661659

P/00/008b 12/11/91
Section 29 (1)
Regulation 3.1 (2)

AUSTRALIA

Patents Act 1990

NOTICE OF ENTITLEMENT

(To be filed before acceptance)

We, RHONE-POULENC RORER INTERNATIONAL (HOLDINGS) INC. of 40 Henlopen Drive, Lewes, Delaware 19958, United States of America, being the applicant in respect of Application No. 80896/91 state the following:-

The Person nominated for the grant of the patent has entitlement from the actual inventors by assignment.

The Person nominated for the grant of the patent has entitlement by virtue of an assignment from Rhône-Poulenc Rorer Pharmaceuticals Inc., who in turn is the assignee of the inventors.

By our Patent Attorneys,
WATERMARK PATENT & TRADEMARK ATTORNEYS

.....

Darryl B. Mischlewski
Registered Patent Attorney

21 December 1994

.....

AU9180896

(12) PATENT ABRIDGMENT (11) Document No. AU-B-80896/91
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 661659

(54) Title
ANTI-THROMBOTIC PEPTIDE AND PSEUDOPEPTIDE DERIVATIVES

International Patent Classificatio, (s)
(51)[5] **C07K 005/06** **A61K 037/02** **C07C 279/18** **C07K 005/02**
C07K 005/08 **A61K 031/195** **A61K 031/215**

(21) Application No. : **80896/91** (22) Application Date : **11.04.91**

(87) PCT Publication Number : **WO92/18117**

(43) Publication Date : **17.11.92**

(44) Publication Date of Accepted Application : **03.08.95**

(71) Applicant(s)
RHONE-POULENC RORER INTERNATIONAL (HOLDINGS), INC.

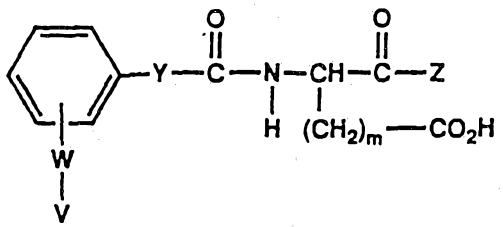
(72) Inventor(s)
SCOTT I KLEIN; BRUCE F. MOLINO; JEFFREY M DENER; MARK CZEKAJ; MICHAEL R BECKER

(74) Attorney or Agent
WATERMARK PATENT & TRADEMARK ATTORNEYS , Locked Bag 5, HAWTHORN VIC 3122

(56) Prior Art Documents
US 4956504
US 4870207
US 4634715

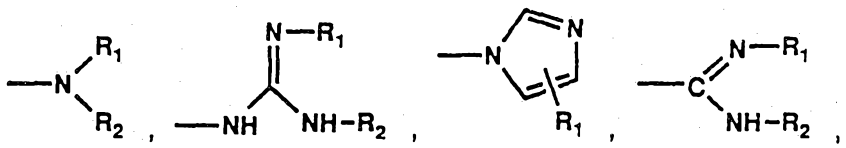
(57) Claim

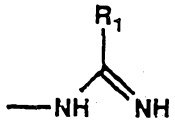
1. A compound of the formula



or a pharmaceutically acceptable salt thereof wherein:

V is

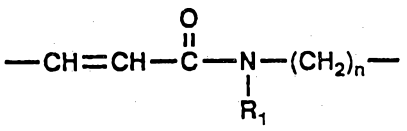



or -C≡N;

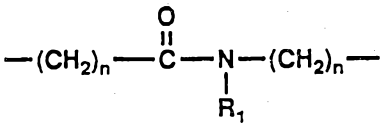
W is $-(CH_2)_n-$, $-CH=CH-(CH_2)_n-$, $-(CH_2)_n-CH=CH-$, $-C\equiv C-(CH_2)_n-$ or $-(CH_2)_n-C\equiv C-$;

.../2

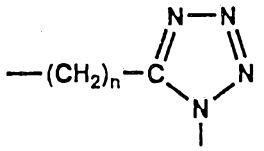
Y is $-(CH_2)_n-$, $-CH=CH-CH_2-$, $-CH_2CH=CH-$, $-CH=CH-$,



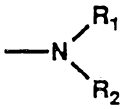
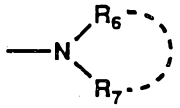
,

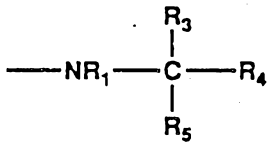


,



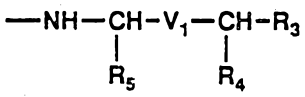
$(CH_2)_n-$, $-CH_2-X_1-$ or $-X_1-CH_2-$ wherein X_1 is O, NH or S;

Z is $-OR_1$,  ,  , a D- or L-amino acid or its corresponding carboximide, a synthetic amino acid of the formula



,

a dipeptide or a dipeptide isostere of the formula

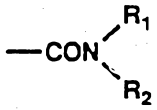
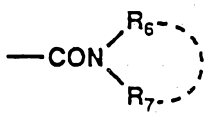


;

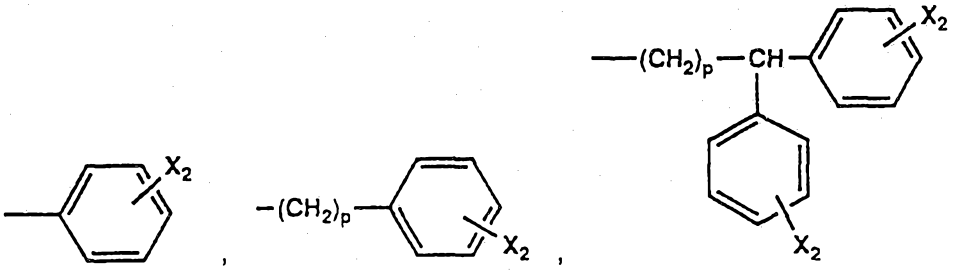
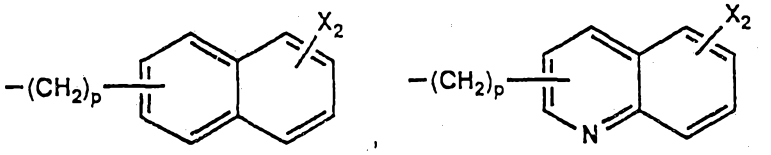
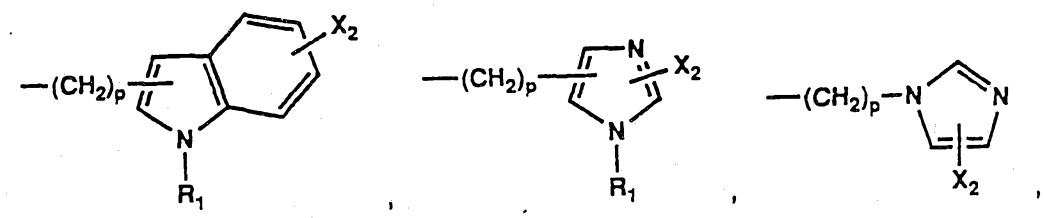
m is 1, 2 or 3;

n is 0 to 6;

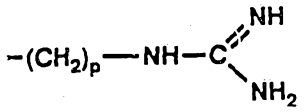
R_1 and R_2 are independently H, alkyl, aryl, aralkyl or allyl;

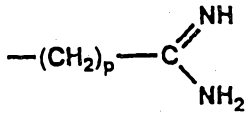
R_3 is H, $-CO_2H$, $-CO_2R_1$, $-CONH_2$,  or  ;

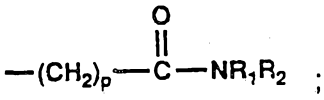
R_4 and R_5 are independently H, alkyl, cycloalkyl, cycloalkylmethyl,

$-(CH_2)_p-OR_1$, $-(CH_2)_p-SR_1$, $-(CH_2)_p-NR_1R_2$,

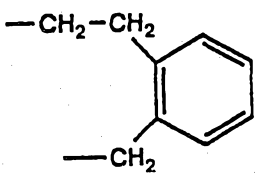



, $-(CH_2)_p-CO_2R_1$ or


;

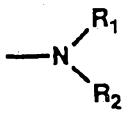
p is 0 to 8;

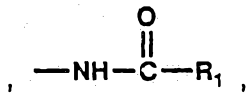
R_6 and R_7 form a ring with the nitrogen to which they are attached and are independently $-(CH_2)_4-$, $(CH_2)_5-$, $-(CH_2)_6-$, $-CH_2CH_2OCH_2CH_2-$,

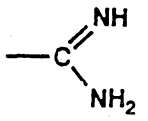
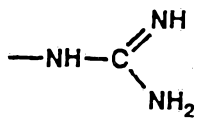
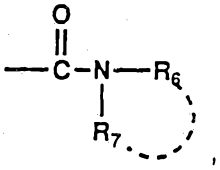


$-CH_2CH_2NR_1CH_2-$ or ;

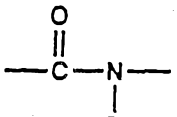
X_2 is H, Cl, Br, F, $-OR_1$, $-NO_2$,


,

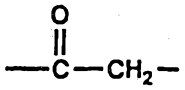

, $-SR_1$,

C_1-C_5 alkyl, phenyl, $-CO_2R_1$,  ,  ,  ,

$-CF_3$ or $-NHSO_2R_1$; and



V_1 is , $-(CH_2)_n-$, $-CH=CH-$, $-CH_2-NH-$, $-CH_2-O-$,

$-CH_2-S-$ or  .

OPI DATE 17/11/92 APPLN. ID 80896/91
AOJP DATE 24/12/92 PCT NUMBER PCT/US91/02471

AU9180896

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 31/195, 31/215, 37/02
C07C 279/00, C07K 5/06
C07K 5/08

A1

(11) International Publication Number: WO 92/18117

(43) International Publication Date: 29 October 1992 (29.10.92)

(21) International Application Number: PCT/US91/02471

(22) International Filing Date: 11 April 1991 (11.04.91)

(71) Applicant *(for all designated States except US)*: RHONE-POULENC RORER INTERNATIONAL (HOLDINGS), INC.[US/US]; ~~40 Henlopen Drive, Lewes, DE 19958 (US)~~. Delaware Corporate Center I / Righter Parkway, Suite 114 Talleyville DE 19803 United States of America

(72) Inventors; and

(75) Inventors/Applicants *(for US only)* : KLEIN, Scott, I. [US/US]; 436 Mill Grove Drive, Audubon, PA 19403 (US). MOLINO, Bruce, F. [US/US]; 2828 North Ford Drive, Hatfield, PA 19440 (US). DENER, Jeffrey, M. [US/US]; 251 West DeKalb Pike, Apt C-1010, King of Prussia, PA 19406 (US). CZEKAJ, Mark [US/US]; 206 Crest Drive, Sellersville, PA 18960 (US). BECKER, Michael, R. [US/US]; 62 Church Road, Norristown, PA 19401 (US).

