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[57] ABSTRACT

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

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ANTICOAGULANT PEPTIDES

ABSTRACT

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

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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. Propylactic administration of anticoagulants is believed to prevent a recurrance 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. Artrial thrombosis, particularly in arteries supplying the heart muscle and brain, is a leading cause of death.

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.

Applicants have 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. The peptides of this invention possess significant anticoagulant activity and their unusual ability to bind only to the recognition site without binding to the cleavage site of thrombin may allow for a scientifically interesting and therapeutically significant adjunct to anticoagulant therapy.

SUMMARY OF THE INVENTION

Peptide derivatives of the formula

$$X\text{-}A_1\text{-}A_2\text{-}A_3\text{-}A_4\text{-}A_5\text{-}A_6\text{-}A_7\text{-}A_8\text{-}A_9\text{-}A_{10}\text{-}Y$$

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 or t-butyloxy carbonyl;

A_1 is a bond or is a peptide containing from 1 to 5 residues of any amino acid;

A_2 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_3 is Glu or Asp;

A_4 is any amino acid;

A_5 is Ile, Val, Leu, Nle, or Thr;

A_6 is Pro, Hyp, 3,4-dehydroPro, thiazolidine-4-carboxylate, Sar, NMePgl or any amino acids having a D-configuration;

A_7 is any amino acid;

A_8 is Glu or Asp;

A_9 is a lipophilic amino acid selected from Tyr, Tyr(SO_3H), Trp, Phe, Leu, Nle, Ile, Val, His and Pro or is a dipeptide containing at least one of these lipophilic amino acids;

A_{10} 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;

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

Pro - proline

Phe - phenylalanine

Trp - tryptophan

Met - methionine

Ser - serine

Thr - threonine

Cys - cysteine

Tyr - tyrosine

Asn - asparagine

Gln - glutamine

Asp - aspartic acid

Glu - glutamic acid

Lys - lysine

Arg - arginine

His - histidine

Nle - norleucine

Hyp - hydroxyproline

3,4-dehydroPro - 3,4-dehydroproline

Tyr(SO_3H) - tyrosine sulfate

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

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, isopropyl, butyl, isobutyl, tert-butyl, pentyl, isopentyl, neo-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 and succinyl. 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, 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 "lipophilic amino acid" includes Tyr, Tyr (SO_3H), Phe, Leu, Nle, Ile, Val, His and Pro.

The natural amino acids with the exception of glycine, contain a chiral carbon atom. Unless otherwise spe-

cifically indicated, the optically active amino acids, referred to herein, are of the L-configuration. 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.

The polypeptides of formula I 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. Illustra-

tively, 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 -His-Asn-Asp-Gly-Asp-,

-Asn-Asp-Gly-Asp-,

-Asp-Gly-Asp-,

-Gly-Asp-,

-Asp-, or a bond.

A_2 is preferably Phe, β-2- or 3-thienylalanine, Tyr, Trp, or pClPhe;

A_3, Glu;

A_4, Glu, Asp, Pro or Ala;

A_5, Ile;

A_6, Pro, Sar, DAla, Hyp or NMePgl;

A_7, Glu, Asp or Ala;

A_8, Glu or Asp;

A_9, Tyr or a dipeptide fragment wherein at least one residue is Tyr or Tyr(SO_3H);

A_{10}, Leu, Asp, Asp-Glu, Leu-Gln, Leu-Pro, or Leu; and

Y, OH or NH_2.

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

A_2, is Phe; B-(2-thienylalanine) or Tyr;

A_3, Glu;

A_4, Gln or Pro;

A_5, Ile;

A_6, Pro;

A_7, Glu;

A_8, Gln or Asp;

A_9, Tyr, Ala-Tyr or Pro;

