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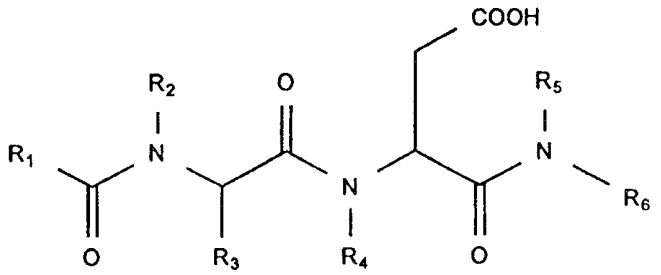
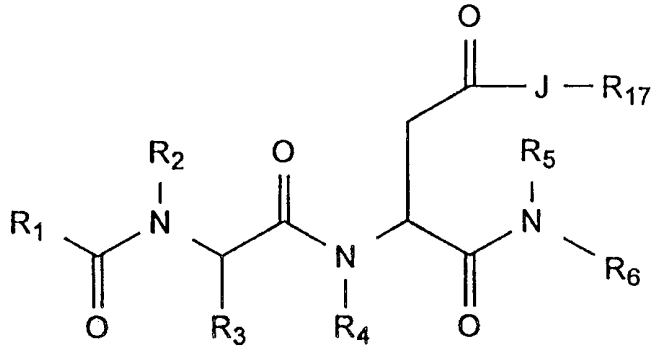
(54) Title: CS-1 PEPTIDOMIMETICS, COMPOSITIONS AND METHODS OF USING THE SAME

(57) Abstract

The present invention contemplates a compound defined by formula (I) and formula (II), that inhibits the binding between the VLA-4 and the fibronectin CS-1 compound. Pharmaceutical compositions containing a contemplated compound and methods for treating immunoinflammatory conditions using the compound are also disclosed.



(I)



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CS-1 PEPTIDOMIMETICS, COMPOSITIONS AND METHODS OF USING THE SAME

Description

Technical Field

The present invention relates to binding of inflammatory cells to endothelial cells that express the CS-1 portion of fibronectin on their surfaces, and more particularly to the inhibition of that binding by peptidomimetic compounds of minimal length.

Background Art

The immune response relies on leukocyte trafficking and immune surveillance as one of the underpinnings of host defense. Not only does this immune surveillance allow leukocytes to recirculate through lymphoid tissues normally, but also permits rapid leukocyte recruitment and extravasation to adjacent tissues at sites of inflammation. The $\alpha 4\beta 1$ (CD49d/CD29, VLA-4) cell adhesion receptor is an active participant in these leukocyte trafficking functions [Hemler, Ann. Rev. Immunol., 8:365-400 (1990); Hemler et al., Immunol. Rev., 114:45-65 (1990)].

The VLA-4 integrin heterodimer was discovered independently by three research groups and identified as a surface antigen on lymphocytes [Sanchez-Madrid et al., Eur. J. Immunol., 16: 1343-1349 (1986); Clayberger et al., J. Immunol., 138:1510-1514 (1987); Hemler et al., J. Biol. Chem., 262:11478-11485 (1987)]. Within the integrin family, VLA-4 is unique on several counts: (I) in contrast to related members of the β1 subfamily, VLA-4 is predominantly expressed on cells of the hematopoietic lineage [Hemler, Ann. Rev. Immunol., 8:365-400 (1990)], and is functionally involved in cell-cell, as well as cell-extracellular matrix (ECM) adhesive interactions [Hemler, Ann. Rev. Immunol., 8:365-400 (1990)]; (ii) despite sequence homology with other integrin α subunits, the α4 subunit stands apart from the two major structural clusters of α subunits because α4 lacks an inserted I-domain, and does not undergo post-translational cleavage near the transmembrane region [Hemler, Ann. Rev. Immunol., 8:365-400 (1990); Hynes, Cell, 69:11-25 (1992)]; and (iii) α4 contains a trypsin-like cleavage site that results in cell type-specific surface expression of at least two different structural variants termed α4-150 and α4-80/70 [Pulido et al., FEBS Lett., 294:121-124 (1991); Teixido et al., J. Biol. Chem., 267:1786-1791 (1992); Rubio et al., Eur. J. Immunol., 22:1099-1102 (1992)].