(74) Agents: BALOGH, Imre, (Jim); Rhone-Poulenc Rorer Inc., 500 Arcola Road, P.O. Box 1200, Collegeville, PA 19426-0107 (US) et al.

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: ANTI-THROMBOTIC PEPTIDE AND PSEUDOPEPTIDE DERIVATIVES

(57) Abstract

Disclosed are novel peptide and pseudopeptide derivatives that inhibit platelet aggregation and thrombos formation in mammalian blood thereby being useful in the prevention and treatment of thrombosis associated with certain disease states, such as, Myocardial infraction, stroke, peripheral arterial disease and disseminated intravascular coagulation.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**ES**	Spain	**MG**	Madagascar
AU	Australia	**FI**	Finland	**ML**	Mali
BB	Barbados	**FR**	France	**MN**	Mongolia
BE	Belgium	**GA**	Gabon	**MR**	Mauritania
BF	Burkina Faso	**GB**	United Kingdom	**MW**	Malawi
BG	Bulgaria	**GN**	Guinea	**NL**	Netherlands
BJ	Benin	**GR**	Greece	**NO**	Norway
BR	Brazil	**HU**	Hungary	**PL**	Poland
CA	Canada	**IT**	Italy	**RO**	Romania
CF	Central African Republic	**JP**	Japan	**RU**	Russian Federation
CG	Congo	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CH	Switzerland			**SE**	Sweden
CI	Côte d'Ivoire	**KR**	Republic of Korea	**SN**	Senegal
CM	Cameroon	**LI**	Liechtenstein	**SU**	Soviet Union
CS	Czechoslovakia	**LK**	Sri Lanka	**TD**	Chad
DE	Germany	**LU**	Luxembourg	**TG**	Togo
DK	Denmark	**MC**	Monaco	**US**	United States of America

ANTI-THROMBOTIC PEPTIDE AND PSEUDOPEPTIDE DERIVATIVES

Background of the Invention

~~This application is a continuation-in-part application of application Serial No.: 475,043, filed February 5, 1990.~~

1. Field of the Invention

This invention relates to novel compounds having anti-thrombotic activity. More particularly, the invention relates to novel peptide and pseudopeptide derivatives that inhibit platelet aggregation and thrombus formation in mammalian blood thereby being useful in the prevention and treatment of thrombosis associated with certain disease states, such as, myocardial infarction, stroke, peripheral arterial disease and disseminated intravascular coagulation.

Haemostasis, the biochemistry of blood coagulation, is an extremely complex and as yet not completely understood phenomena whereby normal whole blood and body tissue spontaneously arrest bleeding from injured blood vessels. Effective haemostasis requires the combined activity of vascular, platelet and plasma factors as well as a controlling mechanism to prevent excessive clotting. Defects, deficiencies, or excesses of any of these components can lead to hemorrhagic or thrombotic consequences.

Platelet adhesion, spreading and aggregation on extracellular matrices are central events in thrombus formation. These events are mediated by a family of platelet adhesive glycoproteins, i.e., fibrinogen, fibronectin, and von Willebrand factor. Fibrinogen is a co-factor for platelet aggregation, fibronectin supports platelet attachments and spreading reactions, and von Willebrand factor is important in platelet attachment to and spreading on subendothelial

matrices. The binding sites for fibrinogen, fibronectin and von Willebrand factor have been located on the platelet membrane glycoprotein complex IIb/IIIa.

Adhesive glycoproteins, like fibrinogen, do not bind with normal resting platelets. However, when a platelet is activated with an agonist such as thrombin or adenosine diphosphate, the platelet changes its shape, perhaps making the GPIIb/IIIa binding site accessible to fibrinogen. The novel molecules described in this invention may block the fibrinogen receptor, thus inhibiting platelet aggregation and subsequent thrombus formation. Pharmaceutical agents and/or compositions possessing such an inhibiting effect may be provided for the prophylaxis and treatment of thrombogenic diseases, such as myocardial infarction, stroke, peripheral arterial disease and disseminated intravascular coagulation.

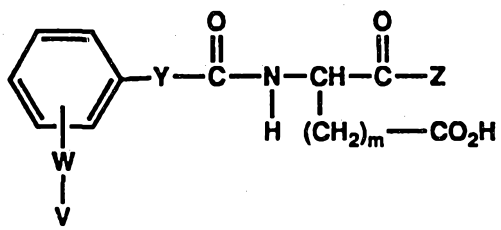
2. Reported Developments

It has been observed that the presence of Arg-Gly-Asp (RGD) is necessary in fibrinogen, fibronectin and von Willebrand factor for their interaction with the cell surface receptor (Ruoslahti E., Pierschbacher, Cell 1986, 44, 517-18). Two other amino acid sequences also seem to take part in the platelet attachment function of fibrinogen, namely, the Gly-Pro-Arg sequence, and dodecapeptide, His-His-Leu-Gly-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val, sequence. Synthetic small peptides containing the RGD or dodecapeptide units show activity: they bind to the platelet receptor and competitively inhibit binding of fibrinogen, fibronection and von Willebrand factor as well as inhibiting aggregation of activated platelets (Plow, et. al. Proc. Natl. Acad. Sci. USA 1985, 82, 8057-61; Ruggeri, et. al. Proc. Natl. Acad. Sci. USA 1986, 5708-12; Ginsberg, et al. J. Biol. Chem. 1985, 260, 3931-36; and Gartner, et. al. J. Biol. Chem. 1987, 260, 11,891-94).

The present invention is directed to novel peptide and pseudopeptide derivatives which inhibit platelet aggregation and subsequent thrombus formation.

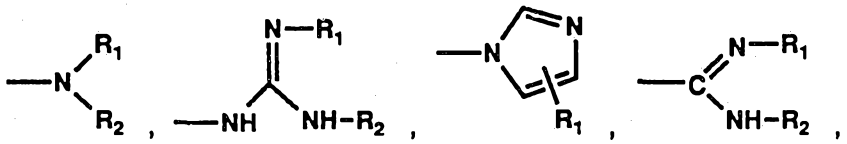
Summary of the Invention

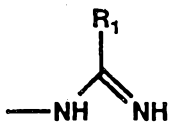
The present invention comprises novel peptide and pseudopeptide derivatives of the general formula:



wherein:

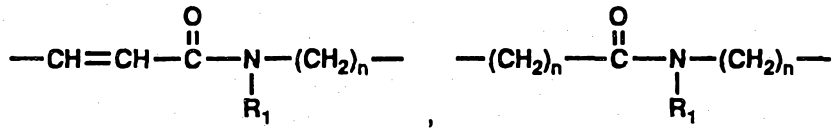
V is

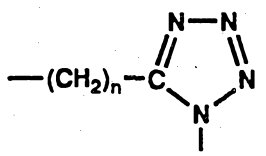


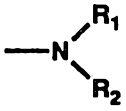
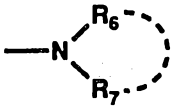

or -C≡N;

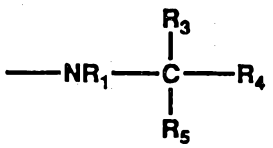
W is $-(CH_2)_n-$, $-CH=CH-(CH_2)_n-$, $-(CH_2)_n-CH=CH-$, $-C\equiv C-(CH_2)_n-$ or $-(CH_2)_n-C\equiv C-$;

Y is $-(CH_2)_n-$, $-CH=CH-CH_2-$, $-CH_2CH=CH-$, $-CH=CH-$,

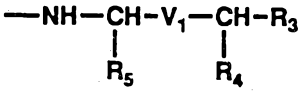



, $-CH_2-X_1-$ or $-X_1-CH_2-$ wherein X_1 is O, NH or S;

Z is $-OR_1$,  ,  , a D- or L-amino acid or its corresponding carboximide, a synthetic amino acid of the formula

 ;

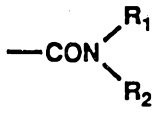
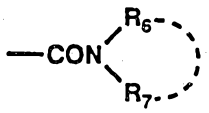
a dipeptide or a dipeptide isostere of the formula

 ;

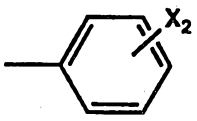
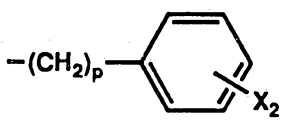
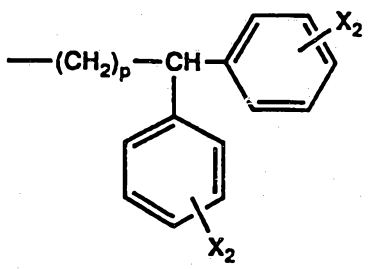
m is 1, 2 or 3;

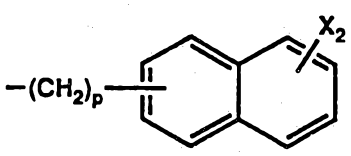
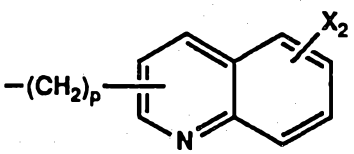
n is 0 to 6;

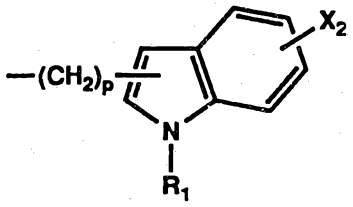
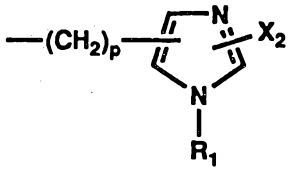
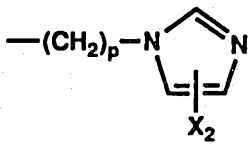
R_1 and R_2 are independently H, alkyl, aryl, aralkyl or allyl;

