BETA-HAIRPIN PEPTIDOMIMETICS AS SELECTIVE ELASTASE INHIBITORS

The present invention provides β-hairpin peptidomimetics which are useful as inhibitors of protease enzymes and are embraced by the general disclosure of , but not specifically disclosed in WO2006/087001 A1.

The β-hairpin peptidomimetics of the invention are compounds of general formula cyclo(Xaa^1-Xaa^2-Thr^3-Xaa^4-Ser^5-Xaa^6-Xaa^7-Xaa^8-Xaa^9-Xaa^{10}-Xaa^{11}-Xaa^{12}-Xaa^{13}-), and pharmaceutically acceptable salts thereof, with Xaa^1, Xaa^2, Xaa^4, Xaa^6, Xaa^7, Xaa^8, Xaa^9, Xaa^{10}, Xaa^{11}, Xaa^{12} and Xaa^{13} being amino acid residues of certain types which are defined in the description and the claims.

These β-hairpin peptidomimetics are useful as inhibitors of protease enzymes and are especially valuable as inhibitors of certain serine proteases such as elastase.

In addition the present invention provides an efficient process by which these compounds can, if desired, be made in library-format.

The β-hairpin peptidomimetics of the invention show high inhibitory activity against human neutrophil elastase while having low inhibitory activity against proteinase 3 and an unexpected low inhibitory activity against porcine pancreatic elastase (PPE). These favourable activity/selectivity profiles depend on the proper choice of certain types of α-amino acid residues and their positions in the monocyclic peptidomimetic.

Inhibitors of proteases are emerging with promising therapeutic uses in the treatment of diseases such as cancers (R. P. Beckett, A. Davidson, A. H. Drummond, M. Whittaker, *Drug Disc. Today* **1996**, *1*, 16-26; L. L. Johnson, R. Dyer, D. J. Hupe, *Curr. Opin. Chem. Biol*. **1998**, *2*, 466-71; D. Leung, G. Abbenante, and D. P. Fairlie, *J. Med. Chem*. **2000**, *43*, 305-341, T. Rockway, *Expert Opin. Ther. Patents* **2003**, *13*, 773-786), parasitic, fungal, and viral infections [e.g. schistosomiasis (M. M. Becker, S. A. Harrop,

J. P. Dalton, B. H. Kalinna, D. P. McManus, D. P. Brindley, *J. Biol. Chem*. **1995**, *270*, 24496-501); *C. albicans* (C. Abad-Zapetero, R. Goldman, S. W. Muchmore, C. Hutchins, K. Stewart, J. Navaza, C. D. Payne, T. L. Ray, *Protein Sci*. **1996**, *5*, 640-52), HIV (A. Wlodawer, J. W. Erickson, *Annu. Rev. Biochem*. **1993**, *62*, 543-85; P. L. Darke, J. R. Huff, *Adv. Pharmacol*. **1994**, *5*, 399-454), hepatitis (J. L. Kim, K. A. Morgenstern, , C. Lin, T. Fox, M. D. Dwyer, J. A. Landro, S. P. Chambers , W. Markland, C. A. Lepre, E. T. O'Malley, S. L. Harbeson, C. M. Rice, M. A. Murcko, P. R. Caron, J. A. Thomson, *Cell*, **1996**, *87*, 343-55; R. A. Love, H. E. Parge, J. A. Wickersham, Z. Hostomsky, N. Habuka, E. W. Moomaw, T. Adachi, Z. Hostomska, *Cell*, **1996**, *87*, 331-342), herpes (W. Gibson, M. R. Hall, Drug. Des. Discov. 1997, 15, 39-47)], and inflammatory, immunological, respiratory (P. R. Bernstein, P. D. Edwards, J. C. Williams, *Prog. Med. Chem*. **1994**, *31*, 59-120; T. E. Hugli, Trends Biotechnol. **1996**, *14*, 409-12,), cardiovascular (M. T. Stubbs, W. A. Bode, *Thromb. Res*. **1993**, *69*, 1-58; H. Fukami et al, *Current Pharmaceutical Design* **1998**, *4*, 439-453), and neurodegenerative defects including Alzheimer's disease (R. Vassar, B. D. Bennett, S. Babu-Kahn, S. Kahn, E. A. Mendiaz, *Science*, **1999**, *286*, 735-41), angiogenesis (M. Kaatinen et al, *Atherosklerosis* **1996**, *123* 1-2, 123-131) and multiple sclerosis (M. Z. Ibrahim et al, *J. Neuroimmunol* **1996**, *70*, 131-138.

Among proteases, serine proteases constitute important therapeutic targets. Serine proteases are classified by their substrate specificity, particularly by the type of residue found at P1, as either trypsin-like (positively charged residues Lys/Arg preferred at P1), elastase-like (small hydrophobic residues Ala/Val at P1), or chymotrypsin-like (large hydrophobic residues Phe/Tyr/Leu at P1). Serine proteases for which protease-inhibitor X-ray crystal data are available on the PDB data base (PDB: www.rcsb.org/pdb) include trypsin, α-chymotrypsin, γ-chymotrypsin, human neutrophil elastase, porcine pancreatic elastase, thrombin, subtilisin, human cytomegalovirus protease, achromobacter protease 1, human cathepsin G, glutamic acid-specific protease, carbopeptidase D, blood coagulation factorVIIa, porcine factor 1XA, mesentericopeptidase, HCV protease, and thermitase. Other serine proteases

which are of therapeutic interest include tryptase, complement convertase, hepatitis C-NS3 protease. Inhibitors of thrombin (e.g. J. L. Metha, L. Y. Chen, W. W. Nichols, C. Mattsson, D. Gustaffson, T. G. P. Saldeen, *J. Cardiovasc. Pharmacol.* **1998**, *31*, 345-51; C. Lila, P. Gloanec, L. Cadet, Y. Herve, J. Fournier, F. Leborgne, T. J. Verbeuren, G. DeNanteuil, *Synth. Comm*. **1998**, *28*, 4419-29) and factor Xa (e.g. J. P. Vacca, *Annu. Rep. Med. Chem*. **1998**, *33*, 81-90) are in clinical evaluation as anti-thrombotics, inhibitors of elastase (J. R. Williams, R. C. Falcone, C. Knee, R. L. Stein, A. M. Strimpler, B. Reaves, R. E. Giles, R. D. Krell, *Am. Rev. Respir. Dis*. **1991**, *144*, 875-83) are in clinical trials for emphysema and other pulmonary diseases, whereas tryptase inhibitors are currently in phase II clinical trials for asthma (C. Seife, *Science* **1997**, *277*, 1602-3), urokinase inhibitors for breast cancer, and chymase inhibitors for heart related diseases. Finally, cathepsin G, elastase and proteinase 3 are intimately involved in the modulation of activities of cytokines and their receptors. Particularly at sites of inflammation, high concentration of these three neutrophil serine proteases (NSPs) are released from infiltrating polymorphonuclear cells in close temporal correlation to elevated levels of inflammatory cytokines, strongly indicating that these proteases are involved in the control of cytokine bioactivity and availability (U. Bank, S. Ansorge, *J. Leukoc. Biol*. **2001**, *69*, 177-90). Thus highly selective inhibitors of elastase constitute valuable targets for novel drug candidates for infectious inflammatory diseases, including lung diseases like chronic obstructive pulmonary disease, acute respiratory distress syndrome, cystic fibrosis and ischemic-reperfusion injury, and in non-infectious processes like glomerulonephritis, arthritis and bullous pemphigoid (H. Ohbayashi, *Epert Opin. Investig. Drugs* **2002**, *11*, 965-980; B. Korkmaz, T. Moreau, F. Gauthier, *Biochimie* **2008**, *90*, 227).

Of the many occurring proteinaceous serine protease inhibitors, one is a 14 amino acid cyclic peptide from sunflower seeds, termed sunflower trypsin inhibitor (**SFTI-1**) (S. Luckett, R. Santiago Garcia, J. J. Barker, A. V. Konarev, P. R. Shewry, A. R. Clarke, R. L. Brady, *J. Mol. Biol*. **1999**, *290*, 525-533; Y.-Q. Long, S.-L. Lee, C.-Y. Lin, I. J. Enyedy, S. Wang, P. Li, R. B. Dickson, P. P. Roller, *Biorg. & Med. Chem. Lett*. **2001**, *11*, 2515-

2519), which shows both sequence and conformational similarity with the trypsin-reactive loop of the Bowman-Birk family of serine protease inhibitors. The inhibitor adopts a β-hairpin conformation when bound to the active site of bovine β-trypsin. **SFTI-1** inhibited β-trypsin (K_i<0.1nM), cathepsin G (K_i ~0.15nM), elastase (K_i ~105 μM), chymotrypsin (K_i ~7.4 μM) and thrombin (K_i ~136mM).

The β-hairpin conformation of the compounds cyclo(-Xaa^1-Xaa^2-Thr^3-Xaa^4-Ser^5-Xaa^6-Xaa^7-Xaa^8-Xaa^9-Xaa^{10}-Xaa^{11}-Xaa^{12}-Xaa^{13}-) is based on the the β-hairpin loop from the naturally occurring peptide combined with an D-amino acid residue at positon 12 and fostered by the conserved amino acid residues Thr and Ser at positions 3 and 5, respectively.

Template-bound hairpin mimetic peptides have been described in the literature (D. Obrecht, M. Altorfer, J. A. Robinson, *Adv. Med. Chem*. **1999**, *4*, 1-68; J. A. Robinson, *Syn. Lett.* **2000**, *4*, 429-441), and serine protnase-inhibiting template-fixed peptidomimetics and methods for their synthesis have been described in International Patent Applications WO2003/054000 A1, WO2006/087001 A1 and in A. Descours, K. Moehle, A. Renard, J.A. Robinson, *ChemBioChem* **2002**, *3*, 318-323 but the previously disclosed molecules do not exhibit such favourable activity/selectivity profiles.