The VLA-4 integrin appears to be one of the earliest adhesion receptors found on CD34-expressing hematopoietic stem cells [Teixido et al., J. Clin. Invest., 90:358-367 (1992)]. However, VLA-4 is expressed only on mature T and B lymphocytes, natural killer (NK) cells, monocytes, basophils and eosinophils, but not on erythrocytes, platelets and neutrophils [Hemler, Ann. Rev. Immunol., 8:365-400

(1990); Gismondi et al., J. Immunol., 146:384-392 (1991); Walsh et al., J. Immunol., 146:3419-3423 (1991); Bochner et al., J. Exp. Med., 173:1553-1556 (1992); Dobrina et al., J. Clin. Invest., 88:20-26 (1991); Weller et al., Proc. Natl. Acad. Sci. USA, 88:7430-7433 (1991)].

To date, most adhesion functions mediated by VLA-4 can be explained by a direct molecular interaction between the VLA-4 integrin and either of two separate counter receptor structures, namely, the cytokine-inducible vascular cell adhesion molecule-1 (VCAM-1) [Elices et al., Cell, 60:577-584 (1990); Rice et al., J. Exp. Med., 171:1369-1374 (1990); Schwartz et al., J. Clin. Invest., 85:2019-2022 (1990); Carlos et al., Blood, 76:965-970 (1990)], and a subset of the ubiquitous ECM protein fibronectin [Wayner et al., J. Cell Biol., 109:1321-1330 (1989); Guan et al., Cell, 60:53-61 (1990); Ferreira et al., J. Exp. Med., 171:351-356 (1990); Elices et al., Cell, 60:577-584 (1990)].

VCAM-1 is a member of the immunoglobulin (Ig) gene superfamily [Osborn et al., Cell, 59: 1203-1211 (1989); Rice et al., Science, 246:1303-1306 (1989)] that is expressed predominantly in vascular endothelium in response to pro-inflammatory cytokines such as IL-1, TNFα, and IL-4 [Osborn et al., Cell, 59:1203-1211 (1989); Rice et al., Science, 246: 1303-1306 (1989); Thornhill et al., J. Immunol., 145:865-872 (1990); Masinovsky et al., J. Immunol., 145:2886-2895 (1990); Thornhill et al., J. Immunol., 146:592-598 (1991); Schleimer et al., J. Immunol., 148:1086-1092 (1992); Birdsall et al., J. Immunol., 148:2717-2723 (1992); Swerlick et al., J. Immunol., 149:798-705 (1992); Briscoe et al., J. Immunol., 149:2954-2960 (1992)]. The VLA-4 binding sites on

VCAM-1 have been mapped to the outermost N-terminal (first) Ig-like region of the 6-Ig-like domain VCAM-1 isoform [Taichman et al., Cell Regul., 2:347-355 (1991); Vonderheide et al., J. Exp. Med., 175: 1433-1442 (1992); Osborn et al., J. Exp. Med., 176:99-107 (1992)], and the first and fourth N-terminal Ig-like regions of the 7-Ig-like domain VCAM-1 isoform [Vonderheide et al., J. Exp. Med., 175:1433-1442 (1992); Osborn et al., J. Exp. Med., 176:99-107 (1992)]. The VLA-4 binding amino acid motif Gln-Ile-Asp-Ser-Pro-Leu (QIDSPL in the single-letter amino acid code) appears to be on an exposed loop, in the three dimensional structure of VCAM-1 [(Jones et al., Nature, 373:539-544 (1995)].

A high affinity peptide recognition sequence for VLA-4 within fibronectin (FN) has also been identified [Wayner et al., J. Cell. Biol., 109:1321-1330 (1989); Ferreira et al., J. Exp. Med., 171:351-356 (1990); Guan et al., Cell, 60:53-61 (1990); Mould et al., J. Biol. Chem., 265:4020-4024 (1990); Garcia-Pardo et al., J. Immunol., 144: 3361-3366 (1990); Komoriya et al., J. Biol. Chem., 266:15075-15079 (1991)]. That sequence comprises a 25-amino acid residue stretch, termed CS-1 [Humphries et al., J. Cell Biol., 103:2637-2647 (1986); Humphries et al., J. Biol. Chem., 262:6886-6892 (1987)].

The FN gene contains three separate exons termed EIIIA, EIIIB and V or IIICS, which are subject to alternative splicing [Hynes, "Fibronectin", Springer-Verlag, New York (1990)]. The presence of additional acceptor and donor splice signals within the IIICS region permits generation of increased diversity in FN by virtue of multiple IIICS polypeptide variants, for instance, five in human FN

[Vibe-Pedersen et al., FEBS Lett., 207:287-291 (1987); Hershberger et al., Mol. Cell. Biol., 10: 662-671 (1990)]. Consequently, only a subset of these molecular variants expresses the 25-amino acid CS-1 sequence recognized by VLA-4 [Wayner et al., J. Cell. Biol., 109:1321-1330 (1989); Guan et al., Cell, 60:53-61 (1990)].

