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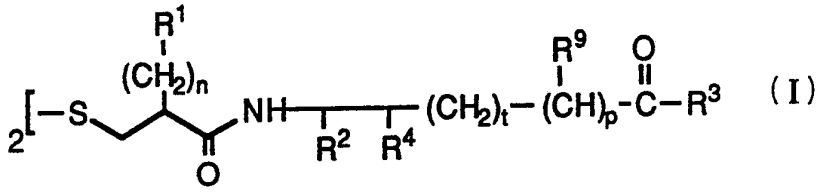
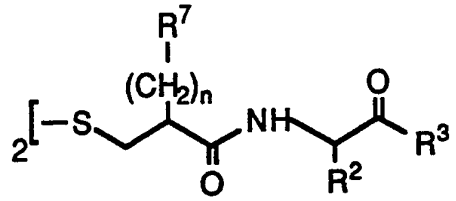


(I)



(II)

(57) Abstract

Novel mercaptoacylamino acid disulfide derivatives of formulae (I) and (II), wherein R^1 is lower alkyl, cyclolower alkyl, aryl or heteroaryl; R^2 is hydrogen; lower alkyl; cyclolower alkyl; lower alkyl substituted with hydroxy, lower alkoxy, mercapto, lower alkylthio, aryl, heteroaryl, aralkyloxy or aralkylthio; aryl; or heteroaryl; R^3 is $-OR^5$ or $-NR^5R^6$; R^4 and R^9 are independently $-(CH_2)_qR^8$; R^5 and R^6 are independently selected from the group consisting of hydrogen, lower alkyl, hydroxy lower alkyl, lower alkoxy lower alkyl and aryl lower alkyl, or R^5 and R^6 together with the nitrogen to which they are attached form a 5-7 membered ring; R^7 is phenyl substituted by 1-3 substituents selected from the group consisting of lower alkyl, lower alkoxy, cycloalkyl, halo, cyano and aminomethyl; R^8 is hydrogen, hydroxy, lower alkoxy, mercapto, lower alkylthio, aryl or heteroaryl; n is 1 or 2; p is 0 or 1; q is 0, 1 or 2; and t is 0 or 1; and the pharmaceutically acceptable salts thereof useful in the treatment of cardiovascular disorders and pain conditions and combinations of mercaptoacylamino acid disulfide derivatives and atrial natriuretic factors or angiotensin converting enzyme inhibitors useful for treating cardiovascular disorders are disclosed.

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DISULFIDE DERIVATIVES OF MERCAPTO-ACYLAMINO ACIDS

BACKGROUND OF THE INVENTION

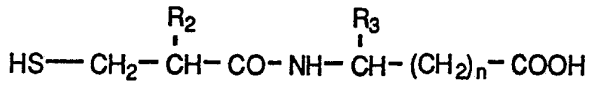
The present invention relates to disulfide derivatives of mercaptoacylamino acids useful in the treatment of cardiovascular disorders and pain conditions.

Cardiovascular disorders which may be treated with compounds of the present invention include hypertension, congestive heart failure, edema and renal insufficiency.

Human hypertension represents a disease of multiple etiologies. Included among these is a sodium and volume dependent low renin form of hypertension. Drugs that act to control one aspect of hypertension will not necessarily be effective in controlling another.

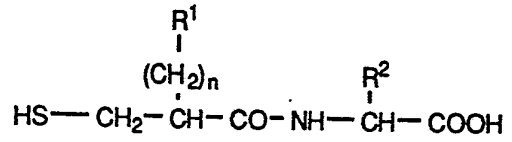
Enkephalin is a natural opiate receptor agonist which is known to produce a profound analgesia when injected into the brain ventricle of rats. It is also known in the art that enkephalin is acted upon by a group of enzymes known generically as enkephalinases, which are also naturally occurring, and is inactivated thereby.

A variety of mercaptoacylamino acids are known as enkephalinase inhibitors useful as analgesics and in the treatment of hypertension. U.S. 4,774,256 discloses compounds of the formula



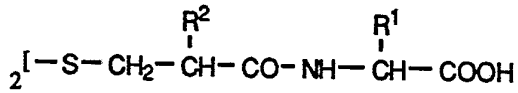
wherein n is 1-15 and R_2 and R_3 are various aryl, arylalkyl and heteroarylalkyl groups. The compounds are disclosed as having enkephalinase inhibiting activity.

U.S. 4,801,609 to Haslanger et al discloses antihypertensive compounds of the formula



wherein n is 0 or 1; R^1 is substituted phenyl and R^2 is substituted alkyl, phenyl or heteroaryl. U.S. 4,513,009 to Roques et al disclose similar compounds wherein R^1 includes alkyl, optionally substituted phenyl and thienyl; and R^2 includes phenyl and substituted alkyl. The compounds are disclosed by Roques et al as principally having enkephalinase inhibitory activity, but are also said to be antihypertensives. U.S. 4,401,677 to Greenberg et al and EPA 38,046 to Wilkinson also disclose compounds of a similar scope, the former disclosing analgesic activity and the latter disclosing a greater specificity for enkephalinase than for angiotensin converting enzyme. U.S. 4,329,495 discloses similar chiral enkephalinase inhibitors wherein R^2 is alkylthioalkyl and either R^1 is substituted phenyl and the -COOH is replaced by $-CH_2OH$ or R^1 is phenyl and the -COOH is present. U.S. 4,500,467 to Kubinyi et al discloses benzoylthio derivatives of compounds similar to those disclosed by Haslanger et al wherein the R^1 side chain contains an NH or sulfur atom.

Disulfide derivatives of mercaptoacylamino acids have been identified as angiotensin convering enzyme inhibitors. U.S. 4,053,651 to Ondetti et al discloses disulfide compounds of the formula



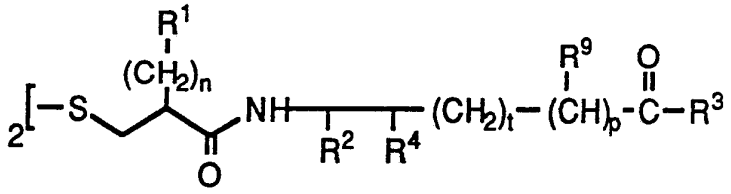
wherein R^2 is either hydrogen, lower alkyl or phenyl lower alkyl and R^1 includes optionally substituted lower alkyl. U.S. 4,228,007, also to Ondetti et al, discloses similar compounds wherein the R^2 side chain contains an oxygen or sulfur atom. U.S. 4,105,776 to Ondetti et al discloses disulfide mercaptoacylproline compounds, and U.S. 4,256,761 to Suh et al discloses disulfide mercapto-acylamino compounds comprising a tertiary amino group.

It is known that the heart secretes a series of peptide hormones called atrial natriuretic factors (ANF) which help to regulate blood pressure, blood volume and the excretion of water, sodium and potassium. ANF were found to produce a short-term reduction in blood pressure and to be useful in the treatment of congestive heart failure. See P. Needleman et al, "Atriopeptin: A Cardiac Hormone Intimately Involved in Fluid, Electrolyte and Blood-Pressure Homeostasis", N. Engl. J. Med., 314, 13 (1986) pp. 828-834, and M. Cantin et al in "The Heart as an Endocrine Gland", Scientific American, 254 (1986) pg. 7681. U.S. 4,740,499 to Olins discloses a method of prolonging the effect of atrial peptides comprising co-administering thiorphan (a compound within the scope of U.S. 4,513,009) or kelatorphan with an atrial peptide.

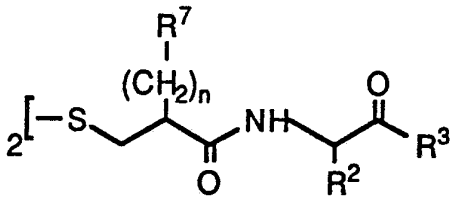
A class of drugs known to be effective in treating some types of hypertension is ACE inhibitors, which compounds are useful in blocking the rise in blood pressure caused by increases in vascular resistance and fluid volume due to the formation of angiotensin II from angiotensin I. For a review of ACE inhibitors, see M. Wyvratt and A. Patchett, "Recent Developments in the Design of Angiotensin Converting Enzyme Inhibitors" in Med. Res. Rev. Vol. 5, No. 4 (1985) pp. 483-531.

SUMMARY OF THE INVENTION

Novel compounds of the present invention are represented by the formulae



I



II

wherein

R^1 is lower alkyl, cyclolower alkyl, aryl or heteroaryl;
R^2 is hydrogen; lower alkyl; cyclolower alkyl; lower alkyl substituted with hydroxy, lower alkoxy, mercapto, lower alkylthio, aryl, heteroaryl, aralkyloxy or aralkylthio; aryl; or heteroaryl;
R^3 is $-OR^5$ or $-NR^5R^6$;
R^4 and R^9 are independently $-(CH_2)_qR^8$;
R^5 and R^6 are independently selected from the group consisting of hydrogen, lower alkyl, hydroxy lower alkyl, lower alkoxy lower alkyl and aryl lower alkyl, or R^5 and R^6 together with the nitrogen to which they are attached form a 5-7 membered ring;
R^7 is phenyl substituted by 1 to 3 substituents selected from the group consisting of lower alkyl, lower alkoxy, cycloalkyl, halo, cyano and aminomethyl;
R^8 is hydrogen, hydroxy, lower alkoxy, mercapto, lower alkylthio, aryl or heteroaryl;
n is 1 or 2;
p is 0 or 1;
q is 0, 1 or 2; and
t is 0 or 1;

and the pharmaceutically acceptable salts thereof.