The ability to generate β-hairpin peptidomimetics using combinatorial and parallel synthesis methods has been established (L. Jiang, K. Moehle, B. Dhanapal, D. Obrecht, J. A. Robinson, *Helv. Chim. Acta*. **2000**, *83*, 3097-3112). The methods described herein allow the synthesis and screening of large hairpin mimetic libraries, which in turn considerably facilitates structure-activity studies, and hence the discovery of new molecules with highly potent and selective serine protease inhibitory activity, particularly with such favourable activity/selectivity profiles as described herein, having compound properties suitable for novel drugs.

Summary

The β-hairpin peptidomimetics of the present invention are a backbone cyclized peptidic compound, built up from 13 amino acid residues, of the general formula cyclo(Xaa1 Xaa2 Thr3 Xaa4 Ser5 Xaa6 Xaa7 Xaa8 Xaa9 Xaa10 Xaa11 Xaa12 Xaa13) (I),

and pharmaceutically acceptable salts thereof,

wherein

Xaa1 is OctGly; Arg; hArg; or Glu(Phenethyl);

Xaa2 is Glu; Nle; hTyr; or Val;

Xaa4 is Ala; or AllylGly;

Xaa6 is Ile;

Xaa7 is Pro;

Xaa8 is Pro;

Xaa9 is Gln; or Tyr;

Xaa10 is Lys;

Xaa11 is hLeu; Ser; Thr; Asn; Tyr; hGln; or His;

Xaa12 is DPro; and

Xaa13 is Pro;

with the proviso that

- if Xaa1 is OctGly, then
  Xaa2 is Glu; or Nle;
  Xaa4 is Ala;
  Xaa6 is Ile;
  Xaa10 is Lys;
  Xaa11 is Ser; Thr; or Asn
  Xaa13 is Pro;

and with the further proviso that

- if Xaa11 is Tyr; or His, then

Xaa1 is Arg; hArg or Glu(Phenethyl). Also disclosed are backbone cyclized peptidic compounds, built up from 13 amino acid residues, of the general formula

cyclo(-Xaa^1-Xaa^2-Thr^3-Xaa^4-Ser^5-Xaa^6-Xaa^7-Xaa^8-Xaa^9-Xaa^{10}-Xaa^{11}-Xaa^{12}-Xaa^{13}-) (I),

and pharmaceutically acceptable salts thereof,

wherein

Xaa^1 is OctGly; Arg; hArg; Cha; Glu(Phenethyl); or Dab(Butanoyl);

Xaa^2 is Glu; Val; Leu; Nle; Phe; hPhe; DiHPhe; Tyr; hTyr; or Trp;

Xaa^4 is Ala; AllylGly; Abu; or Val;

Xaa^6 is Ile; or OctGly;

Xaa^7 is Pro;

Xaa^8 is Pro;

Xaa^9 is Gln; or Tyr;

Xaa^{10} is Lys; or Asn;

Xaa^{11} is hLeu; Ser; hSer; hSer(Me); Thr; alloThr; Asn; Gln; hGln; Dap; Tyr; or His;

Xaa^{12} is ^{D}Pro; and

Xaa^{13} is Pro; Tic; Glu; Asp; Ala; Val; or Lys;

with the proviso that

- if Xaa^1 is OctGly, then
  - Xaa^2 is Glu; or Nle;
  - Xaa^4 is Ala; or Abu;
  - Xaa^6 is Ile; or OctGly;
  - Xaa^{10} is Lys;
  - Xaa^{11} is Ser; Thr; Asn; or Gln;
  - Xaa^{13} is Pro; Tic; Ala; Val; or Lys;

- and/or if Xaa^6 is OctGly, then
  - Xaa^1 is OctGly; Arg; or Cha;
  - Xaa^2 is Glu; or Nle;
  - Xaa^4 is Ala; or Abu;
  - Xaa^{10} is Lys;
  - Xaa^{11} is Ser; Thr; Asn; Gln;
  - Xaa^{13} is Pro; Tic; Ala; Val; or Lys;

and with the further proviso that

- if Xaa^{11} is Tyr; or His, then
  - Xaa^1 is Arg; hArg; or Glu(Phenethyl).

In one aspect, the present invention provides a compound as described herein of the formula I, in free form or in pharmaceutically acceptable salt form, for use as a medicament.

In another aspect, the present invention provides a compound as described herein of the formula I, in free form or in pharmaceutically acceptable salt form, having inhibitory activity against elastase for use in the treatment or prevention of lung cancer; breast cancer; psoriasis; alpha 1 antitrypsin deficiency; pulmonary

emphysema; cystic fibrosis; chronic obstructive pulmonary disease; idiopathic pulmonary fibrosis; bronchiectasis; pulmonary hypertension; arterial pulmonary hypertension; cardiac hypertrophy; myocarditis; acute myocardial infarction; rheumatoid arthritis; osteoarthritis; atherosclerosis; multiple sclerosis; pancreatitis; allergic rhinitis; systemic inflammatory respiratory syndrome; inflammatory dermatoses; inflammatory bowel disease; or Crohn's disease.

In yet another aspect, the present invention provides a pharmaceutical composition comprising a compound or a mixture of compounds as described herein of the formula I, in free form or in pharmaceutically acceptable salt form, and a pharmaceutically inert carrier, particularly in a form suitable for inhalation, for oral, topical, transdermal, injection, buccal, transmucosal, rectal, pulmonary or inhalation administration especially in the form of a tablet, dragee, capsule, solution, liquid, gel, plaster, cream, ointment, syrup, slurry, suspension, powder or suppository.

In yet another aspect, the present invention provides a compound as described herein of the formula I, in free form or in pharmaceutically acceptable salt form, for use as a pharmaceutically active substance having selective protease inhibitory activity, in particular against human neutrophil elastase, and/or anticancer activity and/or anti inflammatory activity and/or anti infective activity and/or anticardiovascular activity and/or antiimmunological activity and/or antineurodegenerative activity.

In yet another aspect, the present invention provides a compound as described herein, in free form or in pharmaceutically acceptable salt form, or a composition as described herein, for use as a medicament having inhibitory activity against elastase for the treatment or prevention of infections or diseases related to such infections; or cancer, such as lung cancer, or breast cancer, mediated or resulting from; or immunological diseases, such as psoriasis, mediated or resulting from; or pulmonary diseases, such as alpha 1 antitrypsin deficiency, pulmonary emphysema, cystic

fibrosis, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, bronchiectasis, pulmonary hypertension, or arterial pulmonary hypertension, mediated or resulting from; or cardiovascular diseases, such as cardiac hypertrophy, myocarditis, or acute myocardial infarction, mediated or resulting from; or neurodegenerative diseases mediated or resulting from; or inflammation or diseases related to inflammation, such as rheumatoid arthritis, osteoarthritis, atherosclerosis, multiple sclerosis, pancreatitis, allergic rhinitis, systemic inflammatory respiratory syndrome, inflammatory dermatoses, inflammatory bowel disease, or Crohn's disease, mediated or resulting from elastase activity; or where immunological reaction is mediated or resulting from elastase activity.

In yet another aspect, the present invention provides the use of a compound as described herein, in free form or in pharmaceutically acceptable salt form, for the manufacture of a medicament having inhibitory activity against elastase for the treatment or prevention of infections or diseases related to such infections; or cancer , such as lung cancer, or breast cancer, mediated or resulting from; or immunological diseases, such as psoriasis, mediated or resulting from; or pulmonary diseases, such as alpha 1 antitrypsin deficiency, pulmonary emphysema, cystic fibrosis, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, bronchiectasis, pulmonary hypertension, or arterial pulmonary hypertension, mediated or resulting from; or cardiovascular diseases, such as cardiac hypertrophy, myocarditis, or acute myocardial infarction, mediated or resulting from; or neurodegenerative diseases mediated or resulting from; or inflammation or diseases related to inflammation, such as rheumatoid arthritis, osteoarthritis, atherosclerosis, multiple sclerosis, pancreatitis, allergic rhinitis, systemic inflammatory respiratory syndrome, inflammatory dermatoses, inflammatory bowel disease, or Crohn's disease, mediated or resulting from elastase activity; or where immunological reaction is mediated or resulting from elastase activity.

The use of a pharmaceutically acceptable amount of a compound or pharmaceutical composition according to any of the claims 1 to 5 or 8 in the manufacture of a medicament for the treatment of an infection or a disease or disorder associated with such an infection resulting from; or cancer mediated or resulting from; or immunological diseases mediated or resulting from; or pulmonary diseases mediated or resulting from; or cardiovascular diseases mediated or resulting from; or neurodegenerative diseases mediated or resulting from; or inflammation mediated or resulting from elastase activity; or where immunological reaction is mediated or resulting from elastase activity, wherein said compound or pharmaceutical composition is to be administered to a subject in need thereof.

In accordance with the present invention these β-hairpin peptidomimetics can be prepared by a process which comprises

(a) coupling an appropriately functionalized solid support with an appropriately N-protected derivative of that amino acid which in the desired end-product corresponds to Xaa^n, wherein n is 13, 8, 7, 6, 5 or 4, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(b) removing the N-protecting group from the product thus obtained;

(c) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product corresponds to Xaa^{n-1}, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(d) removing the N-protecting group from the product obtained in step (c);

(e) effecting steps substantially corresponding to steps (c) and (d) using appropriately N-protected derivatives of amino acids which in the desired end-product are in positions n-2 to 1, any functional group(s) which may be present in said N-protected amino acid derivatives being likewise appropriately protected;

(f) if n is not 13, further effecting steps substantially corresponding to steps (c) and (d) using appropriately N-protected derivatives of amino acids which in the desired end-product are in positions 13 to n + 1, any functional group(s) which may be present in said N-protected amino acid derivatives being likewise appropriately protected;

(g) if desired, before removing the N-protecting group from the product obtained in steps (e) or (f) selectively deprotecting one or several protected functional group(s) present in the molecule and appropriately substituting the reactive group(s) thus liberated by attaching one or several moieties derived from acids, amino acids or amines and removing the N-protecting group from the product obtained;

(h) detaching the product thus obtained from the solid support;

(i) cyclizing the product cleaved from the solid support;

(j) removing any protecting groups present on functional groups of any members of the chain of amino acid residues; and

(k) if desired, converting the product thus obtained into a pharmaceutically acceptable salt or converting a pharmaceutically acceptable, or unacceptable, salt thus obtained into the corresponding free compound or into a different, pharmaceutically acceptable, salt.

