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Docket Number	4-32219A

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UTILITY PATENT APPLICATION TRANSMITTAL AND FEE SHEET

Transmitted herewith for filing under 37 CFR §1.53(b) is the utility patent application of

Applicant (or identifier): KSANDER ET AL.

Title: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Enclosed are:

1. ☒ Specification (Including Claims and Abstract) - 26 pages
2. ☐ Drawings - sheets
3. ☐ Executed Declaration and Power of Attorney (original or copy)
4. ☐ Microfiche Computer Program (appendix)
5. Nucleotide and/or Amino Acid Sequence Submission
   - ☐ Computer Readable Copy
   - ☐ Paper Copy
   - ☐ Statement Verifying Identity of Above Copies
6. ☐ Preliminary Amendment
7. ☐ Assignment Papers (Cover Sheet & Document(s))
8. ☐ English Translation of
9. ☐ Information Disclosure Statement
10. ☐ Certified Copy of Priority Document(s)
11. ☒ Return Receipt Postcard
12. ☒ Other: unsigned Declaration and Application Data Sheet

Filing fee calculation:

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Basic Filing Fee								$ 740
Multiple Dependent Claim Fee ($ 280)								$
Foreign Language Surcharge ($ 900)								$
	For	Number Filed		Number Extra		Rate		
Extra Claims	Total Claims	5	-20	0	x	$ 18	=	$
	Independent Claims	3	-3	0	x	$ 84	=	$
						TOTAL FILING FEE		$ 740

☒ Please charge Deposit Account No. 19-0134 in the name of Novartis Corporation in the amount of $740. An additional copy of this paper is enclosed. The Commissioner is hereby authorized to charge any additional fees under 37 CFR §1.16 and §1.17 which may be required in connection with this application, or credit any overpayment, to Deposit Account No. 19-0134 in the name of Novartis Corporation.

Please address all correspondence to the address associated with Customer No. 001095, which is currently:

Thomas Hoxie
Novartis Pharmaceuticals Corporation
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Please direct all telephone calls to the undersigned at the number given below, and all telefaxes to (862) 778-8064.

Respectfully submitted,

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Correspondence Customer Number:: 001095

APPLICATION INFORMATION

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> Application Two:: 60/349,660
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METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Background of the Invention

The renin angiotensin system is a complex hormonal system comprised of a large molecular weight precursor, angiotensinogen, two processing enzymes, renin and angiotensin converting enzyme (ACE), and the vasoactive mediator angiotensin II (Ang II). See *J. Cardiovasc. Pharmacol.*, Vol. 15, Suppl. B, pp. S1-S5 (1990). The enzyme renin catalyzes the cleavage of angiotensinogen into the decapeptide angiotensin I, which has minimal biological activity on its own and is converted into the active octapeptide Ang II by ACE. Ang II has multiple biological actions on the cardiovascular system, including vasoconstriction, activation of the sympathetic nervous system, stimulation of aldosterone production, anti-natriuresis, stimulation of vascular growth and stimulation of cardiac growth. Ang II functions as a pressor hormone and is involved the pathophysiology of several forms of hypertension.

The vasoconstrictive effects of angiotensin II are produced by its action on the non-striated smooth muscle cells, the stimulation of the formation of the adrenergenic hormones epinephrine and norepinephrine, as well as the increase of the activity of the sympathetic nervous system as a result of the formation of norepinephrine. Ang II also has an influence on electrolyte balance, produces, e.g., anti-natriuretic and anti-diuretic effects in the kidney and thereby promotes the release of, on the one hand, the vasopressin peptide from the pituitary gland and, on the other hand, of aldosterone from the adrenal glomerulosa. All these influences play an important part in the regulation of blood pressure, in increasing both circulating volume and peripheral resistance. Ang II is also involved in cell growth and migration and in extracellular matrix formation.

Ang II interacts with specific receptors on the surface of the target cell. It has been possible to identify receptor subtypes that are termed, e.g., AT 1- and AT 2-receptors. In recent times great efforts have been made to identify substances that bind to the AT 1-receptor. Such active ingredients are often termed Ang II antagonists. Because of the inhibition of the AT 1-receptor such antagonists can be used, e.g., as anti-hypertensives or for the treatment of congestive heart failure, among other indications. Ang II antagonists are therefore understood to be those active ingredients which bind to the AT 1-receptor subtype.

Inhibitors of the renin angiotensin system are well-known drugs that lower blood pressure and exert beneficial actions in hypertension and in congestive heart failure as described. See, e.g, *N. Eng. J. Med.*, Vol. 316, No. 23, pp. 1429-1435 (1987). A large number of peptide and non-peptide inhibitors of the renin angiotensin system are known, the most widely studied being the ACE inhibitors, which includes the drugs captopril, enalapril, lisinopril, benazepril and spirapril. Although a major mode of action of ACE inhibitors involves prevention of formation of the vasoconstrictor peptide Ang II, it has been reported in *Hypertension*, Vol. 16, No. 4, pp. 363-370 (1990), that ACE cleaves a variety of peptide substrates, including the vasoactive peptides bradykinin and substance P. Prevention of the degradation of bradykinin by ACE inhibitors has been demonstrated, and the activity of the ACE inhibitors in some conditions has been reported in *Circ. Res.*, Vol. 66, No. 1, pp. 242-248 (1990), to be mediated by elevation of bradykinin levels rather than inhibition of Ang II formation. Consequently, it cannot be presumed that the effect of an ACE inhibitor is due solely to prevention of angiotensin formation and subsequent inhibition of the renin angiotensin system.

Neutral endopeptidase (EC 3.4.24.11; enkephalinase; atriopeptidase; NEP) is a zinc-containing metalloprotease that cleaves a variety of peptide substrates on the amino terminal side of aromatic amino acids. See *Biochem. J.*, Vol. 241, pp. 237-247 (1987). Substrates for this enzyme include, but are not limited to, atrial natriuretic factors (ANFs), also known as ANPs, brain natriuretic peptide (BNP), met and leu enkephalin, bradykinin, neurokinin A and substance P.

ANPs are a family of vasodilator, diuretic and anti-hypertensive peptides which have been the subject of many recent reports in the literature. See, e.g., *Annu. Rev. Pharm. Tox.*, Vol. 29, pp. 23-54 (1989). One form, ANF 99-126, is a circulating peptide hormone which is released from the heart during conditions of cardiac distension. The function of ANF is to maintain salt and water homeostasis, as well as to regulate blood pressure. ANF is rapidly inactivated in the circulation by at least two processes: a receptor-mediated clearance reported in *Am. J. Physiol.*, Vol. 256, pp. R469-R475 (1989), and an enzymatic inactivation via NEP reported in *Biochem. J.*, Vol. 243, pp. 183-187 (1987). It has been previously demonstrated that inhibitors of NEP potentiate the hypotensive, diuretic, natriuretic and plasma ANF responses to pharmacological injection of ANF in experimental animals. The potentiation of ANF by two specific NEP inhibitors is reported by Sybertz et al., *J. Pharmacol. Exp. Ther.*, Vol. 250, No. 2, pp. 624-631 (1989), and in *Hypertension*, Vol. 15, No. 2, pp. 152-161 (1990), while the potentiation of ANF by NEP in general was disclosed in U.S. Patent No. 4,749,688. In U.S. Patent No. 4,740, 499, Olins disclosed the use of thiorphan and kelatorphan to potentiate atrial peptides. Moreover, NEP inhibitors lower blood pressure and exert ANF-like effects, such as diuresis and increased cyclic guanosine 3',5'-monophosphate (cGMP) excretion in some forms of experimental hypertension. The anti-hypertensive action of NEP inhibitors is mediated through ANF because antibodies to ANF will neutralize the reduction in blood pressure.

Darrow et al. in European Patent Application No. 498361 disclose treating hypertension or congestive heart failure with a combination of certain Ang II antagonists or certain renin inhibitors with certain NEP inhibitors.

Powell et al. in European Patent Application No. 726072 disclose treating hypertension or congestive heart failure with a combination of the Ang II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(*1H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]nonan-4-one with a NEP inhibitor or a dual acting vasopeptidase inhibitor (single molecular entity with both ACE and NEP inhibitory activities). Prolonged and uncontrolled hypertensive vascular disease ultimately leads to a variety of pathological changes in target organs, such as the heart and kidney. Sustained hypertension can lead as well to an increased occurrence of stroke. Therefore, there is a strong need to evaluate the efficacy of anti-hypertensive therapy, an examination of additional cardiovascular endpoints, beyond those of blood pressure lowering, to get further insight into the benefits of combined treatment.

The nature of hypertensive vascular diseases is multifactorial. Under certain circumstances, drugs with different mechanisms of action have been combined. However, just considering any combination of drugs having different mode of action does not necessarily lead to combinations with advantageous effects. Accordingly, there is a need for more efficacious combination therapy which has less deleterious side effects.

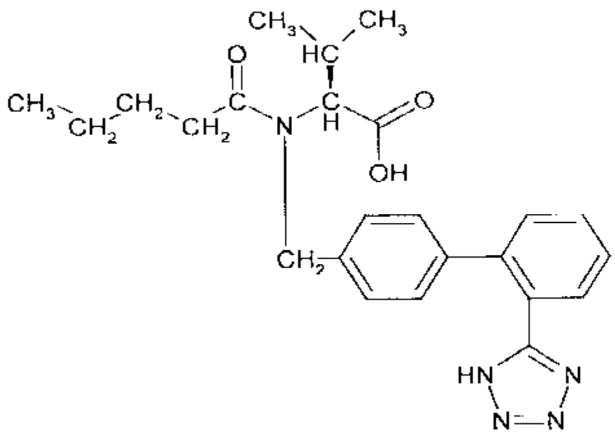
Other objects, features, advantages and aspects of the present invention will become apparent to those of skill from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

Detailed Description of the Preferred Embodiments

In one aspect, the present invention relates to pharmaceutical combinations comprising valsartan or pharmaceutically acceptable salts thereof and a NEP inhibitor or a pharmaceutically effective salts thereof, optionally in the presence of a pharmaceutically acceptable carrier and pharmaceutical compositions comprising them.

In another embodiment, the present invention relates to methods of treating cardiac and renal related conditions by administration of the pharmaceutical composition comprising valsartan plus a NEP inhibitor.

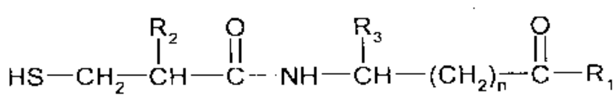
Valsartan is the AT 1-receptor antagonist (*S*)-*N*-(1-carboxy-2-methyl-prop-1-yl)-*N*-pentanoyl-*N*-[2;(*1H*-tetrazol-5-yl)biphenyl-4-yl-methyl]amine of formula (I)



(I)

and is disclosed in EP 0443983 A and U.S. Patent No. 5,399,578, the disclosures of which are incorporated herein in their entirety as if set forth herein.

A NEP inhibitor useful in said combination is a compound of the formula (II)



(II)

and pharmaceutically acceptable salts thereof,

wherein

R_2 is alkyl of 1 to 7 carbons, trifluoromethyl, phenyl, substituted phenyl, $-(CH_2)_{1\text{ to }4}$-phenyl, or $-(CH_2)_{1\text{ to }4}$-substituted phenyl;

R_3 is hydrogen, alkyl of 1 to 7 carbons, phenyl, substituted phenyl, $-(CH_2)_{1\text{ to }4}$-phenyl, or $-(CH_2)_{1\text{ to }4}$-substituted phenyl;

R_1 is hydroxy, alkoxy of 1 to 7 carbons, or NH_2;

n is an integer from 1 to 15; and

the term substituted phenyl refers to a substituent selected from lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4 carbons, lower alkylthio of 1 to 4 carbons, hydroxy, Cl, Br or F.

Preferred selective NEP inhibitors of formula (II) include compounds, wherein

R_2 is benzyl;

R_3 is hydrogen;

n is an integer from 1 to 9; and

R_1 is hydroxy.

Even more preferred selective NEP inhibitors of formula (II) are reported in the literature as SQ 28,603 which is the compound of formula (II), wherein

R_2 is benzyl;

R_3 is hydrogen;

n is one; and

R_1 is hydroxy.

The preparation of the selective NEP inhibitors of formula (II), wherein R_2 is other than trifluoromethyl are disclosed by Delaney et al. in U.S. Patent No. 4,722,810. The preparation of the selective NEP inhibitors of formula (II), wherein R_2 is trifluoromethyl are disclosed by Delaney et al. in U.S. Patent No. 5,223,516.

NEP inhibitors within the scope of the present invention include compounds disclosed in U.S. Patent No. 4,610,816, herein incorporated by reference, including in particular *N*-[*N*-[1(*S*)-carboxyl-3-phenylproplyl]-(*S*)-phenylalanyl]-(*S*)-isoserine and *N*-[*N*-[((*1S*)-carboxy-2-phenyl)ethyl]-(*S*)-phenylalanyl]-β-alanine; compounds disclosed in U.S. Patent No. 4,929,641, in particular, *N*-[2(*S*)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine; SQ 28603 (*N*-[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]-β-alanine), disclosed in South African Patent Application No. 84/0670; UK 69578 (*cis*-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid) and its active enantiomer(s); thiorphan and its enantiomers; retro-thiorphan; phosphoramidon; and

SQ 29072 (7-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]-heptanoic acid). Also suitable for use are any pro-drug forms of the above-listed NEP inhibitors, e.g., compounds in which one or more carboxylic acid groups are esterified.

NEP inhibitors within the scope of the present invention also include the compounds disclosed in U.S. Patent No. 5,217,996, particularly, *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester; the compounds disclosed in EP 00342850, particularly, (*S*)-*cis*-4-[1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid; the compounds disclosed in GB 02218983, particularly, 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo-carbamoyl]cyclopentyl)-2-(2-methoxyethyl)propanoic acid; the compounds disclosed in WO 92/14706, particularly, *N*-(1-(3-(*N*-*t*-butoxycarbonyl-(*S*)-prolylamino)-2(*S*)-*t*-butoxy-carbonylpropyl)cyclopentanecarbonyl)-*O*-benzyl-(*S*)-serine methyl ester; the compounds disclosed in EP 00343911; the compounds disclosed in JP 06234754; the compounds disclosed in EP 00361365, particularly, 4-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid; the compounds disclosed in WO 90/09374, particularly, 3-[1-(*cis*-4-carboxycarbonyl-*cis*-3-butylcyclohexyl-*r*-1-carboamoyl)cyclopentyl]-*2S*-(2-methoxyethoxymethyl)propanoic acid; the compounds disclosed in JP 07157459, particularly, *N*-((*2S*)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine; the compounds disclosed in WO 94/15908, particularly, *N*-(1-(*N*-hydroxycarbamoylmethyl)-1-cyclopentanecarbonyl)-*L*-phenylalanine; the compounds disclosed in U.S. Patent No. 5,273,990, particularly, (*S*)-(2-biphenyl-4-yl)-1-(*1H*-tetrazol-5-yl)ethylamino) methylphosphonic acid; the compounds disclosed in U.S. Patent No. 5,294,632, particularly, (*S*)-5-(*N*-(2-(phosphonomethylamino)-3-(4-biphenyl)propionyl)-2-aminoethyl)tetrazole; the compounds disclosed in U.S. Patent No. 5,250,522, particularly, β-Alanine, 3-[1,1'-biphenyl]-4-yl-*N*-[diphenoxyphosphinyl)methyl]-*L*-alanyl; the compounds disclosed in EP 00636621, particularly, *N*-(2-carboxy-4-thienyl)-3-mercapto-2-benzylpropanamide; the compounds disclosed in WO 93/09101, particularly, 2-(2-mercaptomethyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid; the compounds disclosed in EP 00590442, particularly, ((*L*)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl)-*L*-phenylalanyl)-β-alanine, *N*-[*N*-[(*L*)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine, *N*-[*N*-[(*L*)-1-carboxy-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine, *N*-[2-acetylthiomethyl-3-(2-methyl-phenyl)propionyl]-methionine ethyl ester, *N*-[2-mercaptomethyl-3-(2-methylphenyl)propioyl]-methionine, *N*-[2(*S*)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(*S*)-isoserine, *N*-(*S*)-[3-mercapto-2-(2-methylphenyl)propionyl]-(*S*)-

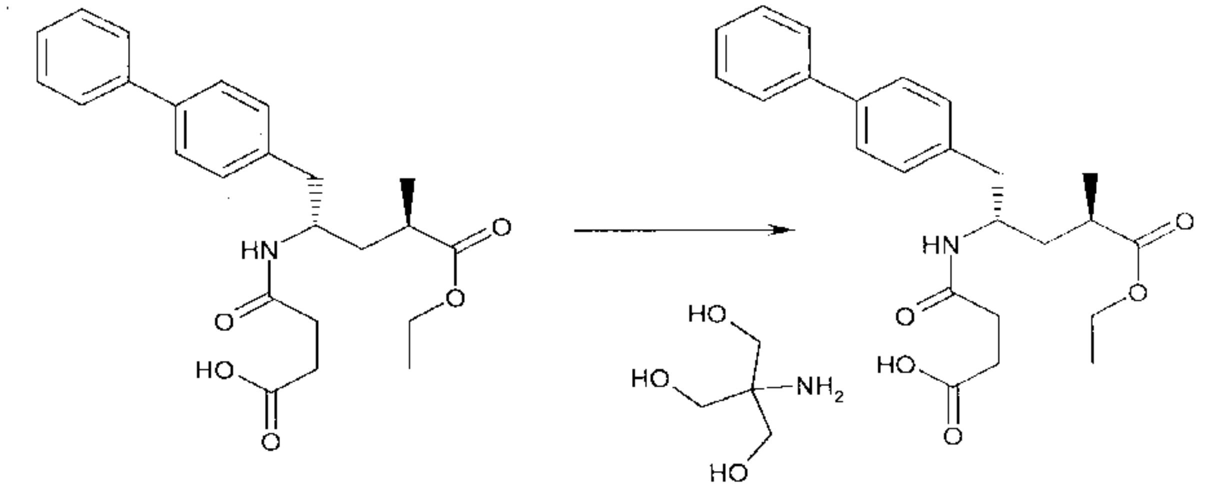
2-methoxy-(*R*)-alanine, *N*-[1-[[1(*S*)-benzyloxycarbonyl-3-phenylpropyl]amino]cyclopentylcarbonyl]-(*S*)-isoserine, *N*-[1-[[1(*S*)-carbonyl-3-phenylpropy]amino]-cyclopentylcarbonyl]-(*S*)-isoserine, 1,1'-[dithiobis-[2(*S*)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-*bis*-(*S*)-isoserine, 1,1'-[dithiobis-[2(*S*)-(2-methylbenzyl)-1-oxo-3,1-propanediyl]]-*bis*-(*S*)-methionine, *N*-(3-phenyl-2-(mercaptomethyl)-propionyl)-(*S*)-4-(methylmercapto)methionine, *N*-[2-acetylthiomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, *N*-[2-mercaptomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, *N*-[1-(2-carboxy-4-phenylbutyl)-cyclopentanecarbonyl]-(*S*)-isoserine, *N*-[1-(acetylthiomethyl)cyclopentane-carbonyl]-(*S*)-methionine ethyl ester, 3(*S*)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amimo-ε-caprolactam; and the compounds disclosed in WO 93/10773, particularly, *N*-(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester.

The compounds to be combined can be present as pharmaceutically acceptable salts. If these compounds have, for example, at least one basic center, they can form acid addition salts. Corresponding acid addition salts can also be formed having, if desired, an additionally present basic center. The compounds having at least one acid group, for example, COOH, can also form salts with bases. Corresponding internal salts may furthermore be formed, if a compound comprises, e.g., both a carboxy and an amino group.

With respect to *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester, preferred salts include the sodium salt disclosed in U.S. Patent No. 5,217,996, the triethanolamine salt and the *tris*(hydroxymethyl)aminomethane salt. Preparation of the triethanolamine salt and the *tris*(hydroxymethyl)aminomethane salt may be carried out as follows:

Triethanolamine

To *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester (349 mg, 0.848 mmol) is added 5 mL of ethyl ether and 0.113 mL (0.848 mmol) of triethanolamine in 1 mL of ethyl acetate. The solid was collected and dried melting at 69-71°C.



Tris(hydroxymethyl) aminomethane

To *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester (3.2 g, 7.78 mmol) is added 32 mL of ethyl acetate and 940 mg (7.78 mmol) *tris*(hydroxymethyl)aminomethane. The suspension is diluted with 45 mL of ethyl acetate and refluxed overnight (~20 hours). The reaction is cooled to 0°C, filtered, solid washed with ethyl acetate and dried melting at 114-115°C.

It has surprisingly been found that, a combination of valsartan and a NEP inhibitor achieves greater therapeutic effect than the administration of valsartan, ACE inhibitors or NEP inhibitors alone and promotes less angioedema than is seen with the administration of a vasopeptidase inhibitor alone. Greater efficacy can also be documented as a prolonged duration of action. The duration of action can be monitored as either the time to return to baseline prior to the next dose or as the area under the curve (AUC) and is expressed as the product of the change in blood pressure in millimeters of mercury (change in mmHg) and the duration of the effect (minutes, hours or days).

Further benefits are that lower doses of the individual drugs to be combined according to the present invention can be used to reduce the dosage, for example, that the dosages need not only often be smaller but are also applied less frequently, or can be used to diminish the incidence of side effects. The combined administration of valsartan or a pharmaceutically acceptable salt thereof and a NEP inhibitor or a pharmaceutically acceptable salt thereof results in a significant response in a greater percentage of treated patients, that is, a greater responder rate results, regardless of the underlying etiology of the condition. This is in accordance with the desires and requirements of the patients to be treated.

It can be shown that combination therapy with valsartan and a NEP inhibitor results in a more effective anti-hypertensive therapy (whether for malignant, essential, reno-vascular, diabetic, isolated systolic or other secondary type of hypertension) through improved efficacy, as well as a greater responder rate. The combination is also useful in the treatment or prevention of heart failure, such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter or detrimental vascular remodeling. It can further be shown that a valsartan and NEP inhibitor therapy proves to be beneficial in the treatment and prevention of myocardial infarction and its sequelae. A valsartan plus NEP inhibitor combination is also useful in treating atherosclerosis, angina (whether stable or unstable), and renal insufficiency (diabetic and non-diabetic). Furthermore, combination therapy using valsartan and a NEP inhibitor can improve endothelial dysfunction, thereby providing benefit in diseases in which normal endothelial function is disrupted, such as heart failure, angina pectoris and diabetes. Furthermore, the combination of the present invention may be used for the treatment or prevention of secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, such as Alzheimer's; glaucoma and stroke.

The person skilled in the pertinent art is fully enabled to select a relevant test model to prove the efficacy of a combination of the present invention in the hereinbefore and hereinafter indicated therapeutic indications.

Representative studies are carried out with a combination of valsartan and *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester, e.g., applying the following methodology:

Drug efficacy is assessed in various animal models including the deoxycorticosterone acetate-salt (DOCA-salt) rat and the spontaneously hypertensive rat (SHR), either maintained on a normal salt diet or with salt loading (4-8% salt in rat chow or 1% NaCl as drinking water).

The DOCA-salt test model utilizes either an acute or chronic study protocol. An acute study procedure involves assessment of the effects of various test substances over a six-hour experimental period using rats with indwelling femoral arterial and venous catheters. The acute study procedure evaluates test substances for their ability to reduce blood pressure during the established phase of DOCA-salt hypertension. In contrast, the chronic study procedure assesses the ability of test substances to prevent or delay the rise in blood pressure during the development phase of DOCA-salt hypertension. Therefore, blood pressure will be monitored in the chronic study procedure by means of a radiotransmitter. The radiotransmitter is surgically implanted into the abdominal aorta of rats, prior to the initiation of DOCA-salt treatment and thus, prior to the induction of hypertension. Blood pressure is chronically monitored for periods of up to six weeks (approximately one week prior to DOCA-salt administration and for five weeks thereafter).

Rats are anesthetized with 2-3% isoflurane in oxygen inhalant followed by Amytal sodium (amobarbital) 100 mg/kg, i.p. The level of anesthesia is assessed by a steady rhythmic breathing pattern.

Acute study procedure:

Rats undergo a unilateral nephrectomy at the time of DOCA implantation. Hair is clipped on the left flank and the back of the neck and scrubbed with sterile alcohol swabs and povidone/iodine. During surgery rats are placed on a heating pad to maintain body temperature at 37°C.

A 20 mm incision is made through the skin and underlying muscle to expose the left kidney. The kidney is freed of surrounding tissue, exteriorized and two ligatures (3-0 silk) are tied securely around the renal artery and vein proximal to their juncture with the aorta. The renal artery and vein are then severed and the kidney removed. The muscle and skin wounds are closed with 4-0 silk suture and stainless steel wound clips, respectively. At the same time, a 15 mm incision is made on the back of the neck and a three-week-release pellet (Innovative Research of America, Sarasota, FL) containing DOCA (100 mg/kg) is implanted subcutaneously (s.c.). The wound is then closed with stainless-steel clips and both wounds are treated with povidone/iodine; the rats are given a post-surgical intramuscular (i.m.) injection of procaine penicillin G (100,000 U) and buprenorphine (0.05-0.1 mg/kg) s.c. The rats are immediately placed on 1% NaCl + 0.2% KCl drinking water; this treatment continues for at least 3 weeks at which time the animals have become hypertensive and available for experimentation.

Forty-eight hours prior to experimentation, animals are anesthetized with isoflurane and catheters are implanted in the femoral artery and vein for measuring arterial pressure, collection of blood and administration of test compounds. Rats are allowed to recover for 48 hours while tethered in a Plexiglas home cage, which also serves as the experimental chamber.

Chronic study procedure:

This procedure is the same as above except that rats are implanted with a radiotransmitter, 7-10 days prior to the unilateral nephrectomy and initiation of DOCA and salt. In addition, rats do not undergo surgery for placement of femoral arterial and venous catheters. Radiotransmitters are implanted as described in Bazil et al., "Telemetric Monitoring of Cardiovascular Parameters in Conscious Spontaneously Hypertensive Rats", *J. Cardiovasc. Pharmacol.*, Vol. 22, pp. 897-905 (1993).

Protocols are then set-up on the computer for measurement of blood pressure, heart rate, etc., at pre-determined time points. Baseline data is collected at various time points and over various time intervals. For example, baseline or pre-dose values usually consist of data collection and averaging over three consecutive, 24-hour time periods prior to drug administration.

Blood pressure, heart rate and activity are determined at various pre-selected time points before, during and after drug administration. All measurements are performed in unrestrained and undisturbed animals. The maximum study time, determined by battery life, could be as long as nine months. For studies of this duration, rats are dosed orally (1-3 mL/kg vehicle), no more than twice daily or drug is administered via the drinking water or mixed with food. For studies of a shorter duration, that is, up to eight weeks, drugs are given via s.c. implanted osmotic minipumps. Osmotic minipumps are selected based on drug delivery rate and time. Valsartan dosages range from 1-10 mg/kg/day and *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester range from 10-50 mg/kg/day.

Additionally, SHRs are utilized to study the effects of valsartan in combination with *N*-(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester. The hypertensive background of the SHR is modified either by chronic salt loading in an effort to suppress the renin angiotensin system (RAS) or chronic salt depletion to activate the RAS in the SHR. These manipulations will be carried out to more extensively evaluate the efficacy of the various test substances. Experiments performed in SHRs are supplied by Taconic Farms, Germantown, NY (Tac:N(SHR)fBR). A radiotelemetric device (Data Sciences International, Inc., St. Paul, MN) is implanted into the lower abdominal aorta of all test animals between the ages of 14-16 weeks of age. All SHRs are allowed to recover from the surgical implantation procedure for at least two weeks prior to the initiation of the experiments. Cardiovascular parameters are continuously monitored via the radiotransmitter and transmitted to a receiver where the digitized signal is then collected and stored using a computerized data acquisition system. Blood pressure (mean arterial, systolic and diastolic pressure) and heart rate are monitored in conscious, freely moving and undisturbed SHR in their home cages. The arterial blood pressure and heart rate are measured every 10 minutes for 10 seconds and recorded. Data reported for each rat represent the mean values averaged over a 24-hour period and are made up of the 144-10 minute samples collected each day. The baseline values for blood pressure and heart rate consist of the average of three consecutive 24-hour readings taken prior to initiating the drug treatments. All rats are individually housed in a temperature and humidity controlled room and are maintained on a 12-hour light dark cycle.

In addition to the cardiovascular parameters, weekly determinations of body weight also are recorded in all rats. Treatments are administered in the drinking water, via daily oral gavage or in osmotic minipumps as stated above. If given in drinking water, water consumption is measured five times per week. Valsartan and *N*-(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester doses for individual rats are then calculated based on water consumption for each rat, the concentration of drug substance in the drinking water and individual body weights. All drug solutions in the drinking water are made up fresh every three to four days. Typical dosages for valsartan in drinking water range from 3-30 mg/kg/day whereas the dosage of *N*-(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester is highly dependent upon the specific agent used. In most situations, a daily dose will not exceed 50 mg/kg/day when administered as the monotherapy. In combination, lower dosages of each agent are used and correspondingly, valsartan is given in the range of 1-30 mg/kg/day and *N*-(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester in dosages below 50 mg/kg/day. However, in cases wherein the responder rate is increased with combination treatment, the dosages are identical to those used as monotherapy.

When drugs are administered by oral gavage, the dose of valsartan ranges from 1-50 mg/kg/day and *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester does not exceed 100 mg/kg/day.

Upon completion of the chronic studies, SHR or DOCA-salt rats are anesthetized and the heart rapidly removed. After separation and removal of the atrial appendages, left ventricle and left plus right ventricle (total) are weighed and recorded. Left ventricular and total ventricular mass are then normalized to body weight and reported. All values reported for blood pressure and cardiac mass represent the group mean ± sem.

Vascular function and structure are evaluated after treatment to assess the beneficial effects of the combination. SHR are studied according to the methods described by Intengan et al., *Circulation*, Vol. 100, No. 22, pp. 2267-2275 (1999). Similarly, the methodology for assessing vascular function in DOCA-salt rats is described in Intengan et al., *Hypertension*, Vol. 34, No. 4, Part 2, pp. 907-913 (1999).

The available results indicate an unexpected therapeutic effect of a combination according to the invention.

In one aspect is the object of this invention to provide a pharmaceutical combination composition, e.g., for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure, such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, such as Alzheimer's, glaucoma and stroke which composition comprises:

(i) the AT 1-antagonists valsartan or a pharmaceutically acceptable salt thereof; and

(ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

In this composition, components (i) and (ii) can be obtained and administered together, one after the other or separately in one combined unit dose form or in two separate unit dose forms. The unit dose form may also be a fixed combination.

A further aspect of the present invention is a method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure, such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, such as Alzheimer's, glaucoma and stroke, comprising administering a therapeutically effective amount of combination of:

(i) the AT 1-antagonists valsartan or a pharmaceutically acceptable salt thereof; and

(ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier to a mammal in need of such treatment.

A therapeutically effective amount of each of the component of the combination of the present invention may be administered simultaneously or sequentially and in any order.

The corresponding active ingredient or a pharmaceutically acceptable salt thereof may also be used in form of a hydrate or include other solvents used for crystallization.

The pharmaceutical compositions according to the invention can be prepared in a manner known *per se* and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including man, comprising a therapeutically effective amount of the pharmacologically active compound, alone or in combination with one or more pharmaceutically acceptable carriers, especially suitable for enteral or parenteral application. Typical oral formulations include tablets, capsules, syrups, elixirs and suspensions. Typical injectable formulations include solutions and suspensions.

The typical pharmaceutically acceptable 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 glycol polymers; betacyclodextrin; fatty alcohols; and hydrolyzed cereal solids, as well as other non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, antioxidants, lubricants, flavoring agents and the like commonly used in pharmaceutical formulations.

The invention also relates to combining separate pharmaceutical compositions in kit form. That is a kit combining two separate units: a valsartan pharmaceutical composition and a NEP inhibitor pharmaceutical composition. The kit form is particularly advantageous when the separate components must be administered in different dosage forms, e.g., parenteral valsartan formulation and oral NEP formulation; or are administered at different dosage intervals.

These pharmaceutical preparations are for enteral, such as oral, and also rectal or parenteral, administration to homeotherms, with the preparations comprising the pharmacological active compound either alone or together with customary pharmaceutical auxiliary substances. For example, the pharmaceutical preparations consist of from about 0.1-90%, preferably of from about 1% to about 80%, of the active compounds. Pharmaceutical preparations for enteral or parenteral administration are, e.g., in unit dose forms, such as coated tablets, tablets, capsules or suppositories and also ampoules. These are prepared in a manner which is known *per se*, e.g., using conventional mixing, granulation, coating, solubulizing or lyophilizing processes. Thus, pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, if desired granulating a mixture which has been obtained, and, if required or necessary, processing the mixture or granulate into tablets or coated tablet cores after having added suitable auxiliary substances.

The dosage of the active compound can depend on a variety of factors, such as mode of administration, homeothermic species, age and/or individual condition.

Preferred dosages for the active ingredients of the pharmaceutical combination according to the present invention are therapeutically effective dosages, especially those which are commercially available.

Normally, in the case of oral administration, an approximate daily dose of from about 1 mg to about 360 mg is to be estimated, e.g., for a patient of approximately 75 kg in weight.

Valsartan is supplied in the form of suitable dosage unit form, e.g., a capsule or tablet, and comprising a therapeutically effective amount, e.g., from about 20 mg to about 320 mg, of valsartan which may be applied to patients. The application of the active ingredient may occur up to three times a day, starting, e.g., with a daily dose of 20 mg or 40 mg of valsartan, increasing via 80 mg daily and further to 160 mg daily up to 320 mg daily. Preferably, valsartan is applied once a day (q.d.) or twice a day (b.i.d.) in heart failure patients with a dose of 80 mg or 160 mg, respectively, each. Corresponding doses may be taken, for example, in the morning, at mid-day or in the evening. Preferred is q.d. or b.i.d. administration in heart failure.

In case of NEP inhibitors, preferred dosage unit forms are, e.g., tablets or capsules comprising, e.g., from about 20 mg to about 800 mg, preferably from about 50 mg to about 700 mg, even more preferably from about 100 mg to about 600 mg and even more preferably from about 100 mg to about 300 mg, administered q.d.

The above doses encompass a therapeutically effective amount of the active ingredients of the present invention.

The following examples illustrate the above-described invention; however, it is not intended to restrict the scope of this invention in any manner.

Formulation Example 1: Film-Coated Tablets

Components	Composition Per Unit (mg)	Standards
Granulation		
Valsartan (= active ingredient)	80.00	
Microcrystalline cellulose/Avicel PH 102	54.00	NF, Ph. Eur
Crospovidone	20.00	NF, Ph. Eur
Colloidal anhydrous silica/colloidal silicon dioxide/Aerosil 200	0.75	Ph. Eur, NF
Magnesium stearate	2.5	NF, Ph. Eur
Blending		
Colloidal anhydrous silica/colloidal silicon dioxide/Aerosil 200	0.75	Ph. Eur, NF
Magnesium stearate	2.00	NF, Ph. Eur
Coating		
Purified water*	–	
DIOLACK Pale Red 00F34899	7.00	
Total Tablet Mass	**167.00**	

*Removed during processing.

The film-coated tablet is manufactured, e.g., as follows:

A mixture of valsartan, microcrystalline cellulose, crospovidone, part of the colloidal anhydrous silica/colloidal silicon dioxide/Aerosile 200, silicon dioxide and magnesium stearate is premixed in a diffusion mixer and then sieve through a screening mill. The resulting mixture is again pre-mixed in a diffusion mixer, compacted in a roller compactor and then sieve through a screening mill. To the resulting mixture, the rest of the colloidal anhydrous silica/colloidal silicon dioxide/Aerosile 200 are added and the final blend is made in a diffusion mixer. The whole mixture is compressed in a rotary tabletting machine and the tablets are coated with a film by using Diolack pale red in a perforated pan.

Formulation Example 2:

Film-coated tablets

Components	Composition Per Unit (mg)	Standards
Granulation		
Valsartan (= active ingredient)	160.00	
Microcrystalline cellulose/Avicel PH 102	108.00	NF, Ph. Eur
Crospovidone	40.00	NF, Ph. Eur
Colloidal anhydrous silica/colloidal silicon dioxide/Aerosil 200	1.50	Ph. Eur, NF
Magnesium stearate	5.00	NF, Ph. Eur
Blending		
Colloidal anhydrous silica/colloidal silicon dioxide/Aerosil 200	1.50	Ph. Eur, NF
Magnesium stearate	4.00	NF, Ph. Eur
Coating		
Opadry® Light Brown 00F33172	10.00	
Total Tablet Mass	**330.00**	

The film-coated tablet is manufactured, e.g., as described in Formulation Example 1.

Formulation Example 3.

Film-coated tablets

Components	Composition Per Unit (mg)	Standards
Core Internal Phase		
Valsartan [= active ingredient]	40.00	
Silica, colloidal anhydrous (colloidal silicon dioxide) [= glidant]	1.00	Ph. Eur, USP/NF
Magnesium stearate [= lubricant]	2.00	USP/NF
Crospovidone [= disintegrant]	20.00	Ph. Eur
Microcrystalline cellulose [= binding agent]	124.00	USP/NF
External Phase		
Silica, colloidal anhydrous (colloidal silicon dioxide) [= glidant]	1.00	Ph. Eur, USP/NF
Magnesium stearate [= lubricant]	2.00	USP/NF
Film Coating		
Opadry Brown 00F16711*	9.40	
Purified water**	–	
Total Tablet Mass	**199.44**	

*The composition of the Opadry brown OOF16711 coloring agent is tabulated below.
**Removed during processing

Opadry® Composition:

Ingredient	Approximate % Composition
Iron oxide, black (C.I. No. 77499, E 172)	0.50
Iron oxide, brown (C.I. No. 77499, E 172	0.50
Iron oxide, red (C.I. No. 77491, E 172)	0.50
Iron oxide, yellow (C.I. No. 77492, E 172)	0.50
Macrogolum (Ph. Eur)	4.00
Titanium dioxide (C.I. No. 77891, E 171)	14.00
Hypromellose (Ph. Eur)	80.00

The film-coated tablet is manufactured, e.g., as described in Formulation Example 1.

Formulation Example 4:

Capsules

Components	Composition Per Unit (mg)
Valsartan [= active ingredient]	80.00
Microcrystalline cellulose	25.10
Crospovidone	13.00
Povidone	12.50
Magnesium stearate	1.30
Sodium lauryl sulphate	0.60
Shell	
Iron oxide, red (C.I. No. 77491, EC No. E 172)	0.123
Iron oxide, yellow (C.I. No. 77492, EC No. E 172)	0.123
Iron oxide, black (C.I. No. 77499, EC No. E 172)	0.245
Titanium dioxide	1.540
Gelatin	74.969
Total Tablet Mass	**209.50**

The tablet is manufactured, e.g., as follows:

Granulation/Drying

Valsartan and microcrystallin cellulose are spray-granulated in a fluidized bed granulator with a granulating solution consisting of povidone and sodium lauryl sulphate dissolved in purified water. The granulate obtained is dried in a fluidized bed dryer.

Milling/Blending

The dried granulate is milled together with crospovidone and magnesium stearate. The mass is then blended in a conical screw type mixer for approximately 10 minutes.

Encapsulation

The empty hard gelatin capsules are filled with the blended bulk granules under controlled temperature and humidity conditions. The filed capsules are de-dusted, visually inspected, weight-checked and quarantined until by Quality Assurance department.

Formulation Example 5:

Capsules

Components	Composition Per Unit (mg)
Valsartan [= active ingredient]	160.00
Microcrystalline cellulose	50.20
Crospovidone	26.00
Povidone	25.00
Magnesium stearate	2.60
Sodium lauryl sulphate	1.20
Shell	
Iron oxide, red (C.I. No. 77491, EC No. E 172)	0.123
Iron oxide, yellow (C.I. No. 77492, EC No. E 172)	0.123
Iron oxide, black (C.I. No. 77499, EC No. E 172)	0.245
Titanium dioxide	1.540
Gelatin	74.969
Total Tablet Mass	**342.00**

The formulation is manufactured, e.g., as described in Formulation Example 4.

Formulation Example 6:

Hard Gelatine Capsule

Components	Composition Per Unit (mg)
Valsartan [= active ingredient]	80.00
Sodium lauryl sulphate	0.60
Magnesium stearate	1.30
Povidone	12.50
Crospovidone	13.00
Microcystalline cellulose	21.10
Total Tablet Mass	**130.00**

Formulation Example 7:

A hard gelatin capsule, comprising as active ingredient, e.g., (*S*)-*N*-(1-carboxy-2-methylprop-1-yl)-*N*-pentanoyl-*N*-[2'(*1H*-tetrazol-5-yl)biphenyl-4-yl-methyl]amine, can be formulated, e.g., as follows:

Components	Composition Per Unit (mg)
(1) Valsartan	80.00
(2) Microcystalline cellulose	110.0
(3) Polyvidone K30	45.2
(4) Sodium lauryl sulfate	1.2
(5) Crospovidone	26.0
(6) Magnesium stearate	2.6

Components (1) and (2) are granulated with a solution of components (3) and (4) in water. The components (5) and (6) are added to the dry granulate and the mixture is filled into size 1 hard gelatin capsules.

All publications and patents mentioned herein are incorporate by reference in their entirety as if set forth in full herein.

What is claimed is:

1. A pharmaceutical composition comprising:

(i) the AT 1-antagonist valsartan or a pharmaceutically acceptable salt thereof; and

(ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of Claim 1, wherein the NEP inhibitor is selected from the group consisting of SQ 28,603, *N*-[*N*-[1(*S*)-carboxyl-3-phenylproplyl]-(*S*)-phenylalanyl]-(*S*)-isoserine, *N*-[*N*-[((*1S*)-carboxy-2-phenyl)ethyl]-(*S*)-phenylalanyl]-β-alanine, *N*-[2(*S*)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine, (*cis*-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid), thiorphan, retro-thiorphan, phosphoramidon, SQ 29072, *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester, (*S*)-*cis*-4-[1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid, 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo carbamoyl]cyclopentyl)-2-(2-methoxyethyl)propanoic acid, *N*-(1-(3-(*N-t*-butoxycarbonyl (*S*)-prolylamino)-2(*S*)-*t*-butoxy-carbonylpropyl)cyclopentanecarbonyl)-*O*-benzyl-(*S*)-serine methyl ester, 4-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid, 3-[1-(*cis*-4-carboxycarbonyl-*cis*-3-butylcyclohexyl-*r*-1-carboamoyl)cyclopentyl]-*2S*-(2-methoxyethoxymethyl)propanoic acid, *N*-((2*S*)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine, *N*-(1-(*N*-hydroxycarbamoylmethyl)-1-cyclopentanecarbonyl)-*L*-phenylalanine, (*S*)-(2-biphenyl-4-yl)-1-(*1H*-tetrazol-5-yl)ethylamino) methylphosphonic acid, (*S*)-5-(*N*-(2-(phosphonomethylamino)-3-(4-biphenyl)propionyl)-2-aminoethyl)tetrazole, β-alanine, 3-[1,1'-biphenyl]-4-yl-*N*-[diphenoxyphosphinyl)methyl]-*L*-alanyl, *N*-(2-carboxy-4-thienyl)-3-mercapto-2-benzylpropanamide, 2-(2-mercaptomethyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid, (*L*)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl)-*L*-phenylalanyl)-β-alanine, *N*-[*N*-[(*L*)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine, *N*-[*N*-[(*L*)-1-carboxy-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine, *N*-[2-acetylthiomethyl-3-(2-methyl-phenyl)propionyl]-methionine ethyl ester, *N*-[2-mercaptomethyl-3-(2-methylphenyl)propioyl]-methionine, *N*-[2(*S*)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(*S*)-isoserine, *N*-(*S*)-[3-mercapto-2-(2-methylphenyl)propionyl]-(*S*)-2-methoxy-(*R*)-alanine, *N*-[1-[[1(*S*)-benzyloxycarbonyl-3-phenylpropyl]amino]cyclopentylcarbonyl]-(*S*)-isoserine,

N-[1-[[1(*S*)-carbonyl-3-phenylpropy]amino]-cyclopentylcarbonyl]-(*S*)-isoserine, 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, *N*-(3-phenyl-2-(mercaptomethyl)-propionyl)-(*S*)-4-(methylmercapto)methionine, *N*-[2-acetylthiomethyl-3-phenyl-propionyl]-3 aminobenzoic acid, *N*-[2-mercaptomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, *N*-[1-(2-carboxy-4-phenylbutyl)-cyclopentanecarbonyl]-(*S*)-isoserine, *N*-[1-(acetylthiomethyl)cyclopentane-carbonyl]-(S)-methionine ethyl ester, 3(S)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amimo-ε-caprolactam and *N*-(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester, or in each case, a pharmaceutically acceptable salt thereof.

3. The pharmaceutical composition of Claim 2, wherein *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester is a triethanolamine or *tris*(hydroxymethyl)aminomethane salt thereof.

4. A kit comprising in separate containers in a single package pharmaceutical compositions comprising in one container a pharmaceutical composition comprising a NEP inhibitor and in a second container a pharmaceutical composition comprising valsartan.

5. A method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure, such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, such as Alzheimer's, glaucoma and stroke, comprising administering a therapeutically effective amount of combination of:

(i) the AT 1-antagonists valsartan or a pharmaceutically acceptable salt thereof; and

(ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier to a mammal in need of such treatment.

6. A method as claimed in Claim 5, wherein the NEP inhibitor is selected from the group consisting of SQ 28,603, *N*-[*N*-[1(*S*)-carboxyl-3-phenylproplyl]-(*S*)-phenylalanyl]-(*S*)-isoserine, *N*-[*N*-[((*1S*)-carboxy-2-phenyl)ethyl]-(*S*)-phenylalanyl]-β-alanine, *N*-[2(*S*)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine, (*cis*-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclopentyl]carbonyl]amino]-cyclohexanecarboxylic acid), thiorphan, retro-thiorphan, phosphoramidon, SQ 29072, *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester, (*S*)-*cis*-4-[1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido]-1-cyclohexanecarboxylic acid, 3-(1-[6-endo-hydroxymethylbicyclo[2,2,1]heptane-2-exo-carbamoyl]cyclopentyl)-2-(2-methoxyethyl)propanoic acid, *N*-(1-(3-(*N*-*t*-butoxycarbonyl-(*S*)-prolylamino)-2(*S*)-*t*-butoxy-carbonylpropyl)cyclopentanecarbonyl)-*O*-benzyl-(*S*)-serine methyl ester, 4-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid, 3-[1-(*cis*-4-carboxycarbonyl-*cis*-3-butylcyclohexyl-r-1-carboamoyl)cyclopentyl]-2*S*-(2-methoxyethoxymethyl)propanoic acid, *N*-((2*S*)-2-(4-biphenylmethyl)-4-carboxy-5-phenoxyvaleryl)glycine, *N*-(1-(*N*-hydroxycarbamoylmethyl)-1-cyclopentanecarbonyl)-*L*-phenylalanine, (*S*)-(2-biphenyl-4-yl)-1-(*1H*-tetrazol-5-yl)ethylamino) methylphosphonic acid, (*S*)-5-(*N*-(2-(phosphonomethylamino)-3-(4-biphenyl)propionyl)-2-aminoethyl)tetrazole, β-alanine, 3-[1,1'-biphenyl]-4-yl-*N*-[diphenoxyphosphinyl)methyl]-*L*-alanyl, *N*-(2-carboxy-4-thienyl)-3-mercapto-2-benzylpropanamide, 2-(2-mercaptomethyl-3-phenylpropionamido)thiazol-4-ylcarboxylic acid, (*L*)-(1-((2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy)carbonyl)-2-phenylethyl)-*L*-phenylalanyl)-β-alanine, *N*-[*N*-[(*L*)-[1-[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]carbonyl]-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine, *N*-[*N*-[(*L*)-1-carboxy-2-phenylethyl]-*L*-phenylalanyl]-(*R*)-alanine, *N*-[2-acetylthiomethyl-3-(2-methyl-phenyl)propionyl]-methionine ethyl ester, *N*-[2-mercaptomethyl-3-(2-methylphenyl)propioyl]-methionine, *N*-[2(*S*)-mercaptomethyl-3-(2-methylphenyl)propanoyl]-(*S*)-isoserine, *N*-(*S*)-[3-mercapto-2-(2-methylphenyl)propionyl]-(*S*)-2-methoxy-(*R*)-alanine, *N*-[1-[[1(*S*)-benzyloxycarbonyl-3-phenylpropyl]amino]cyclopentylcarbonyl]-(S)-isoserine, *N*-[1-[[1(*S*)-carbonyl-3-phenylpropy]amino]-cyclopentylcarbonyl]-(*S*)-isoserine, 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, *N*-(3-phenyl-2-(mercaptomethyl)-propionyl)-(*S*)-4-(methylmercapto)methionine, *N*-[2-acetylthiomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, *N*-[2-mercaptomethyl-3-phenyl-propionyl]-3-aminobenzoic acid, *N*-[1-(2-carboxy-4-phenylbutyl)-cyclopentanecarbonyl]-(*S*)-isoserine, *N*-[1-(acetylthiomethyl)cyclopentane-carbonyl]-(*S*)-methionine ethyl ester, 3(*S*)-[2-(acetylthiomethyl)-3-phenyl-propionyl]amimo-ε-caprolactam and

N-(2-acetylthiomethyl-3-(2-methylphenyl)propionyl)-methionine ethyl ester, and in each case, a pharmaceutically acceptable salt thereof.

7. The method of Claim 6, wherein *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester is a triethanolamine or tris(hydroxymethyl)aminomethane salt thereof.

8. A triethanolamine salt of *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester.

9. A *tris*(hydroxymethyl)aminomethane salt of *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester.

10. A pharmaceutical composition comprising the salt of Claim 8.

11. A pharmaceutical composition comprising the salt of Claim 9.

Abstract of the Disclosure

The invention relates a pharmaceutical composition comprising a combination of:

(i) the AT 1- antagonist valsartan or a pharmaceutically acceptable salt thereof; and

(ii) a NEP inhibitor or a pharmaceutically acceptable salt thereof and optionally a pharmaceutically acceptable carrier and to a method for the treatment or prevention of a condition or disease

selected from the group consisting of hypertension, heart failure, such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, such as Alzheimer's, glaucoma and stroke, comprising administering a therapeutically effective amount of the pharmaceutical composition to a mammal in need thereof.

Case 4-32219A

DECLARATION AND POWER OF ATTORNEY FOR U.S. PATENT APPLICATIONS

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. and

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

the specification of which was filed on as U.S. Application No. .

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims.

I acknowledge my duty to disclose all information which is known by me to be material to the patentability of this application as defined in 37 C.F.R. §1.56.

I hereby claim the benefit under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below and under 35 U.S.C. §365(a) of any PCT international application(s) designating at least one country other than the United States listed below and have also listed below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application the priority of which is claimed for that subject matter:

None

I hereby claim the benefit under 35 USC §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date
60/386,792	June 7, 2002
60/349,660	January 17, 2002

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) listed below and under 35 U.S.C. §365(c) of any PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in said prior application(s) in the manner required by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose all information known by me to be material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date(s) of the prior application(s) and the national or PCT international filing date of this application:

None

I hereby appoint the attorneys and agents associated with Customer No. 001095, respectively and individually, as my attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please address all communications to the address associated with Customer No. 001095, which is currently Thomas Hoxie, Novartis Pharmaceuticals Corporation, Patent and Trademark Dept., One Health Plaza, East Hanover, NJ 07936-1080.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FIRST JOINT INVENTOR:

Full name	:	**Gary Michael Ksander**
Signature	:	____________________
Date	:	____________________ (MM/DD/YY)
Citizenship	:	United States of America
Residence	:	Milford, New Jersey
P.O. Address	:	342 Woolf Road Milford, New Jersey 08848

SECOND JOINT INVENTOR:

Full name	:	**Randy Lee Webb**
Signature	:	____________________
Date	:	____________________ (MM/DD/YY)
Citizenship	:	United States of America
Residence	:	Flemington, New Jersey
P.O. Address	:	17 Honeyman Drive Flemington, New Jersey 08822

IMPORTANT: Before this declaration is signed, the patent application (the specification, the claims and this declaration) must be read and understood by each person signing it, and no changes may be made in the application after this declaration has been signed.

PATENT APPLICATION SERIAL NO. ____________________

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

01/16/2003 DEMMANU1 00000041 190134 10341868

01 FC:1001 750.00 CH
02 FC:1201 168.00 CH

PTO-1556
(5/87)

*U.S. Government Printing Office: 2001 — 481-697/59173

PATENT APPLICATION FEE DETERMINATION RECORD Effective January 1, 2003	Application or Docket Number 4-32219A

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	11	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	11 minus 20=	* 0
INDEPENDENT CLAIMS	5 minus 3 =	* 2
MULTIPLE DEPENDENT CLAIM PRESENT ☐		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐ RATE	FEE	OR	OTHER THAN SMALL ENTITY RATE	FEE
BASIC FEE	$375	OR	BASIC FEE	$750
X$ 9=		OR	X$18=	
X42=		OR	X84=	168
+140=		OR	+280=	
TOTAL		OR	TOTAL	918

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM ☐				

SMALL ENTITY RATE	ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM ☐				

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM ☐				

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
Washington, DC 20231
www.uspto.gov

APPLICATION NUMBER	FILING/RECEIPT DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
10/341,868	01/14/2003	Gary Michael Ksander	4-32219A

CONFIRMATION NO. 8865

FORMALITIES LETTER

OC000000009585353

001095
THOMAS HOXIE
NOVARTIS, PATENT AND TRADEMARK DEPARTMENT
ONE HEALTH PLAZA 430/2
EAST HANOVER, NJ 07936-1080

Date Mailed: 03/03/2003

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The oath or declaration is unsigned.
- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(e) of $130 for a non-small entity, must be submitted with the missing items identified in this letter.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$130** for a Large Entity

- **$130** Late oath or declaration Surcharge.

A copy of this notice MUST be returned with the reply.

Tsehai H

Customer Service Center
Initial Patent Examination Division (703) 308-1202
PART 3 - OFFICE COPY

#7

Case 4-32219A

DECLARATION AND POWER OF ATTORNEY FOR U.S. PATENT APPLICATIONS

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, and

I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

the specification of which was filed on January 14, 2003 as U.S. Application No. **10/341,868**.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims.

I acknowledge my duty to disclose all information which is known by me to be material to the patentability of this application as defined in 37 C.F.R. §1.56.

I hereby claim the benefit under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below and under 35 U.S.C. §365(a) of any PCT international application(s) designating at least one country other than the United States listed below and have also listed below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States for the same subject matter and having a filing date before that of the application the priority of which is claimed for that subject matter:

None

I hereby claim the benefit under 35 USC §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date
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60/349,660	January 17, 2002

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None

I hereby appoint the attorneys and agents associated with Customer No. 001095, respectively and individually, as my attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FIRST JOINT INVENTOR:

Full name	:	**Gary Michael Ksander**
Signature	:	Gary M. Ksander
Date	:	2/20/03 (MM/DD/YY)
Citizenship	:	United States of America
Residence	:	Amherst, New Hampshire
P.O. Address	:	37 The Flume Amherst, New Hampshire 03031

SECOND JOINT INVENTOR:

Full name	:	**Randy Lee Webb**
Signature	:	Randy Lee Webb
Date	:	02/26/03 (MM/DD/YY)
Citizenship	:	United States of America
Residence	:	Flemington, New Jersey
P.O. Address	:	17 Honeyman Drive Flemington, New Jersey 08822

IMPORTANT: Before this declaration is signed, the patent application (the specification, the claims and this declaration) must be read and understood by each person signing it, and no changes may be made in the application after this declaration has been signed.

#3

UNITED STATES PATENT AND TRADEMARK OFFICE

OIPE JC135 MAR 1 0 2003 PATENT & TRADEMARK OFFICE

Commissioner for Patents
Washington, DC 20231
www.uspto.gov

APPLICATION NUMBER	FILING/RECEIPT DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
10/341,868	01/14/2003	Gary Michael Ksander	4-32219A

CONFIRMATION NO. 8865

FORMALITIES LETTER

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NOVARTIS, PATENT AND TRADEMARK DEPARTMENT
ONE HEALTH PLAZA 430/2
EAST HANOVER, NJ 07936-1080

Date Mailed: 03/03/2003

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FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

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- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(e) of $130 for a non-small entity, must be submitted with the missing items identified in this letter.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$130** for a Large Entity

- **$130** Late oath or declaration Surcharge.

03/14/2003 BNGUYEN1 00000070 190134 10341868

01 FC:1051 ~~130.00 CH~~

A copy of this notice MUST be returned with the reply.

Tsehai H

Customer Service Center
Initial Patent Examination Division (703) 308-1202
PART 2 - COPY TO BE RETURNED WITH RESPONSE

3-11-03 #3 MP

CASE 4-32219A

OIPE
MAR 10 2003
PATENT & TRADEMARK OFFICE

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EV 268930367 US — Express Mail Label Number

3/10/03 — Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF

KSANDER ET AL. Group Art Unit: 1614

APPLICATION NO: 10/341,868

FILED: JANUARY 14, 2002

FOR: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Attention: Box Missing Parts
Assistant Commissioner for Patents
Washington, DC 20231

RESPONSE TO NOTICE TO FILE MISSING PARTS

Sir:

The Notice to File Missing Parts of Application - Filing Date Granted dated March 3, 2003 (a copy of which is enclosed) has a shortened statutory time set to expire on May 3, 2003.

In response, applicants now submit an original or copy of a fully executed Declaration and Power of Attorney. Please charge the $130 surcharge fee under 37 CFR §1.16(e) to Deposit Account No. 19-0134 in the name of Novartis Corporation.

The Commissioner is hereby authorized to charge any additional fees under 37 CFR §1.17 which may be required, or credit any overpayment, to Account No. 19-0134 in the name of Novartis Corporation.

A duplicate copy of this letter is provided for charging purposes.

Respectfully submitted,

Greg Ferraro

Gregory D. Ferraro
Attorney for Applicants
Reg. No. 36,134

Novartis
Corporate Intellectual Property
One Health Plaza, Building 430
East Hanover, NJ 07936-1080
(862) 778-7831
GDF:dd
Encl.: executed Declaration and Power of Attorney
Date: 3/10/03

20

11-06-03

GP1614

CASE 4-32219A

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EL 987587051	November 5, 2003
Express Mail Label Number	Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF — Art Unit: 1614

KSANDER ET AL.

APPLICATION NO: 10/341,868

FILED: JANUARY 14, 2002

FOR: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

INFORMATION DISCLOSURE STATEMENT

Sir:

Applicants believe this paper is being filed before the mailing date of a first Office Action on the merits, and so under 37 C.F.R. §1.97(b)(3) no fees are required. If a fee is deemed to be required, the Commissioner is hereby authorized to charge such fee to Deposit Account No. 19-0134.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO-1449.

These references were cited in a search report in a corresponding International application. Copies of these references and the search report are enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO 1449 form(s).

Respectfully submitted,

Gregory D. Ferraro
Attorney for Applicants
Reg. No. 36,134

Novartis
Corporate Intellectual Property
One Health Plaza, Building 430
East Hanover, NJ 07936-1080
(862) 778-7831

Date: November 5, 2003

FORM PTO-1449
(REV. 7-85)

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

ATTY. DOCKET NO.
4-32219A
APPLICATION NO.
10/341,868
APPLICANT
KSANDER ET AL.
FILING DATE
JANUARY 14, 2002

Group
1614

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE
	AA	5,217,996	06/08/93	Ksander	514	533	01/22/92
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						
	AL						

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	OFFICE	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
	AM	WO 01/74348 A2	10/11/01	WIPO			☐	☐
	AN	WO 02/06253	01/24/02	WIPO			☐	☐
	AO	WO 02/092622 A2	11/21/02	WIPO			☐	☐
	AP	0 726 072 A2	08/14/96	Europe			☐	☐
	AQ	0 498 361 A2	08/12/92	Europe			☐	☐

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent pages, Etc.)

	AR	
	AS	
	AT	

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial of reference considered, whether or not citation is in conformance with MPEP 609: Draw a line through citation if not in conformance and not considered. Include a copy of this form with the next communication to applicant.

4-32219 A

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

REVISED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 October 2001 (11.10.2001)

PCT

(10) International Publication Number
WO 01/74348 A2

(51) International Patent Classification: Not classified

(21) International Application Number: PCT/US01/08240

(22) International Filing Date: 15 March 2001 (15.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/194,499 3 April 2000 (03.04.2000) US

(71) Applicant *(for all designated States except US)*: **BRISTOL-MYERS SQUIBB CO.** [US/US]; P.O. Box 4000, Lawrenceville-Princeton Road, Princeton, NJ 08543-4000 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **REEVES, Richard, A.** [US/US]; 4 Western Pine Street, Pennington, NJ 08534 (US). **WOLF, Robert, A.** [US/US]; 8 Crocus Lane, Newtown, PA 18940 (US). **CHANG, Paul, I.** [US/US]; 3750 Morrison Way, Doylestown, PA 18901 (US).

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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(54) Title: VASOPEPTIDASE INHIBITORS TO TREAT ISOLATED SYSTOLIC HYPERTENSION

(57) Abstract:

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13ter.1(c) and Rule 39)

Applicant's or agent's file reference HA0732	IMPORTANT DECLARATION	Date of mailing *(day/month/year)* 21/02/2002
International application No. PCT/US 01/ 08240	International filing date *(day/month/year)* 15/03/2001	(Earliest) Priority date *(day/month/year)* 03/04/2000
International Patent Classification (IPC) or both national classification and IPC		
Applicant BRISTOL-MYERS SQUIBB CO.		

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below

1. ☐ The subject matter of the international application relates to:
   - a. ☐ scientific theories.
   - b. ☐ mathematical theories
   - c. ☐ plant varieties.
   - d. ☐ animal varieties.
   - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
   - f. ☐ schemes, rules or methods of doing business.
   - g. ☐ schemes, rules or methods of performing purely mental acts.
   - h. ☐ schemes, rules or methods of playing games.
   - i. ☐ methods for treatment of the human body by surgery or therapy.
   - j. ☐ methods for treatment of the animal body by surgery or therapy.
   - k. ☐ diagnostic methods practised on the human or animal body.
   - l. ☐ mere presentations of information.
   - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art.

2. [X] The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

   [X] the description [X] the claims ☐ the drawings

3. ☐ The failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions prevents a meaningful search from being carried out:
   - ☐ the written form has not been furnished or does not comply with the standard.
   - ☐ the computer readable form has not been furnished or does not comply with the standard.

4. Further comments:

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Véronique Baillou

Form PCT/ISA/203 (July 1998)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 203

A meaningful search is not possible on the basis of all claims because all claims are directed to - Method for treatment of the human or animal body by therapy - Rule 39.1(iv) PCT

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

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(54) **Composition for the treatment of hypertension and congestive heart failure, containing an angiotensin II antagonist and an endopeptidase inhibitor**

(57) Hypertension and/or congestive heart failure are treated with the combination of the angiotensin II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]nonan-4-one and a selective neutral endopeptidase inhibitor or a dual acting neutral endopeptidase inhibitor.

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Description

Darrow et al. in European Patent Application 498,361 disclose treating hypertension or congestive heart failure with a combination of an angiotensin II antagonist or a renin inhibitor with a neutral endopeptidase inhibitor.

Matsumoto et al., JASN, September 1993, disclose that the combined therapy of an angiotensin II blocker, DUP753, and a neutral endopeptidas inhibitor, candoxatril, may be useful in the treatment of congestive heart failure and renal failure.

Bernhart et al. in United States Patent 5,270,317 disclose a series of N-substituted heterocyclic derivatives which possess angiotensin II antagonist activity. Bernhart et al. disclose that such compounds can be used in the treatment of various cardiovascular complaints, especially hypertension, heart failure, and venous insufficiency, as well as in the treatment of glaucoma, diabetic retinopathy and various complaints of the central nervous system. It is also disclosed that such compound can be used in combination with other active agents such as tranquilizers, beta-blocking compounds, a calcium antagonist, or a diuretic.

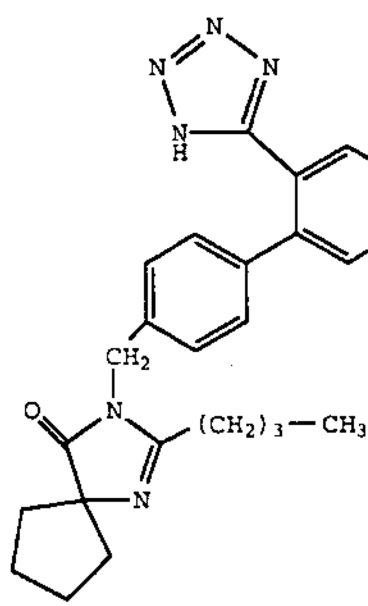
Selective neural endopeptidase inhibitors are taught by Delaney et al. in United States Patents 4,722,810 and 5,223,516 and the use of selective neutral endopeptidase inhibitors alone or in combination with angiotensin converting enzyme inhibitors to treat hypertension are disclosed by Delaney et al. U.K. Patent Application 2,207,351 and by Haslanger et al. in United States Patent 4,749,688. The treatment of congestive heart failure by administration of a combination of a selective neutral endopeptidase inhibitor and an angiotensin converting enzyme inhibitor is disclosed by Seymour in United States Patent 5,225,401.

Compounds possessing both neutral endopeptidase and angiotensin converting enzyme inhibition activity are disclosed by Flynn et al. in United States Patent 5,366,973, European Patent Application 481,522 and PCT Patent Applications WO 93/16103, and WO 94/10193, Warshawsky et al. European Patent Applications 534,363, 534,396 and 534,492, Fournie-Zaluski European Patent Application 524,553, Karanewsky et al. European Patent Application 599,444, Karanewsky European Patent Application 595,610, Robl et al., European Patent Application 629,627, Robl United States Patent 5,362,727 and European Patent Application 657,453.

This invention is directed to the discovery that the angiotensin II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4 -yl]methyl]-1,3-diazaspiro[4.4]nonan-4-one acts synergistically with a selective neutral endopeptidase inhibitor or a dual acting neutral endopeptidase inhibitor as defined below to reduce cardiac preload and afterload and enhance natriureses. The combination of this angiotensin II antagonist and the selective or dual acting neutral endopeptidase inhibitor produced significant reductions in left ventricular end diastolic pressure (LVEDP) and left ventricular systolic pressure (LVSP) that were greater than those produced by either treatment alone. Thus, the combination of this particular angiotensin II antagonist and the selective or dual acting neutral endopeptidase inhibitor is useful in treating hypertension and/or congestive heart failure.

The angiotensin II antagonist employed within this invention is the compound 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4 -yl]methyl]-1,3-diazaspiro[4.4]nonan-4-one having the structural formula

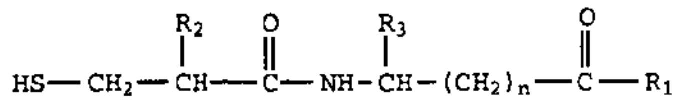
(I)



known in the literature as SR47436, BMS 186295, or irbesartan and pharmaceutically acceptable salts thereof such as the potassium and sodium salts. These angiotensin II antagonists and their method of preparation are disclosed by Bernhart et al. in United States Patent 5,270,317.

The selective neutral endopeptiadase inhibitor for use within this invention are those of the formula

(II)



and pharmaceutically acceptable salts thereof wherein:

R_2 is alkyl of 1 to 7 carbons, trifluoromethyl, phenyl, substituted phenyl, $-(CH_2)_{1\ to\ 4}$-phenyl, or $-(CH_2)_{1\ to\ 4}$-substituted phenyl;

R_3 is hydrogen, alkyl of 1 to 7 carbons, phenyl, substituted phenyl, $-(CH_2)_{1\ to\ 4}$-phenyl, or $-(CH_2)_{1\ to\ 4}$-substituted phenyl;

R_1 is hydroxy, alkoxy of 1 to 7 carbons, or NH_2;

n is an integer from 1 to 15; and

the term substituted phenyl refers to a substituent selected from lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4 carbons, lower alkylthio of 1 to 4 carbons, hydroxy, Cl, Br, or F.

Preferred are the selective neutral endopeptidase inhibitors of formula II wherein:

R_2 is benzyl;

R_3 is hydrogen;

n is an integer from 1 to 9; and

R_1 is hydroxy.

Most preferred for use in this invention is the selective neutral endopeptidase inhibitor of formula II reported in the literature as SQ 28,603 which is the compound of formula II wherein:

R_2 is benzyl;

R_3 is hydrogen;

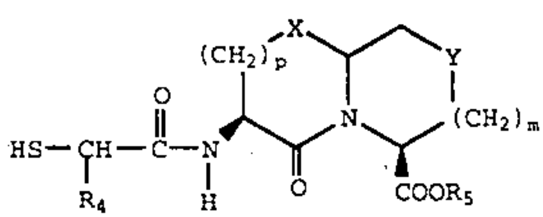
n is one; and

R_1 is hydroxy.

The preparation of the selective neutral endopeptidase inhibitors of formula II wherein R_2 is other than trifluoromethyl are disclosed by Delaney et al. in United States Patent 4,722,810. The preparation of the selective neutral endopeptidase inhibitors of formula II wherein R_2 is trifluoromethyl are disclosed by Delaney et al in United States Patent 5,223,516.

Dual acting neutral endopeptidase inhibitors suitable for use within this invention are compounds which possess both neutral endopeptidase inhibiting activity and angiotensin converting enzyme inhibiting activity. Particularly useful are the dual acting inhibitors of the formula

(III)



and pharmaceutically acceptable salts thereof wherein:

p is one or two;

X is O or S;

m is zero or one;

Y is CH_2, S or O provided that Y is S or O only when m is one;

R_4 is hydrogen, alkyl of 1 to 7 carbons, phenyl, substituted phenyl, $-(CH_2)_{1\ to\ 4}$-phenyl, $-(CH_2)_{1\ to\ 4}$-substituted phenyl, cycloalkyl of 3 to 7 carbons, $-(CH_2)_{1\ to\ 4}$-cycloalkyl of 3 to 7 carbons, heteroaryl, and $-(CH_2)_{1\ to\ 4}$-heteroaryl;

R_5 is hydrogen, alkyl of 1 to 7 carbons, $-(CH_2)_{1\ to\ 4}$-phenyl and $-(CH_2)_{1\ to\ 4}$-substituted phenyl;

the term substituted phenyl refers to a substituent selected from lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4 carbons, lower alkylthio of 1 to 4 carbons, hydroxy, Cl, Br, or F; and the term heteroaryl refers to monocyclic rings of 5 or 6 atoms containing one or two O and S atoms and/or one to four N atoms provided that the total number of heteroatoms in the ring is 4 or less and bicyclic rings wherein the 5 or 6 membered heteroaryl ring as defined above is fused to a benzene or pyridyl ring.

Preferred are the dual acting neutral endopeptidase inhibitors of formula III wherein:

R_4 is benzyl, cyclopropylmethyl, or straight or branched chain alkyl of 3 to 5 carbons;
p is one or two;
X is O or S;
m is zero or one;
Y is CH_2, S, or O provided that Y is S or O when m is one; and
R_5 is hydrogen.

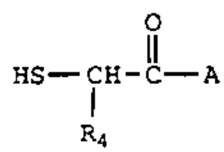
Most preferred for use in this invention is the dual acting neutral endopeptidase inhibitor of formula III wherein:

R_4 is benzyl;
p is two;
Y is S;
m is one;
Y is CH_2 ; and
R_5 is hydrogen.

The dual acting neutral endopeptidase inhibitors of formula III are disclosed in European Patent Application 629,627 of Robl et al.

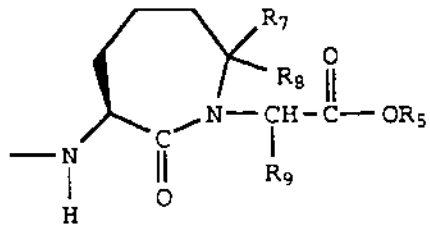
Also useful as neutral endopeptidase inhibitors for use within this invention are the dual acting inhibitors of the formula

(IV)



and pharmaceutically acceptable salts thereof wherein:

A is



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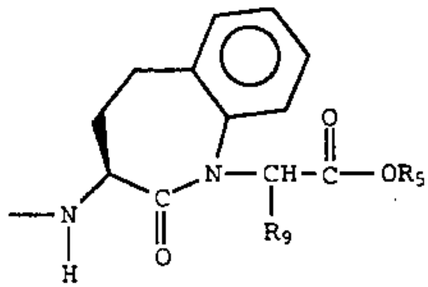

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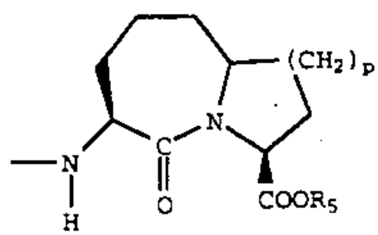
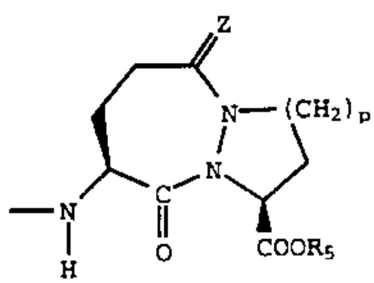


, or



R_4, R_5, and p are as defined above;

R_7 and R_8 are both hydrogen, or both alkyl of 1 to 7 carbons, or R_7 is hydrogen and R_8 is alkyl of 1 to 7 carbons, phenyl, $-(CH_2)_{1\ to\ 4}$-phenyl and $-(CH_2)_{1\ to\ 4}$-substituted phenyl, or R_7 and R_8 taken together with the carbon to which they are attached complete a cycloalkyl of 3 to 5 carbons.

R_9 is hydrogen or alkyl of 1 to 7 carbons.

Z is oxo or two hydrogens.

Preferred are the dual acting neutral endopeptidase inhibitors of formula IV wherein:

R_4 is benzyl;

R_7 and R_8 are both methyl;

R_9 is hydrogen or methyl, especially hydrogen;

p is one or two; and

Z is oxo.

The compounds of formula IV and their method of preparation are disclosed in European Patent Application 599,444 and U.S. Patent Application Serial No. 160,540 filed December 1, 1993.

The angiotensin II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl-1,3-diazaspiro[4.4]nonan-4-one and the selective neutral endopeptidase inhibitor or dual acting neutral endopeptidase inhibitor may be administered from a single dosage form containing both types of compounds, may be administered in separate dosage forms taken at the same time, or may be administered separately on a carefully coordinated schedule. If administered separately, the two compounds can be administered from within several minutes of each other up to about 4 hours apart.

The selective or dual acting neutral endopeptidase inhibitor can be administered at a dosage range of from about 0.03 to about 1000 mg. per kg. of body weight per day with a dosage range of from about 0.3 to about 300 mg. per kg. of body weight per day being preferred. The angiotensin II antagonist can be administered at a dosage range of from about 0.001 to about 50 mg. per kg. of body weight with a dosage range of from about 0.1 to about 10 mg. per kg. of body weight being preferred.

Both compounds can be administered orally, parenterally, or one orally and the other parenterally. Each compound may be administered from one to about four times per day depending upon the duration of activity of the compounds and the severity of the congestive heart failure and/or hypertension being treated.

The compounds can be formulated, in the amounts described above, according to accepted pharmaceutical practice with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., in the particular type of unit dosage form.

Illustrative of the adjuvents which may be incorporated in tablets are the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate or cellulose; a disintegrating agent such as corn starch, potato starch, alginic acid of the like; a lubricant such as stearic acid or magnesium stearate; a sweetening agent such as sucrose, aspartame, lactose or saccharin; a flavoring agent such as orange, peppermint, oil of wintergreen or cherry. When the dosage unit form is a capsule, it may contain in addition to materials of the above type a liquid carrier such as a fatty oil. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain the active compound, water, alcohol or the like as the carrier, glycerol as stabilizer, sucrose as sweetening agent, methyl and propyl parabens as preservatives, a dye and a flavoring such as cherry or orange.

In the following examples, BMS 186295 refers to SR47436, i.e. the compound 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]-methyl]-1,3-diazaspiro[4.4]nonan-4-one, and SQ 28603 refers to the compound (±)-N-[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]-β-alanine.

Example 1

The studies described in this experiment were conducted in male hamsters of the BIO TO-2 strain when they were approximately 260 days of age and weighed on average 115 g. These animals develop a genetic form of cardiomyopathy that progresses uniformly among animals through different stages of heart failure. By 240 - 300 days of age the cardiomyopathic hamsters are characterized (as compared with control hamsters) by low mean arterial pressure, a 40% reduction in cardiac output and a decrease in renal blood flow. They display elevated cardiac filling pressure, depressed ventricular function, increased peripheral vascular resistance and have an 8-10-fold increase in plasma atrial natriuretic peptide concentration. Since most animals at this age do not have gross peripheral edema or elevated plasma renin activity, the cardiomyopathic hamsters were considered to be in compensated heart failure.

The experiments were conducted in conscious, unrestrained, cardiomyphathic hamsters three hours after placement of cardivascular catheters using brief anesthesia. The catheters allowed measurement of mean arterial pressure, left ventricular end diastolic pressure, left ventricular systolic pressure and heart rate, and provided a means for the administration of agents intravenously.

a) Inhibition of The Pressor Response To Angiotensin II

Preliminary experiments were conducted in conscious cardiomyopathic hamsters to determine a dose regimen of BMS 186295 that would nearly completely block the pressor response to angiotensin II for at least two hours. The pressor responses to two challenges of angiotensin II (100 ng/kg, i.v. dissolved in 0.9% sodium chloride, 1 ml/kg) were determined. This dose of angiotensin II produced over a 30% increase in mean arterial pressure. Based on the preliminary experiments, BMS 186295 was administered to 5 cardiomyopathic hamsters at 30 µmol/kg, i.v. followed by continuous i.v. infusion at 1 µmol/kg per min. Challenges of angiotensin II were then repeated at 10- to 30-minute intervals up to 150 minutes following the bolus injection of BMS 186295. The results are shown below.

Minutes	Change in mean arterial pressure, mm Hg
-20′	29±2
-10′	31±3
BMS-186295,	30 µmol/kg, i.v. followed by 1 µmol/kg/min, i.v.
10′	3±2
20′	2±1
30′	3±2
40′	2±1
50′	1±1
60′	4±1
70′	5±2
80′	4±1
90″	3±1
120	3±1
150′	2±1

b) Cardiovascular Effects Of BMS 186295, SQ 28603, And the Combination Of These Agents

In this series of experiments baseline measurements of left ventricular end diastolic pressure, left ventricular systolic pressure and heart rate were determined in groups of conscious cardiomyopathic hamsters. Compounds or vehicle were administered intravenously, and measurements were repeated at 30-minute intervals up to 90 minutes after administration of the last agent. BMS 186295 was administered at 30 µmol/kg, i.v. (0.3 ml) followed by a continuous i.v. infusion at 1 µmol/kg per min (0.01 ml/min). BMS 186295 was prepared in 0.028 M potassium hydroxide and diluted to a final concentration of 0.17 M potassium hydroxide. Potassium hydroxide solution (0.17 M) was administered intravenously to the vehicle group at 0.3 ml followed by a continuous infusion at 0.01 ml/min. SQ 28603 was dissolved in 0.84% sodium bicarbonate and administered at 30 µmol/kg, i.v. This dose of SQ 28603 was previously shown to result in a doubling of plasma atrial natriuretic peptide concentration within 90 minutes in this model. One group of cardiomyopathic hamsters received the combination of BMS 186295 and SQ 28603. In this group BMS 186295 was administered according to the same dosage regimen described above; 30 minutes after the bolus injection of BMS 186295, SQ 28603 was administered at 30 µmol/kg, i.v.

Differences in age, body weight and baseline values among groups were evaluated by analysis of variance. Differences in changes from baseline among groups were evaluated by analysis of covariance with repeated measures and contrasts. The baseline value for each variable was used as the covariate. The level of significance was taken at $P < 0.05$. All data are expressed as mean ± standard error of the mean.

Left Ventricular End Diastolic Pressure (mm Hg)				
Minutes	Vehicle	SQ 28603	BMS 186295	BMS 186295 & SQ 28603
Baseline	19 ± 2	18 ± 3	17 ± 2	21 ± 2
BMS 186295 SQ 28603	18 ± 2	14 ± 3	18 ± 2	20 ± 2
30′	19 ± 1	14 ± 2	16 ± 2	12 ± 1
60′	18 ± 1	17 ± 3	16 ± 2	11 ± 2
90′	16 ± 2	16 ± 3	18 ± 3	10 ± 1

Change From Baseline (mm Hg)				
Minutes after last treatment	Vehicle	SQ 28603	BMS 186295	BMS 186296 & SQ 28603
30	1 ± 1	4 ± 1*	-1 ± 1	-10 ± 2*
60	-1 ± 1	-1 ± 2	-1 ± 1	-10 ± 3*†
90	-3 ± 1	-2 ± 1	1 ± 2	-11 ± 3*†

*P <0.05 vs Vehicle
†P <0.05 vs SQ 28603

Left Ventricular Systolic Pressure (mm Hg)				
Minutes	Vehicle	SQ 28603	BMS 186295	BMS 186295 & SQ 28603
Baseline	111 ± 3	117 ± 5	112 ± 2	107 ± 3
BMS 186295 SQ 28603	111 ± 3	108 ± 5	111 ± 1	104 ± 4
30′	111 ± 5	109 ± 5	114 ± 2	92 ± 2
60′	108 ± 3	105 ± 4	111 ± 2	88 ± 5
90′	105 ± 5	105 ± 5	112 ± 5	89 ± 4

Change From Baseline (mm Hg)				
Minutes After Last Treatment	Vehicle	SQ 28603	BMS 186295	BMS 186296 & SQ 28603
30	-1 ± 3	-8 ± 1*	2 ± 2	-16 ± 3*†
60	-3 ± 1	-12 ± 3*	-1 ± 2	-20 ± 4*†
90	-6 ± 4	-12 ± 2	1 ± 5	-18 ± 4*

*P <0.05 vs Vehicle
†P <0.05 vs SQ 28603

Heart Rate (beats/min)				
Minutes	Vehicle	SQ 28603	BMS 186295	BMS 186295 & SQ 28603
Baseline	350 ± 10	378 ± 12	338 ± 16	364 ± 6
BMS 186295 SQ 28603	365 ± 8	380 ± 7	364 ± 14	366 ± 12
30′	347 ± 15	381 ± 9	363 ± 20	366 ± 11
60′	345 ± 15	366 ± 9	367 ± 18	353 ± 10
90′	354 ± 13	378 ± 8	369 ± 28	351 ± 9

Change From Baseline (mm Hg)				
Minutes after last treatment	Vehicle	SQ 28603	BMS 186295	BMS 186296 & SQ 28603
30	-3 ± 15	3 ± 8	25 ± 9	1 ± 6
60	-5 ± 15	-13 ± 12*	29 ± 7*	-11 ± 9
90	7 ± 13	-1 ± 9	44 ± 11*	-14 ± 5

*P <0.05 vs Vehicle

Discussion of Results

Following the administration of BMS 186295, the pressor responses to angiotensin II were less than 17% of the response before the administration of the inhibitor. These results indicate that nearly complete inhibition of the pressor response to angiotensin II was achieved following the administered dosage regimen of BMS 186295, and suggests effective blockade of the angiotensin II receptors.

The combination of BMS 186295 and SQ 28603 produced cardiovascular effects that were greater than those with either treatment alone. Specifically, the combination caused significant decreases in left ventricular end diastolic pressure and left ventricular systolic pressure with no significant change in heart rate. SQ 28603 produced smaller decreases, whereas BMS 186295 had no significant effects on the measured cardiovascular pressures. Thus, the combination of BMS 186295 and SQ 28603 produced beneficial hemodynamic effects in cardiomyopathic hamsters with compensated heart failure.

Example 2

The studies described in this experiment were conducted in dogs that had been rendered hypertensive by prior unilateral nephrectomy and constriction of the remaining renal artery. This model is characterized by normal basal levels of plasma renin activity and is relatively resistant to the anti-hypertensive activity of angiotensin converting enzyme inhibitors and AT_1 receptor antagonists. Furthermore, the 1-kidney-1-clip (IKIC) hypertensive dogs have normal plasma concentrations of atrial natriutetic peptide and fail to develop depressor responses to neutral endopeptidase inhibitors.

The following experiments were conducted in fasted 1K1C hypertensive dogs lightly restrained in standard canine slings. An indwelling arterial catheter was accessed via a subcutaneous port for measurement of blood pressure via a Gould-Statham pressure transducer. Mean arterial pressure (MAP) was continuously recorded on a Gould chart writer and stored electronically using a Po-Ne-Mah data acquisition system. During each study, urine was collected at 20 minute intervals via a Foley bladder catheter for determination of urine volume. The concentrations of urinary sodium and potassium were measured using ion-selective electrodes and their rates of urinary excretion (µEq/min) were calculated. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined by the renal clearances of exogenous creatinine and para-aminohippuric acid (PAH), respectively. The concentrations of creatinine and PAH in sequential samples of urine and plasma were determined by spectrophotometric assays and the clearances were calculated by the standard formula.

Arterial blood samples were drawn at the end of the control period and at 60 minute intervals thereafter for determination of the plasma concentrations of atrial natriuretic peptide (ANP), cyclic GMP and plasma renin activity (PRA) by separate radioimmunoassays. The plasma and urine samples were preserved and the assays were conducted according to standard radioimmunoassay procedures. Urinary excretion rates of cyclic GMP and ANP were calculated and expressed as pmol/min and fmol/min, respectively.

Four 1K1C hypertensive dogs were treated with the combination of 30 µmol/kg iv of BMS 186295 and 30 µmol/kg iv of SQ 28603. Vehicle (0.84% sodium bicarbonate), 30 µmol/kg iv of SQ 28603 and 30 µmol/kg iv of BMS 186295 were tested in 3 additional groups of 1K1C hypertensive dogs (n=4 to 5/treatment). In each study, baseline measurements were obtained during two 20 minute control periods. One of the treatments was then administered and sampling continued at 20 minute intervals for three hours.

To minimize inter-animal variability, each data point was expressed as the change from the average control value for that parameter. Significant differences among treatments were identified by analysis of variance for repeated measures. Contrasts were calculated to identify significant differences from the effects of v hicle and to compare the combination of SQ 28603 and BMS 186295 to the individual treatments. Results are given as mean ± SEM.

Results

Table 1

Mean Arterial Pressure (mm Hg)				
Time (min) after Treatment	Vehicle (n=5)	SQ 28603 (n=4)	BMS 186295 (n=4)	SQ 28603 + BMS 186295 (n=4)
Control	132±3	132±8	157±7	140±3
	Change from control			
20	3±1	6±1	-1±1	2±2
40	3±2	9±3 *	-10±1 *	2±1 †§
60	2±1	8±3 *	-8±2 *	0±2 †§
80	6±2	8±2	-5±2 *	-1±3 *
100	6±2	5±3	-4±1*	1±2 †
120	6±2	3±4	-1±2 *	2±2
140	7±2	4±2	-1±2 *	6±2 †
160	5±2	5±2	0±2	9±3 †
180	6±3	7±3	-1±3 *	6±4 †

* $p<0.05$ compared to vehicle
† $p<0.05$ compared to BMS 186295
§ $p<0.05$ compared to SQ 28603

BMS 186295 signigicantly reduced mean arterial pressure (MAP) (Table 1) in the conscious 1K1C hypertensive dogs whereas SQ 28603 initially increased MAP. The effects of the combination BMS 186295 and SQ 28603 were not consistently different from those of vehicle.

TABLE 2

Sodium Excretion (µEq/min)				
Time (min) after Treatment	Vehicle (n=5)	SQ 28603 (n=4)	BMS 186295 (n=4)	SQ 28603 + BMS 186295 (n=4)
Control	60±14	40±20	18±2	18±6
	Change from control			
20	-17±10	13±4	23±9	62±31 *†§
40	-16±12	21±16 *	41±13 *	83±34 *†§
60	-12±14	14±9 *	36±8 *	87±18 *†§
80	-11±14	25±13 *	27±6 *	70±16 *†§
100	-12±11	24±12 *	22±3 *	54±12 *†§
120	-16±13	35±19 *	30±4 *	60±21 *†§
140	-10±14	39±18 *	28±7 *	69±22 *†§
160	-4±15	44±19 *	30±7 *	68±16 *†
180	-3±13	44±18 *	30±2 *	74±19 *†§

* $p<0.05$ compared to vehicle
*† $p<0.05$ compared to *vehicle or †BMS186295
*†§ $p<0.05$ compared to *vehicle, †BMS186295 or SQ28603

TABLE 3

Urine Volume (ml/min)				
Time (min) after Treatment	Vehicle (n=5)	SQ 28603 (n=4)	BMS 186295 (n=4)	SQ 28603 + BMS 186295 (n=4)
Control	0.64±0.14	0.38±0.13	0.43±0.16	0.36±0.13
	Change from control			
20	-0.20±0.12	0.29±0.31 *	0.01±0.07	0.32±0.17 *
40	-0.27±0.14	0.15±0.13 *	0.09±0.14 *	0.51±0.22 *†§
60	-0.26±0.14	0.18±0.14 *	0.12±0.07 *	0.51±0.11 *†§
80	-0.26±0.15	0.21±0.15 *	0.05±0.06 *	0.34±0.08 *†
100	-0.23±0.14	0.07±0.08 *	-0.02±0.09 *	0.14±0.08 *
120	-0.30±0.13	0.12±0.13 *	-0.02±0.10 *	0.20±0.07 *
140	-0.25±0.14	0.20±0.10 *	-0.07±0.12 *	0.26±0.09 *†
160	-0.23±0.15	0.24±0.11 *	-0.07±0.16	0.16±0.03 *†
180	-0.22±0.14	0.17±0.06 *	-0.02±0.07 *	0.17±0.03 *

* $p<0.05$ compared to vehicle
*† $p<0.05$ compared to *vehicle or †BMS186295
*†§ $p<0.05$ compared to *vehicle, †BMS186295 or SQ28603

BMS 186295 and SQ 28603 each individually increased sodium excretion (TABLE 2) and urine volume (TABLE 3) in the conscious 1K1C hypertensive dogs. The natriuretic response to the combination of BMS 186295 and SQ 28603 was greater than the activity of either of the compounds administered singly. The increase in the amount of sodium excreted during the 3 hours after simultaneous injections of BMS 186295 and SQ 28603 (12.6±3.4 mEq/3 hr) approximated the sum of the natriuretic responses to BMS 186295 (5.4±1.0 mEq/3 hr) and to SQ 28603 (5.2±2.3 mEq/3 hr) given individually.

TABLE 4

Glomerular Filtration Rate (ml/min)				
Time (min) after Treatment	Vehicle (n=5)	SQ 28603 (n=4)	BMS 186295 (n=4)	SQ 28603 + BMS 186295 (n=4)
Control	50±4	39±5	45±10	46±4
	Change from control			
20	-2±2	6±11	-4±2	-8±7 §
40	0±4	3±2	-2±5	6±3 †
60	2±3	6±5	6±0	11±2 *
80	0±3	5±2	5±2	7±1 *
100	4±3	5±4	3±4	4±4
120	-1±2	5±1	4±3	12±2 *§
140	1±2	7±4	0±5	9±1 *
160	5±4	9±5	0±7	6±2
180	5±3	10±4	0±4	5±5

* $p<0.05$ compared to vehicle
§ $p<0.05$ compared to SQ 28603
† $p<0.05$ compared to BMS 186295
*§ $p<0.05$ compared to *vehicle or §SQ28603

TABLE 5

Effective Renal Plasma Flow (ml/min)				
Time (min) after Treatment	Vehicle (n=5)	SQ 28603 (n=4)	BMS 186295 (n=3)	SQ 28603 + BMS 186295 (n=4)
Control	144±18	123±15	127±41	142±30
	Change from control			
20	-25±10	6±38	-68±30 *	-84±19 *§
40	-27±17	-5±15	-54±35	-64±27 *§
60	-25±16	3±20	-35±24	-51±21 §
80	30±18	-20±6	-13±9	-45±21 †
100	-26±14	-25±10	-1±10	-41±23 †
120	-41±14	-15±6	-8±17 *	-15±8
140	-32±12	-11±12	8±3 *	-12±12
160	-21±13	-1±12	-14±12	-28±14
180	-21±15	-4±9	-12±11	-23±16

* p<0.05 compared to vehicle
*§ p<0.05 compared to *vehicle or §SQ28603
† p<0.05 compared to BMS 186295
§ p<0.05 compared to SQ 28603

The combination of BMS 186295 and SQ 28603 significantly increased GFR (TABLE 4) at several times during the 3 hour test when compared with the effects of vehicle even though effective renal plasma flow (TABLE 5) did not increase. The increase in GFR alone did not account for the full natriuretic response, as indicated by a significantly rise in fractional sodium excretion from 0.26±0.07% to 1.28±0.29%.

TABLE 6

ANP Excretion (fmol/min)				
Time (min) after Treatment	Vehicle (n=5)	SQ 28603 (n=4)	BMS 186295 (n=4)	SQ 28603 + BMS 186295 (n=4)
Control	1.2±0.1	1.9±0.2	1.5±0.6	1.5±0.4
	Change from control			
20	-0.1±0.1	20.8±4.9 *	-0.7±0.5	3.7±1.5
40	-0.1±0.1	24.0±4.6 *	-0.6±0.5	13.8±2.2
60	-0.1±0.1	45.2±24.2 *	-0.2±0.3	40.7±21.1 *†
80	-0.1±0.1	55.5±19.7 *	-0.4±0.3	30.3±8.1 *†§
100	-0.0±0.1	41.0±12.3 *	-0.6±0.4	27.0±10.9 *†
120	-0.2±0.1	48.3±21.5 *	-0.5±0.4	39.4±13.7 *†
140	-0.0±0.1	41.8±14.2 *	-0.6±0.4	37.2±14.9 *†
160	0.3±0.2	36.9±12.4 *	-0.5±0.4	30.1±12.4 *†
180	0.0±0.2	29.0±7.9 *	-0.6±0.4	33.2±10.0 *†§

* $p<0.05$ compared to vehicle

*† $p<0.05$ compared to *vehicle or †BMS186295

*†§ $p<0.05$ compared to *vehicle, †BMS186295 or SQ28603

TABLE 7

Cyclic GMP Excretion (pmol/min)				
Time (min) after Treatment	Vehicle (n=5)	SQ 28603 (n=4)	BMS 186295 (n=4)	SQ 28603 + BMS 186295 (n=4)
Control	1106±85	1017±180	1122±389	1030±170
	Change from control			
20	-177±172	106±295	-372±240	-202±144
40	-185±41	101±280	-277±255	164±94
60	-78±207	338±206	-150±203	432±174
80	-205±231	226±154	-309±199	313±144
100	-180±129	128±178	-266±193	106±97
120	-229±121	117±220	-283±236	139±66
140	-52±195	121±182	-449±206	194±156
160	-24±223	100±246	-391±199	229±62
180	76±246	343±170	-611±346	316±59

Urinary excretion of ANP (TABLE 6) increased significantly after administration of SQ 28603 alone and in combination with BMS 186295, indicating that the NEP inhibitor had prevented the degradation of ANP. Cyclic GMP (TABLE 7), the second messenger of the biological ANP receptor, t nded to increase in the dogs receiving SQ 28603 alone (+32±28 nmol/3 hr) or the combination of BMS 186295 and SQ 28603 (+34±12 nmol/3 hr), but because of the variability of the response, these changes did not achieve statistical significance compared to vehicle (-21±15 nmol/3 hr). These data suggested that the protection of renal ANP contributed to the natriuretic response. BMS 186295 given alone did not affect ANP excretion nor did it alter the ANP response to SQ 28603. Therefore, the enhanced response to the combination of BMS 186295 and SQ 28603 could not be attributed to an additional effect of the angiotensin II antagonist on the renal metabolism of ANP or the resultant accumulation of cyclic GMP.

TABLE 8

Plasma Renin Activity (pmol AI/ml/hr)				
Time (min) after Treatment	Vehicle (n=5)	SQ 28603 (n=4)	BMS 186295 (n=4)	SQ 28603 + BMS 186295 (n=4)
Control	0.45±0.10	0.16±0.02	0.55±0.07	-/90±0.09
	Change from control			
60	-0.09±0.07	-0.07±0.05	1.28±0.51 *	0.39±0.42 †
120	-0.04±0.11	-0.02±0.05	1.49±0.57 *	0.58±0.44 *†
180	-0.03±0.11	-0.01±0.7	1.24±0.62 *	0.36±0.16 †

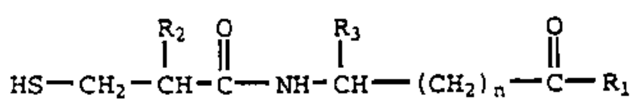
* $p<0.05$ compared to vehicle
† $p<0.05$ compared to BMS 186295
*† $p<0.05$ compared to *vehicle or †BMS186295

Finally, BMS 186295 significantly increased PRA (TABLE 8) indicating that the angiotensin receptor antagonist interrupted the negative feedback of angiotensin II on renin release. The smaller PRA response to BMS 186295 in the presence of SQ 28603 may be attributed to the inhibition of renin release by the increased ANP levels. Alternatively, BMS 186295 may have also activated the intrarenal baroreceptor by virtue of its depressor activity and thereby increased renin secretion.

Claims

1. Use of angiotensin II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]-nonan-4-one or a pharmaceutically acceptable salt thereof and a selective neutral endopeptidase inhibitor or a dual acting neutral endopeptidase inhibitor for manufacturing a medicament for treating hypertension and/or congestive heart failure in a mammalian specie in need of such treatment.

2. The use of Claim 1 wherein said endopeptidase inhibitor is a selective neutral endopeptidase inhibitor of the formula



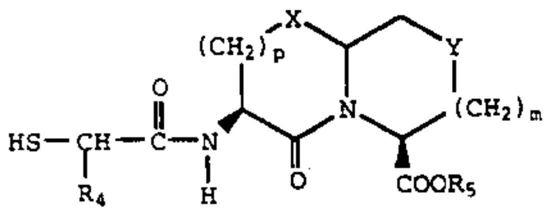
or a pharmaceutically acceptable salt thereof wherein:
R_2 is alkyl of 1 to 7 carbons, trifluoromethyl, phenyl, substituted phenyl, $-(CH_2)_{1\ to\ 4}$-phenyl or $-(CH_2)_{1\ to\ 4}$-substituted phenyl;
R_3 is hydrogen, alkyl of 1 to 7 carbons, phenyl, substituted phenyl, $-(CH_2)_{1\ to\ 4}$-phenyl, or $-(CH_2)_{1\ to\ 4}$-substituted phenyl;
R_1 is hydroxy, alkoxy of 1 to 7 carbons, or NH_2; and
n is an integer from 1 to 15.

3. The method of Claim 2 wherein:
R_2 is benzyl;
R_3 is hydrogen;

n is an integer from 1 to 9; and
R_1 is hydroxy.

4. The use of Claim 2 wherein:
R_2 is benzyl;
R_3 is hydrogen;
n is one; and
R_1 is hydroxy.

5. The use of Claim 1 wherein said endopeptidase inhibitors is a dual acting inhibitor of the formula

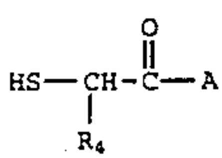


or a pharmaceutically acceptable salt thereof wherein:
p is one or two;
X is O or S;
m is zero or one;
Y is CH_2, S or O provided that Y is S or O only when m is one;
R_4 is hydrogen, alkyl of 1 to 7 carbons, phenyl, substituted phenyl, $-(CH_2)_{1\ to\ 4}$-phenyl, $-(CH_2)_{1\ to\ 4}$-substituted phenyl, cycloalkyl of 3 to 7 carbons, $-(CH_2)_{1\ to\ 4}$-cycloalkyl of 3 to 7 carbons, heteroaryl, and $-(CH_2)_{1\ to\ 4}$-heteroaryl; and
R_5 is hydrogen, alkyl of 1 to 7 carbons, $-(CH_2)_{1\ to\ 4}$-phenyl and $-(CH_2)_{1\ to\ 4}$-substituted phenyl.

6. The use of Claim 5 wherein:
R_4 is benzyl, cyclopropylmethyl, or straight or branched chain alkyl of 3 to 5 carbons;
p is one or two;
X is O or S;
m is zero or one;
Y is CH_2, S, or O provided that Y is S or O when m is one; and
R_5 is hydrogen.

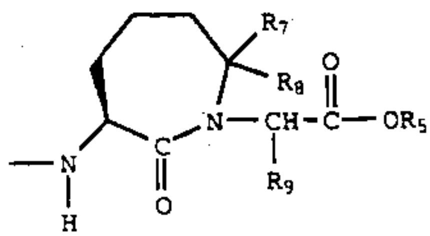
7. The use of Claim 5 wherein:
R_4 is benzyl;
p is two;
Y is S;
m is one;
Y is CH_2 ; and
R_5 is hydrogen.

8. The use of Claim 1 wherein said endopeptidase inhibitor is a dual acting inhibitor of the formula



or a pharmaceutically acceptable salt thereof wherein:

A is



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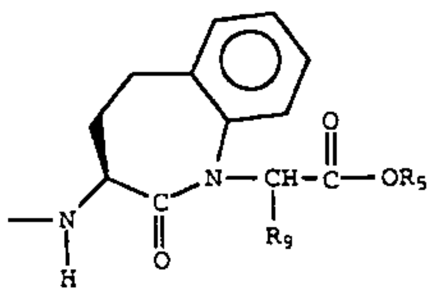

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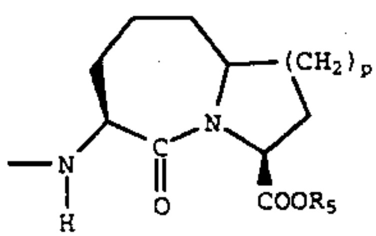

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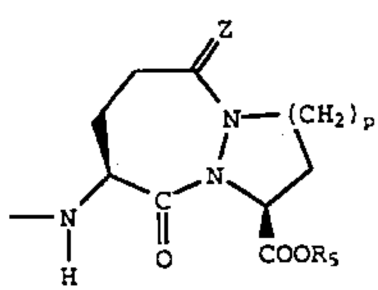


;

p is one or two;

R_4 is hydrogen, alkyl of 1 to 7 carbons, phenyl, substituted phenyl, $-(CH_2)_{1\ to\ 4}$-phenyl, $-(CH_2)_{1\ to\ 4}$-substituted phenyl, cycloalkyl of 3 to 7 carbons, $-(CH_2)_{1\ to\ 4}$-cycloalkyl of 3 to 7 carbons, heteroaryl, and $-(CH_2)_{1\ to\ 4}$-heteroaryl;

R_5 is hydrogen, alkyl of 1 to 7 carbons, $-(CH_2)_{1\ to\ 4}$-phenyl and $-(CH_2)_{1\ to\ 4}$-substituted phenyl;

R_7 and R_8 are both hydrogen, or both alkyl of 1 to 7 carbons, or R_7 is hydrogen and R_8 is alkyl of 1 to 7 carbons, phenyl, $-(CH_2)_{1\ to\ 4}$-phenyl and $-(CH_2)_{1\ to\ 4}$-substituted phenyl, or R_7 and R_8 taken together with the carbon to which they are attached complete a cycloalkyl of 3 to 5 carbons;

R_9 is hydrogen or alkyl of 1 to 7 carbons; and

Z is oxo or two hydrogens.

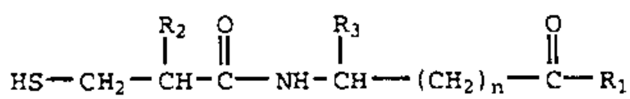
9. The use of Claim 8 wherein:
R_4 is benzyl;
R_7 and R_8 are both methyl;
R_9 is hydrogen or methyl, especially hydrogen;
p is one or two; and
Z is oxo.

10. The use of Claim 1 wherein said angiotensin II antagonist and said selective neutral endopeptidase inhibitor or said dual acting neutral endopeptidase inhibitor are administered from a single dosage form containing both types of compounds.

11. The use of Claim 1 wherein said angiotensin II antagonist and said selective neutral endopeptidase inhibitor or said dual acting neutral endopeptidase inhibitor are administered from separate dosage forms at about the same time.

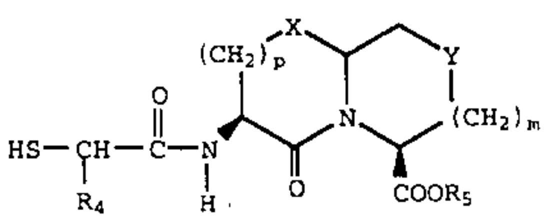
12. The use of Claim 1 wherein said angiotensin II antagonist and said selective neutral endopeptidase inhibitor or said dual acting neutral endopeptidase inhibitor are administered from separate dosage forms at from within several minutes of each other up to about 4 hours apart.

13. A composition useful for treating congestive heart failure and/or hypertension comprising a pharmaceutically acceptable carrier and an effective amount of the angiotensin II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]nonan-4-one or a pharmaceutically acceptable salt thereof and an effective amount of the selective neutral endopeptidase inhibitor of the formula



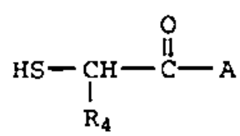
or a pharmaceutically acceptable salt thereof wherein R_1, R_2, R_3 and n are as defined in Claim 2.

14. A composition useful for treating congestive heart failure and/or hypertension comprising a pharmaceutically acceptable carrier and an effective amount of the angiotensin II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]nonan-4-one or a pharmaceutically acceptable salt thereof and an effective amount of the dual acting neutral endopeptidase inhibitor of the formula



or a pharmaceutically acceptable salt thereof wherein X,Y, m, p, R_4, and R_5 are as defined in Claim 5.

15. A composition useful for treating congestive heart failure and/or hypertension comprising a pharmaceutically acceptable carrier and an effective amount of the angiotensin II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)-[1,1'biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]nonan-4-one or a pharmaceutically acceptable salt thereof and an effective amount of the dual acting neutral endopeptidase inhibitor of the formula



or a pharmaceutically acceptable salt thereof wherein A and R_4 are as defined in Claim 8.

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(54) Composition for the treatment of hypertension and congestive heart failure, containing an angiotensin II antagonist and an endopeptidase inhibitor

(57) Hypertension and/or congestive heart failure are treated with the combination of the angiotensin II antagonist 2-butyl-6,7,8,9-tetrahydro-3-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]nonan-4-one and a selective neutral endopeptidase inhibitor or a dual acting neutral endopeptidase inhibitor.

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Application Number EP 96 10 1756

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	EP 0 629 408 A (MERCK SHARP & DOHME ;INST NAT SANTE RECH MED (FR)) 21 December 1994 * abstract * * page 2, line 13 - line 38; claims 1,6,9 *	1,10-12	A61K31/415 A61K45/06
Y	* claims 1,6,9 * ---	2-9, 13-15	
D,X	EP 0 498 361 A (SCHERING CORP) 12 August 1992 * abstract; claims 1,4 *	1-4	
Y	* claims 1,4 * ---	5-15	
D,Y	US 5 270 317 A (BERNHART CLAUDE ET AL) 14 December 1993 * page 3, line 11 - line 33; claims 10-12 * * page 1, line 31 - line 33 * ---	1-15	
Y	EP 0 527 624 A (SQUIBB & SONS INC) 17 February 1993 * abstract; claims 1-4 *	1-15	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
D	& US 5 225 401 A --- -/--	1-15	A61K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	27 October 1997	Gonzalez Ramon, N

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
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L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 96 10 1756

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
Y	WO 92 10097 A (SMITHKLINE BEECHAM CORP) 25 June 1992 * abstract; claim 1 * * page 2, line 28 - line 35 * * page 24, line 33 - line 36 * * page 16; example 8 * ---	1-15	
Y	EP 0 566 157 A (SCHERING CORP) * claims 1-3,7,8,16 * -----	1-15	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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European Patent Office

INCOMPLETE SEARCH SHEET C

Application Number
EP 96 10 1756

Claim(s) searched completely:
-

Claim(s) searched incompletely:
1-15

Reason for the limitation of the search:

In view of the large number of compounds, which are defined by the general definition in the independent claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see Guidelines, Part B, Chapter III, paragraph 3.6).

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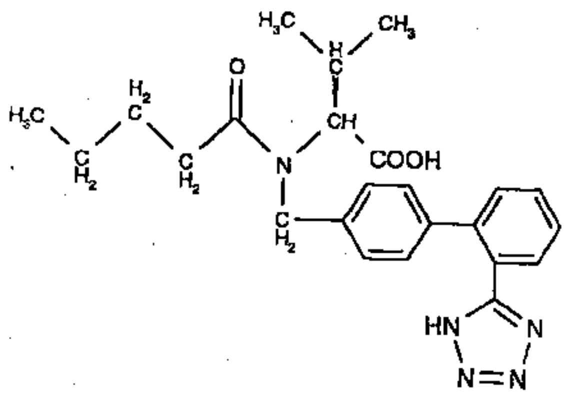
(54) Title: VALSARTAN SALTS

(57) **Abstract:** The invention relates to new salts of valsartan or crystalline, also partly crystalline and amorphous salts of valsartan, the respective production and usage, and pharmaceutical preparations containing such a salt.

VALSARTAN SALTS

The invention relates to new salts of the AT_1 receptor antagonist (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-yl-methyl]-amine (valsartan) of formula



(I).

The active ingredient valsartan is the free acid which is described specifically in EP 0443983, especially in example 16; it has two acidic hydrogen atoms: (i) the hydrogen atom (H atom) of the carboxyl group, and (ii) that of the tetrazole ring. Accordingly, one acidic H atom (primarily the carboxyl H atom) or both acidic H atoms may be replaced by a monovalent or higher valent, e.g. divalent, cation. Mixed salts may also be formed.

EP 443983 does not disclose any specific salts of valsartan. Also, it does not mention any special properties of salts. Meanwhile, the active ingredient valsartan has been introduced as an anti-hypertensive agent in a series of countries under the trade name DIOVAN.

The free acid valsartan has a melting point in a closed crucible of 80 to 95°C and in an open crucible of 105 to 110°C and a melting enthalpy of 12 kJ/mol. The optical rotation is $[\alpha]^{20}_{D} = (-70 \pm 2)^{\circ}$ for a concentration of c = 1% in methanol.

The density of the valsartan crystals and of the salt hydrates was determined by a helium pycnometer (Accupyc 1330 of Micromeritics, Norcross, GA, USA). The density for the crystals of the free acid valsartan is 1.20 ± 0.02.

The X-ray diffraction diagram consists essentially of a very broad, diffuse Xray reflection; the free acid is therefore characterised as almost amorphous under X-ray. The melting point linked with the measured melting enthalpy of 12 kJ/mol unequivocally confirm the existence of a considerable residual arrangement in the particles or structural domains for the free acid valsartan.

There is a need for more stable, e.g. crystalline forms of valsartan, which are even easier to manage in the drying or grinding processes following the final stage of the chemical preparation process and also in the steps for preparing the pharmaceutical formulations. Many futile attempts have been made to find improved forms through salt formation, the forms ideally being as crystalline as possible, as well as physically and chemically stable. Only the salts according to the invention, their solvates and polymorphous forms thereof exhibit the desired improved properties.

The formation of salts of valsartan with the desired advantageous properties has proved to be difficult. In the majority of cases, for example, amorphous salts with little stability are obtained (such as hard foams, waxes or oils). Extensive research has shown that the salts of valsartan according to the invention have proved to be particularly advantageous compared with the free acid valsartan.

The objects of the present invention are salts of valsartan which are selected from the group consisting of the monosodium salt, the monopotassium salt, the dipotassium salt, the magnesium salt, the calcium salt, the bis-diethylammonium salt, the bis-dipropylammonium salt, the bis-dibutylammonium salt, the mono-L-arginine salt, the bis-L-arginine salt, the mono-L-lysine salt and the bis-L-lysine salt, as well as salt mixtures, or respectively, an amorphous form, a solvate, especially hydrate, as well as a polymorphous form thereof, the respective production and usage, and pharmaceutical preparations containing such salts.

The objects of the present invention are salts of valsartan which are selected from the group consisting of the monosodium salt, the monopotassium salt, the dipotassium salt, the magnesium salt, the calcium salt, the bis-diethylammonium salt, the bis-dipropylammonium salt, the bis-dibutylammoniumsalt, the mono-L-arginine salt, the bis-L-arginine salt, the mono-L-lysine salt and the bis-L-lysine salt, or respectively, an amorphous form, a solvate, especially hydrate, as well as a polymorphous form thereof.

Salt mixtures are (i) single salt forms from different cations selected from the above group or (ii) mixtures of those single salt forms which exist for example in the form of conglomerates.

Preferred salts are for example selected from the
mono-sodium salt in amorphous form;
di-sodium salt of valsartan in amorphous or crystalline form, especially in hydrate form, thereof.
Mono-potassium salt of valsartan in amorphous form;
di-potassium salt of valsartan in amorphous or crystalline form, especially in hydrate form, thereof.
calcium salt of valsartan in crystalline form, especially in hydrate form, primarily the tetrahydrate thereof;
magnesium salt of valsartan in crystalline form, especially in hydrate form, primarily the hexahydrate thereof;
calcium/magnesium mixed salt of valsartan in crystalline form, especially in hydrate form;
bis-diethylammonium salt of valsartan in crystalline form, especially in hydrate form;
bis-dipropylammonium salt of valsartan in crystalline form, especially in hydrate form;
bis-dibutylammonium salt of valsartan in crystalline form, especially in hydrate form, primarily the hemihydrate thereof;
mono-L-arginine salt of valsartan in amorphous form;
bis-L-arginine salt of valsartan in amorphous form;
mono-L-lysine salt of valsartan in amorphous form;
bis-L-lysine salt of valsartan in amorphous form.

The salts according to the invention preferably exist in isolated and essentially pure form, for example in a degree of purity of >95%, preferably >98%, primarily >99%. The enantiomer purity of the salts according to the invention is >98%, preferably >99%.

Compared with the free acid, the salts according to the invention, or the amorphous forms, solvates such as salt hydrates, and also the corresponding polymorphous forms thereof, have unexpectedly advantageous properti s. Under given conditions, the crystalline salts and crystalline salt hydrates have a clear melting point which is linked with a marked, endothermic melting enthalpy. The crystalline salts according to the invention are stable and are of better quality than valsartan also during storage and distribution. The amorphous or partially amorphous salts have limited stability, i.e. as the solid, they have a restricted stability range. To be stabilised, they require certain measures which can be achieved for example by galenic formulations.

In addition, both the crystalline and the amorphous salts according to the invention have a high degree of dissociation in water and thus substantially improved water solubility. These properties are of advantage, since on the one hand the dissolving process is quicker and on the other hand a smaller amount of water is required for such solutions. Furthermore, the higher water solubility can, under certain conditions, also lead to increased biological availability of the salts or salt hydrates in the case of solid dosage forms. Improved properties are beneficial especially to the patients. Furthermore, some of the salts according to the invention have proved to be exceptionally physically stable, particularly the alkaline earth salts. For different relative humidities at room temperature and also at a slightly higher temperatures, the salt hydrates according to the invention show practically no water absorption or water loss over a wide range of humidities and for periods of a few hours, e.g. four hours. Also, for example, the melting point of the salts according to the invention will not be changed by storing under different relative humidities.

Improved physicochemical properties of certain salts or certain salt hydrates are of great importance both when they are produced as a pharmaceutically active substance and when producing, storing and applying the galenic preparation. In this way, starting with improved constancy of the physical parameters, an even higher quality of the formulations can be guaranteed. The high stability of the salts or salt hydrates also give the possibility of attaining economic advantages by enabling simpler process steps to be carried out during working up. The high crystallinity of certain salt hydrates allows the use of a choice of analytical methods, especially the various X-ray methods, the usage of which permits a clear and simple analysis of their release to be made. This factor is also of great importance to the quality of the active substance and its galenic forms during production, storage and administration to the patients. In addition, complex provisions for stabilising the active ingredient in the galenic formulations can be avoided.

The invention accordingly relates to crystalline, also partly crystalline and amorphous salts of valsartan.

As well as the solvates, such as hydrates, the invention also relates to polymorphous forms of the salts according to the Invention.

Solvates and also hydrates of the salts according to the invention may be present, for example, as hemi-, mono-, di-, tri-, tetra-, penta-, hexa-solvates or hydrates, respectively. Solvents used for crystallisation, such as alcohols, especially methanol, ethanol, aldehydes, ketones, especially acetone, esters, e.g. ethyl acetate, may be embedded in the crystal grating. The extent to which a selected solvent or water leads to a solvate or hydrate in crystallisation and in the subsequent process steps or leads directly to the free acid is generally unpredictable and depends on the combinations of process conditions and the various interactions between valsartan and the selected solvent, especially water. The respective stability of the resulting crystalline or amorphous solids in the form of salts, solvates and hydrates, as well as the corresponding salt solvates or salt hydrates, must be determined by experimentation. It is thus not possible to focus solely on the chemical composition and the stoichiometric ratio of the molecules in the resulting solid, since under these circumstances both differing crystalline solids and differing amorphous substances may be produced.

The description salt hydrates for corresponding hydrates may be preferred, as water molecules in the crystal structure are bound by strong intermolecular forces and thereby represent an essential element of structure formation of these crystals which, in part, are extraordinarily stable. However, water molecules are also existing in certain crystal lattices which are bound by rather weak intermolecular forces. Such molecules are more or less integrated in the crystal structure forming, but to a lower energetic effect. The water content in amorphous solids can, in general, be clearly determined, as in crystalline hydrates, but is heavily dependent on the drying and ambient conditions. In contrast, in the case of stable hydrates, there are clear stoichiometric ratios between the pharmaceutical active substance and the water. In many cases these ratios do not fulfil completely the stoichiometric value, normally it is approached by lower values compared to theory because of certain crystal defects. The ratio of organic molecules to water molecules for the weaker bound water may vary to a considerable extend, for example, extending over di-, tri- or tetra-hydrates. On the other hand, in amorphous solids, the molecular structure classification of water is not stoichiometric; the classification may however also be stoichiometric only by chance.

In some cases, it is not possible to classify the exact stoichiometry of the water molecules, since layer structures form, e.g. in the alkali metal salts, especially in the potassium salt, so that the embedded water molecules cannot be determined in defined form.

For the crystalline solids having identical chemical composition, the different resulting crystal gratings are summarised by the term polymorphism.

Any reference hereinbefore and hereinafter, to the salts according to the invention is to be understood as referring also to the corresponding solvates, such as hydrates, and polymorphous modifications, and also amorphous forms, as appropriate and expedient.

Especially preferred are the tetrahydrate of the calcium salt of valsartan and the hexahydrate of the magnesium salt of valsartan.

The X-ray diffraction diagram of powders of these two salt hydrates has a number of discrete X-ray reflections, and practically no signs of non-crystalline or amorphous portions. The degree of crystallisation of these defined salt hydrates is therefore surprisingly high. Equally, relatively large crystals may be cultured from certain salt hydrates, and in the crystallographic sense these are single crystals. Such single crystals allow the structure of the solid to be determined. It is effected by computer-aided evaluation of the reflection intensities measured by an X-ray diffractometer.

This process for determining the structure of a crystal enables, under normal conditions such as high physical, chemical and enantiomeric purity of the gauged crystals, a clear determination of the structure to be carried out on a molecular or atomic level, namely symmetry and size of the elementary cells, atom positions and temperature factors, and from the ascertained cell volume, the X-ray-photographic density is shown on the basis of a molecular weight. At the same time, the X-ray-photographic structure determination supplies details of its quality.

The outstanding properties of these two salt hydrates are based on the crystals, which form these salts by incorporating four or six water molecules per valsartan molecule. Thus, practically perfect three-dimensional crystal gratings are produced. These two salts have water solubility that is several times better than the free acid of valsartan, and this is especially surprisingly at high melting points and melting enthalpies, which are eight or five times greater than the free acid. The extraordinary crystal gratings of these two salt hydrates are the basis for the chemical and physical stability of these two compounds.

The particularly notable salt hydrate is the tetrahydrate of the calcium salt of valsartan. In a closed specimen container, for a heating rate of $T_r = 10\ K \cdot min^{-1}$ it has a melting point of 205 ± 1.5 °C and a melting enthalpy of 98 ± 4 $kJ \cdot Mol^{-1}$. The tetrahydrate of the calcium salt of valsartan is not stable at elevated temperatures both in respect of the hydrate water and in respect of the structure of the molecule. The indicated melting point is a hydrate melting point which can only be measured in a closed specimen container. Gold containers with a wall thickness of 0.2 mm were used; after weighing in samples of between 2 and 4 mg salt hydrate, they were sealed by cold welding. These gold containers have an internal free volume of ca. 22 microlitres. The amounts of the sample and the volume of the pressurised containers must be suitably adapted, so that strong dehydration of the salt hydrates cannot take place during measurement of the melting point. The partial pressure of the water at 205° Celsius is ca. 18 bar, so that with an open container in DSC (Differential Scanning Calorimeter) during measurement of the melting point, conversion to the anhydrate takes place. If the data from several heating rates (T_r= 10, 20, 40 $K \cdot min^{-1}$) are extrapolated to a continuously rapid heating rate, a melting point of 213 ± 2 °C and a melting enthalpy of 124 ± 5 $kJ \cdot Mol^{-1}$ result. Both the high hydrate melting point and the amount of the melting enthalpy are an expression of the exceptional stability of the crystal grating of the tetrahydrate of the calcium salt of valsartan. These two thermodynamic characteristics illustrate the advantageous physical properties, compared to the free acid, with the two corresponding data, namely a melting point in the closed system of 90°C and a melting enthalpy of 12 $kJ \cdot Mol^{-1}$. These thermodynamic data, together with the X-ray data, prove the high stability of this crystal grating. They are the foundation for the special physical and chemical resistance of the tetrahydrate of the calcium salt of valsartan.

A measurement of the infrared absorption spectrum of the tetrahydrate of the calcium salt of valsartan in a potassium bromide compressed tablet shows the following significant bands expressed in reciprocal wave numbers (cm^{-1}): 3750 – 3000 (st); 3400 – 2500 (st); 1800 – 1520 (st); 1500 – 1380 (st); 1380 – 1310 (m); 1290 – 1220 (w); 1220 – 1190 (w); 1190 – 1160 (w); 1160 – 1120 (w); 1120 – 1050 (w); 1030 – 990 (m); 989 – 960 (w), 950 – 920 (w); 780 – 715 (m); 710 – 470 (m). The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium; and (st) = strong intensity. Measurement of the infrared spectrum likewise took place by means of ATR-IR (Attenuated Total Reflection-Infrared Spectroscopy) using the instrument Spektrum BX from Perkin-Elmer Corp., Beaconsfield, Bucks, England.

The tetrahydrate of the calcium salt of valsartan has the following absorption bands expressed in reciprocal wave numbers (cm^{-1}):

3594 (w); 3306 (w); 3054 (w); 2953 (w); 2870 (w); 1621 (st); 1578 (m); 1458 (m); 1441 (m); 1417 (m); 1364 (m); 1336 (w); 1319 (w); 1274 (w); 1241 (w); 1211 (w); 1180 (w); 1149 (w); 1137 (w); 1106 (w); 1099 (w); 1012 (m); 1002 (w); 974 (w); 966 (w); 955 (w); 941 (w); 863 (w); 855 (w); 844 (w); 824 (w); 791 (w); 784 (w); 758 (m); 738 (m); 696 (m); 666 (m).

The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium and (st) = strong intensity.

The most intensive absorption bands of the ATR-IR spectroscopy are shown by the following values expressed in reciprocal wave numbers (cm^{-1}): 3306 (w); 1621 (st); 1578 (m); 1458 (m); 1441 (m); 1417 (m); 1364 (m); 1319 (w); 1274 (w); 1211 (w); 1180 (w); 1137 (w); 1012 (m); 1002 (w); 758 (m); 738 (m); 696 (m); 666 (m).

The error margin for all absorption bands of ATR-IR is ± 2 cm^{-1}.

The water content is in theory 13.2% for the tetrahydrate of the calcium salt of valsartan. Using the thermo-scale TGS-2 (Perkin-Elmer Corp. , Norwalk, CT USA) the water content was determined as 12.9 %. A total formula was calculated from this $(C_{24}H_{27}N_5O_3)^{2-}$ Ca^{2+}• (3.9 ± 0.1) H_2O.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the tetrahydrate as a function of temperature, was measured at a heating rate of 10 K•min $^{-1}$. The results are illustrated in table 1.

Table 1

temperature [°C]	weight loss or water loss in %

25	0
50	0
75	0.5
100	3.5
125	10.2
150	12.4
175	12.8
200	12.9
225	12.9
250	13.0
275	13.2

The solubility of the tetrahydrate of the calcium salt of valsartan in water-ethanol mixtures is illustrated in Table 2 for a temperature of 22°C.

Table 2

vol-% ethanol in water	solubility of the tetrahydrate of the calcium salt of valsartan in g/l solution at 22°C
0	9 (pH = 7.4)
10	9
30	14
50	46

A comparison of the solubilities of the two most important salts according to the invention and the free acid in distilled water is illustrated in Table 3.

Table 3

Compound	solubility in g/l solution at 22°C
valsartan	0.17
tetrahydrate of the calcium salt of valsartan	9
hexahydrate of the magnesium salt of valsartan	59

Further characterisation of the tetrahydrate of the calcium salt of valsartan is effected using the interlattice plane intervals determined by a X-ray powder pattern. Measurement of the

X-ray powder patterns was made with a Guinier camera (FR 552 from Enraf Nonius, Delft, NL) on an X-ray film in transmission geometry, using Cu-Kα_1 radiation at room temperature. Evaluation of the films for calculation of the interlattice plane intervals is made both visually and by a Line-Scanner (Johansson Täby, S), and the reflection intensities are determined simultaneously.

The preferred characterisation of the tetrahydrate of the calcium salt of valsartan is obtained from the interlattice plane intervals d of the ascertained X-ray diffraction diagrams, whereby, in the following, average values are indicated with the appropriate error limits.

d in [Å] : 16.1±0.3, 9.9±0.2, 9.4±0.2, 8.03±0.1, 7.71±0.1, 7.03±0.1, 6.50±0.1, 6.33±0.1, 6.20±0.05, 5.87±0.05, 5.74±0.05, 5.67±0.05, 5.20±0.05, 5.05±0.05, 4.95±0.05, 4.73±0.05, 4.55±0.05, 4.33±0.05, 4.15±0.05, 4.12±0.05, 3.95±0.05, 3.91±0.05, 3.87±0.05, 3.35±0.05.

The most intensive reflections in the X-ray diffraction diagram show the following interlattice plane intervals:

d in [Å] : 16.1±0.3, 9.9±0.2, 9.4±0.2, 7.03±0.1, 6.50±0.1, 5.87±0.05, 5.74±0.05, 4.95±0.05, 4.73±0.05, 4.33±0.05, 4.15±0.05, 4.12±0.05, 3.95±0.05.

A preferred method of checking the above-indicated average values of the interlattice plane intervals and intensities measured by experimentation from X-ray diffraction diagrams with a Guinier camera, for a given substance, consists in calculating these intervals and their intensities from the comprehensive single crystal structure determination. This structure determination yields cell constants and atom positions, which enable the X-ray diffraction diagram corresponding to the solid to be calculated by means of computer-aided calculation methods (programme CaRine Crystallography, Université de Compiègne, France). A comparison of these data, namely the interlattice plane intervals and intensities of the most important lines of the tetrahydrate of the calcium salt of valsartan, obtained from measurements with the Guinier camera and from calculating the single crystal data, is illustrated in Table 4.

Table 4

measured		calculated		measured		calculated	
d in [Å]	Intensity	d in [Å]	Intensity	d in [Å]	Intensity	d in [Å]	Intensity
16.10	very strong	16.02	very strong	5.67	very weak	5.658	very weak

9.89	strong	9.88	very strong	5.20	very weak	5.199	very weak
9.38	average	9.37	average	5.05	very weak	5.040	very weak
8.03	weak	8.02	average	4.95	average	4.943	weak
7.71	weak	7.70	weak	4.73	weak	4.724	weak
7.03	average	7.01	average	4.55	weak	4.539	weak
6.50	average	6.49	average	4.33	weak	4.338	weak
6.33	weak	6.33	weak	4.15	strong	4.150	strong
6.20	very weak	6.19	very weak	4.12	weak	4.114	weak
5.87	average	5.862	average	3.95	average	3.941	average
5.74	average	5.738	average	3.35	weak	3.349	weak

The invention relates to the crystalline tetrahydrate of the calcium salt of (S)-N-(1-carboxy-2-methylprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amine, a crystalline solid which is clearly characterised by the data and parameters obtained from single crystal X-ray analysis and X-ray powder patterns. An in-depth discussion of the theory of the methods of single crystal X-ray diffraction and the definition of the evaluated crystal data and the parameters may be found in Stout & Jensen, X-Ray Structure Determination; A Practical Guide, Mac Millian Co., New York, N.Y. (1968) chapter 3.

The data and parameters of the single crystal X-ray structure determination for the tetrahydrate of the calcium salt of valsartan are contained in Table 5.

Table 5

Crystal data and parameters of the tetrahydrate of the calcium salt of valsartan

Crystal data

sum formula	$(C_{24}H_{27}N_6O_3)^{2-}Ca^{2+} \cdot 4H_2O$
molecular mass	545.65
crystal colour	colourless
crystal shape	flat prisms

crystal system	monoclinic
space group	$P2_1$
size of the single crystal	0.42 • 0.39 • 0.17 mm^3
dimensions and angle of elementary cell	a = 10.127(2) Å
	b = 8.596(2) Å
	c = 32.214(6) Å
	α = 90 °
	β = 95.34(3) °
	γ = 90 °
volume of elementary cell	V_c = 2792.1(10) $Å^3$
number of molecules in the elementary cell	4
F (000)	1160
measurement range of cell parameters (Θ)	7.47-16.50 °
calculated density	1.298 ($g•cm^{-3}$)
linear absorption coefficient	0.274 mm^{-1}

X-ray measurement data

diffractometer	Enraf Nonius CAD4
X-radiation (graphite monochromator)	MoKα
wavelength	0.71073
temperature	295 K
scan range (θ)	1.27 - 31.99 °
scan mode	ω / 2 Θ
reflections collected/unique	19384 / 18562
number of significant reflections (I > 2σ(I))	10268
variation in intensity	1.7 %
absorption correction	numeric

Structure refinement

method	full matrix, least squares, F^2
number of parameters	893
agreement index (R)	6.2 %
weighted agreement index (R_W)	14.4 %

S factor (Goodness of fit)	1.085
number of reflections used	18562
treatment of all hydrogen atoms in the molecule, including in the water molecules	all found by difference-Fourier calculation, almost all isotropically refined, a few theoretically fixed (riding)
extinction correction	none
maximum/minimum residual electron density in conclusive difference-Fourier calculation	0.662 / - 0.495 ($e \cdot Å^{-3}$)
absolute structure parameters	0.00 (4)

Computer programmes used

SHELXS 86 (Sheldrick, Göttingen, 1990)
SHELXL 96 (Sheldrick, Göttingen, 1996)
SCHAKAL 86 (Keller, Freiburg 1986)
PLATON (Spek, Acta Cryst., 1990)

The elementary cell is defined by six parameters, namely by the grating constants a, b and c, and by the axial angle, namely by a, β, und γ. In this way, the volume of the elementary cell V_c is determined. A differentiated description of these crystal parameters is illustrated in chapter 3 of Stout & Jensen (see above). The details for the tetrahydrate of the calcium salt of valsartan from the single crystal measurements, especially the atom coordinates, the isotropic thermal parameters, the coordinates of the hydrogen atoms as well as the corresponding isotropic thermal parameters, show that a monoclinic elementary cell exists, its cell content of four formula units Ca^{2+} valsartan^{2-} • 4 H_20 occurring as a result of two crystallographic independent units on two-fold positions.

Given the acentric space group $P2_1$ determined from the single crystal X-ray structure determination, a racemate is ruled out. Thus the enantiomeric purity of the S-configuration for the crystalline tetrahydrate of the calcium salt of (S)-N-(1-carboxy-2-methylprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amine is verified.

An essential feature for the quality of a pure active substance both for the physical-chemical procedures such as drying, sieving, grinding, and in the galenic processes which are carried out with pharmaceutical excipients, namely in mixing processes, in granulation, in spray-drying, in tabletting, is the water absorption or water loss of this active substance depending on temperature and the relative humidity of the environment in question. With certain formulations, free and bound water is without doubt introduced with excipients and/or water is added to the process mass for reasons associated with the respective formulation process. In this way, the pharmaceutical active substance is exposed to free water over rather long periods of time, depending on the temperature of the different activity (partial vapour pressure).

A clear characterisation of this property is achieved by means of isothermal measurements over predetermined time intervals and predetermined relative humidity using dynamic vapour sorption (DVS-1 from the company Surface Measurement Systems LTD, Marlow, Buckinghamshire, UK). Table 6 illustrates the mass change, i.e. the water absorption or loss as a function of relative humidity at 25°C for a sample of 9.5 mg of the tetrahydrate of the calcium salt of valsartan and for a period of 4 hours. The following cycles of changes in relative humidity are shown: 40-90; 90-0; 0-90; 90-0 % relative humidity:

Table 6

relative humidity in %	water absorption or loss in %	relative humidity in %	water absorption or Abgabe in %
40	0.04	10	0.00
50	0.04	0	-0.01
60	0.03	10	0.00
70	0.02	20	0.00
80	0.02	30	0.00
90	0.00	40	0.00
80	0.02	50	0.00
70	0.02	60	0.01
60	0.02	70	0.00
50	0.02	80	-0.01
40	0.02	90	-0.02
30	0.01	0	-0.02
20	0.01	(starting value)	0.00

The measurement error of this sorption method based on thermogravimetry is about 0.1%. Therefore, the tetrahydrate of the calcium salt of valsartan under the conditions employed, which are realistic from a pharmaceutical-galenic point of view, shows no measurable water absorption or loss. This is surprising to a large extent, since the tetrahydrate, which has incorporated about 13% of bound water in the crystal structure, is totally indifferent to water even at extreme values of relative humidity. This property is crucial in the final stages of chemical manufacture and also in practice in all galenic process stages of the different dosage forms. This exceptional stability similarly benefits the patients through the constant availability of the active ingredient.

The intrinsic dissolving rates of the calcium salt of valsartan at pH 1, pH 4.5 and pH 6.8 show improved values over those of valsartan.

The exceptional stability of the calcium salt of valsartan, especially the tetrahydrate thereof, towards water may also be shown in stability tests. In these, the water content of the tetrahydrate of the calcium salt of valsartan remains constant both in an open container and in a sealed ampoule after four weeks at 40°C and 75% relative humidity.

Owing to the advantageous crystallinity of the calcium salt, especially the tetrahydrate thereof, this salt is suitable for pressing directly to form corresponding tablet formulations.

In addition, an improved dissolving profile in a tablet can be assured. In studies of the dissolving profile, it was established that the calcium salt, especially the tetrahydrate thereof, is released by 100% from a film-coated tablet within 15 minutes.

Of the group of new-type crystalline solids, a magnesium salt hydrate of valsartan is preferred, in particular the hexahydrate. The thermal behaviour of this salt hydrate in the region of the melting point shows a certain chemical and physical instability. The thermal data are thus dependent on the measurement conditions. In the sealed gold specimen container with an internal free volume of ca. 22 microlitres, with a sample of 2 to 4 mg and with a heating rate of $T_r = 10\ K \cdot min^{-1}$, the melting point of the hexahydrate of the magnesium salt of valsarten is 132 ± 1.5° Celsius and the melting enthalpy is 56 ± 3 kJ·Mol⁻

[1]. The melting enthalpy which is about 5 times higher than the free acid of valsartan, together with the significantly higher melting point of the hexahydrate of the magnesium salt of valsartan is a measure of the stability of the new-type crystal grating at around room temperature.

The optical rotation of the hexahydrate of the magnesium salt of valsartan in methanol as a 1% solution at 20°C is $[\alpha]^{20}_{D} = -14°$.

A measurement of the infrared absorption spectrum of the hexahydrate of the magnesium salt of valsartan in a potassium bromide compressed tablet shows the following significant bands expressed in reciprocal wave numbers (cm^{-1}): 3800 – 3000 (st); 3000 – 2500 (st); 1800 – 1500 (st); 1500 – 1440 (m); 1440 – 1300 (m); 1280 – 1240 (w); 1240 – 1190 (w); 1190 – 1150 (w); 1120 – 1070 (w); 1050 – 990 (w); 990 – 960 (w); 960 – 920 (w); 920 – 700 (m); 700 – 590 (w); 590 – 550 (w).
The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium; and (st) = strong intensity.

Measurement of the infrared spectrum likewise took place by means of ATR-IR (Attenuated Total Reflection-Infrared Spectroscopy) using the instrument Spektrum BX from Perkin-Elmer Corp., Beaconsfield, Bucks, England.

The hexahydrate of the magnesium salt of valsartan has the following absorption bands expressed in reciprocal wave numbers (cm^{-1}):
3378 (m); 3274 (m); 2956 (m); 2871 (w); 2357 (w); 1684 (w); 1619 (st); 1557 (m); 1464 (m); 1419 (m); 1394 (st); 1374 (m); 1339 (w); 1319 (w); 1300 (w); 1288 (w); 1271 (w) 1255 (w); 1223 (w); 1210 (w); 1175 (m); 1140 (w); 1106 (w); 1047 (w); 1024 (w); 1015 (w); 1005 (w); 989 (w); 975 (w); 955 (w); 941 (w); 888 (w); 856 (w); 836 (m); 820 (w); 766 (st); 751 (m); 741 (st); 732 (st).
The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium and (st) = strong intensity.

The most intensive absorption bands of the ATR-IR spectroscopy are shown by the following values expressed in reciprocal wave numbers (cm^{-1}): 3378 (m); 3274 (m);

2956 (m); 1619 (st); 1557 (m); 1464 (m); 1419 (m); 1394 (st); 1271 (w); 1175 (m); 1015 (w); 975 (w); 836 (m); 766 (st); 751 (m); 741 (st); 732 (st).
The error margin for all absorption bands of ATR-IR is ± 2 cm^{-1}.

The theoretical water content of the hexahydrate of the magnesium salt of valsartan is 19.1%. Using a coupled instrument based on thermogravimetry-Fourier transformation-infrared-spectroscopy (TG-FTIR, IFS 28 from the companies Netzsch Gerätebau GmbH, Selb, Bayern and Bruker Optik GmbH, Karlsruhe), whilst simultaneously measuring the weight loss and identifying the material component given up, using infrared spectroscopy (release of water), the water content was determined at 18.5 %, conforming well with the theoretical value. For the hexahydrate, this corresponds to a molar ratio of 5.8 ± 0.2 mols H_2O per mol magnesium salt.

Table 7 illustrates the water loss of the hexahydrate of the magnesium salt of valsartan depending on temperature, using the weight loss measured in an N_2 atmosphere on a thermogravimetric thermal analysis instrument for a heating rate of 10 K°min^{-1}. From the TG-FTIR measurement, the correlation of the weight loss is assured solely by the release of water.

Table 7

temperature [°C]	weight loss or water release in %
25	0
50	1.2
75	4.2
100	11.0
125	16.7
150	17.7
175	18.3
200	18.5
225	18.7
250	18.9
275	19.3

The hexahydrate of the magnesium salt of valsartan has a solubility in distilled water at 22°C of 59 g per litre of solution for a pH value of 9.3.

The crystalline form of the hexahydrate of the magnesium salt of valsartan is clearly characterised by the interlattice plane intervals calculated from the lines in an X-ray powder pattern. The measurement and analysis methods used are the same as those used for the tetrahydrate of the calcium salt of valsartan.

This preferred characterisation of the hexahydrate of the magnesium salt of valsartan is obtained from the interlattice plane intervals d, whereby, in the following, average values are indicated with the appropriate error limits:
d in [Å]: 19.7±0.3, 10.1±0.2, 9.8±0.2, 7.28±0.1, 6.48±0.1, 6.00±0.1, 5.81±0.1, 5.68±0.1, 5.40±0.05, 5.22 ±0.05, 5.12±0.05, 5.03±0.05, 4.88±0.05, 4.33±0.05, 4.22±0.05, 4.18±0.05, 4.08±0.05, 3.95±0.05, 3.46±0.05, 3.42±0.05.

The most intensive reflections in the X-ray diffraction diagram show the following interlattice plane intervals:
d in [Å] : 19.7±0.3, 10.11±0.2, 9.8±0.2, 7.28±0.1, 5.81±0.05, 5.68±0.05, 5.03±0.05, 4.88±0.05, 4.18±0.05, 4.08±0.05, 3.46 ±0.05.

A preferred method of checking the above-indicated average values of the interlattice plane intervals and intensities measured by experimentation from X-ray diffraction diagrams with a Guinier camera, for a given substance, consists in calculating these intervals and their intensities from the comprehensive single crystal structure determination. This structure determination yields cell constants and atom positions, which enable the X-ray diffraction diagram corresponding to the solid to be calculated by means of computer-aided calculation methods (programme CaRine Crystallography, Université de Compiègne, France). A comparison of these data, namely the interlattice plane intervals and intensities of the most important lines of the hexahydrate of the magnesium salt of valsartan, obtained from measurements with the Guinier camera and from calculating the single crystal data, is illustrated in Table 8.

Table 8

measured		calculated		measured		calculated	
d in [Å]	Intensity	d in [Å]	Intensity	d in [Å]	Intensity	d in [Å]	Intensity
19.7	very strong	19.66	very strong	5.12	weak	5.124	weak
10.11	average	10.09	average	5.03	strong	5.032	very strong
9.83	average	9.84	very strong	4.88	strong	4.878	very strong
7.28	average	7.27	average	4.33	very weak	4.341	weak
6.48	weak	6.46	weak	4.22	weak	4.215	weak
6.00	weak	6.00	weak	4.18	average	4.181	average
5.81	average	5.805	average	4.08	average	4.079	average
5.68	average	5.676	strong	3.95	weak	3.946	weak
5.40	very weak	5.391	very weak	3.46	average	3.463	average
5.22	weak	5.217	weak	3.42	weak	3.428	weak

The invention relates in particular to the crystalline hexahydrate of the magnesium salt of (S)-N-(1-carboxy-2-methylprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amine, a crystalline solid which is clearly characterised by the data and parameters obtained from single crystal X-ray analysis. An in-depth discussion of the theory of the methods of single crystal X-ray diffraction and the definition of the evaluated crystal data and the parameters may be found in Stout & Jensen, X-Ray Structure Determination; A Practical Guide, Mac Millian Co., New York, N.Y. (1968) chapter 3.

The data and parameters of the single crystal X-ray analysis for the magnesium-valsartan-hexahydrate are given in Table 9.

Table 9

<u>Crystal data and parameters of the hexahydrate of the magnesium salt of valsartan</u>

Crystal data

sum formula $(C_{24}H_{27}N_5O_3)^{2-} Mg^{2+} \cdot 6\ H_2O$

molecular mass 565.91

crystal colour	colourless
crystal shape	flat prisms
crystal system	monoclinic
space group	C2
size of the single crystal	0.013 • 0.50 • 0.108 mm^3
dimensions and angle of elementary cell	a = 40.075(8) Å
	b = 7.400(1) Å
	c = 10.275(2) Å
	α = 90 °
	β = 100.85(3) °
	γ = 90 °
volume of elementary cell	V_c = 2992.6(9) $Å^3$
number of molecules in the elementary cell	4
F (000)	1208
measurement range of cell parameters (Θ)	2.82 – 11.15 °
calculated density	1.256 ($g•cm^{-3}$)
linear absorption coefficient	0.114 mm^{-1}

X-ray measurement data

diffractometer	Enraf Nonius CAD4
X-radiation (graphite monochromator)	MoKα
wavelength	0.71073
temperature	295 K
scan range (θ)	1.03 – 26.00 °
scan mode	ω / 2 Θ
reflections collected/unique	5954 / 5868
number of significant reflections (I > 2σ(I))	1341
variation in intensity	<1 %
absorption correction	numeric

Structure refinement

method	full matrix, least squares, F^2

number of parameters	243
agreement index (R)	10.7 %
weighted agreement index (R_W)	13.8 %
S factor (Goodness of fit)	1.001
number of reflections used	5868
determination of hydrogen atoms	majority according to the "riding" model, nine H-atoms from water molecules isotropically refined from difference-Fourier calculation
extinction correction	0.00098 (10)
maximum/minimum residual electron density in final difference-Fourier calculation	0.473 / - 0.614 ($e \cdot Å^{-3}$)
absolute structure parameters	0.0(10)

Computer programmes used

SHELXS 86 (Sheldrick, Göttingen, 1990)

SHELXL 96 (Sheldrick, Göttingen, 1996)

SCHAKAL 86 (Keller, Freiburg 1986)

PLATON (Spek, Acta Cryst., 1990)

The elementary cell is defined by six parameters, namely by the grating constants a, b and c, and by the axial angle, namely by a, β, und γ. In this way, the volume of the elementary cell V_c is determined. A differentiated description of these crystal parameters is illustrated in chapter 3 of Stout & Jensen (see above).

The details for the hexahydrate of the magnesium salt of valsartan from the single crystal measurements, especially the atom coordinates, the isotropic thermal parameters, the coordinates of the hydrogen atoms as well as the corresponding isotropic thermal parameters, show that a monoclinic elementary cell exists, its cell content occurring from four formula units Mg^{2+} Valsartan • 6 H_2O .

Given the acentric space group C2 determined from the single crystal X-ray structure determination, a racemate is ruled out. Thus the enantiomeric purity of the S-configuration for the crystallin hexahydrate of the magnesium salt of valsartan is proved.

Table 10 illustrates the mass change, i.e. the water absorption or loss as a function of relative humidity at 25°C for a sample of 9.5 mg of magnesium-valsartan-hexahydrate and for a period of 4 hours (h). The following cycles of changes in relative humidity are shown: 40-90; 90-0; 0-90; 90-0 % relative humidity:

Table 10

relative humidity in %	water absorption or loss in %	relative humidity in %	water absorption or loss in %
40	0.06	10	-0.12
50	0.14	0	-4.3
60	0.19	10	-0.79
70	0.25	20	-0.14
80	0.41	30	-0.05
90	0.58	40	0.02
80	0.32	50	0.09
70	0.22	60	0.14
60	0.14	70	0.20
50	0.08	80	0.28
40	0.16	90	0.51
30	-0.03	0	-3.68
20	-0.07	(starting value)	-0.01

The measurement error of this sorption method based on thermogravimetry is about 0.1%. Therefore, the hexahydrate of the magnesium salt of valsartan under the conditions employed, which are realistic from a pharmaceutical-galenic point of view, shows weak, reproducible water absorption or water loss in a range of 20 to 80% relative humidity. This is surprising to a large extent, since the hexahydrate, which has incorporated about 19% bound water in the crystal structure, reversibly absorbs or releases water even at extreme values of relative humidity and is relatively insensitive at an average range of relative humidity. This characteristic enables an uncomplicated physical-chemical process to be developed and allows a choice of the b st dosage forms for the patients.

The exceptional stability of the magnesium salt of valsartan, especially the hexahydrate thereof, towards water may also be shown in stability tests In these, the water content of the hexahydrate of the magnesium salt of valsartan remains constant both in an open container and in a sealed ampoule after four weeks at 40°C and 75% relative humidity.

Owing to the advantageous crystallinity of the magnesium salt, especially the hexahydrate thereof, this salt is suitable for pressing directly to form corresponding tablet formulations.

In addition, an improved dissolving profile in a tablet can be assured. In studies of the dissolving profile, it was established that the magnesium salt, especially the hexahydrate thereof, is released by 100% from a film-coated tablet within 15 minutes.

In addition, the magnesium salt of valsartan, especially the hexahydrate thereof, shows an advantageous compression hardness profile.

Calcium/magnesium mixed salts of valsartan also have advantageous properties, for example uniform crystal conglomerates may be produced. These may be advantageously used in the galenic formulation.

The intrinsic dissolving rates of the di-potassium salt of valsartan at pH 1, pH 4.5 and pH 6.8 show improved values over those of valsartan.

A further object of the invention is the preparation of the salts according to the invention.

The salts according to the invention, including amorphous or crystalline forms thereof, may be prepared as follows:

To form the salt, the process is carried out in a solvent system, in which the two reactants, namely the acid valsartan and the respective base, are sufficiently soluble. It is expedient to use a solvent or solvent mixture, in which the resulting salt is only slightly soluble or not soluble at all, in order to achieve crystallisation or precipitation. One variant for the salt according to the invention would be to use a solvent in which this salt is very soluble, and to subsequently add an anti-solvent to this solution, that is a solvent in which the resulting salt has only poor solubility. A further variant for salt crystallisation consists in concentrating the salt solution, for example by heating, if necessary under reduced pressure, or by slowly evaporating the solvent, e.g. at room temperature, or by seeding with the addition of seeding crystals, or by setting up water activity required for hydrate formation.

The solvents that may be used are for example C_1-C_5-alkanols, preferably ethanol and isopropanol, as well as C_1-C_5-dialkylketones, preferably acetone and mixtures thereof with water.

The antisolvents for salt crystallisation may be for example C_3-C_7-alkylnitriles, especially acetonitrile, esters, especially C_2-C_7-alkanecarboxylic acid-C_1-C_5-alkylester, such as ethyl or isopropyl acetate, di-(C_1-C_5-alkyl)-ethers, such as tert.-butylmethylether, furthermore tetrahydrofuran, and C_5-C_8-alkanes, especially pentane, hexane or heptane.

To produce hydrates, a dissolving and crystallising process is used in particular, or a water-equilibrating crystallisation process.

The dissolving and crystallising process is characterised in that
(i) valsartan and the appropriate base are brought to a reaction in a preferably water-containing, organic solvent,
(ii) the solvent system is concentrated, for example by heating, if necessary under reduced pressure and by seeding with seeding crystals or by slowly evaporating, e.g. at room temperature, then crystallisation or precipitation is initiated and
(iii) the salt obtained is isolated.

In the dissolving and crystallising process, the water-containing, organic solvent system employed is advantageously a mixtures of alcohols, such as ethanol, and water, or or alkyl-nitrile, especially acetonitrile, and water.

The equilibrating crystallisation process for producing hydrates is characterised in that
(i) valsartan and the appropriate base are added to a water-containing organic solvent,
(ii) the solvent is concentrated, for example by heating, if necessary under reduced pressure or by slowly evaporating, e.g. at room temperature,
(iii) the residue of evaporation is equilibrated with the required amount of water by

(a) suspending the residue of evaporation, which is advantageously still warm, and which still contains some water, in an appropriate solvent or

(b) by equilibrating the water excess in the solvent;

whereby in a) and b) the existing or added water is present in a quantity in which the water dissolves in the organic solvent and does not form an additional phase; and

(iv) the salt obtained is isolated.

The solvent system used as the water-containing organic solvent advantageously comprises mixtures of suitable alcohols, such as C_1-C_7-alkanols, especially ethanol, and water.

An appropriate solvent for equilibration is, for example, an ester such as C_1-C_7-alkane-carboxylic acid-C_1-C_7-alkylester, especially ethyl acetate, or a ketone such as di-C_1-C_5-alkylketone, especially acetone.

The equilibration process is notable for example for its high yields and outstanding reproducibility.

When producing the mono-alkali metal salts according to the present invention, predominantly amorphous forms are obtained. On the other hand, the di-alkali metal salts and alkaline earth metal salts of the present invention may also be obtained in crystalline form and are in the form of hydrates throughout, from appropriate solvents that are conventionally used in production processes, such as esters, e.g. C_1-C_7-alkanecarboxylic acid-C_1-C_7-alkylesters, especially ethyl acetate, ketones, e.g. di-C_1-C_5-alkylketones, especially acetone, C_3-C_7-alkylnitriles, especially acetonitrile, or ethers, e.g. di-(C_1-C_5-alkyl)-ethers, such as tert.-butylmethylether, also tetrahydrofuran, or mixtures of solvents. By using the dissolving and crystallising process, or the water-equilibrating crystallisation process, the defined hydrates, which are present in crystalline and in polymorphous forms, may be obtained reproducibly.

The preparation of the hydrate-free bis-dialkylammonium salts of the present invention is advantageously effected in one step by using an appropriate solvent which is optionally mixed with an antisolvent. In this way, crystalline salts are obtained.

As a rule, the amino acid salts of the present invention are obtained in amorphous form.

The processes for forming salts are likewise objects of the present invention.

These salts or salt hydrates according to the invention are obtained for example by neutralising the acid valsartan with a base corresponding to the respective cation. This neutralisation is suitably effected in an aqueous medium, e.g. in water or a mixture of water and a solvent in which valsartan is more soluble than in water. Salts with weaker bases may be converted into other salts either by treating with stronger bases or by treating with acids and then neutralising with other bases.

Crystallisation, especially of the alkaline earth salt hydrates, is effected in water or an aqueous medium, which consists of water and at least one solvent that is miscible or partially miscible with water, i.e. not too non-polar, e.g. an alkanol such as methanol, ethanol, propanol, isopropanol, butanol, acetone, methyl ethyl ketone, acetonitrile, DMF, DMSO. The alkanol portion amounts to about 10 to 90, or 20 to 70, advantageously 30 to 50% by volume. For higher alkanols, the less polar solvent may also be present in lower concentrations. Owing to the restricted water-solubility of valsartan, the process frequently takes place in suspensions, or if valsartan is soluble in the other solvent component, in a solution.

In one embodiment, for example to produce the calcium salt of valsartan, an aqueous solution of valsartan is neutralised with a calcium hydroxide solution at room temperature and the solution is left to crystallise. In a preferred procedure, crystallisation is effected from a solvent mixture of water/ethanol, the ethanol proportion amounting to ca. 30 to 50% by volume. In an especially preferred form, crystallisation is effected in a closed system by transporting through a low temperature gradient (especially 1-2°C at 40°C) in 30% by volume of ethanol.

In a preferred variant, crystallisation may be optimised, e.g. accelerated, by adding at least one seed crystal.

The salts according to the invention may be used e.g. in the form of pharmaceutical preparations, which contain the active substance e.g. in a therapeutically effective amount of the active substance, optionally together with a pharmaceutically acceptable carrier, for example with an inorganic or organic, solid or optionally also liquid pharmaceutically acceptable carrier, which is suitable for enteral, e.g. oral, or parenteral administration.

The invention relates in particular to a pharmaceutical composition, especially in a solid dosage unit, preferably for oral administration, optionally together with a pharmaceutically acceptable carrier.

Pharmaceutical preparations of this kind may be used for example for the prophylaxis and treatment of diseases or conditions which may be inhibited by blocking the AT_1 receptor for example

a disease or condition selected from the group consisting of

(a) hypertension, congestive heart failure, renal failure, especially chronic renal failure, restenosis after percutaneous transluminal angioplasty, and restenosis after coronary artery bypass surgery;

(b) atherosclerosis, insulin resistance and syndrome X, diabetes mellitus type 2, obesity, nephropathy, renal failure, e.g. chronic renal failure, hypothyroidism, survival post myocardial infarction (MI), coronary heart diseases, hypertension in the elderly, familial dyslipidemic hypertension, increase of formation of collagen, fibrosis, and remodeling following hypertension (antiproliferative effect of the combination), all these diseases or conditions associated with or without hypertension;

(c) endothelial dysfunction with or without hypertension,

(d) hyperlipidemia, hyperlipoproteinemia, atherosclerosis and hypercholesterolemia, and

(e) glaucoma.

Primary usages are for the treatment of high blood pressure and congestive heart failure, as well as post-myocardial infarction.

The person skilled in the pertinent art is fully enabled to select a relevant and standard animal test model to prove the hereinbefore and hereinafter indicated therapeutic indications and beneficial effects.

The pharmaceutical activities as effected by administration of representatives of the salts of the present invention or of the combination of active agents used according to the present invention can be demonstrated e.g. by using corresponding pharmacological models known in the pertinent art. The person skilled in the pertinent art is fully enabled to select a relevant animal test model to prove the hereinbefore and hereinafter indicated therapeutic indications and beneficial effects.

These beneficial effects can, for example, be demonstrated in the test model as disclosed by G. Jeremic et al. in J. Cardovasc. Pharmacol. 27:347-354, 1996.

For example, the valuable potential of the salts or combinations of the present invention for the prevention and treatment of myocardial infarction can be found using the following test model.

Study design

In the study to be performed, permanent coronary artery occlusion (CAO) in rats is used as a model of acute myocardial infarction. The experiments are carried out with 5 treatment groups characterized by following features:

• sham-operated animals
• CAO + vehicle
• CAO + a salt according to the present invention,
optionally
• CAO + a salt according to the present invention + a combination partner.

During the study following variables are measured:

• infarct size
• LV chamber volume
• interstitial and perivascular collagen density in spared LV myocardium
• COL-I and COL-III protein content in spared LV myocardium by Western blot
• cardiomyocytes cross-sectional area and length in sections of LV myocardium
• plasma concentrations of renin and aldosterone
• urine concentration of sodium, potassium and aldosterone
• blood pressure in conscious animals
• LV and carotid blood pressure in anesthetized animals.

Methodology

Infarct size: Six μm-thick transverse histological sections of the left ventricle are stained with nitroblue tetrazolium and acquired by a B/W XC-77CE CCD video camera (Sony). The resulting image is processed on a KS 300 image analysis system (Carl Zeiss Vision) using a software specifically developed (Porzio *et al.*, 1995). A single operator blinded to treatment interactively defines the boundaries of the interventricular septum, and the infarcted area on each section is semiautomatically identified as the area of unstained ventricular tissue. The software automatically calculates for each component of the ventricular section defined as the chamber, septum, infarcted area, infarcted LV wall and viable LV wall, a set of geometric parameters (Porzio *et al.*, 1995).

Histology: Hearts are fixed in situ, by retrograde perfusion with buffered 4% formaldehyde after arrest in diastole by i.v. injection of 0.5 M KCl. After fixation, the left ventricle (LV) and the free wall of the right ventricle are separately weighed; LV longer diameter is measured with a caliper. LV histological sections are stained with hematoxylin & eosin for qualitative examination and to quantify cardiomyocytes cross-sectional area with a semi-automated image analysis routine. Interstitial collagen deposition in LV is evaluated on Sirius red stained sections with a semi-automated image analysis routine (Masson *et al.*, 1998).

Collagen content in LV spared myocardium: LV tissue in the spared myocardium is homogenized, subjected to PAGE-SDS electrophoresis and electroblotted onto nitrocellulose membrane. The blots are exposed to primary antibodies, i.e. rabbit anti-rat collagen type I or type III antiserum (Chemicon). The primary antibodies are recognized by secondary antibodies conjugated to alkaline phosphatase (for colagen type I) or peroxidase (collagen type III).

Left ventricular chamber volume: LV chamber volume is determined in hearts arrested in diastole (KCl) and fixed in formalin under a hydrostatic pressure equivalent to the measured LV end-diastolic pressure. A metric rod is inserted into the LV to measure LV inner length. The transverse diameters of the LV chamber are measured in two 1-mm thick transverse sections near to the base and the apex of the ventricle (Jeremic *et al.*, 1996). The chamber volume is computed from an equation integrating transverse diameters and inner length.

Syst mic and Left v ntricular hemodynamics: A microtip pressure transducer (Millar SPC-320) connected to a recorder (Windograf, Gould Electronics) is inserted into the right carotid artery to record systolic and diastolic blood pressures. The pressure transducer is advanced into the LV to measure LV systolic (LVSP) and end-diastolic (LVEDP) pressures, the first derivative of LV pressure over time (+dP/dt) and heart rate.

Non-invasive blood pressure: Systolic blood pressure and heart rate are measured by the tail-cuff method (Letica LE 5002) in conscious rats.

Urine electrolytes, hormones: Rats are individually housed in metabolic cages and 24-h urine collected on 1 ml HCl 6N. Water intake is measured. Urine catecholamines are extracted on Bondelut C_{18} columns (Varian), separated by HPLC (Apex-II C18, 3 μm, 50x4.5 mm analytical column, Jones Chromatography) and quantified with an electrochemical detector (Coulochem II, ESA) (Goldstein *et al.*, 1981). Plasma and urine aldosterone, and plasma angiotensin II is determined with specific radioimmunoassays (Aldoctk-2, DiaSorin and Angiotensin II, Nichols Diagnostics). Urine sodium and potassium are measured by flamme photometry.

<u>Sample size</u>

10 animals analyzable in each treatment groups are sufficient to detect biologically significant differences. Only rats with an infarct size of at least 10% of the LV section area are included in the final analysis.

Endothelial dysfunction is being acknowledged as a critical factor in vascular diseases. The endothelium plays a bimodal role as the source of various hormones or by-products with opposing effects: vasodilation and vasoconstriction, inhibition or promotion of growth, fibrinolysis or thrombogenesis, production of anti-oxidants or oxidising agents. Genetically predisposed hypertensive animals with endothelial dysfunction constitute a valid model for assessing the efficacy of a cardiovascular therapy.

Endothelial disfunction is characterized by, for example, increased oxidative stress, causing decreased nitric oxide, increased factors involved in coagulation or fibrinolysis such as plasminogen activating inhibitor-1 (PAI-1), tissue factor (TF), tissue plasminogen activator (tPA), increased adhesion molecules such as ICAM and VCAM, increased growth factors such as bFGF, TGFb, PDGF, VEGF, all factors causing cell growth inflammation and fibrosis.

The treatment e.g. of endothelian dysfunction can be demonstrated in the following pharmacological test:

Material and methods

Male 20-24 week-old SHR, purchased from RCC Ldt (Füllingsdorf, Switzerland), are maintained in a temperature- and light-controlled room with free access to rat chow (Nafag 9331, Gossau, Switzerland) and tap water. The experiment is performed in accordance with the NIH guidelines and approved by the Canton Veterinary office (Bew 161, Kantonales Veterinäramt, Liestal, Switzerland). All rats are treated with the NO synthesis inhibitor L-NAME (Sigma Chemicals) administered in drinking water (50 mg/l) for 12 weeks. The average daily dose of L-NAME calculated from the water consumed was 2.5 mg/kg/d (range 2.1-2.7).

The rats can be divided into 2 or 3 groups: group 1, control (n = e.g. 40); Group 2, a salt according to the present invention; n = e.g. 40); for testing combinations Group 3, combination partner;(n = e.g. 30). The drugs are administered in drinking fluid. The pressure effect of Ang II at 1 mg/kg obtained in controls normotensive rats can be reduced after treatment with a salt according to the present invention (Gervais et al. 1999).

Body weight is measured every week. Systolic blood pressure and heart rate are recorded by tail cuff plethysmography 3 and 2 weeks before starting the study and at 2 weeks after drug administration. Urine is collected over a 24 hour period from rats kept in individual (metabolic) cages the week before starting treatment and at weeks 4 and 12 for volume measurement and protein, creatinine, sodium and potassium determination using standard laboratory methods. At the same time points, blood samples are withdrawn from the retro-orbital plexus (maximum 1 ml) for creatinine, Na^+ and K^+ assays.

Ten rats from each group are sacrificed at 4 weeks for collection of kidney and heart for morphological analysis. The remaining rats are sacrificed at 12 weeks. Cardiac and kidney weight is recorded. Terminal blood sampling is performed in 5 % EDTA at 4 (morphometry study) and 12 (end of the study) weeks for aldosterone, determination by radioimmunoassay using a DPC coat-a-count aldosterone-RIA kit (Bühlmann, Switzerland).

Statistical analysis:

All data are expressed as mean ± SEM. Statistical analysis is performed using a one-way ANOVA, followed by a Duncan's multiple range test and a Newman-Keuls test, 7for comparison between the different groups. Results with a probability value of less than 0.05 are deemed statistically significant.

An improvement of regression of artherosclerosis without effecting the serum lipid levels can, for example, be demonstrated by using the animal model as disclosed by H. Kano et al. in Biochemical and Biophysical Research Communications 259, 414-419 (1999).

That the salts or combinations according to the present invention can be used for the regression of a cholesterol diet-induced atherosclerosis, can be demonstrated using the test model described, e.g., by C. Jiang et al. in Br. J. Pharmacol. (1991), 104, 1033-1037.

That the salts or combinations according to the present invention can be used for the treatment of renal failure, especially chronic renal failure, can be demonstrated using the test model described, e.g., by D. Cohen et al. in Journal of Cardiovascular Pharmacology, 32: 87-95 (1998).

The present pharmaceutical preparations which, if so desired, may contain further pharmacologically active substances, are prepared in a manner known *per se*, for example by means of conventional mixing, granulating, coating, dissolving or lyophilising processes, and contain from about 0.1% to 100%, especially from about 1% to about 50%, of lyophilisates up to 100% of the active substance.

The invention similarly relates to compositions containing the salts according to the invention.

The invention similarly relates to the use of the salts according to the invention preferably for the production of pharmaceutical preparations, especially for the prophylaxis and also for the treatment of diseases or conditions which may be inhibited by blocking the AT_1

receptor. Primary usages are for the treatment of high blood pressure and congestive heart failure, as well as post-myocardial infarction.

The invention similarly relates to the use for the prophylaxis and treatment of diseases or conditions which may be inhibited by blocking the AT_1 receptor, characterised in that a patient, including a human patient, requiring such treatment is administered with a therapeutically effective amount of a salt according to the invention, optionally in combination with at least one composition for the treatment of cardiovascular diseases and related conditions and diseases listed hereinbefore or hereinafter.

The invention similarly relates to combinations, e.g. pharmaceutical combinations, containing a salt of the present invention or in each case a pharmaceutically acceptable salt thereof in combination with at least one composition for the treatment of cardiovascular diseases and related conditions and diseases as listed hereinbefore or hereinafter, or in each case a pharmaceutically acceptable salt thereof. Combinations with other compositions for the treatment of cardiovascular diseases and related conditions and diseases as listed hereinbefore or hereinafter, or in each case a pharmaceutically acceptable salt thereof, are likewise objects of the present invention.

The combination may be made for example with the following compositions, selected from the group consisting of a:

(i) HMG-Co-A reductase inhibitor or a pharmaceutically acceptable salt thereof,

(ii) angiotensin converting enzyme (ACE) Inhibitor or a pharmaceutically acceptable salt thereof,

(iii) calcium channel blocker or a pharmaceutically acceptable salt thereof,

(iv) aldosterone synthase Inhibitor or a pharmaceutically acceptable salt thereof,

(v) aldosterone antagonist or a pharmaceutically acceptable salt thereof,

(vi) dual angiotensin converting enzyme/neutral endopeptidase (ACE/NEP) Inhibitor or a pharmaceutically acceptable salt thereof,

(vii) endothelin antagonist or a pharmaceutically acceptable salt thereof,

(viii) renin inhibitor or a pharmaceutically acceptable salt thereof, and

(ix) diuretic or a pharmaceutically acceptable salt thereof.

