CONVENTION

AUSTRALIA

Patents Act 1990

640502

REQUEST FOR A STANDARD PATENT

The Applicant identified below requests the grant of a patent to the nominated person identified below for an invention described in the accompanying standard complete patent specification.

[70,71]Applicant and Nominated Person:

Merrell Dow Pharmaceuticals Inc.
2110 East Galbraith Road, Cincinnati, Ohio, 45215, UNITED STATES OF AMERICA

[54]Invention Title:

ANALOGS OF HIRUDIN HAVING ANTIPLATELET ACTIVITY

[72]Names Of Actual Inventors:

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Robert James Broersma, Jr

[74]Address for Service:

PHILLIPS ORMONDE & FITZPATRICK
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Melbourne 3000 AUSTRALIA

[31,33,32]

Details of basic application(s):-

557,289	UNITED STATES OF AMERICA	US	24 July 1990

The nominated person is not an opponent or eligible person described in section 33-36 of the Act

18 July 1991

PHILLIPS ORMONDE & FITZPATRICK
Attorneys for:
Merrell Dow Pharmaceuticals Inc.

By: David B Fitzpatrick

Our Ref : 221042

6010

AUSTRALIA

Patents Act 1990

NOTICE OF ENTITLEMENT

I, Gary D. Street

General Patent Counsel

of MERRELL DOW PHARMACEUTICALS INC.

of 2110 East Galbraith Road
Cincinnati, Ohio 45215
United States of America

State the following:

1. The nominated person (applicant) is entitled to the grant of a patent

(i) as assignee of the actual inventor(s)
~~(ii) by contract of employment of the actual inventor(s)~~

2 The basic convention application~~(s)~~ was/~~were~~ the first made in a Convention country in respect of the invention the subject of the application.

Date June 10, 1991

MERRELL DOW PHARMACEUTICALS INC.

By _____
Gary D. Street
General Patent Counsel

To: The Commissioner of Patents

1557A AU

PHILLIPS ORMONDE & FITZPATRICK
Patent and Trademark Attorneys
367 Collins Street
Melbourne, Australia

AU9181110

(12) PATENT ABRIDGMENT (11) Document No. AU-B-81110/91
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 640502

(54) Title
ANALOGS OF HIRUDIN HAVING ANTIPLATELET ACTIVITY

International Patent Classification(s)
(51)[5] A61K 037/64

(21) Application No. : 81110/91 (22) Application Date : 18.07.91

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(31) Number	(32) Date	(33) Country
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(43) Publication Date : 30.01.92

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(71) Applicant(s)
MERRELL DOW PHARMACEUTICALS INC.

(72) Inventor(s)
JOHN LEONARD KRSTENANSKY; ROBERT JAMES BROERSMA JR

(74) Attorney or Agent
PHILLIPS ORMONDE & FITZPATRICK , 367 Collins Street, MELBOURNE VIC 3000

(57) Claim

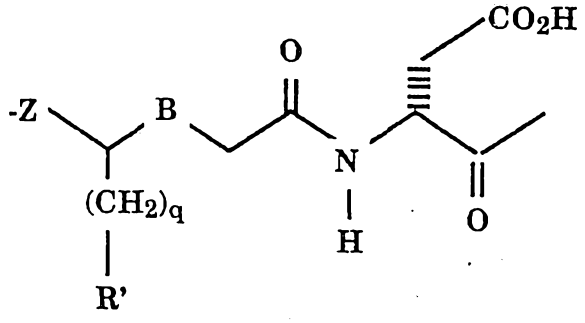
1. A method of treating conditions associated with platelet aggregation in a patient in need thereof which comprises administering to the patient an effective amount of a peptide analog of the formula

$$X\text{-}A_1A_2A_3A_4A_5A_6A_7A_8A_9A_{10}A_{11}\text{-}Y$$

wherein X is an amino terminal residue selected from hydrogen, one or two alkyl groups of from 1 to 6 carbon atoms, one or two acyl groups of from 2 to 10 carbon atoms, carbobenzyloxy, $H_2NC(NH)$-, or t-butyloxy carbonyl group;

A_1 is a bond or is a peptide containing from 1 to 11 residues of any amino acid;

A_2 is a group of one of the the formulae



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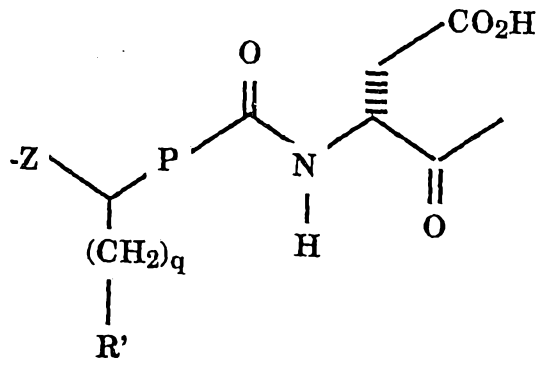
or



wherein Z is a bond or a -NH- or -N(C_1-C_4alkyl)-group;
q is O or an integer of from 1 to 5;
R' is $-NH_2$ or $-N(H)C(=NH)NH_2$;
B is $-C(=O)-N(R)-$, $-P-C(=O)N(H)$, $-N(R)C(=O)-$, $-CH_2N(R)-$, $-C(=O)N(R)CH_2-$, $-CH_2CH_2-$, $-CH=CH-$, $-CH_2-O-$, $-CH_2-S-$, $-CH_2SO-$, or $-CH_2SO_2-$ wherein R is H or a $C_{(1-4)}$alkyl;
P is an ortho-, meta-, or para- phenylene or a 1,2-, 1,3-, or 1,4-cyclohexadiyl;

A_3 is Phe, SubPhe, β-(2- and 3-thienyl)alanine, β-(2-and 3-furanyl)alanine, β-(2-, 3-, and 4-pyridyl)alanine, β-(benzothienyl-2- and 3-yl)alanine, β-(1- and 2-naphthyl)alanine, Tyr or Trp;

A_4 is Glu, Asp, Ser(OSO_3H), Ser(OPO_3H), hSer(OSO_3H), cysteic acid or homocysteic acid;

A_5 is any amino acid;

A_6 is Ile, Val, Leu, Nle, or Phe;

A_7 is Pro, Hyp, 3,4-dehydroPro, thiazolidine-4-carboxylate, Sar, NMePgl or D-Ala;

A_8 is any amino acid;

A_9 is any amino acid;

A_{10} is a lipophilic amino acid selected from Tyr, Trp, Phe, Leu, Nle, Ile, Val, Cha and Pro or is a dipeptide containing at least one of these lipophilic amino acids;

A_{11} is a bond or is a peptide fragment containing from one to five residues of any amino acid; and

Y is a carboxy terminal residue selected from OH, C_1-C_6 alkoxy, amino, mono- or di-(C_1-C_4) alkyl substituted amino, or benzylamino;

or a pharmcaeutically acceptable salt thereof.

