MeOH) (50:1) v/v] yielded the title compound (D9) as a white solid (2.0g) m.p. 131.5-134.0°C.

Observed M^+ 461. $C_{25}H_{39}N_3O_5$ requires M 461.

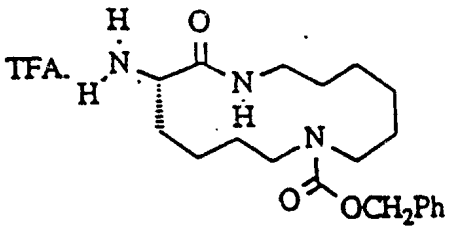
Method B

The aldehyde (D8) (5.8g) in methanol (600 ml) was treated with 5% palladium on charcoal (6g) and hydrogenated at 50 psi for 18h. The suspension was filtered through Kieselguhr and the solvent partly evaporated in vacuo. The concentrated solution (~ 200 ml) was treated with 4A molecular sieves and $NaBH_3CN$ (1.5g). The pH was adjusted to 6 by the addition of concentrated HCl then left stirring overnight. The suspension was filtered through Kieselguhr, and acidified to pH3. The solvent was evaporated in vacuo, and the residue taken up in dichloromethane (100 ml) and washed with aq. 1M NaOH (2 x 60 ml). The organic fraction was dried and the solvent removed under reduced pressure to afford a white solid. The solid was dissolved in THF/H_2O (100 : 30 ml) mixture, treated with benzyl chloroformate (1.37g) and excess

Na_2C0_3. The mixture was stirred for 4h, then the solvent partially evaporated in vacuo. The residue was extracted with dichloromethane (3 x 80 ml), dried and evaporated under reduced pressure to give a white solid. Trituration with diethyl ether and pentane gave the title compound (D9) as a white solid.

Description 10

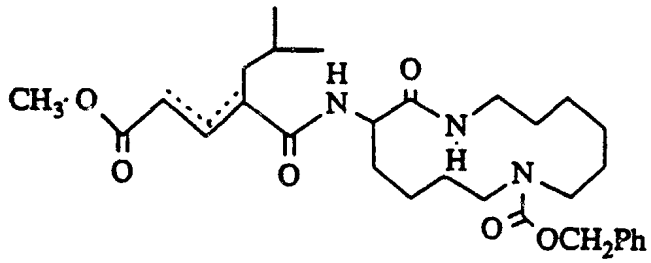
(S)-3-Amino-8-(N-benzyloxycarbonyl)-1,8-diazacyclotetradecan-2-one, trifluoroacetate salt (D10)



A cooled (0°C) solution of the lactam (D9) (9g) in dichloromethane (150ml) was treated with trifluoroacetic acid (50 ml). After 1h the solvent was evaporated under reduced pressure, to afford crude title compound (D10) as an oil. This was used as such without further purification.

Description 11

6-Methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotetradecan-2-one-3-yl]aminocarbonyl]hept-2(and 3)-enoic acids, methyl esters (D11)

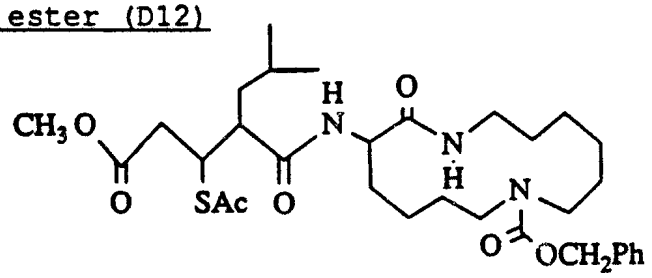


A solution of 4-methoxycarbonyl-2-(2-methylpropyl)but-2-enoic acid (prepared as in EP-A-273689) (5.08g) in anhydrous dichloromethane (200 ml) maintained at 0°C was treated with 1,1,-carbonyldiimidazole (4.12g) in one portion. After 1h at 0°C the mixture was sequentially treated with N,N-diisopropylethylamine (5g) and the crude diazalactam (D10) (0.019 mol) dissolved in dichloromethane (20 ml). The solution was stirred at 0°C for 1h, warmed up to room temperature, and stirring continued overnight. The solution was washed successively with water, 10% citric acid, 10% $NaHCO_3$ and brine, dried over anhydrous magnesium sulphate and evaporated in vacuo. The residue was purified by flash-column chromatography on silica gel, eluting with ethyl acetate-pentane (1:1 v/v) to afford the title compound (D11) as a white solid (5.1g).