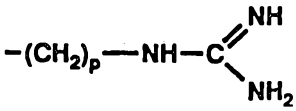
R_3 is H, $-CO_2H$, $-CO_2R_1$, $-CONH_2$,  or  ;

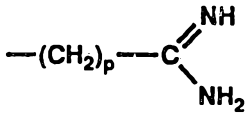
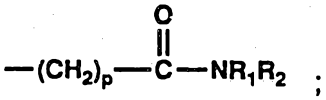
R_4 and R_5 are independently H, alkyl, cycloalkyl, cycloalkylmethyl,

 ,  ,  ,

 ,  ,

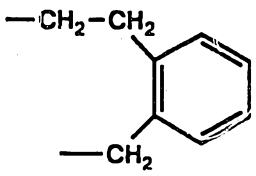




$-(CH_2)_p-OR_1$, $-(CH_2)_p-SR_1$, $-(CH_2)_p-NR_1R_2$, 

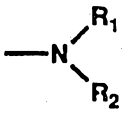
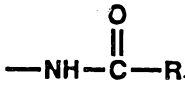
 , $-(CH_2)_p-CO_2R_1$ or  ;

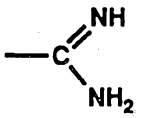
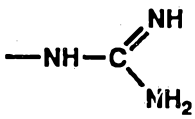
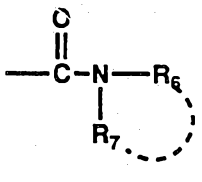
p is 0 to 8;

R_6 and R_7 form a ring with the nitrogen to which they are attached and are $-(CH_2)_4-$, $(CH_2)_5-$, $-(CH_2)_6-$, $-CH_2CH_2OCH_2CH_2-$,

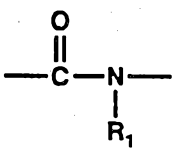


$-CH_2CH_2NR_1CH_2-$ or ;

X_2 is H, Cl, Br, F, $-OR_1$, $-NO_2$,  ,  , $-SR_1$,

C_1-C_5 alkyl, phenyl, $-CO_2R_1$,  ,  ,  ,

$-CF_3$ or $-NHSO_2R_1$;

V_1 is  , $-(CH_2)_n-$, $-CH=CH-$, $-CH_2-NH-$, $-CH_2-O-$,

-CH_2-S- or  ; and pharmaceutically acceptable salts thereof.

Detailed Description of the Invention

In accordance with the present invention, novel compounds are provided which inhibit platelet aggregation by inhibiting fibrinogen binding and other adhesive glycoproteins involved in platelet aggregation and blood clotting to activated platelets. Compounds of the present invention, as tested by methods predictive of anti-thrombotic activity, are believed to be useful in the prevention and treatment of thrombosis associated with certain disease states, such as myocardial infarction, stroke, peripheral arterial disease and disseminated intravascular coagulation.

The present compounds may also be useful for the treatment of certain cancerous diseases since they may interfere with adhesive interactions between cancer cells and the extracellular matrix (Journ. of Biol. Chem., Vol. 262, No. 36 1987, pp. 17703-17711; Science, Vol. 233, 1986, pp. 467-470; and . ., Vol. 57, 59-69, Apr. 1989).

As used above and throughout the description of this invention, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

"Alkyl" means a saturated aliphatic hydrocarbon group which may be straight or branched and having about 1 to about 20 carbon atoms in the chain. Branched means that a lower alkyl group such as methyl, ethyl or propyl. is attached to a linear alkyl chain. Preferred alkyl groups are the "lower alkyl" groups which are those alkyl groups having from 1 to about 6 carbons. Alkyl may be substituted by other moieties such as halogen or alkoxy.

"Halogen" means Cl, Br, I or F.

"Alkoxy" means an alkyl-O- group. Lower alkoxy groups are preferred. Exemplary groups include methoxy, ethoxy, n-propoxy, i-propoxy and n-butoxy.

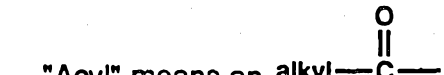
"Aryl" means a mononuclear and polynuclear aromatic hydrocarbon radical which can be substituted or unsubstituted in one or more positions. Examples of aryl groups include phenyl, naphthyl, anthranyl, phenanthranyl, azulyl and the like which can be substituted with one or more of the substituents. Aryl is preferrably substituted or unsubstituted phenyl or naphthyl. Aryl substituents include hydrogen, alkyl, alkoxy, amino, halo, aryl, aryloxy, carboalkoxy, nitro, dialkylamino, trifluoromethyl, thioalkyl and carbamoyl.

"Aralkyl" means an alkyl group substituted by an aryl radical, wherein "aryl" means a phenyl or phenyl substituted with one or more substituents which may be alkyl, alkoxy, amino, nitro, carboxy, carboalkoxy, cyano, alkylamino, halo, hydroxy, hydroxyalkyl, mercapto, alkylthio, acyl or carbamoyl. Exemplary groups include benzyl and phenethyl.

"Carboalkoxy" means an  group. Preferred carboalkoxy groups are those in which the alkyl group is lower alkyl.

"Alkylamino" means an alkyl-NH- group. Preferred groups are lower alkylamino groups.

"Alkylthio" means an alkyl-S- group. Preferred groups are lower alkylthio.

"Acyl" means an  group. Preferred acyl groups are those in which the alkyl group is lower alkyl.

D- and L-amino acids include: Asp, Arg, Ala, Asn, Cys, Gly, Glu, Gln, His, Ile, Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Trp, Tyr and Val.

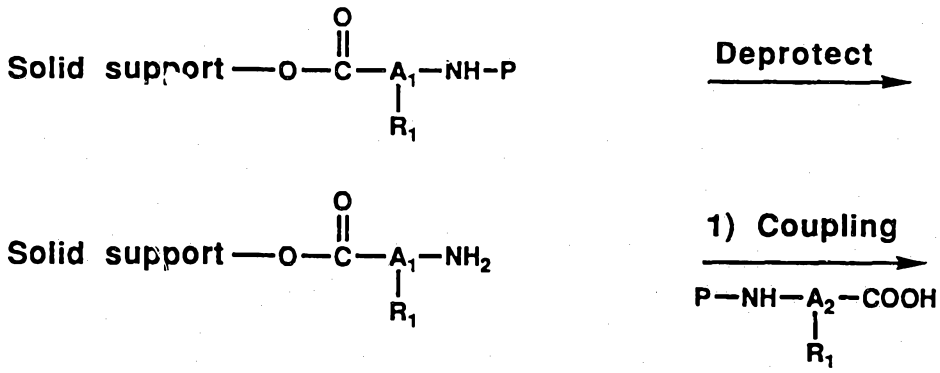
Stereoisomers and diastereomers of the compounds covered by the general formula also constitute a part of the present invention and intended to be covered by the appended claims.

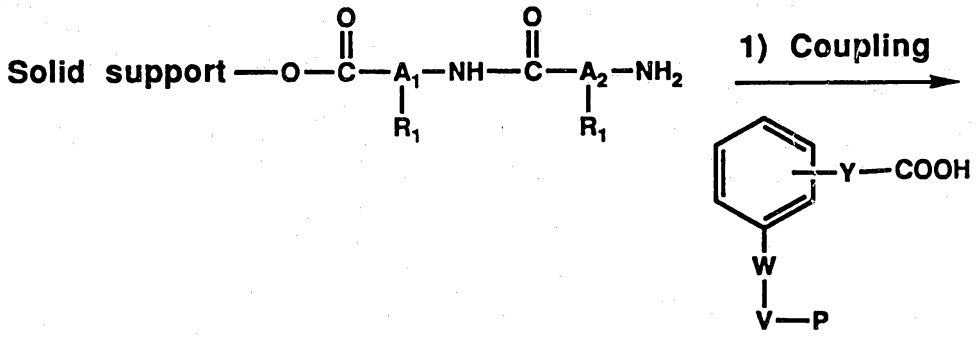
The invention also comprises pharmaceutical compositions useful for the prevention and treatment of thrombosis comprising an aforesaid compound in a pharmaceutically acceptable carrier.

Another aspect of this invention comprises a method for the prevention and treatment of thrombosis associated with the aforesaid diseases.

The compounds of the present invention may be readily prepared by standard solid phase or solution phase peptide synthesis techniques using starting materials and/or intermediates available from chemical supply companies such as Aldrich and Sigma or may be synthesized by standard organic chemical techinques. (H. Paulsen, G. Merz, V. Weichart, "Solid-Phase Synthesis of 0-Glycopeptide Sequences", Angew. Chem. Int. Ed. Engl. 27 (1988); H. Mergler, R. Tanner, J. Gosteli, and P. Grogg, "Peptide Synthesis by a Combination of Solid-Phase and Solution Methods I: A New Very Acid-Labile Anchor Group for the Solid-Phase Synthesis of Fully Protected Fragments". Tetrahedron letters 29, 4005 (1988); Merrifield, R.B., "Solid Phase Synthesis after 25 years: The Design and Synthesis of Antagonists of Glucagon", Makromol. Chem. Macromol. Symp. 19, 31(1988)).

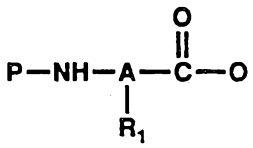
The solid phase method is represented schematically as follows:



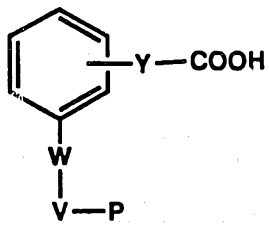


wherein:

the solid support may be, but is not limited to, p-alkoxybenzyl alcohol resin;



is a protected amino acid derivative;



is an N- protected derivative of an amino, amidino or guanidino acid.

In the process of making the desired compound, the amino acid derivatives are added one at a time to the insoluble resin to give the desired dipeptide resin derivative, then the amino or guanidino acid derivative is coupled to the N-terminal of the chain. Any reactive functional groups of these derivatives are blocked by protecting groups to prevent cross reactions during the coupling procedures. These protecting groups include, but are not limited to, tertiary butoxycarbonyl (BOC), carbobenzoxy (CBZ), benzyl, t-butyl,

9-fluorenylmethoxycarbonyl (FMOC) and methoxy-2,3,6-trimethylbenzene-sulfonyl (MTR).