A minimal essential sequence for specific VLA-4 recognition of CS-1 has been identified as the tripeptide Leu-Asp-Val (LDV) [Komoriya et al., J. Biol. Chem., 266:15075-15079 (1991); Wayner et al., J. Cell. Biol., 116:489-497 (1992); Wayner WO 91/03252 published March 21, 1991; Wayner WO 93/12809 published July 8, 1993; and Humphries WO 92/13887, published August 20, 1992] albeit VLA-4 binds to LDV with at least two orders of magnitude lower affinity than to the native CS-1 25-mer. Cystine-linked cyclic peptides that mimic the RGD adhesion signal have also been described as inhibitors of VLA-4 mediated interactions [Nowlin et al., J. Biol. Chem., 268:20352-20359 (1993); Cardarelli et al., J. Biol. Chem., U269U:18668-18673 (1994)].

VLA-4 shares with other members of the β1 integrin subfamily the ability to promote binding and penetration of microbial pathogens into mammalian cells. Thus, specific interactions of β1 integrins with the bacterial protein invasin [Isberg et al., Cell, 60:861-871 (1990); Ennis et al., J. Exp. Med., 177:207-212 (1993)], as well as the protozoan Trypanosoma cruzi [Fernandez et al., Eur. J. Immunol., 23:552-557 (1993)] have been described.

A multitude of in vitro studies suggest interactions of VLA-4 with its two known ligands, VCAM-1 and CS-1 FN, have profound biological significance. For instance, VLA-4 binding to VCAM-1 has been demonstrated in adhesion to cytokine-stimulated vascular endothelium by lymphocytes [Elices et al., Cell, 60:577-584 (1990); Rice et al., J. Exp. Med., 171:1369-1374 (1990); Schwartz et al., J. Clin. Invest., 85:2019-2022 (1990); Carlos et al., Blood, 76:965-970 (1990); Shimizu et al., J. Cell Biol., 113:1203-1212 (1991)], monocytes [Carlos et al., Blood, 77:2266-2271 (1991); Jonjic et al., J. Immunol., 148:2080-2083 (1992)], natural killer (NK) cells [Allavena et al., J. Exp. Med., 173:439-448 (1991)], and eosinophils [Walsh et al., J. Immunol., 146:3419-3423 (1991); Bochner et al., J. Exp. Med., 173:1553-1556 (1992); Dobrina et al., J. Clin. Invest., 88:20-26 (1991); Weller et al., Proc. Natl. Acad. Sci. USA, 88:7430-7433 (1991)]. Because of its involvement in mediating leukocyte-endothelial attachment, VLA-4/VCAM-1 interactions are considered key in inflammation.

The VLA-4/CS-1 interaction, in turn, has been widely documented in hematopoiesis where adhesive interactions between hematopoietic progenitors expressing VLA-4 [Hemler et al., Immunol. Rev., 114:45-65 (1990); Williams et al., Nature, 352:438-441 (1991); Roldan et al., J. Exp. Med., 175:1739-1747 (1992); Sawada et al., J. Immunol., 149:3517-3524 (1992); Wadsworth et al., J. Immunol., 150:847-857 (1993)] and their ECM microenvironment play a critical role in precursor maturation and differentiation. Thus, CS-1 peptides have been shown to inhibit (I) attachment of murine hematopoietic stem cells to ECM derived from bone marrow stroma [Williams et al., Nature, 352:438-441 (1991)], (ii)

immunoglobulin secretion by bone marrow-derived B cell progenitors [Roldan et al., J. Exp. Med., 175:1739-1747 (1992)], (iii) bursal and postbursal development of chicken B cells [Palojoki et al., Eur. J. Immunol., 23:721-726 (1993)], and (iv) thymocyte adhesion and differentiation induced by thymic stromal cell monolayers [Utsumi et al., Proc. Natl. Acad. Sci. USA, 88:5685-5689 (1991); Sawada et al., J. Immunol., 149:3517-3524 (1992)]. VLA-4/CS-1 may also be involved in embryonic development, because CS-1 peptides have been shown to interfere with migration of avian neural crest cells [Dufour et al., EMBO J., 7:2661-2671 (1988)].

In addition to VCAM-1, FN and CS-1 have also been implicated in the pathology of rheumatoid arthritis (RA) [Laffon et al., J. Clin. Invest., 88:546-552 (1992)]. A role for the CS-1 splicing variant of FN has been established in mediating migration of inflammatory cells such as eosinophils across endothelial cell monolayers of VLA-4-expressing leukocytes [Kuijpers et al., J. Exp. Med., 178:279-284 (1993)].