Observed M^+ 543.3307. $C_{30}H_{45}O_6N_3$ requires M 543.3305.

Description 12

3-Acetylmercapto-6-methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotetradecan-2-one-3-yl]aminocarbonyl]heptanoic acid, methyl ester (D12)

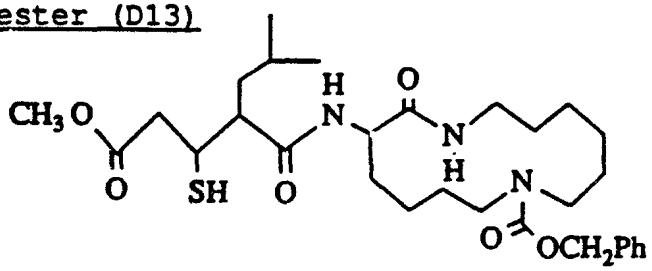


A solution of the esters (D11) (5g) in thiolacetic acid (35 ml) was set aside at room temperature for 18 days and then evaporated to dryness in vacuo. The product was subjected to flash-column chromatography on silica gel using diethyl ether, followed by diethyl ether - ethyl acetate (4:1 v/v) as the eluent. The first fractions to contain solids on evaporation of solvent were combined and triturated with diethyl ether-pentane to afford the title compound (D12) as a single diastereoisomer (Isomer A). Later fractions contained mixtures of diastereoisomers.

Observed M^+ 619. $C_{32}H_{49}N_3O_7S$ requires M 619

Description 13

3-Mercapto-6-methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotetradecan-2-one-3-yl]aminocarbonyl]heptanoic acid, methyl ester (D13)



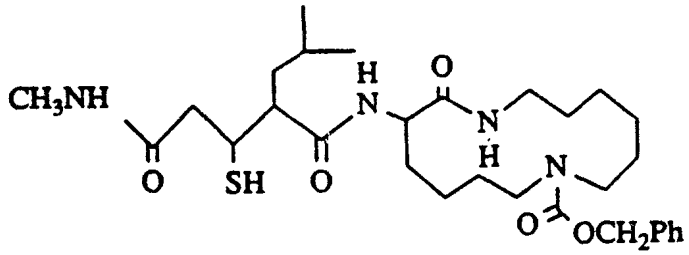
An ice-cooled solution of the diastereoisomeric mixture of esters (D12) (0.215g) in nitrogen-purged methanol (20 ml) was treated with 35% aqueous ammonia (5 ml), and the reaction mixture was stirred under nitrogen for 2h and then evaporated to dryness in vacuo to afford a white solid. The crude product was used without further purification in the preparation of examples 5 and 6.

Observed M^+ 577. $C_{30}H_{47}O_6N_3S$ requires M 577

The above procedure may be repeated with Isomer A (D12) to afford single diastereoisomer (D13). This may then be converted to single diastereoisomers (E5) and (E6).

Description 14

3-Mercapto-6-methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotetradecan-2-one-3-yl]aminocarbonyl]heptanoic acid, methyl amide (D14)

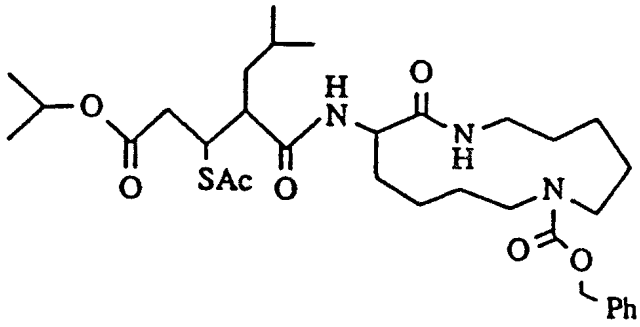


A solution of the methyl ester (D13) (0.04g) was heated in nitrogen-purged methanol (3 ml) containing excess N-methylamine and sodium cyanide (0.013g) at 65°C for 42h in a sealed vessel. The reaction mixture was evaporated to dryness in vacuo and chromatographed on silica gel. Elution with 5% methanol in $CHCl_3$ gave the title compound (0.025g) as a white solid.