Hereinafter follows a list of amino acids which, or the residues of which, are suitable for the purposes of the present invention, the abbreviations corresponding to generally adopted usual practice:

three letter code		one letter code
Ala	L-Alanine	A
Val	L-Valine	V
Leu	L-Leucine	L
Phe	L-Phenylalanine	F
His	L-Histidine	H
Tyr	L-Tyrosine	Y
Trp	L-Tryptophan	W
Lys	L-Lysine	K
Arg	L-Arginine	R
Ser	L-Serine	S
Thr	L-Threonine	T
Asp	L-Aspartic acid	D
Asn	L-Asparagine	N
Glu	L-Glutamic acid	E
Gln	L-Glutamine	Q

Pro	L-Proline	P
AllylGly	L-Allylglycine	
Abu	L-α-Aminobutyric acid	
OctGly	L-Octylglycine	
Cha	L-Cyclohexylalanine	
Nle	L-Norleucine	
hLeu	L-Homo-leucine	
hPhe	L-Homo-phenylalanine	
DiHPhe	L-Dihomo-phenylalanine,	
	(2S)-2-amino-5-phenylpentanoic acid	
hTyr	L-Homo-tyrosine	
Dap	L-2,3-Diaminopropionic acid	
hArg	L-Homo-arginine	
hSer	L-Homo-serine	
hSer(Me)	L-Homo-O-methylserine	
alloThr	(2S, 3S)-2-Amino-3-hydroxy-butyric acid	
hGln	L-Homo-glutamine	
Glu(Phenethyl)	(2S)-2-Amino-5-phenethylamino-5-oxopentanoic acid	
Dab(Butanoyl)	(2S)-2-Amino-4-butanamido-butanoic acid	
Tic	(3S)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid	

In a particular embodiment of the present invention the β-hairpin peptidomimetics are compounds of the general formula **I,**

and pharmaceutically acceptable salts thereof, wherein

Xaa^1 is OctGly; Arg; hArg; or Glu(Phenethyl);

Xaa^2 is Glu; Nle; hTyr; or Val;

Xaa^4 is Ala; or AllylGly;

Xaa^6 is Ile;

Xaa^7 is Pro;

Xaa^8 is Pro;

Xaa^9 is Gln; or Tyr;

Xaa^{10} is Lys;

Xaa^{11} is hLeu; Ser; Thr; Asn; Tyr; hGln; or His;

Xaa^{12} is ^{D}Pro; and

Xaa^{13} is Pro;

with the proviso that

- if Xaa^1 is OctGly, then
- Xaa^2 is Glu; or Nle;
- Xaa^4 is Ala;
- Xaa^6 is Ile;
- Xaa^{10} is Lys;
- Xaa^{11} is Ser; Thr; or Asn
- Xaa^{13} is Pro;

and with the further proviso that

- if Xaa^{11} is Tyr; or His, then
- Xaa^1 is Arg; hArg or Glu(Phenethyl).

In another particular embodiment of the present invention the β-hairpin peptidomimetics are compounds of the general formula **I,** and pharmaceutically acceptable salts thereof, wherein

Xaa^1 is OctGly; Arg; or Glu(Phenethyl);

Xaa^2 is Glu; Nle; hTyr; or Val;

Xaa^4 is Ala;

Xaa^6 is Ile;

Xaa^7 is Pro;

Xaa^8 is Pro;

Xaa^9 is Gln; or Tyr;

Xaa^{10} is Lys;

Xaa^{11} is Ser; Thr; Asn; Tyr; or His;

Xaa^{12} is ^{D}Pro; and

Xaa^{13} is Pro;

with the proviso that

- if Xaa^1 is OctGly, then

  Xaa^2 is Glu; or Nle;

and with the further proviso that

- if Xaa^{11} is Tyr; or His, then

  Xaa^1 is Arg.

In another particular embodiment of the present invention the β-hairpin peptidomimetics are compounds of the general formula **I,** and pharmaceutically acceptable salts thereof, wherein

Xaa^1 is Arg; or hArg;

Xaa^2 is Glu; Val; or hTyr;

Xaa^4 is Ala; or AllylGly;

Xaa^6 is Ile;

Xaa^7 is Pro;

Xaa^8 is Pro;

Xaa^9 is Gln; or Tyr;

Xaa^{10} is Lys;

Xaa^{11} is hLeu; Ser; Thr; or hGln;

Xaa^{12} is ^{D}Pro; and

Xaa^{13} is Pro.

In another particular embodiment of the present invention the β-hairpin peptidomimetic is a compound of the general formula **I,** and pharmaceutically acceptable salts thereof, selected from

Cyclo(-OctGly-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Asn-^{D}Pro-Pro-);
Cyclo(-OctGly-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-Thr-^{D}Pro-Pro-);
Cyclo(-OctGly-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Ser-^{D}Pro-Pro-);
Cyclo(-OctGly-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);
Cyclo(-Arg-hTyr-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Tyr-^{D}Pro-Pro-);
Cyclo(-Arg-Nle-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Tyr-^{D}Pro-Pro-);
Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-His-^{D}Pro-Pro-);
Cyclo(-Glu(Phenethyl)-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Tyr-^{D}Pro-Pro-);
Cyclo(-Arg-Val-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Tyr-^{D}Pro-Pro-).

In another particular embodiment of the present invention the β-hairpin peptidomimetic is a compound of the general formula **I,** and pharmaceutically acceptable salts thereof, selected from

Cyclo(-Arg-Glu-Thr-AllylGly-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);
Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-Ser-^{D}Pro-Pro-);
Cyclo(-hArg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);
Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-hLeu-^{D}Pro-Pro-);
Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);
Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-hGln-^{D}Pro-Pro-);
Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-Arg-Val-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-Arg-hTyr-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-).

The process of the invention can advantageously be carried out as parallel array synthesis to yield libraries of β-hairpin peptidomimetics of the above general formula I. Such parallel synthesis allows one to obtain arrays of numerous (normally 24 to 192, typically 96) compounds of general formula I in high yields and defined purities, minimizing the formation of dimeric and polymeric by-products. The proper choice of the functionalized solid-support (i.e. solid support plus linker molecule), templates and site of cyclization play thereby key roles.

The functionalized solid support is conveniently derived from polystyrene crosslinked with, preferably 1-5%, divinylbenzene; polystyrene coated with polyethyleneglycol spacers (Tentagel®); and polyacrylamide resins (D. Obrecht, J.-M. Villalgordo, "Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries", *Tetrahedron Organic Chemistry Series*, Vol. 17, Pergamon, Elsevier Science, **1998**).

The solid support is functionalized by means of a linker, i.e. a bifunctional spacer molecule which contains on one end an anchoring group for attachment to the solid support and on the other end a selectively cleavable functional group used for the subsequent chemical transformations and cleavage procedures. For the purposes of the present invention two types of linkers are used:

Type 1 linkers are designed to release the amide group under acidic conditions (H. Rink, *Tetrahedron Lett*. **1987**, *28*, 3783-3790). Linkers of this kind form amides of the carboxyl group of the amino acids; examples of resins functionalized by such linker structures include 4-[(((2,4-dimethoxy-phenyl)Fmoc-aminomethyl) phenoxyacetamido) aminomethyl] PS resin, 4-[(((2,4-dimethoxyphenyl) Fmoc-aminomethyl)phenoxy-acetamido) aminomethyl] -4-methyl-benzydrylamine PS

resin (Rink amide MBHA PS Resin), and 4-[(((2,4-dimethoxy-phenyl) Fmoc-aminomethyl)phenoxyacetamido) aminomethyl] benzhydrylamine PS-resin (Rink amide BHA PS resin). Preferably, the support is derived from polystyrene crosslinked with, most preferably 1-5%, divinylbenzene and functionalized by means of the 4-(((2,4-dimethoxyphenyl) Fmoc-aminomethyl)phenoxyacetamido) linker.

Type 2 linkers are designed to eventually release the carboxyl group under acidic conditions. Linkers of this kind form acid-labile esters with the carboxyl group of the amino acids, usually acid-labile benzyl, benzhydryl and trityl esters; examples of such linker structures include 2-methoxy-4-hydroxymethylphenoxy (Sasrin® linker), 4-(2,4-dimethoxyphenyl-hydroxymethyl)-phenoxy (Rink linker), 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid (HMPB linker), trityl and 2-chlorotrityl. Preferably, the support is derived from polystyrene crosslinked with, most preferably 1-5%, divinyl-benzene and functionalized by means of the 2-chlorotrityl linker.

When carried out as parallel array syntheses the processes of the invention can be advantageously carried out as described herein below but it will be immediately apparent to those skilled in the art how these procedures will have to be modified in case it is desired to synthesize one single compound of the invention.

A number of reaction vessels equal to the total number of compounds to be synthesized by the parallel method are loaded with 25 to 1000 mg, preferably 60 mg, of the appropriate functionalized solid support, preferably 1 to 5% cross-linked polystyrene or Tentagel resin.

The solvent to be used must be capable of swelling the resin and includes, but is not limited to, dichloromethane (DCM), dimethylformamide (DMF), N-methylpyrrolidone (NMP), dioxane, toluene, tetrahydrofuran (THF), ethanol (EtOH), trifluoroethanol (TFE), isopropylalcohol and the like. Solvent mixtures containing as at least one component a polar solvent (e.g. 20% TFE/DCM, 35% THF/NMP) are beneficial for

ensuring high reactivity and solvation of the resin-bound peptide chains (G.B. Fields, C.G. Fields, *J. Am. Chem. Soc*. **1991**, *113*, 4202-4207).