The vast body of work suggesting that VLA-4 plays a role in leukocyte trafficking and inflammation has been largely confirmed by in vivo studies using anti-VLA-4 antibodies in various animal models. Essentially, the skin, brain, kidney, lung and gut are targets of a wide variety of VLA-4-dependent inflammatory reactions mostly resulting from recruitment of mononuclear leukocytes and eosinophils.

More specifically, these in vivo studies are as follows: contact hypersensitivity (CH) and delayed type hypersensitivity (DTH) in the mouse and

rat [Ferguson et al., Proc. Natl. Acad. Sci. USA, 88:8072-8076 (1991); Issekutz, Cell Immunol., 138:300-312 (1991); Issekutz, J. Immunol., 147: 4178-4184 (1991); Elices et al., Clin. Exp. Rheumatol., 11:S77-80 (1993); Chisholm, et al., Eur. J. Immunol., 23:682-688 (1993)]; experimental autoimmune encephalomyelitis (EAE) in the mouse and rat [Yednock et al., Nature, 356:63-66 (1992); Baron et al., J. Exp. Med., 177:57-68 (1993)]; nephrotoxic nephritis in the rat [Mulligan et al., J. Clin. Invest., 91:577-587 (1993)]; passive cutaneous anaphylaxis in the guinea pig [Weg et al., J. Exp. Med., 177:561-566 (1993)]; immune complex-induced lung injury in the rat [Mulligan et al., J. Immunol., 150:2401-2406 (1993); Mulligan et al., J. Immunol., 150:2407-2417 (1993)], spontaneous colitis in the monkey [Poldolsky et al., J. Clin. Invest., 92: 372-380 (1993)]; and allergic asthma in multiple species [Lobb, WO 92/13978 published July 22, 1993; Rabb et al., Am. J. Respir. Crit. Care Med., 149:1186-1191 (1994); Pretolani et al., J. Exp. Med., 180:795-805 (1994); Metzger, Springer Sem. Immunopathol., 16:467-478 (1995); Richards et al., Am. J. Resp. Cell. Mol. Biol., 15:172-183 (1996); Sagara et al., Int. Arch. Allergy Immunol., 112:287-294 (1997); Fryer et al., J. Clin. Invest., 99:2036-2044 (1997); Abraham et al., Am. J. Resp. Crit. Care Med. 156:696-703 (1997); Henderson et al., J. Clin. Invest., 100:3038-3092 (1997).

Thus, a preliminary conclusion from in vivo results is that VLA-4 contributes to inflammatory responses that emulate chronic conditions in humans. In an in vivo model of murine contact hypersensitivity, the CS-1 peptide partially inhibited recruitment of T lymphocytes to skin inflammatory sites [Ferguson et al., Proc. Natl.

Acad. Sci. USA, 88:8072-8076 (1991)]. Because the Arg-Gly-Asp peptide from the cell adhesion domain of FN was also inhibitory in this animal model, the authors concluded that emigration of immune T cells to sites of antigenic challenge in the tissue could be facilitated by the interaction of leukocyte integrins with ECM proteins such as FN [Ferguson et al., Proc. Natl.. Acad. Sci. USA, 88:8072-8076 (1991)].

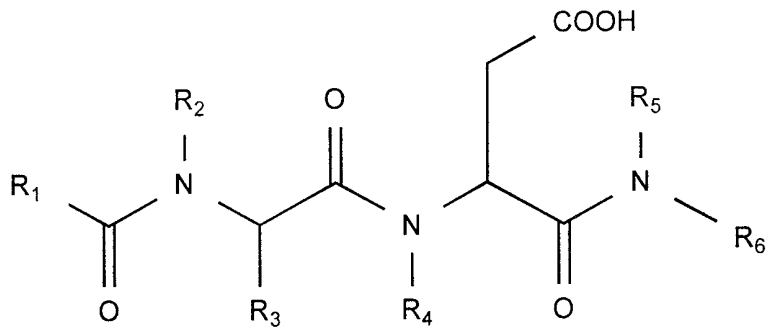
In a more recent study, Elices and coworkers [Elices et al., Clin. Exp. Rheumatol., 11:S77-80 (1993)] were unable to reproduce inhibition of contact hypersensitivity with the native CS-1 peptide. Instead, they found that the CS-1 peptide was rapidly cleared from blood circulation by proteolytic degradation.

The role of VLA-4 and the CS-1 peptide in various chronic and acute immunoinflammatory disease states having been established, it would be of importance if compounds could be found that inhibit the VLA-4-lymphocyte interaction and were other than anti-VLA-4 antibodies that can themselves induce an immune response on repeated administration or the CS-1 peptide that is large and costly to make, and also is subject to rapid degradation.