A_{10}, Gln; Asp; Leu-Pro; a bond; -Leu-Gln- or -Asp-Glu; and

Y, 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 combina-

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tions of these techniques. The solid phase sequential procedure can be performed using established automated methods such as by use of an automated peptide synthesizer. 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 polypeptide, 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, hy-

droxymethylated 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 trifluoroacetic 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, α-nitrophenoxyacetyl and α-chlorobutyryl; (2) aromatic urethan type protecting groups such as benzyloxycarbonyl and substituted benzyloxycarbonyl, such asp-chlorobenzyloxy-

cabbonyl, p-nitrobenzylcarbonyl, 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, adamantyloxycrbonyl and cyclohexyloxycarbonyl; (5) thio urethan type protecting group 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. After coupling agents are (1) carbodiimides (e.g., N,N'-dicyclohexylcarbodiimide and N-ethyl-N'-(Y-dimethylaminopropylcarbodiimide); (2) cyanamides (e.g., N,N-dibenzycyanamide); (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-nitrobenzyloxycarbonyl, 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 anticoagulant dose of a peptide derivative 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 derivative 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.

Anticoagulant therapy is indicated for the treatment and prevention of a variety of thrombotic conditions, particularly coronary artery and cerebrovascular disease. Those experienced in this field are readily aware of the circumstances requiring anticoagulant 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 preparations; or by application to the mucous membranes, such as, that of the nose, throat and bronchial tubes, for example, in an aerosol can containing 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, non-limiting examples.

EXAMPLE 1

Preparation of H-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

The peptide was synthesized by solid-phase methods using 0.1 mmol of a 0.66 mmol/g Boc-Gln-PAM resin. Double symmetrical anhydride couplings were performed with 2.0 mmol Na-Boc-amino acid (Peptides International) except in the case of Boc-Gln, which was coupled by the DCC/HOBT method. The side chain protection utilized was: Asp(Chx), Glu(Bzl), Tyr(2-BrZ). 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 chlo-

ride, acetylated with N-acetylimidazole 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.

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 leaving 101 mg of the desired produce (58% yield based on initial resin substitution). Homogeneity was determined by HPLC and TLC. HPLC Vydac 218TP54 (250 x 4.6 mm) C^{18} column, 2 ml/min, t_o = 1.9 min: time of elution with a 15-40% acetonitrile in 0.1% trifluoroacetic acid linear gradient at 1%/min. (HPLC) is 14.4 min.

TLC: [Merck 5715 20 x 20 cm Silica gel 60 plates (0.25 mm thickness)] n-butanol/acetic acid/water/pyridiene (6:1.2:4.8:6) (TLC I) Rf = 0.42; isopropanol/conc. ammonium hydroxide/water (3:1:1) (TLC II) Rf = 0.32; n-butanol/acetic acid/water (4:5:5) (TLC III) Rf = 0.70. FAB-MS: (M + H) - 1468.4 ± 1 mu (calcd. 1468.6). Amino acid

analysis: (6N HCl hydrolysis: 24 hr. at 106°C): Asx 1.03 (1); Glx (5.05 (5); Pro 1.03 (1); Gly 1.00 (1); Ile 0.97 (1); Leu 1.01 (1); Tyr 0.93 (1); Phe 0.98 (1); NH_3 1.06 (1). M.280 = 1254. 85% peptide content by weight.

In the same manner, the peptides of the following example 2-32 were prepared.

EXAMPLE 2

Ac-Ser-His-Asn-Asp-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 3

H-Asn-Asp-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 4

H-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 5

H-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 6

H-Asp-Phe-Glu-Ala-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 7

H-Asp-Phe-Glu-Glu-Ile-Pro-Ala-Glu-Tyr-Leu-Gln-OH

EXAMPLE 8

H-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Ala-OH

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EXAMPLE 9

H-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Glu-Leu-Ala-OH

EXAMPLE 10

Ac-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 11

Ac-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 12

Suc-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 13

H-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-NH_2

EXAMPLE 14

H-Gly-Asp-Tyr-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 15

H-Gly-Asp-Trp-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 16

H-Gly-Asp-pClPhe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 17

H-Gly-Asp-pNO_2Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 18

H-Gly-Asp-Phe-Glu-Glu-Ile-Sar-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 19

H-Gly-Asp-Phe-Glu-Glu-Ile-DAla-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 20

H-Gly-Asp-Phe-^{D}Glu-Glu-Ile-Hp-^{D}Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 21