Observed $(M+H)^+$ 577. $C_{30}H_{48}O_5N_4S$ requires M 576.

Example 1

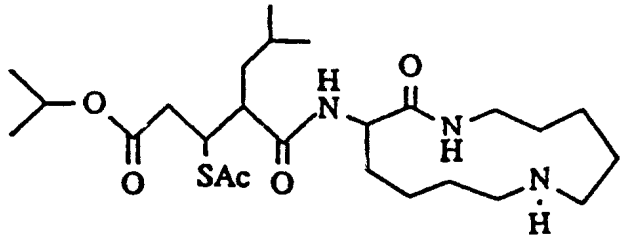
3-Acetylmercapto-6-methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotridecan-2-one-3-yl]aminocarbonyl]heptanoic acid, isopropyl ester (E1)



A solution of the esters (D6) (3g, 5.38 mmol) in thiolacetic acid (35 ml) was set aside at room temperature for 18 days and then evaporated to dryness in vacuo. The product was subjected to flash-column chromatography on silica gel using diethyl ether, followed by diethyl ether-ethyl acetate (4:1) v/v as the eluent. The first fractions to contain solids on evaporation of solvent were combined and recrystallised from diethyl ether-pentane to afford the title compound (E1) (0.32g), m.p. 135-138°C. (Found: C, 62.29; H, 8.03; N, 6.59. $C_{33}H_{51}O_7N_3S$ requires C, 62.53; H, 8.11; N, 6.63%).

Example 2

3-Acetylmercapto-6-methyl-4-[(1,8-diazacyclotridecan-2-one-3-yl)aminocarbonyl]heptanoic acid, isopropyl ester (E2)

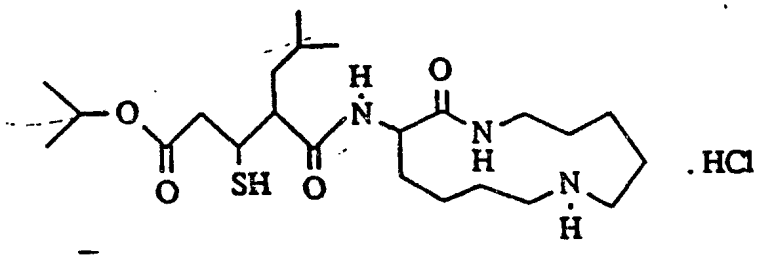


A solution of the lactam (E1) (0.15g, 0.24mmol) in 4.5% formic acid/methanol (4 ml) was added under nitrogen, to a stirred suspension of palladium black (160 mg) in methanol (10 ml). After 2h the mixture was filtered through Kieselguhr and evaporated in vacuo to afford an oil which on standing over pentane-diethyl ether gave the title compound (E2) (0.11g). This was used as such without further purification.

Observed M^+ 499. $C_{25}H_{45}O_5N_3S$ requires M 499.

Example 3

3-Mercapto-6-methyl-4-[(1,8-diazacyclotridecan-2-one-3-yl)aminocarbonyl]heptanoic acid, isopropyl ester hydrochloride salt (E3)



An ice-cooled solution of the amine (E2) (0.1g) in nitrogen-purged methanol (20 ml) was treated with 35% aqueous ammonia (5 ml), and the reaction mixture was stirred under nitrogen for 2h and then evaporated to dryness in vacuo. The product was chromatographed on silica gel using ethyl acetate-chloroform (1:1) v/v then methanol-chloroform (1:5) v/v as the eluent to afford the title compound free base as a clear oil. The product was dissolved in ethanol and treated with a few drops of 1M etheral HCl to afford, on removal of the solvent, the title compound hydrochloride salt (E3) as a white solid (0.08g), m.p. 110-115°C.