Brief Summary of the Invention

Novel CS-1 peptidomimetic inhibitor compounds of Formula IA and Formula IIA, their compositions and methods of use for treating inflammation, asthma and cardiovascular disease.

Formula IA,



wherein,

R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl; the R_1 ring structure can form at R_1, between R_1 and R_2 or between R_1 and R_4 with the proviso that, if the R_1 ring structure forms at R_1, the R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups; the spacer can be optionally substituted by an amino group; the R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6, 5-membered ring wherein, the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups; the R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms, and, if the R_1 ring structure is formed between R_1 and R_4, the heteroatoms are 2 nitrogen atoms; the R_1 ring structure can be conjugated, partially saturated, or saturated; the lower alkyl or lower amino alkyl group can be branched;

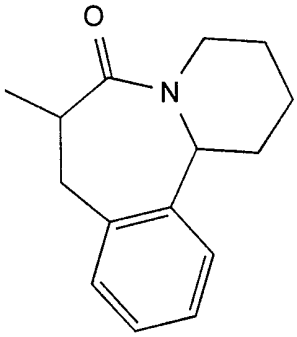
R_2 is a H, lower alkyl, phenyl lower alkyl, or R_2 and R_1 form the R_1 ring structure group;

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl, or di(lower alkyl)thioether; the R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long; the lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched;

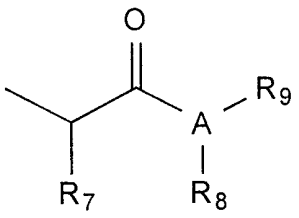
R_4 is a H or R_4 and R_1 form the R_1 ring structure;

R_5 is H or R_5 and R_6 form a R_5 ring structure; the R_5 ring structure is a fused 6,6- ring structure and can be aromatic, partially saturated, or saturated;

R_6 is a benzyl, a 5,6,or 7-membered heterocyclic saturated ring containing 1 or 2 nitrogen atoms optionally substituted by one or more lower alkyl, lower alkyl amide or acyl groups or 1,1 diphenylmethine group, the R_5 ring structure, a group of the formula



or a group of the formula



wherein, A is nitrogen or oxygen;
when A is nitrogen,

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group; the R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long; if the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein, the heteroatom is a nitrogen atom; if the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein, the heteroatoms are 1 or 2 nitrogen atoms; the R_7 ring structure can optionally be substituted by an alcohol, nitro, or lower alkyl ether group;

R_8 is a ring structure, alkyl, alkyl alcohol, or thioalkyl amide group; the ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_8 and R_9 and is an R_8 ring structure; the R_8 ring structure is a 5-, 6- 7- or fused 6,5-membered heterocyclic ring wherein, the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms; the R_8 ring

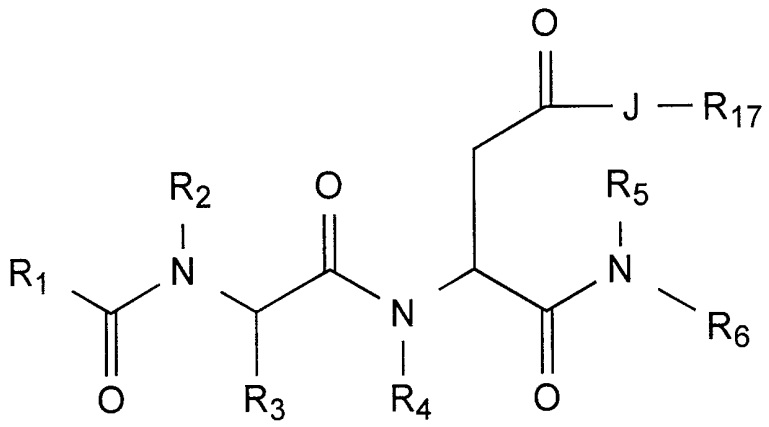
structure optionally can be substituted by one or more lower alkyl, lower dialkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, carboxamide, lower alkyl carboxylic acid, carbonyl, sulfoxide, sulfone or alkyl substituted phenyl sulfonamido groups; the (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups;

R_9 is the R_8 ring structure, a lower alkyl, lower dialkyl, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group; and when A is oxygen:

R_8 is a lower alkyl that can be branched and R_9 is absent; or

a pharmaceutically acceptable salt thereof.