HMG-Co-A reductase inhibitors (also called β-hydroxy-β-methylglutaryl-co-enzyme-A reductase inhibitors) are understood to be those active agents that may be used to lower the lipid levels including cholesterol in blood.

The class of HMG-Co-A reductase inhibitors comprises compounds having differing structural features. For example, mention may be made of the compounds that are selected from the group consisting of atorvastatin, cerivastatin, compactin, dalvastatin, dihydrocompactin, fluindostatin, fluvastatin, lovastatin, pitavastatin, mevastatin, pravastatin, rivastatin, simvastatin, and velostatin, or, in each case, a pharmaceutically acceptable salt thereof.

Preferred HMG-Co-A reductase inhibitors are those agents which have been marketed, most preferred is fluvastatin and pitavastatin or, in each case, a pharmaceutically acceptable salt thereof.

The interruption of the enzymatic degradation of angiotensin I to angiotensin II with so-called ACE-inhibitors (also called angiotensin converting enzyme inhibitors) is a successful variant for the regulation of blood pressure and thus also makes available a therapeutic method for the treatment of congestive heart failure.

The class of ACE inhibitors comprises compounds having differing structural features. For example, mention may be made of the compounds which are selected from the group consisting alacepril, benazepril, benazeprilat, captopril, ceronapril, cilazapril, delapril, enalapril, enaprilat, fosinopril, imidapril, lisinopril, moveltopril, perindopril, quinapril, ramipril, spirapril, temocapril, and trandolapril, or, in each case, a pharmaceutically acceptable salt thereof.

Preferred ACE inhibitors are those agents that have been marketed, most preferred are benazepril and enalapril.

The class of CCBs essentially comprises dihydropyridines (DHPs) and non-DHPs such as diltiazem-type and verapamil-type CCBs.

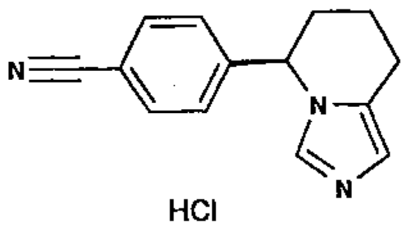
A CCB useful in said combination is preferably a DHP representative selected from the group consisting of amlodipine, felodipine, ryosidine, isradipine, lacidipine, nicardipine, nifedipine, niguldipine, niludipine, nimodipine, nisoldipine, nitrendipine, and nivaldipine, and is preferably a non-DHP representative selected from the group consisting of flunarizine, prenylamine, diltiazem, fendiline, gallopamil, mibefradil, anipamil, tiapamil and verapamil, and in each case, a pharmaceutically acceptable salt thereof. All these CCBs are therapeutically used, e.g. as anti-hypertensive, anti-angina pectoris or anti-arrhythmic drugs. Preferred CCBs comprise amlodipine, diltiazem, isradipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine, and verapamil, or, e.g. dependent on the specific CCB, a pharmaceutically acceptable salt thereof. Especially preferred as DHP is amlodipine or a pharmaceutically acceptable salt, especially the besylate, thereof. An especially preferred representative of non-DHPs is verapamil or a pharmaceutically acceptable salt, especially the hydrochloride, thereof.

Aldosterone synthase inhibitor is an enzyme that converts corticosterone to aldosterone to by hydroxylating cortocosterone to form 18-OH-corticosterone and 18-OH-corticosterone to aldosterone. The class of aldosterone synthase inhibitors is known to be applied for the treatment of hypertension and primary aldosteronism comprises both steroidal and non-steroidal aldosterone synthase inhibitors, the later being most preferred.

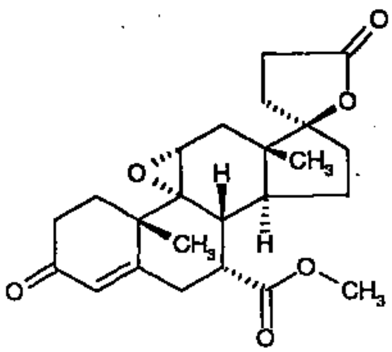
Preference is given to commercially available aldosterone synthase inhibitors or those aldosterone synthase inhibitors that have been approved by the health authorities.

The class of aldosterone synthase inhibitors comprises compounds having differing structural features. For example, mention may be made of the compounds which are selected from the group consisting of the non-steroidal aromatase inhibitors anastrozole, fadrozole (including the (+)-enantiomer thereof), as well as the steroidal aromatase inhibitor exemestane, or, in each case where applicable, a pharmaceutically acceptable salt thereof.

The most preferred non-steroidal aldosterone synthase inhibitor is the (+)-enantiomer of the hydrochloride of fadrozole (US patents 4617307 and 4889861) of formula



A preferred steroidal aldosterone antagonist is eplerenone of the formula



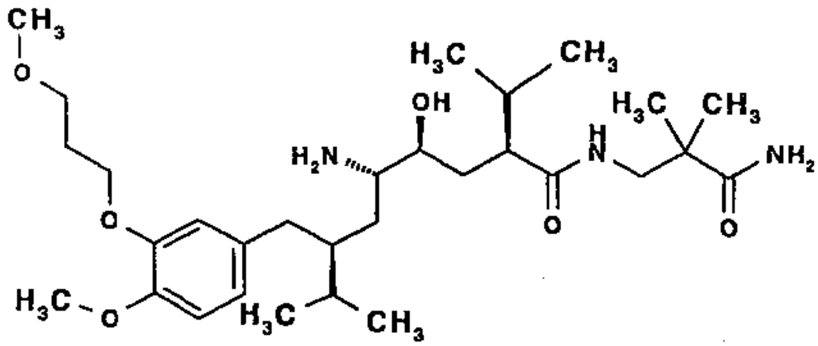
or

spironolactone.

A preferred dual angiotensin converting enzyme/neutral endopetidase (ACE/NEP) inhibitor is, for example, omapatrilate (cf. EP 629627), fasidotril or fasidotrilate, or, if appropriable, a pharmaceutically acceptable salt thereof.

A preferred endothelin antagonist is, for example, bosentan (cf. EP 526708 A), furthermore, tezosentan (cf. WO 96/19459), or in each case, a pharmaceutically acceptable salt thereof.

A renin inhibitor is, for example, a non-peptidic renin inhibitor such as the compound of formula



chemically defined as 2(S),4(S),5(S),7(S)-N-(3-amino-2,2-dimethyl-3-oxopropyl)-2,7-di(1-methylethyl)-4-hydroxy-5-amino-8-[4-methoxy-3-(3-methoxy-propoxy)phenyl]-octanamide. This representative is specifically disclosed in EP 678503 A. Especially preferred is the hemi-fumarate salt thereof.

A diuretic is, for example, a thiazide derivative selected from the group consisting of chlorothiazide, hydrochlorothiazide, methylclothiazide, and chlorothalidon. The most preferred is hydrochlorothiazide.

Preferably, the jointly therapeutically effective amounts of the active agents according to the combination of the present invention can be administered simultaneously or sequentially in any order, separately or in a fixed combination.

The structure of the active agents identified by generic or tradenames may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g. Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference. Any person skilled in the art is fully enabled to identify the active agents and, based on these references, likewise enabled to manufacture and test the pharmaceutical indications and properties in standard test models, both in vitro and in vivo.

The corresponding active ingredients or a pharmaceutically acceptable salts thereof may also be used in form of a solvate, such as a hydrate or including other solvents, used for crystallization.

The compounds to be combined can be present as pharmaceutically acceptable salts. If these compounds have, for example, at least one basic center, they can form acid addition salts. Corresponding acid addition salts can also be formed having, if desired, an additionally present basic center. The compounds having an acid group (for example COOH) can also form salts with bases.

In a variation thereof, the present invention likewise relates to a "kit-of-parts", for example, in the sense that the components to be combined according to the present invention can be dosed independently or by use of different fixed combinations with distinguished amounts of the components, i.e. simultaneously or at different time points. The parts of the kit of parts can then e.g. be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. Preferably, the time intervals are chosen such that the effect on the treated disease or condition in the combined use of the parts is larger than the effect that would be obtained by use of only any one of the components.

The invention furthermore relates to a commercial package comprising the combination according to the present invention together with instructions for simultaneous, separate or sequential use.

Dosaging may depend on various factors, such as mode of application, species, age and/or individual condition. For oral application, the doses to be administered daily are between ca. 0.25 and 10 mg/kg, and for warm-blooded animals with a body weight of ca. 70 kg, preferably between ca. 20 mg and 500 mg, especially 40mg, 80mg, 160mg and 320mg based on the free acid.

The invention is illustrated in particular by the examples and also relates to the new compounds named in the examples and to their usage and to methods for the preparation thereof.

The following examples serve to illustrate the invention without limiting the invention in any way.

For example, the di-potassium salt of valsartan is formed, especially a hydrate thereof. The di-potassium salt is noted in particular for its marked water solubility. The crystalline tetrahydrate of the di-potassium salt of valsartan, with a melting point of 135.0°C, may be mentioned in particular. According to elementary analysis, a certain sample of this hydrate has a water content of 3.72 mols of water per mol of di-potassium salt. For high relative humidity at room temperature, the tetrahydrate is formed and for low values of relative humidity, the anhydrate of the di-potassium salt is formed.

A magnesium salt of valsartan is similarly produced, in this instance as an amorphous solid with 3.4% H_2O. The temperature of glass transition, as a mean value of the stage of the specific heat of 0.85 J • [g • ^{0} C]$^{-1}$ is 167 °C. No melting point is observed. Both facts, namely the glass transition and the absence of a melting point, together with the measured value of the change in specific heat, confirm that this magnesium salt of valsartan is practically 100% amorphous. According to a stereo-specific chromatography method, the enantiomer purity of this amorphous magnesium salt has been determined as 83%.

Example 1:

Production of the calcium salt as the tetrahydrate *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

21.775 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are dissolved at room temperature in 300 ml of ethanol. By careful addition of 300 ml of water, the ethanol concentration is reduced to 50% by volume. Using a magnetic stirrer, 3.89 g of $Ca(OH)_2$ are added slowly in small portions to this clear, slightly acidic (pH 4) solution, so that the pH value temporarily does not exceed a value of ca. 8. Because it absorbs CO_2 from the air, the $Ca(OH)_2$ used contains traces of $CaCO_3$; therefore the added amount includes an excess of 5%. After adding the stoichiometric amount of $Ca(OH)_2$, the pH is ca. 6, and after adding the excess it rises to 7. The solution becomes turbid through the small amount of finely divided $CaCO_3$, which is removed through a folded filter. The product contained in the solution crystallises continuously upon removal of the alcohol content by allowing to stand at room temperature. The procedure can be accelerated by using a flat dish in a recirculating air drier at 40°C. After concentrating to ca. one half, the alcohol content of the solution drops to ca. 10% by volume and most of the product crystallises. It is filtered, rinsed for a short tim with 10% by volume ethanol and dried at 40°C until reaching a constant weight. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt tetrahydrate is obtained.

The melting point for the tetrahydrate of the calcium salt of valsartan, produced according to example 1, for a heating rate of 10 $K{\bullet}min^{-1}$ and in a closed specimen container with a small internal volume is determined as 205°C and the melting enthalpy as 92 $kJ{\bullet}Mol^{-1}$.
The density of the crystals of the calcium-valsartan-tetrahydrate produced according to example 1, determined by a helium pycnometer, is 1.297 $g{\bullet}cm^{-3}$. This value conforms to the theoretically calculated value of 1.298 $g{\bullet}cm^{-3}$ calculated from the single crystal structure.
The optical rotation of the tetrahydrate of the calcium salt of valsartan according to example 1 is measured in methanol as a 1% solution $[a]^{20}_{D} = +1°$.
The enantiomer purity of the salt hydrate produced according to example 1 is determined by a stereo-specific HPLC method. The stereo-specific separation is achieved by a chiral column (Chiral AGP). The enantiomer purity is determined as ee = 100%.

Calculation of the interlattice plane intervals from the X-ray powder pattern taken with a Guinier camera is as follows for the most important lines for this batch of the tetrahydrate of the calcium salt of valsartan:
d in [Å] : 16.27, 9.90, 9.39, 8.04, 7.71, 7.05, 6.49, 6.34, 6.2, 5.87, 5.75, 5.66, 5.20, 5.05, 4.95, 4.73, 4.55, 4.33, 4.15, 4.12, 3.95, 3.91, 3.87, 3.35.

Elementary analysis gives the following measured values of the elements present in calcium-valsartan-tetrahydrate and of water. The water evaluation was carried out at 130°C after expulsion. The findings of the elementary analysis, within the error limits, correspond to the sum formula $(C_{24}H_{27}N_5O_3)^{2-}Ca^{2+} \bullet 4H_2O$.

	% found	% calculated
C	52.82	52.83
H	6.42	6.47
N	12.91	12.83
O	20.20	20.53
water	13.25	13.21

Ca	7.03	7.35

Example 2:

Production of the magnesium salt as the hexahydrate *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

43.55 g of valsartan [(S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine] are dissolved at room temperature in 600 ml of 50% by volume ethanol (from absolute ethanol - see Merck and quarz-bidistilled water). The slightly turbid solution becomes clear after adding a further 50 ml of 50% ethanol. Using a magnetic stirrer, 4.03 g or 0.1 M MgO (Merck p.a.) are slowly added in small portions to this slightly acidic solution with a pH value of 4. The pH value hereby rises to ca. 6. The process is effected with an excess of 10%, i.e. a further 0.40 g of MgO are added. This excess is not fully dissolved, and the pH value rises to ca. 7.5. The small residue is filtered from the solution through a folded filter and washed with 50 ml of 50% ethanol.

The combined clear solution is carefully concentrated at 40°C whilst stirring with a magnetic stirrer in a large crystallisation dish. Towards the end of this procedure, the solution has a tendency to harden into a glassy gel. Scratching with a glass rod induces the *in situ* crystallisation in this phase, which may be recognised by the white colour of the crystalline solid thus formed. The product is dried at 50°C in a recirculating air drier until reaching a constant weight. The yield of magnesium-valsartan-hexahydrate is 53.7 g or 95% based on the valsartan employed as the free acid.

The melting point for the salt hydrate produced according to example2, namely the magnesium-valsartan-hexahydrate, for a heating rate of 10 K•min^{-1} in a sealed sample container with a small internal volume, in an amount of 2.24 mg, was measured at 132°C and the melting enthalpy at 64 kJ•Mol^{-1}.

The density of the crystals of the hexahydrate of the magnesium salt of valsartan produced according to example 2, determined by a helium pycnometer, is 1.273 g•cm^{-3}. This value conforms to the theoretically calculated value of 1.256 g•cm^{-3} calculated from the single crystal structure.

The optical rotation of the magnesium-valsartan-hexahydrate produced according to example 2 is measured in methanol as a 1% solution $[a]^{20}_{D}$ = - 14 °.
The enantiomer purity of the salt hydrate produced according to example 2 is determined by a stereo-specific HPLC method. The stereo-specific separation is achieved by a chiral column (Chiral AGP). The enantiomer purity is determined as ee = 99.6 %.

Calculation of the interlattice plane intervals from the X-ray powder pattern taken with a Guinier camera is as follows for the most important lines for this batch of the magnesium valsartan hexahydrate:
d in [Å]: 19.78, 10.13, 9.84, 7.28, 6.00, 5.81, 5.67, 5.21, 5.04, 4.88, 4.21, 4.18, 4.08, 3.95, 3.46, 3.42.

Elementary analysis gives the following measured values of the elements present in the hexahydrate of the magnesium salt of valsartan and of water. The water evaluation is carried out at 130°C after expulsion. The findings of the elementary analysis, within the error limits, correspond to the sum formula $(C_{24} H_{27} N_5 O_3)^{2-} Mg^{2+} \cdot 6 H_2O$.

	% found	% calculated
C	51.03	50.94
H	7.00	6.95
N	12.45	12.38
O	25.02	25.44
water	19.08	19.10
Mg	4.35	4.29

Example 3:

Production of the hydrate of di-potassium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine (3.5 ± 1.0 mole H_2O)

5 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are dissolved whilst heating gently in 11.5 ml of 2 normal potassium hydroxide solution and mixed with 320 ml of acetonitrile. The mixture is heated for 5 minutes to reflux (turbid solution), left without stirring for 3 days at room temperature (seeding) and then left for 24 hours at 0°C. The mother liquor is decanted. The

crystallisate is washed twice with acetonitrile and then dried in the air for 36 hours until reaching a constant weight. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine dipotassium salt hydrate is obtained (3.7 mols water per mol dipotassium salt). The melting point in a closed specimen container is 135°C.

Elementary analysis: C_{24} H_{27} N_5 O_3 K_2, 3.72 H_2O, molar mass 578.72

	% found	% calculated
C	49.90	49.81
H	5.92	6.00
N	12.14	12.10
O	18.55	18.58
water	11.58	11.58
K	13.50	13.51

X-ray diffraction diagram measured with the diffractometer Scintag Inc., Cupertino, CA 95014, US, using CuKα radiation.

Reflection lines and intensities of the most important lines of the hydrate of the di-potassium salt of valsartan, values given in 2θ in °:

2θ in °	Intensity
4.6	strong
8.8	medium
9.2	strong
11.1	weak
12.5	weak
14.8	strong
15.3	weak
16.4	medium
17.8	strong
18.2	medium
18.4	medium
18.9	m dium

20.4	medium
21.1	weak
21.3	medium
22.3	weak
22.5	strong
23.1	medium
23.9	strong
25.6	weak
26.6	strong
26.9	medium
28.1	medium

Preferred are hydrates comprising the medium and strong intensity peaks.

Table 11:
Crystal data and parameters of the hydrate of the di-potassium salt of valsartan

Crystal data	
sum formula	$(C_{24}H_{27}N_6O_3)^{2-}2K^+ . x H_2O$ (x=3.5±1.0)
molecular mass	574.78
crystal system	orthorhombic
space group	$P2_12_12$
a (Å)	38.555(2)
b (Å)	7.577(1)
c (Å)	10.064(1)
V (Å^3)	2940.0(5)
Z	4
F(000)	1212
$D_{calc.}$ (g.cm^{-3})	1.286
number of reflections for cell parameters	25
θ range for cell parameters (°)	30-38
μ (mm^{-1})	3.24
Temperature (°C)	23
crystal shape	prisms

crystal size (mm)	0.63x0.20x0.14
crystal colour	colourless

Data collection

diffractometer	Enraf Nonius CAD4
radiation (graphite monochromator)	CuKα
wave length (Å)	1.54178
scan mode	ω/2θ
scan range (θ)	3-74
absorption correction	none
number of measured reflections	3450
number of observed reflections (I>2σ(I))	2867
h range	-48→0
k range	-9→0
l range	-12→0
number of standard reflections	3 every 120 mins
variation in intensity	±5%

Structure refinement

refinement method	refinement on F^2, complete matrix
number of parameters	341
R	0.069
R_W	0.182
S	1.57
number of reflections used	2867
treatment of H-atoms	"riding", apart from those of the water molecules ,which were ignored
Δ/σ_{max}	0.24
extinction correction	0.0010(5)
maximum/minimum residual electron density in final difference-Fourier calculation	0.815/-0.676(eÅ^{-3})
absolute structure parameters	-0.02(4)

Programmes used

SHELXS86 (Sheldrick, Göttingen),
XHELXL93 (Sheldrick, Göttingen),
SCHAKAL92 (Keller, Freiburg)

Example 4:

Production of the di-potassium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

25 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are dissolved in 200 ml of ethanol. 50 ml of water are added, the solution cooled to 0°C and then mixed with 57.4 ml of 2 normal potassium hydroxide solution. The mixture is concentrated by evaporation on a rotary evaporator, evaporated again with each of toluene and acetonitrile, and dried in a high vacuum for 15 minutes at 50°C. The product is dissolved in 290 ml of a hot mixture of acetonitrile/water (95:5), mixed with an additional 110 ml of acetonitrile, allowed to cool and seeded at ca. 30°C. The mixture is left to stand for 4 days at room temperature and filtered by suction. The residue is washed with acetonitrile/water (95:5) and dried in a high vacuum at 80°C. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine dipotassium salt is obtained as a white powder. Melting point >300°C.

Elementary analysis: The material obtained is hygroscopic and can be equilibrated in the air ($C_{24}H_{27}N_5O_3K_2$, 3.96 mols H_2O).

	% found	% calculated
C	49.15	49.44
H	6.02	6.04
N	11.91	12.01
O	19.18	19.1
water	12.23	12.24
K	13.4	13.41

Example 5:

Production of the di-sodium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

1 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine is dissolved in 50 ml of ethanol, mixed with 2.3 ml of 2 normal sodium hydroxide solution and concentrated by evaporation, and the residue is evaporated with each of ethanol and ethyl acetate. The white residue is stirred in hot acetonitrile and filtered by suction at room temperature. Drying in a high vacuum at 80°C over night yields (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine disodium salt as a white powder. Melting point from 260°C, brownish discolouration at 295°C.

Elementary analysis: The material obtained (hygroscopic) can be equilibrated in the air ($C_{24} H_{27} N_5 O_3 Na_2$, 5.36 mols H_2O, molar mass 576.05)

	% found	% calculated
C	49.79	50.04
H	6.51	6.60
N	12.00	12.16
O	23.44	23.22
water	16.75	16.76
Na	8.09	7.98

Example 6:

Production of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

5 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are added to a suspension of 0.666 g of magnesium hydroxide in 20 ml of water. 40 ml of methanol are added, then the mixture is stirred for 2 hours at room temperature and concentrated. The residue is dissolved in methanol, filtered through a hard filter, concentrated and evaporated with acetonitrile. The product is stirred with hot

acetonitrile, filtered by suction at room temperature and dried in a high vacuum at 90°C over night. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine magnesium salt is obtained as a white powder. Melting point: The sample becomes brownish upon heating and vitrifies towards 300°C.

Elementary analysis: C_{24} H_{27} N_5 O_3 Mg, 0.89 mols H_2O, molar mass: 473.85

	% found	% calculated
C	61.26	60.83
H	6.13	6.12
N	14.88	14.78
O		13.13
water	3.39	3.38
Mg	4.74	5.13

Example 7:

Production of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

5 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are added to a suspension of 0.851 g of calcium hydroxide in 20 ml of water and then mixed with 200 ml of ethanol. The mixture is stirred for one hour at room temperature, concentrated by evaporation to dryness (re-evaporation with acetonitrile), stirred in hot acetonitrile (with a trace each of ethanol and water) and filtered by suction at room temperature.

0.95 g of the salt are heated to reflux in 20 ml of acetonitrile/water (1:1), whereby the mixture almost dissolves. The mixture is allowed to cool to room temperature, mixed with 20 ml of acetonitrile, filtered by suction and washed twice with acetonitrile/water (1:1) and dried over night in a high vacuum at 80°C. Melting point: from 300°C (decomposition).

Elementary analysis: C_{24} H_{27} N_5 O_3 Ca, 1.71 mols H_2O, molar mass 504.39 (water evaluation carried out after expulsion at 150°C).

	% found	% calculated
C	56.88	57.15
H	6.13	6.08
N	13.89	13.88
O		14.94
water	6.12	6.11
Ca	7.94	7.95

Example 8:

Production of the mono-potassium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

2 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are suspended in 20 ml of water and mixed with 2.296 ml of a 2 normal potassium hydroxide solution. The mixture is stirred for 30 minutes and mixed with 50 ml of ethanol, whereupon a colourless solution is obtained. The mixture is concentrated by evaporation, evaporated once more with acetonitrile and lyophilised from tert.-butanol (with a trace of water).

Elementary analysis (after equilibration in the air). $C_{24} H_{27} N_5 O_3$ Ca, 1.69 mols H_2O, molar mass 504.06 (water evaluation carried out after expulsion at 150°C).

	% found	% calculated
C	57.30	57.19
H	6.35	6.27
N	13.61	13.89
O	14.58	14.89
water	6.04	6.04
K	7.72	7.76

Example 9:

Production of the magnesium salt as the hexahydrate of valsartan by a water-equilibrating process.

1600 g of valsartan and 6820 g of isopropanol are stirred to form a suspension in a mixing container at room temperature, and added to an 80 litre glass receptacle with a stirrer. The mixing container is rinsed with 3919 g of isopropanol in portions and the rinsing solution added to the main mixture. After adding 3800 g of deionised water, the mixture is transformed into a homogeneous solution by stirring. Then, 156.3 g of magnesium oxide, suspended in 1520 g of deionised water, are added and the suspension supplemented with 1000 g of deionised water. By slowly stirring at room temperature, the magnesium oxide goes into solution. The pH value of the resulting solution is ca. 7.2. By adding a further 2.5 g of magnesium oxide in small portions, the pH value is raised to ca. 8.3. The resulting mixture is turbid owing to undissolved particles of unknown type in the magnesium oxide.

This mixture is transferred through a candle filter to a 35 litre enamel boiler and the glass receptacle and the transfer tube are rinsed with 885 g of isopropanol and 1122 g of deionised water. For mild concentration, a vacuum is created in the boiler to an initial theoretical value of 89-100 mbar. With a temperature of the heating medium of 45-50°C and a boiling temperature of the mixture of 37-40°C, a total of 13.66 kg of aqueous isopropanol is distilled. By lowering the distillation pressure to a final value of 10 mbar and simultaneously raising the heating medium temperature to 65°C, the amount of distillate is increased to a total of 17.12 kg. 9300 g of ethyl acetate, followed by 14.9 g of valsartan Mg salt hexahydrate as seeding crystals, are added to the boiler content whilst stirring. Finally, a further 6680 g of ethyl acetate are dispensed in and cooling is effected to room temperature whilst stirring. The stirring procedure is maintained for at least 24 hours. The suspension is then filtered through Büchner filters. A moist filter cake is thus obtained. The boiler is rinsed with 1171 g of ethyl acetate and the rinsing mixture is used to wash the filter cake. Drying of a partial amount on metal sheets in a vacuum drying chamber at 50 mbar pressure and 40°C oven temperature for 6.5 hours until reaching a constant weight yields a dry substance.

The physical data, especially the X-ray powder pattern, correspond to the magnesium hexahydrate salt of example 2.

Example 10:

Production of the calcium salt of valsartan as the tetrahydrate.

1600 g of valsartan and 7000 g of ethanol are stirred to form a suspension in a mixing container at room temperature, and added to a 35 litre enamel boiler with a stirrer. The mixing container is rinsed with 2000 g of ethanol in portions and the rinsing solution added to the main mixture. After adding 9000 g of deionised water, the mixture is transformed into a homogeneous solution by stirring. Then, 272 g of calcium hydroxide, suspended in 1500 g of deionised water, are added and the suspension supplemented with 1300 g of deionised water. By slowly stirring at room temperature, the calcium hydroxide goes into solution. The pH value of the resulting solution is ca. 6.9. By adding a further 9.6 g of calcium hydroxide, the pH value is raised to ca. 10.6. The resulting mixture is turbid owing to undissolved particles (calcium carbonate) in the calcium hydroxide. This mixture is transferred through a candle filter to a 35 litre enamel boiler and the glass receptacle and the transfer tube are rinsed with a solution of 1048 g of ethanol and 1000 g of deionised water. For mild concentration, a vacuum is created in the boiler to a theoretical value of 100-120 mbar. With a temperature of the heating medium of ca. 50°C and a boiling temperature of the mixture of max. 44°C, a total of 11.32 kg of aqueous ethanol is distilled. The dissolved salt crystallises spontaneously during the course of distillation. The suspension present at the end of distillation is cooled to ca. 5°C whilst stirring, and is stirred for ca. 16 hours at 5°C. The suspension is then filtered through Büchner filters. The boiler is rinsed with a mixture of 3600 ml of deionised water and 400 ml of ethanol, the mixture being cooled to 5°C, and the rinsing mixture is used to wash the filter cake. A moist filter cake is thus obtained. Drying of a partial amount on metal sheets in a vacuum drying chamber at 50 mbar pressure and 40°C oven temperature for 24 hours until reaching a constant weight yields a dry substance.

The physical data, especially the X-ray powder pattern, correspond to the calcium tetrahydrate salt of example 1.

Example 11:

Hydrate of valsartan disodium salt (2.4 ± 1.0 mole H_2O):

50 ml of 2N sodium hydroxide solution are added dropwis at ca. 25°C to a solution of 21.5 g of valsartan in 200 ml of isopropanol. The clear solution (pH ca. 7.2) is concentrated under vacuum at ca. 40°C. The amorphous residue of the disodium salt is suspended in 100 ml of isopropanol, and water is removed by concentrating under vacuum once more at ca. 40°C and degassing. The amorphous residue is suspended in 75 ml of acetone and 2 ml of water at ca. 40°C. At ca. 25-30°C, 200 ml of tert.-butylmethylether are added, whereby constituents that are initially smeary are gradually transformed into a crystalline suspension. After stirring over night at ca. 25°C, the suspension is cooled to 10°C and after ca. 1 hour is filtered by suction whilst excluding atmospheric moisture. Washing then takes place with 20 ml of tert.-butylmethylether. The moist filter cake is dried over night at ca. 30 mbar and at 30°C. A colourless, slightly hygroscopic crystal powder is obtained.

Elementary analysis: C_{24} H_{27} N_6 O_3 Na_2, 2.44 mols H_2O

	% found	% calculated
C	55.03	55.07
H	6.16	6.14
N	13.38	13.38
O		16.63
water	8.40	8.41
Na	8.67	8.78

X-ray diffraction diagram (reflection lines and intensities of the most important lines) of the crystalline hydrate of the disodium salt of valsartan measured with the diffractometer Scintag Inc. Cupertino, CA 95014, US, using CuKα radiation:

2θ	Intensity
4.7	strong
9.1	strong
13.3	weak
13.7	weak
15.6	medium
16.4	medium

17.2	medium
17.9	medium
18.7	medium
19.6	medium
21.3	medium
21.9	medium
22.8	strong
24.0	weak
24.8	weak
25.5	weak
26.5	medium
26.8	weak
27.3	weak
27.8	weak
28.6	weak
29.4	weak
29.9	medium

Example 12:

Hydrate of the valsartan dipotassium salt (3.4 ± 1.0 mole H_2O):

6.9 g of potassium carbonate are added at ca. 25°C to the solution of 21.7 g of valsartan in 150 ml of acetone and 20 ml of water. After stirring for 2 hours at ca. 25°C, an almost clear solution is obtained, which is concentrated in a vacuum at ca. 50°C bath temperature. 55 ml of acetone are added to the residue (29.3 g) which contains residual water, and at ca. 35°C, over the course of ca. two hours, a total of 250 ml of tert.-butylmethylether is dispensed in. After stirring at ca. 25°C, the easily stirrable crystal suspension is cooled to 10°C, stirred for at least one hour, filtered by suction and washed with 20 ml of tert.butylmethylether. The moist filter cake is dried over night at ca. 30 mbar and at 30°C. A colourless, slightly hygroscopic crystal powder is obtained.

Elementary analysis: C_{24} H_{27} N_5 O_3 K_2, 3.42 mols H_2O

	% found	% calculated
C	50.37	50.28
H	5.87	5.95
N	12.24	12.22
O		17.92
water	10.76	10.75
K	13.4	13.64

X-ray diffraction diagram measured with the diffractometer Scintag Inc., Cupertino, CA 95014, US using a CuKα radiation.

Reflection lines and intensities of the most important lines of the hydrate of the di-potassium salt of valsartan, values given in 2θ in °:

2θ in °	Intensity
4.9	strong
9.4	strong
11.4	weak
12.8	weak
14.0	weak
15.0	weak
15.6	weak
16.6	medium
18.0	weak
18.5	weak
18.9	weak
20.7	weak
21.5	weak
22.0	weak
22.7	medium
23.3	weak
24.1	medium
25.6	weak
25.8	weak

27.1	medium
29.4	weak

Preferred are hydrates comprising medium and strong intensity peaks.

Example 13:

Valsartan calcium/magnesium mixed salt:

21.5 g of valsartan in 200 ml of isopropanol and 100 ml of water are stirred for ca. 3 hours at ca. 25°C with 1.5 g of magnesium hydroxide and 1.9 g of calcium hydroxide. The practically clear solution is concentrated in a vacuum at ca. 50°C. A total of 240 ml of ethyl acetate is added with stirring to the still warm, semi-solid residue which contains residual water. Upon stirring over night at ca. 25°C, initially sticky constituents are transformed into a homogeneous suspension. The suspension is filtered by suction and washed with 20 ml of ethyl acetate. The moist filter cake is dried in a vacuum at 30-40°C. A colourless crystal powder is obtained.

The X-ray diffraction diagram corresponds to a conglomerate of calcium tetrahydrate and magnesium hexahydrate from example 1 and 2.

Example 14:

Valsartan bis-diethylammonium salt:

1.5 g of diethylamine are added dropwise at ca. 25°C to the solution of 4.35 g of valsartan in 60 ml of acetone. After a short time, crystallisation slowly sets in. After stirring over night, the crystallisate is filtered by suction at ca. 20°C, washed with cold acetone and dried in a vacuum at ca. 50°C. A colourless crystal powder is obtained.

Elementary analysis: $C_{32}H_{51}N_7O_3$, 0.1 mols H_2O

	% found	% calculated
C	65.82	65.84
H	8.90	8.84
N	16.84	16.80

O		8.52
water	0.34	0.34

X-ray diffraction diagram (reflection lines and intensities of the most important lines) of the crystalline bis-diethylammonium salt

2θ	Intensity
4.7	weak
8.5	strong
9.3	strong
10.8	strong
11.3	weak
13.4	strong
14.0	medium
14.3	weak
14.9	medium
17.1	medium
17.4	medium
17.6	medium
18.3	weak
19.0	medium
20.0	weak
21.2	medium
21.6	weak
22.4	medium
22.7	weak
24.9	medium
25.2	weak
27.0	weak

Example 15:

Valsartan bis-dipropylammonium salt:

2.1 g of dipropylamine are added dropwise at 25°C to the solution of 4.35 g of valsartan in 60 ml of acetone. When crystallisation has set in, the temperature is raised for a brief period to 40°C and is allowed to drop to room temperature over ca. 2 hours. After stirring over night, the crystallisate is filtered by suction, washed twice with 15 ml of acetone and dried in a vacuum at ca. 40°C. Granular crystals are obtained.

Elementary analysis: C_{36} H_{69} N_7 O_3, 0.05 mols H_2O

	% found	% calculated
C	67.74	67.69
H	9.32	9.33
N	15.36	15.35
O		7.64
water	0.13	0.14

X-ray diffraction diagram (reflection lines and intensities of the most important lines) of the crystalline bis-dipropylammonium salt

2θ	intensity
8.5	strong
8.9	weak
9.4	strong
10.0	medium
11.2	weak
11.6	weak
12.5	weak
13.2	strong
13.9	strong
14.3	weak
14.7	weak
15.1	weak
15.6	weak
16.0	weak
17.0	medium
17.9	medium
18.7	strong
19.9	weak

20.4	weak
20.6	weak
21.0	strong
21.7	weak
22.3	medium
23.1	strong
24.5	weak
25.5	medium
25.8	weak
26.7	weak
28.6	weak

Example 16:

Bis-dibutylammonium salt of valsartan:

A solution of 2.15 g of valsartan in 30 ml of acetone is mixed with 1.4 g of dibutylamine at ca. 25°C. Crystallisation sets in after a short time, and the thick suspension is gradually diluted with 20 ml of isopropyl acetate over ca. 1 hour. After stirring for 4 hours at ca. 25°C, the crystals are removed by suction, washed twice with 10 ml of isopropyl acetate and dried in a vacuum at 50°C. A colourless, slightly hygroscopic crystal powder is obtained.

Elementary analysis: $C_{40} H_{87} N_7 O_3$, 0.5 mols H_2O

	% found	% calculated
C	68.25	68.30
H	9.79	9.75
N	13.89	13.94
O		8.01
water	1.33	1.33

X-ray diffraction diagram (reflection lines and intensities of the most important lines) of the crystalline bis-dibutylammonium salt

2θ	Intensity
7.5	very strong
8.5	medium
9.7	strong
12.7	strong
13.3	weak
14.1	strong
15.1	medium
16.4	strong
17.7	weak
18.2	weak
19.5	strong
19.9	medium
20.5	medium
21.4	medium
21.9	medium
22.2	medium
22.6	medium
23.0	strong
23.7	weak
24.2	weak
24.7	medium
25.7	medium
26.0	weak
26.5	weak
28.8	weak

<u>Formulation example 1:</u>

Directly compressed tablet:

No.	Ingredient	proportion per batch [g]	proportion per tablet core [mg]
1	valsartan calcium salt tetrahydrate	134.24	80

2	Avicel PH 102 (microcrystalline cellulose)	60.408	36
3	lactose (crystalline)	96.1494	57.3
4	crospovidone	7.551	4.5
5	aerosil 200 (silica, colloidal anhydrous)	0.839	0.5
6	magnesium stearate (vegetable)	6.2086	3.7

Ingredient no. 1 is sieved through a 0.5 mm sieve and mixed for 15 minutes in a Turbula with ingredients 1-6. Tablets are compress using a single punch tablet press with punches of a diameter of 8mm.

Formulation example 2:

Tablet produced by roller compaction:

No.	Ingredient	proportion per batch [g]	proportion per tablet core [mg]
1	valsartan magnesium salt hexahydrate	400	80
2	Avicel PH 102 (microcrystalline cellulose)	270	54
3	crospovidone	75	15
4	aerosil 200 (silica, colloidal anhydrous)	7.5	1.5
5	magnesium stearate	15	3
6	magnesium stearate	7.5	1.5

Ingredients no. 1-5 are mixed for 50 minutes and compacted on a Freund roller compactor. The band is milled and after admixing ingredient no 6, compressed into tablets using a single punch tablet press with punches of a diameter of 8mm.

What we claim is:

1. A salt of valsartan, selected from the group consisting of the monosodium salt, the monopotassium salt, the disodium salt, the dipotassium salt, the magnesium salt, the calcium salt, the bis-diethylammonium salt, the bis-dipropylammonium salt, the bis-dibutylammonium salt, the mono-L-arginine salt, the bis-L-arginine salt, the mono-L-lysine salt and the bis-L-lysine salt, as well as salt mixtures thereof.

2. A salt according to claim 1 in crystalline, partially crystalline or amorphous form.

3. The calcium salt or the magnesium salt of valsartan according to claim 1.

4. The tetrahydrate of the calcium salt of valsartan according to claim 3.

5. The tetrahydrate according to claim 4, characterised by
(i) an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals:
d in [Å]: 16.1±0.3, 9.9±0.2, 9.4±0.2, 7.03±0.1, 6.50±0.1, 5.87±0.05, 5.74±0.05, 4.95±0.05, 4.73±0.05, 4.33±0.05, 4.15±0.05, 4.12±0.05, 3.95±0.05; or
(ii) an ATR-IR spectrum having the following absorption bands expressed in reciprocal wave numbers (cm^{-1}):
1621 (st); 1578 (m); 1458 (m); 1441 (m); 1417 (m); 1364 (m); 1012 (m); 758 (m); 738 (m); 696 (m); 666 (m).

6. The hexahydrate of the magnesium salt of valsartan according to claim 1.

7. The hexahydrate according to claim 6, characterised by
(i) an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals:
d in [Å] : 19.7±0.3, 10.11±0.2, 9.8±0.2, 7.28±0.1, 5.81±0.05, 5.68±0.05, 5.03±0.05, 4.88±0.05, 4.18±0.05, 4.08±0.05, 3.46 ±0.05; or

(ii) an ATR-IR spectrum having the following absorption bands expressed in reciprocal wave numbers (cm^{-1}):
3378 (m); 3274 (m); 2956 (m); 1619 (st); 1557 (m); 1464 (m); 1419 (m); 1394 (st); 1374 (m); 1175 (m); 836 (m); 820 (s); 766 (st); 751 (m); 741 (st); 732 (st).

8. A salt according to one of claims 1-7 in the form of a solvate.

9. A salt according to one of claims 1-8 in the form of a hydrate.

10. A salt according to one of claims 1-9 in a form selected from the group consisting of
(i) a crystalline form;
(ii) a partly crystalline form;
(iii) an amorphous form; and
(iv) a polymorphous form.

11. Pharmaceutical preparation containing a compound according to one of claims 1 to 10 and a pharmaceutically acceptable excipient or additive.

12. Pharmaceutical preparation according to claim 11, containing a salt according to one of claims 1-9 in combination with at least one composition selected from the group consisting of a:
(i) HMG-Co-A reductase inhibitor or a pharmaceutically acceptable salt thereof,
(ii) angiotensin converting enzyme (ACE) inhibitor or a pharmaceutically acceptable salt thereof,
(iii) calcium channel blocker or a pharmaceutically acceptable salt thereof,
(iv) aldosterone synthase inhibitor or a pharmaceutically acceptable salt thereof,
(v) aldosterone antagonist or a pharmaceutically acceptable salt thereof,
(vi) dual angiotensin converting enzyme/neutral endopeptidase (ACE/NEP) inhibitor or a pharmaceutically acceptable salt thereof,
(vii) endothelin antagonist or a pharmaceutically acceptable salt thereof,
(viii) renin inhibitor or a pharmaceutically acceptable salt thereof, and
(ix) diuretic or a pharmaceutically acceptable salt thereof.

13. Use of a compound according to one of claims 1 to 10 in the preparation of a medicament for the prophylaxis or treatment of diseases and conditions which can be inhibited by blocking the AT_1 receptor.

14. Process for the manufacture of a salt according to claim 1, characterised in that
(i) valsartan and the appropriate base are added to a water-containing organic solvent,
(ii) the solvent is concentrated, for example by heating, if necessary under reduced pressure or by slowly evaporating, e.g. at room temperature,
(iii) the residue of evaporation is equilibrated with the required amount of water by
(a) suspending the residue of evaporation, which is advantageously still warm, and which still contains some water, in an appropriate solvent or
(b) by equilibrating the water excess in the solvent;
whereby in a) and b) the existing or added water is present in a quantity in which the water dissolves in the organic solvent and does not form an additional phase; and
(iv) the salt obtained is isolated.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/08253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D257/04 A61K31/41 A61P9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 071 931 A (HUMKE ULRICH) 6 June 2000 (2000-06-06) column 5, line 38-65 ---	1-9
Y	WO 99 67231 A (NICOX SA ;DEL SOLDATO PIERO (IT)) 29 December 1999 (1999-12-29) page 2 -page 5; example 4 ---	1-9
Y	EP 0 443 983 A (CIBA GEIGY AG) 28 August 1991 (1991-08-28) cited in the application page 17, line 47,48; example 16 ---	1-9
P,Y	WO 00 59475 A (LIPOCINE INC) 12 October 2000 (2000-10-12) page 8, line 23 page 14 -page 15 ---	1-9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 November 2001	04/12/2001

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International Application No
PCT/EP 01/08253

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Information on patent family members

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(54) Title: DIPEPTIDE DERIVATIVES

(57) Abstract: Compounds of the formula [formula] wherein,R_1R_1, $COOR_2$,R_7,end x have meaning as defined, such being useful as dual inhibitors of angiotensin converting enzyme and neutral endopeptidase, as well as inhibitors of endothelin converting enzyme.

DIPEPTIDE DERIVATIVES

Summary of the Invention

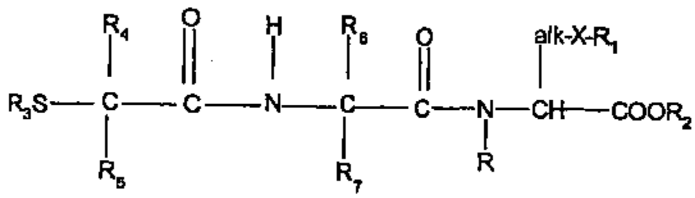
The present invention directed to novel vasopeptidase inhibitors described below which are useful as dual inhibitors of both angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP, EC 3.4.24.11). The compounds of the invention are particularly useful for the treatment and/or the prevention of conditions which are responsive to ACE and NEP inhibition, particularly cardiovascular disorders, such as hypertension, isolated systolic hypertension, renal failure (including edema and salt retention), pulmonary edema, left ventricular hypertrophy, heart failure (including congestive heart failure) and atherosclerosis. The compounds of the Invention are also useful for reducing elevated cholesterol plasma levels in mammals. Furthermore, they also inhibit endothelin converting enzyme (ECE) and are useful for the treatment and/or prevention of conditions which are responsive to ECE inhibition.

By virtue of their inhibition of neutral endopeptidase, the compounds of the invention may also be useful for the treatment of pain, depression, certain psychotic conditions, and cognitive disorders. Other potential indications include the treatment of angina, premenstrual syndrome, Meniere's disease, hyperaldosteronism, hypercalciuria, ascites, glaucoma, asthma and gastrointestinal disorders such as diarrhea, irritable bowel syndrome and gastric hyperacidity.

By virtue of their inhibition of ECE, the compounds of the invention may also be useful for the treatment and/or prevention of endothelin dependent conditions and diseases, including cerebral ischemia (stroke), subarachnoid hemorrhage, traumatic brain injury, cerebral vasospasm, arterial hypertrophy, restenosis, Raynaud's disease, myocardial infarction, obesity; also prostate hyperplasia, migraine, diabetes mellitus (diabetic nephropathy), preeclampsia, glaucoma, and transplantation rejection such as in aorta or solid organ transplantation; as well as erectile dysfunction.

Detailed Description of the Invention

The present invention relates to compounds of formula I



(I)

wherein

R represents hydrogen, lower alkyl, carbocyclic or heterocyclic aryl-lower alkyl or cycloalkyl-lower alkyl;

R_1 represents lower alkyl, cycloalkyl, carbocyclic or heterocyclic aryl, or biaryl; or R_1 represents (cycloalkyl, carbocyclic aryl, heterocyclic aryl or biaryl)-lower alkyl;

alk represents lower alkylene;

R_3 represents hydrogen or acyl;

R_4 represents hydrogen, optionally substituted lower alkyl, carbocyclic or heterocyclic aryl, (carbocyclic or heterocyclic aryl)-lower alkyl, cycloalkyl, cycloalkyl-lower alkyl, biaryl, biaryl-lower alkyl, oxacycloalkyl, thiacycloalkyl, azacycloalkyl, or (oxacycloalkyl, thiacycloalkyl or azacycloalkyl)-lower alkyl;

R_5 represents hydrogen or lower alkyl; or

R_4 and R_5, together with the carbon atom to which they are attached, represent cycloalkylidene, benzo-fused cycloalkylidene; or 5- or 6-membered (oxacycloalkylidene, thiacycloalkylidene or azacycloalkylidene), each optionally substituted by lower alkyl or aryl-lower alkyl;

R_6 represents lower alkyl, carbocyclic or heterocyclic aryl, (carbocyclic or heterocyclic aryl)-lower alkyl, cycloalkyl, cycloalkyl-lower alkyl, biaryl or biaryl-lower alkyl;

R_7 represents lower alkyl, (carbocyclic or heterocyclic aryl)-lower alkyl, cycloalkyl-lower alkyl or biaryl-lower alkyl; or

R_6 and R_7, together with the carbon atom to which they are attached, represent 3- to 10-membered cycloalkylidene which may be substituted by lower alkyl or aryl-lower alkyl or may be fused to a saturated or unsaturated carbocyclic 5- to 7-membered ring; or 5- or

6-membered (oxacycloalkylidene, thiacycloalkylidene or azacycloalkylidene), ach optionally substituted by lower alkyl or aryl-lower alkyl; or 2,2-norbonylidene;

X represents -O-, -S(O)$_n$-, -NHSO$_2$-, or -NHCO-;

n is zero, one or two; and

COOR$_2$ represents carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester;

disulfide derivatives derived from said compounds wherein R$_3$ is hydrogen; and pharmaceutically acceptable salts thereof.

The present invention is also directed to pharmaceutical compositions comprising said compounds; methods for preparation of said compounds; intermediates; and methods of treating disorders in mammals which are responsive to ACE and NEP inhibition by administration of said compounds to mammals in need of such treatment.

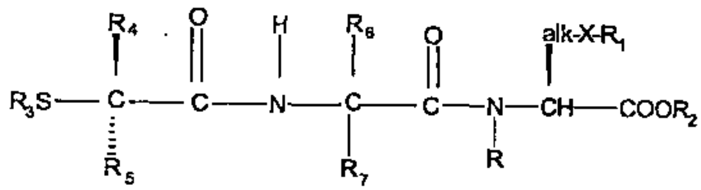
Encompassed by the instant invention are also any prodrug derivatives of compounds of the invention having a free carboxyl, sulfhydryl or hydroxy group, said prodrug derivatives being convertible by solvolysis or under physiological conditions to be the free carboxyl, sulfhydryl and/or hydroxy compounds. Prodrug derivatives are, e.g., the esters of free carboxylic acids and S-acyl and O-acyl derivatives of thiols, alcohols or phenols, wherein acyl has meaning as defined herein.

Pharmaceutically acceptable esters are preferably prodrug ester derivatives, such being convertible by solvolysis or under physiological conditions to the free carboxylic acids of formula I.

Pharmaceutically acceptable prodrug esters are preferably, e.g., lower alkyl esters, aryl-lower alkyl esters, α-(lower alkanoyloxy)-lower alkyl esters such as the pivaloyloxymethyl ester, and α-(lower alkoxycarbonyl, morpholinocarbonyl, piperidinocarbonyl, pyrrolidinocarbonyl or di-lower alkylaminocarbonyl)-lower alkyl esters.

Pharmaceutically acceptable salts are salts derived from pharmaceutically acceptable bases for any acidic compounds of the invention, e.g., those wherein COOR$_2$ represents carboxyl. Such are, e.g., alkali metal salts (e.g., sodium, potassium salts), alkaline earth metal salts (e.g., magnesium, calcium salts), amine salts (e.g., tromethamine salts).

Compounds of formula I, depending on the nature of substituents, possess two or more asymmetric carbon atoms. The resulting diastereomers and optical antipodes are encompassed by the instant invention. The preferr d configuration is indicated in formula Ia.



(Ia)

wherein asymmetric carbons carrying the substituents - alk-X-R_1 and R_4 typically have the S-configuration.

Preferred are the compounds of formula I and Ia wherein R and R_5 represent hydrogen; R_1 represents lower alkyl, C_5- or C_6-cycloalkyl, carbocyclic or heterocyclic aryl, or (carbocyclic or heterocyclic aryl)-lower alkyl; alk represents lower alkylene; X represents -O- or -S(O)$_n$ wherein n represents zero or two; R_3 represents hydrogen or acyl; R_4 represents optionally substituted lower alkyl, oxacycloalkyl, oxacycloalkyl-lower alkyl, or (carbocyclic or heterocyclic aryl)-lower alkyl; R_5 represents hydrogen; or R_4 and R_5 combined with the carbon atom to which they are attached represent C_5 or C_6-cycloalkylidene; R_6 and R_7 represent lower alkyl; or R_6 and R_7, together with the carbon atom to which they are attached, represent 5- or 6-membered cycloalkylidene; $COOR_2$ represents carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester; disulfide derivatives derived from said compounds wherein R_3 is hydrogen; and pharmaceutically acceptable salts thereof.

Further preferred are said compounds of formula I and Ia wherein R and R_5 represent hydrogen; R_1 represents carbocyclic or heterocyclic aryl or (carbocyclic or heterocyclic aryl)-lower alkyl; R_3 represents hydrogen or optionally substituted lower alkanoyl; R_4 represents lower alkyl, cycloalkyl, tetrahydropyranyl or C_1-C_4-lower alkoxy-lower alkyl; R_6 and R_7 both represent C_1-C_4-alkyl and are identical; X represents -O- or -S-; alk represents methylene; $COOR_2$ represents carboxyl, lower alkoxycarbonyl, (di-lower alkylaminocarbonyl)-lower alkoxycarbonyl or (morpholinocarbonyl, piperidinocarbonyl or pyrrolidinocarbonyl)-lower alkoxycarbonyl; and pharmaceutically acceptable salts thereof.

Particularly preferred are said compounds of formula I or Ia wherein R and R_5 represent hydrogen; R_1 represents carbocyclic aryl or carbocyclic aryl-lower alkyl in which carbocyclic aryl represents phenyl or phenyl substituted by one or two of hydroxy, lower alkanoyloxy, lower alkyl, lower alkoxy, trifluoromethyl, trifluoromethoxy or halo; R_3 represents hydrogen, lower alkanoyl or lower alkanoyl substituted by lower alkoxy; R_4 r presents lower alkyl, 4-tetrahydropyranyl or C_1-C_4-low r alkoxy-C_1-C_4-lower alkyl; R_6 and R_7 represent methyl; X represents -O-; alk represents methylene or ethyl ne; and $COOR_2$ represents carboxyl or lower alkoxycarbonyl; and pharmaceutically acceptable salts thereof. An embodiment thereof relates to compounds wherein R_3 represents hydrogen or lower alkanoyl.

Further preferred are the above compounds of formula I or Ia wherein R and R_5 represent hydrogen; R_1 represents phenyl, fluorophenyl, benzyl or fluorobenzyl; R_3 represents hydrogen, lower alkanoyl or lower alkanoyl substituted by lower alkoxy; R_4 represents isopropyl, *tert*-butyl, 1-methoxyethyl or 4-tetrahydropyranyl; R_6 and R_7 represent methyl; X represents -O-; alk represents methylene; and $COOR_2$ represents carboxyl or lower alkoxycarbonyl; and pharmaceutically acceptable salts thereof. An embodiment thereof relates to compounds wherein R_3 represents hydrogen or lower alkanoyl.

Preferred particular embodiments relate to compounds of formula I or Ia wherein R and R_5 represent hydrogen; R_1 represents benzyl; R_3 represents hydrogen, acetyl or methoxyacetyl; R_4 represents isopropyl or *tert*-butyl; R_6 and R_7 represent methyl; X represents -O-; alk represents methylene; and $COOR_2$ represents carboxyl or ethoxycarbonyl; or a pharmaceutically acceptable salt thereof.

The definitions as such or in combination as used herein, unless denoted otherwise, have the following meanings within the scope of the present invention.

Aryl represents carbocyclic or heterocyclic aryl, either monocyclic or bicyclic.

Monocyclic carbocyclic aryl represents optionally substituted phenyl, being preferably phenyl or phenyl substituted by one to three substituents, such being advantageously lower alkyl, hydroxy, lower alkoxy, acyloxy, halogen, cyano, trifluoromethyl, trifluoromethoxy, amino, lower alkanoylamino, lower alkyl-(thio, sufinyl or sulfonyl), lower alkoxycarbonyl, mono- or di-lower alkylcarbamoyl, or mono- or di-lower alkylamino; or phenyl substituted by lower alkylenedioxy.

Bicyclic carbocyclic aryl represents 1- or 2-naphthyl or 1- or 2-naphthyl preferably substituted by lower alkyl, lower alkoxy or halogen.

Monocyclic heterocyclic aryl represents preferably optionally substituted thiazolyl, pyrimidyl, triazolyl, thienyl, furanyl or pyridyl.

Optionally substituted furanyl represents 2- or 3-furanyl or 2- or 3-furanyl preferably substituted by lower alkyl.

Optionally substituted pyridyl represents 2-, 3- or 4-pyridyl or 2-, 3- or 4-pyridyl preferably substituted by lower alkyl, halogen or cyano.

Optionally substituted thienyl represents 2- or 3-thienyl or 2- or 3-thienyl preferably substituted by lower alkyl.

Optionally substituted pyrimidyl represents, e.g., 2-pyrimidyl or 2-pyrimidyl substituted by lower alkyl.

Optionally substituted thiazolyl represents, e.g., -2-thiazolyl or 2-thiazolyl substituted by lower alkyl.

Optionally substituted triazolyl represents, e.g., 1,2,4-triazolyl or 1,2,4-triazolyl preferably substituted by lower alkyl.

Bicyclic heterocyclic aryl represents preferably indolyl, benzothiazolyl, quinolinyl or isoquinolinyl optionally substituted by hydroxy, lower alkyl, lower alkoxy or halogen, advantageously 3-indolyl, 2-benzothiazolyl or 2- or 4-quinolinyl.

Aryl as in aryl-lower alkyl is preferably phenyl or phenyl substituted by one or two of lower alkyl, lower alkoxy, hydroxy, lower alkanoyloxy, halogen, trifluoromethyl, cyano, lower alkanoylamino or lower alkoxycarbonyl; also, optionally substituted naphthyl.

Aryl-lower alkyl is advantageously benzyl or 1- or 2-phenethyl optionally substituted on phenyl by one or two of lower alkyl, lower alkoxy, hydroxy, lower alkanoyloxy, halogen or trifluoromethyl.

The term "lower" referred to herein in connection with organic radicals or compounds respectively defines such with up to and including 7, preferably up and including 4 and advantageously one or two carbon atoms. Such may be straight chain or branched.

Optionally substituted lower alkyl refers to lower alkyl or lower alkyl substituted by, e.g., halo, hydroxy, lower alkoxy, amino, (mono- or di-lower alkyl) amino, acylamino, 1-lower alkyl-piperazino, morpholino, piperidino, pyrrolidino and the like.

Lower alkylene refers to a straight or branched carbon chain having preferably 1 to 4 carbon atoms, which may be substituted, e.g., by lower alkoxy, for example, $-CH_2-$, -CH $(CH_3)-$, $-CH_2CH_2-$ and the lik .

A lower alkyl group preferably contains 1-4 carbon atoms which may be straight chain or branched and represents, for example, ethyl, propyl, butyl or advantageously methyl.

A lower alkoxy group preferably contains 1-4 carbon atoms which may be straight chain or branched and represents, for example, methoxy, propoxy, isopropoxy or advantageously ethoxy.

Cycloalkyl represents a saturated cyclic hydrocarbon radical which preferably contains 5- to 7-ring carbons, preferably cyclopentyl or cyclohexyl.

Oxacycloalkyl represents preferably 5- to 7-membered oxacycloalkyl, e.g., tetrahydropyranyl, such as 4-tetrahydropyranyl.

Thiacycloalkyl represents preferably 5- to 7-membered thiacycloalkyl, e.g., tetrahydrothiopyranyl, such as 4-tetrahydrothiopyranyl.

Azacycloalkyl represents preferably 5- to 7-membered azacycloalkyl, e.g., pyrrolidinyl or piperidinyl in which the nitrogen may be substituted by lower alkyl or aryl-lower alkyl.

The term cycloalkyl-lower alkyl represents preferably (cyclopentyl or cyclohexyl)-methyl, 1- or 2-(cyclopentyl or cyclohexyl)ethyl, 1-, 2- or 3-(cyclopentyl or cyclohexyl)propyl, or 1-, 2-, 3- or 4-(cyclopentyl or cyclohexyl)-butyl. Similarly (oxacyclyl, thiacycloalkyl or azacycloalkyl)-lower alkyl.

A lower alkoxycarbonyl group preferably contains 1 to 4 carbon atoms in the alkoxy portion and represents, for example, methoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl or advantageously ethoxycarbonyl.

Cycloalkylidene is 3- to 10-membered, preferably 5- or 6-membered, and represents a cycloalkane linking group in which the two attached groups are attached to the same carbon of the cycloalkane ring.

5- or 6-membered oxacycloalkylidene represents a tetrahydrofuran or tetrahydropyran linking group, i.e., tetrahydrofuranylidene or tetrahydropyranylidene, in which the two attached groups are attached to the same carbon atom of th respective rings, .g., at the 3- or 4-position thereof.

5- or 6-membered thiacycloalkylidene represents a tetrahydrothiophene or tetrahydrothiopyran linking group in which the two attached groups are attached to the same carbon atom of the respective rings, e.g., at the 3- or 4-position thereof.

5- or 6-membered azacycloalkylidene represents a pyrrolidine or piperidine linking group in which the two attached groups are attached to the same carbon atom of the respective rings, e.g., at the 3- or 4-position thereof, and the nitrogen may be substituted by lower alkyl, e.g., methyl, or by aryl-lower alkyl, e.g., benzyl.

Benzo-fused cycloalkylidene represents, e.g., 1,1- or 2,2-tetralinylidene or 1,1- or 2,2-indanylidene.

Halogen (halo) preferably represents fluoro or chloro, but may also be bromo or iodo.

Acyl is derived from a carboxylic acid and represents preferably optionally substituted lower alkanoyl, carbocyclic aryl-lower alkanoyl, aroyl, lower alkoxycarbonyl or aryl-lower alkoxycarbonyl, advantageously optionally substituted lower alkanoyl, or aroyl.

Lower alkanoyl is preferably acetyl, propionyl, butyryl, or pivaloyl.

Optionally substituted lower alkanoyl, for example, represents lower alkanoyl or lower alkanoyl substituted by, e.g., lower alkoxycarbonyl, lower alkanoyloxy, lower alkanoylthio, lower alkoxy, lower alkylthio, hydroxy, di-lower alkylamino, lower alkanoylamino, morpholino, piperidino, pyrrolidino, 1-lower alkylpiperazino, aryl or heteroaryl.

Aroyl is carbocyclic or heterocyclic aroyl, preferably monocyclic carbocyclic or monocyclic heterocyclic aroyl.

Monocyclic carbocyclic aroyl is preferably benzoyl or benzoyl substituted by lower alkyl, lower alkoxy, halogen or trifluoromethyl.

Monocyclic heterocyclic aroyl is preferably pyridylcarbonyl or thienylcarbonyl.

Acyloxy is preferably optionally substituted lower alkanoyloxy, lower alkoxycarbonyloxy, monocyclic carbocyclic aroyloxy or monocyclic heterocyclic aroyloxy.

Aryl-lower alkoxycarbonyl is preferably monocyclic carbocyclic-lower alkoxycarbonyl, advantageously benzyloxycarbonyl.

Biaryl represents monocarbocyclic aryl substituted by monocyclic carbocyclic or monocyclic heterocyclic aryl, and preferably represents biphenylyl, advantageous 4-biphenylyl optionally substituted on one or both benzene rings by lower alkyl, lower alkoxy, halogen or trifluoromethyl.

Biaryl-lower alkyl is preferably 4-biphenylyl-lower alkyl, advantageously 4-biphenylyl-methyl.

The novel compounds of the invention are ACE inhibitors inhibiting the conversion of angiotensin I to the pressor substance angiotensin II and thus decrease blood pressure in mammals. Furthermore, compounds of the invention demonstrate inhibition of NEP and thus potentiate the cardiovascular (e.g., diuretic and natriuretic) effects of atrial natriuretic factors (ANF). The combined effect is beneficial for the treatment of cardiovascular disorders in mammals, in particular, hypertension, cardiac conditions such as congestive heart failure, and renal failure. A further beneficial effect of the compounds of the invention in the treatment of said cardiovascular disorders is the inhibition of ECE.

The above-cited properties are demonstrable *in vitro* and *in vivo* tests, using advantageously mammals, e.g., mice, rats, dogs, monkeys, or isolated organs, tissues and preparations thereof. Said compounds can be applied *in vitro* in the form of solutions, e.g., preferably aqueous solutions, and *in vivo* either enterally, parenterally, advantageously, orally (p.o.) or intravenously (i.v.), e.g., as a suspension or in aqueous solution. The dosage *in vitro* may range between about 10^{-6} molar and 10^{-9} molar concentrations. The dosage *in vivo* may range, depending on the route of administration, between about 0.01 and 50 mg/kg, advantageously between about 0.1 and 25 mg/kg.

In vitro testing is most appropriate for the free carboxylic acids of the invention. The test compound is dissolved in dimethyl sulfoxide, ethanol or 0.25 M sodium bicarbonate solution, and the solution is diluted with buffer to the desired concentration.

The *in vitro* inhibition of the ACE by the compounds of this invention can be demonstrated by a method analogous to that given in Biochem. Pharmacol., Vol. 20, p.1637 (1971). The buffer for the ACE assay is 300 mM NaCl, 100 mM KH_2PO_4 (pH 8.3). The reaction is initiated by the addition of 100 μL of hippuryl-histidyl-leucine (2 mg/mL) to tubes containing enzyme and drug in a volume of 150 μL and tubes are incubat d for 30 minutes at 37°C. The reaction is terminated by the addition of 0.75 mL 0.6 N NaOH. 100 μL of freshly prepared O-pthaldehyde solution (2 mg/mL in methanol) is added to the tubes, the contents are mixed and allowed to stand at room temperature. After 10 minutes, 100 μL of 6 N HCl is added. The tubes are centrifuged and the supernatant optical density is read at 360 nM. The results are plotted against drug concentration to determine the IC_{50}, i.e., the drug concentration which gives half the activity of the control sample containing no drug.

Typically, the compounds of invention demonstrate an IC_{50} in the range of about 0.1-50 nM for ACE inhibition.

Illustrative of the invention, the compound of Example 6(a) demonstrates an IC_{50} of about 20 nM in the ACE *in vitro* assay.

Inhibition of ACE can be demonstrated *in vivo* on p.o. or i.v. administration by measuring inhibition of the angiotensin I induced pressor response in normotensive rats.

The *in vivo* test for i.v. administered compounds is performed with male, normotensive rats, which are conscious ~~anesthetized with sodium metofan~~. A femoral artery and femoral vein are cannulated respectively for direct blood pressure measurement on i.v. administration of angiotensin I and i.v. or p.o. administration of a compound of this invention. After the basal blood pressure is stabilized, pressor responses to 3 or 4 challenges of 300 ng/kg angiotensin I i.v., at 15 minute intervals, are obtained. Such pressure responses are usually again obtained at 15, 30, 60 and 90 minutes, and then every hour up to 6 hours after i.v. or p.o. administration of the compound to be tested, and compared with the initial responses. Any observed decrease of said pressor response is an indication of ACE inhibition.

Illustrative of the invention, the compound of Example 6(a) inhibits the angiotensin I induced pressor response for 3 hours at a dose of 10 mg/kg i.v. Similarly, the compound of Example 1(a) inhibits the angiotensin I induced pressor response for 6 hours at a dose of 11.8 mg/kg p.o.

The *in vitro* inhibition of NEP (EC 3.4.24.11) can be determined as follows:

NEP 3.4.24.11 activity is determined by the hydrolysis of the substrate glutaryl-Ala-Ala-Phe-2-naphthylamide (GAAP) using a modified procedure of Orlowski and Wilk (1981). The incubation mixture (total volume 125 μL) contains 4.2 μL of protein (rat kidney cortex membranes prepared by method of Maeda et al., 1983), 50 mM tris buffer, pH 7.4 at 25°C, 500 μM substrate (final concentration), and leucine aminopeptidase M (2.5 μg). The mixture is incubated for 10 minutes at 25°C, and 100 μL of fast garnet (250 μg fast garnet/mL of 10% Tween 20 in 1 M sodium acetate pH 4.2) is added. Enzyme activity is measure spectrophotometrically at 540 nm ~~mM~~. One unit of NEP 24.11 activity is defined as 1 nmol of 2-naphthylamine released per minute at 25°C at pH 7.4. IC_{50} values are determined, i.e., the concentration of test compound required for 50% inhibition of the release of 2-naphthylamine.

NEP activity can also be determined using ANF as a substrate. ANF degrading activity is determined by measuring the disappearance of rat-ANF (r-ANF) using a 3-minute reverse phase-HPLC separation. An aliquot of the enzyme in 50 mM tris HCl buffer, pH 7.4, is pre-incubated at 37°C for 2 minutes and the reaction is initiated by the addition of 4 nmol of r-ANF in a total volume of 50 μL. The reaction is terminated after 4 minutes with the addition of 30 μL of 0.27% trifluoroacetic acid (TFA). One unit of activity is defined as the hydrolysis of 1 nmol of r-ANF per minute at 37°C at pH 7.4. IC_{50} values are determined, i.e., the concentration of test compound required for 50% inhibition of the hydrolysis of ANF.

Typically, the compounds of the invention demonstrate an IC_{50} in the range of about 0.1-50 nM for NEP inhibition.

Illustrative of the invention, the compound of Example 6(a) demonstrates an IC_{50} of about 5 nM in the GAAP *in vitro* assay.

The effect of the compounds of the invention on rat plasma ANF concentration can be determined as follows:

Male Sprague-Dawley rats (275-390 g) are anesthetized with ketamine (150 mg/kg)/acepromazine (10%) and instrumented with catheters in the femoral artery and vein to obtain blood samples and infuse ANF, respectively. The rats are tethered with a swivel system and are allowed to recover for 24 hours before being studied in the conscious, unrestrained state.

In the assay, plasma ANF levels are determined in the presence and absence of NEP inhibition. On the day of study, all rats are infused continuously with ANF at 450 ng/kg/min. i.v. for the entire 5 hours of the experiment. Sixty minutes after beginning the infusion, blood samples for baseline ANF measurements are obtained (time 0) and the rats are then randomly divided into groups treated with the test compound or vehicle. Additional blood samples are taken 30, 60, 120, 180 and 240 minutes after administration of the test compound.

Plasma ANF concentrations are determined by a specific radioimmunoassay. The plasma is diluted (x12.5, x25 and x50) in buffer containing: 50 mM tris (pH 6.8), 154 mM NaCl, 0.3% bovine serum albumin, 0.01% EDTA. One hundred microliters of standards [rANF (99-126)] or samples are added to 100 μL of rabbit anti-rANF serum and incubated at 4°C for 16 hours. Ten thousand cpm of [^{125}I]rANF are then added to the reaction mixture which is incubated at 4°C for 16 hours. Ten thousand cpm of [^{125}I]rANF are then added to the reaction mixture which is incubated at 4°C for an additional 24 hours. Goat anti-rabbit IgG serum coupled to paramagnetic particles is added to the reaction mixture and bound [^{125}I]rANF is pelleted by exposing the mixture to an attracting magnetic rack. The supernatant is decanted and the pellets counted in a gamma counter. All determinations are performed in duplicate. Plasma ANF levels are expressed as a percent of those measured in vehicle-treated animals which received ANF alone (450 ng/kg/min. i.v.)

Illustrative of the invention, the compound of Example 1(a) increases plasma ANF levels by about 70% at a dose of 11.8 mg/kg p.o.

The anti-hypertensive activity can be determined, e.g., in the spontaneously hypertensive rat (SHR) and the DOCA-salt hypertensive rat, e.g., according to Bazil et al., J. Cardiovasc. Pharmacol., Vol. 22, pp. 897-905 (1993) and Trapani et al., J. Cardiovasc. Pharmacol., Vol. 14, pp. 419-424 (1989), respectively.

Illustrative of the invention, the compound of example 1(a) reduces mean arterial pressure in conscious SHR at once daily administration of 11.8 mg/kg p.o.

The anti-hypertensive effect can be determined in desoxy-corticosterone acetate (DOCA)-salt hypertensive rats as follows:

DOCA-salt hypertensive rats (280-380 g) are prepared by the standard method. Rats undergowent a unilateral nephrectomy and one week later are implanted with silastic pellets containing 100 mg/kg of DOCA. The rats are maintained on 1% of NaCl/0.2% KCl drinking water for three to five weeks until sustained hypertension is established. The anti-hypertensive activity is evaluated at this time.

Two days before an experiment, the rats are anesthetized with methoxyflurane and instrumented with cathet rs in the femoral artery to measur arterial blood pressure. Forty-eight hours later, baseline arterial pr ssure and heart rate are recorded during a one hour period. The test compound or vehicle is then administered and the same cardiovascular parameters are monitored for an additional 5 hours.

The diuretic (saluretic) activity can be determined in standard diuretic screens, e.g., as described in "New Anti-hypertensive Drugs", Spectrum Publications, pp. 307-321 (1976), or by measuring the potentiation of ANF-induced natriuresis and diuresis in the rat.

The potentiation of the natriuretic effect of ANF can determined as follows:

Male Sprague-Dawley rats (280-360 g) are anesthetized with Inactin (100 mg/kg i.p.) and instrumented with catheters in the femoral artery, femoral vein and urinary bladder to measure arterial pressure, administer ANF and collect urine, respectively. A continuous infusion of normal saline (33 µL/min.) is maintained throughout the experiment to promote diuresis and sodium excretion. The experimental protocol consists of an initial 15-minute collection period (designated as pre-control) followed by three additional collection periods. Immediately after completion of the pre-control period, test compound or vehicle is administered; nothing is done for the next 45 minutes. Then, blood pressure and renal measurements are obtained during a second collection period (designated control, 15 minutes). At the conclusion of this period, ANF is administered (1 µg/kg i.v. bolus) to all animals and arterial pressure and renal parameters are determined during two consecutive 15-minute collection periods. Mean arterial pressure, urine flow and urinary sodium excretion are determined for all collection periods. Blood pressure is measured with a Gould p50 pressure transducer, urine flow is determined gravimetrically, sodium concentration is measured by flame photometry, and urinary sodium excretion is calculated as the product of urine flow and urine sodium concentration.

The *in vitro* inhibition of ECE can be determined as follows:

ECE is partially purified from porcine primary aortic endothelial cells by DE52 anion exchange column chromatography and its activity is quantified by radioimmunoassay (RIA) as described in Anal. Biochem., Vol., 212, pp. 434-436 (1993). Alternatively, the native enzyme can be substituted by a recombinant form of ECE, as described, for example, in Cell, Vol. 78, pp. 473-485 (1994). Human ECE-1 has been described by several groups (Schmidt et al., FEBS Letters, Vol. 356, pp. 238-243 (1994); Kaw et al., 4th Int. Conf. on Endothelin; April 23-25, London (UK) (1995) C6; Valdenaire t al., J. Biol. Ch m., Vol. 270, pp. 29794-29798 (1995); Shimada t al., Biochem. Biophys. Res. Commun., Vol. 207, pp. 807-812 (1995)). The ECE inhibition can be determined as described in Biochem. Mol. Biol. Int., Vol. 31, No. 5, pp. 861-867 (1993), by RIA to measure ET-1 formed from big ET-1.

Alternatively, recombinant human ECE-1 (rhECE-1) can be used, as follows:

Chinese hamster ovary cells expressing rhECE-1 (Kaw et al., 4th Int. Conf. on Endothelin; April 23-25, London (UK), (1995) C6) are cultured in DMEM/F12 medium containing 10% fetal bovine serum and 1x antibiotic-antimycotic. Cells are harvested by scraping, pelleted by centrifugation, and homogenized at 4°C in a buffer containing 5 mM $MgCl_2$, 1 μM pepstatin A, 100 μM leupeptin, 1 mM PMSF, and 20 mM Tris, pH 7.0, with a ratio of 2 mL of buffer/mL of cells. The cell debris is removed by brief centrifugation, and the supernatant is centrifuged again at 100,000 x g for 30 minutes. The resulting pellet is re-suspended in a buffer containing 200 mM NaCl and 50 mM Tes, pH 7.0, at a protein concentration about 15 mg/mL and stored in aliquots at -80°C.

To assess the effect of an inhibitor on ECE-1 activity, 10 μg of protein is pre-incubated with the compound at a desired concentration for 20 minutes at room temperature in 50 mM TES, pH 7.0, and 0.005% Triton X-100 in a volume of 10 μL. Human big ET-1 (5 μL) is then added to a final concentration of 0.2 μM, and the reaction mixture is further incubated for 2 hours at 37°C. The reaction is stopped by adding 500 μL of RIA buffer containing 0.1% Triton X-100, 0.2% bovine serum albumin, and 0.02% NaN_3 in phosphate-buffered saline.

Diluted samples (200 μL) obtained from the above enzyme assay are incubated at 4°C overnight with 25 μL each of [^{125}I]ET-1 (10,000 cpm/tube) and 1:20,000-fold diluted rabbit antibodies that recognize specifically the carboxyl terminal tryptophan of ET-1. Goat anti-rabbit antibodies coupled to magnetic beads (70 μg) are then added to each tube, and the reaction mixture is further incubated for 30 minutes at room temperature. The beads are pelleted using a magnetic rack. The supernatant is decanted, and the radioactivity in the pellet is counted in a gamma counter. Total and nonspecific binding are measured in the absence of non-radioactive ET-1 and anti-ET antibodies, respectively. Under these conditions, ET-1 and big ET-1 displace [^{125}I]ET-1 binding to the antibodies with IC_{50} values of 21 ± 2 and 260,000 ± 66,000 fmol (mean ± SEM, n = 3-5), respectively.

In order to determine the IC_{50} value of an inhibitor, a concentration-response curve of each inhibitor is determined. An IBM-compatible version of ALLFIT program is used to fit data to a one-site model.

ECE inhibition can also be determined *in vivo* by measuring the inhibition of big ET-1-induced pressor response in the anesthesized or conscious rat, as described below. The effect of the inhibitors on the pressor response resulting from big ET-1 challenge is measured in Sprague-Dawley rats as described in Biochem. Mol. Biol. Int., Vol. 31, No. 5, pp. 861-867 (1993). Results are expressed as percent inhibition of the big ET-1-induced pressor response as compared to vehicle.

Male Sprague-Dawley rats are anesthetized with Inactin (100 mg/kg i.p.) and instrumented with catheters in the femoral artery and vein to record mean arterial pressure (MAP) and administer compounds, respectively. A tracheostomy is performed and a cannula inserted into the trachea to ensure airway patency. The body temperature of the animals is maintained at 37 ± 1°C by means of a heating blanket. Following surgery, MAP is allowed to stabilize before interrupting autonomic neurotransmission with chlorisondamine (3 mg/kg i.v.). Rats are then treated with the test compound at 10 mg/kg i.v. or vehicle and challenged with big ET-1 (1 nmol/kg i.v.) 15 and 90 minutes later. Generally, the data are reported as the maximum increase in MAP produced by big ET-1 in animals treated with the test compound or vehicle.

Male Sprague-Dawley rats are anesthetized with methohexital sodium (75 mg/kg i.p.) and instrumented with catheters in the femoral artery and vein to measure MAP and administer drugs, respectively. The catheters are threaded through a swivel system that enables the rats to move freely after regaining consciousness. The rats are allowed to recover from this procedure for 24 hours before initiating the study. On the following day, MAP is recorded via the femoral artery catheter and a test compound or vehicle is administered via the femoral vein. Animals are challenged with big ET-1 at 1 nmol/kg i.v. at various times after dosing. After an adequate washout period, depending upon the dose and regimen, animals can be re-tested at another dose of test compound or vehicle. Generally, the data are reported as the change in MAP produced by big ET-1 at 2-minute intervals in animals treated with the test compound as compared to vehicle.

ECE inhibition can also be determined *in vivo* by measuring the inhibition of the big ET-1 induced pressor response in conscious SHR, e.g. as described in Biochem. Biophys. Res. Commun., Vol. 204, pp. 407-412 (1994).

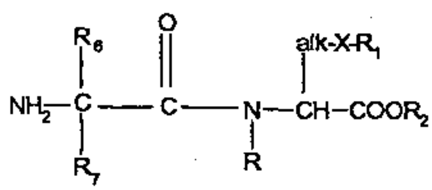
Male SHR (16-18 weeks of age) are administered either test compound or vehicle (1 M $NaHCO_3$) via an osmotic minipump implanted subcutaneously. On day 5, femoral arterial and venous catheters ar placed in anesthetized rats for the measur ment of MAP and for test compound administration, respectively. After a 48-hour recovery period, MAP is recorded (day 7) through the arterial catheter connected to a pressure transducer. Blood pressure and heart rate are allowed to stabilize for 30 minutes before ganglion blockade is performed using chlorisondamine (10 mg/kg i.v.). Approximately 15 minutes later, a bolus dose of big ET-1 (0.25 nmol/kg i.v.) is administered to both vehicle- and test compound-treated rats. The change in blood pressure in response to big ET-1 is then compared between the two groups of rats.

The inhibition of cerebral vasospasm is demonstrated by measuring the inhibition of experimentally induced constriction of basilar cerebral arteries in the rabbit (Caner et al., J. Neurosurg., Vol. 85, pp. 917-922 (1996).

The degree or lack of undesirable immunostimulatory potential of the compounds of the invention can be determined with the murine popliteal lymph node assay described in Toxicology Letters, Vols. 112/113, pp. 453-459 (2000).

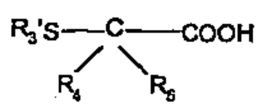
The compounds of the invention, e.g., can be prepared

a) by condensing a compound of formula II



(II)

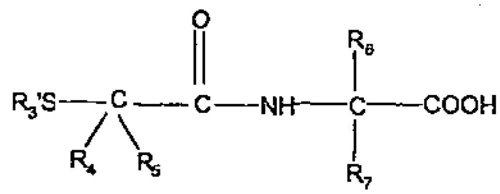
wherein the symbols alk, X, R, R_1, R_6 and R_7 have the meaning as defined above and $COOR_2$ represents esterified carboxyl, with a carboxylic acid of the formula III



(III)

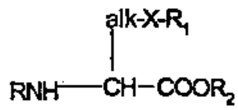
or a reactive functional derivative thereof, wherein R_4 and R_5 have meaning as defined above; R_3' represents hydrogen or a labile S-protecting group, e.g., acyl, t-butyl or optionally substituted benzyl; or

b) by condensing a compound of the formula IV



(IV)

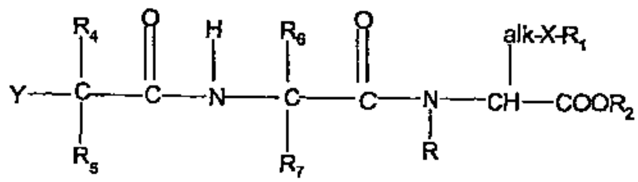
or a reactive functional derivative thereof wherein the symbols R_3', R_4-R_5 and R_6-R_7 have meaning as defined above, with an amino acid ester of the formula V



(V)

wherein alk, X, R and R_1 have meaning as defined above and $COOR_2$ represents esterified carboxyl; or

c) by condensing under basic conditions a compound of the formula VI



(VI)

wherein the symbols R, R_1, $COOR_2$, R_4-R_7, alk and X have meaning as defined above and Y represents a reactive esterified hydroxyl group (e.g., chloro or bromo) as a leaving group, with a compound of the formula

$R_3'SH$ (VII)

or a salt thereof, wherein R_3' represents a labile S-protecting group, e.g., acyl, t-butyl or optionally substituted benzyl; and converting a resulting product to a compound of formula I wherein R_3 is hydrogen;

and in above said process, if temporarily protecting any interfering reactive group(s), removing said protecting group(s), and then isolating the resulting compound of the invention; and, if desired, converting any resulting compound of the invention into another compound of the invention; and/or, if desired, converting a free carboxylic acid function into a pharmaceutically acceptable ester derivative, or converting a resulting ester into the free acid or into another ester derivative; and/or, if desired, converting a resulting free compound into a salt or a resulting salt into the free compound or into another salt, and/or, if desired, separating a mixtur of isomers or racemates, and/or, if desired, resolving a racemate obtained into the optical antipodes.

In starting compounds and intermediates which are convened to the compounds of the invention in manner described herein, functional group present, such as thiol, carboxyl, amino and hydroxy groups, are optionally protected by conventional protecting groups that are common in preparative organic chemistry. Protected thiol, carboxyl, amino and hydroxy groups are those that can be converted under mild conditions into free thiol, carboxyl, amino and hydroxy groups without other undesired side reactions taking place.

The purpose of introducing protecting groups is to protect the functional groups from undesired reactions with reaction components and under the conditions used for carrying out a desired chemical transformation. The need and choice of protecting groups for a particular reaction is known to those skilled in the art and depends on the nature of the functional group to be protected (thiol, carboxyl, amino group, etc.), the structure and stability of the molecule of which the substituent is a part, and the reaction conditions.

Well-known protecting groups that meet these conditions and their introduction and removal are described, for example, in J.F.W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London, N.Y. (1973), T.W. Greene and P.G.M. Wuts, "Protective Groups in Organic Synthesis", Wiley, N.Y. 3rd Ed.(1999), and also in "The Peptides", Vol. I, Schroeder and Luebke, Academic Press, London, N.Y. (1965).

The preparation of compounds of the invention according to process (a) involving the condensation of an amine of formula II with the acid of formula III or a functional reactive derivative thereof, is carried out by methodology well-known for peptide synthesis.

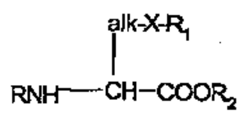
The condensation according to process (a) of an amino ester of formula II with a free carboxylic acid of formula III is carried out advantageously in the presence of a condensing agent such as dicyclohexylcarbodiimide or N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide and hydroxybenzotriazole, 1-hydroxy-7-azabenzotriazole, chlorodimethoxytriazine, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP Reagent), or O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU), and triethylamine or N-methylmorpholine, in an inert polar solvent, such as dimethylformamide or methylene chloride, preferably at room temperature.

The condensation of an amino ester of formula II with a reactive functional derivative of an acid of formula III in the form of an acid halide, advantageously an acid chloride, or mixed anhydride, is carried out in an inert solv nt such as toluene or methylene chloride, advantageously in the presence of a base, e.g., an inorganic base such as potassium carbonate or an organic base such as triethylamine, N-methylmorpholine or pyridine, preferably at room temperature.

Reactive functional derivatives of carboxylic acids of formula III are preferably acid halides (e.g., the acid chloride) and mixed anhydrides, such as the pivaloyl or isobutyloxycarbonyl anhydride, or activated esters such as benzotriazole, 7-azabenzotriazole or hexafluorophenyl ester.

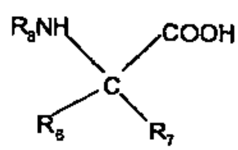
The starting material of formula II can be prepared according to methods described herein and illustrated in the examples.

The preparation of a starting material of formula II involves the acylation of an ester of formula VIII



(VIII)

wherein alk, X, R and R_1 have meaning as defined hereinabove and $COOR_2$ represents esterified carboxyl (e.g., wherein R_2 is lower alkyl or benzyl) with an appropriately N-protected amino acid (or a reactive functional derivative) of formula IX



(IX)

wherein R_6 and R_7 have meaning as defined hereinabove and R_8 is a labile amino protecting group, e.g., t-butoxycarbonyl, to obtain the corresponding N-protected compound of formula II.

The condensation of a compound of formula VIII with a compound of formula IX is carried out by methodology well-known in peptide synthesis, e.g., as described above for the condensation of a compound of formula II with a compound of formula III. The N-protecting group is removed according to methods well-known in the art, e.g., the t-butoxycarbonyl is removed with anhydrous acid such as trifluoroacetic acid or HCl.

The starting amino esters and acids of compounds of formula VIII and IX, respectively, are either known in the art, or if new, can be prepared according to methods well-known in the art, e.g., or illustrated herein. The amino acid esters of formula VIII are preferably the S-enantiomers.

The starting materials of formula III are known, or if new, may be prepared according to conventional methods. The starting materials are prepared, e.g., from the corresponding racemic or optically active α-amino acids, by conversion thereof to the α-bromo derivative followed by displacement thereof with inversion of configuration using the appropriate thiol derivative of formula VII, under basic conditions, for example, as illustrated in European Patent Application No. 524,553 published January 27, 1993. S-debenzylation of the resulting final products is carried out by reductive cleavage, e.g., with Raney nickel in ethanol. S-deacylation is carried out by, e.g., base catalyzed hydrolysis with dilute aqueous sodium hydroxide. Cyclic starting materials of formula III can be prepared by treatment of the cyclic carboxylic acid (e.g., cyclopentanecarboxylic acid) with sulfur in the presence of a strong base such as lithium diethylamide.

The preparation of the compounds of the invention according to process (b) involving the condensation of an acid of formula IV with an amino acid ester of formula V is carried out in a similar fashion to process (a). Similarly, the starting materials of formula IV are prepared by condensation of an acid of formula III with an ester corresponding to gem-disubstituted amino acids of formula IX (wherein R_8 is now hydrogen) under conditions similar to those described above, followed by removal of the carboxyl protecting group.

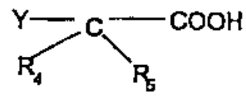
The preparation of the compounds of the invention according to process (c) involving the displacement of a leaving group Y in a compound of formula VI with a thiol derivative R_3'-SH as a salt thereof is carried out according to methods well-known in the art.

A reactive esterified hydroxyl group, represented by Y, is a hydroxyl group esterified by a strong inorganic or organic acid. Corresponding Y groups are in particular halo, for example, chloro, bromo or iodo, also sulfonyloxy groups, such as lower alkyl- or arylsulfonyloxy groups, for example, (methane-, ethane-, benzene- or toluene-) sulfonyloxy groups, also the *trifluoromethylsulfonyloxy group*.

The displacement is carried out in an inert solvent, such as dimethylformamide or methylene chloride in the presence of a base such as potassium carbonate, triethylamine, diisopropylethylamine, N-methylmorpholine, and the like at room or elevated temperature.

Using a salt of R_3'SH (e.g., potassium thioacetate), the reaction is carried out in the absence of a base, in an inert solvent such as tetrahydrofuran or dimethylformamide.

Similarly, the starting materials of formula VI can be prepared by reacting the dipeptide derivative of formula II with an acid of the formula



(X)

wherein R_4 and R_5 and Y have meaning as defined above, under conditions described for process (a).

The compounds of formula X wherein Y is halo, such as the α-bromocarboxylic acids are known and are prepared, e.g., as described in International Application WO 99/55726 published November 4, 1999.

The compounds of the invention and intermediates, e.g., those of formulas II, V and VI, having the side chain alk-X-R_1 are prepared from the corresponding compounds having the alk-X' side chain wherein X' represents amino, hydroxy, thiol or a suitable leaving group according to methodology known in the art and illustrated herein. For example, the acids and esters of formula V can be obtained starting with serine, homoserine, threonine, cysteine and the like, preferably in optically active form.

Certain compounds of the invention and intermediates can be converted to each other according to general reactions well-known in the art.

The free mercaptans may be converted to the S-acyl derivatives by reaction with a reactive derivative of a carboxylic acid (corresponding to R_3 being acyl in formula I), such as an acid anhydride or said chloride, preferably in the presence of a base such as triethylamine in an inert solvent such as acetonitrile or methylene chloride.

Free alcohols and phenols can be converted to the corresponding acyl derivatives, e.g., by reaction with a corresponding acid chloride in the presence of a base, such as triethylamine.

The free mercaptans, wherein R_3 represents hydrogen, may be oxidized to the corresponding disulfides, e.g., by air oxidation or with the use of mild oxidizing agents such as iodine in alcoholic solution. Conversely, disulfides may be reduced to the corresponding mercaptans, e.g., with reducing agents such as sodium borohydride, zinc and acetic acid or tributylphosphine.

Carboxylic acid esters may be prepared from a carboxylic acid by condensation with, e.g., the halide corresponding to R_2-OH, in the presence of a base, or with an excess of the alcohol in the presence of an acid catalyst, according to methods well-known in the art.

Carboxylic acid esters and S-acyl derivatives may be hydrolyzed, e.g., with aqueous alkali such as alkali metal carbonates or hydroxides. S-acyl and ester groups can be selectively removed as illustrated herein.

Preferably, and wherever possible, the preferred isomers of the invention of formula Ia are prepared from pure enantiomers.

In case mixtures of stereoisomers (e.g., diastereomers) are obtained, these can be separated by known procedures such as fractional crystallization and chromatography (e.g., thin layer, column, flash chromatography). Racemic free acids can be resolved into the optical antipodes by fractional crystallization of d- or l-(α-methylbenzylamine, cinchonidine, cinchonine, quinine, quinidine, dehydroabiethylamine, brucine or strychnine) salts and the like. Racemic products, if not diastereoisomers, can first be converted to diastereoisomers with optically active reagents (such as optically active alcohols to form esters) which can then be separated as described above, and, e.g., hydrolyzed to the individual enantiomer. Racemic products can also be resolved by chiral chromatography, e.g., high pressure liquid chromatography using a chiral absorbent; also by enzymatic resolution, e.g., of esters with alkalase.

The above-mentioned reactions are carried out according to standard methods, in the presence or absence of diluents, preferably such as are inert to the reagents and are solvents thereof, of catalysts, alkaline or acidic condensing or said other agents respectively and/or inert to the reagents and are solvents thereof, of catalysts, alkaline or acidic condensing or said other agents respectively and/or inert atmospheres, at low temperatures, room temperature or elevated temperatures, preferably near the boiling point of the solvents used, at atmospheric or superatmospheric pressure.

The invention further includes any variant of said processes, in which an intermediate product obtainable at any stage of the process is used as a starting material and any remaining steps are carried out, or the process is discontinued at any stage thereof, or in which the starting materials are formed under the reaction conditions, or in which the reaction components are used in the form of their salts or optically pure antipodes. Mainly those starting materials should be used in said reactions, that lead to the formation of those compounds indicated above as being preferred.

The present invention additionally relates to the use in mammals of the compounds of the invention and their pharmaceutically acceptable, non-toxic acid addition salts, or pharmaceutical compositions thereof, as medicaments, for inhibiting both ACE and NEP, and, e.g., for the prevention or treatment of cardiovascular disorders such as hypertension, edema, salt retention and congestive heart failure, either alone or in combination with one or more other agents which are useful for the treatment of such disorders. Such may be anti-hypertensive agents, anti-atherosclerotic agents, cardiac agents, diuretic agents, antidiabetic agents, cholesterol-lowering agents and the like. When used in combination with other therapeutic agents such can be administered separately or in a fixed combination.

Examples of therapeutic agents which can be used in combination are angiotensin II receptor antagonists, such as valsartan, losartan, candesartan, eprosartan, irbesartan and telmisartan; β-blockers, such as bisoprolol, propanolol, atenolol, sotalol and metoprolol; renin inhibitors; calcium channel blockers, such as amlodipine, verapamil, diltiazem, bepridil, felodipine, isradipine, nicardipine, nifedipine, nimodipine and nisoldipine; aldosterone synthase inhibitors/aldosterone antagonists, such as eplerenone, (+)-fadrozole (WO 01/76574), spironolactone and canrenone; diuretics, such as furosemide, hydrochlorothiazide, indapamide, metazolone, amiloride and triamterene; vasopressin receptor antagonists, such as OPC 21268, SR 49059, SR121463A, SR49059, VPA985, OPC31260 and YM087; cardiotonic drugs, such as enoximone and levosimendan; endothelin antagonists and ECE inhibitors, such as bosentan, BMS193884, TBC3711 and compounds disclosed in WO 99/55726; anti-atherosclerotic agents, particularly cholesterol lowering agents, such as bile acid sequestrants (e.g., cholestyramine and colestipol); cholesterol absorption inhibitors, such as ezetimibe; fibrates, such as fenofibrate and gemfibrozil; statin HMG CoA reductase inhibitors, such as atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin and pitavastatin; and nicotinic acid derivatives; thyromimetic agents, such as those disclosed in U.S. Patent No. 5,569,674 and WO 00/58279; also antidiabetic agents, such as repaglinide, nateglinide, metformin, rosiglitazone, pioglitazone, glyburide, glipizide, glimepiride, DPP728, LAF237, NH622 and DRF4158.

The present invention also relates to the use of the compounds of the invention for the preparation of pharmaceutical compositions, especially pharmaceutical compositions having ACE and NEP inhibiting activity, and, e.g., anti-hypertensive activity.

The pharmaceutical compositions according the invention are those suitable for enteral, such as oral or rectal, transdermal and parenteral administration to mammals, including man, for the treatment of cardiovascular disorders, such as hypertension, comprising an effective amount of a pharmacologically active compound of the invention or a pharmaceutically acceptable salt thereof, alone or in combination with one or more pharmaceutically acceptable carriers, as well as in combination with other therapeutic agents also useful for the treatment of cardiovascular disorders, as indicated above.

The pharmacologically active compounds of the invention are useful in the manufacture of pharmaceutical compositions comprising an effective amount thereof in conjunction or admixture with excipients or carriers suitable for either enteral or parenteral application. Preferred are tablets and gelatin capsules comprising the active ingredient, together with a) diluents, e.g., lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine; b) lubricants, e.g., silica, talcum, stearic acid, its magnesium or calcium salts and/or polyethyleneglycol; for tablets also c) binders, e.g., magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone; if desired, d) disintegrants, e.g., starches, agar, alginic acid or its sodium salt, or effervescent mixtures; and, if desired, absorbents, colorants, flavors and sweeteners. Injectable compositions are preferably aqueous isotonic solutions or suspensions, and suppositories are advantageously prepared from fatty emulsions or suspensions. Said compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, the compositions may also contain other therapeutically valuable substances. Said compositions are prepared according to conventional mixing, granulating or coating methods, respectively, and contain about 0.1-75%, preferably about 1-50%, of the active ingredient.

Suitable formulations for transdermal application include an effective amount of a compound of the invention with carrier. Advantageous carriers include absorbable pharmacologically acceptable solvents to assist passage through the skin of the host. Characteristically, transdermal devices are in the form of a bandage comprising a backing member, a reservoir containing the compound, optionally with carriers, optionally a rate controlling barrier to deliver the compound to the skin of the host at a controlled and predetermined rate over a prolonged period of time, and means to secure the device to the skin.

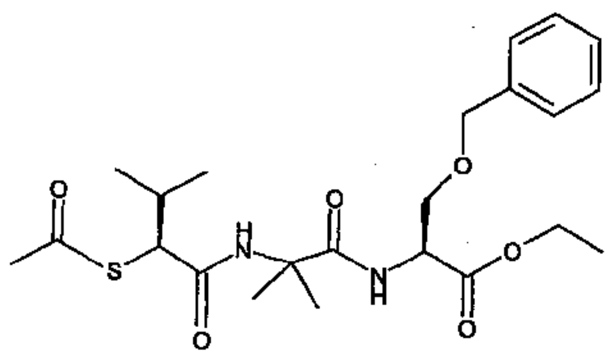
A unit dosage for a mammal of about 50-70 kg may contain between about 10 and 200 mg of the active ingredient. The dosage of active compound is dependent on the species of warm-blooded animal (mammal), the body weight, age and individual condition, and on the form of administration.

The following examples are intended to illustrate the invention and are not to be construed as being limitations thereon. Temperatures are given in degrees Centigrade. If not mentioned otherwise, all evaporations are performed under reduced pressure, preferably between about 15 and 100 mm Hg. Optical rotations (expressed in degrees) are measured at room temperature at 589 nM (D line of sodium) or other wave lengths as specified in the examples. The structure of the compounds are confirmed by standard analytical methods such as mass spectrum, elemental analysis, NMR, IR spectroscopy and the like.

The prefixes R and S are used to indicate the absolute configuration at each asymmetric center.

Example 1

(a) N-[2-[(S)-2-Acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester

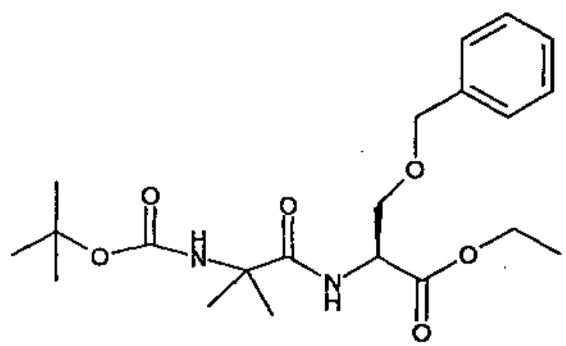


N-[2-[-[(R)-3-bromo-3-methylbutanoylamino]-2-methylproprionyl-O-benzyl-L-serine ethyl ester(4.96 g, 10.5 mmol) is dissolved in tetrahydrofuran (100 mL) and potassium thioacetate (6.00 g, 52.5 mmol) is added. The mixture is stirred at room temperature for 4 hours, then diluted with ethyl acetate (500 mL) and washed with water (100 mL), sodium bicarbonate solution (2 x 100 mL), water (2 x 100 mL) and then brine (50 mL). The solution is dried over sodium sulfate and concentrated *in vacuo*. Th crude material is purified by flash chromatography (silica gel, 3:2 hexane/ethyl acetate) to yield title compound; m.p. 55-57°C; $[\alpha]^{20}_{D}$ - 63.5° (c = 0.99, CH_3OH); MS(M + H):467.

The starting material is prepared as follows:

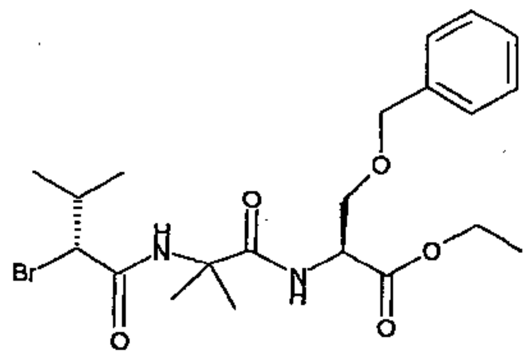
A solution of O-benzyl-L-serine (9.75 g, 50 mmol) in ethanol (200 mL) is saturated with HCl gas for 8 minutes. The mixture is stirred overnight at room temperature, and then concentrated *in vacuo*. The solid is washed with diethyl ether and collected by filtration to yield O-benzyl-L-serine ethyl ester hydrochloride as a white solid.

To a solution of BOC-α-methylalanine (3.05 g, 15 mmol), O-benzyl-L-serine ethyl ester hydrochloride (3.89 g, 15 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 2.88 g, 15 mmol) and 1-hydroxy-7-azabenzotriazole (HOAT, 2.04, 15 mmol) in methylene chloride (150 mL) is added triethylamine (1.52 g, 15 mmol). The mixture is stirred overnight and then concentrated *in vacuo*. The residue is re-dissolved in ethyl acetate and washed with water, 1 N HCl, water, and brine. The solution is dried over sodium sulfate and concentrated to yield N-[2-(BOC-amino)-2-methylpropionyl]-O-benzyl-L-serine ethyl ester of the formula



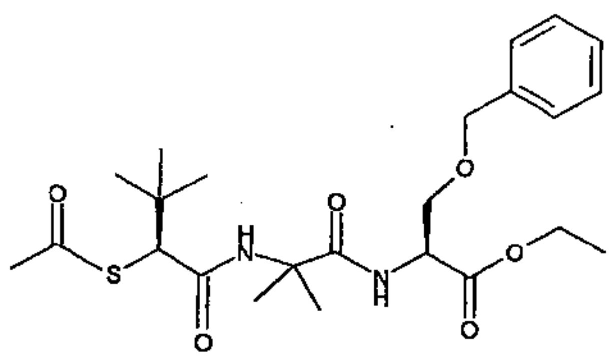
The above carbamate (6.12 g, 15 mmol) is dissolved in methylene chloride (200 mL) and chilled in an ice bath. The solution is saturated with HCl gas for 10 minutes and then stirred at room temperature overnight. The residue is concentrated. Methylene chloride is added and the residue is concentrated again to give N-(2-amino-2-methylpropionyl)-O-benzyl-L-serine ethyl ester hydrochloride as a foam; MS(M+H):309.

To a solution of the above amine hydrochloride (4.90 g, 14 mmol) in methylene chloride (150 mL) is added (R)-2-bromo-3-methylbutanoic acid diisopropyl amine salt (4.03 g, 14 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI, 2.70 g, 14 mmol) and 1-hydroxy-7-azabenzotriazole (HOAT, 1.90 g, 14 mmol). The mixture is stirred at room temperature overnight and then concentrated *in vacuo*. The residue is dissolved in ethyl acetate and washed with water, dilute sodium bicarbonate, water, 1 N HCl, and then brine. The solution is dried over sodium sulfate and concentrated to give a solid. The solid is purified by flash chromatography (silica gel, 2:1 hexane/ethyl acetate) to give N-[2-(R)-2-bromo-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester of the formula

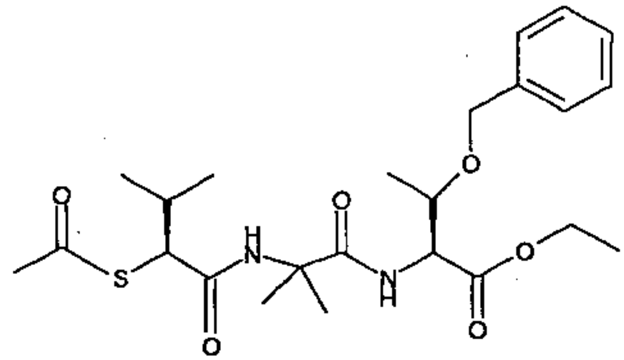


Similarly prepared are:

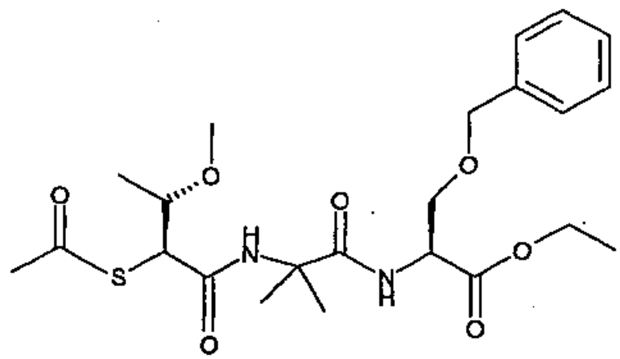
(b) N-[2-[(S)-2-acetylthio-3,3-dimethylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester



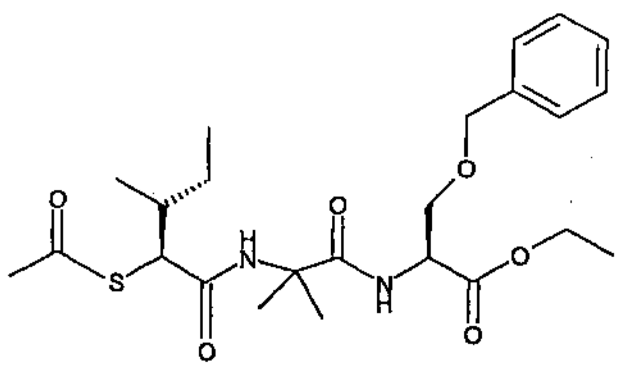
(c) N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-threonine ethyl ester; m.p. 121-122°C.



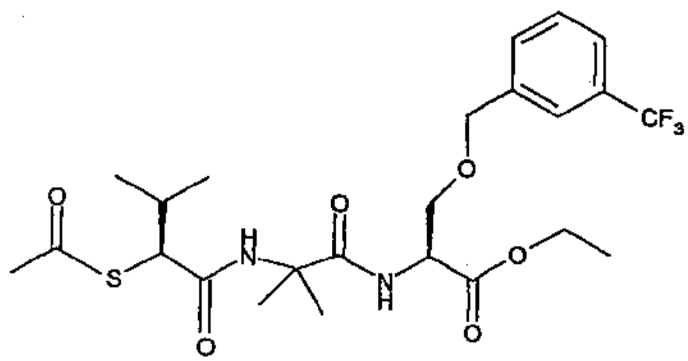
(d) N-[2-[(S)-2-acetylthio-3-methoxybutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; $[\alpha]_D^{20}$ + 14.9° (c = 1.04, DMSO)



(e) N-[2-[(S)]-2-acetylthio-3-methylpentanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; $[\alpha]_D^{20}$ - 6.93° (c = 1.09, CH_3OH)



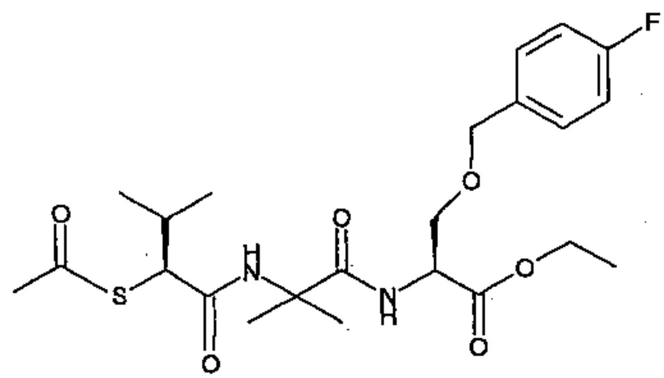
(f) N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-O-(3-trifluoromethylbenzyl)-L-serine ethyl ester



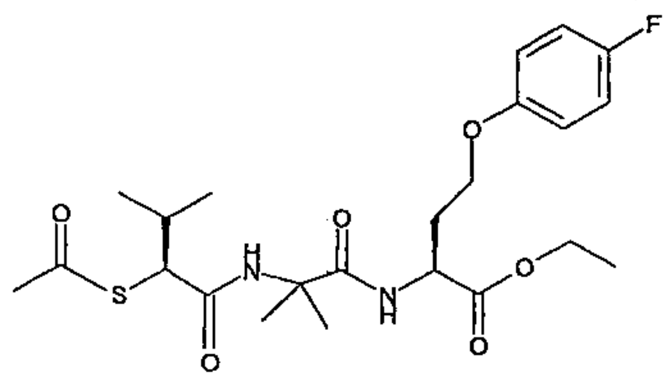
The starting O-(3-trifluoromethylbenzyl)-L-serine ethyl ester hydrochloride is prepared as follows:

To a suspension of sodium hydride (60% in oil, 3.04 g, 76 mmol) in N,N-dimethylformamide (60 mL) at 0°C is added BOC-L-serine (7.80 g, 38 mmol). The mixture is stirred for 1 hour and then m-trifluoromethylbenzyl chloride (7.39 g, 38 mmol) is added. The mixture is allowed to warm to room temperature and is stirred overnight. The mixture is quenched with water. Ethyl acetate is added and the mixture is washed with brine, dried over $MgSO_4$ and concentrated to give a yellow oil which is purified by flash chromatography (SiO_2; hexane/ethyl acetate) to give a clear oil. The residue is dissolved in ethanol (120 mL), the solution is cooled to 0°C and saturated with HCl gas for 5 minutes. The mixture is allowed to warm to room temperature and stirred overnight. The mixture is concentrated to give O-(4-trifluoromethylbenzyl)-L-serine ethyl ester hydrochloride.

(g) N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-O-(4-fluorobenzyl)-L-serine ethyl ester as an oil.



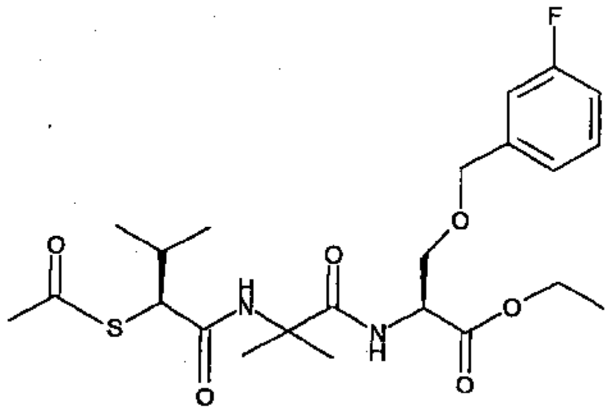
(h) N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-O-(4-fluorophenyl)-L-homoserine ethyl ester as an oil.



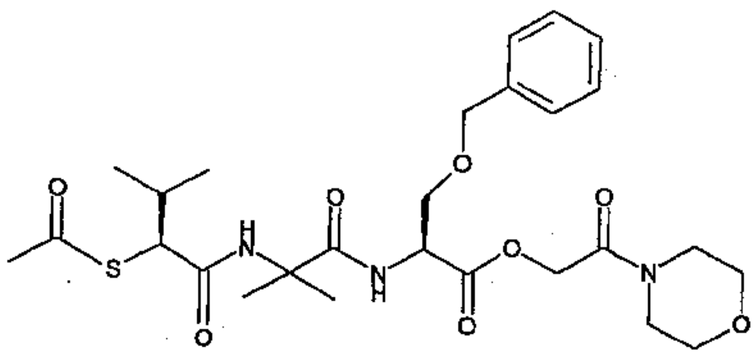
The starting O-(4-fluorophenyl)-L-homoserine ethyl ester hydrochloride is prepared as follows:

To a solution of BOC-L-homoserine t-butyl ester (3.2 g, 11.6 mmol) in tetrahydrofuran is added triphenylphosphine (7.59 g, 29 mmol), p-fluorophenol (2.08 g, 18.6 mmol) and 1,1'-azobis(N,N-dimethylformamide) (3.2 g, 18.6 mmol). The mixture is stirred overnight, washed with brine, dried over $MgSO_4$, and the solvent is removed to give an orange oil. The oil is purified by flash chromatography (SiO_2, 85% hexane/15% ethyl acetate) to give a clear oil which is dissolved in ethanol (100 mL) and the solution is saturated with HCl gas, then stirred overnight. The mixture is concentrated to give O-(4-fluorophenyl)-L-homoserine ethyl ester hydrochloride as a white solid.

(i) N-[2-[(S)-2-acetylthio-3-methyl-butanoylamino]-2-methylpropionyl]-O-(3-fluorophenyl)-L-homoserine ethyl ester



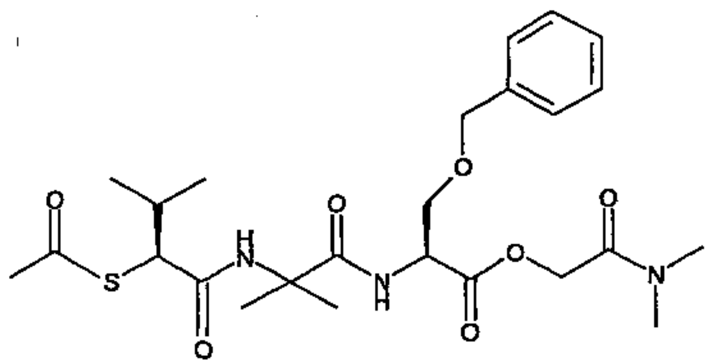
(j) N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine morpholinocarbonylmethyl ester, purified by chromatography on silica gel with hexane, ethyl acetate, methanol (20:70:10) as a white solid.



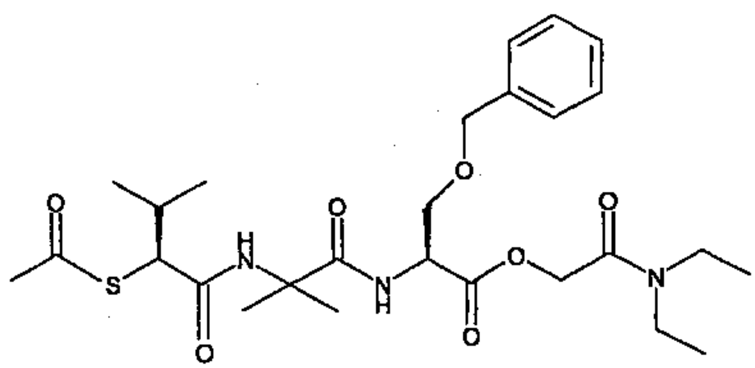
The starting material is prepared as follows:

O-Benzyl-L-serine (10.0 g, 51.3 mmol), di-*tert*-butyl-dicarbonate (11.2 g, 51.4 mmol) and 1 N sodium hydroxide (103 mL, 103 mmol) are stirred together in 100 mL of dioxane at room temperature for 16 hours. The mixture is concentrated *in vacuo*, taken up in water, acidified to pH 1 with 6 N HCl and extracted with ethyl acetate. The organic layer is washed with water, then brine, and dried over anhydrous magnesium sulfate. The mixture is filtered and concentrated *in vacuo* to give BOC-O-benzyl-L-serine as an oil. 4-(2-Chloroacetyl)morpholine (1.22 g, 7.48 mmol) is added to a solution of BOC-O-benzyl-L-serine (2.20 g, 7.46 mmol), triethylamine (0.75 g, 7.43 mmol) and sodium iodide (0.11 g, 0.73 mmol) in 5 mL of N,N-dimethylformamide and the mixture stirred at room temperature for 2 hours. The mixture is diluted with ethyl acetate, washed with wat r, then with brine, and dried over anhydrous magnesium sulfate. The mixture is filtered and concentrated *in vacuo* to give a yellow oil. The oil is chromatographed on silica gel with h xane: thyl acetate:methanol (35:60:5) to give BOC-O-benzyl-L-serine morpholinocarbonylmethyl ester as a colorless oil. HCl gas is bubbled through a solution of the carbamate ester (1.72 g, 4.08 mmol) in methylene chloride (50 mL) for 5 minutes and the mixture is stirred at room temperature for 3 hours. The resulting mixture is concentrated *in vacuo* to yield O-benzyl-L-serine morpholinocarbonylmethyl ester as a foam.

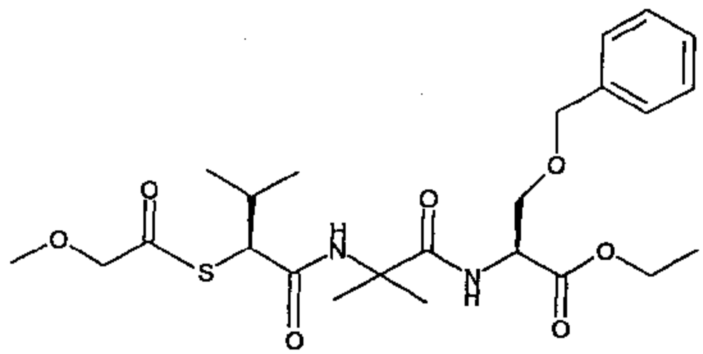
(k) N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine dimethylaminocarbonylmethyl ester, prepared and purified as described for compound of Example 1(j):



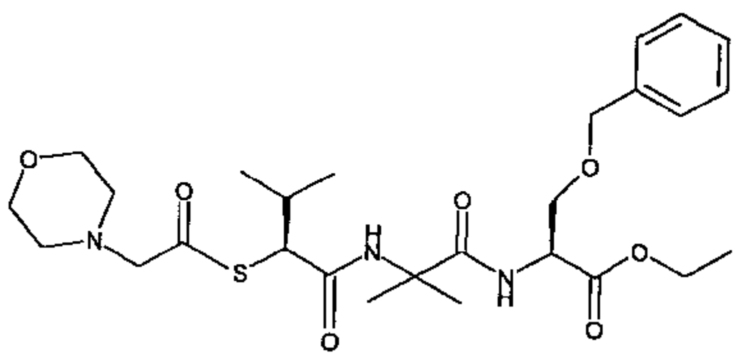
(l) N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine diethylaminocarbonylmethyl ester, prepared as described for compound of Example 1(j) and purified by chromatography on silica gel with hexane, ethyl acetate, methanol (35:60:5).



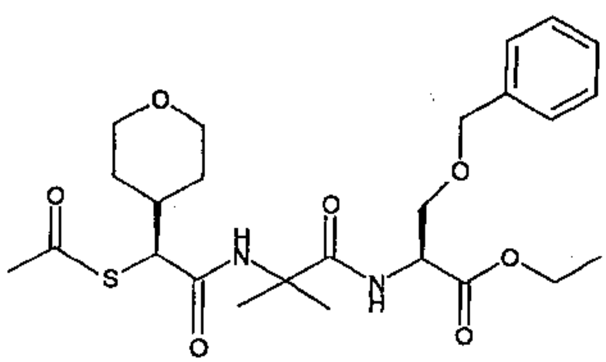
(m) N-[2-[(S)-2-[(methoxyacetyl)thio]-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ster; $[\alpha]_D$ - 55.27°; (c = 1.084, CH_3OH)



(n) N-[2-[(S)-2-[(morpholinoacetyl)thio]-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; $[\alpha]_D$ - 48.61° (c = 1.098, CH_3OH)



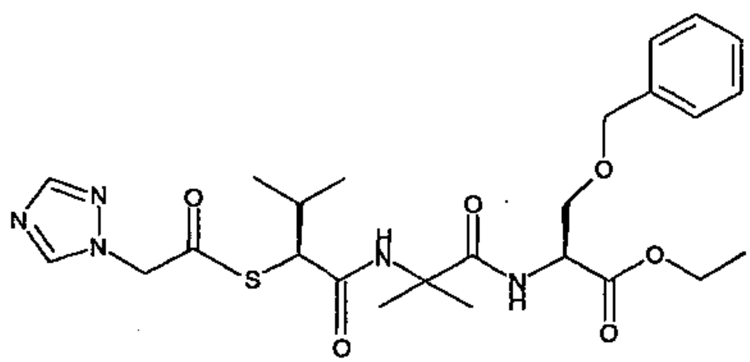
(o) N-[2-[(S)-2-acetylthio-2-(4-tetrahydropyranyl)acetylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; $[\alpha]_D^{20}$ - 55.4° (c = 0.83, DMSO)



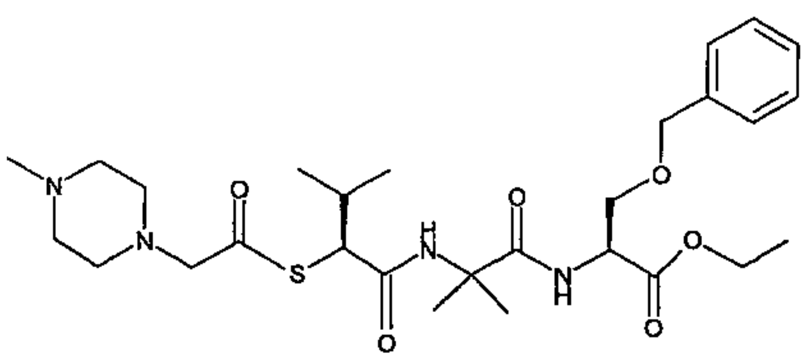
The starting (D)-α-bromo-α-(4-tetrahydropyranyl)-acetic acid can be prepared as follows:

A solution of sodium nitrite (4.71 g, 68.3 mmol) in 35 mL of water is added dropwise to a chilled (0°C) solution of (D)-α-bromo-α-(4-tetrahydropyranyl)-glycine (J. Am. Chem. Soc., Vol. 117, pp. 9375-9376 (1995) (7.05 g, 44.3 mmol) and 48% HBr (aq) (70 mL) in 35 mL of water. Upon completion of the addition, the mixture is allowed to warm to room temperature and stirred at room temperature for 3 hours. The mixture is extracted with ethyl acetate; the organic layer is washed sequentially with water, 5% aqueous sodium thiosulfate, and brine, then dried over anhydrous magnesium sulfate. The mixture is filtered and concentrated *in vacuo* to yield (D)-α-bromo-α-(4-tetrahydropyranyl)-acetic acid as a solid.

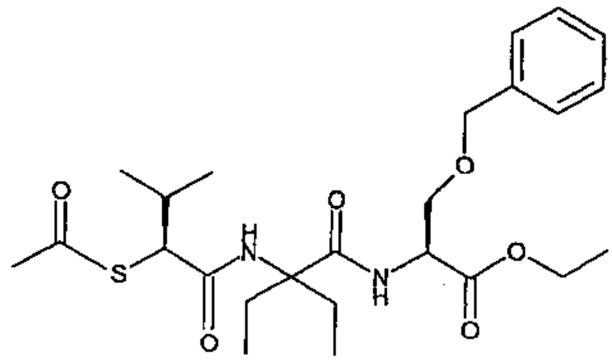
(p) N-[2-[(S)-2-[(1-(1,2,4)-triazolyl)acetylthio]-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; m.p. 106-107°; $[\alpha]_D$ - 61.46° (c = 1.09, CH_3OH)



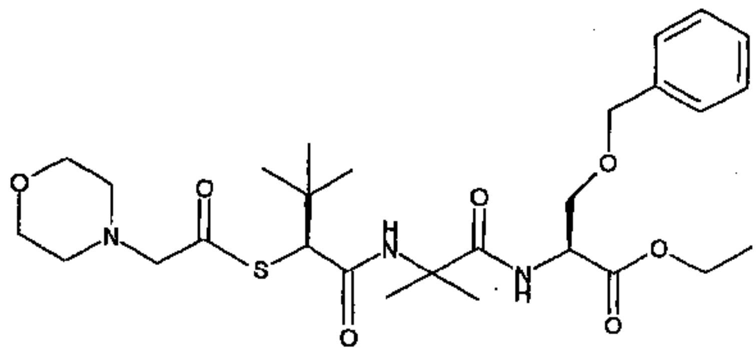
(q) N-[2-[(S)-2-[(4-methylpiperazino)acetylthio]-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; m.p. 95-96°; $[\alpha]_D$ - 48.5° (c = 0.935, CH_3OH)



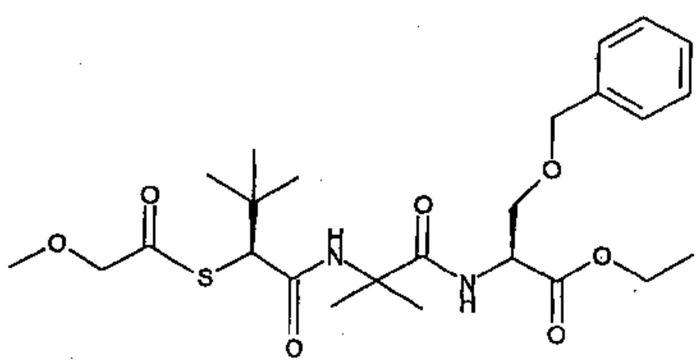
(r) N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-ethylbutanoyl]-O-benzyl-L-serine ethyl ester; $[\alpha]_D$ - 83.6° (c = 1.07, CH_3OH).



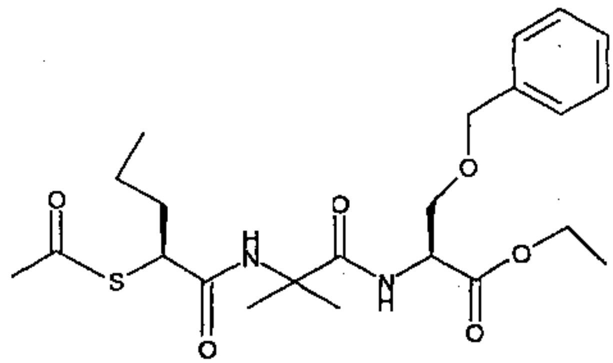
(s) N-[2-[(S)-2-(morpholinoacetylthio)-3,3-dimethylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; $[\alpha]_D$ - 55.5° (c = 1.008, DMSO)



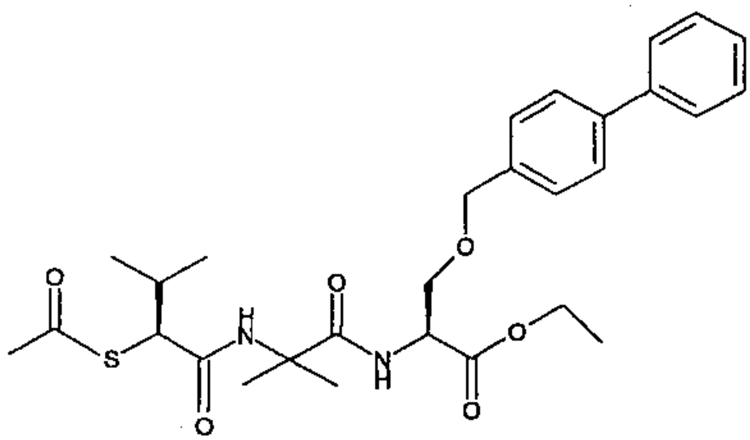
(t) N-[2-[(S)-2-[(methoxyacetyl)thio]-3,3-dimethylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; $[\alpha]_D$ - 61.67° (c = 1.024, DMSO)



(u) N-[2-[(S)-2-(acetylthio)pentanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl est r

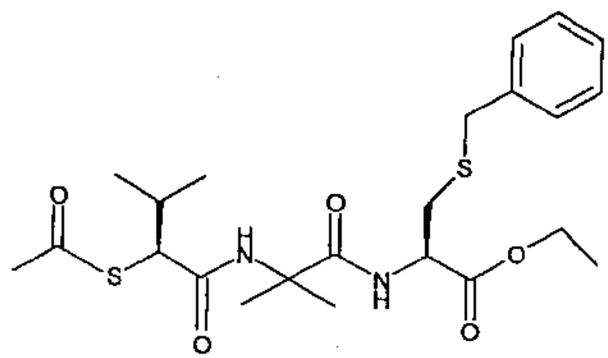


(v) N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-O-(4-biphenylylmethyl)-L-serine ethyl ester



Exampl 2

N-[2-[(S)-2-Acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-S-benzyl-L-cysteine ethyl ester



The title compound is prepared similarly to Example 1 and re-crystallized from methyl t-butyl ether/hexane, m.p. 69-71°C.

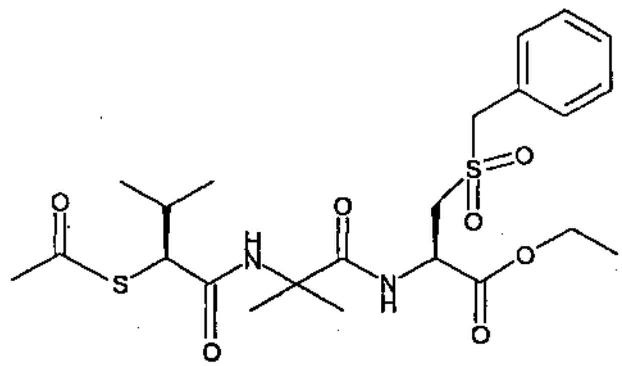
The starting material is prepared as follows:

HCl(g) is bubbled into a solution of BOC-S-benzyl-L-cysteine (9.33 g, 30 mmol) in ethanol (200 mL) for 15 minutes. The container is stoppered and stirred at room temperature overnight. The solvent is evaporated *in vacuo* and the residue stirred in diethyl ether (150 mL) for 1.5 hours to yield S-benzyl-L-cysteine ethyl ester hydrochloride as a solid.

A mixture of S-benzyl-L-cysteine ethyl ester hydrochloride (7.98 g, 29 mmol), BOC-α-methylalanine (5.89 g, 29 mmol), triethylamine (2.93 g, 29 mmol), 1-hydroxybenzotriazole (HOBT, 3.92 g, 29 mmol) and EDCI (5.57 g, 29 mmol) in methylene chloride (200 mL) is stirred under an argon atmosphere at room temperature overnight. The reaction mixture is evaporated to dryness and the residue is dissolved in ethyl acetate (200 mL). The solution is washed with water (50 mL), 1 N HCl (50 mL), water (50 mL), 5% sodium bicarbonate (50 mL), water (50 mL) and finally brine (25 mL). The solution is then dried over sodium sulfate, filtered and evaporated to dryness to give N-[2-(BOC-amino)-2-methylpropionyl]-S-benzyl-L-cysteine ethyl ester.

Example 3

N-[2-[(S)-2-Acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-(S)-2-amino-3-(benzylsulfonyl)-propionic acid ethyl ester



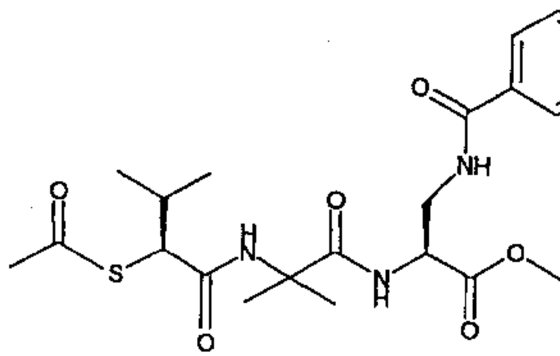
The above compound is prepared similarly to Example 1.

The starting material is prepared as follows:

To a solution of N-[2-(BOC-amino)-2-methylpropionyl]-S-benzyl-L-cysteine ethyl ester (7.21 g, 17 mmol) in methylene chloride (250 mL) under an argon atmosphere is added m-chloro-perbenzoic acid (8.77 g, 51 mmol) and the mixture is stirred overnight at room temperature. The mixture is evaporated to dryness and the residue is dissolved in ethyl acetate (300 mL). The solution is washed with 5% sodium bicarbonate (3 x 50 mL), water (50 mL) and brine (25 mL). The solution is dried over sodium sulfate, filtered and evaporated *in vacuo* to give N-[2-(BOC-amino)-2-methylpropionyl]-(S-)2-amino-3-(benzylsulfonyl)-propionic acid ethyl ester.

Example 4

(a) N[2]-[2-[(S)-2-Acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-(S)-2-amino-3-(benzoylamino)-propionic acid methyl ester



A mixture of benzoyl chloride (0.085 mL, 0.73 mmol), N[2]-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-(S)-2,3-diaminopropionic acid methyl ester hydrochloride (0.29 g, 0.73 mmol) and triethylamine (0.15 mL, 1.49 mmol) in methylene chloride (10 mL) is stirred at room temperature for 16 hours. The reaction mixture is evaporated to dryness *in vacuo*, the residue is dissolved in ethyl acetate, and the solution is washed with water, then with saturated sodium bicarbonate solution and brine, dried over anhydrous magnesium sulfate, and evaporated to dryness to give an oil. The oil is chromatographed on silica gel with hexane, ethyl acetate (50:50) to yield the title compound as a white foam; m.p. 48-54°C.

(b) Similarly prepared is N[2]-[2[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-(S)-2-amino-3-(benzenesulfonamido)propionic acid methyl ester; m.p. 47-51°C; $[\alpha]_D^{20}$ - 41.72 (c = 1.03, CH_3OH).

The starting material is prepared as follows:

A mixture of (S)-2-amino-3-(BOC-amino)-propionic acid methyl ester hydrochloride (4.6 g, 2.1 mmol), N-CBZ-α-methylalanine (5.0 g, 2.1 mmol), HOAT (2.87 g, 2.1 mmol), EDCI (4.02 g, 2.1 mmol) and triethylamine (2.93 g, 2.1 mmol) in methylene chloride (50 mL) is stirred at room temperature for 16 hours. The reaction mixture is washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The resulting oil is chromatographed on silica gel with hexane and ethylacetate (1:1) to yield N[2]-[2-(CBZ-amino)-2-methylpropionyl]-(S)-2-amino-3-(BOC-amino)-propionic acid methyl ester as a white foam; m.p. 100-101°C.

A mixture of the above product (2.14 g, 4.90 mmol) and 10% palladium on charcoal (0.27 g) in ethanol (50 mL) is hydrogenated under 45 psi pressure in a Parr bottle for 4 hours. The mixture is filtered through a pad of Celite and concentrated *in vacuo* to giv N^2-[2-amino-2-methylpropionyl]-(S)-2-amino-3-(BOC-amino)-propionic acid methyl ester hydrochloride as an oil.

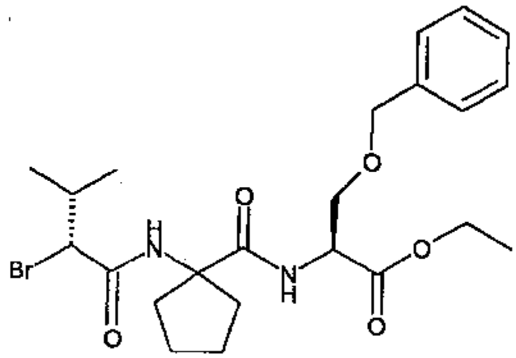
A solution of the above product (2.28 g, 8.09 mmol), (R)-2-bromo-3-methylbutanoic acid diisopropyl amine salt (2.16 g, 7.13 mmol), EDCI (1.43 g, 7.49 mmol) and HOAT (1.15 g, 8.52 mmol) in methylene chloride (75 mL) is stirred at room temperature for 16 hours. The reaction mixture is evaporated to dryness *in vacuo* and the residue taken up in ethyl acetate. The ethyl acetate solution is washed with water, saturated sodium bicarbonate solution and brine, and then dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The resulting oil is chromatographed on silica gel with hexane and ethyl acetate (40:60) to give N^2-[2-[(R)-2-bromo-3-methylbutanoylamino]-2-methylpropionyl]-(S)-2-amino-3-(BOC-amino)-propionic acid methyl ester as a white foam.

A mixture of the above product (1.31 g, 2.82 mmol) and potassium thioacetate (1.28 g, 11.2 mmol) in tetrahydrofuran (50 mL) is stirred at room temperature for 4 hours and diluted with ethyl acetate. The mixture is washed with water, saturated sodium bicarbonate solution, brine and then dried over magnesium sulfate. The reaction mixture is concentrated to dryness *in vacuo* and the resulting oil is chromatographed on silica gel with hexane and ethyl acetate (40:60) to give N-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-(S)-2-amino-3-(BOC-amino)-propionic acid methyl ester.

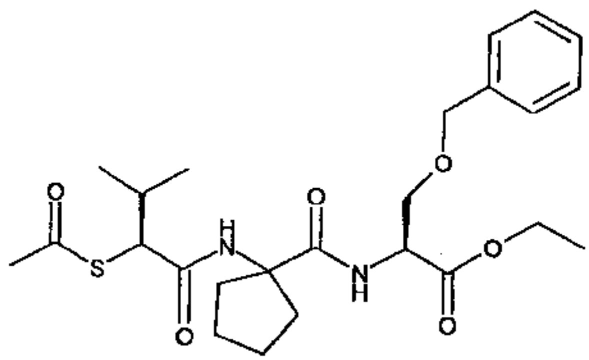
Hydrogen chloride gas is bubbled through a solution of the above compound (1.01 g, 2.19 mmol) in 50 mL of methylene chloride for about 5 minutes, the mixture is stirred at room temperature for 3 hours, and then concentrated *in vacuo* to yield N^2-[2-[(S)-2-acetylthio-3-methylbutanoylamino]-2-methylpropionyl]-(S)-2,3-diaminopropionic acid methyl ester hydrochloride.

Example 5

To a solution of 1-[(R)-2-bromo-3-methylbutanoylamino]cyclopentanecarboxylic acid (1 g, 3.42 mmol), O-benzyl-L-serine ethyl ester hydrochloride (0.89 g, 3.42 mmol), dicyclohexylcarbodiimide (0.7 g, 3.42 mmol) and 1-hydroxy-7-azabenzotriazole (0.47 g, 3.42 mmol) in methylene chloride is added triethylamine (0.48 mL, 3.42 mmol). The mixture is stirred for 24 hours and then washed with brine and concentrated *in vacuo* to give a light yellow oil. The residue is purified by flash chromatography (silica gel hexane/ethyl acetate) to give N-[1-(R)-2-bromo-3-methylbutanoylamino]-cyclopentanecarbonyl]-O-benzyl-L-serine ethyl est r of the formula



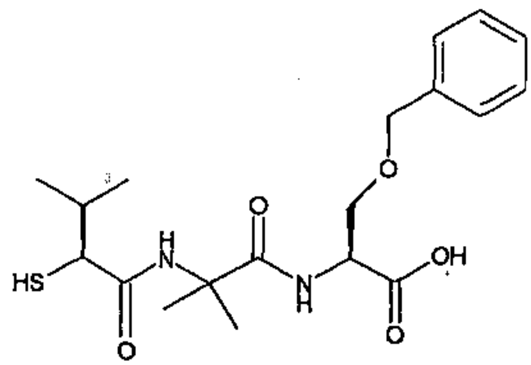
The bromo compound (0.7 g, 1.41 mmol) is dissolved in tetrahydrofuran (50 mL) and potassium thioacetate (0.19 g, 1.69 mmol) is added. The mixture is stirred at room temperature for 18 hours and then diluted with ethyl acetate and washed with brine, dried over magnesium sulfate and concentrated *in vacuo* to give yellow oil. The crude material is purified by flash chromatography (silica gel, hexane/ethyl acetate) to give a semi-solid which is triturated with hexane to yield N-[1-[(S)-2-acetylthio-3-methylbutanoylamino]-cyclopentanecarbonyl]-O-benzyl-L-serine ethyl ester of the formula



The 1-[(R)-2-bromo-3-methylbutanoylamino]cyclopentanecarboxylic acid starting material is prepared essentially by methodology described in WO 99/55726 by condensation of (R)-2-bromo-3-methylbutanoic acid diisopropylamine salt (prepared from L-valine) with cycloleucine methyl ester hydrochloride.

Example 6

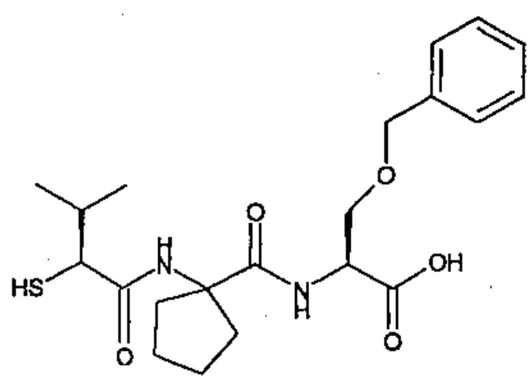
(a) N-[2-[(S)-2-Mercapto-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine



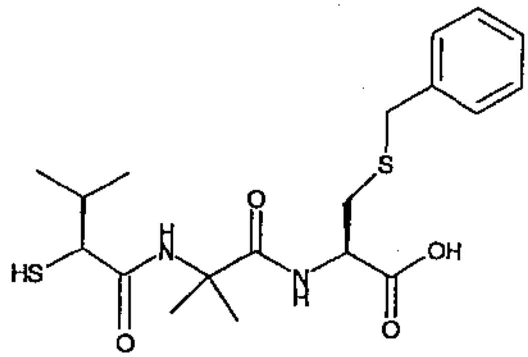
To a solution of the S-acetyl ethyl ester of Example 1 (0.47 g, 1 mmol) in methanol (10 mL) is added 1 N sodium hydroxide (5.0 mL, 5 mmol). The mixture is stirred at room temperature for 4 hours, acidified to pH 1 with 1 N HCl and then concentrated *in vacuo*. To the residue is added ethyl acetate. The mixture is washed with 1 N NaOH. The combined aqueous phase is then acidified and extracted with ethyl acetate. The organic phase is washed with brine, dried over sodium sulfate and then concentrated *in vacuo*. Trituration with hexane yields a white foam; m.p. 57-70°C; $[\alpha]_D^{20}$ - 16.8° (c = 1.032, DMSO); MS(M + H):397.

Similarly prepared are the following:

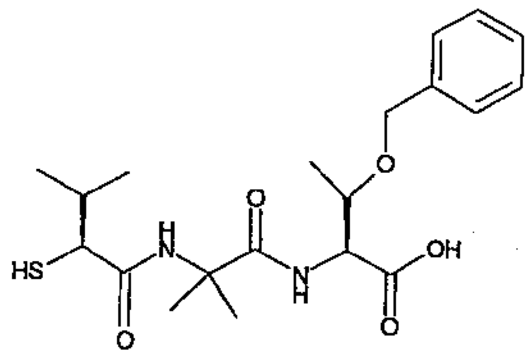
(b) N-[1-[(S)-2-mercapto-3-methylbutanoylamino]-cyclopentanecarbonyl]-O-benzyl-L-serine; m.p. 132-136°C (crystallized from hexane/t-butylmethyl ether)



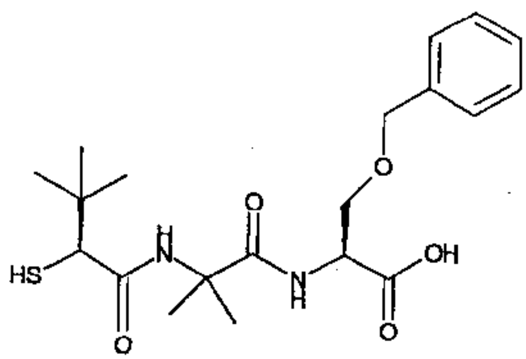
(c) N-[2-[(S)-2-mercapto-3-methylbutanoylamino]-2-methylpropionyl]-S-benzyl-L-cysteine; m.p. 81-87°C; $[\alpha]_D^{20}$ - 37.87 (c = 0.545, DMSO)



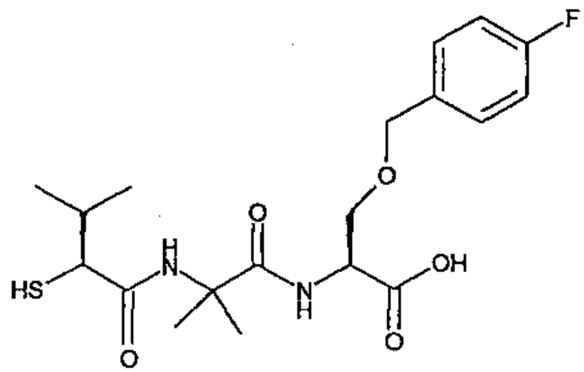
(d) N-[2-[(S)-2-mercapto-3-methylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-threonine; m.p. 61-64°C



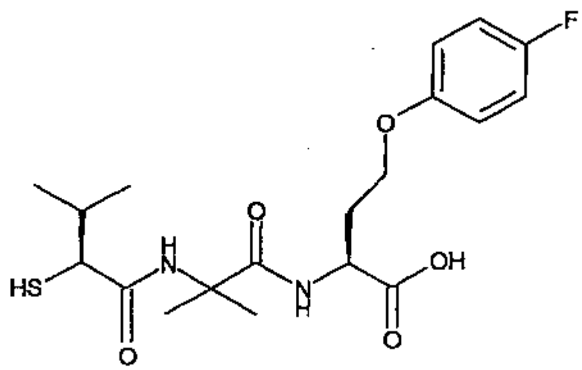
(e) N-[2-[(S)-2-mercapto-3,3-dimethylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine; m.p. 128-130°C; $[\alpha]_D$ - 2.46 (c = 1.06, DMSO)



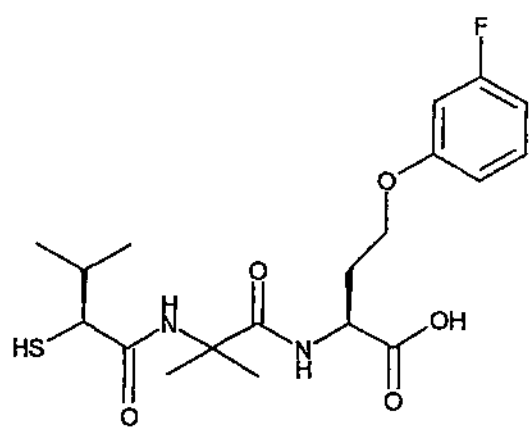
(f) N-[2-[(S)-2-mercapto-3-methylbutanoylamino]-2-methylpropionyl]-O-(4-fluorobenzyl)-L-serine; m.p. 50-54°C



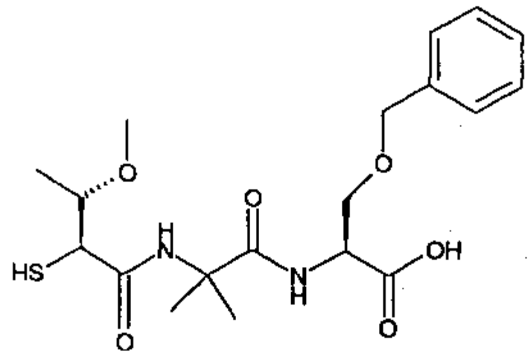
(g) N-[2-[(S)-2-mercapto-3-methylbutanoylamino]-2-methylpropionyl]-O-(4-fluorophenyl)-L-homoserine; m.p. 127-128°C



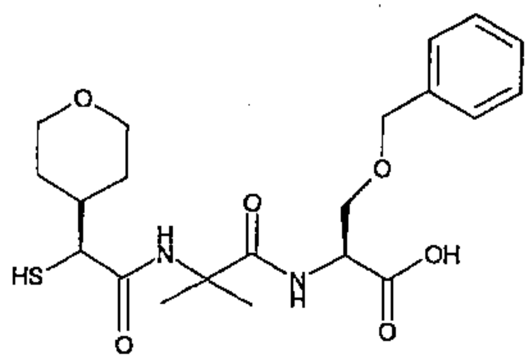
(h) N-[2-[(S)-2-mercapto-3-methylbutanoylamino]-2-methylpropionyl]-O-(3-fluorophenyl)-L-homoserine; m.p. 50-56°C



(i) N-[2-[(S)-2-mercapto-3-methoxybutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine; $[\alpha]_D^{20}$ + 18.85 (c = 0.997, DMSO)

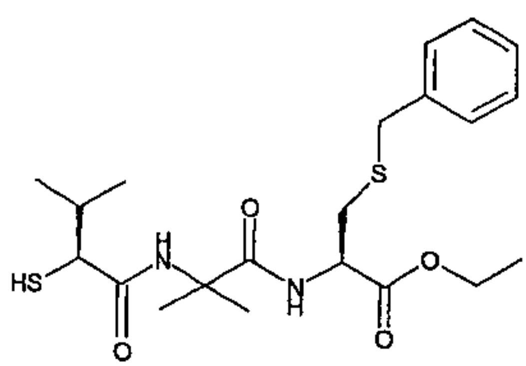


(j) N-[2-[(S)-2-mercapto-2-(4-tetrahydropyranyl)acetylamino]-2-methylpropionyl]-O-benzyl-L-serine; m.p. 184-189°C; $[\alpha]_D^{20}$ - 24.94 (c = 1.013, DMSO)



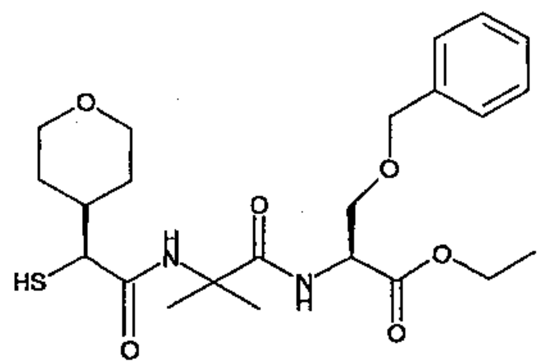
Example 7

(a) N-[2-[(S)-2-mercapto-3-methylbutanoylamino]-2-methylpropionyl]-S-benzyl-L-cysteine ethyl ester

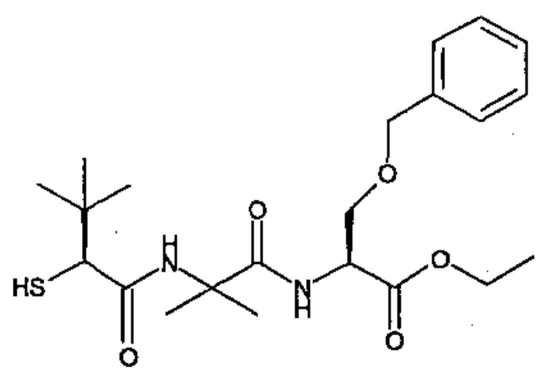


Under an argon atmosphere, the thioacetyl compound of Example 2 (0.48 g, 1.0 mmol) is dissolved in absolut EtOH (5 mL) and treated with 1 N NaOH of (1.0 mL, 1.0 mmol). The mixture is stirred for 4 hours at room temperature before treatment with 1 N HCl until pH 3. The mixture is evaporated to remove most of the EtOH and the aqueous residue is extracted with EtOAc (2 x 10 mL). The combined extracts are washed with H_2O (5 mL) and then with brine solution (5 mL). The solution is dried over Na_2SO_4, filtered and concentrated *in vacuo*. The product solidifies from *tert*-butyl methyl ether/hexane to give product; m.p. 87-91°C.

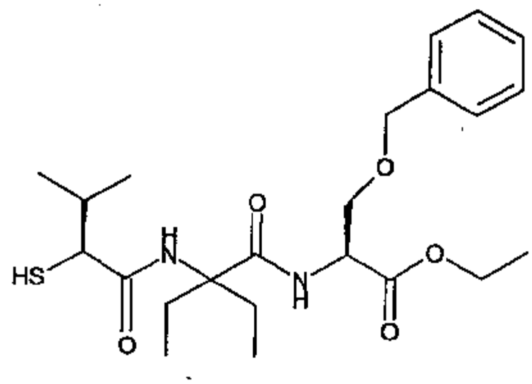
(b) Similarly prepared is N-[2-[(S)-2-mercapto-2-(4-tetrahydropyranyl)acetylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; m.p. 85-93°C; $[\alpha]_D$ - 37.21° (c = 1.012, DMSO)



(c) Similarly prepared is N-[2-[(S)-2-mercapto-3,3-dimethylbutanoylamino]-2-methylpropionyl]-O-benzyl-L-serine ethyl ester; oil; $[\alpha]_D$ - 20.9° (c = 1.025, DMSO)

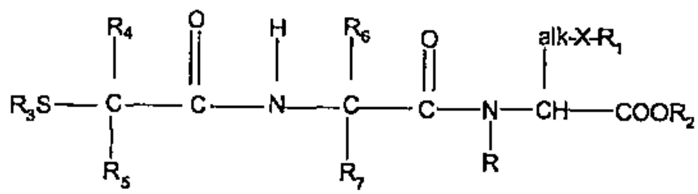


(d) Similarly prepared is N-[2-[(S)-2-mercapto-3-methylbutanoylamino]-2-ethylbutanoyl]-O-benzyl-L-serine ethyl ster; $[\alpha]_D$ - 31.48° (c = 0.955, CH_3OH)



WHAT IS CLAIMED IS:

1. A compound of the formula



(I)

wherein

R represents hydrogen, lower alkyl, carbocyclic or heterocyclic aryl-lower alkyl or cycloalkyl-lower alkyl;

R_1 represents lower alkyl, cycloalkyl, carbocyclic or heterocyclic aryl, or biaryl; or R_1 represents (cycloalkyl, carbocyclic aryl, heterocyclic aryl or biaryl)-lower alkyl;

alk represents lower alkylene;

R_3 represents hydrogen or acyl;

R_4 represents hydrogen, optionally substituted lower alkyl, carbocyclic or heterocyclic aryl, (carbocyclic or heterocyclic aryl)-lower alkyl, cycloalkyl, cycloalkyl-lower alkyl, biaryl, biaryl-lower alkyl; oxacycloalkyl, thiacycloalkyl, azacycloalkyl, or (oxacycloalkyl, thiacycloalkyl or azacycloalkyl)-lower alkyl;

R_5 represents hydrogen or lower alkyl; or

R_4 and R_5, together with the carbon atom to which they are attached, represent cycloalkylidene, benzo-fused cycloalkylidene; or 5- or 6-membered (oxacycloalkylidene, thiacycloalkylidene or azacycloalkylidene), each optionally substituted by lower alkyl or aryl-lower alkyl;

R_6 represents lower alkyl, carbocyclic or heterocyclic aryl, (carbocyclic or heterocyclic aryl)-lower alkyl, cycloalkyl, cycloalkyl-lower alkyl, biaryl or biaryl-lower alkyl;

R_7 represents lower alkyl, (carbocyclic or heterocyclic aryl)-lower alkyl, cycloalkyl-lower alkyl or biaryl-lower alkyl; or

R_6 and R_7, together with the carbon atom to which they are attached, represent 3- to 10-membered cycloalkylidene which may be substituted by lower alkyl or aryl-lower alkyl or may be fused to a saturated or unsaturated carbocyclic 5- to 7-membered ring; or 5- or 6-membered (oxacycloalkylidene, thiacycloalkylidene or azacycloalkylidene), each optionally substituted by lower alkyl or aryl-lower alkyl; or 2,2-norbonylidene;

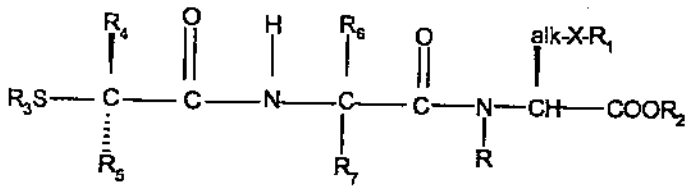
X represents -O-, $-S(O)_n-$, $-NHSO_2-$, or -NHCO-;

n is zero, one or two; and

$COOR_2$ represents carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester;

or a disulfide derivative derived from a said compound wherein R_3 is hydrogen; or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 of the formula



(Ia)

or a disulfide derivative derived from a said compound wherein R_3 is hydrogen; or a pharmaceutically acceptable salt thereof.

3. A compound according to claim 1 wherein R and R_5 represent hydrogen; R_1 represents lower alkyl, C_5- or C_6-cycloalkyl, carbocyclic or heterocyclic aryl, or (carbocyclic or heterocyclic aryl)-lower alkyl; alk represents lower alkylene; X represents -O- or $-S(O)_n$ wherein n represents zero or two; R_3 represents hydrogen or acyl; R_4 represents hydrogen, optionally substituted lower alkyl, oxacycloalkyl, oxacycloalkyl-lower alkyl or (carbocyclic or heterocyclic aryl)-lower alkyl; R_5 represents hydrogen; or R_4 and R_5 combined with the carbon atom to which they are attached represent C_5 or C_6-cycloalkylidene; R_6 and R_7 represent lower alkyl; or R_6 and R_7, together with the carbon atom to which they are attached, represent 5- or 6-membered cycloalkylidene; $COOR_2$ represents carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester; or a disulfide derivatives derived from a said compound wherein R_3 is hydrogen; or a pharmaceutically acceptable salt thereof.

4. A compound according to claim 2 wherein R and R_5 represent hydrogen; R_1 represents lower alkyl, C_5- or C_6-cycloalkyl, carbocyclic or heterocyclic aryl, or (carbocyclic or heterocyclic aryl)-lower alkyl; alk represents lower alkylene; X represents -O- or $-S(O)_n$ wherein n represents zero or two; R_3 represents hydrogen or acyl; R_4 represents hydrogen, optionally substituted lower alkyl, oxacycloalkyl, oxacycloalkyl-lower alkyl or (carbocyclic or heterocyclic aryl)-lower alkyl; R_5 represents hydrogen; or R_4 and R_5 combined

with the carbon atom to which they are attached represent C_5 or C_6-cycloalkylidene; R_6 and R_7 represent lower alkyl; or R_6 and R_7, together with the carbon atom to which they are attached, represent 5- or 6-membered cycloalkylidene; $COOR_2$ represents carboxyl or carboxyl derivatized in form of a pharmaceutically acceptable ester; disulfide derivatives derived from said compounds wherein R_3 is hydrogen; or a pharmaceutically acceptable salt thereof.

5. A compound according to claim 1 wherein R and R_5 represent hydrogen; R_1 represents carbocyclic or heterocyclic aryl or (carbocyclic or heterocyclic aryl)-lower alkyl; R_3 represents hydrogen or optionally substituted lower alkanoyl; R_4 represents lower alkyl, cycloalkyl, tetrahydropyranyl or C_1-C_4-lower alkoxy-lower alkyl; R_6 and R_7 both represent C_1-C_4-alkyl and are identical; X represents -O- or -S-; alk represents methylene; $COOR_2$ represents carboxyl, lower alkoxy-carbonyl, (di-lower alkylaminocarbonyl)-lower alkoxycarbonyl or (morpholinocarbonyl, piperidinocarbonyl or pyrrolidinocarbonyl)-lower alkoxycarbonyl; or a pharmaceutically acceptable salt thereof.

6. A compound according to claim 2 wherein R and R_5 represent hydrogen; R_1 represents carbocyclic or heterocyclic aryl or (carbocyclic or heterocyclic aryl)-lower alkyl; R_3 represents hydrogen or optionally substituted lower alkanoyl; R_4 represents lower alkyl, cycloalkyl, tetrahydropyranyl or C_1-C_4-lower alkoxy-lower alkyl; R_6 and R_7 both represent C_1-C_4-alkyl and are identical; X represents -O- or -S-; alk represents methylene; $COOR_2$ represents carboxyl, lower alkoxy-carbonyl, (di-lower alkylaminocarbonyl)-lower alkoxycarbonyl or (morpholinocarbonyl, piperidinocarbonyl or pyrrolidinocarbonyl)-lower alkoxycarbonyl; or a pharmaceutically acceptable salt thereof.

7. A compound according to claim 1 wherein R and R_5 represent hydrogen; R_1 represents carbocyclic aryl or carbocyclic aryl-lower alkyl in which carbocyclic aryl represents phenyl or phenyl substituted by one or two of hydroxy, lower alkanoyloxy, lower alkyl, lower alkoxy, trifluoromethyl, trifluoromethoxy or halo; R_3 represents hydrogen or lower alkanoyl; R_4 represents lower alkyl, 4-tetrahydropyranyl or C_1-C_4-lower alkoxy-C_1-C_4-lower alkyl; R_6 and R_7 represent methyl; X represents -O-; alk represents methylene or ethylene; and $COOR_2$ represents carboxyl or lower alkoxycarbonyl; or a pharmaceutically acceptable salt thereof.

8. A compound according to claim 2 wherein R and R_5 represent hydrogen; R_1 represents carbocyclic aryl or carbocyclic aryl-lower alkyl in which carbocyclic aryl represents phenyl or phenyl substituted by one or two of hydroxy, lower alkanoyloxy, lower

alkyl, lower alkoxy, trifluoromethyl, trifluoromethoxy or halo; R_3 represents hydrogen or lower alkanoyl; R_4 represents lower alkyl, 4-tetrahydropyranyl or C_1-C_4-lower alkoxy-C_1-C_4-lower alkyl; R_6 and R_7 represent methyl; X represents -O-; alk represents methylene or ethylene; and $COOR_2$ represents carboxyl or lower alkoxycarbonyl; or a pharmaceutically acceptable salt thereof.

9. A compound according to claim 1 wherein R and R_5 represent hydrogen; R_1 represents phenyl, fluorophenyl, benzyl or fluorobenzyl; R_3 represents hydrogen, lower alkanoyl or lower alkanoyl substituted by lower alkoxy; R_4 represents isopropyl, *tert*-butyl, 1-methoxyethyl or 4-tetrahydropyranyl; R_6 and R_7 represent methyl; X represents -O-; alk represents methylene; and $COOR_2$ represents carboxyl or lower alkoxycarbonyl; or a pharmaceutically acceptable salt thereof.

10. A compound according to claim 2 wherein R and R_5 represent hydrogen; R_1 represents phenyl, fluorophenyl, benzyl or fluorobenzyl; R_3 represents hydrogen, lower alkanoyl or lower alkanoyl substituted by lower alkoxy; R_4 represents isopropyl, *tert*-butyl, 1-methoxyethyl or 4-tetrahydropyranyl; R_6 and R_7 represent methyl; X represents -O-; alk represents methylene; and $COOR_2$ represents carboxyl or lower alkoxycarbonyl; or a pharmaceutically acceptable salt thereof.

11. A compound according to claim 10 wherein R and R_5 represent hydrogen; R_1 represents benzyl; R_3 represents hydrogen, acetyl or methoxyacetyl; R_4 represents isopropyl or *tert*-butyl; R_6 and R_7 represent methyl; X represents -O-; alk represents methylene; and $COOR_2$ represents carboxyl or ethoxycarbonyl; or a pharmaceutically acceptable salt thereof.

12. A method of inhibiting both angiotensin converting enzyme and neutral endopeptidase in mammals which comprises administering to a mammal in need thereof an effective amount of a compound according to claim 1.

13. A method of preventing or treating cardiovascular disorders in mammals comprising administering to a mammal in need thereof an effective amount of a compound of claim 1.

14. A method according to claim 12 for the treatment of hypertension, edema, salt retention or congestive heart failure.

15. A pharmaceutical composition comprising an effective amount of a compound of claim 1 in combination with one or more pharmaceutically acceptable carriers.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/092622 A3

(54) **Title:** DIPEPTIDE DERIVATIVES HAVING A N-TERMINAL 2-THIOACYL GROUP AS VASOPEPTIDASE INHIBITORS

(57) **Abstract:** Compounds of formula (I) wherein R, R_1, $COOR_2$, R_3-R_7, alk, and X have meaning as defined, such being useful as dual inhibitors of angiotensin converting enzyme and neutral endopeptidase, as well as inhibitors of endothelin converting enzyme. In a preferred embodiment, R and R_5 represent hydrogen; R_1 represents benzyl; R_3 represents hydrogen, acetyl or methoxyacetyl; R_4 represents isopropyl or *tert*-butyl; R_6 and R_7 represent methyl; X represents -O-; alk represents methylene; and $COOR_2$ represents carboxyl or ethoxycarbonyl.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/05293

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CORIC ET AL: "Optimal recognition of neutral endopeptidase and angiotensin-converting enzyme active sites by mercaptoacyldipeptides as a means to design potent dual inhibitors" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 39, no. 6, 1996, pages 1210-1219, XP002092013 ISSN: 0022-2623	1,2, 12-15
Y	abstract; page 1211, paragraph joining left- and right-hand columns; table 1, in particular compounds 3 and 4	3-11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"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 when the document is taken alone

"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

Date of the actual completion of the international search	Date of mailing of the international search report
26 November 2002	13/12/2002
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer Fausti, S

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 591 891 A (ROQUES BERNARD-PIERRE ET AL) 7 January 1997 (1997-01-07) column 8, lines 1-36; column 9, lines 13-18; compounds of table I on columns 13 and 14, line 9; column 15, line 55 - column 16, line 40; column 17, lines 9-11; column 18, line 64 -column 19, line 9; table III	1,2, 12-15
Y	ROBL J A ET AL: "Recent advances in the design and development of vasopeptidase inhibitors" EXPERT OPINION ON THERAPEUTIC PATENTS, ASHLEY PUBLICATIONS, GB, vol. 9, no. 12, 1999, pages 1665-1677, XP002203837 ISSN: 1354-3776 abstract; page 1670, right-hand column, lines 36-40; compounds 1-30, in particular compounds 9 and 28	3-11
A	WO 99 55726 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); FINK CYNTHIA ANNE) 4 November 1999 (1999-11-04) cited in the application abstract; page 12, lines 14-18; claim 1; examples 6P,12F	1-15
A	WO 99 19346 A (HERGENROEDER STEFAN ;BASF AG (DE); KLING ANDREAS (DE); AMBERG WILH) 22 April 1999 (1999-04-22) abstract; claim 1; examples 1,2	1-15

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 02/05293

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. [X] Claims Nos.: 12-14
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
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. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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.

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

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] 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 claims Nos.:

Remark on Protest

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

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

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

International Application No. PCT/EP 02 05293

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 12-14 are 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/composition.

Continuation of Box I.1

Claims Nos.: 12-14

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/05293

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5591891	A	07-01-1997	FR 2679564	A1	29-01-1993
			AU 2326892	A	23-02-1993
			CA 2113358	A1	04-02-1993
			WO 9302099	A1	04-02-1993
			EP 0524553	A1	27-01-1993
			EP 0595878	A1	11-05-1994
			JP 6509335	T	20-10-1994
			US 5801274	A	01-09-1998
			MX 9204301	A1	01-03-1993
WO 9955726	A	04-11-1999	AU 3819999	A	16-11-1999
			BR 9909805	A	26-12-2000
			CA 2323691	A1	04-11-1999
			CN 1297453	T	30-05-2001
			WO 9955726	A1	04-11-1999
			EP 1073674	A1	07-02-2001
			HU 0101640	A2	28-09-2001
			JP 2002513032	T	08-05-2002
			NO 20005293	A	18-12-2000
			PL 343594	A1	27-08-2001
			SK 15812000	A3	12-03-2001
WO 9919346	A	22-04-1999	DE 19745151	A1	15-04-1999
			AU 9626498	A	03-05-1999
			WO 9919346	A1	22-04-1999
			EP 1023318	A1	02-08-2000
			JP 2001519440	T	23-10-2001
			ZA 9809313	A	13-04-2000

Form PCT/ISA/210 (patent family annex) (July 1992)

(19) Europäisches Patentamt
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(11) Publication number: 0 498 361 A2

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 92101797.6

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(54) Combination of an angiotensin II antagonist or renin inhibitor with a neutral endopeptidase inhibitor.