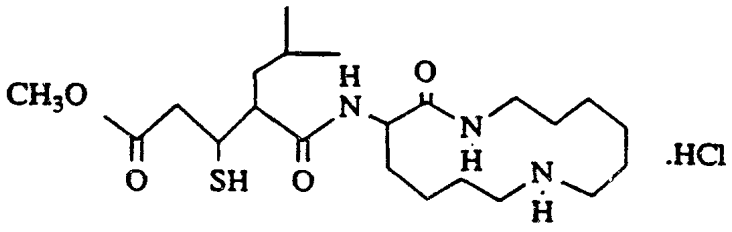
$\delta(CD_3OD)$ 0.89 (3H, d, J=6Hz), 0.91, (3H, d, J=6Hz), 1.23 (6H, d, J=6Hz), 1.3-1.9 (15H, m), 2.42 (1H, dd, J=10, 16Hz), 2.52 (1H, m), 2.71 (1H, dd, J=16, 3Hz), 2.93 (1H, m), 2.99-3.3 (3H, m), 3.1-3.23 (2H, m), 3.65 (1H, m), 4.35 (1H, m), 5.0 (1H, m).

Observed FAB $(M+H)^+$ 458 (free base).

$C_{23}H_{43}N_3O_4S$ requires M 457.

Example 4

3-Mercapto-6-methyl-4-[(1,8-diazacyclotetradecan-2-one-3-yl)aminocarbonyl]heptanoic acid, methyl ester hydrochloride salt (E4)



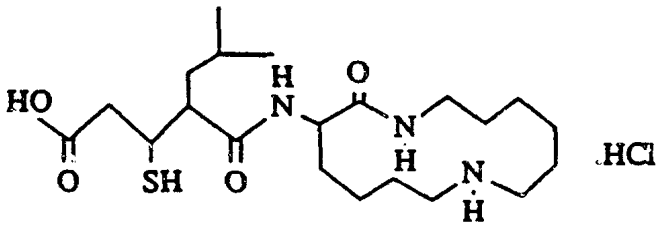
A solution of the lactam (D12) (Isomer A, 0.22g) in glacial acetic acid (3 ml) was treated with 45% HBr in glacial acetic acid (3 ml) and left stirring for 45 minutes at room temperature. The solvent was removed by evaporation in vacuo, and the residue dissolved in methanol (20 ml). The solution was purged with nitrogen for 10 minutes, cooled in an ice bath, then treated with 35% aqueous ammonia (5 ml). The mixture was allowed to stir at 0°C for 0.5h then at room temperature for 3h. The solvent was evaporated under reduced pressure to afford a viscous oil. The residue was diluted with anhydrous methanol (1 ml) and treated dropwise with excess 1M ethereal HCl to afford the title compound as a white solid (0.12g).

Observed M^+ 443 (free base). $C_{22}H_{41}N_3O_4S$ requires M 443.

δ (CD_3OD): 0.6-0.85 (6H, m), 1.2-1.8 (17H, m), 2.32-3.18 (9H, m), 3.5-3.62 (4H, m), 4.25 (1H, m).

Example 5

3-Mercapto-6-methyl-4-[(1,8-diazacyclotetradecan-2-one-3-yl)aminocarbonyl]heptanoic acid, hydrochloride salt (E5)



To a suspension of methyl ester (D13) (0.1 g) in isopropanol (5 ml), previously purged with nitrogen, was

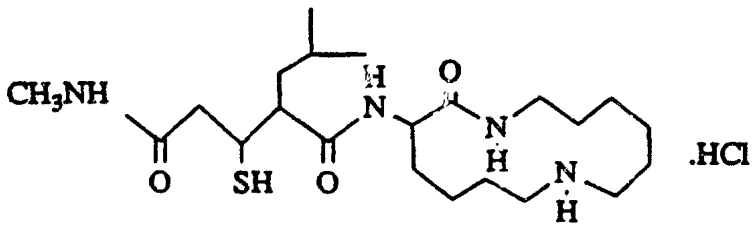
added a solution of sodium hydroxide (0.02g) in water (1 ml) and the resulting solution was stirred at room temperature, under nitrogen for 18h. The solution was acidified with an excess of ethereal-HCl and then evaporated to dryness in vacuo. The residue was diluted with 1N aqueous HCl (4 ml) and extracted with $CHCl_3$ (3 x 5 ml). The combined organic fraction was dried and evaporated to leave a viscous oil which on trituration with ether gave a white solid. The solid was dissolved in glacial acetic acid (5 ml) and treated with 45% hydrogen bromide in glacial acetic acid (2 ml). The mixture was stirred for 1h and then treated with ether to precipitate an oil. The solvent was decanted and residue dissolved in anhydrous methanol (20 ml) and treated with excess 1M ethereal HCl to form a salt. The HCl salt was triturated with ether to give the title compound as a white solid.