A preferred group of compounds of formula I of the present invention is that wherein t is zero, with compounds wherein p and t are both zero being more preferred. Another group of preferred compounds of formula I is that wherein R^4 is hydrogen, hydroxy, methoxy, phenyl or benzyl. Still another preferred group of compounds of formula I is that wherein R^2 is hydrogen or thienyl. Preferred amino acid portions of the compounds of formula I (i.e. the portion $-NH-CH(R^2)-CH(R^4)-(CH_2)_t-(CHR^9)_p-COR^3$) are those wherein p and t are each 0, R^2 is hydrogen and R^4 is hydroxy or methoxy (e.g. isoserine or Q-methyl isoserine); those wherein t is 1, p is 0, R^2 is hydrogen and R^4 is hydroxy (e.g. homo-

isoserine); those wherein p and t are each 0, R^2 is thienyl and R^4 is hydrogen (e.g. β-thienyl-β-alanine); and those wherein p and t are each 0, R^2 is hydrogen and R^4 is phenyl or benzyl.

Other preferred compounds of formula I are those wherein R^1 is phenyl or lower alkyl-substituted phenyl, for example tolyl. Yet another preferred group of compounds is that wherein R^3 is hydroxy or lower alkoxy. A preferred value for n is 1.

Especially preferred compounds of formula I are those wherein R^1 is phenyl or tolyl; n is 1; R^2 is hydrogen or thienyl; R^4 is hydrogen, hydroxy, methoxy, phenyl or benzyl; p is 0; and R^3 is hydroxy or lower alkoxy.

A preferred group of compounds of formula II is that wherein R^7 is lower alkyl-substituted phenyl, especially tolyl. Another preferred group of compounds of formula II is that wherein R^2 is substituted lower alkyl, especially lower alkylthio lower alkyl, and in particular methylthioethyl. A third group of preferred compounds of formula II is that wherein R^3 is hydroxy or lower alkoxy. A preferred value or n is 1.

Especially preferred compounds of formula II are those wherein R^7 is tolyl, R^2 is substituted lower alkyl, particularly loweralkylthio lower alkyl, and R^3 is hydroxy or alkoxy.

The invention also relates to the treatment of cardiovascular diseases with a combination of a mercaptoacylamino acid disulfide derivative of the present invention and an atrial natriuretic factor (ANF) and with a combination of a mercapto-acylamino acid disulfide derivative of the present invention and an angiotensin converting enzyme (ACE) inhibitor.

Other aspects of the invention relate to pharmaceutical compositions comprising a mercaptoacylamino acid disulfide derivative of this invention, alone or in combination with an ANF or an ACE inhibitor, and to methods of treatment of cardiovascular diseases comprising administering a mercaptoacylamino acid disulfide derivative of this invention, alone or in combination with an ANF or an ACE inhibitor, to a mammal in need of such treatment.

Still another aspect of the invention relates to a method of treating pain conditions by administering a mercaptoacylamino acid disulfide derivative of this invention, thereby inhibiting the action of enkephalinase in a mammal and eliciting an analgesic effect. Analgesic pharmaceutical compositions comprising said mercaptoacylamino acid disulfide derivatives are also contemplated.

An additional aspect of the invention relates to a method of treating nephrotoxicity resulting from immunosuppression therapy by administration of a mercaptoacylamino acid disulfide derivative of this invention.

DETAILED DESCRIPTION

As used herein, the term "lower alkyl" means straight or branched alkyl chains of 1 to 6 carbon atoms, and "lower alkoxy" similarly refers to alkoxy groups having 1 to 6 carbon atoms. Cyclolower alkyl means cyclic alkyl groups of 3 to 6 carbon atoms.

"Aryl" means mono-cyclic or fused ring bicyclic carbocyclic aromatic groups having 6 to 10 ring members and "heteroaryl" means mono-cyclic or fused ring bicyclic aromatic groups having 5-10 ring members wherein 1-2 ring members are independently nitrogen, oxygen or sulfur, wherein the carbon ring members of the aryl and heteroaryl groups are substituted by zero to three substituents selected from the group consisting of lower alkyl, hydroxy, halo, lower alkoxy, cyclolower alkyl, cyano, aminomethyl, trifluoromethyl, phenyl, phenoxy or phenylthio. Examples of carbocyclic aryl groups are phenyl, α-naphthyl and β-naphthyl, and examples of heterocyclic aryl groups are furyl, thienyl, pyrrolyl, benzofuryl, benzothienyl, indolyl and pyridyl. All positional isomers, e.g. 2-pyridyl, 3-pyridyl, are contemplated.

"Aralkyloxy" and "aralkylthio" refer to aryl lower alkoxy and aryl lower alkylthio groups, respectively.

"Halo" refers to fluorine, chlorine, bromine or iodine radicals.

Certain compounds of the invention are acidic e.g., those compounds which possess a carboxyl group. These compounds form

pharmaceutically acceptable salts with inorganic and organic bases. Examples of such salts are the sodium, potassium, calcium, aluminum, gold and silver salts. Also included are salts formed with pharmaceutically acceptable amines such as ammonia, alkyl amines, hydroxyalkylamines, N-methylglucamine and the like.

The salts may be formed by conventional means, as by reacting the free acid form of the product with one or more equivalents of the appropriate base or acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the cations of an existing salt for another cation on a suitable ion exchange resin.

Compounds of formulae I and II have at least one asymmetrical carbon atom and therefore include various stereoisomers. The invention includes all such isomers both in pure form and in admixture, including racemic mixtures.

An aspect of the present invention described above relates to the combination of a compound of formulae I and II with an ANF. As indicated by Needleman et al., a number of ANF have been isolated so far, all having the same core sequence of 17 amino acids within a cysteine disulfide bridge, but having different N-termini lengths. These peptides represent N-terminal truncated fragments (21-48 amino acids) of a common preprohormone (151 and 152 amino acids for man and rats, respectively). Human, porcine and bovine carboxy-terminal 28-amino acid peptides are identical and differ from similar peptides in rats and mice in that the former contain a methionine group at position 12 while the latter contain isoleucine. Various synthetic analogs of naturally occuring ANF's also have been found to have comparable biological activity. Examples of ANFs contemplated for use in this invention are α human AP 21 (atriopeptin I), α human AP 28, α human AP 23 (atriopeptin II or APII), α human AP 24, α human AP 25, α human AP 26, α human AP 33, and the corresponding rat sequence of each of the above wherein Met 12 is Ile. See Table I for a comparison of the peptides.

TABLE I

HUMAN PEPTIDE		‡ * ‡
AP	33	LAGPRSLRRSSCFGGRMDRIGAQSGLGCNSFRY
AP	28	SLRRSSCFGGRMDRIGAQSGLGCNSFRY
AP	26	RRSSCFGGRMDRIGAQSGLGCNSFRY
AP	25	RSSCFGGRMDRIGAQSGLGCNSFRY
AP	24	SSCFGGRMDRIGAQSGLGCNSFRY
AP	23	SSCFGGRMDRIGAQSGLGCNSFR
AP	21	SSCFGGRMDRIGAQSGLGCNS

where the amino acids are designated by their single-letter abbreviations, namely

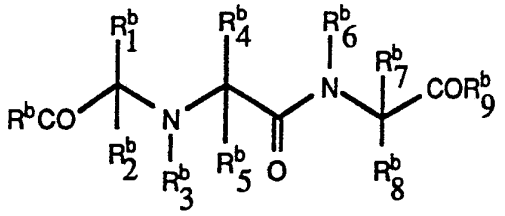
A	Ala	Alanine	M	Met	Methionine
C	Cys	Cysteine	N	Asn	Asparagine
D	Asp	Aspartic acid	P	Pro	Proline
F	Phe	Phenylalanine	Q	Gln	Glutamine
G	Gly	Glycine	R	Arg	Arginine
I	Ile	Isoleucine	S	Ser	Serine
L	Leu	Leucine	Y	Tyr	Tyrosine;

M* is replaced by I (Ile), in the rat peptide; and the two C‡ (Cys) residues are connected by a disulfide bridge.

Another aspect of the invention is the administration of a combination of an ACE inhibitor and a compound of formula I.

Examples of ACE inhibitors are those disclosed in the article by Wyvratt et al., cited above, and in the following U.S. patents: U.S. Patents 4,105,776, 4,468,519, 4,555,506, 4,374,829, 4,462,943, 4,470,973, 4,470,972, 4,350,704, 4,256,761, 4,344,949, 4,508,729, 4,512,924, 4,410,520 and 4,374,847, all incorporated herein by reference; and the following foreign patents or published patent applications:

British Specification No. 2095682 published October 6, 1982 discloses N-substituted-N-carboxyalkyl aminocarbonyl alkyl glycine derivatives which are said to be angiotensin converting enzyme inhibitors and have the formula



either

(A) R^b and $R_9{}^b$ are OH, 1-6C alkoxy, 2-6C alkenyloxy, di-(1-6C alkyl)amino-(1-6C) alkoxy, 1-6C hydroxyalkoxy, acylamino-(1-6C)alkoxy, acyloxy-(1-6C)alkoxy, aryloxy, aryloxy-(1-6C)alkoxy, NH_2, mono- or di-(1-6C alkyl)amino, hydroxyamino or aryl-(1-6C)alkylamino;
$R_1{}^b$-$R_5{}^b$, $R_7{}^b$ and $R_8{}^b$ are 1-20C alkyl, 2-20C alkenyl, 2-20C alkynyl, aryl, aryl-(1-6C) alkyl having 7-12C or heterocyclyl-(1-6C)alkyl having 7-12C;
$R_6{}^b$ is cycloalkyl, polycycloalkyl, partly saturated cycloalkyl or polycycloalkyl, cycloalkyl-(1-6C)alkyl having 3-20C, 6-10C aryl, aryl-(1-6C)alkyl, aryl-(2-6C)alkenyl or aryl-(2-6C) alkynyl; or
$R_2{}^b$ and $R_3{}^b$ together with the C and N atoms to which they are attached or $R_3{}^b$ and $R_5{}^b$ together with the N and C atoms to which they are attached form an N-heterocycle containing 3-5C or 2-4C and a S atom;
all alkyl, alkenyl and alkynyl are optionally substituted by OH, 1-6C alkoxy, thio(sic), 1-6C alkylthio, NH_2, mono- or di(1-6C alkyl)amino, halogen or NO_2;
all 'cycloalkyl' groups (including poly and partially unsaturated) are optionally substituted by halogen, 1-6C hydroxyalkyl, 1-6C alkoxy, amino-(1-6C alkyl)amino, di-(1-6C alkyl)amino, SH, 1-6C alkylthio, NO_2 or CF_3; and

aryl groups are optionally substituted by OH, 1-6C alkoxy, NH_2, mono- or di-(1-6C alkyl) amino, SH, 1-6C alkylthio, 1-6C hydroxyalkyl, 1-6C aminoalkyl, 1-6C thioalkyl, NO_2, halogen, CF_3, OCH_2O, ureido or guanidino; or