(57) Treatment of hypertension or congestive heart failure with a combination of an angiotensin II antagonist or a renin inhibitor with a neutral endopeptidase inhibitor, pharmaceutical compositions comprising said combinations and kits for administering separate pharmaceutical compositions in combination are disclosed, wherein the angiotensin II antagonists include saralasin, sar 1, ile 8 angiotensin II, Dup 753, EXP 6155, EXP 6803 and PD 123319, the renin inhibitors include enalkrein, RO 42-5892, A 65317, CP 80794, ES 1005, ES 8891, SQ 34017, CGP 29287, CGP 38560, SR 43845, U-71038, A 62198, and A 64662, and the neutral endopeptidase inhibitors include N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine, N-[N-[((1S)-carboxy-2-phenyl)ethyl]-(S)-phenylalanyl]-β-alanine; N-[2(S)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine, SQ 28603, UK 69578, SQ 29072, thiorphan, retro-thiorphan and phosphoramidon.

EP 0 498 361 A2

BACKGROUND OF THE INVENTION

The present invention relates to the treatment of hyp rtension and congestive heart failure with a combination of an angiot nsin II antagonist or a r nin inhibitor with a neutral endopeptidas inhibitor.

In a second aspect, this invention relates to a pharmaceutical composition comprising an A II antagonist or a renin inhibitor in combination with an NEP inhibitor and to kits comprising an A II antagonist and an NEP inhibitor or a renin inhibitor and an NEP inhibitor.

The renin angiotensin system is a complex hormonal system comprised of a large molecular weight precursor, angiotensinogen, two processing enzymes, renin and angiotensin converting enzyme (ACE), and a vasoactive mediator, A II. See J. Cardiovasc. Pharmacol., 15(Supp B) (1990) p. S1-S5. The enzyme renin catalyzes the cleavage of angiotensinogen into the decapeptide angiotensin I, which has minimal biological activity on its own and is converted into the active octapeptide A II by ACE. A II has multiple biological actions on the cardiovascular system, including vasoconstriction, activation of the sympathetic nervous system, stimulation of aldosterone production, antinatriuresis, stimulation of vascular growth and stimulation of cardiac growth. A II functions as a pressor hormone and is involved the pathophysiology of several forms of hypertension.

Inhibitors of the renin angiotensin system are well known; such drugs lower blood pressure and exert beneficial actions in hypertension and in congestive heart failure as described, for example, in N. Eng. J. Med.. 316, 23 (1987) p. 1429-1435. A large number of peptide and non-peptide inhibitors of the renin angiotensin system are known, the most widely studied being the ACE inhibitors, which class includes the drugs captopril, enalapril, lisinopril and spirapril. Although a major mode of action of ACE inhibitors involves prevention of formation of the vasoconstrictor peptide A II, it has been reported in Hypertension, 16, 4 (1990) p. 363-370 that ACE cleaves a variety of peptide substrates, including the vasoactive peptides bradykinin and substance P. Prevention of the degradation of bradykinin by ACE inhibitors has been demonstrated, and the activity of the ACE inhibitors in some conditions has been reported in Circ. Res., 66, 1 (1990) p. 242-248 to be mediated by elevation of bradykinin levels rather than inhibition of A II formation. Consequently, it cannot be presumed that the effect of an ACE inhibitor is due solely to prevention of angiotensin formation and subsequent inhibition of the renin angiotensin system.

Neutral endopeptidase (EC 3.4.24.11; enkephalinase; atriopeptidase; NEP) is a zinc-containing metalloprotease which cleaves a variety of peptide substrates on the amino terminal side of aromatic amino acids. See Biochem. J., 241, (1987) p. 237-247. Substrates for this enzyme include, but are not limited to, atrial natriuretic factors (ANF), brain natriuretic peptide, met and leu enkephalin, bradykinin, neurokinin A, and substance P.

ANF are a family of vasodilator, diuretic and antihypertensive peptides which have been the subject of many recent reports in the literature, for example Annu. Rev. Pharm. Tox., 29, (1989) p. 23-54. One form, ANF 99-126, is a circulating peptide hormone which is released from the heart during conditions of cardiac distension. The function of ANF is to maintain salt and water homeostasis as well as to regulate blood pressure. ANF is rapidly inactivated in the circulation by at least two processes: a receptor-mediated clearance reported in Am. J. Physiol., 256 (1989) p. R469-R475 and an enzymatic inactivation via NEP reported in Biochem. J., 243 (1987) p. 183-187. It has been previously demonstrated that inhibitors of NEP potentiate the hypotensive, diuretic, natriuretic and plasma ANF responses to pharmacological injection of ANF in experimental animals. The potentiation of ANF by two specific NEP inhibitors is reported by Sybertz et al in J. Pharmacol. Exp. Ther.. 250. 2 (1989) p. 624-631 and in Hypertension, 15, 2 (1990) p. 152-161, while the potentiation of ANF by NEP in general was disclosed in U.S. patent 4,749,688. In U.S. 4,740,499, Olins disclosed the use of thiorphan and kelatorphan to potentiate atrial peptides. Moreover, NEP inhibitors lower blood pressure and exert ANF-like effects such as diuresis and increased cyclic guanosine 3',5'-monophosphate (cGMP) excretion in some forms of experimental hypertension. The antihypertensive action of NEP inhibitors is mediated through ANF because antibodies to ANF will neutralize the reduction in blood pressure.

U.S. 4,749,688 also established the antihypertensive action of NEP inhibitors and that co-administration of an ACE inhibitor and a NEP inhibitor results in a greater reduction of blood pressure than observed with either agent alone. The antihypertensive effect is best manifested under conditions in which the renin angiotensin system is suppressed, as reported by Sybertz et al in the references cited above. For example, NEP inhibitors reduce blood pressure effectively in the Desoxycorticosteron sodium acetate (DOCA NA) hypertensive rat, a volume-dependent, renin-suppressed model of hypertension, but are less effective under conditions in which the renin angiotensin system is activated, such as in th spontaneously hypertensiv rat (SHR) and in the two kidney Goldblatt hypertension model. Studies in the SHR and in th two-kidn y Goldblatt hypertension model using a prodrug of the NEP inhibitor N-[2(S)-mercaptomethyl-3-(2-m thyl-ph nyl)propionyl]methionine in combination with the ACE inhibitor spirapril demonstrat d the gr ater efficacy of the combination compared to either drug alone. However, this interaction was inhibited in SHR which had been n phrectomized, a manipulation which markedly suppresses renin levels.

An explanation of this interactive effect of ACE inhibitors and NEP inhibitors on blood pressure is that suppression of the renin angiotensin system allows for full expression of the ANF-like antihypertensive effect of the NEP inhibitor. A II and ANF exert opposite effects on the cardiovascular system and it has been proposed by Johnston et al in Am. J. Med., 87, (Supp 6) (1990) p. 6B-24S-6B-28S that these two hormonal systems act to counterbalance one another.

An enhanced effect from a combination of an A II receptor antagonist or a renin inhibitor with an NEP inhibitor is, however, unexpected for several reasons. First, as discussed above, ACE inhibitors exert pharmacological effects other than inhibition of formation of A II. ACE degrades numerous substrates, including bradykinin, neurotensin, and substance P. In some instances, e.g. with bradykinin and substance P, both ACE and NEP will degrade the peptide. Since substance P and bradykinin are vasodilators, an alteration of the metabolism of either of these, or more efficient protection from degradation by inhibiting the two enzymes could account for an enhanced effect. Moreover, although nephrectomy, a maneuver which strikingly reduces plasma renin levels, eliminated the enhanced interaction of the ACE inhibitor and NEP inhibitor, the NEP inhibitor alone did not lower blood pressure in this state. Thus, the interactions of ACE inhibitors and NEP inhibitors are complex and the effect of an A II receptor antagonist or a renin inhibitor in combination with an NEP inhibitor cannot be predicted solely from data obtained from the combination of an ACE inhibitor and NEP inhibitor.

SUMMARY OF THE INVENTION

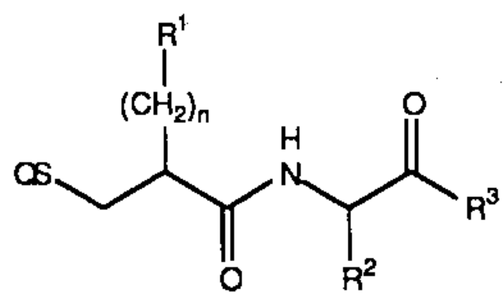
The present invention relates to a method of treating hypertension or congestive heart failure comprising administering an effective amount of a combination of an A II antagonist and a NEP inhibitor to a mammal in need of such treatment. The invention also relates to a method of treating hypertension or congestive heart failure comprising administering an effective amount of a combination of a renin inhibitor and a NEP inhibitor to a mammal in need of such treatment.

Another aspect of the invention relates to pharmaceutical compositions comprising an effective amount of a combination of an A II antagonist and a NEP inhibitor in a pharmaceutically acceptable carrier and to pharmaceutical compositions comprising an effective amount of a combination of a renin inhibitor and a NEP inhibitor in a pharmaceutically acceptable carrier.

Since the present invention relates to a method of treatment comprising a combination of actives wherein the actives may be administered separately, the invention in a third aspect relates to combining separate pharmaceutical compositions in kit form.

DETAILED DESCRIPTION

The NEP inhibitors suitable for use in this invention include, but are not limited to compounds disclosed in U.S. 4,610,816, herein incorporated by reference, including in particular N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine and N-[N-[((1S)-carboxy-2-phenyl)ethyl]-(S)-phenylalanyl]-β-alanine; compounds disclosed in U.S 4,801,609 and 4,929,641, each herein incorporated by reference, including compounds of the formula



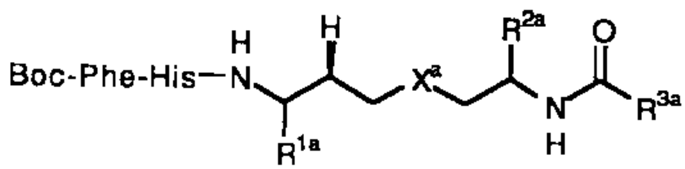
wherein R^1 is phenyl substituted by alkyl, R^2 is alkyl-$S(O)_{0-2}(CH_2)q$, R^3 is OR^7 wherein R^7 is hydrogen or lower alkyl, Q is hydrogen or $R^{10}CO$- wherein R^{10} is alkyl, n is 0-2 and q is 1-4, and in particular N-(2(S)-

mercaptomethyl-3-(2-m thylphenyl)-propionyl]methionine; SQ 28603 (N-[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]-β-alanine), disclos d in South African Patent Application 84/0670; UK 69578 (cis-4-[[[1-[2-carboxy-3-(2-methoxyethoxy)propyl]-cyclop ntyl]carbonyl]amino]-cyclohexan carboxylic acid) and its activ enantiomer(s); thiorphan and its enantiomers; retro-thiorphan; phosphoramidon; and SQ 29072 (7-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]-heptanoic acid). Also suitable for use are any pro-drug forms of the above-listed NEP inhibitors, e.g., compounds in which one or more carboxylic acid groups are esterified.

The A II antagonists suitable for use in this invention include, but are not limited to saralasin; sar 1 (1-(N-methylglycine-angiotensin II); ile 8 angiotensin II (1-de-L-aspartic acid -8-L-isoleucine-angiotensin II); Dup 753 (2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol, monopotassium salt) and active metabolites thereof; EXP 6155 (2-butyl-1-[(4-carboxyphenyl)methyl]-4-chloro-1H-imidazole-5-acetic acid, disodium salt); EXP 6803 (2-butyl-1-[[4-[(2-carboxybenzoyl)amino]phenyl]-methyl]-4-chloro-1H-imidazole-5-acetic acid α-methyl ester, monosodium salt); and PD 123319 (1-(4-dimethylamino-3-methylphenyl)methyl-5-diphenylacetyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid]. Dup 753,EXP 6155 and EXP 6803 are disclosed in European Patent Applications 253,310 and 324,377; PD 123319 is disclosed in European Patent Application 245,637.

The renin inhibitors suitable for use in the present invention include, but are not limited to, enalkrein, RO 42-5892, A 65317 [(2R)-2-benzyl-3-[(2-methoxyethoxymethoxyethyl)methylaminocarbonyl]-propionyl-L-His(2'S,1'R,5S)-3-ethyl-5(1'-hydroxy-2'-amino-3'-cyclohexylpropyl)oxazolidin-2-one amide]; CP 80794; ES 1005 (N-[4-[[1-[[(5-amino-6-hydroxyhexyl)-amino]carbonyl]]-3-methyl-butyl]amino]-2-hydroxy-1-(2-methyl-propyl)-4-oxobutyl]-α-[[3-(1-naphthalenyl)-2-(1-naphthalenylmethyl)-1-oxopropyl]-amino]-1H-imidazole-4-propanamide dihydrochloride); ES 8891 (5-cyclohexyl-2,4,5-trideoxy-N-hexyl-4-[[N-[3-(1-naphthalenyl)-N-(-4-morpholinyl-acetyl)-L-alanyl]-3-(4-thiazolyl)-L-alanyl]amino]-L-threo-pentonamide); SQ 34017; CGP 29287 (carbobenzyloxy-Arg-Arg-Pro-Phe-His-Sta-Ile-His-Lys(BOC)OMe); CGP 38560 (N-[4-[(butylamino)-carbonyl]-1-(cyclohexylmethyl)-2-hydroxy-5-methylhexyl]-α-[[2-[[(1,1-dimethylethyl)-sulfonyl]methyl]-1-oxo-3-phenylpropyl]amino]-1H-imidazole-4-propanamide), disclosed in U.S. 4,758,584; SR 43845 (3-Pyr-(CH_2)-CO-Phe-His-ACHPA-Ile-NH-C$(CH_3$-$(CH_2OH)_2)$; U-71038 (BOC-Pro-Phe-N-MeHis-Leuψ$(CHOHCH_2)$Val-Ile-Amp); A 62198 ([N-(2-methyl-1-oxopropyl)-L-phenylalanyl]-N-[1S,2R,3S]-4-azido-1-(cyclohexylmethyl)-2,3-dihydroxybutyl-L-histidinamide); A 64662 ([N-(3-amino-3-methyl-1-oxobutyl)-4-methoxy-L-phenylalanyl]-N-[1S,2R,3S]-1-(cyclohexyl-methyl)-2,3-dihydroxy-5-methylhexyl-L-histidinamide)and those disclosed by Watkins et al in U.S. 4,906,613, including those disclosed in the following publications, cited therein:

Compounds of the formula



wherein R^{1a} is selected from cyclohexylmethyl, benzyl or butyl; X^a is S or O; R^{2a} is selected from isobutyl, cyclohexylmethyl or benzyl; and R^{3a} is phenethyl. A preferred compound within this class is one wherein R^{1a} is cyclohexylmethyl, R^{2a} is isobutyl, R^{3a} is phenethyl and X^a is S. These compounds are described by Luly et al., Pharmacologist, 27 (3), (1985) p. 260, , and can be prepared by known techniques from known materials.

Other renin inhibitors are disclosed, for example Szelke et al., U.S. Pat. No. 4,424,207 discloses as having the formula

X^b-Y^b-Pro-Phe-His-A^b-B^b-Z^b-W^b (V)

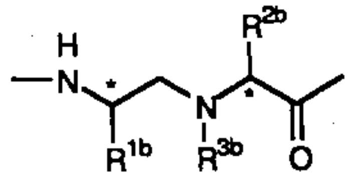
where

Pro, Phe and His may be in substituted form;

X^b = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C_1-C_5); or an L- or D-amino-acyl r sidu , which may its lf be N-prot cted similarly;

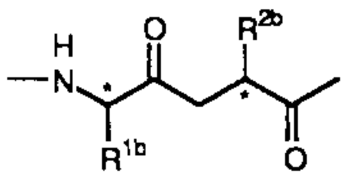
Y^b = D- or L-His or other D- or L-basic or aromatic amino-acetyl r sidue, or is absent;

A^b =



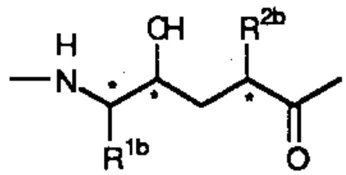
"reduced" isostere bond (VI)

or



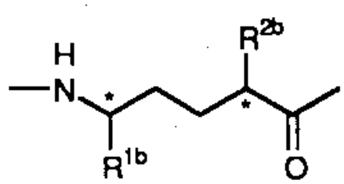
"keto" isostere bond (VII)

or



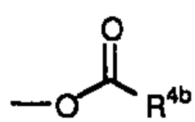
"hydroxy" isostere bond (VIII)

or



"hydrocarbon" isostere bond (IX)

where the configuration at asymmetric centers * is either R or S, where in VIII the hydroxy group may be present as such or protected in ether -OR^{4b} or ester



form where R^{4b} is as given under W below and where R^{1b} and R^{2b}, the same or different = ^{1}Pro(isopropyl), ^{1}Bu(isobutyl), Bz(benzyl) or other lipophilic or aromatic amino-acid side chain;

R^{3b} = -H; lower alkyl (C_1-C_5); or -SO_2Ph, -$SO_2C_6H_4CH_3$(p), Boc, formyl or other N-protecting group;

B^b = D- or L-Val or Ile or other D- or L-lipophilic aminoacyl residue;

Z^b = D- or L-Tyr, Phe, His or other D-or L-aromatic aminoacyl residue; and

W^b =

(a) -OH

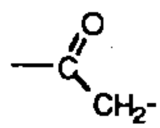
(b) -OR^{4b} where R^{4b} = (1), lower alkyl C_1-C_5(n^1), cycloalkyl C_3-C_7 or Bzi

(c) -NH_2

(c) -NHR^{3b} or -$N(R^{5b})_2$ wher in R^{5b} is an N-protecting group or R^{4b}

(e) L- or D-Lys
(f) L- or D-Arg unprot cted or as the ester or amide
(g) L- or D-Ser and
(h) amino alcohol d rived from (e)-(g) as such or protected in ester or ether form
$Z^b + W^b$ = alcohol derived from
(i) L-Tyr
(ii) L-Phe
(iii) D-Tyr or D-Phe
(iv) His

such peptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds by reduced, $-CH_2-NH-$, keto,



hydroxy, $-CH(OH)-CH_2-$, or hydrocarbon, $-CH_2-CH_2-$ isosteric links and further being in free form or in protected or salt form at one or more remaining peptide, carboxyl, amino, hydroxy or other reactive groups, in particular as their physiologically acceptable acid addition salts at basic centers.

Veber et al., U.S. 4,479,941, discloses renin inhibitor compounds such as
IBU-His-Pro-Phe-His-Sta-Leu-benzylamide;
IBU-His-Pro-Phe-His-Sta-Leu-2-phenylethylamide;
IBU-His-Pro-Phe-His-Sta-Leu-3-phenylpropylamide;
IBU-His-Pro-Phe-His-Sta-Leu-1,2-diphenylethylamide;
BOC-Phe-His-Sta-Leu-(+)[1]-1,2-diphenylethylamide;
BOC-Phe-His-Sta-Leu-(-)-1,2-diphenylethylamide;
BOC-Phe-His-Sta-Leu-benzylamide;
BOC-Phe-His-Sta-Leu-(+)-α-phenylethylamide;
BOC-Phe-His-Sta-Leu-(-)-α-phenylethylamide;
BOC-Phe-His-Sta-Leu-(+)-α-naphthylethylamide;
BOC-Phe-His-Sta-Leu-(-)-α-naphthylethylamide;
BOC-Phe-His-Sta-Leu-p-chlorobenzylamide;
BOC-Phe-His-Sta-Leu-p-methoxybenzylamide;
BOC-Phe-His-Sta-Leu-10,11-dihydro-5H-dibenzo[a,d]-cyclohepteneamide;
BOC-Phe-His-Sta-Leu-D,L-threo-1,2-diphenyl-2-hydroxyethylamide;
BOC-Phe-His-Sta-Leu-Sta;
BOC-Phe-His-AHPPA-Leu-benzylamide;
Acetyl-Phe-His-AHPPA-Leu-benzylamide;
BOC-Phe-His-Sta-Leu-(2-amidomethylpyridine);
BOC-Phe-His-Sta-Leu-(4-amidomethylpyridine);
BOC-Phe-His-Sta-Leu-(4-amido-1-benzylpiperidine);
BOC-Phe-His-Sta-Leu-[N-(3-amidopropyl)diethanolamine];
BOC-Phe-His-AHPPA-Leu-(2-amidomethylpyridine);
BOC-Phe-His-ACHPA-Ile-(2-amidomethylpyridine);
IVA-His-D-Pro-Phe-His-ACHPA-Ile-(2-amidomethylpyridine);
[1] (+) refers to the optical rotation of the amine.

A preferred compound within this class is BOC-Phe-His-Sta-Leu-(4-amido-1-benzyl-piperidine).

Veber et al., U.S. 4,478,826, discloses renin inhibitor compounds such as
tert-Butyloxycarbonyl-His-Pro-Phe-His-Sta-Leu-Leu-Leu-OCH_3,
tert-Butyloxycarbonyl-His-Pro-Phe-His-Sta-Leu-Tyr-NH_2,
iso-Butyryl-His-Pro-Phe-His-Sta-Leu-Phe-Lys-NH_2,
tert-Butyloxycarbonyl-His-Pro-Phe-p-I-Phe-Sta-L u-Phe-NH_2,
iso-Valeryl-His-Pro-Phe-His-Sta-Leu-Val-Phe-NH_2,
His-Pro-Ph -His-Sta-Leu-Phe-NH_2,
iso-Valeryl-His-Pro-Phe-His-Sta-Leu-Phe-NH_2,
Acetyl-Pro-Phe-His-Sta-Leu-Phe-NH_2,

Acetyl-Ph -His-Sta-Leu-Phe-NH_2,
tert-Butyloxycarbonyl-Phe-His-Sta-Leu-Phe-NH_2,
tert-Butyloxycarbonyl-His-Pro-Phe-Phe-Sta-L u-Phe-NH_2,
iso-Butyryl-His-Pro-Phe-His-Sta-Ala-Phe-NH_2,

iso-Butyryl-His-Pro-Phe-His-Sta { Cyclo-hexyl-Phe-NH_2 / Ala

A preferred compound within this class is IVA-His-Pro-Phe-His-Sta-Leu-Phe-NH_2.

Veber et al., U.S. 4,384,994 discloses renin inhibitor compounds such as
N-phenoxyacetyl-L-leucyl-(3S,4S)-statyl-L-valyl-L-phenylalanine;
N-phenoxyacetyl-L-leucyl-(3S,4S)-statyl-L-leucyl-L-phenylalanine
N-phenoxyacetyl-L-leucyl-(4S)-amino-(3S)-hydroxy-5-phenylpentanoyl-L-leucyl-L-phenylalanine;
L-leucyl-(3S,4S)-statyl-L-valyl-L-phenylalanine;
L-leucyl-(3S,4S)-statyl-L-leucyl-L-phenylalanine;
L-leucyl-(4S)-amino-(3S)-hydroxy-5-phenylpentanoyl-L-leucyl-L-phenylalanine;
and the amide and C_{1-4} alkyl ester forms of the above peptides.

Boger et al., U.S. 4,485,099, discloses renin inhibitor compounds such as

IBU-His-Pro-Phe—Lys-Sta-Leu-Phe

IBU-His-Pro-Phe—Orn-Sta-Leu-Phe

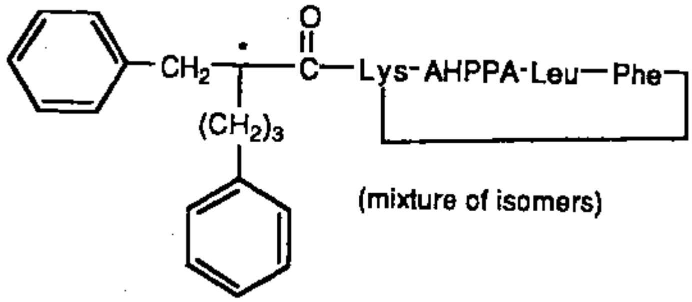
IBU-His-Pro-Phe—DAB-Sta-Leu-Phe-Gly

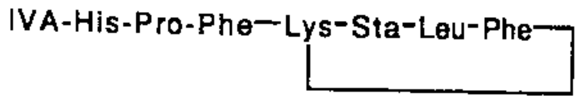
IBU-His-Pro-Phe—HLys-Sta-Leu-Phe

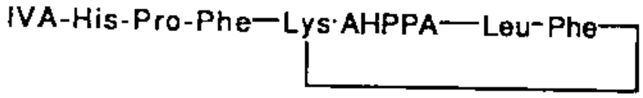
IBU-His-Pro-Phe—Orn-Sta-Leu-Phe-Gly

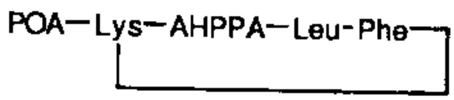
IBU-His-Pro-Phe—Lys-Sta-Leu-Phe-Gly

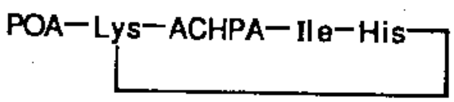
BOC-Phe—Lys-Sta-Leu-Phe

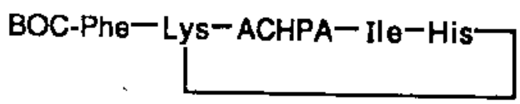


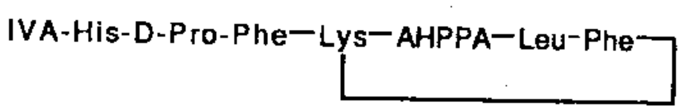


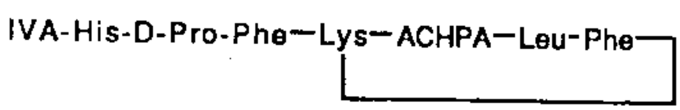


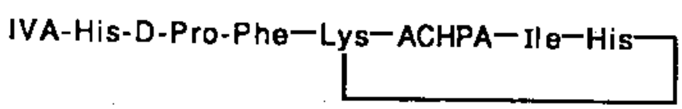




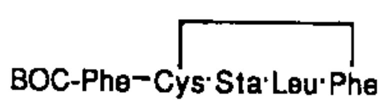




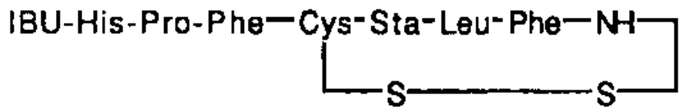


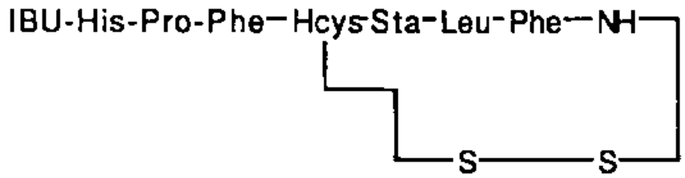


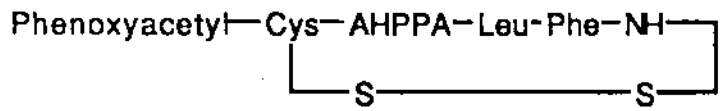
A preferred compound within this class is

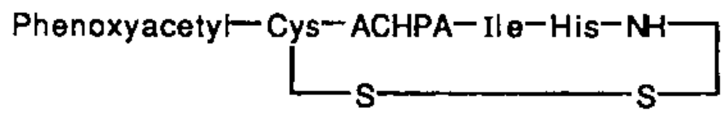


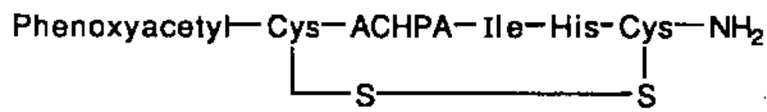
Boger et al., U.S. 4,477,441, discloses renin inhibitor compounds such as

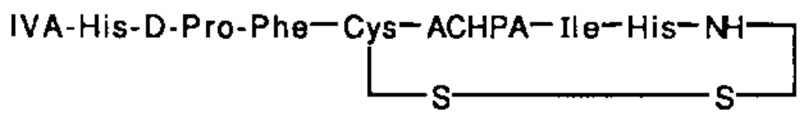


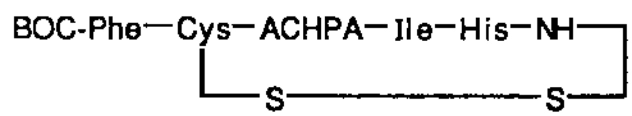




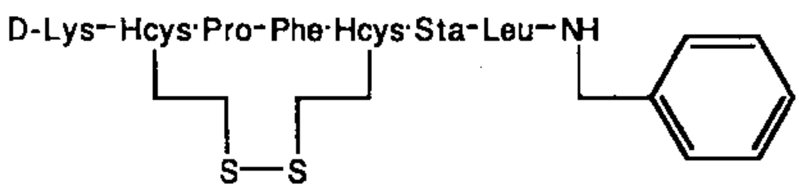


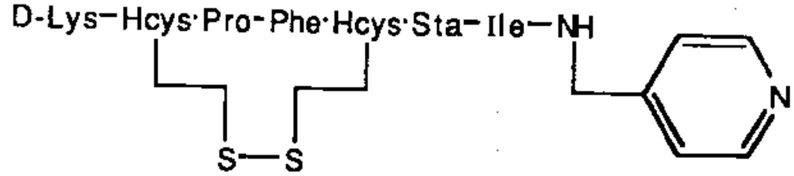


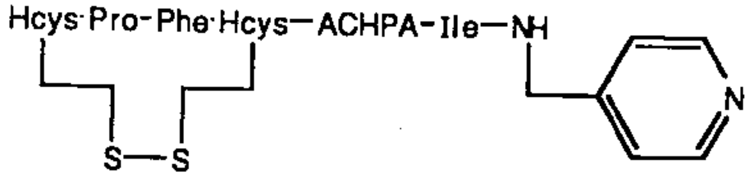


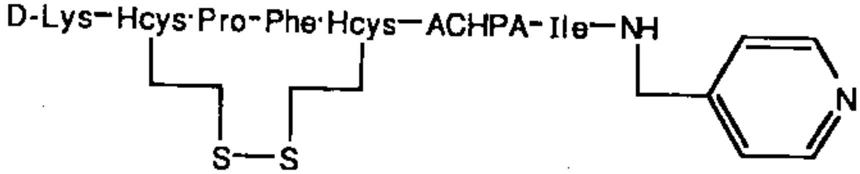


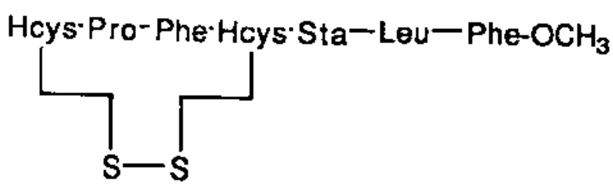
Boger et al., U.S. 4,477,440, discloses renin inhibitor compounds such as

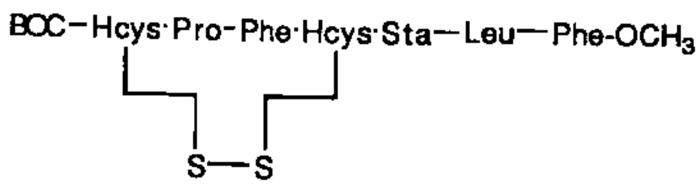


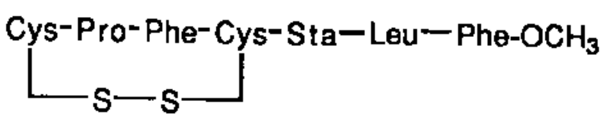

D-Lys−Hcys·Pro−Phe·Hcys·Sta−Ile−NH
N
S—S

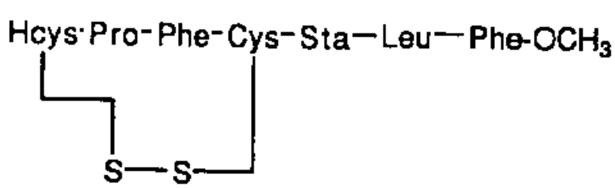

Hcys·Pro−Phe·Hcys−ACHPA−Ile−NH
N
S—S

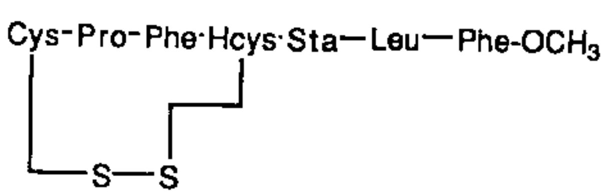

D-Lys−Hcys·Pro−Phe·Hcys−ACHPA−Ile−NH
N
S—S


Hcys·Pro−Phe·Hcys·Sta−Leu−Phe-OCH3
S—S


BOC−Hcys·Pro−Phe·Hcys·Sta−Leu−Phe-OCH3
S—S


Cys−Pro−Phe−Cys−Sta−Leu−Phe-OCH3
S—S

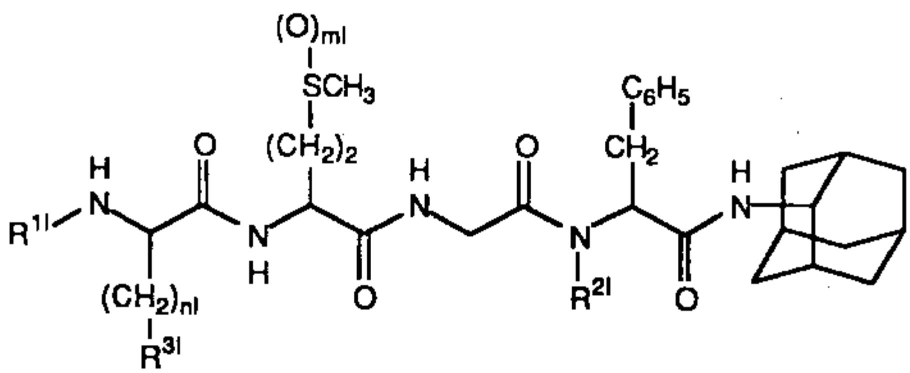

Hcys·Pro−Phe−Cys−Sta−Leu−Phe-OCH3
S—S


Cys−Pro−Phe·Hcys·Sta−Leu−Phe-OCH3
S—S

Boger t al., U.S. 4,470,971, discloses renin inhibitor compounds such as
iso-Butyryl-His-Pro-Ph -His-Sta-Val-His-Gly-NH_2
iso-Butyryl-His-Pro-Ph -His-Sta-Il -His-NH_2
tert-Butyloxycarbonyl-Phe-His-Sta-Ile-His-NH_2
Benzyloxycarbonyl-Phe-His-Sta-Ile-His-NH_2
iso-Valeryl-His-Pro-Phe-His-Sta-Ile-His-NH_2
iso-Valeryl-His-Pro-Phe-His-Sta-Leu-His-NH_2
A preferred compound within this class is IVA-His-Pro-Phe-His-Sta-Ile-His-NH_2.

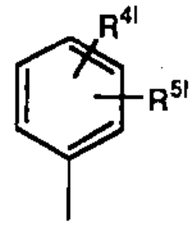
Cazaubon et al., U.S. 4,481,192, discloses renin inhibitor comounds such as BOC-Phe-His-Sta-Ala-Sta-OMe.

Hansen, Jr. et al., U.S. 4,510,085, discloses compounds of the formula

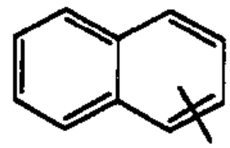


as having renin inhibitory activity
wherein R^{1l} is:
(a) hydrogen; or
(b) alkyl of 1 to 6 carbon atoms, inclusive;
wherein R^{2l} is:
(a) hydrogen; or
(b) alkyl of 1 to 6 carbon atoms, inclusive;
wherein R^{3l} is:

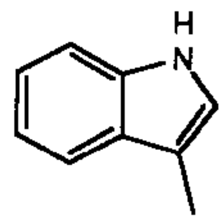
(a)



(b)



(c)



wherein R^{4l} and R^{5l} each being the same or different, are:

(a) hydrogen;

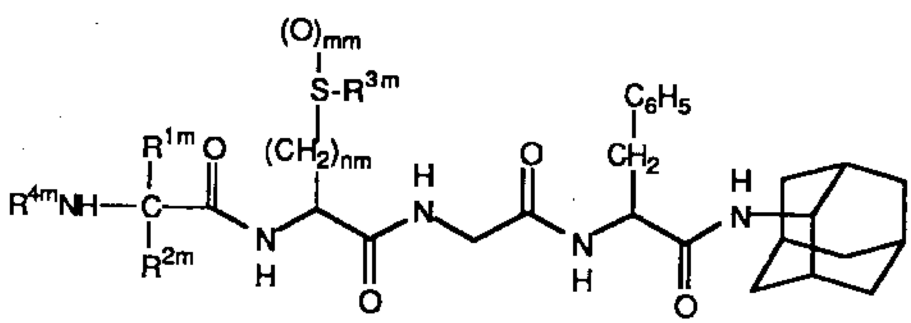
(b) alkyl of 1 to 6 carbon atoms; or

(c) halogen;

wherein ml is 0, 1 or 2,

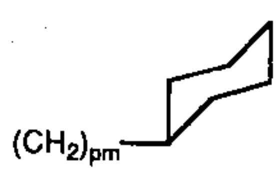
wherein nl is 0 or an integer of from 1 to 4; and the pharmaceutically acceptable salts thereof.

U.S. 4,514,332 discloses renin inhibitory activity for tetrapeptide adamantyl amides of the formula



wherein R^{1m} is:

(a)

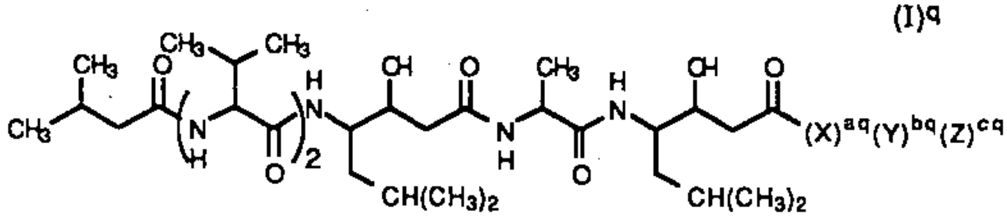


or

(b) straight or branch d chain alkyls of 1 to 6 carbon atoms, inclusive;
wherein R^{2m} is:
(a) hydrog n; or
(b) alkyl of 1 to 3 carbon atoms;
wherein R^{3m} is:
(a) $CH_2C_6H_5$; or
(b) alkyl of 1 to 3 carbon atoms;
wherein R^{4m} is:
(a) hydrogen; or
(b) alkyl of 1 to 6 carbon atoms;
wherein m^m is 0, 1 or 2;
wherein n^m is 1 or 2;
wherein p^m is 0, 1 or 2;
and the stereochemical configuration of each of the optically active amino acid residues may independently be D, L or DL; and the pharmacologically acceptable salts thereof.

Castro et al., U.S. 4,185,096, discloses renin inhibitor compounds such as

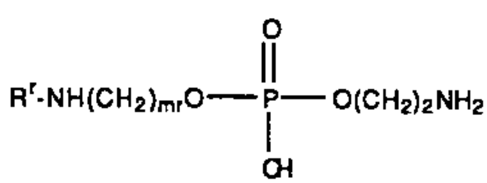
(I)q



as having renin inhibitory activity.

In the general formula $(I)^q$ each of the symbols X^{aq}, Y^{bq} and Z^{cq} which are identical or different, represent an amino-acid residue selected from arginine, glutamic acid, aspartic acid, lysine, histidine or valine, forming with the free carboxyl group of the pepstatin or of the adjacent amino-acid a peptide bond -CONH-; the carboxyl function of the terminal amino-acid may exist in free form or in the form of an ester of an aliphatic alcohol containing 1 to 4 carbon atoms, and the indices aq, bq, and cq are each equal to zero or 1, the sum aq + bq + cq being equal to 1, 2 or 3.

Hayashi et al., U.S. 3,985,875, discloses renin inhibitors of the formula



wherein R^r is an octadeca-9,12-dienoyl, octadeca-9,12,15-trienoyl, 4-(4'-chlorophenoxy)phenoxyacetyl or α-[4-(4'-chlorophenoxy)phenoxy]-propionyl group, and m^r is 2 or3, or their pharmaceutically acceptable acid addition salts.

The following definitions apply throughout the specification: IBU = Iso-butyryl; BOC = Tert-butyloxycarbonyl; AHPPA = (3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid; ACHPA = (3S,4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid; IVA= Iso-valeryl; DAB = 2S-amino-4-aminobutyric acid; HLys = homolysine, 2S-amino-6-aminoheptanoic acid; POA = phenoxyacetyl; Hcys = L-homocysteine.

The above descriptions of classes of renin inhibitors for use in the present invention were taken from the noted patents and publications or abstracts thereof. Reference should be made to such patents and publications thems lves for their full disclosures of such classes and specific compounds within such classes, and as to any typographical rrors or the like which may have occurred in transcription. Also, in describing such renin inhibitors, the superscript l tters a,b,l,m,q and r wer included to distinguish among the various classes of compounds and the variable substituent groups thereof.

The antihypertensive effects of NEP inhibitors, A II antagonists and renin inhibitors, and of combinations of NEP inhibitors with A II antagonists or r nin inhibitors are determined according to the following procedures.

For the DOCA salt hypertension mod l, male Sprague Dawley rats weighing 100-150 g are anesthetized with eth r and the right kidney is removed. Thr pell ts containing Doc ac tate (d soxycorticosterone acetate, DOCA, 25 mg/pellet) are implanted subcutaneously. Animals recover from surgery, are maintained on normal rat chow and are allowed free access to a fluid of 1% NaCl and 0.2% KCl instead of tap water for a period of 25-30 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 the study, animals are again anesthetized with ether and the caudal artery is cannulated for blood pressure measurement. Patency of the caudal artery cannula is maintained with a continuous infusion of dextrose in water at a rate of 0.2 ml/hr. Animals are placed into restraining cages where they recover consciousness. Blood pressure is measured from caudal artery catheter using a Statham pressure transducer attached to a Beckman oscillographic recorder. In addition, a cardiovascular monitoring device (Buxco Eletronics, Inc.) and a digital computer are used to calculate average blood pressures.

After an equilibration period of at least 1.5 hr., animals are dosed subcutaneously (1 ml/kg) with vehicle (methylcellulose, hereinafter MC), NEP inhibitor, A II antagonist, renin inhibitor, a combination of NEP inhibitor and A II antagonist , or a combination of NEP inhibitor and renin inhibitor and blood pressure is monitored for the next 4 hours. The doses of drug are chosen based on amounts previously determined to be effective for inhibition of the respective enzymes.

Two kidney, 1-clip (2K,1C) Goldblatt hypertension is produced in male Sprague Dawley rats as described by DeForrest et al (1984). Rats weighing 180-200 g are anesthetized with ether or Brevital (50 mg/kg, ip) and the left kidney is exposed through a flank incision. A silver clip with an internal diameter of 0.15 mm is placed around the left renal artery. The contralateral kidney remains untouched. The animal is used 3-4 weeks after surgery when sustained hypertension greater than 150 mm Hg is established.

On the day of the experiment, fasted rats are anesthetized with ether and the abdominal aorta is cannulated via the caudal artery with polyethylene tubing. The rats are placed in plastic restrainers and allowed to regain consciousness for at least 90 min. The rats are dosed by oral gavage with a single drug or with a combination of drugs as suspensions in 0.4% methylcellulose vehicle. Blood pressure is recorded continuously from the caudal artery on an oscillographic recorder.

The antihypertensive effect in SHR is determined as follows. Animals are prepared for blood pressure measurement as described above. After stabilization, animals are dosed subcutaneously with test drugs, combinations thereof or placebo and blood pressure is monitored for the next 4 hours.

ANF has been shown to exert beneficial hemodynamic and renal actions in congestive heart failure (CHF) with the exception of the most severe states, in which its actions may be blunted. ANF and the renin angiotensin system also act as physiological antagonists of one another in CHF. Therefore, it is contemplated that the combination of an ANF-potentiating NEP inhibitor and an inhibitor of the renin angiotensin system will be useful in the treatment of CHF. Measurements of the degree of diuresis and natriuresis, as well as hemodynamics, are used to determine the efficacy of the present combination in the treatment of CHF.

The combinations of this invention comprise an NEP inhibitor and an A II antagonist, and an NEP inhibitor and a renin inhibitor. The components of each combination can be administered in the same pharmaceutical composition or by co-administration of separate pharmaceutical compositions. A variety of pharmaceutical dosage forms are suitable, preferably for oral or parenteral administration, although mechanical delivery systems such as transdermal dosage forms are also contemplated.

The daily dosages of the combinations of this invention for treatment of hypertension or congestive heart failure are as follows: for NEP inhibitors, the typical dosage is about 0.3 mg/kg to about 100 mg/kg of mammalian weight per day administered in single or divided doses; for A II antagonists, the typical dosage is about 0.1 mg/kg to about 50 mg/kg of mammalian weight per day administered in single or divided doses; and for renin inhibitors, the typical daily dosage is about 0.1 mg/kg to about 100 mg/kg mammalian weight, administered in single or divided doses. 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, th combinations of this invention can be administered in dosage ranges as follows: for the combination of NEP inhibitor and A II antagonist, about 10 to about 500 mg NEP inhibitor per dose given 1 to 4 times a day, and about 5 to about 100 mg A II antagonist given 1 to 3 times a day; and for the combination of NEP inhibitor and renin inhibitor, about 10 to about 500 mg NEP inhibitor given 1 to 4 times a day, and about 5 to about 600 mg r nin inhibitor giv n 1 to 3 times a day. Where th components of a combination are administer d separately, the number of dos s of each component given per day may not necessarily be the same, .g., wh re one component may hav a greater duration of activity, and will therefore need to be administered less frequently.

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

The typical pharmaceutically acceptable 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 glycol polymers; betacyclodextrin; fatty alcohols; and hydrolyzed cereal solids, as well as other non-toxic 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 and congestive heart failure with combinations of active ingredients wherein said active ingredients can 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: an NEP inhibitor pharmaceutical composition and and A II antagonist pharmaceutical composition in one kit, and an NEP inhibitor pharmaceutical composition and a renin 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 NEP formulation and parenteral A II antagonist formulation) or are administered at different dosage intervals.

Claims

1. A pharmaceutical composition for treating hypertension or congestive heart failure comprising an effective amount of a combination of a neutral endopeptidase inhibitor and either a renin inhibitor or an angiotensin II antagonist, in a pharmaceutically acceptable carrier.

2. A composition of claim 1 wherein:
   the neutral endopeptidase inhibitor is selected from the group consisting of N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine; N-[N-[((1S)-carboxy-2-phenyl)ethyl]-(S)-phenylalanyl]-β-alanine; N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propionyl]methionine; SQ 28603; UK 69578; thiorphan; retro-thiorphan; phosphoramidon; SQ 29072; and the pro-drugs thereof;
   the angiotensin II antagonist is selected from the group consisting of saralasin; sar 1; ile 8 angiotensin II; Dup 753; EXP 6155; EXP 6803; and PD 123319; and
   the renin inhibitor is selected from the group consisting of enalkrein; RO 42-5892; A 65317; CP 80794; ES 1005; ES 8891; SQ 34017; CGP 29287; CGP 38560; SR 43845; U-71038; A 62198; and A 64662.

3. 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 neutral endopeptidase inhibitor, and in a second container a pharmaceutical composition comprising a renin inhibitor or an angiotensin II antagonist.

4. The use of a neutral endopeptidase (NMEP) inhibitor, in combination with either a renin inhibitor or an angiotensin II antagonist, for the preparation of a pharmaceutical composition useful in the treatment of hypertension or congestive heart failure.

5. The use according to claim 4, wherein:
   the neutral endopeptidase inhibitor is selected from the group consisting of N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine; N-[N-[((1S)-carboxy-2-phenyl)ethyl]-(S)-phenylalanyl]-β-alanine; N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propionyl]methionine; SQ 28603; UK 69578; thiorphan; retro-thiorphan; phosphoramidon; SQ 29072; and the pro-drugs thereof;
   the angiotensin II antagonist is selected from th group consisting of saralasin; sar 1; ile 8 angiotensin II; Dup 753; EXP 6155; EXP 6803; and PD 123319; and

the renin inhibitor is s l ct d from the group consisting of enalkrein; RO 42-5892; A 65317; CP 80794; ES 1005; ES 8891; SQ 34017; CGP 29287; CGP 38560; SR 43845; U-71038; A 62198; and A 64662.

6. A composition according to any of claims 1 or 2, wherein:
the NMEP inhibitor is administered at a dosage level of 0.3 mg/kg mammalian weight per day;
the angiotensin II antagonist is administered at a dosage level of 0.1 mg/kg to 50 mg/kg mammalian weight per day; and
the renin inhibitor is administered at a dosage level of 0.1 mg/kg to 100 mg/kg mammalian weight per day.

7. A process for the preparation of a pharmaceutical composition according to any of claims 1, 2 or 6, which comprises mixing a NMEP inhibitor, in combination with either a renin inhibitor or an angiotensin II antagonist, with a pharmaceutically acceptable carrier.

8. A method of treating hypertension or congestive heart failure comprising administering an effective amount of a combination of a neutral endopeptidase inhibitor and either a renin inhibitor or an angiotensin II antagonist to a mammal in need of such treatment.

9. A method of claim 8 wherein:
the neutral endopeptidase inhibitor is selected from the group consisting of N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine; N-[N-[((1S)-carboxy-2-phenyl)ethyl](S)-phenylalanyl]-β-alanine; N-[2(S)-mercaptomethyl-3-(2-methylphenyl)propionyl]methionine; SQ 28603; UK 69578; thiorphan; retro-thiorphan; phosphoramidon; SQ 29072; and pro-drugs thereof;
the angiotensin II antagonist is selected from the group consisting of saralasin; sar 1; ile 8 angiotensin II; Dup 753; EXP 6155; EXP 6803; and PD 123319; and
the renin inhibitor is selected from the group consisting of enalkrein; RO 42-5892; A 65317; CP 80794; ES 1005; ES 8891; SQ 34017; CGP 29287; CGP 38560; SR 43845; U-71038; A 62198; and A 64662.

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(54) Combination of an angiotensin II antagonist or renin inhibitor with a neutral endopeptidase inhibitor.

(57) Treatment of hypertension or congestive heart failure with a combination of an angiotensin II antagonist or a renin inhibitor with a neutral endopeptidase inhibitor, pharmaceutical compositions comprising said combinations and kits for administering separate pharmaceutical compositions in combination are disclosed, wherein the angiotensin II antagonists include saralasin, sar 1, ile 8 angiotensin II, Dup 753, EXP 6155, EXP 6803 and PD 123319, the renin inhibitors include enalkrein, RO 42-5892, A 65317, CP 80794, ES 1005, ES 8891, SQ 34017, CGP 29287, CGP 38560, SR 43845, U-71038, A 62198, and A 64662, and the neutral endopeptidase inhibitors include N-[N-[1(S)-carboxyl-3-phenylpropyl]-(S)-phenylalanyl]-(S)-isoserine, N-[N-[((1S)-carboxy-2-phenyl)ethyl]-(S)-phenylalanyl]-β-alanine; N-[2(S)-mercaptomethyl-3-(2-methylphenyl)-propionyl]methionine, SQ 28603, UK 69578, SQ 29072, thiorphan, retro-thiorphan and phosphoramidon.

EP 0 498 361 A3

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EUROPEAN SEARCH REPORT

Application Number

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	FR-A-2 616 070 (E. R. SQUIBB & SONS, INC) 9 December 1988 * abstract *	1-9	A61K45/06 A61K37/64
A	Dialog 7253314, Embase 8825334; Roque B. P.: "Physiological role of endogenous peptide effectors studied with peptidase inhibitors" & Kidney Int: (USA), 1988, Vol. 34, suppl. 26, p27-33 "abstract"	1-9	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
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CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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CASE 4-32219A

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IN RE APPLICATION OF   Art Unit: 1614

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APPLICATION NO: 10/341,868

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FOR: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Sir:

Applicants believe this paper is being filed before the mailing date of a first Office Action on the merits, and so under 37 C.F.R. §1.97(b)(3) no fees are required. If a fee is deemed to be required, the Commissioner is hereby authorized to charge such fee to Deposit Account No. 19-0134.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the reference cited on the attached form(s) PTO-1449.

A Copy of this reference is enclosed herewith.

The Examiner is requested to consider the foregoing information in relation to this application and indicate that the reference was considered by returning a copy of the initialed PTO 1449 form(s).

Respectfully submitted,

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	AA						
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	AM	WO 03/066606	8/14/03	PCT			☐	☐
	AN						☐	☐
	AO						☐	☐
	AP						☐	☐
	AQ						☐	☐

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent pages, Etc.)

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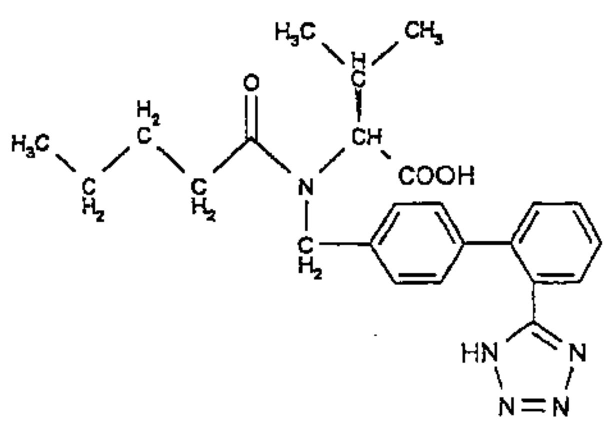
(54) **Title:** SALTS OF VALSARTAN



(I)

(57) **Abstract:** The invention relates to new forms of salts of valsartan or crystalline, also partly crystalline and amorphous salts of valsartan, the respective production and usage, and pharmaceutical preparations containing such a salt. (Formula I).

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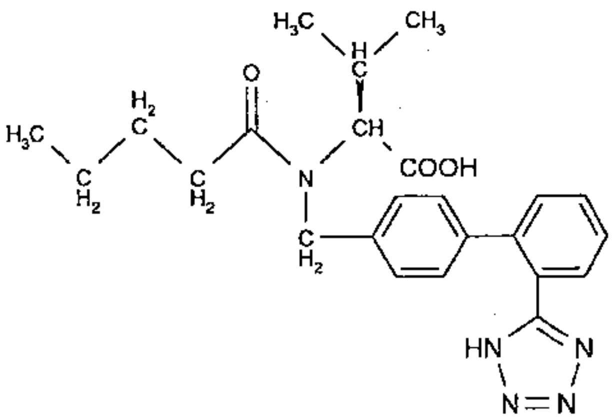
SALTS OF VALSARTAN

The invention relates to additional new salts and salt hydrates of the AT_1 receptor antagonist (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-yl-methyl]-amine (valsartan) of formula



(I).

The active ingredient valsartan is the free acid which is described specifically in EP 0443983, especially in example 16; it has two acidic hydrogen atoms: (i) the hydrogen atom (H atom) of the carboxyl group, and (ii) that of the tetrazole ring. Accordingly, one acidic H atom (primarily the carboxyl H atom) or both acidic H atoms may be replaced by a monovalent or higher valent, e.g. divalent, cation. Mixed salts may also be formed.

EP 443983 does not disclose any specific salts or salt solvates, e.g. hydrates, of valsartan. Also, it does not mention any special properties of salts or salt solvates, e.g. hydrates. Meanwhile, the active ingredient valsartan has been introduced as an anti-hypertensive agent in a series of countries under the trade name DIOVAN.

The free acid valsartan has a melting point in a closed crucible of 80 to 95°C and in an open crucible of 105 to 110°C and a melting enthalpy of 12 kJ/mol. The specific optical rotation is $[\alpha]^{20}_{D} = (-70 \pm 2)^{0}$ measured for a concentration of c = 1% in methanol.

The density of the valsartan crystals and of the salt hydrates was determined by a helium pycnometer (Accupyc 1330 of Micromeritics, Norcross, GA, USA). The density for the crystals of the free acid valsartan is 1.20 ± 0.02.

The X-ray diffraction diagram consists essentially of a very broad, diffuse Xray reflection; the free acid is therefore characterised as almost amorphous under X-ray. The melting point linked with the measured melting enthalpy of 12 kJ/mol unequivocally confirms the existence of a considerable residual arrangement in the particles or structural domains for the free acid valsartan.

There is a need for more stable, e.g. crystalline forms of valsartan, which, for example, are even easier to manage in the drying or grinding processes following the final stage of the chemical preparation process, and also in the steps for preparing the pharmaceutical formulations and lead to an improvement of the process for the manufacture of the drug substance. Many futile attempts have been made to find improved forms through salt formation, the forms ideally being as crystalline as possible, as well as physically and chemically stable. Only the salts according to the present invention including both of the substances assigned here as starting materials, there solvates, e.g. hydrates and polymorphous forms thereof exhibit the desired improved properties.

The formation of salts and salt hydrates of valsartan with the desired advantageous properties has proved to be difficult. In the majority of cases, for example, amorphous salts with little stability are obtained (such as hard foams, waxes or oils). Extensive research has shown that the additional salts and salt hydrates of valsartan according to the invention have proved to be particularly advantageous compared with the free acid valsartan.

The objects of the present invention are salts and salt hydrates of valsartan which are selected from the group of earth alkalimetals consisting of the magnesium salt and the calcium salt, as well as salt mixtures, or respectively, an amorphous form, a solvate, especially hydrate, as well as a polymorphous form thereof, the respective production and use, and pharmaceutical preparations containing such salts.

Salt mixtures are (i) single salt forms from different cations selected from the above group or (ii) mixtures of those single salt forms which exist for example in the form of conglomerates or (III) mixtures of a single salt or a salt hydrate consisting of different physical phases such as several polymorphic forms, of different hydrates or also the anhydrate, of different amorphous forms or (IV) mixtures of any form listed under (I), (II), and (III) with each other.

Preferred salts are for example selected from the
calcium salt of valsartan in crystalline and amorphous forms, especially in hydrate form, primarily the tetrahydrates, the trihydrates, the monohydrate, the di-(calcium salt of valsartan) pentahydrate, the anhydrate, the amorphous forms thereof;
magnesium salt of valsartan in crystalline form, especially in hydrate form, primarily the hexahydrates, the trihydrates, the monohydrate, the anhydrate, the amorphous forms thereof.

The salts according to the invention preferably exist in isolated and essentially pure form, for example in a degree of chemical purity of >95%, preferably >98%, primarily >99%. The enantiomer purity of the salts according to the invention is >98%, preferably >99%.

Compared with the free acid, the salts according to the invention, or the amorphous forms, solvates such as salt hydrates, and also the corresponding polymorphous forms thereof, have unexpectedly advantageous properties. Under given conditions, the crystalline salts and crystalline salt hydrates have a clear melting point which is linked with a marked, endothermic melting enthalpy. The crystalline salts, salt hydrates, amorphous forms and mixtures thereof according to the invention have limited stability, i.e. as the solid, they have a restricted stability range. To be stabilised, they require certain measures which can be achieved for example by galenic formulations.

In addition, both the crystalline and the amorphous salts and salt hydrates according to the invention have a high degree of dissociation in water and thus substantially improved water solubility. These properties are of advantage, since on the one hand the dissolving process is quicker and on the other hand a smaller amount of water is required for such solutions. Furthermore, the higher water solubility can, under certain conditions, also lead to increased biological availability of the salts or salt hydrates in the case of solid dosage forms. Improved properties are beneficial especially to the patients.

The high crystallinity of certain salt hydrates allows the use of a choice of analytical methods, especially the various X-ray methods and/or the infrared spectrum preferably by means of ATR-IR (Attenuated Total Reflection-Infrared Spectroscopy), the usage of both methods permit a clear and simple analysis of their release to be made. This factor is also of great importance to the quality of the active substance and its galenic forms during production, storage and administration to the patients.

The invention accordingly relates to crystalline, also partly crystalline and amorphous salts or salt hydrates of valsartan.

As well as the solvates, such as hydrates, the invention also relates to polymorphous forms of the salts according to the invention.

Solvates and also hydrates of the salts according to the invention may be present, for example, as mono-, di-, tri-, tetra-, penta-, hexa-solvates or hydrates, respectively. Solvates and hydrates may also be consisting in stoichiometric ratios for example, with two, three, four salt molecules per solvate or per hydrate molecule. Another possibility for example, that two salt molecules are stoichiometric related to three, five, seven solvent or hydrate molecules. Solvents used for crystallisation, such as alcohols, especially methanol, ethanol, aldehydes, ketones, especially acetone, esters, e.g. ethyl acetate, may be embedded in the crystal grating. Preferred are pharmaceutically acceptable solvents. The extent to which a selected solvent or water leads to a solvate or hydrate in crystallisation and in the subsequent process steps or leads directly to the free acid is generally unpredictable and depends on the combinations of process conditions and the various interactions between valsartan and the selected solvent, especially water. The respective stability of the resulting crystalline or amorphous solids in the form of salts, salt solvates or salt hydrates, must be determined by experimentation. It is thus not possible to focus solely on the chemical composition and the stoichiometric ratio of the molecules in the resulting solid, since under these circumstances both differing crystalline solids and differing amorphous substances may be produced.

The description salt hydrates for corresponding hydrates may be preferred, as water molecules in the crystal structure are bound by strong intermolecular forces and thereby represent an essential element of structure formation of these crystals which, in part, are extraordinarily stable. However, water molecules are also existing in certain crystal lattices which are bound by rather weak intermolecular forces. Such water molecules are more or less integrated in the crystal structure forming, but to a lower energetic effect. The water content in amorphous solids can, in general, be clearly determined, as in crystalline hydrates, but is heavily dependent on the drying and ambient conditions. In contrast, in the case of stable hydrates, there are clear stoichiometric ratios between the pharmaceutical active substance and the water. In many cases these ratios do not fulfil completely the stoichiometric value, normally it is approached by lower values compared to theory because of imperfection or of certain crystal defects. The ratio of organic molecules to water molecules for the weaker bound water may vary to a considerable extend, for example, even extending from the anhydrous form over mono-, di-, tri- or tetra-hydrates. On the other hand, in amorphous solids, the molecular structure classification of water is not stoichiometric; the classification may however also be stoichiometric only by chance.

In some cases, it is not possible to classify the exact stoichiometry of the water molecules, since layer structures form, e.g. in the alkali metal salts, especially in the potassium salt, so that the embedded water molecules cannot be determined in defined form.

For the crystalline solids having identical chemical composition, the different resulting crystal gratings are summarised by the term polymorphism.

Any reference hereinbefore and hereinafter, to the salts according to the invention is to be understood as referring also to the corresponding solvates, such as hydrates, and polymorphous modifications, and also amorphous forms, as appropriate and expedient.

The particularly preferred salt hydrate is the tetrahydrate of the calcium salt of valsartan in the polymorphic form $\mathbf{A}_{1,Ca}$. In a closed specimen container, for a heating rate of $T_r = 10\ K{\cdot}min^{-1}$ it has a melting point of 190 ± 1.5 °C and a melting enthalpy of 79 ± 4 $kJ{\cdot}Mol^{-1}$. The tetrahydrate of the calcium salt of valsartan $A_{1,Ca}$ is not stable at the melting point both in respect of the hydrate water and therefore in respect of the chemical and physical structure of the molecule. The indicated melting point is a hydrate melting point which can only be measured in a closed specimen container. Gold containers with a wall thickness of 0.2 mm were used; after weighing in samples of between 2 and 4 mg salt hydrate, they were sealed by cold welding. These gold containers have an internal free volume of ca. 22 microlitres. The amounts of the sample and the volume of the pressurised containers must be suitably adapted, so that strong dehydration of the salt hydrates cannot take place during measurement of the melting point. The partial pressure of the water at 191° Celsius is ca. 13 bar, so that with an open container in DSC (Differential Scanning

Calorimeter) during measurement of the melting point, conversion to the anhydrate takes place. Both the high hydrate melting point and the amount of the melting enthalpy are an expression of the exceptional stability of the crystal lattice of the form $A_{1,Ca}$ of the tetrahydrate of the calcium salt of valsartan. These two thermodynamic characteristics illustrate the advantageous physical properties, compared to the free acid, with the two corresponding data, namely a melting point in the closed system of 90°C and a melting enthalpy of 12 kJ·Mol^{-1}. These thermodynamic data, together with the X-ray data, prove the high stability of this crystal lattice. They are the base for the special physical and chemical resistance of the tetrahydrate of the calcium salt of valsartan of the polymorphic form $A_{1,Ca}$.

Measurement of the infrared spectrum likewise took place by means of ATR-IR (Attenuated Total Reflection-Infrared Spectroscopy) using the instrument Spektrum BX from Perkin-Elmer Corp., Beaconsfield, Bucks, England.
The tetrahydrate of the calcium salt of valsartan $A_{1,Ca}$ has the following absorption bands expressed in reciprocal wave numbers (cm^{-1}):
3594 (w); 3307 (w); 3056 (w); 2960 (m); 2871 (w); 1621 (st); 1578 (st); 1459 (m); 1442 (m); 1417 (m); 1407 (m); 1364 (m); 1357 (m); 1319 (m); 1274 (m); 1242 (w); 1211 (m); 1180 (m); 1149 (w); 1137 (m); 1105 (m); 1099 (m); 1012 (m); 1003 (m); 974 (m); 965 (w); 955 (w); 941 (w); 863 (w); 856 (w); 844 (m); 823 (m); 791 (m); 784 (m); 758 (m); 738 (st); 698 (m).
The intensities of the absorption bands are indicated as follows: (w) = weak; (m) = medium and (st) = strong intensity.
The characteristic absorption bands of the ATR-IR spectroscopy for the polymorphic form $A_{1,Ca}$ of the tetrahydrate of the calcium salt of valsartan are shown by the following values expressed in reciprocal wave numbers (cm^{-1}): 3307 (w); 2960 (m); 1621 (st); 1578 (st); 1459 (m); 1442 (m); 1417 (m); 1407 (m); 1364 (m); 1357 (m); 1319 (m); 1274 (m); 1211 (m); 1180 (m); 1137 (m); 1012 (m); 1003 (m); 974 (m); 758 (m); 738 (st); 698 (m).
The error margin for all absorption bands of ATR-IR is $\pm$ 3 cm^{-1}.

The water content is in theory 13.2% for the tetrahydrate of the calcium salt of valsartan. Using the thermobalance TGS-2 (Perkin-Elmer Corp. , Norwalk, CT USA) the water content was determined for the polymorphic form $A_{1,Ca}$ between 25°C and 225°C as 12.3 %. A total formula was calculated from this $(C_{24}H_{27}N_5O_3)^{2-}$ Ca^{2+}• (3.7 $\pm$ 0.2) H_2O.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the tetrahydrate of the calcium salt of valsartan $A_{1,Ca}$ as a function of temperature, was measured at a heating rate of 10 $K \cdot min^{-1}$. The results are illustrated in table 1.

Table 1

temperature [°C]	weight loss or water loss in %
25	1.1 ± 0.5
50	3.3 ± 0.5
75	5.1 ± 0.5
100	9.6 ± 1.0
125	12.2 ± 0.5
150	12.9 ± 0.5
175	13.2 ± 0.5
200	13.3 ± 0.5
225	13.4 ± 0.5
250	13.3 ± 0.5
275	13.7 ± 0.5

An essential feature for the quality of a pure active substance both for the physical-chemical procedures such as drying, sieving, grinding, and in the galenic processes which are carried out with pharmaceutical excipients, namely in mixing processes, in granulation, in spray-drying, in tabletting, is the water absorption or water loss of this active substance depending on temperature and the relative humidity of the environment in question. With certain formulations, free and bound water is without doubt introduced with excipients and/or water is added to the process mass for reasons associated with the respective formulation process. In this way, the pharmaceutical active substance is exposed during the production and galenic processes over time periods of up to several hours or even days to free water of different activity (partial vapour pressure) which is mainly depending on temperature. However, it is easily possible to reach a well-defined hydrate form in the production of the active substance as well as in the formulation of a salt of valsartan after a certain equilibration time under rather constant conditions in respect to temperature and to the relative humidity.

Further characterisation of the tetrahydrate of the calcium salt of valsartan is effected using the interlattice plane intervals determined by a X-ray powder pattern. Measurement of the X-ray powder patterns was made with a Guinier camera (FR 552 from Enraf Nonius, Delft, NL) on a X-ray film in transmission geometry, using Cu-Kα_1 radiation at room temperature. Evaluation of the films for calculation of the interlattice plane intervals is made both visually and by a Line-Scanner (Johansson Täby, S), and the reflection intensities are determined simultaneously.

The preferred characterisation of the tetrahydrate of the calcium salt of valsartan $A_{1,Ca}$ is obtained from the interlattice plane intervals d of the ascertained X-ray diffraction diagrams, whereby, in the following, average values are indicated with the appropriate error limits.

The intensities are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.

d in [Å]: 16.2±0.3 (vst), 11.4±0.2 (vw), 9.9±0.2(w), 9.4±0.2(vw), 8.06±0.1(vw), 7.73±0.1(vw), 7.05±0.1(vw), 6.50±0.05(vw), 6.36±0.05(vw), 5.82±0.05(w), 4.94±0.05(vw), 4.73±0.05(vw), 4.33±0.05(vw), 4.17±0.05(vw), 4.13±0.05(vw), 3.93±0.05(vw).

The characteristic reflections in the X-ray diffraction diagram show the following interlattice plane intervals:

d in [Å]: 16.2±0.3, 11.4±0.2, 9.9±0.2, 9.4±0.2, 8.06±0.1, 7.05±0.1, 6.50±0.05, 5.82±0.05, 4.94±0.05, 4.73±0.05, 4.33±0.05, 4.17±0.05, 4.13±0.05, 3.93±0.05.

Another polymorphic form of the tetrahydrate of the calcium salt of valsartan is the solid state form $A_{2,Ca}$. The melting point of form $A_{2,Ca}$ is 195 ± 1.5 °C and the melting enthalpy is 98 ± 8 $kJ \cdot Mol^{-1}$. The indicated melting point is a hydrate melting point which can only be mesured in a closed specimen container. Gold containers are used and sample weights of between 2 and 4 mg salt hydrate. The heating rate applied is $T_r = 10\ K \cdot min^{-1}$. For details see the explanations given for the form $A_{1,Ca}$. The tetrahydrate of the calcium valsartan salt $A_{2,Ca}$ reveals the following loss of water as a function of temperature using the thermogravimetric instrument TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA) with a heating rate of 10 $K \cdot min^{-1}$, in a water-free N_2 atmosphere, the weight loss is illustrated in Table 2.

Table 2

temperature [°C]	weight loss or water loss in %

25	0 ± 0.3
50	0.1 ± 0.3
75	0.5 ± 0.5
100	4.9 ± 0.5
125	11.2 ± 0.5
150	12.2 ± 0.5
175	12.6 ± 0.5
200	12.7 ± 0.5
225	12.8 ± 0.5
250	12.8 ± 0.5
275	13.0 ± 0.5

The theoretical water content is for a tetrahydrate of the calcium salt of valsartan 13.2%. The tetrahydrate of the form $A_{2,Ca}$ has a bound water content at 225°C determined as a weight loss of 12.8% and the total formula is calculated from this $(C_{24}H_{27}N_5O_3)^{2-}\ Ca^{2+} \cdot (3.9 \pm 0.2)\ H_2O$.

A solid state characterization of the calcium salt of valsartan in form of the tetrahydrate $A_{2,Ca}$ is achieved by a X-ray powder pattern and by the evaluation of the reflections into the interlattice plane intervals. The measurements are throughout made without specific explanations with a Guinier camera (FR 552 from Euraf Nonius, Delft, NL) on an X-ray film in transmission geometry, using Cu-Kα_1 radiation at room temperature. Evaluation of the films for calculation of the interlattice plane intervals is made both visually and by a line scanner (Johansson, Täby, S), and the reflection intensities are determined simultanously. The preferred characterization of the tetrahydrate of the calcium salt of valsartan $A_{2,Ca}$ is obtained from the interlattice plane intervals d of the ascertained X-ray diffraction diagrams, whereby, in the following, values are indicated with the appropriate error limits. The intensities are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.
d in [Å]: 16.2±0.3(vst), 9.9±0.2(w), 9.4±0.2(vw), 8.05±0.1(vw), 7.72±0.1(vw), 7.04±0.1(vw) 6.49±0.05(w), 6.35±0.05(vw), 5.82±0.05(w), 4.94±0.05(vw), 4.73±0.05(vw), 4.34±0.05(vw), 4.13±0.05(m), 3.93±0.05(w), 3.30±0.05(vw).
The characteristic reflections in the X-ray diffraction diagram show the following interlattice plane intervals:

d in [Å]: 16.2±0.3, 9.9±0.2, 9.4±0.2, 8.05±0.1, 7.04±0.1, 6.49±0.05, 5.82±0.05, 4.94±0.05, 4.13±0.05, 3.93±0.05.

A new substance has been found as polymorphic form of a trihydrate of the calcium salt of valsartan assigned with $\mathbf{B_{1,Ca}}$. The melting point of the substance $B_{1,Ca}$ is measured in a closed sample cell with a heating rate of 10 $K \cdot min^{-1}$ as T_{fus} = 175±3°C and the melting enthalpy of the partially crystalline sample is 12 ± 4 $kJ \cdot Mol^{-1}$.
The water content is in theory 10.24% for the trihydrate of the calcium salt of valsartan. Using the thermogravimetric instrument TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA) the water content was determined for the polymorphic form $B_{1,Ca}$ as 9.9±0.4%. A total formula was calculated from this polymorphic form of the trihydrate of the calcium salt of valsartan $(C_{24}H_{27}N_2O_3)^{2-} Ca^{2+} \cdot (2.9 \pm 0.3)\ H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the trihydrate of the calcium salt of valsartan in the polymorphic form $B_{1,Ca}$ as a function of temperature, was measured at a heating rate of 10 $K \cdot min^{-1}$. The results are illustrated in table 3.

Table 3

temperature [°C]	weight loss or water loss in %
25	0.4 ± 0.3
50	2.0 ± 0.5
75	4.0 ± 0.5
100	6.3 ± 0.5
125	8.5 ± 0.5
150	9.5 ± 0.5
175	9.7 ± 0.5
200	9.9 ± 0.5
225	9.9 ± 0.5
250	10.0 ± 0.5
275	10.3 ± 0.5

A solid state characterization of the trihydrate of the calcium salt of valsartan $B_{1,Ca}$ is preferably performed by X-ray powder patterns with the evaluation of the interlattice plane intervals. The measurements have been performed with two samples of the trihydrate $B_{1,Ca}$

of the calcium salt of valsartan and with two different instruments. The first instrument used was a temperature-humidity powder diffraction chamber X'Pert from Philips Analytical X-ray, 7602 Almelo, NL, equipped with a low and medium temperature attachement from Anton Paar GmbH, A-8054 Graz, Austria. The second instrument is a powder diffractometer PW 1710 also from Philips Analytical X-ray, 7602 Almelo, NL. Two parallel measurements with a reference sample, namely a tetrahydrate of the calcium salt of valsartan have been used to calibrate the powder diffractometer PW 1710 with a Guinier camera (FR 552 from Enraf Nonius, Delft, NL) on a X-ray film in transmission geometry, using Cu-Kα_1 radiation. The corrections for the interlattice plane intervals to reach the values of the Guinier camera from the powder diffractometer PW1710 were ranging from +0.55 Å for a d-value of 16Å to +0.02Å for a d-value of 5.7 Å . No correction was necessary for lower d-values.
The characterization of the trihydrate of the calcium salt of valsartan $B_{1,Ca}$ with the interlattice plane intervals d is as such, whereby, in the following values are indicated with the appropriate error limits. The intensities of the d-values are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.
d in [Å]: 16.0±0.3(vst), 11.4±0.2(m), 10.0±0.2(vw), 9.4±0.2(vw), 9.1±0.2(vw), 8.06±0.1(vw), 7.75±0.1(vw), 7.03±0.1(vw), 6.48±0.05(vw), 6.10±0.05(vw), 5.76±0.05(vw), 5.16±0.05(vw), 4.95±0.05(vw), 4.75±0.05(vw), 4.68±0.05(vw), 4.33±0.05(vw).
The characteristic reflections in the X-ray diffraction diagram reveal the following interlattice plane intervals for the form $B_{1,Ca}$:
d in [Å]: 16.0±0.3, 11.4±0.2, 10.0±0.2, 9.4±0.2, 8.06±0.1, 7.75±0.1, 7.03±0.1, 6.48±0.05, 6.10±0.05, 5.16±0.05, 4.75±0.05.

The new polymorphic form $\mathbf{B_{2,Ca}}$ of a trihydrate of the calcium salt of valsartan has a melting point of 197±1.5°C measured in a closed sample cell with a Pyris 1 DSC (Differential Scanning Calorimeter) from Perkin-Elmer Corp., Norwalk, CT USA. The enthalpy of fusion has been determined also from a DSC curve measured also with a heating rate of 10 $K \cdot min^{-1}$ as 62 ± 6 $kJ \cdot Mol^{-1}$. During the DSC measurements of the melting of the trihydrate $B_{2,Ca}$ of the calcium salt of valsartan also a glass transition was observed, as an unequivocal proof of amorphous substance present in this substance. The glass transition temperature was calculated with T_g = 68 ± 20°C as the mid point of a change of the specific heat of the substance, namely the trihydrate $B_{2,Ca}$ of the calcium salt of valsartan. The value for the change of the specific heat was calculated as Δc_p = 0.2 ± 0.1 $J \cdot (g \cdot K)^{-1}$. The amorphicity present in the substance $B_{2,Ca}$ approximated by this value for the change of the specific heat is 18 ± 12%. The crystalline trihydrate $B_{2,Ca}$ of the calcium salt of valsartan is according to the heat of fusion measured with the DSC Pyris 1, the main component is this crystalline product, the amorphous part of the calcium salt of valsartan is a minor part.

The water content of the trihydrate $B_{2,Ca}$ of the calcium salt of valsartan is 10.5±0.5%. The value was measured with a thermogravimteric instrument TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA). The total formula was calculated from this bound water content for the polymorph of the trihydrate $B_{2,Ca}$ as $(C_{24}H_{27}N_5O_3)^{2-}Ca^{2+} \cdot (3.1 \pm 0.3)H_2O$.

Water may also be present in the amorphous part of the substance $B_{2,Ca}$, which is depending on the concentration of the non-crystalline part. This water is within the amorphous part differently bound compared to the water molecules in the hydrate form of the crystalline part. As a first approximation one can state, that the crystalline and the amorphous part are similar in the water concentration in case the last process of reaching the state of the material is not passing the anhydrous form of the calcium salt of valsartan. The explanation for this fact is given with the molecular structure of the calcium salt of valsartan, the same holds for the magnesium salt of valsartan, namely that the salt structure is to a considerable part based on the short range order of the molecular interacting substances valsartan, calcium or magnesium and water which is not free water, however structural bound water. This narrow range molecular structure is rather similar for the crystalline part as for the amorphous part. Of course, in the amorphous material, there is a complete lack of long range order in contrary to the crystalline material were any molecule, in the present case, any molecle in trihydrate $B_{2,Ca}$ calcium salt of valsartan is over neighboring molecules structural interrelated with all the molecules within any single crystal.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the trihydrate $B_{2,Ca}$ as a function of temperature, was measured at a heating rate of 10 $K \cdot min^{-1}$. The results for the polymorph $B_{2,Ca}$ of the trihydrate of the calcium salt of valsartan are illustrated in table 4.

Table 4

Temperature [^{0}C]	Weight loss or water loss in %
25	0.1 ± 0.2

50	0.9 ± 0.3
75	2.2 ± 0.5
100	5.8 ± 0.5
125	8.9 ± 0.5
150	9.9 ± 0.5
175	10.2 ± 0.5
200	10.3 ± 0.5
225	10.5 ± 0.5
250	10.5 ± 0.5
275	10.8 ± 0.5

The solid state characterization of the trihydrate of the calcium salt of valsartan $B_{2,Ca}$ was performed by X-ray powder spectroscopy using two different instruments and two different charges produced with the evaluation of the interlattice plane intervals. The first instrument was a powder diffractometer PW 1710 from Philips Analytical X-ray, 7602 Almelo, NL. The second instrument was a Guinier camera FR 552 from Enraf Nonius, Delft, NL on a X-ray film in transmission geometry, using Cu-Kα_1 radiation. The first instrument has been calibrated with the Guinier camera, the corrections ranging from +0.55Å for a d-value of 16 Å to +0.02 Å for a d-value of 5.7 Å. No corrections were necessary for lower d-values. The characterization of the trihydrate of the calcium salt of valsartan $B_{2,Ca}$ with the interlattice plane intervals is as such, whereby, in the following values are indicated with the appropriate error limits. The intensities of the d values are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.
d in [Å]: 16.2±0.3(vst), 11.5±0.2(w), 9.9±0.2(w), 9.4±0.2(w), 9.0±0.1(vw), 8.13±0.1(vw), 7.78±0.1(vw), 7.04±0.1(vw), 6.50±0.1(vw), 6.09±0.05(vw), 5.79±0.05(vw), 5.18±0.05(vw), 4.95±0.05(vw), 4.74±0.05(vw), 4.16±0.05(w).
The characteristic reflections in the X-ray diffraction diagram show the following interlattice plane intervals:
d in [Å]: 16.2±0.3, 11.5±0.2, 9.9±0.2, 9.4±0.2, 7.04±0.1, 6.50±0.1, 5.79±0.05, 4.74±0.05, 4.16±0.05.

Another polymorph of the trihydrate of the calcium salt of valsartan namely the $B_{3,Ca}$ has a melting point measured with a heating rate of 10K·min^{-1} in a hermetically sealed sample cell of 192±1.5°C. The enthalpy of fusion has been determined also by a DSC measurement with 17±4 kJ·Mol^{-1}.

The glass transition phenomena observed with the DSC at 65°C is revealing a change of the specific heat capacity of sc_p = 0.3 3g^{-1}· K^{-1}. Compared with the change of the specific heat capacity of a 100% amorphous calcium salt of valsartan as a trihydrate the amorphous content of the B_3,ca can be estimated with 50%. Therefore the enthalpy of fusion for the crystalline B_3,ca is 34±10 kJ·Mol^{-1}.

The water content of the polymorphic form $B_{3,Ca}$ for the trihydrate of the calcium salt of valsartan was determined with a thermobalance from Perkin-Elmer Corp., Norwalk, CT USA, named TGS-2 with a value of 9.8±0.5%. The total formula was calculated from this bound water content for the polymorphic from $B_{3,Ca}$ with $(C_{24}H_{27}N_5O_3)^{2-}Ca^{2+} \cdot (2.9\pm0.3)H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the trihydrate $B_{3,Ca}$ as a function of temperature, was measured at a heating rate of 10 K·min^{-1}. The results for the polymorphic form $B_{3,Ca}$ of the trihydrate of the calcium salt of valsartan are illustrated in table 5.

Table 5.

temperature [°C]	weight loss or water loss in %
25	0.3 ± 0.2
50	1.4 ± 0.3
75	2.8 ± 0.5
100	5.7 ± 0.5
125	8.4 ± 0.5
150	9.4 ± 0.5
175	9.6 ± 0.5
200	9.7 ± 0.5
225	9.8 ± 0.5
250	10.0 ± 0.5
275	10.2 ± 0.5

The Guinier camera FR552 with a X-ray film in transmission geometry, using a Cu-Kα_1 radiation from Enraf Nonius, Delft, NL has been installed to characterize at room temperature the crystal lattice by the interlattice plane intervals of the calcium salt of valsartan in form of the trihydrate $B_{3,Ca}$.

The reflections in the X-ray diffraction diagram for the trihydrate of the calcium salt of valsartan $B_{3,Ca}$ reveal the following interlattice plane intervals d, whereby, values are indicated with the appropriate error limits. The intensities of the d-values are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.

d in [Å]: 16.1±0.3(vst), 11.4±0.2(m), 9.9±0.2(w), 9.4±0.2(w), 9.0±0.1(vw), 8.04±0.1(vw), 7.73±0.1(vw), 7.03±0.1(vw), 6.47±0.05(vw), 6.33±0.1(vw), 6.09±0.05(vw), 5.79±0.05(w), 5.17±0.05(vw), 4.95±0.05(vw), 4.73±0.05(vw), 4.48±0.05(vw), 4.33±0.05(vw), 4.15±0.05(vw), 4.11±0.05(vw), 3.94±0.05(vw), 3.61±0.05(vw).

The characteristic reflections in the X-ray diffraction diagram show the following interlattice plane intervals:

d in [Å]: 16.1±0.3, 11.4±0.2, 9.9±0.2, 9.4±0.2, 9.0±0.1, 7.03±0.1, 6.47±0.05, 5.79±0.05, 4.15±0.05, 3.94±0.05.

Measurements of the infrared spectrum were performed by means of ATR-IR (Attenuated Total Reflection-Infrared Spectroscopy) using the instrument Spektrum BX from Perkin-Elmer Corp., Beaconsfield, Bucks, England.

The trihydrate of the calcium salt of valsartan $B_{3,Ca}$ has the following ATR-IR adsorption bands expressed in reciprocal wave numbers (cm^{-1}):

3594(w); 3309(w); 3053(w); 2959(w); 2930(w); 2870(w); 1621(m); 1577(m); 1505(w); 1458(m); 1416(m); 1405(m); 1354(w); 1301(w); 1273(w); 1210(w); 1179(w); 1138(w); 1104(w); 1099(w); 1012(w); 1003(w); 974(w); 941(w); 906(w); 856(w); 841(w); 756(m); 737(m); 667(m).

The intensities of the absorption bands are indicated as follows: (w)=weak, (m)=medium, and (st)=strong intensity.

The characteristic absorption bands of the ATR-IR spectroscopy for the polymorphic form $B_{3,Ca}$ of the trihydrate of the calcium salt of valsartan are shown by the following values expressed in reciprocal wave numbers (cm^{-1}):

3594(w); 2959(w); 1621(st); 1577(m); 1458(m); 1405(m); 1354(w); 1273(w); 1012(w); 756(m); 737(m); 667(m). The error margin for all absorption bands of ATR-IR is ± $3cm^{-1}$.

Additionally, a new substance has been found as the monohydrate of the calcium salt of valsartan $\mathbf{C_{1,Ca}}$.
The bound water content is 3.1±0.3% measured with a thermobalance TGS-2 (Perkin-Emer Corp., Norwalk, CT, USA). The total formula was calculated from the bound water content for the monohydarte $C_{1,Ca}$ as $(C_{24}H_{27}N_5O_3)^{2-}Ca^{2+} \cdot (0.8\pm0.2)H_2O$.

The solid state characterization of the monohydrate of the calcium salt of valsartan $C_{1,Ca}$ was executed by X-ray powder patterns with the evaluation of the interlattice plane intervals. The instrument used was a temperature-humidity powder diffraction chamber X'Pert from Philips Analytical X-ray, 7602 Almelo, NL, equipped with a low and medium temperature attachement from Anton Paar GmbH, A-8054 Graz, Austria.

The characterization of the monohydrate of the calcium salt of valsartan $C_{1,Ca}$ with the interlattice plane intervals d is as such, whereby, in the following values are indicated with the appropriated error limits. The intensities of the d-values are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.
d in [Å]: 16.0±0.3(m), 15.0±0.3(vst), 11.6±0.2(w), 9.9±0.2(vw), 9.4±0.2(vw), 8.02±0.1(vw), 7.53±0.1(vw), 7.02±0.1(vw), 6.47±0.05(vw), 6.11±0.0.5(vw), 4.50±0.05(vw), 4.34±0.05(vw).
The characteristic reflections in the X-ray diffraction diagram show the following interlattice plane intervals:
d in [Å]: 16.0±0.3, 15.0±0.3, 11.6±0.2, 9.4±0.2, 7.53±0.1, 6.11±0.05.

Surprisingly, another new substance has been found, assigned with $\mathbf{D_{1,Ca}}$ beeing the di-(calcium salt of valsartan) pentahydrate. The melting point of this new substance $D_{1,Ca}$ is T_{fus} = 210±2°C measured in a closed sample cell with a heating rate of $10K \cdot min^{-1}$ and with a DSC called Pyris 1 from Perkin-Elmer Corp., Norwalk, CT, USA. With the same instrument and the same procedures as above explained, the heat of fusion was determined. The heat of fusion is for the di-(calcium salt of valsartan) pentahydrate for a 100% crystalline di-(calcium salt of valsartan) pentahydrate is approximated with $94kJ \cdot Mol^{-1}$.

The water content of the di-(calcium salt of valsartan) as pentahydrate was measured with a thermobalance TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA) and gave the value at the plateau of 225°C of 8.1±0.5%. The total formula was elucidated from this bound water content for the substance $D_{1,Ca}$ as $[(C_{24}H_{27}N_5O_3)^{2-}Ca^{2+}]_2 \cdot (4.7\pm0.3)H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the di-(calcium salt of valsartan) pentahydrate $D_{1,Ca}$ as a function of temperature, was measured at a heating rate of 10 $K\cdot min^{-1}$. The results for the di-(calcium salt of valsartan) pentahydrate are illustrated in table 6.

Table 6

Temperature [°C]	Weight loss or water loss in %
25	0.1 ± 0.1
50	1.3 ± 0.3
75	2.8 ± 0.5
100	5.1 ± 0.5
125	7.4 ± 0.5
150	8.0 ± 0.5
175	8.1 ± 0.5
200	8.2 ± 0.5
225	8.2 ± 0.5
250	8.3 ± 0.5
275	8.6 ± 0.5

The solid state characterization of the di-(calcium salt of valsartan) pentahydrate $D_{1,Ca}$ was achieved with a Guinier camera (FR 522 from Enraf Nonius, Delft, NL) on an X-ray film in transmission geometry, using Cu-$K\alpha_1$ radiation at room temperature. Evaluations of the films for calculation of the interlattice plane intervals are made by a line-scanner (Johansson, Täby, S), and the reflection intensities are determined simultaneously. The reflections in the X-ray diffraction diagram could be evaluated to the following interlattice plane intervals d, whereby values are indicated with appropriate error limits. The intensities of the d-values are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.

d in [Å]: 15.5±0.3(vst), 11.5±0.2(st), 9.4±0.2(vw), 9.04±0.1(w), 7.75±0.1(vw), 6.46±0.05(vw), 6.09±0.05(w), 5.82±0.05(vw), 5.66±0.05(vw), 5.16±0.05(vw), 4.76±0.05(vw), 4.48±0.05(vw), 3.83±0.05(vw), 3.60±0.05(vw), 3.36±0.05(vw).

The characteristic reflections in the X-ray diffraction diagram show the following interlattice plane intervals:

d in [Å]: 15.5±0.3, 11.5±0.2, 9.4±0.2, 9.04±0.1, 6.46±0.05, 6.09±0.05, 5.82±0.05, 5.16±0.05, 4.48±0.05, 3.60±0.05.

Another new-type of crystalline, partially amorphous solids are falling into the groups of the magnesium salt hydrate and anhydrate of valsartan. In particular, the hexahydrate of the magnesium salt of valsartan in form of the polymorphic substance $\mathbf{A_{1,Mg}}$ is a preferred substance.

The specific optical rotation of hexahydrates of the magnesium salt of valsartan in water measured with a 1% solution at 20°C is independent of the polymorphic form present as long as it is a hexahydrate $[\alpha]^{D}_{20} = -38°$.

The thermal behaviour of this salt hydrate in the region of the melting point only reveals a certain chemical and physical instability. The thermal data are thus dependent on the measurement conditions. The instrument used for the calorimetric data is throughout a DSC Pyris 1 (Differential Scanning Calorimeter) obtained from Perkin-Elmer Corp., Norwalk, CT USA . The measurements are performed with samples enclosed in a sealed gold specimen container with an internal free volume of ca. 22 microliters, with a sample weight of 2 to 4 mg and with a heating rate of $T_r = 10 K{\cdot}min^{-1}$. The melting point of hexahydrate of the magnesium salt of valsartan in the polymorphic form $A_{1,Mg}$ is 130±3°C and the enthalpy of fusion is 45±5 $kJ{\cdot}Mol^{-1}$. The hexahydrate of the magnesium salt of valsartan as the polymorphic form $A_{1,Mg}$ reveals the following loss of water as a function of temperature in using the method of thermogravimetry. The instrument used was a TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA) and the measurement was performed in a water free atmosphere. The heating rate selected was 10 $K{\cdot}min^{-1}$. The weight loss is illustrated in table 7.

Table 7

Temperature [°C]	Weight loss or water loss in %
25	0.1 ± 0.1
50	1.0 ± 0.3
75	6.8 ± 0.5
100	14.1 ± 0.5

125	15.6 ± 0.5
150	16.4 ± 0.5
175	16.9 ± 0.5
200	17.1 ± 0.5
225	17.3 ± 0.5
250	17.6 ± 0.5
275	18.3 ± 0.5

The theoretical water content is for the hexahydrate of the magnesium salt of valsartan 19.1%. The hexahydrate of the magnesium salt of valsartan in form of the polymorph $A_{1,Mg}$ has a bound water content at 225°C determined as a weight loss of 17.3±0.5%.
The total formula is calculated from this as $(C_{24}H_{27}N_5O_3)^{2-}Mg^{2+} \cdot (5.4\pm0.2)H_2O$.

The solid-state characterization of the magnesium salt of valsartan for the polymorphic form of the hexahydrate $A_{1,Mg}$ is achieved by a X-ray powder pattern and by the evaluation of the reflections into the interlattice plane intervals. The measurements have been made with three different X-ray instruments. The first instrument used is a Guinier camera (FR 522 from Enraf Nonius, Delft, NL) on an X-ray film in transmission geometry, with a Cu-Ka_1 radiation at room temperature. Evaluations of the films for calculation of the interlattice plane intervals are performed with a scanner from Johansson, Täby, S and the reflections intensities are determined simultaneously. The second instrument used for X-ray measurements of the new substance $A_{1,Mg}$ is a temperatur-humidity powder diffraction chamber X'Pert from Philips Analytical X-ray, 7602 Almelo, NL equipped with a low and medium temperature attachement from Anton Paar GmbH, A-8054 Graz. The third instrument applied in the solid state characterization is the powder diffractometer PW1710 from Philips Analytical X-ray. 7602 Almelo, NL. The characterization of the polymorph $A_{1,Mg}$ of the hexahydrate of the magnesium salt of valsartan is achieved from the interlattice plane intervals d of the ascertained X-ray measurements. In the following d values are listed with the appropriate error limits. The intensities are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.
d in [Å]: 19.6±0.3(vst), 16.6±0.3(vw), 10.3±0.2(vw), 9.8±0.2(m), 7.3±0.1(w), 6.9±0.1(vw), 6.01±0.05(w), 5.92±0.05(w), 5.55±0.05(vw), 5.38±0.05(vw), 5.23±0.05(vw), 5.15±0.05(vw), 5.05±0.05(vw), 4.90±0.05(m), 4.54±0.05(vw), 4.22±0.05(vw), 4.13±0.05(vw), 4.07±0.05(w),

3.96±0.05(vw), 3.73±0.05(vw), 3.64±0.05(vw), 3.43±0.05(w), 3.29±0.05(vw), 3.22±0.05(vw), 3.11±0.05(vw).

The characteristic reflections in the X-ray diffraction diagram reveal the following plane intervals:

d in [Å]: 19.6±0.3, 16.6±0.3, 10.3±0.2, 9.8±0.2, 7.3±0.1, 6.01±0.05, 5.92±0.05, 5.55±0.05, 5.38±0.05, 4.90±0.05, 4.13±0.05, 4.07±0.05, 3.43±0.05.

The substance in form of the tetrahydrate $\mathbf{B_{1,Mg}}$ is a partially amorphous solid of the magnesium salt of valsartan. The tetrahydrate $B_{1,Mg}$ shows the following loss of water as a function of temperature measured with a thermobalance TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA). The heating rate selected was $10K\cdot min^{-1}$. The weight loss is tabulated in table 8.

Table 8

Temperature [°C]	Weight loss or water loss in %
25	0 ± 0.1
50	2.1 ± 0.3
75	6.5 ± 0.5
100	9.5 ± 0.5
125	11.1 ± 0.5
150	12.0 ± 0.5
175	12.5 ± 0.5
200	12.8 ± 0.5
225	13.0 ± 0.5
250	13.6 ± 0.5
275	14.3 ± 0.5

The magnesium salt of valsartan in the polymorphic form of the tetrahydrate $B_{1,Mg}$ is showing a bound water content at 225°C of 13.0±0.5%, and as shown for 25°C in Table 8 practically no additional free water is present in the substance $B_{1,Mg}$.The measurements were performed with a thermobalance TGS-2 of the Perkin-Elmer Corp., CT USA. The total formula is therefore calculated as $(C_{24}H_{27}N_5O_3)^{2-}Mg^{2+} \cdot (3.8\pm0.2)H_2O$.

The solid-state characterization of the tetrahydrate of the magnesium salt of valsartan $B_{1,Mg}$ has been performed with an X-ray instrument by a so-called temperature-humidity powder

diffraction chamber X'Pert from Philips Analytical X-ray, 7602 Almelo, NL, equipped with a low and medium temperature attachement from Anton Paar GmbH, A-8054 Graz. Additional X-ray measurements were performed with a powder diffractometer PW 1710 from Philips Analytical X-ray, 7602 Almelo, NL. The crystalline parts of the substance $B_{1,Mg}$ are characterized in the solid state with the interlattice plane intervals d, which are given with appropriate error limits. The intensities are reported in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.
d in [Å]: 15.8±0.3(vst), 11.0±0.2(w), 8.0±0.2(vw).

The new substance $\mathbf{C_{1,Mg}}$ is a the trihydrate of the magnesium salt of valsartan. The water content was measured with a thermobalance TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA). The water content for this substance, namely the trihydrate of the magnesium salt of valsartan $C_{1,Mg}$ is 10.7±0.5%. The total formula is calculated from this $(C_{24}H_{27}N_5O_3)^{2-}Mg^{2+} \cdot (3.0\pm0.3)H_2O$.

The solid-state characterization of the trihydrate of the magnesium salt of valsartan $C_{1,Mg}$ has been performed with X-ray measurements by use of the temperature-humidity powder diffraction chamber X'Pert from Philips Analytical X-ray, 7602 Almelo, NL equipped with a low and medium temperature attachement from Anton Paar GmbH, A-8054 Graz.
The characterization of the substance $C_{1,Mg}$ of the magnesium salt of the valsartan trihydrate is given with the interlattice plane intervals d obtained with X-ray measurements. In the following, d values are listed with the appropriate error limits. The intensities are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.
d in [Å]: 17.9±0.3(m), 10.2±0.2(w), 8.96±0.2(m), 7.18±0.1(w), 6.97±0.1(vw), 6.81±0.1(vw), 6.24±0.05(vw), 5.93±0.05(w), 5.84±0.05(w), 5.72±0.05(vw), 5.59±0.05(vw), 5.42±0.05(m), 5.25±0.05(vw), 5.11±0.05(m), 5.01±0.05(st), 4.82±0.05(w), 4.67±0.05(w), 4.57±0.05(vw), 4.49±0.05(vw), 4.30±0.05(m), 4.19±0.05(vst), 4.13±0.05(vst), 4.02±0.05(vst), 3.88±0.05(vw).
The characteristic reflections in the X-ray diffraction diagram reveal the following plane intervals:
d in [Å]: 17.9±0.3, 10.2±0.2, 8.96±0.2, 7.18±0.1, 5.93±0.05, 5.84±0.05, 5.42±0.05, 5.11±0.05, 5.01±0.05, 4.82±0.05, 4.67±0.05, 4.30±0.05, 4.19±0.05, 4.13±0.05, 4.02±0.05.

The magnesium salt of valsartan is also forming a substance as a monohydrate which is indicated with $D_{1,Mg}$. The water content was measured with a thermobalance TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA). The water content for the monohydrate $D_{1,Mg}$ is 2.8±0.3%. The total formula was calculated from this value with $(C_{24}H_{27}N_5O_3)^{2-}Mg^{2+} \cdot (0.74\pm0.2)H_2O$.

The solid-state characterization of the monohydrate of the magnesium salt of valsartan $D_{1,Mg}$ was achieved with X-ray measurements by use of the temperature-humidity powder diffraction chamber X'Pert from Philips Analytical X-ray, 7602 Almelo, NL. This X-ray instrument is equipped with a low and medium temperature attachement from Anton Paar GmbH, A-8054 Graz.

The characterization of the new substance, namely the monohydrate of the magnesium salt of valsartan $D_{1,Mg}$ is demonstrated with the interlattice plane intervals d of the X-ray investigations. In the following d values are listed with the appropriate error limits. The intensities are given in brackets with the following abbreviations: very strong ≡ vst; strong ≡ st; medium ≡ m; weak ≡ w; and very weak ≡ vw.

d in [Å]: 15.1±0.2(st), 10.9±0.2(w), 10.3±0.2(vw), 7.66±0.1(vw), 7.21±0.1(vw), 5.12±0.05(vw), 4.75±0.05(vw).

The characteristic reflections in the X-ray diffraction diagram for the monohydrate of the magnesium salt of valsartan reveal the following plane intervals:

d in [Å]: 15.1±0.2, 10.9±0.2, 10.3±0.2, 7.66±0.1, 5.12±0.05.

Surprisingly, the crystalline salts of valsartan can be transformed into amorphous or partially amorphous substances. Crystalline and amorphous froms of corresponding chemical entities reveal different physico-chemical properties, related to the different structures of the crystalline and the amorphous form on a molecular level. The main difference is in the threedimensional organization of the solid particals. The crystalline particals or crystals reveal a short distance arrangement of a given number of molecules in well defined crystal lattice positions around each single molecule. All these first neighboring molecules of the set of molecules within an elementry cell of a crystal are within the whole crystal lattice in the same geometrical arrangement. The short distance arrangement of any single molecule is in crystal combined with the low range arrangement. In contrary, an amorphous substances reveals only a short range order for each molecule, however, the long range order is not existing within the amorphous solid particles. A consequence of this structural facts is the completely different behaviour of crystals or of an amorphous substance in heating up, starting at a low temperature within the the solid phases. Any crystalline substance is characterized with a melting point, which might be different for different polymorphs of the same chemical entity, however, together with the enthalpy of fusion the interrelation to the crystalline phase present is proofed. In contrary, an amorphous substance is never revealing a melting point and a enthalpy of fusion. But, in heating up, starting at a low temperature, an amorphous substance corresponds with a glass transition temperature, a temperature for which the molar heat capacity changes over a certain temperature interval. The broadness of this effect is depending on quite different qualities. The enthalpy change in heating up is for a glass transition always a uptake of energy by the sample. The crystalline and amorphous substances are discriminated at room temperature by several spectroscopical methods such as X-ray, Raman, IR. Additionally, a characterization at elevated temperature over the stability region of crystalline substances in the solid phase is also possible with a temperature-humidity powder diffraction chamber. A preferred characterization are the X-ray methods, because the amorphous substances reveal only a broad reflection, however, the crystalline substances are characterized with a discrete set of interlattice plane intervals.

The solid state characterization of the amorphous entity of the calcium salt hydrate of valsartan $\mathbf{E_{1,Ca}}$ is performed with a DSC (Differential Scanning calorimeter) Pyris 1 from Perkin-Elmer Corp., Norwalk, CT USA. The same procedure must be executed as for the crystalline salts of calcium valsartan, namely because of existing salt hydrates, with a bound water content up to 13.2%, the measurements must be made in gold containers with a small internal free volume. In the present case, the gold containers had an internal free volume of ca. 22 microliters. Additional water, so-called free water could be present in the amorphous substance, detectable by a thermobalance as well as by the enthalpy of fusion for bulk water at 0°C. In an open sample pan or with a sample pan with a large internal free volume compared with the sample mass and depending on the water of the substance under investigation, the water evaporates partially or completely in transferring the chemical entity present partially or completely into the corresponding anhydrate or into a hydrate with a lower water content. Gold containers with a wall thickness 0.2mm were used; after weighing the samples between 1.5 and 6mg salt hydrate, they were sealed by cold welding.
The amorphous substance of the calcium salt of valsartan $E_{1,Ca}$ related to the tetrahydrates and the trihydrates has a water content of 11 ± 2%. The water content is given through the laboratory production process. The glass transition was measured with sample weights of 3 - 5 mg in sealed gold containers with a internal free volume of ca. 22 microliters and applying

a heating rate of 10 K·min^{-1}. The glass transition temperature is determined for the amorphous calcium salt of valsartan $E_{1,Ca}$ as T_g = 94±20°C and the change of the specific heat capacity is at the glass transition temperature as Δc_p=0.6±0.3 J·g^{-1}·K^{-1}. No melting point and no enthalpy of fusion could be observed.

The amorphous substance of the calcium salt of valsartan $\mathbf{F_{1,Ca}}$ related to the anhydrate has a water content of 9±2%. The water content is measured using thermogravimetry, in a water-free N_2 atmosphere with a TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA). The glass transition was measured with sample weights of 2 - 4 mg in sealed gold containers with a internal free volume of ca. 22 microliters and applying a heating rate of 10 K·min^{-1}. The glass transition temperature is determined for the amorphous salt of valsartan $F_{1,Ca}$ as T_g = 143±20°C and the change of the specific heat capacity is at the glass transition temperature Δc_p=0.4±0.15 J·g^{-1}·K^{-1}. No melting point and no enthalpy of fusion could be observed. These combined thermodynamic data, melting point and enthalpy of fusion are an absolute prerequisite of a crystalline material or substance.

The amorphous substance of the magnesium salt of valsartan $E_{1,Mg}$ has a water content of 16±3%. The water content is less defined in an amorphous form, as the water molecules in an amorphous substance are weaker bound within the solid structure compared to a crystalline substance forming a hydrate. The water content is measured using thermogravimetry, in a water-free N_2 atmosphere with a TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA). The glass transition was measured with sample weights of 2 - 4 mg in sealed gold containers with an internal free volume of ca. 22 microliters and applying a heating rate of 10 K·min^{-1}. The glass transition temperature is T_g = 78±20°C and the change of the specific heat capacity is at the glass transition temperature Δc_p=0.5±0.25 J·g^{-1}·K^{-1}. No melting point and no enthalpy of fusion could be observed.

Preferred are polymorphic forms that are essentially free of amorphous forms.

A further object of the invention is the preparation of the salts according to the invention.

The salts or salt hydrates according to the invention, including amorphous or crystalline forms thereof, may be prepared as follows:

To form the salt, the process is carried out in a solvent system, in which the two reactants, namely the acid valsartan and the respective base, are sufficiently soluble. It is expedient to use a solvent or solvent mixture, in which the resulting salt is only slightly soluble or not soluble at all, in order to achieve crystallisation or precipitation. One variant for the salt according to the invention would be to use a solvent in which this salt is very soluble, and to subsequently add an anti-solvent to this solution that is a solvent in which the resulting salt has only poor solubility. A further variant for salt crystallisation consists in concentrating the salt solution, for example by heating, if necessary under reduced pressure, in slowly evaporating the solvent, e.g. at room temperature or at a temperature below room temperature, or by seeding with the addition of seeding crystals, or by setting up water activity required for hydrate formation and/or by seeding with the addition of the corresponding seeding crystals. Combinations of these production steps may be appropriately selected.

The solvents that may be used are for example C_1-C_5-alkanols, preferably ethanol and isopropanol, as well as C_1-C_5-dialkylketones, preferably acetone and mixtures thereof with water.

The antisolvents for salt crystallisation may be for example C_3-C_7-alkylnitriles, especially acetonitrile, esters, especially C_2-C_7-alkanecarboxylic acid-C_1-C_5-alkylester, such as ethyl or isopropyl acetate, di-(C_1-C_5-alkyl)-ethers, such as tert.-butylmethylether, furthermore tetrahydrofuran, and C_5-C_8-alkanes, especially pentane, hexane or heptane.

The dissolving and crystallising process is characterised in that

(i) valsartan and the appropriate base are brought to a reaction in a preferably water-containing, organic solvent,

(ii) the solvent system is concentrated, for example by heating, if necessary under reduced pressure and by seeding with seeding crystals or by slowly evaporating, e.g. at room temperature or at elevated temperatures, then crystallisation or precipitation is initiated and

(iii) the salt or salt hydrate obtained is isolated.

In the dissolving and crystallising process, the water-containing, organic solvent system employed is advantageously a mixtures of alcohols, such as ethanol, and water, or alkyl-nitrile, especially acetonitrile, and water.

The equilibrating crystallisation process for producing hydrates is characterised in that

(i) valsartan and the appropriate base are added to a water-containing organic solvent,

(ii) the solvent is concentrated, for example by heating, if necessary under reduced pressure or by slowly evaporating, e.g. at room temperature

(iii) the residue of evaporation is equilibrated with the required amount of water by

(a) suspending the residue of evaporation, which is advantageously still warm, and which still contains some water, in an appropriate solvent or

(b) by equilibrating the water excess in the solvent at a given temperature, or with cooling from a given elevated temperature to a lower one;

whereby in a) and b) the existing or added water is present in a quantity in which the water dissolves in the organic solvent and does not form an additional phase; and

(iv) the salt obtained is isolated.

The solvent system used as the water-containing organic solvent advantageously comprises mixtures of suitable alcohols, such as C_1-C_7-alkanols, especially ethanol, and water.

An appropriate solvent for equilibration is, for example, an ester such as C_1-C_7-alkane-carboxylic acid-C_1-C_7-alkylester, especially ethyl acetate, or a ketone such as di-C_1-C_5-alkylketone, especially acetone.

The equilibration process is notable for example for its high yields and outstanding reproducibility.

Especially, the alkaline earth metal salts of the present invention may be obtained in crystalline form as explained above and are in the form of hydrates, or mixtures of hydrates, or mixtures of hydrates with amorphous forms, from appropriate solvents that are conventionally used in production processes, such as esters, e.g. C_1-C_7-alkanecarboxylic acid-C_1-C_7-alkylesters, especially ethyl acetate, ketones, e.g. di-C_1-C_5-alkylketones, especially acetone, C_3-C_7-alkylnitriles, especially acetonitrile, or ethers, e.g. di-(C_1-C_5-alkyl)-ethers, such as tert.-butylmethylether, also tetrahydrofuran, or mixtures of solvents. By using the dissolving and crystallising process, or the water-equilibrating crystallisation process, the defined hydrates, which are present in crystalline and in polymorphous forms, may be obtained reproducibly.

The processes for forming salts are likewise objects of the present invention.

These salts or salt hydrates according to the invention are obtained for example by neutralising the acid valsartan with a base corresponding to the respective cation. This neutralisation is suitably effected in an aqueous medium, e.g. in water or a mixture of water and a solvent in which valsartan is more soluble than in water. Salts with weaker bases may be converted into other salts either by treating with stronger bases or by treating with acids and then neutralising with other bases.

Crystallisation, especially of the alkaline earth salt hydrates, is effected in water or an aqueous medium, which consists of water and at least one solvent that is miscible or partially miscible with water, i.e. not too non-polar, e.g. an alkanol such as methanol, ethanol, propanol, isopropanol, butanol, acetone, methyl ethyl ketone, acetonitrile, DMF, DMSO. The alkanol portion amounts to about 10% to 99%, or 20% to 90%, advantageously 30% to 70% by volume. For higher alkanols, the less polar solvent may also be present in lower concentrations. Owing to the restricted water-solubility of valsartan, the process frequently takes place in suspensions, or if valsartan is soluble in the other solvent component, in a solution.

In one embodiment, for example to produce the calcium salt of valsartan, an aqueous solution of valsartan is neutralised with a calcium hydroxide solution at room temperature and the solution is left to crystallise. In a preferred procedure, crystallisation is effected from a solvent mixture of water/ethanol, the ethanol proportion amounting to ca. 30% to 50% by volume. In an especially preferred form, crystallisation is effected in a closed system by transporting through a low temperature gradient (especially 1-2°C at 40°C) in 30% by volume of ethanol.

In a preferred variant, crystallisation may be optimised, e.g. accelerated, by adding at least one seed crystal.

To produce a salt of valsartan in a desired form as a hydrate, or an anhydrate and in a specific polymorph, or in a specific amorphous form thereof, a dissolving, chemical reaction, and crystallising process is used in particular, or a water-equilibrating crystallization, or an additional drying-equilibrating process. In the following, the processes consecutive to the dissolving and the chemical reaction shall be outlined:

(i) Transferring the obtained salthydrate, with a given molecular ratio water to salt of valsartan or with a mixture of hydrates, having different molecular ratios of water to salt of valsartan, or as a mixture of hydrates and the anhydrate of the given salt of valsartan, and all these entities and mixtures of entities in a specific polymorphic form, or in a specific amorphous form, or as mixtures of different polymorphs and different amorphous forms with or without a separation from the mother liquid into another liquid phase in which a considerable amount of the solid phase will not be dissolved, however, is present as a suspension. The liquid phase of this suspension is changed stepwise or continously in appropriate conditions such as temperature, pressure, volume, composition in respect to water, solvents, antisolvents in such a way that the salthydrate of choice is generated by a recrystallization process. The recrystallization can be forced by adding at least one seeding crystal.

(ii) Separate the obtained salthydrate in the crystalline state from the mother liquid, or from the liquid phase in which the salthydrate is suspended and transfer the wet cake with or without washing into a drier. The drier preferably used is a moving product drier, such as an example a paddle drier. The conditions in the drier and for the drying process have to be appropriate selected to obtain the salthydrate in the form to be produced.

In a preferred embodiment of the present invention, the different hydrates and polymorphic and amorphic forms thereof can be prepared by using the thermobalance procedure as follows:

Starting from e.g. the $A_{0,Ca}$ or the $A_{0,\,Mg}$ form, respectively,

said forms are (i) dehydrated, e.g. totally or partially, for example, in a thermobalance apparatus e.g. TGS-2, or in a temperature-humidity powder diffraction chamber, e.g. X'Pert, or in Differential Scanning Calorimeter, e.g. DSC Pyris 1;

then (ii) equilibrated by exposure to different relative air humidities over different periods of time,

optionally (iii) relaxed over different periods of time, and

then, if necessarey, (iv) isolated.

The dehydration step is carried out essentially by dehydrating the corresponding starting material in a water free atmosphere, under inert gas, in a defined temperature range and over a defined time intervals. A suitable temperature is from room temperature to 100°C. Suitable time intervals are from 30 minutes to 70 hours.

The equilibration step is carried out by exposing the dehydrated form to different air humidities. Preferred air humidities range from 20% to 70% relative air humidity.

The relaxation period is between 30 minutes to 50 hours. The preferred temperature range for the equilibration step is between 20°C and 25°C.

The form according to the present invention is preferably isolated by crystallisation.

Important conditions among others are the relative humidity of the atmosphere in the drier, the temperature of the atmosphere and the temperature of the dry product, all these parameters as a function of the drying degree and also the drying time interval which also defines the final state of the equilibrated product.
The main driving force for a hydrate formation of a salthydrate of valsartan during the crystallization or precipitation process, or during a recrystallization process as a suspension or as a product in a drying process is the activity of the water in the liquid phase or the partial pressure of the water in the atmosphere of a drier. The composition of the liquid phase in which the salthydrate of valsartan is suspended and its temperature are decisive for the activity of the water. In the drier, the partial pressure of the water is adjusted under equilibrium or non-equilibrium conditions with conditions such as relative humidity of the inlet gas stream, the temperature of the drier and the temperature of the substance dried, the uptake of water or the dehydration of the substance dried, the flow rate of the atmosphere and the mass of the substance dried. Of course, also the ratio of the water molecules to the salt molecules at the beginning and the end of the drying process and the kinetic of the dehydration or hydration are factors influencing the partial pressure of the water in the drier.

An additional thermodynamic parameter, which is decisive for the salt hydrate of valsartan and the polymorphic form of the final state of the product, is the temperature. The thermodynamic stability regions of the salthydrates of valsartan are depending also on temperature, or in other words certain salthydrates of valsartan and polymorphs thereof are only stable for given temperature regions. As an example, a selected salthydrate of valsartan can only be crystallized or can only be recrystallized also from a solution if the temperature is selected properly.

The salts according to the invention may be used e.g. in the form of pharmaceutical preparations, which contain the active substance e.g. in a therapeutically effective amount of the active substance, optionally together with a pharmaceutically acceptable carrier, for example with an inorganic or organic, solid or optionally also liquid pharmaceutically acceptable carrier, which is suitable for enteral, e.g. oral, or parenteral administration.

The invention relates in particular to a pharmaceutical composition, especially in a solid dosage unit, preferably for oral administration, optionally together with a pharmaceutically acceptable carrier.

Pharmaceutical preparations of this kind may be used for example for the prophylaxis and treatment of diseases or conditions which may be inhibited by blocking the AT_1 receptor for example
a disease or condition selected from the group consisting of

(a) hypertension, congestive heart failure, renal failure, especially chronic renal failure, restenosis after percutaneous transluminal angioplasty, and restenosis after coronary artery bypass surgery;

(b) atherosclerosis, insulin resistance and syndrome X, diabetes mellitus type 2, obesity, nephropathy, renal failure, e.g. chronic renal failure, hypothyroidism, survival post myocardial infarction (MI), coronary heart diseases, hypertension in the elderly, familial dyslipidemic hypertension, increase of formation of collagen, fibrosis, and remodeling following hypertension (antiproliferative effect of the combination), all these diseases or conditions associated with or without hypertension;

(c) endothelial dysfunction with or without hypertension,

(d) hyperlipidemia, hyperlipoproteinemia, atherosclerosis and hypercholesterolemia, and

(e) glaucoma.

Primary usages are for the treatment of high blood pressure and congestive heart failure, as well as post-myocardial infarction.

The person skilled in the pertinent art is fully enabled to select a relevant and standard animal test model to prove the hereinbefore and hereinafter indicated therapeutic indications and beneficial effects.

The pharmaceutical activities as effected by administration of representatives of the salts of the present invention or of the combination of active agents used according to the present invention can be demonstrated e.g. by using corresponding pharmacological models known in the pertinent art. The person skilled in the pertinent art is fully enabled to select a relevant animal test model to prove the hereinbefore and hereinafter indicated therapeutic indications and beneficial effects.

These beneficial effects can, for example, be demonstrated in the test model as disclosed by G. Jeremic et al. in J. Cardovasc. Pharmacol. 27:347-354, 1996.

For example, the valuable potential of the salts or combinations of the present invention for the prevention and treatment of myocardial infarction can be found using the following test model.

Study design

In the study to be performed, permanent coronary artery occlusion (CAO) in rats is used as a model of acute myocardial infarction. The experiments are carried out with 5 treatment groups characterized by following features:

- sham-operated animals
- CAO + vehicle
- CAO + a salt according to the present invention,

optionally

- CAO + a salt according to the present invention + a combination partner.

During the study following variables are measured:

- infarct size
- LV chamber volume
- interstitial and perivascular collagen density in spared LV myocardium
- COL-I and COL-III protein content in spared LV myocardium by Western blot
- cardiomyocytes cross-sectional area and length in sections of LV myocardium

- plasma concentrations of renin and aldosterone
- urine concentration of sodium, potassium and aldosterone
- blood pressure in conscious animals
- LV and carotid blood pressure in anesthetized animals.

Methodology

Infarct size: Six μm-thick transverse histological sections of the left ventricle are stained with nitroblue tetrazolium and acquired by a B/W XC-77CE CCD video camera (Sony). The resulting image is processed on a KS 300 image analysis system (Carl Zeiss Vision) using a software specifically developed (Porzio *et al.*, 1995). A single operator blinded to treatment interactively defines the boundaries of the interventricular septum, and the infarcted area on each section is semiautomatically identified as the area of unstained ventricular tissue. The software automatically calculates for each component of the ventricular section defined as the chamber, septum, infarcted area, infarcted LV wall and viable LV wall, a set of geometric parameters (Porzio *et al.*, 1995).

Histology: Hearts are fixed in situ, by retrograde perfusion with buffered 4% formaldehyde after arrest in diastole by i.v. injection of 0.5 M KCl. After fixation, the left ventricle (LV) and the free wall of the right ventricle are separately weighed; LV longer diameter is measured with a caliper. LV histological sections are stained with hematoxylin & eosin for qualitative examination and to quantify cardiomyocytes cross-sectional area with a semi-automated image analysis routine. Interstitial collagen deposition in LV is evaluated on Sirius red stained sections with a semi-automated image analysis routine (Masson *et al.*, 1998).

Collagen content in LV spared myocardium: LV tissue in the spared myocardium is homogenized, subjected to PAGE-SDS electrophoresis and electroblotted onto nitrocellulose membrane. The blots are exposed to primary antibodies, i.e. rabbit anti-rat collagen type I or type III antiserum (Chemicon). The primary antibodies are recognized by secondary antibodies conjugated to alkaline phosphatase (for colagen type I) or peroxidase (collagen type III).

Left ventricular chamber volume: LV chamber volume is determined in hearts arrested in diastole (KCl) and fixed in formalin under a hydrostatic pressure equivalent to the measured LV end-diastolic pressure. A metric rod is inserted into the LV to measure LV inner length.

The transverse diameters of the LV chamber are measured in two 1-mm thick transverse sections near to the base and the apex of the ventricle (Jeremic *et al.*, 1996). The chamber volume is computed from an equation integrating transverse diameters and inner length.

Systemic and Left ventricular hemodynamics: A microtip pressure transducer (Millar SPC-320) connected to a recorder (Windograf, Gould Electronics) is inserted into the right carotid artery to record systolic and diastolic blood pressures. The pressure transducer is advanced into the LV to measure LV systolic (LVSP) and end-diastolic (LVEDP) pressures, the first derivative of LV pressure over time (+dP/dt) and heart rate.

Non-invasive blood pressure: Systolic blood pressure and heart rate are measured by the tail-cuff method (Letica LE 5002) in conscious rats.

Urine electrolytes, hormones: Rats are individually housed in metabolic cages and 24-h urine collected on 1 ml HCl 6N. Water intake is measured. Urine catecholamines are extracted on Bondelut C_{18} columns (Varian), separated by HPLC (Apex-II C18, 3 µm, 50x4.5 mm analytical column, Jones Chromatography) and quantified with an electrochemical detector (Coulochem II, ESA) (Goldstein *et al.*, 1981). Plasma and urine aldosterone, and plasma angiotensin II is determined with specific radioimmunoassays (Aldoctk-2, DiaSorin and Angiotensin II, Nichols Diagnostics). Urine sodium and potassium are measured by flamme photometry.

<u>**Sample size**</u>

10 animals analyzable in each treatment groups are sufficient to detect biologically significant differences. Only rats with an infarct size of at least 10% of the LV section area are included in the final analysis.

Endothelial dysfunction is being acknowledged as a critical factor in vascular diseases. The endothelium plays a bimodal role as the source of various hormones or by-products with opposing effects: vasodilation and vasoconstriction, inhibition or promotion of growth, fibrinolysis or thrombogenesis, production of anti-oxidants or oxidising agents. Genetically predisposed hypertensive animals with endothelial dysfunction constitute a valid model for assessing the efficacy of a cardiovascular therapy.

Endothelial disfunction is characterized by, for example, increased oxidative stress, causing decreased nitric oxide, increased factors involved in coagulation or fibrinolysis such as plasminogen activating inhibitor-1 (PAI-1), tissue factor (TF), tissue plasminogen activator (tPA), increased adhesion molecules such as ICAM and VCAM, increased growth factors such as bFGF, TGFb, PDGF, VEGF, all factors causing cell growth inflammation and fibrosis.

The treatment e.g. of endothelian dysfunction can be demonstrated in the following pharmacological test:

Material and methods

Male 20-24 week-old SHR, purchased from RCC Ldt (Füllingsdorf, Switzerland), are maintained in a temperature- and light-controlled room with free access to rat chow (Nafag 9331, Gossau, Switzerland) and tap water. The experiment is performed in accordance with the NIH guidelines and approved by the Canton Veterinary office (Bew 161, Kantonales Veterinäramt, Liestal, Switzerland). All rats are treated with the NO synthesis inhibitor L-NAME (Sigma Chemicals) administered in drinking water (50 mg/l) for 12 weeks. The average daily dose of L-NAME calculated from the water consumed was 2.5 mg/kg/d (range 2.1-2.7).

The rats can be divided into 2 or 3 groups: group 1, control (n = e.g. 40); Group 2, a salt according to the present invention; n = e.g. 40); for testing combinations Group 3, combination partner;(n = e.g. 30). The drugs are administered in drinking fluid. The pressure effect of Ang II at 1 mg/kg obtained in controls normotensive rats can be reduced after treatment with a salt according to the present invention (Gervais et al. 1999).

Body weight is measured every week. Systolic blood pressure and heart rate are recorded by tail cuff plethysmography 3 and 2 weeks before starting the study and at 2 weeks after drug administration. Urine is collected over a 24 hour period from rats kept in individual (metabolic) cages the week before starting treatment and at weeks 4 and 12 for volume measurement and protein, creatinine, sodium and potassium determination using standard laboratory methods. At the same time points, blood samples are withdrawn from the retro-orbital plexus (maximum 1 ml) for creatinine, Na^+ and K^+ assays.

Ten rats from each group are sacrificed at 4 weeks for collection of kidney and heart for morphological analysis. The remaining rats are sacrificed at 12 weeks. Cardiac and kidney weight is recorded. Terminal blood sampling is performed in 5 % EDTA at 4 (morphometry study) and 12 (end of the study) weeks for aldosterone, determination by radioimmunoassay using a DPC coat-a-count aldosterone-RIA kit (Bühlmann, Switzerland).

Statistical analysis:

All data are expressed as mean ± SEM. Statistical analysis is performed using a one-way ANOVA, followed by a Duncan's multiple range test and a Newman-Keuls test, 7for comparison between the different groups. Results with a probability value of less than 0.05 are deemed statistically significant.

An improvement of regression of artherosclerosis without effecting the serum lipid levels can, for example, be demonstrated by using the animal model as disclosed by H. Kano et al. in Biochemical and Biophysical Research Communications 259, 414-419 (1999).

That the salts or combinations according to the present invention can be used for the regression of a cholesterol diet-induced atherosclerosis, can be demonstrated using the test model described, e.g., by C. Jiang et al. in Br. J. Pharmacol. (1991), 104, 1033-1037.

That the salts or combinations according to the present invention can be used for the treatment of renal failure, especially chronic renal failure, can be demonstrated using the test model described, e.g., by D. Cohen et al. in Journal of Cardiovascular Pharmacology, 32: 87-95 (1998).

The present pharmaceutical preparations which, if so desired, may contain further pharmacologically active substances, are prepared in a manner known *per se*, for example by means of conventional mixing, granulating, coating, dissolving or lyophilising processes, and contain from about 0.1% to 100%, especially from about 1% to about 50%, of lyophilisates up to 100% of the active substance.

The invention similarly relates to compositions containing the salts according to the invention.

The invention similarly relates to the use of the salts according to the invention preferably for the production of pharmaceutical preparations, especially for the prophylaxis and also for the treatment of diseases or conditions which may be inhibited by blocking the AT_1 receptor. Primary usages are for the treatment of high blood pressure and congestive heart failure, as well as post-myocardial infarction.

The invention similarly relates to the use for the prophylaxis and treatment of diseases or conditions which may be inhibited by blocking the AT_1 receptor, characterised in that a patient, including a human patient, requiring such treatment is administered with a therapeutically effective amount of a salt according to the invention, optionally in combination with at least one composition for the treatment of cardiovascular diseases and related conditions and diseases listed hereinbefore or hereinafter.

The invention similarly relates to combinations, e.g. pharmaceutical combinations, containing a salt of the present invention or in each case a pharmaceutically acceptable salt thereof in combination with at least one composition for the treatment of cardiovascular diseases and related conditions and diseases as listed hereinbefore or hereinafter, or in each case a pharmaceutically acceptable salt thereof. Combinations with other compositions for the treatment of cardiovascular diseases and related conditions and diseases as listed hereinbefore or hereinafter, or in each case a pharmaceutically acceptable salt thereof, are likewise objects of the present invention.

The combination may be made for example with the following compositions, selected from the group consisting of a:

(i) HMG-Co-A reductase inhibitor or a pharmaceutically acceptable salt thereof,

(ii) angiotensin converting enzyme (ACE) Inhibitor or a pharmaceutically acceptable salt thereof,

(iii) calcium channel blocker or a pharmaceutically acceptable salt thereof,

(iv) aldosterone synthase inhibitor or a pharmaceutically acceptable salt thereof,

(v) aldosterone antagonist or a pharmaceutically acceptable salt thereof,

(vi) dual angiotensin converting enzyme/neutral endopeptidase (ACE/NEP) inhibitor or a pharmaceutically acceptable salt thereof,

(vii) endothelin antagonist or a pharmaceutically acceptable salt thereof,

(viii) renin inhibitor or a pharmaceutically acceptable salt thereof, and

(ix) diuretic or a pharmaceutically acceptable salt thereof.

HMG-Co-A reductase inhibitors (also called β-hydroxy-β-methylglutaryl-co-enzyme-A reductase inhibitors) are understood to be those active agents that may be used to lower the lipid levels including cholesterol in blood.

The class of HMG-Co-A reductase inhibitors comprises compounds having differing structural features. For example, mention may be made of the compounds that are selected from the group consisting of atorvastatin, cerivastatin, compactin, dalvastatin, dihydrocompactin, fluindostatin, fluvastatin, lovastatin, pitavastatin, mevastatin, pravastatin, rivastatin, simvastatin, and velostatin, or, in each case, a pharmaceutically acceptable salt thereof.

Preferred HMG-Co-A reductase inhibitors are those agents which have been marketed, most preferred is fluvastatin and pitavastatin or, in each case, a pharmaceutically acceptable salt thereof.

The interruption of the enzymatic degradation of angiotensin I to angiotensin II with so-called ACE-inhibitors (also called angiotensin converting enzyme inhibitors) is a successful variant for the regulation of blood pressure and thus also makes available a therapeutic method for the treatment of congestive heart failure.

The class of ACE inhibitors comprises compounds having differing structural features. For example, mention may be made of the compounds which are selected from the group consisting alacepril, benazepril, benazeprilat, captopril, ceronapril, cilazapril, delapril, enalapril, enaprilat, fosinopril, imidapril, lisinopril, moveltopril, perindopril, quinapril, ramipril, spirapril, temocapril, and trandolapril, or, in each case, a pharmaceutically acceptable salt thereof.

Preferred ACE inhibitors are those agents that have been marketed, most preferred are benazepril and enalapril.

The class of CCBs essentially comprises dihydropyridines (DHPs) and non-DHPs such as diltiazem-type and verapamil-type CCBs.

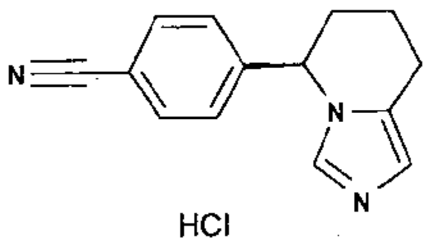
A CCB useful in said combination is preferably a DHP representative selected from the group consisting of amlodipine, felodipine, ryosidine, isradipine, lacidipine, nicardipine, nifedipine, niguldipine, niludipine, nimodipine, nisoldipine, nitrendipine, and nivaldipine, and is preferably a non-DHP representative selected from the group consisting of flunarizine, prenylamine, diltiazem, fendiline, gallopamil, mibefradil, anipamil, tiapamil and verapamil, and in each case, a pharmaceutically acceptable salt thereof. All these CCBs are therapeutically used, e.g. as anti-hypertensive, anti-angina pectoris or anti-arrhythmic drugs. Preferred CCBs comprise amlodipine, diltiazem, isradipine, nicardipine, nifedipine, nimodipine, nisoldipine, nitrendipine, and verapamil, or, e.g. dependent on the specific CCB, a pharmaceutically acceptable salt thereof. Especially preferred as DHP is amlodipine or a pharmaceutically acceptable salt, especially the besylate, thereof. An especially preferred representative of non-DHPs is verapamil or a pharmaceutically acceptable salt, especially the hydrochloride, thereof.

Aldosterone synthase inhibitor is an enzyme that converts corticosterone to aldosterone to by hydroxylating cortocosterone to form 18-OH-corticosterone and 18-OH-corticosterone to aldosterone. The class of aldosterone synthase inhibitors is known to be applied for the treatment of hypertension and primary aldosteronism comprises both steroidal and non-steroidal aldosterone synthase inhibitors, the later being most preferred.

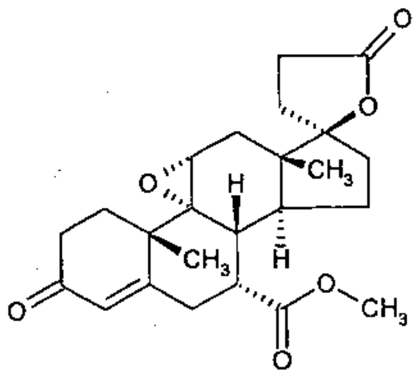
Preference is given to commercially available aldosterone synthase inhibitors or those aldosterone synthase inhibitors that have been approved by the health authorities.

The class of aldosterone synthase inhibitors comprises compounds having differing structural features. For example, mention may be made of the compounds which are selected from the group consisting of the non-steroidal aromatase inhibitors anastrozole, fadrozole (including the (+)-enantiomer thereof), as well as the steroidal aromatase inhibitor exemestane, or, in each case where applicable, a pharmaceutically acceptable salt thereof.

The most preferred non-steroidal aldosterone synthase inhibitor is the (+)-enantiomer of the hydrochloride of fadrozole (US patents 4617307 and 4889861) of formula



A preferred steroidal aldosterone antagonist is eplerenone of the formula



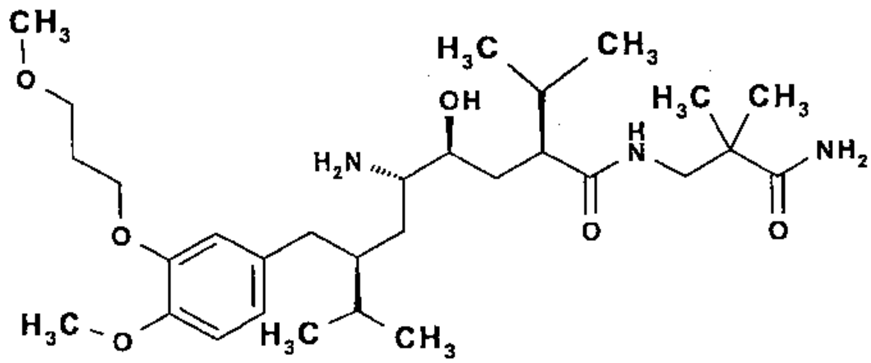
or

spironolactone.

A preferred dual angiotensin converting enzyme/neutral endopetidase (ACE/NEP) inhibitor is, for example, omapatrilate (cf. EP 629627), fasidotril or fasidotrilate, or, if appropriable, a pharmaceutically acceptable salt thereof.

A preferred endothelin antagonist is, for example, bosentan (cf. EP 526708 A), furthermore, tezosentan (cf. WO 96/19459), or in each case, a pharmaceutically acceptable salt thereof.

A renin inhibitor is, for example, a non-peptidic renin inhibitor such as the compound of formula



chemically defined as 2(S),4(S),5(S),7(S)-N-(3-amino-2,2-dimethyl-3-oxopropyl)-2,7-di(1-methylethyl)-4-hydroxy-5-amino-8-[4-methoxy-3-(3-methoxy-propoxy)phenyl]-octanamide. This representative is specifically disclosed in EP 678503 A. Especially preferred is the hemi-fumarate salt thereof.

A diuretic is, for example, a thiazide derivative selected from the group consisting of chlorothiazide, hydrochlorothiazide, methylclothiazide, and chlorothalidon. The most preferred is hydrochlorothiazide.

Preferably, the jointly therapeutically effective amounts of the active agents according to the combination of the present invention can be administered simultaneously or sequentially in any order, separately or in a fixed combination.

The structure of the active agents identified by generic or tradenames may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g. Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference. Any person skilled in the art is fully enabled to identify the active agents and, based on these references, likewise enabled to manufacture and test the pharmaceutical indications and properties in standard test models, both in vitro and in vivo.

The corresponding active ingredients or a pharmaceutically acceptable salts thereof may also be used in form of a solvate, such as a hydrate or including other solvents, used for crystallization.

The compounds to be combined can be present as pharmaceutically acceptable salts. If these compounds have, for example, at least one basic center, they can form acid addition salts. Corresponding acid addition salts can also be formed having, if desired, an additionally present basic center. The compounds having an acid group (for example COOH) can also form salts with bases.

In a variation thereof, the present invention likewise relates to a "kit-of-parts", for example, in the sense that the components to be combined according to the present invention can be dosed independently or by use of different fixed combinations with distinguished amounts of the components, i.e. simultaneously or at different time points. The parts of the kit of parts can then e.g. be administered simultaneously or chronologically staggered, that is at different time points and with equal or different time intervals for any part of the kit of parts. Preferably, the time intervals are chosen such that the effect on the treated disease or condition in the combined use of the parts is larger than the effect that would be obtained by use of only any one of the components.

The invention furthermore relates to a commercial package comprising the combination according to the present invention together with instructions for simultaneous, separate or sequential use.

Dosaging may depend on various factors, such as mode of application, species, age and/or individual condition. For oral application, the doses to be administered daily are between ca. 0.25 and 10 mg/kg, and for warm-blooded animals with a body weight of ca. 70 kg, preferably between ca. 20 mg and 500 mg, especially 40mg, 80mg, 160mg and 320mg based on the free acid.

The invention is illustrated in particular by the examples and also relates to the new compounds named in the examples and to their usage and to methods for the preparation thereof.

The following examples serve to illustrate the invention without limiting the invention in any way.

Production of starting material

Starting materials for all new salthydrates of calcium valsartan and magnesium valsartan have been produced in the following manner. Additionally, the start materials have been characterised by several analytical methods.

Example SM1 (for Starting Material):

Production example as start material for the calcium salt as the tetrahydrate $A_{0,Ca}$ *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

21.775 g of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are dissolved at room temperature in 300 ml of ethanol. By careful addition of 300 ml of water, the ethanol concentration is reduced to 50% by volume. Using a magnetic stirrer, 3.89 g of $Ca(OH)_2$ are added slowly in small portions to this clear, slightly acidic (pH 4) solution, so that the pH value temporarily does not exceed a value of ca. 8. Because it absorbs CO_2 from the air, the $Ca(OH)_2$ used contains traces of $CaCO_3$; therefore the added amount includes an excess of 5%. After adding the stoichiometric amount of $Ca(OH)_2$, the pH is ca. 6, and after adding the excess it rises to 7. The solution becomes turbid through the small amount of finely divided $CaCO_3$, which is removed through a folded filter. The product contained in the solution crystallises continuously upon removal of the alcohol content by allowing to stand at room temperature. The procedure can be accelerated by using a flat dish in a recirculating air drier at 40°C. After concentrating to ca. one half, the alcohol content of the solution drops to ca. 10% by volume and most of the product crystallises. It is filtered, rinsed for a short time with 10% by volume ethanol and dried at 40°C until reaching a constant weight. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt tetrahydrate $A_{0,Ca}$ is obtained.

The melting point for the tetrahydrate of the calcium salt of valsartan $A_{0,Ca}$, produced according to the above given example for the start material, for a heating rate of 10 $K \cdot min^{-1}$, and in a closed specimen container with a small internal volume of ca. 22 microliters is determined as T_{fus} = 205°C and the melting enthalpy as $\Delta_{fus}H$ = 92 $kJ \cdot mol^{-1}$. The density of the crystals of the tetrahydrate $A_{0,Ca}$ of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-

yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-yl-methyl]-amine, produced according to the example for start materials, determined by a helium pycnometer is 1.297 g·cm^3. The specific optical rotation of the tetrahydrate of the calcium salt of valsartan $A_{0,Ca}$ according to this production example is measured at 20°C in methanol as a 1% solution $[\alpha]^{20}_D$ = +1° and in water also at 20°C as a 0.4% solution $[\alpha]^{20}_D$ = -39°.

The enantiomer purity of the salt hydrate produced according to the process for the start materials, namely the tetrahydrate of the calcium salt of valsartan $A_{0,Ca}$ is determined by a stereo-specific HPLC method. The stereo-specific separation is achieved by a chiral column (Chiral AGP). The enantiomer purity for $A_{0,Ca}$ is determined as ee = 100%.

The measurement of the infrared spectrum took place by means of ATR-IR (Attenuated Total Reflection-Infrared Spectroscopy) using the instrument BX from Perkin-Elmer Corp., Beaconsfield, Bucks, England.
The characteristic absorption bands of the ATR-IR spectroscopy are listed below for the tetrahydrate of the calcium salt of valsartan $A_{0,Ca}$ produced according to the example SM1 with the following values expressed in reciprocal wave numbers (cm^{-1}): 3594; 3306; 2954; 1621; 1578; 1458; 1441; 1417; 1364; 1319; 1274; 1211; 1180; 1137; 1012; 1002; 758; 738; 696; 666.
The water content is in theory 13.2% for the tetrahydrate of the calcium salt of valsartan. Using the thermobalance TGS-2 (Perkin-Elmer Corp. , Norwalk, CT USA) the water content was determined as 13.0 %. A total formula was calculated for the tetrahydrate of the calcium salt of valsartan $A_{0,Ca}$ from this as $(C_{24}H_{27}N_5O_3)^{2-}$ Ca^{2+}• 3.9 H_2O.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the tetrahydrate as a function of temperature, was measured at a heating rate of 10 K·min^{-1}. The results for the calcium salt of valsartan tetrahydrate $A_{0,Ca}$ are listed in the following:

Temperature [° C]	weight loss or water loss in %
25	0
50	0
75	0.5
100	3.5

125	10.2
150	12.4
175	12.8
200	12.9
225	13.0
250	13.3
275	13.2

Calculation of the interlattice plane intervals from the X-ray powder pattern taken with a Guinier camera is as follows for the characteristic lines for the batch of the substance $A_{0,Ca}$ as tetrahydrate of the calcium salt of valsartan:
d in [Å]: 16.27, 9.90, 9.39, 8.04, 7.71, 7.05, 6.49, 6.34, 6.20, 5.87, 5.75, 5.66, 5.20, 5.05, 4.95, 4.73, 4.55, 4.33, 4.15, 4.12, 3.95, 3.91, 3.87, 3.35.

Elementary analysis gives the following measured values of the elements present in calcium-valsartan-tetrahydrate and of water. The water evaluation was carried out at 130°C after expulsion. The findings of the elementary analysis, within the error limits, correspond to the sum formula $(C_{24} H_{27} N_5 O_3)^{2-} Ca^{2+} \cdot 4 H_2O$.

	% found	% calculated
C	52.82	52.83
H	6.42	6.47
N	12.91	12.83
O	20.20	20.53
water	13.25	13.21
Ca	7.03	7.35

Example SM2 (for Starting Material):

Production example of the magnesium salt as the hexahydrate $A_{0,Mg}$ *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

43.55 g of valsartan (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are dissolved at room temperature in 600 ml of 50% by volume ethanol (from absolute ethanol - see Merck and quarz-bidistilled water). The slightly turbid solution becomes clear after adding a further 50 ml of 50% ethanol. Using a magnetic stirrer, 4.03 g or 0.1 M MgO (Merck p.a.) are slowly added in small portions to this slightly acidic solution with a pH value of 4. The pH value hereby rises to ca. 6. The process is effected with an excess of 10%, i.e. a further 0.40 g of MgO are added. This excess is not fully dissolved, and the pH value rises to ca. 7.5. The small residue is filtered from the solution through a folded filter and washed with 50 ml of 50% ethanol.

The combined clear solution is carefully concentrated at 40°C whilst stirring with a magnetic stirrer in a large crystallisation dish. Towards the end of this procedure, the solution has a tendency to harden into a glassy gel. Scratching with a glass rod induces the *in situ* crystallisation in this phase, which may be recognised by the white colour of the crystalline solid thus formed. The product is dried at 50°C in a recirculating air drier until reaching a constant weight. The yield of magnesiumsalt as the hexahydrate $A_{0,Mg}$ of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine is 53.7 g or 95% based on the valsartan employed as the free acid.

The melting point of the salt hydrate $A_{0,Mg}$ produced according to the above given procedure, namely the magnesium-valsartan-hexahydrate, for a heating rate of 10 $K{\cdot}min^{-1}$, in a sealed sample container with a small internal volume, in an amount of 2.24mg, was measured at T_{fus} = 132°C and a melting enthalpy at $\Delta_{fus}H$ = 64 $kJ{\cdot}mol^{-1}$.
The density of the crystals of the hexahydrate of the magnesium salt of valsartan produced according to example SM2, determined by a helium pycnometer, is 1.273 $g{\cdot}cm^{-3}$.
The specific optical rotation of the magnesium-valsartan-hexahydrate $A_{0,Mg}$ produced according to the above example for start materials is measured as a 1% solution in methanol $[\alpha]^{20}_{D}$ = -14° and with the same concentration in water as $[\alpha]^{20}_{D}$ = -38°.

The enantiomer purity of the magnesium salt of valsartan hexahydrate $A_{0,Mg}$ produced according to the process for the start materials is determined by a stereo-specific HPLC method. The stereo-specific separation is achieved by a chiral column (Chiral AGP). The enantiomer purity is determined as ee = 99.6%.

The measurement of the infrared spectrum took place by means of ATR-IR (Attenuated Total Reflection-Infrared Spectroscopy) using the instrument Spektrum BX from Perkin Elmer Corp., Beaconsfield, Bucks, England.

The starting material, the hexahydrate of the magnesium salt of valsartan $A_{0,Mg}$ has the following characteristic absorption bands of the ATR-IR spectroscopy listed below with values expressed in reciprocal wave numbers (cm^{-1}): 3374; 3272; 2956; 1619; 1556; 1465; 1420; 1394; 1271; 1175; 1015; 975; 836; 766; 751; 741; 730.

The theoretical water content of the hexahydrate of the magnesium salt of valsartan is 19.1%. Using a coupled instrument based on thermogravimetry-Fourier transformation-infrared-spectroscopy (TG-FTIR, IFS 28 from the companies Netzsch Gerätebau GmbH, Selb, Bayern and Bruker Optik GmbH, Karlsruhe), whilst simultaneously measuring the weight loss and identifying the material component given up, using infrared spectroscopy (release of water), the water content was determined for the hexahydrate of the magnesium salt of valsartan $A_{0,Mg}$ with the weight loss up to the plateau for 225°C a 18.7%. The total formula was calculated for the hexahydrate of the magnesium salt of valsartan $A_{0,Mg}$ from this as $(C_{24}H_{27}N_5O_3)^{2-}Mg^{2+} \cdot 5.9\ H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the hexahydrate as a function of temperature, was measured at a heating rate of 10 $K \cdot min^{-1}$. The results for the magnesium salt of valsartan hexahydrate $A_{0,Mg}$ are listed in the following:

Temperature [° C]	Weight loss or water loss in %
25	0.0
50	1.2
75	4.2
100	11.0
125	16.7
150	17.7
175	18.3
200	18.5
225	18.7
250	18.9
275	19.3

Calculation of the interlattice plane intervals from the X-ray powder pattern taken with a Guinier camera is as follows for the characteristic lines for this batch of the magnesium salt of valsartan hexahydrate $A_{0,Mg}$:
d in [Å]: 19.78, 10.13, 9.84, 7.28, 6.00, 5.81, 5.67, 5.21, 5.04, 4.88, 4.21, 4.18, 4.08, 3.95, 3.46, 3.42.

Elementary analysis gives the following measured values of the elements present in the hexahydrate of the magnesium salt of valsartan and of water. The water evaluation is carried out at 130°C after expulsion. The findings of the elementary analysis, within the error limits, correspond to the sum formula $(C_{24}H_{27}N_5O_3)^{2-}Mg^{2+} \cdot 6H_2O$.

	% found	% calculated
C	51.03	50.94
H	7.00	6.95
N	12.45	12.38
O	25.02	25.44
Water	19.08	19.10
Mg	4.35	4.29

Working Examples:

Example 1
Production of the calcium salt as the tetrahydrate $A_{1,Ca}$ *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

30.18mg of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as tetrahydrate $A_{0,Ca}$ is weighed into a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) and is partially dehydrated at 34°C in a water-free N_2 atmosphere with a flow rate of 50 ml·min^{-1} for a time interval of 50 hours. The observed weight loss, i.e. water loss after the time interval of 50 hours is 7.9%. The water bound at this endpoint for the calcium salt of valsartan was under consideration of the water content for the starting material $A_{0,Ca}$ which is 12.9%, only 5.0%. The consecutive equilibration of the partially dehydrated calcium salt of valsartan is executed in an air atmosphere with a relative humidity of 60% and at a temperature of 23°C. The equilibrated substance obtained is the (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt tetrahydrate $A_{1,Ca}$.

The melting point for the tetrahydrate of the calcium salt of valsartan $A_{1,Ca}$ produced according to example 1 for a heating rate of 10 $K \cdot min^{-1}$ and in a closed specimen container with a small internal volume of ca. 22 microliters and a sample weight of 2.67mg is T_{fus} = 190°C. The enthalpy of fusion for $A_{1,Ca}$ is calculated from the same measurement as explained above with $\Delta_{fus}H = 79 kJ \cdot mol^{-1}$.

The infrared spectrum of the (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt tetrahydrate $A_{1,Ca}$ is measured with a ATR-IR instrument BX from Perkin Elmer Corp., Beaconsfield, Bucks, England.
The characteristic absorption bands of the ATR-IR spectroscopy listed in the following for the tetrahydrate of the calcium salt of valsartan $A_{1,Ca}$ with values expressed in reciprocal wave numbers (cm^{-1}): 3594; 3307; 2960; 1621; 1578; 1459; 1442; 1417; 1407; 1364; 1357; 1319; 1274; 1211; 1180; 1137; 1105; 1099; 1012; 1003; 758; 738; 698.

The water content is in theory 13.2% for a tetrahydrate of the calcium salt of valsartan. Using a thermobalance TGS-2 the water content was determined for the substance produced according to example 1 with 13.4%. An amount of 1.1% H_2O is free and not bound water in the calcium salt of valsartan $A_{1,Ca}$, so the total amount of bound water is 12.3%. A total formula was calculated from this value for $A_{1,Ca}$ as $(C_{24}H_{27}N_5O_3)^{2-} Ca^{2+} \cdot 3.7 H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the tetrahydrate of the calcium salt of valsartan as a function of temperature, was measured at a heating rate of 10 $K \cdot min^{-1}$. The results for the calcium salt of valsartan tetrahydrate $A_{1,Ca}$ are listed as follows:

Temperature [°C]	weight loss or water loss in %
25	1.1
50	3.3
75	5.1
100	9.6
125	12.1

150	12.9
175	13.2
200	13.3
225	13.4
250	13.3
275	13.7

Example 2

Production of the calcium salt as the tetrahydrate $A_{2,Ca}$ *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

32.17mg of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as tetrahydrate $A_{0,Ca}$ is weighed into a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) and is partially dehydrated at 50°C in a water-free N_2 atmosphere with a flow rate of 50 ml·min^{-1} for a time interval of 21 hours. The weight loss, i.e. water loss is observed directly and reached a value of 9.9%. The water bound at this endpoint for the calcium salt of valsartan is under consideration of the water content for the starting material $A_{0,Ca}$ which is 12.9%, only 3%, a value which corresponds with a calcium salt of valsartan monohydrate.
The equilibration of this monohydrate of the calcium salt of valsartan in an air atmosphere with a relative humidity in air of 29% and at a temperature of 23°C is directly observed over a time interval of 46 hours in the thermobalance by a practically equlibrium situation with an uptake of 6.0% H_2O. The final content of bound water is 9.0%, corresponding to 2.6 mole water per molecule of calcium salt of valsartan. The substance, namely the $(C_{24}H_{27}N_5O_3)^{2-}$ $Ca^{2+} \cdot 2.6\ H_2O$ is additionally water equilibrated in an exsiccator with a relative humidity of 90.5%, at a temperature of 23°C and over a time interval of 72 hours. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt tetrahydrate $A_{2,Ca}$ is obtained.

The melting point for the tetrahydrate of the calcium salt of valsartan $A_{2,Ca}$ produced according to example 2, for a heating rate of 10 K·min^{-1} and in a closed specimen container with a small internal volume, and with a sample weight of 1.56mg measured in a DSC Pyris 1 (Differential Scanning calorimeter) is determined as T_{fus} = 195°C and the melting as $\Delta_{fus}H$ = 89 kJ·mol^{-1}.

The water content is in theory 13.2% for a tetrahydrate of the calcium salt of valsartan. Using a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) with a measurement in a water-free N_2 atmosphere, the water content for the substance produced according to example 2 for the temperature interval of 25 to 225°C is determined as 12.6%. A total formula is calculated from this value for $A_{2,Ca}$ as $(C_{24}H_{27}N_5O_3)^{2-} Ca^{2+} \cdot 3.8\ H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the tetrahydrate $A_{2,Ca}$ as a function of temperature, is measured at a heating rate of 10 $K{\cdot}min^{-1}$. The results are listed as follows:

Temperature [°C]	weight loss or water loss in %
25	0
50	0
75	0
100	4.7
125	11.1
150	11.9
175	12.3
200	12.5
225	12.6
250	12.7
275	13.3

Calculation of the interlattice plane intervals from the X-ray powder pattern measured with a Guinier camera is as follows for the characteristic lines for this batch of tetrahydrate of the calcium salt of valsartan $A_{2,Ca}$:
d in [Å] : 16.16, 9.90, 9.40, 8.05, 7.72, 7.04, 6.49, 6.35, 5.82, 4.94, 4.73, 4.13, 3.93.

<u>Example 3</u>
Production of the calcium salt as the trihydrate $B_{1,Ca}$ *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

28.24mg calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as tetrahydrate $A_{0,Ca}$ is placed into an open pan of a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) and has been partially dehydrated at 50°C in a water-free N_2 atmosphere having a flow rate of 50 ml·min^{-1} for a time interval of 28 hours. The weight loss, i.e. water loss is observed directly with the thermobalance and the final stage of dehydration is selected at a water loss of 10.0%. The water bound at this endpoint for the product calcium salt of valsartan was 2.9%, a value which corresponds with 0.8 mole water which is in relation with one mole of calcium salt of valsartan.
The equilibration of this monohydrate is spontanous at a temperature of 22°C and a relative humidity in air of 34% with a relaxation time of about 1 hour. The final equilibration is practically reached after 9 hours with a content of water of 9.7%. The dehydration-hydration process provided the substance (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt trihydrate $B_{1,Ca}$.

The melting point for the trihydrate of the calcium salt of valsartan $B_{1,Ca}$ produced according to example 2, for a heating rate of 10 K·min^{-1} and in a closed specimen container with a small internal volume, and with a sample weight of 3.98mg is determined as T_{fus} = 176°C and the melting enthalpy is $\Delta_{fus}H$ = 7 kJ·mol^{-1}. The crystallinity of the calcium salt of valsartan $B_{1,Ca}$ is ca. 10%.

The water content is determined with a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) with a measurement in a water-free N_2 atmosphere and a heating rate of 10 K·min^{-1}. The water content for the substance $B_{1,Ca}$, produced according to example 3, is for the plateau at the temperature of 225°C determined with 9.7%. The total amount of water bound in $B_{1,Ca}$ is 9.2% calculated from the weight loss at 225°C and the amount of water which is evaporated at 25°C. A total formula is calculated from this value for $B_{1,Ca}$ as $(C_{24}H_{27}N_5O_3)^{2-}$ $Ca^{2+} \cdot 2.7\ H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the trihydrate $B_{1,Ca}$ as a function of temperature, was measured at a heating rate of 10 K·min^{-1}. The measurements are elucidated as follows:

Temperature [°C]	weight loss or water loss in %
25	0.5
50	2.0
75	3.9
100	5.7
125	8.1
150	9.2
175	9.5
200	9.7
225	9.7
250	9.9
275	10.2

Calculated values of the interlattice plane intervals from the X-ray powder pattern measured with powder diffractometer PW 1710 from Philips Analytical X-ray, 7602 Amelo, NL are corrected with reference measurements made with the Guinier camera (FR 552 from Enraf Nonius, Delft, NL). The corrections for the interlattice plane intervals to reach the values measured and calculated for the Guinier camera from the powder diffractometer PW 1710 were ranging from +0.55Å for a d value of 16Å to +0.02Å for a d value of 5.7Å. No corrections are necessary for lower d values.

The interlattice plane intervals are given in the following for the trihydrate of the calcium salt of valsartan $B_{1,Ca}$, produced according to example 3:
d in [Å] : 16.1, 11.5, 10.0, 9.42, 9.12, 8.10, 7.78, 7.03, 6.48, 6.08, 5.76, 5.12, 4.91, 4.72, 4.48, 4.31.

Example 4

Production of the calcium salt as the trihydrate $B_{2,Ca}$ *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

33.84mg calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as tetrahydrate $A_{0,Ca}$ is placed into an open pan of a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) and the tetrahydrate $A_{0,Ca}$ is been partially dehydrated at 61°C in a water-free N_2 atmosphere with a gas flow of 50 ml·min^{-1} over a time interval of 205 minutes. The weight loss, i.e. water loss is observed directly with the thermobalance and the final stage of dehydration is selected to a water loss of 6.4%. The water still bound at this endpoint for the substance of calcium salt of valsartan was 6.5%, a value which corresponds with 1.9 mole water in relation with one mole of calcium salt of valsartan. The equilibration of the dihydrate at 23°C and 22% relative humidity in air with a relaxation time of about 30 minutes revealed a production of a trihydrate. The dehydration-rehydration process provided the substance (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt trihydrate $B_{2,Ca}$.

The melting point for the trihydrate of the calcium salt of valsartan $B_{2,Ca}$ produced according to example 4, for a heating rate of 10 K·min^{-1}, in a closed specimen container with a small internal volume, and with a sample weight of 2.49mg is determined as T_{fus} = 198°C and for the second component T_{fus} = 204°C. The two melting points are elucidated easily, namely, that the produced material is a mixture of the trihydrate $B_{2,Ca}$ and the tetrahydrate $A_{0,Ca}$. The enthalpy of fusion for the two melting peaks reveal the values for the trihydrate $B_{2,Ca}$ of $\Delta_{fus}H$ = 53 kJ·mol^{-1} and for the tetrahydrate $A_{0,Ca}$ of $\Delta_{fus}H$ = 4 kJ·mol^{-1}.

The DSC (Differential Scanning Calorimetry) curve with a sample weight of 2.49mg of the trihydrate $B_{2,Ca}$ calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine with a heating rate of 10 K·min^{-1} in an closed specimen container with a small internal volume reveals in addition to the melting peaks at 198 and 204°C a glass transition as a solid state phenomena related to amorphous substances. The glass transition temperature is determined with a value of T_g = 66°C and the change of the specific heat is for this temperature Δc_p = 0.10 J·(g·K)$^{-1}$. The glass transition temperature observed is an absolute evidence of amorphous material present in the substance produced according to example 4 and the value for the change of the specific heat is a parameter for the quantization of the amorphicity.
An additional amount of estimated 18% is amorphous material, the trihydrate $B_{2,Ca}$ is approximated with the enthalpy of fusion of 53 kJ·mol^{-1} as 78% and the tetrahydrate $A_{0,Ca}$ which has in pure form as starting material the enthalpy of fusion $\Delta_{fus}H$ = 92 kJ·mol^{-1} is approximated in the produced material of example 4 kJ·mol^{-1} with 4%.

The water content is determined with a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) with a measurement in a water-free N_2 atmosphere and a heating rate of 10 K·min^{-1}. The water content for the substance $B_{2,Ca}$, produced according to example 4, is for the plateau of the weight loss at the temperature of 225°C determined with 9.7%. A total formula was calculated from this value for $B_{2,Ca}$ as $(C_{24}H_{27}N_5O_3)^{2-} Ca^{2+} \cdot 2.8\ H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the trihydrate $B_{2,Ca}$ as a function of temperature, is measured at a heating rate of 10 K·min^{-1}. The measurements are elucidated as follows:

Temperature [°C]	Weight loss or water loss in %
25	0
50	0.7
75	1.9
100	5.5
125	8.4
150	9.2
175	9.5
200	9.7
225	9.7
250	9.8
275	10.1

Calculated values of the interlattice plane intervals from the X-ray powder pattern measured with a Guinier camera FR 552 from Euraf Nonius, Delft, NL on a X-rax film in transmission geometry, using Cu-Kα_1 radiation, are obtained for $B_{2,Ca}$. The interlattice plane intervals are given in the following for the trihydrate of the calcium salt of valsartan $B_{2,Ca}$, produced according to example 4:

d in [Å] : 16.2, 11.47, 9.94, 9.44, 9.01,8.13, 7.80, 7.05, 6.50, 6.09, 5.79, 4.95, 4.16, 4.74.

The enantiomer purity of the salt hydrate produced according to example 4 is determined by a stereo-specific HPLC method. The stereo-specific separation is achieved by achiral column (Chiral AGP). The enantiomer purity for the trihydrate of the calcium salt of valsartan $B_{2,Ca}$ is determined as ee = 99.65.

Example 5

Production of the calcium salt as the trihydrate $B_{3,Ca}$ *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

32.15mg calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as tetrahydrate $A_{0,Ca}$ is placed into an open pan of a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) and the tetrahydrate $A_{0,Ca}$ is partially dehydrated at 60°C in a water-free N_2 atmosphere with a gas flow of 50 $ml \cdot min^{-1}$ over a time interval of 255 minutes. The weight loss, i.e. water loss is observed with the thermobalance and the selected final stage of dehydration is 7.0%. The water still bound at this endpoint for the substance of calcium salt of valsartan was 5.9%, a value which corresponds with 1.4 mole water in relation with one mole of calcium salt of valsartan. The rehydration at 23°C and 30% relative humidity in air is observed with a process of a relaxation time of 30 minutes. The dehydration-rehydration process provided the substance (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt trihydrate $B_{3,Ca}$.

The melting point for the trihydrate of the calcium salt of valsartan $B_{3,Ca}$ produced according to example 5, for a heating rate of 10 $K \cdot min^{-1}$, in a closed specimen container with a small internal volume, and with a sample weight of 2.85mg is determined as T_{fus} = 191°C. Additional melting peaks are observed for the material produced according to example 5, namely for 196, 205, and 213°C. The enthalpy of fusion for the different melting peaks are used for an approximation of the quantitative analysis of the material produced according to example 5, namely 87% of the material for the melting point 191°C as $B_{3,Ca}$, 10% of the material for the melting point 196°C as $B_{2,Ca}$, 0.5% of the material for the melting point 205°C as $A_{0,Ca}$, and 3% of the material for the melting point 213°C as $D_{1,Ca}$. The results reveal clearly a material, produced according to example 5, which is dominated by a main component namely $B_{3,Ca}$.

The water content is determined with a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) with a measurement in a water-free N_2 atmosphere and a heating rate of 10 $K \cdot min^{-1}$. The water content for the material with $B_{3,Ca}$ as the main component is for the plateau at the temperature of 225°C determined with 10.1%. A total formula is calculated from for the bound water content of 9.8% for $B_{3,Ca}$ as $(C_{24}H_{27}N_5O_3)^{2-} Ca^{2+} \cdot 2.9\ H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the material, produced according to example 5, with $B_{3,Ca}$ as the dominating component is measured as a function of temperature at a heating rate of 10 $K \cdot min^{-1}$. The measurements reveal the following results:

Temperature [^{0}C]	weight loss or water loss in %
25	0.3
50	1.2
75	2.5
100	5.7
125	8.7
150	9.6
175	9.9
200	10.0
225	10.1
250	10.2
275	10.5

Calculated values of the interlattice plane intervals from the X-ray powder pattern measured with a Guinier camera FR 552 from Euraf Nonius, Delft, NL on a X-rax film in transmission geometry, using Cu-Ka_1 radiation, are obtained for the substance produced according to example 5, with the main component $B_{3,Ca}$.

The interlattice plane intervals are given in the following for the material, produced according to example 5, with the trihydrate of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $B_{3,Ca}$ as the dominating component:

d in [Å] : 16.11, 11.44, 9.90, 9.40, 9.01, 8.04, 7.73, 7.03, 6.47, 6.33, 6.09, 5.80, 5.17, 4.95, 4.73, 4.48, 4.33, 4.15, 4.11, 3.94, 3.61.

The infrared spectrum of the (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt trihydrate $B_{3,Ca}$, as the main component of the material produced according to example 5 is measured with a ATR-IR instrument BX from Perkin Elmer Corp., Beaconsfield, Bucks, England.

The characteristic absorption bands of the ATR-IR spectroscopy are listed in the following for the material produced according to example 5, containing the dominating substance, namely the calcium salt as trihydrate (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $B_{3,Ca}$ with values expressed in reciprocal wave numbers (cm^{-1}): 3594; 3309; 2959; 2930; 2870; 1621; 1577; 1505; 1458; 1416; 1405; 1354; 1273; 1210; 1179; 1138; 1104; 1099; 1012; 1003; 974; 941; 906; 856; 841; 737; 667.

Example 6

Production of the calcium salt as monohydrate $C_{1,Ca}$ *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine

65.5mg calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as tetrahydrate $A_{0,Ca}$ is pressed into an open crucible of a device which allows to set a temperature and a humidity program as function of time and register for selected time intervals the X-ray diffraction pattern (powder diffraction chamber X'Pert from Philips Analytical X-ray, 7602 Almelo, NL). The isothermal temperature is 40°C and the water-free N_2 atmosphere is set at a flow rate of 100 $ml \cdot min^{-1}$. In a parallel production step 4.66 mg (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as tetrahydrate $A_{0,Ca}$ is placed in an open crucible of a thermobalance TGS-2 (Perkin-Elmer Corp., Norwalk, CT USA) and the tetrahydrate $A_{0,Ca}$ is exposed to the following conditions: isothermal temperature 40°C, and to a water-free atmosphere with a flow rate of 50 $ml \cdot min^{-1}$. The substance obtained in both of the devices after 66 hours was the monohydrate of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $C_{1,Ca}$.

The water content was determined with the thermobalance TGS-2. The weight loss, i.e. the water loss after 66 hours in the water-free atmosphere was 9.8%, yielding to a water content in the product $C_{1,Ca}$ of 3.1%. A total formula was calculated from this value for $C_{1,Ca}$ produced according to example 7 as $(C_{24}H_{27}N_5O_3)^{2-} Ca^{2+} \cdot 0.9\ H_2O$.

Calculation of the interlattice plane intervals of the monohydrate of the calcium salt of valsartan $C_{1,Ca}$ had been taken from the X-ray powder patterns measured with the powder diffraction chamber X'Pert from Philips Analytical X-ray, 7602 Almelo, NL. The characteristic lines for the product $C_{1,Ca}$ are listed in the following:

d in [Å] : 15.96, 15.04, 11.56, 9.85, 9.40, 8.02, 7.53, 6.11, 4.49.

Example 7

Production of the di-{calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine} pentahydrate $D_{1,Ca}$ *in situ*

30.65mg of the calcium salt as tetrahydrate of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $A_{0,Ca}$ is placed into an open pan of a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) and the tetrahydrate $A_{0,Ca}$ is exposed at a temperature of 90°C in a water-free atmosphere with a gas flow of 50 $ml \cdot min^{-1}$ over a time interval of 55 minutes. The dehydration of the tetrahydrate reached at the selected final stage a weight loss, i.e. a water loss of 9.7%. The water bound at this endpoint for the product of calcium salt of valsartan was 3.2%, a value which corresponds to 0.9 mole water in relation with one mole of calcium salt of valsartan. The hydration process is performed at 23°C and with a relative humidity in air of 28%. The final equilibration is practically reached after 4 hours. The process provided the product di-{calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine} pentahydrate $D_{1,Ca}$.