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640502

COMPLETE SPECIFICATION
(ORIGINAL)

Class Int. Class

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Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority

Related Art:

Name of Applicant:

Merrell Dow Pharmaceuticals Inc.

Actual Inventor(s):

John Leonard Krstenansky
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Invention Title:

ANALOGS OF HIRUDIN HAVING ANTIPLATELET ACTIVITY

Our Ref : 221042
POF Code: 1432/120371

The following statement is a full description of this invention, including the best method of performing it known to applicant(s):

6006

ANALOGS OF HIRUDIN HAVING ANTIPLATELET ACTIVITY

FIELD OF INVENTION

~~This is a continuation-in-part of application serial number 07/557,289, filed July 24, 1990.~~

This invention relates to peptide analogs having medical use as as anticoagulant and antiplatelet agents.

BACKGROUND OF INVENTION

Anticoagulants are useful therapeutic agents in the pharmacological treatment of, for example, acute deep venous thrombosis, pulmonary embolism, acute arterial embolization of the extremities, myocardial infarction, and disseminated intravascular coagulation. Prophylactic administration of anticoagulants is believed to prevent a recurrence of embolism in patients with rheumatic or arteriosclerotic heart disease and to prevent certain thromboembolic complications of surgery. Administration of anticoagulants has also been indicated in the treatment of coronary artery and cerebrovascular disease. Arterial thrombosis, particularly in arteries supplying the heart muscle and brain, is a leading cause of death.

Antiplatelet agents are useful therapeutic agents in the pharmacological treatment of those platelet associated

thromboembolic diseases that are primarily arterial in origin. For example, antiplatelet agents can be used to prevent reoccurence myocardial infarction and strokes.

Hirudin is a 65 residue polypeptide isolated from the salivary glands of leeches. It is an anticoagulant agent, which is a thrombin specific inhibitor. Although quite potent, clinical use of hirudin isolated from leech extracts seems unlikely because of its limited quantity, expense and allergic reactions which commonly follow administration of any foreign protein of this size.

Applicant has previously discovered a specific region of hirudin that is responsible, at least in part, for its anticoagulant activity. This region has been chemically synthesized and certain of its analogs appear to bind to the recognition site of thrombin but not the enzymatic cleavage site which is spatially separate. Binding of the synthetic peptides competitively prevents binding of the fibrinogen to the recognition site of thrombin, a prerequisite to fibrin production and clot formation.

Several reports have described the ability of the oligopeptide Arg-Gly-Asp and related peptides to inhibit the platelet-dependent thrombus formation. Y. Cadroy, et al., J. Clin. Invest. 84, 939-944 (1989). Applicant has discovered several means of incorporating both the antiplatelet Arg-Gly-Asp fragment and the previously noted Hirudin C-terminal fragment antithrombin analogs into a single entity having both actions.

SUMMARY OF THE INVENTION

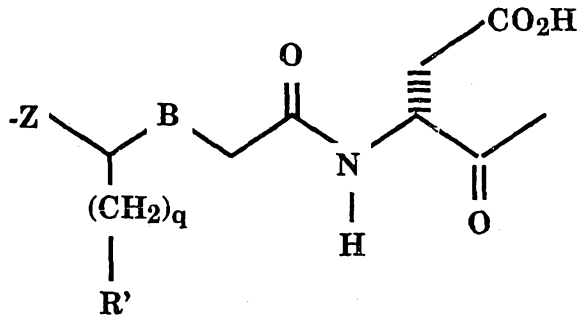
Peptide derivatives of the formula

$$X\text{-}A_1A_2A_3A_4A_5A_6A_7A_8A_9A_{10}A_{11}\text{-}Y$$

wherein X is an amino terminal residue selected from hydrogen, one or two alkyl groups of from 1 to 6 carbon atoms, one or two acyl groups of from 2 to 10 carbon atoms, carbobenzyloxy, $H_2NC(NH)$-, or t-butyloxy carbonyl group;

A_1 is a bond or is a peptide containing from 1 to 11 residues of any amino acid;

A_2 is a group of one of the the formulae



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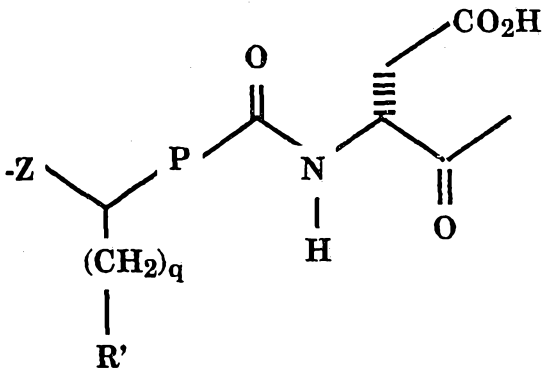


wherein Z is a bond or a -NH- or -N(C_1-C_4alkyl)-group;

q is 0 or an integer of from 1 to 5;

R' is $-NH_2$ or $-N(H)C(=NH)NH_2$;

B is $-C(=O)-N(R)-$, $-P-C(=O)N(H)$, $-N(R)C(=O)-$, $-CH_2N(R)-$, $-C(=O)N(R)CH_2-$, $-CH_2CH_2-$, $-CH=CH-$, $-CH_2-O-$, $-CH_2-S-$, $-CH_2SO-$, or $-CH_2SO_2-$ wherein R is H or a $C_{(1-4)}$alkyl;