With the development of various linkers that release the C-terminal carboxylic acid group under mild acidic conditions, not affecting acid-labile groups protecting functional groups in the side chain(s), considerable progresses have been made in the synthesis of protected peptide fragments. The 2-methoxy-4-hydroxybenzylalcohol-derived linker (Sasrin® linker, Mergler et al., *Tetrahedron Lett*. **1988**, 29 4005-4008) is cleavable with diluted trifluoroacetic acid (0.5-1% TFA in DCM) and is stable to Fmoc deprotection conditions during the peptide synthesis, Boc/tBu-based additional protecting groups being compatible with this protection scheme. Other linkers which are suitable for the process of the invention include the super acid labile 4-(2,4-dimethoxyphenyl-hydroxymethyl)-phenoxy linker (Rink linker, H. Rink, *Tetrahedron Lett.* **1987**, *28*, 3787-3790), where the removal of the peptide requires 10% acetic acid in DCM or 0.2% trifluoroacetic acid in DCM; the 4-(4-hydroxymethyl-3-methoxyphenoxy)butyric acid-derived linker (HMPB-linker, Flörsheimer & Riniker, *Peptides* **1991**, 1990 131) which is also cleaved with 1% TFA/DCM in order to yield a peptide fragment containing all acid labile side-chain protective groups; and, in addition, the 2-chlorotritylchloride linker (Barlos et al., *Tetrahedron Lett.* **1989**, *30*, 3943-3946), which allows the peptide detachment using a mixture of glacial acetic acid/trifluoroethanol/DCM (1:2:7) for 30 min.

Suitable protecting groups for amino acids and, respectively, for their residues are, for example,

- for the amino group (as is present *e.g.* also in the side-chain of lysine)

Cbz	benzyloxycarbonyl
Boc	tert.-butyloxycarbonyl
Fmoc	9-fluorenylmethoxycarbonyl
Alloc	allyloxycarbonyl
Teoc	trimethylsilylethoxycarbonyl

Tcc	trichloroethoxycarbonyl
Nps	o-nitrophenylsulfonyl;
Trt	triphenymethyl or trityl;

- for the carboxyl group (as is present *e.g.* also in the side-chain of aspartic and glutamic acid) by conversion into esters with the alcohol components

tBu	tert.-butyl
Bn	benzyl
Me	methyl
Ph	phenyl
Pac	phenacyl
	allyl
Tse	trimethylsilylethyl
Tce	trichloroethyl;

- for the guanidino group (as is present *e.g.* in the side-chain of arginine)

Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl
Ts	tosyl (i. e. p-toluenesulfonyl)
Cbz	benzyloxycarbonyl
Pbf	pentamethyldihydrobenzofuran-5-sulfonyl;

- for the hydroxy group (as is present *e.g.* in the side-chain of threonine and serine)

tBu	tert.-butyl
Bn	benzyl
Trt	trityl;

The 9-fluorenylmethoxycarbonyl- (Fmoc)-protected amino acid derivatives are preferably used as the building blocks for the construction of the β-hairpin loop mimetics of the invention. For the deprotection, *i.e.* cleaving off of the Fmoc group, 20% piperidine in DMF or 2% DBU/2% piperidine in DMF can be used as well as 25% hexafluoroisopropanol in CH_2Cl_2.

The quantity of the reactant, i. e. of the amino acid derivative, is usually 1 to 20 equivalents based on the milliequivalents per gram (meq/g) loading of the functionalized solid support (typically 0.1 to 2.85 meq/g for polystyrene resins) originally weighed into the reaction tube. Additional equivalents of reactants can be used, if required, to drive the reaction to completion in a reasonable time. The preferred workstations (without, however, being limited thereto) are Labsource's Combi-chem station, Protein Technologies' Symphony and MultiSyn Tech's-Syro synthesizer, the latter additionally equipped with a transfer unit and a reservoir box during the process of detachment of the fully protected linear peptide from the solid support. All synthesizers are able to provide a controlled environment, for example, reactions can be accomplished at temperatures different from room temperature as well as under inert gas atmosphere, if desired.

Amide bond formation requires the activation of the α-carboxyl group for the acylation step. When this activation is being carried out by means of the commonly used carbodiimides such as dicyclohexylcarbodiimide (DCC, Sheehan & Hess, *J. Am. Chem. Soc.* **1955,** *77*, 1067-1068) or diisopropylcarbodiimide (DIC, Sarantakis et al *Biochem. Biophys. Res. Commun.***1976**, *73*, 336-342), the resulting dicyclohexylurea and diisopropylurea is insoluble and, respectively, soluble in the solvents generally used. In a variation of the carbodiimide method 1-hydroxybenzotriazole (HOBt, König & Geiger, *Chem. Ber* **1970**, *103*, 788-798) is included as an additive to the coupling mixture. HOBt prevents dehydration, suppresses racemization of the activated amino acids and acts as a catalyst to improve the sluggish coupling reactions. Certain phosphonium reagents have been used as direct coupling reagents, such as benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP,

Castro et al., *Tetrahedron Lett.* **1975**, *14,* 1219-1222; *Synthesis*, **1976**, 751-752), or benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexaflurophoshate (Py-BOP, Coste et al., *Tetrahedron Lett*. **1990**, *31*, 205-208), or 2-(1H-benzotriazol-1-yl-)1,1,3,3-tetramethyluronium terafluoroborate (TBTU), or hexafluorophosphate (HBTU, Knorr et al., *Tetrahedron Lett.* **1989**, *30*, 1927-1930); these phosphonium reagents are also suitable for *in situ* formation of HOBt esters with the protected amino acid derivatives. More recently diphenoxyphosphoryl azide (DPPA) or O-(7-aza-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TATU) or O-(7-aza-benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU)/7-aza-1-hydroxy benzotriazole (HOAt, Carpino et al., *Tetrahedron Lett.* **1994***, 35*, 2279-2281) or -(6-Chloro-1H-benzotriazol-1-yl-)-N,N,N',N'-1,1,3,3-tetramethyl-uronium tetrafluoro-borate (TCTU), or hexafluorophosphate (HCTU, Marder, Shivo and Albericio: HCTU and TCTU: New Coupling Reagents: Development and Industrial Applications, Poster Presentation, Gordon Conference February 2002) have also been used as coupling reagents as well as 1,1,3,3-bis(tetramethylene)chlorouronium hexafluoro-phosphate (PyClU, especially for coupling N-methylated amino acids, J. Coste, E. Frérot, P. Jouin, B. Castro, *Tetrahedron Lett.* **1991**, *32*, 1967) or pentafluorophenyl diphenyl-phosphinate (S. Chen, J. Xu, *Tetrahedron Lett.* **1991**, *32*, 6711).

Due to the fact that near-quantitative coupling reactions are essential, it is desirable to have experimental evidence for completion of the reactions. The ninhydrin test (Kaiser et al., *Anal. Biochemistry* **1970**, *34*, 595), where a positive colorimetric response to an aliquot of resin-bound peptide indicates qualitatively the presence of the primary amine, can easily and quickly be performed after each coupling step. Fmoc chemistry allows the spectrophotometric detection of the Fmoc chromophore when it is released with the base (Meienhofer et al., *Int. J. Peptide Protein Res.* **1979**, *13*, 35-42).

The resin-bound intermediate within each reaction tube is washed free of excess of retained reagents, of solvents, and of by-products by repetitive exposure to pure solvent(s) by one of the two following methods:

1) The reaction vessels are filled with solvent (preferably 5 mL), agitated for 5 to 300 minutes, preferably 15 minutes, and drained to expel the solvent;

2) The reaction vessels are filled with solvent (preferably 5 mL) and drained into a receiving vessel such as a test tube or vial.

Both of the above washing procedures are repeated up to about 50 times (preferably about 10 times), monitoring the efficiency of reagent, solvent, and by-product removal by methods such as TLC, GC, or inspection of the washings.

The above described procedure of reacting the resin-bound compound with reagents within the reaction wells followed by removal of excess reagents, by-products, and solvents is repeated with each successive transformation until the final resin-bound fully protected linear peptide has been obtained.

Before this fully protected linear peptide is detached from the solid support, it is possible, if desired, to selectively deprotect one or several protected functional group(s) present in the molecule and to appropriately substitute the reactive group(s) thus liberated. To this effect, the functional group(s) in question must initially be protected by a protecting group which can be selectively removed without affecting the remaining protecting groups present. Alloc (allyloxycarbonyl) is an example for an amino protecting group , whereas Allyl is an example for an carboxylic protecting group. Both groups can be selectively removed, e.g. by means of Pd° and phenylsilane in CH_2Cl_2, without affecting the remaining protecting groups, such as Fmoc, present in the molecule. The reactive group thus liberated can then be treated with an agent suitable for introducing the desired substituent. Thus, for example, an amino group can be acylated by means of an acylating agent corresponding to the acyl substituent to be introduced, whereas a carboxylic group can be derivatised by introduction of an amino substituent. Preferably, Alloc or Allyl will be removed by applying 0.2 eq

tetrakis(triphenyl-phosphine)palladium(0) (10 mM) in dry CH_2Cl_2 and 10 eq phenylsilane for 15 min at room temperature. After filtration and washing of the resin the deprotection is completed by repeating the procedure with a fresh solution of reagents. In case of a liberated carboxylic group the subsequent coupling of an amine, for example, can be accomplished, for example, by applying the reagents/reaction conditions for amide bond formation as described above.

Detachment of the fully protected linear peptide from the solid support is achieved by exposing the loaded resin with a solution of the reagent used for cleavage (preferably 3 to 5 mL). Temperature control, agitation, and reaction monitoring are implemented as described above. Via a transfer-unit the reaction vessels are connected with a reservoir box containing reservoir tubes to efficiently collect the cleaved product solutions. The resins remaining in the reaction vessels are then washed 2 to 5 times as above with 3 to 5 mL of an appropriate solvent to extract (wash out) as much of the detached products as possible. The product solutions thus obtained are combined, taking care to avoid cross-mixing. The individual solutions/extracts are then manipulated as needed to isolate the final compounds. Typical manipulations include, but are not limited to, evaporation, concentration, liquid/liquid extraction, acidification, basification, neutralization or additional reactions in solution.