The melting point for the di-{calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine} pentahydrate $D_{1,Ca}$, produced according to example 7, for a heating rate of 10 $K \cdot min^{-1}$, in a closed specimen container with a small internal volume, and with a sample weight of 1.41mg is determined as T_{fus} = 212°C and the melting enthalpy is $\Delta_{fus}H = 15 kJ \cdot Mol^{-1}$.

The water content is determined with a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) with a measurement in a water-free N_2 atmosphere and a heating rate of 10 $K \cdot min^{-1}$. The water content for the substance $D_{1,Ca}$, produced according to example 7, is for the plateau at the temperature of 225°C determined with 8.1%. A total formula is calculated with the amount of bound water which is 8.0% for $D_{1,Ca}$ as $[(C_{24}H_{27}N_5O_3)^{2-}Ca^{2+}]_2 \cdot 4.6\ H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the di-{calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine} pentahydrate $D_{1,Ca}$, produced according to example

7, is measured as a function of temperature at a heating rate of 10 $K \cdot min^{-1}$. The measurements reveal the following results:

Temperature [°C]	Weight loss or water loss in %
25	0.1
50	1.7
75	3.3
100	5.1
125	7.1
150	7.7
175	7.9
200	8.0
225	8.1
250	8.3
275	8.6

The infrared spectrum of the di-{calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine} pentahydrate is measured with a ATR-IR instrument BX from Perkin Elmer Corp., Beaconsfield, Bucks, England. The characteristic absorption bands of the ATR-IR spectroscopy for the di-{calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine} pentahydrate $D_{1,Ca}$ are listed in the following with values expressed in reciprocal wave numbers (cm^{-1}): 3329; 2959; 2930; 2870; 1578; 1506; 1459; 1405; 1354; 1302; 1260; 1208; 1176; 1143; 1104; 1012; 1004; 973; 941; 860; 839; 821; 757; 737; 667.

Calculated values of the interlattice plane intervals from X-ray powder patterns were obtained from a Guinier camera FR 552 from Euraf Nonius, Delft, NL on a X-rax film in transmission geometry, using Cu-$K\alpha_1$ radiation.
The characteristic interlattice plane intervals are given in the following for the the di-{calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine} pentahydrate $D_{1,Ca}$, produced according to example 7:
d in [Å] : 15.46, 11.45, 9.36, 9.04, 7.75,6.46, 6.09, 5.82, 5.66, 5.16, 4.76, 4.48, 3.83, 3.60.

Example 8

Production of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as amorphous substance $E_{1,Ca}$ *in situ.*

3.53mg calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as tetrahydrate $A_{0,Ca}$ is put into a hermetically closed specimen container made from gold with an internal volume of ca. 22 microliters. The starting substance $A_{0,Ca}$ is heated up in a DSC Pyris 1 after cooling to –50°C to 216°C and therefore transfered into the molten phase. The substance is taken out of the gold container after cooling the container to room temperature. (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine calcium salt $E_{1,Ca}$ in amorphous form is obtained.

The substance $E_{1,Ca}$, produced according to example 8, contains 12.9% of water. The thermal characterization of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine in amorphous form $E_{1,Ca}$ with a DSC Pyris 1 (Perkin-Elmer, Norwalk, CT USA) for a heating rate of 10 $K \cdot min^{-1}$ and in a closed specimen container from gold with a small internal volume,reveals a glass transition temperature T_g = 101°C with a change of the specific heat capacity at the temperature region of the melting point of $\Delta c_p = 0.64\ J \cdot (g \cdot K)^{-1}$. No melting point and no enthalpy of fusion is observed up to a temperature of 216°C measured with the DSC Pyris 1 under the same conditions as performed for the glass transition measurements.

The infrared spectrum of the amorphous calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $E_{1,Ca}$ is measured with an ATR-IR instrument BX from Perkin Elmer Corp., Beaconsfield, Bucks, England.
The characteristic absorption bands of the ATR-IR spectroscopy are shown for the amorphous substance $E_{1,Ca}$, produced according to example 8, by the following values expressed in reciprocal wave numbers (cm^{-1}): 3587; 3307; 3182; 3053; 2961; 2870; 2358; 1621; 1578; 1506; 1459; 1441; 1417; 1364; 1319; 1301; 1274; 1211; 1180; 1137; 1105; 1099; 1013; 1003; 974; 941; 864; 856; 844; 823; 758; 738; 666.

Example 9

Production of the calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as amorphous substance $F_{1,Ca}$ *in situ*.

4.14mg of the substance of the calcium salt trihydrate of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $B_{3,Ca}$ is placed in an open crucible of a thermobalance TGS-2 (Perkin-Elmer, Norwalk, CT USA) and heated with a heating rate of 10 $K{\cdot}min^{-1}$ from room temperature up to 225°C. The substance $B_{3,Ca}$ is exposed in the thermobalance to a water-free atmosphere. The dehydrated substance obtained with a weight loss, i.e. water loss of 9.4% at 225°C is consecutively exposed to 31% relative humidity, and 23°C in air and a rehydration over 18 hours lead to the product, namely the amorphous calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $F_{1,Ca}$.

The substance $F_{1,Ca}$, produced according to example 9, is characterized with a DSC Pyris 1 (Perkin-Elmer, Norwalk, CT USA) applying a heating rate of 10 $K{\cdot}min^{-1}$ and using a closed specimen container from gold with a small internal volume. The calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as amorphous substance $F_{1,Ca}$ allowed to elucidate the observed glass transition with the DSC (Differential Scanning Calorimeter). The glass transition temperature is T_g = 139°C and the change of the specific heat capacity at the temperature region of the glass transition is $\Delta c_p = 0.42\ J{\cdot}(g{\cdot}K)^{-1}$. No melting point and no enthalpy of fusion is observed in the DSC when the substance $F_{1,Ca}$ is after cooling to –50°C heated up to 220°C with a heating rate of 10 $K{\cdot}min^{-1}$. Therefore, the substance $F_{1,Ca}$ has a crystallinity which is not detectable with the method applied and the crystallinity is by an estimation of the sensitivity of the DSC Pyris 1 below 1%. The combined thermodynamic data existing, namely melting point and enthalpy of fusion are an absolute prerequisite of a crystalline material or a crystalline substance.

The water content of the amorphous calcium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $F_{1,Ca}$ is determined using thermogravimetry, in a water-free atmosphere. The weight loss, i.e. the water loss for the substance $F_{1,Ca}$ as a function of temperature, is measured at a heating rate of 10 $K{\cdot}min^{-1}$ and the results are listed in the following.

Temperature [°C]	Weight loss or water loss in %
25	0.6
50	3.0
75	5.6
100	7.1
125	7.9
150	8.3
175	8.6
200	8.6
225	8.8

The total formula of $F_{1,Ca}$ is calculated as $(C_{24}H_{27}N_5O_3)^{2-}Ca^{2+}$ containing 8.8% water.

Example 10

Production of the magnesium salt as hexahydrate $A_{1,Mg}$ *in situ* of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine.

28.81mg of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as hexahydrate $A_{0,Mg}$ is placed into an open crucible of a thermobalance TGS-2 (Perkin-Elmer, Norwalk, CT USA) and treated in a water-free N_2 atmosphere at a temperature of 50°C, having a flow rate of 50 $ml \cdot min^{-1}$ for a time interval of 200 minutes. The weight loss, i.e. water loss at the endpoint was 9.4%. The water still bound at this end point for the magnesium salt of valsartan was under consideration of the water content for the start material $A_{0,Mg}$ which is 18.7%, corresponds to 2.6 mole of water in relation with one molecule magnesium salt of valsartan. The substance obtained after this dehydration step is practically a trihydrate, which is exposed in a consecutive step in air to a relative humidity of 31% at 24°C. The uptake of water revealed a relaxation time of about 70 minutes. The substance obtained in reaching an equlibrium condition is the polymorph $A_{1,Mg}$ hexahydrate of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine.

The melting point for hexahydrate of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $A_{1,Mg}$, produced according to example 10, for a heating rate of 10 $K \cdot min^{-1}$, and measured in a closed specimen container with a small volume of ca. 22 microliters, and with a sample weight of 1.92mg is T_{fus} = 134°C. The enthalpy of fusion measured also with a DSC Pyris 1 is for $A_{1,Mg}$, produced according to example 10, $\Delta_{fus}H$ = 46kJ·Mol^{-1}.

The water content is in theory 19.1% for the hexahydrate of the magnesium salt of valsartan. The water content of the hexahydrate of the magnesium salt of valsartan of the polymorph $A_{1,Mg}$ is 17.4%, measured as weight loss for the plateau at 225°C. The total formula calculated from this as the polymorph of the hexahydrate $A_{1,Mg}$ is $(C_{24}H_{27}N_5O_3)^{2-}\ Mg^{2+} \cdot 5.5\ H_2O$.

Using thermogravimetry, in a water-free N_2 atmosphere, the weight loss, i.e. the water loss for the polymorph of the hexahydrate $A_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine produced according to example 7, is as a function of temperature, measured at a heating rate of 10 K·min^{-1} as follows:

Temperature [°C]	Weight loss or water loss in %
25	0
50	0.9
75	6.8
100	14.3
125	15.7
150	16.5
175	17.0
200	17.2
225	17.4
250	17.8
275	18.3

The solid state characterization of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine for the polymorph of the hexahydrate $A_{1,Mg}$ is achieved by a X-ray powder pattern and by the evaluation of the reflections into the interlattice plane intervals. The measurements are made with a Guinier camera and the calculated lines for $A_{1,Mg}$, namely the polymorph of the hexahydrate of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine are expressed in interlattice plane intervals as follows:

d in [Å] : 19.58, 16.63, 10.30, 9.83, 7.40, 6.83, 6.01, 5.93, 5.52, 5.34, 5.20, 5.11, 5.02, 4.87, 4.51, 4.13, 4.06, 3.95, 3.73, 3.63, 3.42.

The enantiomer purity of the salt hydrate produced according to example 10, namely $A_{1,Mg}$ is determined by a stereo-specific HPLC method. The enantiomer purity is determined as ee = 99.63%.

Example 11

Production of a material as mixture of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as tetrahydrate $B_{1,Mg}$ and the amorphous substance $E_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine and the crystalline substance as monohydrate $D_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine.

71.4mg of magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as hexahydrate $A_{0,Mg}$ is brought into an open crucible of a device which allows to set a temperature and a humidity program as function of time and register for selected time intervals the X-ray diffraction pattern (powder diffraction chamber X'Pert from Philips Analytical X-ray, 7602 Almelo, NL). The isothermal temperature is set at 35°C and the water-free N_2 atmosphere is achieved with a flow rate of 100 $ml \cdot min^{-1}$. In a parallel production step 5.36mg magnesium salt of valsartan as hexahydrate $A_{0,Mg}$ is filled into an open crucible of a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA) and the start material was exposed to practically identical conditions as the start material in the X-ray device, namely an isothermal temperature of 35°C and a water-free atmosphere with a flow rate 50 $ml \cdot min^{-1}$. The substance obtained after 42 hours in the thermobalance is the monohydrate $D_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine. The substance obtained in the powder diffraction chamber X'Pert is determined by the interlattice plane intervals as monohydrate $D_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine.

The bound water content of the monohydrate $D_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine produced according to example 11 is determined with the thermobalance TGS-2 and is 2.8%. The total formula for $D_{1,Mg}$ is calculated from this as $(C_{24}H_{27}N_5O_3)^{2-} Mg^{2+} \cdot 0.74\ H_2O$. Calculation of the interlattice plane intervals from the X-ray powder pattern taken with temperature-humidity powder diffraction chamber X'Pert is for the most important lines of the monohydrate $D_{1,Mg}$ of the magnesium valsartan:
d in [Å] : 15.10, 10.87, 10.27, 7.66, 7.21, 5.12, 4.75.

The substance, namely the monohydrate $D_{1,Mg}$ is kept for additional 35 hours at 35°C in a water-free atmosphere in the thermobalance as well as in the powder diffraction chamber X'Pert. Both of the substances obtained after 70 hours from the beginning of the treatment in the two different devices revealed according to the thermobalance and the X-ray diffraction pattern the existence of the monohydrate $D_{1,Mg}$ of the magnesium salt of valsartan. After 70 hours both of the substances were exposed to a higher relative humidity. In the X-ray device X'Pert the conditions were 26°C and the relative humidity 45%. In the thermobalance the conditions were 23°C and 30% relative humidity in air. Both of the materials obtained, produced according to example 11 are mixtures of the tetrahydrate $B_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine and the amorphous magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine $E_{1,Mg}$.

The solid state characterization of the material produced finally after equilibration according to example 11 is performed with a DSC Pyris 1 from Perkin-Elmer Corp., Norwalk, CT USA. The glass transition is measured with a sample weight of 2.57mg in a sealed gold container with a small internal volume of ca. 22 microliters and applying a heating rate of 10 $K \cdot min^{-1}$. The glass transition temperature for the amorphous magnesium salt of valsartan $E_{1,Mg}$ as a part of the material produced according to example 11 is T_g = 100°C and the change of the specific heat $\Delta c_p = 0.3\ J \cdot (g \cdot K)^{-1}$.

The water content of the material produced according to the example 11 within the powder diffraction chamber X'Pert is for the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine 13.0% measured with a thermobalance TGS-2. The total formula is approximated from this content of water for the crystalline part $B_{1,Mg}$ of the material produced according to example 11 as $(C_{24}H_{27}N_5O_3)^{2-}$ $Mg^{2+} \cdot 3.8\ H_2O$.

The material, produced according to example 11 within the powder diffraction chamber X'Pert with the main component $E_{1,Mg}$ and the second component $B_{1,Mg}$ shows the following loss of water as a function of temperature measured with a thermobalance TGS-2 (Perkin-Elmer Corp. Norwalk, CT USA). The heating rate selected was 10 K·min^{-1}. The weight loss is tabulated as follows:

Temperature [° C]	weight loss or water loss in %
25	0
50	2.1
75	6.3
100	9.4
125	11.1
150	12.0
175	12.3
200	12.6
225	13.0
250	13.5
275	14.2

The crystalline part $B_{1,Mg}$ as the tetrahydrate of the magnesium salt of valsartan has been characterized with calculated plane intervals from X-ray measurements performed with a temperature-humidity powder diffraction chamber. The characteristic lines for the crystalline part of this material are listed as follows:

d in [Å] : 15.82, 11.02, 8.03.

Measurements of the infrared spectrum took place by means of ATR-IR (Attenuated Total Reflection – Infrared Spectroscopy) using the instrument BX. The following characteristic absorption bands expressed in reciprocal wave numbers (cm^{-1}) for the material produced according to example 11 within the thermobalance TGS-2 of Perkin-Elmer Corp., namely the amorphous from $E_{1,Mg}$ as the main component and the crystalline form $B_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine:

3182; 2960; 2870; 1596; 1508; 1460; 1406; 1359; 1302; 1264; 1206; 1174; 1104; 1013; 1005; 975; 941; 845; 819; 785; 738; 666.

Example 12

Production of the trihydrate $C_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine.

76.3mg of magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as hexahydrate $A_{0,Mg}$ is pressed into an open crucible of a device which allows to set a temperature and a humidity program as function of time and register for selected time intervals the X-ray diffraction pattern (powder diffraction chamber X'Pert). The isothermal temperature is 28°C and the water-free N_2 atmosphere is set at a flow rate of 100 ml·min^{-1}. In a parallel production step 4.75mg of magnesium salt of valsartan as hexahydrate $A_{0,Mg}$ is placed in an open crucible of a thermobalance TGS-2. The atmosphere in the thermobalance is water-free and the instrument is flashed with a N_2 flow of 50 ml·min^{-1}. The substance obtained after 13 hours is the trihydrate $C_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine.

The water content of the substance $C_{1,Mg}$ is determined with a thermobalance TGS-2. The weight loss, i.e. water loss after 13 hours in a water-free atmosphere at a temperature of 28°C is 8.5%, yielding a bound water content in the product $C_{1,Mg}$ of 10.0%. A total formula is calculated from this value for $C_{1,Mg}$ as $(C_{24}H_{27}N_5O_3)^{2-} Mg^{2+} \cdot 2.8\ H_2O$.

Calculation of the interlattice plane intervals is taken from X-ray powder patterns measured with a powder diffraction chamber X'Pert. The characteristic lines for the trihydrate $C_{1,Mg}$ of the magnesium salt of valsartan are as follows:
d in [Å] : 17.94, 10.23, 8.96, 7.18, 6.97, 6.81, 6.24, 5.93, 5.84, 5.72, 5.59, 5.42, 5.25, 5.11, 5.01, 4.82, 4.67, 4.57, 4.49, 4.30, 4.19, 4.13, 4.02, 3.88.

Example 13

Production of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine in amorphous form $E_{1,Mg}$.

4.02 mg magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine as hexahydrate $A_{0,Mg}$ is filled into a sample pan of a DSC Pyris 1 and the substance was cooled to –50°C and heated up to 145°C. The cooling rate was 100 $K \cdot min^{-1}$ and heating was 10 $K \cdot min^{-1}$. After cooling the molten substance to room temperature the substance $E_{1,Mg}$ was obtained as amorphous form of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine.

The characterization of the substance $E_{1,Mg}$, produced according to example 14 is performed with the DSC Pyris 1. The cooling of the obtained substance $E_{1,Mg}$ to –50°C and the heating up to 145°C in a DSC Pyris 1 with a heating rate of 10 $K \cdot min^{-1}$ in a sealed gold container with a small internal volume revealed a glass transition phenomena. The glass transition temperature measured for the amorphous form $E_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine is T_g = 73°C and the change of the specific heat capacity is $\Delta c_p = 0.53\ J \cdot (g \cdot K)^{-1}$.

The water content is measured in a water-free N_2 atmosphere and at a heating rate of 10 $K \cdot min^{-1}$ using a thermobalance TGS-2. The sample weight is 2.5mg and the water content is determined for the amorphous form $E_{1,Mg}$ of the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine, produced according to example 13 at the plateau of the weight loss for a temperature of 225°C with 15.5%. The total formula of $E_{1,Mg}$ was calculated as $(C_{24}H_{27}N_5O_3)^{2-} Mg^{2+}$ containing 15.5% water.

Measurements of the infrared spectrum took place by means of ATR-IR (Attenuated Total Reflexion-Infrared Spectroscopy) using an instrument BX. The following most important absorption bands expressed in reciprocal wave numbers (cm^{-1}) characterize the magnesium salt of (S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amine in amorphous form $E_{1,Mg}$ produced according to example 13:
3189; 2959; 2871; 2356; 1589; 1507; 1459; 1405; 1358; 1299; 1263; 1206; 1174; 1104; 1013; 1005; 974; 942; 841; 736; 668.

<u>Formulation example 1:</u>

Directly compressed tablet:

No.	Ingredient	proportion per batch [g]	proportion per tablet core [mg]
1	valsartan calcium salt tetrahydrate according to the present invention	134.24	80
2	Avicel PH 102 (microcrystalline cellulose)	60.408	36
3	lactose (crystalline)	96.1494	57.3
4	crospovidone	7.551	4.5
5	aerosil 200 (silica, colloidal anhydrous)	0.839	0.5
6	magnesium stearate (vegetable)	6.2086	3.7

Ingredient no. 1 is sieved through a 0.5 mm sieve and mixed for 15 minutes in a Turbula with ingredients 1-6. Tablets are compress using a single punch tablet press with punches of a diameter of 8mm.

Formulation example 2:

Tablet produced by roller compaction:

No.	Ingredient	proportion per batch [g]	proportion per tablet core [mg]
1	valsartan magnesium salt hexahydrate according to the present invention	400	80
2	Avicel PH 102 (microcrystalline cellulose)	270	54
3	crospovidone	75	15
	aerosil 200 (silica, colloidal anhydrous)	7.5	1.5
5	magnesium stearate	15	3
6	magnesium stearate	7.5	1.5

Ingredients no. 1-5 are mixed for 50 minutes and compacted on a Freund roller compactor. The band is milled and after admixing ingredient no 6, compressed into tablets using a single punch tablet press with punches of a diameter of 8mm.

What is claimed is:

1. A salt of valsartan selected from (i) polymorphs of the tetrahydrate,
(ii) polymorphs of the trihydrate,
(iii) the monohydrate, and
(iv) the di-(calcium salt of valsartan) pentahydrate,
in each case of the calcium salt of valsartan, and the anhydrate thereof;
and selected from
(i) a polymorphic form of the hexahydrate,
(ii) the trihydrate,
(iii) the monohydrate, and
(iv) the tetrahydrate;
in each case of the magnesium salt of valsartan, and the anhydrate thereof.

2. A salt according to claim 1 in crystalline, partially crystalline or amorphous form.

3. The tetrahydrate of the calcium salt of valsartan according to claim 1, characterised by (i) an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals: d in [Å]: 16.2±0.3, 11.4±0.2, 9.9±0.2, 9.4±0.2, 8.06±0.1, 7.05±0.1, 6.50±0.05, 5.82±0.05, 4.94±0.05, 4.73±0.05, 4.33±0.05, 4.17±0.05, 4.13±0.05, 3.93±0.05 or
(ii) an ATR-IR spectrum having the following absorption bands expressed in reciprocal wave numbers (cm^{-1}): 2960 (m); 1621 (st); 1578 (st); 1459 (m); 1442 (m); 1417 (m); 1407 (m); 1364 (m); 1357(m); 1012 (m); 758 (m); 738 (st); 698 (m).

4. The tetrahydrate of the calcium salt of valsartan according to claim 1, characterised by an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals: d in [Å]: 16.2±0.3, 9.9±0.2, 9.4±0.2, 8.05±0.1, 7.04±0.1, 6.49±0.05, 5.82±0.05, 4.94±0.05, 4.13±0.05, 3.93±0.05.

5. The trihydrate of the calcium salt of valsartan according to claim 1, characterised by an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals:

d in [Å]: 16.0±0.3, 11.4±0.2, 10.0±0.2, 9.4±0.2, 8.06±0.1, 7.75±0.1, 7.03±0.1, 6.48±0.05, 6.10±0.05, 5.16±0.05, 4.75±0.05.

6. The trihydrate of the calcium salt of valsartan according to claim 1, characterised by an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals: d in [Å]: 16.2±0.3, 11.5±0.2, 9.9±0.2, 9.4±0.2, 7.04±0.1, 6.50±0.1, 5.79±0.05, 4.74±0.05, 4.16±0.05, 3.96±0.05.

7. The trihydrate of the calcium salt of valsartan according to claim 1, characterised by an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals:
(i) d in [Å]: 16.1±0.3, 11.4±0.2, 9.9±0.2, 9.4±0.2, 9.0±0.1, 7.03±0.1, 6.47±0.05, 5.79±0.05, 4.15±0.05, 3.94±0.05; or
(ii) an ATR-IR spectrum having the following absorption bands expressed in reciprocal wave numbers (cm^{-1}): 1621(st); 1577(m); 1458(m); 1405(m); 1354(w); 1273(w); 1012(w); 756(m); 737(m); 667(m).

8. The monohydrate of the calcium salt of valsartan according to claim 1, characterised by an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals: d in [Å]: 16.0±0.3, 15.0±0.3, 11.6±0.2, 9.4±0.2, 7.53±0.1, 6.11±0.05.

9. The pentahydrate of the di-(calcium salt of valsartan) according to claim 1, characterised by an x-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals: d in [Å]: 15.5±0.3, 11.5±0.2, 9.4±0.2, 9.04±0.1, 6.46±0.05, 6.09±0.05, 5.82±0.05, 5.16±0.05, 4.48±0.05, 3.60±0.05.

10. An amorphous calcium salt of valsartan according to claim 1, characterised by heating up from a temperature far below 0°C in open or closed sample pans up to 220°C or higher temperatures by a glass transition temperature with a change of the specific heat capacity and showing no melting point and no enthalpy of fusion.

11. The amorphous calcium salt of valsartan according to claim 1, characterised by
(i) a water content of 11 ± 2%
(ii) a glass transition of 94 ± 20°C

(iii) no melting point
(iv) no enthalpy of fusion.

12. The amorphous calcium salt of valsartan according to claim 1, characterised by
(i) a water content of 9 ± 2%
(ii) a glass transition of 143 ± 20°C
(iii) no melting point
(iv) no enthalpy of fusion.

13. The hexahydrate of the magnesium salt of valsartan according to claim 1, characterised by an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals: d in [Å]: 19.6±0.3, 16.6±0.3, 10.3±0.2, 9.8±0.2, 7.3±0.1, 6.01±0.05, 5.92±0.05, 5.55±0.05, 5.38±0.05, 4.90±0.05, 4.13±0.05, 4.07±0.05, 3.43±0.05.

14. The tetrahydrate of the magnesium salt of valsartan according to claim 1, characterised by an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals: d in [Å]: 15.8±0.3, 11.0±0.2, 8.0±0.2.

15. The trihydrate of the magnesium salt of valsartan according to claim 1, characterised by an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals: d in [Å]: 17.9±0.3, 10.2±0.2, 8.96±0.2, 7.18±0.1, 5.93±0.05, 5.84±0.05, 5.42±0.05, 5.11±0.05, 5.01±0.05, 4.82±0.05, 4.67±0.05, 4.30±0.05, 4.19±0.05, 4.13±0.05, 4.02±0.05.

16. The monohydrate of the magnesium salt of valsartan according to claim 1, characterised by an X-ray powder pattern taken with a Guinier camera comprising the following interlattice plane intervals: d in [Å]: 15.1±0.2, 10.9±0.2, 10.3±0.2, 7.66±0.1, 5.12±0.05.

17. An amorphous magnesium salt of valsartan according to claim 1, characterised by heating up from a temperature far below 0°C in open or closed sample pans up to 220°C or higher temperatures by a glass transition temperature with a change of the specific heat capacity and showing no melting point and no enthalpy of fusion.

18. The amorphous magnesium salt of valsartan according to claim 1, characterised by
(i) a water content of 16 ± 3%
(ii) a glass transition of 78 ± 20°C
(iii) no melting point
(iv) no enthalpy of fusion.

19. A salt according to claim 1 in a form selected from the group consisting of
(i) a crystalline form;
(ii) a partly crystalline form;
(iii) an amorphous form; and
(iv) a polymorphous form.

20. A salt according to claim 1 in the form of a solvate.

21. A salt according to claim 1 in the form of a hydrate.

22. A salt according to claim 1 in the form of an anhydrate.

23. A pharmaceutical composition comprising a salt according to claim 1 and a pharmaceutically acceptable excipient or additive.

24. Pharmaceutical preparation according to claim 23, further comprising at least one compound selected from the group consisting of a:
(i) HMG-Co-A reductase inhibitor or a pharmaceutically acceptable salt thereof,
(ii) angiotensin converting enzyme (ACE) Inhibitor or a pharmaceutically acceptable salt thereof,
(iii) calcium channel blocker or a pharmaceutically acceptable salt thereof,
(iv) aldosterone synthase inhibitor or a pharmaceutically acceptable salt thereof,
(v) aldosterone antagonist or a pharmaceutically acceptable salt thereof,
(vi) dual angiotensin converting enzyme/neutral endopeptidase (ACE/NEP) inhibitor or a pharmaceutically acceptable salt thereof,
(vii) endothelin antagonist or a pharmaceutically acceptable salt thereof,
(viii) renin inhibitor or a pharmaceutically acceptable salt thereof, and
(ix) diuretic or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/01047

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D257/04 A61K31/41
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07D A61K
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02 06253 A (NOVARTIS ERFIND VERWALT GMBH ;MARTI ERWIN (CH); NOVARTIS AG (CH);) 24 January 2002 (2002-01-24) abstract examples e.g. example 10 claims 1,3-7 page 2, paragraph 2 - paragraph 4 -----	1-24

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"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 when the document is taken alone

"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

Date of the actual completion of the international search	Date of mailing of the international search report
3 April 2003	13. 06. 03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stix, E

Form PCT/ISA/210 (second sheet) (July 1992)

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

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

2. [ ] Claims Nos.:
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. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

see additional sheet

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

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

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] 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 claims Nos.:

1-2(part),3,4,10-12(part),19-21(part),23-24(part)

Remark on Protest

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

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

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-2 (part),3,4,10-12(part),19-21(part),23-24(part)

   Option (i): Polymorphs of the tetrahydrate of the calcium salt of valsartan

2. Claims: 1-2 (part),5-7,10-12(part),19-21(part),23-24(part)

   Option (ii): Polymorphs of the trihydrate of the calcium salt of valsartan

3. Claims: 1-2 (part),8,10-12(part),19-21(part),23-24(part)

   Option (iii): Polymorphs of the monohydrate of the calcium salt of valsartan

4. Claims: 1-2 (part),9,10-12(part),19-21(part),23-24(part)

   Option (iv): Polymorphs of the pentahydrate of the dicalcium salt of valsartan

5. Claims: 1-2 (part),13,17-21(part),23-24(part)

   Option (i'): Polymorphic form of the hexahydrate of the magnesium salt of valsartan

6. Claims: 1-2 (part),15,17-21(part),23-24(part)

   Option (ii'): Polymorphic form of the trihydrate of the magnesium salt of valsartan

7. Claims: 1-2 (part),16,17-21(part),23-24(part)

   Option (iii'): Polymorphic form of the monohydrate of the magnesium salt of valsartan

8. Claims: 1-2 (part),14,17-21(part),23-24(part)

   Option (iv'): Polymorphic form of the tetrahydrate of the magnesium salt of valsartan

9. Claims: 1-2 (part),10-12(part),17-21(part),22,23-24(part)

   Polymorphic form of the anhydrate of the magnesium or calcium salt of valsartan

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/01047

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0206253	A	24-01-2002	AU	8967201 A	30-01-2002
			WO	0206253 A1	24-01-2002
			EP	1313714 A1	28-05-2003
			NO	20030232 A	17-01-2003

Form PCT/ISA/210 (patent family annex) (July 1992)

10-05-04 JFW 1614

CASE 4-32219A

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EV 540154701	October 4, 2004
Express Mail Label Number	Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF — Art Unit: 1614

KSANDER ET AL.

APPLICATION NO: 10/341,868

FILED: JANUARY 14, 2003

FOR: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

STATUS REQUEST

Sir:

Applicants respectfully inquire as to the status of the above-identified application.

Respectfully submitted,

Gregory D. Ferraro
Attorney for Applicants
Reg. No. 36,134

Novartis
Corporate Intellectual Property
One Health Plaza, Building 430
East Hanover, NJ 07936-1080
(862) 778-7831

Date:October 4, 2004

12-01-04

1614
JFW

CASE 4-32219A

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EV 457867920US — Express Mail Label Number
11/30/04 — Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
KSANDER ET AL.
APPLICATION NO: 10/341,868
FILED: JANUARY 14, 2003
FOR: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Art Unit: 1614
Examiner: Criares, T.

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Sir:

Prior to the Examination of the above-identified application, Applicants respectfully request the following amendment be entered and the claims considered in light thereof.

Amendments to the claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

This listing of the claims will replace all prior versions, and listings, of claims in the application.

1. (currently amended) A pharmaceutical composition comprising:

(i) the AT 1-antagonist valsartan or a pharmaceutically acceptable salt thereof; and

(ii) a the NEP inhibitor *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester or (2R,4S)-5-Biphenyl -4-yl-4(3-carboxy-propionyl amino)-2-methyl-pentanoic acid ora pharmaceutically acceptable salts thereof and a pharmaceutically acceptable carrier.

2. (cancel)

3. (currently amended) The pharmaceutical composition of Claim 21, wherein *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester is a triethanolamine or *tris*(hydroxymethyl)aminomethane salt thereof.

4. (currently amended) A kit comprising in separate containers in a single package pharmaceutical compositions comprising in one container a pharmaceutical composition comprising a NEP inhibitor *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester or (2R,4S)-5-Biphenyl -4-yl-4(3-carboxy-propionyl amino)-2-methyl-pentanoic acid or pharmaceutically acceptable salts thereof and in a second container a pharmaceutical composition comprising valsartan.

5. (currently amended) A method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure, such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, such as Alzheimer's, glaucoma and stroke, comprising administering a therapeutically effective amount of combination of:

(i) the AT 1-antagonists valsartan or a pharmaceutically acceptable salt thereof; and

(ii) a the NEP inhibitor *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester or its active metabolite or (2R,4S)-5-Biphenyl -4-yl-4(3-carboxy-propionyl amino)-2-methyl-pentanoic acid or a pharmaceutically acceptable salts thereof and a pharmaceutically acceptable carrier to a mammal in need of such treatment.

6. (cancel)

7. (currently amended) The method of Claim 65, wherein *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester is a triethanolamine or tris(hydroxymethyl)aminomethane salt thereof.

8. (previously presented) A triethanolamine salt of *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester.

9. (previously presented) A *tris*(hydroxymethyl)aminomethane salt of *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester.

10. (previously presented) A pharmaceutical composition comprising the salt of Claim 8.

11. (previously presented) A pharmaceutical composition comprising the salt of Claim 9.

REMARKS

Consideration of the above-identified application as amended is requested. Claims 1, 3-5 and 7-11 remain in this application. Claims 1, 3-5 and 7 have been amended. These amendments do not introduce new matter into the application, since the compound (2R,4S)-5-Biphenyl -4-yl-4(3-carboxy-propionyl amino)-2-methyl-pentanoic acid is the active metabolite of *N*-(3-carboxy-1-oxopropyl)-(*4S*)-*p*-phenylphenylmethyl)-4-amino-*2R*-methylbutanoic acid ethyl ester and is disclosed in U.S. Patent No. 5,217,996 which is disclosed on page 6 of the specification and incorporated into the present application by reference.

In view of the foregoing, Applicant submits the Application is now in condition for allowance and respectfully requests early notice to that effect.

Respectfully submitted,

Gregory D. Ferraro
Attorney for Applicants
Reg. No. 36,134

Novartis
Corporate Intellectual Property
One Health Plaza, Building 104
East Hanover, NJ 07936-1080
(862) 778-7831

Date: November 29, 2004

PATENT APPLICATION FEE DETERMINATION RECORD
Effective January 1, 2003

Application or Docket Number
10/341868

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	11	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	11 minus 20=	* 0
INDEPENDENT CLAIMS	5 minus 3 =	* 2
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐ OR OTHER THAN SMALL ENTITY

SMALL ENTITY RATE	FEE		OTHER THAN SMALL ENTITY RATE	FEE
BASIC FEE	$375	OR	BASIC FEE	$750
X$ 9=		OR	X$18=	
X42=		OR	X84=	168
+140=		OR	+280=	
TOTAL		OR	TOTAL	918

CLAIMS AS AMENDED - PART II

11/30/04

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 9	Minus	** 20	= —
Independent	* 5	Minus	*** 5	= —
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY RATE	ADDITIONAL FEE		OTHER THAN SMALL ENTITY RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	—
X42=		OR	X84=	—
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	—

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
***If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

MULTIPLE DEPENDENT CLAIM
FEE CALCULATION SHEET
(FOR USE WITH FORM PTO-875)

SERIAL NO. 10/341868 | FILING DATE

APPLICANT(S)

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
1	1					
2	—	—				
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TOTAL IND.	5	←		←		←
TOTAL DEP.	4					
TOTAL CLAIMS	9					

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	IND.	DEP.	IND.	DEP.	IND.	DEP.
51						
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99						
100						
TOTAL IND.		←		←		←
TOTAL DEP.						
TOTAL CLAIMS						

PTO-2050 (3-78) *MAY BE USED FOR ADDITIONAL CLAIMS OR AMENDMENTS U.S. DEPARTMENT of COMMERCE Patent and Trademark Office

8-16-05

1614

CASE 4-32219A

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EV722485384US — Express Mail Label Number

8/15/05 — Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF

Art Unit: 1614

KSANDER ET AL.

APPLICATION NO: 10/341,868

FILED: JANUARY 14, 2003

FOR: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

STATUS REQUEST

Sir:

Applicants respectfully inquire as to the status of the above-identified application.

Respectfully submitted,

Gregory D. Ferraro
Attorney for Applicants
Reg. No. 36,134

Novartis
Corporate Intellectual Property
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(862) 778-7831

Date: August 15, 2005

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/341,868	01/14/2003	Gary Michael Ksander	4-32219A	8865

1095 7590 08/29/2005

NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 104/3
EAST HANOVER, NJ 07936-1080

EXAMINER	
KIM, JENNIFER M	

ART UNIT	PAPER NUMBER
1617	

DATE MAILED: 08/29/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

DATE: 08/29/2005

NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 104/3
EAST HANOVER, NJ 07936-1080

To: Applicant of Serial Number 10341868 (Art Unit 1617)

It is estimated that this application will receive an Office action in approximately 3 months. This is an estimate that is based on the current inventory level of applications filed in this art area and the current staffing levels in this Art Unit. The USPTO is dedicated to minimizing first action and total pendency, and in art areas with high new application inventories, we are targeting resources to help address these backlogs. Thank you for your inquiry.

Customer Service Office in Technology Center: 1600

Phone Number: 571-272-1600
Central Fax Number: 571-273-8300

Applicant/Attorney Contact Information:

Phone Number:
Fax Number:

PTOLFA1 (Rev. 07/05)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/341,868	01/14/2003	Gary Michael Ksander	4-32219A	8865

1095 7590 09/20/2005

NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 104/3
EAST HANOVER, NJ 07936-1080

EXAMINER	
KIM, JENNIFER M	
ART UNIT	**PAPER NUMBER**
1617	

DATE MAILED: 09/20/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Office Action Summary	Application No.	Applicant(s)	
	10/341,868	KSANDER ET AL.	
	Examiner	Art Unit	
	Jennifer Kim	1617	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 January 2003*.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-5,7-11* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1,3-5 and 7-11* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1,3, 4, 8 –11 are drawn to a pharmaceutical composition comprising AT 1-antagonist valsartan and the specific NEP inhibitors set forth in claims 1,3, 4, 8 and 9, classified in class 514, subclass 222.8.

II. Claims 5 and 7, drawn to a method for the treatment or prevention of a condition or disease set forth in claim 5 administering a pharmaceutical composition comprising AT 1-antagonist valsartan and the specific NEP inhibitors set forth in claims 1,3, 4, 8 and 9, classified in class 514, subclass 222.8.

The inventions are distinct, each from the other because of the following reasons:

Inventions Group I and Group II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product (MPEP § 806.05(h)). In the instant case the product as claimed can be used in a materially different process of using that product since the product can be used to treat psychotic conditions.

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art because of their recognized divergent subject matter, restriction for examination purposes as indicated is proper.

If Applicants elect Group II, following election of species is required:

This application contains claims directed to the following patentably distinct species of the claimed invention: Various conditions or disease set forth in claim 5 (i.e. hypertension, heart failure, Alzheimer, glaucoma, diabetic nephrophay.. etc.).

Applicants are required under 35 U.S.C. 121 to elect a single ultimate disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, a condition or disease is generic.

Applicants are advised that a reply to this requirement must include an identification of the species that is elected consonant with this requirement, and a listing of all claims readable thereon, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise include all the limitations of an allowed generic claim as provided by 37 CFR 1.141. If claims are added after the election, applicant must indicate which are readable upon the elected species. MPEP § 809.02(a).

Should applicants traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other invention.

> The examiner has required restriction between product and process claims. Where applicant elects claims directed to the product, and a product claim is subsequently found allowable, withdrawn process claims that depend from or otherwise include all the limitations of the allowable product claim will be rejoined in accordance with the provisions of MPEP § 821.04. **Process claims that depend from or otherwise include all the limitations of the patentable product** will be entered as a matter of right if the amendment is presented prior to final rejection or allowance, whichever is earlier. Amendments submitted after final rejection are governed by 37 CFR 1.116; amendments submitted after allowance are governed by 37 CFR 1.312.
>
> In the event of rejoinder, the requirement for restriction between the product claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103, and 112. Until an elected product claim is found allowable, an otherwise proper restriction requirement between product claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowed product claim will not be rejoined. See "Guidance on Treatment of Product and Process Claims in light of *In re Ochiai, In re Brouwer* and 35 U.S.C. § 103(b)," 1184 O.G. 86 (March 26, 1996). Additionally, in order to retain the right to rejoinder in accordance with the above policy, Applicant is advised that the process claims should be amended during prosecution either to maintain dependency on the product claims or to otherwise include the limitations of the product claims. **Failure to do so may result in a loss of the right to rejoinder.**

Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Kim whose telephone number is 571-272-0628. The examiner can normally be reached on Monday through Friday 6:30 am to 3 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Sreenivasan Padmanabhan can be reached on 571-272-0629. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Sreenivasan Padmanabhan
Supervisory Examiner
Art Unit 1617

Jmk
September 19, 2005

10.03.05

1617

CASE 4-32219A

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EV722487495US — Express Mail Label Number

September 30, 2005 — Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
KSANDER ET AL.
APPLICATION NO: 10/341,868
FILED: JANUARY 14, 2003
FOR: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Art Unit: 1617
Examiner: Kim, Jennifer M

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

RESPONSE TO RESTRICTION REQUIREMENT

Sir:

Responsive to the Office Action dated September 20, 2005, for which the time to respond extends to and includes October 20, 2005, Applicants elects Group 1, claims 1, 3, 4 and 8-11 for prosecution, with traverse.

In view of the foregoing, Applicants submit the Application is now in condition for allowance and respectfully requests early notice to that effect.

Respectfully submitted,

Gregory D. Ferraro
Attorney for Applicants
Reg. No. 36,134

Novartis
Corporate Intellectual Property
One Health Plaza, Building 104
East Hanover, NJ 07936-1080
(862) 778-7831

Date: September 30, 2005

PATENT APPLICATION FEE DETERMINATION RECORD
Effective January 1, 2003

Application or Docket Number
10/341868

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	11	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	11 minus 20=	* 0
INDEPENDENT CLAIMS	5 minus 3 =	* 2
MULTIPLE DEPENDENT CLAIM PRESENT		☐

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE ☐ OR OTHER THAN SMALL ENTITY

RATE	FEE		RATE	FEE
BASIC FEE	$375	OR	BASIC FEE	$750
X$ 9=		OR	X$18=	
X42=		OR	X84=	168
+140=		OR	+280=	
TOTAL		OR	TOTAL	918

CLAIMS AS AMENDED - PART II

9-30-05

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* 9	Minus	** 20	= —
Independent	* 5	Minus	*** 5	= —
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

SMALL ENTITY OR OTHER THAN SMALL ENTITY

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	—
X42=		OR	X84=	—
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	—

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	* SPARE	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				☐

RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X$ 9=		OR	X$18=	
X42=		OR	X84=	
+140=		OR	+280=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

STIC Search Report
Biotech-Chem Library

USPTO

STIC Database Tracking Number: 174376

TO: Jennifer Kim
Location: REM-4B02/4B15
Art Unit: 1617
Wednesday, December 28, 2005

Case Serial Number: 10/341868

From: Paul Schulwitz
Location: Biotech-Chem Library
REM-1A65
Phone: 571-272-2527

Paul.schulwitz@uspto.gov

Search Notes

Examiner Kim,

Please review the attached search results.
If you have any questions or if you would like to refine the search query, please feel free to contact me at any time.

Thank you for using STIC search services!

Paul Schulwitz
Technical Information Specialist
REM-1A65
571-272-2527

sira Search and Information Resources Administration

STIC SEARCH RESULTS FEEDBACK FORM

USPTO

Biotech-Chem Library

Questions about the scope or the results of the search? Contact ***the searcher or contact:***

Mary Hale, Information Branch Supervisor
Remsen Bldg. 01 D86
571-272-2507

Voluntary Results Feedback Form

- *I am an examiner in Workgroup:* ☐ *Example: 1610*
- *Relevant prior art **found**, search results used as follows:*
  - ☐ 102 rejection
  - ☐ 103 rejection
  - ☐ Cited as being of interest.
  - ☐ Helped examiner better understand the invention.
  - ☐ Helped examiner better understand the state of the art in their technology.

  Types of relevant prior art found:
  - ☐ Foreign Patent(s)
  - ☐ Non-Patent Literature
    (journal articles, conference proceedings, new product announcements etc.)
- *Relevant prior art **not found:***
  - ☐ Results verified the lack of relevant prior art (helped determine patentability).
  - ☐ Results were not useful in determining patentability or understanding the invention.

Comments:

Drop off or send completed forms to STIC-Biotech-Chem Library Remsen Bldg.

This listing of the claims will replace all prior versions, and listings, of claims in the application.

1. (currently amended) A pharmaceutical composition comprising:

(i) the AT 1-antagonist valsartan or a pharmaceutically acceptable salt thereof; and

(ii) a the NEP inhibitor N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester or (2R,4S)-5-Biphenyl -4-yl-4(3-carboxy-propionyl amino)-2-methyl-pentanoic acid ora pharmaceutically acceptable salts thereof and a pharmaceutically acceptable carrier.

149709-62-6

Family Search

2. (cancel)

3. (currently amended) The pharmaceutical composition of Claim 21, wherein N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester is a triethanolamine or *tris*(hydroxymethyl)aminomethane salt thereof.

4. (currently amended) A kit comprising in separate containers in a single package pharmaceutical compositions comprising in one container a pharmaceutical composition comprising a NEP inhibitor N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methylbutanoic acid ethyl ester or (2R,4S)-5-Biphenyl -4-yl-4(3-carboxy-propionyl amino)-2-methyl-pentanoic acid or pharmaceutically acceptable salts thereof and in a second container a pharmaceutical composition comprising valsartan.

5. (currently amended) A method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure, such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction, such as Alzheimer's, glaucoma and stroke, comprising administering a therapeutically effective amount of combination of:

(i) the AT 1-antagonists valsartan or a pharmaceutically acceptable salt thereof; and

(ii) ~~a~~ the NEP inhibitor *N*-(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester or its active metabolite or (2R,4S)-5-Biphenyl-4-yl-4(3-carboxy-propionyl amino)-2-methyl-pentanoic acid or ~~a~~ pharmaceutically acceptable salts thereof and a pharmaceutically acceptable carrier to a mammal in need of such treatment.

6. (cancel)

7. (currently amended) The method of Claim ~~6~~5, wherein *N*-(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester is a triethanolamine or tris(hydroxymethyl)aminomethane salt thereof.

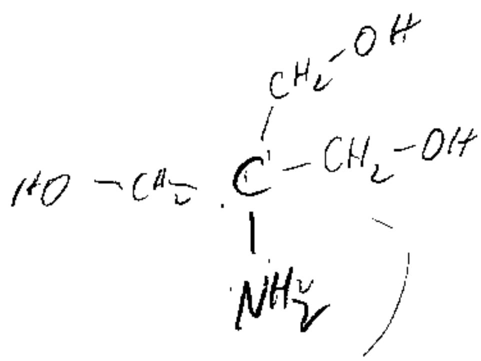
Cl.3

8. (previously presented) A triethanolamine salt of *N*-(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester. 565453-98-7

9. (previously presented) A *tris*(hydroxymethyl)aminomethane salt of *N*-(3-carboxy-1-oxopropyl)-(4*S*)-*p*-phenylphenylmethyl)-4-amino-2*R*-methylbutanoic acid ethyl ester. 565453-99-8

10. (previously presented) A pharmaceutical composition comprising the salt of Claim 8.

11. (previously presented) A pharmaceutical composition comprising the salt of Claim 9.



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=> d his ful

     (FILE 'HOME' ENTERED AT 10:55:23 ON 28 DEC 2005)

     FILE 'REGISTRY' ENTERED AT 10:55:28 ON 28 DEC 2005
                E N-(3-CARBOXY-1-OXOPROPYL/CN
                D COST
L1       264222 SEA ABB=ON  PLU=ON  "OXOPROPYL"
                D COS
L2            0 SEA ABB=ON  PLU=ON  "OXOPROPYL"(3A)"PHENYLPHENYLMETHYL"
L3            1 SEA ABB=ON  PLU=ON  149709-62-6/RN
                SEL RN
L4            3 SEA ABB=ON  PLU=ON  149709-62-6/CRN
                D SCA
L5            4 SEA ABB=ON  PLU=ON  L3 OR L4
L6              STR  149709-62-6
L7            0 SEA FAM SAM L6
L8            5 SEA FAM FUL L6
                D SCA

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L9            0 SEA ABB=ON  PLU=ON  L5 OR L8

     FILE 'HCAPLUS' ENTERED AT 11:11:10 ON 28 DEC 2005
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L11           2 SEA ABB=ON  PLU=ON  L8
L12           3 SEA ABB=ON  PLU=ON  (L10 OR L11)

     FILE 'REGISTRY' ENTERED AT 11:13:00 ON 28 DEC 2005
                D SCA L4

     FILE 'HCAPLUS' ENTERED AT 11:13:09 ON 28 DEC 2005
                D IBIB 1-3
                DIS

     FILE 'REGISTRY' ENTERED AT 11:15:30 ON 28 DEC 2005
L13             STR L6
L14           3 SEA SSS SAM L13
                D SCA
L15          27 SEA SSS FUL L13

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L16           3 SEA ABB=ON  PLU=ON  L15
L17           0 SEA ABB=ON  PLU=ON  L16 NOT L12
L18         813 SEA ABB=ON  PLU=ON  BEILSTEIN
                S L13

     FILE 'REGISTRY' ENTERED AT 11:17:19 ON 28 DEC 2005
L19          27 SEA SSS FUL L13

     FILE 'HCAPLUS' ENTERED AT 11:17:20 ON 28 DEC 2005
L20           3 SEA ABB=ON  PLU=ON  L19

     FILE 'BEILSTEIN' ENTERED AT 11:17:33 ON 28 DEC 2005
L21          18 SEA SSS FUL L13
L22          18 SEA ABB=ON  PLU=ON  L21 NOT L19
                D L22 IDE ALLREF 10
L23          10 SEA ABB=ON  PLU=ON  L22 AND KSANDER?/AU
L24           8 SEA ABB=ON  PLU=ON  L22 NOT L23
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              D IDE ALLREF 5

     FILE 'MARPAT' ENTERED AT 11:22:57 ON 28 DEC 2005
L25            0 SEA SSS SAM L13
L26            1 SEA SSS FUL L13
L27            0 SEA ABB=ON  PLU=ON  L26 NOT L20
L28            1 SEA ABB=ON  PLU=ON  L12 OR L20

     FILE 'MEDLINE, EMBASE, BIOSIS, USPATFULL, USPAT2' ENTERED AT 11:24:04 ON
     28 DEC 2005
L29            3 SEA ABB=ON  PLU=ON  L19
L30            0 SEA ABB=ON  PLU=ON  L20 NOT L29

     FILE 'HCAPLUS' ENTERED AT 11:25:16 ON 28 DEC 2005
L*** DEL       0 S L30 NOT L29


     FILE HOME

     FILE REGISTRY
     Property values tagged with IC are from the ZIC/VINITI data file
     provided by InfoChem.

     STRUCTURE FILE UPDATES:    27 DEC 2005  HIGHEST RN 870676-46-3
     DICTIONARY FILE UPDATES:   27 DEC 2005  HIGHEST RN 870676-46-3

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     **************************************************************
     *                                                            *
     * The CA roles and document type information have been removed from *
     * the IDE default display format and the ED field has been added,   *
     * effective March 20, 2005.  A new display format, IDERL, is now    *
     * available and contains the CA role and document type information. *
     *                                                            *
     **************************************************************

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     REGISTRY includes numerically searchable data for experimental and
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     http://www.cas.org/ONLINE/UG/regprops.html

     FILE CAOLD
     FILE COVERS 1907-1966
     FILE LAST UPDATED:  01 May 1997 (19970501/UP)

       This file contains CAS Registry Numbers for easy and accurate
       substance identification.  Title keywords, authors, patent
       assignees, and patent information, e.g., patent numbers, are
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  now searchable from 1907-1966.  TIFF images of CA abstracts
  printed between 1907-1966 are available in the PAGE
  display formats.

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FILE HCAPLUS

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FILE COVERS 1907 - 28 Dec 2005  VOL 144 ISS 1
FILE LAST UPDATED: 27 Dec 2005  (20051227/ED)

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 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

FILE BEILSTEIN
FILE LAST UPDATED ON OCTOBER 10, 2005

FILE COVERS 1771 TO 2005.
 FILE CONTAINS  9,363,954 SUBSTANCES

>>>PLEASE NOTE: Reaction Data and substance data are stored in
   separate documents and can not be searched together in one query.
   Reaction data for BEILSTEIN compounds may be displayed
   immediately with the display codes PRE (preparations) and REA
   (reactions).  A substance answer set retrieved after the search
   for a chemical name, a compounds with available reaction
   information by combining with PRE/FA, REA/FA or more generally
   with RX/FA. The BEILSTEIN Registry Number (BRN) is the link
   between a BEILSTEIN compound and belonging reactions. For mo
   detailed reaction searches BRNs can be searched as reaction
   partner BRNs Reactant BRN (RX.RBRN) or Product BRN (RX.PBRN).<<<

>>> FOR SEARCHING PREPARATIONS SEE HELP PRE  <<<

****************************************************************
* PLEASE NOTE THAT THERE ARE NO FORMATS FREE OF COST.           *
* SET NOTICE FEATURE: THE COST ESTIMATES CALCULATED FOR SET NOTICE  *
* ARE BASED ON THE HIGHEST PRICE CATEGORY. THEREFORE; THESE     *
* ESTIMATES MAY NOT REFLECT THE ACTUAL COSTS.                   *
* FOR PRICE INFORMATION SEE HELP COST                           *
****************************************************************
    NEW
* PATENT NUMBERS (PN) AND BABS ACCESSION NUMBERS (BABSAN) CAN NOW BE
```

SEARCHED, SELECTED AND TRANSFERRED.
* NEW DISPLAY FORMATS ALLREF, ALLP AND BABSAN SHOW ALL REFERENCES, ALL PATENT REFERENCES, OR ALL BABS ACCESSION NUMBERS FOR A COMPOUND AT A GLANCE.

FILE MARPAT
FILE CONTENT: 1988-PRESENT (VOL 143 ISS 26 (20051223/ED)

MOST RECENT CITATIONS FOR PATENTS FROM FIVE MAJOR ISSUING AGENCIES
(COVERAGE TO THESE DATES IS NOT COMPLETE):

```
US    6949561 27 SEP 2005
DE 1020040544 15 SEP 2005
EP    1582199 05 OCT 2005
JP 2005320486 17 OCT 2005
WO 2005097137 20 OCT 2005
```

Expanded G-group definition display now available.

New CAS Information Use Policies, enter HELP USAGETERMS for details.

MARPATpreviews will be removed from STN on December 31, 2005.

FILE MEDLINE
FILE LAST UPDATED: 27 DEC 2005 (20051227/UP). FILE COVERS 1950 TO DATE.

On December 11, 2005, the 2006 MeSH terms were loaded.

The MEDLINE reload for 2006 will soon be available. For details on the 2005 reload, enter HELP RLOAD at an arrow promt (=>).
See also:

http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate

FILE EMBASE
FILE COVERS 1974 TO 22 Dec 2005 (20051222/ED)

EMBASE has been reloaded. Enter HELP RLOAD for details.

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE BIOSIS
FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 21 December 2005 (20051221/ED)

FILE USPATFULL
FILE COVERS 1971 TO PATENT PUBLICATION DATE: 27 Dec 2005 (20051227/PD)
FILE LAST UPDATED: 27 Dec 2005 (20051227/ED)
HIGHEST GRANTED PATENT NUMBER: US6981281
HIGHEST APPLICATION PUBLICATION NUMBER: US2005283878
CA INDEXING IS CURRENT THROUGH 27 Dec 2005 (20051227/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 27 Dec 2005 (20051227/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Oct 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Oct 2005

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>>>  USPAT2 is now available.  USPATFULL contains full text of the     <<<
>>>  original, i.e., the earliest published granted patents or         <<<
>>>  applications.  USPAT2 contains full text of the latest US         <<<
>>>  publications, starting in 2001, for the inventions covered in     <<<
>>>  USPATFULL.  A USPATFULL record contains not only the original     <<<
>>>  published document but also a list of any subsequent              <<<
>>>  publications.  The publication number, patent kind code, and      <<<
>>>  publication date for all the US publications for an invention     <<<
>>>  are displayed in the PI (Patent Information) field of USPATFULL   <<<
>>>  records and may be searched in standard search fields, e.g., /PN, <<<
>>>  /PK, etc.                                                         <<<

>>>  USPATFULL and USPAT2 can be accessed and searched together        <<<
>>>  through the new cluster USPATALL.  Type FILE USPATALL to          <<<
>>>  enter this cluster.                                               <<<
>>>                                                                    <<<
>>>  Use USPATALL when searching terms such as patent assignees,       <<<
>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                           <<<
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This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE USPAT2

FILE COVERS 2001 TO PUBLICATION DATE: 27 Dec 2005 (20051227/PD)
FILE LAST UPDATED: 27 Dec 2005 (20051227/ED)
HIGHEST GRANTED PATENT NUMBER: US2004267271
HIGHEST APPLICATION PUBLICATION NUMBER: US2005283875
CA INDEXING IS CURRENT THROUGH 27 Dec 2005 (20051227/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 27 Dec 2005 (20051227/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Oct 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Oct 2005

USPAT2 is a companion file to USPATFULL. USPAT2 contains full text of the latest US publications, starting in 2001, for the inventions covered in USPATFULL. USPATFULL contains full text of the original published US patents from 1971 to date and the original applications from 2001. In addition, a USPATFULL record for an invention contains a complete list of publications that may be searched in standard search fields, e.g., /PN, /PK, etc.

USPATFULL and USPAT2 can be accessed and searched together through the new cluster USPATALL. Type FILE USPATALL to enter this cluster.

Use USPATALL when searching terms such as patent assignees, classifications, or claims, that may potentially change from the earliest to the latest publication.

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L13            STR
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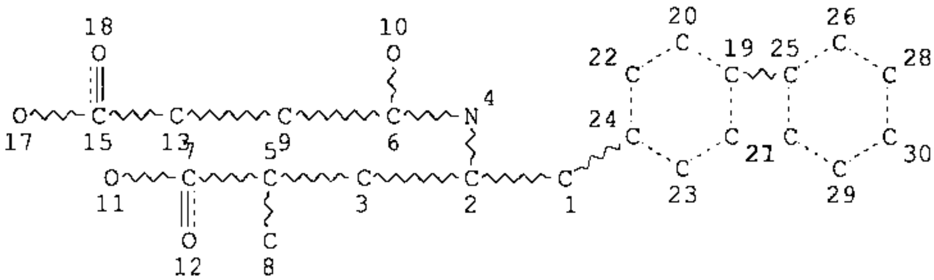

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NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS  28

STEREO ATTRIBUTES: NONE
L19          27 SEA FILE=REGISTRY SSS FUL L13
L20           3 SEA FILE=HCAPLUS ABB=ON  PLU=ON  L19


=> d l20 ibib abs hitstr 1-3

L20  ANSWER 1 OF 3  HCAPLUS  COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER:         2003:570812  HCAPLUS
DOCUMENT NUMBER:          139:138733
TITLE:                    Pharmaceutical compositions comprising valsartan and
                          neutral endopeptidase inhibitors
INVENTOR(S):              Webb, Randy Lee; Ksander, Gary Michael
PATENT ASSIGNEE(S):       Novartis A.-G., Switz.; Novartis Pharma G.m.b.H.
SOURCE:                   PCT Int. Appl., 31 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.          KIND    DATE          APPLICATION NO.        DATE
     ---------------     ----    --------      --------------------   --------
     WO 2003059345        A1     20030724      WO 2003-EP415          20030116
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
             HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LT, LU,
             LV, MA, MD, MK, MN, MX, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SE,
             SG, SK, TJ, TM, TN, TR, TT, UA, US, UZ, VC, VN, YU, ZA, ZW
         RW: AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE,
             DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI,
             SK, TR
     US 2003144215        A1     20030731      US 2003-341868         20030114
     CA 2472399           AA     20030724      CA 2003-2472399        20030116
     EP 1467728           A1     20041020      EP 2003-704413         20030116
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
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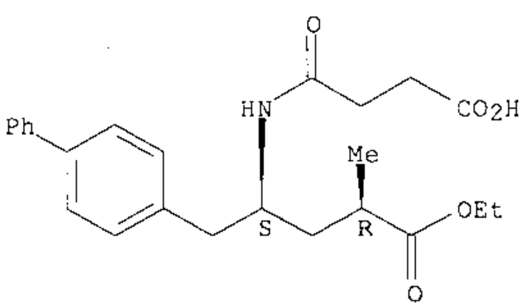
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK					
BR 2003006907	A	20041221	BR 2003-6907		20030116
JP 2005514441	T2	20050519	JP 2003-559507		20030116
ZA 2004005117	A	20050622	ZA 2004-5117		20040628
NO 2004003380	A	20041007	NO 2004-3380		20040813
PRIORITY APPLN. INFO.:			US 2002-349660P	P	20020117
			US 2002-386792P	P	20020607
			WO 2003-EP415	W	20030116

AB The invention relates a pharmaceutical composition comprising a combination of (i) the AT-1 antagonist valsartan or a pharmaceutically acceptable salt thereof and (ii) a NEP (neutral endopeptidase) inhibitor or a pharmaceutically acceptable salt thereof and optionally a pharmaceutically acceptable carrier and to a method for the treatment or prevention of a condition or disease selected from the group consisting of hypertension, heart failure such as (acute and chronic) congestive heart failure, left ventricular dysfunction and hypertrophic cardiomyopathy, diabetic cardiac myopathy, supraventricular and ventricular arrhythmias, atrial fibrillation, atrial flutter, detrimental vascular remodeling, myocardial infarction and its sequelae, atherosclerosis, angina (whether unstable or stable), renal insufficiency (diabetic and non-diabetic), heart failure, angina pectoris, diabetes, secondary aldosteronism, primary and secondary pulmonary hypertension, renal failure conditions, such as diabetic nephropathy, glomerulonephritis, scleroderma, glomerular sclerosis, proteinuria of primary renal disease, and also renal vascular hypertension, diabetic retinopathy, the management of other vascular disorders, such as migraine, peripheral vascular disease, Raynaud's disease, luminal hyperplasia, cognitive dysfunction (such as Alzheimer's), glaucoma and stroke, comprising administering a therapeutically effective amount of the pharmaceutical composition to a mammal in need thereof.

IT 149709-62-6 565453-98-7 565453-99-8
RL: PEP (Physical, engineering or chemical process); PYP (Physical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(pharmaceutical compns. comprising valsartan and neutral endopeptidase inhibitors)

RN 149709-62-6 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, ethyl ester, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

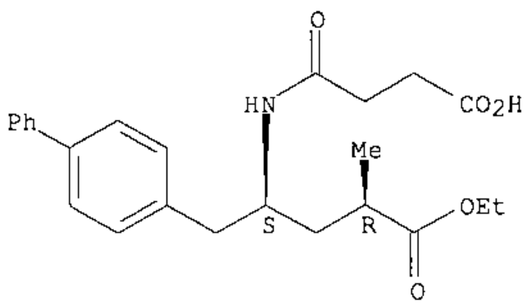


RN 565453-98-7 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, ethyl ester, (αR,γS)-, compd. with 2,2',2''-nitrilotris[ethanol] (1:1) (9CI) (CA INDEX NAME)

CM 1

CRN 149709-62-6
CMF C24 H29 N O5

Absolute stereochemistry.



CM 2

CRN 102-71-6
CMF C6 H15 N O3

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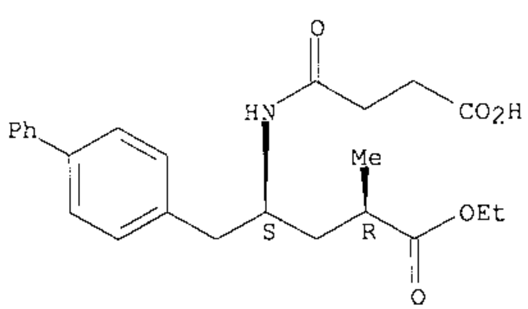
RN 565453-99-8 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, ethyl ester, (αR,γS)-, compd. with
2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1) (9CI) (CA INDEX NAME)

CM 1

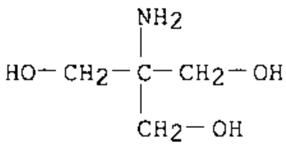
CRN 149709-62-6
CMF C24 H29 N O5

Absolute stereochemistry.



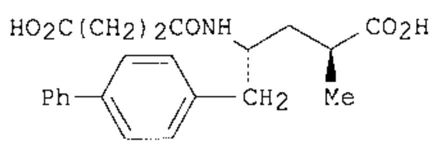
CM 2

CRN 77-86-1
CMF C4 H11 N O3

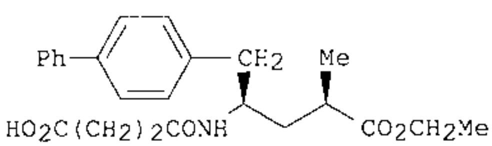


REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L20 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1995:538778 HCAPLUS
DOCUMENT NUMBER: 122:281426
TITLE: Dicarboxylic Acid Dipeptide Neutral Endopeptidase Inhibitors
AUTHOR(S): Ksander, Gary M.; Ghai, Raj D.; deJesus, Reynalda; Diefenbacher, Clive; Yuan, Andrew; Berry, Carol; Sakane, Yumi; Trapani, Angelo
CORPORATE SOURCE: Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ, 079C1, USA
SOURCE: Journal of Medicinal Chemistry (1995), 38(10), 1689-700
CODEN: JMCMAR; ISSN: 0022-2623
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
GI



I



II

AB The synthesis of three series of dicarboxylic acid dipeptide neutral endopeptidase 24.11 (NEP) inhibitors is described. In particular, the amino butyramide I exhibited potent NEP inhibitory activity (IC50 = 5.0 nM) in vitro and in vivo. Blood levels of I were determined using an ex vivo method by measuring plasma inhibitory activity in conscious rats, mongrel dogs, and cynomolgus monkeys. Free drug concns. were 10-1500 times greater than the inhibitory constant for NEP over the course of a 6 h experiment
A good correlation of free drug concns. was obtained when comparing values determined by the ex vivo anal. to those calculated from direct HPLC measurements.

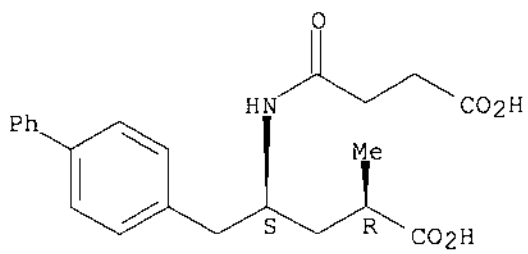
Plasma atrial natriuretic factor (exogenous) levels were elevated in rats and dogs after oral administration of II. Urinary volume and urinary sodium excretion were also potentiated in anesthetized dogs treated with I.

IT 149709-44-4P
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); MFM (Metabolic formation); PRP (Properties); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation); PROC (Process); USES (Uses)
(dicarboxylic acid dipeptide neutral endopeptidase inhibitors in relation to pharmacokinetics and pharmacol. and structure)

RN 149709-44-4 HCAPLUS

CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

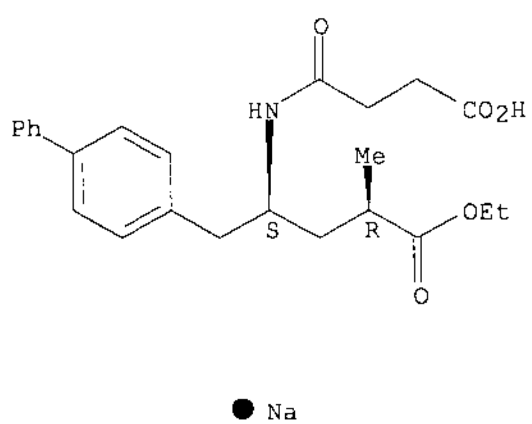


IT 149690-05-1P
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); RCT (Reactant); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); PROC (Process); RACT (Reactant or reagent); USES (Uses)
(dicarboxylic acid dipeptide neutral endopeptidase inhibitors in relation to pharmacokinetics and pharmacol. and structure)

RN 149690-05-1 HCAPLUS

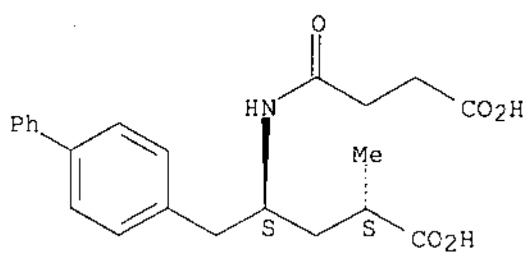
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, α-ethyl ester, monosodium salt, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



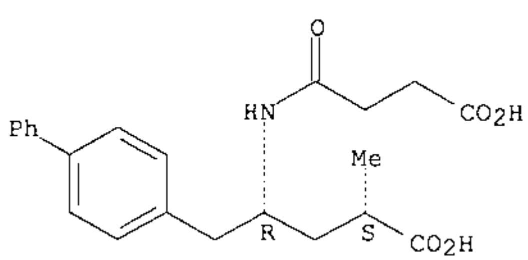
IT 149709-45-5P 149709-47-7P 149709-48-8P
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(dicarboxylic acid dipeptide neutral endopeptidase inhibitors in relation to pharmacokinetics and pharmacol. and structure)
RN 149709-45-5 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, [S-(R*,R*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



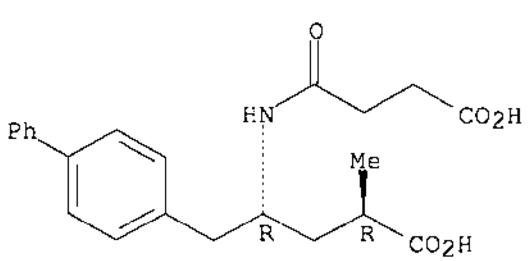
RN 149709-47-7 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, [R-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



RN 149709-48-8 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, [R-(R*,R*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

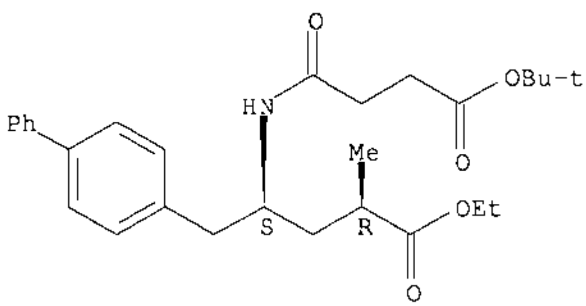


IT 149709-64-8P 149709-65-9P 162972-31-8P

162972-32-9P 162972-33-0P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(dicarboxylic acid dipeptide neutral endopeptidase inhibitors in relation to pharmacokinetics and pharmacol. and structure)

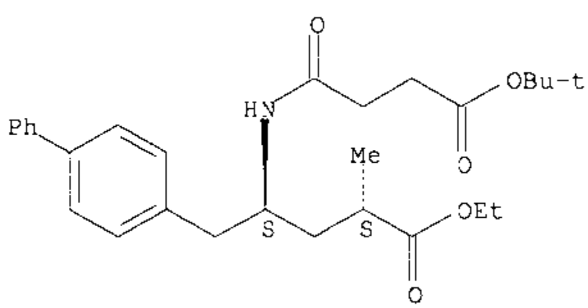
RN 149709-64-8 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[[4-(1,1-dimethylethoxy)-1,4-dioxobutyl]amino]-α-methyl-, ethyl ester, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

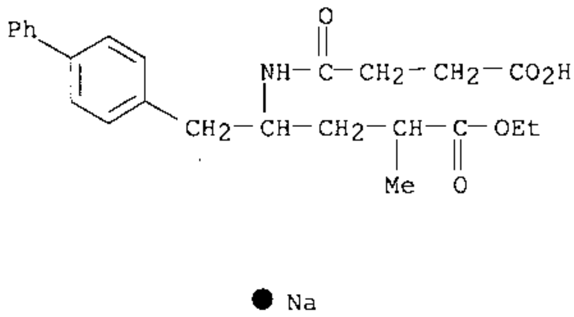


RN 149709-65-9 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[[4-(1,1-dimethylethoxy)-1,4-dioxobutyl]amino]-α-methyl-, ethyl ester, [S-(R*,R*)]- (9CI) (CA INDEX NAME)

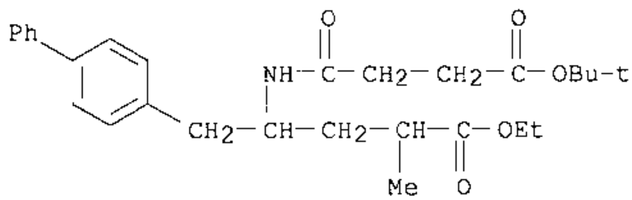
Absolute stereochemistry.



RN 162972-31-8 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, α-ethyl ester, monosodium salt (9CI) (CA INDEX NAME)

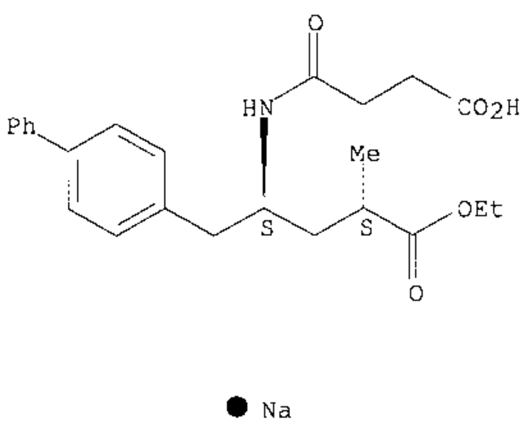


RN 162972-32-9 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[[4-(1,1-dimethylethoxy)-1,4-dioxobutyl]amino]-α-methyl-, ethyl ester (9CI) (CA INDEX NAME)



RN 162972-33-0 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, ethyl ester, monosodium salt, [S-(R*,R*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



L20 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1993:670810 HCAPLUS
DOCUMENT NUMBER: 119:270810
TITLE: Preparation of biaryl substituted 4-amino-butyric acid amides
INVENTOR(S): Ksander, Gary
PATENT ASSIGNEE(S): Ciba-Geigy Corp., USA

SOURCE: U.S., 13 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5217996	A	19930608	US 1992-824132	19920122
EP 555175	A1	19930811	EP 1993-810016	19930113
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
AU 9331842	A1	19930729	AU 1993-31842	19930115
AU 666902	B2	19960229		
JP 05310664	A2	19931122	JP 1993-5908	19930118
CA 2087652	AA	19930723	CA 1993-2087652	19930120
ZA 9300421	A	19930722	ZA 1993-421	19930121
NO 9300193	A	19930723	NO 1993-193	19930121
HU 63376	A2	19930830	HU 1993-166	19930121
US 5354892	A	19941011	US 1993-8031	19930125

PRIORITY APPLN. INFO.: US 1992-824132 A 19920122
OTHER SOURCE(S): MARPAT 119:270810

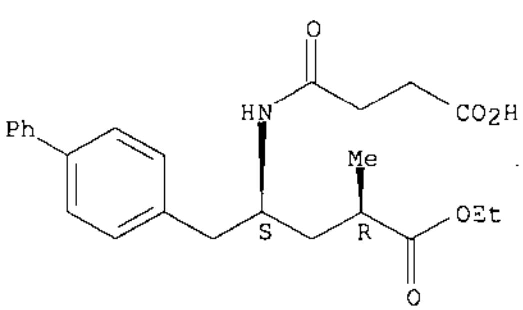
AB Title compds. RO2CCHMeCH2CH(CH2C6H4Ph-4)NHCO(CH2)2CO2R' (R, R' = H, C1-4 alkyl (substituted) PhCH2 on Ph, prodrug ester, salt), neutral endopeptidase inhibitors and thus useful for treatment of cardiovascular disorders, are prepared α-Tert-BOC-(R)-tyrosine Me ester and pyridine in CH2Cl2 were cooled at 0-5°, trifluoromethanesulfonic anhydride was added to give Me (R)-2-(tert-butoxycarbonylamino)-3-[4-(trifluoromethylsulfonyloxy)phenyl]propionate which in 11 steps was converted into Na N-(3-carboxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino-2R-methyl]butanoic acid Et ester (I). I at 1-30 mg/kg, s.c., produced significant increase in plasma atrial natriuretic factor level and significant reduction in blood pressure in DOCA-salt hypertensive rat model. Pharmaceutical capsules comprising I are given.

IT 149709-62-6P 149709-63-7P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(preparation and reaction of, preparation of neutral endopeptidase inhibitors)

RN 149709-62-6 HCAPLUS

CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-α-methyl-, ethyl ester, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

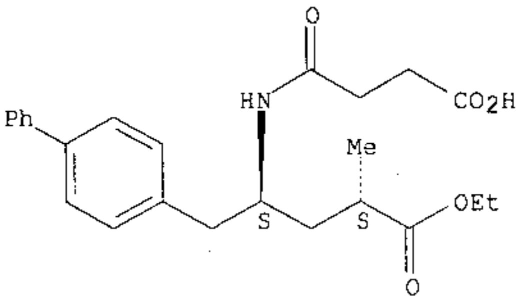
Absolute stereochemistry.



RN 149709-63-7 HCAPLUS

CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, ethyl ester, [S-(R*,R*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

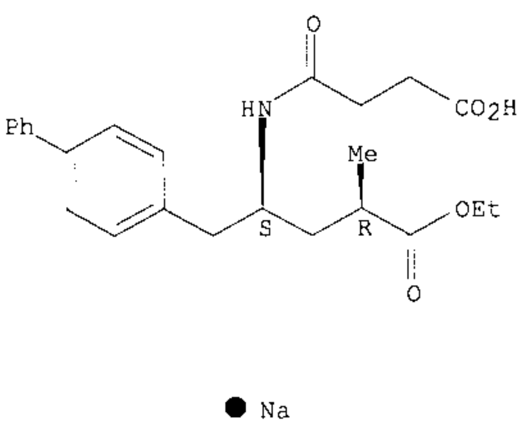


IT 149690-05-1P 149690-06-2P 149690-10-8P
149690-11-9P 149709-44-4P 149709-45-5P
149709-46-6P 149709-47-7P 149709-48-8P
149709-49-9P 149709-53-5P 149709-64-8P
149709-65-9P 149818-96-2P 149818-97-3P
RL: SPN (Synthetic preparation); PREP (Preparation)
(preparation of, as neutral endopeptidase inhibitor)

RN 149690-05-1 HCAPLUS

CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, α-ethyl ester, monosodium salt, [S-(R*,S*)]- (9CI)
(CA INDEX NAME)

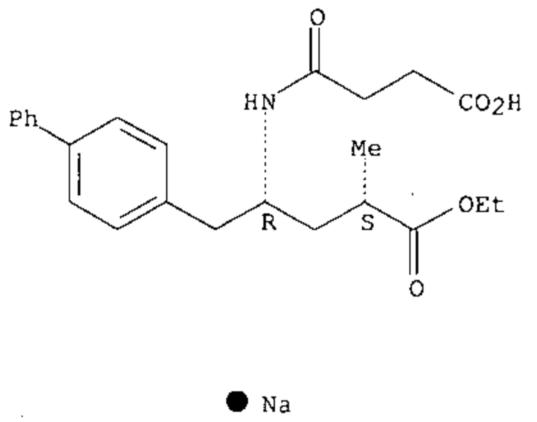
Absolute stereochemistry.



RN 149690-06-2 HCAPLUS

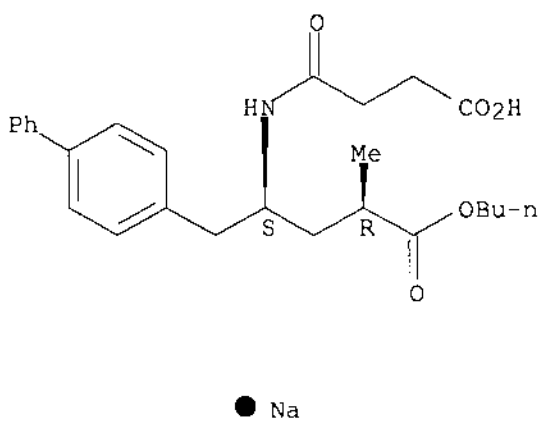
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, α-ethyl ester, monosodium salt, [R-(R*,S*)]- (9CI)
(CA INDEX NAME)

Absolute stereochemistry.



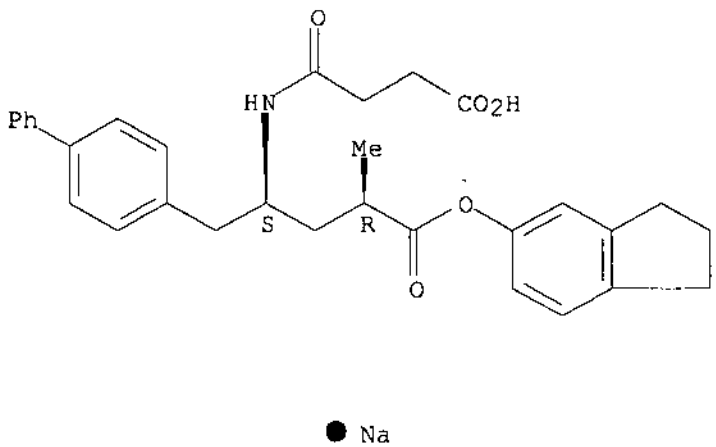
RN 149690-10-8 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, α-butyl ester, monosodium salt, [S-(R*,S*)]- (9CI)
(CA INDEX NAME)

Absolute stereochemistry.



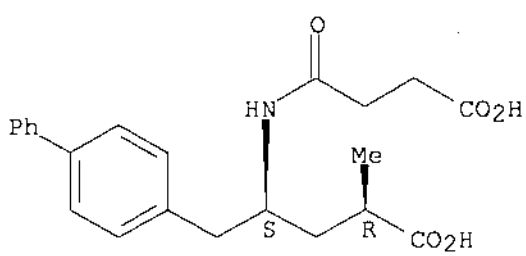
RN 149690-11-9 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, α-(2,3-dihydro-1H-inden-5-yl) ester, monosodium
salt, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



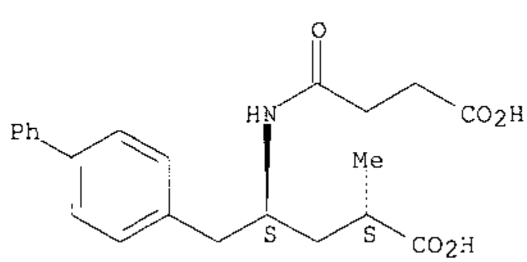
RN 149709-44-4 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



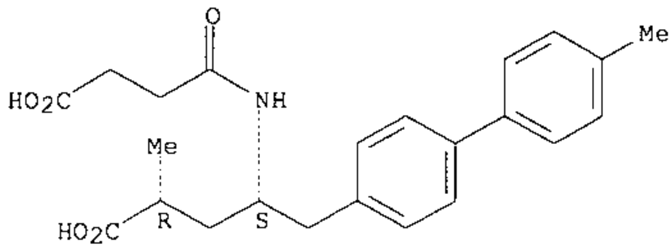
RN 149709-45-5 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, [S-(R*,R*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



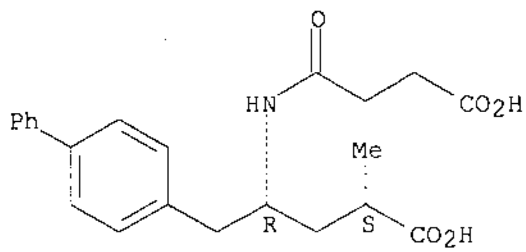
RN 149709-46-6 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α,4'-dimethyl-, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



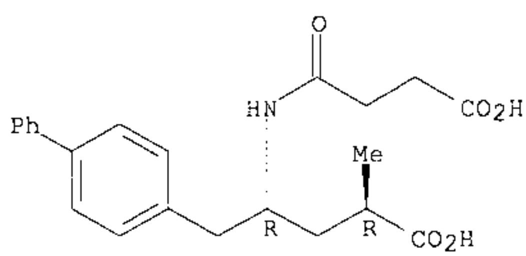
RN 149709-47-7 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, [R-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



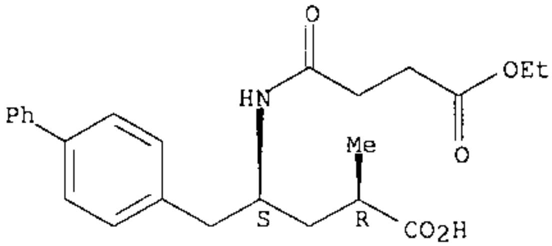
RN 149709-48-8 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, [R-(R*,R*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

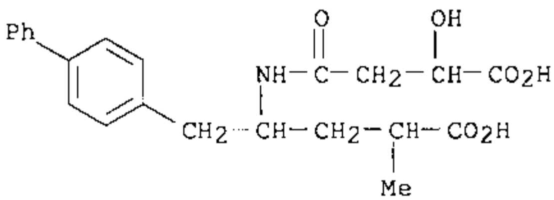


RN 149709-49-9 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(4-ethoxy-1,4-dioxobutyl)amino]-
α-methyl-, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

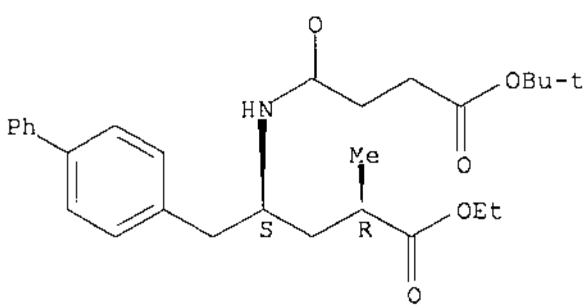


RN 149709-53-5 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-3-hydroxy-1-oxopropyl)amino]-α-methyl- (9CI) (CA INDEX NAME)



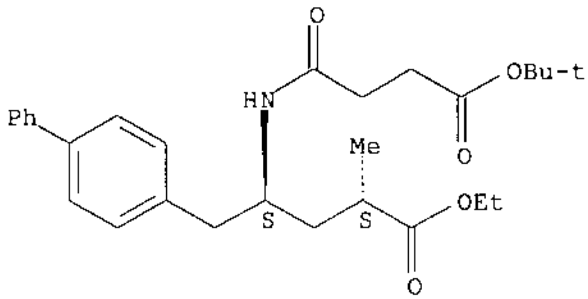
RN 149709-64-8 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[[4-(1,1-dimethylethoxy)-1,4-dioxobutyl]amino]-α-methyl-, ethyl ester, [S-(R*,S*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



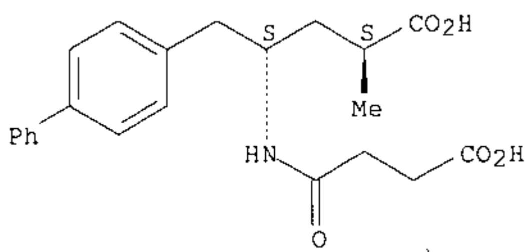
RN 149709-65-9 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[[4-(1,1-dimethylethoxy)-1,4-dioxobutyl]amino]-α-methyl-, ethyl ester, [S-(R*,R*)]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



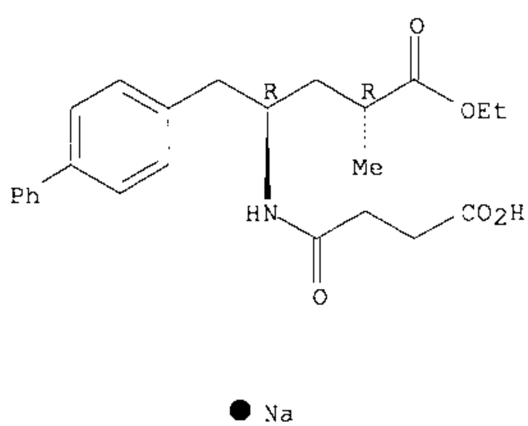
RN 149818-96-2 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, (R*,R*)- (9CI) (CA INDEX NAME)

Relative stereochemistry.



RN 149818-97-3 HCAPLUS
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, α-ethyl ester, monosodium salt, (R*,R*)- (9CI) (CA
INDEX NAME)

Relative stereochemistry.



FILE HOME

FILE STNGUIDE
FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Dec 30, 2005 (20051230/UP).

FILE ADISCTI

FILE COVERS 1998 TO 30 Dec 2005 (20051230/ED)

FILE LAST UPDATED: 30 DEC 2005 (20051230/ED)

FILE ADISINSIGHT

FILE COVERS 1998 TO 29 Dec 2005 (20051229/ED)
FILE LAST UPDATED: 29 DEC 2005 (20051229/ED)

FILE ADISNEWS

FILE COVERS 1983 TO 5 Jan 2006 (20060105/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

FILE BIOSIS
FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 4 January 2006 (20060104/ED)

FILE BIOTECHNO
FILE LAST UPDATED: 7 JAN 2004 <20040107/UP>
FILE COVERS 1980 TO 2003.

\>>> BIOTECHNO IS NO LONGER BEING UPDATED AS OF 2004 <<<

\>>> SIMULTANEOUS LEFT AND RIGHT TRUNCATION AVAILABLE IN /CT AND BASIC INDEX <<<

FILE CAPLUS

FILE COVERS 1907 - 5 Jan 2006 VOL 144 ISS 2
FILE LAST UPDATED: 4 Jan 2006 (20060104/ED)

Effective October 17, 2005, revised CAS Information Use Policies apply. They are available for your review at:

http://www.cas.org/infopolicy.html

FILE DDFB
\>>> FILE COVERS 1964 TO 1982 - CLOSED FILE <<<

FILE DGENE
FILE LAST UPDATED: 30 DEC 2005 <20051230/UP>

```
DGENE CURRENTLY CONTAINS 7,596,625 BIOSEQUENCES

>>> NEW DISPLAY FIELDS LS AND LS2 (LEGAL STATUS DATA FROM
    THE INPADOC DATABASE) AVAILABLE IN DGENE  -  SEE NEWS <<<

>>> ONLINE THESAURUS AVAILABLE IN /PACO <<<

>>> DOWNLOAD THE DGENE WORKSHOP MANUAL:
http://www.stn-international.de/training_center/bioseq/dgene_wm.pdf

>>> DOWNLOAD COMPLETE DGENE HELP AS PDF:
http://www.stn-international.de/training_center/bioseq/dgene_help.pdf   <<

>>> DOWNLOAD DGENE BLAST/GETSIM FREQUENTLY ASKED QUESTIONS:
 http://www.stn-international.de/service/faq/dgenefaq.pdf    <<<

FILE DISSABS
FILE COVERS 1861 TO 20 DEC 2005 (20051220/ED)

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MATERIALS OR THEIR USE.

FILE DRUGB
 >>>  FILE COVERS 1964 TO 1982 - CLOSED FILE  <<<

FILE DRUGMONOG2

 FILE IS CURRENT THROUGH 9 Dec 2005 (20051209/ED)

   ###################################################################
   #                                                                 #
   #                     !!! ATTENTION !!!                           #
   #                                                                 #
   #  Welcome to DRUGMONOG2.  This file is available to all users.   #
   #  To access drug pricing information, use DRUGMONOG, accessible  #
   #  only to pharmaceutical organizations for reasons of            #
   #  confidentiality.                                               #
   #                                                                 #
   #  If you already have subscription status on any of the IMSworld#
   #  files on STN and belong to a pharmaceutical organization, you  #
   #  should automatically have access to DRUGMONOG.  If you belong  #
   #  to a pharmaceutical organization and would like to use         #
   #  DRUGMONOG, please contact your STN Help Desk.  If you do not   #
   #  need pricing information, use DRUMONOG2.                       #
   #                                                                 #
   #  See HELP SUBSCRIPTION for more information.                    #
   ###################################################################

FILE DRUGU
FILE LAST UPDATED: 23 DEC 2005       <20051223/UP>
>>>  DERWENT DRUG FILE (SUBSCRIBER)  <<<

>>>  FILE COVERS 1983 TO DATE  <<<
>>>  THESAURUS AVAILABLE IN /CT  <<<


FILE EMBAL

FILE COVERS CURRENT RECORDS AND IS UPDATED DAILY
FILE LAST UPDATED: 5 JAN 2006 (20060105/ED)

FILE EMBASE
```

```
 FILE COVERS 1974 TO 29 Dec 2005 (20051229/ED)

 EMBASE has been reloaded. Enter HELP RLOAD for details.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

FILE ESBIOBASE
FILE LAST UPDATED: 3 JAN 2006       <20060103/UP>
FILE COVERS 1994 TO DATE.

>>>  SIMULTANEOUS LEFT AND RIGHT TRUNCATION AVAILABLE IN
     /CC, /ORGN, AND /ST  <<<

FILE IFIPAT
FILE COVERS 1950 TO PATENT PUBLICATION DATE: 3 Jan 2006 (20060103/PD)
FILE LAST UPDATED: 4 Jan 2006 (20060104/ED)
HIGHEST GRANTED PATENT NUMBER: US6983486
HIGHEST APPLICATION PUBLICATION NUMBER: US2005289677
UNITERM INDEXING IS AVAILABLE IN THE IFIUDB FILE
UNITERM INDEXING LAST UPDATED: 31 Oct 2005 (20051031/UP)
INDEXING CURRENT THROUGH PAT PUB DATE: 27 May 2004 (20040527/PD)

IFIPAT reloaded on 9/22/05.  Enter HELP RLOAD for details.


FILE IMSDRUGNEWS
FILE COVERS 1995 TO 16 Dec 2005 (20051216/ED)

 ###################################################################
 #                                                                 #
 #                    !!! ATTENTION !!!                            #
 #                                                                 #
 #  Welcome to IMSDRUGNEWS.  This is the Drug News file from       #
 #  IMSworld Publications.                                         #
 #                                                                 #
 #  For detailed information regarding the printed version         #
 #  of this file, please contact IMS HEALTH Customer Services      #
 #  directly by phone at +44(0)20-7393-5888, or email              #
 #  globaldirect@uk.imshealth.com.                                 #
 #                                                                 #
 #  See HELP SUBSCRIPTION for more information.                    #
 ###################################################################

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

 The file name was changed from DRUGNL to IMSDRUGNEWS on 7 Dec. 2003.
 The file name DRUGNL is now an alias for IMSDRUGNEWS.

FILE IMSPRODUCT
 FILE COVERS 1982 TO 2 Dec 2005 (20051202/ED)

###################################################################
#                                                                 #
#                    !!! ATTENTION !!!                            #
#                                                                 #
#  Welcome to IMSPRODUCT.  A special subscriber rate is           #
#  available to purchasers of the IMSworld publication,           #
#  Drug Launches.                                                 #
#                                                                 #
#  For detailed information regarding eligibility and             #
#  authorization for this subscriber discount, please contact     #
#  IMS HEALTH Customer Services directly by phone                 #
#  at +44(0)20-7393-5888, or email globaldirect@uk.imshealth.com #
#  See HELP SUBSCRIPTION for more information.                    #
#                                                                 #
###################################################################
```

```
The file name was changed from DRUGLAUNCH to IMSPRODUCT on 7 Dec. 2003.
The file name DRUGLAUNCH is now an alias for IMSPRODUCT.

FILE IPA
FILE COVERS 1970 TO 29 DEC 2005 (20051229/ED)

This file contains CAS Registry Numbers for easy and accurate
substance identification.

FILE JICST-EPLUS
FILE COVERS 1985 TO 28 DEC 2005 (20051228/ED)

THE JICST-EPLUS FILE HAS BEEN RELOADED TO REFLECT THE 1999 CONTROLLED
TERM (/CT) THESAURUS RELOAD.

FILE KOSMET
FILE LAST UPDATED: 2 JAN 2006       <20060102/UP>
FILE COVERS 1968 TO DATE.

>>> SIMULTANEOUS LEFT AND RIGHT TRUNCATION IS AVAILABLE
    IN THE BASIC INDEX (/BI) FIELD  <<<

FILE LIFESCI
FILE COVERS 1978 TO 20 Dec 2005 (20051220/ED)

FILE MEDLINE
 FILE LAST UPDATED: 4 JAN 2006 (20060104/UP).  FILE COVERS 1950 TO DATE.

 On December 11, 2005, the 2006 MeSH terms were loaded.

 The MEDLINE reload for 2006 will soon be available.  For details
 on the 2005 reload, enter HELP RLOAD at an arrow promt (=>).
 See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
    http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

 OLDMEDLINE is covered back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2006 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate

FILE NAPRALERT

FILE COVERS 1650 TO 8 AUG 2005 (20050808/ED)

This file contains CAS Registry Numbers for easy and accurate
substance identification.

The NAPRALERT File is no longer being updated. ******

FILE NLDB
FILE COVERS 1988 TO 5 JAN 2006 (20060105/ED)

FILE NUTRACEUT
FILE LAST UPDATED: 21 DEC 2005       <20051221/UP>
FILE COVERS MAY 1996 TO DATE

FILE PASCAL
FILE LAST UPDATED: 19 DEC 2005       <20051219/UP>
FILE COVERS 1977 TO DATE.

>>> SIMULTANEOUS LEFT AND RIGHT TRUNCATION IS AVAILABLE
```

IN THE BASIC INDEX (/BI) FIELD <<<

FILE PCTGEN
FILE LAST UPDATED: 5 JAN 2006 <20060105/UP>
MOST RECENT PCT PUB DATE: 5 JAN 2006 <20060105/PD>

PCTGEN CURRENTLY CONTAINS 4,419,239 BIOSEQUENCES

>>> DOWNLOAD THE PCTGEN WORKSHOP MANUAL:
http://www.stn-international.de/training_center/bioseq/pctgen_wm.pdf

>>> DOWNLOAD COMPLETE PCTGEN HELP AS PDF:
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>>> DOWNLOAD RUN BLAST/GETSIM FREQUENTLY ASKED QUESTIONS:
http://www.stn-international.de/service/faq/dgenefaq.pdf <<<

FILE PHARMAML
FILE LAST UPDATED: 4 JAN 2006 <20060104/UP>
FILE COVERS 1992 TO DATE

<<< DISPLAY PRICES FOR THE MOST CURRENT 4-WEEKS INFORMATION
DIFFER FROM THE PREVIOUS ONES ==> see HELP COST >>>

FILE PHIC
FILE COVERS CURRENT RECORDS AND IS UPDATED DAILY
FILE LAST UPDATED: 5 JAN 2006 (20060105/ED)

FILE PHIN
FILE COVERS 1980 TO 3 JAN 2006 (20060103/ED)

FILE SCISEARCH

FILE COVERS 1974 TO 4 Jan 2006 (20060104/ED)

SCISEARCH has been reloaded, see HELP RLOAD for details.

FILE TOXCENTER

FILE COVERS 1907 TO 3 Jan 2006 (20060103/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

New CAS Information Use Policies, enter HELP USAGETERMS for details.

TOXCENTER has been enhanced with new files segments and search fields.
See HELP CONTENT for more information.

TOXCENTER thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.
See http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html
for a description of changes.

FILE USPATFULL
FILE COVERS 1971 TO PATENT PUBLICATION DATE: 3 Jan 2006 (20060103/PD)
FILE LAST UPDATED: 3 Jan 2006 (20060103/ED)
HIGHEST GRANTED PATENT NUMBER: US6983486
HIGHEST APPLICATION PUBLICATION NUMBER: US2005289677
CA INDEXING IS CURRENT THROUGH 3 Jan 2006 (20060103/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 3 Jan 2006 (20060103/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Oct 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Oct 2005

```
>>> USPAT2 is now available.  USPATFULL contains full text of the     <<<
>>> original, i.e., the earliest published granted patents or         <<<
>>> applications.  USPAT2 contains full text of the latest US         <<<
>>> publications, starting in 2001, for the inventions covered in     <<<
>>> USPATFULL.  A USPATFULL record contains not only the original     <<<
>>> published document but also a list of any subsequent              <<<
>>> publications.  The publication number, patent kind code, and      <<<
>>> publication date for all the US publications for an invention     <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL   <<<
>>> records and may be searched in standard search fields, e.g., /PN, <<<
>>> /PK, etc.                                                         <<<

>>> USPATFULL and USPAT2 can be accessed and searched together        <<<
>>> through the new cluster USPATALL.  Type FILE USPATALL to          <<<
>>> enter this cluster.                                               <<<
>>>                                                                   <<<
>>> Use USPATALL when searching terms such as patent assignees,       <<<
>>> classifications, or claims, that may potentially change from      <<<
>>> the earliest to the latest publication.                           <<<

This file contains CAS Registry Numbers for easy and accurate
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FILE USPAT2

FILE COVERS 2001 TO PUBLICATION DATE: 5 Jan 2006 (20060105/PD)
FILE LAST UPDATED: 5 Jan 2006 (20060105/ED)
HIGHEST GRANTED PATENT NUMBER: US2004192897
HIGHEST APPLICATION PUBLICATION NUMBER: US2006004269
CA INDEXING IS CURRENT THROUGH 5 Jan 2006 (20060105/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 5 Jan 2006 (20060105/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED:  Oct 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Oct 2005

USPAT2 is a companion file to USPATFULL.  USPAT2 contains full text
of the latest US publications, starting in 2001, for the inventions
covered in USPATFULL.  USPATFULL contains full text of the original
published US patents from 1971 to date and the original applications
from 2001.  In addition, a USPATFULL record for an invention contains
a complete list of publications that may be searched in standard
search fields, e.g., /PN, /PK, etc.

USPATFULL and USPAT2 can be accessed and searched together through
the new cluster USPATALL.  Type FILE USPATALL to enter this cluster.

Use USPATALL when searching terms such as patent assignees,
classifications, or claims, that may potentially change from the
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FILE REGISTRY
Property values tagged with IC are from the ZIC/VINITI data file
provided by InfoChem.

STRUCTURE FILE UPDATES:    4 JAN 2006  HIGHEST RN 871209-00-6
DICTIONARY FILE UPDATES:   4 JAN 2006  HIGHEST RN 871209-00-6

New CAS Information Use Policies, enter HELP USAGETERMS for details.

TSCA INFORMATION NOW CURRENT THROUGH JULY 14, 2005

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

*******************************************************************
*                                                                 *
* The CA roles and document type information have been removed from *
* the IDE default display format and the ED field has been added,   *
```

```
     * effective March 20, 2005.  A new display format, IDERL, is now    *
     * available and contains the CA role and document type information. *
     *                                                                   *
     *******************************************************************

     Structure search iteration limits have been increased.  See HELP SLIMITS
     for details.

     REGISTRY includes numerically searchable data for experimental and
     predicted properties as well as tags indicating availability of
     experimental property data in the original document.  For information
     on property searching in REGISTRY, refer to:

     http://www.cas.org/ONLINE/UG/regprops.html

=> d his

     (FILE 'HOME' ENTERED AT 13:53:28 ON 05 JAN 2006)

     FILE 'STNGUIDE' ENTERED AT 13:53:36 ON 05 JAN 2006

     FILE 'HOME' ENTERED AT 13:53:40 ON 05 JAN 2006

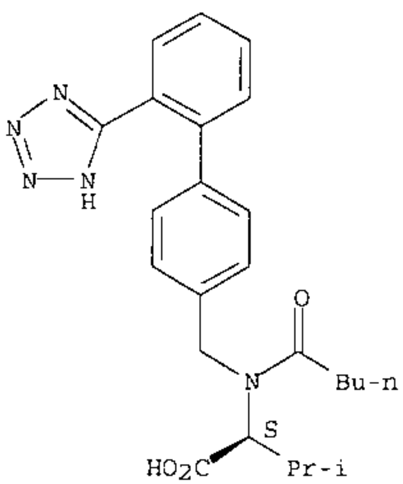
     FILE 'ADISCTI, ADISINSIGHT, ADISNEWS, BIOSIS, BIOTECHNO, CAPLUS, DDFB,
     DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ESBIOBASE,
     IFIPAT, IMSDRUGNEWS, IMSPRODUCT, IPA, JICST-EPLUS, KOSMET, LIFESCI,
     MEDLINE, NAPRALERT, NLDB, NUTRACEUT, PASCAL, ...' ENTERED AT 13:53:49 ON
     05 JAN 2006
L1            2 S 565453-99-8/RN
L2            2 DUP REM L1 (0 DUPLICATES REMOVED)
L3            2 S 565453-98-7/RN

     FILE 'REGISTRY' ENTERED AT 13:55:46 ON 05 JAN 2006
L4            1 S VALSARTAN/CN

     FILE 'USPATFULL' ENTERED AT 13:56:18 ON 05 JAN 2006
L5          227 S L4
L6          114 S L5 AND (CARDIOVASCULAR AND HYPERTENSION)
L7            0 S L6 AND VARSARTAN/AB
L8            0 S L6 AND VARSARTAN
L9           11 S L6 AND VALSARTAN/AB
L10           3 S L9 AND PD<2003
L11          25 S VALSARTAN/AB
L12           1 S L1 AND (CARDIOVASCULAR OR HYPERTENSION)/AB
L13          33 S L5 AND (VALSARTAN (P) HYPERTENSION)
L14          33 DUP REM L13 (0 DUPLICATES REMOVED)
L15          33 S L14
L16           3 S L14 AND PD<2002
L17          16 S VALSARTAN/TI
L18           1 S L17 AND PD<2002
L19           1 S L18 AND HYPERTENSION
L20           0 S L19 AND CARDIOVASCULAR
```

L4 ANSWER 1 OF 1 REGISTRY
RN 137862-53-4 REGISTRY
ED Entered STN: 13 Dec 1991
CN L-Valine, N-(1-oxopentyl)-N-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN CGP 48933
CN Diovan
CN Nisis
CN Tareg
CN **Valsartan**
FS STEREOSEARCH
DR 186597-74-0
MF C24 H29 N5 O3
CI COM
SR CA
LC STN Files: ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CANCERLIT, CAPLUS, CASREACT, CBNB, CEN, CHEMCATS, CIN, DDFU, DIOGENES, DRUGU, EMBASE, IMSDRUGNEWS, IMSPATENTS, IMSRESEARCH, IPA, MEDLINE, MRCK*, PATDPASPC, PHAR, PROMT, PROUSDDR, PS, RTECS*, SCISEARCH, SYNTHLINE, TOXCENTER, USAN, USPAT2, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

815 REFERENCES IN FILE CA (1907 TO DATE)
10 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
821 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=>

L19 ANSWER 1 OF 1 USPATFULL on STN
AN 2001:162871 USPATFULL
TI Solid oral dosage forms of **valsartan**
IN Wagner, Robert Frank, Neshanic Station, NJ, United States
Katakuse, Yoshimitsu, Hirakata, Japan
Taike, Takashi, Kobe, Japan
Yamato, Fujiki, Takarazuka, Japan
Kohlmeyer, Manfred, Basel, Switzerland
PA Novartis AG, Basel, Switzerland (non-U.S. corporation)
PI US 6294197 B1 20010925 <--
WO 9749394 19971231
AI US 1999-202805 19990507 (9)
WO 1997-EP3172 19970618
19990507 PCT 371 date
19990507 PCT 102(e) date
PRAI GB 1996-13470 19960627
DT Utility
FS GRANTED
EXNAM Primary Examiner: Page, Thurman K.; Assistant Examiner: Di Nola-Baron, Liliana
LREP Tso, Diane P.
CLMN Number of Claims: 53
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 687
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
TI Solid oral dosage forms of **valsartan**
PI US 6294197 B1 20010925 <--
WO 9749394 19971231
SUMM . . . age, sex or race and is also well tolerated. Its combination with HCTZ is also known for the treatment of **hypertension**.
SUMM . . . mg with hydrochlorothiazide in a dose range from about 6 to 60 mg, is suitable for more efficient treatment of **hypertension**.
With these dose ranges of the combined active agents, valsartan is found to have a greater efficacy in reducing elevated. . .
SUMM Hydrochlorothiazide is a known therapeutic agent which is useful in the treatment of **hypertension**.
SUMM . . . blood pressure, either systolic or diastolic or both. The conditions for which the instant invention is useful include, without limitation, **hypertension** (whether of the malignant, essential, reno-vascular, diabetic, isolated systolic, or other secondary type), congestive heart failure, angina (whether stable or. . .
CLM What is claimed is:
26. A method of treating **hypertension**, congestive heart failure, angina, myocardial infarction, arteriosclerosis, diabetic nephropathy, diabetic cardiac myopathy, renal insufficiency, peripheral vascular disease, left ventricular hypertrophy,. . .
37. A method of treating **hypertension**, congestive heart failure, angina, myocardial infarction, arteriosclerosis, diabetic nephropathy, diabetic cardiac myopathy, renal insufficiency, peripheral vascular disease, stroke, left ventricular. . .

=>

L3 ANSWER 171 OF 177 PROMT

AN 96:59745 PROMT

TI Making the next choice easier
Introduced new cardiovascular drug called Lotrel

SO Med Ad News, (1 Jan 1996) pp. 3.
ISSN: 0745-0907.

LA English

WC 1083

FULL TEXT IS AVAILABLE IN THE ALL FORMAT

AB Lotrel, a combination of amlodipine and benazepril, is indicated as second-line therapy for **hypertension**. Amlodipine already is on the market as the single-ingredient calcium channel blocker Norvasc, marketed by Pfizer Inc. Benazepril already is. . .

TX Lotrel, a combination of amlodipine and benazepril, is indicated as second-line therapy for **hypertension**. Amlodipine already is on the market as the single-ingredient calcium channel blocker Norvasc, marketed by Pfizer Inc. Benazepril already is. . .

About 50 million Americans have **hypertension**. Of those treated, half are prescribed either a calcium channel blocker or an ACE inhibitor. Despite the proven efficacy of. . .

Lotrel is the first calcium channel blocker and ACE inhibitor combination therapy for the treatment of **hypertension**. This could change relatively soon, however. At least two pharmaceutical companies, Merck & Co. Inc. and Hoechst Marion Roussel Inc.,. . .

A new drug application was filed with regulatory authorities Dec. 31, 1994, for an indication as a second-line therapy to treat **hypertension**.

Mr. . . . is referring to a new Ciba product ready for filing. A new drag application for an angiotensin-II antagonist, brand named **Valsartan**, is being prepared. Phase III clinical trials of the product, as a treatment for **hypertension**, have been completed.

Indication: Second-line, combination treatment of **hypertension**

L5 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN
AN 2003:570812 CAPLUS
DN 139:138733
TI Pharmaceutical compositions comprising valsartan and neutral endopeptidase inhibitors
IN Webb, Randy Lee; Ksander, Gary Michael
PA Novartis A.-G., Switz.; Novartis Pharma G.m.b.H.
SO PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2003059345	A1	20030724	WO 2003-EP415	20030116
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LT, LU, LV, MA, MD, MK, MN, MX, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SE, SG, SK, TJ, TM, TN, TR, TT, UA, US, UZ, VC, VN, YU, ZA, ZW				
RW: AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR				
US 2003144215	A1	20030731	US 2003-341868	20030114
CA 2472399	AA	20030724	CA 2003-2472399	20030116
EP 1467728	A1	20041020	EP 2003-704413	20030116
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
BR 2003006907	A	20041221	BR 2003-6907	20030116
JP 2005514441	T2	20050519	JP 2003-559507	20030116
ZA 2004005117	A	20050622	ZA 2004-5117	20040628
NO 2004003380	A	20041007	NO 2004-3380	20040813
PRAI US 2002-349660P	P	20020117		
US 2002-386792P	P	20020607		
WO 2003-EP415	W	20030116		

RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
IT 107-95-9, β-Alanine 36357-77-4, Phosphoramidon 76721-89-6, Thiorphan 82154-09-4, retro-Thiorphan 83861-02-3 100845-83-8, SQ28603 105262-04-2 115406-23-0 122222-44-0, SQ 29072 123122-55-4 123898-42-0 123984-67-8 123985-34-2 123985-36-4 129093-37-4 133153-38-5 135925-65-4 137613-73-1 139994-51-7 139994-53-9 144505-58-8 144933-39-1 145707-85-3 145775-14-0 145841-10-7 149705-07-7 **149709-62-6** 150055-94-0 153037-29-7 154116-31-1 158894-60-1 161952-07-4 565453-90-9 565453-91-0 565453-92-1 565453-93-2 565453-94-3 565453-95-4 565453-96-5 565453-97-6 **565453-98-7** **565453-99-8**
RL: PEP (Physical, engineering or chemical process); PYP (Physical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(pharmaceutical compns. comprising valsartan and neutral endopeptidase inhibitors)

L5 ANSWER 2 OF 4 USPATFULL on STN
AN 2003:207854 USPATFULL
TI Methods of treatment and pharmaceutical composition
IN Ksander, Gary Michael, Amherst, NH, UNITED STATES
Webb, Randy Lee, Flemington, NJ, UNITED STATES
PI US 2003144215 A1 20030731
AI US 2003-341868 A1 20030114 (10)
PRAI US 2002-386792P 20020607 (60)
US 2002-349660P 20020117 (60)
DT Utility
FS APPLICATION
LREP THOMAS HOXIE, NOVARTIS, CORPORATE INTELLECTUAL PROPERTY, ONE HEALTH PLAZA 430/2, EAST HANOVER, NJ, 07936-1080

CLMN   Number of Claims: 11
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 946
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
IT   107-95-9, β-Alanine   36357-77-4, Phosphoramidon   76721-89-6,
        Thiorphan   82154-09-4, retro-Thiorphan   83861-02-3   100845-83-8,
        SQ28603   105262-04-2   115406-23-0   122222-44-0, SQ 29072   123122-55-4
        123898-42-0   123984-67-8   123985-34-2   123985-36-4   125132-63-0
        129093-37-4   133153-38-5   135925-65-4   137613-73-1   139994-51-7
        139994-53-9   144505-58-8   144933-39-1   145707-85-3   145775-14-0
        145841-10-7   149705-07-7   150055-94-0   153037-29-7   154116-31-1
        158894-60-1   161952-07-4   565453-90-9   565453-91-0   565453-92-1
        565453-93-2   565453-94-3   565453-95-4   565453-96-5   565453-97-6
        565453-98-7 565453-99-8
          (pharmaceutical compns. comprising valsartan and neutral endopeptidase
          inhibitors)

L5   ANSWER 3 OF 4 USPATFULL on STN
AN   94:88833 USPATFULL
TI   Biaryl substituted 4-amino-butyric acid amides
IN   Ksander, Gary, Milford, NJ, United States
PA   Ciba-Geigy Corporation, Ardsley, NY, United States (U.S. corporation)
PI   US 5354892   19941011
AI   US 1993-8031   19930125 (8)
DCD   20100608
RLI   Continuation of Ser. No. US 1992-824132, filed on 22 Jan 1992, now
        patented, Pat. No. US 5217996
DT   Utility
FS   Granted
EXNAM   Primary Examiner: Dees, Jose G.; Assistant Examiner: Frazier, Barbara S.
LREP   Gruenfeld, Norbert
CLMN   Number of Claims: 10
ECL   Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 1239
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
IT   128779-47-5P   149690-12-0P   149690-13-1P   149709-56-8P   149709-57-9P
        149709-58-0P   149709-59-1P   149709-60-4P   149709-61-5P
        149709-62-6P   149709-63-7P   149818-98-4P
          (preparation and reaction of, preparation of neutral endopeptidase inhibitors)

L5   ANSWER 4 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 1
AN   1993:670810 CAPLUS
DN   119:270810
TI   Preparation of biaryl substituted 4-amino-butyric acid amides
IN   Ksander, Gary
PA   Ciba-Geigy Corp., USA
SO   U.S., 13 pp.
     CODEN: USXXAM
DT   Patent
LA   English
FAN.CNT 1

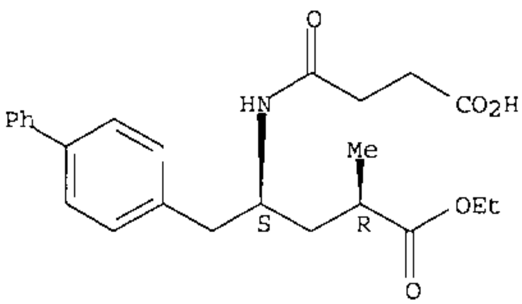
	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5217996	A	19930608	US 1992-824132	19920122
	EP 555175	A1	19930811	EP 1993-810016	19930113
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
	AU 9331842	A1	19930729	AU 1993-31842	19930115
	AU 666902	B2	19960229		
	JP 05310664	A2	19931122	JP 1993-5908	19930118
	CA 2087652	AA	19930723	CA 1993-2087652	19930120
	ZA 9300421	A	19930722	ZA 1993-421	19930121
	NO 9300193	A	19930723	NO 1993-193	19930121
	HU 63376	A2	19930830	HU 1993-166	19930121
	US 5354892	A	19941011	US 1993-8031	19930125
PRAI	US 1992-824132	A	19920122		

OS   MARPAT 119:270810

IT 128779-47-5P 149690-12-0P 149690-13-1P 149709-56-8P 149709-57-9P
149709-58-0P 149709-59-1P 149709-60-4P 149709-61-5P
149709-62-6P 149709-63-7P 149818-98-4P
RL: RCT (Reactant); SPN (Synthetic preparation); PREP (Preparation); RACT (Reactant or reagent)
(preparation and reaction of, preparation of neutral endopeptidase inhibitors)