Upon completion of each coupling reaction, the N-terminal amino protecting group is removed by standard procedures and the deprotected amino group coupled to a derivative having a free carboxylic acid function. The procedure is repeated until the desired product derivative is formed. The final product is obtained by deprotection and cleavage of the product from the resin by standard techniques.

Alternatively, the compounds of the present invention may be prepared in solution, i.e., without using a solid support. In a manner that is similar to the solid phase synthesis, starting with a protected amino acid derivative with a free N-terminal amino group, the protected derivatives are coupled, then deprotected using standard procedures.

The invention will now be explained further by the following illustrative examples:

EXAMPLE 1

N-[3-(2-guanidinoethyl)benzoyl]-L-aspartyl-L-valine

A. A solution of 2.0 g of 3-trifluoromethylphenylacetonitrile in 5 ml of ether was added dropwise to a solution of 0.50 g of lithium aluminum hydride in 20 ml of ether, while cooling at 0°C. The mixture was then stirred at room temperature for four hours and then quenched by sequential addition of 0.5 ml of water, 0.5 ml of 15% sodium hydroxide solution and 1.5 ml of water. The mixture was filtered and the filtrate dried over magnesium sulfate. The filtered solution was acidified with a 1N hydrogen chloride solution in ether and the solid which precipitated was collected to give 2-(3-trifluoromethylphenyl)ethyl amine hydrochloride.

B. 1.38 g of 2-(3-trifluoromethylphenyl)ethyl amine hydrochloride was heated at 100°C in 3.5g concentrated sulfuric acid for three hours according to the method of Nikolaus, or disclosed in U.S. Patent 3,792,048, which is incorporated herein by reference. The cooled solution was diluted with 100 ml

of ether and the resulting precipitate collected to give 3-(2-aminoethyl)benzoic acid as the sulfate salt.

C. 1.38 g of the amine salt product from Example 1B was dissolved in 10 ml water and 1N sodium hydroxide solution was added to bring the pH up to 7. The guanidine was then prepared essentially by the method of Miller, et. al., Synthesis, 777(1986), which is incorporated herein by reference. To the amine solution was added 0.849g of potassium carbonate, then 0.76g aminoimino-methanesulfonic acid was added, portionwise, over 10 minutes. The mixture was stirred at room temperature for four hours. Upon reduction of the volume by half, in vacuo, a precipitate formed which was collected and recrystallized from water. The solid was suspended in 20% aqueous tetrahydrofuran. 1N hydrogen chloride in ether was added to give a homogeneous solution which was evaporated in vacuo. The residue was crystallized from methanol/ether to give 3-(2-guanidinoethyl)benzoic acid hydrochloride.

D. 0.89 g N-(9-fluorenylmethoxycarbonyl)-L-valine-p-alkoxybenzyl alcohol resin ester (containing approximately 0.5 mmol of amino acid) was deprotected by shaking with 10 ml of a solution of 20% piperidine in dimethylformamide for one hour. The mixture was filtered and the resin derivative washed with methylene chloride to give L-valine-p-alkoxy benzyl alcohol resin ester.

E. The product from Example 1D was shaken with 0.822g N-FMOC-L-aspartic acid-β-t-butyl ester, 0.38 g 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC), 0.270g 1-hydroxybenzotriazole (HOBT) and 0.28 ml of triethylamine in 10 ml of dimethylformamide for two hours. The mixture was filtered and the resin derivative washed with methylene chloride. The resin derivative was then deprotected as in Example 1D to give L-aspartyl-β-t-butyl ester-L-valine-p-alkoxybenzyl alcohol resin ester.

F. 0.25g 3-(2-guanidinoethyl)benzoic acid hydrochloride was shaken with the product from Example 1E, 0.191g EDC, 0.135g HOBT and 0.14 ml triethylamine in 10 ml of dimethylformamide for two hours. The mixture was filtered and washed with methylene chloride. The β-t-butyl ester blocking group was removed, and the product cleaved from the resin, by treating with 95% trifluoroacetic acid (10 ml) for two hours. The resin was removed by filtration and the filtrate diluted with water, washed with ethyl acetate and

lyophilized to give N-3-[2-(guanidinoethyl)benzoyl]-L-aspartyl-L-valine as the trifluoroacetate salt.

EXAMPLE 2

N-[4-(2-guanidinoethyl)benzoyl]-L-aspartyl-L-valine

A. When 4-trifluoromethylphenylacetonitrile was treated in a manner similar to that in Example 1A, 2-(4-trifluoromethylphenyl)ethyl amine hydrochloride was obtained.

B. When the amine from Example 2A was treated in a manner similar to that in Example 1B, 4-(2-aminoethyl)benzoic acid sulfate was obtained.

C. When the benzoic acid derivative from Example 2B was treated in a manner similar to that in Example 2C, 4-(2-guanidinoethyl)benzoic acid hydrochloride was obtained.

D. 0.272g 4-(2-guanidinoethyl)benzoic acid hydrochloride and L-aspartyl-β-t-butyl ester-L-valine-p-alkoxybenzyl alcohol resin ester (prepared from 1.0g N-FMOC-L-valine-p-alkoxybenzyl resin ester as in Example 1D,E) were reacted together in the presence of 0.214g EDC, 0.151g HOBT, and 0.16 ml triethylamine in 10 ml of dimethylformamide in a manner similar to that of Example 1F. The product was deprotected and cleaved from the resin as in Example 1F to give N-[4-(2-guanidinoethyl)benzoyl]-L-aspartyl-L-valine trifluoroacetate.

EXAMPLE 3

N-(3-guanidinobenzoyl)-L-aspartyl-L-valine

A. 3-guanidinobenzoic acid was prepared from 3-aminobenzoic acid by the method of Miller, et. al., cited in Example 1C. The guanidine was treated with ethereal hydrogen chloride to give 3-guanidinobenzoic acid hydrochloride.

B. 3-guanidinobenzoic acid hydrochloride was treated in a manner similar to that in Examples 1F and 2D to give N-(3-guanidinobenzoyl)-L-aspartyl-L-valine.

EXAMPLE 4

N-(4-guanidinomethylbenzoyl)-L-aspartyl-L-valine

A. 4-guanidinomethylbenzoic acid hydrochloride was prepared from 4-aminomethylbenzoic acid in a manner similar to that of Example 3A.

B. 4-guanidinomethylbenzoic acid hydrochloride was treated in a manner similar to that of Examples 1F and 2D to give N-(4-guanidinomethylbenzoyl)-L-aspartyl-L-valine trifluoroacetate.

EXAMPLE 5

N-(3-guanidinomethylbenzoyl)-L-aspartyl-L-valine

A. 3-guanidinomethylbenzoic acid hydrochloride was prepared from 3-aminomethylbenzoic acid in a manner similar to that of Example 3A.

B. 3-guanidinomethylbenzoic acid hydrochloride was treated in a manner similar to that of Examples 1F and 2D to give N-(3-guanidinomethylbenzoyl)-L-aspartyl-L-valine as the trifluoroacetate salt.

EXAMPLE 6

N-(4-guanidinobenzoyl)-L-aspartyl-L-valine

A. 4-guanidinobenzoic acid hydrochloride was prepared from 4-aminobenzoic acid in a manner similar to that of Example 3A.

B. 4-guanidinobenzoic acid hydrochloride was treated in a manner similar to that of Examples 1F and 2D to give N-(4-guanidinobenzoyl)-L-aspartyl-L-valine as the trifluoroacetate salt.

EXAMPLE 7

N-[3-(2-aminoethyl)benzoyl]-L-aspartyl-L-valine

A. One equivalent of 3-(2-aminoethyl)benzoic acid (prepared as in Example 1B) is stirred with one equivalent of di-t-butyl-dicarbonate in the presence of two equivalents of sodium carbonate in tetrahydrofuran/water (1:1). The reaction mixture is evaporated to remove the tetrahydrofuran and the aqueous is acidified with dilute hydrochloric acid. The product is extracted into ethyl acetate and the solution is dried, then evaporated to give N-tert-butoxycarbonyl-3-(2-aminoethyl)benzoic acid.

B. If N-BOC-3-(2-aminoethyl)benzoic acid is substituted for 3-(2-guanidinoethyl)benzoic acid hydrochloride in Example 1F, and treated similarly, then N-[3-(2-aminoethyl)benzoyl]-L-aspartyl-L-valine is obtained as the trifluoroacetate salt.

EXAMPLE 8

N-(4-guanidinophenylacetoyl)-L-aspartyl-L-valine

A. If 4-aminophenylacetic acid is substituted for 3-(2-aminoethyl)benzoic acid in Example 1C, and treated similarly, then 4-guanidinophenylacetic acid hydrochloride is obtained.

B. If 4-guanidinophenylacetic acid hydrochloride is substituted for the benzoic acid in Example 1F and treated similarly, then N-(4-guanidinophenylacetoyl)-L-aspartyl-L-valine is obtained as the trifluoroacetate salt.

EXAMPLE 9

N-(4-aminophenylacetoyl)-L-aspartyl-L-valine

A. If 4-aminophenylacetic acid is substituted for the benzoic acid in Example 7A, and treated similarly, then N-tert-butoxycarbonyl-4-aminophenylacetic acid is obtained.

B. If N-BOC-4-aminophenylacetic acid is substituted for 3-(2-guanidinoethyl)benzoic acid hydrochloride in Example 1F, and treated similarly, then N-(4-aminophenylacetoyl)-L-aspartyl-L-valine is obtained as the trifluoroacetate salt.

EXAMPLE 10

4-Guanidinocinnamoyl-L-aspartyl-L-valine trifluoroacetate

A. To a solution of 5g (25 mmol) of 4-aminocinnamic acid hydrochloride in 25 ml of water containing 6.92g (50 mmol) of potassium carbonate was added 3.11g (25 mmol) of aminoiminomethanesulfonic acid (AIMSA) portionwise at room temperature. The reaction mixture was stirred 24 hours at room temperature and the product was filtered off and washed with cold water. The white solid was dissolved in 1N aqueous hydrochloric acid and concentrated in vacuo to give 4-guanidinocinnamic acid hydrochloride as a white solid.