Observed $(M)^+-H_2S$ 395 (free base). $C_{21}H_{39}N_3O_4S$ requires M 429.

δ (CD_3OD): 0.9 (6H, m), 1.2-2.0 (17H, m), 2.45-3.3 (9H, m), 3.65 (1H, m), 4.35 (1H, m).

Example 6

3-Mercapto-6-methyl-4-[(1,8-diazacyclotetradecan-2-one-3-yl)aminocarbonyl]heptanoic acid, methyl amide hydrochloride salt (E6)



A solution of amide (D14) (0.02g) in glacial acetic acid (3 ml) was treated with 45% hydrogen bromide in glacial acetic acid (1 ml). The mixture was stirred for 1h and then treated with ether to precipitate a solid. The solvent was decanted, and the residue dissolved in anhydrous methanol (2 ml) and treated with excess 1M ethereal HCl. The HCl salt that formed was triturated with ether to give the title compound as a yellow solid.

Observed $(MH)^+-H_2S$ 409 (free base). $C_{22}H_{42}N_4O_3S$ requires M 442.

δ (CD_3OD): 0.85 (6H, m), 1.25-2.0 (17H, m), 2.4-3.3 (12H, m), 3.65 (1H, m), 4.35 (1H, m).

Example 7

Pharmaceutical compositions for oral administration are prepared by combining the following:

1) Solid Dosage Formulation

	% w/w
Compound of Example 1	10%
Magnesium stearate	0.5%
Starch	2.0%
HPM cellulose	1.0%
Microcrystalline cellulose	86.5%

The mixture may be compressed to tablets, or filled into hard gelatin capsules.

The tablet may be coated by applying a suspension of film former (e.g. HPM cellulose), pigment (e.g. titanium dioxide) and plasticiser (e.g. diethyl phthalate) and drying the film by evaporation of the solvent. The film coat can comprise 2.0% to 6.0% of the tablet weight, preferably about 3.0%.

2) Capsule

	%w/w
Compound of Example 1	20%
Polyethylene glycol	80%

The medicinal compound is dispersed or dissolved in the liquid carrier, with the thickening agent added, if present. The formulation is then enclosed in a soft gelatin capsule by suitable technology.

Example 8

A pharmaceutical composition for parenteral administration is prepared by combining the following:

Compound of Example 1	1.0%
Saline	99.0%

The solution is sterilised and sealed in sterile containers.

COLLAGENASE INHIBITOR ASSAY

The test is performed essentially as in Cawston and Barrett, Anal. Biochem. 99, 340-345 (1979). Compounds for testing are dissolved in methanol by sonication and added to collagenase (purified from culture supernatants from the human lung fibroblast cell line, WI-38) in buffer. To ensure that thiol collagenase inhibitors remain unoxidised, β-mercaptoethanol may be incorporated in the methanol solvent and/or the diluent buffers to give a final concentration of 9.6×10^{-5}M. The minimal direct effect of β-mercaptoethanol on the degradation of collagen by human collagenase is controlled for. After a 5 min pre-incubation at 37°C, the assay tubes are cooled to 4°C and ^{3}H-acetylated rat skin type I collagen is added. The assay tubes are incubated at 37°C overnight. The ^{3}H-collagen forms insoluble fibrils, which are the substrate for the enzyme.

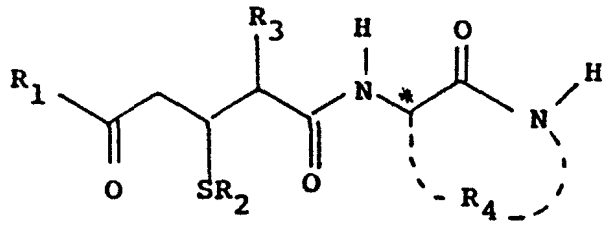
To terminate the assay, the assay tubes are spun at 12000 rpm for 15 minutes. Undigested ^{3}H-collagen is pelleted, while digested ^{3}H-collagen is found as soluble peptides in the supernatant. A sample of the supernatant is taken for liquid scintillation counting.