The solutions containing fully protected linear peptide derivatives which have been cleaved off from the solid support and neutralized with a base, are evaporated. Cyclization is then effected in solution using solvents such as DCM, DMF, dioxane, THF and the like. Various coupling reagents which were mentioned earlier can be used for the cyclization. The duration of the cyclization is about 6-48 h, preferably about 16 h. The progress of the reaction is followed, e. g. by RP-HPLC (Reverse Phase High Performance Liquid Chromatography). Then the solvent is removed by evaporation, the fully protected cyclic peptide derivative is dissolved in a solvent which is not miscible with water, such as DCM, and the solution is extracted with water or a

mixture of water-miscible solvents, in order to remove any excess of the coupling reagent.

Finally, the fully protected peptide derivative is treated with 95% TFA, 2.5% H_2O, 2.5% TIS or another combination of scavengers for effecting the cleavage of protecting groups. The cleavage reaction time is commonly 30 minutes to 12 h, preferably about 2.5 h.

Alternatively, the detachment and complete deprotection of the fully protected peptide from the solid support can be achieved manually in glass vessels.

After full deprotection, for example, the following methods can be used for further work-up:

1) The volatiles are evaporated to dryness and the crude peptide is dissolved in 20% AcOH in water and extracted with isopropyl ether or other solvents which are suitable therefore. The aqueous layer is collected and evaporated to dryness, and the fully deprotected peptide, cyclo(-Xaa^1-Xaa^2-Thr^3-Xaa^4-Ser^5-Xaa^6-Xaa^7-Xaa^8-Xaa^9-Xaa^{10}-Xaa^{11}-Xaa^{12}-Xaa^{13}-), is obtained as final product;

2) The deprotection mixture is concentrated under vacuum. Following precipitation of the fully deprotected peptide in diethylether at preferably 0 °C the solid is washed up to about 10 times, preferably 3 times, dried, and the the fully deprotected peptide, cyclo(-Xaa^1-Xaa^2-Thr^3-Xaa^4-Ser^5-Xaa^6-Xaa^7-Xaa^8-Xaa^9-Xaa^{10}-Xaa^{11}-Xaa^{12}-Xaa^{13}-), is obtained as final product.

Depending on its purity, the final product as obtained above can be used directly for biological assays, or has to be further purified, for example by preparative HPLC.

As mentioned earlier, it is thereafter possible, if desired, to convert the fully deprotected cyclic product thus obtained into a pharmaceutically acceptable salt or to convert a pharmaceutically acceptable, or unacceptable, salt thus obtained into

the corresponding free compound or into a different, pharmaceutically acceptable, salt. Any of these operations can be carried out by methods well known in the art.

The β-hairpin peptidomimetics of the invention can be used in a wide range of applications where inflammatory diseases or pulmonary diseases or infections or immunological diseases or cardiovascular diseases or neurodegenerative diseases are mediated or resulting from serine protease activity, or where cancer is mediated or resulting from serine protease activity. For the control or prevention of a given illness or disease amenable to treatment with protease inhibitors, the β-hairpin peptidomimetics or the invention may be administered per se or may be applied as an appropriate formulation together with carriers, diluents or excipients well known in the art.

When used to treat, prevent, modulate or remodel diseases such as alpha 1 antitrypsin deficiency (AATD), pulmonary emphysema, rheumatoid arthritis, osteoarthritis, atherosclerosis, psoriaris, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), idiopathic pulmanory fibrosis (IPF), bronchiectasis, bronchodilation, chronic bronchitis, multiple sclerosis, acute respiratory distress syndrome (ARDS), acute lung injury (ALI), pulmonary hypertension (PH), arterial pulmonary hypertension (PAH), pancreatitis, asthma, allergic rhinitis, inflammatory dermatoses, postangioplasty restenosis, systemic inflammatory respiratory syndrome (SIRS), ischemia reperfusion injury, cardiac hypertrophy, myocarditis, acute myocardial infarction (AMI), heart failure, cardiac transplant, inflammatory bowel disease (IBD), colitis, Crohn's disease, adaptive colitis or cancer such as, but not limited to, lung cancer, breast cancer, or cancer related to angiogenesis or metastasis, the β-hairpin peptidomimetics of the invention can be administered singly, as mixtures of several β-hairpin peptidomimetics, in combination with other anti-inflammatory agents, or antimicrobial agents or anti-cancer agents and/or in combination with other pharmaceutically active agents. The β-hairpin

peptidomimetics of the invention can be administered per se or as pharmaceutical compositions.

Pharmaceutical compositions comprising β-hairpin peptidomimetics of the invention may be manufactured by means of conventional mixing, dissolving, granulating, coated tablet-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxilliaries which facilitate processing of the active β-hairpin peptidomimetics of the invention into preparations which can be used pharmaceutically. Proper formulation depends upon the method of administration chosen.

For topical administration the β-hairpin peptidomimetics of the invention may be formulated as solutions, gels, ointments, creams, suspensions, etc. as are well-known in the art.

Systemic formulations include those designed for administration by injection, e.g. subcutaneous, intravenous, intramuscular, intrathecal or intraperitoneal injection, as well as those designed for transdermal, transmucosal, oral or pulmonary administration.

For injections, the β-hairpin peptidomimetics of the invention may be formulated in adequate solutions, preferably in physiologically compatible buffers such as Hink's solution, Ringer's solution, or physiological saline buffer. The solutions may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the β-hairpin peptidomimetics of the invention may be in powder form for combination with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.
For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation as known in the art.

For oral administration, the compounds can be readily formulated by combining the active β-hairpin peptidomimetics of the invention with pharmaceutically acceptable carriers well known in the art. Such carriers enable the β-hairpin peptidomimetics of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, powders etc., for oral ingestion by a patient to be treated. For oral formulations such as, for example, powders, capsules and tablets, suitable excipients include fillers such as sugars, such as lactose, sucrose, mannitol and sorbitol; cellulose preparations such as maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP); granulating agents; and binding agents. If desired, desintegrating agents may be added, such as cross-linked polyvinylpyrrolidones, agar, or alginic acid or a salt thereof, such as sodium alginate. If desired, solid dosage forms may be sugar-coated or enteric-coated using standard techniques.

For oral liquid preparations such as, for example, suspensions, elixirs and solutions, suitable carriers, excipients or diluents include water, glycols, oils, alcohols, etc. In addition, flavoring agents, preservatives, coloring agents and the like may be added.

For buccal administration, the composition may take the form of tablets, lozenges, etc., formulated as known in the art.

For administration by inhalation, the β-hairpin peptidomimetics of the invention are conveniently delivered in form of an aeorosol spray from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluromethane, carbon dioxide or another suitable gas. In the case of a pressurized aerosol the dose unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the β-hairpin

peptidomimetics of the invention and a suitable powder base such as lactose or starch.

The compounds may also be formulated in rectal or vaginal compositions such as suppositories together with appropriate suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the β-hairpin peptidomimetics of the invention may also be formulated as depot preparations. Such long acting formulations may be administered by implantation (e.g. subcutaneously or intramuscularly) or by intramuscular injection. For the manufacture of such depot preparations the β-hairpin peptidomimetics of the invention may be formulated with suitable polymeric or hydrophobic materials (e.g. as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble salts.

In addition, other pharmaceutical delivery systems may be employed such as liposomes and emulsions well known in the art. Certain organic solvents such as dimethylsulfoxide may also be employed. Additionally, the β-hairpin peptidomimetics of the invention may be delivered using a sustained-release system, such as semipermeable matrices of solid polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic agent, additional strategies for protein stabilization may be employed.

As the β-hairpin peptidomimetics of the invention contain charged residues, they may be included in any of the above described formulations as such or as pharmaceutically acceptable salts. Pharmaceutically acceptable salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free forms.

Particluarly suitable pharmaceutically acceptable salts include salts with carboxylic, phosphonic, sulfonic and sulfamic acids, e.g. acetic acid, propionic acid, octanoic acid, decanoic acid, dodecanoic acid, glycolic acid, lactic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, malic acid, tartaric acid, citric acid, amino acids, such as glutamic acid or aspartic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, cyclohexanecarboxylic acid, adamantanecarboxylic acid, benzoic acid, salicylic acid, 4-aminosalicylic acid, phthalic acid, phenylacetic acid, mandelic acid, cinnamic acid, methane- or ethane-sulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 2-naphthalenesulfonic acid, 1,5-naphthalenedisulfonic acid, 2-, 3- or 4-methyl-benzenesulfonic acid, methylsulfuric acid, ethylsulfuric acid, dodecylsulfuric acid, N-cyclohexylsulfamic acid, N-methyl-, N-ethyl- or N-propyl-sulfamic acid, and other organic protonic acids, such as ascorbic acid. Suitable inorganic acids are for example hydrohalic acids, such as hydrochloric acid, sulfuric acid and phosphoric acid.

The β-hairpin peptidomimetics of the invention, or compositions thereof, will generally be used in an amount effective to achieve the intended purpose. It is to be understood that the amount used will depend on a particular application.

For topical administration to treat or prevent diseases amenable to treatment with beta hairpin mimetics a therapeutically effective dose can be determined using, for example, the in vitro assays provided in the examples. The treatment may be applied while the disease is visible, or even when it is not visible. An ordinary skilled expert will be able to determine therapeutically effective amounts to treat topical diseases without undue experimentation.

For systemic administration, a therapeutically effective dose can be estimated initially from in vitro assays. For example, a dose can be formulated in animal models to achieve a circulating β-hairpin peptidomimetic concentration range that includes the

IC_{50} as determined in the cell culture. Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be determined from in vivo data, e.g. animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amounts for applications as serine protease inhibitory agents may be adjusted individually to provide plasma levels of the β-hairpin peptidomimetics of the invention which are sufficient to maintain the therapeutic effect. Therapeutically effective serum levels may be achieved by administering multiple doses each day.

In cases of local administration or selective uptake, the effective local concentration of the β-hairpin peptidomimetics of the invention may not be related to plasma concentration. One having the ordinary skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

The amount of β-hairpin peptidomimetics of the invention administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgement of the prescribing physician.

Normally, a therapeutically effective dose of the β-hairpin peptidomimetics of the invention described herein will provide therapeutic benefit without causing substantial toxicity.