H-Gly-Asp-Phe-^{D}Glu-Glu-Ile-NMePgl-Glu-^{D}Glu-Tyr-Leu-Gln-OH

EXAMPLE 22

H-Gly-Asp-Phe-^{D}Glu-Pro-Ile-Pro-^{D}Glu-Asp-Ala-Tyr-Asp-^{D}Glu-OH

EXAMPLE 23

Suc-Phe-^{D}Glu-Pro-Ile-Pro-^{D}Glu-Glu-Tyr-Leu-Pro-NH_2

EXAMPLE 24

H-Gly-Asp-Phe-^{D}Glu-Pro-Ile-Pro-Glu-Asp-Ala-Tyr-Pro-NH_2

EXAMPLE 25

Suc-Phe-^{D}Glu-Pro-Ile-Pro-Glu-Asp-Ala-Tyr-Pro-NH_2

EXAMPLE 26

H-Asp-Phe-Glu-Pro-Ile-Pro-^{D}Glu-Glu-Ala-Tyr-Gln-OH

EXAMPLE 27

H-Asp-Phe-Glu-Glu-Ile-Pro-Glu-^{D}Glu-Ala-Tyr-Glu-OH

EXAMPLE 28

H-Asp-Phe-^{D}Glu-Glu-Ile-Pro-^{D}Glu-Asp-Ala-Tyr-Gln-OH

EXAMPLE 29

Suc-Phe-^{D}Glu-Pro-Ile-Pro-Glu-Glu-Tyr-Leu-Pro-NH_2

EXAMPLE 30

H-Gly-Asp-D-(2-thienyl)alanyl-Glu-Glu-Ile-Pro-Glu-Gln-Tyr-Leu-Gln-OH

EXAMPLE 31

H-Gly-Ala-O-methyltyrosyl-Gln-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH

EXAMPLE 32

Suc-Phe-Gln-Pro-Ile-Pro-Glu-Glu-Pro-OH

The peptides of examples 2-32 have the following properties:

EXAMPLE NO.	Amino Acids Analysis (6N HCl Hydrolysis; 24 Hrs at 106° C)										
	His	Asx	Ser	Glx	Pro	Ala	Gly	Ile	Leu	Tyr	Phe
2	0.95(1)	3.12(3)	0.88(1)	5.15(5)	1.11(1)		1.03(1)	0.95(1)	1.00(1)	0.84(1)	0.99(1)
3		3.03(3)		5.02(5)	1.03(1)		1.01(1)	0.97(1)	1.02(1)	0.93(1)	0.99(1)
4		1.02(1)		5.04(5)	1.01(1)			0.98(1)	1.03(1)	0.89(1)	1.03(1)
5				5.05(5)	1.04(1)			0.97(1)	1.01(1)	0.93(1)	0.99(1)
6		0.94(1)		4.48(4)	0.94(1)	0.88(1)		0.94(1)	0.98(1)	0.89(1)	0.95(1)
7		0.94(1)		4.46(4)	0.96(1)	0.89(1)		0.92(1)	0.98(1)	0.87(1)	0.98(1)
8		1.03(1)		4.00(4)	1.08(1)	1.02(1)		0.99(1)	1.01(1)	0.88(1)	0.99(1)
9		1.03(1)		4.83(5)	1.08(1)	1.01(1)		0.99(1)	1.04(1)		1.01(1)
10		1.03(1)		5.02(5)	1.00(1)			0.96(1)	1.01(1)	0.94(1)	1.03(1)
11		0.99(1)		5.02(5)	0.98(1)		0.98(1)	0.98(1)	1.03(1)	1.02(1)	1.00(1)
12				5.10(5)	1.01(1)			0.92(1)	1.03(1)	0.91(1)	1.02(1)
13		1.04(1)		3.93(4)	1.11(1)			0.95(1)	1.03(1)	0.90(1)	1.02(1)
14		0.99(1)		5.09(5)	1.00(1)		0.99(1)	1.00(1)	0.97(1)	1.89(2)	
15		0.60(1)		4.87(5)	1.14(1)		1.04(1)	0.97(1)	1.02(1)	0.94(1)	
16		1.10(1)		4.97(5)	1.07(1)		0.99(1)	0.94(1)	1.05(1)	0.88(1)	
17		0.41(1)		5.03(5)	0.99(1)		1.00(1)	0.96(1)	1.02(1)	0.99(1)	
18		1.01(1)		4.99(5)			1.00(1)	1.02(1)	1.03(1)	0.90(1)	1.05(1)
19		1.03(1)		4.95(5)		0.98(1)	1.03(1)	1.03(1)	1.02(1)	0.92(1)	1.03(1)
20		1.07(1)		4.94(5)			1.02(1)	1.01(1)	1.02(1)	0.93(1)	1.03(1)
21		1.09(1)		4.96(5)			1.08(1)	0.96(1)	0.94(1)	0.89(1)	1.09(1)
22		2.42(3)		3.07(3)	2.24(2)		1.05(1)	1.06(1)		1.01(1)	1.09(1)