Formula IIA,



wherein,

R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl; the R_1 ring structure can form at R_1, between R_1 and R_2 or between R_1 and R_4 with the proviso that, if the R_1 ring structure forms at R_1, the R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups; the spacer can be optionally substituted by an amino group; the R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6, 5-membered ring wherein, the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups; the R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms, and, if the R_1 ring structure is formed between R_1 and R_4, the heteroatoms are 2 nitrogen atoms; the R_1 ring structure can be conjugated, partially saturated, or saturated; the lower alkyl or lower amino alkyl group can be branched;

R_2 is a H, lower alkyl, phenyl lower alkyl, or R_2 and R_1 form the R_1 ring structure group;

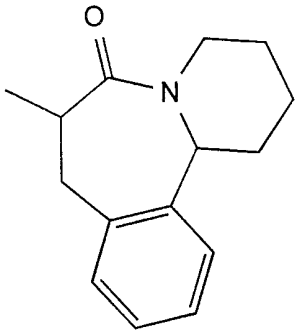
R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl, or di(lower alkyl)thioether; the R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long; the lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched;

R_4 is a H or R_4 and R_1 form the R_1 ring structure;

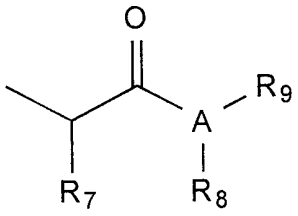
R_5 is H or R_5 and R_6 form a R_5 ring structure; the R_5 ring structure is a fused 6,6- ring

structure and can be aromatic, partially saturated, or saturated;

R_6 is a benzyl, a 5,6,or 7-membered heterocyclic saturated ring containing 1 or 2 nitrogen atoms optionally substituted by one or more lower alkyl, lower alkyl amide or acyl groups or 1,1 diphenylmethine group, the R_5 ring structure, a group of the formula



or a group of the formula



wherein:

A is nitrogen or oxygen;

when A is nitrogen;

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group; the R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl

group 0 to about 3 carbon atoms long; if the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein, the heteroatom is a nitrogen atom; if the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein, the heteroatoms are 1 or 2 nitrogen atoms; the R_7 ring structure can optionally be substituted by an alcohol, nitro, or lower alkyl ether group;

R_8 is a ring structure, alkyl, alkyl alcohol, or thioalkyl amide group; the ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_8 and R_9 and is an R_8 ring structure; the R_8 ring structure is a 5-, 6- 7- or fused 6,5-membered heterocyclic ring wherein, the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms; the R_8 ring structure optionally can be substituted by one or more lower alkyl, lower dialkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carboxamide, carbonyl, sulfoxide, sulfone or alkyl substituted phenyl sulfonamido groups; the (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups;

R_9 is the R_8 ring structure, a lower alkyl, lower dialkyl, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group;
when A is oxygen:

R_8 is a lower alkyl that can be branched and R_9 is absent;

J is oxygen or sulfur; and

R_{17} is alkyl, alkyl optionally substituted by a hydroxyl, phenyl or phenyl sulfonyl, di(lower alkyl)sulfide, (lower alkoxy)lower alkyl, [(lower alkoxy)lower alkoxy]lower alkyl, (lower alkylcarbonyloxy)lower alkyl, [N-(lower alkyl)aminocarbonyl]lower alkyl, {[(N-(lower alkyl)](N-(lower alkoxy)} amino-carbonyl)lower alkyl,(N,N-di(lower alkyl)aminocarbonyl)lower alkyl, (N'-morpholinocarbonyl)lower alkyl, (benzyloxycarbonyl)methyl, 1-((O-((lower alkylcarbonato))eth-1-yl group; 2-oxo-1,3-dioxolen-4-ylmethyl group, cyclopentyl, cyclohexyl, cycloheptyl, phenyl, 1,3- dioxan-2-yl, 3-tetrahydropyranyl, (4-hydroxybutyric)lacton-3-yl, phthalidyl, or fused 6,5-membered aromatic, partially aromatic, or non-aromatic ring, wherein said ring is connected to J either directly by a bond or indirectly by a lower alkyl group; or

a pharmaceutically-acceptable salt thereof.

A contemplated method corresponds to a method of treating inflammation, asthma and cardiovascular disease by administering an above-described compound.

Brief Description of the Drawings

In the drawings forming a portion of this disclosure:

Fig. 1 is a graph illustrating the in vitro binding inhibition of VLA-4-bearing Jurkat cells to the solid phase-bound CS-1 compound (SEQ ID NO:1) by that compound itself and shorter compounds having portions of the CS-1 compound sequence. Data are shown as percentages relative to the indicated "Standard" (SEQ ID NO:3). Data for compounds with deletions at the "N-terminus" of compound B12 (SEQ ID NO:2) are shown to the left of the Standard, and data for compounds with deletions at the "C-terminus" of compound B12 are shown to the right of the standard. Compound sequences are in single letter code.