Toxicity of the β-hairpin peptidomimetics of the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the LD_{50} (the dose lethal to 50% of the population) or the LD_{100} (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in humans. The dosage of the β-hairpin peptidomimetics of the invention lies preferably within a range of

circulating concentrations that include the effective dose with little or no toxicity. The dosage may vary within the range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dose can be chosen by the individual physician in view of the patient's condition (see, e.g. Fingl et al. **1975**, In: *The Pharmacological Basis of Therapeutics*, Ch.1, p.1).

The present invention may also include compounds, which are identical to the compounds of the general formula cyclo(-Xaa^1-Xaa^2-Thr^3-Xaa^4-Ser^5-Xaa^6-Xaa^7-Xaa^8-Xaa^9-Xaa^{10}-Xaa^{11}-Xaa^{12}-Xaa^{13}-), except that one or more atoms are replaced by an atom having an atomic mass number or mass different from the atomic mass number or mass usually found in nature, e.g. compounds enriched in ^2H (D), ^3H, ^{11}C, ^{14}C, ^{129}I etc. These isotopic analogs and their pharmaceutical salts and formulations are considered useful agents in the therapy and/or diagnostic, for example, but not limited to, where a fine-tuning of *in vivo* half-life time could lead to an optimized dosage regimen.

The following Examples illustrate the present invention but are not to be construed as limiting its scope in any way.

Examples

1. Peptide synthesis

Coupling of the first protected amino acid residue to the resin

1 g (1.4 mMol) 2-chlorotritylchloride resin (1.4 mMol/g; 100 – 200 mesh, copoly(styrene-1% DVB) polymer matrix; Barlos et al. *Tetrahedron Lett*. **1989**, *30*, 3943-3946) was filled into a dried flask. The resin was suspended in CH_2Cl_2 (5 mL) and allowed to swell at room temperature under constant shaking for 30 min. A solution of 0.98 mMol (0.7 eq) of the first suitably protected amino acid residue (see below) in CH_2Cl_2 (5 mL) mixed with 960 µl (4 eq) of diisopropylethylamine (DIEA) was added. After shaking the reaction mixture for 4 h at 25 °C, the resin was filtered off and washed successively with CH_2Cl_2 (1x), DMF (1x) and CH_2Cl_2 (1x). A solution of CH_2Cl_2/MeOH/DIEA (17/2/1, 10 mL) was added to the resin and the suspension was shaken for 30 min. After filtration the resin was washed in the following order with CH_2Cl_2 (1x), DMF (1x), CH_2Cl_2 (1x), MeOH (1x), CH_2Cl_2 (1x), MeOH (1x), CH_2Cl_2 (2x), Et_2O (2x) and dried under vacuum for 6 hours.

Loading was typically 0.6-0.7 mMol/g.

The following preloaded resins were prepared:
Fmoc-Ser(tBu) -O-2-chlorotrityl resin and Fmoc-Pro-O-2-chlorotrityl resin.

The synthesis was carried out employing a Syro-peptide synthesizer (MultiSynTech) using 24-96 reaction vessels. In each vessel 0.04 mMol of the above resin was placed and the resin was swollen in CH_2Cl_2 and DMF for 15 min, respectively. The following reaction cycles were programmed and carried out:

Step	Reagent	Time
1	DMF, wash	5x1 min
2	20% piperidine/DMF	1x5 min, 1x15 min
3	DMF, wash	5x1 min
4	3.6 eq Fmoc amino acid, 3.6 eq HOAT/DMF	
	+3.6 eq DIC/DMF	1x40 min
5	DMF, wash	1x1 min
6	3.6 eq Fmoc amino acid, 3.6 eq HOAT/DMF	
	+3.6 eq HATU	
	+7.2 eq DIPEA	1x40 min

Unless indicated otherwise, the final coupling of an amino acid was followed by Fmoc deprotection by applying steps 1-3 of the above described reaction cycle.

The appropriately protected amino acid building blocks are commercially available or can be synthesized as known in the art.

Attachment of phenethylamine to carboxylic group-bearing side chain

Procedure A

Attachment of phenethylamine to selectively deprotected linear peptides on resin:

To remove the allyl-protecting group from the carboxy function present in the resin bound peptide the latter (0.04 mMol) was swollen in freshly distilled CH_2Cl_2 for at least 15 min followed by adding 0.2 eq tetrakis(triphenyl-phosphine)palladium(0) (10 mM) in dry CH_2Cl_2 and 10 eq phenylsilane. After shaking the reaction mixture for 15 min at room temperature, the resin was filtered off and a fresh solution of reagents was added to repeat the procedure. Following subsequent washing of the resin with CH_2Cl_2, DMF and Et_2O, the resin was swollen again in CH_2Cl_2 and the

attachment of phenethylamine was accomplished by subsequently adding a mixture of 3.6 eq of phenethylamine and 3.6 eq HOAt dissolved in DMF and 3.6 eq DIC dissolved in DMF allowing the reaction mixture to stand for 1 h disrupted only by occasionally stirring. After filtration and washing of the resin three times with DMF, the coupling was completed by repeating the procedure with a fresh solution of a mixture of 3.6 eq of the same amine and 3.6 eq HOAt dissolved in DMF and a mixture of 3.6 eq HATU and 7.2 eq DIPEA in DMF.

Cyclization and work up of backbone cyclized peptides

Cleavage of the fully protected peptide fragment

After completion of the synthesis, the resin (0.04 mMol) was suspended in 1 mL (0.13 mMol, 3.4 eq) of 1% TFA in CH_2Cl_2 (*v/v*) for 3 minutes, filtered, and the filtrate was neutralized with 1 mL (0.58 mMol, 14.6 eq) of 10% DIEA in CH_2Cl_2 (*v/v*). This procedure was repeated three times to ensure completion of the cleavage. The filtrate was evaporated to dryness and a sample of the product was fully deprotected by using a cleavage mixture containing 95% trifluoroacetic acid (TFA), 2.5% water and 2.5% triisopropylsilane (TIS) to be analyzed by reverse phase-HPLC (C_{18} column) and ESI-MS to monitor the efficiency of the linear peptide synthesis.

Cyclization of the linear peptide

The fully protected linear peptide (0.04 mMol) was dissolved in DMF (4 μMol/mL). Then 30.4 mg (0.08 mMol, 2 eq) of HATU, 10.9 mg (0.08 mMol, 2 eq) of HOAt and 28 μl (0.16 mMol, 4 eq) DIEA were added, and the mixture was vortexed at 25°C for 16 hours and subsequently concentrated under high vacuum. The residue was partitioned between CH_2Cl_2 and H_2O/CH_3CN (90/10: *v/v*). The CH_2Cl_2 phase was evaporated to yield the fully protected cyclic peptide.

Full deprotection of the cyclic peptide

The cyclic peptide obtained was dissolved in 3 mL of the cleavage mixture containing 82.5% trifluoroacetic acid (TFA), 5% water, 5% thioanisole, 5% phenol and 2.5% ethanedithiole (EDT). The mixture was allowed to stand at 25 °C for 2.5 hours and thereafter concentrated under vacuum. After precipitation of the cyclic fully deprotected peptide in diethylether (Et_2O) at 0 °C the solid was washed twice with Et_2O and dried.

After purification of the crude products via preparative HPLC the peptides were lyophilized (white powders) and analysed by the following analytical methods:

Analytical method A for Examples 5-10, 16-18:

Analytical HPLC retention times (RT, in minutes) were determined using a Ascentis Express C18 column, 50 x 3.0 mm, (cod. 53811-U- Supelco) with the following solvents A (H_2O + 0.1% TFA) and B (CH_3CN + 0.01% TFA) and the gradient: 0-0.05 min: 97% A, 3% B; 4.95 min: 3% A 97% B; 5.35 min: 3% A, 97% B; 5.40 min: 97% A, 3% B. Flow rate = 1.3 mL/min; UV_Vis = 220 nm.

Analytical method B for Examples 1-4:

Analytical HPLC retention times (RT, in minutes) were determined using a Ascentis Express C18 column, 50 x 3.0 mm, (cod. 53811-U- Supelco) with the following solvents A (H_2O + 0.1% TFA) and B (CH_3CN + 0.01% TFA) and the gradient: 0-0.05 min: 97% A, 3% B; 3.40 min: 33% A, 67% B; 3.45 min: 3% A, 97% B; 3.65 min: 3% A, 97% B; 3.70 min: 97% A, 3% B. Flow rate = 1.3 mL/min; UV_Vis = 220 nm.

Analytical method C for Examples 11-15:

Analytical HPLC retention times (RT, in minutes) were determined using a Xselect CSH C18 XP column, 100 x 3.0 mm, (cod. 186006107, Waters) with the following solvents A (H_2O + 0.1% TFA) and B (CH_3CN + 0.01% TFA) and the gradient: 0-0.05 min: 95% A,

5% B; 10.05 min: 3% A, 97% B; 12.05 min: 3% A, 97% B; 12.10 min: 95% A, 5% B. Flow rate = 0.6 mL/min; UV_Vis = 220 nm.

Examples 1-13 are shown in *Table 1*. The peptides were synthesized as follows: Starting resin was Fmoc-Ser(tBu)-O-2-chlorotrityl resin, which was prepared as described above. To that resin Xaa^4, finally at position 4, was grafted. The linear peptide was synthesized on solid support according to the procedure described above in the following sequence: Resin-Ser^5-Xaa^4-Thr^3-Xaa^2-Xaa^1-Xaa^{13}-Xaa^{12}-Xaa^{11}-Xaa^{10}-Xaa^9-Xaa^8-Xaa^7-Xaa^6. Following a final Fmoc deprotection as described above, the peptide was cleaved from the resin, cyclized, deprotected and purified as indicated above.

The HPLC-retention times and UV-purities, determined using the analytical method as described above, are shown in *Table 1*.

Examples 14, 16 are shown in *Table 1*. The peptides were synthesized as follows: Starting resin was Fmoc-Pro-O-2-chlorotrityl resin, which was prepared as described above. To that resin Xaa^{12}, finally at position 12, was grafted. The linear peptide was synthesized on solid support according to the procedure described above in the following sequence: Resin-Pro^{13}-Xaa^{12}-Xaa^{11}-Xaa^{10}-Xaa^9-Xaa^8-Xaa^7-Xaa^6-Ser^5-Xaa^4-Thr^3-Xaa^2-Xaa^1. Following a final Fmoc deprotection as described above, the peptide was cleaved from the resin, cyclized, deprotected and purified as indicated above.