*(Me)Tyr coelutes but was not quantitated.

EXAMPLE NO.	Amino Acids Analysis (6N HCl Hydrolysis; 24 Hrs at 106° C)										
	His	Asx	Ser	Glx	Pro	Ala	Gly	Ile	Leu	Tyr	Phe
23											
24		2.01(2)		2.03(2)	3.02(3)	1.00(1)	1.00(1)	0.95(1)		0.99(1)	0.99(1)
25		1/07(1)		2.03(2)	3.00(3)	1.00(1)		0.95(1)		0.96(1)	0.99(1)
26		1.00(1)		4.06(4)	2.01(2)	0.98(1)		0.97(1)		1.00(1)	0.99(1)
27		1.00(1)		5.04(5)	1.02(1)	0.97(1)		0.98(1)		0.99(1)	1.00(1)
28		2.01(1)		4.03(4)	1.01(1)	1.00(1)		0.96(1)		0.99(1)	1.01(1)
29				3.08(3)	3.00(3)			0.96(1)	1.01(1)	0.95(1)	
30		1.03(1)		5.03(5)	0.99(1)		1.01(1)	0.95(1)	1.03(1)	0.96(1)	
31		0.97(1)		5.17(5)	1.00(1)		0.95(1)	0.93(1)	0.98(1)	*	
32				3.05(3)	3.00(3)			0.96(1)	0.99(1)		

*(Me)Tyr coelutes but was not quantitated.

Physical Characteristics						
EXAMPLE NO.	HPLC t_r (min) (15-40% gradient)	TLC I (Rf)	TLC II (Rf)	TLC III (Rf)	FAB-MS (M + H)	ε_{280}
2					1963	
3	14.3	0.33	0.33	0.70	1698	1203
4	14.2	0.44	0.39	0.74	1412	1395
5	12.6	0.51	0.43	0.72	1296	1210
6	14.3	0.54	0.78	0.84	1354	1235
7	14.2	0.51	0.72	0.83	1354	1309
8	18.5	0.53	0.77	0.82	1355	1177
9	12.5	0.42	0.57	0.77	1321	N.D.
10	17.1				1453	1548
11	16.7				1511	1538
12	18.4				1397	1438
13	14.0				1282	1341
14	12.6				1485	2693
15	15.3				1507	3691
16	15.7				1502	2191
17	15.0				1514	7380
18	12.9				1442	739
19	15.6				1442	1285
20	11.8				1484	1092
21	19.0				1518	1490
22	10.0				1496	1135
23	17.8				1333	1160
24	10.7				1347	1318

Physical Characteristics						
EXAMPLE NO.	HPLC t_r (min) (15-40% gradient)	TLC I (Rf)	TLC II (Rf)	TLC III (Rf)	FAB-MS (M + H)	ε_{280}
25	13.7				1276	1187
26	10.0				1337	1831
27	9.9				1370	1305
28	8.8				1355	1265
29	17.8				1333	1004
30	13.6				1474	1330
31	14.8				1530*	2373
32	12.6				1057	N.D.