(B) R^b and $R_9{}^b$ are H or 1-6C alkoxy;

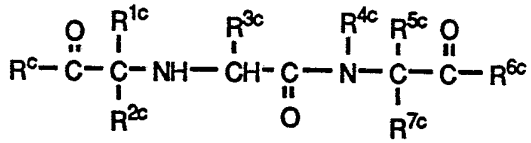
$R_1{}^b$ and $R_2{}^b$ are H, 1-6C alkyl, aryl-(1-6C) alkyl having 7-12C or heterocyclyl-(1-6C) alkyl having 6-12C;

$R_3{}^b$-$R_5{}^b$, $R_7{}^b$ and $R_8{}^b$ are H or 1-6C alkyl;

$R_6{}^b$ is cycloalkyl, polycycloalkyl, partly saturated cycloalkyl or polycycloalkyl, cycloalkyl-(1-6C) alkyl having 3-20C, aryl or aryl-(1-6C) alkyl; and

aryl has 6-10C and is optionally substituted by 1-6C alkyl, 2-6C alkenyl, 2-6C alkynyl, OH, 1-6C alkoxy, NH_2, mono- or di-(1-6C alkyl) amino, SH, 1-6C alkylthio, 1-6C hydroxyalkyl, 1-6C aminoalkyl, 1-6C thioalkyl, NO_2, halogen, CF_3, OCH_2O, ureido or guanidino;

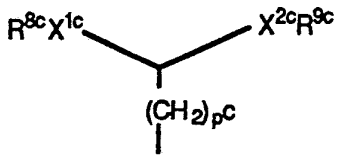
European Patent Application 0 050 800 published May 5, 1982 discloses carboxyalkyl dipeptides derivatives which are said to be angiotensin converting enzyme inhibitors and have the formula



or a pharmaceutically acceptable salt thereof, wherein R^c and R^{6c} are the same or different and are hydroxy, lower alkoxy, lower alkenyloxy, dilower alkylamino lower alkoxy, acylamino lower alkoxy, acyloxy lower alkoxy, aryloxy, aryllower alkoxy, amino, lower alkylamino, dilower alkylamino, hydroxyamino, aryllower alkylamino, or substituted aryloxy or substituted aryllower alkoxy wherein the substituent is methyl, halo or methoxy; R^{1c} is hydrogen, alkyl of from 1 to 10 carbon atoms, substituted lower alkyl wherein the substituent is hydroxy, lower alkoxy, aryloxy, substituted aryloxy, heteroaryloxy, substituted heteroaryloxy, amino, lower alkylamino, diloweralkylamino, acylamino, arylamino, substituted arylamino, guanidino, imidazolyl, indolyl, lower alkylthio, arylthio,

substituted arylthio, carboxy, carbamoyl, lower alkoxy carbonyl, aryl, substituted aryl, aralkyloxy, substituted aralkyloxy, aralkylthio or substituted aralkylthio, wherein the aryl or heteroaryl portion of said substituted aryloxy, heteroaryloxy, arylamino, arylthio, aryl, aralkyloxy, aralkylthio group is substituted with a group selected from halo, lower alkyl, hydroxy, lower alkoxy, amino, aminomethyl, carboxyl, cyano, or sulfamoyl; R^{2c} and R^{7c} are the same or different and are hydrogen or lower alkyl; R^{3c} is hydrogen, lower alkyl, phenyl lower alkyl, aminoethylphenyl lower alkyl, hydroxyphenyl lower alkyl, hydroxy lower alkyl, acylamino lower alkyl, amino lower alkyl, dimethylamino lower alkyl, guanidino lower alkyl, imidazolyl lower alkyl, indolyl lower alkyl, or lower alkyl thio lower alkyl; R^{4c} and R^{5c} are the same or different and are hydrogen, lower alkyl or Z^c, or R^{4c} and R^{5c} taken together form a group represented by Q^c, U^c, V^c, Y^c, D^c or E^c, wherein;

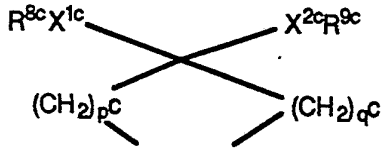
Z^c is



wherein X^{1c} and X^{2c} independent of each other are O, S or CH_2, R^{8c} and R9c independent of each other are lower alkyl, lower alkenyl, lower alkynyl, cycloalkyl having 3 to 8 carbon atoms, hydroxy lower alkyl, or $-(CH_2)_{n^c}Ar^c$, wherein n^c is 0, 1, 2 or 3 and Ar^c is unsubstituted or substituted phenyl, furyl, thienyl or pyridyl, wherein said substituted phenyl, furyl, thienyl or pyridyl groups are substituted with at least one group that is independently selected from C_1 to C_4 alkyl, lower alkoxy, lower alkylthio, halo, CF_3 and hydroxy, or R^{8c} and R^{9c} taken together form a bridge W^c, wherein W^c is a single bond or a methylene bridge or a substituted methylene bridge when at least one of X^{1c} and X^{2c} is methylene, or W^c is an alkylene or substituted alkylene bridge having 2 or 3 carbon atoms, said substituted methylene bridge or said substituted alkylene bridge having one or two substituents selected from lower alkyl, aryl and aryl lower alkyl groups, and p^c is 0, 1 or 2; with the proviso that at least one of R^{4c} and R^{5c} is Z^c, with the proviso that if R^{4c} is Z^c and p^c is 0 then X^{1c} and X^{2c} must both be methylene, and with the proviso that

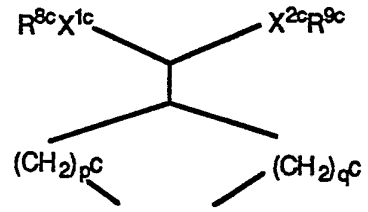
if X^{1c} and X^{2c} are both methylene then R^{8c} and R^{9c} must form an alkylene bridge W^c;

Q^c is



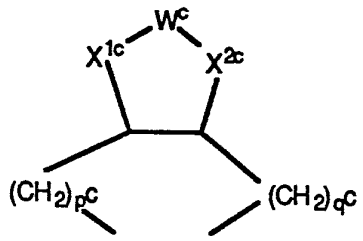
wherein R^{8c}, R^{9c}, X^{1c} and X^{2c} are as defined above, p^c is 0, 1 or 2, q^c is 0, 1 or 2, with the proviso that the sum of p^c and q^c must be 1, 2 or 3, with the proviso that if p^c is 0 then X^{1c} and X^{2c} must be methylene, and with the proviso that if X^{1c} and X^{2c} are methylene then R^{8c} and R^{9c} taken together form a bridge W^c, wherein W^c is as defined above;

V^c is



wherein R^{8c}, R^{9c}, X^{1c} and X^{2c} are as defined above, p^c is 0, 1 or 2 and q^c is 0, 1 or 2, with the proviso that the sum of p^c and q^c is 1, 2 or 3, with the proviso that if X^{1c} and X^{2c} are CH_2 then R^{8c} and R^{9c} taken together form a bridge W^c, wherein W^c is as defined above;

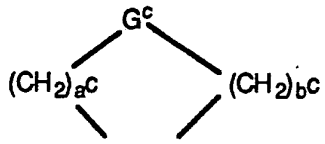
U^c is



wherein W^c is as defined above (except that W^c may also be a methylene bridge when X^{1c} and X^{2c} are oxygen or sulfur), X^{1c} and X^{2c} are as defined above, p^c is 0, 1 or 2, q^c is 0, 1 or 2, with the proviso that

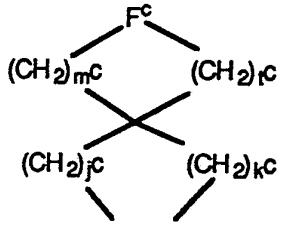
the sum of p^c and q^c is 1 or 2, and with the proviso that if p^c is 0, X^{1c} must be CH_2;

Y^c is



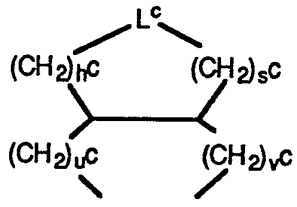
wherein G^c is oxygen, sulfur or CH_2, a^c is 2, 3 or 4 and b^c is 1, 2, 3, 4 or 5, with the proviso that the sum of a^c and b^c is 5, 6 or 7 or G^c is CH_2, a^c is 0, 1, 2 or 3, b^c is 0, 1, 2 or 3 with the proviso that the sum of a^c and b^c is 1, 2 or 3, with the proviso that the sum of a^c and b^c may be 1, 2 or 3 only if R^{1c} is lower alkyl substituted with aralkylthio or aralkyloxy;

D^c is



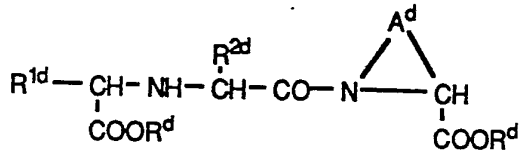
wherein F^c is O or S, j^c is 0, 1 or 2 and k^c is 0, 1 or 2, with the proviso that the sum of j^c and k^c must be 1, 2 or 3, and m^c is 1, 2 or 3 and t^c is 1, 2 or 3, with the proviso that the sum of m^c and t^c must be 2, 3 or 4;