```
L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2005 ACS on STN
RN   149709-62-6  REGISTRY
ED   Entered STN:  01 Sep 1993
CN   [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
     α-methyl-, ethyl ester, [S-(R*,S*)]- (9CI)  (CA INDEX NAME)
FS   STEREOSEARCH
MF   C24 H29 N O5
CI   COM
SR   CA
LC   STN Files:   CA, CAPLUS, USPATFULL
```

Absolute stereochemistry.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

2 REFERENCES IN FILE CA (1907 TO DATE)
2 REFERENCES IN FILE CAPLUS (1907 TO DATE)

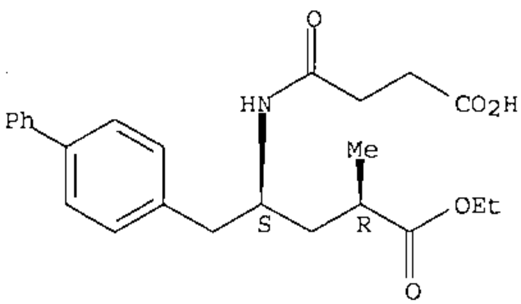
=>

L2 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2005 ACS on STN
RN **565453-98-7** REGISTRY
ED Entered STN: 13 Aug 2003
CN [1,1'-Biphenyl]-4-pentanoic acid, γ-[(3-carboxy-1-oxopropyl)amino]-
α-methyl-, ethyl ester, (αR,γS)-, compd. with
2,2',2''-nitrilotris[ethanol] (1:1) (9CI) (CA INDEX NAME)
FS STEREOSEARCH
MF C24 H29 N O5 . C6 H15 N O3
SR CA
LC STN Files: CA, CAPLUS, USPATFULL

CM 1

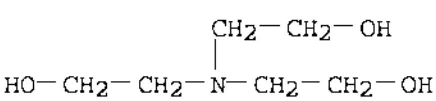
CRN 149709-62-6
CMF C24 H29 N O5

Absolute stereochemistry.



CM 2

CRN 102-71-6
CMF C6 H15 N O3



1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=>

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/341,868	01/14/2003	Gary Michael Ksander	4-32219A	8865

1095 7590 01/12/2006

NOVARTIS
CORPORATE INTELLECTUAL PROPERTY
ONE HEALTH PLAZA 104/3
EAST HANOVER, NJ 07936-1080

EXAMINER	
KIM, JENNIFER M	
ART UNIT	PAPER NUMBER
1617	

DATE MAILED: 01/12/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Office Action Summary	Application No.	Applicant(s)	
	10/341,868	KSANDER ET AL.	
	Examiner	Art Unit	
	Jennifer Kim	1617	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 September 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-5 and 7-11* is/are pending in the application.

4a) Of the above claim(s) *5 and 7* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,4 and 8-11* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *11/5/2003;6/1/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Applicant's election of Group 1, claims 1, 3, 4 and 8-11, drawn to a pharmaceutical composition comprising AT-1 antagonist valsartan and the specific NEP inhibitors in the reply filed on September 30, 2005 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Accordingly, claims 5 and 7 are withdrawn from consideration since they are non-elected invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 8-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ksander (U.S.Patent No. 5,217,996) of record.

Ksander teaches the compound, 4-[N-(3-carboxy-1-oxo-propyl)amino]-4-(p-phenylphenylmethyl)-2-methylbutanoic acid ethyl ester, the (2R,4S)antipode thereof (also known as N-(3-caroxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino2R-methylbutanoic acid ethyl ester) is a pharmacologically potent neutral endopeptidase enzyme inhibitor and it is useful for the treatment of cardiovascular disorders such as **hypertension**. (column 9, lines 5-15, column 12, lines 1-10, claims 1-22). Ksander teaches ammonium salts, mono-, di- or tri-lower **(alkyl or hydroxyalkyl)-ammonium salts** (e.g. **triethanolammonium**) are suitable pharmaceutically acceptable salts of the compound. (column 5, lines 35-45).

Ksander does not illustrate the specific salt form of the compound set forth in claims 8 and 9.

It would have been obvious to one of ordinary skill in the art to employ triethanolamine salt of the compound because Ksander teaches that triethanolamine salt is pharmaceutically acceptable salt of the compound. One would have been motivated to employ the pharmaceutically acceptable salt of the compound e.g. **triethanolammonium** as taught by Ksander. Further, the specified salt (tris(hydroxymethyl)aminomethane salt) of the compound set forth in claim 9 is obvious because Ksander teaches the any ammonium salts, including tri-lower **(alkyl or hydroxyalkyl)-ammonium salt** is pharmaceutically acceptable and the antihypertensive utility is retained. Therefore, one of ordinary skill in the art would have been motivated to employ any one of ammonium salts, including tri-lower **(alkyl or hydroxyalkyl)-ammonium salt** including (tris(hydroxymethyl)aminomethane salt) in order to achieve an expected benefit of formulating the compound with its pharmaceutically acceptable salt useful for antihypertensive effect taught by Ksander. Accordingly, the instant claim is obvious therefrom.

Claims 1, 3 and 4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ksander (U.S.Patent No. 5,217,996) of record and Buhlmayer et al. (U.S.Patent No. 5,399,578).

Ksander teaches a pharmaceutical composition comprising the compound, 4-[N-(3-carboxy-1-oxo-propyl)amino]-4-(p-phenylphenylmethyl)-2-methylbutanoic acid ethyl ester, the (2R,4S)antipode thereof (also known as N-(3-caroxy-1-oxopropyl)-(4S)-p-phenylphenylmethyl)-4-amino2R-methylbutanoic acid ethyl ester) is a pharmacologically potent neutral endopeptidase enzyme (NEP) inhibitor and it is useful for the treatment of cardiovascular disorders such as **hypertension**. (column 9, lines 5-15, column 12, lines 1-10, claims 1-22). Ksander teaches ammonium salts, mono-, di- or tri-lower **(alkyl or hydroxyalkyl)-ammonium salts** (e.g. **triethanolammonium**) are suitable pharmaceutically acceptable salts of the compound. (column 5, lines 35-45).

Buhlmayer et al. teach valsartan is useful for an anti-hypertensive treatment. (abstract, claims).

The claims differ from the cited references in claiming a pharmaceutical composition comprising combination of the specific NEP inhibitor and valsartan. To employ combinations of specific NEP inhibitor and valsartan would have been obvious because all the components are well known individually for treating hypertension. One of ordinary skill in the art would have been motivated to combine specific NEP inhibitor and valsartan in a single composition in order to achieve an expected benefit of antihypertensive effect of the combination. The motivation for combining the components flows from their individually known common utility (see In re Kerkhoven, 205 USPQ 1069(CCPPA 1980)). Thus, the claims fail to patentably distinguish over the state of the art as represented by the cited references.

None of the claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Kim whose telephone number is 571-272-0628. The examiner can normally be reached on Monday through Friday 6:30 am to 3 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Sreenivasan Padmanabhan can be reached on 571-272-0629. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Sreenivasan Padmanabhan
Supervisory Examiner
Art Unit 1617

Jmk
January 6, 2006

Notice of References Cited	Application/Control No. 10/341,868	Applicant(s)/Patent Under Reexamination KSANDER ET AL.	
	Examiner Jennifer Kim	Art Unit 1617	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,399,578	03-1995	Buhlmayer et al.	514/381
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

FORM PTO-1449 (REV. 7-85)
U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

ATTY. DOCKET NO. 4-32219A
APPLICATION NO. 10/341,868
APPLICANT KSANDER ET AL.
FILING DATE JANUARY 14, 2002
Group 1614

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE
*	AA	5,217,996	06/08/93	Ksander	514	533	01/22/92
	AB						
	AC						
	AD						
	AE						
	AF						
	AG						
	AH						
	AI						
	AJ						
	AK						
	AL						

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	OFFICE	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
	AM	WO 01/74348 A2	10/11/01	WIPO			☐	☐
	AN	WO 02/06253	01/24/02	WIPO			☐	☐
	AO	WO 02/092622 A2	11/21/02	WIPO			☐	☐
	AP	0 726 072 A2	08/14/96	Europe			☐	☐
	AQ	0 498 361 A2	08/12/92	Europe			☐	☐

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent pages, Etc.)

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EXAMINER

DATE CONSIDERED 1/6/2006

*EXAMINER: Initial of reference considered, whether or not citation is in conformance with MPEP 609: Draw a line through citation if not in conformance and not considered. Include a copy of this form with the next communication to applicant.

FORM PTO-1449 (REV. 7-85)
U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

Sheet 1 of 1

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

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PATENT & TRADEMARK OFFICE

ATTY. DOCKET NO.
4-32219A
APPLICATION NO.
10/341,868
APPLICANT
KSANDER ET AL.
FILING DATE
JANUARY 14, 2003
Group
1614

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE
	AA						
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FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	OFFICE	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
7	AM	WO 03/066606	8/14/03	PCT			☐	☐
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	AO						☐	☐
	AP						☐	☐
	AQ						☐	☐

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent pages, Etc.)

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	AT	

EXAMINER [signature]

DATE CONSIDERED 1/6/2006

*EXAMINER: Initial of reference considered, whether or not citation is in conformance with MPEP 609: Draw a line through citation if not in conformance and not considered. Include a copy of this form with the next communication to applicant.

Index of Claims	Application/Control No.	Applicant(s)/Patent under Reexamination
	10/341,868	KSANDER ET AL.
	Examiner	Art Unit
	Jennifer Kim	1617

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
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Claim		Date								
Final	Original	9/20/05	1/6/06							
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Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination
	10/341,868	KSANDER ET AL.
	Examiner	Art Unit
	Jennifer Kim	1617

SEARCHED			
Class	Subclass	Date	Examiner
514	533	1/6/2006	JMK
514	381	1/6/2006	JMK

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)	DATE	EXMR
Inventor search	1/6/2006	JMK
STN (medicine, registry); WEST	1/6/2006	JMK
STIC	12/15/2005	JMK

U.S. Patent and Trademark Office

Part of Paper No. 01062006

04-06-06

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PATENT & TRADEMARK OFFICE

TDW 1617

CASE 4-32219A

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10	
EV727274665US	4/4/06
Express Mail Label Number	Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
KSANDER ET. AL.
APPLICATION NO: 10/341,868
FILED: JANUARY 14, 2003
FOR: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Art Unit: 1617
Examiner: Kim, Jennifer M

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

FEE LETTER FOR INFORMATION DISCLOSURE STATEMENT

Sir:

Please charge Deposit Account No. 19-0134 in the name of Novartis in the amount of $180 for payment of the fee pursuant to 37 CFR §1.17(p) for the submission of an Information Disclosure Statement under 37 CFR §1.97(c).

An additional copy of this paper is here enclosed. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 19-0134 in the name of Novartis.

Respectfully submitted,

Greg Ferraro

Gregory D. Ferraro
Attorney for Applicants
Reg. No. 36,134

Novartis
Corporate Intellectual Property
One Health Plaza, Building 104
East Hanover, NJ 07936-1080
(862) 778-7831

Date: April 3, 2006

CASE 4-32219A

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EV727274665US
Express Mail Label Number

4/4/06
Date of Deposit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
KSANDER ET AL.
APPLICATION NO: 10/341,868
FILED: JANUARY 14, 2003
FOR: METHODS OF TREATMENT AND PHARMACEUTICAL COMPOSITION

Art Unit: 1617
Examiner: Kim, Jennifer M

MS: Amendment
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Sir:

This paper is supplemental to the Information Disclosure Statement filed November 5, 2003 and June 1, 2004. Since it is being filed in accordance with 37 C.F.R. §1.97(c), a letter for payment of fee set forth in 37 C.F.R. §1.17(p) is enclosed.

In accordance with 37 C.F.R. §1.56, applicants wish to call the Examiner's attention to the references cited on the attached form(s) PTO-1449.

Copies of these references are enclosed herewith.

04/07/2006 SFELEKE1 00000010 190134 10341868
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The Examiner is requested to consider the foregoing information in relation to this application and indicate that each reference was considered by returning a copy of the initialed PTO 1449 form(s).

Respectfully submitted,

Gregory D. Ferraro
Attorney for Applicants
Reg. No. 36,134

Novartis
Corporate Intellectual Property
One Health Plaza, Building 104
East Hanover, NJ 07936-1080
(862) 778-7831

Date: April 3, 2006

FORM PTO-1449
(REV. 7-85)

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

ATTY. DOCKET NO.
4-32219A
APPLICATION NO.
10/341,868
APPLICANT
KSANDER ET AL.
FILING DATE
JANUARY 14, 2003

Group
1617

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE
	AA	US 4,610,816	09/09/86	Berger	549	452	06/15/84
	AB	US 4,722,810	02/02/88	Delaney et al.	260	402.5	08/13/86
	AC	US 4,740,499	04/26/88	Olins	514	13	07/28/86
	AD	US 4,749,688	06/07/88	Haslanger et al.	514	19	06/20/86
	AE	US 4,929,641	05/29/90	Haslanger et al.	514	506	05/11/88
	AF	US 5,217,996	06/08/93	Ksander	514	533	01/22/92
	AG	US 5,223,516	06/29/93	Delaney et al.	514	339	04/24/91
	AH	US 5,273,990	12/28/93	De Lombaert	514	381	09/03/92
	AI	US 5,294,632	03/15/94	Erion et al.	514	381	10/09/92
	AJ	US 5,399,578	03/21/95	Bühlmayer et al.	514	381	12/29/92
	AK	US 5,520,522	05/28/96	Rathore et al.	417	322	09/21/94
	AL						

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	OFFICE	CLASS	SUBCLASS	TRANSLATION YES	NO
	AM	EP 0 342 850	11/23/89	Europe			☐	☐
	AN	EP 0 343 911	11/29/89	Europe			☐	☐
	AO	EP 0 361 365	04/04/90	Europe			☐	☐
	AP	EP 0 443 983	08/28/91	Europe			☐	☐
	AQ	EP0636621B1	3/12/97	Europe			☐	☐

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	AR	Almeida et al., "Clearance Function of Type C Receptors of Atrial Natriuretic Factor in Rats", *Am J Physiol*, Vol. 256, pp. R469-R475 (1989).
	AS	Bazil, Krulan and Webb, "Telemetric Monitoring of Cardiovascular Parameteres in Conscious Spontaneously Hypertensive Rats", *J Cardiovasc Pharmacol*, Vol. 22, pp. 897-905 (1993).
	AT	Consensus Trial Study Group, "Effects of Enalapril on Mortality in Severe Congestive Heart Failure", *N Eng J Med*, Vol. 316, No. 23, pp. 1429-1435 (1987).

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial of reference considered, whether or not citation is in conformance with MPEP 609. Draw a line through citation if not in conformance and not considered. Include a copy of this form with the next communication to applicant.

FORM PTO-1449
(REV. 7-85)

U.S. DEPARTMENT OF COMMERCE
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INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

ATTY. DOCKET NO. 4-32219A
APPLICATION NO. 10/341,868
APPLICANT KSANDER ET AL.
FILING DATE JANUARY 14, 2003
Group 1617

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	OFFICE	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
	CA	EP 0 636 621	02/01/95	Europe			☐	☐
	CB	GB 2 218 983	11/29/89	United Kingdom			☐	☐
	CC	WO 90/09374	08/23/90	WIPO			☐	☐
	CD	WO 92/14706	09/03/92	WIPO			☐	☐
	CE	WO 93/09101	05/13/93	WIPO			☐	☐
	CF	WO 93/10773	06/10/93	WIPO			☐	☐
	CG	WO 94/15908	07/1/94	WIPO (English Abstract)			☐	☐
	CH						☐	☐
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EXAMINER INITIAL		OTHER DOCUMENTS (Including Author, Title, Date, Pertinent pages, Etc.)
	DA	Stephenson et al., "The hydrolysis of a-human atrial natriuretic peptide by pig kindney microvillar membranes is initiated by endopeptidase-24.11 *Biochem J*, Vol. 243, pp. 183-187(1987).
	DB	Erdös, "Angiotensin I Converting Enzyme and the Changes in Our Concepts Through the Years" – Lewis K. Dahl Memorial Lecture, *Hypertension*, Vol. 16, No. 4, pp. 363-370 (1990).
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	DD	Intengan, Thibault, Li and Schiffrin, "Resistance Artery Mechanics, Structure, and Extracellular Components in Spontaneously Hypertensive Rats", *Circulation*, Vol. 100, No. 22, pp. 2267-2275 (1999).
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	DF	Stephenson and Kenny, "Metabolism of Neuropeptides", *Biochem J*, Vol. 241, pp. 237-247 (1987).
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	DH	Sybertz et al., "Atrial Natriuretic Factor-Potentiating and Antihypertensive Activity of SCH 34826", *Hypertension*, Vol. 15, No. 2, pp. 152-161 (1990).
	DI	Williford, Sharma, Korth and Sheu, "Spatial Heterogeneity of Intracellular Ca^{2+} Concentration in Nonbeating Guinea Pig Ventricular Myocytes", *Circ Res*, Vol. 66, No. 1, pp. 241-249 (1990).
	DJ	Zannad, "The Emerging Role of ACE Inhibitors in the Treatment of Cardiovascular Disease", *J Cardiovasc Pharmacol*, Vol. 15, Suppl. 2, pp. S1, S5 (1990).
	DK	CAPLUS Abstract AN 1986:573042`- Taub et al., f ZA8400670, 9/25//1985
	DL	CAPLUS Abstract AN 1995: 931230- Sugano et al., JP 07157459, 06/20/1995,
	DM	CAPLUS Abstract AN 1995:412660 – Yamada et al., JP 06234754, 8/23/1994
	DN	

EXAMINER	DATE CONSIDERED

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Europäisches Patentamt
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(19)

(11) Publication number: **0 342 850 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 89304698.7

(51) Int. Cl.⁴: C07C 103/737

(22) Date of filing: 09.05.89

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(43) Date of publication of application: 23.11.89 Bulletin 89/47

(84) Designated Contracting States: AT BE CH DE ES FR GB GR IT LI LU NL SE

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(84) GB

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235 East 42nd Street
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(84) BE CH DE ES FR GR IT LI LU NL SE AT

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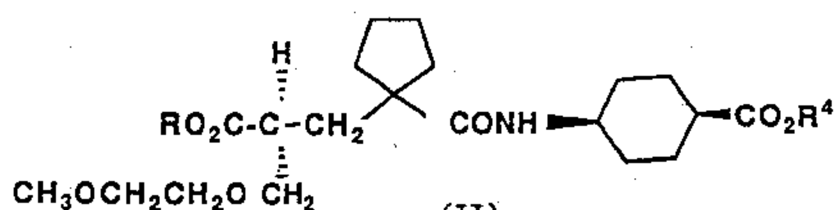
(74) Representative: Moore, James William, Dr.
Pfizer Limited Ramsgate Road
Sandwich Kent CT13 9NJ(GB)

(54) Enantiomeric glutaramide diuretic agents.

(57) S Enantiomeric diuretic agent of the formula:



(II)

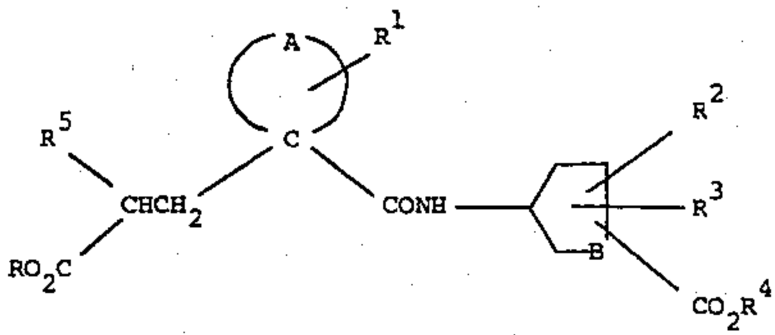
wherein each R and R^4 is H or one of R and R^4 is H and the other is a biolabile ester group, a process for their preparation and intermediates therefor.

EP 0 342 850 A1

ENANTIOMERIC GLUTARAMIDE DIURETIC AGENTS

This invention relates to certain spiro-substituted glutaramide derivatives which are diuretic agents having utility in a variety of therapeutic areas including the treatment of various cardiovascular disorders such as hypertension and heart failure.

According to the specification of our European patent application 0274234 we describe and claim a series of spiro-substituted glutaramide derivatives of the formula:



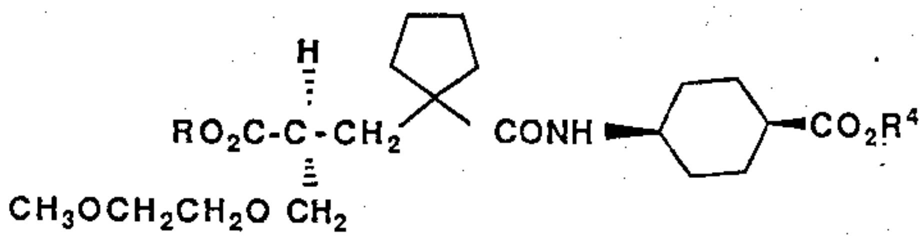
(I)

wherein A completes a 4 to 7 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be fused to a further saturated or unsaturated 5 or 6 membered carbocyclic ring;
B is $(CH_2)_m$ wherein m is an integer of from 1 to 3; each of R and R^4 is independently H, C_1-C_6 alkyl, benzyl or an alternative biolabile ester-forming group;
R^1 is H or C_1-C_4 alkyl;
R^2 and R^3 are each independently H, OH, C_1-C_4 alkyl or C_1-C_4 alkoxy;
and R^5 is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl(C_2-C_6 alkynyl), C_3-C_7 cycloalkyl, C_3-C_7 cycloalkenyl, C_1-C_6 alkoxy, -NR^6R^7, -NR^8COR^9, -$NR^8SO_2R^9$ or a saturated heterocyclic group;
or C_1-C_6 alkyl substituted by one or more substituents chosen from halo, hydroxy, C_1-C_6 alkoxy, C_2-C_6 hydroxyalkoxy, C_1-C_6 alkoxy(C_1-C_6 alkoxy), C_3-C_7 cycloalkyl, C_3-C_7 cycloalkenyl, aryl, aryloxy, aryloxy(C_1-C_4 alkoxy), heterocyclyl, heterocyclyloxy, -NR^6R^7, -NR^8COR^9, -$NR^8SO_2R^9$, -$CONR^6R^7$, -SH, -$S(O)_pR^{10}$, -COR^{11} or -CO_2R^{12};
wherein R^6 and R^7 are each independently H, C_1-C_4 alkyl, C_3-C_7 cycloalkyl (optionally substituted by hydroxy or C_1-C_4 alkoxy), aryl, aryl(C_1-C_4 alkyl), C_2-C_6 alkoxyalkyl, or heterocyclyl; or the two groups R^6 and R^7 are taken together with the nitrogen to which they are attached to form a pyrrolidinyl, piperidino, morpholino, piperazinyl or N-(C_1-C_4 alkyl)-piperazinyl group;
R^8 is H or C_1-C_4 alkyl;
R^9 is C_1-C_4 alkyl, CF_3, aryl, aryl(C_1-C_4 alkyl), aryl(C_1-C_4 alkoxy), heterocycyl, C_1-C_4 alkoxy or NR^6R^7 wherein R^6 and R^7 are as previously defined;
R^{10} is C_1-C_4 alkyl, aryl, heterocyclyl or NR^6R^7 wherein R^6 and R^7 are as previously defined;
R^{11} is C_1-C_4 alkyl, C_3-C_7 cycloalkyl, aryl or heterocyclyl;
R^{12} is H or C_1-C_4 alkyl;
and p is 0, 1 or 2;
and pharmaceutically acceptable salts thereof and bioprecursors therefor.

The compounds are inhibitors of the zinc-dependent, neutral endopeptidase E.C.3.4.24.11. This enzyme is involved in the breakdown of several peptide hormones, including atrial natriuretic factor (ANF), which is secreted by the heart and which has potent vasodilatory, diuretic and natriuretic activity. Thus, by inhibiting the neutral endopeptidase E.C.3.4.24.11, the compounds can potentiate the biological effects of ANF and, in particular, the compounds are diuretic agents having utility in the treatment of a number of disorders, including hypertension, heart failure, angina, renal insufficiency, premenstrual syndrome, cyclical oedema, Menières disease, hyperaldosteroneism (primary and secondary) and hypercalciuria. In addition, because of their ability to potentiate the effects of ANF the compounds have utility in the treatment of glaucoma. As a further result of their ability to inhibit the neutral endopeptidase E.C.3.4.24.11 the compounds of the invention may have activity in other therapeutic areas including for example the treatment of asthma, inflammation, pain, epilepsy, affective disorders, dementia and geriatric confusion, obesity and gastrointestinal disorders (especially diarrhoae and irritable bowel syndrome), the modulation of gastric acid secretion and the treatment of hyperreninaemia.

Particularly preferred compounds according to European patent application 0274234 are:
cis-4-{1-[2-carboxy-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid and biolabile ester derivatives thereof, including in particular the indanyl ester:
cis-4-{1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

It will be noted that the two above compounds have an asymmetric carbon atom and therefore exist as R and S enantiomeric forms. We have now separated the isomers and unexpectedly discovered that the biological activity resides exclusively in the (+) enantiomer of diacid (II) ($R = R^4 = H$), to which we have assigned the S configuration. The R enantiomer is virtually inactive. Thus the present invention provides S enantiomeric compounds of the formula:-



(II)

wherein each of R and R^4 is H or one of R and R^4 is H and the other is a biolabile ester group, said enantiomer being substantially free of the R enantiomer.

By substantially free of the R enantiomer is meant that the compounds of formula (II) contain less than 10%, and preferably less than 5% of the R enantiomer.

The term biolabile ester-forming group is well understood in the art as meaning a group which provides an ester which can be readily cleaved in the body to liberate the corresponding diacid of formula (II) wherein R and R^4 are both H. Examples of such esters include, in particular,
ethyl
benzyl
1-(2,2-diethylbutyryloxy)ethyl
2-ethylpropionyloxymethyl
1-(2-ethylpropionyloxy)ethyl
1-(2,4-dimethylbenzoyloxy)ethyl
α-benzoyloxybenzyl
1-(benzoyloxy)ethyl
2-methyl-1-propionyloxy-1-propyl
2,4,6-trimethylbenzoyloxymethyl
1-(2,4,6-trimethylbenzoyloxy)ethyl
pivaloyloxymethyl
phenethyl
phenpropyl
2,2,2-trifluoroethyl
1- or 2-naphthyl
2,4-dimethylphenyl
4-t-butylphenyl
and 5-indanyl.

Of these a particular preferred biolabile ester-forming group is 5-indanyl.

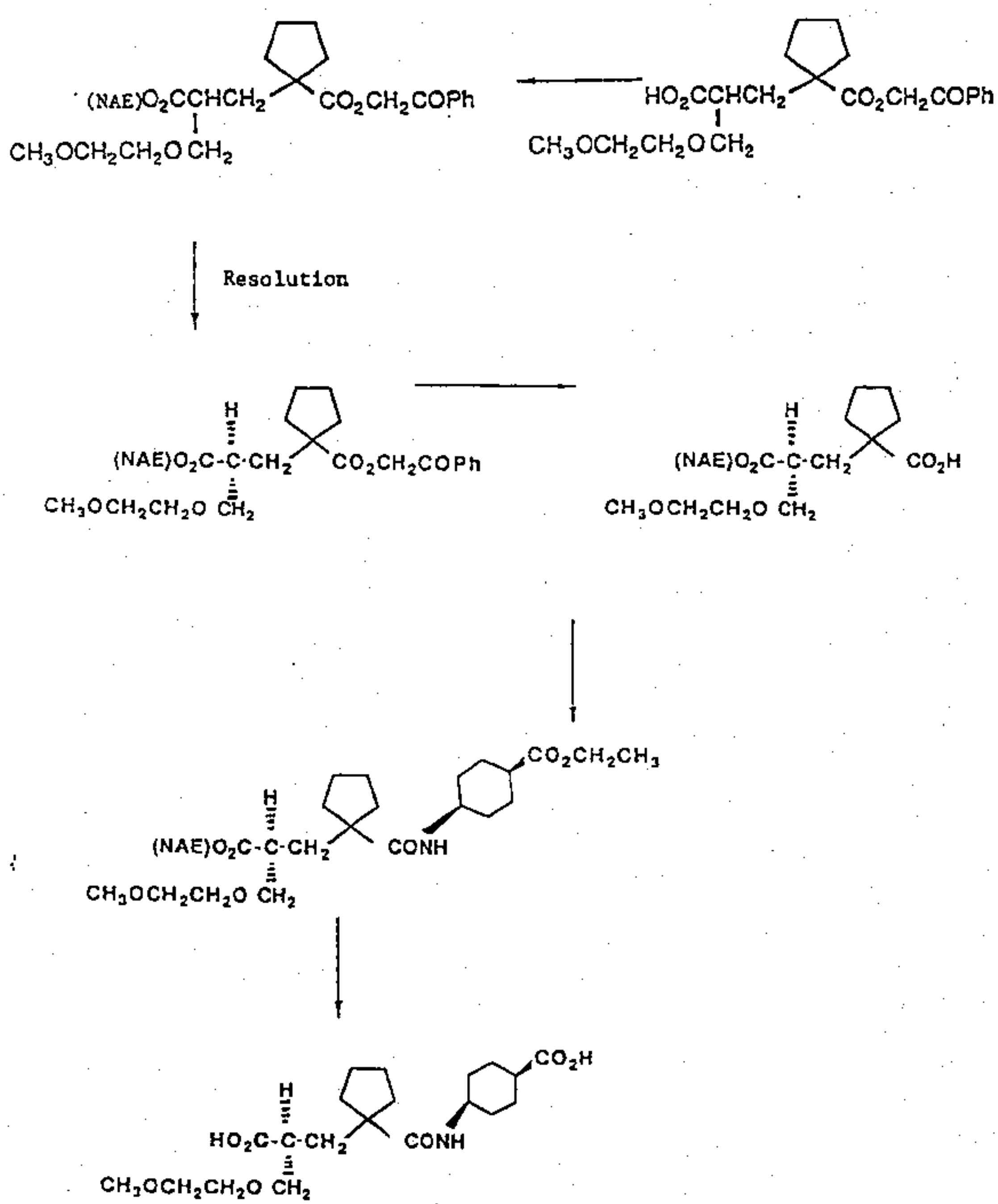
Thus particularly preferred individual compounds according to the invention are:
(S)-cis-4-{1-[2-carboxy-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid and
(S)-cis-4-{1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-

cyclohexanecarboxylic acid

The compounds of formula (II) are prepared generally following the synthetic procedures already disclosed in European patent application 0274234 but incorporating a resolution step at some convenient point in the synthetic sequence. Such resolution may be achieved by known techniques such as by fractional crystallisation of a salt formed with an optically active base or by chromatographic resolution of a diastereoisomeric derivative, such as, for example, an ester formed by reaction with an optically active alcohol.

Thus, in one process for preparation of the bis-acid of formula (II) wherein R and R^4 are both H, the following synthetic procedure may be employed wherein (NAE) indicates the N-acetyl-(1R,2S)-ephedrine ester:-

Scheme 1

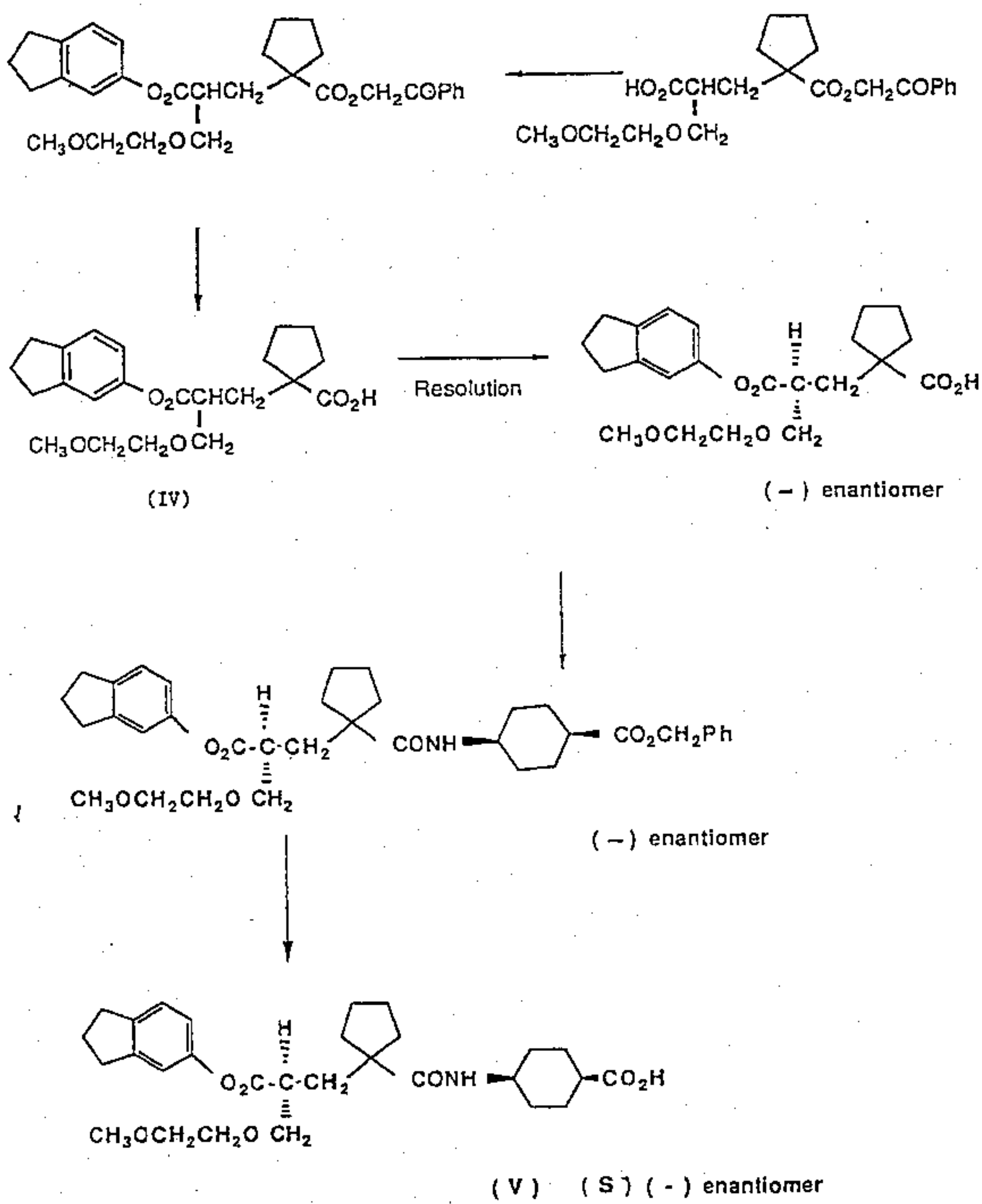


(III) (S) (+) enantiomer

In this process N-acetyl-(1R,2S)-ephedrine is coupled to 2-(2-methoxyethoxymethyl)-3-[1-(phenacyloxycarbonyl)cyclopentyl]propanoic acid (prepared as described in European patent application no. 0274234) using, for example, a carbodiimide coupling reaction. Resolution of the resulting diester product may be achieved by chromatography on silica. The required separated diastereoisomer is treated with zinc in glacial acetic acid to remove the phenacyl ester group and the product is coupled with cis-4-aminocyclohexane carboxylic acid ethyl ester, again using a carbodiimide coupling reaction. The ester groups are finally removed by catalytic hydrogenation followed by mild alkaline hydrolysis to yield the dicarboxylic acid (III) as its dextrorotatory S enantiomer.

In a further process for preparing the compound of formula (II) wherein R is 5-indanyl and R[4] is H the following synthetic sequence may be followed:-

Scheme 2

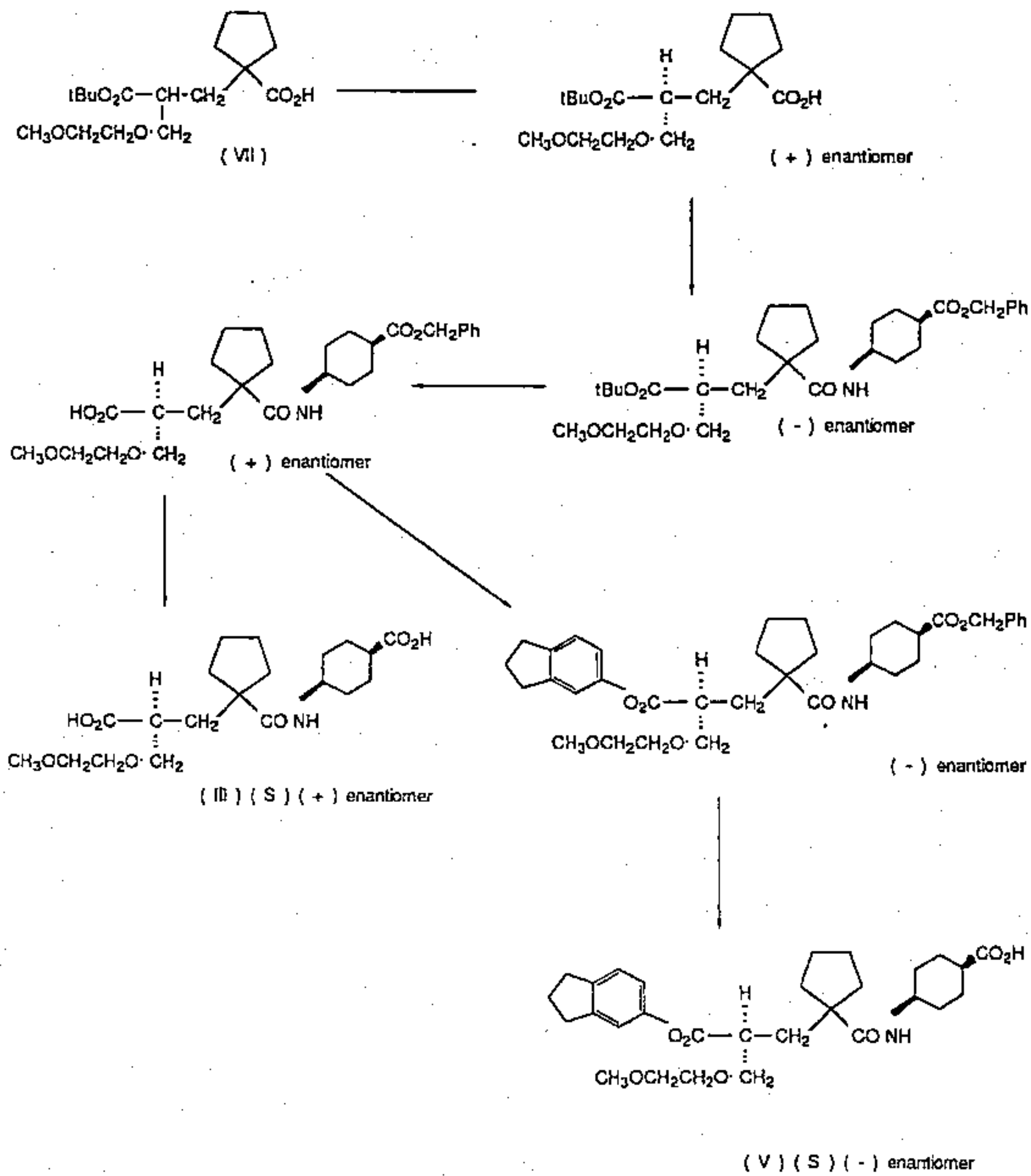


In this process, a similar sequence is followed but the indanyl ester (IV) is resolved by fractional crystallisation of its (+) pseudoephedrine salt. A solution of the separated salt is acidified and the free carboxylic acid isolated as the pure S(-) enantiomer. Other salts which can be used as resolving agents for this step include for example, salts with 1-cinchonidine, 1-ephedrine, S(-)alpha-methylbenzylamine, (S,S) (+)2-amino-1-phenyl-1,3-propanediol, L-phenylalaninol and dehydroabietylamine. The absolute stereochemistry was established to be S by comparison with material prepared by asymmetric synthesis. Optical purity was established by chiral NMR assay. This product is coupled with benzyl cis-4-amino-1-cyclohexanecarboxylate as previously described and the benzyl group subsequently removed by catalytic hydrogenation to yield the laevotrotatory S enantiomeric indanyl ester (V).

Enzymatic hydrolysis of this product was shown to give the dextrorotatory S enantiomer of the diacid (III).

Another process for obtaining either the dicarboxylic acid of formula (III) or its indanyl ester of formula (V) is shown in Scheme 3. In this process 1-[2-tert-butoxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxylic acid is resolved by fractional crystallisation of its (+) pseusoephedrine salt. The alternative salts identified above identified above may also be employed in this step. The (+) enantiomer is then coupled to benzyl cis-4-aminocyclohexane carboxylate using, for example, propanephophonic acid cyclic anhydride as the condensing agent. The t-butyl ester group is removed by treatment with trifluoroacetic acid to yield the mono benzyl ester. This may

Scheme 3



then either be subjected to catalytic hydrogenation to yield the dicarboxylic acid (III), or esterified with 5-indanol followed by catalytic hydrogenation to yield the indanyl ester (V).

Appropriate reagents and conditions for the various coupling and deprotection steps, described above, together with procedures for determining the biological activity of the products of formula (I) and appropriate pharmaceutical compositions and dosage ranges for their use are described in European patent application no. 0274234.

The invention will now be more particularly illustrated by reference to the following experimental examples. The purity of compounds was routinely monitored by thin layer chromatography using Merck Kieselgel 60 F_{254} plates. ^{1}H-Nuclear magnetic resonance spectra were recorded using a Nicolet QE-300 spectrometer and were in all cases consistent with the proposed structures.

EXAMPLE 1

(2S)-(2-Methoxyethoxymethyl)-3-[1-(phenacyloxycarbonyl)cyclopentyl]propanoic acid N-acetyl-(1R,2S)-ephedrine ester

N,N′-Dicyclohexylcarbodiimide (5.66 g, 24.5 mmole) was added to an ice cold, stirred solution of N-acetyl-(1R,2S)-ephedrine (4.24 g, 20.46 mmole), 2-(2-methoxyethoxymethyl)-3-[1-(phenacyloxycarbonyl)-cyclopentyl]propanoic acid (8.43 g, 21.48 mmole) and 4-dimethylaminopyridine (1.23 g, 10 mmole) in dry methylene chloride (100 ml). After one hour the solution was allowed to warm to ambient temperature and stirred for $2\frac{1}{2}$ days. The suspension was filtered, the solvent evaporated under reduced pressure and the residue partitioned between diethyl ether and water. The organic layer was washed sequentially with 0.5 N hydrochloric acid, water, saturated aqueous sodium bicarbonate, and water. Drying ($MgSO_4$) and evaporation gave the crude mixture of diastereoisomers as an oil (12.5 g), which was chromatographed on silica eluting with hexane containing increasing proportions of ethyl acetate (4:6 to 1:9). The faster running component, having Rf 0.45 (silica; ethyl acetate) was the desired diastereoisomer and was obtained following evaporation of the relevant fractions as a gum (5.21 g, 44%). $[\alpha]_D^{25}$ -34.1°, $[\alpha]_{365}^{25}$ - 111.0° (c = 1.0, CH_2Cl_2). Found: C,68.19; H,7.59; N,2.46. $C_{33}H_{43}NO_9$ requires C,68.14; H,7.45, N,2.41%

The other diastereoisomer had an Rf of 0.35 (silica; ethyl acetate); $[\alpha]_D^{25}$ - 21.5°, $[\alpha]_{365}^{25}$ -67.3° (c = 1.0, CH_2Cl_2).

EXAMPLE 2

(2S)-(2-Methoxyethoxymethyl)-3-(1-carboxycyclopentyl)propanoic acid N-acetyl-(1R,2S)-ephedrine ester

A solution of (2S)-(2-methoxyethoxymethyl)-3-[1-(phenacyloxycarbonyl)cyclopentyl]propanoic acid N-acetyl-(1R,2S)-ephedrine ester (5.17 g, 8.89 mmole) in glacial acetic acid (40 ml) was stirred with activated zinc dust (3.0 g, 47.7 mmole) at room temperature under nitrogen for two hours. The mixture was filtered and the filtrate evaporated to dryness under vacuum, traces of acetic acid being removed by azeotroping with toluene. The residue was dissolved in diethyl ether and the solution extracted with 1N sodium hydroxide solution (12 ml) and washed with water. The combined extracts were acidified with concentrated hydrochloric acid and extracted with diethyl ether. The ether extracts were washed with saturated brine, dried ($MgSO_4$) and evaporated to give the title product as a thick oil (4.03 g, 98%). Found: C,63.96; H,8.21; N,2.87. $C_{25}H_{37}NO_7$ (0.3 H_2O) requires C,64.03; H,8.08; N,2.99%. $[\alpha]_D^{25}$ -34.9°, $[\alpha]_{365}^{25}$ -115.4° (c = 1.03, CH_2Cl_2).

EXAMPLE 3

3-{1-[(cis-4-Ethoxycarbonylcyclohexyl)carbamoyl]cyclopentyl}-(2S)-(2-methoxyethoxymethyl)propanoic acid N-acetyl-(1R,2S)-ephedrine ester

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (3.32 g, 17.34 mmole) was added to an ice cold stirred mixture of the product of Example 2 (3.98 g, 8.58 mmole), cis-4-aminocyclohexanecarboxylic acid ethyl ester hydrochloride (2.70 g, 13 mmole), 1-hydroxybenzotriazole (1.17 g, 8.67 mmole) and N-methylmorpholine (3.07 g, 30.34 mmole) in dry methylene chloride (30 ml). After 15 minutes the mixture was allowed to warm to ambient temperature and to stand overnight. The solvent was evaporated under vacuum and the residue partitioned between diethyl ether and water. The organic layer was washed sequentially with water, 2N-hydrochloric acid, water, saturated aqueous sodium bicarbonate and water. The solution was dried ($MgSO_4$) and the solvent evaporated to give a gum which was chromatographed on silica eluting with ethyl acetate. Further chromatography of the product containing fractions on silica eluting with a mixture of hexane and ethyl acetate (15:85) gave the title compound as a gum (4.65 g, 88%). $[\alpha]_D^{25}$ -30.3°, $[\alpha]_{365}^{25}$ -101.3° (c = 1.01, CH_2Cl_2). Found: C,66.16; H,8.66; N,4.45. $C_{34}H_{52}N_2O_8$ requires C,66.21;

H,8.50; N,4.54%.

EXAMPLE 4

(S)-cis-4-{1-[2-Carboxy-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid

The diester product from Example 3 (4.52 g, 7.33 mmole) in a mixture of ethanol (50 ml) and water (50 ml) was hydrogenated over 10% palladium on charcoal catalyst (2.5 g) at 60 p.s.i. (4.1 bar) at room temperature for 24 hours. The mixture was filtered and the filtrate evaporated under reduced pressure. The residue was taken up in diethyl ether and the mono-ester product was extracted into 1N sodium hydroxide (30 ml) the ether being washed with water (30 ml). The combined aqueous extracts were washed with diethyl ether and allowed to stand at room temperature for three days. The solution was saturated with salt, acidified with concentrated hydrochloric acid and extracted with methylene chloride. The organic extract was washed with saturated brine, dried ($MgSO_4$) and the solvent evaporated. Recrystallisation from a mixture of hexane and ethyl acetate gave the title product as a white solid (2.32 g, 79%), m.p. 107.5-108°C. $[\alpha]_D^{25}$ +2.7°, $[\alpha]_{365}^{25}$ +5.1° (c = 1.58, CH_2Cl_2). Found: C,60.18; H,8.44; N,3.82. $C_{20}H_{33}NO_7$ requires C,60.13; H,8.33; N,3.51%.

EXAMPLE 5

Phenacyl 1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxylate

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (31.1 g, 0.1625 mole) was added to a stirred solution of 2-(2-methoxyethoxymethyl)-3-[1-(phenacyloxycarbonyl)cyclopentyl]propanoic acid (49 g, 0.125 mole), 5-indanol (83.6 g, 0.625 mole), 1-hydroxybenzotriazole hydrate (18.6 g, 0.1375 mole) and N-methylmorpholine (16.3 g, 0.1625 mole), in methylene chloride (100 ml). The solution was stirred at ambient temperature for 18 hours, diluted with further methylene chloride (300 ml) and washed sequentially with water (2 x 100 ml), 2N hydrochloric acid (2 x 100 ml) and saturated aqueous sodium bicarbonate (2 x 100 ml). Drying ($MgSO_4$) and evaporation gave an oil (129 g) which was chromatographed on silica (1 kg) eluting with hexane containing increasing proportions of ethyl acetate (4:1 to 2:1) to give the title diester as a pale yellow oil (54.5 g; 86%), Rf. 0.54 (silica; hexane, ethyl acetate (2:1).

EXAMPLE 6

1-[2-(5-Indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxylic acid

Activated zinc dust (36 g, 0.554 mole) was added portionwise over 45 minutes to a stirred solution of the diester from Example 5 (54 g, 0.106 mole) in glacial acetic acid (378 ml), the temperature being allowed to rise to 32°C. After stirring for 18 hours a further portion of activated zinc dust (36 g, 0.554 mole) was added and the mixture stirred for another hour. The reaction mixture was filtered and the filtrate was evaporated to an oil (46 g) which was chromatographed on silica (500 g) eluting with hexane containing increasing proportions of ethyl acetate (4:1 to 1:1) to give the title ester as a colourless oil (37.8 g, 91.5%) Rf. 0.23 (silica; hexane, ethyl acetate 2:1).

This product could be further characterized as its isopropylamine salt m.p. 76-8°C (hexane). Found: C,66.19; H,8.64; N,3.04. $C_{23}H_{39}NO_6$ requires C,66.79; H,8.75; N,3.12%.

EXAMPLE 7

(S)-1-[2-(5-Indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxylic acid

A hot solution of (+)pseudoephedrine (1.98 g) in ethyl acetate (6 ml) was run into a cooled and stirred solution of 1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxylic acid (4.68 g) in toluene (6 ml), the temperature being allowed to rise to 35 °C. The resulting clear solution was chilled to induce crystallisation and granulated at 5 °C for several hours. Filtration and drying gave the crude (+)-pseudoephedrine salt of the (S)-acid (4.0 g, 60%) as a white solid m.p. 98-102 °C. Recrystallisation of 3.5 g of this material from a mixture of toluene (10.5 ml) and ethyl acetate (10.5 ml) gave the (+)-pseudoephedrine salt of the title compound (2.2 g, 62.8% recovery) as white crystals m.p. 111-3 °C, $[\alpha]_D$ + 25.1° (c=5, MeOH). Found: C,69.19; H,8.20; N,2.38. $C_{32}H_{45}NO_7$ requires C,69.16; H,8.16; N,2.51%.

A sample of this salt (2 g) was suspended in a mixture of hexane (5 ml), ethyl acetate (5 ml) and water (10 ml) and concentrated hydrochloric acid was added dropwise to adjust the pH of the aqueous phase to 1.5. The two phases of the solution were separated, and the aqueous phase was washed with a 1:1 ethyl acetate-hexane mixture (10 ml). Evaporation of the combined organic layers gave the title compound as a colourless oil (1.2 g, 85% from salt), $[\alpha]_D$ - 3.5° (c=5, MeOH), Rf. 0.41 (silica; toluene, acetic acid 8:2). Found: C,67.25; H,7.77. $C_{22}H_{30}O_6$ requires C,67.67; H,7.74%. A chiral NMR assay of this product showed it to be substantially pure S enantiomer containing only 4% of the R enantiomer.

EXAMPLE 8

(S)-Benzyl cis-4-{1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylate

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (337.5 mg, 1.76 mmole) was added to a stirred solution of (S)-1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxylic acid (625 mg, 1.6 mmole), benzyl cis-4-amino-1-cyclohexanecarboxylate p-toluenesulphonate. (700 mg, 1.73 mmole), 1-hydroxybenzotriazole hydrate (240 mg, 1.78 mmole) and N-methylmorpholine (560 mg, 5.5 mmole) in methylene chloride (3.75 ml). The solution was stirred at ambient temperature for eighteen hours, evaporated under vacuum and the residue partitioned between diethyl ether and water. The organic extract was washed sequentially with 1N hydrochloric acid, saturated aqueous sodium bicarbonate, and water. Drying ($MgSO_4$) and evaporation gave an oil (0.9 g) which was chromatographed on silica (25 g) eluting with hexane containing increasing proportions of ethyl acetate (4:1 to 3:1) to give the required diester as an oil (830 mg, 86%) $[\alpha]_D$ - 3.3° (c=1, MeOH), Rf. 0.52 (silica: ethyl acetate). Found: C,70.32; H,7.74; N,2.19. $C_{36}H_{47}NO_7(0.5\ H_2O)$ requires C,70.33; H,7.87; N,2.28%.

EXAMPLE 9

(S)-cis-4-{1-[2-(5-Indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid

A solution of (S)-benzyl cis-4-{1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylate (597 mg, 0.986 mmole) in 5% aqueous ethanol (10 ml) was hydrogenated over 10% palladium on charcoal catalyst (60 mg) at 60 p.s.i. (4.1 bar) and room temperature for 3.5 hours. The catalyst was removed by filtration and the filtrate evaporated under vacuum. The residue was dissolved in diethyl ether (50 ml) and the solution was clarified by filtration, and concentrated to low volume (about 5 ml) when crystallisation occurred. After granulation, filtration and drying gave the title ester (390 mg, 77%) as white crystals m.p. 107-9 °C, $[\alpha]_D$ - 5.8° (c=1, MeOH), Rf. 0.40 (silica; toluene, dioxan, acetic acid 90:24:5). Found: C,67.45; H,8.18; N,2.63. $C_{29}H_{41}NO_7$ requires C,67.55; H,8.01; N,2.72%.

EXAMPLE 10

(S)-1-[2-(tert-Butoxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxylic acid.

A solution of 1-[2-(tert-butoxycarbonyl)-3-(2-methoxyethoxy) propyl]-1-cyclopentanecarboxylic acid (110.1 g, 0.333 mole) in hexane (550 ml) was treated with (+) pseudoephedrine (55.1 g, 0.333 mole) and the mixture was heated to reflux. The resulting solution was cooled to induce crystallisation, and stirred at 5°C for 1 hour to granulate the crystals. After overnight refrigeration at 5°C, filtration, washing with hexane (200 ml) and drying gave the crude (+) pseudoephedrine salt of the (S) acid (89.9 g, 54.4%) as a white solid m.p. 76°-80°C. Recrystallisation of 30 g of this material twice from hexane (225 ml) gave the (+)-pseudoephedrine salt of the title compound (21.45 g, 71.5% recovery) as white crystals m.p. 86-7°C, $[\alpha]_D$ + 34.9° (c=1, MeOH). Found: C,65.21; H,9.23; N,2.91. $C_{27}H_{45}NO_7$ requires C,65.42; H,9.15; N,2.82%.

A sample of this salt (10 g) was suspended in hexane (50 ml), and treated with 2N hydrochloric acid (15 ml) (the pH of the aqueous phase was 1.5). The two phases of the solution were separated, and the hexane phase was washed with water (15 ml). Evaporation of the organic layer gave the title compound as a colourless oil (6.3 g, 94% from salt), $[\alpha]_D$ + 2.9° (c=2, MeOH), Rf. 0.44 (silica; diethyl ether, hexane, acetic acid (75:25:1) Found: C,61.41; H,9.17. $C_{17}H_{30}O_6$ requires C,61.79; H,9.15%. A chiral NMR assay of this product showed it to be substantially pure (S) enantiomer containing only 3% of the (R) enantiomer.

EXAMPLE 11

(S)-Benzyl cis-4-{1-[2-(tert-butoxycarbonyl)-3-(2-methoxyethoxy) propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylate.

A solution of (S)-1-[2-(tert-butoxycarbonyl)-3-(2-methoxyethoxy) propyl]-1-cyclopentanecarboxylic acid (6.61 g, 0.02 mole) in methylene chloride (40 ml) was treated with benzyl cis-4-amino-1-cyclohexane carboxylate p-toluenesulphonate (8.11g, 0.02 mole) and water (26 ml) adjusted to pH 9.5 with 5N aqueous sodium hydroxide. To the stirred two phase solution was added propanephosphonic acid cyclic anhydride (17.8 g of commerical 50% w/w solution in methylene chloride, 0.028 mole) over 45 minutes with dropwise addition of 5N aqueous sodium hydroxide solution to maintain the pH at 8.5. The mixture was stirred for 18 hours and treated with further benzyl cis-4-amino-1-cyclohexanecarboxylate p-toluenesulphonate (2.03 g, 0.005 mole) and propanephosphonic acid cyclic anhydride (12.7 g 50% w/w solution, 0.02 mole), maintaining the pH of the aqueous phase at 8.5 by addition of 5N aqueous sodium hydroxide solution. After stirring for another hour the phases were separated and the organic phase was washed with water (20 ml) and evaporated to an oil (13.04 g) which was chromatographed on silica (300 g). Elution with hexane containing increasing proportions of ethyl acetate (4:1 to 7:3) gave the required diester as an oil (8.12 g, 79.1%) $[\alpha]_D$ - 0.4° (c=2, MeOH), Rf. 0.55 (silica: ethyl acetate).

EXAMPLE 12

(S)-Benzyl cis-4-{1-[2-carboxy-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylate.

To (S)-benzyl cis-4-{1-[2-tert-butoxycarbonyl)-3-(2-methoxyethoxy) propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylate (50 g, 0.0917 mole) was added trifluoroacetic acid (100 ml; 1.298 mole) with stirring and cooling to maintain the temperature below 25°C. The solution was allowed to stand for 18 hours, evaporated under vacuum, and the residue (50.2 g) dissolved in ethyl acetate (250 ml). The solution was washed with water (250 ml) adjusted to pH 3.0 with a little saturated aqueous sodium carbonate solution, and then with further water (30 ml). The organic layer was evaporated to give the title compound as a pale amber oil (44.19 g, 98.4%), $[\alpha]_D$ + 0.9°, (c=1, MeOH), Rf. 0.76

(silica;methylene chloride, methanol, acetic acid 90:10:1).

EXAMPLE 13

(S)-Benzyl cis-4-{1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy) propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylate.

A solution of (S)-benzyl cis-4-{1-[2-carboxy-3-(2-methoxyethoxy) propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylate (12.2 g, 0.025 mole) in methylene chloride (12.2 ml) was treated with 5-indanol (6.7 g, 0.05 mole) and then with 1-propanephosphonic acid cyclic anhydride (52.3 g of commerical 50% w/w solution in methylene chloride, 0.0825 mole). The solution was stirred for 17 hours at ambient temperature, and washed sequentially with water (50 ml), 0.5M aqueous potassium hydroxide (20 ml) and water (12 ml). Drying ($MgSO_4$) and evaporation gave an oil (16.54 g) which was chromatographed on silica (60 g) eluting with hexane containing increasing proportions of ethyl acetate (3:1 to 1:1) to give the title diester as a pale yellow oil (10.9 g; 72.1%), $[\alpha]_D$ - 3.3° (c=1, MeOH), Rf. 0.52 (silica; ethylacetate), R_f 0.35 (silica; ethyl acetate, toluene 1:1).
This material is identical to that described in Example 8, and is converted in identical manner to (S)-cis-4-{1-[2-(5-indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid (as described in Example 9).

EXAMPLE 14

(S)-cis-4-{1-[2-Carboxy-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

A solution of (S)-benzyl cis-4-{1-[2-carboxy-3-(2-methoxyethoxy) propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylate (4.0 g, 8.18 mmole) in 5% aqueous ethanol (20 ml) was hydrogenated over 5% palladium on charcoal catalyst (0.4 g 50% wet catalyst) at 60 p.s.i. (4.1 bar) and room temperature for 18 hours. The catalyst was removed by filtration and the filtrate was evaporated under vacuum. The residue (3.42 g) was recrystallised from ethyl acetate (13.7 ml) to give the title diacid (2.15 g, 63%) as white crystals m.p. 108.5°-9.1° C, $[\alpha]_D$ + 1.4° (c=1, MeOH), Rf. 0.55 (silica; methylene chloride, methanol, acetic acid 90:10:1). Found C,60.11; H,8.34; N,3.36. $C_{20}H_{33}NO_7$ requires C,60.13; H,8.33; N,3.51%.
This material is identical to that described in Example 4. A chiral NMR assay of this product showed it substantially pure (S) enantiomer containing only 3% of the (R) enantiomer.

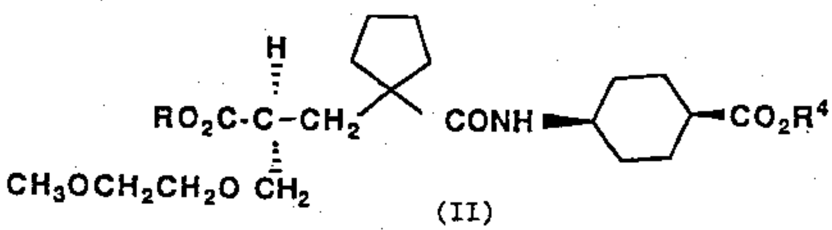
ACTIVITY DATA

The activity of the racemate and separated enantiomers of the cis-4-{1-[2-carboxy-3-(2-methoxyethoxy)-propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid was assessed by measuring their ability to inhibit the neutral endopeptidase E.C.3.4.24.11 in vitro or to induce natriuresis in mice in vivo following the procedure described in European patent application 0274234.

Enantiomer	IC_{50} against E.C.3.4.24.11 (molar)	Natriuresis in mouse (i.v.)
(±) R,S	4.8 x 10^{-8}	active at 3 mg/kg
(±) S	3.9 x 10^{-8}	active at 1.5 mg/kg
(-) R	less than 10^{-5}	inactive at 3 mg/kg

Claims

1. An S enantiomeric compound of the formula:



(II)

and pharmaceutically acceptable salts thereof,
wherein each R and R^4 is H, or one of R and R^4 is H and the other is a biolabile ester group, said enantiomer being substantially free of the R-enantiomer.

2. A compound of the formula (II) wherein said biolabile ester group is:-
ethyl
benzyl
1-(2,2-diethylbutyryloxy)ethyl
2-ethylpropionyloxymethyl
1-(2-ethylpropionyloxy)ethyl
1-(2,4-dimethylbenzoyloxy)ethyl
α-benzoyloxybenzyl
1-(benzoyloxy)ethyl
2-methyl-1-propionyloxy-1-propyl
2,4,6-trimethylbenzoyloxymethyl
1-(2,4,6-trimethylbenzoyloxy)ethyl
pivaloyloxymethyl
phenethyl
phenpropyl
2,2,2-trifluoroethyl
1- or 2-naphthyl
2,4-dimethylphenyl
4-t-butylphenyl
and 5-indanyl.

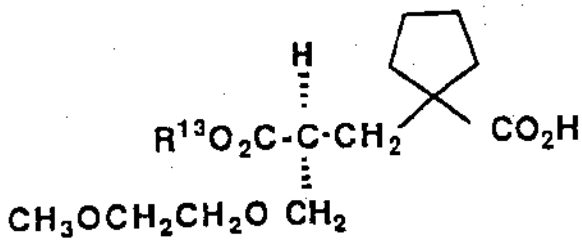
3. (S)-cis-4-{1-[2-(5-Indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

4. (-)-cis-4-{1-[2-(5-Indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

5. (S)-cis-4-{1-[2-Carboxy-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

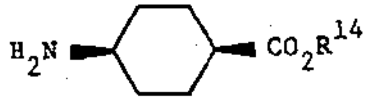
6. (+)-cis-4-{1-[2-Carboxy-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

7. A process for preparing a compound as claimed in claim 1 which comprises the steps of
(a) coupling an S enantiomeric compound of the formula:-



---(VI)

with a compound of the formula:



wherein R^{13} and R^{14} are as defined for R and R^4 other than H, or are selectively removable carboxylic acid protecting groups, and

(b) removing one or both of R^{13} and R^{14} to yield the mono-ester or dicarboxylic acid product of formula (II);

or

(c) removing R^{13}, esterifying the product to provide a biolabile ester group and removing R^{14} to yield the product of formula (II) wherein R^4 is H and R is a biolabile ester group.

8. A process as claimed in claim 7 wherein R^{13} forms an N-acetyl-(1R,2S)-ephedrine ester and R^{14} is ethyl and the ester groups are removed by hydrogenation followed by hydrolysis to yield the compound of formula (II) wherein R and R^4 are both H.

9. A process as claimed in claim 7 wherein R^{13} is indanyl and R^{14} is benzyl and said benzyl group is removed to yield the compound of formula (II) wherein R is 5-indanyl and R^4 is H.

10. A process as claimed in claim 7 wherein R^{13} is tert-butyl and R^{14} is benzyl and said groups are removed to yield the compound of formula (II) wherein R and R^4 are both H.

11. A process as claimed in claim 7 wherein R^{13} is tert-butyl and R^{14} is benzyl and said tert-butyl group is removed, the product esterfied to provide a biolabile ester group at R and the benzyl group is removed.

12. A pharmaceutical composition comprising a compound as claimed in any one of claims 1 to 6, or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable diluent or carrier.

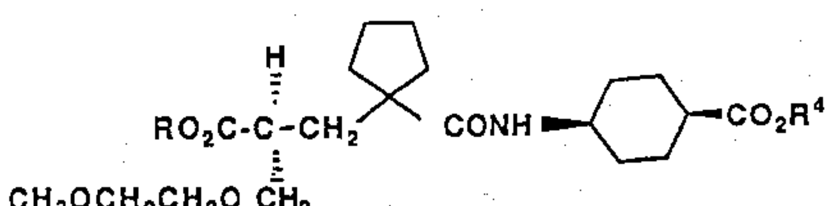
13. A compound as claimed in any one of claims 1 to 6, or a pharmaceutically acceptable salt thereof, for use in medicine, in particular for use as a diuretic agent for the treatment of hypertension and heart failure.

14. A compound of the formula (VI) as defined in claim 7.

15. A compound as claimed in claim 14 wherein R^{13} is 5-indanyl, tert-butyl or forms a N-acetyl-(1R,2S)-ephedrine ester.

Claims for the following Contracting State: ES

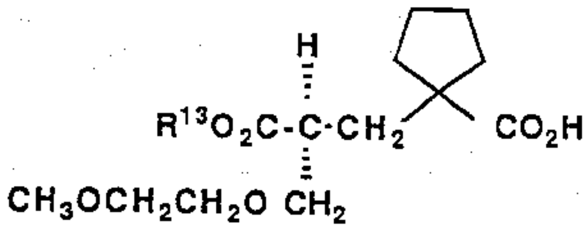
1. A process for preparing an S enantiomeric compound of the formula:



(II)

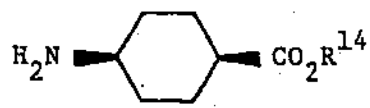
and pharmaceutically acceptable salts thereof, wherein each R and R^4 is H, or one of R and R^4 is H and the other is a biolabile ester group, said enantiomer being substantially free of the R-enantiomer, which comprises the steps of

(a) coupling an S enantiomeric compound of the formula:-



—(VI)

with a compound of the formula:



wherein R^{13} and R^{14} are as defined for R and R^4 other than H, or are selectively removable carboxylic acid protecting groups, and

(b) removing one or both of R^{13} and R^{14} to yield the mono-ester or dicarboxylic acid product of formula (II),

or

(c) removing R^{13}, esterifying the product to provide a biolabile ester group and removing R^{14} to yield the product of formula (II) wherein R^4 is H and R is a biolabile ester group.

2. A process as claimed in claim 1 wherein R^{13} forms an N-acetyl-(1R,2S)-ephedrine ester and R^{14} is ethyl and the ester groups are removed by hydrogenation followed by hydrolysis to yield the compound of formula (II) wherein R and R^4 are both H.

3. A process as claimed in claim 1 wherein R^{13} is idanyl and R^{14} is benzyl and said benzyl group is removed to yield the compound formula (II) wherein R is 5-indanyl and R^4 is H.

4. A process as claimed in claim 1 wherein R^{13} is tert-butyl and R^{14} is benzyl and said groups are removed to yield the compound of formula (II) wherein R and R^4 are both H.

5. A process as claimed in claim 1 wherein R^{13} is tert-butyl and R^{14} is benzyl and said tert-butyl group is removed, the product esterfied to provide a biolabile ester group at R and the benzyl group is removed.

6. A process as claimed in claim 1 wherein said biolabile ester group is:-

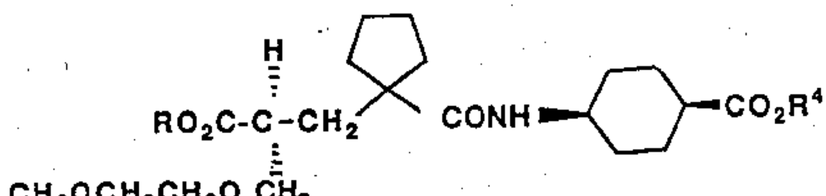
ethyl
benzyl
1-(2,2-diethylbutyryloxy)ethyl
2-ethylpropionyloxymethyl
1-(2-ethylpropionyloxy)ethyl
1-(2,4-dimethylbenzoyloxy)ethyl
α-benzoyloxybenzyl
1-(benzoyloxy)ethyl
2-methyl-1-propionyloxy-1-propyl
2,4,6-trimethylbenzoyloxymethyl
1-(2,4,6-trimethylbenzoyloxy)ethyl
pivaloyloxymethyl
phenethyl
phenpropyl
2,2,2-trifluoroethyl
1- or 2-naphthyl
2,4-dimethylphenyl
4-t-butylphenyl
or 5-indanyl.

7. A process as claimed in claim 1, claim 2 or claim 4 for producing the compound: (S)-cis-4-{1-[2-Carboxy-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

8. A process as claimed in claim 1, claim 3 or claim 5, for producing the compound: (S)-cis-4-{1-[2-(5-Indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

Claims for the following Contracting State: GR

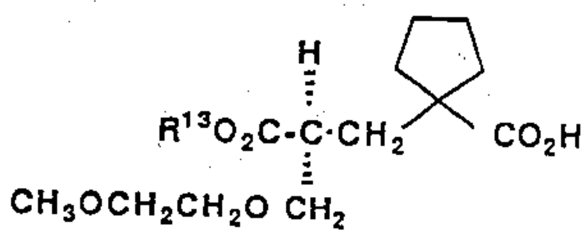
1. A process for preparing an S enantiomeric compound of the formula:



(II)

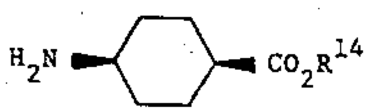
and pharmaceutically acceptable salts thereof, wherein each R and R^4 is H, or one of R and R^4 is H and the other is a biolabile ester group, said enantiomer being substantially free of the R-enantiomer, which comprises the steps of

(a) coupling an S enantiomeric compound of the formula:-



---(VI)

with a compound of the formula:



wherein R^{13} and R^{14} are as defined for R and R^4 other than H, or are selectively removable carboxylic acid protecting groups, and

(b) removing one or both of R^{13} and R^{14} to yield the mono-ester or dicarboxylic acid product of formula (II);

or

(c) removing R^{13}, esterifying the product to provide a biolabile ester group and removing R^{14} to yield the product of formula (II) wherein R^4 is H and R is a biolabile ester group.

2. A process as claimed in claim 1 wherein R^{13} forms an N-acetyl-(1R,2S)-ephedrine ester and R^{14} is ethyl and the ester groups are removed by hydrogenation followed by hydrolysis to yield the compound of formula (II) wherein R and R^4 are both H.

3. A process as claimed in claim 1 wherein R^{13} is idanyl and R^{14} is benzyl and said benzyl group is removed to yield the compound formula (II) wherein R is 5-indanyl and R^4 is H.

4. A process as claimed in claim 1 wherein R^{13} is tert-butyl and R^{14} is benzyl and said groups are removed to yield the compound of formula (II) wherein R and R^4 are both H.

5. A process as claimed in claim 1 wherein R^{13} is tert-butyl and R^{14} is benzyl and said tert-butyl group is removed, the product esterfied to provide a biolabile ester group at R and the benzyl group is removed.

6. A process as claimed in claim 1 wherein said biolabile ester group is:-
ethyl
benzyl
1-(2,2-diethylbutyryloxy)ethyl
2-ethylpropionyloxymethyl
1-(2-ethylpropionyloxy)ethyl
1-(2,4-dimethylbenzoyloxy)ethyl
α-benzoyloxybenzyl
1-(benzoyloxy)ethyl
2-methyl-1-propionyloxy-1-propyl
2,4,6-trimethylbenzoyloxymethyl
1-(2,4,6-trimethylbenzoyloxy)ethyl
pivaloyloxymethyl
phenethyl
phenpropyl
2,2,2-trifluoroethyl
1- or 2-naphthyl
2,4-dimethylphenyl
4-t-butylphenyl
or 5-indanyl.

7. A process as claimed in claim 1, claim 2 or claim 4 for producing the compound: (S)-cis-4-{1-[2-Carboxy-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

8. A process as claimed in claim 1, claim 3 or claim 5, for producing the compound: (S)-cis-4-{1-[2-(5-Indanyloxycarbonyl)-3-(2-methoxyethoxy)propyl]-1-cyclopentanecarboxamido}-1-cyclohexanecarboxylic acid.

9. A compound of the formula (VI) as defined in claim 1.

10. A compound as claimed in claim 9 wherein R^{13} is 5-indanyl, tert-butyl or forms a N-acetyl-(1R,2S)-ephedrine ester.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 30 4698

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
A,P D	EP-A-0 274 234 (PFIZER LIMITED) * claims * -----	1,2,7-15	C 07 C 103/737 A 61 K 31/16
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			C 07 C 103/00 A 61 K 31/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	28-06-1989	RUFET J.M.A.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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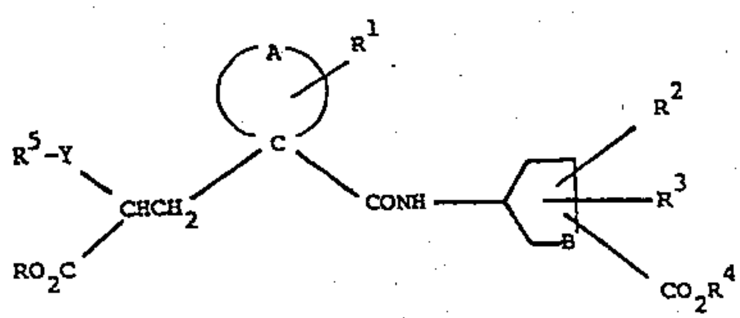
(54) **Cycloalkyl-substituted glutaramide diuretic agents.**

(57) Compounds having the formula:



(I)

wherein A completes a 4 to 7 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be fused to a further carbocyclic ring; B is $(CH_2)_m$ wherein m is 1 to 3; R and R^4 are

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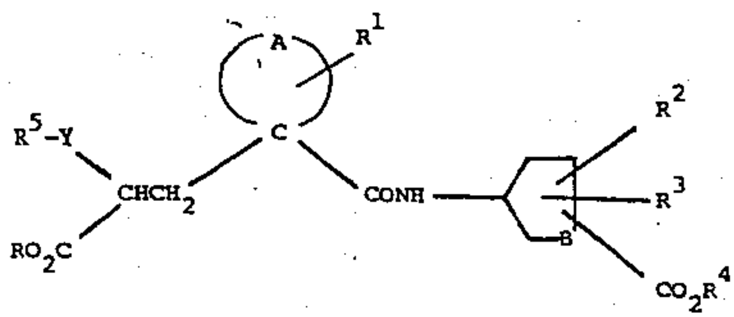
H, C_1-C_6 alkyl, benzyl or biolabile ester-forming groups; R^1 is H or C_1-C_4 alkyl; R^2 and R^3 are each H, OH, C_1-C_6 alkyl or C_1-C_6 alkoxy, or are linked together and are $(CH_2)_r$ wherein r is 1 to 4; Y is an optional alkylene group of from 1 to 6 carbon atoms which may be straight or branched-chain;
and R^5 is R^6CONR^9-, $R^6SO_2NR^9$-, R^6CO_2-, R^6CO-, R^6SO_q-, R^7NR^9CO-, $R^7NR^9SO_2$- or R^7OCO-; wherein R^6 is a group of the formula $R^8(R^{10}R^{11}C\text{-}CONR^9)_nR^{10}R^{11}C$-; R^7 is a group of the formula $R^{10}R^{11}R^{12}C$- and R^9 is H, C_1-C_6 alkyl, aryl, C_3-C_7 cycloalkyl, heterocyclyl, aryl(C_1-C_6 alkyl) or heterocyclyl(C_1-C_6 alkyl); wherein R^8 is R^9CONR^9-, $R^9SO_2NR^9$-, $R^{13}R^{14}N\text{-}(CH_2)_p$-, or R^9O-, R^{10} and R^{11} are H or C_1-C_6 alkyl; or R^{10} is H and R^{11} is C_1-C_6 alkyl which is substituted by OH, SH, SCH_3, NH_2, aryl(C_1-C_6 alkyl)OCONH-, NH_2CO-, CO_2H, guanidino, aryl, or heterocyclyl; or the two groups R^{10} and R^{11} are joined to form a five or 6 membered carbocyclic ring which may be saturated, mono-unsaturated, optionally substituted by C_1-C_4 alkyl or fused to a further carbocylic ring; or R^8 and R^{11} are linked to form a 2-(N-COR^9-4-aminopyrrolidinyl) group; R^{12} is $R^{13}R^{14}NCO$-, R^9OCO-, R^9OCH_2- or heterocyclyl, R^{13} and R^{14} are H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, aryl, aryl(C_1-C_6 alkyl), C_2-C_6 alkoxyalkyl, amino (C_1-C_6 alkyl), heterocyclyl or heterocyclyl(C_1-C_6 alkyl); or the two groups R^{13} and R^{14} form a pyrrolidinyl, piperidino, morpholino, piperazinyl, N-(C_1-C_4 alkyl) piperazinyl, pyrrolyl, imidazolyl, pyrazolyl or triazolyl group; n is 0 or 1; p is 0 or 1 to 6; and q is 0, 1 or 2; and pharmaceutically acceptable salts thereof and bioprecursors therefor, are diuretic agents of value in the treatment of hypertension, heart failure and renal insufficiency.

Cycloalkyl-substituted Glutaramide Diuretic Agents

This invention relates to a series of cycloalkyl-substituted glutaramide derivatives which are diuretic agents having utility in a variety of therapeutic areas including the treatment of various cardiovascular disorders such as hypertension, heart failure and renal insufficiency.

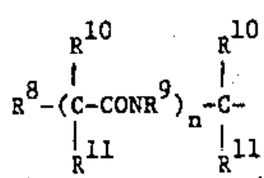
The compounds are inhibitors of the zinc-dependent, neutral endopeptidase E.C.3.4.24.11. This enzyme is involved in the breakdown of several peptide hormones, including atrial natriuretic factor (ANF), which is secreted by the heart and which has potent vasodilatory, diuretic and natriuretic activity. Thus, the compounds of the invention, by inhibiting the neutral endopeptidase E.C.3.4.24.11, can potentiate the biological effects of ANF, and in particular the compounds are diuretic agents having utility in the treatment of a number of disorders, including hypertension, heart failure, angina, renal insufficiency, premenstrual syndrome, cyclical oedema, Menières disease, hyperaldosteronism (primary and secondary) pulmonary oedema, ascites, and hypercalciuria. In addition, because of their ability to potentiate the effects of ANF the compounds have utility in the treatment of glaucoma. As a further result of their ability to inhibit the neutral endopeptidase E.C.3.4.24.11 the compounds of the invention may have activity in other therapeutic areas including for example the treatment of asthma, inflammation, pain, epilepsy, affective disorders, dementia and geriatric confusion, obesity and gastrointestinal disorders (especially diarrhoea and irritable bowel syndrome), the modulation of gastric acid secretion and the treatment of hyperreninaemia and leukemia.

The compounds are of the formula:

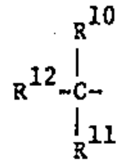


(I)

wherein A completes a 4 to 7 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be fused to a further saturated or unsaturated 5 or 6 membered carbocyclic ring;
B is $(CH_2)_m$ wherein m is an integer of from 1 to 3;
each of R and R^4 is independently H, C_1-C_6 alkyl, benzyl or an alternative biolabile ester-forming group;
R^1 is H or C_1-C_4 alkyl;
R^2 and R^3 are each independently H, OH, C_1-C_6 alkyl or C_1-C_6 alkoxy; or R^2 and R^3 are linked together and are $(CH_2)_r$ wherein r is an integer of from 1 to 4;
Y is an optional alkylene group of from 1 to 6 carbon atoms which may be straight or branched-chain;
and R^5 is R^6CONR^9-, $R^6SO_2NR^9$-, R^6CO_2-, R^6CO-, R^6SO_q-, R^7NR^9CO-, $R^7NR^9SO_2$- or R^7OCO-;
wherein R^6 is a group of the formula:



R^7 is a group of the formula:



and R^9 is H, C_1-C_6 alkyl, aryl, C_3-C_7 cycloalkyl, heterocyclyl, aryl(C_1-C_6 alkyl) or heterocyclyl(C_1-C_6 alkyl); wherein R^8 is R^9CONR^9-, $R^9SO_2NR^9$-, $R^{13}R^{14}N$-$(CH_2)_p$- or R^9O-, wherein each R^9 is as previously defined above;

R^{10} and R^{11} are each independently H or C_1-C_6 alkyl; or R^{10} is H and R^{11} is C_1-C_6 alkyl which is substituted by OH, SH, SCH_3, NH_2, aryl(C_1-C_6 alkyl)OCONH-, NH_2CO-, CO_2H, guanidino, aryl, or heterocyclyl; or the two groups R^{10} and R^{11} are joined together to form, with the carbon atom to which they are attached, a 5 or 6 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be substituted by C_1-C_4 alkyl or fused to a further 5 or 6 membered saturated or unsaturated carbocyclic ring;

or R^{10} is H, n is 0 and R^8 and R^{11} are linked to form a 2-(N- COR^9-4-aminopyrrolidinyl) group;

R^{12} is $R^{13}R^{14}NCO$-, R^9OCO-, R^9OCH_2- or heterocyclyl, wherein R^9 is as previously defined above;

R^{13} and R^{14} are each independently H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, aryl, aryl(C_1-C_6 alkyl), C_2-C_6 alkoxyalkyl, amino(C_1-C_6 alkyl), heterocyclyl or heterocyclyl(C_1-C_6 alkyl); or the two groups R^{13} and R^{14} are taken together to form, with the nitrogen to which they are attached, a pyrrolidinyl, piperidino, morpholino, piperazinyl, N-(C_1-C_4 alkyl)piperazinyl, pyrrolyl, imidazolyl, pyrazolyl or triazolyl group;

n is 0 or 1;

p is 0 or an integer of from 1 to 6;

and q is 0, 1 or 2;

and pharmaceutically acceptable salts thereof and bioprecursors therefor.

In the above definition, unless otherwise indicated, alkyl groups having three or more carbon atoms may be straight or branched-chain. The term aryl as used herein means an aromatic hydrocarbon group such as phenyl, naphthyl or biphenyl which may optionally be substituted, for example with one or more OH, CN, CF_3, C_1-C_4 alkyl, C_1-C_4 alkoxy, halo, carbamoyl, aminosulphonyl, amino, mono or di(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Halo means fluoro, chloro, bromo or iodo.

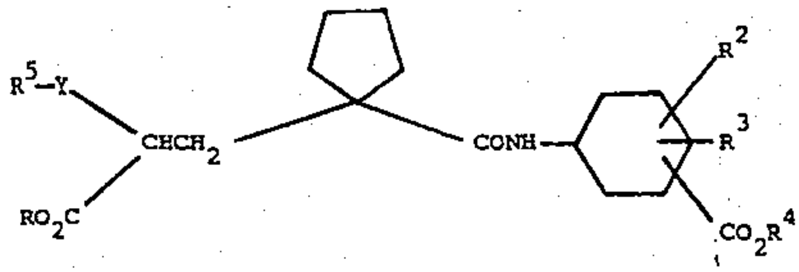
The term heterocyclyl means a 5 or 6 membered nitrogen, oxygen or sulphur containing heterocyclic group which, unless otherwise stated, may be saturated or unsaturated and which may optionally include a further oxygen or one to three nitrogen atoms in the ring and which may optionally be benzofused or substituted with for example, one or more halo, C_1-C_4 alkyl, hydroxy, carbamoyl, benzyl, oxo, amino or mono or di-(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Particular examples of heterocycles include pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, furanyl, tetrahydrofuranyl, tetrahydropyranyl, dioxanyl, thienyl, oxazolyl, isoxazolyl, thiazolyl, indolyl, isoindolinyl, quinolyl, quinoxalinyl, quinazolinyl and benzimidazolyl, each being optionally substituted as previously defined.

The compounds of formula (I) may contain several asymmetric centres and thus they can exist as enantiomers and diastereomers. The invention includes both mixtures and the separated individual isomers. The substituents R^2, R^3 and CO_2R^4 may have cis or trans geometry relative to the amide attachment.

The pharmaceutically acceptable salts of the compounds of formula (I) containing an acidic centre are those formed with bases which form non-toxic salts. Examples include the alkali metal salts such as the sodium, potassium or calcium salts or salts with amines such as diethylamine. Compounds having a basic centre can also form acid addition salts with pharmaceutically acceptable acids. Examples include the hydrochloride hydrobromide, sulphate or bisulphate, phosphate or hydrogen phosphate, acetate, citrate, fumarate, gluconate, lactate, maleate, succinate and tartrate salts.

The term bioprecursor in the above definition means a pharmaceutically acceptable biologically degradable derivative of the compound of formula (I) which, upon administration to an animal or human being, is converted in the body to produce a compound of the formula (I).

A preferred group of compounds of the formula (I) are those wherein A is $(CH_2)_4$, R^1 is H and B is $(CH_2)_2$, i.e. compounds of the formula (II) below wherein R, R^2, R^3, R^4, Y and R^5 are as previously defined for formula (I):



(II)

Also preferred are those compounds of formulae (I) and (II) wherein R and R^4 are both H (diacids) as well as biolabile mono and di-ester derivatives thereof wherein one or both of R and R^4 is a biolabile ester-forming group.

The term biolabile ester-forming group is well understood in the art as meaning a group which provides an ester which can be readily cleaved in the body to liberate the corresponding diacid of formula (I) wherein R and R^4 are both H. A number of such ester groups are well known, for example in the penicillin area or in the case of the ACE-inhibitor antihypertensive agents.

In the case of the compounds of formulae (I) and (II) such biolabile pro-drug esters are particularly advantageous in providing compounds of the formula (I) suitable for oral administration. The suitability of any particular ester-forming group can be assessed by conventional animal or in vitro enzyme hydrolysis studies. Thus, desirably for optimum effect, the ester should only be hydrolysed after absorption, accordingly, the ester should be resistant to hydrolysis before absorption by digestive enzymes but should be readily hydrolyzed by for example, liver enzymes. In this way the active diacid is released into the bloodstream following oral absorption.

In addition to lower alkyl esters (particularly ethyl) and benzyl esters, suitable biolabile esters include alkanoyloxyalkyl esters, including alkyl, cycloalkyl and aryl substituted derivatives thereof, aryloxyalkyl esters, aroyloxyalkyl esters, aralkyloxyalkyl esters, arylesters, aralkylesters, and haloalkyl esters wherein said alkanoyl or alkyl groups have from 1 to 8 carbon atoms and are branched or straight chain and said aryl groups are phenyl, naphthyl or indanyl optionally substituted with one or more C_1-C_4 alkyl or C_1-C_4 alkoxy groups or halo atoms.

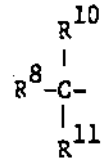
Thus examples of R and R^4 when they are biolabile ester-forming groups other than ethyl and benzyl include: 1-(2,2-diethylbutyryloxy)ethyl, 2-ethylpropionyloxymethyl, 1-(2-ethylpropionyloxy)ethyl, 1-(2,4-dimethylbenzoyloxy)ethyl, α-benzoyloxybenzyl, 1-(benzoyloxy)ethyl, 2-methyl-1-propionyloxypropyl, 2,4,6-trimethylbenzoyloxymethyl, 1-(2,4,6-trimethylbenzoyloxy)ethyl, pivaloyloxymethyl, phenethyl, phenpropyl, 2,2,2-trifluoroethyl, 1- or 2-naphthyl, 2,4-dimethylphenyl, 4-t-butylphenyl, 5-(4-methyl-1,3-dioxalynyl-2-onyl)-methyl and 5-indanyl.

Particularly preferred biolabile ester-forming groups are ethyl, benzyl, 2,4-dimethylphenyl, 4-t-butyl-phenyl and 5-indanyl.

Compounds of the formulae (I) and (II) wherein one or both of R and R^4 are C_1-C_6 alkyl, particularly ethyl, or benzyl, are also active by virtue of their hydrolysis in vivo, and, in addition, are valuable intermediates for the preparation of the diacids wherein R and R^4 are both H.

In a further group of preferred compounds of formula (II), R, R^2 and R^4 are each H. R^3 is preferably H or C_4-C_6 alkyl especially n-butyl. Particularly preferred are those compounds wherein the carboxy group CO_2R^4 is attached at the 3- or 4-position of the cyclohexane ring, most especially those compounds having cis-stereochemistry relative to the amide group.

In one aspect of the invention R^5 is R^6CONR^9, or R^7NR^9CO-wherein R^9 is H and R^6 is a group of the formula:



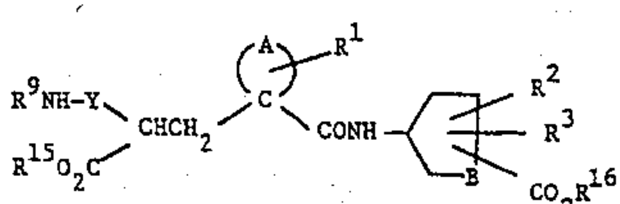
Particularly wherein R^8 is (C_1-C_6 alkyl)CONH-, arylCONH-, or (C_1-C_6 alkyl)SO_2NH-, R^{10} is H and R^{11} is C_1-C_4 alkyl, benzyl or amino(C_1-C_6 alkyl).

In a further particular and preferred aspect of the invention Y is methylene and R^5 is N^2-substituted-L-lysyl-amino, particularly where said substituent is N^2-acetyl, N^2-benzoyl, N^2-naphthoyl or N^2-methanesulphonyl; thus preferred compounds are:

2-(N^2-acetyl-L-lysylaminomethyl)-3-{1-[(cis-4-carboxycyclohexyl)carbamoyl]cyclopentyl} propanoic acid;
2-(N^2-benzoyl-L-lysylaminomethyl)-3-{1-[cis-4-carboxycyclohexyl)carbamoyl]cyclopentyl} propanoic acid,
2-(N^2-naphthoyl-L-lysylaminomethyl)-3- {1-[(cis-4-carboxy-cyclohexyl)carbamoyl]cyclopentyl} propanoic acid,
2-(N^2-acetyl-L-lysylaminomethyl)-3 {1-[cis-4-carboxy-cis-3-butyl-cyclohexyl)carbamoyl]cyclopentyl} propanoic acid,
2-(N^2-acetyl-L-lysylaminomethyl)-3 {1-[cis-4-carboxy-trans-3-butyl-cyclohexyl)carbamoyl]cyclopentyl} propanoic acid,
2-(N^2-methanesulphonyl-L-lysylaminomethyl)-3- {1-[cis-4-carboxy-cis-3-(3-methylbutyl)-cyclohexyl)-carbamoyl]cyclopentyl} propanoic acid, and 2-(N^2-methanesulphonyl-L-lysylaminomethyl)-3 {1-[cis-4-carboxy-cis-3-butyl-cyclohexyl)carbamoyl]cyclopentyl} propanoic acid.

The compounds of formula (I) are prepared by a number of different processes according to the invention:

(a) In one process, the compounds of formula (I) wherein R^5 is R^6CONR^9 are prepared by a process which involves acylating an amine of the formula:-

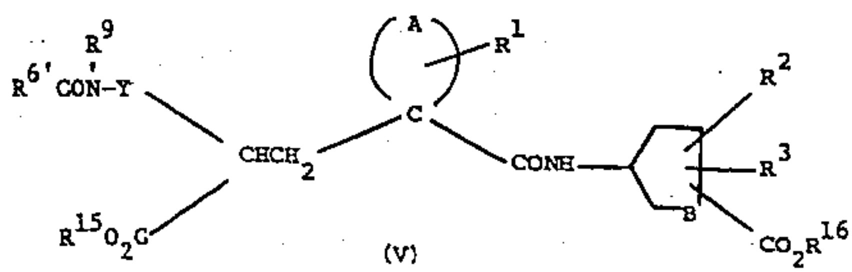


(III)

wherein A, B, Y, R^1, R^2, R^3 and R^9 are as previously defined and R^{15} and R^{16} are as previously defined for R and R^4 excluding H, or they are conventional carboxylic acid protecting groups; by reaction with an acid of the formula:

R^6-CO_2H -- (IV)

wherein R^6 is as previously defined, and wherein any reactive groups therein are optionally protected, to yield a compound of the formula:



(V)

wherein $R^{6'}$ is as previously defined for R^6 with any reactive groups therein optionally protected; and subsequently removing any protecting groups, if present, and, if desired, hydrolysing the ester product to yield the carboxylic acids wherein R and R^4 are H.

The reaction of the compounds of formula (III) and (IV) is achieved using conventional amide coupling techniques. Thus in one process the reaction is achieved with the reactants dissolved in an organic solvent, e.g. dichloromethane, using a carbodiimide condensing agent, for example 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, or N,N′-dicyclohexylcarbodiimide, advantageously in the presence of 1-hydroxybenzotriazole and an organic base such as N-methylmorpholine. The reaction is generally complete after a period of from 12 to 24 hours at room temperature and the product is then isolated by conventional procedures, i.e. by washing with water or filtration to remove the urea biproduct and evaporation of the solvent. The product may be further purified by crystallisation or chromatography, if necessary. The compounds of formula (V) include compounds of formula (I) wherein R and R^4 are C_1-C_6 alkyl or benzyl.

The diesters of formula (V) may be further reacted to give the monoester or diacid derivatives of formula (I) wherein one or both of R and R^4 are H. The conditions used will depend on the precise nature of the groups R^{15} and R^{16} present in the compound of formula (V) and a number of variations are possible. Thus for example when both of R^{15} and R^{16} are benzyl, hydrogenation of the product will yield the diacid of formula (I) wherein R and R^4 are both H. Alternatively if R^{15} is benzyl and R^{16} is alkyl, hydrogenation will yield a monoester product. This can then be hydrolysed, if desired, to again yield the diacid product. When one of R^{15} and R^{16} is t-butyl, treatment of the compound of formula (V) with trifluoroacetic acid yields the corresponding acid. The diester product wherein R^{15} and R^{16} are benzyl or lower alkyl can also be treated with trimethylsilyl iodide to produce the dicarboxylic acid product. If some other carboxylic acid protecting group is used for R^{15} or R^{16} then clearly appropriate conditions for its removal must be employed in the final step to give the ester or diacid product of formula (I). In the case where the ring A or the substituent R^5 is unsaturated, the deprotection must be effected by non-reductive methods, thus for example if either of R and R^4 is benzyl, they may be removed by treatment with trimethylsilyl iodide.

Finally any protecting groups which may be present in $R^{6'}$ are removed by methods appropriate to the particular group used. Thus, for example, if an amino group is present in R^6, this may be protected as the benzyloxycarbonylamino group, the benzyloxycarbonyl group being removed in the final step by catalytic hydrogenation.

Compounds of the formula (I) where one or both of R and R^4 are biolabile ester forming groups are prepared following similar procedures, starting with a compound of the formula (III) wherein R^{15} and/or R^{16} are biolabile ester forming groups.

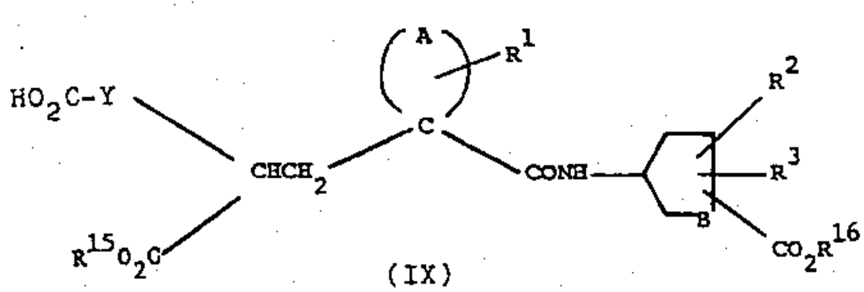
In each case the product may be obtained as the free carboxylic acid or it may be neutralised with an appropriate base and isolated in salt form.

The starting cycloalkyl-substituted glutaric acid mono esters of formula III may be prepared by a number of different processes as described in our European patent application no. 0274234.

The acids of formula IV are generally known compounds which are either commercially available or they may be prepared following literature precedents.

(b) In a further process, compounds of the formula (I) wherein R^5 in $R^6SO_2NR^9$- are prepared by an entirely analogous procedure by reaction of a sulphonyl halide of formula R^6SO_2-hal with the amine of formula (III).

(c) Compounds of the formula (I) wherein R^5 is R^7NR^9CO- are prepared by reaction of a compound of the formula:

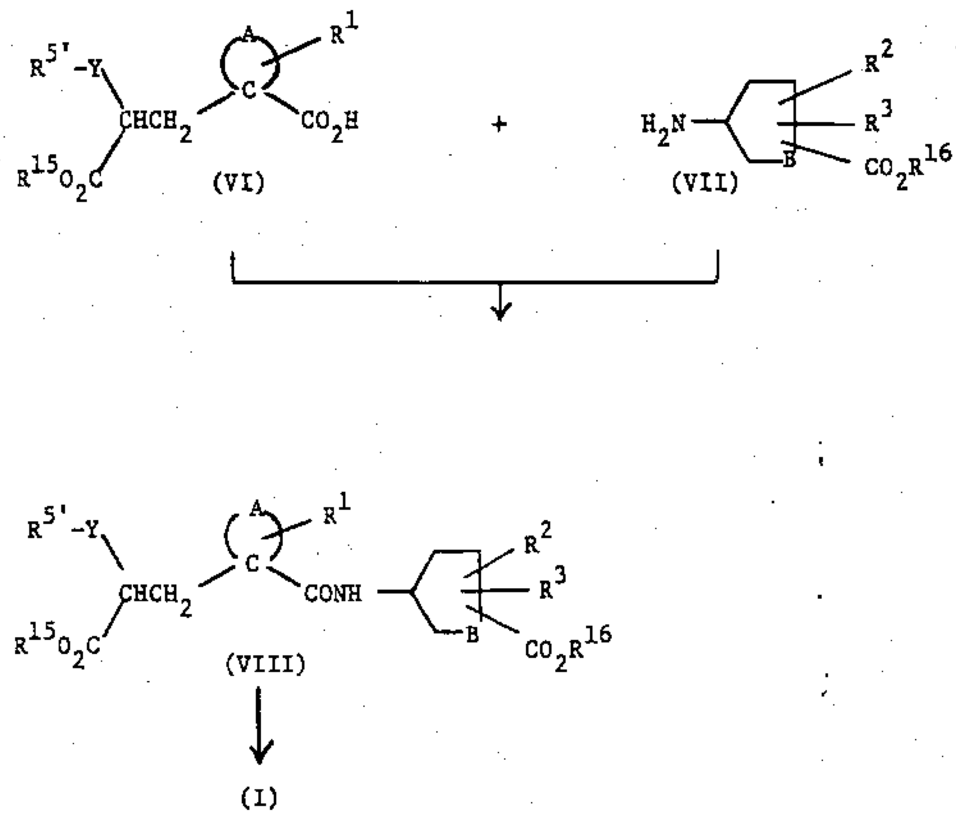


(IX)

wherein A, B, Y, R^1, R^2, R^3, R^{15} and R^{16} are as previously defined, by reaction with an amine of the formula R^7R^9NH, followed by removal of any protecting groups which may be present and hydrolysis of the ester product to yield the carboxylic acids wherein R and R^4 are H.

The reaction of the compound of formula (IX) and the amine may be achieved using the amide coupling techniques already described under process (a) above. The subsequent steps are also as previously described. The compounds of formula (IX) are prepared following the procedures described in our European patent application 0274234 to provide the corresponding benzyl ester (where R^5 is $C_6H_5CH_2OCO$-), catalytic hydrogenation gives the carboxylic acid of formula (IX). The amines of formula R^7R^9NH are generally derived from the naturally occurring amino acids with appropriate protection of reactive side chains.

(d) In a further process compounds of formula (I) wherein R^5 is R^6CONR^9 or wherein R^5 is R^6CO_2-, R^6CO-, R^6SO_q-, $R^7NR^9SO_2$- or R^7OCO are prepared following the synthetic procedure described in our European patent application no. 0274234, i.e. using the following process:-



wherein A, B, Y, R^1, R^2, R^3, R^{15} and R^{16} are as previously defined and $R^{5'}$ is as defined by R^5 with any reactive groups therein optionally protected.

The protecting and coupling techniques required are as previously described. The compounds of formulae (VI) and (VII) may be prepared following the general procedures described in the above-mentioned European patent application.

As previously mentioned, the compounds of the invention are potent inhibitors of the neutral endopeptidase (E.C.3.4.24.11). This enzyme is involved in the breakdown of a number of peptide hormones and, in particular it is involved in the breakdown of atrial natriuretic factor (ANF). This hormone consists of a family of related natriuretic peptides, secreted by the heart, of which the major circulating form in humans is known to be the 28 amino-acid peptide referred to as α-hANP (see for example G. A. Sagnella and G. A. MacGreggor, Nature, 1984, 309, 666 and S. A. Atlas and others, Nature, 1984, 309, 717-725). Thus, the compounds of the invention, by preventing the degradation of ANF, by endopeptidase E.C.3.4.24.11 can potentiate its biological effects and the compounds are thus diuretic and natriuretic agents of utility in a number of disorders as previously described.

Activity against neutral endopeptidase E.C.3.4.24.11 is assessed using a procedure based on the assay described by J. T. Gafford, R. A. Skidgel, E. G. Erdos and L. B. Hersh, Biochemistry, 1983, 32, 3265-3271. The method involves determining the concentration of compound required to reduce by 50% the rate of release of radiolabelled hippuric acid from hippuryl-L-phenylalanyl-L-arginine by a neutral endopeptidase preparation from rat kidney.

The activity of the compounds as diuretic agents is determined by measuring their ability to increase urine output and sodium ion excretion in saline loaded conscious mice. In this test, male mice (Charles River CDI, 22-28 g) are acclimatised and starved overnight in metabowls. The mice are dosed intravenously via the tail vein, with the test compound dissolved in a volume of saline solution equivalent to 2.5% of body weight. Urine samples are collected each hour for two hours in pre-weighed tubes and analysed for electrolyte concentration. Urine volume and sodium ion concentration from the test animals are compared to a control group which received only saline.

For administration to man in the curative or prophylactic treatment of hypertension, congestive heart failure or renal insufficiency, oral dosages of the compounds will generally be in the range of from 4-800 mg daily for an average adult patient (70 kg). Thus for a typical adult patient, individual tablets or capsules contain from 2 to 400 mg of active compound. In a suitable pharmaceutically acceptable vehicle or carrier for administration singly, or in multiple doses, once or several times a day. Dosages for intravenous administration would typically be within the range 1 to 400 mg per single dose as required. In practice the physician will determine the actual dosage which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case but there can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

For human use, the compounds of the formula (I) can be administered alone, but will generally be administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they may be administered orally in the form of tablets containing such excipients as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavouring or colouring agents. They may be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood.

The compounds may be administered alone but may also be administered together with such other agents as the physician shall direct to optimise control of blood pressure or to treat congestive heart failure, renal insufficiency or other disorders in any particular patient in accordance with established medical practice. Thus the compounds can be co-administered with a variety of cardiovascular agents, for example with an ACE inhibitor such as captopril or enalapril to facilitate the control of blood pressure in treatment of hypertension; or with digitalis, or another cardiac stimulant or with an ACE inhibitor, for the treatment of congestive heart failure. Other possibilities include co-administration with a calcium antagonist (e.g. nifedipine, amlodipine or diltiazem) a beta-blocker (e.g. atenolol) or an alpha-blocker (e.g. prazosin or doxazosin) as shall be determined by the physician as appropriate for the treatment of the particular patient or condition involved.

In addition to the above, the compounds may also be administered in conjunction with exogenous ANF, or a derivative thereof or related peptide or peptide fragment having diuretic/natriuretic activity or with other ANF-gene related peptides (e.g. as described by D. L. Vesely et al, Biochem. Biophys. Res. Comm., 1987, 143, 186).

Thus in a further aspect the invention provides a pharmaceutical composition comprising a compound of the formula (I), or a pharmaceutically acceptable salt thereof or bioprecursor therefor, together with a pharmaceutically acceptable diluent or carrier.

The invention also includes a compounds of the formula (I), or a pharmaceutically acceptable salt thereof or bioprecursor therefor, for use in medicine, particularly for use as a diuretic agent for the treatment of hypertension, congestive heart failure or renal insufficiency in a human being.

The invention further includes the use of a compound of the formula (I) for the manufacture of a medicament for the treatment of hypertension, heart failure, angina, renal insufficiency, premenstrual syndrome, cyclical oedema, Menières disease, hyperaldosteronism, pulmonary oedema, ascites, hypercalciuria, glaucoma, asthma, inflammation, pain, epilepsy, affective disorders, dementia and geriatric confusion, obesity, gastrointestinal disorders (including diarrhoea), hyperreninaemia, leukemia, and the modulation of gastric acid secretion.

The preparation of the compounds of the invention will now be more particularly illustrated by reference to the following experimental examples. The purity of compounds was routinely monitored by thin layer chromatography using Merck Kieselgel 60 F_{254} plates. ^{1}H-Nuclear magnetic resonance spectra were recorded using a Nicolet QE-300 spectrometer and were in all cases consistent with the proposed structures.

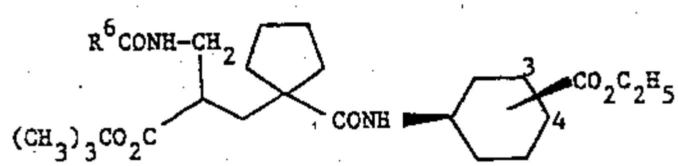
EXAMPLE 1

2-(N^2-Acetyl-N^6-benzyloxycarbonyl-L-lysyl-aminomethyl)-3-{1-[(cis-4-ethoxycarbonyl-cyclohexyl)-carbamoyl]cyclopentyl} propanoic acid t-butyl ester

1-Hydroxybenztriazole (207 mg, 1.53 mmole) and N-methylmorpholine (235 mg, 2.36 mmole) were added to a stirred solution of N^2-acetyl-N^6-benzyloxycarbonyl-L-lysine (456 mg, 1.41 mmole) in dry dichloromethane at 0° C, followed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (361 mg). The solution was stirred at 0° C for 20 minutes and 3-{1-[(cis-4-ethoxycarbonylcyclohexyl)carbamoyl]cyclopentyl}-2-(aminomethyl)propanoic acid t-butyl ester (500 mg, 1.18 mmole) in dichloromethane (10 ml) was added in one portion and the reaction mixture allowed to warm to room temperature and stirred for 16 hours. The solution was concentrated under vacuum to a volume of 10 ml and partitioned between ethyl acetate and water. The organic phase was washed with water (2 x 50 ml), 2M hydrochloric acid (50 ml, 2 x 25 ml), sodium bicarbonate solution (2 x 25 ml) and brine and then dried ($MgSO_4$) and the solvent evaporated. The residue was chromatographed on silica eluting with ethyl acetate to give the title compound (610mg, 71%). Found: C,63.34; H,8.61; N,7.45. $C_{39}H_{60}N_4O_9$ (0.25 H_2O) requires C,63.48; H,8.33; N,7.59%.

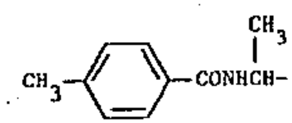
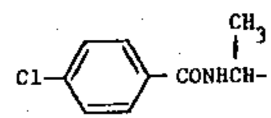
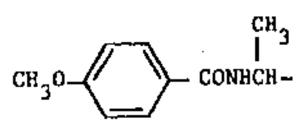
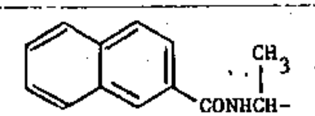
EXAMPLES 2-27

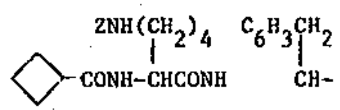
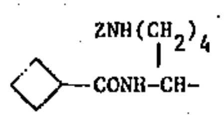
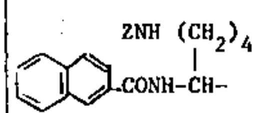
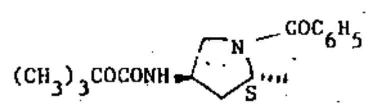
The following compounds were prepared by the general method of Example 1 using as starting materials either 3-{1-[(cis-4-ethoxycarbonyl-cyclohexyl)carbamoyl]cyclopentyl}-2-(aminomethyl)propanoic acid t-butyl ester(see Preparation 1) or the 3-ethoxycarbonyl isomer (see Preparation 2) and reacting with the appropriate acid of formula IV instead of N^2-acetyl-N^6-benzyloxycarbonyl-L-lysine.



Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
2	# $ZNH(CH_2)_4$ | $C_6H_5CONHCH-$	4	66.59 (66.81	7.81 7.90	7.23 7.08)
3	$C_6H_5CH_2$ | $CH_3CONHCH-$	4	66.00 (66.53	8.38 8.38	6.86 6.85)
4	$C_6H_5CH_2$ | $C_6H_5CONHCH-$	4	69.06 (69.31	8.16 7.90	6.18 6.22)
5	CH_3 | $CH_3CONHCH-$	4	61.57 (62.54	8.64 8.81	7.69 7.82)
6	CH_3 | $C_6H_5CONHCH-$	4	64.57 (66.08	8.21 8.24	6.68 7.01)
7	$(CH_3)_2CH$ | $CH_3CONHCH-$	4	63.57 (63.69	9.11 9.09	7.26 7.43)

Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
8	$(CH_3)_2CH$ – $C_6H_5CONHCH-$ (with $(CH_3)_2CH$ on the CH)	4	66.15 (66.96	8.67 8.51	6.85 6.69)
9	CH_3CONH– 	3	64.69 (64.44	8.94 8.90	7.27 7.27)
10	C_6H_5CONH– 	3	67.71 (67.58	8.24 8.35	6.34 6.57)
11	$CH_3CONHC(CH_3)_2-$	3	62.59 (63.13	8.80 8.95	7.63 7.62)
12	$C_6H_5CONHC(CH_3)_2-$	3	66.08 (66.53	8.49 8.38	6.75 6.85)

Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
13		3	66.41 (66.53	8.35 8.38	6.63 6.85)
14	$(CH_3)_2CHCONHCH(CH_3)-$	3	63.26 (63.69	9.28 9.09	7.16 7.43)
15		3	61.98 (62.23	7.78 7.68	6.27 6.40)(1)
16		3	64.67 (64.84	8.78 8.16	6.59 6.67)
17		3	67.94 (68.39	7.81 7.91	6.38 6.47)
18	$ZNH(CH_2)_4$, $C_6H_5CH_2$ $C_6H_5CONH-CH$ — $CONHCH-$	3	67.02 (67.21	7.57 7.66	7.20 7.40)(2)

Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
19	$ZNH(CH_2)_4$ $C_6H_5CH_2$ $CH_3CONH-CH$ CONHCH-	3	66.02 (65.80	8.06 7.94	7.94 7.99)
20		3	65.70 (65.57	7.98 8.09	7.46 7.50)(3)
21		3	64.47 (65.60	8.21 8.39	7.22 7.29)
22		3	68.27 (68.55	7.77 7.67	6.56 6.66)
23		3	63.84 (64.00	8.13 8.20	7.37 7.45)(2)
24	$ZNH(CH_2)_4$ $CH_3SO_2NH-CH-$	3	58.65 (59.66	7.84 7.91	7.17 7.32)

Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
25	ZNH | $ZNH(CH_2)_4$-CH-	3	63.29 (63.29	7.73 7.59	6.49 6.49)(4)
26	$ZNH(CH_2)_4$ | CH_3CONH-CH-	3	63.65 (64.26	8.39 8.30	7.63 7.69)
27	$ZNH(CH_2)_4$ | C_6H_5CONH-CH-	3	66.95 (66.81	7.95 7.90	6.75 7.08)

Z = $C_6H_5CH_2OCO-$

(1) 0.25 mole $CH_3CO_2C_2H_5$

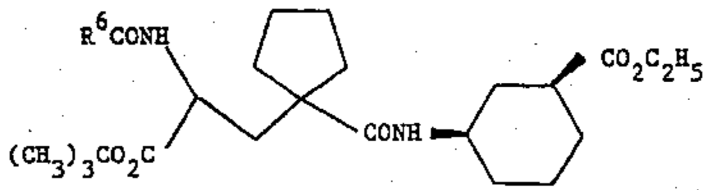
(2) 0.5 H_2O

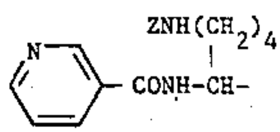
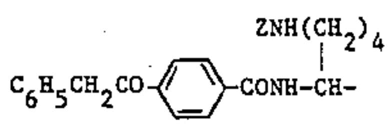
(3) 1.0 H_2O

(4) 0.5 mole CH_2Cl_2

EXAMPLES 28-29

The following compounds were prepared following the general method of Example 1 using as starting material 3-{1-[cis-3-ethoxycarbonyl-cyclohexyl)carbamoyl]cyclopentyl}-2-aminopropanoic acid and reacting with the apropriate acid of formula IV.

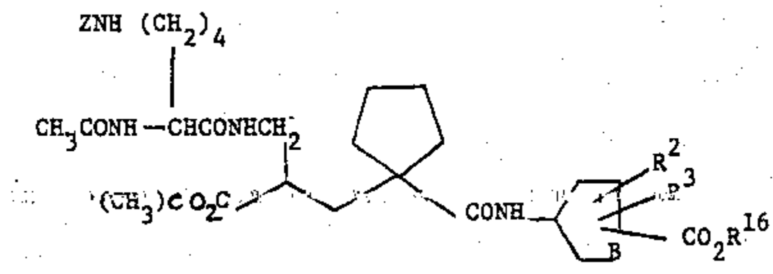


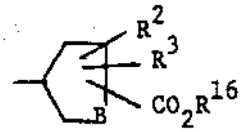
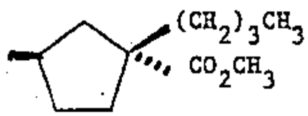
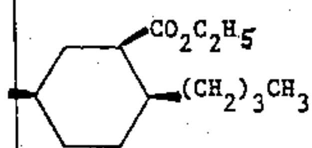
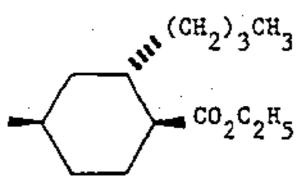
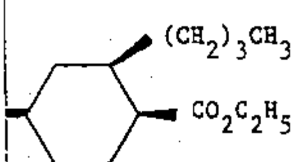
Example	R^6	Analysis % (Theoretical in brackets) C	H	N
28		64.17 (64.10	7.71 7.69	8.79 8.90)[1]
29		67.73 (68.00	7.62 7.53	6.16 6.34)

(1) hemihydrate

EXAMPLES 30-33

The following compounds were prepared following the general method of Example 1 using the appropriate amine of formula (III) and coupling with N^2-acetyl-N^6-benzyloxycarbonyl-L-lysine.



Example		Analysis % (Theoretical in brackets) C	H	N
30	 R,S	64.82 (64.60	8.83 8.66	7.18 7.18) (1)
31		63.69 (63.59	8.43 8.81	6.94 6.90) (2)
32	 diastereoisomers	65.95 (65.79	8.90 8.73	6.83 7.14
		64.52 (64.31	8.33 8.78	6.43 6.97) (3)
33		65.94 (65.79	8.83 8.73	7.14 7.14)

(1) Hemihydrate (2) 1.5 hydrate (3) hydrate

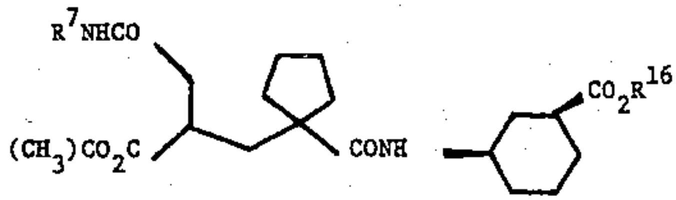
EXAMPLE 34

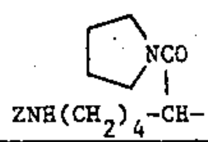
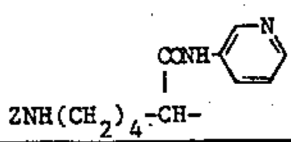
N-[4-{1-[cis-3-t-butoxycarbonylcyclohexyl)-carbamoyl]cyclopentyl}-3-(t-butoxycarbonyl)-butanoyl] phenylalanine t-butyl ester.

1-Hydroxybenztriazole (76 mg, 0.576 mmol) and N-methylmorpholine (0.5 ml, 4.5 mmole) were added to a stirred solution of 3-{1-[(cis-3-t-butoxycarbonylcyclohexyl)-carbamoyl]cyclopentyl} -2-(carboxymethyl)-propanoic acid t-butyl ester (219.6 mg, 0.457 mmol) in dry dichloromethane (20 ml) at 0° C followed by 1-ethyl-3-(dimethylaminopropyl)carbodimide (94 mg). The solution was stirred at 0° C for 20 minutes and phenylalanine t-butyl ester (128 mg, 0.5 mmol) in dry dichloromethane (5 ml) added in one portion, and the reaction allowed to warm up to room temperature and stirred for 16 hours. The solution was concentrated under vacuum to a volume of 10 ml, and partitioned between ethyl acetate and water. The organic phase was washed with water (2 x 25 ml), 2 M hydrochloric acid (2 x 10 ml), sodium bicarbonate solution (2 x 10 ml) and brine, and dried ($MgSO_4$) and the solvent evaporated to yield the title compound as an oil (325 mg, 100%). Found: C,66.59; H,8.92; N,4.25. $C_{39}H_{60}N_2O_8$. H_2O requires C,66.63; H,8.89; N,3.49%

EXAMPLES 35-38

The following compounds were prepared following the procedure of Example 34 using as starting material the appropriate 3-{1-[cis-3-alkoxycarbonyl-cyclohexyl)carbamoyl]cyclopentyl}-2-carboxy-methyl-propanoic acid t-butyl ester and reacting with the appropriate amine of formula R^7R^9NH.



Example	R^7	R^{16}	Analysis % (Theoretical in brackets) C	 H	 N
35	$(CH_3)_2NCO$ | $ZNH(CH_2)_4CH-$	$-C(CH_3)_3$	63.39 (63.39	8.38 8.38	6.95 6.98)[(1)]
36	$(CH_3)_2CHNHCO$ | $ZNH(CH_2)_4-CH-$	$-C_2H_5$	63.83 (63.96	8.40 8.59	7.01 6.63)
37		$-C_2H_5$	65.17 (65.60	8.39 8.39	7.10 7.29)
38		$-C_2H_5$	63.17 (63.11	7.73 7.54	8.28 8.48)

(1) 0.4 mole CH_2Cl_2

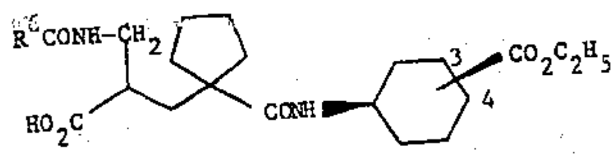
EXAMPLE 39

2-(N^2-Acetyl-N^6-benzyloxycarbonyl-L-lysyl-aminomethyl)-3-{1-[(cis-4-ethoxycarbonylcyclohexyl)carbamoyl]-cyclopentyl}propanoic acid

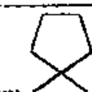
The t-butyl ester from Example 1 (571 mg, 0.783 mmol) was dissolved in a mixture of trifluoroacetic acid (2 ml) and dichloromethane (1 ml). The solution was kept at 4 °C overnight, then concentrated to dryness under vacuum, and the residue was azeotroped six times with dichloromethane. The resulting crude product was then taken up in ethyl acetate and washed with water until the washings were neutral. The organic phase was dried ($MgSO_4$) and evaporated under vacuum to afford the title compound (442 mg, 84%) as a white foam. Found: C,61.89; H,7.76; N,7.93. $C_{35}H_{52}N_4O_9$ (0.5 H_2O) requires C,61.25; H,7.69; N,8.22%.

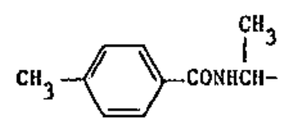
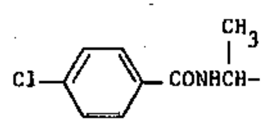
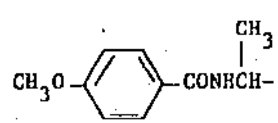
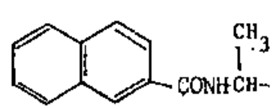
EXAMPLES 40 to 65

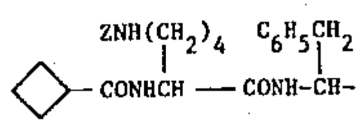
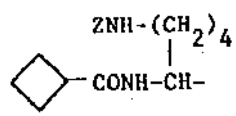
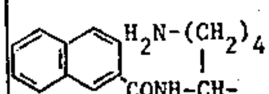
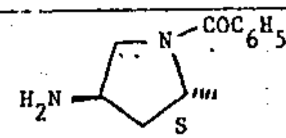
The following compounds were prepared following the procedure of Example 39 but using as starting material the appropriate t-butyl ester of Examples 2 to 27.



Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
40	$ZNH(CH_2)_4$ $C_6H_5CONHCH-$	4	65.02 (65.37	7.33 7.41	7.60 7.61)
41	$C_6H_5CH_2$ $CH_3CONHCH-$	4	64.18 (64.61	7.54 7.77	7.63 7.54)
42	$C_6H_5CH_2$ $C_6H_5CONHCH-$	4	66.94 (67.83	7.34 7.32	6.65 6.78)
43	CH_3 $CH_3CONHCH-$	4	49.96 (49.69	6.49 6.26	6.50 6.44)(1)
44	CH_3 $C_6H_5CONHCH-$	4	63.67 (64.07	7.80 7.60	7.52 7.73)
45	$(CH_3)_2CH$ $CH_3CONHCH-$	4	60.89 (61.27	8.58 8.50	8.14 8.25)

Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
46	$(CH_3)_2CH$ / $C_6H_5CONHCH$	4	64.88 (65.12	7.35 7.93	7.34 7.35)
47	CH_3CONH 	3	62.11 (62.16	8.44 8.31	7.95 8.06)
48	C_6H_5CONH 	3	65.49 (65.85	7.80 7.77	6.99 7.20)
49	$CH_3CONH-CH(CH_3)-CH(CH_3)-$	3	60.20 (60.58	8.46 8.34	8.30 8.48)
50	$C_6H_5CONH-CH(CH_3)-CH(CH_3)-$	3	64.86 (64.61	7.91 7.77	7.24 7.54)

Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
51		3	64.61 (64.61	7.96 7.77	7.33 7.54)
52	$(CH_3)_2CHCONHCH(CH_3)-$	3	61.08 (61.27	8.52 8.50	7.91 8.25)
53		3	60.24 (60.25	7.19 6.97	6.90 7.27)
54		3	62.19 (62.32	7.59 7.58	7.15 7.27)(2)
55		3	66.10 (66.09	7.21 7.33	6.97 7.01)(3)
56	$C_6H_5CONHCH[(CH_2)_4NHZ]$ — $CONH-CH(CH_2C_6H_5)-$	3	63.70 (63.65	6.87 6.98	7.53 7.56)(4)

Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
57	$ZNH(CH_2)_4$ $C_6H_5CH_2$ / $CH_3CONHCH$ —— CONH-CH-	3	63.83 (63.75	7.59 7.54	8.32 8.45)(5)
58		3	65.41 (65.63	7.66 7.62	8.01 8.14)
59		3	62.16 (64.02	7.95 7.92	7.62 7.80)
60		3 (8)	gum Rf 0.33 (silica)		
61		3	58.34 (58.25	7.38 7.41	8.60 8.77)(6)

Example	R^6	$-CO_2C_2H_5$ attachment	Analysis % (Theoretical in brackets) C	H	N
62	$ZNH(CH_2)_4$ | $CH_3SO_2NH-CH-$	3	57.40 (57.61	7.45 7.41	7.89 7.90)
63	ZNH | $ZNH(CH_2)_4-CH$	3	63.20 (63.02	7.49 7.26	7.23 7.13)
64	$ZNH(CH_2)_4$ | $CH_3CONH-CH-$	3	60.54 (60.79	7.85 7.63	7.94 8.07)
65	$ZNH(CH_2)_4$ | $C_6H_5CONH-CH-$	3	64.53 (64.79	7.29 7.35	7.36 7.54)(9)

(1) 1.5 mole CF_3CO_2H

(2) 0.25 mole H_2O

(3) 0.33 mole H_2O

(4) 0.5 mole CH_2Cl_2

(5) 0.5 mole H_2O

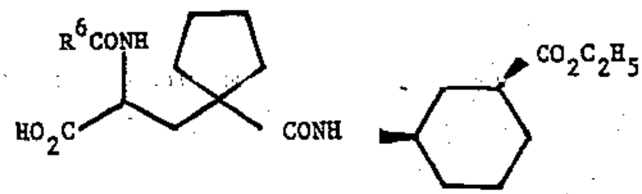
(6) HCl in CH_2Cl_2 at 0°C for 3 hours used instead of trifluoroacetic acid. Product isolated as $.HCl.H_2O$.

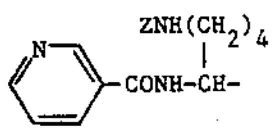
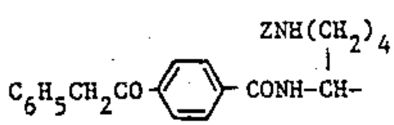
(7) 0.1 mole CH_2Cl_2.

(8) From Example 106.

EXAMPLES 66-67

The following compounds were prepared following the general method of Example 39 using as starting material the appropriate t-butyl ester of Examples 28 and 29.

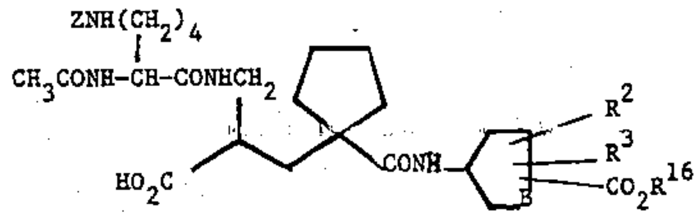


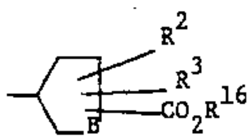
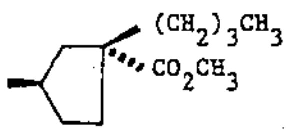
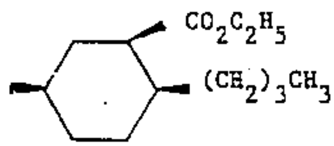
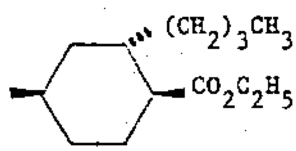
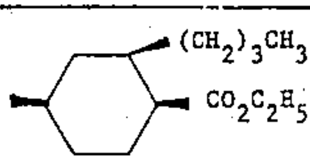
Example	R^6	Analysis % (Theoretical in brackets) C H N
66		61.79 7.19 9.39 (62.07 7.20 9.52)(1)
67		foam

(1) 0.75 H_2O

EXAMPLES 68-71

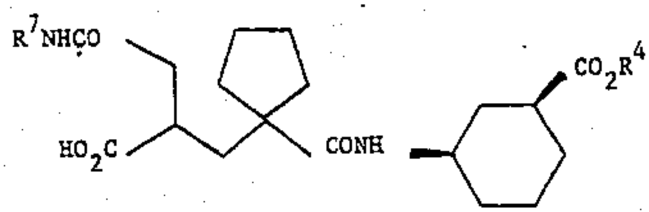
The following compounds were prepared by the general method of Example 39 using the appropriate t-butyl ester of Examples 30 to 33.

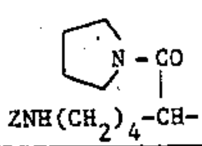
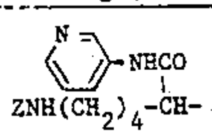


Example		Analysis % (Theoretical in brackets) C H N
68		60.74 7.97 7.42 (61.05 7.85 7.40)(1)
69		62.64 8.22 7.47 (62.71 8.37 7.50)
70		two diastereoisomers isolated as gums
71		gum

EXAMPLES 72-76

The following compounds were prepared by the general method of Example 39 using the appropriate t-butyl ester of Examples 34-38.



Example	R^7	R^4	Analysis % (Theoretical in brackets) C H N
72	CO_2H | $C_6H_5CH_2$-CH-	H	61.49 7.29 4.64 (62.13 7.19 4.28)(1)
73	$(CH_3)_2NCO$ | $ZNH(CH_2)_4$-CH-	H	60.98 7.47 7.86 (60.95 7.71 8.36)(2)
74	$(CH_3)_2CHNHCO$ | $ZNH(CH_2)_4$-CH-	C_2H_5	59.78 7.65 7.14 (59.79 7.53 7.40)
75		C_2H_5	60.58 7.55 7.23 (60.70 7.55 7.33)
76		C_2H_5	62.01 7.01 8.86 (61.81 7.08 9.16)

(1) 0.5 mole $CH_3CO_2C_2H_5$ (2) 0.6 H_2O

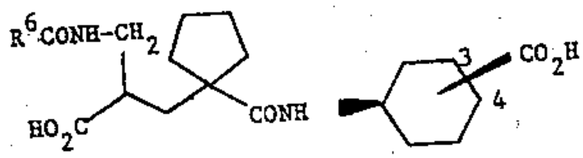
EXAMPLE 77

2-(N[2]-Acetyl-N[6]-benzyloxycarbonyl-L-lysyl-aminomethyl)-3-{1-[(cis-4-carboxycyclohexyl)carbamoyl]-cyclopentyl} propanoic acid

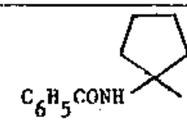
The ethyl ester from Example 39 (404 mg, 0.600 mmol) was dissolved in 2M sodium hydroxide solution (10 ml), and the resulting solution was kept at room temperature overnight. The reaction mixture was diluted to 30 ml and extracted with diethyl ether. The aqueous phase was acidified to pH 1 with 2M hydrochloric acid and extracted with ethyl acetate (3 x 25 ml). The organic phase was dried ($MgSO_4$) and evaporated to afford the title compound (375 mg, 97%) as a white foam. Found: C,58.30; H,7.24; N,8.20. $C_{33}H_{48}N_4O_9$. (0.25 CH_2Cl_2, 1.25 H_2O) requires C,58.00; H,7.17; N,8.14%.

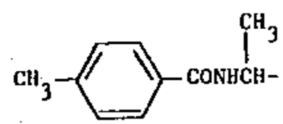
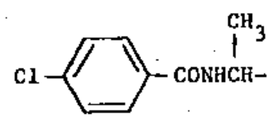
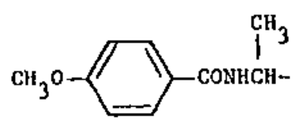
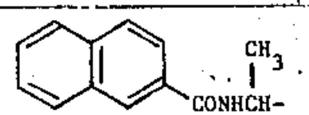
EXAMPLES 78-96

The following compounds were prepared following the procedure of Example 77 but using as starting material the appropriate ethyl ester of Examples 40 to 58.



Example	R^6	$-CO_2H$ attachment	Analysis % (Theoretical in brackets) C	H	N
78	$ZNH(CH_2)_4$ | $C_6H_5CONHCH-$	4	63.42 (63.94	7.16 7.34	7.48 7.68 (1)
79	$C_6H_5CH_2$ | $CH_3CONHCH-$	4	61.34 (61.50	7.47 7.39	7.57 7.65) (2)
80	$C_6H_5CH_2$ | $C_6H_5CONHCH-$	4	65.39 (65.49	6.84 7.08	6.92 6.94) (3)
81	CH_3 | $CH_3CONHCH-$	4	52.87 (52.67	6.66 6.97	8.34 8.01) (4)
82	CH_3 | $C_6H_5CONHCH-$	4	60.78 (60.82	7.19 7.11	7.73 7.82) (5)
83	$(CH_3)_2CH$ | $CH_3CONHCH-$	4	58.16 (58.16	8.03 8.08	8.18 8.43) (6)

Example	R^6	$-CO_2H$ attachment	Analysis % (Theoretical in brackets) C	H	N
84	$(CH_3)_2CH$–, $C_6H_5CONHCH-$	4	63.30 (63.49	7.75 7.70	7.17 7.25)(7)
85		3	59.52 (59.74	8.04 8.02	8.07 8.36)(1)
86		3	64.68 (64.84	7.62 7.44	7.15 7.56)
87	$CH_3CONH-C(CH_3)_2-$	3	55.66 (56.09	7.71 7.80	8.31 8.44)(8)
88	$C_6H_5CONHC(CH_3)_2-$	3	62.77 (63.13	7.57 7.63	7.31 7.44)(9)

Example	R^6	$-CO_2H$ attachment	Analysis % (Theoretical in brackets) C	H	N
89		3	62.38 (62.43	7.47 7.49	7.60 7.80)(1)
90	$(CH_3)_2CHCONHCH(CH_3)-$	3	58.73 (58.75	8.21 8.22	8.32 8.57)(1)
91		3	57.76 (58.00	6.66 6.67	7.30 7.52)(1)
92		3	60.81 (60.63	7.25 7.27	7.57 7.58)(1)
93		3	64.52 (64.48	7.15 7.08	6.98 7.09)(10)
94	$C_6H_5CONH-CH[(CH_2)_4NHZ]-CONH-CH(CH_2C_6H_5)-$	3	65.12 (65.41	7.07 7.01	7.88 8.12)(1)

Example	R^6	$-CO_2H$ attachment	Analysis % (Theoretical in brackets) C	H	N
95	$ZNH(CH_2)_4$ $C_6H_5CH_2$ $CH_3CONH-CH$ —— $CONH-CH-$	3	63.19 (62.98	7.27 7.30	8.11 8.74)(1)
96	$ZNH(CH_2)_4$ $C_6H_5CH_2$ $-CONH-CH$ —— $CONH-CH-$	3	64.24 (64.26	7.42 7.43	8.11 8.33)(1)

(1) 0.5 mole H_2O;

(2) 0.125 mole CH_2Cl_2; 0.5 mole H_2O;

(3) 0.75 mole H_2O;

(4) 0.5 mole CH_2Cl_2; 0.25 mole CF_3CO_2H;

(5) 0.2 mole CH_2Cl_2; 0.25 mole H_2O;

(6) 0.125 mole CH_2Cl_2; 0.33 mole H_2O;

(7) 0.4 mole $CH_3CO_2C_2H_5$;

(8) 0.25 mole CH_2Cl_2; 0.5 mole H_2O;

(9) 0.28 mole $CH_3CO_2C_2H_5$; 0.14 mole H_2O;

(10) 0.2 mole $CH_3CO_2C_2H_5$; 0.5 mole H_2O.

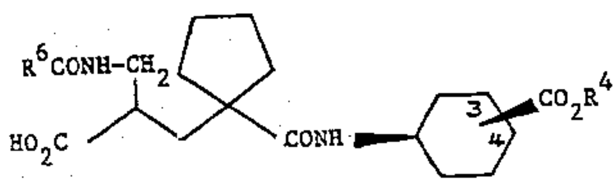
EXAMPLE 97

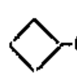
2-(N^2-Acetyl-L-lysyl-aminomethyl)-3-{1-[(cis-4-carboxycyclohexyl)carbamoyl]cyclopentyl} propanoic acid

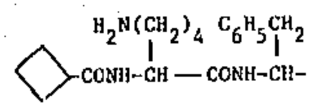
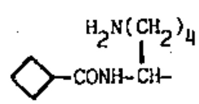
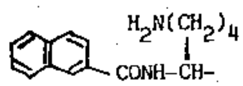
A solution of the product from Example 77 (200 mg, 0.31 mmol) in a mixture of ethanol (27 ml) and water (3 ml) was reduced on 10% palladium on charcoal (20 mg) under 50 p.s.i. (3.46 bar) of hydrogen for 1½ hours. The solution was filtered and the solvent evaporated under vacuum, and the residue azeotroped with dichloromethane (6 x) to afford the title compound (161 mg, 100%) as a white solid, m.p. 161-163° C. Found: C,56.80; H,8.49; N,9.22. $C_{25}H_{42}N_4O_7.H_2O$ requires C,56.80; H,8.39; N,10.60%.

EXAMPLES 98-110

The following compounds were prepared following the procedure of Example 97 but using as starting material the appropriate amino-protected ethyl ester or acid.



Example	R^6	$-CO_2R^4$ attachment	Analysis % (Theoretical in brackets) C	H	N
98	$H_2N(CH_2)_4$ / $C_6H_5CH_2CONH-CH-$	$4-CO_2H$	60.64 (60.58	7.84 7.79	8.77 9.42)(1)
99	$H_2N(CH_2)_4$ $C_6H_5CH_2$ / $C_6H_5CONHCH$ —$CONH-CH-$	$3-CO_2C_2H_5$	64.48 (64.29	7.96 7.76	8.21 9.14)(1)
100	$H_2N(CH_2)_4$ $C_6H_5CH_2$ / $C_6H_5CONHCH$ — $CONH-CH-$	$3-CO_2H$	63.57 (63.48	7.68 7.51	8.46 9.49)(1)
101	$H_2N(CH_2)_4$ $C_6H_3CH_2$ / CH_3CONH CH —$CONH-CH-$	$3-CO_2C_2H_5$	61.74 (61.43	8.27 8.16	9.20 9.95)(1)
102	$H_2N(CH_2)_4$ $C_6H_5CH_2$ / CH_3CONH CH —$CONH-CH-$	$3-CO_2H$	60.54 (60.42	7.93 7.91	9.97 10.36)(1)
103	$H_2N(CH_2)_4$ $C_6H_5CH_2$ / $-CONHCH$ —$CONH-CH-$	$3-CO_2C_2H_5$	64.03 (64.13	8.41 8.21	8.48 9.59)(2)

Example	R^6	$-CO_2R^4$ attachment	Analysis % (Theoretical in brackets) C	H	N
104		$3-CO_2H$	62.16 (62.08	8.08 8.03	9.58 9.78)(1)
105		$3-CO_2C_2H_5$	58.87 (60.88	8.50 8.53	8.88 9.41)(3)
106	 (6)	$3-CO_2C_2H_5$	gum		
107	$H_2N(CH_2)_4$ / $CH_3SO_2NH-CH-$	$3-CO_2C_2H_5$	51.75 (52.50	8.07 7.89	8.83 9.33)(4)
108	NH_2 / $H_2N(CH_2)_4-CH-$	$3-CO_2C_2H_5$	57.07 (59.15	8.85 8.84	10.12 10.99)(5)
109	$H_2N(CH_2)_4$ / $CH_3CONH-CH-$	$3-CO_2C_2H_5$	55.63 (57.32	8.42 8.73	9.27 9.91)(6)

Example	R^6	$-CO_2R^4$ attachment	Analysis % (Theoretical in brackets) C	H	N
110	$H_2N(CH_2)_4$ | $C_6H_5CONH-CH-$	$3-CO_2C_2H_5$	62.46 (62.12	8.17 8.15	8.65 9.06)(1)

(1) Hydrate

(2) $0.25H_2O$

(3) 0.2 mole CH_2Cl_2

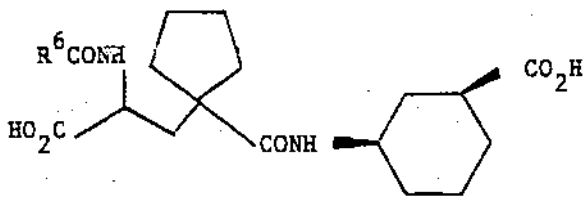
(4) 0.25 mole H_2O, 0.25 mole CH_2Cl_2

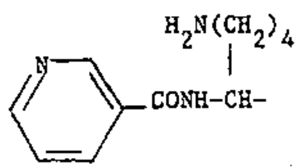
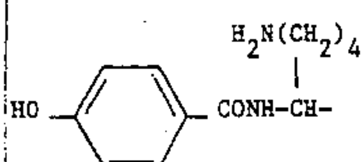
(5) 0.25 mole H_2O, 0.1 CH_2Cl_2

(6) From Example 22, t-butyl ester.

EXAMPLES 111-112

The following compounds were prepared from Examples 66 and 67 by the hydrolysis of the ester group following the general procedure of Example 77 followed by catalytic hydrogenation or by treatment with HBr in glacial acetic acid to remove the benzyloxycarbonyl protecting group.

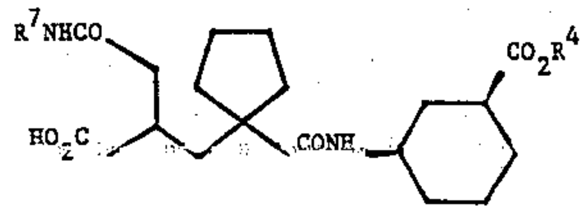


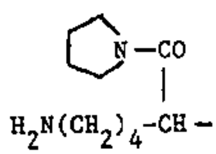
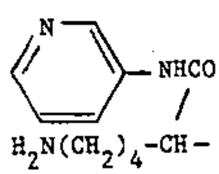
Example	R^6	Analysis % (Theoretical in brackets) C	H	N
111		59.24 (59.14	7.62 7.44	12.15 12.32)(1)
112		foam		

(1) 0.5 mole H_2O

EXAMPLES 113-119

The following Examples were prepared from Examples 73 to 76 by catalytic hydrogenation according to the procedure of Example 97, followed, in the case of the ethyl esters, by hydrolysis according to the procedure of Example 77. The diacids were isolated by ion-exchange chromatography eluting with aqueous pyridine.

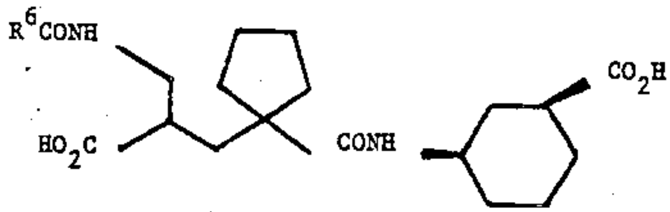


Example	R^7	R^4	Analysis % (Theoretical in brackets) C	H	N
113	$(CH_3)_2NCO$ | $H_2N(CH_2)_4$-CH-	H	55.62	8.37	7.95
			(55.62	9.57	7.95)(1)
114	$(CH_3)_2CHNHCO$ | $H_2N(CH_2)_4$-CH-	C_2H_5	60.10	8.71	8.34
			(59.82	8.79	8.67
115		H	56.27	8.37	9.79
			(56.42	8.77	9.74)
116		C_2H_5	59.52	8.51	8.53
			(59.35	8.76	8.92)
117		H	59.14	8.46	9.35
			(58.97	8.53	9.48
118		C_2H_5	59.02	7.81	10.55
			(58.88	7.75	10.33)
119		H	56.79	8.01	9.08
			(60.72	7.55	12.21)

(1) 3.3 moles C_2H_5OH, 1.5 mole H_2O

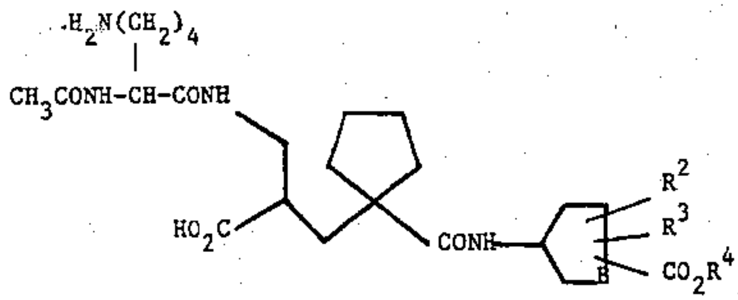
EXAMPLES 120-126

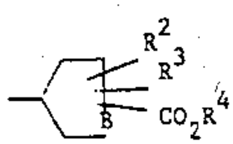
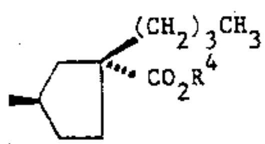
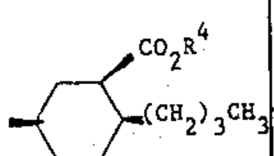
The following compounds were prepared by hydrolysis of the appropriate ester of Examples 61 or 105-110 following the procedure of Example 77. The products were isolated by ion-exchange chromatography eluting with aqueous pyridine.


R6CONH
CO2H
HO2C
CONH

EXAMPLES 127-134

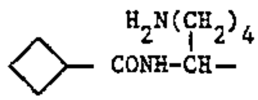
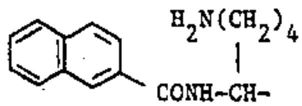
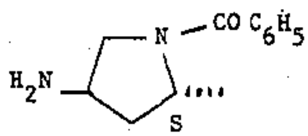
The following compounds were prepared from Examples 68-71 by catalytic hydrogenation according to the procedure of Example 97 to yield the esters (R^4 = CH_3 or C_2H_5) followed by hydrolysis following the procedure of Example 77 to yield the corresponding acids (R^4 = H).



Example		R^4	Analysis % (Theoretical in brackets) C	H	N
127		CH_3	59.19 (58.85	8.93 9.13	8.62 9.15) (1)
128		H	60.12 (60.01	9.00 8.94	9.88 9.65) (2)
129		C_2H_5	60.05 (60.13	8.97 8.81	8.55 8.93) (3)
130		H	58.59 (58.54	8.97 9.08	8.85 9.26) (4)

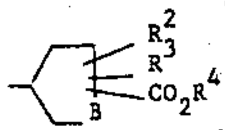
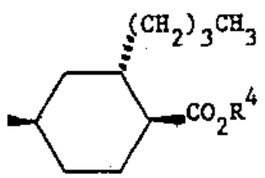
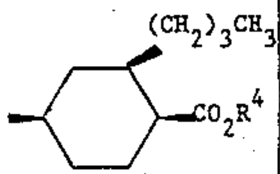
(1) 1.75 H_2O (2) 0.75 H_2O (3) 0.4 mole CH_2Cl_2

(4) 1.5 H_2O, 0.25 C_2H_5OH

Example	R^6	Analysis % (Theoretical in brackets) C	H	N
120		57.36 (59.13	8.29 8.51	9.28 9.85)[(1)]
121		64.43 (64.64	7.60 7.50	8.95 8.87)[(2)]
122		61.54 (61.58	7.59 7.30	9.90 9.90)[(2)]
123	$H_2N(CH_2)_4$ | CH_3SO_2NH-CH-	50.32 (52.38	7.86 7.77	9.04 10.18)[(3)]
124	NH_2 | $H_2N(CH_2)_4$-CH-	53.61 (57.30	8.55 8.68	10.69 11.62)[(4)]
125	$H_2N(CH_2)_4$ | CH_3CONH-CH-	56.57 (56.79	8.55 8.39	10.19 10.60)[(1)]
126	$H_2N(CH_2)_4$ | C_6H_5CONH-CH-	60.85 (60.67)	7.96 8.05	8.69 9.13)[(5)]

(1) hydrate (2) 0.5 mole H_2O (3) 0.2 mole H_2O

(4) 0.75 mole H_2O (5) 1.0 mole H_2O 0.5 mole C_2H_5OH

Example		R^4	Analysis % (Theoretical in brackets) C	H	N
131		C_2H_5	products obtained as gums		
132		H	(I) 60.40	8.80	9.65(1)
			(II) 60.64	8.87	9.80(1)
			(60.50	8.93	9.73)
			(two diasteroisomers)		
133		C_2H_5	gum		
134		H	60.29	9.11	9.68
			(60.50	8.93	9.73) (1)

(1) 0.5 H_2O

EXAMPLE 135

2-(N^2-Methanesulphonyl-L-lysyl-aminomethyl)-3-{1-[(cis-4-carboxy-cis-3-butylcyclohexyl)carbamoyl]-cyclopentyl} propanoic acid

1. 3-(1-Carboxycyclopentyl)-2-aminopropanoic acid t-butyl ester

3-(1-Carboxycyclopentyl)-2-(dibenzylaminomethyl)propanoic acid t-butyl ester hydrochloride (2.0 g; 41 mmol) in ethanol (160 ml) and triethylamine (20 ml) was hydrogenated over palladium (from 20% $Pd(OH)_2/C$; 20 g) at 60 p.s.i. (4.1 bar). After eighteen hours the mixture was filtered through arbicel, the solvent evaporated and the residue dried azeotropically with toluene. The required primary amino acid triethylamine salt, containing one mole equivalent of triethylamine hydrochloride was thus obtained as a white solid (18.21 g).

2. 2-(N^2-Benzyloxycarbonyl-N^6-t-butoxycarbonyl-L-lysyl-aminomethyl)-3-[1-(1-carboxycyclopentyl)]propanoic acid t-butyl ester (diastereoisomer)

The above product (8.24g;20.1 mmole) and N^2-benzyloxy-carbonyl-N^6-t-butoxycarbonyl-L-lysine 4-nitrophenyl ester (9.50 g, 18.9 mmole) were dissolved in dry methylene chloride (70 ml). The solution was stirred and after cooling to 10°C, triethylamine (2.64 ml, 18.9 mmol) was added. After half an hour the mixture became homogenous and was allowed to stand at room temperature overnight. The solution was then washed with 1M citric acid followed by water, dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel (500 g) eluting with increasing proportions of ethyl acetate in hexane (2:1 to 4:1) and finally with ethylacetate, hexane, acetic acid (4:1:0.05). The required mixture of diastereoisomers was thus obtained as a colourless gum (10.5 g). The isomers were then separated by chromatography on silica gel (1 kg) eluting with a mixture of toluene, isopropanol and diethylamine (10:2:1). The diethylamine salt of the required more polar diastereoisomer was obtained as an orange foam (3.01 g), which was dissolved in ethyl acetate and washed with 1M citric acid and brine. Drying over $MgSO_4$ and evaporation gave the free acid as a yellow foam (2.81 g). Found: C,62.89; H,8.29; N,6.69. $C_{33}H_{51}N_3O_9$ requires C,62.54; H,8.11; N,6.63%. Additional chromatography of a small sample on silica eluting with increasing proportions of ethyl acetate in hexane (2:3 to 17:3) gave a cream powder $[\alpha]_D^{25}$ - 2.8°, $[\alpha]_{365}^{25}$ -3.6° (c = 0.5, ethanol).

3. 2-(N^2-Benzyloxycarbonyl-N^6-t-butoxycarbonyl-L-lysyl-aminomethyl)-3- {1-[(cis-4-ethoxycarbonyl-cis-3-butylcyclohexyl)-carbamoyl]cyclopentyl} propanoic acid t-butyl ester

Coupling of the above product from step 2 (650 mg; 1.03 mmole) with c-4-amino-c-2-butyl-r-1-cyclohexane carboxylic acid ethyl ester hydrochloride (271 mg; 1.03 mmole) as described in Example 1 followed by chromatography on silica eluting with increasing proportions of ethyl acetate in hexane (7:3 to 4:6) gave the required product as a pale foam (630 mg; 73%). Found: C,64.37; H,8.98; N,6.81. $C_{46}H_{74}N_4O_{10}$ (0.75 H_2O) requires C,64.50; H,8.88; N,6.54%.

4. 2-(N^2-Methanesulphonyl-L-lysyl-aminomethyl)-3-{1-[(cis-4-carboxy-cis-3-butylcyclohexyl)carbamoyl]-cyclopentyl} propanoic acid

i) The above product from step 3 (620 mg; 0.735 mmole) in ethanol (18 ml) and water (2 ml) was hydrogenated over 5% palladium on carbon (200 mg) at 50 p.s.i. (3.5 bar). After three hours the mixture was filtered through Arbicel and evaporated to dryness giving a white foam (520 mg; 95%).

ii) Methane sulphonyl chloride (0.11 ml; 1.41 mmole) was added dropwise to an ice cold stirred solution of the above product (500 mg; 0.67 mmole) and N-methylmorpholine (0.16 ml; 1.4 mmole) in dry methylene chloride (15 ml). After three hours more methane sulphonyl chloride (0.03 ml) and N-methylmorpholine (0.04 ml) were added and the mixture kept at 0°C overnight. The mixture was then washed in succession with water, saturated aqueous sodium bicarbonate and water, dried over $MgSO_4$ and evaporated to give the crude product which was chromatographed on silica gel. Elution with increasing proportions of ethyl acetate in hexane (4:6 to 1:9) gave the required methanesulphonyl derivative as a colourless foam (460 mg; 87%). Rf. 0.15 (ethylacetate, hexane 1:1).

iii) Treatment of the above product (440 mg; 0.56 mmole) with trifluoroacetic acid as described in Example 39, followed by hydrolysis with 1N sodium hydroxide (4.5 ml) at 50-55°C for 65 hours and adsorption on ion-exchange resin 50W-X8 eluting with 10% aqueous pyridine gave the required diacid as a foam. Trituration with acetonitrile afforded a white powder (245 mg; 73%). Found: C,54.92; H,8.29; N,9.12. $C_{28}H_{50}N_4O_8S$ (0.5 H_2O) requires C,54.97; H,8.40; N,9.16%.

EXAMPLE 136

2-(N^2-Methanesulphonyl-L-lysyl-aminomethyl)-3- {1-[(cis-4-carboxy-cis-3-(3-methylbutyl)-cyclohexyl)-carbamoyl]cyclopentyl} propanoic acid

This compound was prepared following the procedure of Example 135 but using c-4-amino-c-2-(3-methylbutyl)-r-1-cyclohexane carboxylic acid in step 3. The product was obtained as a cream powder. Found: C,56.07; H,8.22; N,8.95. $C_{29}H_{52}N_4O_8S$ requires C,56.47; H,8.50; N,9.08%.

Preparation 1

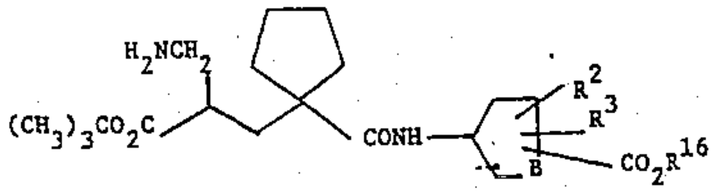
3-{1-[(cis-4-Ethoxycarbonyl-cyclohexyl)carbamoyl]cyclopentyl}-2-(aminomethyl)propanoic acid t-butyl ester

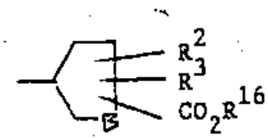
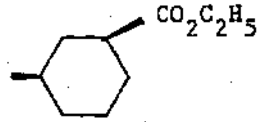
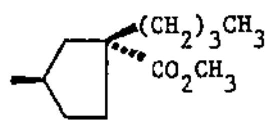
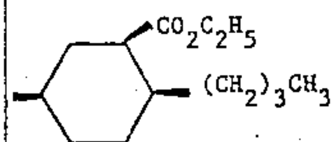
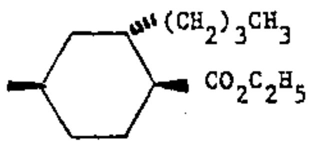
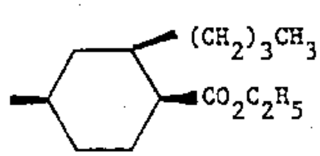
(a) A solution of 2-(bromomethyl)propenoic acid t-butyl ester (20 g, 90.5 mmole) in dry acetonitrile (360 ml), cooled to 0 °C, was treated with solid potassium carbonate (15.63 g, 113 mmole), followed by a solution of dibenzylamine (17.83 g, 90.5 mmole) in dry acetonitrile (600 ml), producing a 10 °C exotherm. The reaction was stirred at 0 °C for 0.5 hours, followed by 1 hour at room temperature, and then partitioned between water and diethyl ether. The ether phase was washed again with water, dried (sodium sulphate) and evaporated to yield the crude product (31 g) which was filtered through a pad of silica, eluting with hexane/CH_2Cl_2 (1:1), to yield 2-(dibenzylaminomethyl)propenoic acid t-butyl ester (23.8 g, 78%) as a solid, m.p. 62-63 °C. Found: C,78.09; H,8.20; N,4.18. $C_{22}H_{27}NO_2$ requires C,78.3; H,8.06; N,4.15%.

(b) To a stirred solution of diisopropylamine (14.98 g, 20.75 ml, 148 mmole) in dry tetrahydrofuran (250 ml) cooled to -30 °C under nitrogen, was added dropwise, n-butyl lithium (59.3 ml of a 2.5 M solution, 148 mmole), keeping the temperature below -20 °C. The reaction was stirred at -20 °C for 1 hour, then cooled to -30 °C and cyclopentanecarboxylic acid (8.05 g, 7.65 ml, 70.6 mmole) added dropwise in a small amount of dry tetrahydrofuran. The reaction mixture was stirred at 0 °C for two hours, during which time a white precipitate formed. The solution was then cooled to -70 °C, and a solution of 2-(dibenzylaminomethyl)-propenoic acid t-butyl ester (23.8 g, 70.6 mmol) in dry tetrahydrofuran (35 ml) was added dropwise. The reaction was left overnight (below -40 °C), and then poured into iced hydrochloric acid (4.2 eq, final pH =

and evaporated to yield the crude product (31 g) which was filtered through a pad of silica, eluting with hexane/CH_2Cl_2 (1:1), to yield 2-(dibenzylaminomethyl)propenoic acid t-butyl ester (23.8 g, 78%) as a solid, m.p. 62-63 °C. Found: C,78.09; H,8.20; N,4.18. $C_{22}H_{27}NO_2$ requires C,78.3; H,8.06; N,4.15%.

(b) To a stirred solution of diisopropylamine (14.98 g, 20.75 ml, 148 mmole) in dry tetrahydrofuran (250 ml) cooled to -30 °C under nitrogen, was added dropwise, n-butyl lithium (59.3 ml of a 2.5 M solution, 148 mmole), keeping the temperature below -20 °C. The reaction was stirred at -20 °C for 1 hour, then cooled to

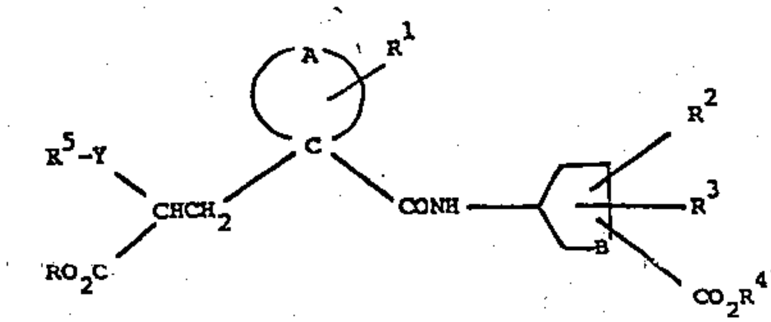


Preparation		Analysis % (Theoretical in brackets) C	H	N
2		60.46 (61.88	9.11 9.05	6.09 6.19)
3		66.34 (66.28	10.02 9.95	5.67 5.95)(1)
4		gum Rf 0.69 (silica; CH_2Cl_2, CH_3OH, CH_3CO_2H, 90:10:1)		
5		67.10 (67.46	10.09 10.06	5.69 5.83)
6		67.07 (67.46	10.06 10.06	5.71 5.83)

(1) 0.25 mole H_2O

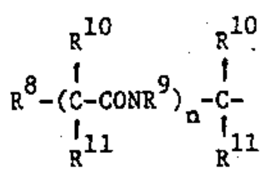
Claims

1. A compound having the formula:

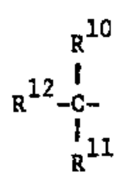


(I)

wherein A completes a 4 to 7 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be fused to a further saturated or unsaturated 5 or 6 membered carbocyclic ring;
B is $(CH_2)_m$ wherein m is an integer of from 1 to 3;
each of R and R^4 is independently H, C_1-C_6 alkyl, benzyl or an alternative biolabile ester-forming group;
R^1 is H or C_1-C_4 alkyl;
R^2 and R^3 are each independently H, OH, C_1-C_6 alkyl or C_1-C_6 alkoxy; or R^2 and R^3 are linked together and are $(CH_2)_r$ wherein r is an integer of from 1 to 4;
Y is an optional alkylene group of from 1 to 6 carbon atoms which may be straight or branched-chain;
and R^5 is R^6CONR^9-, $R^6SO_2NR^9$-, R^6CO_2-, R^6CO-, R^6SO_q-, R^7NR^9CO-, $R^7NR^9SO_2$- or R^7OCO-;
wherein R^6 is a group of the formula:



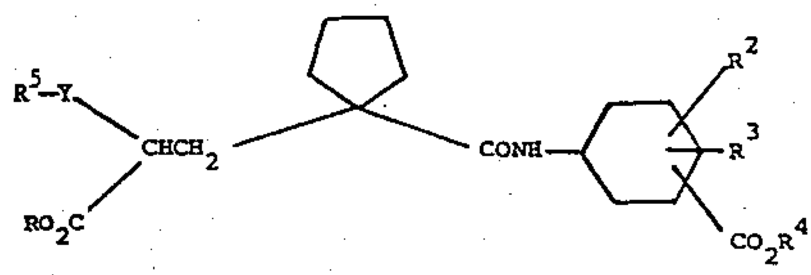
R^7 is a group of the formula:



and R^9 is H, C_1-C_6 alkyl, aryl, C_3-C_7 cycloalkyl, heterocyclyl, aryl(C_1-C_6 alkyl) or heterocyclyl(C_1-C_6 alkyl);
wherein R^8 is R^9CONR^9-, $R^9SO_2NR^9$-, $R^{13}R^{14}N$-$(CH_2)_p$-, or R^9O-, wherein each R^9 is as previously defined above;
R^{10} and R^{11} are each independently H or C_1-C_6 alkyl;
or R^{10} is H and R^{11} is C_1-C_6 alkyl which is substituted by OH, SH, SCH_3, NH_2, aryl(C_1-C_6 alkyl)OCONH-, NH_2CO-, CO_2H, guanidino, aryl, or heterocyclyl; or the two groups R^{10} and R^{11} are joined together to form, with the carbon atom to which they are attached, a 5 or 6 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be substituted by C_1-C_4 alkyl or fused to a further 5 or 6 membered saturated or unsaturated carbocyclic ring;

or R^{10} is H, n is 0 and R^8 and R^{11} are linked to form a 2-(N-COR^9-4-aminopyrrolidinyl) group; R^{12} is $R^{13}R^{14}$NCO-, R^9OCO-, R^9OCH_2- or heterocyclyl, wherein R^9 is as previously defined above; R^{13} and R^{14} are each independently H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, aryl, aryl(C_1-C_6 alkyl), C_2-C_6 alkoxyalkyl, amino(C_1-C_6 alkyl), heterocyclyl or heterocyclyl(C_1-C_6 alkyl); or the two groups R^{13} and R^{14} are taken together to form, with the nitrogen to which they are attached, a pyrrolidinyl, piperidino, morpholino, piperazinyl, N-(C_1-C_4 alkyl)piperazinyl, pyrrolyl, imidazolyl, pyrazolyl or triazolyl group;
n is 0 or 1;
p is 0 or an integer of from 1 to 6;
and q is 0, 1 or 2;
and pharmaceutically acceptable salts thereof and bioprecursors therefor.

2. A compound as claimed in claim 1 wherein A is $(CH_2)_4$, R^1 is H and B is $(CH_2)_2$ having the formula:



(II)

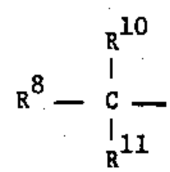
3. A compound as claimed in claim 1 or claim 2 wherein R and R^4 are both H.

4. A compound as claimed in claim 1 or claim 2 wherein one of R and R^4 is H and the other is C_1-C_6 alkyl, benzyl or an alternative biolabile ester-forming group.

5. A compound as claimed in claim 4 wherein said alternative biolabile ester forming group is 1-(2,2-diethylbutyryloxy)ethyl, 2-ethylpropionyloxymethyl, 1-(2-ethylpropionyloxy)ethyl, 1-(2,4-dimethylbenzoyloxy)-ethyl,α-benzoyloxybenzyl, 1-(benzoyloxy)ethyl, 2-methyl-1-propionyloxy-propyl, 2,4,6-trimethylbenzoyloxymethyl, 1-(2,4,6-trimethylbenzoyloxy)ethyl, pivaloyloxymethyl, phenethyl, phenpropyl, 2,2,2-trifluoroethyl, 1- or 2-napthyl, 2,4-dimethylphenyl, 4-t-butylphenyl, 5-(4-methyl-1,3-dioxalynyl-2-onyl)methyl or 5-indanyl.

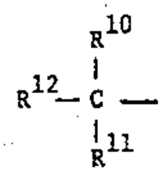
6. A compound as claimed in any previous claim wherein R^5 is R^6CONR^9-, R^7NR^{9}CO-, wherein R^6, R^7 and R^9 are as previously defined.

7. A compound as claimed in claim 6 wherein R^5 is R^6CONR^9-, R^9 is H and R^6 is a group of the formula:



wherein R^8 is (C_1-C_6 alkyl)CONH-, arylCONH or C_1-C_6 alkyl)SO_2NH-, R^{10} is H and R^{11} is C_1-C_4 alkyl, benzyl or amino (C_1-C_6 alkyl).

8. A compound as claimed in claim 6 wherein R^5 is R^7NR^{9}CO-wherein R^8 is H and R^7 is a group of the formula



wherein R^{12} is HO_2C, (C_1-C_6 alkyl)NHCO-, (C_1-C_6 alkyl)$_2$NCO-, arylNHCO- or 1-pyrrolidinoyl, R^{10} is H and R^{11} is benzyl or amino(C_1-C_6 alkyl).

9. A compound is claimed as claim 7 wherein R^5 is N^2-acetyl-L-lysyl-amino, N^2-benzoyl-L-lysyl-amino, N^2-naphthoyl-L-lysyl-amino, or N^2-methanesulphonamido-L-lysyl-amino.

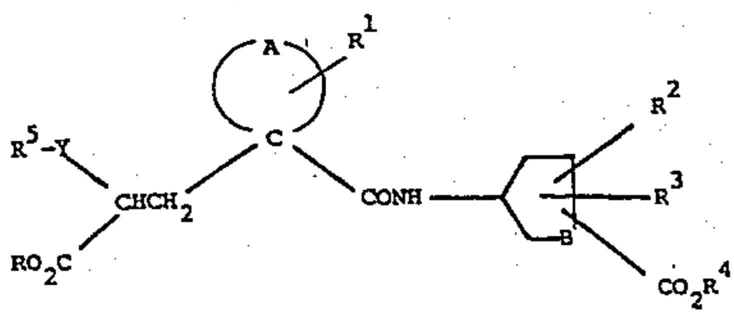
10. A compound as claimed in claim 7 wherein said compound is-:
2-(N^2-acetyl-L-lysylaminomethyl)-3-{1-[(cis-4-carboxy-cyclohexyl)carbamoyl]cyclopentyl}propanoic acid,
2-(N^2-benzoyl-L-lysylaminomethyl)-3-{1-[(cis-4-carboxy-cyclohexyl)carbamoyl]cyclopentyl}propanoic acid,
2-(N^2-naphthoyl-L-lysylaminomethyl)-3-{1-[(cis-4-carboxy-cyclohexyl)carbamoyl]cyclopentyl}propanoic acid,
2-(N^2-acetyl-L-lysylaminomethyl)-3-{1-[cis-4-carboxy-cis-3-butyl-cyclohexyl)carbamoyl]-cyclopentyl}propanoic acid.
2-(N^2-acetyl-L-lysylaminomethyl)-3{1-[cis-4-carboxy-trans-3-butyl-cyclohexyl)carbamoyl]-cyclopentyl}propanoic acid,
2-(N^2-methanesulphonyl-L-lysylaminomethyl)-3{1-[cis-4-carboxy-cis-3-(methylbutyl)-cyclohexyl)carbamoyl]-cyclopentyl} propanoic acid, or
2-(N^2-methanesulphonyl-L-lysylaminomethyl)-3{1-[cis-4-carboxy-cis-3-butyl-cyclohexyl)carbamoyl]-cyclopentyl}propanoic acid.

11. A pharmaceutical composition comprising a compound of the formula (I) or (II) as claimed in any one of claims 1 to 10 or a pharmaceutically acceptable salt thereof or bioprecursor therefor, together with a pharmaceutically acceptable diluent or carrier.

12. A compound of the formula (I) or (II) as claimed in any of claims 1 to 10 or a pharmaceutically acceptable salt thereof or bioprecursor therefor, for use in medicine, particularly for the treatment of hypertension, heart failure or renal insufficiency.

Claims for the following Contracting States: ES, GR

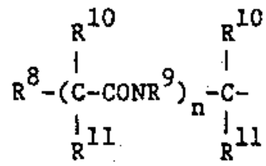
1. A process for preparing a compound having the formula:



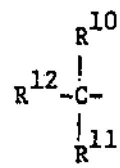
(I)

wherein A completes a 4 to 7 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be fused to a further saturated or unsaturated 5 or 6 membered carbocyclic ring;
B is $(CH_2)_m$ wherein m is an integer of from 1 to 3;
each of R and R^4 is independently H, C_1-C_6 alkyl, benzyl or an alternative biolabile ester-forming group;
R^1 is H or C_1-C_4 alkyl;
R^2 and R^3 are each independently H, OH, C_1-C_6 alkyl or C_1-C_6 alkoxy; or R^2 and R^3 are linked together and

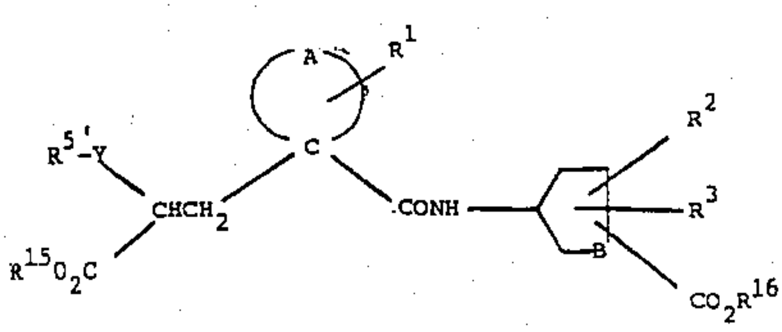
are$(CH_2)_r$ wherein r is an integer of from 1 to 4;
Y is an optional alkylene group of from 1 to 6 carbon atoms which may be straight or branched-chain;
and R^5 is R^6CONR^9-, $R^6SO_2NR^9$-, R^6CO_2-, R^6CO-, R^6SO_q-, R^7NR^9CO-, $R^7NR^9SO_2$- or R^7OCO-;
wherein R^6 is a group of the formula:



R^7 is a group of the formula:



and R^9 is H, C_1-C_6 alkyl, aryl, C_3-C_7 cycloalkyl, heterocyclyl, aryl(C_1-C_6 alkyl) or heterocyclyl(C_1-C_6 alkyl);
wherein R^8 is R^9CONR^9-, $R^9SO_2NR^9$-, $R^{13}R^{14}N$-$(CH_2)_p$-, or R^9O-, wherein each R^9 is as previously defined above;
R^{10} and R^{11} are each independently H or C_1-C_6 alkyl;
or R^{10} is H and R^{11} is C_1-C_6 alkyl which is substituted by OH, SH, SCH_3, NH_2, aryl(C_1-C_6 alkyl)OCONH-, NH_2CO-, CO_2H, guanidino, aryl, or heterocyclyl; or the two groups R^{10} and R^{11} are joined together to form, with the carbon atom to which they are attached, a 5 or 6 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be substituted by C_1-C_4 alkyl or fused to a further 5 or 6 membered saturated or unsaturated carbocyclic ring;
or R^{10} is H, n is O and R^8 and R^{11} are linked to form a 2-(N-COR^9-4-aminopyrrolidinyl) group;
R^{12} is $R^{13}R^{14}NCO$-, R^9OCO-, R^9OCH_2- or heterocyclyl, wherein R^9 is as previously defined above;
R^{13} and R^{14} are each independently H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, aryl, aryl(C_1-C_6 alkyl), C_2-C_6 alkoxyalkyl, amino(C_1-C_6 alkyl), heterocyclyl or heterocyclyl(C_1-C_6 alkyl); or the two groups R^{13} and R^{14} are taken together to form, with the nitrogen to which they are attached, a pyrrolidinyl, piperidino, morpholino, piperazinyl, N-(C_1-C_4 alkyl)piperazinyl, pyrrolyl, imidazolyl, pyrazolyl or triazolyl group;
n is 0 or 1;
p is 0 or an integer of from 1 to 6;
and q is 0, 1 or 2;
which comprises subjecting a compound of the formula:



wherein R^{15} and R^{16} are as previously defined for R and R^4 excluding H, or they are conventional carboxylic acid protecting groups and $R^{5'}$ is as defined for R^5 with any reactive groups therein optionally protected;
to a deprotection and/or hydrolysis and/or hydrogenation or other deprotection reaction to remove and

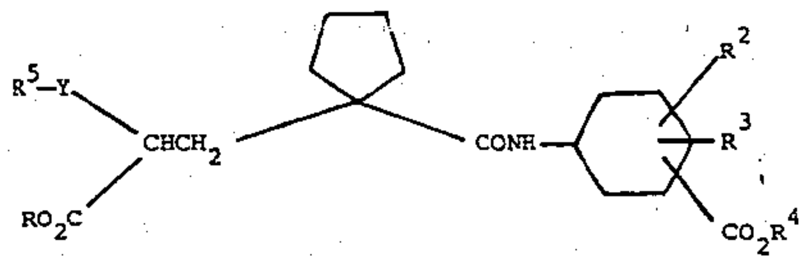
protecting group present in $R^{5'}$ and to remove one or both of R^{15} and R^{16} to yield the corresponding mono-ester or dicarboxylic acid of formula (I) wherein one or both of R and R^4 are hydrogen;
and optionally forming a pharmaceutically acceptable salt of the product.

2. A process as claimed in claim 1 wherein R^{15} is t-butyl and said group is removed by treatment with trifluoroacetic acid.

3. A process as claimed in claim 1 wherein R^{16} is C_1-C_4 alkyl and said group is removed by treatment with aqueous alkali.

4. A process as claimed in claim 1 wherein said protecting group present in $R^{5'}$ is a benzyloxycarbonyl amino-protecting group and said group is removed by catalytic hydrogenation.

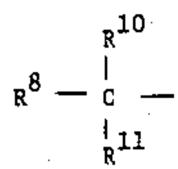
5. A process as claimed in claim 1 wherein said compound is of formula:



wherein R, R^2, R^3, R^4 and R^5 are as previously defined.

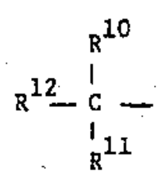
6. A process as claimed in any previous claim wherein R^5 is R^6CONR^9-, or R^7NR^9CO-, wherein R^6, R^7 and R^9 are as previously defined.

7. A process as claimed in claim 6 wherein R^5 is R^6CONR^9-, R^9 is H and R^6 is a group of the formula:



wherein R^8 is (C_1-C_6 alkyl)CONH-, arylCONH-or C_1-C_6 alkyl)SO_2NH-, R^{10} is H and R^{11} is C_1-C_4 alkyl, benzyl or amino(C_1-C_6 alkyl).

8. A process as claimed in claim 6 wherein R^5 is R^7NR^9CO-wherein R^9 is H and R^7 is a group of the formula



wherein R^{12} is HO_2C, (C_1-C_6 alkyl)NHCO-, (C_1-C_6 alkyl)$_2$NCO-, arylNHCO- or 1-pyrrolidinoyl, R^{10} is H and R^{11} is benzyl or amino (C_1-C_6 alkyl).

9. A process as claimed in claim 7 wherein R^5 is N^2-acetyl-L-lysyl-amino, N^2-benzoyl-L-lysyl-amino, N^2-naphthoyl-L-lysyl-amino, or N^2-methanesulphonamido-L-lysyl-amino.

10. A process as claimed in claim 7 wherein said compound is-:
2-(N^2-acetyl-L-lysylaminomethyl)-3-{1-[(cis-4-carboxy-cyclohexyl)carbamoyl]cyclopentyl}propanoic acid,
2-(N^2-benzoyl-L-lysylaminomethyl)-3-{1-[(cis-4-carboxy-cyclohexyl)carbamoyl]cyclopentyl}propanoic acid,
2-(N^2-naphthoyl-L-lysylaminomethyl)-3-{1-[(cis-4-carboxy-cyclohexyl)carbamoyl]cyclopentyl}propanoic acid,
2-(N^2-acetyl-L-lysylaminomethyl)-3-{1-[cis-4-carboxy-cis-3-butyl-cyclohexyl)carbamoyl]-cyclopentyl}propanoic acid.
2-(N^2-acetyl-L-lysylaminomethyl)-3{1-[cis-4-carboxy-trans-3-butyl-cyclohexyl)carbamoyl]-cyclopentyl}propanoic acid,

2-(N^{2}-methanesulphonyl-L-lysylaminomethyl)-3{1-[cis-4-carboxy-cis-3-(3-methylbutyl)-cyclohexyl)-carbamoyl]cyclopentyl} propanoic acid, or
2-(N^{2}-methanesulphonyl-L-lysylaminomethyl)-3{1-[cis-4-carboxy-cis-3-butyl-cyclohexyl)carbamoyl]-cyclopentyl}propanoic acid.

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(19)

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(12) **EUROPEAN PATENT APPLICATION**

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(22) Date of filing: 23.05.89

(51) Int. Cl.5: C07C 103/737, A61K 31/215, C07K 5/06, C07D 213/82, A61K 31/455, C07D 295/18, A61K 31/40, C07D 207/14

(30) Priority: 27.05.88 GB 8812597

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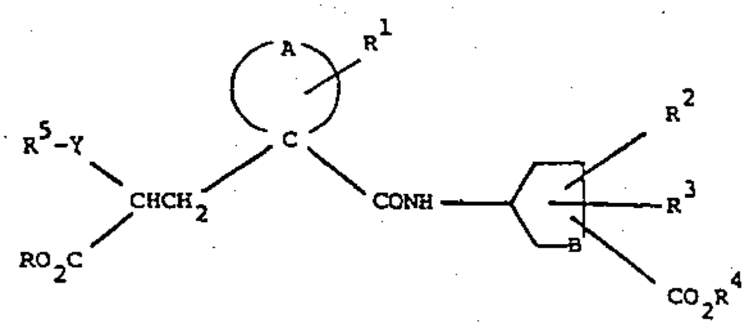
(54) **Cycloalkyl-substituted glutaramide diuretic agents.**

(57) Compounds having the formula:



(I)

wherein A completes a 4 to 7 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be fused to a further carbocyclic ring; B is $(CH_2)_m$ wherein m is 1 to 3; R and R^4 are H,

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C_1-C_6 alkyl, benzyl or biolabile ester-forming groups; R^1 is H or C_1-C_4 alkyl; R^2 and R^3 are each H, OH, C_1-C_6 alkyl or C_1-C_6 alkoxy, or are linked together and are $(CH_2)_r$ wherein r is 1 to 4; Y is an optional alkylene group of from 1 to 6 carbon atoms which may be straight or branched-chain;
and R^5 is R^6CONR^9-, $R^6SO_2NR^9$-, R^6CO_2-, R^6CO-, R^6SO_q-, R^7NR^9CO-, $R^7NR^9SO_2$- or R^7OCO-; wherein R^6 is a group of the formula $R^8(R^{10}R^{11}C\text{-}CONR^9)_nR^{10}R^{11}C$-; R^7 is a group of the formula $R^{10}R^{11}R^{12}C$- and R^9 is H, C_1-C_6 alkyl, aryl, C_3-C_7 cycloalkyl, heterocyclyl, aryl(C_1-C_6 alkyl) or heterocyclyl(C_1-C_6 alkyl); wherein R^8 is R^9CONR^9-, $R^9SO_2NR^9$-, $R^{13}R^{14}N\text{-}(CH_2)_p$-, or R^9O-, R^{10} and R^{11} are H or C_1-C_6 alkyl; or R^{10} is H and R^{11} is C_1-C_6 alkyl which is substituted by OH, SH, SCH_3, NH_2, aryl(C_1-C_6 alkyl)OCONH-, NH_2CO-, CO_2H, guanidino, aryl, or heterocyclyl; or the two groups R^{10} and R^{11} are joined to form a five or 6 membered carbocyclic ring which may be saturated, mono-unsaturated, optionally substituted by C_1-C_4 alkyl or fused to a further carbocylic ring; or R^8 and R^{11} are linked to form a 2-(N-COR^9-4-aminopyrrolidinyl) group; R^{12} is $R^{13}R^{14}NCO$-, R^9OCO-, R^9OCH_2- or heterocyclyl, R^{13} and R^{14} are H, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, aryl, aryl(C_1-C_6 alkyl), C_2-C_6 alkoxyalkyl, amino (C_1-C_6 alkyl), heterocyclyl or heterocyclyl(C_1-C_6alkyl); or the two groups R^{13} and R^{14} form a pyrrolidinyl, piperidino, morpholino, piperazinyl, N-(C_1-C_4 alkyl) piperazinyl, pyrrolyl, imidazolyl, pyrazolyl or triazolyl group; n is 0 or 1; p is 0 or 1 to 6; and q is 0, 1 or 2; and pharmaceutically acceptable salts thereof and bioprecursors therefor, are diuretic agents of value in the treatment of hypertension, heart failure and renal insufficiency.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 30 5180

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	US-B-3 862 57 (H.E. ALBURN) * Claim 1 * - - -	1	C 07 C 103/737 A 61 K 31/215 C 07 K 5/06 C 07 D 213/82 A 61 K 31/455 C 07 D 295/18 A 61 K 31/40 C 07 D 207/14
P,X	EP-A-0 274 234 (PFIZER LTD) * Claims * - - - - -	1-9,11,12	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 07 C 103/00 C 07 D 295/00

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	08 November 90	SANCHEZ Y GARCIA J.M

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same category
A: technological background
O: non-written disclosure
P: intermediate document
T: theory or principle underlying the invention

E: earlier patent document, but published on, or after the filing date
D: document cited in the application
L: document cited for other reasons

&: member of the same patent family, corresponding document

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European Patent Office
Office européen des brevets

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EUROPEAN PATENT APPLICATION

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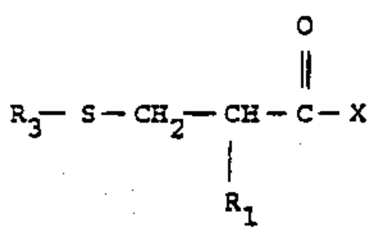
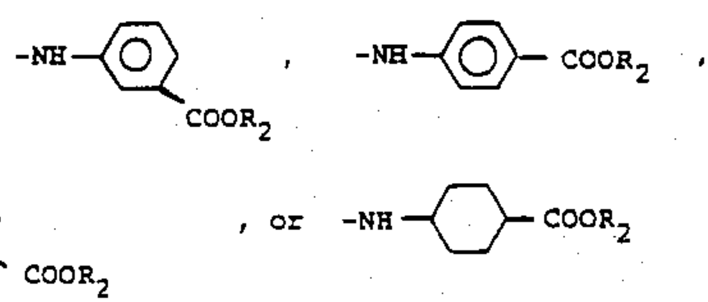
(54) **Aminobenzoic and aminocyclohexane-carboylic acid compounds, compositions, and their method of use.**

(57) Compounds of the formula



wherein X is



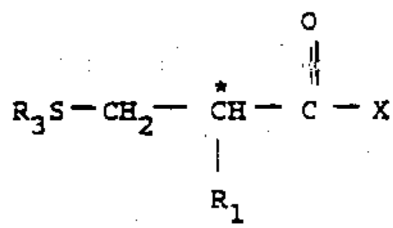
inhibit the action of neutral endopeptidase. As a result, such compounds produce diuresis, natriuresis, and lower blood pressure as well as being useful in the treatment of congestive heart failure, relieving pain, and diarrhea when administered to a mammalian host.

EP 0 361 365 A1

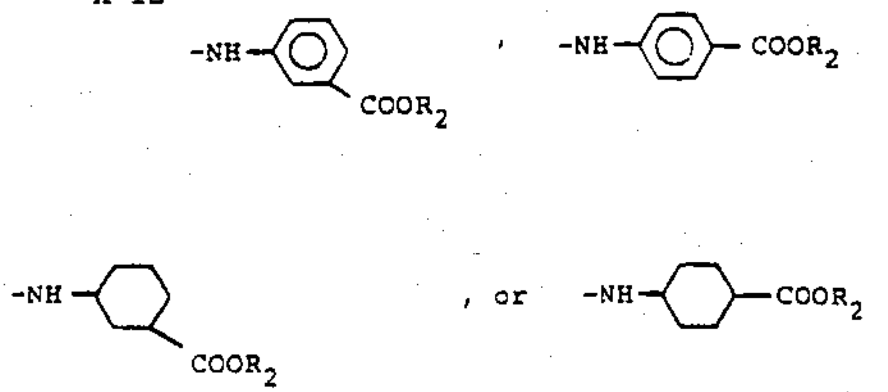
AMINOBENZOIC AND AMINOCYCLOHEXANECARBOYLIC ACID COMPOUNDS, COMPOSITIONS, AND THEIR METHOD OF USE

This invention is directed to reducing blood pressure and producing diuresis and natriuresis, as well as treating congestive heart failure, pain, and/or diarrhea by administering a pharmaceutical composition containing a neutral endopeptidase inhibitor of formula I and salts thereof

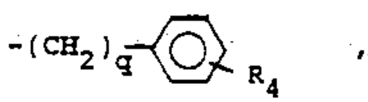
(I)



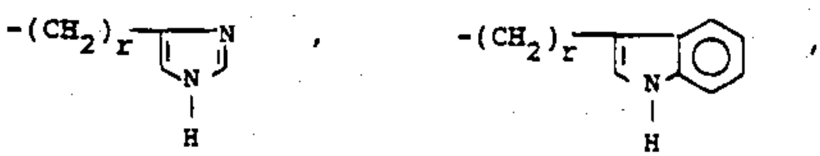
X is



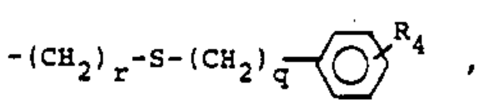
R_1 is hydrogen, lower alkyl, halo substituted lower alkyl,



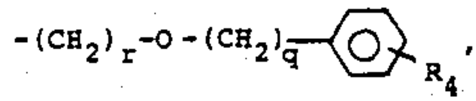
$-(CH_2)_r$-cycloalkyl, $-(CH_2)_r$-(α-napthyl), $-(CH_2)_r$-(β-naphthyl),

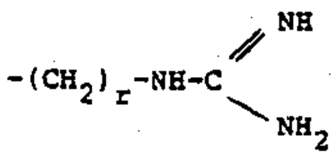


$-(CH_2)_r-NH_2$, $-(CH_2)_r-SH$, $-(CH_2)_r$-S-lower alkyl, $-(CH_2)_r-OH$,



$-(CH_2)_r$-O-lower alkyl,

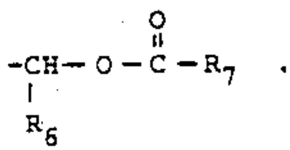




or

$-(CH_2)_r-\overset{O}{\overset{\|}{C}}-NH_2$.

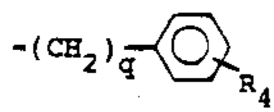
R_2 is hydrogen, lower alkyl, benzyl, benzhydryl, a salt forming ion, or



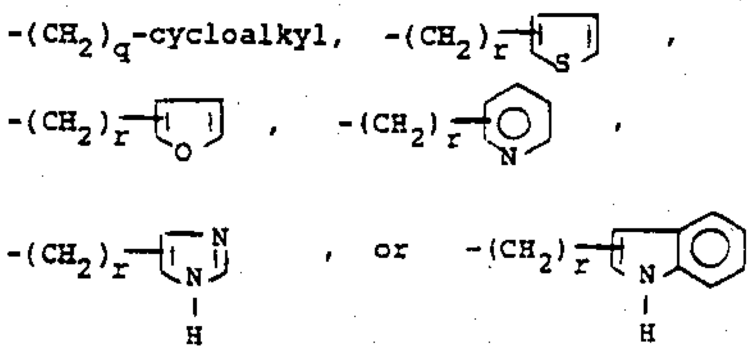
R_3 is hydrogen

or $R_5-\overset{O}{\overset{\|}{C}}-$.

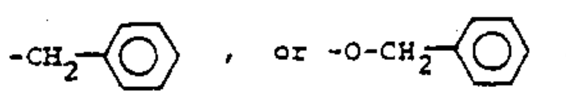
R_5 is lower alkyl,



$-(CH_2)_q-$(α-naphthyl), $-(CH_2)_q-$(β-naphthyl),



R_4 is hydrogen, lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4 carbons, lower alkylthio of 1 to 4 carbons, halo, hydroxy, CF_3, phenyl,



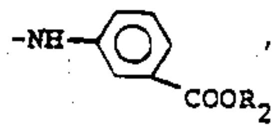
R_6 is hydrogen, lower alkyl, cycloalkyl, or phenyl.

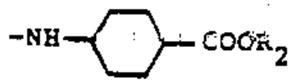
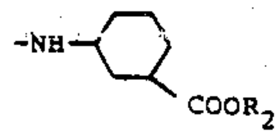
R_7 is hydrogen, lower alkyl, lower alkoxy, or phenyl.

r is an integer from 1 to 4.

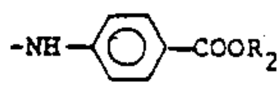
q is zero or an integer from 1 to 7.

This invention is also directed to the novel compounds of formula I wherein X is



 , or 

and R_1, R_2 and R_3 are as defined above and the novel compounds of formula I wherein X is



and R_1, R_2, and R_3 are as defined above except that R_1 is not methyl.

This invention in its broadest aspects relates to the method of lowering blood pressure and producing diuresis and natriuresis by administering a pharmaceutical composition containing a neutral endopeptidase inhibitor of formula I. This invention is also directed to the novel compounds of formula I wherein R_1 is other than methyl or when R_1 is methyl, X is 3-aminobenzoic acid, 3- or 4-aminocyclohexanecarboxylic acid.

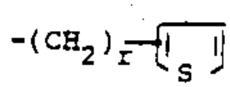
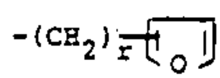
The term lower alkyl used in defining various symbols refers to straight or branched chain radicals having up to seven carbons. The preferred lower alkyl groups are straight or branched chain of up to four carbons. Similarly the terms lower alkoxy and lower alkylthio refer to such lower alkyl groups attached to an oxygen or sulfur.

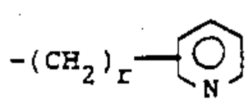
The term cycloalkyl refers to saturated rings of 4 to 7 carbons atoms with cyclopentyl and cyclohexyl being most preferred.

The term halogen refers to chloro, bromo , fluoro, and iodo.

The term halo substituted lower alkyl refers to such lower alkyl groups described above in which one or more hydrogens have been replaced by chloro, bromo or fluoro groups such as trifluoromethyl, which is preferred, pentafluoroethyl, 2,2,2-trichloroethyl, chloromethyl, bromomethyl, etc.

The symbols

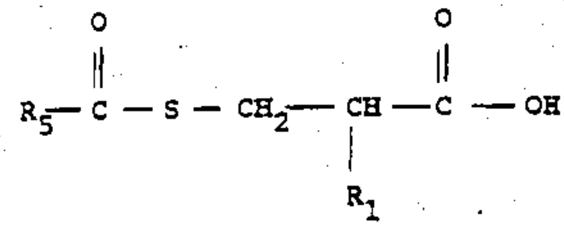
 ,  ,

 ,

etc., represent that the alkylene bridge is attached to an available carbon atom.

The compounds of formula I can be prepared by coupling an acylthio carboxylic acid of the formula

(II)



to the aminobenzoic acid ester or aminocyclohexanecarboxylic acid ester of the formula (III) HX .

The aminobenzoic acid ester or aminocyclohexanecarboxylic acid ester of formula III can be employed as the hydrochloride salt and methyl is the preferred ester group. The acylthio carboxylic acid of formula II is preferably converted to an activated form such as an acid chloride, mixed anhydride etc. The reaction is preferably carried out in the presence of diisopropylethylamine.

The resulting acylthio aminobenzoic or aminocyclohexanecarboxylic acid ester can be hydrolyzed by treating with a base such as sodium hydroxide to remove the acyl group and the R_2 ester group and yield the desired mercaptan products of formula I, i.e., R_2 and R_3 are both hydrogen.

Alternatively, the aminobenzoic or aminocyclohexanecarboxylic acid of formula III, i.e., R_2 is hydrogen, can be coupled directly to the activated form of the carboxylic acid of formula II by first treating the aminobenzoic or aminocyclohexanecarboxylic acid with bis(trimethylsilyl) trifluoroacetamide. The resulting acylthio aminobenzoic or aminocyclohexanecarboxylic acid can be treated with ammonia to remove the acyl group and yield the desired mercaptan products of formula I, i.e., R_3 is hydrogen.

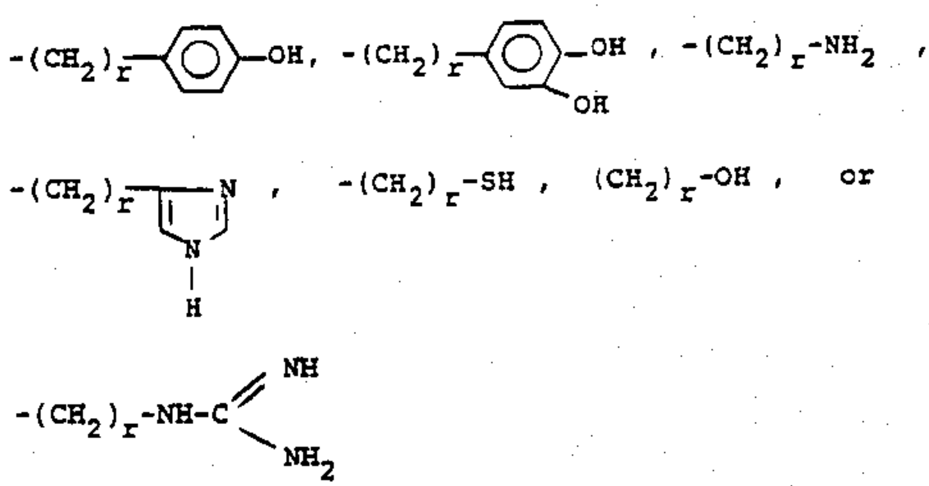
Of course, the mercaptan products of formula I can be acylated with an acid chloride of the formula

(IV) $R_5-\overset{O}{\overset{\|}{C}}-Cl$

to introduce other acyl groups.

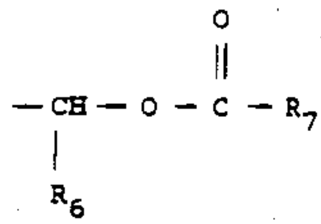
The acylthio carboxylic acids of formula II are described in various literature and patent references. For example, the carboxylic acids wherein R_1 is hydrogen, lower alkyl, phenyl, or phenyl-lower alkyl are described by Ondetti et al. in U.S. Patent 4,105,776, the carboxylic acids wherein R_1 is alkylthioalkylene are described by Ondetti et al. in U.S. Patent 4,116,962, the carboxylic acids wherein R_1 is carbamoylalkylene are described by Ondetti in U.S. Patent 4,091,024, the carboxylic acids wherein R_1 is aminoalkylene or guanidinylalkylene are described by Ondetti et al. in U.S. Patent 4,113,715, the carboxylic acids wherein R_1 is trifluoromethyl are described by Ondetti et al. in U.S. Patent 4,154,935, etc.

In the above reactions if R_1 and/or R_5 is



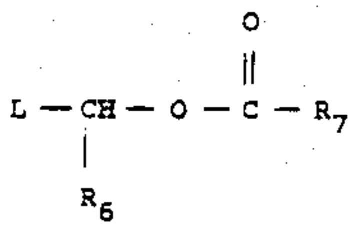
then the hydroxyl, amino imidazolyl, mercaptan, or guanidinyl function should be protected during the coupling reaction. Suitable protecting groups include benzyloxycarbonyl, t-butoxycarbonyl, benzyl, benzhydryl, trityl, etc., and nitro in the case of guanidinyl. The protecting group is removed by treatment with acid or other known methods following completion of the reaction.

The ester products of formula I wherein R_2 is



can be obtained by treating the product of formula I wherein R_2 is hydrogen with a molar equivalent of a compound of the formula

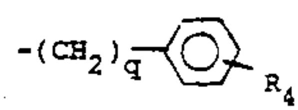
(V)



wherein L is a leaving group such as chlorine, bromine, toluenesulfonyloxy, etc., in the presence of base.

Preferred compounds of this invention are those of formula I wherein

R_1 is straight or branched chain alkyl of 2 to 4 carbons,



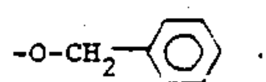
wherein q is zero or an integer from 1 to 4, or trifluoromethyl.

R_2 is hydrogen or an alkali metal salt ion.

R_3 is hydrogen or

$R_5-\overset{O}{\overset{\|}{C}}-$, especially hydrogen.

R_4 is hydrogen or



R_5 is methyl or phenyl, especially methyl.

Also, preferred as intermediates are the compounds of formula I wherein

R_1 is as defined above.

R_2 is methyl

R_3 is

$R_5-\overset{O}{\overset{\|}{C}}-$.

R_5 is methyl or phenyl, especially methyl.

R_4 is as defined above.

The compounds of formula I wherein R_2 is hydrogen form salts with a variety of inorganic or organic bases. The nontoxic, pharmaceutically acceptable salts are preferred, although other salts are also useful in isolating or purifying the product. Such pharmaceutically acceptable salts include alkali metal salts such as sodium, potassium or lithium, alkaline earth metal salts such as calcium or magnesium, and salts derived from amino acids such as arginine, lysine, etc. The salts are obtained by reacting the acid form of the compound with an equivalent of the base supplying the desired ion in a medium in which the salt precipitates or in aqueous medium and then lyophilizing.

As shown above, the compounds of formula I wherein R_1 is other than hydrogen contain asymmetric centers as represented by the * in formula I. An additional asymmetric center is present in the ester products when R_6 is other than hydrogen. Thus, the compounds of formula I can exist in diastereomeric forms or in mixtures thereof. The above described processes can utilize racemates, enantiomers or diastereomers as starting materials. When diastereomeric products are prepared, they can be separated by conventional chromatographic or fractional crystallization methods.

Human as well as other mammalian atria contain specific granules which have been found to contain a precursor to a family of peptides collectively called atrial natriuretic factor (deBold, Science, Vol. 230, p. 767-770, 1985). The biologically active segments of this precursor which circulate in the blood are 21-28 amino acid peptides called atrial natriuretic peptides. These peptides cause diuresis, natriuresis, and relaxation of smooth muscle in blood vessels and other tissues (Needleman et al., Hypertension, Vol. 7, p. 469 - 482, 1985). The putative circulating hormone in man is a 28 amino acid peptide called human ANF 99 - 126. Exogeneous administration of this peptide to man has been reported to cause diuresis, natriuresis, and a fall in blood pressure(Richards et al., Hypertension, Vol. 7, p. 812 - 817, 1985).

The compounds of formula I inhibit the activity of neutral endopeptidase (EC 3.4.24.11), a membrane-bound zinc metallopeptidase found in many tissues including the brain and kidney. Neutral endopeptidase hydrolyzes peptide bonds which are on the amino terminal side of hydrophobic amino acid residues. Atrial natriuretic peptides have been shown to be cleaved at the Cys^{105}-Phe^{106} bond by the action of neutral endopeptidase (Delaney et al., Fed. Proc. 46, p. 1296, 1987; Stephenson et al. Biochem. J., Vol. 243, p. 183 - 187, 1987). Cleavage of rat ANF 103 - 126 at Cys^{105}-Phe^{106} results in diminishing of its vasorelaxant (Bergey et al. Fed. Proc.46, p. 1296, 1987) and natriuretic, diuretic and depressor activities (Seymour et al., Fed.Proc.46, p. 1296, 1987). Stephensen et al. reported that the hydrolysis of human ANF 99 - 126 by pig kidney microvillar membranes <u>in vitro</u> was suppressed by the neutral endopeptidase inhibitor, phosphoramidon.

While not limiting the scope of this invention to a specific theory or mechanism of action, inhibition of neutral endopeptidase is believed to result in reduced inactivation of exogenously administered or endogenous atrial natriuretic peptides. Thus the compounds of formula I are useful in the treatment of hypertension, congestive heart failure, renal failure or hepatic cirrhosis. Diuresis, natriuresis, and blood pressure reduction are produced in a mammalian host such as man by the administration of from about 1 mg. to about 100 mg. per kg. of body weight per day, preferably from about 1 mg to about 50 mg. per kg. of body weight per day, of one or more neutral endopeptidase inhibitors of formula I or a pharmaceutically acceptable salt thereof. The neutral endopeptidase inhibitors of formula I are preferably administered orally, but parenteral routes such as subcutaneous, intramuscular, and intravenous can also be employed. The daily dose can be administered singly or can be divided into two to four doses administered throughout the day.

The neutral endopeptidase inhibitors of formula I can also be administered in combination with other blood pressure lowering agents. For example, the neutral endopeptidase inhibitors of formula I can be combined for dual administration with an angiotensin converting enzyme (ACE) inhibitor such as captopril, zofenopril, fosinopril, enalapril, lisinopril, etc. Such combination would be at a weight ratio of endopeptidase inhibitor to ACE inhibitor of from about 1:10 to about 10:1.

The neutral endopeptidase inhibitors of formula I can also be administered in combination with human ANF 99 - 126. Such combination would contain the inhibitor of formula I at from about 1 to about 100 mg. per kg. of body weight and the human ANF 99 - 126 at from about 0.001 to about 0.1 mg. per kg. of body weight.

The neutral endopeptidase inhibitors of formula I or pharmaceutically acceptable salts thereof can also be administered to a mammalian host such as man to inhibit the degradation of endogenous opioid pentapeptides, [Met^5]-enkephalin (Try-Gly-Gly-Phe-Met) and [Leu^5]-enkephalin (Try-Gly-Gly-Phe-Leu), in the brain or in peripheral tissues. Due to its role in the degradation of enkephalinase, brain endopeptidase has often been referred to as "enkephalinase." Enkephalins are neurotransmitters which decrease the perception of pain (Hughes, et al., Nature, Vol. 258, December 1975, p. 577 - 579). These endogenous opioid peptides are functionally inactivated by cleavage of their Gly^3-Phe^4 peptide bonds by neutral endopeptidase located at nerve terminals in the brain where enkephalins are released (Malfroy, et al., Nature, Vol. 276, November 1978, p. 523 - 526). Neutral endopeptidase inhibitors enhance the recovery of endogenous enkephalins released from isolated brain slices (Patey, et al., Science, Vol. 212, June 1981, p. 1153 - 1155) and cause analgesia in mice that is reversed by the opiate antagonist naloxone (Roques, et al., Nature, Vol. 288, November 1980, p. 286 - 288). Inhibitors of neutral endopeptidase also show naloxone -reversible antidiarrheal effects in rats (Marcais - Collado, et al., European Journal of Pharmacology, Vol. 144, p. 125 - 132, 1987).

Thus, the compounds of formula I or a pharmaceutically acceptable salt thereof can be administered as an analgesic or antidiarrheal agent to patients orally or parenterally in an effective amount within the daily dosage range of from about 0.1 to about 25 mg. of compound per kg. of patient body weight. Administration can be once daily or in 2 to 4 divided daily doses.

The inhibitors of formula I and other pharmaceutically acceptable ingredients can be formulated for the above described pharmaceutical uses. Suitable compositions for oral administration include tablets, capsules, and elixirs, and suitable compositions for parenteral administration include sterile solutions and suspensions. About 10 to 500 mg. of active ingredient is compounded with physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavoring, etc., in a unit dose form as called for by accepted pharmaceutical practice.

The following examples are illustrative of the invention. Temperatures are given in degrees centigrade.

Example 1

4-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid

a) 4-Aminobenzoic acid, methyl ester, hydrochloride

Thionyl chloride (5.84 ml., 80 mmole) was added, dropwise with stirring, to a cold suspension of 4-aminobenzoic acid (5.49 g., 40 mmole) in methanol (100 ml.). The addition was done at a rate so as to maintain the temperature between -5° and -10°. After the addition was completed, the mixture was allowed to warm to room temperature and stirred overnight. The mixture was then concentrated *in vacuo* to give a white solid which was twice triturated in ether to yield 7.25 g. of 4-aminobenzoic acid, methyl ester, hydrochloride as a white solid; m.p. 189 - 192°. TLC (silica gel; n-butanol:acetic acid:water, 4:1:1) R_f = 0.77.

b) 3-Acetylthio-2-benzylpropanoic acid

Benzyl malonic acid (13 g., 67 mmole) was mixed with 40% aqueous dimethylamine (7.6 g., 68 mmole) and 37% formalin (5.4 g., 68 mmole) in water (150 ml.). The voluminous solid that formed in 15 minutes was filtered after 2 hours, washed with water, and dried partially in air to give 20.8 g. of solid. The solid was melted in an oil bath (170°) and heated for 10 minutes until amine evolution stopped and bubbling had virtually ceased. The cooled product, a mobile liquid, was acidified with 10% potassium bisulfate, extracted with hexane, dried (Na_2SO_4), and evaporated to give 6.3 g. of solid. The aqueous filtrates were allowed to stand overnight and were then heated at 100° on a steam cone until bubbling ceased (2 hours). Cooling, acidification, and extraction gave another 1.2 g. of solid for a total of 7.5 g. of benzylacrylic acid.

A solution of benzylacrylic acid (5.96 g., 40 mmole) in thiolacetic acid (10 ml.) was stirred for 1 hour at room temperature and then heated on a steam bath for one hour. The thiolacetic acid was removed by vacuum distillation, and the resulting dark yellow oil was poured into saturated sodium bicarbonate (much bubbling) and extracted with ethyl acetate (2 x 40 ml.). The aqueous portion was acidified to a pH of about 3 with 10% potassium bisulfate, and reextracted with ethyl acetate (3 x 40 ml.). These extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo* to give 6.44 g. of yellow oil 3-acetylthio-2-benzylpropanoic acid.

c) 4-[[2-[(Acetylthio)methyl]-1-oxo-3-phenylpropyl]amino]benzoic acid, methyl ester