Fig. 2 is a graph with data obtained and expressed similarly to those of Fig. 1. Here, binding inhibition by further, still shorter deletion compounds, is illustrated.

Fig. 3 is another graph of binding data obtained as discussed in Fig. 1. This graph utilizes an ordinate that is on a log scale. These data are arranged into five groups and are again shown relative to the indicated Standard compound (SEQ ID NO:3), with D-proline being shown as "p". The data of the right-hand-most two groups illustrate the effects of three different X groups on a single indicated compound and of three Z groups in which X is phenyl acetyl (ϕAc) and Z is as shown, respectively.

Fig. 4 shown in two panels as Fig. 4A and Fig. 4B illustrates the effects of a contemplated compound in treating asthma in the rabbit. Fig. 4A shows the percent change in dynamic compliance (C_{dyn}) over a six-hour time period immediately following the onset of induced asthma attacks. Data for rabbits treated by a nebulized composition containing the inhibitor compound N-phenyl acetyl-Leu-Asp-Phe-morpholinamide are shown as open circles, whereas data for untreated rabbits are shown with darkened circles; both circles including error bars. The ordinate is in units of percent change from the initial dynamic compliance value, whereas the abscissa is in units of hours after challenge.

Fig. 4B shows results obtained for the percent change in lung resistance (L_R) from the same study, with data being presented as in Fig. 4A. The ordinate is in units of percent change from the original lung resistance value, whereas the abscissa is in hours.

Fig. 5 is a graph showing the results of a study of the effect of the inhibitor compound N-phenyl acetyl-Leu-Asp-Phe-D-Pro-NH_2 on delayed type hypersensitivity measured in ears of 14 mice. After immunization, one group of seven mice was treated with only a saline solution provided by an implanted pump over a 24-hour time period and challenged. The other immunized group of seven mice was similarly challenged, but each was treated with an aqueous pharmaceutical composition containing the above inhibitor compound for the same 24-hour time period, also supplied by implanted pumps. The ordinate is in units of inches of swelling diameter at the challenge site.

Fig. 6 is a graph showing averaged clinical scores for six mice each in two evaluations of treatments of experimental autoimmune encephalomyelitis (EAE). Darkened circles are for treatments using the inhibitor compound N-phenyl acetyl-Leu-Asp-Phe-D-Pro-NH_2, whereas points shown as darkened squares are for treatments using the control sequence compound of Example 6. The ordinant shows the averaged score for the six mice in each study, whereas the abscissa is in days after initiation of EAE.

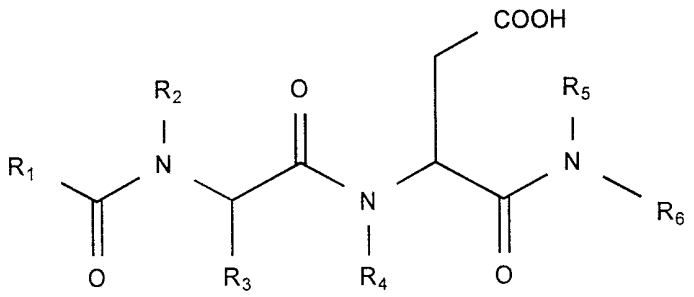
Fig. 7 is a graph showing the percentage of change in lung resistance, SR_L, from base line for the asthmatic sheep model depicted as described in Fig. 4B. The nebulized composition here contained N-phenyl acetyl-Leu-Asp-Phe-D-Pro-NH_2 in open circles and the control sequence of that compound (Example 6) in darkened circles, including error bars where appropriate.

Detailed Description of the Invention

The present invention contemplates a compound, a prodrug compound, a composition containing such a compound, and a method of using such a compound. A contemplated compound inhibits binding between the CS-1 of fibronectin and the inflammatory cell VLA-4 surface receptor, and is therefore sometimes referred to herein as an inhibitor compound.

A. Compounds

The present invention contemplates a compound and compositions that inhibits CS-1 binding to the VLA-4 receptor, e.g., a compound of Formula-IA.



Formula IA

wherein:

R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl; the R_1 ring structure can form at R_1, between R_1 and R_2 or between R_1 and R_4 with the proviso that, if the R_1 ring structure forms at R_1, the R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming one or more alkyl,

N-amido, N-sulfonimido, N-urea, N-carboxyl groups; the spacer can be optionally substituted by an amino group; the R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein, the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups; the R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms, and, if the R_1 ring structure is formed between R_1 and R_4, the heteroatoms are 2 nitrogen atoms; the R_1 ring structure can be conjugated, partially saturated, or saturated; the lower alkyl or lower amino alkyl group can be branched.