E^c is

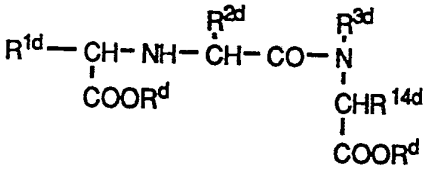


wherein L^c is O or S, u^c is 0, 1 or 2 and v^c is 0, 1 or 2, with the proviso that the sum of u^c and v^c must be 1 or 2, and h^c is 1 or 2 and s^c is 1 or 2, with the proviso that the sum of h^c and s^c must be 2 or 3;

European Patent Application 0 079 522 published May 25, 1983 discloses N-carboxymethyl(amidino) lysyl-proline compounds which are said to be angiotensin converting enzyme inhibitors and have the formula where



$(I)^d$

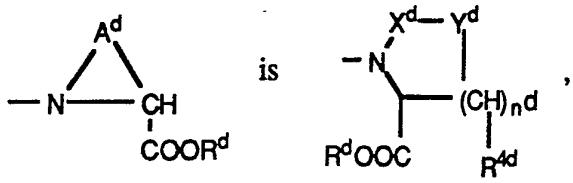


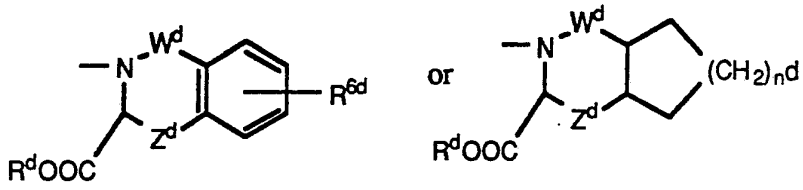
$(Ia)^d$

wherein:

R^d and R^{2d} are independently hydrogen; loweralkyl; aralkyl; or aryl;

R^{1d} is hydrogen; branched or straight chain C_{1-12} alkyl and alkenyl; C_3-C_9 cycloalkyl and benzofused alkyl; substituted loweralkyl where the substituents are halo, hydroxy loweralkoxy, aryloxy, amino, mono- or diloweralkylamino, acylamino, arylamino, guanidino, mercapto, loweralkylthio, arylthio, carboxy, carboxamido, or loweralkoxycarbonyl; aryl; substituted aryl where the substituents are loweralkyl, loweralkoxy, or halo; arloweralkyl; arloweralkenyl; heteroarloweralkyl; heteroarloweralkenyl; substituted arloweralkyl, substituted arloweralkenyl, substituted heteroarloweralkyl, or substituted heteroarloweralkenyl where the aryl and heteroaryl substituents are halo, dihalo, loweralkyl, hydroxy, loweralkoxy, amino, aminoloweralkyl, acylamino, mono- or diloweralkylamino, carboxyl, haloloweralkyl, nitro, cyano, or sulfonamido, and where the loweralkyl portion of arloweralkyl may be substituted by amino, acylamino, or hydroxyl;





where:where:

X^d and Y^d taken together are $-CH_2-CH_2-$; $-CH(R^{5d})-S-$; $-C(O)-CH_2-$; $-CH_2-C(O)-$; $-C(O)-O-$; $-C(O)-S-$; $-CH_2-CH(OR^{4d})-$; $-C(O)-N(R^{4d})-$; or $-CH_2-C(R^{4d})-R^{5d}$;

R^{4d} is hydrogen; loweralkyl; aryl; substituted aryl;

R^{5d} is hydrogen; loweralkyl; aryl or substituted aryl;

n^d is 1 to 3;

W^d is absent; $-CH_2-$; or $-C(O)-$;

Z^d is $-(CH_2)_m{}^d$, where m^d is 0 to 2, provided that m^d may not be 0 and W^d may not be absent at the same time; and

R^{6d} is hydrogen; loweralkyl; halo; or OR^{4d};

R^{2d} is $----(CH_2)_r{}^d----B^d----(CH_2)_s{}^d----NR^{7d}R^{15d}$

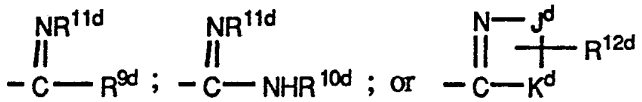
where

r^d and s^d are independently 0 to 3;

B^d is absent; -O-; -S-; or $-NR^{8d}$;

where R^{8d} is hydrogen; loweralkyl; alkanoyl; or aroyl; and

R^{7d} is



where

R^{9d} is loweralkyl; aralkyl; aryl; heteroaryl; or heteroarloweralkyl and these groups substituted by hydroxy, lower alkoxy or halo; carboxyl; carboxamido; nitromethenyl.

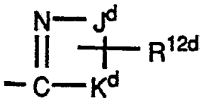
R^{10d} is hydrogen; loweralkyl; aryl; or amidino;

R^{11d} is hydrogen; loweralkyl; cyano; amidino; aryl; aroyl; loweralkanoyl; $-C(O)-NHR^{13d}$;$-C(O)-OR^{13d}$; $-NO_2$; $-SO_2NH_2$; or SO_2R^{13d};

R^{12d} is hydrogen; loweralkyl; halo; aralkyl; amino; cyano; mono- or diloweralkylamino; or OR^{4d};

R^{13d} is hydrogen; loweralkyl; or aryl;

R^{15d} is hydrogen; lower alkyl; aralkyl; or aryl;

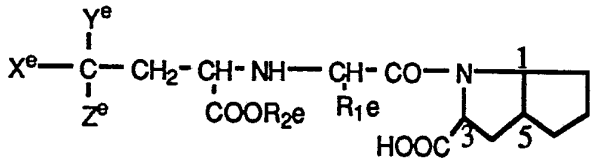


constitute a basic heterocycle of 5 or 6 atoms or benzofused analogs thereof and optionally containing 1-3 N atoms, an oxygen, a sulfur, an S=O, or an SO_2 group optionally substituted by amino, lower alkyl amino, diloweralkyl amino, lower alkoxy, or aralkyl groups;

R^{3d} is C_{3-8} cycloalkyl and benzofused C_{3-8} cycloalkyl; perhydrobenzofused C_{3-8} cycloalkyl; aryl; substituted aryl; heteraryl; substituted heteroaryl;

R^{14d} is hydrogen or loweralkyl; and, a pharmaceutically acceptable salt thereof;

European Patent 79022 published May 18, 1983 discloses N-amino acyl-azabicyclooctane carboxylic acid derivatives which have the formula

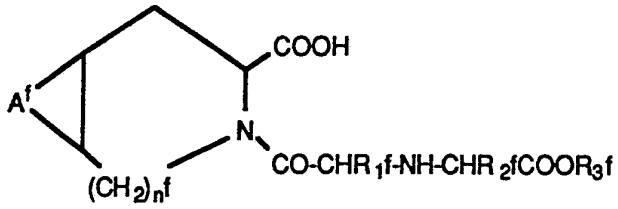


hydrogen atoms at ring positions 1 and 5 are cis to each other and the 3-carboxy group has the endo orientation;

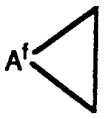
R_1^e is H, allyl, vinyl or the side chain of an optionally protected naturally occurring α-amino acid;

$R_2^e$is H, 1-6C alkyl, 2-6C alkenyl or aryl(1-C alkyl);
Y^e is H or OH and Z^e is H, or Y^e and Z^e together oxygen;
X^e is 1-6C alkyl, 2-6C alkenyl, 5-9C cycloalkyl, 6-12C aryl (optionally substituted by one to three 1-4C alkyl or alkoxy, OH, halo, nitro, amino (optionally substituted by one or two 1-4C alkyl), or methylenedioxy) or indol-3-yl);

European Patent 46953 published March 10, 1982 discloses N-amino acyl-indoline and tetrahydro isoquinoline carboxylic acids which are angiotensin coverting enzyme inhibitors and have the formula



n^f is 0 or 1;

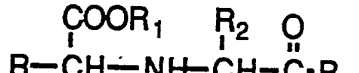


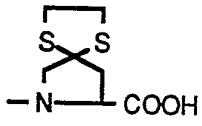
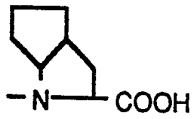
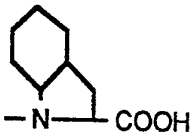
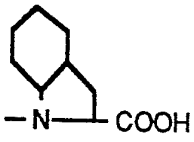
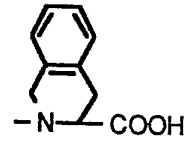
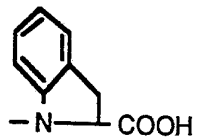
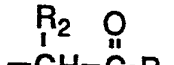
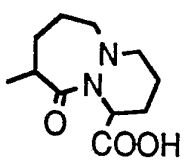
is a benzene or cyclohexane ring:

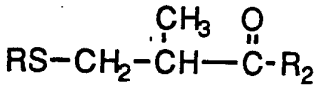
R_1^f and R_2^f are each 1-6C alkyl, 2-6C alkenyl, 5-7C cycloalkyl, 5-7C cycloalkenyl, 7-12C cycloalkylalkyl, optionally partially hydrogenated 6-10C aryl, 7-14C aralkyl or 5-7 membered monocyclic or 8-10 membered bicyclic heterocyclyl containing 1 or 2 S or O and/or 1-4N atoms; all R_1^f and R_2^f groups are optionally substituted,
R_3^f is H, 1-6C alkyl, 2-6C alkenyl or 7-14C aralkyl.

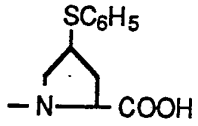
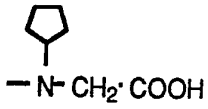
The following Table II lists ACE inhibitors preferred for use in the combination of this invention.