The activity of collagenase inhibitors (IC_{50}: 50% inhibitory concentration) is expressed as that concentration of compound that inhibits a known (standard) concentration of enzyme by 50%.

The compounds of Examples E1-E6 had IC_{50} values in the range $4.7 \times 10^{-6} - 9.7 \times 10^{-8}$M.

Claims

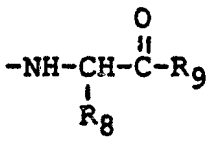
1. A compound of formula (I) or a salt, solvate or hydrate thereof



(I)

in which,

R_1 is -OH; alkoxy; aryloxy or aralkyloxy in each of which the aryl group is optionally substituted; $-NR_6R_7$, where each of R_6 and R_7 is independently hydrogen or alkyl, or R_6 and R_7 together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring with an optional oxygen or sulphur atom or an optionally substituted second nitrogen atom in the ring; or a group



where R_8 is hydrogen; alkyl optionally substituted by -OH, alkoxy, $-NR_6R_7$ as defined for R_1, guanidine, $-CO_2H$, $-CONH_2$, -SH, or -S-alkyl; or $-CH_2$-Ar where Ar is

optionally substituted aryl; and R_9 is alkoxy; OH; or $-NR_6R_7$ as defined for R_1;

R_2 is hydrogen; C_{2-8} alkanoyl; or optionally substituted aroyl;

R_3 is C_{3-6} alkyl; and

R_4 is $-(CH_2)_p-X-(CH_2)_q-$ where p is an integer from 1 to 9, q is an integer from 2 to 10, and the moiety $-(CH_2)_p-$ is adjacent to the carbon atom marked with an asterisk in formula (I), and X is $-NR_5-$ where R_5 is selected from hydrogen, C_{1-6} alkyl, C_{2-6} alkanoyl, C_{1-6} alkoxycarbonyl and aroyl, aralkyl or aralkyloxycarbonyl in each of which the aryl moiety is optionally substituted.

2. A compound according to claim 1 in which R_1 is hydroxy, C_{1-4} alkoxy or C_{1-6} alkylamino.

3. A compound according to claim 1 or 2 in which R_2 is hydrogen, acetyl or Ph-C(=O)- in which Ph is an optionally substituted phenyl group.

4. A compound according to any one of claims 1 to 3 in which R_3 is n-butyl, iso-butyl or sec-butyl.

5. A compound according to any one of claims 1 to 4 in which R_4 is a group $-(CH_2)_p-X-(CH_2)_q-$ where p and q have values such that R_4 forms part of an 11- to 16-membered azalactam structure and X is $-NR_5-$ where R_5 is hydrogen, methyl, benzyl, t-butoxycarbonyl or benzyloxycarbonyl.

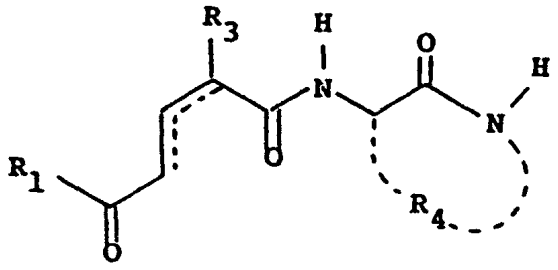
6. A compound according to any one of claims 1 to 5 in which R_1 is hydroxy, methoxy, iso-propyloxy or methylamino, R_2 is hydrogen or acetyl, R_3 is iso-butyl, and R_4 is $-(CH_2)_p-X-(CH_2)_q-$ where p is 4 and q is 5 or p is 4 and q is 6 and X is a group $-NR_5-$ where R_5 is hydrogen.

7. A compound according to any one of claims 1 to 6 in which the centre marked with an asterisk in formula (I) has the (S)-configuration.