Oxalyl chloride (0.68 ml., 7.8 mmole) was added to a solution of 3-acetylthio-2-benzylpropanoic acid

(1.79 g., 7.5 mmole) in ether (15 ml.). This mixture was cautiously treated with a catalytic amount (2 drops) of dimethyl formamide, and then stirred at room temperature for 1 hour. The mixture was concentrated in vacuo, producing an oil which was dissolved in tetrahydrofuran (15 ml.) and again concentrated in vacuo. The resulting residue was disssolved in methylene chloride (20 ml.) and added dropwise over 10 minutes to a cold (-5°), stirred suspension of 4-aminobenzoic acid, methyl ester, hydrochloride (1.52 g., 8.1 mmole) and diisopropylethylamine (2.94 ml., 16.9 mmole) in dichloromethane (20 ml.). After stirring in the cold (-5° C) for 2.5 hours, the mixture was allowed to warm to room temperature and allowed to stir overnight. The mixture was concentrated in vacuo and the residue was taken up into ethyl acetate (100 ml.) and filtered to remove diisopropylethylamine, hydrochloride. The filtrate was washed sequentially with 10% potassium bisulfate, water, 5% sodium bicarbonate, water, and 50% brine (3 x 30 ml. each). The organic layer was dried (Na_2SO_4) and concentrated to yield 3.0 g. of a yellow oil. This oil was applied to a column of 300 g. of Merck silica gel (230 - 400 mesh) and eluted with hexane/ethyl acetate (2:1) to give 1.13 g. of 4-[[2-[(acetylthio)methyl]-1-oxo-3-phenylpropyl]amino]benzoic acid, methyl ester as an off-white solid; m.p. 97 - 99° (sinters at greater than 92°). TLC (silica gel; ethyl acetate:hexane, 1:1) R_f = 0.49.

d) 4-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid

The methyl ester product from part (c) (1.13 g., 3.04 mmole) was dissolved in methanol (20 ml.) and then chilled in an ice bath under nitrogen. 1N Sodium hydroxide (9.5 ml., 3 equiv.) was added dropwise to this solution over 10 minutes. The mixture was stirred at 0° for 10 minutes and then allowed to warm to room temperature and stirred for 3 hours. The mixture was concentrated in vacuo to remove the methanol. The residue (a white suspension) was diluted with water (40 ml.) and extracted with chloroform (2 x 15 ml.). The organic layer was concentrated in vacuo and the residue was taken up in 1N sodium hydroxide (40 ml.) and extracted with chloroform (2 x 15 ml.). Both aqueous extracts were combined and acidified to a pH of about 1.5 with concentrated HCl. The resulting white suspension was extracted with ethyl acetate (3 x 40 ml.). These extracts were combined, washed with water and brine (3 x 40 ml. each), dried (Na_2SO_4), and concentrated to give 910 mg. of an off-white solid. Recrystallization from chloroform yields a solid that was dissolved in methanol and filtered through a cellulose micro-filter to yield 580 mg. of 4-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid as an off-white solid; m.p. 178-179°. TLC (silica gel; benzene:acetic acid, 4:1) R_f = 0.43 (trace at 0.36).

Anal. calc'd. for $C_{17}H_{17}NO_3S$:				
	C, 64.74;	H, 5.43;	N, 4.44;	S, 10.17
Found:	C, 64.65;	H, 5.50;	N, 4.47;	S, 10.17.

Example 2

(S)-4-[(3-Mercapto-2-methyl-1-oxopropyl)amino]benzoic acid

a) (S)-4-[[3-Acetylthio)-2-methyl-1-oxopropyl]amino]benzoic acid, methyl ester

A suspension of 4-aminobenzoic acid, methyl ester, hydrochloride (6.75 g., 36.0 mmole) and diisopropylethylamine (9.5 g., 73.5 mmole) in dry methylene chloride (80 ml.) was cooled to -10° under nitrogen and treated dropwise with a solution of (D)-3-(acetylthio)-2-methylpropanoyl chloride (6.0 g., 32.7 mmole) in dry methylene chloride (80 ml.). After the addition was completed, the mixture was stirred cold for an additional 2.5 hours and then allowed to warm to ambient temperature overnight. The reaction mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate (300 ml.) and the solution was washed with 50 ml. portions of 10% aqueous potassium bisulfate, water, 5% aqueous sodium bicarbonate, water, and brine, dried ($MgSO_4$), and concentrated in vacuo to give 9.7 g. of crude material as a white solid. Flash chromatography on Merck 9385 silica gel (970 g.) eluting with 3:1 hexanes:ethyl acetate gave 6.87 g. of (S)-4-[[(3-acetylthio)-2-methyl-1-oxopropyl]amino]benzoic acid, methyl ester as a white solid; m.p. 140 - 141°. TLC (silica gel; hexanes:ethyl acetate, 3:1) R_f = 0.14.

Anal. calc'd. for $C_{14}H_{17}NO_4S$:				
	C, 56.93;	H, 5.80;	N, 4.74;	S, 10.86
Found:	C, 56.96;	H, 5.81;	N, 4.74;	S, 10.98.

b) (S)-4-[(3-Mercapto-2-methyl-1-oxopropyl)amino]benzoic acid

A solution of the methyl ester product from part (a) (6.85 g., 23.2 mmole) in methanol (160 ml.) was cooled in an ice bath under nitrogen and treated dropwise with 1N sodium hydroxide solution (69.6 ml., 69.6 mmole). After the addition was completed, the reaction mixture was stirred cold for 15 minutes and then allowed to warm to ambient temperature overnight. The reaction mixture was concentrated in vacuo to remove the methanol. The aqueous layer remaining was diluted with water (200 ml.) and washed with chloroform (2 x 70 ml.). The aqueous layer was acidified to pH 1 with concentrated HCl and extracted with 3 x 200 ml. of ethyl acetate. The combined organic extract was washed with 70 ml. of water and brine, dried ($MgSO_4$) and concentrated in vacuo to yield 5.15 g. of crude material. Flash chromatography in 2 batches on a column of Merck 9385 silica gel (300 g.) eluting with dichloromethane:methanol:acetic acid, 60:1:1 gave 2 g. of (S)-4-[(3-mercapto-2-methyl-1-oxopropyl)amino]benzoic acid as a white solid; m.p. 222 - 224°; $[\alpha]_D$ = -90.2° (c = 0.5, methanol). TLC (silica gel; benzene:acetic acid, 4:1) R_f = 0.33 (minor impurity at 0.24).

Anal. calc'd. for $C_{11}H_{13}NO_3S$:					
	C, 55.21;	H, 5.48;	N, 5.85;	S, 13.40;	SH, 13.82
Found:	C, 54.99;	H, 5.56;	N, 5.75;	S, 13.01;	SH, 13.52.

Example 3

4-[[2-(Mercaptomethyl)-1-oxopentyl]amino]benzoic acid

a) 2-Propyl-3-acetylthiopropionic acid

A solution of potassium hydroxide pellets (11.22 g., 200 mmole) in absolute ethanol (120 ml.) was added to a stirred solution of diethyl propylmalonate (22.22 g., 100 mmole) in absolute ethanol (60 ml.) over a period of 30 minutes. After the mixture stood overnight at room temperature, the crystalline precipitate was filtered off. This material (10 g., 43 mmole) was dissolved in water (35 ml.) and a solution of potassium hydroxide pellets (2.6 g., 64 mmole) in water (50 ml.) was added and the reaction mixture was refluxed for 2 hours. The mixture was cooled to 5°, concentrated HCl (8.9 ml., 107 mmole) was added over 20 minutes while keeping the temperature below 10°, and the mixture was extracted with ether (3 x 75 ml.). The combined ether extracts were washed with saturated sodium chloride solution, dried ($MgSO_4$), and concentrated under reduced pressure to give 6.05 g. of n-propyl malonic acid, m.p. 94 - 95°.

n-Propyl malonic acid (3.0 g., 20 mmole) was dissolved in water (15 ml.) and neutralized by the slow addition of 25% aqueous dimethylamine. An additional amount of n-propyl malonic acid (3.0 g., 20 mmole) was added with stirring and the resulting solution was cooled to 0°. Aqueous formaldehyde (4.16 ml.of 33% solution) was added with cooling and stirring. After standing overnight the solution precipitated 3.42 g. of crystalline propyl (dimethylaminomethyl) malonic acid; m.p. 119 - 120°.

The propyl (dimethylaminomethyl) malonic acid (3.42 g., 17 mmole) was suspended in water (10 ml.) and the solution was neutralized by the addition of 10% aqueous sodium hydroxide. The resulting solution was refluxed overnight under a nitrogen atmosphere, then cooled and acidified with concentrated HCl (10 ml.). The mixture was extracted with ether (3 x 50 ml.), and the combined extracts were washed with water (1 x 20 ml.) and extracted with saturated sodium bicarbonate (3 x 30 ml.). The combined sodium bicarbonate extracts were acidified with concentrated HCl and extracted with ether (3 x 50 ml.). The ether extracts were washed with saturated sodium chloride (2 x 20 ml.), dried (Na_2SO_4), and concentrated under reduced pressure to give 1.55 g. of propylacrylic acid as an oily crude product.

The crude propylacrylic acid (1.55 g., 13 mmole) and thiolacetic acid (1.42 g., 18 mmole) with the addition of a few crystals of 2,2′-azobis[2-methylpropanenitrile] were refluxed for 4 hours and allowed to stand at room temperature for 20 hours. The reaction mixture was concentrated under reduced pressure, and residual thiolacetic acid was chased with toluene to give 1.51 g. of 2-propyl-3-acetylthiopropionic acid.

b) 4-[[2-[(Acetylthio)methyl]-1-oxopentyl]amino]benzoic acid, methyl ester

2-Propyl-3-acetylthiopropionic acid (1.91 g., 10 mmole) was dissolved in freshly distilled ether (20 ml.) and cooled to -5°. Oxalyl chloride (0.88 ml., 10 mmole) was added dropwise followed by N,N-dimethylformamide (3 drops). The ice bath was removed and the reaction was allowed to warm to room temperature as gas evolved. After stirring at room temperature for 3 hours, the reaction was a clear yellow solution with a small amount of a gummy yellow precipitate. The solvent was removed in vacuo and the residue was chased with tetrahydrofuran. The resulting yellow oil was dissolved in dichloromethane (10 ml.), cooled to 0°, and added to a solution of 4-aminobenzoic acid, methyl ester (1.52 g., 10 mmole) and diisopropylethylamine (1.75 ml., 10 mmole) in dry dichloromethane (30 ml.) at 0°. After stirring at 0° (10 minutes) and at room temperature (17 hours), the reaction was washed with saturated sodium bicarbonate, HCl (1 M), and saturated sodium chloride. The organic phase was dried ($MgSO_4$), filtered, and the solvent removed in vacuo to give 2.56 g. of the desired product as a yellow solid. Recrystallization from dichloromethane and hexane provides 2.2 g. of 4-[[2-[(acetylthio)methyl]-1-oxopentyl]amino]benzoic acid, methyl ester as white needles; m.p. 102.5 - 103.5°.

c) 4-[[2-(Mercaptomethyl)-1-oxopentyl]amino]benzoic acid

Sodium hydroxide (1M, 25 ml., 25 mmole) and methanol (25 ml.) were degassed with argon and added to 4-[[2-[(acetylthio)methyl]-1-oxopentyl]amino]benzoic acid, methyl ester (2.0 g., 6.18 mmole) under argon. Upon stirring for 10 minutes all of the starting material dissolved and the clear yellow reaction was allowed to stir for 16 hours at room temperature. The reaction mixture was washed with ether, acidified to pH 1 with concentrated HCl, and extracted with ethyl acetate. The organic extracts were combined, dried ($MgSO_4$), filtered, and the solvent removed. The white solid residue was purified by flash chromatography (150 g. of Whatman LPS-1; 5% acetic acid, 20% ethyl acetate, 75% hexane) to give 1.34 g. of 4-[[2-(mercaptomethyl)-1-oxopentyl]amino]benzoic acid as a white solid; m.p. 207.5 - 208.5°. TLC (silica gel; 5% acetic acid, 40% ethyl acetate, 55% hexane) r_f = 0.39.

Anal. calc'd. for $C_{13}H_{17}NO_3S$:					
	C, 58.40;	H, 6.41;	N, 5.24;	S, 11.99;	SH, 12.37
Found:	C, 58.49;	H, 6.47;	N, 5.21;	S, 11.79;	SH, 12.32.

Example 4

4-[[2-(Mercaptomethyl)-3-methyl-1-oxobutyl]amino]benzoic acid

a) 2-[(Acetylthio)methyl]-3-methylbutanoic acid

Potassium hydroxide (100 g., 1.79 mole) was dissolved with cooling in distilled water (100 ml.). Diethyl isopropyl malonate (100 ml., 488 mmole) was added and the two phase reaction mixture was heated at 70° for 18 hours. The homogeneous, clear brown reaction mixture was cooled to room temperature, acidified to a pH of 1 with concentrated HCl, and extracted with ethyl acetate. The organic extracts were dried ($MgSO_4$), filtered, and the solvent removed in vacuo to yield 80.79 g. of crude product. Recrystallization from isopropyl ether and hexane provides 67.5 g. of isopropyl malonic acid as a white solid.

Isopropyl malonic acid (65 g., 450 mmole) was dissolved in distilled water (400 ml.) and 37% aqueous formaldehyde (37.9 g., 470 mmole) and 40% aqueous dimethylamine (52.6 g., 470 mmole) were added and the mixture was stirred for 16 hours. The clear yellow solution was then heated at 90° for 12 hours during which time a gas evolved. After cooling to room temperature, the reaction was acidified to pH 1 with concentrated HCl, and extracted with ether and dichloromethane. The combined organic extracts were dried ($MgSO_4$), filtered and the solvent removed in vacuo to give 42.84 g. of isopropyl acrylic acid as a yellow oil.

Thiolacetic acid (22 ml., 307 mmole) and isopropyl acrylic acid (10 g., 87.6 mmole) were combined and heated at 80° for 70 minutes and then allowed to stir at room temperature overnight. The thiolacetic acid was removed by distillation in vacuo (aspirator, 40 - 45°) and the residue was chased several times with toluene to yield 20.04 g. of a clear yellow liquid. A portion (6.67 g.) of this material was purified by flash chromatography (300 g. of Whatman LPS-1, 15% acetone and hexane) to provide 3.896 g. of 2-[(acetylthio)-methyl]-3-methylbutanoic acid as a yellow oil.

b) 4-[[2-[(Acetylthio)methyl]-3-methyl-1-oxobutyl]amino]benzoic acid, methyl ester

The 2-[(acetylthio)methyl]-3-methylbutanoic acid (1.556 g., 8.18 mmole) was dissolved in freshly distilled ether (20 ml.) and cooled to -5°. Oxalyl chloride (0.71 ml., 8.18 mmole) was added dropwise followed by N,N-dimethylformamide (3 drops). The ice bath was removed and the reaction was allowed to warm to room temperature as gas evolved. After stirring at room temperature for 1.66 hours, the reaction was a clear yellow solution with a small amount of gummy yellow precipitate. The solvent was removed in vacuo and the residue was chased with tetrahydrofuran. The resulting yellow oil was dissolved in dichloromethane (10 ml.), cooled to 0°, and added to a solution of 4-aminobenzoic acid, methyl ester (1.24 g. 8.18 mmole) and diisopropylethyl amine (1.42 ml., 8.18 mmole) in dry dichloromethane (30 ml.) at 0°. After stirring 10 minutes at 0° and then 16 hours at room temperature, the reaction was washed with saturated sodium bicarbonate, 1.0 M HCl, and saturated sodium chloride. The organic phase was dried ($MgSO_4$), filtered, and the solvent removed in vacuo to give 2.22 g. of 4-[[2-[(acetylthio)methyl]-3-methyl-1-oxobutyl]amino]benzoic acid, methyl ester as a yellow solid.

c) 4-[[2-(Mercaptomethyl)-3-methyl-1-oxobutyl]amino]benzoic acid

Sodium hydroxide (1.0 M, 25 ml., 25 mmole) and methanol (25 ml.) were degassed with argon and added to 4-[[2-[(acetylthio)methyl]-3-methyl-1-oxobutyl]amino]benzoic acid, methyl ester (2.0 g., 6.18 mmole) under argon. Upon stirring for 10 minutes all of the starting material dissolved and the clear yellow reaction was allowed to stir for 5 hours at room temperature. The reaction mixture was then acidified to pH of 1 with concentrated HCl and extracted with ethyl acetate. The organic extracts were combined, dried ($MgSO_4$), filtered, and then the solvent removed. The white solid residue was purified by flash chromatography (160 g. of Whatman LPS-1; 5% acetic acid, 20% ethyl acetate, 75% hexane) to yield 238 mg. of 4-[[2-(mercaptomethyl)-3-methyl-1-oxobutyl]amino]benzoic acid as a white solid; m.p. 250 - 270° (dec.). TLC (silica gel; 5% acetic acid, 40% ethyl acetate, 55% hexane) R_f = 0.34.

Anal. calc'd. for $C_{13}H_{17}NO_3S$:					
	C, 58.40;	H, 6.41;	N, 5.24;	S, 11.99;	SH, 12.37
Found:	C, 58.41;	H, 6.45;	N, 5.05;	S, 11.78;	SH, 12.27.

Example 5

4-[[2-(Mercaptomethyl)-4-methyl-1-oxopentyl]amino]benzoic acid

a) 4-[[2-[(Acetylthio)methyl]-4-methyl-1-oxopentyl]amino]benzoic acid, methyl ester

2-[(Acetylthio)methyl]-4-methylpentanoic acid (1.5 g., 7.34 mmole) [prepared as described by Sundeen et al. in U.S. Patent 4,235,885 in Example 1] was dissolved in freshly distilled ether (15 ml.) and cooled to -5°. Oxalyl chloride (0.64 ml., 7.34 mmole) was added dropwise followed by N,N-dimethylformamide (3 drops). The ice bath was removed and the reaction was allowed to warm to room temperature as gas evolved. After stirring at room temperature for one hour, the reaction was a clear yellow solution with a small amount of gummy precipitate. The solvent was removed in vacuo and the residue was chased with tetrahydrofuran. The resulting yellow oil was dissolved in dichloromethane (10 ml.), cooled to 0°, and added to a solution of 4-aminobenzoic acid, methyl ester (1.11 g., 7.34 mmole) and diisopropylethylamine (1.42 ml., 8.18 mmole) in dry dichloromethane (20 ml.) at 0°. After stirring for 10 minutes at 0° and then for one hour at room temperature, the reaction was washed with saturated sodium bicarbonate, 1.0 M HCl, and saturated sodium chloride. The organic phase was dried ($MgSO_4$), filtered, and the solvent was removed in vacuo to yield 2.16 g. of crude product as a yellow solid. Purification by flash chromatography (130 g. of Whatman LPS-1, 20% ethyl acetate, hexane) followed by a second chromatography (100 g. of Whatman LPS-1, 18% ethyl acetate, hexane) gave 1.89 g. of 4-[[2-[(acetylthio)methyl]-4-methyl-1-oxopentyl]amino]-benzoic acid, methyl ester. TLC (silica gel; 20% ethyl acetate, hexane) R_f = 0.20.

b) 4-[[2-(Mercaptomethyl)-4-methyl-1-oxopentyl]amino]benzoic acid

The methyl ester product from part (a) (1.89 g., 5.33 mmole) was dissolved in methanol (35 ml.) and degassed by bubbling argon through the mixture. Sodium hydroxide (1.0 M, 16 ml, 16 mmole) was added and the reaction was allowed to stir for 2 hours at room temperature. The reaction mixture was concentrated in vacuo; and the residue was dissolved in water (200 ml.), and washed with dichloromethane. The aqueous layer was acidified to a pH of 1 with concentrated HCl and extracted with ethyl acetate. The organic extracts were combined, dried ($MgSO_4$), filtered, and the solvent removed. The white residue was purified by flash chromatography (130 g. of Whatman LPS-1, 5% acetic acid, 10% ethyl acetate, 85% hexane) to give 1.25 g. of 4-[[2-(mercaptomethyl)-4-methyl-1-oxopentyl]amino]benzoic acid as a white solid; m.p. 200 - 201°. TLC (silica gel; 35% ethyl acetate, 5% acetic acid, 60% hexane) R_f = 0.66.

Anal. calc'd. for $C_{14}H_{19}NO_3S$:					
	C, 59.76;	H, 6.81;	N, 4.98;	S, 11.39;	SH, 11.75
Found:	C, 59.66;	H, 6.96;	N, 4.90,	S, 11.45;	SH, 11.79.

Example 6

4-[[2-(Mercaptomethyl)-1-oxobutyl]amino]benzoic acid

a) 2-[(Acetylthio)methyl]butanoic acid

Diethylethyl malonate (20 g., 106.3 mmole) was placed in a flask with potassium hydroxide (22.9 g., 408.1 mmole) and water (18.3 ml.). The reaction was stirred at reflux for 27 hours then cooled to room temperature. The reaction was diluted with water (200 ml.), washed with ethyl acetate, acidified to pH 1 with concentrated HCl, and extracted with ethyl acetate. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield a yellow solid which was recrystallized from isopropyl ether and hexane to yield 46.7 g. of ethylmalonic acid as a yellow crystalline solid; m.p. 112°.

The ethylmalonic acid (45 g., 340 mmole) was dissolved in water (300 ml.), and 37% aqueous formaldehyde (29 g., 358 mmole) and dimethylamine (49 g., 358 mmole) were added. The solution was stirred at room temperature for 24 hours and then the reaction was heated to 80° until the solid dissolved and the evolution of gas ceased. After 5 hours, the reaction was acidified to pH 1 with concentrated HCl, extracted with dichloromethane, dried ($MgSO_4$), filtered, and concentrated in vacuo (bath temperature of 20°) to give 16.2 g. of ethyl acrylic acid as a clear colorless liquid.

A mixture of the ethyl acrylic acid (16 g., 159.8 mmole) and thiolacetic acid (40 ml., 559.3 mmole) was stirred for one hour at room temperature and then at 80° for 2 hours. The reaction was cooled to room temperature and the thiolacetic acid was azeotroped with toluene in vacuo (aspirator) to give 25 g. of a yellow oil. Purification of 5 g. of this material by flash chromatography (Whatman LPS-1; 15% acetone, hexane) yields 4.46 g. of 2-[(acetylthio)methyl]butanoic acid.

b) 4-[[2-[(Acetylthio)methyl]-1-oxobutyl]amino]benzoic acid, methyl ester

A solution of 2-[(acetylthio)methyl]butanoic acid (1.87 g., 10.61 mmole) in freshly distilled ether (15 ml.), under argon, was cooled to 0°. Oxalyl chloride (0.93 ml., 10.61 mmole) and N,N-dimethylformamide (catalytic amount) were added and the yellow solution was stirred at room temperature. After 2 hours, the reaction was concentrated in vacuo and chased with tetrahydrofuran. The resulting yellow oil was dissolved in dichloromethane (10 ml.), cooled to 0°, and added to a solution of 4-aminobenzoic acid, methyl ester (1.6 g., 10.61 mmole) and diisopropylethylamine (1.85 ml., 10.61 mmole) in dichloromethane (20 ml.) at 0°. The orange solution was warmed to room temperature and stirred under argon for 2 hours. The reaction mixture was then washed with saturated sodium bicarbonate, 1.0 M HCl, and saturated sodium chloride. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield an orange solid. Purification by flash chromatography (140 g. of Whatman LPS-1 silica gel; 23% ethyl acetate, hexane) yielded a white solid; m.p. 109 -111°. Further purification by recrystallization from dichloromethane and hexane yielded 2.22 g. of 4-[[2-[(acetylthio)methyl]-1-oxobutyl]amino]benzoic acid, methyl ester as a white solid.

Anal. calc'd. for $C_{15}H_{19}NO_4S$:				
	C, 58.23;	H, 6.19;	N, 4.53;	S, 10.36
Found:	C, 58.32;	H, 6.21;	N, 4.40;	S, 10.05.

c) 4-[[2-(Mercaptomethyl)-1-oxobutyl]amino]benzoic acid

The methyl ester product from part (b) (1.73 g., 56.59 mmole) was dissolved in methanol (37 ml.), degassed with argon, and cooled to 0°. Over a ten minute period, 1.0 N sodium hydroxide (16.8 ml., 16.8 mmole) was added, and the reaction was again degassed with argon and stirred for 2 hours under argon at room temperature. The reaction was then concentrated in vacuo, diluted with water (50 ml.), acidified to pH 1 with concentrated HCl, and extracted with ethyl acetate. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield 1.33 g. of a white solid. Purification by flash chromatography (85 g. of Whatman LPS-1; 12% ethyl acetate, 5% acetic acid, 83% hexane) yielded the product eluting with the starting material. This material was redissolved in 1.0 N sodium hydroxide and extracted with chloroform. The aqueous phase was acidified to pH 1, extracted with ethyl acetate, dried ($MgSO_4$), and concentrated in vacuo to a white solid. Purification by flash chromatography (146 g. of Whatman LPS-1; 10% ethyl acetate, 5% acetic acid, 85% hexane) yielded 1.24 g. of 4-[[2-(mercaptomethyl)-1-oxobutyl]amino]benzoic acid as a white solid; m.p. 216 - 217° (dec.). TLC (silica gel; 35% ethyl acetate, 5% acetic acid, 60% hexane) R_f = 0.61.

Anal. calc'd. for $C_{12}H_{15}NO_3S$:					
	C, 56.67;	H, 5.99;	N, 5.51;	S, 12.61;	SH, 13.00
Found:	C, 56.65;	H, 6.15;	N, 5.30;	S, 12.57;	SH, 13.26.

Example 7

3-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid

a) 3-[[2-[(Acetylthio)methyl]-1-oxo-3-phenylpropyl]amino]benzoic acid, methyl ester

3-Acetylthio-2-benzylpropanoic acid (2.0 g., 8.84 mmole) was dissolved in freshly distilled ether (20 ml.) and cooled to -5°. Oxalyl chloride (0.77 ml., 8.84 mmole) was added dropwise followed by N,N-dimethylformamide (3 drops). The ice bath was removed and the reaction was allowed to warm to room temperature as gas evolved. After stirring at room temperature for 1.5 hours, the reaction was a clear yellow solution with a small amount of gummy yellow precipitate. The solvent was removed *in vacuo* and the residue was chased with tetrahydrofuran. The resulting yellow-green oil was dissolved in freshly distilled dichloromethane (10 ml.), cooled to 0°, and added to a solution of 3-aminobenzoic acid, methyl ester (1.13 g., 8.84 mmole) and diisopropylethylamine (1.54 ml., 8.84 mmole) in dry dichloromethane (20 ml.) at 0°. After stirring for ten minutes at 0° and then overnight at room temperature, the reaction was washed with saturated sodium bicarbonate, 1.0 M HCl, and saturated sodium chloride. The organic phase was dried ($MgSO_4$), filtered, and the solvent was removed *in vacuo* to yield 2.77 g. of crude product as a brown oil. Purification by flash chromatography (120 g. of Whatman LPS-1; 20% ethyl acetate, hexane) yielded 1.76 g. of 3-[[2-[(acetylthio)methyl]-1-oxo-3- phenylpropyl]amino]benzoic acid, methyl ester as a tan solid; m.p. 101 - 102.5°. TLC (silica gel; 20% ethyl acetate, hexane) R_f = 0.13.

b) 3-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid

The methyl ester product from part (a) (1.53 g., 4.11 mmole) was dissolved in 12 ml. of methanol, sodium hydroxide (1.0 M, 12 ml., 12 mmole) was added, and the reaction was degassed *in vacuo* and placed under argon. After stirring for 2.5 hours at room temperature, an additional amount of sodium hydroxide (8 ml.) was added. After stirring for an additional 2 hours, the reaction was acidified to pH 1 with concentrated hydrochloric acid. The methanol was removed *in vacuo* and the aqueous residue was extracted with ethyl acetate. The organic extracts were combined, dried ($MgSO_4$), filtered, and the solvent removed. The residue was purified by flash chromatography (100 g. of Whatman LPS-1, 5% acetic acid, 20% ethyl acetate, 75% hexane) to yield 1.1 g. of 3-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]-benzoic acid as a white solid; m.p. 173 - 175°. TLC (silica gel; 5% acetic acid, 40% ethyl acetate, 55% hexane) R_f = 0.33.

Anal. calc'd. for $C_{17}H_{17}NO_3S$:	
	C, 64.74; H, 5.43; N, 4.44; S, 10.17; SH, 10.49
Found:	C, 64.89; H, 5.42; N, 4.37; S, 10.18; SH, 10.67.

Example 8

(S)-3-[(3-Mercapto-2-methyl-1-oxopropyl)amino]benzoic acid

a) (S)-3-[[3-[(Acetylthio)methyl]-2-methyl-1-oxopropyl]amino]benzoic acid, methyl ester

A mixture of 3-aminobenzoic acid, methyl ester (1.01 g., 6.7 mmole) and diisopropylethylamine (1.3 ml., 7.4 mmole) was dissolved in freshly distilled dichloromethane (20 ml.). After cooling to 0°, D-3-(acetylthio)-2-methylpropanoyl chloride (1.0 ml., 6.7 mmole) was added dropwise and the reaction was allowed to stir and warm to room temperature over 15 hours. The reaction mixture was then washed with saturated sodium bicarbonate, 1.0 M HCl, and saturated sodium chloride solutions. The organic phase was then dried ($MgSO_4$), filtered, and the solvent removed to give 2.12 g. of crude product as a brown oil. Purification by flash chromatography (100 g. of Whatman LPS-1; 20% ethyl acetate, hexane) yielded 1.62 g. of (S)-3-[[3-[(acetylthio)methyl]-2-methyl-1-oxopropyl]amino]benzoic acid, methyl ester as a white, glassy solid. TLC (silica gel; 20% ethyl acetate, hexane) R_f = 0.09.

b) (S)-3-[(3-Mercapto-2-methyl-1-oxopropyl)amino]benzoic acid

The methyl ester product from part (a) (1.62 g., 5.49 mmole) was dissolved in methanol (22 ml.), sodium hydroxide (1.0 M, 22 ml., 22 mmole) was added and the reaction was degassed *in vacuo* and placed under argon. After stirring for 2 hours at room temperature, the reaction was washed with ethyl acetate. The aqueous layer was acidified to pH 1 with concentrated HCl and extracted with ethyl acetate. The organic extracts were combined, dried ($MgSO_4$), filtered, and the solvent removed. The residue was purified by flash chromatography (110 g. of Whatman LPS-1; 5% acetic acid, 20% ethyl acetate, 75% hexane) to yield 968 mg. of (S)-3-[(3-mercapto-2-methyl-1-oxopropyl)amino]benzoic acid as a white solid; m.p. 195 - 196°; $[\alpha]_D$ = -73.6° (c = 0.72, methanol). TLC (silica gel; 5% acetic acid, 40% ethyl acetate, 55% hexane) R_f = 0.31.

Anal. calc'd. for $C_{11}H_{13}NO_3S$:					
	C, 55.21;	H, 5.48;	N, 5.85;	S, 13.40;	SH, 13.82
Found:	C, 55.22;	H, 5.37;	N, 5.89;	S, 13.13;	SH, 13.99.

Example 9

3-[[2-(Mercaptomethyl)-4-methyl-1-oxopentyl]amino]benzoic acid

a) 3-Aminobenzoic acid, methyl ester, hydrochloride

A suspension of 3-aminobenzoic acid (10 g., 72.9 mmole) in methanol (200 ml.) was cooled to -10° and treated dropwise with thionyl chloride (17.3 g. 146 mmole) keeping the temperature below -5°. After the addition was complete, the reaction mixture was allowed to warm to ambient temperature overnight. The mixture was concentrated *in vacuo*, and the residue was twice triturated with ether to give 13.2 g. of 3-aminobenzoic acid, methyl ester, hydrochloride as a white solid; m.p. 207 -216°. TLC (silica gel; chloroform:methanol:acetic acid, 18:1:1) R_f = 0.70.

b) 3-[[2-[(Acetylthio)methyl]-4-methyl-1-oxopentyl]amino]benzoic acid, methyl ester

A solution of 2-[(acetylthio)methyl]-4-methylpentanoic acid (2.85 g., 13.95 mmole) in dry ether (30 ml.) under nitrogen was treated with oxalyl chloride (1.77 g., 13.95 mmole) followed by N,N-dimethylformamide (3 drops). The reaction mixture was stirred at room temperature for 2 hours, concentrated in vacuo, and then twice concentrated from dry tetrahydrofuran (30 ml.) to give 2-[(acetylthio)methyl]-4-methylpentanoyl chloride.

A solution of 3-aminobenzoic acid, methyl ester, hydrochloride (2.62 g., 13.95 mmole) in dry dichloromethane (20 ml.) was treated with diisopropylethylamine (1.8 g., 13.95 mmole), and the reaction mixture was cooled to -5° under nitrogen. This mixture was treated concurrently with a solution of the above 2-[-(acetylthio)methyl]-4-methylpentanoyl chloride in dichloromethane (20 ml.) and with diisopropylethylamine (1.8 g., 13.95 mmole). The mixture was stirred cold for 2 hours and then allowed to come to ambient temperature overnight. The reaction mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate (100 ml.), and this solution was washed with 25 ml. portions of 10% potassium bisulfate, water, 5% sodium bicarbonate, water, and brine, dried ($MgSO_4$), and concentrated in vacuo to give 4.6 g. of crude product. Flash chromatography (400 g. of Merck 9385 silica gel, eluting with 7:1 petroleum ether:acetone) gives 3.8 g. of 3-[[2-[(acetylthio)methyl]-4-methyl-1-oxopentyl] amino]benzoic acid, methyl ester as an oil. TLC (silica gel; petroleum ether:acetone, 5:1) R_f = 0.23.

c) 3-[[2-(Mercaptomethyl)-4-methyl-1-oxopentyl]amino]benzoic acid

A solution of the methyl ester product from part (b) (3.7 g., 10.9 mmole) in methanol (80 ml.) was cooled in an ice bath under nitrogen and treated dropwise with 1N sodium hydroxide (32.9 ml., 32.9 mmole). The reaction mixture was stirred cold for 15 minutes and then allowed to warm to ambient temperature overnight. The methanol was removed in vacuo, and the aqueous residue was diluted with water (100 ml.). The mixture was acidified with concentrated HCl and extracted with 3 x 75 ml. of ethyl acetate. The organic extract was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo to give 2.9 g. of crude product. Flash chromatography [300 g. of Merck 9385 silica gel; eluting with toluene:acetic acid (10:1)] gave 2.02 g. of 3-[[2-(mercaptomethyl)-4-methyl-1-oxopentyl]amino]benzoic acid as a white solid; m.p. 206 - 210°.

Anal. calc'd. for $C_{14}H_{19}NO_3S$:					
	C, 59.76;	H, 6.81;	N, 4.98;	S, 11.39;	SH, 11.75
Found:	C, 60.06;	H, 6.78;	N, 4.94;	S, 11.17;	SH, 11.45.

Example 10

4-[[3,3,3-Trifluoro-2-(mercaptomethyl)-1-oxopropyl]amino]benzoic acid

a) 2-[(Acetylthio)methyl]-3,3,3-trifluoropropanoyl chloride

α-Trifluoromethyl acrylic acid (10 g., 71 mmole) [prepared as described in J. Chem Soc., 1954, p. 371] was cooled in a salt-ice water bath, stirred, and treated portionwise with 97% thiolacetic acid (5.7 ml., 75 mmole). After the addition, the yellow liquid was stored in the cold for one hour, allowed to warm to room temperature, and distilled to yield 14 g. of 2-[(acetylthio)methyl]-3,3,3-trifluoropropanoic acid as a light yellow oil; b.p. 149 - 153° (13 mm.).

This acid (7.0 g., 32 mmole) was treated with redistilled thionyl chloride (18 ml., 25 mmole) and the mixture was refluxed for 3 hours. After removing excess thionyl chloride on a rotary evaporator, the residue was distilled to give 2-[(acetylthio)methyl]-3,3,3-trifluoropropanoyl chloride as a pale yellow oil; b.p. 80 - 82° (16 mm.).

b) 4-[[3,3,3-Trifluoro-2-[(acetylthio)methyl]-1-oxopropyl]amino]benzoic acid

4-Aminobenzoic acid (685 mg., 5 mmole) was suspended in acetonitrile (10 ml.). Bis(trimethylsilyl)-trifluoroacetamide (4 ml.) was added and the solid went into solution immediately. The resulting solution was stirred for 3 hours at room temperature. The 2-[(acetylthio)methyl]-3,3,3-trifluoropropanoyl chloride (1 g., 4.26 mmole) in acetonitrile (2 ml.) was added, and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated on a rotary evaporator. Water (20 ml.) was added to the residue and this mixture was stirred for 15 minutes. It was then partitioned between ethyl acetate and 5% potassium bisulfate. The ethyl acetate solution was washed with brine and concentrated to a yellow solid. This was chromatographed on Merck silica (300 ml.) eluting with dichloromethane:methanol:acetic acid, 40:1:1 to give 810 mg of 4-[[3,3,3-trifluoro-2-[(acetylthio)methyl]-1-oxopropyl]amino]benzoic acid as a white solid. TLC (silica gel; dichloromethane:methanol:acetic acid, 40:1:1) R_f = 0.72.

c) 4-[[3,3,3-Trifluoro-2-(mercaptomethyl)-1-oxopropyl]amino]benzoic acid

The product from part (b) (810 mg., 2.42 mmole) was stirred at 0° under argon with concentrated ammonium hydroxide (1.6 ml.) and water (3.5 ml.) for 15 minutes. 5% Potassium bisulfate (100 ml.) was added and the resulting solution was extracted with ethyl acetate. The combined ethyl acetate layers were washed with brine, dried ($MgSO_4$), and concentrated to a beige solid which was then chromatographed on Whatman LPS-1 silica (300 ml.) using dichloromethane:methanol:acetic acid (40:1:1) as the eluant to give 270 mg. of 4-[[3,3,3-trifluoro-2-(mercaptomethyl)-1-oxopropyl]amino]benzoic acid as a white solid; m.p. 217°; $[\alpha]_D$ = +2.2° (c = 0.23, methanol). TLC(silica gel; dichloromethane: methanol:acetic acid, 20:1:1) R_f = 0.50.

Anal. calc'd. for $C_{11}H_{10}F_3NO_3S$						
	C, 45.05;	H, 3.44;	N, 4.78;	S, 10.93;	SH, 11.28;	F, 19.44
Found:	C, 45.41;	H, 3.39;	N, 4.64;	S, 11.08;	SH, 11.62;	F, 19.66.

Example 11

3-[[3,3,3-Trifluoro-2-(mercaptomethyl)-1-oxopropyl]amino]benzoic acid

a) 3-[[3,3,3-Trifluoro-2-[(acetylthio)methyl]-1-oxopropyl]amino]benzoic acid

3-Aminobenzoic acid (2.0 g., 14.58 mmole) was placed in a flask under argon and suspended in dry acetonitrile (15 ml.). The suspension was cooled to 0° and bis(trimethylsilyl)trifluoroacetamide (7.7 ml., 29.2 mmole) was added. Within 20 minutes all of the solid had dissolved and the 2-[(acetylthio)methyl]-3,3,3-trifluoropropanoyl chloride (1.49 g., 6.35 mmole) was added dropwise. The reaction was allowed to stir and warm to room temperature overnight. The reaction mixture was partitioned between ethyl acetate and 1.0 M HCl and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$), filtered, and the solvent removed to yield 2.0 g. of crude product as a white solid. The residue was purified by flash chromatography (100 g. of Whatman LPS-1, 5% acetic acid, 20% ethyl acetate, and 75% hexane) to yield 1.54 g. of 3-[[3,3,3-trifluoro-2-[(acetylthio)methyl]-1-oxopropyl]amino]benzoic acid as a white solid; m.p. 205.5 - 206.5°.

b) 3-[[3,3,3-Trifluoro-2-(mercaptomethyl)-1-oxopropyl]amino]benzoic acid

The product from part (a) (500 mg., 1.49 mmole) was placed in a flask under argon and aqueous ammonium hydroxide (1.2 ml., 4.0 M) was added. The solid dissolved and the reaction became clear yellow. After stirring for 40 minutes, the reaction was acidified with 1.0 M HCl and then extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and the solvent was removed to yield 381 mg. of crude product as a yellow solid. Purification by preparative HPLC (YMC S345 50 x 200 mm column, 50 - 74% methanol in water containing 0.1% trifluoroacetic acid linear gradient over 50 minutes, 18.6 ml./min. flow rate, UV detection at 220 nm) gave 140 mg. of 3-[[3,3,3-trifluoro-2-(mercaptomethyl)-1-oxopropyl]-amino]benzoic acid as a white solid; m.p. 234 - 236° (dec.). TLC(silica gel; 5% acetic acid, 40% ethyl acetate, 55% hexane) R_f = 0.33.

Anal. calc'd. for $C_{11}H_{10}F_3NO_3S$:						
	C, 45.05;	H, 3.44;	N, 4.78;	F, 19.44;	S, 10.93;	SH, 11.28
Found:	C, 44.87;	H, 3.38;	N, 4.67;	F, 19.05;	S, 10.73;	SH, 11.58.

Example 12

4-[(3-Mercapto-1-oxo-2-phenylpropyl)amino]benzoic acid

a) 3-(Acetylthio)-2-phenylpropanoic acid

Thiolacetic acid (5.32 g., 70 mmole) was added dropwise to a solution of atropic acid (7.4 g., 50 mmole) in chloroform (75 ml.). The solution was allowed to stand overnight at room temperature. The solvent was removed on the rotary evaporator and the semi-solid residue was triturated with hexane to 9.3 g. of product; m.p. 96 - 98°. Recrystallization from cyclohexane gave 3-(acetylthio)-2-phenylpropanoic acid as a solid; m.p. 94 - 96°.

Anal. calc'd. for $C_{11}H_{12}O_3S$:			
	C, 58.91;	H, 5.40;	S, 14.30
Found:	C, 58.69;	H, 5.40;	S, 14.20.

b) 4-[[3-(Acetylthio)-1-oxo-2-phenylpropyl]amino]benzoic acid, methyl ester

A solution of 3-(acetylthio)-2-phenylpropanoic acid (2.55 g., 11.37 mmole) in distilled ether (15 ml.) under argon was cooled to 0°. Oxalyl chloride (0.99 ml., 11.37 mmole) and N,N-dimethylformamide (3 drops) were added and the yellow solution was stirred at room temperature. After 2 hours, the reaction was concentrated in vacuo and chased with tetrahydrofuran. The resulting yellow oil was dissolved in dichloromethane (10 ml.), cooled to 0°, and added to a solution of 4-aminobenzoic acid, methyl ester (1.72 g., 11.37 mmole) and diisopropylethylamine (1.98 ml., 11.37 mmole) in dichloromethane (15 ml.) at 0°. The orange solution was warmed to room temperature and stirred under argon for 1 hour. The reaction mixture was washed with saturated sodium bicarbonate, 1N HCl, and saturated sodium chloride. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield an orange foam. Purification by flash chromatography (445 g. of Whatman LPS-1; 23% ethyl acetate, hexane) yielded 3.66 g. of 4-[[3-(acetylthio)-1-oxo-2-phenylpropyl]amino]benzoic acid, methyl ester as a white solid. TLC (silica gel; 30% ethyl acetate, hexane) R_f = 0.30.

c) 4-[(3-Mercapto-1-oxo-2-phenylpropyl)amino]benzoic acid

The methyl ester product from part (b) (2.94 g., 8.56 mmole) was dissolved in methanol (57 ml.), degassed with argon, and cooled to 0°. Over a ten minute period 1.0 N sodium hydroxide (26 ml., 26 mmole) was added, the reaction was again degassed with argon and stirred at room temperature under argon. Additional sodium hydroxide was added after one hour (17 ml., 17 mmole), and two hours (17 ml., 17 mmole), then the reaction was stirred for 45 minutes. The reaction was concentrated in vacuo, dissolved in water, acidified to pH 1 with concentrated HCl, and extracted with ethyl acetate. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to yield 2.39 g. of a white solid. Purification three times by flash chromatography (160 g. of Whatman LPS-1; 15% ethyl acetate, 5% acetic acid, 80% hexane) yielded 330 mg. of 4-[(3-mercapto-1-oxo-2-phenylpropyl)amino]benzoic acid as an off white solid; m.p. 240 - 242° TLC (silica gel; ethyl acetate:acetic acid:hexane, 50:5:45) R_f = 0.58.

Anal. calc'd. for $C_{16}H_{15}NO_3S$:					
	C, 63.77;	H, 5.02;	N, 4.65;	S, 10.64;	SH, 10.97
Found:	C, 64.05;	H, 5.35;	N, 4.68;	S, 10.40;	SH, 11.07.

Example 13

4-[[2-(Mercaptomethyl)-1-oxo-4-phenylbutyl]amino]benzoic acid

a) 2-[(Acetylthio)methyl]-4-phenylbutanoic acid

Phenethyl bromide (30 g., 160 mmole) was added, all at once, to a solution of sodium metal (6.5 g., 280 mmole) and diethyl malonate (45 g., 280 mmole) in absolute ethanol (90 ml.). After stirring for 30 minutes at room temperature, a white precipitate formed and the reaction temperature rose to 40°. Stirring was continued overnight, then the mixture was heated at reflux for 6 hours. The solvent was removed in vacuo and the residue was partitioned between water (250 ml.) and ether (3 x 200 ml.). The organic extracts were combined, washed with brine, and dried ($MgSO_4$). Removal of the ether in vacuo yielded an amber colored, viscous residue (46.2 g.). This material was distilled and the fraction boiling at 161 - 163° 5 mm Hg. was collected to give 19.5 g. of diethyl(phenylethyl)malonate.

Diethyl(phenylethyl) malonate (114.6 g., 430 mmole) was treated with 10% aqueous sodium hydroxide (600 ml.) and heated under reflux with stirring for 5 hours, cooled, and allowed to stand overnight. The reaction was then refluxed for an additional 3 hours. The resulting clear solution was made strongly acidic with 20% aqueous HCl and the white precipitate that formed was filtered to yield 82.6 g. of (phenylethyl) malonic acid as a white solid; m.p. 128 - 130° (dec.).

A suspension of the (phenylethyl) malonic acid (78.8 g., 380 mmole) in water (860 ml.) was treated with 40% aqueous dimethylamine (410 mmole) and formalin (33.3 g., 410 mmole). A clear solution formed promptly, and the mixture was allowed to stand overnight at room temperature. The resulting white precipitate was filtered, the filtrate was acidified with 10% potassium bisulfate, and the solid that formed was filtered and combined with the first crop to give 86.4 g. of phenylethyl dimethylaminomethyl malonic acid; m.p. 120 - 122° (dec.).

This malonic acid (86.4 g., 330 mmole) was mixed with water (3 l.) and heated under reflux for 1.5 hours during which carbon dioxide evolved and a clear solution was formed. The solution was cooled, acidified with potassium bisulfate, and the resulting white precipitate was filtered and dried to give 48.5 g. of (phenylethyl) acrylic acid as a white solid; m.p. 51 - 52° (dec.).

A mixture of the (phenylethyl) acrylic acid (17.6 g.) and thiolacetic acid (50 ml.) was stirred for 24 hours at room temperature during which time a clear solution formed. The solution was allowed to stand at room temperature for an additional week and then the excess thiolacetic acid was removed by water aspiration followed by a hot water bath and vacuum pump. The solid yellow residue was heated on a steam cone under high vacuum and then triturated in hexane to give 18.2 g. of white crystalline 2-[(acetylthio)methyl]-4-phenylbutanoic acid; m.p. 52 - 53°.

b) 4-[[2-[(Acetylthio)methyl]-1-oxo-4-phenylbutyl]amino]benzoic acid, methyl ester

A solution of 2-[(acetylthio)methyl]-4-phenylbutanoic acid (2.72 g., 10.8 mmole) in dry ether (25 ml.) under nitrogen was treated with oxalyl chloride (1.37 g., 10.8 mmole) followed by N,N-dimethylformamide (2 drops), and the mixture was stirred at ambient temperature. After one hour, the reaction mixture was concentrated in vacuo, and was then twice dissolved in toluene and concentrated in vacuo.

A solution of 4-aminobenzoic acid, methyl ester (1.63 g., 10.8 mmole) in dry dichloromethane (15 ml.) was cooled to -5° under nitrogen. The solution was treated concurrently with a solution of the above acid chloride in dry dichloromethane (15 ml.) and distilled diisopropylethylamine (1.39 g., 10.9 mmole). The reaction mixture was stirred cold for 2 hours, and then was allowed to warm to ambient temperature overnight. The mixture was concentrated in vacuo. The residue was dissolved in ethyl acetate (75 ml.) and washed with 25 ml. portions of 10% potassium bisulfate, water, 5% sodium bicarbonate, water, and brine, dried ($MgSO_4$), and concentrated in vacuo to give 4.3 g. of crude product. Flash chromatography on 400 g. of Merck 9385 silica gel, eluting with 6:1 petroleum ether:acetone gave 3.6 g. of partially purified product. A second flash chromatography on 350 g. of Merck 9385 silica gel, eluting with 10:1 toluene:acetone gave 3.25 g. of 4-[[2-[(acetylthio)methyl]-1-oxo-4-phenylbutyl]amino[benzoic acid, methyl ester as as white solid; m.p. 88 - 93°. TLC (silica gel; toluene:acetone, 10:1) R_f = 0.53.

c) 4-[[2-(Mercaptomethyl)-1-oxo-4-phenylbutyl]amino]benzoic acid

A solution of the methyl ester product from part (b) (3.2 g., 8.3 mmole) in methanol (65 ml.) was cooled in an ice bath under nitrogen and treated dropwise with 1N sodium hydroxide solution (24.9 ml., 24.9 mmole). The reaction mixture was stirred cold for 15 minutes, and then was allowed to warm to ambient temperature overnight. The methanol was removed in vacuo. The remaining aqueous mixture was diluted with water (80 ml.) and washed with chloroform (2 x 30 ml.). The aqueous portion was acidified with concentrated HCl and extracted with ethyl acetate (3 x 65 ml.). The organic extract was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo to give 2.5 g. of crude product. Flash chromatography on 250 g. of Merck 9385 silica gel eluting with (10:1) toluene:acetone gave 1.35 g. of partially purified material. Three flash chromatographies on 125 g. columns of Merck 9385 silica gel eluting with (100:1:1) chloroform:methanol:acetic acid followed by a final flash chromatography on 100 g. of Merck 9385 silica gel eluting with (20:2:1) hexanes:ethyl acetate:acetic acid yielded 484 mg. of 4-[[2-(mercaptomethyl)-1-oxo-4-phenylbutyl]amino]benzoic acid as a white solid; m.p. 184 - 186°. TLC (silica gel; chloroform:methanol:acetic acid, 50:1:1) R_f = 0.31.

Anal. calc'd. for $C_{18}H_{19}NO_3S \cdot 0.26\ H_2O$:					
	C, 64.70;	H, 5.89;	N, 4.19;	S, 9.60;	SH, 9.90
Found:	C, 64.51;	H, 5.93;	N, 4.38;	S, 9.66;	SH, 9.91.

Example 14

4-[[2-(Mercaptomethyl)-1-oxo-3-[4-(phenylmethoxy)phenyl]propyl]amino]benzoic acid

a) 2-[(Acetylthio)methyl]-3-[4-(phenylmethoxy)phenyl]propanoic acid

A mixture of p-hydroxybenzaldehyde (91.8 g., 750 mmole), diethyl malonate (120 g., 750 mmole), acetic acid (20 ml.), and piperidine (6 ml.) in dry benzene (250 ml.) was heated under reflux for 7 hours and then allowed to cool to ambient temperature overnight. The solution was cooled in an acetone-dry ice bath and the solid that separated was filtered to give 138.1 g. of (p-hydroxybenzylidene) malonic acid, diethyl ester; m.p. 88 - 90°.

A solution of (p-hydroxybenzylidene) malonic acid, diethyl ester (26.4 g., 100 mmole) in absolute ethanol (150 ml.) was treated with 10% palladium on carbon catalyst (1 g.) and shaken on a Parr apparatus pressurized with hydrogen for 2.5 hours. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The identical procedure was repeated twice with 52.8 g. (200 mmole) and 58.9 g. (220 mmole) of (p-hydroxybenzylidene) malonic acid, diethyl ester to give a total of 88.9 g. of (p-hydroxybenzyl) malonic acid, diethyl ester as a solid; m.p. 47 - 48°.

A solution of (p-hydroxybenzyl) malonic acid, diethyl ester (13.3 g., 50 mmole) in 1N sodium hydroxide (50 ml.) and 95% ethanol (50 ml.) was treated under nitrogen with benzyl bromide (8.5 g., 50 mmole), and the reaction mixture was stirred at ambient temperature overnight. The ethanol was removed in vacuo. The resulting suspension was extracted twice with ether (200 ml.). The ether extract was washed with brine (100 ml.), dried ($MgSO_4$), and concentrated in vacuo to yield 7.7 g. of [p-(benzyloxy)benzyl] malonic acid, diethyl ester as a yellow oil. The aqueous layer was acidified and extracted with ether. This ether extract was dried ($MgSO_4$) and concentrated in vacuo to yield 4.0 g. of [p-(benzyloxy)benzyl] malonic acid, monoethyl ester; m.p. 70 - 80°.

A suspension of the above diethyl ester product (7.7 g., 22 mmole) and the monoethyl ester product (4.0 g., 12 mmole) in 10% sodium hydroxide (100 ml.) was heated under reflux for 5 hours. The solution was filtered, cooled and acidified with 20% HCl. The resulting white solid was collected to give 7.1 g. of [p-(benzyloxy)benzyl] malonic acid as a white crystalline solid; m.p. 149 - 151° (dec.).

A mixture of the above malonic acid product (94.6 g., 320 mmole), 40% aqueous dimethylamine (37.7 g., 330 mmole), and 37% aqueous formaldehyde (26.7 g., 330 mmole) in water (700 ml.) was stirred overnight at ambient temperature. The crystalline solid that separates was filtered to give 104.6 g. of [p-(benzyloxy)benzyl][(dimethylamino)methyl] malonic acid; m.p. 114 - 116° (dec.).

A solution of [p-(benzyloxy)benzyl][(dimethylamino)methyl] malonic acid (78 g., 220 mmole) in water (3 l.) was heated under reflux for one hour (gas evolves), and then was refrigerated overnight. The mixture was acidified with 10% potassium bisulfate and the resulting white precipitate was extracted into ether. The ether extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to a solid residue. This solid was triturated with petroleum ether and filtered to give 41 g. of [p-(benzyloxy)benzyl] acrylic acid; m.p. 107 - 110°.

This acrylic acid (26.8 g., 100 mmole) was added portionwise to thiolacetic acid (50 ml.) at ambient temperature, resulting in a fine suspension. The mixture was warmed briefly at 50°, and the resulting clear solution was stirred at ambient temperature under nitrogen for 72 hours. The crystalline precipitate was filtered and triturated with hexane to give 29.8 g. of crude product; m.p. 117 - 120°. Recrystallization from cyclohexane yielded pure 2-[(acetylthio)methyl]-3-[4-(phenylmethoxy)phenyl]propanoic acid; m.p. 121 - 123° (dec.). TLC (silica gel; ethyl acetate) R_f = 0.71.

Anal. calc'd. for $C_{19}H_{20}O_4S$:	C, 66.26;	H, 5.85;	S, 9.31;
Found:	C, 66.32;	H, 5.90;	S, 8.83.

b) 4-[[2-[(Acetylthio)methyl]-1-oxo-3-[4-(phenylmethoxy)phenyl]propyl]amino]benzoic acid, methyl ester

2-[(Acetylthio)methyl]-3-[4-(phenylmethoxy)phenyl]propanoic acid (2.0 g., 5.81 mmole) was dissolved in freshly distilled ether (15 ml.) and cooled to -5°. Oxalyl chloride (0.74 ml., 5.81 mmole) was added dropwise followed by N,N-dimethylformamide (4 drops). The ice bath was removed and the reaction was allowed to warm to room temperature as gas evolved. After stirring at room temperature for 2 hours, the reaction was a clear yellow solution with a small amount of gummy yellow precipitate. The solvent was removed in vacuo and the residue was chased with tetrahydrofuran. The resulting yellow oil was dissolved in dichloromethane (10 ml.), cooled to 0°, and added to a solution of 4-aminobenzoic acid, methyl ester (0.88 g., 5.81 mmole) and diisopropylethyl amine (1.01 ml., 5.81 mmole) in dry dichloromethane (20 ml.) at 0°. After 3 hours, the reaction was washed with saturated sodium bicarbonate, 1.0 M HCl, and saturated sodium chloride. The organic phase was dried ($MgSO_4$), filtered, and the solvent removed in vacuo to yield 2.5 g. of crude product as an orange foam. Purification by flash chromatography (200 g. of Whatman LPS - 1; 26% ethyl acetate, hexane) yielded 1.92 g. of 4-[[2-[(acetylthio)methyl]-1-oxo-3-[4-phenylmethoxy)-phenyl]propyl]amino]benzoic acid, methyl ester as an off-white solid. TLC (silica gel; 30% ethyl acetate, 70% hexane) R_f = 0.18.

c) 4-[[2-(Mercaptomethyl)-1-oxo-3-[4-(phenylmethoxy)phenyl]propyl]amino]benzoic acid

The methyl ester product from part (b) (1.72 g., 3.6 mmole) was suspended in methanol (24 ml.) and degassed by bubbling argon through the mixture. The reaction was cooled to 0° and 1.0 M sodium hydroxide (11.0 ml., 11.0 mmole) was added. After 1 hour, solid remained and additional methanol (4 ml.) and sodium hydroxide (3.6 ml., 3.6 mmole) were added. After stirring for 2 hours, the reaction was still heterogeneous and freshly distilled tetrahydrofuran (48 ml.) was added at which time all the solid dissolved. After stirring for 5.5 hours, another aliquot of sodium hydroxide (3.6 ml., 3.6 mmole) was added. The reaction was stirred for 1.5 hours then concentrated in vacuo and the residue was dissolved in water (100 ml.). The solution was acidified to pH 1 with concentrated HCl and extracted with ethyl acetate. The organic extracts were combined, dried ($MgSO_4$), filtered, and the solvent removed. The off-white solid was purified by flash chromatography (140 g. of Whatman LPS - 1; 5% acetic acid, 25% ethyl acetate, 70% hexane) to yield 1.34 g. of partially purified product. This solid was repurified by flash chromatography (42 g. of Whatman LPS-1; 5% acetic acid, 18% ethyl acetate, 77% hexane) to give 90 mg. of 4-[[2-(mercaptomethyl)-1-oxo-3-[4-(phenylmethoxy)phenyl]propyl]amino]benzoic acid as a white solid; m.p. 189 - 191° (dec.). TLC (silica gel; ethyl acetate:acetic acid:hexane, 25:5:70) R_f = 0.28.

Anal., calc'd. for $C_{24}H_{23}NO_4S \cdot 2.92\ H_2O$:				
	C, 60.79;	H, 6.13;	N, 2.95;	S, 6.76
Found:	C, 60.50;	H, 5.77;	N, 3.18;	S, 6.46.

Example 15

4-[[2-[(Acetylthio)methyl]-1-oxo-3-phenylpropyl]amino]benzoic acid

A solution of 2-[(acetylthio)methyl]-3-phenylpropanoic acid (15.06 g., 63.2 mmole) in dry tetrahydrofuran (200 ml.) under nitrogen was treated dropwise with oxalyl chloride (8.02 g., 63.2 mmole). The resulting solution was treated with N,N-dimethylformamide (5 drops) and stirred at ambient temperature for one hour. The reaction mixture was concentrated to dryness in vacuo. The residue was twice concentrated from dry tetrahydrofuran (100 ml.), and then used without further purification.

A solution of 4-aminobenzoic acid (8.67 g., 63.2 mmole) in dry acetonitrile (125 ml.) was treated with bis(trimethylsilyl)trifluoroacetamide (38.8 g., 150 mmole, 2.4 equivalents), and the mixture was stirred at ambient temperature for 30 minutes. The reaction mixture was cooled in an ice-bath, treated dropwise with a solution of the above acid chloride (nominally 63.2 mmole) in dry acetonitrile (125 ml.), and then allowed to warm to ambient temperature overnight. The reaction mixture was concentrated in vacuo, and the residue was partitioned between 400 ml. each of ethyl acetate and water. The organic layer was extracted with 5% sodium bicarbonate (3 x 250 ml.). The aqueous extract was acidified to a pH of 1 with concentrated HCl and extracted with ethyl acetate (3 x 400 ml.). The organic extract was washed with brine, dried ($MgSO_4$), and then concentrated in vacuo. The original ethyl acetate layer from the bicarbonate extractions was combined with the final organic extract to give 34.0 g. of slightly yellow solid. Two recrystallizations from hot chloroform/hexane gave 19.85 g. of 4-[[2-[(acetylthio)methyl]-1-oxo-3-phenylpropyl]amino]benzoic acid as a white crystalline solid; m.p. 184 - 186°. TLC (silica gel; toluene:acetic acid, 10:1) R_f = 0.28.

Anal. calc'd. for $C_{19}H_{19}NO_4S$:				
	C, 63.85;	H, 5.36;	N, 3.92;	S, 8.97
Found:	C, 63.51;	H, 5.25;	N, 3.86;	S, 9.07.

Example 16

4-[[2-[(Benzoylthio)methyl]-1-oxo-3-phenylpropyl]amino]benzoic acid

a) 2-[(Benzoylthio)methyl]-3-phenylpropanoic acid

A mixture of benzyl acrylic acid (13.7 g., 85 mmole) [prepared as described in Example 1(b)] and thiobenzoic acid (15 ml., 127 mmole) in dichloromethane (170 ml.) was stirred under argon at reflux temperature for 3 days, after which it was concentrated in vacuo. The residue was recrystallized twice from ether hexane to give 4.7 g. of 2-[(benzoylthio)methyl]-3-phenylpropanoic acid as a white solid: m.p. 99 - 102°.

Anal. calc'd. for $C_{17}H_{16}O_2S$:	C, 67.98;	H, 5.37;	S, 10.67
Found:	C, 67.89;	H, 5.34;	S, 10.70.

b) 4-[[2-[(Benzoylthio)methyl]-1-oxo-3-phenylpropyl]amino]benzoic acid

A solution of 2-[(benzoylthio)methyl]-3- phenylpropanoic acid (874 mg., 2.91 mmole) in tetrahydrofuran (10 ml.) under nitrogen was treated with oxalyl chloride (369 mg., 2.91 mmole) and then one drop of dimethylformamide was added cautiously. The reaction mixture was stirred at ambient temperature for one hour, and then concentrated in vacuo. The residue was taken up in tetrahydrofuran (10 ml.) and concentrated in vacuo twice to give the desired acid chloride.

A solution of 4-aminobenzoic acid (399 mg., 2.91 mmole) in dry acetonitrile (10 ml.) under nitrogen was treated with bis(trimethylsilyl) trifluoroacetamide (1.8 g., 6.98 mmole, 2.4 equiv.), and the mixture was cooled in an ice bath and treated dropwise with the above acid chloride (2.91 mmole) in dry acetonitrile (10 ml.). The mixture was allowed to warm to ambient temperature overnight. The reaction mixture was concentrated in vacuo. The residue was partitioned between 20 ml. each of ethyl acetate and water. The organic layer was further extracted with 5% sodium bicarbonate (3 x 10 ml.). The combined bicarbonate extract was acidified to pH 1 with concentrated HCl and extracted with ethyl acetate (3 x 20 ml.). A 500 mg. amount of solid insoluble in ethyl acetate was filtered off and saved. The combined ethyl acetate extract was concentratd in vacuo, and the solid residue was twice triturated from methanol and filtered to give a 260 mg. portion of crude product. The solid was triturated with acetonitrile and filtered to give 190 mg. of solid. Some solid which precipitated out of the original ethyl acetate solution after the bicarbonate extractions was filtered to afford an additional 250 mg. of material. The 500 mg., 190 mg., and 250 mg. batches of solid were combined and recrystallized from hot acetonitrile to give 323 mg. of product as a white solid. A second 76 mg. crop was collected for a total yield of 399 mg. of 4-[[2-[(benzoylthio)methyl]-1-oxo-3-phenylpropyl]amino]benzoic acid as a white solid; m.p. 223 - 225°; $[\alpha]_D$ = +0.6° (c = 0.5, dimethylformamide). TLC (silica gel; toluene:acetic acid, 20:1) R_f = 0.29.

Anal. calc'd. for $C_{24}H_{21}NO_4S \cdot 0.15 H_2O$:	C, 68.27;	H, 5.09;	N, 3.32;	S, 7.59
Found:	C, 68.09;	H, 4.90;	N, 3.50;	S. 7.59.

Example 17

4-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid

The product of Example 1 was also prepared as follows.

A solution of 4-[[2-[(acetylthio)methyl]-1-oxo-3-phenylpropyl]amino]benzoic acid (12.7 g., 35.53 mmole in water (90 ml.) and concentrated ammonia (50 ml.) was flushed with argon, and stirred stoppered at ambient temperature. After 30 minutes, the reaction mixture was washed with ethyl acetate (3 x 75 ml.). The aqueous layer was acidified to a pH of 1 with concentrated HCl, and extracted with ethyl acetate (3 x 75 ml.). The combined organic extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give 11.4 g. of crude product. This material was triturated with hexanes and filtered to give 10.6 g. of 4-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid as a white solid; m.p. 179-181°. TLC (silica gel; hexane:ethyl acetate: acetic acid, 12:7:1) R_f = 0.46 (minor impurity at 0.23).

Anal. calc'd. for $C_{17}H_{17}NO_3S$:				
	C, 64.74;	H, 5.43;	N, 4.44;	S, 10.17
Found:	C, 64.73;	H, 5.40;	N, 4.43;	S, 10.11.

Example 18

(cis)-4-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]cyclohexanecarboxylic acid

a) (cis)-4-Aminocyclohexanecarboxylic acid, methyl ester, monohydrochloride

Thionyl chloride (1.46 ml., 20 mmole) was added dropwise to a stirred suspension of (cis)-4-aminocyclohexanecarboxylic acid (1.43g., 10 mmole) [prepared as described by Villani et al., J. Org. Chem., Vol. 29. p. 2585 - 2587, 1964] in methanol (25 ml.) under argon, maintaining the reaction temperature at -5° to -10° during the addition. The resulting solution was allowed to warm to room temperature overnight. The solvent was then evaporated and the white residue was chased with methanol, then toluene, and then heated with acetonitrile (approximately 40 ml.). The crystals that separated upon cooling were filtered to give 1.74 g. of (cis)-4-aminocyclohexanecarboxylic acid, methyl ester, monohydrochloride as white needles; m.p. 185 - 187°. TLC (silica gel; n-butanol:acetic acid:water, 4:1:1) R_f = 0.43.

Anal. calc'd. for $C_8H_{16}NO_2Cl$:				
	C, 49.61;	H, 8.33;	N, 7.23;	Cl. 18.30
Found:	C, 49.76;	H, 8.42;	N, 7.29;	Cl. 17.91.

b) (cis)-4-[[2-[(Acetylthio)methyl]-1-oxo-3-phenylpropyl]amino]cyclohexanecarboxylic acid, methyl ester

A solution of 3-acetylthio-2-benzylpropanoic acid (166 g., 7 mmole) in ether (14 ml.) under nitrogen was treated with oxalyl chloride (0.61 ml., 7 mmole) followed by a drop of dimethylformamide and the mixture was stirred at room temperature for one hour. The solvent was evaporated and the residue was chased twice with dry tetrahydrofuran (15 ml.) to give the desired acid chloride.

A suspension of (cis)-4-aminocyclohexanecarboxylic acid, methyl ester, monohydrochloride (1.35 g., 7 mmole) in dichloromethane (25 ml.) at -5° under nitrogen was treated concurrently with a solution of the above acid chloride (nominally 7 mmole) in dichloromethane (14 ml.) and diisopropylethylamine (2.44 ml., 14 mmole). After the addition was complete, the reaction mixture was stirred for an additional hour, and then allowed to warm to room temperature overnight. The reaction mixture was concentrated and the residue was taken up in ethyl acetate (50 ml.). The mixture was filtered to remove diisopropylethylamine hydrochloride and the resulting solution was washed with 10% potassium bisulfate, water, 5% sodium bicarbonate, water and brine, then dried ($MgSO_4$) and evaporated to give 2.51 g. of residue. This residue was flash chromatographed on silica gel (Merck 9385, 250 g.) eluting with petroleum ether:acetone (5:1) to yield 2.30 g. of (cis)-4-[[2-[(acetylthio)methyl]-1-oxo-3-phenylpropyl]amino]cyclohexanecarboxylic acid, methyl ester as a white solid; m.p. 86 - 92°. TLC (silica gel; petroleum ether:acetone, 3:1) R_f - 0.52.

Anal. calc'd. for $C_{20}H_{27}NO_4S$:				
	C, 63.63;	H, 7.21;	N, 3.71;	S, 8.49
Found:	C, 63.57;	H, 7.39;	N, 3.81;	S, 8.81.

c) (cis)-4-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]cyclohexanecarboxylic acid

A solution of the methyl ester product from part (b) (2.28 g., 6.04 mmole) in methanol (18 ml.) under nitrogen was cooled in an ice bath and treated with 1N sodium hydroxide dropwise over 20 minutes. The mixture was allowed to warm to room temperature and stirred for 30 hours. The mixture was concentrated. The aqueous residue was diluted with water (50 ml.) and washed with chloroform (3 x 15 ml.). The aqueous portion was acidified to pH of 1 with concentrated HCl and extracted with ethyl acetate (3 x 30 ml.). The combined organic extract was washed with water and brine, dried ($MgSO_4$), and evaporated to give 1.9 g. of a white foamy solid. Flash chromatography on silica gel (Merck 9385, 200 g.) eluting with toluene:acetic acid (10:1) yielded 1.15 g. of (cis)-4-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]-cyclohexanecarboxylic acid as an amorphous white solid; m.p. 126 - 132°. TLC (silica gel; toluene:acetic acid, 5:1) R_f = 0.39.

Anal. calc'd. for $C_{17}H_{23}NO_3S \cdot 0.1\ H_2O$:					
	C, 63.17;	H, 7.23;	N, 4.33;	S, 9.92;	SH,10.23
Found:	C, 63.49;	H, 7.38;	N, 4.40;	S,10.12;	SH, 9.84.

In a similar manner, by employing (trans)-4-aminocyclohexanecarboxylic acid [prepared as described by Johnston et al., Jour. of Med. Chem., Vol. 20, p 279 - 290, 1977] in the above procedure one obtains (trans)-4-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]cyclohexanecarboxylic acid.

Also, by employing (cis)-3-aminocyclohexanecarboxylic acid and (trans)-3-aminocyclohexanecarboxylic acid (also disclosed by Johnston et al.) in the above procedure, one obtains (cis)-3-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]cyclohexanecarboxylic acid and (trans)-3-[[2-(mercaptomethyl)-1-oxo-3-phenylpropyl]amino]cyclohexanecarboxylic acid.

Example 19

(cis)-4-[[3,3,3-Trifluoro-2-(mercaptomethyl)-1-oxopropyl]amino]cyclohexanecarboxylic acid

a) (cis)-4-[[3,3,3-Trifluoro-2-[(acetylthio)methyl]-1-oxopropyl]amino]cyclohexanecarboxylic acid

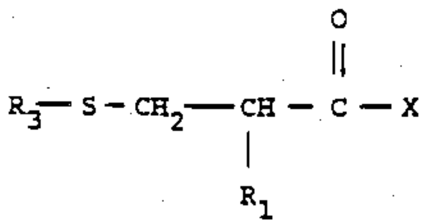
A suspension of (cis)-4-aminocyclohexanecarboxylic acid (2.15 g., 15 mmole) in dry acetonitrile (distilled from calcium hydride) under argon was cooled to 0° and bis(trimethylsilyl) trifluoroacetamide (8 ml., 30 mmole) was added. The reaction mixture was allowed to stir and warm to room temperature overnight. An additional 4 ml. of bis(trimethylsilyl)trifluoroacetamide was added. After an additional 4 hours of stirring, dimethylformamide (6 ml.) was added to bring the remaining solid into solution. After 4 hours, almost all of the solid had dissolved. The mixture was cooled to 5° and a solution of 2-[(acetylthio)methyl]-3,3,3-trifluoropropanoyl chloride [3.52 g., 15 mmole, prepared as described in Example 10(a)] in acetonitrile (7 ml.) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 36 hours. The solvents were removed in vacuo. The residue was partitioned between water (50 ml.) and ethyl acetate (30 ml.) and the organic layer was separated. The aqueous layer was extracted twice more with ethyl acetate, and the combined ethyl extract was washed with brine, dried ($MgSO_4$) and evaporated to give 8.2 g. of a yellow residue. A portion of this crude product (5.85 g.) was triturated with ethyl acetate:hexane (1:2) and the white solid that separated was filtered and washed with ethyl acetate:hexane (1:2 and then 2:1) to give 0.9 g. of pure (cis)-4-[[3,3,3-trifluoro-2-[(acetylthio)methyl]-1-oxopropyl]amino]-cyclohexanecarboxylic acid as a white solid; m.p. 171 - 174°. TLC (silica gel; toluene:acetic acid, 10:1) R_f = 0.20.

b) (cis)-4-[[3,3,3-Trifluoro-2-(mercaptomethyl)-1-oxopropyl]amino]cyclohexanecarboxylic acid

Treatment of the product from part (a) with concentrated ammonium hydroxide according to the procedure of Example 10(c) yields (cis)-4-[[3,3,3-trifluoro-2-(mercaptomethyl)-1-oxopropyl]amino]-cyclohexanecarboxylic acid.

Examples 20 - 50

Following the procedures of Examples 1 - 19 the following additional examples within the scope of this invention can be obtained:



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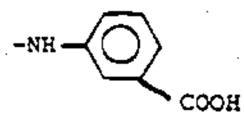
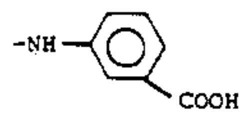
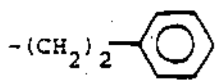
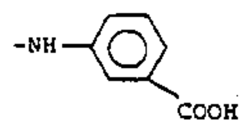
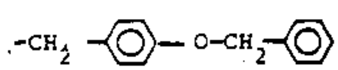
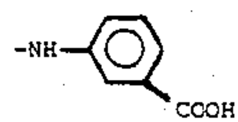
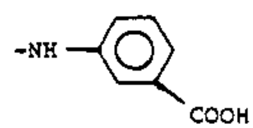
20	H	$-C_2H_5$	
21	H	$-CH(CH_3)_2$	
22	H		
23	H		
24	H	$-C_3H_7$	

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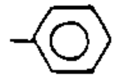
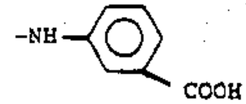
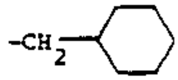
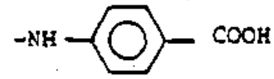
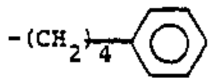
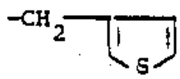
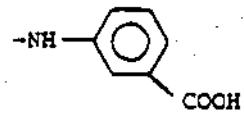
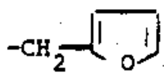
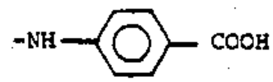
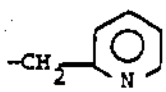
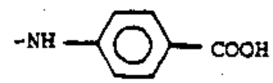
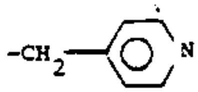
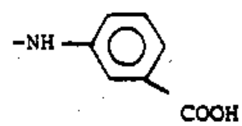
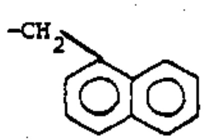
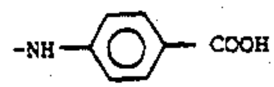
25	H		
26	H		
27	H		
28	H		
29	H		
30	H		
31	H		
32	H		

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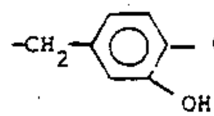
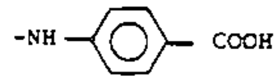
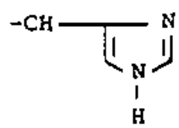
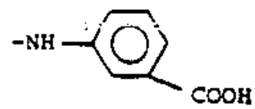
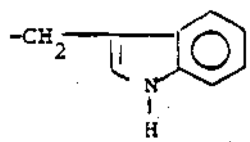
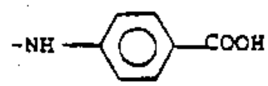
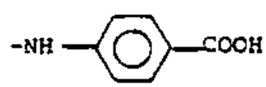
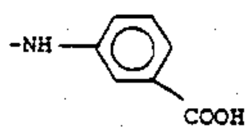
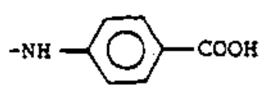
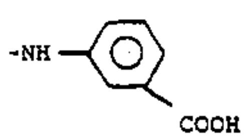
33	H		
34	H		
35	H		
36	H	$-(CH_2)_4-NH_2$	
37	H	$-(CH_2)_2-S-CH_3$	
38	H	$-(CH_2)_3-NH-C(=NH)-NH_2$	
39	H	$-CH_2-C(=O)-NH_2$	

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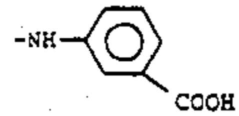
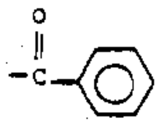
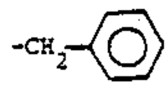
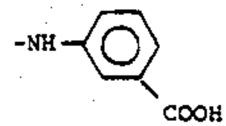
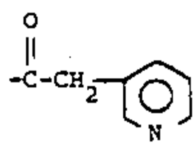
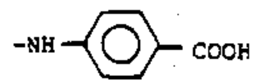
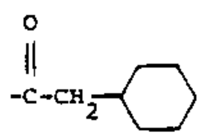
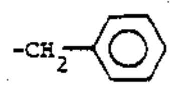
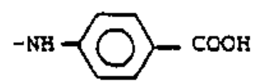
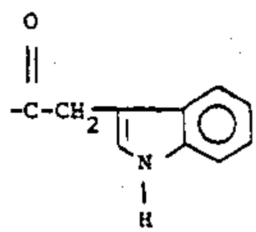
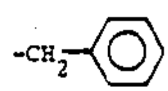
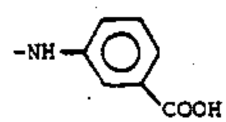
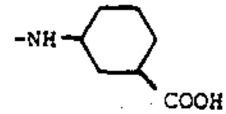
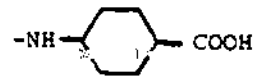
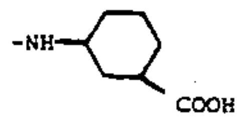
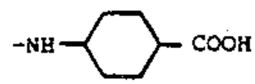
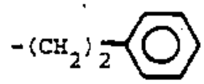
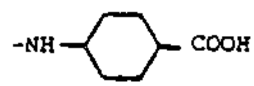
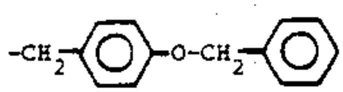
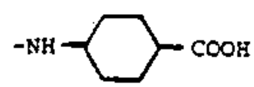
40	$-\overset{O}{\overset{\|\|}{C}}-CH_3$	$-CH-(CH_3)_2$	
41			
42		$-C_2H_5$	
43			
44			

Ex	R_3	R_1	X
45	H	$-CF_3$	
46	H	$-CH(CH_3)_2$	
47	H	$-CH_2CH(CH_3)_2$	
48	H	$-C_2H_5$	
49	H		
50	H		

Example 51

1000 tablets each containing the following ingredients:

4-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid	100 mg.
Cornstarch	50 mg.
Gelatin	7.5 mg.
Avicel (microcrystalline cellulose)	25 mg.
Magnesium stearate	2.5 mg.
	185 mg.

are prepared from sufficient bulk quantities by mixing the product of Examples 1 or and cornstarch with an aqueous solution of the gelatin. The mixture is dried and ground to a powder. The Avicel and then the magnesium stearate are admixed with granulation. This mixture is then compressed in a tablet press to form 1000 tablets each containing 100 mg. of active ingredient. This same procedure can be employed to prepare tablets containing 50 mg. of active ingredient.

Similarly, tablets containing 50 mg. or 100 mg. of the product of any of Examples 2 to 16 and 18 to 50 can be prepared.

Example 52

Two piece #1 gelatin capsules are filled with a mixture of the following igredients:

3-[[2-(Mercaptomethyl)-1-oxo-3-phenylpropyl]amino]benzoic acid	100 mg.
Magnesium stearate	7 mg.
Lactose	193 mg.
	300 mg.

In a similar manner, capsules containing 100 mg. of the product of any of Examples 1 to 6, 8 to 16, and 18 to 50 can be prepared.

Example 53

An injectable solution is prepared as follows:

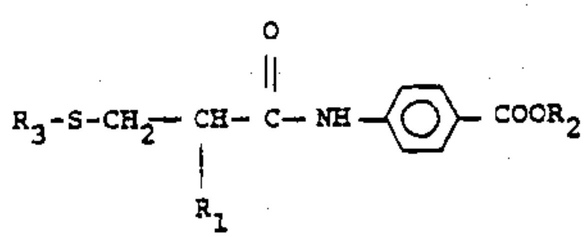
3-[[3,3,3-Trifluoro-2-(mercaptomethyl)-1-oxopropyl]amino]benzoic acid	500 g.
Methyl paraben	5 g.
Propyl paraben	1 g.
Sodium chloride	25 g.
Water for injection	5 l.

The active substance, preservatives and sodium chloride are dissolved in 3 liters of water for injection and then the volume is brought up to 5 liters. The solution is filtered through a sterile filter and aseptically filled into presterilized vials with rubber closures. Each vial contains 5 ml. of solution in a concentration of 100 mg. of active ingredient per ml. of solution for injection.

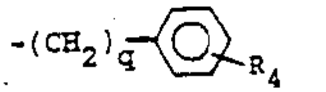
In a similar manner, an injectable solution containing 100 mg. of active ingredient per ml. of solution can be prepared for the product of any of Examples 1 to 10, 12 to 16, and 18 to 50.

Claims

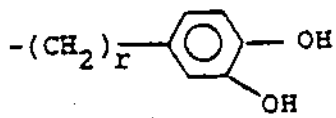
1. A compound of the formula



or a pharmaceutically acceptable salt thereof wherein:
R_1 is hydrogen, straight or branched chain lower alkyl of 2 to 7 carbons, halo substituted lower alkyl,


,

$-(CH_2)_r$-cycloalkyl, $-(CH_2)_r$-(α-naphthyl), $-(CH_2)_r$-(β-naphthyl),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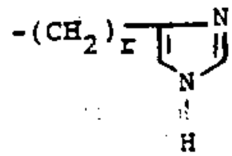
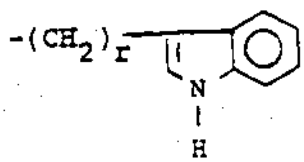
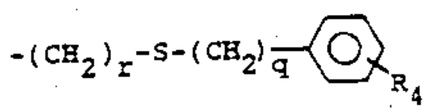
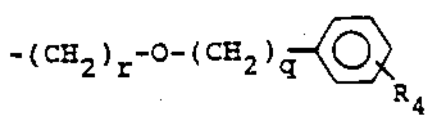

,


,

$-(CH_2)_r-NH_2$, $-(CH_2)_r-SH$, $-(CH_2)_r$-S-lower alkyl,


,

$-(CH_2)_r-OH$, $-(CH_2)_r$-O-lower alkyl,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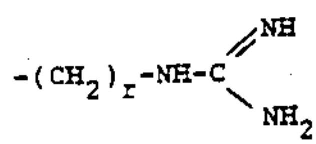
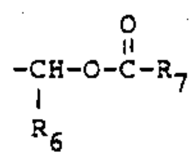

,

or

$-(CH_2)_r-\overset{O}{\overset{\|}{C}}-NH_2$;

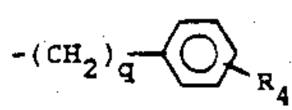
R_2 is hydrogen, lower alkyl, benzyl, benzhydryl, a pharmaceutically acceptable salt forming ion, or


;

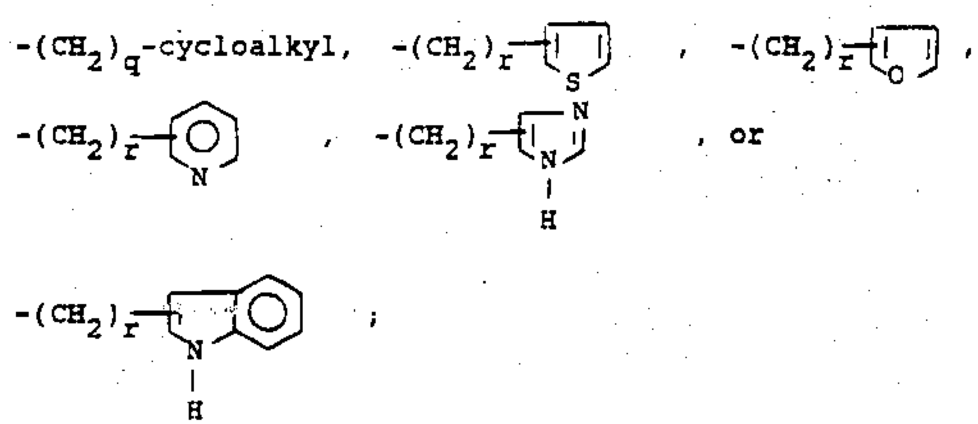
R_3 is hydrogen or

$R_5-\overset{O}{\overset{\|}{C}}-$;

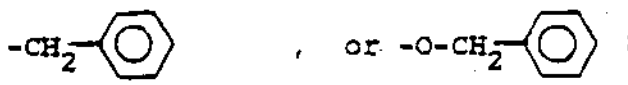
R_5 is lower alkyl,


,

-$(CH_2)_q$-(α-naphthyl), -$(CH_2)_q$-(β-naphthyl),

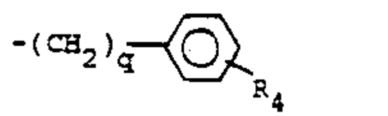


R_4 is hydrogen, lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4 carbons, lower alkylthio of 1 to 4 carbons, halo, hydroxy, CF_3, phenyl,



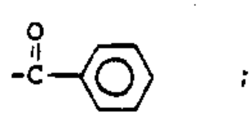
R_6 is hydrogen, lower alkyl, cycloalkyl, or phenyl;
R_7 is hydrogen, lower alkyl, lower alkoxy or phenyl;
r is an integer from 1 to 4; and
q is zero or an integer from 1 to 7.

2. A compound of Claim 1 wherein:
R_1 is straight or branched chain alkyl of 2 to 4 carbons,

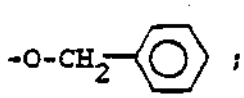


or trifluoromethyl;
R_2 is hydrogen, methyl, or an alkali metal salt ion;
R_3 is hydrogen,

$-\overset{O}{\overset{\|}{C}}-CH_3$ or



R_4 is hydrogen or

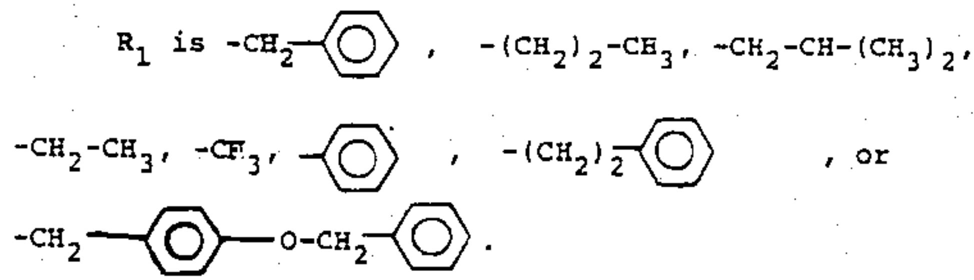


q is zero or an integer from 1 to 4.

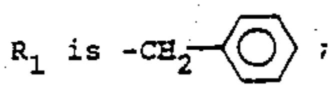
3. A compound of Claim 2 wherein:

R_2 is hydrogen; and
R_3 is hydrogen.

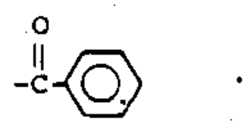
4. A compound of Claim 3 wherein:



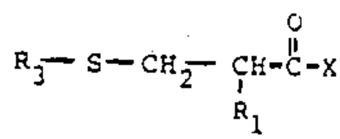
5. A compound of Claim 2 wherein:



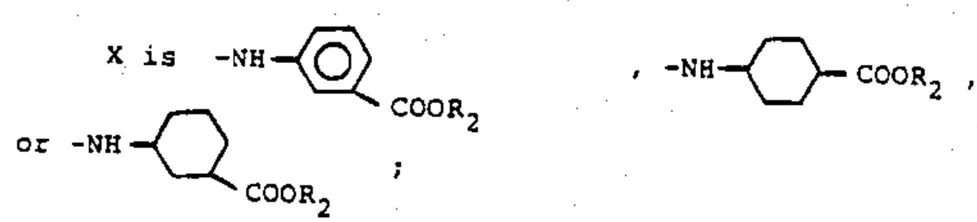
R_2 is hydrogen; and
R_3 is
$-\overset{C}{\overset{\|}{C}}-CH_3$ or



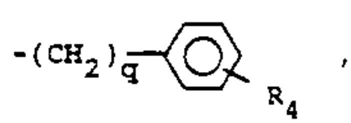
6. A compound of the formula



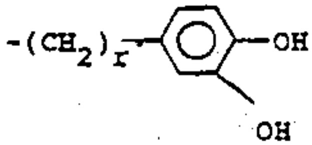
or a pharmaceutically acceptable salt thereof wherein:



R_1 is hydrogen, lower alkyl, halo substituted lower alkyl,



$-(CH_2)_r$-cycloalkyl, $-(CH_2)_r$-(α-naphthyl), $-(CH_2)_r$-(β-naphthyl),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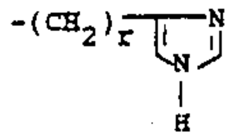
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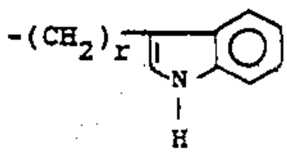
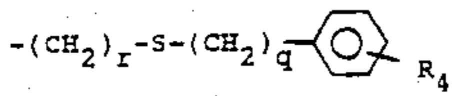
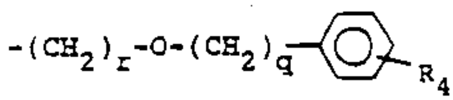


$-(CH_2)_r-NH_2$, $-(CH_2)_r-SH$, $-(CH_2)_r$-S-lower alkyl,


,

$-(CH_2)_r-OH$, $-(CH_2)_r$-O-lower alkyl,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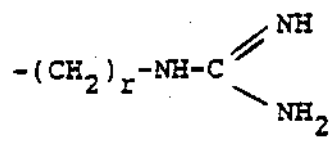
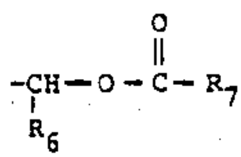

,

or

$-(CH_2)_r-\overset{O}{\overset{\|}{C}}-NH_2$;

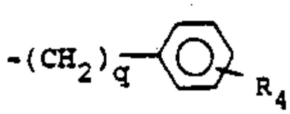
R_2 is hydrogen, lower alkyl, benzyl, benzhydryl, a pharmaceutically acceptable salt forming ion, or


;

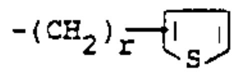
R_3 is hydrogen or

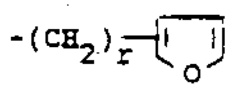
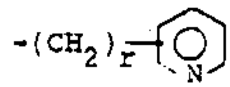
$R_5-\overset{O}{\overset{\|}{C}}-$;

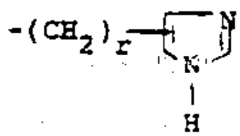
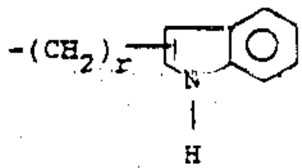
R_5 is lower alkyl,


,

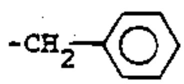
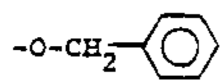
$-(CH_2)_q$-(α-naphthyl), $-(CH_2)_q$-(β-naphthyl),

$-(CH_2)_q$-cycloalkyl,  ,

 ,  ,

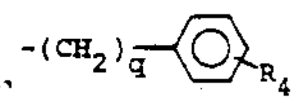
 , or  ;

R_4 is hydrogen, lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4 carbons, lower alkylthio of 1 to 4 carbons, halo, hydroxy, CF_3, phenyl,

 , or  ;

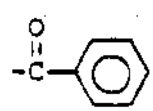
R_6 is hydrogen, lower alkyl, cycloalkyl, or phenyl;
R_7 is hydrogen, lower alkyl, lower alkoxy, or phenyl;
r is an integer from 1 to 4; and
q is zero or an integer from 1 to 7.

7. A compound of Claim 6 wherein:
R_1 is straight or branched chain alkyl of 2 to 4 carbons,

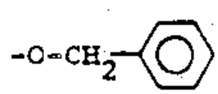
 ,

or trifluoromethyl;
R_2 is hydrogen, methyl, or an alkali metal salt ion;
R_3 is hydrogen,

$-\overset{O}{\overset{\|}{C}}-CH_3$ or

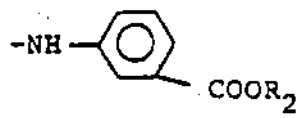
 ;

R_4 is hydrogen or

 ;

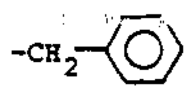
q is zero or an integer from 1 to 4.

8. A compound of Claim Claim 7 wherein:
X is



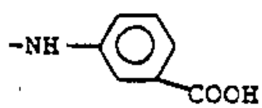
9. A compound of Claim 8 wherein:
R_2 is hydrogen; and
R_3 is hydrogen.

10. A compound of Claim 9 wherein:
R_1 is

,

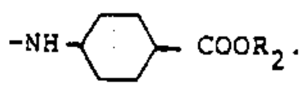
$-CH_2-CH-(CH_3)_2$, or CF_3.

11. A compound of Claim 6 wherein:
X is

;

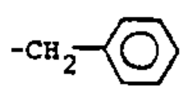
R_1 is methyl; and
R_3 is hydrogen.

12. A compound of Claim 7 wherein:
X is



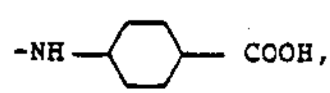
13. A compound of Claim 12 wherein:
R_2 is hydrogen; and
R_3 is hydrogen.

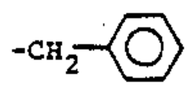
14. A compound of Claim 13 wherein:
R_1 is



or CF_3.

15. A compound of Claim 7 wherein

X is ,

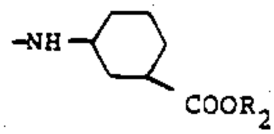
R_1 is ,

and
R_3 is

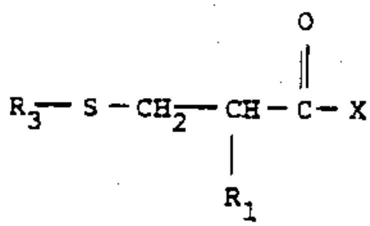
$-\overset{O}{\overset{\|}{C}}-CH_3$.

16. A compound of Claim 7 wherein

X is

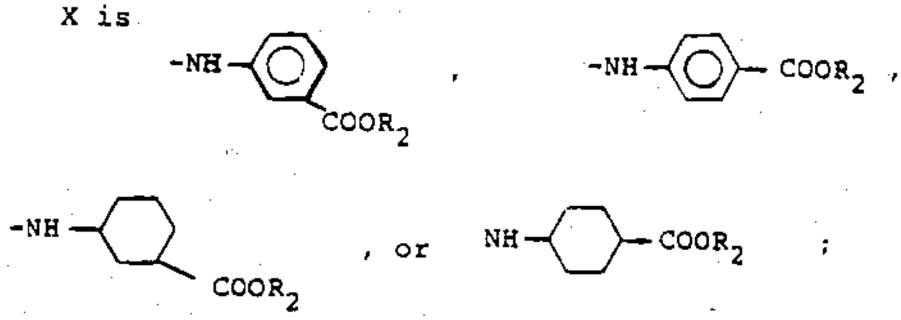


17. A pharmaceutical composition useful for reducing blood pressure and producing diuresis and natriuresis as well as treating congestive heart failure, pain and/or diarrhea in a mammalian host comprising a pharmaceutically acceptable carrier and an endopeptidase inhibiting compound of the formula

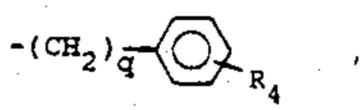


or a pharmaceutically acceptable salt thereof wherein:

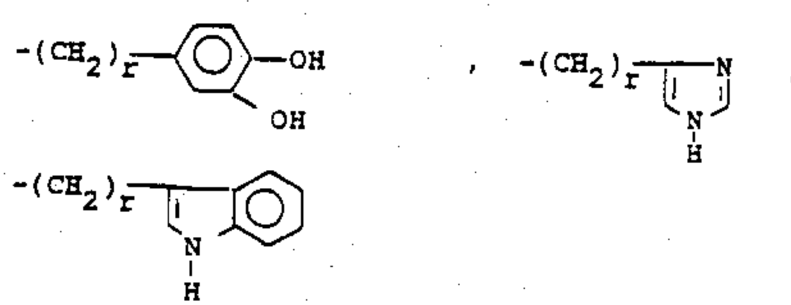
X is



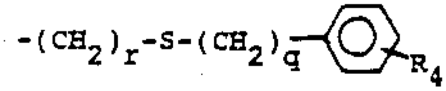
R_1 is hydrogen, lower alkyl, halo substituted lower alkyl,



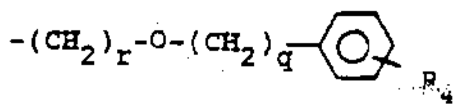
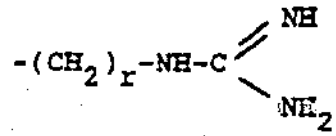
$-(CH_2)_r$-cycloalkyl, $-(CH_2)_r$-(α-naphthyl), $-(CH_2)_r$-(β-naphthyl),



$-(CH_2)_r-NH_2$, $-(CH_2)_r-SH$, $-(CH_2)_r-S$-lower alkyl,

,

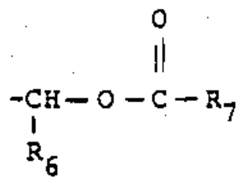
$-(CH_2)_r-OH$, $-(CH_2)_r-O$-lower alkyl,

, ,

or

$-(CH_2)_r-\overset{O}{\overset{\|}{C}}-NH_2$;

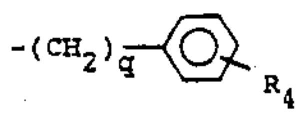
R_2 is hydrogen, lower alkyl, benzyl, benzhydryl, a pharmaceutically acceptable salt forming ion, or

;

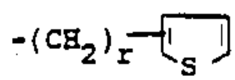
R_3 is hydrogen or

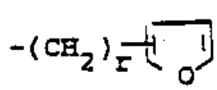
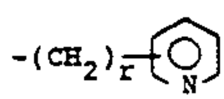
$R_5-\overset{O}{\overset{\|}{C}}-$;

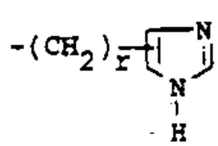
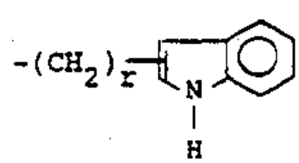
R_5 is lower alkyl,

,

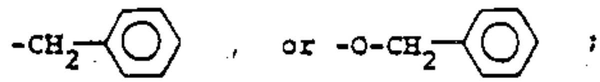
$-(CH_2)_q$-(α-naphthyl), $-(CH_2)_q$-(βnaphthyl),

$-(CH_2)_q$-cycloalkyl, ,

, ,

, or ;

R_4 is hydrogen, lower alkyl of 1 to 4 carbons, lower alkoxy of 1 to 4 carbons, lower alkylthio of 1 to 4 carbons, halo, hydroxy, CF_3, phenyl,



R_6 is hydrogen, lower alkyl, cycloalkyl, or phenyl;
R_7 is hydrogen, lower alkyl, lower alkoxy, or phenyl;
r is an integer from 1 to 4; and
q is zero or an integer from 1 to 7.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 89 11 7683

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X,Y	US-A-4 132 802 (T.A. MARTIN) * Column 1, line 45 - column 2, line 33; column 6, lines 61,62 *	1,6,11	C 07 C 323/60 C 07 C 327/32 A 61 K 31/195
Y	CHEMICAL ABSTRACTS, vol. 67, no. 5, 31th July 1967, page 2036, abstract no. 21570j, Columbus, Ohio, US; G.M. AIRAPETYAN et al.: "Preparation of some derivatives of beta-mercaptopropionic acid, cystamine, and cycteamine and study of their radioprotective activity", & IZV. AKAD. NAUK SSSR, SER. KHIM. 1967(2),334-41 * Page 2036, column 2, line 18 *	1-3	
A	BE-A- 890 948 * Claim 1 *	1-3,6-11	
A	EP-A-0 115 997 (ROUSSEL-UCLAF) * Page 15, example 43; claim 1 *		

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 07 C 323/00
C 07 C 327/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	06-12-1989	ZAROKOSTAS K.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0401)

AP

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CH-4125 Riehen (CH)
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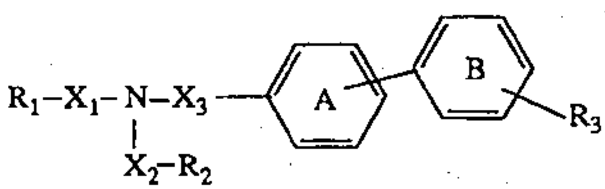
Friedensgasse 36
CH-4056 Basel (CH)

(54) **Acyl compounds.**

(57) Verbindungen der Formel



(I),

worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO, SO_2 oder -O-C(=O)-, wobei das Kohlenstoffatom der Carbonylgruppe an das in der Formel I eingezeichnete Stickstoffatom gebunden ist, steht; X_2 einen gegebenenfalls durch Hydroxy, Carboxy, Amino, Guanidino, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest oder einen zweiwertigen cycloaliphatischen Kohlenwasserstoffrest bedeutet, wobei ein Kohlenstoffatom des aliphatischen Kohlenwasserstoffrestes zusätzlich durch einen zweiwertigen aliphatischen Kohlenwasserstoffrest überbrückt sein kann; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, 1H-Tetrazol-5-yl, Pyridyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 einen zweiwertigen aliphatischen Kohlenwasserstoff bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind; in freier Form oder in Salzform, sind in an sich bekannter Weise herstellbar und können beispielsweise als Azneimittelwirkstoffe verwendet werden.

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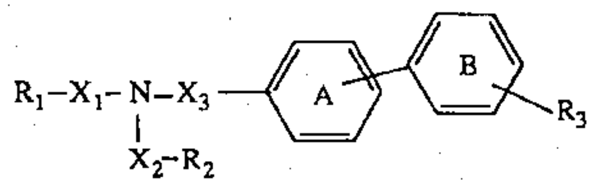
ACYLVERBINDUNGEN

Die Erfindung betrifft Verbindungen der Formel



(I),

worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO, SO_2 oder -O-C(=O)-, wobei das Kohlenstoffatom der Carbonylgruppe an das in der Formel I eingezeichnete Stickstoffatom gebunden ist, steht; X_2 einen gegebenenfalls durch Hydroxy, Carboxy, Amino, Guanidino, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest oder einen zweiwertigen cycloaliphatischen Kohlenwasserstoffrest bedeutet, wobei ein Kohlenstoffatom des aliphatischen Kohlenwasserstoffrestes zusätzlich durch einen zweiwertigen aliphatischen Kohlenwasserstoffrest überbrückt sein kann; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, 1H-Tetrazol-5-yl, Pyridyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 einen zweiwertigen aliphatischen Kohlenwasserstoff bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind; in freier Form oder in Salzform, ein Verfahren zur Herstellung dieser Verbindungen, die Verwendung dieser Verbindungen und pharmazeutische Präparate, enthaltend eine solche Verbindung I in freier Form oder in Form eines pharmazeutisch verwendbaren Salzes.

Die Verbindungen I können als, insbesondere pharmazeutisch verwendbare, Salze vorliegen. Weisen die Verbindungen I z. B. mindestens ein basisches Zentrum auf, können sie Säureadditionssalze bilden. Diese werden beispielsweise mit starken anorganischen Säuren, wie Mineralsäuren, z.B. Schwefelsäure, einer Phosphorsäure oder einer Halogenwasserstoffsäure, mit starken organischen Carbonsäuren, wie gegebenenfalls, z.B. durch Halogen, substituierten C_1-C_4-Alkancarbonsäuren, z.B. Essigsäure, wie gegebenenfalls ungesättigten Dicarbonsäuren, z.B. Oxal-, Malon-, Bernstein-, Malein-, Fumar-, Phthal- oder Terephthalsäure, wie Hydroxycarbonsäuren, z.B. Ascorbin-, Glykol-, Milch-, Äpfel-, Wein- oder Zitronensäure, wie Aminosäuren, z.B. Asparagin- oder Glutaminsäure, oder wie Benzoesäure, oder mit organischen Sulfonsäuren, wie gegebenenfalls, z.B. durch Halogen, substituierten C_1-C_4-Alkan- oder Aryl-sulfonsäuren, z.B. Methan- oder p-Toluolsulfonsäure, gebildet. Entsprechende Säureadditionssalze können auch mit einem gegebenenfalls zusätzlich vorhandenen basischen Zentrum gebildet werden. Ferner können die Verbindungen I mit mindestens einer aciden Gruppe (beispielsweise COOH oder 5-Tetrazolyl) Salze mit Basen bilden. Geeignete Salze mit Basen sind beispielsweise Metallsalze, wie Alkali- oder Erdalkalimetallsalze, z.B. Natrium-, Kalium- oder Magnesiumsalze, oder Salze mit Ammoniak oder einem organischen Amin, wie Morpholin, Thiomorpholin, Piperidin, Pyrrolidin, einem Mono-, Di- oder Triniederalkylamin, z. B. Ethyl-, tert.-Butyl-, Diethyl-, Diisopropyl-, Triethyl-, Tributyl- oder Dimethyl-propyl-amin, oder einem Mono-, Di- oder Trihydroxyniederalkylamin, z.B. Mono-, Di- oder Triethanolamin. Weiterhin können entsprechende innere Salze gebildet werden. Umfasst sind ferner für pharmazeutische Verwendungen nicht geeignete Salze, die beispielsweise für die Isolierung bzw. Reinigung von freien Verbindungen I oder deren pharmazeutisch verwendbaren Salzen eingesetzt werden.

Ein aliphatischer Kohlenwasserstoffrest ist beispielsweise Niederalkyl, Niederalkenyl oder in zweiter Linie Niederalkinyl.

Ein durch Halogen oder Hydroxy substituierter aliphatischer Rest bedeutet beispielsweise Halogenniederalkyl, -niederalkenyl, -niederalkinyl, Hydroxyniederalkyl, -niederalkenyl oder -niederalkinyl.

Ein cycloaliphatischer Kohlenwasserstoffrest stellt insbesondere Cycloalkyl und in zweiter Linie Cycloalkenyl dar.

Als araliphatischer Rest kommt insbesondere Phenylniederalkyl, ferner Phenylniederalkenyl oder -niederalkinyl in Frage.

Ein zweiwertiger Kohlenwasserstoffrest, der ein C-Atom eines aliphatischen Restes X_2 überbrückt, bedeutet beispielweise C_2-C_8-Alkylen, insbesondere C_4-C_5-Alkylen.

Ein cycloaliphatischer Rest bedeutet beispielsweise einen gegebenenfalls ein- oder ferner mehrfach, z.B.

zweifach, z.B. durch gegebenenfalls verestertes oder amidiertes Carboxy oder gegebenenfalls acetalisiertes Formyl, substituiertes Cycloalkyl bzw. in zweiter Linie Cycloalkenyl.

Ein aromatischer Rest bedeutet beispielsweise einen carbocyclischen oder heterocyclischen aromatischen Rest, insbesondere Phenyl oder insbesondere einen entsprechenden 5- oder 6-gliedrigen und monocyclischen Rest, der bis zu vier gleiche oder verschiedene Heteroatome, wie Stickstoff-, Sauerstoff- bzw. Schwefelatome, vorzugsweise ein, zwei, drei oder vier Stickstoffatome, ein Sauerstoff- oder ein Schwefelatom, aufweist. Entsprechende 5-gliedrige Heteroarylreste sind z.B. monoaza-, diaza-, triaza-, tetraaza-, monooxa- oder monothia-cyclische Arylreste, wie Pyrrolyl, Pyrazolyl, Imidazolyl, Triazolyl, Tetrazolyl, Furyl und Thienyl, während als entsprechende 6-gliedrige Reste insbesondere Pyridyl in Frage kommt. Entsprechende aromatische Reste sind gegebenenfalls ein- oder mehrfach, z.B. zwei- oder dreifach, substituiert, beispielsweise durch gleiche oder verschiedene Reste, z.B. ausgewält aus: Halogen, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R und einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest, der gegebenenfalls durch -O- unterbrochen ist sowie gegebenenfalls zusätzlich, z.B. durch gegebenenfalls verestertes oder amidiertes Carboxy oder gegebenenfalls acetalisiertes Formyl, substituiert ist.

Ein zweiwertiger aliphatischer Kohlenwasserstoffrest (X_2) bedeutet beispielsweise Alkylen oder Alkyliden.

Ein zweiwertiger cycloaliphatischer Kohlenwasserstoffrest bedeutet beispielweise Cycloalkylen.

Verestertes Carboxy bedeutet beispielsweise Carboxy, welches durch einen Alkohol verestert ist, der sich von einem aliphatischen oder araliphatischen Kohlenwasserstoffrest ableitet, wie Niederalkyl, Phenylniederalkyl, Niederalkenyl und in zweiter Linie Niederalkinyl, und der gegebenenfalls durch -O- unterbrochen ist, wie Niederalkoxyniederalkyl, -niederalkenyl und -niederalkinyl. Beispielhaft seien Niederalkoxy-, Phenylniederalkoxy-, Niederalkenyloxy- und Niederalkoxyniederalkoxy-carbonyl genannt.

Amidiertes Carboxy ist beispielsweise Carbamoyl, in welchem die Aminogruppe gegebenenfalls durch einen aliphatischen oder araliphatischen Kohlenwasserstoffrest mono- oder unabhängig voneinander disubstituiert oder durch einen zweiwertigen aliphatischen Kohlenwasserstoffrest, der gegebenenfalls durch -O- unterbrochen oder an zwei benachbarten C-Atomen mit einem Benzolring kondensiert ist, insbesondere Niederalkylen oder Niederalkylenoxyniederalkylen, disubstituiert ist. Als Beispiele für entsprechend substituierte Aminogruppen seien Niederalkyl-, Niederalkenyl-, Niederalkinyl-, Phenylniederalkyl-, Phenylniederalkenyl-, Phenylniederalkinyl-, Diniederalkyl-, N-Niederalkyl-N-phenylniederalkyl- und Diphenylniederalkyamino sowie Chinol-1-yl, Isochinol-2-yl, Niederalkylen- und Niederalkylenoxyniederalkylen-amino genannt.

Substituiertes Amino hat die im Zusammenhang mit substituiertem Carbamoyl angegebenen Bedeutungen und bedeutet weiterhin Acylamino, wie Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl- oder Benzolsulfonylamino.

Acetalisiertes Formyl stellt beispielsweise Diniederalkoxymethyl oder Oxyniederalkylenoxymethylen dar.

Verethertes Hydroxy bedeutet z.B. mit einem aliphatischen Alkohol verethertes Hydroxy, insbesondere Niederalkoxy oder Niederalkenyloxy, und steht ebenso für einen Phenylniederalkoxy- oder Phenoxyrest.

In N-substituiertem Sulfamoyl hat die substituierte Aminogruppe die im Zusammenhang mit substituiertem Carbamoyl angegebenen Bedeutungen.

Ein aliphatischer Kohlenwasserstoffrest, der durch -O- unterbrochen ist, bedeutet insbesondere Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl, Niederalkenyloxyniederalkyl, -niederalkenyl oder -niederalkinyl.

Vor- und nachstehend sind ungesättigte aliphatische, cycloaliphatische und araliphatische Substituenten in erster Linie nicht über das C-Atom, von dem eine Mehrfachbindung ausgeht, mit einem aromatischen Rest verknüpft.

(Hetero-)Aromatische Reste sind insbesondere, sofern nicht abweichend definiert, jeweils unsubstituiert oder ein- oder mehrfach, z.B. zwei- oder dreifach, insbesondere z.B. durch einen Substituenten ausgewählt aus Halogen, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R und einen gegebenenfalls, z.B. durch Halogen oder Hydroxy, substituierten Kohlenwasserstoffrest, der gegebenenfalls durch -O- unterbrochen ist, substituiert.

Die Ringe A und B stellen in erster Linie ein 4-Biphenylyl-, ferner 2- oder 3- Biphenylylringsystem dar, wobei der Rest R_3 vorzugsweise in ortho-Position des Ringes B lokalisiert ist. Entsprechend sind die Ringe A und B gegebenenfalls ein- oder mehrfach, z.B. zwei- oder dreifach, substituiert, beispielsweise durch gleiche oder verschiedene Reste z.B. ausgewählt aus: Halogen, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R und einen gegebenenfalls durch Halogen oder Hydroxy substituierten Kohlenwasserstoffrest, der gegebenenfalls durch -O- unterbrochen ist.

Die vor- und nachstehend verwendeten Allgemeindefinitionen haben, sofern nicht abweichend definiert, folgende Bedeutungen:

Der Ausdruck "Nieder" bedeutet, dass entsprechende Gruppen und Verbindungen jeweils insbesondere bis und mit 7, vorzugsweise bis und mit 4, Kohlenstoffatome enthalten.

Halogen ist insbesondere Halogen mit Atomnummer bis und mit 35, wie Fluor, Chlor oder Brom, und umfasst ferner Iod.

Alkanoyl ist beispielsweise Niederalkanoyl und bedeutet insbesondere C_2-C_7-Alkanoyl, wie Acetyl, Propionyl, Butyryl, Isobutyryl oder Pivaloyl. Bevorzugt ist C_2-C_5-Alkanoyl.

Halogenalkylsulfamoyl bedeutet insbesondere Halogen-C_1-C_7-alkansulfamoyl und ist z.B. Trifluormethan-, Difluormethan-,1,1,2-Trifluorethan- oder Heptafluorpropansulfamoyl. Bevorzugt ist Halogen-C_1-C_4-alkansulfamoyl.

Niederalkyl bedeutet insbesondere C_1-C_7-Alkyl, z.B. Methyl, Ethyl, n-Propyl, Isopropyl, n-Butyl, Isobutyl, sek.-Butyl, tert.-Butyl, und umfasst ferner entsprechende Pentyl-, Hexyl- und Heptylreste. Bevorzugt ist C_1-C_4-Alkyl.

Niederalkenyl bedeutet insbesondere C_3-C_7-Alkenyl und ist z.B. 2-Propenyl oder 1-,2- oder 3-Butenyl. Bevorzugt ist C_3-C_5-Alkenyl.

Niederalkinyl ist insbesondere C_3-C_7-Alkinyl und bedeutet vorzugsweise Propargyl.

Halogenniederalkyl bedeutet insbesondere Halogen-C_1-C_4-alkyl, wie Trifluormethyl, 1,1,2-Trifluor-2-chlorethyl oder Chlormethyl.

Halogenniederalkenyl bedeutet insbesondere Halogen-C_3-C_5-alkenyl, wie 3-Chlorallyl.

Halogenniederalkinyl ist insbesondere Halogen-C_3-C_5-alkinyl, wie 3-Chlorpropargyl.

Hydroxyniederalkyl bedeutet insbesondere Hydroxy-C_1-C_4-alkyl, wie Hydroxymethyl, 2-Hydroxyethyl oder 3-Hydroxypropyl.

Hydroxyniederalkenyl bedeutet insbesondere Hydroxy-C_3-C_5-alkenyl, wie 3-Hydroxyallyl.

Hydroxyniederalkinyl bedeutet insbesondere Hydroxy-C_3-C_5-alkinyl, wie 3-Hydroxypropargyl.

Cycloalkyl ist insbesondere C_3-C_7-Cycloalkyl und bedeutet z.B. Cyclopropyl, Cyclobutyl, Cyclopentyl, Cyclohexyl und Cycloheptyl. Bevorzugt ist Cyclopentyl und Cyclohexyl.

Cycloalkenyl ist insbesondere C_3-C_7-Cycloalkenyl und bedeutet vorzugsweise Cyclopent-2-, -3-enyl, Cyclohex-2- und -3-en-yl.

Phenylniederalkyl ist insbesondere Phenyl-C_1-C_4-alkyl und bedeutet vorzugsweise Benzyl, 1- und 2-Phenethyl, während Phenylniederalkenyl und Phenylniederalkinyl insbesondere Phenyl-C_3-C_5-alkenyl und -alkinyl bedeuten, insbesondere 3-Phenylallyl und 3-Phenylpropargyl.

Pyrrolyl ist z.B. 2- oder 3-Pyrrolyl. Pyrazolyl ist 3- oder 4-Pyrazolyl. Imidazolyl ist 2- oder 4-Imidazolyl. Triazolyl ist z.B. 1,3,5-1H-Triazol-2-yl oder 1,3,4-Triazol-2-yl. Tetrazolyl ist z.B. 1,2,3,4-Tetrazol-5-yl, Furyl ist 2- oder 3-Furyl und Thienyl ist 2- oder 3-Thienyl, während als Pyridyl 2-, 3- und 4-Pyridyl in Frage kommt.

Alkylen bedeutet insbesondere C_1-C_{10}-Alkylen oder Niederalkylen, wie C_1-C_7-Alkylen, und ist geradkettig oder verzweigt und bedeutet insbesondere Methylen, Ethylen, Propylen und Butylen sowie 1,2-Propylen, 2-Methyl- 1,3-propylen und 2,2-Dimethyl-1,3-propylen. Bevorzugt ist C_1-C_5-Alkylen.

Alkyliden bedeutet insbesondere C_2-C_{10}-Alkyliden, wie Ethyliden, 1,1- oder 2,2-Propyliden, ferner 1,1- oder 2,2-Butyliden oder 1,1-, 2,2- oder 3,3-Pentyliden. Bevorzugt ist C_2-C_5-Alkyliden.

Cycloalkylen ist insbesondere C_3-C_7-Cycloalkylen und bedeutet z.B. 1,2-Cyclopropylen, 1,2- oder 1,3-Cyclobutylen, 1,2-, 1,3-Cyclopentylen, 1,2-, 1,3- oder 1,4-Cyclohexylen und 1,2-, 1,3- oder 1,4-Cycloheptylen. Bevorzugt ist 1,3-Cyclopentylen und 1,4-Cyclohexylen.

Niederalkoxy bedeutet insbesondere C_1-C_7-Alkoxy und ist z.B. Methoxy, Ethoxy, n-Propyloxy, Isopropyloxy, n-Butyloxy, Isobutyloxy, sek.-Butyloxy, tert.-Butyloxy und umfasst ferner entsprechende Pentyloxy-, Hexyloxy- und Heptyloxyreste. Bevorzugt ist C_1-C_4-Alkoxy.

Niederalkoxyniederalkyl bedeutet insbesondere C_1-C_4-Alkoxy-C_1-C_4-alkyl, wie 2-Methoxy-ethyl, 2-Ethoxy-ethyl, 2-n-Propyloxy-ethyl oder Ethoxymethyl.

Niederalkoxyniederalkenyl bzw. -niederalkinyl bedeutet insbesondere C_1-C_5-Alkoxy-C_3-C_5-alkenyl bzw. -alkinyl.

Niederalkoxycarbonyl bedeutet insbesondere C_2-C_8-Alkoxycarbonyl und ist z.B. Methoxy-, Ethoxy-, Propyloxy- oder Pivaloyloxy-carbonyl. Bevorzugt ist C_2-C_5-Alkoxycarbonyl.

Phenylniederalkoxycarbonyl bedeutet insbesondere Phenyl-C_1-C_4-alkoxy-carbonyl und ist z.B. Benzyloxy-, 1- oder 2-Phenylethoxy-, 3-Phenylpropyloxy- oder 4-Phenylbutyloxy-carbonyl. Bevorzugt ist Benzyloxycarbonyl.

Niederalkenyloxycarbonyl bedeutet insbesondere C_3-C_5-Alkenyloxy-carbonyl, vorzugsweise Allyloxycarbonyl, während Niederalkinyloxycarbonyl insbesondere C_3-C_5-Alkinyloxy-carbonyl, wie Propargyloxycarbonyl, bedeutet.

Niederalkoxyniederalkoxycarbonyl bedeutet insbesondere C_1-C_4-Alkoxy-C_1-C_4-alkoxycarbonyl, vorzugsweise Ethoxy-ethoxycarbonyl, Methoxyethoxycarbonyl und Isopropyloxy-ethoxycarbonyl.

Niederalkylenoxyniederalkylen bedeutet insbesondere C_1-C_4-Alkylenoxy-C_2-C_4-alkylen, vorzugsweise Ethylenoxyethylen.

Niederalkylamino bedeutet insbesondere C_1-C_7-Alkylamino und ist z.B. Methyl-, Ethyl-, n-Propyl- und Isopropyl-amino. Bevorzugt ist C_1-C_4-Alkylamino.

Niederalkenylamino bedeutet vorzugsweise C_3-C_5-Alkylamino, wie Allyl- und Methallylamino.

Niederalkinylamino bedeutet vorzugsweise C_3-C_5-Alkinylamino, wie Propargylamino.

Phenylniederalkylamino bedeutet vorzugsweise Phenyl-C_1-C_4-alkylamino, insbesondere Benzyl-, 1- und 2-Phenylethylamino.

Phenylniederalkenylamino bedeutet vorzugsweise Phenyl-C_3-C_5-alkenyl-amino, insbesondere 3-Phenylallylamino und 3-Phenylmethallylamino.

Phenylniederalkenylamino bedeutet vorzugsweise Phenyl-C_3-C_5-alkinylamino, insbesondere 3-Phenylpropargylamino.

Diniederalkylamino bedeutet insbesondere Di-C_1-C_4-alkylamino, wie Dimethyl-, Diethyl-, Di-n-propyl-, Methyl-propyl-, Methyl-ethyl-, Methyl-butyl-amino und Dibutylamino.

N-Niederalkyl-N-phenylniederalkylamino bedeutet insbesondere N-C_1-C_4-Alkyl-N-phenyl-C_1-C_4-alkylamino, vorzugsweise Methyl-benzyl-amino und Ethyl-benzyl-amino.

Diphenylniederalkylamino bedeutet insbesondere Di-phenyl-C_1-C_4-alkyl-amino, vorzugsweise Dibenzylamino.

Niederalkylenamino bedeutet insbesondere C_2-C_6-Alkylenamino, vorzugsweise Pyrrolidin-1-yl oder Piperidin-1-yl.

Niederalkylenoxyniederalkylenamino bedeutet insbesondere C_2-C_3-Alkylenoxy-C_2-C_3-alkylenamino, insbesondere Morpholino.

Niederalkanoylamino bedeutet insbesondere C_1-C_5-Alkanoylamino, wie Formyl-, Acetyl-, Propionyl-, Butyryl- oder Pivaloylamino. Bevorzugt ist C_2-C_5-Alkanoylamino.

Phenylniederalkanoylamino bedeutet insbesondere Phenyl-C_2-C_5-alkanoylamino, wie Phenylacetyl- oder Phenylpropionylamino.

Niederalkansulfonylamino bedeutet insbesondere C_1-C_7-Alkansulfonylamino, wie Methan-, Ethan-, Propan- oder Butansulfonylamino. Bevorzugt ist C_1-C_4-Alkansulfonylamino.

Niederalkenyloxy bedeutet insbesondere C_3-C_7-Alkenyloxy und ist z.B. Allyloxy oder But-2-en- oder But-3-enyloxy. Bevorzugt ist C_3-C_5-Alkenyloxy.

Phenylniederalkoxy bedeutet insbesondere Phenyl-C_1-C_4-alkoxy, wie Benzyloxy, 1- oder 2-Phenylethoxy, 3-Phenylpropyloxy oder 4-Phenylbutyloxy.

Niederalkenyloxyniederalkyl bedeutet insbesondere C_3-C_5-Alkenyloxy-C_1-C_4-alkyl, wie 2-Allyloxyethyl, und Niederalkenyloxyniederalkenyl bzw. -niederalkinyl bedeutet insbesondere C_3-C_5-Alkenyloxy-C_3-C_5-alkenyl bzw. -alkinyl.

Ausgedehnte pharmakologische Untersuchungen haben ergeben, dass die Verbindungen I und ihre pharmazeutisch verwendbaren Salze z. B. ausgeprägte Angiotensin-II-antagonisierende Eigenschaften aufweisen.

Bekanntlich hat Angiotensin-II starke vasokonstriktorische Eigenschaften und stimuliert ausserdem die Aldosteronsekretion und bewirkt somit eine deutliche Natrium/Wasser-Retention. Die Folge der Angiotensin-II-Aktivität manifestiert sich unter anderem in einer Erhöhung des Blutdrucks. Die Bedeutung von Angiotensin-II-Antagonisten besteht darin, durch kompetitive Hemmung der Bindung von Angiotensin-II an die Rezeptoren die durch Angiotensin-II bewirkten vasokonstriktorischen und die Aldosteronsekretion-stimulierenden Effekte zu unterdrücken.

Die Angiotensin-II-antagonisierenden Eigenschaften der Verbindungen der Formel I und ihrer pharmazeutisch verwendbaren Salze können im Angiotensin-II-Bindungstest erfasst werden. Dabei werden glatte Muskelzellen der Ratte aus homogenisierter Rattenaorta verwendet. Das feste Zentrifugat wird in 50 mM Tris-Puffer (pH 7,4) unter Einsatz von Peptidaseinhibitoren suspendiert. Die Proben werden 60 Minuten bei 25°C mit ^{125}I-Angiotensin-II (0,175 nM) und einer variierenden Konzentration an Angiotensin-II oder an Testsubstanz inkubiert. Die Inkubation wird dann durch Zugabe von mit eiskaltem Phosphat gepuffertem Kochsalz beendet und es wird durch Whatman GF/F Filter filtriert. Die Filter werden mit einem Gamma-Zähler gezählt. Aus der Dosis-Wirkungs-Kurve werden die IC_{50}-Werte bestimmt. Für die Verbindungen der Formel I und ihre pharmazeurisch verwendbaren Salze werden IC_{50}-Werte ab etwa 10 nM ermittelt.

Zur Bestimmung der Angiotensin-II induzierten Vasokonstriktion können Untersuchungen an dem isolierten Kaninchen-Aortaring herangezogen werden. Hierzu werden von jeder Brust Aortaringe präpariert und zwischen 2 parallelen Klammern bei einer anfänglich bestehenden Spannung von 2 g fixiert. Anschliessend werden die Ringe bei 37°C in 20 ml eines Gewebebades getaucht und mit einem Gemisch aus 95 % O_2 und 5 % CO_2 begast. Die isometrischen Reaktionen werden gemessen. In 20-minütigen Intervallen werden die Ringe abwechselnd mit 10 nM Angiotensin-II (Hypertensin-CIBA) und 5 nM Noradrenalinchlorid stimuliert. Anschliessend werden die Ringe mit ausgewählten Konzentrationen der Testsubstanzen vor der Behandlung mit den Agonisten inkubiert. Die Daten werden mit einem Buxco Digitalcomputer analysiert. Die Konzentrationen, die eine 50%-ige Hemmung der Anfangskontrollwerte bewirken, werden als IC_{50}-Werte angegeben. Für die Verbindungen der Formel I und ihre pharmazeutisch verwendbaren Salze werden IC_{50}-Werte ab etwa 5 nM bestimmt.

Dass die Verbindungen der Formel I und ihre pharmazeutisch verwendbaren Salze durch Angiotensin-II induzierten Bluthochdruck reduzieren können, kann im Testmodell der normotensiven, narkotisierten Ratte verifiziert werden. Nach Kalibration der Präparationen mit jeweils 0,9 % NaCl (1 ml/kg i.v.), Noradrenalin (1 µg/kg i.v.) bzw. Angiotensin-II (0,3 µg/kg i.v.) werden steigende Dosen (3-6) der Testsubstanz durch Bolusinjektion intravenös injiziert, worauf nach jeder Dosis in 5 Minuten-Intervallen Angiotensin-II bzw. Noradrenalin appliziert wird. Der Blutdruck wird direkt in der Halsschlagader gemessen und mit einem on-line Datenerfassungssystem aufgezeichnet (Buxco). Die Spezifität des Angiotensin-II-Antagonismus wird angezeigt durch die selektive Hemmung des von Angiotensin-II, nicht aber des durch Noradrenalin hervorgerufenen Druckeffektes. In diesem Testmodell zeigen die Verbindungen der Formel I und ihre pharmazeutisch verwendbaren Salze ab einer Dosis von etwa 0,3 mg/kg i.v. einen hemmenden Effekt.

Auch im Testmodell der renalen hypertensiven Ratte kann die antihypertensive Aktivität der Verbindungen der Formel I und ihrer pharmazeutisch verwendbaren Salze manifestiert werden. Bei männlichen Ratten wird durch Verengung einer renalen Arterie gemäss der Goldblatt-Methode Bluthochdruck erzeugt. Den Ratten werden mittels einer Magensonde Dosen der Testsubstanz verabreicht. Kontrolltiere erhalten ein äquivalentes Volumen an Lösungsmittel. Blutdruck und Herzschlag werden indirekt an wachen Tieren nach der Schwanzklemm-Methode von Gerold et al. [Helv. Physiol. Acta 24 (1966), 58] vor Verabreichung der Testsubstanz bzw. des Lösungsmittels sowie während des Verlaufs der Experimente in Intervallen gemessen. Der ausgeprägte antihypertensive Effekt kann ab einer Dosis von etwa 30 mg/kg p.o. nachgewiesen werden.

Dementsprechend können die Verbindungen der Formel I und ihre pharmazeutisch verwendbaren Salze z.B. als Wirkstoffe in Antihypertensiva verwendet werden, welche z.B. zur Behandlung von Bluthochdruck sowie von Herzinsuffizienz eingesetzt werden. Ein Erfindungsgegenstand ist somit die Verwendung der Verbindungen der Formel I und ihrer pharmazeutisch verwendbaren Salze zur Herstellung von entsprechenden Arzneimitteln und zur therapeutischen Behandlung von Bluthochdruck sowie von Herzinsuffizienz. Bei der Herstellung der Arzneimittel ist auch die gewerbsmässige Herrichtung der Wirksubstanzen eingeschlossen.

Die Erfindung betrifft in erster Linie Verbindungen der Formel I und ihre Salze, worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO oder SO_2 steht; X_2 einen gegebenenfalls durch Hydroxy, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest oder einen zweiwertigen cycloaliphatischen Kohlenwasserstoffrest bedeutet, wobei ein Kohlenstoffatom des aliphatischen Kohlenwasserstoffrestes zusätzlich durch einen zweiwertigen aliphatischen Kohlenwasserstoffrest überbrückt sein kann; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 einen zweiwertigen aliphatischen Kohlenwasserstoff bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind.

Die Erfindung betrifft insbesondere Verbindungen der Formel I und ihre Salze, worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO oder SO_2 steht; X_2 einen gegebenenfalls durch Hydroxy, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest bedeutet; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 $-CH_2-$ bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind.

Die Erfindung betrifft insbesondere Verbindungen der Formel I und ihre Salze, worin R_1 Niederalkyl, Niederalkenyl, Niederalkinyl, Halogenniederalkyl, -niederalkenyl, -niederalkinyl, Hydroxyniederalkyl, -niederalkenyl, -niederalkinyl, Cycloalkyl, Cycloalkenyl, Phenylniederalkyl, Phenylniederalkenyl oder Phenylniederalkinyl bedeutet; X_1 für CO oder SO_2 steht; X_2 Alkylen oder Alkyliden bedeutet, die gegebenenfalls durch Hydroxy, einen Cycloalkyl-, Cycloalkenyl-, einen Phenylrest oder einen 5- oder 6-gliedrigen, monocyclischen heteroaromatischen Rest mit bis zu vier gleichen oder verschiedenen Heteroatomen substituiert sind, wobei die cyclischen Reste ihrerseits gegebenenfalls substituiert sind durch Carboxy, welches gegebenenfalls verestert ist mit einem Alkohol, der sich von Niederalkyl, Phenylniederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl ableitet, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen; R_2 Carboxy, welches gegebenenfalls verestert ist mit einem Alkohol, der sich von Niederalkyl, Phenylniederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl ableitet, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Amino, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl-, Benzolsulfonyl-amino, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen, Hydroxy, Niederalkoxy, Niederalkenyloxy, Phenylniederalkoxy, Phenoxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 und R für Wasserstoff, Niederalkyl, Niederalkenyl oder Niederalkinyl steht, Niederalkanoyl, Sulfamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3-CH_2- bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenniederalkylsulfamoyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B unabhängig voneinander jeweils gegebenenfalls substituiert sind durch einen oder mehrere Substituenten ausgewählt aus Halogen, Hydroxy, Niederalkoxy, Niederalkenyloxy, jeweils gegebenenfalls durch Halogen oder Hydroxy substituiertes Niederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl, -niederalkinyl, Niederalkenyloxyniederalkyl, -niederalkenyl und -niederalkinyl.

Die Erfindung betrifft insbesondere Verbindungen der Formel I und ihre Salze, worin X_2 Alkylen oder Alkyliden bedeutet, die gegebenenfalls durch Hydroxy, einen Cycloalkyl-, Cycloalkenyl-, einen Phenylrest oder einen 5- oder 6-gliedrigen, monocyclischen heteroaromatischen Rest mit bis zu vier gleichen oder verschiedenen Heteroatomen substituiert sind, wobei ein C-Atom von Alkylen bzw. Alkyliden durch C_2-C_6-Alkylen überbrückt sein kann und wobei die cyclischen Reste ihrerseits gegebenenfalls substituiert sind durch Carboxy, welches gegebenenfalls verestert ist mit einem Alkohol, der sich von Niederalkyl, Phenylniederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl ableitet, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Formyl, Diniederalkoxymethyl oder durch Oxyniederalkylenoxymethylen, oder X_2 C_3-C_7-Cycloalkylen bedeutet; X_3 Niederalkylen oder Niederalkyliden bedeutet; und die Variablen X_1, R_1, R_2, R_3 die unmittelbar vorstehend angegebenen Bedeutungen haben und die (hetero-)aromatischen Ringe einschliesslich der Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können.

Die Erfindung betrifft insbesondere Verbindungen der Formel I und ihre Salze, worin R_1 Niederalkyl, Niederalkenyl, Halogenniederalkyl, -niederalkenyl, Hydroxyniederalkyl, 3-bis 7-gliedriges Cycloalkyl oder Phenylniederalkyl bedeutet; X_1 für CO, SO_2 oder -O-C(=O)-, wobei das Kohlenstoffatom der Carbonylgruppe an das in der Formel I eingezeichnete Stickstoffatom gebunden ist, steht; X_2 C_1-C_{10}-Alkylen oder C_1-C_7-Alkyliden, die gegebenenfalls substituiert sind durch Hydroxy, Carboxy, Amino, Guanidino, einen 3- bis 7-gliedrigen Cycloalkyl-, 3- bis 7-gliedrigen Cycloalkenyl-, Phenyl-, Pyrrolyl-, Pyrazolyl-, Imidazolyl-, Triazolyl-, Tetrazolyl-, Furyl-, Thienyl- oder Pyridylrest, welche ihrerseits gegebenenfalls zusätzlich durch Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl oder Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Formyl, Diniederalkoxymethyl oder Oxyniederalkylenoxymethylen substituiert sein können; R_2 Carboxy, Niederalkoxy-, Phenylniederalkoxy-, Niederalkenyloxy-, Niederalkoxyniederalkoxy-carbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen, das gegebenenfalls an zwei benachbarten Kohlenstoffatomen mit einem Benzolring kondensiert ist, oder Niederalkylenoxyniederalkylen disubstituiert ist, Amino, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl-, Benzolsulfonyl-amino, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 und R für Niederalkyl steht, Niederalkanoyl, Sulfamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 Methylen ist; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenniederalkylsulfamoyl bedeutet; (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls zusätzlich substituiert sind durch einen oder mehrere Substituenten ausgewählt aus Halogen, Hydroxy, Niederalkoxy, jeweils gegebenenfalls durch Halogen oder Hydroxy substituiertes Niederalkyl bzw. Niederalkoxyniederalkyl.

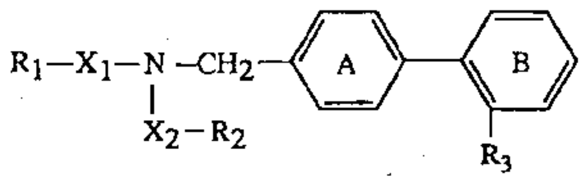
Die Erfindung betrifft insbesondere Verbindungen der Formel I und ihre Salze, worin R_1 Niederalkyl, Niederalkenyl, Halogenniederalkyl, -niederalkenyl, Hydroxyniederalkyl, 3- bis 7-gliedriges Cycloalkyl oder Phenylniederalkyl bedeutet; X_1 für CO oder SO_2 steht; X_2 C_1-C_{10}-Alkylen oder C_1-C_7-Alkyliden, die gegebenenfalls substituiert sind durch Hydroxy, einen 3- bis 7-gliedrigen Cycloalkyl-, 3- bis 7-gliedrigen Cycloalkenyl-, Phenyl-, Pyrrolyl-, Pyrazolyl-, Imidazolyl-, Triazolyl-, Tetrazolyl-, Furyl-, Thienyl- oder Pyridylrest, welche ihrerseits gegebenenfalls zusätzlich durch Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl oder Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Formyl, Diniederalkoxymethyl oder Oxyniederalkylenoxymethylen substituiert sein können; R_2 Carboxy, Niederalkoxy-, Phenylniederalkoxy-, Niederalkenyloxy-, Niederalkoxyniederalkoxy-carbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Amino, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl-, Benzolsulfonyl-amino, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 und R für Niederalkyl steht, Niederalkanoyl, Sulfamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 Methylen ist; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenniederalkylsulfamoyl bedeutet; (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls zusätzlich substituiert sind durch einen oder mehrere Substituenten ausgewählt aus Halogen, Hydroxy, Niederalkoxy, jeweils gegebenenfalls durch Halogen oder Hydroxy substituiertes Niederalkyl bzw. Niederalkoxyniederalkyl.

Die Erfindung betrifft insbesondere Verbindungen der Formel I und ihre Salze, worin X_2 C_1-C_{10}-Alkylen oder C_1-C_7-Alkyliden, die gegebenenfalls substituiert sind durch Hydroxy, einen 3- bis 7-gliedrigen Cycloalkyl-, 3- bis 7-gliedrigen Cycloalkenyl-, Phenyl-, Pyrrolyl-, Pyrazolyl-, Imidazolyl-, Triazolyl-, Tetrazolyl-, Furyl-, Thienyl- oder Pyridylrest, welche ihrerseits gegebenenfalls zusätzlich durch Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl oder Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Formyl, Diniederalkoxymethyl oder durch Oxyniederalkylenoxymethylen substituiert sein können, wobei ein C-Atom von Alkylen bzw. Alkyliden durch C_2-C_8-Alkylen überbrückt sein kann, oder X_2 C_3-C_7-Cycloalkylen bedeutet; X_3 Niederalkylen oder Niederalkyliden bedeutet und die Variablen X_1, R_1, R_2, R_3 die unmittelbar vorstehend angegebenen Bedeutungen haben und die (hetero-)aromatischen Ringe einschliesslich der Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können.

Die Erfindung betrifft insbesondere Verbindungen der Formel I und ihre Salze, worin die Variablen R_1, X_1, R_3 die jeweils vorstehend angegebenen Bedeutungen haben; X_2 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, Phenyl oder Imidazolyl substituiertes Niederalkylen oder Niederalkyliden bedeutet und R_2 Carboxy, Niederalkoxy-, Phenylniederalkoxy-, Niederalkoxyniederalkoxy-carbonyl, Carbamoyl, welches gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Amino, Niederalkanoyl-, Phenylniederalkanoyl-, Niederalkansulfonylamino, Hydroxy, Niederalkoxy, Phenylniederalkoxy oder Phenoxy bedeutet; X_3-CH_2- bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch einen oder mehrere Substituenten ausgewählt aus Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl, Hydroxyniederalkyl oder Niederalkoxyniederalkyl substituiert sind.

Die Erfindung betrifft insbesondere Verbindungen der Formel I und ihre Salze, worin X_2 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, 7-gliedriges Cycloalkenyl, Phenyl oder Imidazolyl substituiertes Niederalkylen oder Niederalkyliden bedeutet, wobei ein C-Atom von Niederalkylen bzw. Niederalkyliden durch C_2-C_6-Alkylen überbrückt sein kann, oder X_2 C_3-C_7-Cycloalkylen bedeutet; und die Variablen X_1, X_3, R_1, R_2, R_3 die unmittelbar vorstehend angegebenen Bedeutungen haben, die Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können.

Die Erfindung betrifft insbesondere Verbindungen der Formel

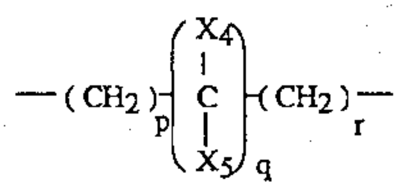


(Ia)

und ihre Salze, worin die Variablen R_1, X_1, X_2, R_2 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben und die Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können.

Die Erfindung betrifft insbesondere Verbindungen der Formel Ia und ihre Salze, worin X_2 gegebenenfalls durch Hydroxy oder 3- bis 7-gliedriges Cycloalkyl substituiertes Niederalkylen oder Niederalkyliden bedeutet, wobei ein C-Atom von Niederalkylen bzw. Niederalkyliden durch C_2-C_6-Alkylen, insbesondere C_4-C_5-Alkylen, überbrückt sein kann, oder worin X_2 C_3-C_7-Cycloalkylen bedeutet, und die Variablen R_1, X_1, R_2 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben und die Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können.

Die Erfindung betrifft insbesondere Verbindungen der Formel Ia und ihre Salze, worin X_2 für die Gruppe der Formel



(Ib)

steht, in der p für 0 oder 1, q für 1 und r für 0 oder 1 stehen oder in der p für 1 bis 8 und q sowie r jeweils für 0 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, Phenyl oder Imidazolyl substituiertes Niederalkyl oder Phenyl bedeutet und X_5 Wasserstoff oder Niederalkyl bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, Amino, Niederalkanoylamino, Phenylniederalkanoylamino oder Niederalkansulfonylamino bedeutet; und die Variablen R_1, X_1 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind.

Die Erfindung betrifft insbesondere Verbindungen der Formel Ia und ihre Salze, worin X_2 für die Gruppe der Formel Ib steht, in der p für 0 oder 1, q für 1 und r für 0 oder 1 stehen oder in der p für 1 bis 8 und q sowie r jeweils für 0 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, Phenyl oder Imidazolyl substituiertes Niederalkyl oder Phenyl bedeutet und X_5 Wasserstoff oder Niederalkyl bedeutet; oder X_4 und X_5 gemeinsam für C_2-C_8-Alkylen, insbesondere C_4-C_5-Alkylen, stehen, oder X_2 C_3-C_7-Cycloalkylen, insbesondere C_5-C_6-Cycloalkylen, bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, Amino, Niederalkanoylamino, Phenylniederalkanoylamino oder Niederalkansulfonylamino bedeutet; und die Variablen R_1, X_1 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind.

Die Erfindung betrifft insbesondere Verbindungen der Formel Ia und ihre Salze, worin R_1 Niederalkyl, insbesondere C_3-C_5-Alkyl, oder Niederalkenyl, insbesondere C_3-C_5-Alkenyl, bedeutet; X_1 für CO oder ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r für 0 oder 1 und q für 1 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, wie Cyclohexyl, durch gegebenenfalls durch Halogen oder Hydroxy substituiertes Phenyl oder Imidazolyl, wie 4-Imidazolyl, substituiertes Niederalkyl, insbesondere C_1-C_4-Alkyl, oder Phenyl bedeutet; X_5 Wasserstoff oder Niederalkyl, wie C_1-C_4-Alkyl, bedeutet oder X_4 und X_5 gemeinsam für C_2-C_8-Alkylen, wie C_4-C_5-Alkylen, bedeuten, oder X_2 C_3-C_7-Cycloalkylen, wie C_5-C_6-Cycloalkylen, wie 1,4-Cyclohexylen, bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, wie C_2-C_5-Alkoxycarbonyl, Phenylniederalkoxycarbonyl, wie Phenyl-C_1-C_4-alkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, wie C_1-C_4-Alkoxy-C_2-C_5-alkoxycarbonyl, Hydroxy oder Niederalkoxy, wie C_1-C_4-Alkoxy, bedeutet; R_3 Carboxy oder 5-Tetrazolyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind.

Die Erfindung betrifft insbesondere Verbindungen der Formel Ia und ihre Salze, worin R_1 Niederalkyl, insbesondere C_3-C_5-Alkyl, oder Niederalkenyl, insbesondere C_3-C_5-Alkenyl, bedeutet; X_1 für CO oder ferner SO_2

steht; X_2 für die Gruppe der Formel Ib steht, in der p und r für 0 oder 1 und q für 1 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, wie Cyclohexyl, durch gegebenenfalls durch Halogen oder Hydroxy substituiertes Phenyl oder Imidazolyl, wie 4-Imidazolyl, substituiertes Niederalkyl, insbesondere C_1-C_4-Alkyl, oder Phenyl bedeutet; X_5 Wasserstoff oder Niederalkyl, wie C_1-C_4-Alkyl, bedeutet; R_2 Carboxy, Niederalkoxycarbonyl,wie C_2-C_5-Alkoxycarbonyl, Phenylniederalkoxycarbonyl, wie Phenyl-C_1-C_4-alkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, wie C_1-C_4-Alkoxy-C_2-C_5-alkoxycarbonyl, Hydroxy oder Niederalkoxy, wie C_1-C_4-Alkoxy, bedeutet; R_3 Carboxy oder 5-Tetrazolyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind.

Die Erfindung betrifft insbesondere Verbindungen der Formel Ia und ihre Salze, worin R_1 Niederalkyl, insbesondere C_3-C_5-Alkyl, oder ferner Niederalkenyl, insbesondere C_3-C_5-Alkenyl, bedeutet; X_1 für CO oder ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p für 1-8 und q sowie r für 0 stehen; R_2 Hydroxy, Niederalkoxy, wie C_1-C_4-Alkoxy, Phenylniederalkoxy, wie Phenyl-C_1-C_4-alkoxy, Phenoxy, Niederalkanoylamino, wie C_1-C_4-Alkanoylamino, Phenylniederalkanoylamino, wie Phenyl-C_1-C_4-alkanoylamino, Niederalkansulfonylamino, wie C_1-C_4-Alkansulfonylamino, bedeutet; R_3 Carboxy oder in erster Linie 5-Tetrazolyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind.

Die Erfindung betrifft in erster Linie Verbindungen der Formel Ia und ihre Salze, worin R_1 C_3-C_5-Alkyl oder in zweiter Linie C_3-C_5-Alkenyl, bedeutet; X_1 für CO, ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r unabhängig voneinander für 0 oder 1 und q für 1 stehen; X_4 C_1-C_4-Alkyl, wie Methyl, Ethyl, Propyl, Isopropyl, 1- oder 2-Butyl, Hydroxy-C_1-C_4-alkyl, wie Hydroxymethyl, C_3-C_7-Cycloalkyl-C_1-C_4-alkyl, wie Cyclohexylmethyl, Phenyl-C_1-C_4-alkyl, wie Benzyl, oder Imidazolyl-C_1-C_4-alkyl, wie Imidazol-4-yl-methyl, bedeutet; X_5 Wasserstoff oder C_1-C_4-Alkyl, wie Methyl, bedeutet; oder X_4 und X_5 gemeinsam für Tetramethylen, ferner Pentamethylen stehen; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl, ferner Phenyl-C_1-C_4-alkoxycarbonyl, wie Benzyloxycarbonyl, bedeutet; R_3 Carboxy oder insbesondere 5-Tetrazolyl bedeutet.

Die Erfindung betrifft in erster Linie Verbindungen der Formel Ia und ihre Salze, worin R_1 C_3-C_5-Alkyl oder in zweiter Linie C_3-C_5-Alkenyl, bedeutet; X_1 für CO, ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r jeweils für 0 oder 1 und q für 1 stehen; X_4 C_1-C_4-Alkyl, wie Methyl, Ethyl, Propyl, Isopropyl, 1- oder 2-Butyl, Hydroxy-C_1-C_4-alkyl, wie Hydroxymethyl, C_3-C_7-Cycloalkyl-C_1-C_4-alkyl, wie Cyclohexylmethyl, Phenyl-C_1-C_4-alkyl, wie Benzyl, oder Imidazolyl-C_1-C_4-alkyl, wie Imidazol-4-yl-methyl, bedeutet; X_5 Wasserstoff bedeutet; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl, ferner Phenyl-C_1-C_4-alkoxycarbonyl, wie Benzyloxycarbonyl, bedeutet; R_3 Carboxy oder 5-Tetrazolyl bedeutet.

Die Erfindung betrifft in erster Linie Verbindungen der Formel Ia und ihre Salze, worin R_1 C_3-C_5-Alkyl, wie Propyl, Butyl oder Pentyl, bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der q und r für 0 und p für 1 bis 3, insbesondere 2, stehen, oder in der p und q für 1 und r für 0 stehen; X_4 C_1-C_4-Alkyl, wie Methyl, Ethyl, Propyl, Isopropyl, 1- oder 2-Butyl, bedeutet; X_5 Wasserstoff oder C_1-C_4-Alkyl, wie Methyl, bedeutet; R_2 Carboxy, C_2-C_5-Alkoxycarbonyl, wie Methoxy- oder Ethoxycarbonyl, bedeutet; R_3 Carboxy oder 5-Tetrazolyl bedeutet.

Die Erfindung betrifft in erster Linie Verbindungen der Formel Ia und ihre Salze, worin R_1 C_3-C_5-Alkyl, wie Propyl, Butyl oder Pentyl, bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der p für 0 oder 1, r für 0 und q für 1 stehen; X_4 C_1-C_4-Alkyl, wie Methyl, Ethyl, Propyl, Isopropyl, 1- oder 2-Butyl, bedeutet; X_5 Wasserstoff oder C_1-C_4-Alkyl, wie Methyl oder Ethyl, bedeutet oder X_4 und X_5 gemeinsam für Tetramethylen oder Pentamethylen stehen; R_2 Carboxy, C_2-C_5-Alkoxycarbonyl, wie Methoxy- oder Ethoxycarbonyl, bedeutet; R_3 5-Tetrazolyl bedeutet.

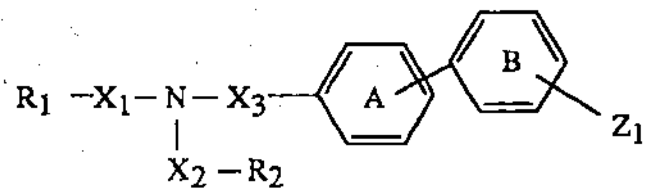
Die Erfindung betrifft in erster Linie Verbindungen der Formel Ia und ihre Salze, worin R_1 C_3-C_5-Alkyl, wie Propyl, Butyl oder Pentyl, bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der p 0 oder 1 und r für 0 und q für 1 stehen; X_4 und X_5 gemeinsam für Tetramethylen, ferner Pentamethylen stehen; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl, wie Methoxy- oder Ethoxycarbonyl, bedeutet; R_3 5-Tetrazolyl bedeutet.

Die Erfindung betrifft in erster Linie Verbindungen der Formel Ia und ihre Salze, worin R_1 C_3-C_5-Alkyl, wie Propyl, Butyl oder Pentyl, bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der p und r für 0 oder 1 und q für 1 stehen; X_4 C_1-C_4-Alkyl, wie Methyl, Ethyl, Propyl, Isopropyl, 1- oder 2-Butyl, bedeutet; X_5 Wasserstoff bedeutet; R_2 Carboxy, C_2-C_5-Alkoxycarbonyl, wie Methoxy- oder Ethoxycarbonyl, bedeutet; R_3 5-Tetrazolyl bedeutet.

Die Erfindung betrifft insbesondere die in den Beispielen aufgeführten neuen Verbindungen sowie die dort beschriebenen Herstellungsweisen.

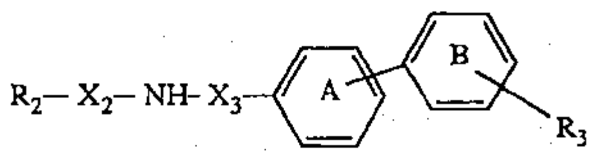
Gegenstand der Erfindung sind auch Verfahren zur Herstellung der erfindungsgemässen Verbindungen. Die Herstellung von Verbindungen der Formel I und ihrer Salze erfolgt in an sich bekannter Weise und ist z.B. dadurch gekennzeichnet, dass man

a) in einer Verbindung der Formel



(II)

oder einem Salz davon, worin Z_1 einen in R_3 überführbaren Rest bedeutet, Z_1 in R_3 überführt oder
b) eine Verbindung der Formel R_1-X_1OH (IIIa), ein reaktionsfähiges Derivat davon oder ein Salz davon mit einer Verbindung der Formel



(IIIb)

oder einem Salz davon umsetzt und jeweils, wenn erwünscht, eine verfahrensgemäss oder auf andere Weise erhältliche Verbindung I in freier Form oder in Salzform in eine andere Verbindung I überführt, ein verfahrensgemäss erhältliches Gemisch von Isomeren auftrennt und das gewünschte Isomere isoliert und/oder eine verfahrensgemäss erhältliche freie Verbindung I in ein Salz oder ein verfahrensgemäss erhältliches Salz einer Verbindung I in die freie Verbindung I oder in ein anderes Salz überführt.

Salze von Ausgangsmaterialien, die mindestens ein basisches Zentrum aufweisen, beispielsweise der Formel IIIb, sind entsprechende Säureadditionssalze, während Salze von Ausgangsstoffen, die eine acide Gruppe aufweisen, beispielsweise der Formel (IIIa), als Salze mit Basen vorliegen, jeweils wie in Zusammenhang mit entsprechenden Salzen der Formel I vorstehend aufgeführt.

In die Variable R_3 überführbare Reste Z_1 stellen beispielsweise Cyano, Mercapto, Halogen, die Gruppe -N_2^+ A^-, in der A^- ein von einer Säure abgeleitetes Anion bedeutet, Amino sowie von COOH, SO_3H, PO_3H_2 oder PO_2H_2 verschiedene funktionell abgewandelte Formen sowie N-geschütztes 5-Tetrazolyl.

Reaktionsfähige Derivate von Verbindungen der Formel IIIa sind beispielsweise davon abgeleitete aktivierte Ester oder reaktionsfähige Anhydride, ferner reaktionsfähige cyclische Amide.

Die vor- und nachstehend in den Varianten beschriebenen Umsetzungen werden in an sich bekannter Weise durchgeführt, z.B. in Ab- oder üblicherweise in Anwesenheit eines geeigneten Lösungs- oder Verdünnungsmittels oder eines Gemisches derselben, wobei man je nach Bedarf unter Kühlen, bei Raumtemperatur oder unter Erwärmen, z.B. in einem Temperaturbereich von etwa -80°C bis zur Siedetemperatur des Reaktionsmediums, vorzugsweise von etwa -10° bis etwa +200°C, und, falls erforderlich, in einem geschlossenen Gefäss, unter Druck, in einer Inertgasatmosphäre und/oder unter wasserfreien Bedingungen arbeitet.

Verfahrensvariante a):

In 5-Tetrazolyl R_3 überführbare Reste Z_1 sind beispielsweise Cyano oder geschütztes 5-Tetrazolyl.

Zur Herstellung von Verbindungen der Formel I, worin R_3 5-Tetrazolyl bedeutet, geht man beispielsweise von Ausgangsmaterial der Formel II aus, worin Z_1 Cyano bedeutet, und setzt dieses mit einem Azid, wie HN_3 oder insbesondere einem Salz, wie Alkalimetallsalz, davon oder mit einem Organozinnazid, wie Tri(nieder)alkyl- oder Triarylzinnazid, um. Bevorzugte Azide sind beispielsweise Natrium- und Kaliumazid sowie Tri-C_1-C_4-alkyl-, z.B. Triethyl- oder Tributylzinnazid, und Triphenylzinnazid. Bevorzugt wird die Tetrazol-5-yl-Bildung mit solchen Verbindungen der Formel II durchgeführt, worin R_2 von Carboxy verschieden ist.

Als Schutzgruppen von geschütztem 5-Tetrazolyl kommen die üblicherweise in der Tetrazolchemie verwendeten Schutzgruppen in Frage, insbesondere Triphenylmethyl, gegebenenfalls, z.B. durch Nitro, substituiertes Benzyl, wie 4-Nitrobenzyl, Niederalkoxymethyl, wie Methoxy- und Ethoxymethyl, Niederalkylthiomethyl, wie Methylthiomethyl, Silyl, wie Triniederalkylsilyl, z.B. Dimethyl-tert-butyl- und Triisopropyl-silyl, sowie 2-Cyanoethyl, ferner Niederalkoxyniederalkoxymethyl, wie 2-Methoxyethoxymethyl, Benzyloxymethyl sowie Phenacyl.

Die Abspaltung der Schutzgruppen erfolgt in Anlehnung an bekannte Methoden, beispielsweise wie in J. Green, Protective Groups in Organic Synthesis, Wiley-Interscience (1980) beschrieben. So wird z.B. die Triphenylmethylgruppe üblicherweise durch Hydrolyse, insbesondere in Gegenwart einer Säure, oder Hydrogenolyse in Gegenwart eines Hydrierungskatalysators, 4-Nitrobenzyl z.B. durch Hydrogenolyse in Gegenwart eines Hydrierungskatalysators, Methoxy- oder Ethoxymethyl z.B. durch Behandeln mit einem Triniederalkyl-, wie Triethyl- oder Tributyl-zinn-bromid, Methylthiomethyl z.B. durch Behandeln mit Trifluoressigsäure, Silyreste z.B. durch Behandeln mit Fluoriden, wie Tetraniederalkylammoniumfluoriden, z.B. Tetrabutylammoniumfluorid, oder Alkalimetallfluoriden, z.B. Natriumfluorid, oder 2-Cyanoethyl z.B. durch Hydrolyse, beispielsweise mit Natronlauge, 2-Methoxyethoxymethyl z.B. durch Hydrolyse, z.B. mit Salzsäure, Benzyloxymethyl und Phenacyl z.B. durch Hydrogenolyse in Gegenwart eines Hydrierungskatalysators abgespalten.

Ein in R_3= SO_3H überführbarer Rest ist beispielsweise die Mercaptogruppe. Eine solche Gruppe aufweisende Ausgangsverbindungen der Formel II werden beispielsweise durch an sich bekannte Oxidationsverfahren zu solchen Verbindungen der Formel I oxidiert, worin R_3 SO_3H ist. Als Oxidationsmittel kommen beispielsweise anorganische Persäuren, wie Persäuren von Mineralsäuren, z.B. Periodsäure oder Perschwefelsäure, organische Persäuren, wie entsprechende Percarbon- oder Persulfonsäuren, z.B. Perameisen-, Peressig-, Trifluorperessig- bzw. Perbenzoesäure oder p-Toluolpersulfonsäure, oder Gemische aus Wasserstoffperoxid und Säuren, z.B. Gemisch aus Wasserstoffperoxid mit Essigsäure, in Betracht.

Häufig führt man die Oxidation in Gegenwart von geeigneten Katalysatoren durch, wobei als Katalysatoren geeignete Säuren, wie gegebenenfalls substituierte Carbonsäuren, z.B. Essigsäure oder Trifluoressigsäure, oder Übergangsmetalloxide, wie Oxide von Elementen der VII. Nebengruppe, z.B. Vanadium-, Molybdän- oder Wolframoxid, zu nennen sind. Die Oxidation wird unter milden Bedingungen, z.B. bei Temperaturen von etwa -50° bis etwa +100°C, durchgeführt.

Unter einer in $R_3 = PO_3H_2$ überführbaren Gruppe ist beispielsweise eine Gruppe $N_2^+ A^-$ zu verstehen, wobei A^- für ein Anion einer Säure, wie Mineralsäure, steht. Derartige Diazoniumverbindungen werden beispielsweise in an sich bekannter Weise mit einem P(III)-Halogenid, wie PCl_3 oder PBr_3, umgesetzt und hydrolytisch aufgearbeitet, wobei solche Verbindungen der Formel I erhältlich sind, worin R_3 PO_3H_2 ist.

Als in Halogenalkylsulphamoyl R_3 überführbarer Rest Z_1 kommt beispielsweise primäres Amino in Frage.

Zur Herstellung von Verbindungen der Formel I, worin R_3 Halogenalkylsulphamoyl bedeutet, setzt man beispielsweise entsprechende Aniline mit einer üblicherweise reaktionsfähig veresterten Halogenalkylsulfonsäure um, wobei gegebenenfalls in Gegenwart einer Base gearbeitet wird. Als bevorzugte reaktionsfähig veresterte Halogensulfonsäure kommt das entsprechende Halogenid, wie Chlorid oder Bromid, in Frage.

Ein in R_3 = COOH überführbarer Rest Z_1 steht beispielsweise für funktionell abgewandeltes Carboxy, wie Cyano, verestertes oder amidiertes Carboxy, Hydroxymethyl oder Formyl.

Verestertes Carboxy ist beispielsweise mit einem gegebenenfalls substituierten aliphatischen, cycloaliphatischen oder aromatischen Alkohol verestertes Carboxy. Ein aliphatischer Alkohol ist beispielsweise ein Niederalkanol, wie Methanol, Ethanol, Propanol, Isopropanol, n-Butanol, sec- oder tert.-Butanol, während als cycloaliphatischer Alkohol beispielsweise ein 3- bis 8-gliedriges Cycloalkanol, wie Cyclopentanol, -hexanol oder -heptanol, in Frage kommt. Ein aromatischer Alkohol ist beispielsweise ein Phenol oder heterocyclischer Alkohol, welche jeweils gegebenenfalls substituiert sein können, insbesondere Hydroxypyridin, z.B. 2-, 3- oder 4-Hydroxypyridin. Carboxy kann ebenfalls mit einem silyliertem Alkohol verestert sein und bedeutet insbesondere Tri-(C_1-C_4-)-alkylsilyl-(C_1-C_4-)alkoxy-carbonyl, insbesondere Trimethylsilylethoxycarbonyl.

Amidiertes Carboxy ist beispielsweise Carbamoyl, durch Hydroxy, Amino oder gegebenenfalls substituiertes Phenyl monosubstituiertes, durch Niederalkyl mono- oder disubstituiertes oder durch 4- bis 7-gliedriges Alkylen bzw. 3-Aza-, 3-Niederalkylaza-, 3-Oxo- oder 3-Thiaalkylen disubstituiertes Carbamoyl. Als Beispiele sind Carbamoyl, N-Mono- oder N,N-Diniederalkylcarbamoyl, wie N-Methyl-, N-Ethyl-, N,N-Dimethyl-, N,N-Diethyl- oder N,N-Dipropylcarbamoyl, Pyrrolidino- oder Piperidinocarbonyl, Morpholino-, Piperazino- bzw. 4-Methylpiperazino- sowie Thiomorpholinocarbonyl, Anilinocarbonyl oder durch Niederalkyl, Niederalkoxy und/oder Halogen substituiertes Anilinocarbonyl zu nennen.

Bevorzugtes funktionell abgewandeltes Carboxy ist beispielsweise Niederalkoxycarbonyl, wie Methoxy- oder Ethoxycarbonyl, Tri-(C_1-C_4-)-alkylsilyl-(C_1-C_4-)alkoxy-carbonyl, insbesondere Trimethylsilylethoxycarbonyl, oder Cyano. Verbindungen der Formel I, worin R_3 Carboxy ist, können beispielsweise ausgehend von Verbindungen der Formel II, worin Z_1 funktionell abgewandeltes Carboxy bedeutet, in an sich bekannter Weise, beispielsweise durch Hydrolyse, insbesondere in Gegenwart einer Base, im Falle von entsprechenden Tri-(C-C-)alkylsilyl-(C-C-)alkoxy-carbonylderivaten z.B. durch Behandeln mit einem Ammoniumfluorid, wie Tetraniederalkylammonium-, z.B. Tetra-n-butyl-ammonium-fluorid, oder im Falle von Benzyloxycarbonylderivaten durch Hydrogenolyse in Gegenwart eines Hydrierungskatalysators, bzw. ausgehend von solchen Verbindungen der Formel II, worin Z_1 Hydroxymethyl oder Formyl bedeutet, unter Verwendung üblicher Oxidationsmittel, durch Oxidation hergestellt werden.

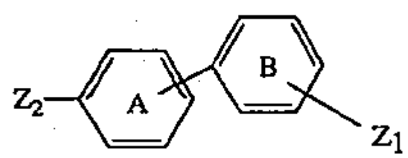
Die Oxidation erfolgt beispielsweise in einem inerten Lösungsmittel, wie einer Niederalkancarbonsäure z.B. Essigsäure, einem Keton, z.B. Aceton, einem Ether, z.B. Tetrahydrofuran, einem heterocyclischen Aromaten, z.B. Pyridin, oder Wasser oder einem Gemisch davon, erforderlichenfalls unter Kühlen oder Erwärmen, z.B. von etwa 0° bis etwa 150°C. Als Oxidationsmittel kommen beispielsweise oxidierende Übergangsmetallverbindungen, insbesondere solche mit Elementen der I., VI., oder VIII. Nebengruppe, in Frage. Als Beispiele seien genannt: Silberverbindungen, wie Silbernitrat, -oxid oder -picolinat, Chromverbindungen, wie Chromtrioxid oder Kaliumdichromat, Manganverbindungen, wie Kaliumpermanganat, Tetrabutylammonium- oder Benzyl(triethyl)ammoniumpermanganat. Weitere Oxidationsmittel sind beispielsweise geeignete Verbindungen mit Elementen der 4. Hauptgruppe, wie Bleidioxid, oder Halogen-Sauerstoff-Verbindungen, wie Natriumiodat oder Kaliumperiodat.

So wird beispielsweise Hydroxymethyl und Formyl zu Carboxy R_3 oxidiert.

Vorzugsweise eignet sich diese Variante zur Herstellung solcher Verbindungen der Formel I, worin die Variablen Bedeutungen haben, die von ungesättigten Resten verschieden sind.

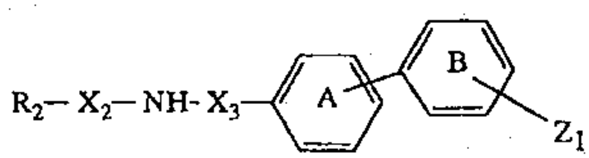
Als Basen kommen beispielsweise Alkalimetall-hydroxide, -hydride, -amide, -alkanolate, -carbonate, -triphenylmethylide, -diniederalkylamide, -aminoalkylamide oder -niederalkylsilylamide, Naphthalinamine, Niederalkylamine, basische Heterocyclen, Ammoniumhydroxide, sowie carbocyclische Amine in Frage. Beispielhaft seien Natriumhydroxid, -hydrid, -amid, Natriummethylat, -ethylat, Kalium-tert-butylat, -carbonat, Lithium-triphenylmethylid, -diisopropylamid, Kalium-3-(aminopropyl)-amid, -bis-(trimethylsilyl)-amid, Dimethylaminonaphthalin, Di- oder Triethylamin, oder Ethyl-diisopropylamin, N-Methyl-piperidin, Pyridin, Benzyltrimethyl-ammoniumhydroxid, 1,5-Diazabicyclo[4.3.0]non-5-en (DBN) sowie 1,8-Diaza-bicyclo[5.4.0] undec-7-en (DBU) genannt.

Das Ausgangsmaterial der Formel II ist beispielsweise zugänglich, indem man eine Verbindung der Formel R_2-X_2-NH_2 (IIa) mit einer Verbindung der Formel



(IIb),

worin Z_2 für -X_3-Z_4 und Z_4 für reaktionsfähiges verestertes Hydroxy steht, beispielsweise in Gegenwart einer Base, umsetzt und die so erhältliche Verbindung der Formel



(IIc)

im nächsten Reaktionsschritt mit einer Verbindung der Formel IIIa, z.B. analog Variante b), umsetzt.

Reaktionsfähiges verestertes Hydroxy Z_4 ist insbesondere mit einer starken anorganischen Säure oder organischen Sulfonsäure verestertes Hydroxy, beispielsweise Halogen, wie Chlor, Brom oder Iod, Sulfonyloxy, wie Hydroxysulfonyloxy, Halogensulfonyloxy, z.B. Fluorsulfonyloxy, gegebenenfalls, z.B. durch Halogen, substituiertes C_1-C_7-Alkansulfonyloxy, z.B. Methan- oder Trifluormethansulfonyloxy, C_5-C_7-Cycloalkansulfonyloxy, z.B. Cyclohexansulfonyloxy, oder gegebenenfalls, z.B. durch C_1-C_7-Alkyl oder Halogen, substituiertes Benzolsulfonyloxy, z.B. p-Brombenzol- oder p-Toluolsulfonyloxy.

Verbindungen der Formel IIb ihrerseits sind beispielsweise aus EP 253,310 bekannt oder können in an sich bekannter Weise hergestellt werden. Verbindungen der Formel (IIa) sind im wesentlichen bekannt oder sind analog an sich bekannter Herstellungsverfahren zugänglich.

Verfahrensvariante b):

Aktivierte Ester von Verbindungen der Formel IIIa sind insbesondere am Verknüpfungskohlenstoffatom des veresterden Restes ungesättigte Ester, z.B. vom Vinylester-Typ, wie Vinylester (erhältlich z.B. durch Umesterung eines entsprechenden Esters mit Vinylacetat; Methode des aktivierten Vinylesters), Carbamoylvinylester (erhältlich z.B. durch Behandeln der entsprechenden Säure mit einem Isoxazoliumreagens; 1,2-Oxazolium- oder Woodward-Methode) oder 1-Niederalkoxyvinylester (erhältlich z.B. durch Behandeln der entsprechenden Säure mit einem Niederalkoxyacetylen; Ethoxyacetylen-Methode), oder Ester vom Amidinotyp, wie N,N'-disubstituierte Amidinoester (erhältlich z.B. durch Behandeln der entsprechenden Säure mit einem geeigneten N,N'-disubstituierten Carbodiimid, z.B. N,N'-Dicyclohexylcarbodiimid; Carbodiimid-Methode) oder N,N-disubstituierte Amidinoester (erhältlich z.B. durch Behandeln der entsprechenden Säure mit einem N,N-disubstituierten Cyanamid; Cyanamid-Methode), geeignete Arylester, insbesondere durch elektronenanziehende Substituenten substituierte Phenylester (erhältlich z.B. durch Behandeln der entsprechenden Säure mit einem geeignet substituierten Phenol, z.B. 4-Nitrophenol, 4-Methylsulfonylphenol, 2,4,5-Trichlorphenol, 2,3,4,5,6-Pentachlorphenol oder 4-Phenyldiazophenol, in Gegenwart eines Kondensationsmittels, wie N,N'-Dicyclohexylcarbodiimid; Methode der aktivierten Arylester), Cyanmethylester (erhältlich z.B. durch Behandeln der entsprechenden Säure mit Chloracetonitril in Gegenwart einer Base; Cyanmethylester-Methode), Thioester, insbesondere gegebenenfalls, z.B. durch Nitro, substituierte Phenylthioester (erhältlich z.B. durch Behandeln der entsprechenden Säure mit gegebenenfalls, z.B. durch Nitro, substituierten Thiophenolen, u.a. mit Hilfe der Anhydrid- oder Carbodiimid-Methode; Methode der aktivierten Thiolester) oder insbesondere Amino- oder Amidoester (erhältlich z.B. durch Behandeln der entsprechenden Säure mit einer N-Hydroxyamino- bzw. N-Hydroxyamido-Verbindung und deren aktivierten Derivaten, z.B. N-Hydroxysuccinimid, N-Hydroxypiperidin, N-Hydroxyphthalimid, N-Hydroxy-5-norbornen- oder norbornan-2,3-dicarbonsäureimid, 1-Hydroxybenzotriazol bzw. Benzotriazol-1-yloxy-phosphoniumsalzen oder Benzotriazol-1-yluroniumsalzen, oder 3-Hydroxy-3,4-dihydro-1,2,3-benzotriazin-4-on, z.B. nach der Anhydrid- oder Carbodiimid-Methode; Methode der aktivierten N-Hydroxyester).

Anhydride von Säuren können symmetrische oder vorzugsweise gemischte Anhydride dieser Säuren sein, z.B. Anhydride mit anorganischen Säuren, wie Säurehalogenide, insbesondere Säurechloride (erhältlich z.B. durch Behandeln der entsprechenden Säure mit Thionylchlorid, Phosphorpentachlorid oder Oxalylchlorid; Säurechloridmethode), Azide (erhältlich z.B. aus einem entsprechenden Säureester über das entsprechende Hydrazid und dessen Behandlung mit salpetriger Säure; Azidmethode), Anhydride mit Kohlensäurehalbestern, z.B. Kohlensäureniederalkylhalbestern (erhältlich z.B. durch Behandeln der entsprechenden Säure mit Chlorameisensäureniederalkylestern oder mit einem 1-Niederalkoxycarbonyl-2-niederalkoxy-1,2-dihydrochinolin, z.B. 1-Ethoxycarbonyl-2-ethoxy-1,2-dihydrochinolin; Methode der gemischten O-Alkylkohlensäureanhydride), Anhydride mit dihalogenierter, insbesondere dichlorierter Phosphorsäure (erhältlich z.B. durch Behandeln der entsprechenden Säure mit Phosphoroxychlorid; Phosphoroxychlorid-methode), Anhydride mit anderen Phosphorsäurederivaten (z.B. solchen, die man mit Phenyl-N-phenylphosphoramidochloridat erhalten kann) oder mit Phosphorigsäurederivaten, oder Anhydride mit organischen Säuren, wie gemischte Anhydride mit organischen Carbonsäuren (erhältlich z.B. durch Behandeln der entsprechenden Säure mit einem gegebenenfalls substituierten Niederalkan- oder Phenylniederalkancarbonsäurehalogenid, z.B. Phenylessigsäure-, Pivalinsäure- oder Trifluoressigsäurechlorid; Methode der gemischten Carbonsäureanhydride) oder mit organischen Sulfonsäuren (erhältlich z.B. durch Behandeln eines Salzes, wie eines Alkalimetallsalzes, der entsprechenden Säure mit einem geeigneten organischen Sulfonsäurehalogenid, wie Niederalkan- oder Aryl-, z.B. Methan- oder p-Toluolsulfonsäurechlorid; Methode der gemischten Sulfonsäureanhydride), sowie symmetrische Anhydride (erhältlich z.B. durch Kondensation der entsprechenden Säure in Gegenwart eines Carbodiimids oder von 1-Diethylaminopropin; Methode der symmetrischen Anhydride).

Geeignete cyclische Amide sind insbesondere Amide mit fünfgliedrigen Diazacyclen aromatischen Charakters, wie Amide mit Imidazolen, z.B. Imidazol (erhältlich z.B. durch Behandeln der entsprechenden Säure mit N,N'-Carbonyldiimidazol; Imidazol-Methode), oder Pyrazolen, z.B. 3,5-Dimethylpyrazol (erhältlich z.B. über das Säurehydrazid durch Behandeln mit Acetylaceton; Pyrazolid-Methode).

Die Kondensation zur Herstellung der Amidbindung kann in an sich bekannter Weise durchgeführt werden, beispielsweise wie in Standardwerken, wie Houben-Weyl, "Methoden der organischen Chemie", 4. Auflage, Band 15/II, Georg Thieme Verlag, Stuttgart 1974, "The Peptides" (Herausg. E. Gross und J. Meienhofer), Band 1 und 2, Academic Press, London und New York, 1979/1980, oder M. Bodanszky, "Principles of Peptide Synthesis", Springer-Verlag, Berlin 1984, beschrieben.

Die Kondensation kann in Gegenwart eines der üblichen Kondensationsmittel durchgeführt werden. Uebliche Kondensationsmittel sind z.B. Carbodiimide, beispielsweise Diethyl-, Dipropyl-, N-Ethyl-N'-(3-dimethylaminopropyl)-carbodiimid oder insbesondere Dicyclohexylcarbodiimid, ferner geeignete Carbonylverbindungen, beispielsweise Carbonyldiimidazol, 1,2-Oxazoliumverbindungen, z.B. 2-Ethyl-5-phenyl-1,2-oxazolium-3'-sulfonat und 2-tert-Butyl-5-methylisoxazoliumperchlorat, oder eine geeignete Acylaminoverbindung, z.B. 2-Ethoxy-1-ethoxycarbonyl- 1,2-dihydrochinolin, ferner aktivierte Phosphorsäurederivate, z.B. Diphenylphosphorylazid, Diethylphosphorylcyanid, Phenyl-N-phenylphosphoramidochloridat, Bis-(2-oxo-3-oxazolidinyl)-phosphinsäurechlorid oder 1-Benzotriazolyloxy-tris-(dimethylamino)-phosphonium-hexafluorophosp hat.

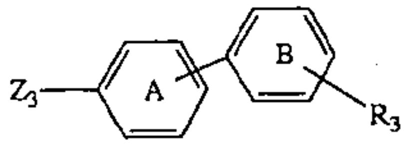
Gewünschtenfalls wird eine organische Base zugegeben, z.B. ein Triniederalkylamin mit voluminösen Resten, z.B. Ethyldiisopropylamin, oder eine heterocyclische Base, z.B. Pyridin, 4-Dimethylaminopyridin oder bevorzugt N-Methylmorpholin.

Die Kondensation von Säureanhydriden mit Aminen kann z.B. in Gegenwart von anorganischen Carbonaten, z.B. Alkalimetallcarbonaten oder -hydrogencarbonaten, wie Natrium- oder Kaliumcarbonat oder -hydrogencarbonat (üblicherweise zusammen mit einem Sulfat), erfolgen.

Die Kondensation wird vorzugsweise in einem inerten, polaren, aprotischen, vorzugsweise wasserfreien, Lösungsmittel oder Lösungsmittelgemisch durchgeführt, beispielsweise in einem Carbonsäureamid, z.B. Formamid oder Dimethylformamid, einem halogenierten Kohlenwasserstoff, z.B. Methylenchlorid, Tetrachlorkohlenstoff oder Chlorbenzol, einem Keton, z.B. Aceton, cyclischen Ether, z.B. Tetrahydrofuran, einem Ester, z.B. Essigsäureethylester, oder einem Nitril, z.B. Acetonitril, oder in Mischungen davon, gegebenenfalls bei erniedrigter oder erhöhter Temperatur, z.B. in einem Temperaturbereich von etwa -40°C bis etwa +100°C, bevorzugt von etwa -10°C bis etwa +50°C, und gegebenenfalls unter Inertgas-, z.B. Stickstoffatmosphäre.

Reaktionsfähige Säurederivate können auch in situ gebildet werden.

Das Ausgangsmaterial der Formel IIIb kann man beispielsweise herstellen, indem man eine Verbindung der Formel IIa mit einer Verbindung der Formel



(IIIc),

worin Z_3 -X_3-Z_4 und Z_4 reaktionsfähiges verestertes Hydroxy bedeutet, insbesondere in Gegenwart einer der vorstehend aufgeführten Basen, umsetzt. Zur Herstellung von Verbindungen der Formel IIIb, worin X_3 -CH_2- bedeutet, geht man z.B. von Verbindungen der Formel IIa aus und setzt diese mit Verbindungen der Formel IIIc um, worin Z_3 Formyl bedeutet. Die so erhältlichen Schiff'schen Basen werden anschliessend mit Hilfe eines Reduktionsmittels, wie Natriumcyanoborhydrid, reduziert.

Reaktionsfähiges verestertes Hydroxy Z_4 ist insbesondere mit einer starken anorganischen Säure oder organischen Sulfonsäure verestertes Hydroxy, beispielsweise Halogen, wie Chlor, Brom oder Iod, Sulfonyloxy, wie Hydroxysulfonyloxy, Halogensulfonyloxy, z.B. Fluorsulfonyloxy, gegebenenfalls, z.B. durch Halogen, substituiertes C_1-C_7-Alkansulfonyloxy, z.B. Methan- oder Trifluormethansulfonyloxy, C_5-C_7-Cycloalkansulfonyloxy, z.B. Cyclohexansulfonyloxy, oder gegebenenfalls, z.B. durch C_1-C_7-Alkyl oder Halogen, substituiertes Benzolsulfonyloxy, z.B. p-Brombenzol- oder p-Toluolsulfonyloxy.

Eine verfahrensgemäss erhältliche erfindungsgemässe Verbindung kann in an sich bekannter Weise in eine andere erfindungsgemässe Verbindung übergeführt werden.

Eine Hydroxy aufweisende erfindungsgemässe Verbindung kann nach an sich bekannten Methoden verethert werden. Die Veretherung kann z.B. mit einem Alkohol, wie gegebenenfalls substituiertem Niederalkanol, oder einem reaktionsfähigen Ester desselben erfolgen. Als reaktionsfähige Ester der gewünschten Alkohole kommen beispielsweise solche mit starken anorganischen oder organischen Säuren in Frage, wie entsprechende Halogenide, Sulfate, Niederalkansulfonate oder gegebenenfalls substituierte Benzolsulfonate, z.B. Chloride, Bromide, Iodide, Methan-, Benzol- oder p-Toluol-sulfonate, in Betracht. Die Veretherung kann z.B. in Gegenwart einer Base, eines Alkalimetallhydrids, -hydroxids, -carbonats oder eines Amins, erfolgen. Umgekehrt können entsprechende Ether, wie Niederalkoxyverbindungen, z.B. mittels starker Säuren, wie Mineralsäuren, z.B. den Halogenwasserstoffsäuren Brom- oder Iodwasserstoffsäure, die vorteilhaft in Form von Pyridiniumhalogeniden vorliegen können, oder mittels Lewissäuren, z.B. Halogeniden von Elementen der 3. Hauptgruppe oder der entsprechenden Nebengruppen, gespalten werden. Diese Umsetzungen können, falls erforderlich, unter Kühlen oder Erwärmen, z.B. einem Temperaturbereich von etwa -20° bis etwa 100°C, in An- oder Abwesenheit eines Lösungs- oder Verdünnungsmittels, unter Inertgas und/oder unter Druck und gegebenenfalls in einem geschlossenen Gefäss, durchgeführt werden.

Hydroxymethylgruppen aufweisende erfindungsgemässe Verbindungen können beispielsweise ausgehend von entsprechenden Carboxy oder verestertes Carboxy aufweisenden Verbindungen hergestellt werden, wobei entsprechende Verbindungen in an sich bekannter Weise reduziert werden, z.B. durch Reduktion mit einem gegebenenfalls komplexen Hydrid, wie einem Hydrid gebildet aus einem Element der 1. und 3. Hauptgruppe des Periodensystems der Elemente, z.B. Boranat oder Alanat, beispielsweise Lithiumborhydrid, Lithium-, Diisobutylaluminiumhydrid (gegebenenfalls ist ein nachgelagerter Reduktionsschritt unter Verwendung von Alkalimetall-, wie Natriumcyanoborhydrid, erforderlich), ferner Diboran.

Falls ein aromatischer Strukturbestandteil durch (Nieder-)Alkylthio substituiert ist (in $S(O)_m$-R steht m für 0), kann man dieses auf übliche Weise zu entsprechendem (Nieder-)-Alkansulfinyl bzw. - sulfonyl oxidieren. Als geeignetes Oxidationsmittel für die Oxidation zur Sulfoxidstufe kommen beispielsweise anorganische Persäuren, wie Persäuren von Mineralsäuren, z.B. Periodsäure oder Perschwefelsäure, organische Persäuren, wie entsprechende Percarbon- oder Persulfonsäuren, z.B. Perameisen-, Peressig-, Trifluorperessig- bzw. Perbenzoesäure oder p-Toluolpersulfonsäure, oder Gemische aus Wasserstoffperoxid und Säuren, z.B. Gemisch aus Wasserstoffperoxid mit Essigsäure, in Betracht.