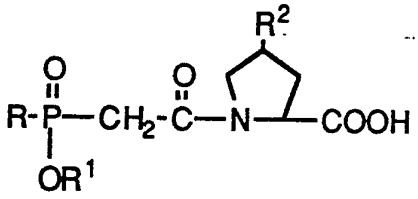
TABLE II
PREFERRED ACE INHIBITORS

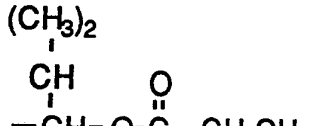


	R	R_1	R_2	R_3
spirapril	$C_6H_5CH_2CH_2$-	Et	CH_3	
enalapril	$C_6H_5CH_2CH_2$-	Et	CH_3	prolyl
ramipril	$C_6H_5CH_2CH_2$-	Et	CH_3	
perindopril	$CH_3CH_2CH_2$-	Et	CH_3	
indolapril	$C_6H_5CH_2CH_2$-	Et	CH_3	
lysinopril	$C_6H_5CH_2CH_2$-	H	$NH_2(CH_2)_4$-	prolyl
quinapril	$C_6H_5CH_2CH_2$-	Et	CH_3	
pentopril (NH = CH_2)	CH_3	Et	CH_3	
cilazapril	$C_6H_5CH_2CH_2$-	H	 is	

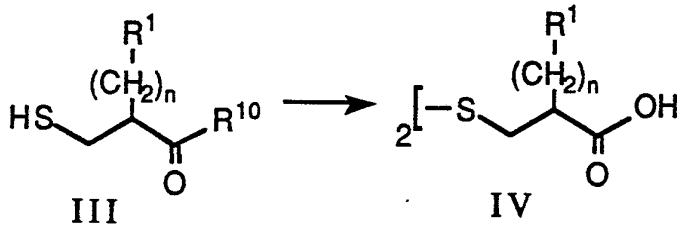


	R	R_2
captopril	H	prolyl
zofenopril	C_6H_5CO-	
pivalopril	$(CH_3)_3C$-C(=O)-	

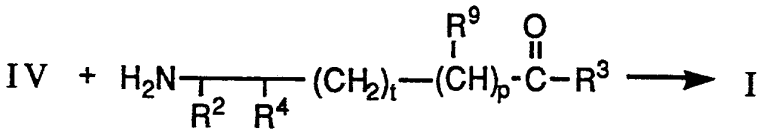


	R	R^1	R^2
fosinopril	C_6H_5-$(CH_2)_4$-		C_6H_5-

Compounds of the present invention can be made by methods well known to those skilled in the art. For example, a mercaptan of formula III can be oxidized to the disulfide and converted to an acid of formula IV, then amidated with an amino acid, amino ester or amino amide of formula V to obtain a compound of formula I:

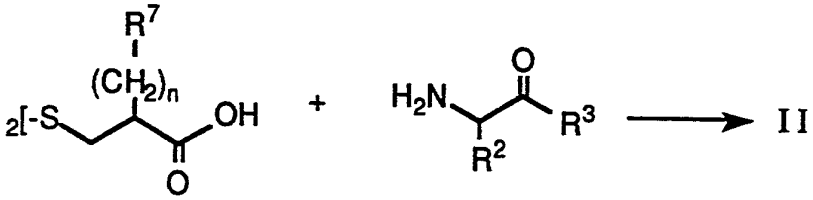


III IV



V

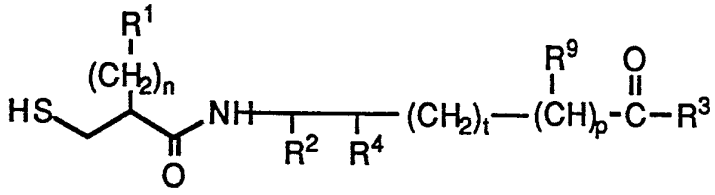
wherein n, p, t, R^1, R^2, R^3, R^4 and R^9 are as defined above and R^{10} is OH or a group convertible to OH, e.g. ethoxy or benzyloxy. An analogous procedure using an acid of formula VI and an amine of formula VII can be used to prepare compounds of formula II:



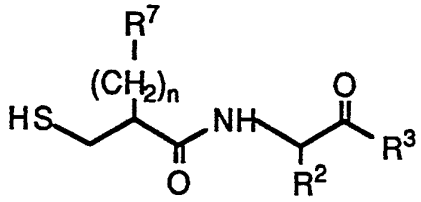
VI VII

wherein n, R^2, R^3, and R^7 are as defined above.

A second method for preparing compounds of the present invention above comprises oxidizing a mercapto-acylamino acid of formula Ia or IIa to obtain the disulfide:



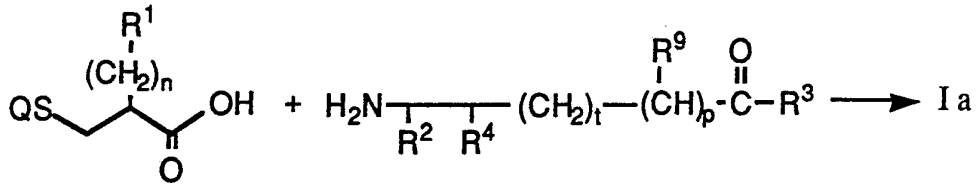
Ia



IIa

The reaction can be carried out in an organic solvent such as ethanol using an oxidizing agent such as iodine.

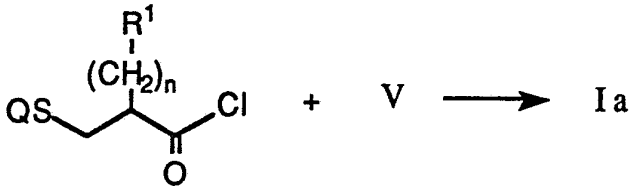
Starting materials of formulae Ia and IIa can be made by methods well known to those skilled in the art. A typical general procedure for preparing compounds of formula Ia is to combine a propionic acid, IIIa (i.e., a compound of formula III wherein R^{10} is OH), with an amino acid, amino ester or amino amide, V, under typical peptide coupling conditions, using, for example, a coupling agent such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC):



IIIa V

wherein Q is a sulfur-protecting group such as acetyl or benzoyl and R^3 is as defined above. To obtain compounds of formula Ia, the sulfur protecting group can be removed by conventional means, e.g. removal of an acetyl or benzoyl group can be accomplished by treating with sodium hydroxide, then acidifying with HCl.

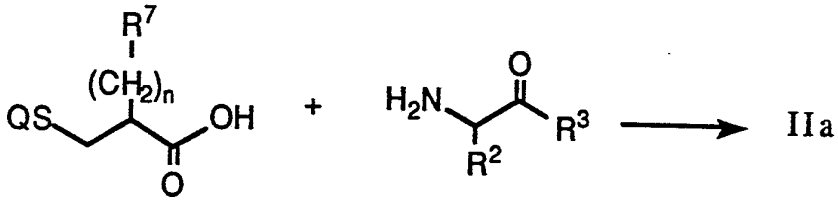
Alternatively, the propionic acid (IIIa) can be converted by known methods (e.g. treatment with thionyl chloride) to the corresponding acid chloride (IIIb), and the acid chloride can be reacted with the amino acid, amino ester or amino amide in the presence of a base such as triethylamine to obtain, after deprotection, a compound of formula Ia:



IIIb

For compounds of formula V wherein R^4 is hydroxy, it may be desirable to protect such a group during the reaction, e.g. with a t-butoxycarbonyl or benzyloxycarbonyl group.

Similar reactions may be used to prepare compounds of formula IIa, using a propionic acid of formula VIa (or the corresponding acid chloride) wherein Q is as defined above, and an amino acid, amino ester or amide of formula VII:



VIa VII

It is apparent that using methods well known to those skilled in the art, compounds of formula Ia and IIa can be converted to different compounds of formula Ia and IIa, respectively, by appropriate reaction of the R^3 variable, e.g. an acid can be converted to an amide or an ester to an amide.

Compounds of formula III, IIIa, and V to VII are known in the art or can be prepared by methods well known in the art.

We have found that the novel compounds of the present invention are effective in treating cardiovascular disorders such as congestive heart failure, edema, renal insufficiency and various types of hypertension, particularly volume expanded hypertension. These novel compounds enhance both the magnitude and duration of the antihypertensive and natriuretic effects of endogenous ANF. Administration of a combination of a mercaptoacylamino acid disulfide derivative and an ACE inhibitor provides an antihypertensive and anti-congestive heart failure effect greater than either the mercapto-acylamino acid disulfide derivative or ACE inhibitor alone. Administration of a combination of a mercaptoacylamino acid disulfide derivative of formula I or II and an exogenous ANF or ACE inhibitor is therefore particularly useful in treating hypertension or congestive heart failure.

In addition to the compound aspect, the present invention therefore also relates to treating cardiovascular disorders with a mercaptoacylamino acid disulfide derivative of formula I or II or with a mercaptoacylamino acid disulfide derivative of formula I or II in combination with an ANF or an ACE inhibitor, which methods comprise administering to a mammal in need of such treatment an amount of the mercaptoacylamino acid disulfide derivative effective to treat hypertension or congestive heart failure or an amount of a combination of a mercaptoacylamino acid disulfide derivative and ANF or ACE inhibitor effective to treat hypertension or congestive heart failure. The drug or combination of drugs is preferably administered in a pharmaceutically acceptable carrier, e.g. for oral or parenteral administration. The combinations of drugs may be co-administered in a single composition, or components of the combination therapy may be administered separately. Where the components are administered separately, any convenient combination of dosage forms may be used, e.g. oral mercaptoacylamino acid disulfide derivative/oral ANF, oral mercaptoacylamino acid disulfide derivative/parenteral ACE inhibitor, parenteral mercaptoacylamino acid disulfide derivative/oral ANF, parenteral mercaptoacylamino acid disulfide derivative/parenteral ACE inhibitor.

When the components of a combination of a mercaptoacylamino acid disulfide derivative and an ANF are administered separately, it is preferred that the mercaptoacylamino acid disulfide derivative be administered first.

The present invention also relates to a pharmaceutical composition comprising a mercaptoacylamino acid disulfide derivative for use in treating hypertension or congestive heart failure, to a pharmaceutical composition comprising both a mercaptoacylamino acid disulfide derivative and an ANF and to a pharmaceutical composition comprising both a mercaptoacylamino acid disulfide derivative and an ACE inhibitor.