8. A compound according to claim 1 which is selected from the group consisting of:
3-acetylmercapto-6-methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotridecan-2-one-3-yl]aminocarbonyl]heptanoic acid, isopropyl ester,
3-acetylmercapto-6-methyl-4-[(1,8-diazacyclotridecan-2-one-3-yl)aminocarbonyl]heptanoic acid, isopropyl ester,
3-mercapto-6-methyl-4-[(1,8-diazacyclotridecan-2-one-3-yl)aminocarbonyl]heptanoic acid, isopropyl ester hydrochloride salt,
3-mercapto-6-methyl-4-[(1,8-diazacyclotetradecan-2-one-3-yl)aminocarbonyl]heptanoic acid, methyl ester hydrochloride salt,
3-mercapto-6-methyl-4-[(1,8-diazacyclotetradecan-2-one-3-yl)aminocarbonyl]heptanoic acid, hydrochloride salt,
3-mercapto-6-methyl-4-[(1,8-diazacyclotetradecan-2-one-3-yl)aminocarbonyl]heptanoic acid methyl amide hydrochloride salt,
3-acetylmercapto-6-methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotetradecan-2-one-3-yl]aminocarbonyl]heptanoic acid, methyl ester,
3-mercapto-6-methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotetradecan-2-one-3-yl]aminocarbonyl]heptanoic acid, methyl ester, or

3-mercapto-6-methyl-4-[[8-(N-benzyloxycarbonyl)-1,8-diazacyclotetradecan-2-one-3-yl]aminocarbonyl]heptanoic acid, methyl amide.

9. A process for the preparation of a compound according to claim 1 which process comprises the reaction of a compound of formula (II):

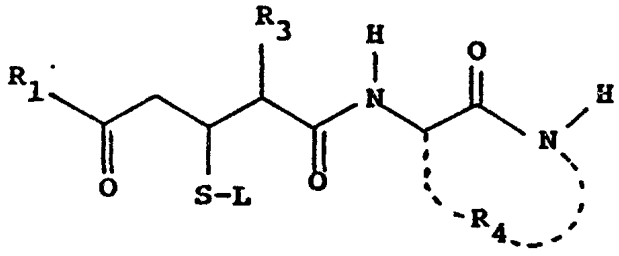


(II)

wherein R_1, R_3 and R_4 are as defined in formula (I), with a thiol of formula (III):

$$L - SH \qquad (III)$$

wherein L is a conventional sulphur protection group, to give a compound of formula (IV):



(IV)

wherein R_1, R_3 and R_4 are as defined in formula (I) and L is as defined in formula (III); and subsequently as necessary

- cleaving the group L and/or R_5 to give a compound of formula (I) in which R_2 and/or R_5 is hydrogen;

- converting the group R_2 in a compound of formula (I) into another group R_2;

- where appropriate converting the group R_5 in a compound of formula (I) into another group R_5.

10. A pharmaceutical composition comprising a compound according to any one of claims 1 to 8 or a pharmaceutically acceptable salt, solvate or hydrate thereof, and a pharmaceutically acceptable carrier.

11. The use of a compound according to any one of claims 1 to 8 or a pharmaceutically acceptable salt, solvate or hydrate thereof, in the manufacture of a medicament for the treatment of conditions in which degradation of connective tissue and other proteinaceous components of the body occurs.

12. A method of treatment of conditions in which degradation of connective tissue and other proteinaceous compounds of the body occurs which comprises administration to a host in need thereof an effective amount of a compound according to any one of claims 1 to 8 or a pharmaceutically acceptable salt, solvate or hydrate thereof.

13. Compounds of formula (I), processes for their preparation, pharmaceutical compositions containing them or methods of treatment involving them, substantially as hereinbefore described with reference to the Examples.

DATED this 3rd day of August, 1993
Beecham Group p.l.c.
By Its Patent Attorneys
DAVIES COLLISON CAVE

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01749

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 D 243/08 C 07 D 245/02 C 07 D 247/00
A 61 K 31/55 A 61 K 31/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 D 243/00 C 07 D 245/00 C 07 D 247/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0273689 (BEECHAM) 6 July 1988, see the whole document (cited in the application) ---	1-16
P,A	EP,A,0438223 (BEECHAM) 24 July 1991, see the whole document -----	1-16

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
06-01-1992	03. 02. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Danielle van der Haas

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claim 16 is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound.

Please see rule 39.1(iv) - PCT

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101749 SA 51885

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 21/01/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0273689	06-07-88	AU-B- 599736	26-07-90
		AU-A- 8292687	01-09-88
		JP-A- 63215660	08-09-88
		US-A- 4937243	26-06-90
EP-A- 0438223	24-07-91	AU-A- 6931791	18-07-91