B. A solution of 0.27g (1.12 mmol) of 4-guanidinocinnamic acid hydrochloride, 0.21g (1.12 mmol) of EDC, 0.15g (1.12 mmol) of HOBT and 0.16 ml of triethylamine in 10 ml of DMF was added to the L-aspartyl-β-t-butyl ester-L-valine-p-alkoxybenzyl alcohol resin (prepared as described in Example 1E) and shaking was continued for 1 hour. The solution was removed and the resin was washed with DMF (2 x 20 ml) and methylene chloride (3 x 10 ml).

A solution containing 9.5 ml of trifluoroacetic acid, 0.5 ml of water and several drops of 1,2-ethanedithiol was added to the resin and shaking was continued for 2 hours. The resin was filtered off and washed with trifluoroacetic acid. The filtrate was concentrated in vacuo. The residue was taken up in 0.5N acetic acid and washed with ethyl acetate (3 x 50 ml). The aqueous layer was lyophilized to a white powder which was purified by reverse phase (RP) HPLC using a C-18 column and a methanol/water gradient to give 4-guanidinocinnamoyl-L-aspartyl-L-valine trifluoroacetate.

EXAMPLE 11

4-Guanidinobenzoyl-N-ethylglycyl-L-aspartyl-L-valine trifluoroacetate

A. To 14.8 g of a 50% aqueous solution of glyoxylic acid was added 50 ml of water. The resulting solution was cooled to 0°C and treated with 10 ml of a 70% solution of ethylamine in water added by dropwise addition over 15 minutes. The reaction mixture was transferred to a Parr bottle, then 10% palladium on carbon was added and the reaction vessel was shaken under hydrogen at 44 psi for 24 hours. The reaction mixture was filtered through a celite pad and the filtrate was concentrated in vacuo to give a tan oil. The oil was treated with 1N aqueous HCl and concentrated in vacuo to give a solid which was recrystalized from acetic acid.

3.65 g of N-ethyl glycine hydrochloride was stirred in 35 ml of water. This was treated with 8.31 g of sodium carbonate and cooled to 0°C, followed by the dropwise addition of 6.77 g of 9-fluorenylmethyl chloroformate in 15 ml of tetrahydrofuran (THF). The reaction mixture was allowed to slowly warm to room temperature and stirred for 24 hours. The THF was removed in vacuo and the residue was diluted with water and extracted with ether. The aqueous fraction was acidified to pH < 2 with 1N aqueous HCl and extracted with ethyl acetate. The organic extracts (ethylacetate) were dried, filtered and concentrated to give N^{α}-FMOC-N^{α}-ethyl glycine as a white solid. All FMOC protected substituted glycines were made by this procedure simply by substituting another amine for ethyl amine in this procedure.

B. L-aspartyl-β-t-butyl ester-L-valine-p-alkoxybenzyl alcohol resin ester was prepared as described in Example 1E and treated with 0.33g of FMOC-N-ethyl glycine, from the preceding procedure, 0.191g EDC, 0.135g HOBT and 0.14 ml of triethylamine in 10 ml of DMF for two hours. The mixture was filtered and washed with methylene chloride. The FMOC protecting group was removed by the procedure described in Example 1D to give N-ethyl glycyl-L-aspartic acid-β-t-butylester-L-valine-p-alkoxybenzyl alcohol resin ester.

C. 0.204g of 4-guanidinobenzoic acid hydrochloride prepared as described in Example 6A was dissolved in 5 ml of DMF and treated with 0.10g of triethylamine. The solution was cooled to 0°C and 0.25g of bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) was added. The reaction mixture was stirred at 0°C for 5 minutes than the peptide resin from Example 11B was added. Shaking was continued for 2 hours at room temperature. Applying the procedure for removal of the peptide from the resin described in Example 1F,

resulted in 4-guanidinobenzoyl-N-ethyl glycyl-L-aspartyl-L-valine as the trifluoroacetate salt.

EXAMPLE 12

4-Guanidinocinnamoyl-N-ethyl glycyl-L-aspartyl-L-valine

This compound was prepared in a similar manner to the compound prepared in Example 11, by replacing 4-guanidinobenzoic acid hydrochloride in Example 11C with 4-guanidinocinnamic acid hydrochloride (prepared as described in Example 10A).

EXAMPLE 13

4-Guanidinohomocinnamoyl-L-aspartyl-L-valine

A. 4-Guanidinophenethyl alcohol was prepared from 4-aminophenethyl alcohol using the procedure described in Example 1C.

B. To a solution of 1.28g (7.15 mmol) of 4-guanidinophenethyl alcohol in 7.15 ml of a 4N aqueous sodium hydroxide and 35.75 ml of acetone was added 1.8g (7.15 mmol) of 4-methoxy-2,3,5-trimethylbenzenesulfonylchloride (Mtr protecting group) in 7.15 ml of acetone, dropwise at 0°C. After 4 hours acetone was removed in vacuo and the residue was diluted with water and brought to pH<5 with 1N aqueous hydrochloric acid. The mixture was extracted with ethyl acetate and the organic extracts were dried, filtered and concentrated to give 4-(N^g-Mtr-guanidino)-phenethyl alcohol.

1.75g (4.47 mmol) of the 4-(N^g-Mtr-guanidino)-phenethyl alcohol was added in a single portion to 1.44g (6.7 mmol) of pyridinium chlorochromate in 50 ml of methylene chloride. The resulting mixture was allowed to stir for 2 hours at room temperature and was then filtered through silica gel using 25% ethyl acetate in hexanes as eluent. The recovered aldehyde (1.20g , 3.1 mmol) was dissolved in 40 ml of chloroform and 1.03g (3.1 mmol) of methyl triphenylphosphoranylidine acetate was added in a single portion. The resulting solution was heated at reflux for 24 hours. Chloroform was removed in vacuo and the residue was taken up in ether and filtered. The filtrate was

concentrated in vacuo and subjected to flash chromatography to provide 4-(Nᵍ-Mtr-guanidino)-homocinnamic acid methyl ester.

The methyl ester (1.07g, 2.4 mmol) was dissolved in 16 ml of methanol and 4 ml of 1N aqueous sodium hydroxide. The solution was allowed to stir 3 hours at 60°C. Methanol was removed in vacuo and the residue was diluted with water, brought to pH<2 with 1N aqueous hydrochloric acid and extracted with ethyl acetate. The organic extracts were dried, filtered and concentrated to provide 4-(Nᵍ-Mtr-guanidino)-homocinnamic acid.

C. 4-(Nᵍ-Mtr-guanidino)-homocinnamic acid, when substituted for 4-guanidinocinnamic acid in Example 10B, was coupled to L-aspartyl-β-t-butyl ester-L-valine-p-alkoxybenzyl alcohol resin (which was prepared as described in Example 1E). The peptide was cleaved from the resin and deprotected when the 95% trifluoroacetic acid/5% water/5% ethanediol method of Example 10B was employed and the reaction time was extended to 24 hours. The isolation and purification of the peptide proceeded as described in Example 10B to give 4-guanidinohomocinnamoyl-L-aspartyl-L-valine trifluoroacetate was a white powder.

EXAMPLE 14

4-(Imidazol-1-yl)-cinnamoyl-L-aspartyl-L-valine trifluoroacetate

A. 4-(Imidazol-1-yl)-cinnamic acid hydrochloride salt was prepared according to the procedure of Lizuka, et al. described in U.S. Patent No. 4,226,878.

B. The 4-(imidazol-1-yl)-cinnamic acid hydrochloride salt when substituted for 4-guanidinocinnamic acid hydrochloride in Example 10B gave 4-(imidazol-1-yl)-cinnamoyl-L-aspartyl-L-valine trifluoroacetate.

EXAMPLE 15

4-Guanidinocinnamoyl-L-aspartyl-L-leucine amide trifluoroacetate

A. 0.50g of N-t-butoxycarbonyl(BOC)-L-leucine-p-methyl benzhydrylamine (MBHA) resin (containing 0.71 mmol of amino acid per gram of resin) was shaken with 50% trifluoroacetic acid in methylene chloride for 1 hour to remove the BOC group. The mixture was filtered and the resin washed successively with methylene chloride (CH_2Cl_2), 50% CH_2Cl_2 in dimethylformamide (DMF), 20% triethylamine in CH_2Cl_2, 50% CH_2Cl_2 in DMF and finally CH_2Cl_2 to give L-leucine MBHA resin.

B. The resin from Example 14A was shaken with 0.45g of N-BOC-β-cyclohexyl ester-L-aspartic acid, 0.19g of HOBT, 0.27g of EDC and 198 µl of triethylamine in 10 ml of DMF for 2 hours. The mixture was filtered and the resin was washed with DMF (4 x 10 ml) followed by treatment with 10 ml of 50% trifluoroacetic acid in CH_2Cl_2 for 1 hour. The resin was filtered and then washed with the same sequence of solvents listed in Example 14A to give L-aspartic acid-β-cyclohexyl ester-L-leucine-MBHA resin.

C. The resin from Example 14B was shaken with 0.34g of 4-guanidino-cinnamic acid, HCl, 0.19g of HOBT, 0.27g of EDC and 198 µl of triethylamine in 10 ml of DMF overnight. The resin was filtered and then washed with DMF and CH_2Cl_2. The peptide was cleaved from the resin and deprotected at the same time by treatment with hydrofluoric acid and lyophilized to give 200 mg of crude product. This was taken up into 150 ml of water, filtered and the filtrate was washed with ethyl acetate. The aqueous portion was then frozen and lyophilized to give a white powder which was purified by reverse phase HPLC using a C-18 reverse phase column and a methanol/water gradient. The purified fractions were lyophilized to give 4-guanidinocinnamoyl-L-aspartyl-L-leucine amide trifluoroacetate as a white powder.

Utilizing analogous procedures described in Examples 1-15 the following compounds are made:

4-Guanidinocinnamoyl-L-aspartyl-L-norvaline trifluoroacetate.

4-Guanidinocinnamoyl-N-ethyl-glycyl-L-aspartyl-L-valine ditrifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-norleucine trifluoroacetate.

4-Guanidinocinnamoyl-sarcosyl-L-aspartyl-L-leucine trifluoroacetate.

4-Guanidinocinnamoyl-β-alanyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-sarcosyl-L-aspartyl-L-isoleucine trifluoroacetate.

4-Guanidinocinnamoyl-sarcosyl-L-aspartyl-L-arginine ditrifluoroacetate.