The antihypertensive effect of mercaptoacylamino acid disulfide derivatives was determined according to the following procedure:

Male Sprague Dawley rats weighing 100-150 g were anesthetized with ether and the right kidney was removed. Three pellets containing DOC acetate (desoxycorticosterone acetate, DOCA, 25 mg/pellet) were implanted subcutaneously. Animals recovered from surgery, were maintained on normal rat chow and were allowed free access to a fluid of 1% NaCl and 0.2% KCl instead of tap water for a period of 17-30 days. This procedure results in a sustained elevation in blood pressure and is a slight modification of published procedures (e.g. Brock et al., 1982) that have been used to produce DOCA salt hypertension in the rat.

On the day of study, animals were again anesthetized with ether and the caudal artery was cannulated for blood pressure measurement. Patency of the caudal artery cannula was maintained with a continuous infusion of dextrose in water at a rate of 0.2 ml/hr. Animals were placed into restraining cages where they recovered consciousness. Blood pressure was measured from caudal artery catheter using a Statham pressure transducer attached to a Beckman oscillographic recorder. In addition, a cardiovascular monitoring device (Buxco Electronics, Inc.) and a digital computer were used to calculate average blood pressures.

After an equilibration period of at least 1.5 hr., animals were dosed subcutaneously (1 ml/kg) with vehicle (methylcellulose, hereinafter MC) or mercaptoacylamino acid disulfide derivative and blood pressure was monitored for the next 4 hours.

A similar procedure can be used to determine the effect of mercaptoacylamino acid disulfide derivatives in combination with ACE inhibitors.

The antihypertensive effect of mercaptoacylamino acid disulfide derivatives in combination with ANF can be determined according to the following procedures:

Male spontaneously hypertensive rats (SHR), 16-18 weeks old, 270-350 g, are anesthetized with ether and the abdominal aorta is cannulated through the tail artery. The animals are then placed into restrainers to recover from anesthesia (in less than 10 min.) and remain inside throughout the experiments. Through a pressure transducer (Gould P23 series) analog blood pressure signals are registered on a Beckman 612 recorder. A Buxco digital computer is used to obtain mean arterial pressures. Patency of the arterial cannula is maintained with a continuous infusion of 5% dextrose at 0.2 ml/hr. Animals are allowed a 90-min equilibration period. The animals first undergo a challenge with an ANF such as atriopeptin II (AP II) or AP28 30 µg/kg iv and at the end of 60 min. are treated with drug vehicle or a mercapto-acylamino acid subcutaneously. A second ANF challenge is administered 15 min. later and blood pressure is monitored for the next 90 min.

The antihypertensive effect in SHR of mercaptoacylamino acid disulfide derivatives and ACE inhibitors, alone and in combination, can be determined as follows:

Animals are prepared for blood pressure measurement as described above. After stabilization, animals are dosed subcutaneously or orally with test drugs or placebo and blood pressure is monitored for the next 4 hr.

The compounds having structural formulae I and II have also been found to inhibit the activity of enzymes designated enkephalinases. The compounds are particularly useful for the inhibition of enkephalinase A, which is derived from the striata of both rats and humans. In in vitro tests, using test procedures for enkephalinase A inhibition well known to those skilled in the art, selected compounds having structural formula I and II have been found to inhibit the activity of the aforementioned enzyme. Therefore, the present invention also relates to a method of inhibiting the action of enkephalinases in a mammal thereby to elicit an analgesic effect with a compound of formula I or II, and to analgesic pharmaceutical compositions comprising compounds of formula I or II.

The use of atrial natriuretic peptides in the treatment of nephrotoxicity associated with the immunosuppressive cyclosporin was reported by Capasso et al in the American Journal of Hypertension, 3, 3 (1990), p. 204-210. Since compounds of this invention enhance endogenous ANF, they can be used alone to treat nephrotoxicity, or they can be administered in combination with exogenous ANF.

The compositions of this invention comprise a mercapto-acylamino acid disulfide derivative or a mercaptoacylamino acid disulfide derivative and an ANF or a mercaptoacylamino acid disulfide derivative and an ACE inhibitor in combination with a pharmaceutically acceptable carrier for administration to mammals. A variety of pharmaceutical forms is suitable, preferably for oral or parenteral administration, although mechanical delivery systems such as transdermal dosage forms are also contemplated.

The daily dose of the compound or combinations of this invention for treatment of hypertension or congestive heart failure is as follows: for mercaptoacylamino acid disulfide derivatives alone the typical dosage is 0.1 to 10 mg/kg of mammalian weight per day administered in single or divided dosages; for the combination of mercaptoacylamino acid disulfide derivative and an ANF, the typical dosage is 0.1 to 10 mg of mercaptoacylamino acid disulfide derivative/ kg mammalian weight per day in single or divided dosages plus 0.001 to 0.1 mg ANF/kg of mammalian weight per day, in single or divided dosages, and for the combination of mercaptoacylamino acid disulfide derivative and an ACE inhibitor, the typical dosage is 0.1 to 10 mg of mercaptoacylamino acid disulfide derivative/kg mammalian weight per day in single or divided dosages plus 0.1 to 30 mg ACE inhibitor/kg of mammalian weight per day in single or divided dosages. The exact dose of any component or combination to be administered is determined by the attending clinician and is dependent on the potency of the compound administered, the age, weight, condition and response of the patient.

Generally, in treating humans having hypertension or congestive heart failure, the compounds or combinations of this

invention may be administered to patients in a dosage range as follows: for treatment with mercaptoacylamino acid disulfide derivatives alone, about 5 to about 500 mg per dose given 1 to 4 times a day, giving a total daily dose of about 5 to 2000 mg per day; for the combination of mercaptoacylamino acid disulfide derivative and ANF, about 5 to about 500 mg mercaptoacylamino acid disulfide derivative per dose given 1 to 4 times a day and about 0.001 to about 1 mg ANF given 1 to 6 times a day (total daily dosage range of 5 to 2000 mg day and .001 to 6 mg/day, respectively); and for the combination of a mercaptoacylamino acid disulfide derivative and an ACE inhibitor, about 5 to about 500 mg mercaptoacylamino acid disulfide derivative per dose given 1 to 4 times a day and about 5 to about 50 mg ACE inhibitor given 1 to 3 times a day (total daily dosage range of 5to 2000 mg/day and 5 to 150 mg/day, respectively). Where the components of a combination are administered separately, the number of doses of each component given per day may not necessarily be the same, e.g. where one component may have a greater duration of activity, and will therefore need to be administered less frequently.

To produce an analgesic effect, compounds of this invention will be administered in a dosage range of from about 1 to about 100 mg/kg. The doses are to be administered at intervals of from 3 to 8 hours. However, the quantity and frequency of dosage will depend upon such factors as the severity of the pain, the general physical condition of the patient, the age and weight of the patient, and other factors recognized by the skilled clinician.

For treatment of edema, renal insufficiency or nephrotoxicity associated with immunosuppressive therapy, dosage ranges of the compounds of this invention are the same as for treatment of hypertension with the use of mercapto-acylamino acid disulfide derivatives of this invention alone or in combination with ANF.

Typical oral formulations include tablets, capsules, syrups, elixirs and suspensions. Typical injectable formulations include solutions and suspensions.

The typical acceptable pharmaceutical carriers for use in the formulations described above are exemplified by: sugars such as lactose, sucrose, mannitol and sorbitol, starches such as cornstarch, tapioca starch and potato starch; cellulose and derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and methyl cellulose; calcium phosphates such as dicalcium phosphate and tricalcium phosphate; sodium sulfate; calcium sulfate; polyvinylpyrrolidone, polyvinyl alcohol; stearic acid; alkaline earth metal stearates such as magnesium stearate and calcium stearate, stearic acid, vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil and corn oil; non-ionic, cationic and anionic surfactants; ethylene gylcol polymers; beta-cyclodextrin; fatty alcohols and hydrolyzed cereal solids; as well as other nontoxic compatible fillers, binders, disintegrants, buffers, preservatives, antioxidants, lubricants, flavoring agents, and the like commonly used in pharmaceutical formulations.

Since the present invention relates to treatment of hypertension or congestive heart failure with a combination of active ingredients wherein said active ingredients may be administered separately, the invention also relates to combining separate pharmaceutical compositions in kit form. That is, two kits are contemplated, each combining two separate units: a mercapto-acylamino acid disulfide derivative pharmaceutical composition and an ANF pharmaceutical composition in one kit and a mercapto-acylamino acid disulfide derivative pharmaceutical composition and an ACE inhibitor pharmaceutical composition in a second kit. The kit form is particularly advantageous when the separate components must be administered in different dosage forms (e.g. oral and parenteral) or are administered at different dosage intervals.

Following are examples of procedures for preparing compounds of formulae I and 2.

PREPARATION 1

N-[3-MERCAPTO-2(S)-(2-METHYLBENZYL)PROPIONYL]-(S)-METHIONINE ETHYL ESTER

To N-[3-acetylthio-2(S)-(2-methylbenzyl)propionyl]-(S)-methionine ethyl ester (1.00 g), add 6.8% ammonia in absolute ethanol (20 ml) and stir for 3 hr. Concentrate the resulting solution and dry the resulting residue in vacuo to obtain a white solid, m.p. 73-76°C, $[\alpha]_D^{26}$ = -7.6° (MeOH).

PREPARATION 2

N-[3-MERCAPTOMETHYL-2(S)-(2-METHYLBENZYL) PROPIONYL]-(S)-ISOSERINE ETHYL ESTER

Under a nitrogen atmosphere, add 7.8% w/w NH_3 in EtOH to N-[2(S)-acetylthiomethyl-3-(2-methylphenyl)propionyl]-(S)-isoserine ethyl ester (2.62 g) and stir for 15 hr. Concentrate the reaction mixture in vacuo to obtain a colorless oil. Treat the oil with degassed water and concentrate in vacuo to obtain the title compound as a colorless oil, $[\alpha]_D^{26}$=+60.7°C (MeOH).