*(M + Na)

CLAIMS:

1. A peptide derivative of the formula

$$X\text{-}A_1\text{-}A_2\text{-}A_3\text{-}A_4\text{-}A_5\text{-}A_6\text{-}A_7\text{-}A_8\text{-}A_9\text{-}A_{10}\text{-}Y$$

wherein X is a hydrogen, or one or two acyl groups of from 2 to 10 carbon atoms;

A_1 is Gly-Asp, Ser-His-Asn-Asp-Gly-Asp, Asn-Asp-Gly-Asp, Asp, Gly-Ala or a bond,

A_2 is Phe, β-(2- and 3-thienyl)alanine, Tyr, or Trp;

A_3 is Glu;

A_4 is Glu, Ala or Gln

A_5 is Ile;

A_6 is Pro, Hyp, 3,4-dehydroPro, Sar, NMePgl, or D-Ala;

A_7 is Glu, Ala or Gln;

A_8 is Glu, Asp, or Ala;

A_9 is a lipophilic amino acid selected from Tyr, Leu, and Pro or is a dipeptide containing at least one of these lipophilic amino acids;

A_{10} is a Leu-Gln, Leu-Ala, Leu, Asp-Glu, Pro, Gln, Glu, Asp, D-Asp, Phe-Lys, CH_2, D-Lys, Leu-Pro, Leu-Orn, Tyr-Glu, Tyr-Gln, Lys or a bond.

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Y is a carboxy terminal residue selected from OH, and amino.

2. A peptide derivative of Claim 1 wherein A_2 is Phe, β-(2- or 3-thienyl)alanine, or Tyr.

3. A peptide derivative of Claim 1 wherein A_3 is Glu.

4. A peptide derivative of Claim 1 wherein A_4 is Glu, Ala, or Pro.

5. A peptide derivative of Claim 1 wherein A_5 is Ile.

6. A peptide derivative of Claim 1 wherein A_6 is Pro.

7. A peptide derivative of Claim 1 wherein A_7 is Glu or Ala.

8. A peptide derivative of Claim 1 wherein A_8 is Glu or Asp.

9. A peptide derivative of Claim 1 wherein A_9 is Tyr-Leu or Ala-Tyr.

10. A peptide derivative of Claim 1 wherein A_{10} is Gln, Asp, Pro, a bond, Asp-Glu, Glu or Ala.

11. A peptide derivative of Claim 1 wherein X is H, acetyl, or succinyl.

12. A peptide derivative of Claim 1 wherein Y is OH or NH_2.

13. A peptide derivative of Claim 1 wherein A_1 is

-Ser-His-Asn-Asp-Gly-Asp,

Asn-Asp-Gly-Asp,

Asp-Gly-Asp,

Gly-Asp,

Asp, or

a bond.

14. A peptide derivative of Claim 1 wherein

X is H,

A_1 is -Gly-Asp-,

A_2 is Phe,

A_3 is Glu,

A_4 is Glu,

A_5 is Ile,

A_6 is Pro,

A_7 is Glu,

A_8 is Glu,

A_9 is Tyr-Leu,

A_{10} is Gln, and

Y is OH.

15. A peptide derivative of Claim 1 wherein

X is Suc,

A_1 is a bond,

A_2 is Phe,

A_3 is Gly,

A_4 is Glu,

A_5 is Ile,

A_6 is Pro,

A_7 is Glu,

A_8 is Glu,

A_9 is Tyr-Leu,

A_{10} is Gln, and

Y is OH.

16. A peptide derivative of Claim 1 wherein

X is H

A_1 is Gly-Asp,

A_2 is Phe,

A_3 is Glu,

A_4 is Pro,

A_5 is Ile,

A_6 is Pro,

A_7 is Glu,

A_8 is Asp,

A_9 is Ala-Tyr,

A_{10} is Asp-Glu, and

Y is OH.

17. A peptide derivative of Claim 1 wherein

X is Suc,

A_1 is a bond,

A_2 is Phe,

A_3 is Glu,

A_4 is Pro,

A_5 is Ile,

A_6 is Pro,

A_7 is Glu,

A_8 is Glu,

A_9 is Tyr-Leu,

A_{10} is Pro, and

Y is NH_2.

18. A peptide derivative of Claim 1 which is Suc-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-Gln-OH.

19. A method of reducing blood coagulation in a patient in need thereof which comprises administering an anticoagulant effective amount of a peptide derivative of Claim 1 and a pharmaceutically acceptable carrier.

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