P is an ortho-, meta-, or para- phenylene or a 1,2-, 1,3-, or 1,4-cyclohexadiyl;

A_3 is Phe, SubPhe, β-(2- and 3-thienyl)alanine, β-(2-and 3-furanyl)alanine, β-(2-, 3-, and 4-pyridyl)alanine, β-(benzothienyl-2- and 3-yl)alanine, β-(1- and 2-naphthyl)alanine, Tyr or Trp;

A_4 is Glu, Asp, Ser(OSO_3H), Ser(OPO_3H), hSer(OSO_3H), cysteic acid or homocysteic acid;

A_5 is any amino acid;

A_6 is Ile, Val, Leu, Nle, or Phe;

A_7 is Pro, Hyp, 3,4-dehydroPro, thiazolidine-4-carboxylate, Sar, NMePgl or D-Ala;

A_8 is any amino acid;

A_9 is any amino acid;

A_{10} is a lipophilic amino acid selected from Tyr, Trp, Phe, Leu, Nle, Ile, Val, Cha and Pro or is a dipeptide containing at least one of these lipophilic amino acids;

A_{11} is a bond or is a peptide fragment containing from one to five residues of any amino acid; and

Y is a carboxy terminal residue selected from OH, C_1-C_6 alkoxy, amino, mono- or di-(C_1-C_4) alkyl substituted amino, or benzylamino;

and the pharmaceutically acceptable salts thereof are useful anticoagulant agents.

DETAILED DESCRIPTION OF THE INVENTION

The following common abbreviations of the amino acids are used throughout this specification:

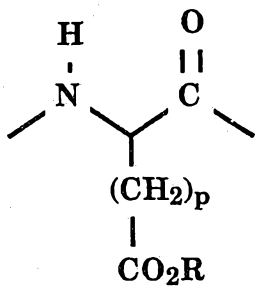
Gly - glycine
Ala - alanine
Val - valine
Leu - leucine
Ile - isoleucine
Cha - cyclohexylalanine
Orn - ornithine
Pro - proline
Phe - phenylalanine
Trp - tryptophan
Met - methionine
Ser - serine
Thr - threonine
Cys - cysteine
Tyr - tyrosine
Asn - asparagine
Gln - glutamine
Asp - aspartic acid
Glu - glutaminc acid
Lys - lysine
Hly - homolysine
Arg - arginine
Har - homoarginine
His - histidine
Nle - norleucine
Hyp - hydroxyproline
Glt - glutaryl
Mal - maleyl
Npa -β-(2-naphthyl)alanine
3,4-dehydroPro - 3,4-dehydroproline
Tyr(SO_3H) - tyrosine sulfate
D-Glu - D-isomer of glutamic acid

Pgl - phenylglycine
NMePgl - N-methyl-phenylglycine
Sar - sarcocine (N-methylglycine)
pSubPhe - para substituted phenylalanine
SubPhe - ortho, meta, or para, mono- or di- substituted phenylalanine
DAla - D-alanine
Ac - acetyl
Suc - succinyl
pClPhe - para-chloro-phenylalanine
pNO_2Phe - para-nitro-phenylalanine
Tyr(Me) - O'-methyl-4-tyrosine
5GP - 5-guanidinopentyl
5AP - 5-aminopentyl

An alkyl group and the alkyl portion of an alkoxy group is taken to include straight, branched, or cyclic alkyl groups, for example, methyl, ethyl, propyl, isopro- pyl, butyl, isobutyl, tert-butyl, pentyl, isopentyl, sec-pentyl, cyclopentyl, hexyl, isohexyl, cyclohexyl and cyclopentylmethyl. An acyl group of from 2 to 10 carbon atoms is taken to include straight, branched, cyclic, saturated and unsaturated acyl groups having 1 or 2 carbonyl moieties per group, for example, acetyl, benzoyl succinyl, maleyl, and glutaryl. A halogen group is a fluoro, chloro, bromo or iodo group.

The term "any amino acid" as used herein includes the naturally occurring amino acids as well as other "non-protein" α-amino acids commonly utilized by those in the peptide chemistry arts when preparing synthetic analogs of naturally occurring peptides. The naturally occurring amino acids are glycine, alanine, valine, leucine,isoleucine, serine, methionine, threonine, phenylalanine, tyrosine, tryptophan, cysteine, proline, histidine, aspartic acid, asparagine, glutamic acid, glutamine, arginine, ornithine,

and lysine. Examples of "non-protein" α-amino acids are norleucine, norvaline, alloisoleucine, homoarginine, thiaproline, dehydroproline, hydroxyproline (Hyp), homoserine, Ser(OSO_3H), hSer(OSO_3H), cysteic acid, homocysteic acid, cyclohexylglycine (Chg), α-amino-n-butyric acid (Aba), cyclohexylalanine (Cha), aminophenylbutyric acid (Pba), phenylalanines substituted at the ortho, meta, or paraposition of the phenyl moiety with one or two of the following, a (C_1-C_4) alkyl, (C_1-C_4) alkoxy, halogen, or nitro groups or substituted with a methylenedioxy group, β-2- and 3-thienylalanine, β-2- and 3-furanylalanine, β-2-, 3-, and 4-pyridylalanine, β-(benzothienyl-2- and 3-yl)alanine, β-(1- and 2-naphthyl)alanine, O-alkylated derivates of serine, threonine, or tyrosine, S-alkylated cysteine, the O-sulfate ester of tyrosine, 3,5-diiodotyrosine and the D-isomers of the naturally occurring amino acids. The term "any amino acid" is also intended to encompass those naturally ocurring and non-protein α-amino acids of the formulae



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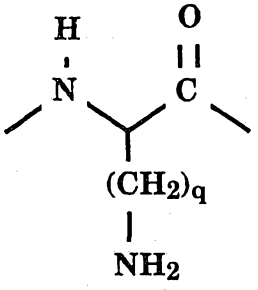

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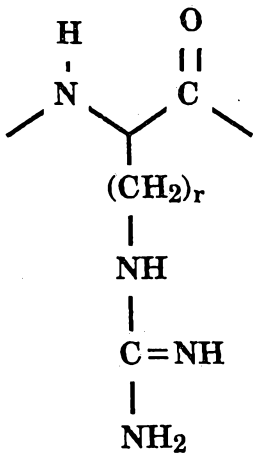
and



4

wherein p, q, and r are each independently an integer of from 1 to 5 and wherein R is a hydrogen or a (C_1-C_4)alkyl group.