EXAMPLE 1

1,1'-[DITHIOBIS-[2(S)-(2-METHYLBENZYL)-1-OXO-3,1-PROPANEDIYL]]BIS-(S)-METHIONINE DIETHYL ESTER

To the product of Preparation 1 (0.89 g) in absolute EtOH (30 ml), add 1% iodine solution in absolute EtOH (~32 ml) dropwise (until light brown solution is obtained). Evaporate the ethanol and partition the residue between diethyl ether/1% sodium thiosulfate solution. Partition the organic solution with brine. Dry ($MgSO_4$) and

concentrate to obtain a white solid, m.p. 74-79°C, $[\alpha]_D^{26}$ = -176.4° (MeOH).

EXAMPLE 2

1,1'-[DITHIOBIS-[2(S)-(2-METHYLBENZYL)-1-OXO-3,1-PROPANEDIYL]]BIS-(S)-METHIONINE

Add 1N NaOH (1.3 ml) to the product of Example 1 (0.46 g) and stir the resulting mixture for 3 hr. Add 0.1N HCl and extract with EtOAc. Concentrate the dried ($MgSO_4$) EtOAc and chromatograph the residue on preparative thin layer plates (4 x 1000μ) using CH_2Cl_2:NH_4OH:MeOH 70:27:3 as eluant to obtain a light yellow solid, m.p. 53-58°C, $[\alpha]_D^{26}$ = -177.8° (MeOH).

EXAMPLE 3

1,1'-[DITHIOBIS-[2(S)-(2-METHYLBENZYL)-1-OXO-3,1-PROPANEDIYL]]BIS-(S)- ISOSERINE DIETHYL ESTER

Dissolve the product of Preparation 2 (1.72 g) in a 1% iodine solution in EtOH (70ml) and stir the reaction mixture at room temperature for 18 hr. Concentrate the mixture in vacuo and add EtOAc (800 ml). Partition the EtOAc solution with 5% sodium bicarbonate solution,1% sodium thiosulfate solution and then brine. Concentrate the dried ($MgSO_4$) EtOAc solution in vacuo to give a white solid. Place the white solid on a column of flash silica gel (200 ml) and elute with EtOAc:hexane 1:1 (2000ml), 3:2 (2000 ml), 3:1 (2000 ml), EtOAc (1000 ml) and EtOAc:MeOH 4:1 (2000 ml) to give a white solid. Place this white solid on a column of flash silica gel (120 g) and elute with CH_2Cl_2:MeOH 99:1 (2000 ml), 49:1 (2000 ml) and 19:1 (2000 ml) to obtain the title compound as a white solid, mp 137-8°C, $[\alpha]^{26}_D$=-1.5° (MeOH).

EXAMPLE 4

1,1'-[DITHIOBIS-[2(S)-(2-METHYLBENZYL)-1-OXO-3,1-PROPANEDIYL]]BIS-(S)-ISOSERINE

Add 1N NaOH (1.1 ml) to the product of Example 3 (0.324 g) in MeOH (15 ml) and stir for 2 hr. Concentrate the reaction mixture under a stream of nitrogen and add 1N HCl (2 ml) to give a white precipitate. Filter and wash the solid with water and dry in vacuo to obtain the title compound as a white solid, m.p. 121-3° C, $[\alpha]_D^{26}$=-30.3° (MeOH).

Using the test procedures described above, compounds of examples 1 to 4 were found to produce a drop in blood pressure (ΔBP) in the DOCA salt model and in the ANF potentiation procedure:

	ANF Potentiation			DOCA Salt		
Compound	ΔBP (mmHg)	mg/kg	route	ΔBP (mmHg)	mg/kg	route
Example 1	0	30	sc	15	3	po
Example 2	27	30	sc	57	10	po
				41	1	po
Example 3	29	30	sc	44	10	po
				30	1	po
				31	0.1	po
Example 4	24	30	sc	31	10	po
				21	1	po
				0	0.1	po

(sc = subcutaneous; po = oral)

The following formulations exemplify some of the dosage forms of the compositions of this invention. In each, the term "active ingredient" designates a compound of formula I or II, preferably 1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]bis-(S)-methionine. However, this compound can be replaced by equally effective amounts of other compounds of formula I or II.

Example A

Tablets

No.	Ingredient	mg/tablet	mg/tablet
1	Active Compound	100	500
2	Lactose USP	122	113
3	Corn Starch, Food Grade, as a 10% Paste in Purified Water	30	40
4	Corn Starch, Food Grade	45	40
5	Magnesium Stearate	3	7
	Total	300	700

Method of Manufacture

Mix Items Nos. 1 and 2 in suitable mixer for 10-15 minutes. Granulate the mixture with Item No. 3. Mill the damp granules through a coarse screen (e.g., 1/4", 0.63 cm) if necessary. Dry the damp granules. Screen the dried granules if necessary and mix with Item No. 4 and mix for 10-15 minutes. Add Item No. 5 and mix for 1-3 minutes. Compress the mixture to appropriate size and weigh on a suitable tablet machine.

Example B

Capsules

No.	Ingredient	mg/capsule	mg/capsule
1	Active Compound	100	500
2	Lactose USP	106	123
3	Corn Starch, Food Grade	40	70
4	Magnesium Stearate NF	4	7
	Total	250	700

Method of Manufacture

Mix Item Nos. 1, 2 and 3 in a suitable blender for 10-15 minutes. Add Item No. 4 and mix for 1-3 minutes. Fill the mixture into

suitable two-piece hard gelatin capsules on a suitable encapsulating machine.

Example C

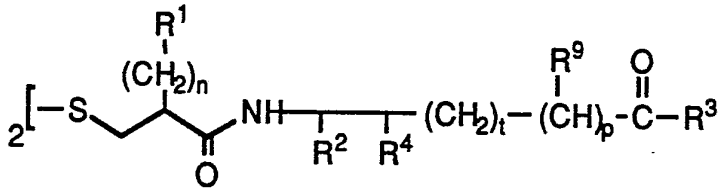
Parenteral Preparation

Ingredient	mg/vial	mg/vial
Active Compound Sterile Powder	100	500

For reconstitution add sterile water for injection or bacteriostatic water for injection.

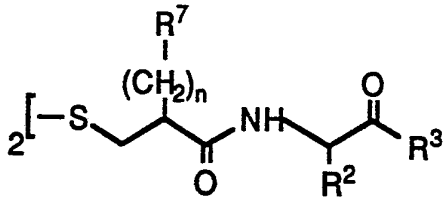
We Claim:

1. A compound having the structural formula



I

or



II

wherein

R^1 is lower alkyl, cyclolower alkyl, aryl or heteroaryl;

R^2 is hydrogen; lower alkyl; cyclolower alkyl; lower alkyl substituted with hydroxy, lower alkoxy, mercapto, lower alkylthio, aryl, heteroaryl, aralkyloxy or aralkylthio; aryl; or heteroaryl;

R^3 is $-OR^5$ or $-NR^5R^6$;

R^4 and R^9 are independently $-(CH_2)_qR^8$;

R^5 and R^6 are independently selected from the group consisting of hydrogen, lower alkyl, hydroxy lower alkyl, lower alkoxy lower alkyl and aryl lower alkyl, or R^5 and R^6 together with the nitrogen to which they are attached form a 5-7 membered ring;

R^7 is phenyl substituted by 1 to 3 substituents selected from the group consisting of lower alkyl, lower alkoxy, cycloalkyl, halo, cyano and aminomethyl;

R^8 is hydrogen, hydroxy, lower alkoxy, mercapto, lower alkylthio, aryl or heteroaryl;

n is 1 or 2;

p is 0 or 1;

q is 0, 1 or 2; and

t is 0 or 1;

or a pharmaceutically acceptable salt thereof.

2. A compound of formula I of claim 1 wherein R^1 is phenyl or lower alkyl substituted phenyl and n is 1.

3. A compound of formula I of claim 1 wherein R^3 is hydroxy or lower alkoxy.

4. A compound of formula I of claim 1 wherein p is zero.

5. A compound of formula I of claim 1 wherein R^4 is hydrogen, hydroxy, methoxy, phenyl or benzyl.

6. A compound of formula I of claim 1 wherein R^2 is hydrogen or thienyl.

7. A compound of formula I of claim 1 wherein p and t are each zero, R^2 is hydrogen and R^4 is hydroxy or methoxy.

8. A compound of formula II of claim 1 wherein R^7 is lower alkyl-substituted phenyl and n is 1.

9. A compound of formula II of claim 1 wherein R^2 is loweralkylthio lower alkyl.

10. A compound of claim 1 which is:

1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-isoserine diethyl ester;

1,1'-[dithiobis-[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-bis-(S)-isoserine;

1,1'-[dithiobis[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]bis-(S)-methionine diethyl ester;

1,1'-[dithiobis[2(S)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]bis-(S)-methionine.

11. A pharmaceutical composition for treating hypertension, congestive heart failure, edema, renal insufficiency, nephrotoxicity or pain comprising an effective amount of a compound of claim 1, alone or in combination with an atrial nartiuretic factor or an angiotensin converting enzyme inhibitor, in a pharmaceutically acceptable carrier.

12. A composition of claim 11 wherein the atrial natriuretic peptide is chosen from α human AP 21, α human AP 28, α human AP 23, α human AP 24, α human AP 25, α human AP 26, α human AP 33, and the corresponding atrial peptides wherein the methionine at position 12 is replaced by isoleucine.

13. A composition of claim 11 wherein the angiotensin converting enzyme inhibitor is selected from: spirapril, enalapril, ramipril, perindopril, indolapril, lysinopril, quinapril, pentopril, cilazapril, captopril, zofenopril, pivalopril and fosinopril.