The HPLC-retention times and UV-purities, determined using the analytical method as described above, are shown in *Table 1*.

Example 15 is shown in *Table 1*. The peptide was synthesized as follows: Starting resin was Fmoc-Pro-O-2-chlorotrityl resin, which was prepared as described above. To that resin Xaa^{12}, finally at position 12, was grafted. The linear peptide was synthesized on solid support according to the procedure described above in the following sequence: Resin-Pro^{13}-Xaa^{12}-Xaa^{11}-Xaa^{10}-Xaa^9-Xaa^8-Xaa^7-Xaa^6-Ser^5-Xaa^4-Ser^3-Xaa^2-Glu^1. Before the last Fmoc-deprotection *procedure A* was applied to attach

phenylethylamine to the side chain of Glu^1. Following a final Fmoc deprotection as described above, the peptide was cleaved from the resin, cyclized, deprotected and purified as indicated above.

The HPLC-retention times and UV-purities, determined using the analytical method as described above, are shown in *Table 1*.

Examples 17-18 are shown in *Table 1*. The peptides were synthesized as follows: Starting resin was Fmoc-Pro-O-2-chlorotrityl resin, which was prepared as described above. To that resin Xaa^7, finally at position 7, was grafted. The linear peptide was synthesized on solid support according to the procedure described above in the following sequence: Resin-Pro^8-Xaa^7-Xaa^6-Ser^5-Xaa^4-Thr^3-Xaa^2-Xaa^1-Xaa^{13}-Xaa^{12}-Xaa^{11}-Xaa^{10}-Xaa^9. Following a final Fmoc deprotection as described above, the peptide was cleaved from the resin, cyclized, deprotected and purified as indicated above.

The HPLC-retention times and UV-purities, determined using the analytical method as described above, are shown in *Table 1*.

Table 1: Examples

Ex.	Xaa[1] a)	Xaa[2] a)	Xaa[3] a)	Xaa[4] a)	Xaa[5] a)	Xaa[6] a)	Xaa[7] a)	Xaa[8] a)	Xaa[9] a)	Xaa[10] a)	Xaa[11] a)	Xaa[12] a)	Xaa[13] a)	Purity [%]	MS b)	RT [min]
1	OctGly	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Asn	^{D}Pro	Pro	76	1430.2	2.30[c)]
2	OctGly	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Tyr	Lys	Thr	^{D}Pro	Pro	61	1452.2	2.63[c)]
3	OctGly	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Ser	^{D}Pro	Pro	73	1403.1	2.36[c)]
4	OctGly	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Thr	^{D}Pro	Pro	72	1417.1	2.47[c)]
5	Arg	hTyr	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Tyr	^{D}Pro	Pro	77	757.5	1.72
6	Arg	Nle	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Tyr	^{D}Pro	Pro	79	725.4	1.81
7	Arg	Glu	Thr	AllylGly	Ser	Ile	Pro	Pro	Gln	Lys	Thr	^{D}Pro	Pro	70	715.5	1.63
8	Arg	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Tyr	Lys	Ser	^{D}Pro	Pro	88	712.9	1.58
9	hArg	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Thr	^{D}Pro	Pro	74	709.4	1.49
10	Arg	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	hLeu	^{D}Pro	Pro	70	1429.8	1.67
11	Arg	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Thr	^{D}Pro	Pro	86	702.4	3.04[d)]
12	Arg	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Tyr	Lys	hGln	^{D}Pro	Pro	94	740.4	3.19[d)]
13	Arg	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Tyr	Lys	Thr	^{D}Pro	Pro	88	720.0	3.28[d)]
14	Arg	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Tyr	Lys	His	^{D}Pro	Pro	67	737.9	3.00[d)]
15	Glu(Phen)[e)]	Glu	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Tyr	^{D}Pro	Pro	77	771.5	4.39[d)]
16	Arg	Val	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Thr	^{D}Pro	Pro	77	687.5	1.75

Table 1, continued: Examples

Ex.	Xaa[1] a)	Xaa[2] a)	Xaa[3] a)	Xaa[4] a)	Xaa[5] a)	Xaa[6] a)	Xaa[7] a)	Xaa[8] a)	Xaa[9] a)	Xaa[10] a)	Xaa[11] a)	Xaa[12] a)	Xaa[13] a)	Purity [%]	MS b)	RT [min]
17	Arg	hTyr	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Thr	^{D}Pro	Pro	77	726.5	1.76
18	Arg	Val	Thr	Ala	Ser	Ile	Pro	Pro	Gln	Lys	Tyr	^{D}Pro	Pro	78	718.5	1.79

a) Abbreviations of amino acid see listing above.
b) MS: either $[M+1H]^{1+}$ or $[M+2H]^{2+}$.
c) Analytical method B
d) Analytical method C
e) Glu(Phen) = Glu(Phenethyl)

2. Biological methods

2.1. Preparation of the peptide samples

Lyophilized peptides were weighed on a Microbalance (Mettler MT5) and dissolved in DMSO to a final concentration of 10 mM. Stock solutions were kept at +4 °C, light protected. The biological assays were carried out under assay conditions having less than 1% DMSO unlike indicated otherwise.

2.2. Inhibition of human neutrophil elastase

The ability of the peptides of the invention to inhibit the hydrolysis activity of human neutrophil elastase (Serva Electrophoresis, Germany) using the synthetic tetrapeptidic substrate MeOSuc-AAPV-pNA (Bachem, Switzerland) was determined as follows:

The above substrate (0.3mM) and human neutrophil elastase (10nM) were incubated at 37°C with serial dilutions of the peptides (1% DMSO final) in assay buffer (50mM Tris, pH 8, 300mM NaCl, 0.01% Tween20). The release of pNA was followed by monitoring the change in absorbance at 405nm for 30 minutes. Control assays with the same assay set-up as above, but without peptide, ran linearly. The dose-response data were fitted to the 4-parameter Hill equation providing the IC_{50} value using Graphpad (Prism 5).

2.3. Inhibition of porcine pancreatic elastase

The ability of the peptides of the invention to inhibit the hydrolysis activity of porcine pancreatic elastase (Sigma, USA) using the synthetic tripeptidic substrate MeOSuc-AAA-pNA (Bachem, Switzerland) was determined as follows:

The above substrate (1mM) and human porcine pancreatic elastase (15nM) were incubated at 37°C with serial dilutions of the peptides (0.5% DMSO final) in assay

buffer (50mM Tris, pH8, 100mM NaCl, 0.01% Tween20). The release of pNA was followed by monitoring the change in absorbance at 405nm for 30 minutes. Control assays with the same assay set-up as above, but without peptide, ran linearly. The dose-response data were fitted to the 4-parameter Hill equation providing the IC_{50} value using Graphpad (Prism 5).

2.4. Inhibition of human proteinase 3

The inactivation of human proteinase 3 (Elastin Products Company, USA) by the peptides of the invention using synthetic tripeptidic substrate Boc-Ala-Ala-Nva-SBzl (Elastin Products Company, USA) was determined as follows:

The above substrate (1mM), 4,4′-dithiodipyridine (250µM) and human proteinase 3 (10nM) were incubated at 37°C with serial dilutions of the peptides (0.5% DMSO final) in assay buffer (50mM Tris, pH7.4, 150mM NaCl, 0.01% Tween20). The reaction process was followed by monitoring the change in absorbance at 340nm for 30 minutes. Control assays with the same assay set-up as above, but without peptide, ran linearly. The dose-response data were fitted to the 4-parameter Hill equation providing the IC_{50} value using Graphpad (Prism 5).

3.0 Results

The results of the experiments described under 2.2 - 2.4, above, are indicated in *Table 2* herein below.

Table 2:

Ex.	Human neutrophil elastase (hNE) IC_{50} [nM]	hNE IC_{50} SD [nM]	Porcine pancreatic elastase (PPE) IC_{50} [µM]	PPE IC_{50} SD [µM]	Human proteinase 3 (hPr3) IC_{50} [µM]	hPr3 IC_{50} SD [µM]	hNE/PPE selectivity	hNE/hPr3 selectivity
1	5.3	0.3	1.55	0.47	1.81	0.1	292	342
2	5.4	1.0	0.53	0.13	1.52	0.79	98	282
3	5.5	0.4	0.79	0.28	1.44	0.14	144	262
4	7.9	3.7	2.75	0.44	2.15	0.88	348	272
5	12.7	0.7	1.75	0.01	1.71	0.66	138	135
6	15.5	0.8	2.69	0.26	2.37	0.26	174	153
7	14.9	0.1	59.0	4.5	80.2	28	3960	5383
8	16.4	7.9	45.2	1.3	88.1	16.8	2756	5372
9	23.7	7.4	83.4	2.2	70.1	46.5	3519	2958
10	30	7.5	>100	n.d.	>100	n.d.	>3333	>3333
11	12.5	1.1	>100	n.d.	>100	n.d.	>8000	>8000
12	10.0	5.2	>100	n.d.	>100	n.d.	>10000	>10000
13	30.6	6.5	>100	n.d.	>100	n.d.	>3268	>3268
14	20.3	9.2	4.65	0.9	77.6	15.4	229	3823

Table 2, continued:

Ex.	Human neutrophil elastase (hNE) IC_{50} [nM]	hNE IC_{50} SD [nM]	Porcine pancreatic elastase (PPE) IC_{50} [µM]	PPE IC_{50} SD [µM]	Human proteinase 3 (hPr3) IC_{50} [µM]	hPr3 IC_{50} SD [µM]	hNE/PPE selectivity	hNE/hPr3 selectivity
15	18.6	9.2	1.62	0.4	6.69	2.4	87	360
16	26.4	11.6	>100	n.d.	>100	n.d.	>3788	>3788
17	15.6	9.2	66.4	7.8	83.9	11.4	4256	5378
18	6.9	2.9	7.2	1.8	1.8	0.1	1043	261

n.d. = not determined

CLAIMS

1. A backbone cyclized peptidic compound, built up from 13 amino acid residues, of the general formula

cyclo(-Xaa^1-Xaa^2-Thr^3-Xaa^4-Ser^5-Xaa^6-Xaa^7-Xaa^8-Xaa^9-Xaa^{10}-Xaa^{11}-Xaa^{12}-Xaa^{13}-)

(I),

and pharmaceutically acceptable salts thereof,

wherein

Xaa^1 is OctGly; Arg; hArg; or Glu(Phenethyl);

Xaa^2 is Glu; Nle; hTyr; or Val;

Xaa^4 is Ala; or AllylGly;

Xaa^6 is Ile;

Xaa^7 is Pro;

Xaa^8 is Pro;

Xaa^9 is Gln; or Tyr;

Xaa^{10} is Lys;

Xaa^{11} is hLeu; Ser; Thr; Asn; Tyr; hGln; or His;

Xaa^{12} is ^{D}Pro; and

Xaa^{13} is Pro;

with the proviso that

- if Xaa^1 is OctGly, then
  Xaa^2 is Glu; or Nle;
  Xaa^4 is Ala;
  Xaa^6 is Ile;
  Xaa^{10} is Lys;
  Xaa^{11} is Ser; Thr; or Asn
  Xaa^{13} is Pro;

and with the further proviso that

- if Xaa^{11} is Tyr; or His, then
  Xaa^{1} is Arg; hArg or Glu(Phenethyl).