The term "lipophilic amino acid" includes Tyr, Phe, Leu, Nle, Ile, Val, His and Pro.

The natural amino acids with the exception of glycine, contain a chiral carbon atom. Unless otherwise specifically indicated, the optically active amino acids, referred to herein, are of the L-configuration, including those amino acids depicted by formulae 2, 3, and 4. For example, any of the amino acids of the A_1 or A_{10} group can be of the D- or L-configuration. As is customary, the structure of peptides written out herein is such that the amino terminal end is on the left side of the chain and the carboxy terminal end is on the right side of the chain. As is also customary when using the three-letter code for the amino acids, a three-letter code begining with an upper case letter indicates the L-confuguration and a three-letter code beginning with a lower-case letter indicates the D-configuration.

The polypeptides of formula 1 can form pharmaceutically acceptable salts with any non-toxic, organic or inorganic acid. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulphuric and phosphoric acid and acid metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids which form suitable salts include the mono, di and tricarboxylic acids. Illustrative of such acids are, for example, acetic, glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric. malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, benzoic, hydroxybenzoic, phenylacetic, cinnamic, salicylic, 2-phenoxybenzoic and sulfonic acids such as methane sulfonic acid and 2-hydroxyethane sulfonic acid. Salts of the

carboxy terminal amino acid moiety include the non-toxic carboxylic acid salts formed with any suitable inorganic or organic bases. Illustratively, these salts include those of alkali metals, as for example, sodium and potassium; alkaline earth metals, such as calcium and magnesium; light metals of Group IIIA including aluminum; and organic primary, secondary and tertiary amines, as for example, trialkylamines, including triethylamine, procaine, dibenzylamine, 1-ethenamine, N,N'-dibenzylethylenediamine, dihydroabietylamine, N-(lower)alkylpiperidine, and any other suitable amine.

As with any generic group of chemical compounds, certain groups are preferred. Applicants prefer those peptide derivatives of formula 1 wherein

X is hydrogen, acetyl, or succinyl.

Also preferred are those formula 1 compounds wherein

A_1 is Thr-Pro-Lys-Pro-Gln-Ser-His-Asn-Asp-Gly-Asp,
-Ser-Thr-Pro-Asn-Pro-Glu-Ser-His-Asn-Asn-Gly-Asp-,
-His-Asn-Asp-Gly-Asp-,
-Asn-Asp-Gly-Asp-,
-Asp-Gly-Asp-,
-Gly-Asp-,
-Asp-, or a bond;

A_2 is as defined above wherein R' is $-N(H)C(=NH)NH_2$, Z is -NH- or a bond, B is $-C(=O)-N(H)-$,

A_3 is preferably Phe, β-2- or 3-thienylalanine, Tle, Trp, Npa, pClPhe or Tyr(Me);

q, an integer of from 2 to 4;

A_4, Glu;

A_5, Glu, Asp, Pro or Ala;

A_6, Ile, Leu;

A_7, Pro, Sar, D-Ala, Hyp or NMePgl;

A_8, Glu, Gln, Asp or Ala;

A_9, Glu, Asp or Ala;

A_{10}, Pro, Ala-Tyr, Ala-Cha, Tyr-Cha, Tyr-Leu, Ala-Phe, Tyr-Tyr;

A_{11}, Glu, glu, Asn, Asp-Glu, Pro, Gln, Ala, a bond, D-Lys, Lys, asp or Orn; and

Y, OH or NH_2.

Applicants also prefer those compounds wherein

X is hydrogen;

A_1 is a bond;

A_2 is of the second represented structure and Z is a bond, q is O, and R' and P are as defined above;

A_3 is preferably Phe, β-2- or 3-thienylalanine, Tyr, Trp, Npa, pClPhe or Tyr(Me);

q, an integer of from 2 to 4;

A_4, Glu;

A_5, Glu, Asp, Pro or Ala;

A_6, Ile, Leu;

A_7, Pro, Sar, D-Ala, Hyp or NMePgl;

A_8, Glu, Gln, Asp or Ala;

A_9, Glu, Asp or Ala;

A_{10}, Pro, Ala-Tyr, Ala-Cha, Tyr-Cha, Tyr-Leu, Ala-Phe, Tyr-Tyr;

A_{11}, Glu, glu, Asn, Asp-Glu, Pro, Gln, Ala, a bond, D-Lys, Lys, asp or Orn; and

Y, OH or NH_2.

Especially preferred are those peptide derivatives of formula 1 wherein either X is succinyl or hydrogen and A_1 is Gly-Asp or Asp or X is succinyl and A_1 is a bond and wherein

Z, is -NH- or a bond;

R' is $H_2NC(=NH)NH$-;

q, 3;

B -C(=O)NH-;

A_3, Phe, Tyr, Tyr(Me) or Trp;;

A_4, Glu;

A_5, Glu or Pro;

A_6, Ile;
A_7, Pro;
A_8, Glu;
$A_8$9 Glu or Asp;
A_{10}, Tyr-Leu, Ala-Tyr, Tyr-Tyr, Ala-Phe, Ala-Cha or Pro;
A_{11}, Gln; Asp; Pro; a bond; D-Asp, D-Lys, glu or -Asp-Glu; and
Y, OH or NH_2.

Applicants also especially prefer those peptide derivatives of formula 1 wherein

X is hydrogen;
A_1 is a bond;
A_2 is the second represented structure and Z is a bond, q is O, P is paraphenylene or 1,4-cyclohexadiyl, and R' is $-NH_2$ or $-N(H)C(=NH)NH_2$;
A_3 is Phe or Trp;
A_4 is Glu or Pro;
A_5 is Glu or Pro;
A_6 is Ile;
A_7 is Pro;
A_8 is Glu'
A_9 is Glu or Asp;
A_{10} is Ala or Cha;
A_{11} is glu; and
Y is OH or NH_2.