14. A kit comprising in separate containers in a single package pharmaceutical compositions for use in combination to treat hypertension, congestive heart failure or nephrotoxicity in mammals which comprises in one container a pharmaceutical composition comprising a mercapto-acylamino acid disulfide derivative of claim 1 and in a second container a pharmaceutical composition comprising an atrial natriuretic factor.

15. A kit comprising in separate containers in a single package pharmaceutical compositions for use in combination to treat hypertension or congestive heart failure in mammals which comprises in one container a pharmaceutical composition comprising a mercapto-acylamino acid disulfide derivative of claim 1 and in a second container

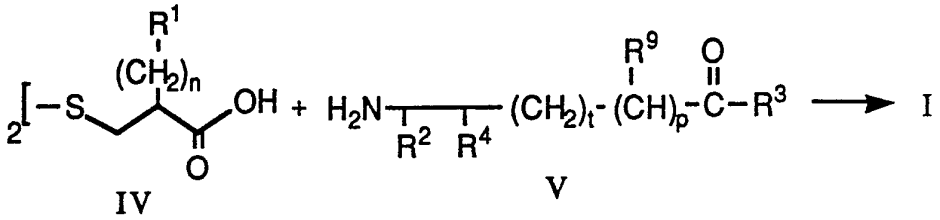
a pharmaceutical composition comprising an angiotensin converting enzyme inhibitor.

16. A method for treating hypertension, congestive heart failure, edema, renal insufficiency nephrotoxicity or pain in mammals comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 1, alone or in combination with an atrial natriuretic factor or an angiotensin converting enzyme inhibitor.

17. A method for preparing a pharmaceutical composition comprising admixing a compound of claim 1 with a pharmaceutically acceptable carrier.

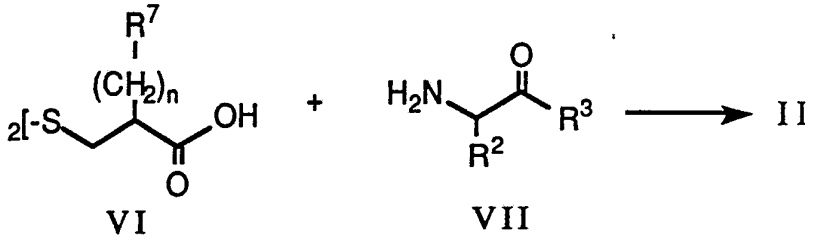
18. The use of a compound of claim 1 for the manufacture of a medicament for treating hypertension, congestive heart failure, edema, renal insufficiency, nephrotoxicity or pain.

19. A process for the preparation of a compound of formula I or II as defined in claim 1, wherein R^1, R^2, R^3, R^4, R^7, R^9, n, p and t are as defined in claim 1, selected from the following processes A, B, C, and D:
Process A for preparing compounds of formula I comprising amidating an acid of formula IV with an amine of formula V:



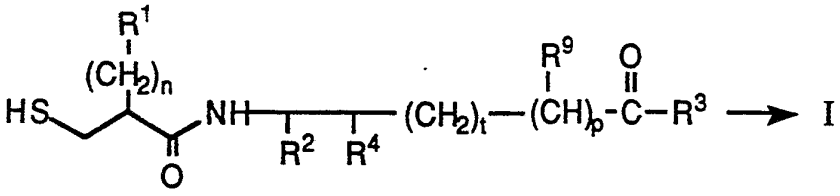
IV V I

Process B for preparing compounds of formula II comprising amidating an acid of formula VI with an amine of formula VII:



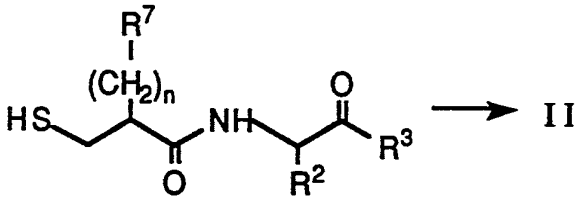
VI VII II

Process C for preparing compounds of formula I comprising oxidizing a mercaptoacylamino acid of formula Ia:



Ia

Process D for preparing compounds of formula II comprising oxidizing a mercaptoacylamino acid of formula IIa:

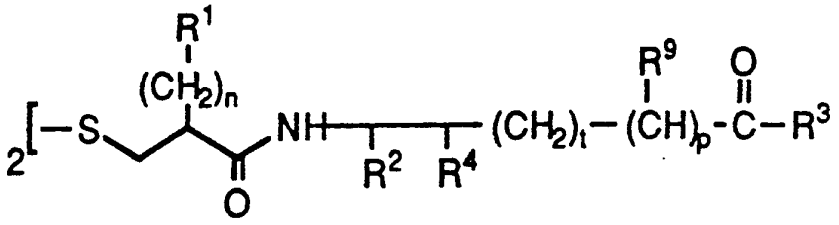


IIa

wherein each process is followed by isolation of the preferred isomer, if desired, and removal of the protecting groups, if necessary, to yield the desired product, and if desired, preparation of a salt thereof.

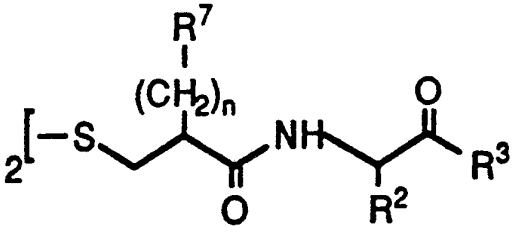
AMENDED CLAIMS
[received by the International Bureau
on 5 November 1991(05.11.91);
original claim 1 amended; other claims unchanged(1 page)]

1. A compound having the structural formula



I

or



II

wherein

R^1 is lower alkyl, cyclolower alkyl, aryl or heteroaryl;

R^2 is hydrogen; lower alkyl; cyclolower alkyl; lower alkyl substituted with hydroxy, lower alkoxy, mercapto, lower alkylthio, aryl, heteroaryl, aralkyloxy or aralkylthio; aryl; or heteroaryl;

R^3 is $-OR^5$ or $-NR^5R^6$;

R^4 and R^9 are independently $-(CH_2)_qR^8$;

R^5 and R^6 are independently selected from the group consisting of hydrogen, lower alkyl, hydroxy lower alkyl, lower alkoxy lower alkyl and aryl lower alkyl, or R^5 and R^6 together with the nitrogen to which they are attached form a 5-7 membered ring;

R^7 is phenyl substituted by 1 to 3 substituents selected from the group consisting of lower alkyl, lower alkoxy, cycloalkyl, cyano and aminomethyl;

R^8 is hydrogen, hydroxy, lower alkoxy, mercapto, lower alkylthio, aryl or heteroaryl;

n is 1 or 2;

p is 0 or 1;

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/03251

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 C 323/60 A 61 K 31/16

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 C 323/00

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]
X	Chemical Abstracts, vol. 97, 19 July 1982, (Columbus, Ohio, US), M.C. Fournie-Zaluski et al.: "Study of crucial components in enkephalinase inhibitors and synthesis of photoaffinity labels and tritiated derivatives", see page 339, abstract 19653q, & Pept.: Synth., Struct., Funct., Proc. Am. Pept. Symp., 7th 1981, 425-8, & 11th Collective Index, p. 30649CS, "Glycine, N,N'-[dithiobis[2-[(3,5-dibromophenyl)-methyl]-1-oxo-3,1-propanediyl]]bis"	1
A	US,A,4173704 (ONDETTI et al.) 6 November 1979, see the whole document	1-15,17-19
A	FR,A,2556721 (ROUSSEL-UCLAF) 21 June 1985, see examples; claims	1-15,17-19

-/-

° 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
29-08-1991	26. 09. 91
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: Danielle van der Haas

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	CH,A, 637374 (E.R. SQUIBB & SONS) 29 July 1977, see the whole document ---	1-15,17 -19
A	US,A,4329495 (BINDRA) 11 May 1982, see examples; claims ---	1-15,17 -19
A	EP,A,0136883 (E.R. SQUIBB & SONS) 10 April 1985, see examples; claims ---	1-15,17 -19
A	Chemical Abstracts, vol. 111, 27 November 1989, (Columbus, Ohio, US), F. Gimenez et al.: "Methods for determining thiorphan in aqueous solution. Application to the stability assay of pharmaceutical preparations", see page 453, abstract 201730x, & Ann. Pharm. Fr. 1989, 46(6), 347-54, & Chemical Substance Index, p. 4697CS, "Glycine N,N'-[dithiobis[1-oxo-2-(phenylmethyl)-3,1-propane diyl]]-bis-" ---	1-15,17 -19
A,P	EP,A,0339441 (SCHERING) 24 October 1990, see examples; claims -----	1-15,17 -19

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 16 because they relate to subject matter not required to be searched by this Authority, namely:

Please see rule 39.1(iv) - PCT:
Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

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. US 9103251

SA 47882

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 24/09/91
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
US-A- 4173704	06-11-79	US-A-	4053651	11-10-77
		AU-B-	513622	11-12-80
		AU-A-	2410677	12-10-78
		BE-A-	854458	10-11-77
		CA-A-	1119177	02-03-82
		CH-A-	621763	27-02-81
		CH-A-	620202	14-11-80
		DE-A,C	2717548	01-12-77
		FR-A,B	2372624	30-06-78
		FR-A,B	2367741	12-05-78
		GB-A-	1577415	22-10-80
		JP-C-	1354089	24-12-86
		JP-A-	52136117	14-11-77
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		US-A-	4112119	05-09-78
		US-A-	4339600	13-07-82
		US-A-	4140786	20-02-79
		US-A-	4140797	20-02-79
		US-A-	4199512	22-04-80
FR-A- 2556721	21-06-85	None		
CH-A- 637374	29-07-83	None		
US-A- 4329495	11-05-82	AT-T-	E8881	15-08-84
		AU-B-	529511	09-06-83
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		AU-A-	3377884	18-04-85
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP-A- 60136554	20-07-85
		US-A- 4722810	02-02-88
EP-A- 0339441	02-11-89	DE-A- 3814374	09-11-89
		JP-A- 2043607	14-02-90
		US-A- 4961650	09-10-90