4-Guanidinohomocinnamoyl-aspartyl valine trifluoroacetate.

4-(Imidazol-1-yl)-cinnamoyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-aspartyl-N^{Im}-methyl-D,L-tryptophan trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-histidine ditrifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-D-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-β-(2-naphthyl)alanine trifluoroacetate.

4-Guanidinocinnamoyl-sarcosyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinobenzoyl-N-ethylglycyl-L-aspartyl-L-valine trifluoroacetate.

4-Amidinobenzoyl-sarcosyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinobenzoyl-N-propylglycyl-L-aspartyl-L-valine trifluoroacetate.

4-(4-Guanidinophenyl)butyryl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinobenzoyl-sarcosyl-L-aspartyl-L-valine trifluoroacetate.

3-Guanidinobenzoylglycyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinobenzoylglycyl-L-aspartyl-L-valine trifluoroacetate.

α-Cyano-para-toluyl-L-aspartyl-L-valine.

4-Guanidinocinnamoyl-L-aspartic acid-α-isobutyl amide trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-tryptophan amide hydrogen fluoride.

4-Guanidinocinnamoyl-L-aspartyl-L-lysine amide dihydrogen fluoride.

4-Guanidinomethyl cinnamoyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-leucine-amide hydrogen fluoride.

4-Guanidnocinnamoyl-L-aspartyl-L-valine amide trifluoroacetate.

3-Guanidinocinnamoyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-proline trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-arginine ditrifluoroacetate.

4-Amidinocinnamoyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-asparagine trifluoroacetate.

4-Guanidinocinnamoyl-D-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-aspartic acid trifluoroacetate.

4-Guanidinocinnamoyl-glycyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-lysine ditrifluoroacetate.

4-(4-Guanidinophenyl)butyryl-L-aspartyl-L-valine trifluoroacetate.

4-Dimethylaminocinnamoyl-L-aspartyl-L-valine.

4-Aminocinnamoyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-threonine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-tryptophan trifluoroacetate.

4-Guanidinophenylthioacetoyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-serine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-tyrosine trifluoroacetate.

4-Guanidinophenoxyacetoyl-L-aspartyl-L-valine trifluoroacetate.

4'-Guanidinooxaniloyl-L-aspartyl-L-valine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-leucine trifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-isoleucyl-L-arginine ditrifluoroacetate.

4-Guanidinocinnamoyl-L-aspartyl-L-arginyl-L-isoleucine.

4-Guanidinocinnamoyl-N-(ethyl)-glycyl-L-aspartyl-L-isoleucine trifluoroacetate.

4-Guanidinocinnamoyl-N-(ethyl)-glycyl-L-aspartyl-L-leucine trifluoroacetate.

[2-(5'-Guanidinopentyl)benzoyl]-L-aspartyl-L-valine.

[2-(6'-Guanidinohexyl)benzoyl]-L-aspartyl-L-valine.

E-[2-(5'-guanidinopent-1'-enyl)benzoyl]-L-aspartyl-L-valine.

E-[2-(6'-guanidinohex-1'-enyl)benzoyl]-L-aspartyl-L-valine.

[2-(5'-Guanidinopent-1'-ynyl)benzoyl]-L-aspartyl-L-valine.

[2-(6'-Guanidinohex-1'-ynyl)benzoyl]-L-aspartyl-L-valine.

[2-(5'-Guanidinopentyl)phenyl]acetyl-L-aspartyl-L-valine.

[2-(4'-Guanidinobutyl)phenyl]acetyl-L-aspartyl-L-valine.

E-[2-(5'-guanidinopent-1'-enyl)phenyl]acetyl-L-aspartyl-L-valine.

E-[2-(4'-guanidinobut-1'-enyl)phenyl]acetyl-L-aspartyl-L-valine.

[2-(5'-Guanidinopent-1'-ynyl)phenyl]acetyl-L-aspartyl-L-valine.

[2-(4'-Guanidinobut-1'-ynyl)phenyl]acetyl-L-aspartyl-L-valine.

4-Guanidinocinnamoyl-L-aspartyl-D-α-benzyl-arginine-(D-ornithine).

4-Guanidinocinnamoyl-L-aspartyl-L-α-benzyl-arginine-(L-ornithine).

Compounds of the present invention were tested for inhibition of platelet aggregation using the following procedures:

I. Inhibition of Radiolabeled (^{125}I) Fibrinogen Binding Assay, which is essentially based on the method described in Proc. Natl. Acad. Sci. USA Vol. 83, pp. 5708-5712, Aug. 1986, and is as follows.

Platelets are washed free of plasma constituents by the albumin density-gradient technique. In each experimental mixture platelets in modified Tyrode's buffer are stimulated with human α-thrombin at 22-25°C for 10 minutes (3.125×10^{11} platelets per liter and thrombin at 0.1 NIH units/ml). Hirudin is then added at a 25-fold excess for 5 minutes before addition of the radiolabeled ligand and any competing ligand. After these additions, the final platelet count in the mixture is 1×10^{11}/liter. After incubation for an additional

30 minutes at 22-25°C, bound and free ligand are separated by centrifuging 50 µl of the mixture through 300 µl of 20% sucrose at 12,000xg for 4 minutes. The platelet pellet is then separated from the rest of the mixture to determine platelet-bound radioactivity. Nonspecific binding is measured in mixtures containing an excess of unlabeled ligand. When binding curves are analyzed by Scatchard analysis, nonspecific binding is derived as a fitted parameter from the binding isotherm by means of a computerized program. To determine the concentration of each inhibitory compound necessary to inhibit 50% of fibrinogen binding to thrombin-stimulated platelets (IC_{50}), each compound is tested at 0.176µgmol/1iter (60µg/ml). The IC_{50} is derived by plotting residual fibrinogen binding against the logarithm of the sample compound's concentration.

II. Inhibition of Fibrinogen - Mediated Platelet Aggregation, which is essentially based on the method described in Blood, Vol. 66, No. 4, Oct. 1985, pp. 846-952, and is as follows.

Human Platelets were isolated from freshly drawn whole blood and were suspended in 0.14 mol/L NaCl, 2.7 mmol/L K11, 12 mmol/L $NaHC0_3$, 0.42 mmol/L Na_2HP0_4, 0.55 mmol/L glucose, and 5 mmol/L Hepes, pH 7.35 at 2 x 10^8 platelets/ml. The suspension was incubated at 37°C. An aliquot of 0.4 ml of platelet suspension was activated by human thrombin at a final concentration of 2µg/ml of thrombin for one minute. After one minute the reaction was stopped by a thrombin inhibitor. Serial dilution of the compound being tested was then added to the activated platelet, the reaction was allowed to proceed for one minute, followed by the addition of human fibrinogen at a final concentration of 60µ/ml of fibrinogen. Platelet aggregation was then recorded by an aggregometer. Rate of aggregation was used to calculate IC_{50}.

Representative results of platelet aggregation inhibition are shown in Table I.

TABLE I

	Inhibition of ^{125}I-Fibrinogen Binding	Inhibition of Fibrinogen Mediated Platelet Aggregation	% inhibition at
	IC_{50}(μM)	IC_{50}(μM)	25 μM
3-(2-Guanidinoethyl)benzoyl-L-aspartyl-L-valine	115.0	33.1	37.0
4-(2-Guanidinoethyl)benzoyl-L-aspartyl-L-valine	10.9	7.7	85.0
4-Guanidinocinnamoyl-L-aspartyl-L-valine	2.0	2.8	-
4-Guanidinomethylphenylacetoyl-L-aspartyl-L-valine	*	-	42.0
3-(4-Guanidinophenyl)propanoyl-L-aspartyl-L-valine	4.5	14.0	65.0
4-(3-Guanidinopropyl)benzoyl-L-aspartyl-L-valine	44.0	7.6	83.3
4-Guanidinophenylacetoyl-L-aspartyl-L-valine	20.0	-	43.0
4-Guanidinobenzoyl-L-aspartyl-L-valine	*	-	33.0

* no inhibition of ^{125}I-Fibrinogen binding observed at concentrations of 50 μM or lower.

TABLE I (continued)

	Inhibition of ^{125}I-Fibrinogen Binding	Inhibition of Fibrinogen Mediated Platelet Aggregation	% inhibition at
	IC_{50}(μM)	IC_{50}(μM)	25 μM
3-Guanidinomethylbenzoyl-L-aspartyl-L-valine	*	30.2	53.0
4-Guanidinomethylbenzoyl-L-aspartyl-L-valine	22.0	-	16.0
3-Guanidinobenzoyl-L-aspartyl-L-valine	*	-	6.3
3-(Guanidinomethyl)phenylacetoyl-L-aspartyl-L-valine	-	-	15.2
3-(3-Guanidinopropyl)benzoyl-L-aspartyl-L-valine	*	-	52.0
4-Guanidinocinnamoyl-D-aspartyl-L-valine	38.0	~50.0	33
4-Guanidinocinnamoyl-L-aspartyl-D-valine	30.0	25.0	50
3-Guanidinocinnamoyl-L-aspartyl-L-valine	>200.0	-	7
4-Guanidinocinnamoyl-L-glutamyl-L-valine	>200.0	-	2

* no inhibition of ^{125}I-Fibrinogen binding observed at concentrations of 50 μM or lower.

TABLE I (continued)

	Inhibition of ^{125}I-Fibrinogen Binding	Inhibition of Fibrinogen Mediated Platelet Aggregation	% inhibition at
	IC_{50}(μM)	IC_{50}(μM)	25 μM
4-Amidinocinnamoyl-L-aspartyl-L-valine	50.0	-	36
4-(Imidazol-1-yl)-cinnamoyl-L-aspartyl-L-valine	>200.0	-	-
4-Guanidinocinnamoyl-glycyl-L-aspartyl-L-valine	5.0	6.6	75
4-Guanidinocinnamoyl-sarcosyl-L-aspartyl-L-valine	0.10	2.1	98
4-Guanidinohomocinnamoyl-L-aspartyl-L-valine	54.0	-	-
4-Guanidinocinnamoyl-L-aspartyl-L-leucine	4.8	1.7	90
4-Guanidinocinnamoyl-L-aspartyl-L-leucine amide	30.0	-	33
4-Guanidinocinnamoyl-L-aspartyl-L-arginine	0.34	0.66	96
4-Guanidinocinnamoyl-L-aspartyl-L-tryptophan	0.38	0.80	93

* no inhibition of ^{125}I-Fibrinogen binding observed at concentrations of 50 μM or lower.