The proteins of this invention can be prepared by a variety of procedures readily known to those skilled in the art. Such procedures include the solid phase sequential and block synthesis, gene cloning and combinations of these techniques. The solid phase sequential procedure can be performed using established automated methods such as by use of an automated peptide sythesizer. In this procedure an α-amino protected amino acid is bound to a resin support. The resin support employed can be any suitable resin

conventionally employed in the art for the solid phase preparation of polypeptides, preferably polystyrene which has been cross-linked with from 0.5 to about 3 percent divinyl benzene, which has been either chloromethylated or hydroxymethylated to provide sites for ester formation with the initially introduced α-amino protected amino acid.

An example of a hydroxymethyl resin is described by Bodanszky, et al., Chem. Ind. (London) 38, 1597-98 (1966). A chloromethylated resin is commercially available from Bio Rad Laboratories, Richmond, California, and the preparation of such a resin is described by Stewart et al., "Solid Phase Peptide Synthesis" (Freeman & Co., San Francisco 1969), Chapter 1, pp. 1-6. The protected amino acid can be bound to the resin by the procedure of Gisin, Helv. Chem Acta, 56, 1476 (1973). Many resin bound, protected amino acids are commercially available. As an example, to prepare a polypeptide of this invention wherein the carboxy terminal end is a Thr residue, a tert-butyloxycarbonyl (Boc) protected Thr bound to a benzylated, hydroxymethylated phenylacetamidomethyl (PAM) resin can be used and is commercially available.

Following the coupling of the α-amino protected amino acid to the resin support, the protecting group is removed using any suitable procedure such as by using trifluoro-acetic acid in methylene chloride, trifluoroacetic acid alone, or HCl in dioxane. The deprotection is carried out at a temperature of between 0°C and room temperature. Other standard cleaving reagents and conditions for removal of specific α-amino protecting groups may be used. After removal of the α-amino protecting group the other amino protected amino acids are coupled step-wise in the desired order. Alternatively, multiple amino acid groups may be coupled by the solution method prior to coupling with the resin supported amino acid sequence.

The α-amino protecting group employed with each amino acid introduced into the polypeptide sequence may be any such protecting group known to the art. Among the classes of α-amino protecting groups contemplated are (1) acyl type protecting groups such as: formyl, trifluoroacetyl, phthalyl, toluenesulfonyl (tosyl), benzenesulfonyl, nitrophenylsulfenyl, tritylsulfenyl, o-nitrophenoxyacetyl and α-chlorobutyryl; (2) aromatic urethan type protecting groups such as benzyloxycarbonyl and substituted benzyloxycarbonyl, such as p-chlorobenzyloxycarbonyl, p-nitrobenzyl- carbonyl, p-bromobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, 1-(p-biphenylyl)-1-methylethoxycarbonyl, α, α-dimethyl-3,5-dimethoxybenzyloxycarbonyl and benzhydryloxycarbonyl; (3) aliphatic urethan protecting groups such as tert-butyloxycarbonyl (Boc), diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl and allyloxycarbonyl; (4) cycloalkyl urethan type protecting groups such as cyclopentyloxycarbonyl, adamantyloxycarbonyl and cyclohexyloxycarbonyl; (5) thio urethan type protecting groups such as phenylthiocarbonyl; (6) alkyl type protecting groups such as triphenylmethyl (trityl) and benzyl; and (7) trialkylsilane groups such as trimethylsilane. The preferred α-amino protecting group is tert-butyloxycarbonyl.

The selection of an appropriate coupling reagent is within the skill of the art. A particularly suitable coupling reagent where the amino acid to be added is Gln, Asn or Arg is N,N'-diisopropylcarbodiimide and 1-hydroxybenzotriazole. The use of these reagents prevents nitrile and lactam formation. Other coupling agents are (1) carbodiimides (e.g., N,N'-dicyclohexylcarbodiimide and N-ethyl-N'-(γ-dimethylaminopropylcarbodiimide); (2) cyanamides (e.g., N,N-dibenzylcyanamide); (3) ketenimines; (4) isoxazolium salts (e.g., N-ethyl-5-phenyl-isoxazolium-3'-sulfonate; (5) monocyclic nitrogen containing heterocyclic

amides of aromatic character containing one through four nitrogens in the ring such as imidazolides, pyrazolides, and 1,2,4-triazolides. Specific heterocyclic amides that are useful include N,N'-carbonyldiimidazole and N,N-carbonyl-di-1,2,4-triazole; (6) alkoxylated acetylene (e.g., ethoxyacetylene); (7) reagents which form a mixed anhydride with the carboxyl moiety of the amino acid (e.g., ethylchloroformate and isobutylchloroformate) or the symmetrical anhydride of the amino acid to be coupled (e.g., Boc-Ala-O-Ala-Boc) and (8) nitrogen containing heterocyclic compounds having a hydroxy group on one ring nitrogen (e.g., N-hydroxyphthalimide, N-hydroxysuccinimide and 1-hydroxybenzotriazole). Other activating reagents and their use in peptide coupling are described by Kapoor, J. Pharm. Sci., 59, pp. 1-27 (1970). Applicants prefer the use of the symmetrical anhydride as a coupling reagent for all amino acids except Arg, Asn and Gln.

Each protected amino acid or amino acid sequence is introduced into the solid phase reactor in about a four-fold excess and the coupling is carried out in a medium of dimethylformamide: methylene chloride (1:1) or in dimethylformamide alone or preferably methylene chloride alone. In cases where incomplete coupling occurs, the coupling procedure is repeated before removal of the α-amino protecting group, prior to the coupling of the next amino acid in the solid phase reactor. The success of the coupling reaction at each stage of the synthesis is monitored by the ninhydrin reaction as described by E. Kaiser et al, Analyt. Biochem. 34, 595 (1970).

After the desired amino acid sequence has been obtained, the peptide is removed from the resin. This can be done by hydrolysis such as by treatment of the resin bound polypeptide with a solution of dimethyl sulfide, p-cresol and thiocresol in dilute aqueous hydrofluoric acid.