Häufig führt man die Oxidation in Gegenwart von geeigneten Katalysatoren durch, wobei als Katalysatoren geeignete Säuren, wie gegebenenfalls substituierte Carbonsäuren, z.B. Essigsäure oder Trifluoressigsäure, oder Übergangsmetalloxide, wie Oxide von Elementen der VII. Nebengruppe, z.B. Vanadium-, Molybdän- oder Wolframoxid, zu nennen sind. Die Oxidation wird unter milden Bedingungen, z.B. bei Temperaturen von etwa -50° bis etwa +100°C, durchgeführt.

Die Oxidation zur Sulfonstufe kann man auch mit Distickstofftetroxid als Katalysator in Gegenwart von Sauerstoff bei tiefen Temperaturen entsprechend durchführen, ebenso wie die direkte Oxidation des (Nieder-)Alkylthio zum (Nieder-)Alkansulfonyl. Jedoch setzt man hierbei üblicherweise das Oxidationsmittel im Überschuss ein.

Weist eine der Variablen Amino auf, können entsprechende Verbindungen der Formel I, ihre Tautomeren oder Salze in an sich bekannter Weise N-alkyliert werden; ebenso können Carbamoyl bzw. Carbamoyl aufweisende Reste N-alkyliert werden. Die (Aryl-)-Alkylierung erfolgt z.B. mit einem reaktiven Ester eines (Aryl-)C_1-C_7-Alkylhalogenids, z.B. -bromid oder -iodid, (Aryl-)C_1-C_7-Alkylsulfonat, z.B. methansulfonat oder -p-toluolsulfonat, oder einem Di-C_1-C_7-alkylsulfat, z.B. Dimethylsulfat, vorzugsweise unter basischen Bedingungen, wie in Gegenwart von Natronlauge oder Kalilauge, und vorteilhaft eines Phasentransfer-Katalysators, wie Tetrabutylammoniumbromid oder Benzyltrimethylammoniumchlorid, wobei indes stärker basische Kondensationsmittel, wie Alkalimetallamide, -hydride oder -alkoholate, z.B. Natriumamid, Natriumhydrid oder Natriumethanolat, erforderlich sein können. Ebenso kann Amino in an sich bekannter Weise, z.B analog Variante b), acyliert werden.

In Verbindungen der Formel I, die als Substituenten eine veresterte oder amidierte Carboxygruppe aufweisen, kann man eine solche Gruppe z.B. mittels Hydrolyse, z.B. in Gegenwart eines basischen Mittels, oder eines sauren Mittels, wie einer Mineralsäure, in eine freie Carboxygruppe überführen. Tert-Butyloxycarbonyl beispielsweise kann weiterhin z.B. in an sich bekannter Weise, wie durch Behandeln mit Trihalogen-, wie Trifluoressigsäure, und Benzyloxycarbonyl z.B. durch katalytische Hydrierung in Gegenwart eines Hydrierungskatakysators, z.B. in der nachstehend beschriebenen Weise, in Carboxy überführt werden.

Ferner kann man in Verbindungen der Formel I, die als Substituenten eine Carboxygruppe aufweisen, insbesondere sofern R_3 von Carboxy verschieden ist, diese z.B. durch Behandeln mit einem Alkohol, wie einem Niederalkanol, in Gegenwart eines geeigneten Veresterungsmittels, wie eines sauren Reagens, z.B. einer anorganischen oder organischen Säure oder einer Lewissäure, z.B. Zinkchlorid, oder eines wasserbindenden Kondensationsmittels, z.B. eines Carbodiimids, wie N,N'-Dicyclohexyl-carbodiimid, oder durch Behandeln mit einem Diazoreagens, wie mit einem Diazoniederalkan, z.B. Diazomethan, in eine veresterte Carboxygruppe überführen. Diese kann man auch erhalten, wenn man Verbindungen der Formel I, worin die Carboxygruppe in freier Form oder in Salz-, wie Ammonium- oder Metall-, z.B. Alkalimetall-, wie Natrium- oder Kaliumsalzform vorliegt, mit einem reaktionsfähigen Ester eines (C_1-C_7-)Alkylhalogenid, z.B. Methyl- oder Ethyl-bromid oder -iodid, oder einem organischen Sulfonsäureester, wie einem entsprechenden (C_1-C_7-)Alkylester, z.B. Methansulfonsäure- oder p-Toluolsulfonsäuremethylester oder -ethylester, behandelt.

Verbindungen der Formel I, die als Substituenten eine veresterte Carboxygruppe aufweisen, kann man durch Umesterung, z.B. durch Behandeln mit einem Alkohol, üblicherweise einem höheren als dem der veresterten Carboxygruppe im Ausgangsmaterial entsprechenden Alkohol, in Gegenwart eines geeigneten Umesterungsmittels, wie eines basischen Mittels, z.B. eines Alkalimetall-(C_1-C_7-)alkanoats, -(C_1-C_7-)alkanolats oder -cyanids, wie Natriumacetat, -methanolat, -ethylat, -tert-butanolat oder -cyanid, oder eines geeigneten sauren Mittels, gegebenenfalls unter Entfernung des entstehenden Alkohols, z.B. durch Destillation, in andere Esterverbindungen der Formel I umestern. Man kann auch von entsprechenden, sogenannten aktivierten Estern der Formel I ausgehen, die als Substituenten eine aktivierte veresterte Carboxygruppe aufweisen (siehe unten), und diese durch Behandeln mit einem (C_1-C_7-)Alkanol, in einen anderen Ester umwandeln.

Man kann in Verbindungen der Formel I, die als Substituenten die Carboxylgruppe enthalten, diese auch zuerst in ein reaktionsfähiges Derivat, wie ein Anhydrid, inkl. ein gemischtes Anhydrid, wie ein Säurehalogenid, z.B. -chlorid (z.B. durch Behandeln mit einem Thionylhalogenid, z.B. -chlorid), oder ein Anyhdrid mit einem Ameisensäureester, z.B. -(C_1-C_7-)alkylester (z.B. durch Behandeln eines Salzes, wie eines Ammonium- oder Alkalimetallsalzes, mit einem Halogen-, wie Chlorameisensäureester, wie (C_1-C_7-)Alkyl-ester), oder in einen aktivierten Ester, wie Cyanmethyl-, Nitrophenyl-, z.B. 4-Nitrophenyl-, oder Polyhalogenphenyl-, z.B. Pentachlorphenylester (z.B. durch Behandeln mit einer entsprechenden Hydroxyverbindung in Gegenwart eines geeigneten Kondensationsmittels, wie N,N'-Dicyclohexyl-carbodiimid) überführen, und ein solches reaktionsfähiges Derivat dann mit einem Amin umsetzen und so zu Amidverbindungen der Formel I gelangen, die als Substituenten eine amidierte Carboxygruppe aufweisen. Dabei kann man diese direkt oder über Zwischenverbindungen erhalten; so kann man z.B. einen aktivierten Ester, wie einen 4-Nitrophenylester, einer Verbindung der Formel I mit einer Carboxygruppe zuerst mit einem 1-unsubstituierten Imidazol umsetzen und die so entstandene 1-Imidazolylcarbonylverbindung mit einem Amin in Reaktion bringen. Man kann aber auch andere, nicht-aktivierte Ester, wie (C_1-C_7-)Alkylester von Verbindungen der Formel I, die als Substituenten z.B. (C_2-C_8-)Alkoxycarbonyl aufweisen, mit Aminen zur Reaktion bringen.

Weist ein aromatischer Ring als Substituenten ein Wasserstoffatom auf, so kann dieses mit Hilfe eines Halogenierungsmittels in üblicher Weise durch ein Halogenatom ersetzt, z.B. mit Brom, Hypobromsäure, Acylhypobromite oder andere organische Bromverbindungen, z.B. N-Bromsuccinimid, N-Bromacetamid, N-Bromphthalimid, Pyridiniumperbromid, Dioxandibromid, 1,3-Dibrom-5,5-dimethylhydantoin, 2,4,4,6-Tetrabrom-2,5-cyclohexandien-1-on, bromiert bzw. mit elementarem Chlor, z.B. in einem halogenierten Kohlenwasserstoff, wie Chloroform, und unter Kühlen, z.B. bis auf etwa -10° bis etwa +100°C, chloriert werden.

Enthält ein aromatischer Ring in den erfindungsgemässen Verbindungen eine Aminogruppe, so kann diese in üblicher Weise diazotiert werden, z.B. durch Behandeln mit einem Nitrit, z.B. Natriumnitrit, in Gegenwart einer geeigneten Protonsäure, z.B. Mineralsäure, wobei die Reaktionstemperatur vorteilhaft unter etwa 5°C gehalten wird.

Die so erhältliche, in Salzform vorliegende Diazoniumgruppe kann man nach analogen Verfahren beispielsweise wie folgt substituieren: durch die Hydroxygruppe analog der Phenolverkochung in Gegenwart von Wasser, durch eine Alkoxygruppe durch Behandeln mit einem entsprechenden Alkohol, wobei Energie zugeführt werden muss; durch das Fluoratom analog der Schiemann-Reaktion bei der Thermolyse von entsprechenden Diazoniumtetrafluorboraten; durch die Halogenatome Chlor, Brom oder Iod sowie die Cyanogruppe analog der Sandmeyer-Reaktion bei der Umsetzung mit entsprechenden Cu(I)-Salzen, zunächst unter Kühlen, z.B. auf etwa unter 5°C, und anschliessendem Erhitzen, z.B. auf etwa 60° bis etwa 150°C.

Enthalten die Verbindungen der Formel I ungesättigte Reste, wie (Nieder-)Alkenyl oder (Nieder-)Alkinylgruppierungen, können diese in an sich bekannter Weise in gesättigte Reste überführt werden. So erfolgt beispielsweise die Hydrierung von Mehrfachbindungen durch katalytische Hydrierung in Gegenwart von Hydrierungskatalysatoren, wobei hierfür z.B. Nickel, wie Raney-Nickel, sowie Edelmetalle bzw. deren Derivate, z.B. Oxide, geeignet sind, wie Palladium, Platinoxid, die gegebenenfalls auf Trägermaterialien, z.B. auf Kohle oder Calciumcarbonat, aufgezogen sein können. Die Hydrierung kann vorzugsweise bei Drucken zwischen 1 und etwa 100 at und bei Raumtemperatur zwischen etwa -80° bis etwa 200°C, vor allem zwischen Raumtemperatur und etwa 100°C, durchgeführt werden. Die Reaktion erfolgt zweckmässig in einem Lösungsmittel, wie Wasser, einem Niederalkanol, z.B. Ethanol, Isopropanol oder n-Butanol, einem Ether, z.B. Dioxan, oder einer Niederalkancarbonsäure, z.B. Essigsäure.

Weiterhin kann in Verbindungen der Formel I, worin z.B. einer der Reste R_1 und/oder X_2 Halogen, wie Chlor, aufweist, Halogen durch Umsetzung mit einem gegebenenfalls substituierten Amin, einem Alkohol oder Mercaptan ausgetauscht werden.

Die Erfindung betrifft insbesondere die in den Beispielen beschriebenen Verfahren.

Salze von Verbindungen der Formel I können in an sich bekannter Weise hergestellt werden. So erhält man beispielsweise Säureadditionssalze von Verbindungen der Formel I durch Behandeln mit einer Säure oder einem geeigneten Ionenaustauscherreagenz. Salze können in üblicher Weise in die freien Verbindungen überführt werden, Säureadditionssalze z.B. durch Behandeln mit einem geeigneten basischen Mittel.

Je nach Verfahrensweise bzw. Reaktionsbedingungen können die erfindungsgemässen Verbindungen mit salzbildenden, insbesondere basischen Eigenschaften, in freier Form oder bevorzugt in Form von Salzen erhalten werden.

Infolge der engen Beziehung zwischen der neuen Verbindung in freier Form und in Form ihrer Salze sind im Vorausgegangenen und nachfolgend unter der freien Verbindung oder ihren Salzen sinn- und zweckgemäss gegebenenfalls auch die entsprechenden Salze bzw. die freie Verbindung zu verstehen.

Die neuen Verbindungen einschliesslich ihrer Salze von salzbildenden Verbindungen können auch in Form ihrer Hydrate erhalten werden oder andere zur Kristallisation verwendete Lösungsmittel einschliessen.

Die neuen Verbindungen können, je nach der Wahl der Ausgangsstoffe und Arbeitsweisen, in Form eines der möglichen Isomeren oder als Gemische derselben, z.B. je nach der Anzahl der asymmetrischen Kohlenstoffatome, als reine optische Isomere, wie Antipoden, oder als Isomerengemische, wie Racemate, Diastereoisomerengemische oder Racematgemische, vorliegen. Beispielsweise weisen Verbindungen der Formel Ia, worin X_2 für die Gruppe der Formel Ib, in welcher q für 1 steht und X_4 und X_5 unterschiedliche Bedeutungen haben, steht, ein asymmetrisches C-Atom auf. In entsprechenden Verbindungen der Formel I, worin R_2 beispielsweise gegebenenfalls verestertes oder amidiertes Carboxy oder gegebenenfalls verethertes Hydroxy bedeutet, weist das betreffende asymmetrische C-Atom der Partialstruktur der Formel -X_2-R_2 vorzugsweise die S-Konfiguration auf.

Erhaltene Racemate und Diastereomerengemische können auf Grund der physikalischchemischen Unterschiede der Bestandteile in bekannter Weise in die reinen Isomeren oder Racemate aufgetrennt werden, beispielsweise durch fraktionierte Kristallisation. Erhaltene Racemate lassen sich ferner nach bekannten

Methoden in die optischen Antipoden zerlegen, beispielsweise durch Umkristallisation aus einem optisch aktiven Lösungsmittel, Chromatographie an chiralen Adsorbentien, mit Hilfe von geeigneten Mikroorganismen, durch Spaltung mit spezifischen, immobilisierten Enzymen, über die Bildung von Einschlussverbindungen, z.B. unter Verwendung chiraler Kronenether, wobei nur ein Enantiomeres komplexiert wird, oder durch Überführung in diastereomere Salze, z.B. durch Umsetzung eines basischen Endstoffracemats mit einer optisch aktiven Säure, wie Carbonsäure, z.B. Wein- oder Apfelsäure, oder Sulfonsäure, z.B. Camphersulfonsäure, und Trennung des auf diese Weise erhaltenen Diastereomerengemisches, z.B. auf Grund ihrer verschiedenen Löslichkeiten, in die Diastereomeren, aus denen das gewünschte Enantiomere durch Einwirkung geeigneter Mittel freigesetzt werden kann. Vorteilhaft isoliert man das wirksamere Enantiomere.

Die Erfindung betrifft auch diejenigen Ausführungsformen des Verfahrens, nach denen man von einer auf irgendeiner Stufe des Verfahrens als Zwischenprodukt erhältlichen Verbindung ausgeht und die fehlenden Schritte durchführt oder einen Ausgangsstoff in Form eines Derivates bzw. Salzes und/oder seiner Racemate bzw. Antipoden verwendet oder insbesondere unter den Reaktionsbedingungen bildet.

Beim Verfahren der vorliegenden Erfindung werden vorzugsweise solche Ausgangsstoffe verwendet, welche zu den eingangs als besonders wertvoll geschilderten Verbindungen führen. Neue Ausgangsstoffe, die speziell für die Herstellung der erfindungsgemässen Verbindungen entwickelt wurden, ihre Verwendung und Verfahren zu ihrer Herstellung bilden ebenfalls einen Gegenstand der Erfindung, wobei die Variablen R, R_1, R_2, R_3, X_1, X_2, X_3, X_4, X_5, m, p, q, und r die für die jeweils bevorzugten Verbindungsgruppen der Formel I angegebenen Bedeutungen haben. Insbesondere sind Verbindungen der Formel IIa, ihre Tautomeren und Salze, worin Z_1 Cyano bedeutet, als Ausgangsmaterial bevorzugt.

Die Erfindung betrifft ebenfalls die Verwendung der Verbindungen der Formel I oder von pharmazeutisch verwendbaren Salzen von solchen Verbindungen mit salzbildenden Eigenschaften, insbesondere als pharmakologische, in erster Linie Angiotensin-II-antagonisierende Wirksubstanzen. Dabei kann man sie, vorzugsweise in Form von pharmazeutisch verwendbaren Zubereitungen, in einem Verfahren zur prophylaktischen und/oder therapeutischen Behandlung des tierischen oder menschlichen Körpers, insbesondere als Angiotensin-II-Antagonisten, verwenden.

Die Erfindung betrifft gleichfalls pharmazeutische Präparate, die die erfindungsgemässen Verbindungen oder pharmazeutisch verwendbare Salze derselben als Wirkstoffe enthalten, sowie Verfahren zu ihrer Herstellung.

Bei den erfindungsgemässen pharmazeutischen Präparaten, welche die erfindungsgemässe Verbindung oder pharmazeutisch verwendbare Salze davon enthalten, handelt es sich um solche zur enteralen, wie oralen, ferner rektalen, und parenteralen Verabreichung an Warmblüter(n), wobei der pharmakologische Wirkstoff allein oder zusammen mit einem pharmazeutisch anwendbaren Trägermaterial enthalten ist. Die tägliche Dosierung des Wirkstoffes hängt von dem Alter und dem individuellen Zustand sowie von der Applikationsweise ab.

Die neuen pharmazeutischen Präparate enthalten z.B. von etwa 10 % bis etwa 80 %, vorzugsweise von etwa 20 % bis etwa 60 %, des Wirkstoffs. Erfindungsgemässe pharmazeutische Präparate zur enteralen bzw. parenteralen Verabreichung sind z.B. solche in Dosiseinheitsformen, wie Dragées, Tabletten, Kapseln oder Suppositorien, ferner Ampullen. Diese werden in an sich bekannter Weise, z.B. mittels konventioneller Misch-, Granulier-, Dragier-, Lösungs- oder Lyophilisierungsverfahren hergestellt. So kann man pharmazeutische Präparate zur oralen Anwendung erhalten, indem man den Wirkstoff mit festen Trägerstoffen kombiniert, ein erhaltenes Gemisch gegebenenfalls granuliert, und das Gemisch bzw. Granulat, wenn erwünscht oder notwendig, nach Zugabe von geeigneten Hilfsstoffen zu Tabletten oder Dragée-Kernen verarbeitet.

Geeignete Trägerstoffe sind insbesondere Füllstoffe, wie Zucker, z.B. Lactose, Saccharose, Mannit oder Sorbit, Cellulosepräparate und/oder Calciumphosphate, z.B. Tricalciumphosphat oder Calciumhydrogenphosphat, ferner Bindemittel, wie Stärkekleister, unter Verwendung z.B. von Mais-, Weizen-, Reis- oder Kartoffelstärke, Gelatine, Tragakanth, Methylcellulose und/oder Polyvinylpyrrolidon, wenn erwünscht, Sprengmittel, wie die obengenannten Stärken, ferner Carboxymethylstärke, quervernetztes Polyvinylpyrrolidon, Agar, Alginsäure oder ein Salz davon, wie Natriumalginat. Hilfsmittel sind in erster Linie Fliess-, Fliessregulier- und Schmiermittel, z.B. Kieselsäure, Talk, Stearinsäure oder Salze davon, wie Magnesium- oder Calciumstearat, und/oder Polyethylenglykol. Dragée-Kerne werden mit geeigneten, gegebenenfalls Magensaftresistenten Überzügen versehen, wobei man u.a. konzentrierte Zuckerlösungen, welche gegebenenfalls arabischen Gummi, Talk, Polyvinylpyrrolidon, Polyethylenglykol und/oder Titandioxid enthalten, Lacklösungen in geeigneten organischen Lösungsmitteln oder Lösungsmittelgemische oder, zur Herstellung von Magensaft-resistenten Überzügen, Lösungen von geeigneten Cellulosepräparaten, wie Acetylcellulosephthalat oder Hydroxypropylmethylcellulosephthalat, verwendet. Den Tabletten oder Dragée-Überzügen können Farbstoffe oder Pigmente, z.B. zur Identifizierung oder zur Kennzeichnung verschiedener Wirkstoffdosen, beigefügt werden.

Weitere oral anwendbare pharmazeutische Präparate sind Steckkapseln aus Gelatine, sowie weiche, geschlossene Kapseln aus Gelatine und einem Weichmacher, wie Glycerin oder Sorbitol. Die Steckkapseln können den Wirkstoff in Form eines Granulates, z.B. im Gemisch mit Füllstoffen, wie Lactose, Bindemitteln, wie Stärken, und/oder Gleitmitteln, wie Talk oder Magnesiumstearat, und gegebenenfalls Stabilisatoren, enthalten. In weichen Kapseln ist der Wirkstoff vorzugsweise in geeigneten Flüssigkeiten, wie fetten Ölen, Paraffinöl oder flüssigen Polyethylenglykolen, gelöst oder suspendiert, wobei ebenfalls Stabilisatoren zugefügt sein können.

Als rektal anwendbare pharmazeutische Präparate kommen z.B. Suppositorien in Betracht, welche aus einer Kombination des Wirkstoffs mit einer Suppositoriengrundmasse bestehen. Als Suppositoriengrundmasse eignen sich z.B. natürliche oder synthetisch Triglyceride, Paraffinkohlenwasserstoffe, Polyethylenglyckole oder höhere Alkanole. Ferner können auch Gelatine-Rektalkapseln verwendet werden, die eine Kombination des Wirkstoffs mit einem Grundmassenstoff enthalten. Als Grundmassenstoffe kommen z.B. flüssige Triglyceride, Polyethylenglykole oder Paraffinkohlenwasserstoffe in Frage.

Zur parenteralen Verabreichung eignen sich in erster Linie wässrige Lösungen eines Wirkstoffs in wasserlöslicher Form, z.B. eines wasserlöslichen Salzes, ferner Suspensionen des Wirkstoffs, wie entsprechende ölige Injektionssuspensionen, wobei man geeignete lipophile Lösungsmittel oder Vehikel, wie fette Öle, z.B. Sesamöl, oder synthetische Fettsäureester, z.B. Ethyloleat oder Triglyceride, verwendet oder wässrige Injektionssuspensionen, welche viskositätserhöhende Stoffe, z.B. Natrium-carboxymethylcellulose, Sorbit und/oder Dextran, und gegebenenfalls auch Stabilisatoren enthalten.

Die Dosierung des Wirkstoffes hängt von der Warmblüter-Spezies, dem Alter und dem individuellen Zustand sowie der Applikationsweise ab. Im Normalfall ist für einen etwa 75 kg schweren Patienten bei oraler Applikation eine ungefähre Tagesdosis von etwa 10 mg bis etwa 250 mg zu veranschlagen.

Die nachfolgenden Beispiele illustrieren die oben beschriebene Erfindung; sie sollen jedoch diese in ihrem Umfang in keiner Weise einschräken. Temperaturen werden in Celsiusgraden angegeben.

Folgende Laufmittelsysteme für die Chromatogaphie werden in den nachfolgenden Beispielen verwendet:

Neutrale Systeme		
N1	Ethylacetat/Hexan	2:1
N2	Ethylacetat/Hexan	1:1
N3	Ethylacetat/Hexan	1:2
N4	Ethylacetat/Hexan	1:4
N5	Ethylacetat/Hexan	1:9
N6	CH_2Cl_2/Methanol	95:5
N7	CH_2Cl_2/Methanol	9:1
N8	CH_2Cl_2/Methanol	4:1
N9	CH_2Cl_2/Methanol	2:1
N10	CH_2Cl_2/Methanol	1:1
Basische Systeme		
B1	CH_2Cl_2/Methanol/konzentriertes NH_3	40:10:1
B2	CH_2Cl_2/Methanol/konzentriertes NH_3	50:10:1
B3	CH_2Cl_2/Methanol/konzentriertes NH_3	60:10:1
B4	CH_2Cl_2/Methanol/konzentriertes NH_3	80:10:1
B5	CH_2Cl_2/Methanol/konzentriertes NH_3	100:10:1
B6	Ethylacetat/Ethanol/konzentriertes NH_3	24:12:4
B7	Toluol/Isopropanol/konzentriertes NH_3	170:30:2
Saure Systeme		
S1	CH_2Cl_2/Methanol/Wasser/Essigsäure	150:50:10:1
S2	Toluol/Isopropanol/Essigsäure	170:30:2

Mit basischen (d. h. konzentriertes Ammoniak enthaltenden) Laufmittelsystemen chromatographierte Produkte mit sauren funktionellen Gruppen werden nach der Chromatographie in einem organischen Lösungsmittel, z. B. in Diethylether, Essigsäureethylester oder Dichlormethan, aufgenommen. Sodann wird dieses organische Gemisch nacheinander mit (ca. 1 N-) Salzsäure, Wasser und gesättigter Natriumchloridlösung gewaschen und die organische Phase getrocknet und eingedampft. Auf diese Weise erhält man das Produkt mit der freigesetzten sauren funktionellen Gruppe.

Beispiel 1: N-Carboxymethyl-N-pentanoyl-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-ymethyl]-amin

1,2 g N-(2'-Cyanobiphenyl-4-ylmethyl)-N-methoxycarbonylmethyl-N-pentanoyl-amin, 2,18 g Tributylzinnazid und 40 ml Xylol werden 24 Stunden unter Rückfluss erhitzt. Dann wird das Reaktionsgemisch eingeengt, der Rückstand mit 1 N-Natronlauge versetzt, dieses Gemisch 10 Stunden bei Raumtemperatur gerührt und dann mit Diethylether extrahiert, die wässrige Phase sauer gestellt und anschliessend mit Diethylether extrahiert, diese zweite etherische Phase mit Sole gewaschen, getrocknet und eingeengt und das Rohprodukt mittels Flashchromatographie (100 g Kieselgel; System B1) gereinigt. Amorphes Produkt [R_f-Wert: 0,29 (CH_2Cl_2/Methanol/konzentriertes Ammoniak = 30:10:1)].

Das Ausgangsmaterial kann beispielsweise wie folgt erhalten werden:

a) 2'-Cyano-4-formyl-biphenyl

250 g 4-Brommethyl-2'-cyano-biphenyl, 150 g Natriumacetat und 2,5 l Eisessig werden über Nacht unter Rückfluss erhitzt. Das Gemisch wird anschliessend im Hochvakuum eingeengt und der Rückstand in Ethylacetat aufgenommen. Man extrahiert nacheinander mit Wasser, Natriumhydrogencarbonatlösung und Sole und dampft am Rotationsverdampfer ein. Das Rohprodukt wird in 3,1 l Ethanol gelöst, die Lösung mit 430 ml 2 N-Natronlauge versetzt, das Gemisch über Nacht bei Raumtemperatur gerührt und dann ein-

geengt und der Rückstand in Ethylacetat aufgenommen. Das Gemisch wird nacheinander mit Wasser und Sodalösung gewaschen und eingeengt. Der Rückstand wird in Hexan suspendiert, die Suspension abgenutscht und der Filterkuchen gewaschen und 20 Stunden bei 60° im Hochvakuum getrocknet. Man erhält so das 2'-Cyano-4-hydroxymethyl-biphenyl in Form eines weissen Pulvers [^{1}H-NMR (DMSO-d_6): 4,58 ppm (d, 2 H); 5,3 ppm (t, 1 H); 7,6 bis 8,0 ppm (m, 8 H)].

Eine Lösung von 53 ml Oxalylchlorid in 2 l Dichlormethan wird auf -60° gekühlt. Bei dieser Temperatur wird eine Lösung von 88 ml Dimethylsulfoxid in 150 ml Dichlormethan zugetropft und das Gemisch 2 Minuten nachgerührt. Dann wird bei -60° eine Lösung von 117 g 2'-Cyano-4-hydroxymethyl-biphenyl in 1 l Dichlormethan zugetropft. Nach beendeter Zugabe (nach ca. 5 Minuten) wird das Gemisch 15 Minuten nachgerührt. Dann werden 390 ml Triethylamin zugetropft. Man rührt das Gemisch 2 Minuten bei -60° nach, lässt es dann auf Raumtemperatur erwärmen und giesst es auf Wasser. Das Gemisch wird mit Dichlormethan extrahiert und die organische Phase nacheinander mit verdünnter Salzsäure und Sole gewaschen, getrocknet und eingeengt. Der Rückstand wird in Hexan suspendiert, die Suspension abgenutscht, der Filterkuchen gewaschen und das so erhaltene Produkt im Hochvakuum bei 60° getrocknet (Elementaranalyse: 80,7 % C; 4,5 % H; 6,7 % N; 7,7 % O).

b) N-(2'-Cyanobiphenyl-4-ylmethyl)-N-methoxycarbonylmethyl-amin

Ein Gemisch aus 2,0 g 2'-Cyano-4-formyl-biphenyl, 1,22 g 2-Aminoethansäuremethylesterhydrochlorid, 9,6 g Molekularsieb 5A und 26 ml Tetrahydrofuran wird 36 Stunden bei Raumtemperatur gerührt und dann auf 0 bis 5° abgekühlt. Es werden 680 mg Natriumcyanoborhydrid (90 %), gelöst in 4,8 ml Methanol, zugegeben. Das Gemisch wird 24 Stunden bei Raumtemperatur gerührt und dann im Vakuum eingeengt. Das Rohprodukt wird mittels Flashchromatographie (180 g Kieselgel; Essigsäureethylester/Petrolether = 1:1) gereinigt [^{1}H-NMR (DMSO-d_6): 3,63 ppm (s, 3 H); 3,79 ppm (s, 2 H); 7,4 bis 8,0 ppm (m, 10 H); 2,6 ppm (1 H)].

c) N-(2'-Cyanobiphenyl-4-ylmethyl)-N-methoxycarbonylmethyl-N-pentanoyl-amin

0,96 g N-(2'-Cyanobiphenyl-4-ymethyl)-N-methoxycarbonylmethyl-amin werden in 9 ml Dichlormethan gelöst. Die Lösung wird mit 1,7 ml Triethylamin und anschliessend bei 0° mit 1,5 ml Pentanoylchlorid versetzt. Man rührt bei Raumtemperatur über Nacht und dampft dann zur Trockne ein: Der Rückstand wird in Diethylether aufgenommen und das etherische Gemisch nacheinander mit Natriumhydrogencarbonatlösung und Sole gewaschen. Flashchromatographie (180 g Kieselgel; Essigsäureethylester/Petrolether = 1: 1) liefert das Produkt in Form eines weissen Pulvers [R_f-Wert: 0,68 (System N2)].

Beispiel 2: (S)-N-(1-Carboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Analog Beispiel 1 wird ausgehend von 1,24 g N-Valeryl-N- [(2'-cyanobiphenyl-4-yl)methyl] -(L)-alaninmethylester und 2,73 g Tributylzinnazid wird nach Flashchromatographie (B3) und anschliessendem Umkristallisieren aus Essigester das Produkt als weisses Pulver erhalten. Smp.: 115° (Zers.).

Das Ausgangsmaterial kann beispielsweise wie folgt erhalten werden:

a) N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-alaninmethylester ausgehend von 2,0 g 2'-Cyanobiphenyl-4-carbaldehyd, 1,34 g (L)-Alaninmethylester-Hydrochlorid, 680 mg Natriumcyanoborhydrid und 2,4 g Molekularsieb 5 A und anschliessender Flashchromatographie mit dem System N3. (DC: System N1) R_f-Wert: 0,59.

b) N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl] -(L)-alaninmethylester ausgehend von 1,65 g N-[(2'-Cyanobiphenyl)-methyl]-(L)-alaninmethylester, 2,7 ml Triethylamin und 2,35 ml n-Valeriansäurechlorid und anschliessender Flashchromatographie (N2). (DC:System N2) R_f-Wert: 0,62.

Beispiel 3: (S)-N-(1-Methoxycarbonylethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amin

0,3 g Säure aus Beispiel 2 werden in 5 ml Methylalkohol gelöst, mit 0,5 ml Salzsäure in Methylalkohol versetzt und während 24 Stunden bei Raumtemperatur gerührt. Das Reaktionsgemisch wird darauf eingeengt, in Methylenchlorid aufgenommen, mit Wasser extrahiert, die organische Phase getrocknet und am Rotationsverdampfer eingeengt. Nach Flashchromatographie (B1) erhält man das Produkt. Smp. des amorphen Materials: 57-59°.

Beispiel 4:
N-[1-Carboxy-2-(4-fluorphenyl)-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amin

Ausgehend von 2,3 g N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(DL)-p-fluorphenylalaninmethylester und 3,25 g Tributylzinnazid wird nach Flashchromatographie (B1) das Produkt nach Lyophilisation aus tert.-Butylalkohol erhalten. FAB-MS: m/e = 502 $(M+H)^+$.

Das Ausgangsmaterial kann beispielsweise wie folgt erhalten werden:

N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(DL)-p-fluorphenylalaninmethylester ausgehend von 2,33 g 2'-Cyanobiphenyl-4-carbaldehyd, 2,63 g (DL)-p-Fluorphenylalaninmethylester, 790 mg Natriumcyanoborhydrid und 11,0 g Molekularsieb 5 A und anschliessender Flashchromatographie mit System N3. (DC: System N2) R_f-Wert: 0,36.

N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(DL)-p-fluorphenylalaninmethylester ausgehend von 2,1 g N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(DL)-p-fluorphenylalaninmethylester, 1,0 ml Triethylamin und 0,85 ml n-Valeriansäurechlorid und anschliessender Flashchromatographie (N3). (DC: System N2) R_f-Wert: 0,64.

Beispiel 5:
N-[2-(4-Fluorphenyl)-1-methoxycarbonyl-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Analog Beispiel 3 ausgehend von 1,29 g N-Valeryl-N-[(2'-(1H-tetrazol-5-yl)-biphenyl-4-yl)-methyl]-(DL)-p-fluorphenylalanin gemäss Beispiel 4. FAB-MS: m/e = 516 $(M+H)^+$.

Beispiel 6:
N-[2-(4-Fluorphenyl)-1-hydroxymethyl-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

0,5 g N-Valeryl-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]-(DL)-p-fluorphenylalanin-methylester aus Beispiel 5 werden in 5 ml Tetrahydrofuran bei -70° mit 1,9 ml Diisobutylaluminiumhydrid versetzt. Nach 20 Minuten gibt man 0,2 ml Methylalkohol zu und lässt auf Raumtemperatur aufwärmen. Das Reaktionsgemisch wird mit Ether und Wasser versetzt, die organische Phase abgetrennt, mit Sole gewaschen, getrocknet und eingeengt. Flashchromatographie (B2) liefert den entsprechenden Aldehyd. Dieser wird bei 0° in 5 ml Ethylalkohol mit 27 mg Natriumborhydrid versetzt und während 3,5 Stunden bei dieser Temperatur gerührt. Nach Abfiltrieren und Einengen wird das Produkt durch Flashchromatographie (N8) und Lyophilisieren aus tert.-Butylalkohol erhalten. FAB-MS: m/e= 488 $(M+H)^+$.

Beispiel 7: N-(2'-Carboxybiphenyl-4-ylmethyl)-N-[1-carboxy-2-(4-fluorphenyl)-ethyl]-N-pentanoyl-amin

N-Valeryl-N-[(2'-carboxybiphenyl-4-yl)-methyl]-(DL)-p-fluorphenylalanin-methylester werden in 10 ml Methylalkohol und 3 ml Wasser mit 0,45 ml 2N NaOH versetzt. Man rührt über Nacht bei Raumtemperatur und neutralisiert anschliessend mit 0,45 ml 2N Salzsäure. Nach Flashchromatographie (B1) und Lyophilisieren aus tert.-Butanol erhält man das amorphe Produkt. FAB-MS: m/e= 478 $(M+H)^+$.

Beispiel 8:
N-(2'-Carboxybiphenyl-4-ylmethyl)-N-[2-(4-fluorphenyl)-1-methoxy-carbonyl-ethyl]-N-pentanoyl-amin

840 mg N-Valeryl-N-[(2'-(trimethylsilylethoxycarbonyl)biphenyl-4-yl)-methyl]-(DL)-p-fluorphenylalaninmethylester werden in 10 ml Dimethylformamid mit 15,6 ml einer 0,5 M Lösung von Tetrabutylammoniumfluorid in Tetrahydrofuran versetzt und über Nacht bei Raumtemperatur gerührt. Das Reaktionsgemisch wird eingeengt, in Essigester aufgenommen, mit Wasser und Sole gewaschen, getrocknet und eingeengt. Nach Flashchromatographie (B4) und Lyophilisieren aus tert.-Butanol erhält man das Produkt. FAB-MS: m/e= 492 $(M+H)^+$.

Das Ausgangsmaterial kann beipielsweise wie folgt erhalten werden:

14,2 g 4-Methyl-2'-carboxybiphenyl (EP 253,310) werden in 60 ml Acetonitril und 10,7 ml Pyridin gelöst und 11,4 ml Trimethylsilylethanol zugegeben. Man versetzt mit bei 0° 15,1 g Dicyclohexylcarbodiimid und rührt bei dieser Temperatur während 3 Stunden. Darauf wird das Reaktionsgemisch im Hochvakuum eingedampft, mit Ether versetzt und Dicyclohexylharnstoff abfiltriert. Nach Flashchromatographie (Essigester/Hexan 95:5) erhält man das 4-Methyl-2'-(trimethylsilylethoxycarbonyl)biphenyl als leicht gelbliches Oel. (DC: Essigester-/Hexan 95:5) R_f-Wert: 0.42.

312 mg 4-Methyl-2'-(trimethylsilylethoxycarbonyl)biphenyl, 178 mg N-Bromsuccinimid, 5 mg Azoisobutyronitril und 15 ml Tetrachlorkohlenstoff werden eine Stunde zum Rückfluss erhitzt. Nach Abkühlen wird das Gemisch eingedampft. Flashchromatographie (Essigester/Hexan 95:5) liefert 4-Brommethyl-2'-(trimethylsilylethoxycarbonyl)biphenyl als leicht gelbliches Oel. ^{1}H-NMR ($CFCl_3$): 0 ppm (s, 9 H), 0,7 ppm (t, 2 H), 4,5 ppm (s, 2 H), 7,1-8 ppm Aromaten.

2,8 g 4-Brommethyl-2'-(trimethylsilylethoxycarbonyl)biphenyl und 1,17 g wasserfreies Natriumacetat werden in Eisessig über Nacht bei 65° gerührt und anschliessend 3 Stunden unter Rückfluss gekocht. Das

Reaktionsgemisch wird eingedampft, der Rückstand in Essigester aufgenommen, mit Wasser und Natriumhydrogencarbonat gewaschen, die organische Phase getrocknet und eingeengt. Der Rückstand wird in 25 ml Ethanol vorgelegt, 6,3 ml 1N NaOH zugegeben und 30 Minuten bei Raumtemperatur gerührt. Das Gemisch wird im Vakuum eingedampft, mit Essigester versetzt, mit Wasser und Sole gewaschen, getrocknet und eingedampft. Flashchromatographie (N4) liefert 4-Hydroxymethyl-2-(trimethylsilylethoxycarbonyl)biphenyl als farbloses Oel. ^{1}H-NMR (DMSO): 0 ppm (s, 9 H), 0,75 ppm (t, 2 H), 4,1 ppm (t, 2 H), 4,73 ppm (d, 2 H), 5,27 ppm (t, 1H), 7,2-7,7 ppm Aromaten.

2'-(Trimethylsilylethoxycarbonyl)biphenyl-4-carbaldehyd wird analog Beispiel 1 a) erhalten ausgehend von 6,5 g 4-Hydroxymethyl-2'-(trimethylsilylethoxycarbonyl)-biphenyl, 1,87 ml Oxalylchlorid, 3,1 ml Dimethylsulfoxid und 13,8 ml Triethylamin und anschliessender Flashchromatographie mit Methylenchlorid. ^{1}H-NMR ($CDCl_3$): 0 ppm (s, 9 H), 0,8 ppm (t, 2 H), 4,2 ppm (t, 2 H), 7,2-8,1 ppm Aromaten, 10,1 ppm (s, 1 H).

Analog Beispiel 1 b) erhält man ausgehend von 1,0 g 2'-(Trimethylsilylethoxycarbonyl)-biphenyl-4-carbaldehyd, 3,0 g Molekularsieb 5 A, 0,715 g (D,L)-p-Fluorphenylalaninmethylester-Hydrochlorid und 215 mg Natriumcyanoborhydrid und anschliessender Flashchromatographie (N3) N-[(2'-(Trimethylsilylethoxy-carbonyl)biphenyl-4-yl)-methyl]-(D,L)-p-fluorphenylalanin-methylester. (DC: N3) R_f-Wert: 0,64.

Analog Beispiel 1 c) erhält man ausgehend von 0,8 g N-[(2'-(Trimethylsilylethoxycarbonyl)biphenyl-4-yl)-methyl]-(D,L)-p-fluorphenylalanin-methylester, 0,29 ml Triethylamin und 0,25 ml Valerylchlorid nach Flashchromatographie (N3) N-Valeryl-N-[(2'-(trimethylsilylethoxycarbonyl)biphenyl-4-yl)-methyl] -(D,L)-p-fluorphenylalaninmethylester. (DC: N3) R_f-Wert = 0,65.

Beispiel 9: (S)-N-(2'-Carboxybiphenyl-4-ylmethyl)-N-(1-hydroxymethyl-2-phenylethyl)-N-pentanoyl-amin

290 mg N-[3-(p-Fluorphenyl)-1-hydroxy-2-propyl]-N-[2'-(trimethylsilylethoxycarbonyl)-4-yl-methyl]-valeriansäureamid werden in 3 ml Dimethylformamid während 20 Stunden bei Raumtemperatur mit 5,82 ml einer 0,5 molaren Lösung von Tetrabutylammoniumfluorid in Tetrahydrofuran behandelt. Das Gemisch wird im Vakuum eingeengt, in Essigester aufgenommen, mit Wasser und Sole gewaschen und eingeengt. Nach Flashchromatographie (N7) und Lyophilisation erhält man das Produkt als weisses Pulver. FAB-MS: m/e= 446 $(M+H)^+$.

Das Ausgangsmaterial kann beispielsweise wie folgt erhalten werden:

Analog Beispiel 1 b) erhält man ausgehend von 1,5 g 2'-(Trimethylsilylethoxycarbonyl)-biphenyl-4-carbaldehyd, 4,5 g Molekularsieb 5 A, 0,694 g (D,L)-3-Phenyl-2-amino-propan-1-ol und 321 mg Natriumcyanoborhydrid nach Flashchromatographie (B5) N-[(2'-(Trimethylsilylethoxycarbonyl)biphenyl-4-yl)-methyl] -3-(p-fluorphenyl)-2-aminopropan-1-ol. ^{1}H-NMR (DMSO): 0 ppm (2 s, 9 H), 0,73 ppm (2 t, 2 H), 2 ppm (b, 1 H), 2,73 ppm (m, 3 H), 3,3 ppm (m, 2 H), 3,83 ppm (s, 2 H), 4,1 ppm (2 t, 2 H), 4,6 ppm (t, 1 H), 7,15-7,8 ppm, m (8 H).

Analog Beispiel 1 c) erhält man ausgehend von 365 mg N-[(2'-(Trimethylsilylethoxycarbonyl)biphenyl-4-yl)-methyl]-3-(phenyl)-2-amino-propan-1-ol, 0,136 ml Triethylamin, 0,112 ml Valerylchlorid und anschliessender Flashchromatographie (N3) N-[3-(Phenyl)-1-hydroxy-2-propyl]-N-[(2'-(trimethylsilylethoxycarbonyl)-4-yl-methyl]-valeriansäureamid. FAB-MS: m/e= 546 $(M+H)^+$.

Beispiel 10:
(S)-N-(2'-Carboxybiphenyl-4-ylmethyl)-N-(1-hydroxymethyl-2-imidazol-4-yl-ethyl)-N-pentanoyl-amin

Analog Beispiel 9 erhält man das Produkt ausgehend von 272 mg N-[3-(Imidazol-4-yl)-1-hydroxy-2-propyl]-N-[(2'-(trimethylsilylethoxycarbonyl)biphenyl-4-yl)-methyl]-valeriansäureamid und 5,54 ml Tetrabutylammoniumfluoridlösung nach Flashchromatographie (B1). FAB-MS $(M+H)^+$=436.

Das Ausgangsmaterial kann beispielsweise analog Beispiel 9 wie folgt erhalten werden:

Umsetzung von 1,5 g 2'-(Trimethylsilylethoxycarbonyl)biphenyl-4-carbaldehyd, 0,984 g 3-(Imidazol-4-yl)-2-(S)-amino-propan-1-ol-dihydrochlorid, 321 mg Natriumcyanoborhydrid und 4,5 g Molekularsieb 5 A liefert nach Flashchromatographie (B5) N-[(2'-(Trimethylsilylethoxycarbonyl)biphenyl-4-yl)-methyl]-3-(imidazol-4-yl)-2-aminopropan-1-ol. (DC) R_f-Wert (0,36).

Umsetzung von 0,45 g N-[(2'-(Trimethylsilylethoxycarbonyl)biphenyl-4-yl)-methyl]-3-(imidazol-4-yl)-2-(S)-amino-propan-1-ol, 0,152 ml Triethylamin und 0,132 ml Valerylchlorid liefert nach Flashchromatographie (Methylenchlorid-Methanol-conc. Ammoniak: 120-10-1) N-[3-(Imidazol-4-yl)-1-hydroxy-2-propyl]-N-[(2'-(trimethylsilylethoxycarbonyl)biphenyl-4-yl)-methyl]-valeriansäureamid. Bei der Aufarbeitung wird die wässrige Phase leicht basisch gestellt. FAB-MS: m/e= 536 $(M+H)^+$.

Beispiel 11: (R)-N-(1-Carboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Analog Beispiel 1 wird das Produkt hergestellt ausgehend von 0,84 g N-Valeryl-N-[(2'-cyano-biphenyl-4-yl)-methyl]-(D)-alanin-methylester und 731 mg Tributylzinnazid und anschliessender Flashchromatographie (B1). FAB-MS: m/e = 408 (M+H)+.

Das Ausgangsmaterial kann beispielsweise analog Beispiel 1 b) erhalten werden:

Umsetzung von 2,0 g 2'-Cyanobiphenyl-4-carbaldehyd, 9,6 g Molekularsieb 5 A, 1,34 g (D)-Alaninmethylester-Hydrochlorid und 680 mg Natriumcyanoborhydrid liefert nach Flashchromatographie (N3) N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(D)-alaninmethylester. ^{1}H-NMR (DMSO): 1,21 ppm (d, 3 H), 3,63 ppm (s, 3 H), 3,75 ppm (dd, 1 H), 4,56 ppm (d, 2 H), 4,58 ppm (d, 2 H), 5,31 ppm (t, 1 H), 7,4-8 ppm Aromaten.

Umsetzung analog Beispiel 1 c) von 1,25 g N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(D)-alaninmethylester, 2,1 ml Triethylamin und 1,8 ml n-Valeriansäurechlorid liefert nach Flashchromatographie (N3) N-Valeryl-N-[(2'-cyano-biphenyl-4-yl)-methyl]-(D)-alaninmethylester (DC: N2) R_f-Wert: 0,61.

Beispiel 12:
(1S),(2S)-N-(1-Carboxy-2-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Das Produkt kann ausgehend von 2,0 g N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-isoleucinmethylester und 3,19 g Tributylzinnazid und anschliessender Flashchromatographie (B1) hergestellt werden. FAB-MS (M+H)+ = 450.

Das Ausgangsmaterial kann beispielsweise analog Beispiel 1 b) erhalten werden:

Die Umsetzung von 2,0 g 2'-Cyanobiphenyl-4-carbaldehyd, 9,6 g Molekularsieb 5 A, 1,76 g (L)-Isoleucinmethylester-Hydrochlorid und 680 mg Natriumcyanoborhydrid liefert nach Flashchromatographie (Essigester-Hexan 1:3) den N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-isoleucinmethylester. ^{1}H-NMR (DMSO): 1,21 ppm (d, 3 H), 3,63 ppm (s, 3 H), 3,75 (dd, 1 H), 4,56 ppm (d, 2 H), 4,58 ppm (d, 2 H), 5,31 ppm (t, 1 H), 7,4-8 ppm Aromaten.

Die Umsetzung analog Beispiel 1 c) von 1,80 g N-[(2'-Cyanobiphenyl)-4-yl-methyl]-(L)-isoleucinmethylester, 2,7 ml Triethylamin und 2,35 ml n-Valeriansäurechlorid liefert nach Flashchromatographie (N4) den N-Valeryl-N-[(2'-cyano-biphenyl-4-yl)-methyl]-(L)-isoleucinmethylester. (DC: N3) R_f-Wert: 0,43.

Beispiel 13:
(1S),(2S)-N-(1-Methoxycarbonyl-2-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Das Produkt kann erhalten werden analog Beispiel 3 ausgehend von 200 mg N-Valeryl-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]-(L)-isoleucin und anschliessender Flashchromatographie (B1). FAB-MS: m/e= 464 (M+H)+.

Beispiel 14: (S)-N-(1-Carboxybut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Das Produkt kann analog Beispiel 1 ausgehend von 0,30 g N-Valeryl-N-[(2'-cyano-biphenyl-4-yl)-methyl]-(L)-norvalin-methylester und 490 mg Tributylzinnazid und anschliessender Flashchromatographie (B1) hergestellt werden. FAB-MS (M+H)+ = 436.

Das Ausgangsmaterial kann beispielsweise analog Beispiel 1 b) erhalten werden: Die Umsetzung von 2,0 g 2'-Cyanobiphenyl-4-carbaldehyd, 9,6 g Molekularsieb 5 A, 1,34 g (L)-Norvalinmethylester-Hydrochlorid und 680 mg Natriumcyanoborhydrid liefert nach Flashchromatographie (N3) den N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-norvalin-methyl-ester. ^{1}H-NMR (DMSO): 0,83 ppm (t, 3 H), 1,33 ppm (m, 2 H), 1,55 ppm (m, 2 H), 3,62 ppm (s, 3 H), 3,1 ppm (m, 1 H), 7,3-8 ppm Aromaten.

Die Umsetzung analog Beispiel 1 c) von 1,5 g N-[(2'-Cyanobiphenyl)-4-yl)-methyl]-(L)-norvalinmethylester, 2,35 ml Triethylamin und 2, 15 ml n-Valeriansäurechlorid liefert nach Flashchromatographie (Essigester-Hexan: 1-3) den N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-norvalinmethylester (DC:B1) R_f-Wert: 0,9.

Beispiel 15:
(S)-N-(1-Methoxycarbonylbut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amin

Das Produkt kann analog Beispiel 3 erhalten werden ausgehend von 200 mg der Verbindung gemäss Beispiel 14 und anschliessender Flashchromatographie (B1). FAB-MS: m/e=464 (M+H)+.

Beispiel 16:
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amin

Das Produkt kann hergestellt werden ausgehend von 1,40 g N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-valinmethylester und 2,25 g Tributylzinnazid und anschliessender Flashchromatographie (B1). FAB-MS $(M+H)^+$ = 436, Schmelzintervall 105-115° (aus Ethylacetat).

Das Ausgangsmaterial kann beispielsweise analog Beispiel 1 b) erhalten werden:

Umsetzung von 0,5 g 2'-Cyanobiphenyl-4-carbaldehyd, 2,5 g Molekularsieb 5 A, 0,815 g (L)-Valinmethylester-Hydrochlorid und 180 mg Natriumcyanoborhydrid liefert nach Flashchromatographie (N3) den N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-valinmethylester. (DC: N3) R_f-Wert: 0,5.

Umsetzung analog Beispiel 1 c) von 1,15 g N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-valinmethylester, 0,625 ml Triethylamin und 0,56 ml n-Valeriansäurechlorid liefert nach Flashchromatographie (N3) den N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-valinmethylester. (DC: N2) R_f-Wert: 0,63.

Beispiel 17: (S)-N-(1-Carboxyethyl)-N-hexanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Das Produkt kann hergestellt werden ausgehend von 2,4 g N-Caproyl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-alaninmethylester und 4,05 g Tributylzinnazid und anschliessender Flashchromatographie (B1). FAB-MS: m/e= 422 $(M+H)^+$.

Das Ausgangsmaterial kann beispielsweise analog Beispiel 2 erhalten werden:

Umsetzung von 2,0 g N-[(2'-Cyanobiphenyl)-4-yl-methyl]-(L)-alaninmethylester, 1,23 ml Triethylamin, und 1,22 ml n-Caproylchlorid liefert den N-Caproyl-ageN- [(2'-cyanobiphenyl-4-yl)-methyl]-(L)-alanin-methylester. (DC: N2) R_f-Wert: 0,5.

Beispiel 18: (S)-N-Butanoyl-N-(1-carboxyethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Das Produkt kann hergestellt werden ausgehend von 2,25 g N-Butyryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-alaninmethylester und 4,11 g Tributylzinnazid und anschliessender Flashchromatographie (B1). FAB-MS: m/e = 394 $(M+H)^+$.

Das Ausgangsmaterial kann beispielsweise analog Beispiel 2 erhalten werden:

Umsetzung von 2,0 g N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-alaninmethylester, 1,23 ml Triethylamin und 0,92 ml n-Buttersäurechlorid liefert den N-Butyryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]- (L)-alanin-methylester. (DC: N2) R_f-Wert: 0,5.

Beispiel 19: (S)-N-(1-Carboxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Das Produkt kann hergestellt werden ausgehend von 0,68 g N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-2-aminobuttersäuremethylester und 1,15 g Tributylzinnazid. Kristallisation aus Ether. Smp.: 102- 104°. FAB-MS $(M+H)^+$ = 422.

Das Ausgangsmaterial kann beispielsweise analog Beispiel 1 b) erhalten werden:

Umsetzung von 3,0 g 2'-Cyanobiphenyl-4-carbaldehyd, 14,5 g Molekularsieb 5 A, 2,23 g (L)-2-Aminobuttersäure-Hydrochlorid und 1075 mg Natriumcyanoborhydrid liefert nach Flashchromatographie (N3) den N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-2-aminobuttersäuremethylester. ^{1}H-NMR (DMSO): 0,88 ppm (t, 3 H), 1,62 ppm (m, 2 H), 2,53 ppm (b, 1 H), 3,15 ppm (m, 1 H), 3,63 ppm (s, 3 H), 3,62 ppm (d, 2 H), 3,81 ppm (d, 1 H).

Umsetzung analog Beispiel 1 c) von 0,54 g N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-2Aminobuttersäuremethylester, 0,33 ml Triethylamin und 0,29 ml N-Valeriansäurechlorid liefert den N-Valeryl-N-[(2'-cyano-biphenyl-4-yl)-methyl]-(L)-2-aminobuttersäuremethylester. (DC: N2) R_f-Wert: 0,52.

Beispiel 20:
(S)-N-(1-Carboxy-2-cyclohexyl-ethyl)-N-pentanoyl-N-(2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Das Produkt kann hergestellt werden ausgehend von 4,0 g N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-cyclohexylalaninmethylester und 5,8 g Tributylzinnazid und anschliessender Flashchromatographie (B1). FAB-MS $(M+H)^+$ = 490.

Das Ausgangsmaterial kann beispielsweise analog Beispiel 1 b) erhalten werden:

Umsetzung von 9,35 g 2'-Cyanobiphenyl-4-carbaldehyd, 46 g Molekularsieb 5 A, 10,0 g (L)-Cyclohexylalaninmethylester-Hydrochlorid und 3,3 g Natriumcyanoborhydrid liefert nach Flashchromatographie (N3) den N-[(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-cyclohexylalaninmethylester. (DC: N3) R_f-Wert: 0,45.

Umsetzung analog Beispiel 1 c) von 9,0 g N-(2'-Cyanobiphenyl-4-ylmethyl)-(L)-cyclohexylalaninmethylester, 4,33 g Triethylamin und 3,75 ml n-Valeriansäurechlorid liefert nach Flashchromatographie (N3) den N-Valeryl-N-[(2'-cyano-biphenyl-4-yl)-methyl](L)-cyclohexylalanin-ester-methylester. (DC: N3) R_f-Wert: 0,55.

Beispiel 21:
(S)-N-(2-Cyclohexyl-1-methoxycarbonyl-ethyl)-N-pentanoyl-N-[2'-(1-H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Das Produkt kann erhalten werden analog Beispiel 3 ausgehend von 1,02 g der Verbindung aus Beispiel 20. FAB-MS: m/e= 504 $(M+H)^+$.

Beispiel 22:
(R)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Das Produkt kann hergestellt werden analog Beispiel 11 ausgehend von 3,8 g N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(D)-valinmethylester und 6;17 g Tributylzinnazid und anschliessender Flashchromatographie (N8). FAB-MS $(M+H)^+$ = 436.

Das Augangsmaterial kann beispielsweise analog Beispiel 1 b) erhalten werden:

Umsetzung von 4,0 g 2'-Cyanobiphenyl-4-carbaldehyd, 19,3 g Molekularsieb 5 A, 3,8 g (D)-Valinmethylester-Hydrochlorid und 1,43 g Natriumcyanoborhydrid liefert nach Flashchromatographie (N3) den N-[(2'-cyanobiphenyl-4-yl)-methyl]-(D)-Valinmethylester. (DC: N2) R_f-Wert: 0,56.

Umsetzung analog Beispiel 1 c) von 3,2 g N-[(2'-Cyanobiphenyl)-4-yl-methyl]-(D)-valinmethylester, 1,82 ml Triethylamin und 1,6 ml N-Valeriansäurechlorid liefert nach Flashchromatographie (N2) den N-Valeryl-N-[(2'-cyano-biphenyl-4-yl)methyl]-(D)-valinmethylester. FAB-MS: m/e= 407 $(M+H)^+$.

Beispiel 23: N-(2-Methoxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Unter Ueberleiten eines schwachen Stickstoffstromes wird eine Lösung von 1,6 g (4,5 mMol) rohem N-[(2'-Cyanobiphenyl-4-yl)-methyl]-N-(2-methoxyethyl)-valeriansäureamid und 1,8 g (5,5 mMol) Tri-n-butylzinnazid in 15 ml o-Xylol während 20-24 Stunden unter Rückfluss erhitzt. Nach dem Abkühlen wird die Lösung mit ca. 30 ml Toluol verdünnt, mit 15 ml 1N wässriger Natronlauge versetzt und während 2 Stunden intensiv gerührt. Die wässrige Phase wird abgetrennt und mit 16 ml 1N wässriger Salzsäure sauer gestellt. Das ausgefällte Produkte wird durch Extraktion mit Aethylacetat isoliert. Man erhält so die rohe Titelverbindung als Oel, das aus wenig Ethylacetat kristallisiert, Smp. 120-122°.

Das Ausgangsmaterial kann beispielsweise wie folgt hergestellt werden:

a) 4-[N-(2-Methoxyethyl)-aminomethyl]-2'-cyanobiphenyl

Eine Lösung von 5,45 g (20 mMol) 4-Brommethyl-2'-cyanobiphenyl in 40 ml 1,4-Dioxan wird mit 7,5 g (100 mMol) 2-Methoxyethylamin versetzt und hierauf 8-10 Stunden unter Rückfluss zum Sieden erhitzt. Nach dem gründlichen Eindampfen im Wasserstralvakuum wird der Eindampfrückstand in 60 ml 2N Salzsäure gelöst und mit 60 ml Ether extrahiert. Die salzsaure Lösung wird abgetrennt und mit conc. Natronlauge alkalisch gestellt. Das ausgefallene Oel wird mit Ether extrahiert, die Etherlösung mit Wasser gewaschen, über Magnesiumsulfat getrocknet und eingedampft. Man erhält so die rohe Titelverbindung als Oel, das in wenig Ether gelöst und mit einer methanolischen Lösung von Salzsäure-Gas versetzt wird. Das so erhaltene kristalline Hydrochlorid wird aus 2-Propanol umkristallisiert und schmilzt bei 174-176°.

b) N-[(2'-Cyanobiphenyl-4-yl)methyl]-N-(2-methoxyethyl)-n-valeri ansäureamid

Zu einem Gemisch von 3,7 g (12,2 mMol) 4-[N-(2-Methoxyethyl)-aminomethyl]2'-cyanobiphenyl-Hydrochlorid und 3,1 g (31 mMol) Triethylamin in 50 ml 1,4-Dioxan werden unter Rühren und Kühlen mit Eiswasser 1,5 g (15 mMol) n-Valerylchlorid getropft. Die Suspension wird 4-6 Stunden bei Raumtemperatur gerührt. Nach dem Eindampfen im Wasserstrahlvakuum wird das Reaktionsgemisch zwischen 20 ml Wasser und 200 ml Ethylacetat verteilt. Die organische Phase wird nacheinander mit je 10 ml 2N Salzsäure, gesättigter $NaHCO_3$-Lösung und Sole gewaschen, über Magnesiumsulfat getrocknet und im Vakuum eingedampft. Die so erhaltene Titelverbindung wird als Oel erhalten (R_f-Wert: 0,51 im System B7) und kann roh weiter umgesetzt werden.

Beispiel 24: N-(2-Benzyloxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

6,5 g (15,2 mMol) rohes N-(2-Benzyloxyethyl)-N-[(2'-cyanobiphenyl-4-yl)-methyl]n-valeriansäureamid und 6,1 g Tri-n-butylzinnazid werden analog Beispiel 23 umgesetzt und aufgearbeitet. Man erhält so die rohe Titelverbindung, die nach Umkristallisation aus wenig Ethylacetat bei 109-110° schmilzt.

Das Ausgangsmaterial kann beispielsweise auf folgende Weise hergestellt werden:

a) 4-[N-(2-Benzyloxyethyl)-aminomethyl]-2'-cyanobiphenyl

Analog Beispiel 23 a) erhält man aus 2-Benzyloxyethylamin (J. Am. Pharm. Assoc., Sci. Ed. 1952, 41, 257) die Titelverbindung nach flash-chromatographischer Reinigung (Silicagel; Toluol-Methanol 19:1) als gelbliches Oel, das im DC im System B7 einen R_f-Wert von 0,48 aufweist.

b) N-(2-Benzyloxyethyl)-N-[(2'-cyanobiphenyl-4-yl)methyl]-n-vale riansäureamid

Analog Beispiel 26 b) erhält man aus 4-[N-(2-Benzyloxyethyl)-aminomethyl]-2'-cyanobiphenyl die Titelverbindung. Sie weist im DC-System B7 einen R_f-Wert von 0,71 auf und kann roh weiterverwendet werden.

Beispiel 25: N-(3-Methoxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Analog Beispiel 23 erhält man aus 2,1 g (5,8 mMol) rohem N-[(2'-Cyanobiphenyl-4-yl)methyl]-N-(3-methoxypropyl)-n-valeriansäureamid und 2,3 g (6,9 mMol) Tri-n-butylzinnazid in 20 ml o-Xylol und flash-chromatographischer Reinigung die Titelverbindung als dickflüssiges Oel mit einem R_f-Wert von 0,33 im DC-System B6.

Das Ausgangsmaterial kann beispielsweise auf folgende Weise hergestellt werden:

a) 4-[N-(3-Methoxypropyl)-aminomethyl]-2'-cyanobiphenyl

Analog Beispiel 23 a) erhält man aus 3-Methoxypropylamin die Titelverbindung, die ein Hydrochlorid vom Smp. 183-184° bildet (aus 2-Propanol-Ether).

b) N-[(2'-Cyanobiphenyl-4-yl)-methyl]-N-(3-methoxypropyl)-n-vale riansäureamid

Analog Beispiel 23 b) erhält man aus 25 a) die Titelverbindung. Sie weist im DC-System B7 einen R_f-Wert von 0,55 auf und kann roh weiter umgesetzt werden.

Beispiel 26: N-(3-Benzyloxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

5,8 g (13 mMol) der Verbindung 26 b) und 5,3 g (16 mMol) Tri-n-butylzinnazid werden analog Beispiel 23 umgesetzt und aufgearbeitet. Man erhält so die rohe Titelverbindung als Oel, das aus wenig 2-Propanol-Ether zur Kristallisation gebracht wird und dann bei 112-115° schmilzt.

Das Ausgangsmaterial kann beispielsweise auf folgende Weise hergestellt werden:

a) 4-[N-(3-Benzyloxypropyl)-aminomethyl]-2'-cyanobiphenyl

Eine Lösung von 6,0 g (22 mMol) 4-Brommethyl-2'-cyanobiphenyl, 5,8 g (35 mMol) 3-Benzyloxypropylamin und 3,6 g Triethylamin in 50 ml 1,4-Dioxan wird 18 Stunden unter Rückfluss zum Sieden erhitzt. Nach Aufarbeitung analog Beispiel 23 a) erhält man ein Oel, das nach flashchromatographischer Reinigung (Ethanol:Ethylacetat 1:4) die Titelverbindung ergibt (DC-System B7; Rf-Wert 0,39).

b) N-(3-Benzyloxypropyl)-N-[(2'-cyanobiphenyl-4-yl)methyl]-n-val eriansäureamid

2,0 g (16,7 mMol) n-Valerylchlorid werden unter Kühlung mit einem Wasserbad unter Rühren in eine Lösung von 5,5 g (15,4 mMol) der Verbindung 26 a) und 4,0 g Triethylamin in 40 ml 1,4-Dioxan getropft. Das Reaktionsgemisch wird 5-10 Stunden bei Raumtemperatur gerührt und wie in Beispiel 23 b) aufgearbeitet. Man erhält so die Titelverbindung als Oel (R_f im System B7: 0,51), das für die weitere Umsetzung genügend rein ist.

Beispiel 27: N-(2-Hydroxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Eine Lösung von 2,6 g (5,5 mMol) der in Beispiel 24 beschriebenen Verbindung in 90 ml 1,4-Dioxan wird unter Zusatz von insgesamt 2,0 g Palladium-auf-Kohle-Katalysator (5 %) bei Raumtemperatur solange hydriert, bis in einer DC-Kontrolle (System B6) keine Ausgangsverbindung mehr festzustellen ist (ca. 70 Stunden). Der Katalysator wird abfiltriert, das Filtrat im Vakuum eingedampft und der Rückstand in Ethylacetat gelöst. Durch Waschen der Ethylacetat-Lösung mit Wasser, Trocknen und Eindampfen im Vakuum erhält man einen farblosen Schaum, dessen ^{1}H-NMR-Spektrum mit der Struktur der Titelverbindung übereinstimmt und der einen R_f-Wert von 0,60 aufweist (DC-System B6).

Beispiel 28: N-(3-Hydroxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

2,7 g (5,5 mMol) der in Beispiel 26 beschriebenen Verbindung werden analog Beispiel 27 hydriert und aufgearbeitet. Man erhält ein gelbliches Oel, das nach flashchromatographischer Reinigung (System S2) die Titelverbindung als farblosen Schaum ergibt, die einen R_f-Wert von 0,26 aufweist (System S2).

Beispiel 29:
N-(1-Methoxycarbonyl-1-methyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Eine Lösung von 9,4 g (24 mMol) rohem 2-Amino-N- [(2'-cyanobiphenyl-4-yl)methyl]-2-methyl-N-valeryl-propansäure-methylester und 9,7 g (29 mMol) Tri-n-butylzinnazid in 120 ml o-Xylol wird 30 Stunden unter Rückfluss zum Sieden erhitzt und dann analog Beispiel 23 aufgearbeitet. Die so als Oel erhaltene, rohe Titelverbindung wird zur Reinigung mit dem System B6 flashchromatographiert. Die so erhaltene Titelverbindung bildet einen Schaum und zeigt einen R_f-Wert von 0,39 (System B6).

Das Ausgangsprodukt kann beispielsweise auf folgende Weise erhalten werden:

a) 2-Amino-N-[(2'-cyanobiphenyl-4-yl)methyl]-2-methyl-propansäur e-methylester

Ein Gemisch von 10,9 g (40 mMol) 4-Brommethyl-2'-cyanobiphenyl, 18,4 g (120 mMol) 2-Amino-2-methylpropansäuremethylester-hydrochlorid (D. Leibfritz et al., Tetrahedron 1982, 38, 2165) und 22 g Kaliumcarbonat in 100 ml Dimethylformamid wird 18-20 Stunden unter Rühren in einem Bad von 80° erwärmt. Die Suspension wird filtriert, das Filtrat im Vakuum eingedampft und der Rückstand zwischen 200 ml Ethylacetat und 50 ml Wasser verteilt. Die organische Phase wird abgetrennt, mit je 30 ml Wasser und Sole gewaschen, getrocknet und eingedampft. Man erhält so die rohe Titelverbindung. Sie bildet ein Hydrochlorid vom Smp. 170-175° (aus 2-Propanol).

b) 2-Amino-N-[(2'-cyanobiphenyl-4-yl)methyl]-2-methyl-N-valerylp ropionsäuremethylester

Eine Lösung von 7,4 g (24 mMol) der Verbindung 29 a) (als Base) und 3,7 g (29 mMol) Ethyldiisopropylamin in 100 ml Methylenchlorid wird unter Rühren tropfenweise mit 3,5 g (29 mMol) Valerylchlorid versetzt. Das Reaktionsgemisch wird 20-25 Stunden bei Raumtemperatur gerührt, bis kein Ausgangsamin mehr im DC festzustellen ist (System B7). Aufarbeitung analog Beispiel 23 b) ergibt die rohe Titelverbindung als gelbliches Oel mit R_f 0,40 (System B7), welches roh weiterverwendet wird.

Beispiel 30: N-(2-Carboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

393 mg N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3-amino-propansäureethylester werden analog Beispiel 1 umgesetzt. Das Rohprodukt wird an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 95:5 gereinigt, R_f = 0,15 (System N8).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) 3-[(2'-Cyano-biphenyl-4-yl)methylamino]-propansäureethylester

wird analog Beispiel 1 b) aus 4,145 g 2'-Cyanobiphenyl-4-carbaldehyd und 3,135 g 3-Amino-propansäureethylester-hydrochlorid erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 95:5 gereinigt, R_f = 0,21 (System N6).

b) N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3-amino-propänsä ure-ethylester

wird analog Beispiel 1 c) aus 1,542 g 3-[(2'-Cyano-biphenyl-4-yl)methylamino]-propansäureethylester erhalten, R_f = 0,66 (System N6).

Beispiel 31: N-(2-Carboxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

785 mg rac-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3-amino-2-methyl-propansäuremethylester werden analog Beispiel 1 umgesetzt und extraktiv gereinigt, R_f = 0,29 (Sytem N8).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) rac-3-Amino-2-methyl-propansäure-methylester-hydrochlorid

wird aus 10,312 g rac-3-Amino-2-methylpropansäure in 100 ml Methanol durch tropfenweise Zugabe von 7,3 ml Thionylchlorid erhalten, R_f = 0,30 (System N8).

b) rac-3-[(2'-Cyano-biphenyl-4-yl)methylamino]-2-methyl-propansäuremethylester

wird analog Beispiel 1 b) aus 4,145 g 2'-Cyanobiphenyl-4-carbaldehyd und 3,072 g rac-3-Amino-2-methylpropansäuremethylester-hydrochlorid erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 97:3 gereinigt, R_f = 0,31 (System N6).

c) rac-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3-amino-2-me thyl-propansäuremethylester

wird analog Beispiel 1 c) aus 1,542 g rac-3-[(2'-Cyano-biphenyl-4-yl)methylamino]-2-methyl-propansäuremethylester erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 98:2 gereinigt, R_f = 0,66 (System N6).

Beispiel 32: N-(1-Carboxy-1-methyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amin

Eine Lösung von 2,5 g (6 mMol) des in Beispiel 29 beschriebenen Esters in 30 ml Methanol wird mit 3,5 ml wässriger Natronlauge (20 %) versetzt und solange unter Rückfluss und Rühren zum Sieden erhitzt (ca. 35-40 Std.), bis der Ausgangsester im DC (System B6) nicht mehr nachzuweisen ist. Die Lösung wird klar filtriert, das Methanol wird im Vakuum abgedampft und die verbleibende wässrige Lösung mit conc. Salzsäure auf pH 1-2 gebracht. Das ausgefallene Produkt wird mit 200 ml Ethylacetat extrahiert, die organische Phase abgetrennt, mit Sole gewaschen und über $MgSO_4$ getrocknet. Das nach dem Abdampfen des Lösungsmittels isolierte Rohprodukt wird mittels eines Gemisches Methylenchlorid 360 ml, Methanol 40 ml, Wasser 4 ml, Essigsäure 2 ml flashchromatographisch gereinigt. Die einheitlich nur das Produkt enthaltenden Fraktionen werden vereinigt eingedampft und ergeben die Titelverbindung als farblosen Schaum, der im DC (System wie oben erwähnt) einen R_f-Wert von 0,33 aufweist.

Beispiel 33: N-(5-Hydroxypent-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Eine Lösung von 6,5 g (17 mMol) rohem N-[(2'-Cyanobiphenyl-4-yl)methyl]-N-(5hydroxypentyl)-n-valeriansäureamid und 6,8 g (20,4 mMol) Tri-n-butylzinnazid in 70 ml o-Xylol wird analog Beispiel 23 umgesetzt und aufgearbeitet. Das so erhaltene Rohprodukt wird durch Flash-Chromatographie (System B6) gereinigt. Die das Produkt (R_f-Wert 0,20) enthaltenden Fraktionen werden eingedampft. Aus dem so isolierten Ammoniumsalz der Titelverbindung wird das freie Tetrazol mittels 1N Salzsäure freigesetzt und mit Aethylacetat extrahiert. Man erhält so die Titelverbindung als gelblichen, glasartigen Feststoff vom R_f-Wert 0,20 (System B6), der aus Ethylacetat kristallin erhalten wird, Smp. 117-118°.

Das Ausgangsmaterial kann beispielsweise auf folgende Weise hergestellt werden:

a) 4-[N-(5-Hydroxypentyl)-aminomethyl]-2'-cyanobiphenyl

Eine Lösung von 6,8 g (25 mMol) 4-Brommethyl-2'-cyanobiphenyl und 12,9 g (125 mMol) 5-Amino-1-pentanol in 50 ml 1,4-Dioxan wird 2-3 Stunden unter Rückfluss zum Sieden erhitzt. Aufarbeitung analog Beispiel 23 a) unter Verwendung von Ethylacetat als Lösungsmittel ergibt die Titelverbindung als Hydrochlorid vom Smp. 189-190° (aus 2-Propanol).

b) N-[(2'-Cyanobiphenyl-4-yl)methyl]-N-(5-hydroxypentyl)-n-valer iansäureamid

Aus 5,1 g (17,3 mMol) der Verbindung 33 a) und 2,3 g (19 mMol) n-Valerylchlorid erhält man unter Verwendung von 9 ml Ethyldiisopropylamin und 50 ml Methylenchlorid analog Beispiel 26b) die Titelverbindung als Oel vom R_f 0,36 (System B7), welches ohne weitere Reinigung weiter umgesetzt wird.

Beispiel 34: N-(1-Carboxyprop-2-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

3,390 g rac-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3-amino-butansäureethylester werden analog Beispiel 1 umgesetzt und extraktiv gereinigt, R_f = 0,30 (System N8).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) rac-3-[(2'-Cyano-biphenyl-4-yl)methylamino]-butansäure-ethyle ster

wird analog Beispiel 1 b) aus 4,145 g 2'-Cyanobiphenyl-4-carbaldehyd und 4,634 ml rac-3-Amino-butansäure-ethylester erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 98:2 gereinigt, R_f = 0,25 (System N6).

b) rac-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3-amino-buta nsäure-ethylester

wird analog Beispiel 1 c) aus 7,070 g rac-3-[(2'-Cyano-biphenyl-4-yl)methylamino]butansäure-ethylester erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 99: 1 gereinigt, R_f = 0,36 (System N6).

Beispiel 35:
N-(2-Ethoxycarbonyl-3-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

2,194 g rac-N- [(2'-Cyano-biphenyl-4-yl)methyl] -N-valeryl-2-(aminomethyl)-3-methylbutansäure-ethylester werden analog Beispiel 1 umgesetzt und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH gereinigt, R_f = 0,48 (System N8).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) rac-2-[(2'-Cyano-biphenyl-4-yl)methylaminomethyl]-3-methyl-bu tansäure-ethylester

wird analog Beispiel 1 b) aus 4,145 g 2'-Cyanobiphenyl-4-carbaldehyd und 3,180 g rac-2-Aminomethyl-3-methyl-butansäure-ethylester (Miyazaki et al. J. pharm. Soc. Jpn. 77, 415 (1957)) erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 97:3 gereinigt, R_f = 0,48 (System N6).

b) rac-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-2-(aminomethyl)-3-methyl-butansäure-ethylester

wird analog Beispiel 1 c) aus 2,519 g rac-2-[(2'-Cyano-biphenyl-4-yl)methylaminomethyl]-3-methyl-butansäureethylester erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 99: 1 gereinigt, R_f = 0,67 (System N6).

Beispiel 36: N-(2-Carboxy-3-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

980 mg rac-N-[(2'-(1H-Tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-2-(aminomethyl)-3-methyl-butansäureethylester werden in 3,1 ml 2N NaOH während 72 Stunden auf 100° erhitzt. Neutralisieren mit 3,1 ml 2N HCl und extrahieren mit CH_2Cl_2 liefert das Produkt, R_f = 0,30 (System N8).

Beispiel 37:
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

4,2 g N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)methyl]-(L)-valinbenzylester werden in 40 ml Xylol mit 5,7 g Tri-n-butyl-zinnazid während 24 Stunden zum Rückfluss erhitzt. Darauf wird zur Trockene eingedampft. Das Rohprodukt wird anschliessend in 40 ml Dioxan aufgenommen, mit 400 mg Palladiumkohle (5%) versetzt und unter Normaldruck bis zur Sättigung hydriert. Es wird vom Katalysator abfiltriert, eingedampft, in Ether aufgenommen und das Produkt mit 18 ml 1N NaOH und 100 ml Wasser extrahiert. Die wässrige Phase wird mit Ether gewaschen und nach Ansäuern mit einem Ueberschuss an 1N Salzsäure mit Essigester extrahiert. Umkristallisieren aus Diisopropylether liefert das reine Produkt vom Smp. 116-117°.

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) N-(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valinbenzylester

4,38 g 2'-Cyanobiphenyl-4-carbaldehyd, 8,03 g (L)-Valinbenzylester-Toluolsulfonsäuresalz und 25 g Molekularsieb 5A werden in 80 ml Tetrahydrofuran während 36 Stunden bei Raumtemperatur gerührt und dann auf 0° abgekühlt. Es werden 2, 19 g Natriumcyanoborhydrid (90%), gelöst in 10 ml Methanol, zugegeben, 24 Stunden bei Raumtemperatur gerührt und dann im Vakuum eingeengt. Das Reaktionsgemisch wird darauf filtriert, das Filtrat eingeengt, der Rückstand in Methylenchlorid aufgenommen, dreimal mit Wasser gewaschen, gertrocknet und eingeengt. Der Rückstand wird in Wasser aufgenommen und mit einem Ueberschuss konzentrierter Salzsäure versetzt. Das Produkt wird als Hydrochlorid ausgefällt und abfiltriert. Nach Umkristallisieren aus Essigester/Hexan 1:1 erhält man das reine Produkt vom Smp. 153-155°.

b) N-Valeryl-N-[(2'cyanobiphenyl-4-yl)methyl]-(L)-valinbenzylest er

5,5 g N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valinbenzylester-Hydrochlorid, 4,33 g Diisopropylethylamin und 3 ml Valerylchlorid werden bei Raumtemperatur während 36 Stunden gerührt und anschliessend zur Trockene eingedampft. Der Rückstand wird in Ether aufgenommen, Mit Natriumbicarbonat und Sole gewaschen. Das Rohprodukt wird ohne Reinigung weiterverarbeitet.

Beispiel 38: In analoger Weise wie vorstehend beschrieben kann man auch die folgenden Verbindungen herstellen:

1. N-(3-Phenoxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]amin;
2. N-[2-(4-Hydroxyphenyl)ethyl] -N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
3. N-[3-(4-Hydroxyphenyl)prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
4. N-(8-Hydroxyokt-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]amin;
5. N-(2-Methansulfonylaminoethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
6. N-(3-Acetylaminoprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
7. N-(2-Methoxy-2-oxo-1-phenyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
8. N-(4-Hydroxybut-2-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]amin;
9. N-(2-Hydroxy-1 -phenyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin; und
10. N-[3-(4-Hydroxybenzylcarbonylamino)prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin.

Beispiel 39:
N-(2-Ethoxycarbonyl-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

3.75 g N-[(2'-Cyano-biphenyl-4-yl)-methyl]-N-valeryl-1-aminomethyl-c yclopentan-1-carbonsäure-ethylester werden in 200 ml Xylol mit 10.4 g Tri-n-butylzinnazid versetzt und während 41 h zum Rückfluss erhitzt. Darauf wird im Vakuum eingedampft, der Rückstand in 50 ml 2N NaOH-Lösung aufgenommen und 3 mal mit Ether extrahiert. Die wässrige Phase wird sodann mit 30 ml 4N Salzsäure angesäuert und mit Dichlormethan extrahiert. Das Produkt wird durch Eindampfen der zuvor über Na_2SO_4 getrockneten organischen Phase als farbloser Schaum erhalten, R_f= 0.53 (System N 8). MS (FAB): m/e 490 (M^++H).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) 1-Aminomethyl-cyclopentan-1-carbonsäure-ethylester wird erhalten durch hydrieren von 33 g 1-Cyanocyclopentan-1-carbonsäure-ethylester (Alfred Bader Chemicals) in 330 ml Ethanol, der ca. 4% Ammoniak enthält, in Gegenwart von 10 g Raney-Nickel bei 45°C und unter Normaldruck. Nach Abfiltrieren vom Katalysator und Entfernen der Lösungsmittel im Vakuum wird das Produkt durch Destillation erhalten, Sdp. 71-74°C bei 0.75 mbar.

b) N-[(2'-Cyano-biphenyl-4-yl)-methyl]-1-aminomethyl-cyclopentan-1-carbonsäure-äthylester wird analog Beispiel 1 b) aus 4.15 g 2'-Cyanobiphenyl-4-carbaldehyd und 4.15 g 1-Aminomethyl-cyclopentan-1-carbonsäure-ethylester erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2/-MeOH (99.5:0.5) gereinigt, R_f = 0.38 (System N 6).

c)N-[(2'-cyano-biphenyl-4-yl)-methyl]-N-valeryl-1-aminomethyl-cyclopentan-1-carbonsäure-äthylester wird analog Beispiel 1 c) aus 4.70 g N-[(2'-Cyano-biphenyl-4-yl)methyl]-1-aminomethyl-cyclopentan-1-carbonsäure-ethylester erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 99.5:0.5 gereinigt, R_f = 0.69 (System N 6).

Beispiel 40:
N-(2-Carboxy-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

0.979 g N-[(2'-(1H-Tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-1-am inomethyl-cyclopentan-1-carbonsäure-ethylester werden in 10 ml Ethanol gelöst, mit 4 ml 2 N NaOH-Lösung versetzt und während 23 h zum Rückfluss erhitzt. Nach Abkühlen auf Raumtemperatur und Zugabe von 4.5 ml 2N Salzsäure wird eingedampft und das Produkt durch Chromatographie an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 95:5 isoliert, R_f = 0.35 (System N 8). MS (FAB): m/e 462 (M^++H).

Beispiel 41: N-(3-Ethoxycarbonylcyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin und N-(3-Carboxycyclohexyl)-N-pentanoyl-N-(2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

0.661 g N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3-amin o-cyclohexan-1-carbonsäure-ethylester werden analog Beispiel 1 umgesetzt und extraktiv gereinigt. Das Rohprodukt wird an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 95:5 gereinigt, R_f = 0.33 (System N 8) für die Säure und R_f = 0.67 (System N 8) für den Ester. MS (FAB): m/e 462 (M^++H), 484 (M^++Na) bzw. m/e 490 (M^+ +H), 512 (M^++Na).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) rac-3-[2'-Cyano-biphenyl-4-yl)methylamino]-cyclohexan-1-carbonsäure-ethylester wird aus 2.711 g 4-Brom-methyl-2'-cyano-biphenyl und 2.055 g 3-Amino-cyclohexan-1-carbonsäure-ethylester in Gegenwart von N-Methyl-morpholin bei 10 minütigem Erhitzen auf 160°C erhalten. Das Rohprodukt wird an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 9: 1 gereinigt, R_f = 0.73 (System N 8).

b) rac-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3- amino-cyclohexan-1-carbonsäure-ethylester wird analog Beispiel 1 c) aus 0.766 g rac-3-[(2'-Cyano-biphenyl-4-yl)methylamino]-cyclohexan-1-carbonsäure-eth ylester erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 99.5:0.5 gereinigt, R_f = 0.56 (System N 6).

Beispiel 42: cis-N-(4-Carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

2.700 g cis-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-4-amino-cycl ohexan-1carbonsäure-ethylester werden analog Beispiel 1 umgesetzt und extraktiv gereinigt. R_f = 0.40 (System N 8). MS (FAB): m/e 462 (M^++H).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) cis-4-[(2'-Cyano-biphenyl-4-yl)methylamino]-cyclohexan-1-carbonsäure-ethylester wird analog Beispiel 1 b) aus 4.145 g 2'-Cyanobiphenyl-4-carbaldehyd und 5.137 g 4-Amino-cyclohexan-1-carbonsäure-ethylester erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 99.5:0.5 gereinigt, R_f = 0.18 (System N 6).

b) cis-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-4-amino-cycl ohexan-1 -carbonsäure-ethylester wird analog Beispiel 1c aus 2.540 g cis-4-[(2'-Cyano-biphenyl-4-yl)methylamino]-cyclohexan-1-carbonsäure-ethylester erhalten und an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH 98:2 gereinigt, R_f = 0.32 (System N 6).

Beispiel 43:
cis-N-(2-Ethoxycarbonylcyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

1.350 g rac-cis-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-2-amino- cyclohexan-1-carbonsäure-ethylester werden analog Beispiel 1 umgesetzt. Das Rohprodukt wird an Kieselgel 60 (40-63 μm) mit CH_2Cl_2-MeOH

95:5 gereinigt, R_f = 0.53 (System N 8). MS (FAB): m/e 490 (M^++H).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) rac-cis-2-[(2'-Cyano-biphenyl-4-yl)methylamino]-cyclohexan-1- carbonsäure-ethylester wird analog Beispiel 1 b) aus 4.145 g 2'-Cyanobiphenyl-4-carbaldehyd und 5.137 g rac-cis-2-Amino-cyclohexan-1-carbonsäure-ethylester erhalten und an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 99:1 gereinigt, R_f = 0.24 (System N 6).

b) rac-cis-N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-2-amino- cyclohexan-1-carbonsäure-ethylester wird analog Beispiel lc) aus 2.110 g rac-cis-2-[(2'-Cyano-biphenyl-4-yl)methylamino]-cyclohexan-1-carbonsäure-äthylester erhalten und an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 98:2 gereinigt, R_f = 0.35 (System N 6).

Beispiel 44: cis-N-(2-Carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

649 mg rac-cis-N-[(2'-(1H-Tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-2-aminocyclohexan-1-carbonsäure-ethylester werden zusammen mit 10 ml Ethanol und 2 ml 2 N NaOH während 18 Std. auf 80° erhitzt. Die Mischung wird mit 2 ml 2 N HCl neutralisiert und eingedampft. Das Rohprodukt wird an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH (95:5) gereinigt, R_f = 0.30 (System N 8). MS (FAB): m/e 462 (M^++H), 484 (M^++Na).

Beispiel 45:
N-(2-Ethoxycarbonyl-2-ethyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

3.28 g N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-2-aminomethyl-2- ethyl-buttersäure-ethylester werden analog Beispiel 1 umgesetzt und extraktiv gereinigt. R_f=0.52 (System N 8). MS (FAB): m/e 492 (M^++H), 514 (M^++Na).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) 2-Aminomethyl-2-ethyl-buttersäure-ethylester wird erhalten durch hydrieren von 12.83 g 2-Ethyl-2-cyano-buttersäure-ethylester (Pfaltz &. Bauer Inc.) in 130 ml Ethanol, der 4% Ammoniak enthält, in Gegenwert von 4 g Raney-Nickel bei 44°C und unter Normaldruck. Nach Abtrennen vom Katalysator wird im Vakuum eingedampft und die dabei zurückbleibende Flüssigkeit im Vakuum destilliert. Sdp. 60-61°C bei 0.70 mbar.

b) N-[(2'-Cyano-biphenyl-4-yl)methyl]-2-aminomethyl-2-ethyl-butt ersäure-ethylester wird aus 2.711 g 4-Brom-methyl-2'-cyano-biphenyl und 4.332 g 2-Aminomethyl-2-ethyl-buttersäure-ethylester analog Beispiel 41 a) erhalten und an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 97:3 gereinigt, R_f = 0.54 (System N 6).

c) N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-2-aminomethyl-2- ethyl-buttersäure-ethylester wird analog Beispiel 1c) aus 3.256 g N-[(2'-Cyano-biphenyl-4-yl) methyl]-2-aminomethyl-2-ethyl-buttersäure-ethylester erhalten und an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 99: 1 gereinigt, R_f = 0.67 (System N 6).

Beispiel 46:
N-(2-Ethoxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

4.21 g N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3-amino-2,2-dime thylpropionsäure-ethylester werden analog Beispiel 1 umgesetzt. Das Rohprodukt wird an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 9: 1 gereinigt, R_f = 0.60 (System N 8). MS (FAB): m/e 464 (M^++H), 486 (M^++Na).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) N-[(2'-Cyano-biphenyl-4-yl)methyl]-3-amino-2,2-dimethyl-propi onsäure-ethylester wird aus 2.711 g 4-Brom-methyl-2'-cyano-biphenyl und 3.630 g 3-Amino-2,2-dimethylpropionsäure-ethylester analog Beispiel 41a) erhalten und als Rohprodukt weiterverwendet, R_f = 0.54 (System N 6).

b) N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-3-amino-2,2-dime thyl-propionsäure-ethylester wird analog Beispiel 1c) aus 3.36 g N-[(2'-Cyano-biphenyl-4-yl)methyl]3-amino-2,2-dimethyl-propionsäure-ethylester erhalten und extraktiv gereinigt, R_f = 0.63 (System N 6).

Beispiel 47:
N-(2-[2-(4-Hydroxyphenyl)ethylaminocarbonyl]-2,2-tetramethylen-ethyl)N-pentanoyl-N-(2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

0.507 g N-[(2'-(1H-Tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-1-am inomethylcyclopentan-1-carbonsäure wird in 4 ml DMF gelöst und mit 0.210 g Tyramin-hydrochlorid, 0.225 ml Hünig-Base und 0.164 g HOBT versetzt. Das Gemisch wird auf 0°C gekühlt und es werden 0.274 g EDCI hinzugefügt. Nach 48 stündigem Rühren bei Raumtemperatur wird im Vakuum eingedampft, der Rückstand in 75 ml Essigester aufgenommen und mit 25 ml 1 N Salzsäure gewaschen. Die organische Phase wird über Na_2SO_4 getrocknet und im Vakuum von den Lösungsmittel befreit. Das so erhaltene Rohprodukt wird an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 95:5 gereinigt, R_f = 0.43 (System N 8). MS (FAB): m/e 581 (M^++H), 603 (M^++Na).

Beispiel 48:
(S)-N-{1-[2-(4-Hydroxyphenyl)ethylaminocarbonyl]-2-methyl-prop-1-yl}-N-pentanoyl-N-(2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

0,5 g der Verbindung aus Beispiel 16, 0,21 g Tyramin Hydrochlorid, 0,225 ml N-Aethyldiisopropylamin, 0,164 g 1-Hydroxybenzotriazol und 0,296 g Dicyclohexylcarbodiimid werden während 48 h in 4 ml DMF bei Raumtemperatur gerührt. Nach Abdampfen des Lösungsmittels im Vakuum wird der Rückstand während 1 h in einem Gemisch von 4 ml CH_2Cl_2-MeOH-AcOH 94:3:3 verrührt. Nach Eindampfen wird mittels Flashchromatographie aufgetrennt (100 g, System N6). Nach Lyophilisieren aus tert.-Butanol erhält man das Produkt als amorphes Pulver. FAB-MS: m/e = 555 $(M+H)^+$.

Beispiel 49:
(S)-N-(1-Carboxy-2,2-dimethyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Ausgehend von 240 mg N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-tert.-leuci nmethylester und 399 mg Tributylzinnazid wird nach Flashchromatographie (B2) das Produkt erhalten. Smp. 122-124°.

Das Ausgangsmaterial kann beispielsweise wie folgt erhalten werden:

a) N-(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-tert.-leucinmethylester ausgehend von 2,5 g 2'-Cyanbiphenyl-4-carbaldehyd, 4,39 g (L)-tert.-Leucinmethylester Hydrochlorid, 895 mg Natriumcyanoborhydrid (85 %) und 12,5 g Molekularsieb 5A und anschliessender Flashchromatographie mit System N3. (DC-System N2) R_f-Wert: 0,58.

b) N-Valeryl-N-[(2'-cyanobiphenyl-4-yl)-methyl]-(L)-tert.-leucinmethylester ausgehend von 1,2 g N-(2'-Cyanobiphenyl-4-yl)-methyl]-(L)-tert.-leucinmethylester, 0,65 ml Triethylamin und 0,565 ml n-Valeriansäurechlorid und anschliessender Flashchromatographie (N4). (DC-System N3) R_f-Wert: 0,56.

Beispiel 50:
(S)-N-(1-Methoxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

0,8 g N-Valeryl-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]-(L)-valinmethylester wird erhalten analog Beispiel 3 ausgehend von 4,4 g N-Valeryl-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]-(L)-valin die in MeOH/HCl verestert werden. Flashchromatographie (Essigester/Hexan 1:3). FAB-MS: m/e = 450 $(M+H)^+$.

Beispiel 51:
(S)-N-(1-Hydroxymethyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

0,8 g N-Valeryl-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]-(L) -valinmethylester werden in 30 ml THF gelöst, bei 5°C mit 83 mg Lithiumborhydrid versetzt und während 24 h bei Raumtemperatur gerührt. Das Reaktionsgemisch wird darauf eingeengt, mit Wasser versetzt, mit Salzsäure auf pH 2 gestellt, wobei eine weisse Fällung eintritt. Es wird mit Essigester extrahiert, mit Wasser und Sole gewaschen, getrocknet und schliesslich mittels Flashchromatographie aufgetrennt (CH_2Cl_2-MeOH 5: 1). FAB-MS:m/e = 422 $(M+H)^+$.

Beispiel 52: N-(4-Phenoxybut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

3,3 g (7,5 mMol) rohes N-[(2'-Cyanobiphenyl-4-yl)-methyl]-N-(4-phenoxybutyl)-n-valeriansäureamid und 3,0 g (9 mMol) Tri-n-butylzinnazid werden analog Beispiel 23 umgesetzt und aufgearbeitet. Man erhält so die Titelverbindung, die durch Flashchromatographie (Toluol-Methanol 4: 1) noch gereinigt wird, als dickflüssiges Oel, R_f 0,50 (System B6).

Das Ausgangsmaterial kann beispielsweise auf folgende Weise hergestellt werden:

a) 4-[N-(4-Phenoxybutyl)-aminomethyl]-2'-cyanobiphenyl.

Analog Beispiel 23a erhält man aus 4-Phenoxybutylamin die Titelverbindung, deren Hydrochlorid bei 103-104° schmilzt (aus Isopropanol-Aethylacetat).

b) N-[(2'-Cyanobiphenyl-4-yl)-methyl]-N-(4-phenoxybutyl)-n-valer iansäureamid.

Analog Beispiel 23b erhält man aus der unter a) beschriebenen Verbindung die Titelverbindung als gelbes Oel vom R_f-Wert 0,71 (System B7), das roh weiterverwendet wird.

Beispiel 53:
N-(2-Hydroxy-1-phenyl-2-oxo-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Analog Beispiel 1 werden 11,0 g (21 mMol) N-[(2'-Cyanobiphenyl-4-yl)-methyl]-N-valeryl-phenylglycin-benzylester mit 8,5 g (25,5 mMol) Tri-n-butylzinnazid in 60 ml o-Xylol umgesetzt und anschliessend 3 Stunden mit 100 ml 2-n.KOH hydrolysiert. Durch Ansäuern der wässrigen Phase mit 2-n.Salzsäure und Extraktion mit Toluol erhält man die rohe Titelverbindung, die aus wenig Toluol kristallin erhalten wird. Die so erhaltenen Kristalle vom Smp. 145-148 ° enthalten 1/3 Mol-Aequivalent Toluol.

Das Ausgangsmaterial kann beispielsweise wie folgt hergestellt werden:

a) rac. N-[(2'-Cyanobiphenyl-4-yl)-methyl]-phenylglycin-benzylester

24,8 g (60 mMol) rac. Phenylglycin-benzylester-tosylat und 8,2 g (30 mMol) 4-Brommethyl-2'-cyanobiphenyl werden zusammen mit 15,5 g Diisopropylethylamin (Hünigbase) in 60 ml DMF 2 Stunden unter Rühren bei 80° gehalten. Das Reaktionsgemisch wird dann auf Eiswasser gegossen und mit Aethylacetat extrahiert. Das Aethylacetat wird abgetrennt und mit 2-n.Salzsäure verrührt. Das ölig ausfallende Hydrochlorid der Titelverbindung wird abgetrennt, mit Sodalösung in die Base übergeführt und roh weiterverwendet (R_f 0,65 in System B7).

b) N-[(2'-Cyanobiphenyl-4-yl)-methyl]-N-valeryl-phenylglycin-ben zylester

9,4 g (21,7 mMol) der rohen, unter a) beschriebenen Verbindung wird zusammen mit 5,7 g (44 mMol) Hünigbase in 45 ml Methylenchlorid gelöst und mit 3,14 g (26 mMol) Valeriansäurechlorid versetzt. Die Lösung wird 30-40 Stunden stehen gelassen. Aufarbeitung analog Beispiel Ic ergibt die rohe Titelverbindung als dickflüssiges Oel mit Rf-Wert 0,73 (System B7), welches roh weiterverwendet wird.

Beispiel 54:
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amin

Eine Lösung von 21,1 g (40 mMol) N-[(2'-(1H-Tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-(L)-valinbenzylester in 210 ml Methanol wird unter Zusatz von 4 g Pd/C (10 %) bis zur Aufnahme der berechneten Menge Wasserstoff bei Raumtemperatur hydriert (24 Stunden). Durch Filtration und Eindampfen der Lösung erhält man die rohe Säure. Sie wird zwischen 80 ml 2-n.Kalilauge und 50 ml Aether verteilt. Die wässrige Phase wird abgetrennt, sauer gestellt und die Titelverbindung durch Extraktion mit Ethylacetat isoliert. Sie wird aus Ethylacetat kristallin erhalten und zeigt einen Schmelzintervall von 105-115 ° und eine optische Drehung $[\alpha]_D^{20}$-69,95°±0,05° (c = 1 % in Methanol).

Das Ausgangsmaterial kann beispielsweise wie folgt hergestellt werden:

a) N-[(2'-Cyanobiphenyl-4-yl)-methyl] -(L)-valinbenzylester

Eine Lösung von 13,6 g (50 mMol) 4-Brommethyl-2'-cyanobiphenyl, 22,8 g (60 mMol) (L)-ValOBz-Tosylat und 34 ml Hünigbase in 100 ml DMF wird 1 Stunde bei 80° gerührt. Das Reaktionsgemisch wird dann abgekühlt, auf 300 ml Eiswasser gegossen und mit 150 ml Ethylacetat extrahiert. Durch Waschen des Extraktes mit wässriger Kaliumbicarbonatlösung, Trocknen und Eindampfen erhält man die rohe Titelverbindung als Oel, das ein Hydrochlorid vom Smp. 172-173° bildet.

b) N-[(2'-Cyanobiphenyl-4-yl)-methyl]-N-valeryl-(L)-valinbenzyle ster

6,2 g (15,5 mMol) N-[(2'-Cyanobiphenyl-4-yl)-methyl] -(L)-valinbenzylester und 8,0 ml Hünigbase, gelöst in 50 ml Methylenchlorid werden unter Rühren mit 2,3 ml Valeriansäurechlorid versetzt und analog Beispiel 29b weiterbearbeitet. Man erhält so die Titelverbindung als gelbes Oel, das roh weiterverarbeitet wird (R_f-Wert 0,51, Toluol-Methanol 19: 1)

c) (S)-N-(1-Benzyloxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

6,6 g (13,6 mMol) roher N-[(2'-Cyanobiphenyl-4-yl)-methyl]-N-valeryl-(L)-valinbenzylester und 6,0 g (18 mMol) Tributylzinnazid werden in 75 ml o-Xylol 48 Stunden unter Rühren zum Sieden erhitzt. Nach 24 Stunden erfolgt ein Zusatz von 2,0 g Tributylzinnazid. Aufarbeitung analog Beispiel 23 unter Verwendung von 110 ml 1-n.Kalilauge während 20 Minuten ergibt die Titelverbindung als gelbliches Oel, das einen R_f-Wert von 0,40 (System S2) und eine optische Drehung $[\alpha]_{20}^{D}$ - 36,6° (c = 1 % in Methanol) aufweist.

Beispiel 55:
(S)-N-(1-Benzyloxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Eine Lösung von 91 g (ca. 100 mMol) rohem N-[(2'-(1-Triphenylmethyl-tetrazol-5-yl)biphenyl-4-yl)methyl]-

N-valeryl-(L)-valinbenzylester in 300 ml Dioxan wird bei 60° mit 300 ml 1-n.Salzsäure versetzt und 2 Stunden bei 60° gehalten. Das Dioxan wird hierauf im Vakuum abgedampft und die wässrige Phase mit 2-n.Kalilauge alkalisch gestellt. Neutrale Teile werden mit Aether extrahiert. Die Wasserphase ergibt durch Ansäuern und Extraktion mit Ethylacetat die rohe Titelverbindung als Oel (R_f 0,40 im System S2).

Das Ausgangsmaterial kann beispielsweise wie folgt hergestellt werden:

a) N-[(2'-(1-Triphenylmethyl-tetrazol-5-yl)biphenyl-4-yl)methyl] -(L)-valinbenzylester

Analog Beispiel 57a erhält man aus 4-Brommethyl-2'-(1-triphenylmethyl-tetrazol-5-yl)biphenyl die Titelverbindung (R_f 0,78 im System B6), die roh weiterverwendet wird.

b) N-[(2'-(1-Triphenylmethyl-tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-(L)-valinbenzylester

Die unter a) erwähnte Verbindung wird mit 2,5 Aequivalenten Valeriansäurechlorid und 5 Aequivalenten Hünigbase in Methylenchlorid analog Beispiel 29b umgesetzt und aufgearbeitet. Die so erhaltene Titelverbindung wird roh weiterumgesetzt.

Beispiel 56: N-Butanoyl-N-(1-carboxy-1-methyl-ethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Eine Lösung von 2,1 g (4,2 mMol) 2-Amino-N-butyryl-2-methyl-N-[(2'-(1H-tetrazol-5-yl) biphenyl-4-yl)methyl]-propansäurebenzylester in 20 ml Methanol wird unter Zusatz von 0,2 g Pd/C (10 %) bei 5 bar Anfangsdruck hydriert, bis der Ausgangsbenzylester im DC (System B6, S2) nicht mehr nachzuweisen ist. Durch Filtrarion, Abdampfen des Lösungsmittels und Umkristallisation des Rückstandes aus CH_3CN erhält man die Titelverbindung vom Smp. 187- 189°.

Das Ausgangsmaterial kann beispielsweise wie folgt hergestellt werden:

a) 2-Amino-N-(2'-cyanobiphenyl-4-ylmethyl)-2-methyl-propansäure-benzylester

Analog Beispiel 29a erhält man unter Verwendung von 2-Amino-2-methyl-propansäurebenzylester-tosylat die Titelverbindung, die ein Hydrochlorid vom Smp. 200-202° (Ethylacetat-4-n.HCl in absolutem Ethanol) bildet.

b) 2-Amino-N-butyryl-N-(2'-cyanobiphenyl-4-ylmethyl)-2-methylpropansäure-benzylester

Eine Lösung von 6,3 g (15 mMol) des Hydrochlorids der unter a) beschriebenen Verbindung und 10,2 ml (60 mMol) Hünigbase in 60 ml Methylenchlorid wird mit 1,8 g (16 mMol) Buttersäurechlorid versetzt und über Nacht gerührt. Durch weitere Zusätze von Säurechlorid und Hünigbase wird die Reaktion vervollständigt. Aufarbeitung analog Beispiel 23b ergibt die Titelverbindung, die roh weiterumgesetzt wird.

c) 2-Amino-N-butyryl-2-methyl-N-[(2'-(1H-tetrazol-5-yl)biphenyl 4-yl)-methyl]-propansäure-benzylester

Aus der unter b) beschriebenen Verbindung (6 g, roh) und 5,2 g Tributylzinnazid in 50 ml o-Xylol erhält man analog Beispiel 23 die Titelverbindung vom Smp. 203-204° (aus Ethylacetat).

Beispiel 57: N-(4-Hydroxybut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Analog Beispiel 33 erhält man aus N-[(2'-Cyanobiphenyl-4-yl)methyl]-N-(4-hydroxybutyl)-n-valeriansäureamid die Titelverbindung vom Smp. 110-111° (aus Ethylacetat).

Das Ausgangsmaterial kann beispielsweise wie folgt hergestellt werden:

a) 4-[N-(4-Hydroxybutyl)-aminomethyl]-2'-cyanobiphenyl

Analog Beispiel 33a) erhält man unter Verwendung von 4-Aminobutanol die Titelverbindung als Oel (R_f 0,18 in System B7), das roh weiterverwendet wird.

b) N-[(2'-Cyanobiphenyl-4-yl)-methyl]-N-(4-hydroxybutyl)-n-valeriansäureamid

Analog Beispiel 33b) erhält man aus der unter a) beschriebenen Verbindung die Titelverbindung als Oel (R_f 0,37) das roh weiter umgesetzt wird.

Beispiel 58:

(S)-N-(1-Benzyloxycarbonyl-2-methyl-prop-1-yl)-N-[3-brom-2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-N-pentanoyl-amin

Eine Lösung von 4,5 g (8 mMol) N-[(3-Brom-2'-cyanobiphenyl-4-yl)methyl]-N-valeryl(L)-valinbenzylester und 3,4 g (10,4 mMol) Tributylzinnazid in 50 ml Xylol wird 20 Stunden unter Rückfluss zum Sieden erhitzt. Aufarbeitung analog Beispiel 54 und "flash"-Chromatographische Reinigung (Toluol-Methanol 4: 1) ergibt die Titelverbindung als farblosen Schaum (R_f-Wert 0,57, System S2).

Das Ausgangsmaterial kann beispielsweise wie folgt hergestellt werden:

a) 3'-Brom-4'-methyl- 1,1'-biphenyl-2-carbonitril

Eine Suspension von 21,0 g (0,157 Mol) wasserfreiem Aluminiumchlorid in 800 ml Tetrachloräthan wird mit 25,0 g (0,129 Mol) 4'-Methyl-1,1'-biphenyl-2-carbonitril versetzt und unter Rühren auf 60° Innentemperatur gebracht. Sobald das Aluminiumchlorid in Lösung gegangen ist, wird bei 60° Innentemperatur eine Lösung von 20,7 g (0,129 Mol) Brom in 100 ml Tetrachloräthan zugetropft. Das Reaktionsgemisch wird 24 Stunden bei 60° gerührt. Nach Zusatz von weiteren 6,2 g Aluminiumchlorid und Erwärmen auf 60-70° lässt sich in DC (Toluol) kein Ausgangsmaterial mehr feststellen. Das Reaktionsgemisch wird hierauf unter Eiskühlung mit 20 ml conc. Salzsäure zersetzt, die organische Phase abgetrennt und im Vakuum eingedampft. Der dunkle Rückstand wird in Aethylacetat gelöst, mit Wasser und Natriumcarbonat-Lösung gewaschen, getrocknet ($MgSO_4$) und eingedampft. Das Rohprodukt wird flash-chromatographisch gereinigt, wodurch 22,0 g (62 % d. Th.) der Titelverbindung erhalten werden, Smp. 104-106° (aus Cyclohexan).

b) 3'-Brom-4'-brommethyl-1,1'-biphenyl-2-carbonitril

In eine Lösung von 8,9 g (0,033 Mol) 3'-Brom-4'-methyl-1,1'-biphenyl-2-carbonitril in 900 ml Tetrachloräthan werden nach Zugabe von 0,1 g Benzoylperoxid unter UV-Bestrahlung bei 100-110° 5,6 g (0,035 Mol) Brom, gelöst in 20 ml Tetrachloräthan, getropft. Nach 30 Minuten wird das Reaktionsgemisch abgekühlt und im Vakuum eingedampft. Der kristalline Rückstand wird aus Aethylacetat umkristallisiert und ergibt 4,1 g der Titelverbindung vom Smp. 152-153°.

c) N-[(3-Brom-2'-cyanobiphenyl-4-yl)methyl]-(L)-valin-benzyleste r

Eine Lösung von 4,63 g (12,2 mMol) (L)-Valinbenzylester-tosylat und 4,8 ml Hünig-Base in 20 ml DMF wird mit einer Lösung von 3,3 g (9,4 mMol) der unter b) beschriebenen Verbindung versetzt und 4 Stunden bei 100° gerührt. Aufarbeitung analog Beispiel 54a und "flash"-chromatographische Reinigung (n-Hexan-Ethylacetat 4: 1) führen zur Titelverbindung als rotbraunem Oel mit R_f 0,21 (n-Hexan-Ethylacetat 4: 1).

d) N-[(3-Brom-2'-cyanobiphenyl-4-yl)methyl]-N-valeryl-(L)-valin benzylester

Aus der unter c) erwähnten Verbindung erhält man analog Beispiel 54b die Titelverbindung als gelbes Oel mit R_f 0,17 (n-Hexan-Ethylacetat).

Beispiel 59:
(S)-N-[3-Brom-2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-amin

Eine Lösung von 2,4 g (4 mmol) N-[(3-Brom-2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-(L)-valin-benzylester in 90 ml Dioxan wird unter Zusatz von 1,2 g Pd/C (10 %) bei 5 bar und Zimmertemperatur bis zur Aufnahme der berechneten Menge Wasserstoff hydriert. Nach dem Eindampfen der filtrierten Lösung wird der Eindampfrückstand in 2-n.Natronlauge gelöst, mit Aether extrahiert und die Wasserphase mit 2-n.Salzsäure sauer gestellt. Durch Extraktion mit Ethylacetat, Trocknen und Eindampfen erhält man die Titelverbindung als farblosen Schaum (R_f 0,40, System S2), FAB-MS: m/e = 514 $(M+H)^+$.

Beispiel 60: N-(2-Acetylaminoethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

9,9 g (22 mMol) rohes N-(2-Acetylamino-ethyl)-N-[2'-cyanobiphenyl-4-yl)methyl]-n-valeriansäureamid und 12,3 g (37 mMol) Tributylzinnazid werden in 100 ml Xylolgemisch 30 Stunden unter Rückfluss erhitzt. Der sich abscheidende Niederschlag wird nach dem Abkühlen durch Dekantieren isoliert und anschliessend durch Verrühren zwischen 100 ml Ether und 100 ml 1-n.Kalilauge in Lösung gebracht (3-4 Stunden). Aus der wässrigen, alkalischen Phase wird die Titelverbindung durch Ansäuern mit 2-n.HCl und Extraktion mit viel Ethylacetat isoliert und durch "flash"-Chromatographie (System S2) gereinigt. Man erhält so die Titelverbindung als Feststoff mit einem Schmelzintervall von 74-80°.

Das Ausgangsmaterial kann beispielsweise wie folgt hergestellt werden:

a) N-[2-2'-Cyanobiphenyl-4-yl)methylamino)ethyl]-acetamid

Aus 9,2 g (90 mMol) 2-Aminoethylacetamid und 8,1 g (30 mMol) 4-Brommethyl-2'-cyanobiphenyl in 100 ml Dioxan erhält man analog Beispiel 23a die Titelverbindung als Oel, das roh weiterverwendet wird.

b) N-[(2-Acetylamino-ethyl)-N-[(2'-cyanobiphenyl-4-yl)methyl]-n-valeriansäureamid

Eine Lösung von 4,2 g (8,8 mMol) der unter a) erwähnten Verbindung und 5,0 ml Hünig-Base in 40 ml Methylenchlorid wird mit 2,4 g (20 mMol) Valeriansäurechlorid versetzt und 24 Stunden unter Rückfluss zum Sieden erhitzt. Aufarbeitung analog Beispiel 23b und "flash"-chromatographische Reinigung (n-Hexan-Ethylacetat 4:1)ergeben die Titelverbindung als gelbes Oel mit R_f 0,17 (n-Hexan-Ethylacetat 4:1).

Beispiel 61:
N-[2-(n-Butoxycarbonyl)-2,2-tetramethylen-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-am in

0,490 g N-[(2'-(1H-Tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-1-aminomethyl-cyclopentan-1-carbonsäure wird in 20 ml 1-Butanol gelöst, mit Molekularsieb 4Å sowie 0,5 ml 4N Salzsäure versetzt und 48 Stunden zum Rückfluss erhitzt. Das Reaktionsgemisch wird im Vakuum eingedampft und an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 95:5 gereinigt, R_f = 0,73 (System N8). MS(FAB): m/e 518 (M^++H), 540 (M^++Na).

Beispiel 62:
N-(2-Ethoxycarbonyl-2,2-pentamethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

8,70 g N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-1-aminomethyl-cyclohexan-1carbonsäureethylester werden analog Beispiel 1 umgesetzt. Das Rohprodukt wird an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 95:5 gereinigt, R_f = 0,66 (System N8). MS (FAB): m/e 504 (M^++H), 526 (M^++Na), 542 (M^++K).

Das Ausgangsmaterial kann beispielsweise folgendermassen hergestellt werden:

a) 1-Aminomethyl-cyclohexan-1-carbonsäureethylester wird erhalten durch hydrieren von 72,08g 1-Cyano-cyclohexan-1-carbonsäureethylester (T. Kurihara et al. Tet. Lett. 1976, 2455) in 600 ml Aethanol, der ca. 4 % Ammoniak enthält, in Gegenwart von 20 g Raney-Nickel bei 45°C und unter Normaldruck. Nach Entfernen des Katalysators und Lösungsmittels wird das Produkt durch Destillation erhalten, Siedepunkt 72-75°C bei 0,3 mbar.

b) N-[(2'-Cyano-biphenyl-4-yl)methyl]-1-aminomethyl-cyclohexan-1-carbonsäureethylester wird analog Beispiel 41a) aus 5,422 g 4-Brommethyl-2'-cyano-biphenyl und 9,264 g 1-Aminomethyl-cyclohexan-1-carbonsäureethylester erhalten und an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 97,5:2,5 gereinigt, R_f = 0,67 (System N6).

c) N-[(2'-Cyano-biphenyl-4-yl)methyl]-N-valeryl-1-aminomethyl-1 -carbonsäureethylester wird analog Beispiel 1c) aus 7,12 g N-[(2'-Cyano-biphenyl-4-yl)methyl]-1-aminomethylcyclohexan-1-carbonsäureethylester erhalten und extraktiv gereinigt, R_f = 0,68 (System N6).

Beispiel 63:
N-(2-Benzylaminocarbonyl-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

0,507 g N-[(2'-(1H-Tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-1-aminomethyl-cyclopentan-1-carbonsäure wird analog Beispiel 48 mit 0,214 g Benzylamin umgesetzt und das Rohprodukt wird an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 95:5 gereinigt, R_f = 0,49 (System N8). MS (FAB): m/e 551 (M^++H), 573 (M^++Na).

Beispiel 64: N-(2-Carboxy-2-ethyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

1,146 g N-[(2'-(1H-Tetrazol-5-yl)biphenyl-4-yl)methyl]-N-valeryl-2-aminomethyl-2-ethyl-buttersäure-ethylester werden in 10 ml Ethanol gelöst, mit 4,66 ml 2N NaOH-Lösung versetzt und 20 Stunden zum Rückfluss erhitzt. Nach Abkühlen auf Raumtemperatur und Zugabe von 4,66 ml 2N Salzsäure wird eingedampft. Das Produkt wird durch Chromatographie an Kieselgel 60 (40-63 µm) mit CH_2Cl_2-MeOH 80:20 isoliert, R_f = 0,38 (System N8). MS (FAB): m/e 486 (M^++Na), 502 (M^++K).

Beispiel 65:
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-ethoxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

0.34 g N-Carboethoxy-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl] -(L)-valin-benzylester und 0.17 g Palladiumkohle (10%) werden in 10 ml Tetrahydrofuran unter Normaldruck 20 Stunden bis zur Sättigung hydriert. Es wird vom Katalysator abfiltriert und das Rohprodukt wird mittels Flashchromatographie (25 g Kieselgel, Fliessmittel B1) gereinigt. Amorphes Produkt FAB-MS:m/e = 424 ($M+H^+$).

Das Ausgangsmaterial kann beispielsweise wie folgt erhalten werden:

a) N-Carboethoxy-N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin-ben zylester
10.0 g N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin-benzylester werden in 150 ml Chloroform gelöst und bei 0° mit 8.2 ml Diisopropylethylamin versetzt. Man gibt 2.4 ml Chlorameisensäureethylester zu und erhitzt während 3 Stunden zum Rückfluss. Das Reaktionsgemisch wird mit 0.1 M-Salzsäure und Sole gewaschen, getrocknet und eingeengt. Amorphes Produkt. DC (System N3) R_f-Wert: 0.45.

b) N-Carboethoxy-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methy l]-(L)-valin-benzylester
10.0 g N-Carboethoxy-N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin-ben zylester und 9.2 g Tributylzinnazid werden in 150 ml Xylol 18 Stunden zum Rückfluss erhitzt. Das Reaktionsgemisch wird eingeengt und der Rückstand während 15 Minuten in 5M etherischer Salzsäure verrührt. Man engt wieder ein, löst den Rückstand in Ether und extrahiert mit kalter 4M Kalilauge. Die Wasserphase wird sauer gestellt und mit Essigester extrahiert. Diese Essigesterphase wird mit Sole gewaschen, über Magnesiumsulfat getrocknet und

eingeengt. Das Rohprodukt wird mittels Flashchromatographie (250 g Kieselgel, Fliessmittel N6) gereinigt. Amorphes Produkt, DC (System N6) R_f-Wert: 0.22.

Beispiel 66:
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-propyloxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Analog Beispiel 1 ausgehend von 0.14 g N-Carbopropoxy-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]-(L)-valin und 0.07 g Palladiumkohle wird nach Flashchromatographie (B1) das amorphe Produkt erhalten. FAB-MS: m/e = 438 (M+H⁺).

Das Ausgangsmaterial kann beispielsweise wie folgt erhalten werden:

a) N-Carbopropoxy-N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin -benzylester ausgehend von 1.0 g N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin-benzylester 0.8 ml Diisopropylethylamin und 0.34 ml Chlorameisensäurepropylester und anschliessender Flashchromatographie mit System N3. Amorphes Produkt. DC (System N2) R_f-Wert: 0.38.

b) N-Carbopropoxy-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methy l]-(L)-valin-benzylester ausgehend von 1.04 g N-Carbopropoxy-N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)- valin-benzylester und 1.1 g Tributylzinnazid und anschliessender Flashchromatographie mit dem System N6. Amorphes Produkt, DC (System N6) R_f-Wert: 0.21.

Beispiel 67:
(S)-N-Butyloxycarbonyl-N-(1-Carboxy-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Analog Beispiel 1 ausgehend von 0.40 g N-Carbobutoxy-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]-(L)-valin und 0.20 g Palladiumkohle wird nach Flashchromatographie (B1) das amorphe Produkt erhalten. FAB-MS: m/e = 452 (M+H⁺).

Das Ausgangsmaterial kann beispielsweise wie folgt erhalten werden:

a) N-Carbobutoxy-N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin -benzylester ausgehend von 1.0 g N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin-benzylester 0.8 ml Diisopropylethylamin und 0.34 ml Chlorameisensäurebutylester und anschliessender Flashchromatographie mit System N3. Amorphes Produkt. DC (System N2) R_f-Wert: 0.41.

b) N-Carbobutoxy-N- [(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]- (L)-valin-benzylester ausgehend von 1.05 g N-Carbobutoxy-N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin-benzylester und 1.05 g Tributylzinnazid und anschliessender Flashchromatographie mit dem System N6. Amorphes Produkt, DC (System N6) R_f-Wert: 0.17.

Beispiel 68:
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-methoxycarbonyl-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin

Analog Beispiel 1 ausgehend von 2.40 g N-Carbomethoxy-N-[(2'-(1H-tetrazol-5-yl) biphenyl-4-yl)-methyl]-(L)-valin und 0.50 g Palladiumkohle wird nach Flashchromatographie (B1) das amorphe Produkt erhalten. FAB-MS: m/e = 410 (M+H⁺).

Das Ausgangsmaterial kann beispielsweise wie folgt erhalten werden:

a) N-Carbomethoxy-N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin -benzylester ausgehend von 4.0 g N-[(2'-Cyanobiphenyl-4-yl)methyl]-(L)-valin-benzylester 3.3 ml Diisopropylethylamin und 0.78 ml Chlorameisensäuremethylester und anschliessender Flashchromatographie mit System N3. Amorphes Produkt. DC (System N3) R_f-Wert: 0.34.

b) N-Carbomethoxy-N-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl] -(L)-valin-benzylester ausgehend von 3.21 g N-Carbomethoxy-N-[(2'-Cyanobiphenyl-4-yl) methyl]-(L)-valin-benzylester und 3.50 g Tributylzinnazid und anschliessender Flashchromatographie mit dem System N6. Amorphes Produkt, DC (System N6) R_f-Wert: 0.26.

Beispiel 69:

In analoger Weise wie in Beispiel 47 beschrieben kann man auch das N-(2-Diethylaminocarbonyl-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [R_f-Wert: 0,47 (System N8)] herstellen.

Beispiel 70:

In analoger Weise wie in Beispiel 47 beschrieben kann man auch das N-(2-Methyl-2-morpholin-4-ylcarbonyl-propyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [R_f-Wert: 0,61 (System N8)] herstellen.

Beispiel 71:

In analoger Weise wie in Beispiel 64 beschrieben kann man auch das N-(2-Carboxy-2-methyl-propyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [R_f-Wert: 0,39 (System N8)] herstellen.

Beispiel 72:

In analoger Weise wie in Beispiel 40 beschrieben kann man auch das N-(2-Carboxy-2,2-pentamethylenethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl] -amin [R_f-Wert: 0,33 (System N8)] herstellen.

Beispiel 73:

Eine Lösung von 1,5 g (2,8 mmol) N-(1-Benzyloxycarbonylcyclopentyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin in 20 ml Dioxan wird unter Zusatz von 0,3 g Pd/C (10%) in analoger Weise wie in Beispiel 56 beschrieben hydriert. Nach Reinigung durch Flash-Chromatographie (Kieselgel; System S2) erhält man das N-(1-Carboxycyclopentyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin in Form eines Schaumes [R_f-Wert: 0,29 (System S2)].

Das Ausgangsmaterial kann z. B. wie folgt hergestellt werden:

a) Ein Gemisch aus 2,72 g (10 mmol) 4-Brommethyl-2'-cyano-biphenyl, 2,63 g (12 mmol) 1-Aminocyclopentancarbonsäurebenzylester, 3,4 ml (20 mmol) Hünigbase und 10 ml N,N-Dimethylformamid wird unter Rühren 2 Stunden auf 130 bis 140° (Badtemperatur) erhitzt. Nach dem Abkühlen wird das Reaktionsgemisch auf 50 ml Eiswasser gegossen. Durch Extraktion mit Ethylacetat erhält man das rohe N-(1-Benzyloxycarbonylcyclopentyl)-N-(2'-cyanobiphenyl-4-ylmethyl)-amin, das ein zwischen 180 und 182° (Ethanol/Diethylether) schmelzendes Hydrochlorid bildet.

b) Eine Lösung von 2,9 g (6,5 mmol) N-(1-Benzyloxycarbonylcyclopentyl)-N-(2'-cyanobiphenyl-4-ylmethyl)-amin-hydrochlorid und 4,4 ml (26 mmol) Hünigbase in 50 ml Ethylacetat wird mit 1,1 g (9 mmol) Pentanoylchlorid versetzt und das Gemisch 15 Stunden bei 25 bis 30° gerührt. Nach Zusatz von weiteren 0,5 g Pentanoylchlorid wird weitere 8 Stunden gerührt. Das Reaktionsgemisch wird dann mit 10 ml wässriger Ammoniaklösung (5%) versetzt und 0,5 Stunden gerührt. Die Ethylacetatphase wird abgetrennt, nacheinander mit 2 N-Salzsäure, Wasser und Natriumhydrogencarbonatlösung gewaschen, getrocknet und eingedampft. Man erhält so das N-(1-Benzyloxycarbonylcyclopentyl)-N-(2'-cyanobiphenyl-4-ylmethyl)-N-pentanoyl-amin in Form eines braunen Oels [R_f-Wert: 0,53 (System B7)], das in roher Form weiterumgesetzt wird.

c) Ein Gemisch aus 3,2 g (6,5 mmol) N-(1-Benzyloxycarbonylcyclopentyl)-N-(2'-cyanobiphenyl-4-ylmethyl)-N-pentanoyl-amin, 3,3 g (9,8 mmol) Tributylzinnazid und 35 ml o-Xylol wird 24 Stunden unter Rückfluss erhitzt. Aufarbeitung des Gemisches in analoger Weise wie in Beispiel 23 beschrieben ergibt das N-(1-Benzyloxycarbonylcyclopentyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin in Form eines gelben Oels [R_f-Wert: 0,37 (System S2)], das in roher Form weiterumgesetzt werden kann.

Beispiel 74:

Eine Lösung von 2,4 g (4,3 mmol) N-(1-Benzyloxycarbonylcyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin in 40 ml Dioxan wird unter Zusatz von 0,5 g Pd/C (10%) in analoger Weise wie in Beispiel 73 beschrieben hydriert und aufgearbeitet. Man erhält so das N-(1-Carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin in Form von farblosen Kristallen (aus Ethylacetat), die zwischen 134 und 136° schmelzen.

Das Ausgangsmaterial kann z. B. wie folgt hergestellt werden:

a) Das N-(1-Benzyloxycarbonylcyclohexyl)-N-(2'-cyanobiphenyl-4-ylmethyl)-amin, das ein zwischen 164 und 166° (Isopropanol) schmelzendes Hydrochlorid bildet, erhält man in analoger Weise wie in Beispiel 73a) beschrieben.

b) Eine Lösung von 2,9 g (6,8 mmol) N-(1-Benzyloxycarbonylcyclohexyl)-N-(2'-cyanobiphenyl-4-ylmethyl)-amin und 4,4 ml (26 mmol) Hünigbase in 50 ml Ethylacetat wird mit 1,1 g (9 mmol) Pentanoylchlorid versetzt und das Gemisch 24 Stunden unter Rückfluss erhitzt. Nach dem Abkühlen wird das Reaktionsgemisch mit 20 ml wässriger Ammoniaklösung (2 N) versetzt und 1 Stunde gerührt. Die organische Phase wird abgetrennt, nacheinander mit 2 N-Salzsäure, gesättigter Natriumhydrogencarbonatlösung und Sole gewaschen, getrocknet und eingedampft. Man erhält so das N-(1-Benzyloxycarbonylcyclohexyl)-N-(2'-cyanobiphenyl-4-ylmethyl)-N-pentanoyl-amin in Form eines braunen Oels [R_f-Wert: 0,44 (Toluol/Methanol = 19:1)], das in roher Form weiterumgesetzt wird.

c) Ein Gemisch aus 3,3 g (6,5 mmol) N-(1-Benzyloxycarbonylcyclohexyl)-N-(2'-cyanobiphenyl-4-ylmethyl)-N-pentanoyl-amin, 4,1 g (12,3 mmol) Tributylzinnazid und 30 ml o-Xylol wird 44 Stunden unter Rückfluss erhitzt. Aufarbeitung des Gemisches in analoger Weise wie in Beispiel 23 beschrieben ergibt das N-(1-Benzyloxycarbonylcyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin in Form von hellbraunen Kristallen, die zwischen 189 und 190° (aus Ethylacetat/Diethylether) schmelzen.

Beispiel 75:

In analoger Weise wie in Beispiel 74 beschrieben kann man auch das N-(1-Carboxy-1-ethyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [heller Schaum; R_f-Wert: 0,35 (System S2)] herstellen.

Beispiel 76:

170 mg (S)-N-(1-Benzyloxycarbonyl-5-benzyloxycarbonylamino-pent-1-yl)N-pentanoyl-N- [2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin werden in 5 ml Methanol gelöst. Die Lösung wird mit 170 mg Palladium-/Kohle (10%) versetzt und das Gemisch unter Normaldruck und bei Raumtemperatur bis zur Sättigung hydriert. Das Gemisch wird über Hyflo filtriert und das Filtrat eingedampft, wodurch das reine (S)-N-(5-Amino-1-carboxy-pent-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin in Form eines weissen Schaumes erhalten wird [MS (FAB): m/z = 465, $(M + H)^+$].

Das Ausgangsmaterial kann z. B. wie folgt hergestellt werden:

a) 5,0 g (S)-2-Amino-6-benzyloxycarbonylamino-hexansäwebenzylester werden in 250 ml N,N-Dimethylformamid gelöst. Die Lösung wird mit 4,33 ml N,N-Diisopropyl-N-ethyl-amin versetzt und das Gemisch auf 80° erwärmt, 30 Minuten gerührt, mit 4,44 g 4-Brommethyl-2'-(1-triphenylmethyl-1H-tetrazol-5-yl)-biphenyl versetzt, 16 Stunden bei 80° gerührt und dann eingedampft. Der Rückstand wird mit Wasser und Ethylacetat aufgearbeitet. Die organische Phase wird getrocknet und mittels Flashchromatographie gereinigt (200 g Kieselgel; System N4). Das (S)-N-(1-Benzyloxycarbonyl-5-benzyloxycarbonylamino-pent-1-yl)-N-[2'-(1-triphenylmethyl-1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin wird in Form eines braunen Oels erhalten [R_f-Wert: 0,18 (System N3)].

b) 1,1 g (S)-N-(1-Benzyloxycarbonyl-5-benzyloxycarbonylamino-pent-1-yl)-N-[2'-(1-triphenylmethyl-1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin werden in 20 ml CH_2Cl_2 gelöst. Die Lösung wird auf 0° gekühlt und mit 0,408 ml N,N-Diisopropyl-N-ethyl-amin und anschliessend mit 0,29 ml Pentanoylchlorid versetzt. Man rührt das Gemisch 15 Minuten in einem Eisbad und dann 16 Stunden bei Raumtemperatur. Das Gemisch wird dann mit CH_2Cl_2 verdünnt, nacheinander mit 1 N-Natronlauge, 1 N-Salzsäure, Wasser und Sole gewaschen und über $MgSO_4$ getrocknet. Nach Reinigung mittels Flashchromatographie (200 g Kieselgel; System N3) erhält man das (S)-N-(1-Benzyloxycarbonyl-5-benzyloxycarbonylamino-pent-1-yl)-N-pentanoyl-N-[2'-(1-triphenylmethyl-1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin in Form eines bräunlichen Oels [R_f-Wert: 0,34 (System N2)].

c) 1,07 g (S)-N-(1-Benzyloxycarbonyl-5-benzyloxycarbonylamino-pent-1-yl)-N-pentanoyl-N-[2'-(1-triphenylmethyl-1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin werden in 15 ml Dioxan gelöst. Diese Lösung wird mit 1,5 ml einer Lösung von Chlorwasserstoff in Dioxan (7 N) versetzt und das Gemisch 4,5 Stunden bei 40° gerührt, eingedampft und mittels Flashchromatographie gereinigt (200 g Kieselgel; System N6). Man erhält so das (S)-N-(1 -Benzyloxycarbonyl-5-benzyloxycarbonylamino-pent-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [R_f-Wert: 0,42 (System N7)].

Beispiel 77:

Ein Gemisch aus 3,64 g N-Butansulfonyl-N-(2'-cyanobiphenyl-4-ylmethyl)-N-(2-ethoxycarbonyl-2,2-pentamethylen-ethyl)-amin, 5,0 g Tributylzinnazid und 20 ml o-Xylol wird 15 Stunden unter Rückfluss erhitzt. Nach dem Abkühlen wird das Gemisch eingedampft. Der Rückstand wird mit 20 ml methanolischer Salzsäure (3 N) versetzt und das Gemisch 1 Stunde gerührt und dann eingedampft. Der Rückstand wird in Diethylether aufgenommen. Die Etherlösung wird mit 1 N-Natronlauge extrahiert. Die wässrige Phase wird mit konzentrierter Salzsäure auf pH 3 angesäuert und mit CH_2Cl_2 extrahiert. Die vereinigten organischen Phasen werden über $MgSO_4$ getrocknet und eingeengt. Der Rückstand wird durch Flashchromatographie gereinigt (220 g Kieselgel; CH_2Cl_2/Aceton = 9:1). Kristallisation aus Pentan liefert das N-Butansulfonyl-N-(2-ethoxycarbonyl-2,2-pentamethylen-ethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [Smp.: 121° (Zersetzung)].

Das Ausgangsmaterial kann z. B. wie folgt hergestellt werden:

a) 3,0 g 1-Aminomethyl-1-ethoxycarbonyl-cyclohexan werden in 25 ml $CHCl_3$ gelöst. Die Lösung wird bei Raumtemperatur mit 0,7 ml Butansulfonylchlorid versetzt. Das Gemisch wird 5 Stunden unter Rückfluss erhitzt und nach dem Abkühlen eingedampft. Der Rückstand wird in Diethylether aufgenommen. Die etherische Phase wird nacheinander mit 1 N-Salzsäure und Wasser extrahiert, über $MgSO_4$ getrocknet und eingedampft. Der gelbe harzige Rückstand, das rohe N-Butansulfonyl-N-(2-ethoxycarbonyl-2,2-pentamethylen-ethyl)-amin [R_f-Wert: 0,64 (System N2)], kann ohne weitere Reinigung weiterumgesetzt werden.

b) 3,75 g N-Butansulfonyl-N-(2-ethoxycarbonyl-2,2-pentamethylen-ethyl)-amin werden in 30 ml Tetrahydrofuran gelöst. Die Lösung wird mit einem Eisbad gekühlt und mit 309 mg Natriumhydrid-Dispersion (80% in Oel) versetzt. Nach dem Erwärmen auf Raumtemperatur werden 3,5 g 4-Brommethyl-2'-cyano-biphenyl zugegeben. Das Gemisch wird 30 Stunden bei Raumtemperatur und dann 4 Stunden bei 60° gerührt und nach dem Abkühlen eingeengt. Der Rückstand wird in Diethylether aufgenommen. Die etherische Phase wird nacheinander mit 1 N-Salzsäure und Wasser extrahiert, getrocknet und eingeengt. Flashchromatographie des Rückstands (300 g Kieselgel; Hexan/tert.-Butylmethylether = 4:1) liefert das reine N-Butansulfonyl-N-(2'-cyanobiphenyl-4-ylmethyl)-N-(2-ethoxycarbonyl-2,2-pentamethylen-ethyl)-amin in Form eines gelben Harzes [R_f-Wert: 0,46 (Hexan/tert.-Butylmethylether = 1:1)].

Beispiel 78:

1,8 g N-Butansulfonyl-N-(2-ethoxycarbonyl-2,2-pentamethylen-ethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin werden in 50 ml Methanol/Wasser (1:1) aufgenommen. Das Gemisch wird mit 5,0 g Natriumhydroxid versetzt, 20 Stunden unter Rückfluss erhitzt, auf Raumtemperatur abgekühlt, mit Wasser verdünnt und mit Ethylacetat extrahiert. Die wässrige Phase wird mit konzentrierter Salzsäure auf pH 3 angesäuert, mit NaCl gesättigt und mit CH_2Cl_2 extrahiert. Die vereinigten organischen Phasen werden getrocknet und eingedampft. Umkristallisation aus Diethylether/Hexan liefert das reine N-Butansulfonyl-N-(2-carboxy-2,2-pentamethylen-ethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [Smp.: 123° (Zersetzung)].

Beispiel 79:

In analoger Weise wie in Beispiel 77 beschrieben kann man auch das N-Butansulfonyl-N-(2-ethoxycarbonyl-2-methyl-prop-1 -yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 104°) herstellen.

Beispiel 80:

In analoger Weise wie in Beispiel 78 beschrieben kann man auch das N-Butansulfonyl-N-(2-carboxy-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 137°) herstellen.

Beispiel 81:

In analoger Weise wie in Beispiel 77 beschrieben kann man auch das (S)-N-Butansulfonyl-N-(1-tert.-butoxycarbonylethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin herstellen, ausgehend von (S)-2-Aminopropansäure-tert.-butylester.

Beispiel 82:

750 mg (S)-N-Butansulfonyl-N-(1-tert.-butoxycarbonylethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin werden 24 Stunden bei 0° mit salzsaurem Eisessig (1,9 N) behandelt. Eindampfen des Gemisches und Flashchromatographie des Rückstands (100 g Kieselgel; CH_2Cl_2/Ethylacetat/Toluol/Ameisensäure = 40:40:20:4) liefert das (S)-N-Butansulfonyl-N-(1-carboxyethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin in Form eines weissen amorphen Pulvers [Smp.: 90° (Zersetzung bei 127°)].

Beispiel 83:

In analoger Weise wie in den Beispielen 77 und 37 beschrieben kann man auch das (S)-N-Butansulfonyl-N-(1-carboxy-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [Smp.: 103° (Zersetzung)] herstellen, ausgehend von (S)-2-Amino-3-methyl-butansäurebenzylester-p-toluolsulfonat.

Beispiel 84:

In analoger Weise wie in Beispiel 48 beschrieben kann man auch das (S)-N-(1-Aminocarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 177 bis 178°) herstellen.

Beispiel 85:

In analoger Weise wie in Beispiel 48 beschrieben kann man auch das (S)-N-(2-Methyl-1-methylaminocarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 183 bis 184°) herstellen.

Beispiel 86:

In analoger Weise wie in Beispiel 48 beschrieben kann man auch das (S)-N-(1-Dimethylaminocarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol1-5-yl)biphenyl-4-ylmethyl] -amin (Smp.: 179 bis 180°) herstellen.

Beispiel 87:

In analoger Weise wie in Beispiel 48 beschrieben kann man auch das (S)-N-(2-Methyl-1-morpholin-4-ylcarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [Smp.: 130° (Zersetzung)] herstellen.

Beispiel 88:

In analoger Weise wie in Beispiel 8 beschrieben kann man auch das (S)-N-(2'-Carboxybiphenyl-4-ylmethyl)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoylamin (Smp.: 66 bis 68°) herstellen.

Beispiel 89:

In analoger Weise wie in Beispiel 16 beschrieben kann man auch das (S)-N-(1,2-Dicarboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 303 bis 305°) herstellen.

Beispiel 90:

In analoger Weise wie in Beispiel 16 beschrieben kann man auch das (S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-(5-oxopent-1-en-5-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 108 bis 109°) herstellen.

Beispiel 91:

In analoger Weise wie vorstehend beschrieben kann man auch die folgenden Verbindungen herstellen:

1. (S)-N-(1-Carboxy-3-phenyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 124 bis 125°);
2. (S)-N-(2-Cyclohexyl-1-hydroxymethyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 86 bis 87°);
3. (R)-N-(1-Methoxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 77 bis 78°);
4. (S)-N-(2-Hydroxy-1-methoxycarbonyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
5. N-Pentanoyl-N-(1H-tetrazol-5-ylmethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
6. N-Pentanoyl-N-pyrid-3-ylmethyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
7. (S)-N-(1-Carboxy-4-guanidino-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl] -

amin-hydrochlorid [R_f-Wert: 0,34 (CH_2Cl_2/CH_3OH/konzentriertes Ammoniak = 20:10:1)];
8. N-(2-Hydroxy-1-methoxycarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
9. N-(1-Benzyloxycarbonyl-1-methyl-ethyl)-N-butanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 203 bis 204°);
10. (S)-N-(1-Carboxy-3-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: >300°);
11. N-(1-Carboxy-2-hydroxy-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
12. (S)-N-(1-Carboxy-2-hydroxy-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
13. (S)-N-[2-Methyl-1-(2-phenylethylaminocarbonyl)-prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 109 bis 111 °);
14. (S)-N-(2-Benzyloxy-1-hydroxymethyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl) -amin;
15. (S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-3-ylmethyl]-amin (Smp.: 78 bis 79°);
16. (S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[3'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 97 bis 98°);
17. (S)-N-[2-Methyl-1-(1,2,3,4-tetrahydrochinol-1-ylcarbonyl)-prop-1 -yl] -N-pentanoylN-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 100 bis 110°);
18. (S)-N-(2-Methyl-1-piperidin-1-ylcarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 100°);
19. (S)-N-[2-Methyl-1-(1,2,3,4-tetrahydroisochinol-2-ylcarbonyl)-prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin (Smp.: 122°);
20. N-(2-Hydroxymethyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl] -amin [R_f-Wert: 0,45 (CH_2Cl_2/CH_3OH = 4:1)];
21. N-Ethoxycarbonyl-N-(2-ethoxycarbonyl-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl] -amin [R_f-Wert: 0,64 (CH_2Cl_2/CH_3OH = 4:1)]; und
22. N-(2-Carboxy-2-methyl-prop-1-yl)-N-ethoxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin [R_f-Wert: 0,32 (CH_2Cl_2/CH_3OH = 4:1)].

Beispiel 92:

Tabletten, enthaltend je 50 mg Wirkstoff, z.B. (S)-N-(1-Carboxy-2-methylprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, können wie folgt hergestellt werden:

Zusammensetzung (für 10000 Tabletten):

Wirkstoff	500,0 g
Lactose	500,0 g
Kartoffelstärke	352,0 g
Gelatine	8,0 g
Talk	60,0 g
Magnesiumstearat	10,0 g
Siliciumdioxid (hochdispers)	20,0 g
Ethanol	q. s.

Der Wirkstoff wird mit der Lactose und 292 g Kartoffelstärke vermischt, die Mischung mit einer alkoholischen Lösung der Gelatine befeuchtet und durch ein Sieb granuliert. Nach dem Trocknen mischt man den Rest der Kartoffelstärke, den Talk, das Magnesiumstearat und das hochdisperse Siliciumdioxid zu und presst das Gemisch zu Tabletten von je 145,0 mg Gewicht und 50,0 mg Wirkstoffgehalt, die gewünschtenfalls mit Teilkerben zur feineren Anpassung der Dosierung versehen sein können.

Beispiel 93:

Lacktabletten, enthaltend je 100 mg Wirkstoff, z.B. (S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, können wie folgt hergestellt werden:

Zusammensetzung (für 1000 Tabletten):

Wirkstoff	100,00 g
Lactose	100,00 g
Maisstärke	70,00 g
Talk	8,50 g
Calciumstearat	1,50 g
Hydroxypropylmethylcellulose	2,36 g
Schellack	0,64 g
Wasser	q. s.
Dichlormethan	q. s.

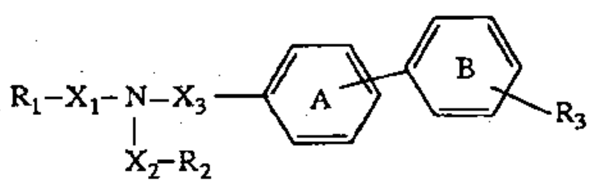
Der Wirkstoff, die Lactose und 40 g der Maisstärke werden gemischt und mit einem Kleister, hergestellt aus 15 g Maisstärke und Wasser (unter Erwärmen), befeuchtet und granuliert. Das Granulat wird getrocknet, der Rest der Maisstärke, der Talk und das Calciumstearat werden zugegeben und mit dem Granulat vermischt. Das Gemisch wird zu Tabletten (Gewicht: 280 mg) verpresst und diese mit einer Lösung der Hydroxypropylmethylcellulose und des Schellacks in Dichlormethan lackiert (Endgewicht der Lacktablette: 283 mg).

Beispiel 94:

In analoger Weise wie in den Beispielen 92 und 93 beschrieben können auch Tabletten und Lacktabletten, enthaltend eine andere Verbindung der Formel I oder ein pharmazeutisch verwendbares Salz einer Verbindung der Formel I, z.B. gemäss einem der Beispiele 1 bis 91, hergestellt werden.

Patentansprüche

1. Eine Verbindung der Formel



(I),

worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO, SO_2 oder -O-C(=O)-, wobei das Kohlenstoffatom der Carbonylgruppe an das in der Formel I eingezeichnete Stickstoffatom gebunden ist, steht; X_2 einen gegebenenfalls durch Hydroxy, Carboxy, Amino, Guanidino, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest oder einen zweiwertigen cycloaliphatischen Kohlenwasserstoffrest bedeutet, wobei ein Kohlenstoffatom des aliphatischen Kohlenwasserstoffrestes zusätzlich durch einen zweiwertigen aliphatischen Kohlenwasserstoffrest überbrückt sein kann; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, 1H-Tetrazol-5-yl, Pyridyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 einen zweiwertigen aliphatischen Kohlenwasserstoff bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind; in freier Form oder in Salzform.

2. Eine Verbindung gemäss Anspruch 1 der Formel I, worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO oder SO_2 steht; X_2 einen gegebenenfalls durch Hydroxy, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest oder einen zweiwertigen cycloaliphatischen Kohlenwasserstoffrest bedeutet, wobei ein Kohlenstoffatom des aliphatischen Kohlenwasserstoffrestes zusätzlich durch einen zweiwertigen aliphatischen Kohlenwasserstoffrest überbrückt sein kann; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, gegebenenfalls verethertes

Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 einen zweiwertigen aliphatischen Kohlenwasserstoff bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind, in freier Form oder in Salzform.

3. Eine Verbindung gemäss Anspruch 1 der Formel I, worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO oder SO_2 steht; X_2 einen gegebenenfalls durch Hydroxy, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest bedeutet; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3-CH_2- bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind, in freier Form oder in Salzform.

4. Eine Verbindung gemäss Anspruch 1 der Formel I, worin R_1 Niederalkyl, Niederalkenyl, Niederalkinyl, Halogenniederalkyl, -niederalkenyl, -niederalkinyl, Hydroxyniederalkyl, -niederalkenyl, -niederalkinyl, Cycloalkyl, Cycloalkenyl, Phenylniederalkyl, Phenylniederalkenyl oder Phenylniederalkinyl bedeutet; X_1 für CO oder SO_2 steht; X_2 Alkylen oder Alkyliden bedeutet, die gegebenenfalls durch Hydroxy, einen Cycloalkyl-, Cycloalkenyl-, einen Phenylrest oder einen 5- oder 6-gliedrigen, monocyclischen heteroaromatischen Rest mit bis zu vier gleichen oder verschiedenen Heteroatomen substituiert sind, wobei die cyclischen Reste ihrerseits gegebenenfalls substituiert sind durch Carboxy, welches gegebenenfalls verestert ist mit einem Alkohol, der sich von Niederalkyl, Phenylniederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl ableitet, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen; R_2 Carboxy, welches gegebenenfalls verestert ist mit einem Alkohol, der sich von Niederalkyl, Phenylniederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl ableitet, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Amino, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl-, Benzolsulfonyl-amino, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen, Hydroxy, Niederalkoxy, Niederalkenyloxy, Phenylniederalkoxy, Phenoxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 und R für Wasserstoff, Niederalkyl, Niederalkenyl oder Niederalkinyl steht, Niederalkanoyl, Sulfamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 -CH2- bedeutet; und R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenniederalkylsulfamoyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B unabhängig voneinander jeweils gegebenenfalls substituiert sind durch einen oder mehrere Substituenten ausgewählt aus Halogen, Hydroxy, Niederalkoxy, Niederalkenyloxy, jeweils gegebenenfalls durch Halogen oder Hydroxy substituiertes Niederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl, -niederalkinyl, Niederalkenyloxyniederalkyl, -niederalkenyl und -niederalkinyl, in freier Form oder in Salzform.

5. Eine Verbindung gemäss Anspruch 1 der Formel I, worin X_2 Alkylen oder Alkyliden bedeutet, die gegebenenfalls durch Hydroxy, einen Cycloalkyl-, Cycloalkenyl-, einen Phenylrest oder einen 5- oder 6-gliedrigen, monocyclischen heteroaromatischen Rest mit bis zu vier gleichen oder verschiedenen Heteroatomen substituiert sind, wobei ein C-Atom von Alkylen bzw. Alkyliden durch C_2-C_6-Alkylen überbrückt sein kann und wobei die cyclischen Reste ihrerseits gegebenenfalls substituiert sind durch Carboxy, welches gegebenenfalls verestert ist mit einem Alkohol, der sich von Niederalkyl, Phenylniederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl ableitet, Carbamoyl, in dem die

Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Formyl, Diniederalkoxymethyl oder durch Oxyniederalkylenoxymethylen, oder X_2 C_3-C_7-Cycloalkylen bedeutet; X_3 Niederalkylen oder Niederalkyliden bedeutet; die Variablen X_1, R_1, R_2, und R_3 die unmittelbar vorstehend angegebenen Bedeutungen haben; und die (hetero-)aromatischen Ringe einschliesslich der Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

6. Eine Verbindung gemäss Anspruch 1 der Formel I, worin R_1 Niederalkyl, Niederalkenyl, Halogenniederalkyl, -niederalkenyl, Hydroxyniederalkyl, 3- bis 7-gliedriges Cycloalkyl oder Phenylniederalkyl bedeutet; X_1 für CO, SO_2 oder -O-C(=O)-, wobei das Kohlenstoffatom der Carbonylgruppe an das in der Formel I eingezeichnete Stickstoffatom gebunden ist, steht; X_2 C_1-C_{10}-Alkylen oder C_1-C_7-Alkyliden, die gegebenenfalls substituiert sind durch Hydroxy, Carboxy, Amino, Guanidino, einen 3- bis 7-gliedrigen Cycloalkyl-, 3- bis 7-gliedrigen Cycloalkenyl-, Phenyl-, Pyrrolyl-, Pyrazolyl-, Imidazolyl-, Triazolyl-, Tetrazolyl-, Furyl-, Thienyl- oder Pyridylrest, welche ihrerseits gegebenenfalls zusätzlich durch Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl oder Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Formyl, Diniederalkoxymethyl oder Oxyniederalkylenoxymethylen substituiert sein können; R_2 Carboxy, Niederalkoxy-, Phenylniederalkoxy-, Niederalkenyloxy-, Niederalkoxyniederalkoxy-carbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen, das gegebenenfalls an zwei benachbarten Kohlenstoffatomen mit einem Benzolring kondensiert ist, oder Niederalkylenoxyniederalkylen disubstituiert ist, Amino, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl-, Benzolsulfonyl-amino, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 und R für Niederalkyl steht, Niederalkanoyl, Sulfamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 Methylen ist; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenniederalkylsulfamoyl bedeutet; und (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls zusätzlich substituiert sind durch einen oder mehrere Substituenten ausgewählt aus Halogen, Hydroxy, Niederalkoxy, jeweils gegebenenfalls durch Halogen oder Hydroxy substituiertes Niederalkyl bzw. Niederalkoxyniederalkyl, in freier Form oder in Salzform.

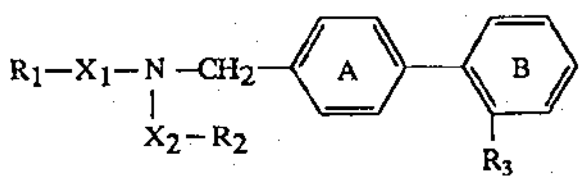
7. Eine Verbindung gemäss Anspruch 1 der Formel I, worin R_1 Niederalkyl, Niederalkenyl, Halogenniederalkyl, -niederalkenyl, Hydroxyniederalkyl, 3- bis 7-gliedriges Cycloalkyl oder Phenylniederalkyl bedeutet; X_1 für CO oder SO_2 steht; X_2 C_1-C_{10}-Alkylen oder C_1-C_7-Alkyliden, die gegebenenfalls substituiert sind durch Hydroxy, einen 3- bis 7-gliedrigen Cycloalkyl-, 3- bis 7-gliedrigen Cycloalkenyl-, Phenyl-, Pyrrolyl-, Pyrazolyl-, Imidazolyl-, Triazolyl-, Tetrazolyl-, Furyl-, Thienyl- oder Pyridylrest, welche ihrerseits gegebenenfalls zusätzlich durch Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl oder Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Formyl, Diniederalkoxymethyl oder Oxyniederalkylenoxymethylen substituiert sein können; R_2 Carboxy, Niederalkoxy-, Phenylniederalkoxy-, Niederalkenyloxy-, Niederalkoxyniederalkoxy-carbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Amino, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl-, Benzolsulfonyl-amino, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 und R für Niederalkyl steht, Niederalkanoyl, Sulfamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 Methylen ist; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenniederalkylsulfamoyl bedeutet; und (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls zusätzlich substituiert sind durch einen oder mehrere Substituenten ausgewählt aus Halogen, Hydroxy, Niederalkoxy, jeweils gegebenenfalls durch Halogen oder Hydroxy substituiertes Niederalkyl bzw. Niederalkoxyniederalkyl, in freier Form oder in Salzform.

8. Eine Verbindung gemäss Anspruch 1 der Formel I, worin X_2 C_1-C_{10}-Alkylen oder C_1-C_7-Alkyliden, die gegebenenfalls substituiert sind durch Hydroxy, einen 3- bis 7-gliedrigen Cycloalkyl-, 3- bis 7-gliedrigen Cycloalkenyl-, Phenyl-, Pyrrolyl-, Pyrazolyl-, Imidazolyl-, Triazolyl-, Tetrazolyl-, Furyl-, Thienyl- oder Pyridylrest, welche ihrerseits gegebenenfalls zusätzlich durch Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl oder Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Formyl, Diniederalkoxymethyl oder durch Oxyniederalkylenoxymethylen substituiert sein können, wobei ein C-Atom von Alkylen bzw. Alkyliden durch C_2-C_6-Alkylen überbrückt sein kann, oder X_2 C_3-C_7-Cycloalkylen bedeutet; X_3 Niederalkylen oder Niederalkyliden bedeutet; die Variablen X_1, R_1, R_2, R_3 die unmittelbar vorstehend angegebenen Bedeutungen haben; und die (hetero-)aromatischen Ringe einschliesslich der Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

9. Eine Verbindung gemäss Anspruch 1 der Formel I, worin die Variablen R 1, X_1, R_3 die jeweils vorstehend angegebenen Bedeutungen haben; X_2 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, Phenyl oder Imidazolyl substituiertes Niederalkylen oder Niederalkyliden bedeutet und R_2 Carboxy, Niederalkoxy-, Phenylniederalkoxy-, Niederalkoxyniederalkoxy-carbonyl, Carbamoyl, welches gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Amino, Niederalkanoyl-, Phenylniederalkanoyl-, Niederalkansulfonylamino, Hydroxy, Niederalkoxy, Phenylniederalkoxy oder Phenoxy bedeutet; X_3 -CH_2- bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch einen oder mehrere Substituenten ausgewählt aus Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl, Hydroxyniederalkyl oder Niederalkoxyniederalkyl substituiert sind, in freier Form oder in Salzform.

10. Eine Verbindung gemäss Anspruch 1 der Formel I, worin X_2 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, 7-gliedriges Cycloalkenyl, Phenyl oder Imidazolyl substituiertes Niederalkylen oder Niederalkyliden bedeutet, wobei ein C-Atom von Niederalkylen bzw. Niederalkyliden durch C_2-C_6-Alkylen überbrückt sein kann, oder X_2 C_3-C_7-Cycloalkylen bedeutet; die Variablen X_1, X_3, R_1, R_2 und R_3 die unmittelbar vorstehend angegebenen Bedeutungen haben; und die Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

11. Eine Verbindung gemäss Anspruch 1 der Formel



(Ia),

worin die Variablen R_1, X_1, X_2, R_2 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben und die Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

12. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin X_2 gegebenenfalls durch Hydroxy oder 3- bis 7-gliedriges Cycloalkyl substituiertes Niederalkylen oder Niederalkyliden bedeutet, wobei ein C-Atom von Niederalkylen bzw. Niederalkyliden durch C_2-C_6-Alkylen, insbesondere C_4-C_5-Alkylen, überbrückt sein kann, oder worin X_2 C_3-C_7-Cycloalkylen bedeutet; die Variablen R_1, X_1, R_2 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben; und die Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

13. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin X_2 für die Gruppe der Formel

$$-(CH_2)_p-\left(\begin{array}{c} X_4 \\ | \\ C \\ | \\ X_5 \end{array}\right)_q-(CH_2)_r- \quad \text{(Ib)}$$

steht, in der p für 0 oder 1, q für 1 und r für 0 oder 1 stehen oder in der p für 1 bis 8 und q sowie r jeweils für 0 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, Phenyl oder Imidazolyl substituiertes Niederalkyl oder Phenyl bedeutet; und X_5 Wasserstoff oder Niederalkyl bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, Amino, Niederalkanoylamino, Phenylniederalkanoylamino oder Niederalkansulfonylamino bedeutet; und die Variablen R_1, X_1 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

14. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin X_2 für die Gruppe der Formel Ib steht, in der p für 0 oder 1, q für 1 und r für 0 oder 1 stehen oder in der p für 1 bis 8 und q sowie r jeweils für 0 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, Phenyl oder Imidazolyl substituiertes Niederalkyl oder Phenyl bedeutet; und X_5 Wasserstoff oder Niederalkyl bedeutet; oder X_4 und X_5 gemeinsam für C_2-C_6-Alkylen, insbesondere C_4-C_5-Alkylen, stehen; oder X_2 C_3-C_7-Cycloalkylen, insbesondere C_5-C_6-Cycloalkylen, bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl; Niederalkoxyniederalkoxycarbonyl, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, Amino, Niederalkanoylamino, Phenylniederalkanoylamino oder Niederalkansulfonylamino bedeutet; und die Variablen R_1, X_1 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

15. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin R_1 Niederalkyl, insbesondere C_3-C_5-Alkyl, oder Niederalkenyl, insbesondere C_3-C_5-Alkenyl, bedeutet; X_1 für CO oder ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r für 0 oder 1 und q für 1 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, wie Cyclohexyl, durch gegebenenfalls durch Halogen oder Hydroxy substituiertes Phenyl oder Imidazolyl, wie 4-Imidazolyl, substituiertes Niederalkyl, insbesondere C_1-C_4-Alkyl, oder Phenyl bedeutet; und X_5 Wasserstoff oder Niederalkyl, wie C_1-C_4-Alkyl, bedeutet; oder X_4 und X_5 gemeinsam C_2-C_6-Alkylen, wie C_4-C_5-Alkylen, bedeuten; oder X_2 C_3-C_7-Cycloalkylen, wie C_5-C_6-Cycloalkylen, bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, wie C_2-C_5-Alkoxycarbonyl, Phenylniederalkoxycarbonyl, wie Phenyl-C_1-C_4-alkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, wie C_1-C_4-Alkoxy-C_2-C_5-alkoxycarbonyl, Hydroxy oder Niederalkoxy, wie C_1-C_4-Alkoxy, bedeutet; und R_3 Carboxy oder 5-Tetrazolyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

16. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin R_1 Niederalkyl, insbesondere C_3-C_5-Alkyl, oder Niederalkenyl, insbesondere C_3-C_5-Alkenyl, bedeutet; X_1 für CO oder ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r für 0 oder 1 und q für 1 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, durch gegebenenfalls durch Halogen oder Hydroxy substituiertes Phenyl oder Imidazolyl, wie 4-Imidazolyl, substituiertes Niederalkyl, insbesondere C_1-C_4-Alkyl, oder Phenyl bedeutet; und X_5 Wasserstoff oder Niederalkyl, wie C_1-C_4-Alkyl, bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, wie C_2-C_5-Alkoxycarbonyl, Phenylniederalkoxycarbonyl, wie Phenyl-C_1-C_4-alkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, wie C_1-C_4-Alkoxy-C_2-C_5-alkoxycarbonyl, Hydroxy oder Niederalkoxy, wie C_1-C_4-Alkoxy, bedeutet; und R_3 Carboxy oder 5-Tetrazolyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

17. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin R_1 Niederalkyl, insbesondere C_3-C_5-Alkyl, oder ferner Niederalkenyl, insbesondere C_3-C_5-Alkenyl, bedeutet; X_1 für CO oder ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p für eine ganze Zahl von 1 bis 8 und q sowie r für 0 stehen; R_2 Hydroxy, Niederalkoxy, wie C_1-C_4-Alkoxy, Phenylniederalkoxy, wie Phenyl-C_1-C_4-alkoxy, Phenoxy, Niederalkanoylamino, wie C_1-C_4-Alkanoylamino, Phenylniederalkanoylamino, wie Phenyl-C_1-C_4-alkanoylamino, Niederalkansulfonylamino, wie C_1-C_4-Alkansulfonylamino, bedeutet; und R_3 Carboxy oder in erster Linie 5-Tetrazolyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

18. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin R_1 C_3-C_5-Alkyl oder in zweiter Linie C_3-C_5-Alkenyl, bedeutet; X_1 für CO, ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r unabhängig voneinander für 0 oder 1 und q für 1 stehen; X_4 C_1-C_4-Alkyl, Hydroxy-C_1-C_4-alkyl, C_3-C_7-Cycloalkyl-C_1-C_4-alkyl, Phenyl-C_1-C_4-alkyl oder Imidazolyl-C_1-C_4-alkyl bedeutet; und X_5 Wasserstoff oder C_1-C_4-Alkyl bedeutet; oder X_4 und X_5 gemeinsam für Tetramethylen, ferner Pentamethylen stehen; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl, ferner Phenyl-C_1-C_4-Alkoxycarbonyl bedeutet; und R_3 Carboxy oder insbesondere 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

19. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin R_1 C_3-C_5-Alkyl oder in zweiter Linie C_3-C_5-Alkenyl bedeutet; X_1 für CO, ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r jeweils für 0 oder 1 und q für 1 stehen; X_4 C_1-C_4-Alkyl, Hydroxy-C_1-C_4-alkyl, C_3-C_7-Cycloalkyl-C_1-C_4-alkyl, Phenyl-C_1-C_4-alkyl oder Imidazolyl-C_1-C_4-alkyl bedeutet; und X_5 Wasserstoff bedeutet; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl, ferner Phenyl-C_1-C_4-alkoxycarbonyl bedeutet, und R_3 Carboxy oder 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

20. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin R_1 C_3-C_5-Alkyl bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der q und r für 0 und p für 1 bis 3, insbesondere für 2, stehen oder in der p und q für 1 und r für 0 stehen; X_4 C_1-C_4-Alkyl bedeutet; X_5 Wasserstoff oder C_1-C_4-Alkyl bedeutet; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl bedeutet; und R_3 Carboxy oder 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

21. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin R_1 C_3-C_5-Alkyl bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der p für 0 oder 1, r für 0 und q für 1 stehen; X_4 C_1-C_4-Alkyl bedeutet; und X_5 Wasserstoff oder C_1-C_4-Alkyl bedeutet; oder X_4 und X_5 gemeinsam für Tetramethylen oder Pentamethylen stehen; R_2 Carboxy, oder C_2-C_5-Alkoxycarbonyl bedeutet; und R_3 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

22. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin R_1 C_3-C_5-Alkyl bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der p für 0 oder 1, r für 0 und q für 1 stehen; X_4 und X_5 gemeinsam für Tetramethylen, ferner Pentamethylen stehen; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl bedeutet; und R_3 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

23. Eine Verbindung gemäss Anspruch 1 der Formel Ia, worin R_1 C_3-C_5-Alkyl bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der p und r für 0 oder 1 und q für 1 stehen; X_4 C_1-C_4-Alkyl bedeutet; und X_5 Wasserstoff bedeutet; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl bedeutet; und R_3 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

24. (S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, in freier Form oder in Salzform.

25. N-(2-Carboxy-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl] -amin, in freier Form oder in Salzform.

26. N-(2-Carboxy-2-ethyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, in freier Form oder in Salzform.

27. (S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-ethoxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl] in, in freier Form oder in Salzform.

28. N-(1-carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, in freier Form oder in Salzform.

29. (S)-N-(1-Carboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-2-ethyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,

(S)-N-(1-Hydroxymethyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-2,2-pentamethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-propyloxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-carboxy-2-methyl-propyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-carboxy-2,2-pentamethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-aminocarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin oder
(S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-(5-oxopent-1-en-5-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, jeweils in freier Form oder in Salzform.

30. N-Carboxymethyl-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Methoxycarbonylethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[1-Carboxy-2-(4-fluorphenyl)-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[2-(4-Fluorphenyl)-1-methoxycarbonyl-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[2-(4-Fluorphenyl)-1-hydroxymethyl-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2'-Carboxybiphenyl-4-ylmethyl)-N-[1-carboxy-2-(4-fluorphenyl)-ethyl]-N-pentanoyl-amin,
N-(2'-Carboxybiphenyl-4-ylmethyl)-N-[2-(4-fluorphenyl)-1-methoxycarbonyl-ethyl]-N-pentanoyl-amin,
(S)-N-(2'-Carboxybiphenyl-4-ylmethyl)-N-(1-hydroxymethyl-2-phenyl-ethyl)-N-pentanoyl-amin,
(S)-N-(2'-Carboxybiphenyl-4-ylmethyl)-N-(1-hydroxymethyl-2-imidazol-4-yl-ethyl)-N-pentanoyl-amin,
(R)-N-(1-Carboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(1S),(2S)-N-(1-Carboxy-2-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(1S),(2S)-N-(1-Methoxycarbonyl-2-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxybut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Methoxycarbonylbut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxyethyl)-N-hexanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-Butanoyl-N-(1-carboxyethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-cyclohexyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Cyclohexyl-1-methoxycarbonyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(R)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Methoxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Benzyloxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Methoxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Benzyloxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Hydroxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Methoxycarbonyl-1-methyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Carboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Carboxyprop-1-yl)-N-pentanoyl-N- [2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxy-1-methyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(5-Hydroxypent-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxyprop-2-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-3-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Carboxy-3-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Phenoxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[2-(4-Hydroxyphenyl)ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[3-(4-Hydroxyphenyl)prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(8-Hydroxyoct-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Methansulfonylaminoethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Acetylaminoprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Methoxy-2-oxo-1-phenyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,

N-(4-Hydroxybut-2-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxy-1-phenyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[3-(4-Hydroxybenzylcarbonylamino)prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Ethoxycarbonylcyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
cis-N-(4-Carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
cis-N-(2-Ethoxycarbonylcyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
cis-N-(2-Carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-{2-[2-(4-Hydroxyphenyl)ethylaminocarbonyl]-2,2-tetramethylen-ethyl}-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N- {1-[2-(4-Hydroxyphenyl)ethylaminocarbonyl]-2-methyl-prop-1-yl}-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2,2-dimethyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Methoxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(4-Phenoxybut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxy-1-phenyl-2-oxo-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Benzyloxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Butanoyl-N-(1-carboxy-1-methyl-ethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(4-Hydroxybut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Benzyloxycarbonyl-2-methyl-prop-1-yl)-N-[3-bromo-2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-N-pentanoyl-amin,
(S)-N-[3-Brom-2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-amin,
N-(2-Acetylaminoethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[2-(n-Butoxycarbonyl)-2,2-tetramethylen-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Benzylaminocarbonyl-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-Butyloxycarbonyl-N-(1-Carboxy-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-methoxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Diethylaminocarbonyl-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Methyl-2-morpholin-4-ylcarbonyl-propyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxycyclopentyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxy-1-ethyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(5-Amino-1-carboxy-pent-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Butansulfonyl-N-(2-ethoxycarbonyl-2,2-pentamethylen-ethyl)-N-[2'-(thyl]-amin,
N-Butansulfonyl-N-(2-carboxy-2,2-pentamethylen-ethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Butansulfonyl-N-(2-ethoxycarbonyl-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Butansulfonyl-N-(2-carboxy-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-Butansulfonyl-N-(1-tert.-butoxycarbonylethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-Butansulfonyl-N-(1-carboxyethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-Butansulfonyl-N-(1-carboxy-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Methyl-1-methylaminocarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Dimethylaminocarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Methyl-1-morpholin-4-ylcarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,

(S)-N-(2'-Carboxybiphenyl-4-ylmethyl)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-amin,