2. A compound according to claim 1 of the formula I wherein

Xaa^{1} is OctGly; Arg; or Glu(Phenethyl);

Xaa^{2} is Glu; Nle; hTyr; or Val;

Xaa^{4} is Ala;

Xaa^{6} is Ile;

Xaa^{7} is Pro;

Xaa^{8} is Pro;

Xaa^{9} is Gln; or Tyr;

Xaa^{10} is Lys;

Xaa^{11} is Ser; Thr; Asn; Tyr; or His;

Xaa^{12} is ^{D}Pro; and

Xaa^{13} is Pro;

with the proviso that

- if Xaa^{1} is OctGly, then
  Xaa^{2} is Glu; or Nle;

and with the further proviso that

- if Xaa^{11} is Tyr; or His, then
  Xaa^{1} is Arg.

3. A compound according to claim 1 of the formula I wherein

Xaa^{1} is Arg; or hArg;

Xaa^{2} is Glu; Val; or hTyr;

Xaa^{4} is Ala; or AllylGly;

Xaa^{6} is Ile;

Xaa^{7} is Pro;

Xaa^8 is Pro;

Xaa^9 is Gln; or Tyr;

Xaa^{10} is Lys;

Xaa^{11} is hLeu; Ser; Thr; or hGln;

Xaa^{12} is ^{D}Pro; and

Xaa^{13} is Pro.

4. A compound according to claim 1 which is selected from

Cyclo(-OctGly-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Asn-^{D}Pro-Pro-);

Cyclo(-OctGly-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-OctGly-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Ser-^{D}Pro-Pro-);

Cyclo(-OctGly-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-Arg-hTyr-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Tyr-^{D}Pro-Pro-);

Cyclo(-Arg-Nle-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Tyr-^{D}Pro-Pro-);

Cyclo(-Arg-Glu-Thr-AllylGly-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-Ser-^{D}Pro-Pro-);

Cyclo(-hArg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-hLeu-^{D}Pro-Pro-);

Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-hGln-^{D}Pro-Pro-);

Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-Arg-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Tyr-Lys-His-^{D}Pro-Pro-);

Cyclo(-Glu(Phenethyl)-Glu-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Tyr-^{D}Pro-Pro-);

Cyclo(-Arg-Val-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-Arg-hTyr-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Thr-^{D}Pro-Pro-);

Cyclo(-Arg-Val-Thr-Ala-Ser-Ile-Pro-Pro-Gln-Lys-Tyr-^{D}Pro-Pro-).

5. A compound according to anyone of claims 1 to 4 of the formula I, in free form or in pharmaceutically acceptable salt form, for use as a medicament.

6. A compound according to anyone of claims 1 to 4 of the formula I, in free form or in pharmaceutically acceptable salt form, having inhibitory activity against elastase for use in the treatment or prevention of lung cancer; breast cancer; psoriasis; alpha 1 antitrypsin deficiency; pulmonary emphysema; cystic fibrosis; chronic obstructive pulmonary disease; idiopathic pulmonary fibrosis; bronchiectasis; pulmonary hypertension; arterial pulmonary hypertension; cardiac hypertrophy; myocarditis; acute myocardial infarction; rheumatoid arthritis; osteoarthritis; atherosclerosis; multiple sclerosis; pancreatitis; allergic rhinitis; systemic inflammatory respiratory syndrome; inflammatory dermatoses; inflammatory bowel disease; or Crohn's disease.

7. A pharmaceutical composition comprising a compound or a mixture of compounds according to any one of claims 1 to 4 of the formula I, in free form or in pharmaceutically acceptable salt form, and a pharmaceutically inert carrier, particularly in a form suitable for inhalation, for oral, topical, transdermal, injection, buccal, transmucosal, rectal, pulmonary or inhalation administration especially in the form of a tablet, dragee, capsule, solution, liquid, gel, plaster, cream, ointment, syrup, slurry, suspension, powder or suppository.

8. A compound according to anyone of claims 1 to 4 of the formula I, in free form or in pharmaceutically acceptable salt form, for use as a pharmaceutically active substance having selective protease inhibitory activity, in particular against human neutrophil elastase, and/or anticancer activity and/or anti inflammatory activity and/or anti infective activity and/or anticardiovascular activity and/or antiimmunological activity and/or antineurodegenerative activity.

9. A compound according to any one of claims 1 to 4, in free form or in pharmaceutically acceptable salt form, or a composition according to claim 8, for use as a medicament having inhibitory activity against elastase for the treatment or prevention of infections or diseases caused by such infections; or cancer, such as

lung cancer, or breast cancer, mediated or resulting from; or immunological diseases, such as psoriasis, mediated or resulting from; or pulmonary diseases, such as alpha 1 antitrypsin deficiency, pulmonary emphysema, cystic fibrosis, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, bronchiectasis, pulmonary hypertension, or arterial pulmonary hypertension, mediated or resulting from; or cardiovascular diseases, such as cardiac hypertrophy, myocarditis, or acute myocardial infarction, mediated or resulting from; or neurodegenerative diseases mediated or resulting from; or inflammation or diseases caused by inflammation, such as rheumatoid arthritis, osteoarthritis, atherosclerosis, multiple sclerosis, pancreatitis, allergic rhinitis, systemic inflammatory respiratory syndrome, inflammatory dermatoses, inflammatory bowel disease, or Crohn's disease, mediated or resulting from elastase activity; or where immunological reaction is mediated or resulting from elastase activity.

10. The use of a compound according to any one of claims 1 to 4, in free form or in pharmaceutically acceptable salt form, for the manufacture of a medicament having inhibitory activity against elastase for the treatment or prevention of infections or diseases caused by such infections; or cancer , such as lung cancer, or breast cancer, mediated or resulting from; or immunological diseases, such as psoriasis, mediated or resulting from; or pulmonary diseases, such as alpha 1 antitrypsin deficiency, pulmonary emphysema, cystic fibrosis, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, bronchiectasis, pulmonary hypertension, or arterial pulmonary hypertension, mediated or resulting from; or cardiovascular diseases, such as cardiac hypertrophy, myocarditis, or acute myocardial infarction, mediated or resulting from; or neurodegenerative diseases mediated or resulting from; or inflammation or diseases caused by inflammation, such as rheumatoid arthritis, osteoarthritis, atherosclerosis, multiple sclerosis, pancreatitis, allergic rhinitis, systemic inflammatory respiratory syndrome, inflammatory dermatoses, inflammatory bowel disease, or Crohn's disease,

mediated or resulting from elastase activity; or where immunological reaction is mediated or resulting from elastase activity.

11. The use of a pharmaceutically acceptable amount of a compound or pharmaceutical composition according to any of the claims 1 to 4 or 7 in the manufacture of a medicament for the treatment of an infection or a disease or disorder associated with such an infection resulting from; or cancer mediated or resulting from; or immunological diseases mediated or resulting from; or pulmonary diseases mediated or resulting from; or cardiovascular diseases mediated or resulting from; or neurodegenerative diseases mediated or resulting from; or inflammation mediated or resulting from elastase activity; or where immunological reaction is mediated or resulting from elastase activity, wherein said compound or pharmaceutical composition is to be administered to a subject in need thereof.

12. A process for the manufacture of a compound as defined in any one of the claims 1 to 4 of the formula I comprising the steps of

(a) coupling an appropriately functionalized solid support with an appropriately N-protected derivative of that amino acid which in the desired end-product corresponds to Xaa^n, wherein n is 13, 8, 7, 6, 5 or 4, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(b) removing the N-protecting group from the product thus obtained;

(c) coupling the product thus obtained with an appropriately N-protected derivative of that amino acid which in the desired end-product corresponds to Xaa^{n-1}, any functional group which may be present in said N-protected amino acid derivative being likewise appropriately protected;

(d) removing the N-protecting group from the product obtained in step (c);

(e) effecting steps substantially corresponding to steps (c) and (d) using appropriately N-protected derivatives of amino acids which in the desired end-product are in positions n-2 to 1, any functional group(s) which may be

present in said N-protected amino acid derivatives being likewise appropriately protected;

(f) if n is not 13, further effecting steps substantially corresponding to steps (c) and (d) using appropriately N-protected derivatives of amino acids which in the desired end-product are in positions 13 to n + 1, any functional group(s) which may be present in said N-protected amino acid derivatives being likewise appropriately protected;

(g) detaching the product thus obtained from the solid support;

(h) cyclizing the product cleaved from the solid support;

(i) removing any protecting groups present on functional groups of any members of the chain of amino acid residues; and

(j) if desired, converting the product thus obtained into a pharmaceutically acceptable salt or converting a pharmaceutically acceptable, or unacceptable, salt thus obtained into the corresponding free compound or into a different, pharmaceutically acceptable, salt.