As is known in the art of solid phase peptide synthesis many of the amino acids bear functionalities requiring protection during the chain preparation. The use and selection of the appropriate protecting group is within the ability of those skilled in the art and will depend upon the amino acid to be protected and the presence of other protected amino acid residues on the peptide. The selection of such a side chain protecting group is critical in that it must be one which is not removed by cleavage during cleavage of the protecting group of the α-amino moiety. For example, suitable side chain protecting groups for lysine are benzyloxycarbonyl and substituted benzyloxycarbonyl, said substituent being selected from halo (e.g., chloro, bromo, fluoro) and nitro (e.g., 2-chlorobenzyloxycarbonyl, p-nitrobenzyloxy-carbonyl, 3,4-dichlorobenzyloxycarbonyl), tosyl, t-amyloxycarbonyl, t-butyloxycarbonyl and diisopropylmethoxycarbonyl. The alcoholic hydroxyl group of threonine and serine can be protected with an acetyl, benzoyl, tert-butyl, trityl, benzyl, 2,6-dichlorobenzyl or benzyloxycarbonyl group. The preferred protecting group is benzyl.

These groups can be removed by procedures well known in the art. Typically protecting group removal is done after the peptide chain synthesis is complete but the protecting groups can be removed at any other appropriate time.

The antiplatelet dose of a peptide analog of this invention is from 0.2 mg/kg to 250 mg/kg of patient body weight per day depending on the patient, the severity of the thromobotic condition to be treated and the peptide analog selected. The suitable dose for a particular patient can be readily determined. Preferably from 1 to 4 daily doses would be administered typically with from 5 mg to 100 mg of active compound per dose.

Antiplatelet therapy is indicated for the prevention of recurrence of myocardial infarction and stroke. as well as other disease conditions associated with platelet aggregation. Those experienced in this field are readily aware of the circumstances requiring anticoagulant and antiplatelet therapy. The term "patient" used herein is taken to mean mammals such as primates, including humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice.

Although some of the peptide derivatives may survive passage through the gut following oral administration, applicants prefer non-oral administration, for example, subcutaneous, intravenous, intramuscular or intraperitoneal; administration by depot injection; by implant preparation; or by application to the mucous membranes, such as, that of the nose, throat and bronchial tubes, for example, in an aerosol can containg a peptide derivative of this invention in a spray or dry powder form.

For parenteral administration the compounds may be administered as injectable dosages of a solution or suspension of the compound in a physiologically acceptable diluent with a pharmaceutical carrier which can be a sterile liquid such as water and oils with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. Illustrative of oils which can be employed in these preparations are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, and mineral oil. In general, water, saline, aqueous dextrose and related sugar solutions, ethanol and glycols such as propylene glycol or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions.

The compounds can be administered in the form of a depot injection or implant preparation which may be formulated in such a manner as to permit a sustained release of the active ingredient. The active ingredient can be compressed into pellets or small cylinders and implanted subcutaneously or intramuscularly as depot injections or implants. Implants may employ inert materials such as biodegradable polymers or synthetic silicones, for example, Silastic, silicone rubber manufactured by the Dow-Corning Corporation.

EXAMPLES

This invention is illustrated by the following, nonlimiting examples.

EXAMPLE 1

Preparation of 5GP-Gly-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

The peptide was synthesized by solid-phase methods using 0.1 mmol of a 0.66 mmol/g Boc-(Bzl)D-Glu- resin. Double symmetrical anhydride couplings were performed with 2.0 mmol Nα-Boc-amino acid (Peptides International). The side chain protection utilized was: Asp(Chx), Trp(CHO), Glu(Bzl). Upon completion of the synthesis the Nα-Boc protection was removed with 50% trifluoroacetic acid in methylene chloride. The resin was washed three times with methylene chloride, neutralized with three washings of 10% diisopropylethylamine in methylene chloride, washed three times with methylene chloride, and dried *in vacuo*. The peptide was deprotected and cleaved from the resin with HF containing 2% anisole at 0°C, for 35 min. The HF was removed *in vacuo* at 0°C, the peptide precipitated with ethyl ether, extracted from the resin with 30% aqueous acetic acid and lyophilized.

Half of this crude 5-AP analog was treated with O-methylisourea at 5°C for 16 hours to yield the crude 5-GP analog.

The peptide was purified by desalting on a 92 x 2.6 cm Sephadex G-15 column in 5% aqueous acetic acid and lyophilized. Preparative HPLC was performed on a C^{18} Vydac 218TP1010 (250 x 10 mm) column with 24% acetonitrile in 0.1% aqueous trifluoroacetic acid at 5 ml/min. The major peak was collected and lyophilized. Homogeneity was determined by HPLC and TLC.

In the same manner, the peptides of the following example 2-6 were prepared.

EXAMPLE 2

5AP-Gly-Asp-Trp-Glu-Glu-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

EXAMPLE 3

Arg-Gly-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

EXAMPLE 4

5AP-Gly-Asp-Tyr(OCH_3)-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

EXAMPLE 5

Suc-Tyr-Glu-Pro-Ile-Pro-Arg-Gly-Asp-Phe-glu-OH

EXAMPLE 6

5GP-Gly-Asp-Tyr(OCH_3)-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

EXAMPLE 7

4-Aminomethylbenzoyl-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

EXAMPLE 8

4-Guanidinomethylbenzoyl-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

EXAMPLE 9

4-Aminomethyl-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

EXAMPLE 10

4-Guanidinomethylcyclohexylcarbonyl-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

EXAMPLE 11

4-Aminomethylcyclohexylcarbonyl-Gly-Asp-Phe-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

EXAMPLE 12

4-Guanidinomethylcyclohexylcarbonyl-Gly-Asp-Phe-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH

The peptides of examples 1 - 12 have the following properties:

EXAMPLE No.	Amino Acids Analysis (6N HCl Hydrolysis; 24 Hrs at 105°C)						
	B	Z	Arg	Pro	Ala	Gly	Ile
1	0.64	4.06	1.07	2.01	1.01	0.96	0.96
2	0.60	4.07	1.08	1.85	1.08	0.99	0.84
3	0.66	4.08	1.02	1.97	1.02	0.99	0.96
4	0.98	4.09	1.04	1.95	1.04	0.98	0.96
5	1.02	4.09	0.97	1.97	0.97	0.98	1.01
6	0.99	1.96	1.02	1.98	1.02	0.99	0.97