TABLE I (continued)

	Inhibition of ^{125}I-Fibrinogen Binding $IC_{50}(\mu M)$	Inhibition of Fibrinogen Mediated Platelet Aggregation $IC_{50}(\mu M)$	% inhibition at 25 µM
4-Guanidinocinnamoyl-L-aspartyl-L-tryptophan amide	8.2	4.2	93
4-Guanidinocinnamoyl-L-aspartyl-L-phenylalanine	1.5	0.85	97
4-Guanidinocinnamoyl-L-aspartyl-L-asparagine	34.0	25.0	50
4-Guanidinocinnamoyl-L-aspartyl-L-serine	5.6	28.0	-
4-Guanidinocinnamoyl-L-aspartyl-L-tyrosine	2.2	1.3	85
4-Guanidinocinnamoyl-L-aspartyl-L-alanine	10.0	5.9	92
4-Guanidinocinnamoyl-L-aspartyl-L-aspartic acid	47.0	>75.0	22
4-Guanidinocinnamoyl-L-aspartyl-L-lysine	4.0	1.6	87
4-Guanidinocinnamoyl-L-aspartyl-L-lysine amide	10.5	11.4	75
4-Guanidinocinnamoyl-L-aspartyl-L-histidine	6.0	4.0	90

* no inhibition of ^{125}I-Fibrinogen binding observed at concentrations of 50 µM or lower.

TABLE I (continued)

	Inhibition of ^{125}I-Fibrinogen Binding	Inhibition of Fibrinogen Mediated Platelet Aggregation	% inhibition at
	IC_{50}(μM)	IC_{50}(μM)	25 μM
4-Guanidinobenzoyl-glycyl-L-aspartyl-L-valine	6.4	23.4	-
3-Guanidinobenzoyl-glycyl-L-aspartyl-L-valine	*	-	40
4-Guanidinobenzoyl-sarcosyl-L-aspartyl-L-valine	3.2	1.2	95
4-Amidinobenzoyl-sarcosyl-L-aspartyl-L-valine	110.0	-	17
4-Guanidinobenzoyl-N-ethylglycyl-L-aspartyl-L-valine	0.17	1.1	97
4-Guanidinobenzoyl-N-propylglycyl-L-aspartyl-L-valine	0.46	7.9	86
4-(4-Guanidinophenyl)-butyryl-L-aspartyl-L-valine	5.8	1.8	94

* no inhibition of ^{125}I-Fibrinogen binding observed at concentrations of 50 μM or lower.

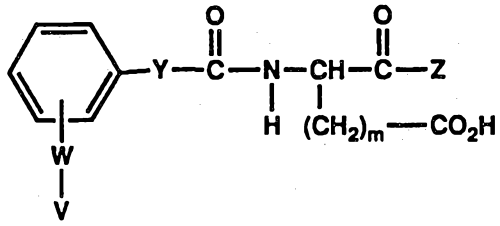
The compounds of the present invention may be orally or parenterally administered to mammals. The compound may be incorporated into pharmaceutical formulations having excipients suitable for these administrations and which do not adversely react with the compounds, for example, water, vegetable oils, certain alcohols and carbohydrates, gelatin and magnesium stearate. The pharmaceutical formulations containing an active compound of the present invention may be made into: tablets, capsules, elixirs, drops or suppositories for enteral administration; and solutions, suspensions or emulsions for parenteral administration.

In general, compounds of this invention is administered in dosages of approximately 1 to 200 mg per dosage unit or higher. The daily dosage is approximately 0.02-5 mg/kg of body weight. It is to be understood, however, that the particular dose for each patient as usually depends on very diverse factors, such as the age, body weight, general condition of health, sex, diet and the like of the patient, on the time and route of administration, on the rate of excretion, on the combination of medicaments and on the severity of the disease.

Having described the invention, it will be apparent to one of ordinary skill in the art that changes and modifications can be made thereto without departing from the spirit and scope of the invention as set forth herein.

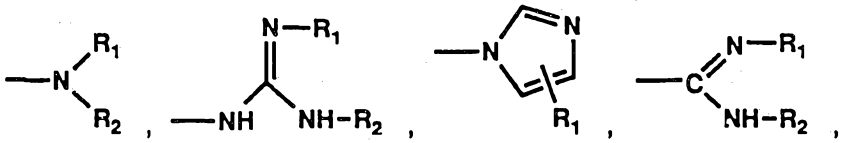
What is Claimed is:

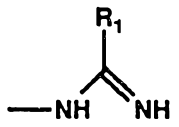
1. A compound of the formula



or a pharmaceutically acceptable salt thereof wherein:

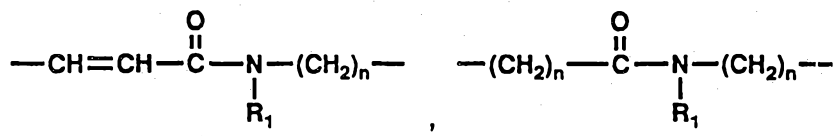
V is

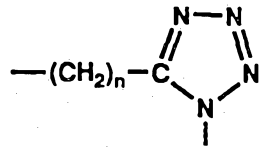


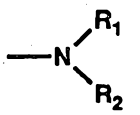
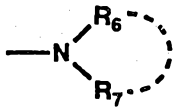

or -C≡N;

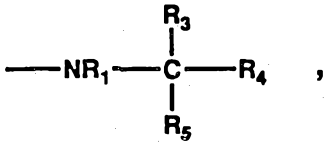
W is $-(CH_2)_n-$, $-CH=CH-(CH_2)_n-$, $-(CH_2)_n-CH=CH-$, $-C\equiv C-(CH_2)_n-$ or $-(CH_2)_n-C\equiv C-$;

Y is $-(CH_2)_n-$, $-CH=CH-CH_2-$, $-CH_2CH=CH-$, $-CH=CH-$,

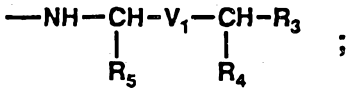



, $-CH_2-X_1-$ or $-X_1-CH_2-$ wherein X_1 is O, NH or S;

Z is -OR_1,  ,  , a D- or L-amino acid or its corresponding carboxamide, a synthetic amino acid of the formula

 ,

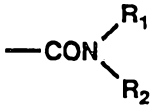
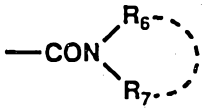
a dipeptide or a dipeptide isostere of the formula

 ;

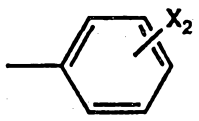
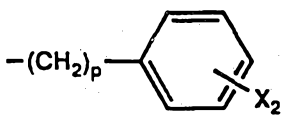
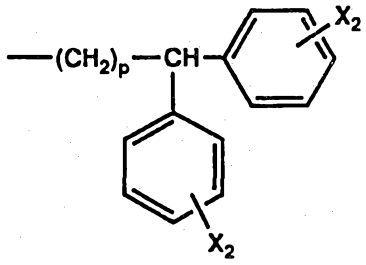
m is 1, 2 or 3;

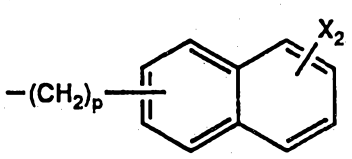
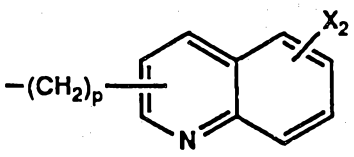
n is 0 to 6;

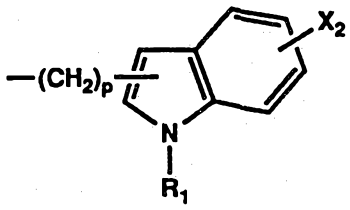
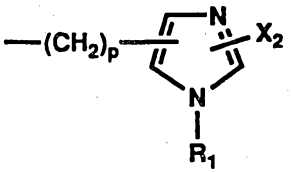
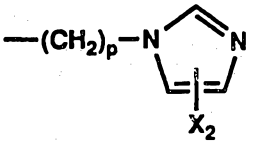
R_1 and R_2 are independently H, alkyl, aryl, aralkyl or allyl;

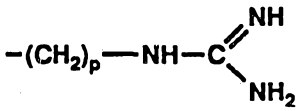
R_3 is H, -CO_2H, -CO_2R_1, -$CONH_2$,  or  ;

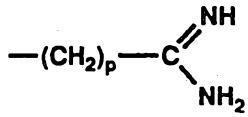
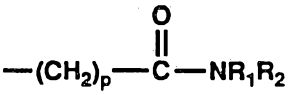
R_4 and R_5 are independently H, alkyl, cycloalkyl, cycloalkylmethyl,

 ,  ,  ,

 ,  ,

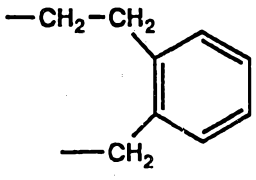




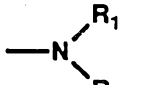
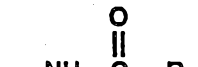
$-(CH_2)_p-OR_1$, $-(CH_2)_p-SR_1$, $-(CH_2)_p-NR_1R_2$, 

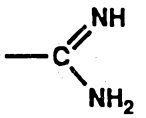
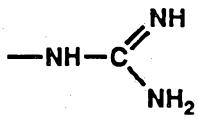
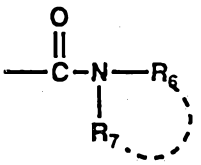
 , $-(CH_2)_p-CO_2R_1$ or  ;

p is 0 to 8;

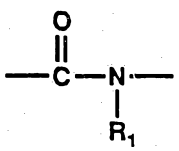
R_6 and R_7 form a ring with the nitrogen to which they are attached and are independently $-(CH_2)_4-$, $(CH_2)_5-$, $-(CH_2)_6-$, $-CH_2CH_2OCH_2CH_2-$,

$-CH_2CH_2NR_1CH_2-$ or  ;

X_2 is H, Cl, Br, F, $-OR_1$, $-NO_2$,  ,  , $-SR_1$,

C_1-C_5 alkyl, phenyl, $-CO_2R_1$,  ,  ,  ,

$-CF_3$ or $-NHSO_2R_1$; and

V_1 is  , $-(CH_2)_n-$, $-CH=CH-$, $-CH_2-NH-$, $-CH_2-O-$,

$-CH_2-S-$ or $-\overset{O}{\overset{\|}{C}}-CH_2-$.