(S)-N-(1,2-Dicarboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-3-phenyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Cyclohexyl-1-hydroxymethyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(R)-N-(1-Methoxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Hydroxy-1-methoxycarbonyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Pentanoyl-N-(1H-tetrazol-5-ylmethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Pentanoyl-N-pyrid-3-ylmethyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-4-guanidino-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxy-1-methoxycarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Benzyloxycarbonyl-1-methyl-ethyl)-N-butanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-3-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxy-2-hydroxy-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-hydroxy-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N- [2-Methyl-1-(2-phenylethylaminocarbonyl)-prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Benzyloxy-1-hydroxymethyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-3-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[3'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-[2-Methyl-1-(1,2,3,4-tetrahydrochinol-1-ylcarbonyl)-prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Methyl-1-piperidin-1-ylcarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-[2-Methyl-1-(1,2,3,4-tetrahydroisochinol-2-ylcarbonyl)-prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxymethyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Ethoxycarbonyl-N-(2-ethoxycarbonyl-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin oder
N- (2-Carboxy-2-methyl-prop-1-yl)-N-ethoxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
jeweils in freier Form oder in Salzform.

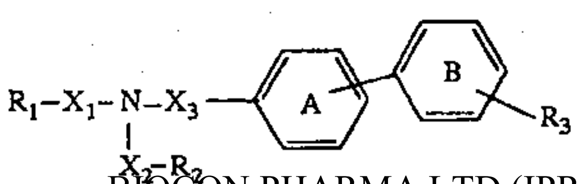
31. Eine Verbindung gemäss einem der Ansprüche 1 bis 30, in freier Form oder in Form eines pharmazeutisch verwendbaren Salzes, zur Anwendung in einem Verfahren zur therapeutischen Behandlung des menschlichen oder tierischen Körpers.

32. Eine Verbindung gemäss einem der Ansprüche 1 bis 31, in freier Form oder in Form eines pharmazeutisch verwendbaren Salzes, zur Anwendung als Antihypertensivum.

33. Ein pharmazeutisches Präparat, als Wirkstoff enthaltend eine Verbindung gemäss einem der Ansprüche 1 bis 32, in freier Form oder in Form eines pharmazeutisch verwendbaren Salzes, gegebenenfalls neben üblichen pharmazeutischen Hilfsstoffen.

34. Ein antihypertensiv wirksames pharmazeutisches Präparat gemäss Anspruch 33, dadurch gekennzeichnet, dass man einen antihypertensiv wirksamen Wirkstoff wählt.

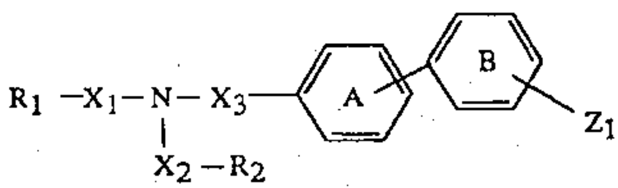
35. Verfahren zur Herstellung einer Verbindung der Formel



(I),

worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO, SO_2 oder -O-C(=O)-, wobei das Kohlenstoffatom der Carbonylgruppe an das in der Formel I eingezeichnete Stickstoffatom gebunden ist, steht; X_2 einen gegebenenfalls durch Hydroxy, Carboxy, Amino, Guanidino, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest oder einen zweiwertigen cycloaliphatischen Kohlenwasserstoffrest bedeutet, wobei ein Kohlenstoffatom des aliphatischen Kohlenwasserstoffrestes zusätzlich durch einen zweiwertigen aliphatischen Kohlenwasserstoffrest überbrückt sein kann; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, 1H-Tetrazol-5-yl, Pyridyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 einen zweiwertigen aliphatischen Kohlenwasserstoff bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind; in freier Form oder in Salzform, dadurch gekennzeichnet, dass man

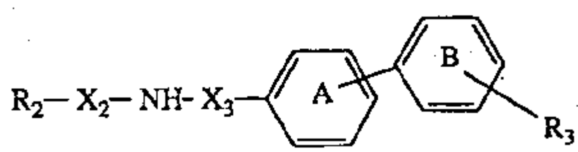
a) in einer Verbindung der Formel



(II)

oder einem Salz davon, worin Z_1 einen in R_3 überführbaren Rest bedeutet, Z_1 in R_3 überführt oder

b) eine Verbindung der Formel R_1-X_1OH (IIIa), ein reaktionsfähiges Derivat davon oder ein Salz davon mit einer Verbindung der Formel



(IIIb)

oder einem Salz davon umsetzt und jeweils, wenn erwünscht, eine verfahrensgemäss oder auf andere Weise erhältliche Verbindung I in freier Form oder in Salzform in eine andere Verbindung I überführt, ein verfahrensgemäss erhältliches Gemisch von Isomeren auftrennt und das gewünschte Isomere isoliert und/oder eine verfahrensgemäss erhältliche freie Verbindung I in ein Salz oder ein verfahrensgemäss erhältliches Salz einer Verbindung I in die freie Verbindung I oder in ein anderes Salz überführt.

36. Verfahren zur Herstellung eines pharmazeutischen Präparats gemäss Anspruch 33 oder 34, dadurch gekennzeichnet, dass man den Wirkstoff, gegebenenfalls unter Beimischung von üblichen pharmazeutischen Hilfsstoffen, zu einem pharmazeutischen Präparat verarbeitet.

37. Verfahren gemäss Anspruch 36 zur Herstellung eines antihypertensiv wirksamen pharmazeutischen Präparats gemäss Anspruch 34, dadurch gekennzeichnet, dass man einen antihypertensiv wirksamen Wirkstoff wählt.

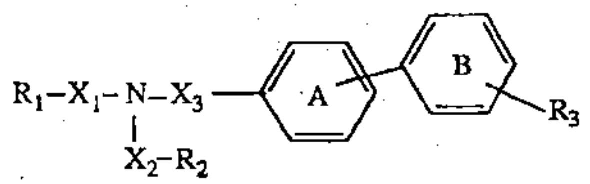
38. Verwendung einer Verbindung gemäss einem der Ansprüche 1 bis 32, in freier Form oder in Form eines pharmazeutisch verwendbaren Salzes, zur Herstellung eines pharmazeutischen Präparats.

39. Verwendung einer Verbindung gemäss einem der Ansprüche 1 bis 32, in freier Form oder in Form eines pharmazeutisch verwendbaren Salzes, zur Herstellung eines pharmazeutischen Präparats auf nicht-chemischem Wege.

40. Verwendung einer Verbindung gemäss Anspruch 38 oder 39 zur Herstellung eines Antihypertensivums.

Patentansprüche für folgenden Vertragsstaaten: ES und GR

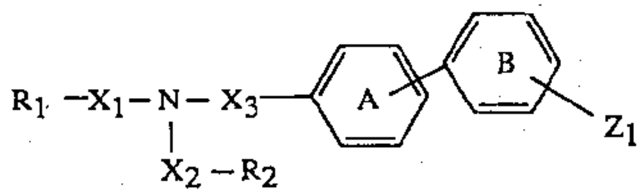
1. Verfahren zur Herstellung einer Verbindung der Formel



(I),

worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO, SO_2 oder -O-C(=O)-, wobei das Kohlenstoffatom der Carbonylgruppe an das in der Formel I eingezeichnete Stickstoffatom gebunden ist, steht; X_2 einen gegebenenfalls durch Hydroxy, Carboxy, Amino, Guanidino, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest oder einen zweiwertigen cycloaliphatischen Kohlenwasserstoffrest bedeutet, wobei ein Kohlenstoffatom des aliphatischen Kohlenwasserstoffrestes zusätzlich durch einen zweiwertigen aliphatischen Kohlenwasserstoffrest überbrückt sein kann; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, 1H-Tetrazol-5-yl, Pyridyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 einen zweiwertigen aliphatischen Kohlenwasserstoff bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind; in freier Form oder in Salzform, dadurch gekennzeichnet, dass man

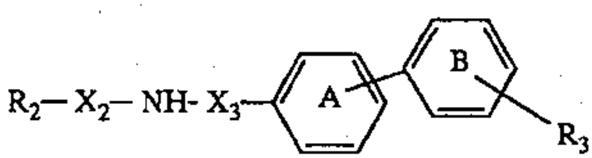
a) in einer Verbindung der Formel



(II)

oder einem Salz davon, worin Z_1 einen in R_3 überführbaren Rest bedeutet, Z_1 in R_3 überführt oder

b) eine Verbindung der Formel R_1-X_1OH (IIIa), ein reaktionsfähiges Derivat davon oder ein Salz davon mit einer Verbindung der Formel



(IIIb)

oder einem Salz davon umsetzt und jeweils, wenn erwünscht, eine verfahrensgemäss oder auf andere Weise erhältliche Verbindung I in freier Form oder in Salzform in eine andere Verbindung I überführt, ein verfahrensgemäss erhältliches Gemisch von Isomeren auftrennt und das gewünschte Isomere isoliert und/oder eine verfahrensgemäss erhältliche freie Verbindung I in ein Salz oder ein verfahrensgemäss erhältliches Salz einer Verbindung I in die freie Verbindung I oder in ein anderes Salz überführt.

2. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel I, worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO oder SO_2 steht; X_2 einen gegebenenfalls durch Hydroxy, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest oder einen zweiwertigen cycloaliphatischen Kohlenwasserstoffrest bedeutet, wobei ein Kohlenstoffatom des aliphatischen Kohlenwasserstoffrestes zusätzlich durch

einen zweiwertigen aliphatischen Kohlenwasserstoffrest überbrückt sein kann; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 einen zweiwertigen aliphatischen Kohlenwasserstoff bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind, in freier Form oder in Salzform.

3. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel I, worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO oder SO_2 steht; X_2 einen gegebenenfalls durch Hydroxy, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest bedeutet; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3-CH_2 bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind, in freier Form oder in Salzform.

4. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel I, worin R_1 Niederalkyl, Niederalkenyl, Niederalkinyl, Halogenniederalkyl, -niederalkenyl, -niederalkinyl, Hydroxyniederalkyl, -niederalkenyl, -niederalkinyl, Cycloalkyl, Cycloalkenyl, Phenylniederalkyl, Phenylniederalkenyl oder Phenylniederalkinyl bedeutet; X_1 für CO oder SO_2 steht; X_2 Alkylen oder Alkyliden bedeutet, die gegebenenfalls durch Hydroxy, einen Cycloalkyl-, Cycloalkenyl-, einen Phenylrest oder einen 5- oder 6-gliedrigen, monocyclischen heteroaromatischen Rest mit bis zu vier gleichen oder verschiedenen Heteroatomen substituiert sind, wobei die cyclischen Reste ihrerseits gegebenenfalls substituiert sind durch Carboxy, welches gegebenenfalls verestert ist mit einem Alkohol, der sich von Niederalkyl, Phenylniederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl ableitet, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen; R_2 Carboxy, welches gegebenenfalls verestert ist mit einem Alkohol, der sich von Niederalkyl, Phenylniederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl ableitet, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Amino, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl-, Benzolsulfonyl-amino, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen, Hydroxy, Niederalkoxy, Niederalkenyloxy, Phenylniederalkoxy, Phenoxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 und R für Wasserstoff, Niederalkyl, Niederalkenyl oder Niederalkinyl steht, Niederalkanoyl, Sulfamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 -CH_2- bedeutet; und R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenniederalkylsulfamoyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B unabhängig voneinander jeweils gegebenenfalls substituiert sind durch einen oder mehrere Substituenten ausgewählt aus Halogen, Hydroxy, Niederalkoxy, Niederalkenyloxy, jeweils gegebenenfalls durch Halogen oder Hydroxy substituiertes Niederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl, -niederalkinyl, Niederalkenyloxyniederalkyl, -niederalkenyl und -niederalkinyl, in freier Form oder in Salzform.

5. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel I, worin X_2 Alkylen oder Alkyliden bedeutet, die gegebenenfalls durch Hydroxy, einen Cycloalkyl-, Cycloalkenyl-, einen Phenylrest oder einen 5- oder 6-gliedrigen, monocyclischen heteroaromatischen Rest mit bis zu vier gleichen oder verschiedenen Heteroatomen substituiert sind, wobei ein C-Atom von Alkylen bzw. Alkyliden durch C_2-C_6-Alkylen

überbrückt sein kann und wobei die cyclischen Reste ihrerseits gegebenenfalls substituiert sind durch Carboxy, welches gegebenenfalls verestert ist mit einem Alkohol, der sich von Niederalkyl, Phenylniederalkyl, Niederalkenyl, Niederalkinyl, Niederalkoxyniederalkyl, -niederalkenyl oder -niederalkinyl ableitet, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Niederalkenyl, Niederalkinyl, Phenylniederalkyl, Phenylniederalkenyl, Phenylniederalkinyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Formyl, Diniederalkoxymethyl oder durch Oxyniederalkylenoxymethylen, oder X_2 C_3-C_7-Cycloalkylen bedeutet; X_3 Niederalkylen oder Niederalkyliden bedeutet; die Variablen X_1, R_1, R_2, und R_3 die unmittelbar vorstehend angegebenen Bedeutungen haben; und die (hetero-)aromatischen Ringe einschliesslich der Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

6. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel I, worin R_1 Niederalkyl, Niederalkenyl, Halogenniederalkyl, -niederalkenyl, Hydroxyniederalkyl, 3- bis 7-gliedriges Cycloalkyl oder Phenylniederalkyl bedeutet; X_1 für CO, SO_2 oder -O-C(=O)-, wobei das Kohlenstoffatom der Carbonylgruppe an das in der Formel I eingezeichnete Stickstoffatom gebunden ist, steht; X_2 C_1-C_{10}-Alkylen oder C_1-C_7-Alkyliden, die gegebenenfalls substituiert sind durch Hydroxy, Carboxy, Amino, Guanidino, einen 3- bis 7-gliedrigen Cycloalkyl-, 3- bis 7-gliedrigen Cycloalkenyl-, Phenyl-, Pyrrolyl-, Pyrazolyl-, Imidazolyl-, Triazolyl-, Tetrazolyl-, Furyl-, Thienyl- oder Pyridylrest, welche ihrerseits gegebenenfalls zusätzlich durch Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl oder Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Formyl, Diniederalkoxymethyl oder Oxyniederalkylenoxymethylen substituiert sein können; R_2 Carboxy, Niederalkoxy-, Phenylniederalkoxy-, Niederalkenyloxy-, Niederalkoxyniederalkoxy-carbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen, das gegebenenfalls an zwei benachbarten Kohlenstoffatomen mit einem Benzolring kondensiert ist, oder Niederalkylenoxyniederalkylen disubstituiert ist, Amino, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl-, Benzolsulfonyl-amino, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 und R für Niederalkyl steht, Niederalkanoyl, Sulfamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 Methylen ist; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenniederalkylsulfamoyl bedeutet; und (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls zusätzlich substituiert sind durch einen oder mehrere Substituenten ausgewählt aus Halogen, Hydroxy, Niederalkoxy, jeweils gegebenenfalls durch Halogen oder Hydroxy substituiertes Niederalkyl bzw. Niederalkoxyniederalkyl, in freier Form oder in Salzform.

7. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel I, worin R_1 Niederalkyl, Niederalkenyl, Halogenniederalkyl, -niederalkenyl, Hydroxyniederalkyl, 3- bis 7-gliedriges Cycloalkyl oder Phenylniederalkyl bedeutet; X_1 für CO oder SO_2 steht; X_2 C_1C_{10}-Alkylen oder C_1-C_7-Alkyliden, die gegebenenfalls substituiert sind durch Hydroxy, einen 3- bis 7-gliedrigen Cycloalkyl-, 3- bis 7-gliedrigen Cycloalkenyl-, Phenyl-, Pyrrolyl-, Pyrazolyl-, Imidazolyl-, Triazolyl-, Tetrazolyl-, Furyl-, Thienyl- oder Pyridylrest, welche ihrerseits gegebenenfalls zusätzlich durch Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl oder Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Formyl, Diniederalkoxymethyl oder Oxyniederalkylenoxymethylen substituiert sein können; R_2 Carboxy, Niederalkoxy-, Phenylniederalkoxy-, Niederalkenyloxy-, Niederalkoxyniederalkoxy-carbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Amino, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert oder durch Niederalkylen- oder Niederalkylenoxyniederalkylen disubstituiert ist, Niederalkanoyl-, Phenylniederalkanoyl-, Benzoyl-, Niederalkansulfonyl-, Benzolsulfonyl-amino, Formyl, Diniederalkoxymethyl, Oxyniederalkylenoxymethylen, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 und R für Niederalkyl steht, Niederalkanoyl, Sulfamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 Methylen ist; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenniederalkylsulfamoyl bedeutet; und (hetero-)aromatische Reste einschliesslich der Ringe A

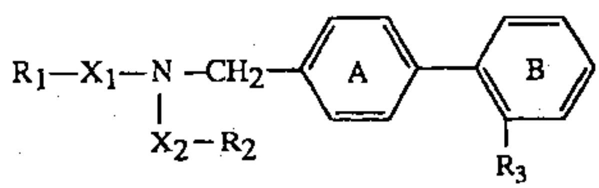
und B jeweils gegebenenfalls zusätzlich substituiert sind durch einen oder mehrere Substituenten ausgewählt aus Halogen, Hydroxy, Niederalkoxy, jeweils gegebenenfalls durch Halogen oder Hydroxy substituiertes Niederalkyl bzw. Niederalkoxyniederalkyl, in freier Form oder in Salzform.

8. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel I, worin X_2 C_1-C_{10}-Alkylen oder C_1-C_7-Alkyliden, die gegebenenfalls substituiert sind durch Hydroxy, einen 3- bis 7-gliedrigen Cycloalkyl-, 3- bis 7-gliedrigen Cycloalkenyl-, Phenyl-, Pyrrolyl-, Pyrazolyl-, Imidazolyl-, Triazolyl-, Tetrazolyl-, Furyl-, Thienyl- oder Pyridylrest, welche ihrerseits gegebenenfalls zusätzlich durch Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Carbamoyl, in dem die Aminogruppe gegebenenfalls durch Niederalkyl oder Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Formyl, Diniederalkoxymethyl oder durch Oxyniederalkylenoxymethylen substituiert sein können, wobei ein C-Atom von Alkylen bzw. Alkyliden durch C_2-C_8-Alkylen überbrückt sein kann, oder X_2 C_3-C_7-Cycloalkylen bedeutet; X_3 Niederalkylen oder Niederalkyliden bedeutet; die Variablen X_1, R_1, R_2, R_3 die unmittelbar vorstehend angegebenen Bedeutungen haben; und die (hetero-)aromatischen Ringe einschliesslich der Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

9. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel I, worin die Variablen R_1, X_1, R_3 die jeweils vorstehend angegebenen Bedeutungen haben; X_2 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, Phenyl oder Imidazolyl substituiertes Niederalkylen oder Niederalkyliden bedeutet und R_2 Carboxy, Niederalkoxy-, Phenylniederalkoxy-, Niederalkoxyniederalkoxy-carbonyl, Carbamoyl, welches gegebenenfalls durch Niederalkyl, Phenylniederalkyl mono- oder unabhängig voneinander disubstituiert ist, Amino, Niederalkanoyl-, Phenylniederalkanoyl-, Niederalkansulfonylamino, Hydroxy, Niederalkoxy, Phenylniederalkoxy oder Phenoxy bedeutet; X_3 -CH_2- bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch einen oder mehrere Substituenten ausgewählt aus Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl, Hydroxyniederalkyl oder Niederalkoxyniederalkyl substituiert sind, in freier Form oder in Salzform.

10. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel I, worin X_2 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, 7-gliedriges Cycloalkenyl, Phenyl oder Imidazolyl substituiertes Niederalkylen oder Niederalkyliden bedeutet, wobei ein C-Atom von Niederalkylen bzw. Niederalkyliden durch C_2-C_8-Alkylen überbrückt sein kann, oder X_2 C_3-C_7-Cycloalkylen bedeutet; die Variablen X_1, X_3, R_1, R_2 und R_3 die unmittelbar vorstehend angegebenen Bedeutungen haben; und die Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

11. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel



(Ia),

worin die Variablen R_1, X_1, X_2, R_2 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben und die Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

12. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin X_2 gegebenenfalls durch Hydroxy oder 3- bis 7-gliedriges Cycloalkyl substituiertes Niederalkylen oder Niederalkyliden bedeutet, wobei ein C-Atom von Niederalkylen bzw. Niederalkyliden durch C_2-C_6-Alkylen, insbesondere C_4-C_5-Alkylen, überbrückt sein kann, oder worin X_2 C_3-C_7-Cycloalkylen bedeutet; die Variablen R_1, X_1, R_2 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben; und die Ringe A und B wie unmittelbar vorstehend angegeben substituiert sein können, in freier Form oder in Salzform.

13. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin X_2 für die Gruppe der Formel

$$-(CH_2)_p\left(\begin{array}{c}X_4\\ |\\ C\\ |\\ X_5\end{array}\right)_q(CH_2)_r- \qquad (Ib)$$

steht, in der p für 0 oder 1, q für 1 und r für 0 oder 1 stehen oder in der p für 1 bis 8 und q sowie r jeweils für 0 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, Phenyl oder Imidazolyl substituiertes Niederalkyl oder Phenyl bedeutet; und X_5 Wasserstoff oder Niederalkyl bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, Amino, Niederalkanoylamino, Phenylniederalkanoylamino oder Niederalkansulfonylamino bedeutet; und die Variablen R_1, X_1 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

14. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin X_2 für die Gruppe der Formel Ib steht, in der p für 0 oder 1, q für 1 und r für 0 oder 1 stehen oder in der p für 1 bis 8 und q sowie r jeweils für 0 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, Phenyl oder Imidazolyl substituiertes Niederalkyl oder Phenyl bedeutet; und X_5 Wasserstoff oder Niederalkyl bedeutet; oder X_4 und X_5 gemeinsam für C_2-C_6-Alkylen, insbesondere C_4-C_5-Alkylen, stehen; oder X_2 C_3-C_7-Cycloalkylen, insbesondere C_5-C_6-Cycloalkylen, bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, Phenylniederalkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, Hydroxy, Niederalkoxy, Phenylniederalkoxy, Phenoxy, Amino, Niederalkanoylamino, Phenylniederalkanoylamino oder Niederalkansulfonylamino bedeutet; und die Variablen R_1, X_1 und R_3 die jeweils vorstehend angegebenen Bedeutungen haben; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

15. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin R_1 Niederalkyl, insbesondere C_3-C_5-Alkyl, oder Niederalkenyl, insbesondere C_3-C_5-Alkenyl, bedeutet; X_1 für CO oder ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r für 0 oder 1 und q für 1 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, wie Cyclohexyl, durch gegebenenfalls durch Halogen oder Hydroxy substituiertes Phenyl oder Imidazolyl, wie 4-Imidazolyl, substituiertes Niederalkyl, insbesondere C_1-C_4-Alkyl, oder Phenyl bedeutet; und X_5 Wasserstoff oder Niederalkyl, wie C_1-C_4-Alkyl, bedeutet; oder X_4 und X_5 gemeinsam C_2-C_6-Alkylen, wie C_4-C_5-Alkylen, bedeuten; oder X_2 C_3-C_7-Cycloalkylen, wie C_5-C_6-Cycloalkylen, bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, wie C_2-C_5-Alkoxycarbonyl, Phenylniederalkoxycarbonyl, wie Phenyl-C_1-C_4-alkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, wie C_1-C_4-Alkoxy-C_2-C_5-alkoxycarbonyl, Hydroxy oder Niederalkoxy, wie C_1-C_4-Alkoxy, bedeutet; und R_3 Carboxy oder 5-Tetrazolyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

16. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin R_1 Niederalkyl, insbesondere C_3-C_5-Alkyl, oder Niederalkenyl, insbesondere C_3-C_5-Alkenyl, bedeutet; X_1 für CO oder ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r für 0 oder 1 und q für 1 stehen; X_4 gegebenenfalls durch Hydroxy, 3- bis 7-gliedriges Cycloalkyl, durch gegebenenfalls durch Halogen oder Hydroxy substituiertes Phenyl oder Imidazolyl, wie 4-Imidazolyl, substituiertes Niederalkyl, insbesondere C_1-C_4-Alkyl, oder Phenyl bedeutet; und X_5 Wasserstoff oder Niederalkyl, wie C_1-C_4-Alkyl, bedeutet; R_2 Carboxy, Niederalkoxycarbonyl, wie C_2-C_5-Alkoxycarbonyl, Phenylniederalkoxycarbonyl, wie Phenyl-C_1-C_4-alkoxycarbonyl, Niederalkoxyniederalkoxycarbonyl, wie C_1-C_4-Alkoxy-C_2-C_5-alkoxycarbonyl, Hydroxy oder Niederalkoxy, wie C_1-C_4-Alkoxy, bedeutet; und R_3 Carboxy oder 5-Tetrazolyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

17. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin R_1 Niederalkyl, insbesondere C_3-C_5-Alkyl, oder ferner Niederalkenyl, insbesondere C_3-C_5-Alkenyl, bedeutet; X_1 für CO oder ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p für eine ganze Zahl von 1 bis 8 und q sowie r für 0 stehen; R_2 Hydroxy, Niederalkoxy, wie C_1-C_4-Alkoxy, Phenylniederalkoxy, wie Phenyl-C_1-C_4-alkoxy, Phenoxy, Niederalkanoylamino, wie C_1-C_4-Alkanoylamino, Phenylniederalkanoylamino, wie Phenyl-C_1-C_4-alkanoylamino, Niederalkansulfonylamino, wie C_1-C_4-Alkansulfonylamino, bedeutet; und R_3 Carboxy oder in erster Linie 5-Tetrazolyl bedeutet; wobei (hetero-)aromatische Reste einschliesslich der Ringe A und B jeweils gegebenenfalls durch Halogen, Trifluormethyl, Hydroxy, Niederalkoxy, Niederalkyl oder Hydroxyniederalkyl substituiert sind, in freier Form oder in Salzform.

18. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin R_1 C_3-C_5-Alkyl oder in zweiter Linie C_3-C_5-Alkenyl, bedeutet; X_1 für CO, ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r unabhängig voneinander für 0 oder 1 und q für 1 stehen; X_4 C_1-C_4-Alkyl, Hydroxy-C_1-C_4-alkyl, C_3-C_7-Cycloalkyl-C_1-C_4-alkyl, Phenyl-C_1-C_4-alkyl oder Imidazolyl-C_1-C_4-alkyl bedeutet; und X_5 Wasserstoff oder C_1-C_4-Alkyl bedeutet; oder X_4 und X_5 gemeinsam für Tetramethylen, ferner Pentamethylen stehen; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl, ferner Phenyl-C_1-C_4-alkoxycarbonyl bedeutet; und R_3 Carboxy oder insbesondere 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

19. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin R_1 C_3-C_5-Alkyl oder in zweiter Linie C_3-C_5-Alkenyl bedeutet; X_1 für CO, ferner SO_2 steht; X_2 für die Gruppe der Formel Ib steht, in der p und r jeweils für 0 oder 1 und q für 1 stehen; X_4 C_1-C_4-Alkyl, Hydroxy-C_1-C_4-alkyl, C_3-C_7-Cycloalkyl-C_1-C_4-alkyl, Phenyl-C_1-C_4-alkyl oder Imidazolyl-C_1-C_4-alkyl bedeutet; und X_5 Wasserstoff bedeutet; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl, ferner Phenyl-C_1-C_4-alkoxycarbonyl bedeutet; und R_3 Carboxy oder 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

20. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin R_1 C_3-C_5-Alkyl bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der q und r für 0 und p für 1 bis 3, insbesondere für 2, stehen oder in der p und q für 1 und r für 0 stehen; X_4 C_1-C_4-Alkyl bedeutet; X_5 Wasserstoff oder C_1-C_4-Alkyl bedeutet; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl bedeutet; und R_3 Carboxy oder 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

21. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin R_1 C_3-C_5-Alkyl bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der p für 0 oder 1, r für 0 und q für 1 stehen; X_4 C_1-C_4-Alkyl bedeutet; und X_5 Wasserstoff oder C_1-C_4-Alkyl bedeutet; oder X_4 und X_5 gemeinsam für Tetramethylen oder Pentamethylen stehen; R_2 Carboxy, oder C_2-C_5-Alkoxycarbonyl bedeutet; und R_3 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

22. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin R_1 C_3-C_5-Alkyl bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der p für 0 oder 1, r für 0 und q für 1 stehen; X_4 und X_5 gemeinsam für Tetramethylen, ferner Pentamethylen stehen; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl bedeutet; und R_3 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

23. Verfahren gemäss Anspruch 1 zur Herstellung einer Verbindung der Formel Ia, worin R_1 C_3-C_5-Alkyl bedeutet; X_1 für CO steht; X_2 für die Gruppe der Formel Ib steht, in der p und r für 0 oder 1 und q für 1 stehen; X_4 C_1-C_4-Alkyl bedeutet; und X_5 Wasserstoff bedeutet; R_2 Carboxy oder C_2-C_5-Alkoxycarbonyl bedeutet; und R_3 5-Tetrazolyl bedeutet, in freier Form oder in Salzform.

24. Verfahren gemäss Anspruch 1 zur Herstellung von (S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, in freier Form oder in Salzform.

25. Verfahren gemäss Anspruch 1 zur Herstellung von N-(2-Carboxy-2,2-tetramethylenethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, in freier Form oder in Salzform.

26. Verfahren gemäss Anspruch 1 zur Herstellung von N-(2-Carboxy-2-ethyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, in freier Form oder in Salzform.

27. Verfahren gemäss Anspruch 1 zur Herstellung von (S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-ethoxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, in freier Form oder in Salzform.

28. Verfahren gemäss Anspruch 1 zur Herstellung von N-(1-carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, in freier Form oder in Salzform.

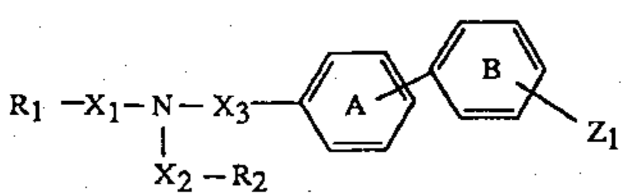
29. Verfahren gemäss Anspruch 1 zur Herstellung von (S)-N-(1-Carboxyethyl)-N-pentanoyl-N- [2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-2-ethyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Hydroxymethyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-2,2-pentamethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-propyloxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-carboxy-2-methyl-propyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-carboxy-2,2-pentamethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-aminocarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin oder
(S)-N-(1-carboxy-2-methyl-prop-1-yl)-N-(5-oxopent-1-en-5-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin, jeweils in freier Form oder in Salzform.

30. Verfahren gemäss Anspruch 1 zur Herstellung von N-Carboxymethyl-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Methoxycarbonylethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[1-Carboxy-2-(4-fluorphenyl)-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[2-(4-Fluorphenyl)-1-methoxycarbonyl-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[2-(4-Fluorphenyl)-1-hydroxymethyl-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2'-Carboxybiphenyl-4-ylmethyl)-N-[1-carboxy-2-(4-fluorphenyl)-ethyl]-N-pentanoyl-amin,
N-(2'-Carboxybiphenyl-4-ylmethyl)-N-[2-(4-fluorphenyl)-1-methoxycarbonyl-ethyl]-N-pentanoyl-amin,
(S)-N-(2'-Carboxybiphenyl-4-ylmethyl)-N-(1-hydroxymethyl-2-phenyl-ethyl)-N-pentanoyl-amin,
(S)-N-(2'-Carboxybiphenyl-4-ylmethyl)-N-(1-hydroxymethyl-2-imidazol-4-yl-ethyl)-N-pentanoyl-amin,
(R)-N-(1-Carboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(1S),(2S)-N-(1-Carboxy-2-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(1S),(2S)-N-(1-Methoxycarbonyl-2-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxybut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Methoxycarbonylbut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxyethyl)-N-hexanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-Butanoyl-N-(1-carboxyethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-cyclohexyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Cyclohexyl-1-methoxycarbonyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(R)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Methoxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Benzyloxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Methoxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Benzyloxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Hydroxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Methoxycarbonyl-1-methyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Carboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Carboxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxy-1-methyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,

N-(5-Hydroxypent-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxyprop-2-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Ethoxycarbonyl-3-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Carboxy-3-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Phenoxyprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[2-(4-Hydroxyphenyl)ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[3-(4-Hydroxyphenyl)prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(8-Hydroxyoct-1-yl)-N-pentanoyl-N- [2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Methansulfonylaminoethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Acetylaminoprop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Methoxy-2-oxo-1-phenyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(4-Hydroxybut-2-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxy-1-phenyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[3-(4-Hydroxybenzylcarbonylamino)prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl1)biphenyl-4-ylmeth yl]-amin,
N-(3-Ethoxycarbonylcyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(3-Carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]amin,
cis-N-(4-Carboxycyclohexyl)-N-pentanoyl-N- [2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
cis-N-(2-Ethoxycarbonylcyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
cis-N-(2-Carboxycyclohexyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-{2-[2-(4-Hydroxyphenyl)ethylaminocarbonyl]-2,2-tetramethylen-ethyl}-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-{1-[2-(4-Hydroxyphenyl)ethylaminocarbonyl]-2-methyl-prop-1-yl}-N-pentanoyl-N-[2'-(1H-tetrazol-5 -yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2,2-dimethyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Methoxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(4-Phenoxybut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxy-1-phenyl-2-oxo-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Benzyloxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Butanoyl-N-(1-carboxy-1-methyl-ethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N- (4-Hydroxybut-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Benzyloxycarbonyl-2-methyl-prop-1-yl)-N-[3-bromo-2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-N -pentanoyl-amin,
(S)-N-[3-Brom-2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-amin,
N-(2-Acetylaminoethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-[2-(n-Butoxycarbonyl)-2,2-tetramethylen-ethyl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethy l]-amin,
N-(2-Benzylaminocarbonyl-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylme thyl]-amin,
(S)-N-Butyloxycarbonyl-N-(1-Carboxy-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-methoxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Diethylaminocarbonyl-2,2-tetramethylen-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylme thyl]-amin,
N-(2-Methyl-2-morpholin-4-ylcarbonyl-propyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxycyclopentyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxy-1-ethyl-prop-1-yl)-N-pentanoyl-N- [2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(5-Amino-1-carboxy-pent-1-yl)-N-pentanoyl-N- [2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Butansulfonyl-N-(2-ethoxycarbonyl-2,2-pentamethylen-ethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmet hyl]-amin,
N-Butansulfonyl-N-(2-carboxy-2,2-pentamethylen-ethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-am in,
N-Butansulfonyl-N-(2-ethoxycarbonyl-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-a

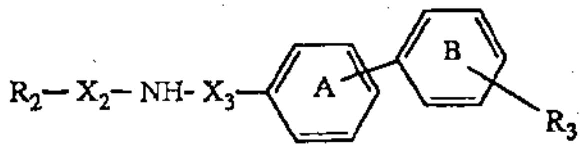
min,
N-Butansulfonyl-N-(2-carboxy-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-Butansulfonyl-N-(1-tert.-butoxycarbonylethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-Butansulfonyl-N-(1-carboxyethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-Butansulfonyl-N-(1-carboxy-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Methyl-1-methylaminocarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmet hyl]-amin,
(S)-N-(1-Dimethylaminocarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylm ethyl]-amin,
(S)-N-(2-Methyl-1-morpholin-4-ylcarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylm ethyl]-amin,
(S)-N-(2'-Carboxybiphenyl-4-ylmethyl)-N-(1-carboxy-2-methyl-prop-1-yl)-N-pentanoyl-amin,
(S)-N-(1,2-Dicarboxyethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-3-phenyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Cyclohexyl-1-hydroxymethyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-am in,
(R)-N-(1-Methoxycarbonyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin;
(S)-N-(2-Hydroxy-1-methoxycarbonyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-am in,
N-Pentanoyl-N-(1H-tetrazol-5-ylmethyl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Pentanoyl-N-pyrid-3-ylmethyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-4-guanidino-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxy-1-methoxycarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-am in,
N-(1-Benzyloxycarbonyl-1-methyl-ethyl)-N-butanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-3-methyl-but-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-(1-Carboxy-2-hydroxy-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-hydroxy-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-[2-Methyl-1-(2-phenylethylaminocarbonyl)-prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl -4-ylmethyl]-amin,
(S)-N-(2-Benzyloxy-1-hydroxymethyl-ethyl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-am in,
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-3-ylmethyl]-amin,
(S)-N-(1-Carboxy-2-methyl-prop-1-yl)-N-pentanoyl-N-[3'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
(S)-N-[2-Methyl-1-(1,2,3,4-tetrahydrochinol-1-ylcarbonyl)-prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl) biphenyl-4-ylmethyl]-amin,
(S)-N-(2-Methyl-1-piperidin-1-ylcarbonyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmet hyl]-amin,
(S)-N-[2-Methyl-1-(1,2,3,4-tetrahydroisochinol-2-ylcarbonyl)-prop-1-yl]-N-pentanoyl-N-[2'-(1H-tetrazol-5 -yl)biphenyl-4-ylmethyl]-amin,
N-(2-Hydroxymethyl-2-methyl-prop-1-yl)-N-pentanoyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
N-Ethoxycarbonyl-N-(2-ethoxycarbonyl-2-methyl-prop-1-yl)-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin oder
N-(2-Carboxy-2-methyl-prop-1-yl)-N-ethoxycarbonyl-N-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]-amin,
jeweils in freier Form oder in Salzform.

31. Verfahren zur Herstellung eines pharmazeutischen Präparats, dadurch gekennzeichnet, dass man
a) in einer Verbindung der Formel

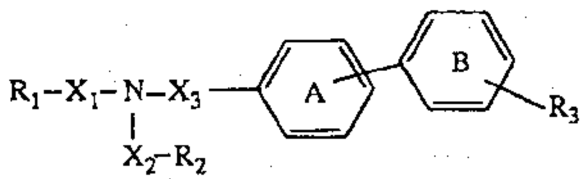

(II)

oder einem Salz davon, worin Z_1 einen in R_3 überführbaren Rest bedeutet, Z_1 in R_3 überführt oder

b) eine Verbindung der Formel R_1-X_{10}H (IIIa), ein reaktionsfähiges Derivat davon oder ein Salz davon mit einer Verbindung der Formel


(IIIb)

oder einem Salz davon umsetzt und jeweils, wenn erwünscht, eine verfahrensgemäss oder auf andere Weise erhältliche Verbindung I in freier Form oder in pharmazeutisch verwendbarer Salzform in eine andere Verbindung I überführt, ein verfahrensgemäss erhältliches Gemisch von Isomeren auftrennt und das gewünschte Isomere isoliert und/oder eine verfahrensgemäss erhältliche freie Verbindung I in ein pharmazeutisch verwendbares Salz oder ein verfahrensgemäss erhältliches pharmazeutisch verwendbares Salz einer Verbindung I in die freie Verbindung I oder in ein anderes pharmazeutisch verwendbares Salz überführt und eine auf diese Weise erhaltene Verbindung der Formel


(I),

worin R_1 einen gegebenenfalls durch Halogen oder Hydroxy substituierten aliphatischen Kohlenwasserstoffrest oder einen cycloaliphatischen oder araliphatischen Kohlenwasserstoffrest bedeutet; X_1 für CO, SO_2 oder -O-C(=O)-, wobei das Kohlenstoffatom der Carbonylgruppe an das in der Formel I eingezeichnete Stickstoffatom gebunden ist, steht; X_2 einen gegebenenfalls durch Hydroxy, Carboxy, Amino, Guanidino, einen cycloaliphatischen oder aromatischen Rest substituierten zweiwertigen aliphatischen Kohlenwasserstoffrest oder einen zweiwertigen cycloaliphatischen Kohlenwasserstoffrest bedeutet, wobei ein Kohlenstoffatom des aliphatischen Kohlenwasserstoffrestes zusätzlich durch einen zweiwertigen aliphatischen Kohlenwasserstoffrest überbrückt sein kann; R_2 gegebenenfalls verestertes oder amidiertes Carboxy, gegebenenfalls substituiertes Amino, gegebenenfalls acetalisiertes Formyl, 1H-Tetrazol-5-yl, Pyridyl, gegebenenfalls verethertes Hydroxy, $S(O)_m$-R, wobei m für 0, 1 oder 2 steht und R Wasserstoff oder einen aliphatischen Kohlenwasserstoffrest bedeutet, Alkanoyl, gegebenenfalls N-substituiertes Sulfamoyl oder PO_nH_2 bedeutet, wobei n für 2 oder 3 steht; X_3 einen zweiwertigen aliphatischen Kohlenwasserstoff bedeutet; R_3 Carboxy, 5-Tetrazolyl, SO_3H, PO_2H_2, PO_3H_2 oder Halogenalkylsulfamoyl ist; und die Ringe A und B unabhängig voneinander gegebenenfalls substituiert sind; in freier Form oder in pharmazeutisch verwendbarer Salzform, gegebenenfalls unter Beimischung von üblichen pharmazeutischen Hilfsstoffen, zu einem pharmazeutischen Präparat verarbeitet.

32. Verfahren zur Herstellung eines pharmazeutischen Präparats, dadurch gekennzeichnet, dass man eine Verbindung, erhältlich gemäss einem der Ansprüche 1 bis 30, in freier Form oder in Form eines pharmazeutisch verwendbaren Salzes, gegebenenfalls unter Beimischung von üblichen pharmazeutischen Hilfsstoffen, zu einem pharmazeutischen Präparat verarbeitet.

33. Verfahren gemäss Anspruch 31 oder 32 zur Herstellung eines antihypertensiv wirksamen pharmazeutischen Präparats, dadurch gekennzeichnet, dass man einen antihypertensiv wirksamen Wirkstoff wählt.

34. Verwendung einer Verbindung, erhältlich gemäss einem der Ansprüche 1 bis 30, in freier Form oder in Form eines pharmazeutisch verwendbaren Salzes, zur Herstellung eines pharmazeutischen Präparats.

35. Verwendung einer Verbindung, erhältlich gemäss einem der Ansprüche 1 bis 30, in freier Form oder in Form eines pharmazeutisch verwendbaren Salzes, zur Herstellung eines pharmazeutischen Präparats auf nichtchemischem Wege.

36. Verwendung einer Verbindung gemäss Anspruch 34 oder 35 zur Herstellung eines Antihypertensivums.

Europäisches Patentamt

EUROPÄISCHER RECHERCHENBERICHT

Nummer der Anmeldung

EP 91810098.3

EINSCHLÄGIGE DOKUMENTE			
Kategorie	Kennzeichnung des Dokuments mit Angabe, soweit erforderlich, der maßgeblichen Teile	Betrifft Anspruch	KLASSIFIKATION DER ANMELDUNG (Int Cl 5)
A	EP - A2 - 0 253 310 (E.I. DU PONT DE NEMOURS) * Zusammenfassung; Beispiele 85-182; Ansprüche; Seiten 277-288 * --	1,11, 31-40	C 07 D 257/04 C 07 C 233/47 C 07 C 231/00 C 07 D 233/64 A 61 K 31/41 A 61 K 31/195
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			RECHERCHIERTE SACHGEBIETE (Int Cl 5)
			C 07 D 257/00 C 07 C 233/00 C 07 C 231/00 C 07 D 233/00

Der vorliegende Recherchenbericht wurde für alle Patentansprüche erstellt.

Recherchenort	Abschlußdatum der Recherche	Prüfer
WIEN	03-06-1991	KÖRBER

KATEGORIE DER GENANNTEN DOKUMENTEN

X : von besonderer Bedeutung allein betrachtet
Y : von besonderer Bedeutung in Verbindung mit einer anderen Veröffentlichung derselben Kategorie
A : technologischer Hintergrund
O : nichtschriftliche Offenbarung
P : Zwischenliteratur
T : der Erfindung zugrunde liegende Theorien oder Grundsätze
E : älteres Patentdokument, das jedoch erst am oder nach dem Anmeldedatum veröffentlicht worden ist
D : in der Anmeldung angeführtes Dokument
L : aus andern Gründen angeführtes Dokument
& : Mitglied der gleichen Patentfamilie, übereinstimmendes Dokument

EPA Form 1503 03 82

AQ

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(54) **Beta-mercapto-propanamide derivatives useful in the treatment of cardiovascular diseases**

Beta-mercapto-propanamidderivate verwendbar zur Behandlung kardiovaskularer Krankheiten oder Erkrankungen

Dérivés des propanamid-bêta mercapto utiles dans le traitement des maladies du système cardiovasculaire

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WO-A-93/09101

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Description

The present invention relates to β-mercapto-propanamide derivatives useful in the treatment of cardiovascular diseases and, more particularly, it relates to N-heteroaryl substituted β-mercapto-propanamide derivatives useful in the treatment of cardiovascular diseases as inhibitors of the metabolism of vasoactive peptides.

The pharmacologic interest towards the study of molecules which inhibit the metabolism of vasoactive peptides derives from the role that said peptides exert on the cardiocirculatory system.

For instance, among the inhibitors of the metabolism of vasoactive peptides, the so-called NEP-inhibitors and ECE-inhibitors hold particular interest.

In particular, NEP-inhibitors are able to inhibit neutral endopeptidase enzyme (NEP), also called enkephalinase, which is responsible for the inactivation, not only of endogenous enkephaline, but also of atrial natriuretic factor (ANF), a vasodilator hormone secreted by heart.

ECE-inhibitors, instead, are able to inhibit endothelin converting enzyme (ECE), which is responsible for the transformation of big-endothelin into endothelin, a 21 amino acid peptide with vasoconstrictor activity.

Therefore, both ECE-inhibitors and NEP-inhibitors are useful in therapy in the treatment of hypertension, renal failure and congestive heart failure.

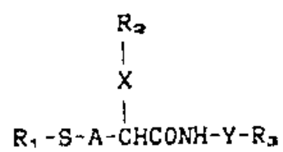
The molecule which is considered the parent of ECE-inhibitors is phosphoramidon [N-[N-[[(6-deoxy-α-L-mannopyranosyl)oxy]hydroxyphosphinyl]-L-leucyl]-L-tryptophan], first isolated as microbial metabolite [Umezawa et al., Tetrahedron Letters, No. 1, pages 97-100, (1972)] and subsequently studied as inhibitor of the metabolism of vasoactive peptides [see, for instance, Matsumura et al., European Journal of Pharmacology, 185 (1990), 103-106].

The molecule which is considered the parent of NEP-inhibitors is thiorphan [DL-(3-mercapto-2-benzylpropanoyl) glycine], first described by Roques et al. in Nature, Vol. 288, pages 286-288, (1980).

Several molecules with NEP-inhibitory activity, other than thiorphan, are described in the literature.

Some of them are chemically related to the structure of β-mercapto-propanamides.

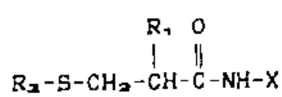
The International patent application No. WO 93/09101 (Fujisawa Pharmaceutical Co. Ltd.) describes β-mercapto-propanamides of formula



wherein R_1 is hydrogen or a protecting group; R_2 is a lower alkyl or a phenyl optionally substituted by a lower alkylenedioxy; R_3 is tetrazolyl, thiazolyl or thiadiazolyl optionally substituted by acyl or acyl-lower alkyl groups; A is a lower alkylene; X is a lower alkylene or S and Y is a single bond or a lower alkylene.

These compounds are NEP-inhibitors.

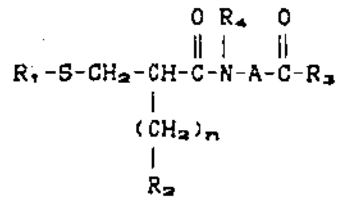
The European patent application No. 0361365 (E. R. Squibb & Sons, Inc.) describes β-mercapto-propanamides of formula



wherein R_1 is, among others, hydrogen, alkyl, haloalkyl, aryl or arylalkyl; X is a phenyl or a cyclohexyl, substituted in 3 or 4 by a $COOR_2$ group; R_2 is hydrogen, alkyl, benzyl, benzhydryl, etc.; R_3 is hydrogen or acyl.

These compounds are NEP-inhibitors.

The European patent application No. 0364767 (Schering Corporation) describes β-mercapto-propanamides of formula

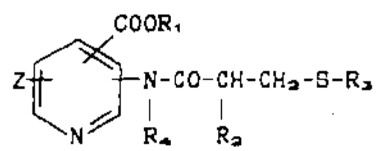


wherein R_1 is hydrogen or acyl; R_2 is aryl or heteroaryl; $-COR_3$ is a carboxylic, ester or amide residue; n is 0-3; R_4 is hydrogen, alkyl or arylalkyl and A is a group selected among optionally substituted phenyl, naphthyl, diphenyl, phenoxyphenyl, phenylthiophenyl, phenylmethylphenyl and pyridyl.

These compounds are able to potentiate the anti-hypertensive and natriuretic action of endogenous ANF and are useful in the treatment of congestive heart failure and of hypertension.

Other examples of the compounds known in the literature, which are structurally related to the class of β-mercapto-propanamides, do not present instead an activity on the cardiocirculatory system, but in general on the central nervous system.

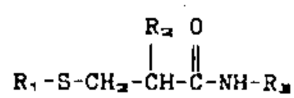
The European patent N. 0110484 (SIMES Società Italiana Medicinali e Sintetici S.p.A., now Zambon Group S.p.A.) describes, among others, β-mercapto-propanamides of formula



wherein Z is hydrogen, alkyl, halogen, alkoxy; R_1 is hydrogen, alkyl, arylalkyl, aryl; R_2 is hydrogen, alkyl, arylalkyl; R_3 is hydrogen or acyl; R_4 is hydrogen or alkyl.

These compounds are useful as analgesics, anti-hypertensives, for the treatment of drug addiction and of psychological disturbances. The European patent application No. 0136883 (E.R. Squibb & Sons, Inc.) describes mercapto-alkanoyl and acylmercaptoalkanoyl compounds which possess enkephalinase inhibition activity and are useful as analgesic agents.

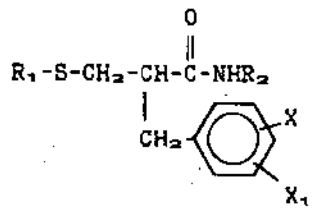
The European patent application N. 0115997 (Roussel-Uclaf) describes, among others, β-mercapto-propanamides of formula



wherein R_1 is hydrogen or acyl; R_2 is, among others, hydrogen, optionally substituted alkyl, aryl or arylalkyl; R_3 is a heterocycle selected among thiazolyl, 4,5-dihydrothiazolyl, pyridyl, oxazolyl, isoxazolyl, imidazolyl, pirimidyl, tetrazolyl, benzimidazolyl, benzothiazolyl or benzoxazolyl optionally substituted by alkyl or R_3 is a phenyl optionally substituted by a radical selected among alkyl, alkoxy, hydroxy, nitro, halogen, trifluoromethyl, carboxymethyl, alkoxycarbonylmethyl, arylalkoxy, amino, monoalkylamino, dialkylamino.

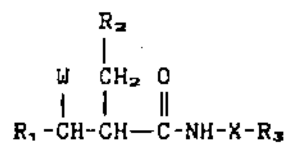
These compounds are useful as analgesics.

The European patent application N. 0280627 (Roussel-Uclaf) describes α-mercaptomethyl-benzenepropanamides of formula



wherein R_1 is hydrogen or acyl; X and X_1 are hydrogen, alkyl, alkoxy, hydroxy, halogen or trifluoromethyl; R_2 is pyrrolidinyl, morpholinyl, piperidinyl, piperazinyl, tetrahydrothiazinyl or hexahydroazepinyl optionally substituted by one or more alkyl, alkoxy, hydroxy, nitro, trifluoromethyl, acyl groups and halogen. These compounds are endowed with analgesic, psychotropic, antidepressant and anxiolythic activity.

The European patent application N. 0318859 (Dainippon Pharmaceutical Co. Ltd.) describes β-mercapto-propanamides of formula

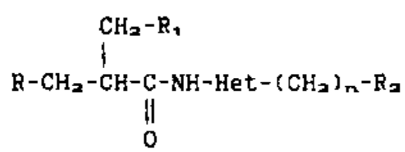


wherein R_1 is a SH group or a biological precursor thereof; W is hydrogen, alkyl or arylalkyl; R_2 is aryl, heterocycle or alkyl, optionally substituted; X is a cycloalkylene, cycloalkylidene or a phenylene, optionally substituted or fused with another ring; R_3 is a carboxyl or a biological precursor thereof.

These compounds are useful as analgesics.

We have now found β-mercapto-propanamides derivatives N-substituted by a 5 membered heterocycle which are endowed with a remarkable NEP-inhibitory activity and ECE-inhibitory activity.

Therefore, object of the present invention are β-mercapto-propanamides of formula



(I)

wherein

R is a mercapto group or an R_3COS group convertible into the organism to the mercapto group; R_3 is a C_1-C_4 alkyl group;

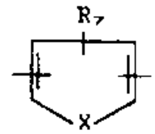
R_1 is a hydrogen atom, a phenyl group or a 5 or 6 membered heterocycle containing 1 or 2 heteroatoms selected among nitrogen, oxygen and sulphur, optionally substituted by one or two groups selected among C_1-C_4 alkyl or alkoxy groups, hydroxy, halogen and trifluoromethyl groups;

R_2 is a carboxylic group or a $COOR_4$ or

group convertible into the organism to the carboxylic group; R_4 is a C_1-C_4 alkyl group or a phenylalkyl having from 1 to 4 carbon atoms in the alkyl moiety; R_5 and R_6, the same or different, are hydrogen atoms, C_1-C_4 alkyl or C_6-C_7 cycloalkyl groups;

n is 0 or 1;

Het is a 5-membered heterocycle of formula



wherein X is an oxygen or sulphur atom or an NH group; R_7 is a hydrogen atom, a C_1-C_4 alkyl group or a phenyl optionally substituted by C_1-C_4 alkoxy groups;

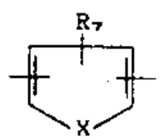
and their pharmaceutically acceptable salts.

The compounds of formula I have at least an asymmetric carbon atom and may therefore exist in the form of stereoisomers. The compounds of formula I in the form of stereoisomeric mixture as well as in the form of single stereoisomers are object of the present invention.

The compounds of formula I are endowed with both NEP-inhibitory and ECE-inhibitory activity and are useful in the treatment of cardiovascular diseases such as hypertension, renal failure and congestive heart failure.

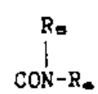
In the present description, unless otherwise specified, with the term C_1-C_4 alkyl we intend a straight or branched C_1-C_4 alkyl such as methyl, ethyl, n.propyl, isopropyl, n.butyl, isobutyl, sec.butyl and t.butyl; with the term C_5-C_7 cycloalkyl we intend cyclopentyl, cyclohexyl and cycloheptyl; with the term C_1-C_4 alkoxy we intend a straight or branched C_1-C_4 alkoxy such as methoxy, ethoxy, n.propoxy, isopropoxy, n.butoxy, isobutoxy, sec.butoxy and t.butoxy. With the term 5- or 6-membered heterocycle containing 1 or 2 heteroatoms selected among nitrogen, oxygen and sulphur we intend a heterocycle preferably selected among thiazole, oxazole, isothiazole, isoxazole, pyrazole, imidazole, thiophene, pyrrole and pyridine. Preferred compounds are the compounds of formula I wherein R is a mercapto group or an R_3COS group wherein R_3 is methyl; R_2 is a carboxylic group.

Still more preferred compounds are the compounds of formula I wherein R is a mercapto group or an R_3COS group wherein R_3 is methyl; R_2 is a carboxylic group; R_1 is phenyl or pyridyl, optionally substituted by a C_1-C_4 alkyl or alkoxy group or by a halogen atom and Het is a heterocycle of formula



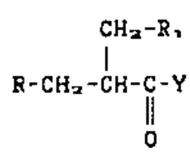
wherein X is an oxygen or sulphur atom or an NH group and R_7 is a hydrogen atom.

It is evident that the compounds of formula I, wherein R is an R_3COS group convertible into the organism to the mercapto group or R_2 is a $COOR_4$ or



group, convertible into the organism to the carboxylic group, are biological precursors (pro-drugs) of the corresponding compounds of formula I wherein R is a mercapto group (R=SH) and R_2 is a carboxylic group (R_2=COOH).

The preparation of the compounds of formula I, object of the present invention, is carried out by reacting a derivative of the β-mercapto-propionic acid of formula



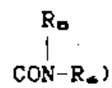
(II)

wherein R and R_1 have the above reported meanings and Y is a halogen atom, preferably chlorine or bromine; and a compound of formula

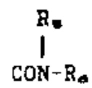
$$H_2N\text{-Het-}(CH_2)_n\text{-}R_2 \qquad (III)$$

wherein R_2, Het and n have the above reported meanings;
in a suitable solvent, in the presence of a base; followed by optional hydrolysis.

Preferably the intermediates of formula II and III are used in a protected form ($R=R_3COS$ and $R_2=COOR_4$ or

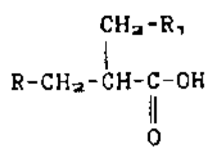


affording thus the corresponding compounds of formula I wherein $R=R_3COS$ and $R_2=COOR_4$ or



from which, by hydrolysis, the compounds of formula I wherein R=SH and R_2=COOH are obtained.

The compounds of formula II are known or easily prepared according to conventional methods (see for instance the British patent n. 1576161 in the name of Squibb E.R. & Sons Inc.) from the corresponding acids of formula



(IV)

wherein R and R_1 have the above reported meanings.

Also the intermediates of formula III are known or easily prepared with known methods.

For a bibliographic reference to the preparation of the compounds of formula III see for instance Michel Sy et al., Bull. Soc. Chim. Fr., 1276-1277, (1963) and Moses Lee et al., J. Org. Chem., 53, No. 9, 1855-1859, (1988).

The compounds of formula I in the form of single stereoisomers are prepared by stereoselective synthesis or by separation of the stereoisomeric mixture according to conventional techniques.

The compounds of formula I are active as NEP-inhibitors and ECE-inhibitors and are useful in the treatment of cardiovascular diseases such as hypertension, renal failure and congestive heart failure. The NEP-inhibitory activity of the compounds of formula I was evaluated by means of in vitro tests as percentage of inhibition in the formation of [^{3}H]-Tyr-Gly-Gly, a metabolite of [^{3}H][Leu^5]-enkephaline (see example 26).

The inhibitory activity, expressed as IC_{50} (nM), of the compounds of formula I resulted to be substantially comparable with that of the reference compounds.

Thiorphan, the compound N-(3-carboxyphenyl)-3-mercapto-2-benzyl-propanamide, described in the aforementioned European patent application No. 0361365 (E.R. Squibb & Sons, Inc.) and the compound N-(4-carboxymethyl-2-thiazolyl)-3-mercapto-2-benzyl-propanamide, described in the aforementioned International patent application No. WO 93/09101 (Fujisawa Pharmaceutical Co. Ltd.) were used as reference compounds (see table 1).

The ECE-inhibitory activity of the compounds of formula I was evaluated by means of in vitro tests for the inhibition of endothelin formation and resulted to be significantly greater than that of phosphoramidon (see example 26).

For the practical use in therapy the compounds of formula I can be formulated in solid or liquid pharmaceutical compositions, suitable for oral or parenteral administration.

Therefore the pharmaceutical compositions containing one or more compounds of formula I, as active ingredient, in admixture with a carrier for pharmaceutical use are a further object of the present invention.

Specific examples of the pharmaceutical compositions according to the present invention are tablets, coated tablets, capsules, granulates, solutions and suspensions suitable for oral administration, solutions and suspensions suitable for parenteral administration.

The pharmaceutical compositions object of the present invention may contain one or more compounds of formula I in association with other active ingredients such as for instance ACE-inhibitors. The pharmaceutical compositions object of the present invention are prepared according to conventional techniques.

The daily dose of compound of formula I will depend on different factors such as the seriousness of the disease, the individual response of the patient, the use of biological precursors and the kind of formulation but it is usually comprised between 0.1 mg and 50 mg per Kg of body weight in a single dose or divided into more daily doses.

With the aim of better illustrating the present invention the following examples are now given.

Example 1

Preparation of N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-benzylpropanamide (compound 1)

3-Acetylthio-2-benzyl-propionic acid (2.9 g; 12 mmoles) and dimethylformamide (3 drops) were dissolved in thionyl chloride (3 ml).

After 16 hours at room temperature the solvent was evaporated under vacuum and the residue was collected twice with toluene (10 ml), evaporating to dryness each time.

The obtained oil was dissolved in toluene (30 ml) and the solution was cooled with ice. Then a solution of 4-amino-2-ethoxycarbonyl-thiophene (1.8 g; 10.5 mmoles) and triethylamine (1.69 ml) in toluene (37 ml) was added dropwise.

After 5 hours under stirring at room temperature, the reaction mixture was diluted with water (30 ml) and extracted with ethyl acetate.

The organic phase was dried on sodium sulphate and the solvent was evaporated under vacuum.

The oil was purified by chromatography (silica gel, eluent n.hexane: ethyl acetate=7:3) affording N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-benzyl-propanamide (1.4 g; 32.2% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.35 (t, 3H); 2.32 (s, 3H); 2.68 (m, 1H); 2.85-3.30 (m, 4H); 4.32 (q, 2H); 7.20 (m, 5H); 7.46 (d, 1H); 7.69 (d, 1H).

Example 2

Preparation of N-(2-carboxy-4-thienyl)-3-mercapto-2-benzyl-propanamide (compound 2)

A solution of N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-benzyl-propanamide (1.35 g; 34 mmoles), prepared as described in example 1, and sodium hydroxide (0.407 g; 10.2 mmoles) in water (5.76 ml) and methanol (14 ml) was kept under stirring for 16 hours at 20°C under nitrogen.

Methanol was evaporated under vacuum and the mixture was acidified with diluted hydrochloric acid to pH about 4.

After extraction with ethyl acetate, the organic phase was washed with water and dried on sodium sulphate.

By evaporating the solvent under vacuum an oil was obtained which crystallizes from methylene chloride:hexane=1:9, affording N-(2-carboxy-4-thienyl)-3-mercapto-2-benzyl-propanamide (0.43 g; 39.4% yield).

m.p. 174-177°C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.32 (t, 1H); 2.53-2.92 (m, 5H); 7.11-7.30 (m, 5H); 7.62 (d, 1H); 7.70 (d, 1H).

Example 3

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-benzyl-propanamide (compound 3)

By working in a way similar to that described in example 1 but substituting 4-amino-2-ethoxycarbonyl-thiophene with 4-amino-2-ethoxycarbonyl-pyrrole, N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2--benzyl-propanamide was obtained (55.6% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.30 (t, 3H); 2.32 (s, 3H); 2.66 (m, 1H); 2.80-3.30 (m, 4H); 4.27 (q, 2H); 6.52 (dd, 1H); 7.22 (m, 5H); 7.37 (dd, 1H).

Example 4

Preparation of N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-benzylpropanamide (compound 4)

By working in a way similar to that described in example 2, after chromatography on silica gel (eluent CH_2Cl_2:CH_3OH:CH_3COOH=90:10:1) and crystallization from CH_2Cl_2:hexane=1:2, N-(2-carboxy-4-pyrrolyl)-3-mercapto-

2-benzyl-propanamide (4.93 g; 46.3% yield) white crystalline solid was obtained.

m.p. 169-172°C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.22 (t, 1H); 2.55-2.94 (m, 5H); 6.56 (dd, 1H); 7.11-7.30 (m, 6H); 9.84 (bs, 1H); 11.41 (bs, 1H).

Example 5

Preparation of ethyl 2-ethoxycarbonyl-3-(3-pyridyl)-propionate

Diethyl malonate (10.176 ml; 67.1 mmoles) was added dropwise to a solution obtained by dissolving metallic sodium (1.543 g; 67.1 mmoles) in anhydrous ethanol (20 ml) heated at 50°C.

The solution was kept under stirring at 50°C for 30 minutes and then cooled at room temperature.

3-Chloromethyl-pyridine (5 g; 39.2 mmoles) was added dropwise and the reaction mixture was heated under reflux for 90 minutes.

After evaporating the mixture under vacuum, the residue was collected with ethyl acetate and evaporated to dryness.

The obtained crude was purified by silica gel chromatography (eluent hexane:ethyl acetate=1:1) affording ethyl 2-ethoxycarbonyl-3-(3-pyridyl)-propionate (4.83 g; 49% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.18 (t, 6H); 3.19 (d, 2H); 3.60 (t, 1H); 4.13 (q, 4H); 7.12-7.21 (m, 1H); 7.51 (dt, 1H); 8.41-8.47 (m, 2H).

Example 6

Preparation of 2-carboxy-3-(3-pyridyl)-propionic acid

A solution of potassium hydroxide at 85% (96.8 g; 1.47 moles) in water (300 ml) was added to a solution of ethyl 2-ethoxycarbonyl-3--(3-pyridyl)-propionate (168 g; 0.668 moles), prepared as described in example 5, in dioxane (1680 ml).

The reaction mixture was kept under stirring at room temperature for 4 hours.

The reaction mixture was then neutralized by adding hydrochloric acid 12 N (122.5 ml) and evaporated to dryness under vacuum.

The residue was collected with ethanol (4x750 ml) and the mixture was kept at boiling temperature before filtering off the precipitate.

The solution was evaporated to dryness under vacuum and a crude product (128 g) was obtained which, crystallized from ethanol (1000 ml), afforded 2-carboxy-3-(3-pyridyl)-propionic acid (93.5 g; 72% yield).

m.p. 128-129°C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 3.40 (d, 2H); 3.64 (t, 1H); 7.26-7.33 (m, 1H); 7.67 (dt, 1H); 8.37-8.43 (m, 2H).

Example 7

Preparation of 2-(3-pyridylmethyl)-propenoic acid

An aqueous solution 7.9 N of dimethylamine (2.28 ml; 0.018 moles) was added at 10°C to 2-carboxy-3-(3-pyridyl)-propionic acid (3.5 g; 0.018 moles), prepared as described in example 6.

The reaction mixture was cooled at 0°C and formaldehyde (1.48 g; 0.018 moles) was added dropwise.

At the end, the reaction mixture was kept under stirring at room temperature overnight.

By evaporating to dryness under vacuum and by heating the obtained residue at 125°C under vacuum for 4 hours, a crude was obtained which, chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH:CH_3COOH= 90:10:1), afforded 2-(3-pyridylmethyl)-propenoic acid (1.8 g; 61.3% yield).

m.p. 101-102°C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 3.58 (s, 2H); 5.62 (s, 1H); 6.15 (s, 1H); 7.25-7.38 (m, 1H); 7.60 (dt, 1H); 8.42 (m, 2H).

Example 8

Preparation of 3-acetylthio-2-(3-pyridylmethyl)-propionic acid

A mixture of 2-(3-pyridylmethyl)-propenoic acid (10 g; 0.061 moles), prepared as described in example 7, and thioacetic acid (4.56 ml; 0.064 moles) was heated at 100°C for 1 hour.

The reaction mixture was then evaporated to dryness under vacuum and the residue was purified by silica gel chromatography (eluent CH_2Cl_2:CH_3OH:CH_3COOH=95:5:0.5) obtaining oily 3-acetylthio-2-(3-pyridylmethyl)-propionic acid (10.5 g; 72% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.17 (s, 3H); 2.37-2.57 (m, 5H); 6.66 (dd, 1H); 6.83 (dt, 1H); 8.19 (d, 2H).

Example 9

Preparation of N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (compound 5)

A solution of 3-acetylthio-2-(3-pyridylmethyl)-propionic acid (1 g; 4.2 mmoles), prepared as described in example 8, in thionyl chloride (5 ml) and in the presence of dimethylformamide (1 drop) was left at room temperature for 12 hours.

Said mixture was diluted with pyridine (10 ml) and added dropwise to a solution of 4-amino-2-ethoxycarbonylthiophene (0.65 g; 3.78 mmoles) in pyridine (5 ml).

After 3 hours at room temperature the reaction mixture was evaporated to dryness under vacuum and the residue was collected with water (20 ml) and extracted with ethyl acetate (3x20 ml).

The collected organic phases were dried on sodium sulphate and evaporated to dryness under vacuum.

The obtained crude was chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH=95:5) obtaining an oil which, collected with ethyl ether and filtered, afforded N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (0.57 g; 38.5% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.33 (t, 3H); 2.33 (s, 3H); 2.72-3.27 (m, 5H); 4.30 (q, 2H); 7.18 (dd, 1H); 7.52 (m, 2H); 7.79 (d, 1H); 8.13 (d, 1H); 8.38 (dd, 1H); 9.58 (s, 1H).

Example 10

Preparation of N-(2-carboxy-4-thienyl)-3-mercapto-2-(3-pyridylmethyl)-propanamide (compound 6)

A solution of sodium hydroxide 10.8 N (0.437 ml; 0.0047 moles) in water (5 ml) was added to a solution of N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (0.57 g; 1.45 mmoles), prepared as described in example 9, in methanol (10 ml).

The reaction mixture was kept under stirring at room temperature for 12 hours.

At the end, it was evaporated to dryness under vacuum and the residue was collected with water (10 ml) and washed with ethyl acetate.

The aqueous phase was acidified to pH 4 with hydrochloric acid 1 N and subsequently extracted with ethyl acetate.

The organic phase was dried on sodium sulphate and evaporated to dryness under vacuum; the obtained crude was collected with ethyl ether and filtered affording N-(2-carboxy-4-thienyl)-3-mercapto-2--(3-pyridylmethyl)-propanamide (0.1 g; 21.4% yield).

m.p. 115-118°C

Mass (Chemical ionization, isobutane): (M^++H): 323

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.57-2.91 (m, 5H); 7.27 (dd, 1H); 7.52-7.63 (dt, 1H); 7.72 (d, 1H); 8.37 (dd, 2H); 10.39 (s, 1H).

Example 11

Preparation of ethyl 3-(4-chlorophenyl)-2-diethoxyphosphinyl-propionate

Sodium hydride (3.12 g; 0.130 moles) was added dropwise to a solution of ethyl diethoxyphosphinylacetate (37 ml; 0.186 moles) in anhydrous dimethylformamide (150 ml), kept at 0°C under nitrogen atmosphere.

After 3 hours at a temperature of 0-5°C, a solution of 4-chlorobenzyl chloride (20 g; 0.124 moles) in dimethylformamide (90 ml) was added at 0°C.

At the end, the reaction mixture was kept under stirring at room temperature for 48 hours, diluted with water (400 ml) containing concentrate hydrochloric acid (5 ml) and extracted with ethyl acetate (3x50 ml).

The collected organic phases were washed twice with water (50 ml), dried on sodium sulphate and evaporated to dryness under vacuum.

The residue was distilled in Vigreaux column (0.7 mm Hg; 165°C) obtaining oily ethyl 3-(4-chlorophenyl)-2-diethoxyphosphinyl-propionate (19 g; 44% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.13 (t, 3H); 1.33 (t, 6H); 3.05-3.24 (m, 3H); 4.01-4.22 (m, 6H); 7.07-7.23 (m, 4H).

Example 12

Preparation of ethyl 2-(4-chlorobenzyl)-acrylate

Potassium carbonate (10 g; 0.072 moles) was added to a solution of ethyl 3-(4-chlorophenyl)-2-diethoxyphosphinyl-propionate (22 g; 0.065 moles), prepared as described in example 11, in formaldehyde (40 ml).

The reaction mixture was heated under reflux for 4 hours.

At the end, it was diluted with water (100 ml), extracted with ethyl acetate (3x50 ml), dried on sodium sulphate and evaporated to dryness under vacuum.

The obtained crude which was purified by distillation (8 mm Hg; 150°C) afforded ethyl 2-(4-chlorobenzyl)-acrylate (8.45 g; 58% yield) as oil.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.23 (t, 3H); 3.58 (s, 2H); 4.15 (q, 2H); 5.44 (d, 1H); 6.21 (s, 1H); 7.07-7.26 (m, 4H).

Example 13

Preparation of 2-(4-chlorobenzyl)-propenoic acid

A solution of sodium hydroxide 12 N (3.8 ml; 0.0456 moles) was added to a solution of ethyl 2-(4-chlorobenzyl)-acrylate (8.45 g; 0.038 moles), prepared as described in example 12, in methanol (40 ml).

The reaction mixture was kept under stirring at room temperature for 24 hours.

Methanol was evaporated under vacuum and the formed precipitate was collected with water (50 ml); the mixture was acidified to pH 2 with concentrate hydrochloric acid.

By extracting with ethyl acetate (3x30 ml), drying the collected organic phases on sodium sulphate and evaporating to dryness under vacuum, 2-(4-chlorobenzyl)-propenoic acid (6.6 g; 88% yield) was obtained.

m.p. 76-86°C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.78 (s, 2H); 4.79 (d, 1H); 6.06 (s, 1H); 6.59-6.68 (m, 4H).

Example 14

Preparation of 3-acetylthio-2-(4-chlorobenzyl)-propionic acid

By working in a way similar to that described in example 8 and by using 2-(4-chlorobenzyl)-propenoic acid (6.7 g; 0.034 moles), prepared as described in example 13, and thioacetic acid (3.64 ml; 0.051 moles), a crude was obtained which chromatographed on silica gel (eluent ligroin:ethyl acetate=1:1) afforded 3-acetylthio-2-(4--chlorobenzyl)-propionic acid (4.36 g; 47% yield) as oil.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.32 (s, 3H); 2.71-3.10 (m, 5H); 7.08-7.28 (m, 4H)

Example 15

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(4-chlorobenzyl)-propanamide (compound 7)

A solution of 3-acetylthio-2-(4-chlorobenzyl)-propionic acid (4.36 g; 0.016 moles), prepared as described in example 14, in thionyl chloride (5 ml), in the presence of dimethylformamide (2 drops), was kept at room temperature and under nitrogen atmosphere for 24 hours.

After that, the excess of thionyl chloride was removed by azeotropic distillation with toluene.

Said reaction mixture was added dropwise at 0°C and under nitrogen atmosphere to a solution of 4-amino-2-ethoxycarbonyl-pyrrole (2.46 g; 0.016 moles) and triethylamine (1.7 g; 0.017 moles) in toluene (40 ml).

After 3 hours at room temperature the reaction mixture was evaporated under vacuum and the residue was collected with ethyl ether and filtered.

The solid was crystallized from ethyl acetate:ligroin=1:2 and N-(2--ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(4-chlorobenzyl)-propanamide (3.5 g; 53.5% yield) was obtained.

m.p. 141-144°C

^{1}H-NMR (200 MHz, DMSO-d_6) : δ (ppm): 1.25 (t, 3H); 2.29 (s, 3H); 2.69-3.01 (m, 5H); 4.20 (q, 2H); 6.61 (m, 1H); 7.11-7.35 (m, 5H); 9.89 (s, 1H); 11.60 (s, 1H).

Example 16

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-2-(4-chlorobenzyl)-3--mercapto-propanamide (compound 8)

A solution of triethylamine (0.68 ml; 4.89 mmoles) in methanol (10 ml) was added to a solution of N-(2-ethoxycarbonyl-4-pyrrolyl)-3--acetylthio-2-(4-chlorobenzyl)-propanamide (1 g; 2.45 mmoles), prepared as described in example 15, in methanol (20 ml).

The reaction mixture was kept under stirring at room temperature for 3 hours, then it was acidified to pH 3 with acetic acid and diluted with water (20 ml).

After extraction with ethyl acetate (3x30 ml), the collected organic phases were dried on sodium sulphate and evaporated to dryness under vacuum.

The obtained crude was chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH=95:5), further collected with CH_2Cl_2:ligroin=1:1 and filtered affording N-(2-ethoxycarbonyl-4-pyrrolyl)-2-(4-chlorobenzyl)-3-mercapto-propanamide (0.63 g; 70% yield).

m.p. 140-143°C

Mass (Chemical ionization, isobutane): (M^++H): 367

^{1}H-NMR (200 MHz, $CDCl_3$) : δ (ppm): 1.30 (t, 3H); 2.49-3.03 (m, 5H); 4.28 (q, 2H); 6.59 (t, 1H); 7.03-7.24 (m, 5H); 7.36 (t, 1H); 9.09 (bs, 1H).

Example 17

Preparation of N-(2-carboxy-4-pyrrolyl)-2-(4-chlorobenzyl)-3-mercapto-propanamide (compound 9)

By working in a way similar to that described in example 10 and by using N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(4-chlorobenzyl)-propanamide (1 g; 2.45 mmoles), prepared as described in example 15, a crude was obtained which, chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH:CH_3COOH=90:10:1) and further collected with toluene: ligroin=1:1 and filtered, afforded N-(2-carboxy-4-pyrrolyl)-2-(4-chlorobenzyl)-3-mercapto-propanamide (0.5 g; 60.2% yield). Mass (Chemical ionization, isobutane): (M^++H) : 339 ^{1}H-NMR (200 MHz, DMSO-d_6) : δ (ppm): 2.46-2.86 (m, 5H); 6.56 (s, 1H); 7.11-7.32 (m, 5H); 9.73 (s, 1H); 11.38 (bs, 1H).

Example 18

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (compound 10)

N-hydroxysuccinimide (0.962 g; 8.36 mmoles) and dicyclohexylcarbodiimide (1.72 g; 8.36 mmoles) were added to a solution of 3-acetylthio-2-(3-pyridylmethyl)-propionic acid (2 g; 8.36 mmoles), prepared as described in example 8, in dioxane (50 ml).

The reaction mixture was kept under stirring at room temperature for 2 hours.

At the end, the formed precipitate was filtered off and the solution was evaporated to dryness under vacuum.

The residue was collected with chloroform (20 ml) and the solution was filtered and evaporated to dryness; this procedure was repeated twice.

The residue collected again with dioxane (20 ml), was added to a solution of 4-amino-2-ethoxycarbonyl-pyrrole (1.29 g; 8.36 mmoles) in dioxane (20 ml).

The reaction mixture was kept under stirring at room temperature for 16 hours.

After said time, it was diluted with water (40 ml) and extracted with ethyl acetate (3x30 ml).

The collected organic phases were washed twice with water (30 ml) dried on sodium sulphate and evaporated to dryness under vacuum affording a crude which was chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH=95:5).

N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (0.6 g; 19.3% yield) was thus obtained.

Mass (Chemical ionization, isobutane): (H^++H): 376

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.23 (t, 3H); 2.30 (s, 3H); 2.74-3.18 (m, 5H); 4.20 (q, 2H); 6.55 (t, 1H); 7.10-7.18 (dd, 1H); 7.39 (t, 1H); 7.49 (dt, 1H); 8.12 (d, 1H); 8.29 (dd, 1H); 9.49 (s, 1H); 9.71 (bs, 1H).

Example 19

Preparation of N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-(3-pyridylmethyl)-propanamide (compound 11)

A solution of sodium hydroxide (0.131 g; 3.28 mmoles) in water (10 ml) was added to a solution of N-(2-ethoxy-carbonyl-4-pyrrolyl)-3--acetylthio-2-(3-pyridylmethyl)-propanamide (0.56 g; 1.49 mmoles), prepared as described in example 18, in methanol (10 ml).

The reaction mixture was kept under reflux for 6 hours and sodium hydroxide (0.065 g; 1.64 mmoles) was therein added again.

After 12 hours at room temperature, methanol was evaporated and the residue was diluted with water (20 ml) while pH was brought to 7 by adding sodium bicarbonate.

The mixture was evaporated to dryness and by chromatography on silica gel (eluent CH_2Cl_2:CH_3OH:NH_3 79:15:1) a crude was obtained which, collected with chloroform:ethyl ether, afforded N-(2-carboxy--4-pyrrolyl)-3-mercapto-2-(3-pyridylmethyl)-propanamide (80 mg; 17.6% yield).

m.p 85-90°C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.55-2.89 (m, 5H); 6.49 (m, 1H); 7.09 (m, 1H); 7.20-7.30 (dd, 1H); 7.51-7.60 (dd, 1H); 8.36 (d, 2H); 9.82 (s, 1H); 11.23 (bs, 1H).

Example 20

Preparation of ethyl 2-diethoxyphosphinyl-3-(3-methoxyphenyl)-propionate

By working in a way similar to that described in example 11 and by using ethyl diethoxyphosphinylacetate (59 g; 0.26 moles), sodium hydride at 60% (9.33 g; 0.233 moles) and 3-methoxybenzyl chloride (20.62 g; 0.13 moles), ethyl 2-diethoxyphosphinyl-3-(3-methoxyphenyl)-propionate (34 g; 76% yield) was obtained.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.12 (t, 3H); 1.32 (t, 6H); 3.10-3.32 (m, 3H); 3.75 (s, 3H); 4.08-4.22 (m, 6H); 6.69-6.78 (m, 3H); 7.10-7.22 (m, 1H).

Example 21

Preparation of ethyl 2-(3-methoxybenzyl)-acrylate

By working in a way similar to that described in example 12 and by using ethyl 2-diethoxyphosphinyl-3-(3-methoxyphenyl)-propionate (34 g; 0.0987 moles), prepared as described in example 20, ethyl 2-(3-methoxybenzyl)-acrylate (21.5 g; 98.9% yield) was obtained.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.25 (t, 3H); 3.69 (s, 2H); 3.77 (s, 3H); 4.17 (q, 2H); 5.45 (d, 1H); 6.21 (s, 1H); 6.70-6.80 (m, 3H); 7.14-7.23 (m, 1H).

Example 22

Preparation of 2-(3-methoxybenzyl)-propenoic acid

By working in a way similar to that described in example 13 and by using ethyl 2-(3-methoxybenzyl)-acrylate (10 g; 0.0454 moles), prepared as described in example 21, 2-(3-methoxybenzyl)-propenoic acid (7 g; 80.2% yield) was obtained.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 3.59 (s, 2H); 3.78 (s, 3H); 5.58 (d, 1H); 6.37 (s, 1H); 6.72-6.81 (t, 3H); 7.16-7.25 (m, 1H).

Example 23

Preparation of 3-acetylthio-2-(3-methoxybenzyl)-propionic acid

By working in a way similar to that described in example 14 and by using 2-(3-methoxybenzyl)-propenoic acid (6.2 g; 0.0323 moles), prepared as described in example 22, a crude was obtained which, chromatographed on silica gel (eluent hexane:ethyl acetate=1:1), afforded 3-acetylthio-2-(3-methoxybenzyl)-propionic acid (3.5 g; 40.4% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.32 (s, 3H); 2.77-3.13 (m, 5H); 3.78 (s, 3H); 6.65-6.78 (m, 3H); 7.12-7.22 (m, 1H).

Example 24

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3--methoxybenzyl)-propanamide (compound 12)

By working in a way similar to that described in example 15 and by using 3-acetylthio-2-(3-methoxybenzyl)-propionic acid (3.9 g; 0.0145 moles), prepared as described in example 23, thionyl chloride (1.3 ml) and a solution of 4-amino-2-ethoxycarbonyl-pyrrole (2.24 g; 0.0145 moles) in pyridine (200 ml), a crude was obtained which, chromatographed on silica gel (eluent ligroin:ethyl acetate=7:3) and further crystallized from ligroin:ethyl acetate=1:1, afforded N-(2--ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-methoxybenzyl)-pro-panamide (2 g; 34% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.30 (t, 3H); 2.30 (s, 3H); 2.62-3.18 (m, 5H); 3.70 (s, 3H); 4.27 (q, 2H); 6.52 (dd, 1H); 6.65-6.77 (m, 3H); 7.06-7.23 (m 2H); 7.37 (dd, 1H); 8.95 (bs, 1H).

Example 25

Preparation of N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-(3-methoxybenzyl)-propanamide (compound 13)

By working in a way similar to that described in example 17 and by using N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-methoxybenzyl)-propanamide (0.98 g; 2.42 mmoles), prepared as described in example 24, a crude was obtained which, chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH:CH_3COOH:90:10:1) and collected with ligroin:ethyl acetate=1:1 afforded N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-(3-methoxybenzyl)-propanamide (0.520 g; 64.2% yield) as white solid.

m.p. 153-158°C

Mass (Chemical ionization, isobutane): (H^++H): 335

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.46-2.89 (m, 5H); 3.65 (s, 3H); 6.53 (m, 1H); 6.72 (m, 3H); 7.10-7.20 (m, 2H); 9.83 (s, 1H); 11.32 (bs, 1H).

Example 26

pharmacological activity

a) In vitro NEP-inhibitory activity

The NEP-inhibitory activity in vitro was evaluated according to the method reported in the literature by C. Llorens et al., Eur. J. Pharmacol., 69, (1981), 113-116.

Membranes from kidney cortex were prepared according to the following procedure.

By working at 0-4°C the kidneys were removed from killed male Sprague-Dawley rats weighing approximately 300 g.

Cortex was carefully dissected, finely minced and suspended in homogenization buffer (10 mM sodium phosphate pH 7.4 containing 1 mM $MgCl_2$, 30 mM NaCl, 0.02% NaN_3) 1:15 weight/volume.

The tissue was then homogenized for 30 seconds using an Ultra-Turrax homogenizer.

Approximately 10 ml of homogenate were layered over 10 ml of sucrose (41% weight/volume) and centrifuged at 31200 rpm for 30 minutes at 4°C in a fixed angle rotor.

The membranes were collected from the buffer/sucrose interface, washed twice with 50 mM TRIS/HCl buffer (pH 7.4) and resuspended into the same buffer for storage.

The membranes were stored in small aliquots at -80°C until use. The NEP-inhibitory activity was evaluated by using the following method.

Aliquots of the membrane suspension prepared as above described (concentration 5 μg/ml of proteins) were preincubated in the presence of an aminopeptidase inhibitor (Bestatin - 1 mM) for 10 minutes at 30°C.

[^{3}H][Leu^5]-enkephaline (15 nM) and buffer TRIS/HCl pH 7.4 (50 mH) were added in order to obtain a final volume of 100 μl.

Incubation (20 minutes at 30°C) was stopped by adding 0.1 M HCl (100 μl).

The formation of the metabolite [^{3}H]Tyr-Gly-Gly was quantified by chromatography on polystyrene columns (Porapak Q).

The percentage of inhibition of the metabolite formation in the membrane preparations treated with the compounds of formula I and the reference compounds in comparison to the untreated membrane preparations was expressed as IC_{50} value (nM).

The used reference compounds were:

N-(3-mercapto-2-benzyl-1-oxo-propyl)glycine (thiorphan)
N-(3-carboxyphenyl)-3-mercapto-2-benzyl-propanamide (compound R-1)
N-(4-carboxymethyl-2-thiazolyl)-3-mercapto-2-benzyl-propanamide (compound R-2).

b) In vitro ECE-inhibitory activity

The ECE-inhibitory activity in vitro was evaluated according to the method reported in the literature by M. Auget et al., Eur. J. Pharmacol., 224, (1992), 101-102.

Male New Zealand rabbits (2.5-3 Kg) were sacrificed with an excess of pentobarbital and blood was drawn.

The left saphenous artery was removed and cleaned of the surround ing tissue, cut into 2-3 mm lenght rings and suspended in 25 ml baths containing Krebs-Henseleit solution at 37°C and oxygenated with O_2 containing 5% CO_2. This solution was composed of (mM); NaCl, 118; KCl, 4.7; $Cacl_2$, 2.5; KH_2PO_4, 1.2; $HgSO_4$, 1.2; $NaHCO_3$, 2.5; glucose, 11. The preparations were kept under ten-sion and readjusted to 1 g during the equilibration period (1 hour).

After said period, the preparations were exposed to a submaximal concentration of norepinephrine 1 μM which was repeated every 30 minutes until the response was stable. A concentration of acetylcholine 10 μM on the contraction of norepinephrine verified the presence of the endothelium.

After 30 minutes from the last contraction due to norepinephrine, a concentration of human Big endothelin 3×10^{-8}M was administered.

After reaching the plateau the preparations were washed for 30 minutes and a concentration 1 μM of the compound to be tested or of its vehicle was administered keeping it in contact for 30 minutes, after that a concentration of Big endothelin 3×10^{-8}M was administered again. The percentage of ECE-inhibition was expressed as IC_{50} value (nM).

The values of NEP-inhibitory activity and ECE-inhibitory activity for some representative compounds of formula I are reported in the following table 1.

Table 1

NEP-inhibitory activity expressed as IC_{50} value (nM) of the compounds 2, 4, 6, 9 and 13 in comparison to thiorphan, compound R-1 and compound R-2 and ECE-inhibitory activity expressed as IC_{50} value (nM) of the above mentioned compounds in comparison to phosphoramidon.

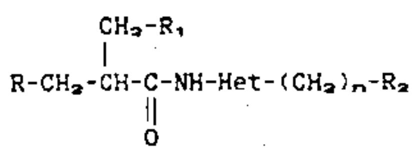
Compound	NEP-inhibitory activity IC_{50} (nM)	ECE-inhibitory activity IC_{50} (nM)
thiorphan	8.3	---
R-1	3.12	---
R-2	8.8	---
phosphoramidon	---	50
compound 2	1.5	2
compound 4	2.1	2
compound 6	12.6	1
compound 9	2.7	4
compound 13	5.0	3

The results reported in table 1 clearly show that the compounds of formula I, object of the present invention, are endowed with both NEP-inhibitory activity and ECE-inhibitory activity.

In particular, the NEP-inhibitory activity of the compounds of formula I is substantially comparable with that of the reference compounds and the ECE-inhibitory activity is significantly greater than that of phosphoramidon.

Claims

1. A compound of formula



(I)

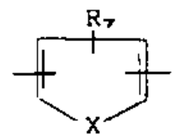
wherein

- R is a mercapto group or an R_3COS group convertible into the organism to the mercapto group; R_3 is a C_1-C_4 alkyl group;
- R_1 is a hydrogen atom, a phenyl group or a 5 or 6 membered heterocycle containing 1 or 2 heteroatoms selected among nitrogen, oxygen and sulphur, optionally substituted by one or two groups selected among C_1-C_4 alkyl or alkoxy groups, hydroxy, halogen and trifluoromethyl groups;
- R_2 is a carboxylic group or a $COOR_4$ or

group convertible into the organism to the carboxylic group; R_4 is a C_1-C_4 alkyl group or a phenylalkyl having from 1 to 4 carbon atoms in the alkyl moiety; R_5 and R_6, the same or different, are hydrogen atoms, C_1-C_4 alkyl or C_5-C_7 cycloalkyl groups;

<u>n</u> is 0 or 1;

Het is a 5-membered heterocycle of formula

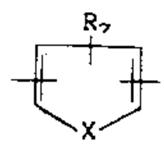


wherein X is an oxygen or sulphur atom or an NH group; R_7 is a hydrogen atom, a C_1-C_4 alkyl group or phenyl optionally substituted by C_1-C_4 alkoxy groups;

and its pharmaceutically acceptable salts.

2. A compound according to claim 1 wherein R is a mercapto group or an R_3COS group wherein R_3 is methyl; R_2 is a carboxylic group.

3. A compound according to claim 1 wherein R is a mercapto group or an R_3COS group wherein R_3 is methyl; R_2 is a carboxylic group; R_1 is phenyl or pyridyl, optionally substituted by a C_1-C_4 alkyl or alkoxy group or by a halogen atom and Het is a heterocycle of formula

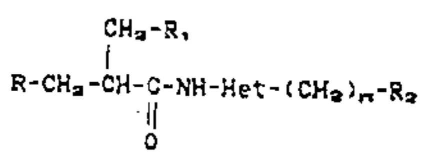


wherein X is an oxygen or sulphur atom or an NH group and R_7 is a hydrogen atom.

4. A pharmaceutical composition containing a therapeutically effective amount of one or more compounds of formula I in admixture with a carrier for pharmaceutical use.

Patentansprüche

1. Eine Verbindung der Formel



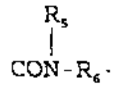
(I)

worin

R eine Mercaptogruppe oder eine im Organismus in die Mercaptogruppe umwandelbare R_3COS-Gruppe bedeutet; R_3 für eine C_1-C_4-Alkylgruppe steht;

R_1 für ein Wasserstoffatom, eine Phenylgruppe oder einen 5 oder 6 gliedrigen Heterocyclus steht, gegebenenfalls substituiert durch eine oder zwei Gruppen, die aus C_1-C_4-Alkyl oder Alkoxygruppen, Hydroxyl-, Halogen- und Trifluormethylgruppe(n) ausgewählt sind, wobei der Heterocyclus 1 oder 2 Heteroatome enthält, die ausgewählt sind aus Stickstoff, Sauerstoff und Schwefel;

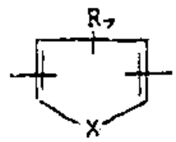
R_2 eine Carboxylgruppe oder eine im Organismus in die Carboxylgruppe umwandelbare $COOR_4$- oder



Gruppe bedeutet, R_4 für eine C_1-C_4-Alkylgruppe oder Phenylalkyl steht, welches im Alkylrest 1 bis 4 Kohlenstoffatome besitzt; R_5 und R_6 gleich oder verschieden sind und Wasserstoff, C_1-C_4-Alkyl- oder C_5-C_7-Cycloalkylgruppen bedeuten;

n für 0 oder 1 steht;

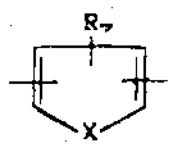
Het einen 5-gliedrigen Heterocyclus der Formel



bedeutet, worin X für ein Sauerstoff- oder Schwefelatom steht oder eine NH-Gruppe bedeutet; R_7 für ein Wasserstoffatom, eine C_1-C_4-Alkylgruppe oder ein Phenyl, gegebenenfalls durch C_1-C_4-Alkoxygruppen substituiert.

2. Eine Verbindung gemäß Patentanspruch 1, worin R eine Mercaptogruppe oder eine R_3COS-Gruppe bedeutet, worin R_3 für Methyl steht; R_2 eine Carboxylgruppe bedeutet.

3. Eine Verbindung gemäß Patentanspruch 1, worin R für eine Mercaptogruppe oder eine R_3COS-Gruppe steht, worin R_3 Methyl bedeutet; R_2 eine Carboxylgruppe bedeutet; R_1 für Phenyl oder Pyridyl steht, gegebenenfalls substituiert durch eine C_1-C_4-Alkyl- oder Alkoxygruppe oder durch ein Halogenatom, und Het einen Heterocyclus der Formel

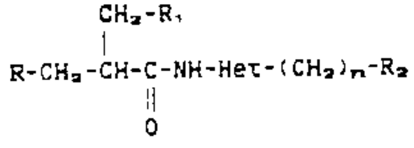


bedeutet, worin X für ein Sauerstoff- oder Schwefelatom steht oder eine NH-Gruppe bedeutet und R_7 für ein Wasserstoffatom steht.

4. Eine pharmazeutische Zusammensetzung enthaltend eine therapeutisch wirksame Menge einer oder mehrerer Verbindungen der Formel I vermischt mit einem Träger für pharmazeutische Anwendung.

Revendications

1. Composé de formule :



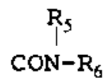
(I)

dans laquelle :

R est un groupe mercapto ou un groupe R_3COS convertible dans l'organisme en groupe mercapto, R_3 est un groupe alkyle en C_1-C_4 ;

R_1 est un atome d'hydrogène, un groupe phényle ou un hétérocycle pentagonal ou hexagonal contenant 1 ou 2 hétéroatomes choisis parmi l'azote, l'oxygène et le soufre, éventuellement substitué par un ou deux groupes choisis parmi les groupes alkyle et alcoxy en C_1-C_4 et les groupes hydroxy, halogène et trifluorométhyle ;

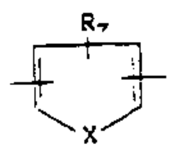
R_2 est un groupe carboxylique ou un groupe $COOR_4$ ou



convertible dans l'organisme en groupe carboxylique, R_4 est un groupe alkyle en C_1-C_4 ou un groupe phénylalkyle comportant de 1 à 4 atomes de carbone dans le fragment alkyle, R_5 et R_6, identiques ou différents, sont des atomes d'hydrogène ou des groupes alkyle en C_1-C_4 ou cycloalkyle en C_5-C_7;

n est égal à 0 ou 1;

Het est un hétérocycle pentagonal de formule :

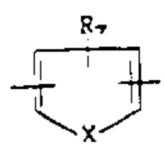


dans laquelle X est un atome d'oxygène ou de soufre ou un groupe NH, R_7 est un atome d'hydrogène, un groupe alkyle en C_1-C_4 ou un groupe phényle éventuellement substitué par des groupes alcoxy en C_1-C_4 ;

et ses sels pharmaceutiquement acceptables.

2. Composé suivant la revendication 1, dans lequel R est un groupe mercapto ou un groupe R_3COS dans lequel R_3 est du méthyle et R_2 est un groupe carboxylique.

3. Composé la revendication 1, dans lequel R est un groupe mercapto ou un groupe R_3COS dans lequel R_3 est du méthyle, R_2 est un groupe carboxylique, R_1 est un groupe phényle ou pyridyle, éventuellement substitué par un groupe alkyle ou alcoxy en C_1-C_4 ou par un atome d'halogène et Het est un hétérocycle de formule :



dans laquelle X est un atome d'oxygène ou de soufre ou un groupe NH et R_7 est un atome d'hydrogène.

4. Composition pharmaceutique contenant une quantité thérapeutiquement efficace d'un ou plusieurs composés de formule I en mélange avec un support pour utilisation pharmaceutique.

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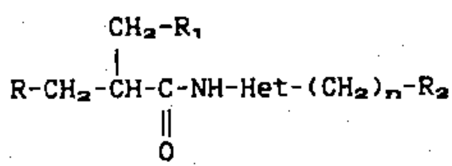
(54) Beta-mercapto-propanamide derivatives useful in the treatment of cardiovascular diseases.

(57) Compounds of formula



(I)

wherein R, R_1, R_2, Het and n have the meanings reported in the description, processes for their preparation and pharmaceutical compositions which contain them as active ingredients are described. The compounds of formula I are useful in the treatment of cardiovascular diseases.

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The present invention relates to β-mercapto-propanamide derivatives useful in the treatment of cardiovascular diseases and, more particularly, it relates to N-heteroaryl substituted β-mercapto-propanamide derivatives useful in the treatment of cardiovascular diseases as inhibitors of the metabolism of vasoactive peptides.

The pharmacologic interest towards the study of molecules which inhibit the metabolism of vasoactive peptides derives from the role that said peptides exert on the cardiocirculatory system.

For instance, among the inhibitors of the metabolism of vasoactive peptides, the so-called NEP-inhibitors and ECE-inhibitors hold particular interest.

In particular, NEP-inhibitors are able to inhibit neutral endopeptidase enzyme (NEP), also called enkephalinase, which is responsible for the inactivation, not only of endogenous enkephaline, but also of atrial natriuretic factor (ANF), a vasodilator hormone secreted by heart.

ECE-inhibitors, instead, are able to inhibit endothelin converting enzyme (ECE), which is responsible for the transformation of big-endothelin into endothelin, a 21 amino acid peptide with vasoconstrictor activity.

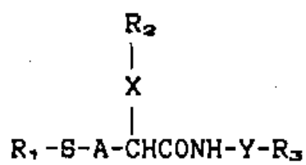
Therefore, both ECE-inhibitors and NEP-inhibitors are useful in therapy in the treatment of hypertension, renal failure and congestive heart failure.

The molecule which is considered the parent of ECE-inhibitors is phosphoramidon [N-[N-[[(6-deoxy-α-L-mannopyranosyl)oxy]hydroxyphosphinyl]-L-leucyl]-L-tryptophan], first isolated as microbial metabolite [Umezawa et al., Tetrahedron Letters, No. 1, pages 97-100, (1972)] and subsequently studied as inhibitor of the metabolism of vasoactive peptides [see, for instance, Matsumura et al., European Journal of Pharmacology, 185 (1990), 103-106].

The molecule which is considered the parent of NEP-inhibitors is thiorphan [DL-(3-mercapto-2-benzylpropanoyl)glycine], first described by Roques et al. in Nature, Vol. 288, pages 286-288, (1980). Several molecules with NEP-inhibitory activity, other than thiorphan, are described in the literature.

Some of them are chemically related to the structure of β-mercapto-propanamides.

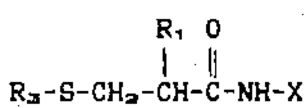
The International patent application No. WO 93/09101 (Fujisawa Pharmaceutical Co. Ltd.) describes β-mercapto-propanamides of formula



wherein R_1 is hydrogen or a protecting group; R_2 is a lower alkyl or a phenyl optionally substituted by a lower alkylenedioxy; R_3 is tetrazolyl, thiazolyl or thiadiazolyl optionally substituted by acyl or acyl-lower alkyl groups; A is a lover alkylene; X is a lower alkylene or S and Y is a single bond or a lower alkylene.

These compounds are NEP-inhibitors.

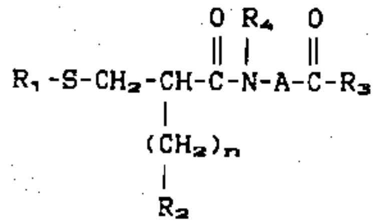
The European patent application No. 0361365 (E. R. Squibb & Sons, Inc.) describes β-mercapto-propanamides of formula



wherein R_1 is, among others, hydrogen, alkyl, haloalkyl, aryl or arylalkyl; X is a phenyl or a cyclohexyl, substituted in 3 or 4 by a $COOR_2$ group; R_2 is hydrogen, alkyl, benzyl, benzhydryl, etc.; R_3 is hydrogen or acyl.

These compounds are NEP-inhibitors.

The European patent application No. 0364767 (Schering Corporation) describes β-mercapto-propanamides of formula

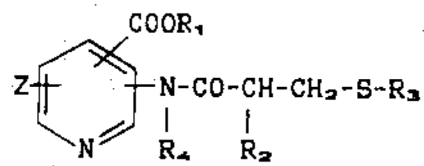


wherein R_1 is hydrogen or acyl; R_2 is aryl or heteroaryl; $-COR_3$ is a carboxylic, ester or amide residue; n is 0-3; R_4 is hydrogen, alkyl or arylalkyl and A is a group selected among optionally substituted phenyl, naphthyl, diphenyl, phenoxyphenyl, phenylthiophenyl, phenylmethylphenyl and pyridyl.

These compounds are able to potentiate the anti-hypertensive and natriuretic action of endogenous ANF and are useful in the treatment of congestive heart failure and of hypertension.

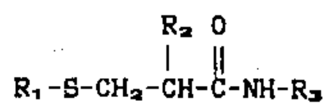
Other examples of the compounds known in the literature, which are structurally related to the class of β-mercapto-propanamides, do not present instead an activity on the cardiocirculatory system, but in general on the central nervous system.

The European patent N. 0110484 (SIMES Società Italiana Medicinali e Sintetici S.p.A., now Zambon Group S.p.A.) describes, among others, β-mercapto-propanamides of formula



wherein Z is hydrogen, alkyl, halogen, alkoxy; R_1 is hydrogen, alkyl, arylalkyl, aryl; R_2 is hydrogen, alkyl, arylalkyl; R_3 is hydrogen or acyl; R_4 is hydrogen or alkyl.

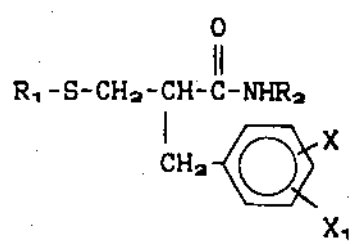
These compounds are useful as analgesics, anti-hypertensives, for the treatment of drug addiction and of psychological disturbances. The European patent application N. 0115997 (Roussel-Uclaf) describes, among others, β-mercapto-propanamides of formula



wherein R_1 is hydrogen or acyl; R_2 is, among others, hydrogen, optionally substituted alkyl, aryl or arylalkyl; R_3 is a heterocycle selected among thiazolyl, 4,5-dihydrothiazolyl, pyridyl, oxazolyl, isoxazolyl, imidazolyl, pirimidyl, tetrazolyl, benzimidazolyl, benzothiazolyl or benzoxazolyl optionally substituted by alkyl or R_3 is a phenyl optionally substituted by a radical selected among alkyl, alkoxy, hydroxy, nitro, halogen, trifluoromethyl, carboxymethyl, alkoxycarbonylmethyl, arylalkoxy, amino, monoalkylamino, dialkylamino.

These compounds are useful as analgesics.

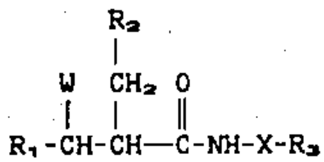
The European patent application N. 0280627 (Roussel-Uclaf) describes α-mercaptomethyl-benzenepropanamides of formula



wherein R_1 is hydrogen or acyl; X and X_1 are hydrogen, alkyl, alkoxy, hydroxy, halogen or trifluoromethyl; R_2 is pyrrolidinyl, morpholinyl, piperidinyl, piperazinyl, tetrahydrothiazinyl or hexahydroazepinyl optionally substituted by one or more alkyl, alkoxy, hydroxy, nitro, trifluoromethyl, acyl groups and halogen.

These compounds are endowed with analgesic, psychotropic, antidepressant and anxiolythic activity.

The European patent application N. 0318859 (Dainippon Pharmaceutical Co. Ltd.) describes β-mercapto-propanamides of formula

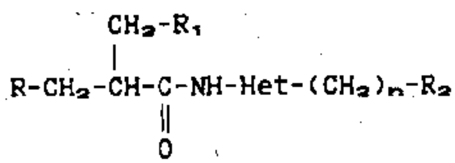


wherein R_1 is a SH group or a biological precursor thereof; W is hydrogen, alkyl or arylalkyl; R_2 is aryl, heterocycle or alkyl, optionally substituted; X is a cycloalkylene, cycloalkylidene or a phenylene, optionally substituted or fused with another ring; R_3 is a carboxyl or a biological precursor thereof.

These compounds are useful as analgesics.

We have now found β-mercapto-propanamides derivatives N-substituted by a 5 membered heterocycle which are endowed with a remarkable NEP-inhibitory activity and ECE-inhibitory activity.

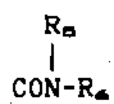
Therefore, object of the present invention are β-mercapto-propanamides of formula



(I)

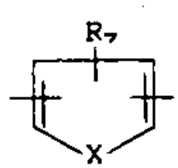
wherein

- R is a mercapto group or an R_3COS group convertible into the organism to the mercapto group; R_3 is a C_1-C_4 alkyl group;
- R_1 is a hydrogen atom, a phenyl group or a 5 or 6 membered heterocycle containing 1 or 2 heteroatoms selected among nitrogen, oxygen and sulphur, optionally substituted by one or two groups selected among C_1-C_4 alkyl or alkoxy groups, hydroxy, halogen and trifluoromethyl groups;
- R_2 is a carboxylic group or a $COOR_4$ or



group convertible into the organism to the carboxylic group; R_4 is a C_1-C_4 alkyl group or a phenylalkyl having from 1 to 4 carbon atoms in the alkyl moiety; R_5 and R_6, the same or different, are hydrogen atoms, C_1-C_4 alkyl or C_5-C_7 cycloalkyl groups;

- n is 0 or 1;
- Het is a 5-membered heterocycle of formula



wherein X is an oxygen or sulphur atom or an NH group; R_7 is a hydrogen atom, a C_1-C_4 alkyl group or a phenyl optionally substituted by C_1-C_4 alkoxy groups;

and their pharmaceutically acceptable salts.

The compounds of formula I have at least an asymmetric carbon atom and may therefore exist in the form of stereoisomers.

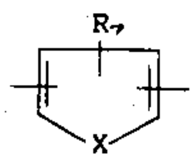
The compounds of formula I in the form of stereoisomeric mixture as well as in the form of single stereoisomers are object of the present invention.

The compounds of formula I are endowed with both NEP-inhibitory and ECE-inhibitory activity and are useful in the treatment of cardiovascular diseases such as hypertension, renal failure and congestive heart failure.

In the present description, unless otherwise specified, with the term C_1-C_4 alkyl we intend a straight or branched C_1-C_4 alkyl such as methyl, ethyl, n.propyl, isopropyl, n.butyl, isobutyl, sec.butyl and t.butyl; with the term C_5-C_7 cycloalkyl we intend cyclopentyl, cyclohexyl and cycloheptyl; with the term C_1-C_4 alkoxy we intend a straight or branched C_1-C_4 alkoxy such as methoxy, ethoxy, n.propoxy, isopropoxy, n.butoxy, isobutoxy, sec.butoxy and t.butoxy. With the term 5- or 6-membered heterocycle containing 1 or 2 heteroatoms selected among nitrogen, oxygen and sulphur we intend a heterocycle preferably selected among thiazole, oxazole, isothiazole, isoxazole, pyrazole, imidazole, thiophene, pyrrole and pyridine.

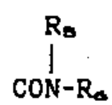
Preferred compounds are the compounds of formula I wherein R is a mercapto group or an R_3COS group wherein R_3 is methyl; R_2 is a carboxylic group.

Still more preferred compounds are the compounds of formula I wherein R is a mercapto group or an R_3COS group wherein R_3 is methyl; R_2 is a carboxylic group; R_1 is phenyl or pyridyl, optionally substituted by a C_1-C_4 alkyl or alkoxy group or by a halogen atom and Het is a heterocycle of formula



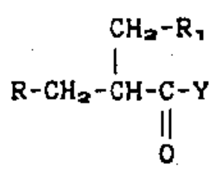
wherein X is an oxygen or sulphur atom or an NH group and R_7 is a hydrogen atom.

It is evident that the compounds of formula I, wherein R is an R_3COS group convertible into the organism to the mercapto group or R_2 is a $COOR_4$ or



group, convertible into the organism to the carboxylic group, are biological precursors (pro-drugs) of the corresponding compounds of formula I wherein R is a mercapto group (R=SH) and R_2 is a carboxylic group (R_2=COOH).

The preparation of the compounds of formula I, object of the present invention, is carried out by reacting a derivative of the β-mercapto-propionic acid of formula



(II)

wherein R and R_1 have the above reported meanings and Y is a halogen atom, preferably chlorine or bromine;
and a compound of formula

H_2N-Het-$(CH_2)_n$-R_2 (III)

wherein R_2, Het and n have the above reported meanings;
in a suitable solvent, in the presence of a base; followed by optional hydrolysis.

Preferably the intermediates of formula II and III are used in a protected form ($R = R_3COS$ and $R_2 = COOR_4$ or

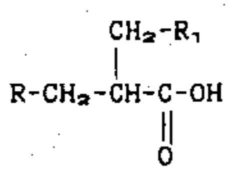
$$\begin{array}{c} R_5 \\ | \\ CON\text{-}R_6 \end{array})$$

affording thus the corresponding compounds of formula I wherein $R = R_3COS$ and $R_2 = COOR_4$ or

$$\begin{array}{c} R_5 \\ \\ CON\text{-}R_6 \end{array}$$

from which, by hydrolysis, the compounds of formula I wherein $R = SH$ and $R_2 = COOH$ are obtained.

The compounds of formula II are known or easily prepared according to conventional methods (see for instance the British patent n. 1576161 in the name of Squibb E.R. & Sons Inc.) from the corresponding acids of formula



(IV)

wherein R and R_1 have the above reported meanings.

Also the intermediates of formula III are known or easily prepared with known methods.

For a bibliographic reference to the preparation of the compounds of formula III see for instance Michel Sy et al., Bull. Soc. Chim. Fr., 1276-1277, (1963) and Moses Lee et al., J. Org. Chem., 53, No. 9, 1855-1859, (1988).

The compounds of formula I in the form of single stereoisomers are prepared by stereoselective synthesis or by separation of the stereoisomeric mixture according to conventional techniques.

The compounds of formula I are active as NEP-inhibitors and ECE-inhibitors and are useful in the treatment of cardiovascular diseases such as hypertension, renal failure and congestive heart failure.

The NEP-inhibitory activity of the compounds of formula I was evaluated by means of in vitro tests as percentage of inhibition in the formation of [^{3}H]-Tyr-Gly-Gly, a metabolite of [^{3}H][Leu^5]-enkephaline (see example 26).

The inhibitory activity, expressed as IC_{50} (nM), of the compounds of formula I resulted to be substantially comparable with that of the reference compounds.

Thiorphan, the compound N-(3-carboxyphenyl)-3-mercapto-2-benzyl-propanamide, described in the aforementioned European patent application No. 0361365 (E.R. Squibb & Sons, Inc.) and the compound N-(4-carboxymethyl-2-thiazolyl)-3-mercapto-2-benzyl-propanamide, described in the aforementioned International patent application No. WO 93/09101 (Fujisawa Pharmaceutical Co. Ltd.) were used as reference compounds (see table 1).

The ECE-inhibitory activity of the compounds of formula I was evaluated by means of in vitro tests for the inhibition of endothelin formation and resulted to be significantly greater than that of phosphoramidon (see example 26).

For the practical use in therapy the compounds of formula I can be formulated in solid or liquid pharmaceutical compositions, suitable for oral or parenteral administration.

Therefore the pharmaceutical compositions containing one or more compounds of formula I, as active ingredient, in admixture with a carrier for pharmaceutical use are a further object of the present invention.

Specific examples of the pharmaceutical compositions according to the present invention are tablets, coated tablets, capsules, granulates, solutions and suspensions suitable for oral administration, solutions and suspensions suitable for parenteral administration.

The pharmaceutical compositions object of the present invention may contain one or more compounds of formula I in association with other active ingredients such as for instance ACE-inhibitors. The pharmaceutical compositions object of the present invention are prepared according to conventional techniques.

The daily dose of compound of formula I will depend on different factors such as the seriousness of the disease, the individual response of the patient, the use of biological precursors and the kind of formulation but it is usually comprised between 0.1 mg and 50 mg per Kg of body weight in a single dose or divided into more daily doses.

With the aim of better illustrating the present invention the following examples are now given.

Example 1

Preparation of N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-benzyl-propanamide (compound 1)

3-Acetylthio-2-benzyl-propionic acid (2.9 g; 12 mmoles) and dimethylformamide (3 drops) were dissolved in thionyl chloride (3 ml). After 16 hours at room temperature the solvent was evaporated under vacuum and the residue was collected twice with toluene (10 ml), evaporating to dryness each time.

The obtained oil was dissolved in toluene (30 ml) and the solution was cooled with ice. Then a solution of 4-amino-2-ethoxycarbonyl-thiophene (1.8 g; 10.5 mmoles) and triethylamine (1.69 ml) in toluene (37 ml) was added dropwise.

After 5 hours under stirring at room temperature, the reaction mixture was diluted with water (30 ml) and extracted with ethyl acetate.

The organic phase was dried on sodium sulphate and the solvent was evaporated under vacuum.

The oil was purified by chromatography (silica gel, eluent n.hexane: ethyl acetate=7:3) affording N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-benzyl-propanamide (1.4 g; 32.2% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.35 (t, 3H); 2.32 (s, 3H); 2.68 (m, 1H); 2.85-3.30 (m, 4H); 4.32 (q, 2H); 7.20 (m, 5H); 7.46 (d, 1H); 7.69 (d, 1H).

Example 2

Preparation of N-(2-carboxy-4-thienyl)-3-mercapto-2-benzyl-propanamide (compound 2)

A solution of N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-benzyl-propanamide (1.35 g; 34 mmoles), prepared as described in example 1, and sodium hydroxide (0.407 g; 10.2 mmoles) in water (5.76 ml) and methanol (14 ml) was kept under stirring for 16 hours at 20°C under nitrogen.

Methanol was evaporated under vacuum and the mixture was acidified with diluted hydrochloric acid to pH about 4.

After extraction with ethyl acetate, the organic phase was washed with water and dried on sodium sulphate.

By evaporating the solvent under vacuum an oil was obtained which crystallizes from methylene chloride:hexane=1:9, affording N-(2-carboxy-4-thienyl)-3-mercapto-2-benzyl-propanamide (0.43 g; 39.4% yield).

m.p. 174-177°C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.32 (t, 1H); 2.53-2.92 (m, 5H); 7.11-7.30 (m, 5H); 7.62 (d, 1H); 7.70 (d, 1H).

Example 3

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-benzyl-propanamide (compound 3)

By working in a way similar to that described in example 1 but substituting 4-amino-2-ethoxycarbonyl-thiophene with 4-amino-2-ethoxycarbonyl-pyrrole, N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-benzyl-propanamide was obtained (55.6% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.30 (t, 3H); 2.32 (s, 3H); 2.66 (m, 1H); 2.80-3.30 (m, 4H); 4.27 (q, 2H); 6.52 (dd, 1H); 7.22 (m, 5H); 7.37 (dd, 1H).

Example 4

Preparation of N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-benzylpropanamide (compound 4)

By working in a way similar to that described in example 2, after chromatography on silica gel (eluent CH_2Cl_2:CH_3OH:CH_3COOH = 90:10:1) and crystallization from CH_2Cl_2:hexane = 1:2, N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-benzyl-propanamide (4.93 g; 46.3% yield) white crystalline solid was obtained.
m.p. 169-172 °C
^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.22 (t, 1H); 2.55-2.94 (m, 5H); 6.56 (dd, 1H); 7.11-7.30 (m, 6H); 9.84 (bs, 1H); 11.41 (bs, 1H).

Example 5

Preparation of ethyl 2-ethoxycarbonyl-3-(3-pyridyl)-propionate

Diethyl malonate (10.176 ml; 67.1 mmoles) was added dropwise to a solution obtained by dissolving metallic sodium (1.543 g; 67.1 mmoles) in anhydrous ethanol (20 ml) heated at 50 °C.

The solution was kept under stirring at 50 °C for 30 minutes and then cooled at room temperature.

3-Chloromethyl-pyridine (5 g; 39.2 mmoles) was added dropwise and the reaction mixture was heated under reflux for 90 minutes.

After evaporating the mixture under vacuum, the residue was collected with ethyl acetate and evaporated to dryness.

The obtained crude was purified by silica gel chromatography (eluent hexane:ethyl acetate = 1:1) affording ethyl 2-ethoxycarbonyl-3-(3-pyridyl)-propionate (4.83 g; 49% yield).
^{1}H-NMR (200 MHz, $CDCl_3$); δ (ppm): 1.18 (t, 6H); 3.19 (d, 2H); 3.60 (t, 1H); 4.13 (q, 4H); 7.12-7.21 (m, 1H); 7.51 (dt, 1H); 8.41-8.47 (m, 2H).

Example 6

Preparation of 2-carboxy-3-(3-pyridyl)-propionic acid

A solution of Potassium hydroxide at 85% (96.8 g; 1.47 moles) in water (300 ml) was added to a solution of ethyl 2-ethoxycarbonyl-3-(3-pyridyl)-propionate (168 g; 0.668 moles), prepared as described in example 5, in dioxane (1680 ml).

The reaction mixture was kept under stirring at room temperature for 4 hours.

The reaction mixture was then neutralized by adding hydrochloric acid 12 N (122.5 ml) and evaporated to dryness under vacuum.

The residue was collected with ethanol (4x750 ml) and the mixture was kept at boiling temperature before filtering off the precipitate.

The solution was evaporated to dryness under vacuum and a crude product (128 g) was obtained which, crystallized from ethanol (1000 ml), afforded 2-carboxy-3-(3-pyridyl)-propionic acid (93.5 g; 72% yield).
m.p. 128-129 °C
^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 3.40 (d, 2H); 3.64 (t, 1H); 7.26-7.33 (m, 1H); 7.67 (dt, 1H); 8.37-8.43 (m, 2H).

Example 7

Preparation of 2-(3-pyridylmethyl)-propenoic acid

An aqueous solution 7.9 N of dimethylamine (2.28 ml; 0.018 moles) was added at 10 °C to 2-carboxy-3-(3-pyridyl)-propionic acid (3.5 g; 0.018 moles), prepared as described in example 6.

The reaction mixture was cooled at 0 °C and formaldehyde (1.48 g; 0.018 moles) was added dropwise.

At the end, the reaction mixture was kept under stirring at room temperature overnight.

By evaporating to dryness under vacuum and by heating the obtained residue at 125 °C under vacuum for 4 hours, a crude was obtained which, chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH:CH_3COOH = 90:10:1), afforded 2-(3-pyridylmethyl)-propenoic acid (1.8 g; 61.3% yield).
m.p. 101-102 °C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 3.58 (s, 2H); 5.62 (s, 1H); 6.15 (s, 1H); 7.25-7.38 (m, 1H); 7.60 (dt, 1H); 8.42 (m, 2H).

Example 8

Preparation of 3-acetylthio-2-(3-pyridylmethyl)-propionic acid

A mixture of 2-(3-pyridylmethyl)-propenoic acid (10 g; 0.061 moles), prepared as described in example 7, and thioacetic acid (4.56 ml; 0.064 moles) was heated at 100 °C for 1 hour.

The reaction mixture was then evaporated to dryness under vacuum and the residue was purified by silica gel chromatography (eluent CH_2Cl_2:CH_3OH:CH_3COOH = 95:5:0.5) obtaining oily 3-acetylthio-2-(3-pyridylmethyl)-propionic acid (10.5 g; 72% yield).
^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.17 (s, 3H); 2.37-2.57 (m, 5H); 6.66 (dd, 1H); 6.83 (dt, 1H); 8.19 (d, 2H).

Example 9

Preparation of N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (compound 5)

A solution of 3-acetylthio-2-(3-pyridylmethyl)-propionic acid (1 g; 4.2 mmoles), prepared as described in example 8, in thionyl chloride (5 ml) and in the presence of dimethylformamide (1 drop) was left at room temperature for 12 hours.

Said mixture was diluted with pyridine (10 ml) and added dropwise to a solution of 4-amino-2-ethoxycarbonyl-thiophene (0.65 g; 3.78 mmoles) in pyridine (5 ml).

After 3 hours at room temperature the reaction mixture was evaporated to dryness under vacuum and the residue was collected with water (20 ml) and extracted with ethyl acetate (3x20 ml).

The collected organic phases were dried on sodium sulphate and evaporated to dryness under vacuum.

The obtained crude was chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH = 95:5) obtaining an oil which, collected with ethyl ether and filtered, afforded N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (0.57 g; 38.5% yield).
^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.33 (t, 3H); 2.33 (s, 3H); 2.72-3.27 (m, 5H); 4.30 (q, 2H); 7.18 (dd, 1H); 7.52 (m, 2H); 7.79 (d, 1H); 8.13 (d, 1H); 8.38 (dd, 1H); 9.58 (s, 1H).

Example 10

Preparation of N-(2-carboxy-4-thienyl)-3-mercapto-2-(3-pyridylmethyl)-propanamide (compound 6)

A solution of sodium hydroxide 10.8 N (0.437 ml; 0.0047 moles) in water (5 ml) was added to a solution of N-(2-ethoxycarbonyl-4-thienyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (0.57 g; 1.45 mmoles), prepared as described in example 9, in methanol (10 ml).

The reaction mixture was kept under stirring at room temperature for 12 hours.

At the end, it was evaporated to dryness under vacuum and the residue was collected with water (10 ml) and washed with ethyl acetate.

The aqueous phase was acidified to pH 4 with hydrochloric acid 1 N and subsequently extracted with ethyl acetate.

The organic phase was dried on sodium sulphate and evaporated to dryness under vacuum; the obtained crude was collected with ethyl ether and filtered affording N-(2-carboxy-4-thienyl)-3-mercapto-2-(3-pyridylmethyl)-propanamide (0.1 g; 21.4% yield).
m.p. 115-118 °C
Mass (Chemical ionization, isobutane): (M^+ + H): 323
^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.57-2.91 (m, 5H); 7.27 (dd, 1H); 7.52-7.63 (dt, 1H); 7.72 (d, 1H); 8.37 (dd, 2H); 10.39 (s, 1H).

Example 11

Preparation of ethyl 3-(4-chlorophenyl)-2-diethoxyphosphinyl-propionate

Sodium hydride (3.12 g; 0.130 moles) was added dropwise to a solution of ethyl diethoxyphosphinylacetate (37 ml; 0.186 moles) in anhydrous dimethylformamide (150 ml), kept at 0°C under nitrogen atmosphere.

After 3 hours at a temperature of 0-5°C, a solution of 4-chlorobenzyl chloride (20 g; 0.124 moles) in dimethylformamide (90 ml) was added at 0°C.

At the end, the reaction mixture was kept under stirring at room temperature for 48 hours, diluted with water (400 ml) containing concentrate hydrochloric acid (5 ml) and extracted with ethyl acetate (3x50 ml).

The collected organic phases were washed twice with water (50 ml), dried on sodium sulphate and evaporated to dryness under vacuum.

The residue was distilled in Vigreaux column (0.7 mm Hg; 165°C) obtaining oily ethyl 3-(4-chlorophenyl)-2-diethoxyphosphinyl-propionate (19 g; 44% yield).
^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.13 (t, 3H); 1.33 (t, 6H); 3.05-3.24 (m, 3H); 4.01-4.22 (m, 6H); 7.07-7.23 (m, 4H).

Example 12

Preparation of ethyl 2-(4-chlorobenzyl)-acrylate

Potassium carbonate (10 g; 0.072 moles) was added to a solution of ethyl 3-(4-chlorophenyl)-2-diethoxyphosphinyl-propionate (22 g; 0.065 moles), prepared as described in example 11, in formaldehyde (40 ml).

The reaction mixture was heated under reflux for 4 hours.

At the end, it was diluted with water (100 ml), extracted with ethyl acetate (3x50 ml), dried on sodium sulphate and evaporated to dryness under vacuum.

The obtained crude which was purified by distillation (8 mm Hg; 150°C) afforded ethyl 2-(4-chlorobenzyl)-acrylate (8.45 g; 58% yield) as oil.
^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.23 (t, 3H); 3.58 (s, 2H); 4.15 (q, 2H); 5.44 (d, 1H); 6.21 (s, 1H); 7.07-7.26 (m, 4H).

Example 13

Preparation of 2-(4-chlorobenzyl)-propenoic acid

A solution of sodium hydroxide 12 N (3.8 ml; 0.0456 moles) was added to a solution of ethyl 2-(4-chlorobenzyl)-acrylate (8.45 g; 0.038 moles), prepared as described in example 12, in methanol (40 ml).

The reaction mixture was kept under stirring at room temperature for 24 hours.

Methanol was evaporated under vacuum and the formed precipitate was collected with water (50 ml); the mixture was acidified to pH 2 with concentrate hydrochloric acid.

By extracting with ethyl acetate (3x30 ml), drying the collected organic phases on sodium sulphate and evaporating to dryness under vacuum, 2-(4-chlorobenzyl)-propenoic acid (6.6 g; 88% yield) was obtained.
m.p. 78-86°C
^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.78 (s, 2H); 4.79 (d, 1H); 6.06 (s, 1H); 6.59-6.68 (m, 4H).

Example 14

Preparation of 3-acetylthio-2-(4-chlorobenzyl)-propionic acid

By working in a way similar to that described in example 8 and by using 2-(4-chlorobenzyl)-propenoic acid (6.7 g; 0.034 moles), prepared as described in example 13, and thioacetic acid (3.64 ml; 0.051 moles), a crude was obtained which chromatographed on silica gel (eluent ligroin:ethyl acetate = 1:1) afforded 3-acetylthio-2-(4-chlorobenzyl)-propionic acid (4.36 g; 47% yield) as oil.
^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.32 (s, 3H); 2.71-3.10 (m, 5H); 7.08-7.28 (m, 4H).

Example 15

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(4-chlorobenzyl)-propanamide (compound 7)

A solution of 3-acetylthio-2-(4-chlorobenzyl)-propionic acid (4.36 g; 0.016 moles), prepared as described in example 14, in thionyl chloride (5 ml), in the presence of dimethylformamide (2 drops), was kept at room temperature and under nitrogen atmosphere for 24 hours. After that, the excess of thionyl chloride was removed by azeotropic distillation with toluene.

Said reaction mixture was added dropwise at 0 °C and under nitrogen atmosphere to a solution of 4-amino-2-ethoxycarbonyl-pyrrole (2.46 g; 0.016 moles) and triethylamine (1.7 g; 0.017 moles) in toluene (40 ml).

After 3 hours at room temperature the reaction mixture was evaporated under vacuum and the residue was collected with ethyl ether and filtered.

The solid was crystallized from ethyl acetate:ligroin = 1:2 and N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(4-chlorobenzyl)-propanamide (3.5 g; 53.5% yield) was obtained.

m.p. 141-144 °C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 1.25 (t, 3H); 2.29 (s, 3H); 2.69-3.01 (m, 5H); 4.20 (q, 2H); 6.61 (m, 1H); 7.11-7.35 (m, 5H); 9.89 (s, 1H); 11.60 (s, 1H).

Example 16

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-2-(4-chlorobenzyl)-3-mercapto-propanamide (compound 8)

A solution of triethylamine (0.68 ml; 4.89 mmoles) in methanol (10 ml) was added to a solution of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(4-chlorobenzyl)-propanamide (1 g; 2.45 mmoles), prepared as described in example 15, in methanol (20 ml).

The reaction mixture was kept under stirring at room temperature for 3 hours, then it was acidified to pH 3 with acetic acid and diluted with water (20 ml).

After extraction with ethyl acetate (3x30 ml), the collected organic phases were dried on sodium sulphate and evaporated to dryness under vacuum.

The obtained crude was chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH = 95:5), further collected with CH_2Cl_2:ligroin = 1:1 and filtered affording N-(2-ethoxycarbonyl-4-pyrrolyl)-2-(4-chlorobenzyl)-3-mercapto-propanamide (0.63 g; 70% yield).

m.p. 140-143 °C

Mass (Chemical ionization, isobutane): (M^+ + H): 367

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.30 (t, 3H); 2.49-3.03 (m, 5H); 4.28 (q, 2H); 6.59 (t, 1H); 7.03-7.24 (m, 5H); 7.36 (t, 1H); 9.09 (bs, 1H).

Example 17

Preparation of N-(2-carboxy-4-pyrrolyl)-2-(4-chlorobenzyl)-3-mercapto-propanamide (compound 9)

By working in a way similar to that described in example 10 and by using N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(4-chlorobenzyl)-propanamide (1 g; 2.45 mmoles), prepared as described in example 15, a crude was obtained which, chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH:CH_3COOH = 90:10:1) and further collected with toluene: ligroin = 1:1 and filtered, afforded N-(2-carboxy-4-pyrrolyl)-2-(4-chlorobenzyl)-3-mercapto-propanamide (0.5 g; 60.2% yield).

Mass (Chemical ionization, isobutane): (M^+ + H): 339

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.46-2.86 (m, 5H); 6.56 (s, 1H); 7.11-7.32 (m, 5H); 9.73 (s, 1H); 11.38 (bs, 1H).

Example 18

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (compound 10)

N-hydroxysuccinimide (0.962 g; 8.36 mmoles) and dicyclohexylcarbodiimide (1.72 g; 8.36 mmoles) were added to a solution of 3-acetylthio-2-(3-pyridylmethyl)-propionic acid (2 g; 8.36 mmoles), prepared as described in example 8, in dioxane (50 ml).

The reaction mixture was kept under stirring at room temperature for 2 hours.

At the end, the formed precipitate was filtered off and the solution was evaporated to dryness under vacuum.

The residue was collected with chloroform (20 ml) and the solution was filtered and evaporated to dryness; this procedure was repeated twice.

The residue, collected again with dioxane (20 ml), was added to a solution of 4-amino-2-ethoxycarbonylpyrrole (1.29 g; 8.36 mmoles) in dioxane (20 ml).

The reaction mixture was kept under stirring at room temperature for 16 hours.

After said time, it was diluted with water (40 ml) and extracted with ethyl acetate (3x30 ml).

The collected organic phases were washed twice with water (30 ml), dried on sodium sulphate and evaporated to dryness under vacuum affording a crude which was chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH = 95:5).

N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (0.6 g; 19.3% yield) was thus obtained.

Mass (Chemical ionization, isobutane): (M^+ + H): 376

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.23 (t, 3H); 2.30 (s, 3H); 2.74-3.18 (m, 5H); 4.20 (q, 2H); 6.55 (t, 1H); 7.10-7.18 (dd, 1H); 7.39 (t, 1H); 7.49 (dt, 1H); 8.12 (d, 1H); 8.29 (dd, 1H); 9.49 (s, 1H); 9.71 (bs, 1H).

Example 19

Preparation of N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-(3-pyridylmethyl)-propanamide (compound 11)

A solution of sodium hydroxide (0.131 g; 3.28 mmoles) in water (10 ml) was added to a solution of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-pyridylmethyl)-propanamide (0.56 g; 1.49 mmoles), prepared as described in example 18, in methanol (10 ml).

The reaction mixture was kept under reflux for 6 hours and sodium hydroxide (0.065 g; 1.64 mmoles) was therein added again.

After 12 hours at room temperature, methanol was evaporated and the residue was diluted with water (20 ml) while pH was brought to 7 by adding sodium bicarbonate.

The mixture was evaporated to dryness and by chromatography on silica gel (eluent CH_2Cl_2:CH_3OH:NH_3 = 79:15:1) a crude was obtained which, collected with chloroform:ethyl ether, afforded N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-(3-pyridylmethyl)-propanamide (80 mg; 17.6% yield).

m.p. 85-90 °C

^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.55-2.89 (m, 5H); 6.49 (m, 1H); 7.09 (m, 1H); 7.20-7.30 (dd, 1H); 7.51-7.60 (dd, 1H); 8.36 (d, 2H); 9.82 (s, 1H); 11.23 (bs, 1H).

Example 20

Preparation of ethyl 2-diethoxyphosphinyl-3-(3-methoxyphenyl)-propionate

By working in a way similar to that described in example 11 and by using ethyl diethoxyphosphinylacetate (59 g; 0.26 moles), sodium hydride at 60% (9.33 g; 0.233 moles) and 3-methoxybenzyl chloride (20.62 g; 0.13 moles), ethyl 2-diethoxyphosphinyl-3-(3-methoxyphenyl)-propionate (34 g; 76% yield) was obtained.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.12 (t, 3H); 1.32 (t, 6H); 3.10-3.32 (m, 3H); 3.75 (s, 3H); 4.08-4.22 (m, 6H); 6.69-6.78 (m, 3H); 7.10-7.22 (m, 1H).

Example 21

Preparation of ethyl 2-(3-methoxybenzyl)-acrylate

By working in a way similar to that described in example 12 and by using ethyl 2-diethoxyphosphinyl-3-(3-methoxyphenyl)-propionate (34 g; 0.0987 moles), prepared as described in example 20, ethyl 2-(3-methoxybenzyl)-acrylate (21.5 g; 98.9% yield) was obtained.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.25 (t, 3H); 3.69 (s, 2H); 3.77 (s, 3H); 4.17 (q, 2H); 5.45 (d, 1H); 6.21 (s, 1H); 6.70-6.80 (m, 3H); 7.14-7.23 (m, 1H).

Example 22

Preparation of 2-(3-methoxybenzyl)-propenoic acid

By working in a way similar to that described in example 13 and by using ethyl 2-(3-methoxybenzyl)-acrylate (10 g; 0.0454 moles), prepared as described in example 21, 2-(3-methoxybenzyl)-propenoic acid (7 g; 80.2% yield) was obtained.
^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 3.59 (s, 2H); 3.78 (s, 3H); 5.58 (d, 1H); 6.37 (s, 1H); 6.72-6.81 (t, 3H); 7.16-7.25 (m, 1H).

Example 23

Preparation of 3-acetylthio-2-(3-methoxybenzyl)-propionic acid

By working in a way similar to that described in example 14 and by using 2-(3-methoxybenzyl)-propenoic acid (6.2 g; 0.0323 moles), prepared as described in example 22, a crude was obtained which, chromatographed on silica gel (eluent hexane:ethyl acetate = 1:1), afforded 3-acetylthio-2-(3-methoxybenzyl)-propionic acid (3.5 g; 40.4% yield).
^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 2.32 (s, 3H); 2.77-3.13 (m, 5H); 3.78 (s, 3H); 6.65-6.78 (m, 3H); 7.12-7.22 (m, 1H).

Example 24

Preparation of N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-methoxybenzyl)-propanamide (compound 12)

By working in a way similar to that described in example 15 and by using 3-acetylthio-2-(3-methoxybenzyl)-propionic acid (3.9 g; 0.0145 moles), prepared as described in example 23, thionyl chloride (1.3 ml) and a solution of 4-amino-2-ethoxycarbonyl-pyrrole (2.24 g; 0.0145 moles) in pyridine (200 ml), a crude was obtained which, chromatographed on silica gel (eluent ligroin:ethyl acetate = 7:3) and further crystallized from ligroin: ethyl acetate = 1:1, afforded N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-methoxybenzyl)-propanamide (2 g; 34% yield).
^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm): 1.30 (t, 3H); 2.30 (s, 3H); 2.62-3.18 (m, 5H); 3.70 (s, 3H); 4.27 (q, 2H); 6.52 (dd, 1H); 6.65-6.77 (m, 3H); 7.06-7.23 (m, 2H); 7.37 (dd, 1H); 8.95 (bs, 1H).

Example 25

Preparation of N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-(3-methoxybenzyl)-propanamide (compound 13)

By working in a way similar to that described in example 17 and by using N-(2-ethoxycarbonyl-4-pyrrolyl)-3-acetylthio-2-(3-methoxybenzyl)-propanamide (0.98 g; 2.42 mmoles), prepared as described in example 24, a crude was obtained which, chromatographed on silica gel (eluent CH_2Cl_2:CH_3OH:CH_3COOH = 90:10:1) and collected with ligroin:ethyl acetate = 1:1 afforded N-(2-carboxy-4-pyrrolyl)-3-mercapto-2-(3-methoxybenzyl)-propanamide (0.520 g; 64.2% yield) as white solid.
m.p. 153-158 °C
Mass (Chemical ionization, isobutane): (M^+ + H): 335
^{1}H-NMR (200 MHz, DMSO-d_6): δ (ppm): 2.46-2.89 (m, 5H); 3.65 (s, 3H); 6.53 (m, 1H); 6.72 (m, 3H); 7.10-7.20 (m, 2H); 9.83 (s, 1H); 11.32 (bs, 1H).

Example 26

Pharmacological activity

a) In vitro NEP-inhibitory activity
The NEP-inhibitory activity in vitro was evaluated according to the method reported in the literature by C. Llorens et al., Eur. J. Pharmacol., 69, (1981), 113-116.
Membranes from kidney cortex were prepared according to the following procedure.
By working at 0-4 °C the kidneys were removed from killed male Sprague-Dawley rats weighing

approximately 300 g.

Cortex was carefully dissected, finely minced and suspended in homogenization buffer (10 mM sodium phosphate pH 7.4 containing 1 mM $MgCl_2$, 30 mM NaCl, 0.02% NaN_3) 1:15 weight/volume.

The tissue was then homogenized for 30 seconds using an Ultra-Turrax homogenizer.

Approximately 10 ml of homogenate were layered over 10 ml of sucrose (41% weight/volume) and centrifuged at 31200 rpm for 30 minutes at 4 °C in a fixed angle rotor.

The membranes were collected from the buffer/sucrose interface, washed twice with 50 mM TRIS/HCl buffer (pH 7.4) and resuspended into the same buffer for storage.

The membranes were stored in small aliquots at -80 °C until use.

The NEP-inhibitory activity was evaluated by using the following method.

Aliquots of the membrane suspension prepared as above described (concentration 5 µg/ml of proteins) were preincubated in the presence of an aminopeptidase inhibitor (Bestatin - 1 mM) for 10 minutes at 30 °C.

[^{3}H][Leu^5]-enkephaline (15 nM) and buffer TRIS/HCl pH 7.4 (50 mM) were added in order to obtain a final volume of 100 µl.

Incubation (20 minutes at 30 °C) was stopped by adding 0.1 M HCl (100 µl).

The formation of the metabolite [^{3}H]Tyr-Gly-Gly was quantified by chromatography on polystyrene columns (Porapak Q).

The percentage of inhibition of the metabolite formation in the membrane preparations treated with the compounds of formula I and the reference compounds in comparison to the untreated membrane preparations was expressed as IC_{50} value (nM).

The used reference compounds were:

N-(3-mercapto-2-benzyl-1-oxo-propyl)glycine (thiorphan)

N-(3-carboxyphenyl)-3-mercapto-2-benzyl-propanamide (compound R-1)

N-(4-carboxymethyl-2-thiazolyl)-3-mercapto-2-benzyl-propanamide (compound R-2).

b) In vitro ECE-inhibitory activity

The ECE-inhibitory activity in vitro was evaluated according to the method reported in the literature by M. Auget et al., Eur. J. Pharmacol., 224, (1992), 101-102.

Male New Zealand rabbits (2.5-3 Kg) were sacrificed with an excess of pentobarbital and blood was drawn.

The left saphenous artery was removed and cleaned of the surrounding tissue, cut into 2-3 mm lenght rings and suspended in 25 ml baths containing Krebs-Henseleit solution at 37 °C and oxygenated with O_2 containing 5% CO_2. This solution was composed of (mM); NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 2.5; glucose, 11. The preparations were kept under tension and readjusted to 1 g during the equilibration period (1 hour).

After said period, the preparations were exposed to a submaximal concentration of norepinephrine 1 µM which was repeated every 30 minutes until the response was stable. A concentration of acetylcholine 10 µM on the contraction of norepinephrine verified the presence of the endothelium.

After 30 minutes from the last contraction due to norepinephrine, a concentration of human Big endothelin $3x10^{-8}$M was administered. After reaching the plateau the preparations were washed for 30 minutes and a concentration 1 µM of the compound to be tested or of its vehicle was administered keeping it in contact for 30 minutes, after that a concentration of Big endothelin $3x10^{-8}$M was administered again. The percentage of ECE-inhibition was expressed as IC_{50} value (nM).

The values of NEP-inhibitory activity and ECE-inhibitory activity for some representative compounds of formula I are reported in the following table 1.

Table 1

NEP-inhibitory activity expressed as IC_{50} value (nM) of the compounds 2, 4, 6, 9 and 13 in comparison to thiorphan, compound R-1 and compound R-2 and ECE-inhibitory activity expressed as IC_{50} value (nM) of the above mentioned compounds in comparison to phosphoramidon.

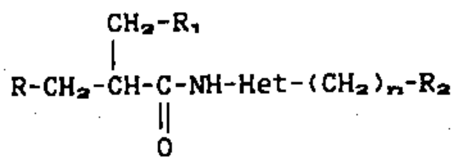
Compound	NEP-inhibitory activity IC_{50} (nM)	ECE-inhibitory activity IC_{50} (nM)
thiorphan	8.3	---
R-1	3.12	---
R-2	8.8	---
phosphoramidon	---	50
compound 2	1.5	2
compound 4	2.1	2
compound 6	12.6	1
compound 9	2.7	4
compound 13	5.0	3

The results reported in table 1 clearly show that the compounds of formula I, object of the present invention, are endowed with both NEP-inhibitory activity and ECE-inhibitory activity.

In particular, the NEP-inhibitory activity of the compounds of formula I is substantially comparable with that of the reference compounds and the ECE-inhibitory activity is significantly greater than that of phosphoramidon.

Claims

1. A compound of formula



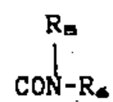
(I)

wherein

R is a mercapto group or an R_3COS group convertible into the organism to the mercapto group; R_3 is a C_1-C_4 alkyl group;

R_1 is a hydrogen atom, a phenyl group or a 5 or 6 membered heterocycle containing 1 or 2 heteroatoms selected among nitrogen, oxygen and sulphur, optionally substituted by one or two groups selected among C_1-C_4 alkyl or alkoxy groups, hydroxy, halogen and trifluoromethyl groups;

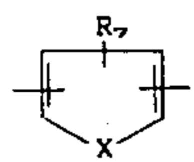
R_2 is a carboxylic group or a $COOR_4$ or



group convertible into the organism to the carboxylic group; R_4 is a C_1-C_4 alkyl group or a phenylalkyl having from 1 to 4 carbon atoms in the alkyl moiety; R_5 and R_6, the same or different, are hydrogen atoms, C_1-C_4 alkyl or C_5-C_7 cycloalkyl groups;

n is 0 or 1;

Het is a 5-membered heterocycle of formula

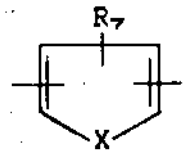


wherein X is an oxygen or sulphur atom or an NH group; R_7 is a hydrogen atom, a C_1-C_4 alkyl group or phenyl optionally substituted by C_1-C_4 alkoxy groups;

and its pharmaceutically acceptable salts.

2. A compound according to claim 1 wherein R is a mercapto group or an R_3COS group wherein R_3 is methyl; R_2 is a carboxylic group.

3. A compound according to claim 1 wherein R is a mercapto group or an R_3COS group wherein R_3 is methyl; R_2 is a carboxylic group; R_1 is phenyl or pyridyl, optionally substituted by a C_1-C_4 alkyl or alkoxy group or by a halogen atom and Het is a heterocycle of formula



wherein X is an oxygen or sulphur atom or an NH group and R_7 is a hydrogen atom.

4. A pharmaceutical composition containing a therapeutically effective amount of one or more compounds of formula I in admixture with a carrier for pharmaceutical use.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 11 1584

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,Y	EP-A-0 318 859 (DAINIPPON PHARMACUTICAL CO. LTD.) 7 June 1989 * page 1, line 2; claim 1 *	1-4	C07D409/12 A61K31/44 C07D333/38 A61K31/38 C07D207/34 A61K31/40 C07D401/12
D,Y	EP-A-0 361 365 (E.R. SUIBB & SONS, INC.) 4 April 1990 * page 1, line 3; claim 1 *	1-4	
D,Y	EP-A-0 364 767 (SCHERING CORPORATION) 25 April 1990 * page 1, line 4 - line 15; claim 1 *	1-4	
D,Y	EP-A-0 110 484 (SIMES S.P.A) 13 June 1984 * page 4, line 11 - line 12; claim 1 *	1-4	
A	EP-A-0 136 883 (E.R. SUIBB & SONS, INC.) 10 April 1985 * claim 1 *	1-4	
D,A	EP-A-0 280 627 (ROUSSEL-UCLAF) 31 August 1988 * page 4, line 33 - line 39; claim 1 *	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A,D	WO-A-93 09101 (FUJISAWA PHARMACEUTICAL CO.) 13 May 1993 * claim 1 *	1-4	C07D A61K
D,A	EP-A-0 115 997 (ROUSSEL-UCLAF) 15 August 1984 * page 6, line 10 - line 11; claim 1 *	1-4	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	6 September 1994	Gettins, M

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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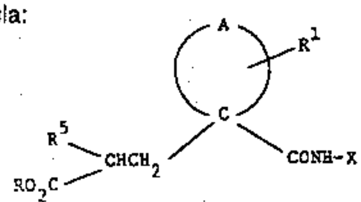
C214 C22Y C225 C226 C227 C25Y C253 C280
C281 C30Y C34Y C342 C36Y C360 C361 C364
C366 C367 C368 C593 C603 C62X C628 C638
C65X C658 C668 C678 C80Y C802
U1S S2414

(56) Documents cited
None

(58) Field of search
UK CL (Edition J) C2C CKM
Chemical Abstracts (CAS On-line)

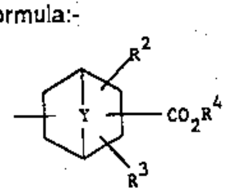
(54) **Spiro-substituted glutaramides as diuretics**

(57) Compounds of the formula:

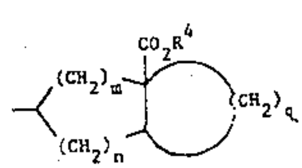


(I)

wherein A completes a 4 to 7 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be fused to a further saturated or unsaturated 5 or 6 membered carbocyclic ring ;
X is a bridged cyclic group of the formula:-



wherein Y is O, CH_2 or $(CH_2)_2$, or a bicyclic group of the formula:-



wherein each of n and m is independently 1 or 2 and q is an integer of from 3 to 5;
each of R and R^4 is independently H, C_1-C_6 alkyl, benzyl or an alternative biolabile ester-forming group;
R^1 is H or C_1-C_4 alkyl;
R^2 and R^3 are each independently H, OH, C_1-C_4 alkyl or C_1-C_4 alkoxy;
and R^5 is a substituent;
and pharmaceutically acceptable salts thereof and bioprecursors therefor.
The compounds are diuretic agents having utility in the treatment of cardiovascular disorders such as hypertension and heart failure.

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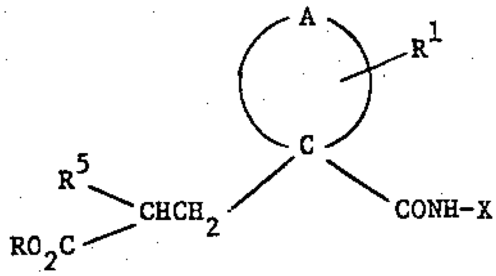
This invention relates to a series of spiro-substituted glutaramide derivatives which are diuretic agents having utility in a variety of therapeutic areas including the treatment of various cardiovascular disorders such as hypertension and heart failure.

The compounds are inhibitors of the zinc-dependent, neutral endopeptidase E.C.3.4.24.11. This enzyme is involved in the breakdown of several peptide hormones, including atrial natriuretic factor (ANF), which is secreted by the heart and which has potent vasodilatory, diuretic and natriuretic activity. Thus, the compounds of the invention, by inhibiting the neutral endopeptidase E.C.3.4.24.11, can potentiate the biological effects of ANF. Thus, in particular the compounds are diuretic agents having utility in the treatment of a number of disorders, including hypertension, heart failure, angina, renal insufficiency, premenstrual syndrome, cyclical oedema, Menières disease, hyperaldosteronism (primary and secondary), pulmonary oedema, ascites and hypercalciuria. In addition, because of their ability to potentiate the effects of ANF the compounds have utility in the treatment of glaucoma. As a further result of their ability to inhibit the neutral endopeptidase E.C.3.4.24.11 the compounds of the invention may have activity in other therapeutic areas including for example the treatment of asthma, inflammation, pain, epilepsy, affective disorders, dementia and geriatric confusion, obesity and gastrointestinal disorders (especially diarrhoea and irritable bowel syndrome), the modulation of gastric acid secretion and the treatment of hyperreninaemia

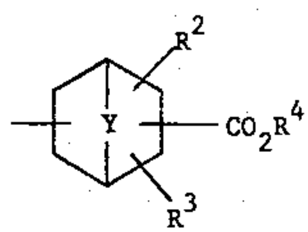
The compounds are of the formula:



(I)

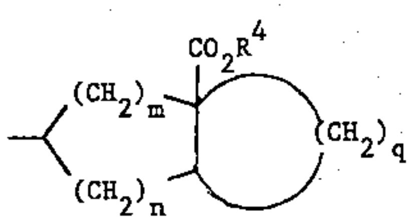
wherein A completes a 4 to 7 membered carbocyclic ring which may be saturated or mono-unsaturated and which may optionally be fused to a further saturated or unsaturated 5 or 6 membered carbocyclic ring;

X is a bridged cyclic group of the formula:-



wherein Y is O, CH_2 or $(CH_2)_2$,

or a bicyclic group of the formula:-



wherein each of n and m is independently 1 or 2 and q is an integer of from 3 to 5;

each of R and R^4 is independently H, C_1-C_6 alkyl, benzyl or an alternative biolabile ester-forming group;

R^1 is H or C_1-C_4 alkyl;

R^2 and R^3 are each independently H, OH, C_1-C_4 alkyl or C_1-C_4 alkoxy;

and R^5 is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, aryl(C_2-C_6 alkynyl), C_3-C_7 cycloalkyl, C_3-C_7 cycloalkenyl, C_1-C_6 alkoxy, $-NR^6R^7$, $-NR^8COR^9$, $-NR^8SO_2R^9$ or a saturated heterocyclic group;

or C_1-C_6 alkyl substituted by one or more substituents chosen from halo, hydroxy, C_1-C_6 alkoxy, C_2-C_6 hydroxyalkoxy, C_1-C_6 alkoxy(C_1-C_6 alkoxy), C_3-C_7 cycloalkyl, C_3-C_7 cycloalkenyl, aryl, aryloxy, aryloxy(C_1-C_4 alkoxy), heterocyclyl, heterocyclyloxy, $-NR^6R^7$, $-NR^8COR^9$, $-NR^8SO_2R^9$, $-CONR^6R^7$, -SH, $-S(O)_pR^{10}$, $-COR^{11}$ or $-CO_2R^{12}$;

wherein R^6 and R^7 are each independently H, C_1-C_4 alkyl, C_3-C_7 cycloalkyl (optionally substituted by hydroxy or C_1-C_4 alkoxy), aryl, aryl(C_1-C_4 alkyl), C_2-C_6 alkoxyalkyl, or heterocyclyl; or the two groups R^6 and R^7 are taken together with the nitrogen to which they are attached to form a pyrrolidinyl, piperidino, morpholino, piperazinyl or N-(C_1-C_4 alkyl)-piperazinyl group;

R^8 is H or C_1-C_4 alkyl;

R^9 is C_1-C_4 alkyl, CF_3, aryl, aryl(C_1-C_4 alkyl), aryl(C_1-C_4 alkoxy), heterocycyl, C_1-C_4 alkoxy or NR^6R^7 wherein R^6 and R^7 are as previously defined;

R^{10} is C_1-C_4 alkyl, aryl, heterocyclyl or NR^6R^7 wherein R^6 and R^7 are as previously defined;

R^{11} is C_1-C_4 alkyl, C_3-C_7 cycloalkyl, aryl or heterocyclyl;

R^{12} is H or C_1-C_4 alkyl;

and p is 0, 1 or 2;

and pharmaceutically acceptable salts thereof and bioprecursors therefor.

In the above definition, unless otherwise indicated, alkyl groups having three or more carbon atoms may be straight or branched-chain. The term aryl as used herein means an aromatic hydrocarbon group such as phenyl or naphthyl which may optionally be substituted with, for example, one or more OH, CN, CF_3, C_1-C_4 alkyl, C_1-C_4 alkoxy, halo, carbamoyl, aminosulphonyl, amino, mono or di(C_1-C_4 alkyl) amino or (C_1-C_4 alkanoyl)amino groups. Halo means fluoro, chloro, bromo or iodo.

The term heterocyclyl means a 5 or 6 membered nitrogen, oxygen or sulphur containing heterocyclic group which, unless otherwise stated, may be saturated or unsaturated and which may optionally include a further oxygen or one to three nitrogen atoms in the ring and which may optionally be benzofused or substituted with for example, one or more halo, C_1-C_4 alkyl, hydroxy, carbamoyl, benzyl, oxo, amino or mono or di-(C_1-C_4 alkyl)amino or

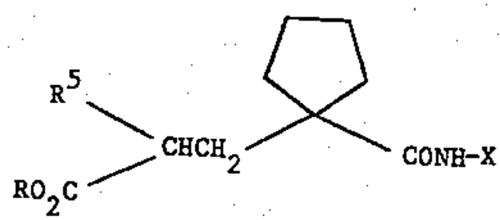
(C_1-C_4 alkanoyl)amino groups. Particular examples of heterocycles include pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, furanyl, tetrahydrofuranyl, tetrahydropyranyl, dioxanyl, thienyl, oxazolyl, isoxazolyl, thiazolyl, indolyl, isoindolinyl, quinolyl, quinoxalinyl, quinazolinyl and benzimidazolyl, each being optionally substituted as previously defined.

The compounds of formula (I) may contain several asymmetric centres and thus they can exist as enantiomers and diastereomers. The invention includes both the separated individual isomers as well as mixtures of isomers.

The pharmaceutically acceptable salts of the compounds of formula (I) containing an acidic centre are those formed with bases which form non-toxic salts. Examples include the alkali metal salts such as the sodium, potassium or calcium salts or salts with amines such as diethylamine. Compounds having a basic centre can also form acid addition salts with pharmaceutically acceptable acids. Examples include the hydrochloride hydrobromide, sulphate or bisulphate, phosphate or hydrogen phosphate, acetate, citrate, fumarate, gluconate, lactate, maleate, succinate and tartrate salts.

The term bioprecursor in the above definition means a pharmaceutically acceptable biologically degradable derivative of the compound of formula (I) which, upon administration to an animal or human being, is converted in the body to produce a compound of the formula (I).

A preferred group of compounds of the formula (I) are those wherein A is $(CH_2)_4$ and R^1 is H, i.e. compounds of the formula (II) wherein R, R^5, and X are as previously defined for formula (I):



(II)

Also preferred are those compounds of formulae (I) and (II) wherein R and R^4 are both H (diacids) as well as biolabile mono and di-ester derivatives thereof wherein one or both of R and R^4 is a biolabile ester-forming group.

The term biolabile ester-forming group is well understood in the art as meaning a group which provides an ester which can be readily cleaved in the body to liberate the corresponding diacid of formula (I) wherein R and R^4 are both H. A number of such ester groups are well known, for example in the penicillin area or in the case of the ACE-inhibitor antihypertensive agents.

In the case of the compounds of formulae (I) and (II) such biolabile pro-drug esters are particularly advantageous in providing compounds of the formula (I) suitable for oral administration. The suitability of any particular ester-forming group can be assessed by conventional animal or *in vitro* enzyme hydrolysis studies. Thus, desirably for optimum effect, the ester should only be hydrolysed after absorption, accordingly, the ester should be resistant to hydrolysis before absorption by digestive enzymes but should be readily hydrolyzed by for example, liver enzymes. In this way the active diacid is released into the bloodstream following oral absorption.

In addition to lower alkyl esters (particularly ethyl) and benzyl esters, suitable biolabile esters include alkanoyloxyalkyl esters, including alkyl, cycloalkyl and aryl substituted derivatives thereof, aryloxyalkyl esters, aroyloxyalkyl esters, aralkyloxyalkyl esters, arylesters, aralkylesters, and haloalkyl esters wherein said alkanoyl or alkyl groups have from 1 to 8 carbon atoms and are branched or straight chain and said aryl groups are phenyl, naphthyl or indanyl optionally substituted with one or more C_1-C_4 alkyl or C_1-C_4 alkoxy groups or halo atoms.

Thus examples of R and R^4 when they are biolabile ester-forming groups other than ethyl and benzyl include: 1-(2,2-diethylbutyryloxy)ethyl, 2-ethylpropionyloxymethyl 1-(2-ethylpropionyloxy)ethyl, 1-(2,4-dimethylbenzoyloxy)ethyl, α-benzoyloxybenzyl, 1-(benzoyloxy)ethyl, 2-methyl-1-propionyloxy-propyl, 2,4,6-trimethylbenzoyloxymethyl