Physical Characteristics	
EXAMPLE NO.	FAB-MS (M+H)
1	1564.5
2	1523.3
3	1580.0
4	1514.1
5	1322.9
6	1556.4

EXAMPLE NO.	Bovine Antithrombin IC_{50} (μM)	Dog Antiplatelet IC_{50} (μM)
1	1.6	6
2	1.7	250
3	2.8	7
4	5.4	280
5	15	42
6	5.9	10
7	2.1	
8	1.3	
9	3.5	
10	2.3	

~~WHAT IS CLAIMED IS:~~

The claims defining the invention are as follows:

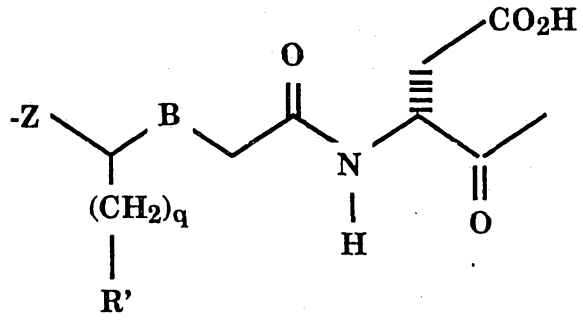
1. A method of treating conditions associated with platelet aggregation in a patient in need thereof which comprises administering to the patient an effective amount of a peptide analog of the formula

$$X\text{-}A_1A_2A_3A_4A_5A_6A_7A_8A_9A_{10}A_{11}\text{-}Y$$

wherein X is an amino terminal residue selected from hydrogen, one or two alkyl groups of from 1 to 6 carbon atoms, one or two acyl groups of from 2 to 10 carbon atoms, carbobenzyloxy, $H_2NC(NH)$-, or t-butyloxy carbonyl group;

A_1 is a bond or is a peptide containing from 1 to 11 residues of any amino acid;

A_2 is a group of one of the the formulae



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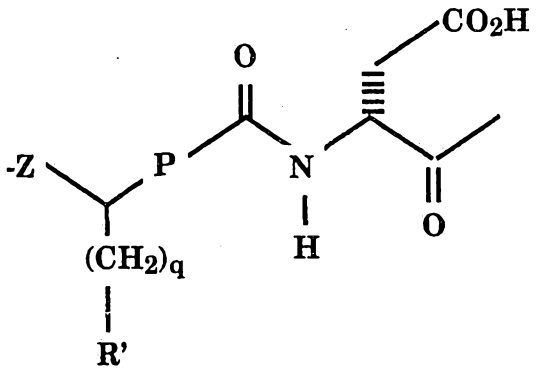


wherein Z is a bond or a -NH- or -N(C_1-C_4alkyl)-group;

q is 0 or an integer of from 1 to 5;

R' is $-NH_2$ or $-N(H)C(=NH)NH_2$;

B is $-C(=O)-N(R)-$, $-P-C(=O)N(H)$, $-N(R)C(=O)-$, $-CH_2N(R)-$, $-C(=O)N(R)CH_2-$, $-CH_2CH_2-$, $-CH=CH-$, $-CH_2-O-$, $-CH_2-S-$, $-CH_2SO-$, or $-CH_2SO_2-$ wherein R is H or a $C_{(1-4)}$alkyl;

P is an ortho-, meta-, or para- phenylene or a 1,2-, 1,3-, or 1,4-cyclohexadiyl;

A_3 is Phe, SubPhe, β-(2- and 3-thienyl)alanine, β-(2-and 3-furanyl)alanine, β-(2-, 3-, and 4-pyridyl)alanine, β-(benzothienyl-2- and 3-yl)alanine, β-(1- and 2-naphthyl)alanine, Tyr or Trp;

A_4 is Glu, Asp, Ser(OSO_3H), Ser(OPO_3H), hSer(OSO_3H), cysteic acid or homocysteic acid;

A_5 is any amino acid;

A_6 is Ile, Val, Leu, Nle, or Phe;

A_7 is Pro, Hyp, 3,4-dehydroPro, thiazolidine-4-carboxylate, Sar, NMePgl or D-Ala;

A_8 is any amino acid;

A_9 is any amino acid;

A_{10} is a lipophilic amino acid selected from Tyr, Trp, Phe, Leu, Nle, Ile, Val, Cha and Pro or is a dipeptide containing at least one of these lipophilic amino acids;

A_{11} is a bond or is a peptide fragment containing from one to five residues of any amino acid; and

Y is a carboxy terminal residue selected from OH, C_1-C_6 alkoxy, amino, mono- or di-(C_1-C_4) alkyl substituted amino, or benzylamino;

or a pharmcaeutically acceptable salt thereof.

2. The method of claim 1 wherein A_3 is Phe, β-(2- or 3-thienyl)alanine, or Tyr.

3. The method of claim 1 wherein A_4 is Glu.

4. The method of claim 1 wherein A_5 is Glu, Ala, or Pro.

5. The method of claim 1 wherein A_6 is Ile.

6. The method of claim 1 wherein A_7 is Pro.

7. The method of claim 1 wherein A_8 is Glu or Ala.

8. The method of claim 1 wherein A_9 is Glu or Asp.

9. The method of claim 1 wherein A_{10} is Tyr-Leu, Ala-Tyr, or Ala-Cha.

10. The method of claim 1 wherein A_{11} is Gln, Asp, Pro, a bond, Asn, Asp-Glu, Glu, Ala, D-Lys, Lys, D-Asp, D-Glu or Orn.

11. The method of claim 1 wherein X is H, acetyl, or succinyl.

12. The method of claim 1 wherein Y is OH or NH_2.

13. The method of claim 1 wherein

R' is $-N(H)C(=NH)NH_2$;
X is NH or a bond;
B is -C)=O)N(H)-; and
q is an integer of from 2 to 4.