2. A compound of Claim 1 wherein said D- or L-amino acid is selected from the group consisting of: Asp, Arg, Ala, Asn, Cys, Gly, Glu, Gln, His, Ile, Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Trp, Tyr and Val.

3. A compound of claim 1 wherein:

R_1 and R_2 are independently hydrogen or phenyl;

R_3 is H or $-CO_2H$;

R_4 and R_5 are independently H, alkyl or cycloalkyl;

m is 1;

n is 0;

p is 1; and

R_6 and R_7 are $-(CH_2)_4-$ forming a ring with the nitrogen to which they are attached.

4. A stereoisomeric compound of claim 1.

5. A diastereomeric compound of claim 1.

6. A pharmaceutical composition for the prophylaxis or treatment of abnormal thrombus formation in a mammal comprising a pharmaceutically acceptable carrier and a pharmaceutically active amount of a compound of claim 1.

7. A method of preventing or treating thrombus formation in a mammal comprising the administration of a composition of claim 6.

8. A compound of claim 1 which is:

N-[3-(2-guanidinoethyl)benzoyl]-L-aspartyl-L-valine;

N-[4-(2-guanidinoethyl)benzoyl]-L-aspartyl-L-valine;

N-(3-guanidinobenzoyl)-L-aspartyl-L-valine;

N-(4-guanidinomethylbenzoyl)-L-aspartyl-L-valine; or

N-(3-guanidinomethylbenzoyl)-L-aspartyl-L-valine.

9. A compound of claim 1 which is:

N-(4-guanidinobenzoyl)-L-aspartyl-L-valine;

N-[3-(2-aminoethyl)benzoyl]-L-aspartyl-L-valine;

N-(4-guanidinophenylacetoyl)-L-aspartyl-L-valine;

N-(4-aminophenylacetoyl)-L-aspartyl-L-valine; or

4-guanidinocinnamoyl-L-aspartyl-L-valine trifluoroacetate.

10. A compound of claim 1 which is:

4-Guanidinobenzoyl-N-ethyl glycyl-L-aspartyl-L-valine trifluoroacetate;

4-guanidinocinnamoyl-N-ethyl glycyl-L-aspartyl-L-valine;

4-Guanidinohomocinnamoyl-L-aspartyl-L-valine;

4-(imidazol-1-yl)-cinnamoyl-L-aspartyl-L-valine trifluoroacetate; or

4-guanidinocinnamoyl-L-aspartyl-L-leucine amide trifluoroacetate.

11. A compound of claim 1 which is:

4-Guanidinocinnamoyl-L-aspartyl-L-norvaline trifluoroacetate;

4-Guanidinocinnamoyl-N-(ethyl)-glycyl-L-aspartyl-L-valine ditrifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-norleucine trifluoroacetate;

4-Guanidinocinnamoyl-sarcosyl-L-aspartyl-L-leucine trifluoroacetate; or

4-Guanidinocinnamoyl-β-alanyl-L-aspartyl-L-valine trifluoroacetate.

12. A compound of claim 1 which is:

4-Guanidinocinnamoyl-L-sarcosyl-L-aspartyl-L-isoleucine trifluoroacetate;

4-Guanidinocinnamoyl-sarcosyl-L-aspartyl-L-arginine ditrifluoroacetate;

4-Guanidinohomocinnamoyl-aspartyl valine trifluoroacetate;

4-(Imidazol-1-yl)-cinnamoyl-L-aspartyl-L-valine trifluoroacetate; or

4-Guanidinocinnamoyl-aspartyl-N^{Im}-methyl-D,L-tryptophan trifluoroacetate.

13. A compound of claim 1 which is:

4-Guanidinocinnamoyl-L-aspartyl-L-histidine ditrifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-D-valine trifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-β-(2-naphthyl)alanine trifluoroacetate;

4-Guanidinocinnamoyl-sarcosyl-L-aspartyl-L-valine trifluoroacetate; or

4-Guanidinobenzoyl-N-ethylglycyl-L-aspartyl-L-valine trifluoroacetate.

14. A compound of claim 1 which is:

4-Amidinobenzoyl-sarcosyl-L-aspartyl-L-valine trifluoroacetate;

4-Guanidinobenzoyl-N-propylglycyl-L-aspartyl-L-valine trifluoroacetate;

4-(4-Guanidinophenyl)butyryl-L-aspartyl-L-valine trifluoroacetate;

4-Guanidinobenzoyl-sarcosyl-L-aspartyl-L-valine trifluoroacetate; or

3-Guanidinobenzoylglycyl-L-aspartyl-L-valine trifluoroacetate.

15. A compound of claim 1 which is:

4-Guanidinobenzoylglycyl-L-aspartyl-L-valine trifluoroacetate;

α-Cyano-para-toluyl-L-aspartyl-L-valine;

4-Guanidinocinnamoyl-L-aspartic acid-α-isobutyl amide trifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-tryptophan amide hydrogen fluoride; or

4-Guanidinocinnamoyl-L-aspartyl-L-lysine amide dihydrogen fluoride.

16. A compound of claim 1 which is:

4-Guanidinomethyl cinnamoyl-L-aspartyl-L-valine trifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-leucine-amide hydrogen fluoride;

4-Guanidnocinnamoyl-L-aspartyl-L-valine amide trifluoroacetate;

3-Guanidinocinnamoyl-L-aspartyl-L-valine trifluoroacetate; or

4-Guanidinocinnamoyl-L-aspartyl-L-proline trifluoroacetate.

17. A compound of claim 1 which is:

4-Guanidinocinnamoyl-L-aspartyl-L-arginine ditrifluoroacetate;

4-Amidinocinnamoyl-L-aspartyl-L-valine trifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-asparagine trifluoroacetate;

4-Guanidinocinnamoyl-D-aspartyl-L-valine trifluoroacetate; or

4-Guanidinocinnamoyl-L-aspartyl-L-aspartic acid trifluoroacetate.

18. A compound of claim 1 which is:

4-Guanidinocinnamoyl-glycyl-L-aspartyl-L-valine trifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-lysine ditrifluoroacetate;

4-(4-Guanidinophenyl)butyryl-L-aspartyl-L-valine trifluoroacetate;

4-Dimethylaminocinnamoyl-L-aspartyl-L-valine; or

4-Aminocinnamoyl-L-aspartyl-L-valine trifluoroacetate.

19. A compound of claim 1 which is:

4-Guanidinocinnamoyl-L-aspartyl-L-threonine trifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-tryptophan trifluoroacetate;

4-Guanidinophenylthioacetoyl-L-aspartyl-L-valine trifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-serine trifluoroacetate; or

4-Guanidinocinnamoyl-L-aspartyl-L-tyrosine trifluoroacetate.

20. A compound of claim 1 which is:

4-Guanidinophenoxyacetoyl-L-aspartyl-L-valine trifluoroacetate;

4'-Guanidinooxaniloyl-L-aspartyl-L-valine trifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-leucine trifluoroacetate;

4-Guanidinocinnamoyl-L-aspartyl-L-isoleucyl-L-arginine ditrifluoroacetate; or

4-Guanidinocinnamoyl-L-aspartyl-L-arginyl-L-isoleucine.

21. A compound of claim 1 which is:

4-Guanidinocinnamoyl-N-(ethyl)-glycyl-L-aspartyl-L-isoleucine trifluoroacetate;

4-Guanidinocinnamoyl-N-(ethyl)-glycyl-L-aspartyl-L-leucine trifluoroacetate;

[2-(5'-Guanidinopentyl)benzoyl]-L-aspartyl-L-valine;

[2-(6'-Guanidinohexyl)benzoyl]-L-aspartyl-L-valine; or

E-[2-(5'-guanidinopent-1'-enyl)benzoyl]-L-aspartyl-L-valine.

22. A compound of claim 1 which is:

E-[2-(6'-guanidinohex-1'-enyl)benzoyl]-L-aspartyl-L-valine;

[2-(5'-Guanidinopent-1'-ynyl)benzoyl]-L-aspartyl-L-valine;

[2-(6'-Guanidinohex-1'-ynyl)benzoyl]-L-aspartyl-L-valine;

[2-(5'-Guanidinopentyl)phenyl]acetyl-L-aspartyl-L-valine; or

[2-(4'-Guanidinobutyl)phenyl]acetyl-L-aspartyl-L-valine.

23. A compound of claim 1 which is:

E-[2-(5'-guanidinopent-1'-enyl)phenyl]acetyl-L-aspartyl-L-valine;

E-[2-(4'-guanidinobut-1'-enyl)phenyl]acetyl-L-aspartyl-L-valine; or

[2-(5'-Guanidinopent-1'-ynyl)phenyl]acetyl-L-aspartyl-L-valine.

24. A compound of claim 1 which is:

[2-(4'-Guanidinobut-1'-ynyl)phenyl]acetyl-L-aspartyl-L-valine;

4-Guanidinocinnamoyl-L-aspartyl-D-α-benzyl-arginine-(D-ornithine); or

4-Guanidinocinnamoyl-L-aspartyl-L-α-benzyl-arginine-(L-ornithine).

INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/02471

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

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

IPC(5): A61K 31/195, 31/215, 37/02; C07C 279/00 C07K 5/06, 5/08

US.Cl: 514/19, 563; 530/331; 560/34; 562/439

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	514/18, 19, 563; 530/331; 560/34; 562/439

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US, A, 4,105,789 (Ondetti et al.) 08 August 1978, see abstract.	1-24
A	US, A, 4,379,764 (Fujii et al.) 12 April 1983, see abstract.	1-24
A	US, A, 4,634,715 (Greenlee et al.) 06 January 1987, see abstract.	1-24
A	US, A, 4,870,207 (Umezawa et al.) 26 September 1989, see entire document.	1-24
A	US, A, 4,956,504 (Takeuchi et al.) 11 September 1990, see abstract.	1-24

* Special categories of cited documents: [15]

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

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

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

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

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

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

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

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

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
17 July 1991	08 OCT 1991
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	Lester L. Lee

Form PCT/ISA/210 (second sheet) (May 1986)