14. The method of claim 1 wherein

X is H or succinyl;
A_1 is a bond;
R' is $-N(H)C(=NH)NH_2$;
Z is -NH or a bond;
q is 3;
B is -C(=O)NH-;
A_3 is Phe, Tyr, Tyr(Me), or Trp;
A_4 is Glu;
A_5 is Glu or Pro;
A_6 is Ile;
A_7 is Pro;

A_8 is Glu;
A_9 is Glu or Asp;
A_{10} is -Ala-Cha;
A_{11} is glu; and
Y is OH.

15. A method of claim 1 wherein the peptide analog is
5GP-Gly-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

16. A method of claim 1 wherein the peptide analog is
5AP-Gly-Asp-Trp-Glu-Glu-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

17. A method of claim 1 wherein the peptide analog is
H-Arg-Gly-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

18. A method of claim 1 wherein the peptide analog is
5AP-Gly-Asp-Tyr(Me)-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

19. A method of claim 1 wherein the peptide analog is
5GP-Gly-Asp-Tyr(Me)-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

20. A method of claim 1 wherein the peptide analog is
4-Aminomethylbenzoyl-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

21. A method of claim 1 wherein the peptide analog is 4-Guanidinomethylbenzoyl-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

22. A method of claim 1 wherein the peptide analog is 4-Aminomethyl-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

23. A method of claim 1 wherein the peptide analog is 4-Guanidinomethylcyclohexylcarbonyl-Asp-Trp-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

24. A method of claim 1 wherein the peptide analog is 4-Aminomethylcyclohexylcarbonyl-Gly-Asp-Phe-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

25. A method of claim 1 wherein the peptide analog is 4-Guanidinomethylcyclohexylcarbonyl-Gly-Asp-Phe-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-glu-OH.

26. A method of preparing a pharmaceutical composition for use in treating conditions associated with platelet aggregation which comprises adding a peptide analog of the formula

$$X\text{-}A_1A_2A_3A_4A_5A_6A_7A_8A_9A_{10}A_{11}\text{-}Y$$

wherein X is an amino terminal residue selected from hydrogen, one or two alkyl groups of from 1 to 6 carbon atoms, one or two acyl groups of from 2 to 10 carbon atoms, carbobenzyloxy, $H_2NC(NH)$-, or t-butyloxy carbonyl group;

A_1 is a bond or is a peptide containing from 1 to 11 residues of any amino acid;

A_2 is a group of one of the the formulae

wherein Z is a bond or a -NH- or -N(C_1-C_4alkyl)-group;

q is 0 or an integer of from 1 to 5;

R' is $-NH_2$ or $-N(H)C(=NH)NH_2$;

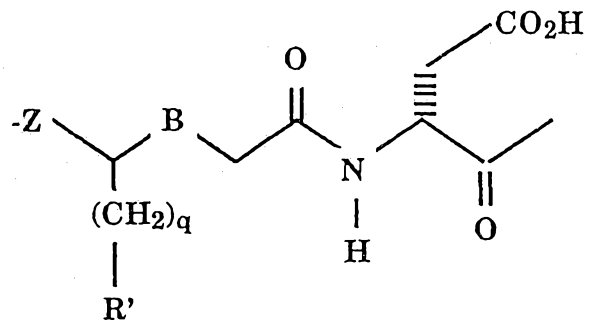
B is $-C(=O)-N(R)-$, $-P-C(=O)N(H)$, $-N(R)C(=O)-$, $-CH_2N(R)-$, $-C(=O)N(R)CH_2-$, $-CH_2CH_2-$, $-CH=CH-$, $-CH_2-O-$, $-CH_2-S-$, $-CH_2SO-$, or $-CH_2SO_2-$ wherein R is H or a $C_{(1-4)}$alkyl;

P is an ortho-, meta-, or para- phenylene or a 1,2-, 1,3-, or 1,4-cyclohexadiyl;

A_3 is Phe, SubPhe, β-(2- and 3-thienyl)alanine, β-(2-and 3-furanyl)alanine, β-(2-, 3-, and 4-pyridyl)alanine, β-(benzothienyl-2- and 3-yl)alanine, β-(1- and 2-naphthyl)alanine, Tyr or Trp;

A_4 is Glu, Asp, $Ser(OSO_3H)$, $Ser(OPO_3H)$, $hSer(OSO_3H)$, cysteic acid or homocysteic acid;

A_5 is any amino acid;



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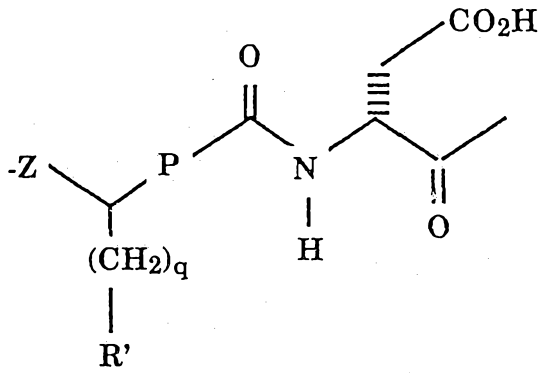

A_6 is Ile, Val, Leu, Nle, or Phe;

A_7 is Pro, Hyp, 3,4-dehydroPro, thiazolidine-4-carboxylate, Sar, NMePgl or D-Ala;

A_8 is any amino acid;

A_9 is any amino acid;

A_{10} is a lipophilic amino acid selected from Tyr, Trp, Phe, Leu, Nle, Ile, Val, Cha and Pro or is a dipeptide containing at least one of these lipophilic amino acids;

A_{11} is a bond or is a peptide fragment containing from one to five residues of any amino acid; and

Y is a carboxy terminal residue selected from OH, C_1-C_6 alkoxy, amino, mono- or di-(C_1-C_4) alkyl substituted amino, or benzylamino;

or a pharmaceutically acceptable salt thereof to a pharmaceutically acceptable carrier.

27. A method as claimed in claim 1 substantially as hereinbefore described with reference to any one of the examples.

DATED: 27 May, 1993

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MERRELL DOW PHARMACEUTICALS INC.

David B Fitzpatrick

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ABSTRACT OF THE DISCLOSURE

This invention relates to peptide derivatives which are useful anticoagulant agents.