R_2 is a H, lower alkyl, phenyl lower alkyl, or R_2 and R_1 form the R_1 ring structure group.

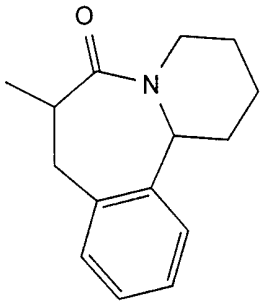
R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl, or di(lower alkyl)thioether; the R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long; the lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_4 is a H or R_4 and R_1 form the R_1 ring structure.

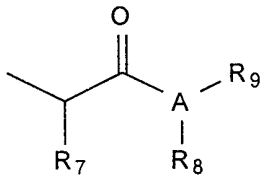
R_5 is H or R_5 and R_6 form a R_5 ring structure; the R_5 ring structure is a fused 6,6- ring structure and can be aromatic, partially saturated, or saturated.

R_6 is a benzyl, a 5,6,or 7-membered heterocyclic saturated ring containing 1 or 2 nitrogen atoms optionally substituted by one or more

lower alkyl, lower alkyl amide or acyl groups or 1,1 diphenylmethine group, the R_5 ring structure, a group of the formula



or a group of the formula



wherein:

A is nitrogen or oxygen;

when A is nitrogen;

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group; the R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long; if the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein, the heteroatom is a nitrogen atom; if the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a

5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein, the heteroatoms are 1 or 2 nitrogen atoms; the R_7 ring structure can optionally be substituted by an alcohol, nitro, or lower alkyl ether group.

R_8 is a ring structure, alkyl, alkyl alcohol, or thioalkyl amide group; the ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_8 and R_9 and is an R_8 ring structure; the R_8 ring structure is a 5-, 6- 7- or fused 6,5-membered heterocyclic ring wherein, the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms; the R_8 ring structure optionally can be substituted by one or more lower alkyl, lower dialkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, carboxamide, lower alkyl carboxylic acid, carbonyl, sulfoxide, sulfone or alkyl substituted phenyl sulfonamido groups; the (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups;

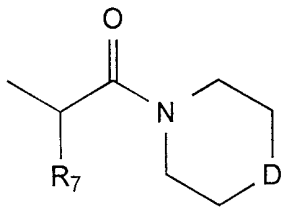
R_9 is the R_8 ring structure, a lower alkyl, lower dialkyl, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group; and

when A is oxygen:

R_8 is a lower alkyl that can be branched and R_9 is absent.

Preferred compounds of Formula IA are where R_2 is lower alkyl, or phenyl lower alkyl.

Also preferred are compounds of Formula IA are where R_6 is



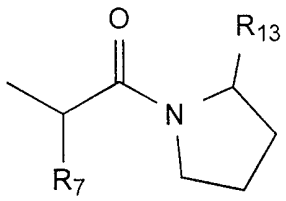
where D is carbon optionally substituted by one or more lower alkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, or alkyl substituted phenyl sulfonamido groups, or

where, D is a nitrogen atom optionally substituted by a lower alkyl, amino carbonyl lower alkyl wherein the nitrogen atom of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, lower alkyl alcohol, lower hydroxy alkyl ether, or lower alkyl carboxylic acid groups, or

where, D is an oxygen atom, or

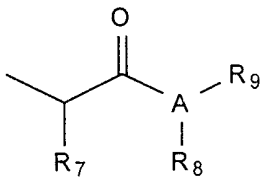
where, D is a sulfur atom optionally forming a sulfoxide or sulfone.

Also preferred are compounds of Formula IA are where R_6 is



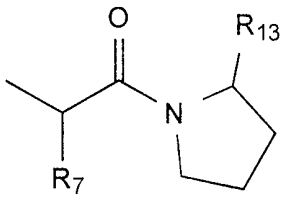
wherein, R_{13} is a lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, carboxamide, or H group.

Also preferred are compounds of Formula IA where R_6 is as described above, provided that in the formula:



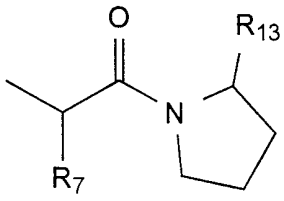
the R_8 ring structure is: not a 5-membered heterocyclic ring; more preferably, not a 5-membered heterocyclic ring where the heteroatoms are 1 or 2 nitrogen atoms; more preferably, not a 5-membered heterocyclic ring where the heteroatoms is 1 nitrogen atom; more preferably, not a 5-membered heterocyclic nitrogen ring that is substituted as decribed above; and, more preferably, not a 5-membered heterocyclic nitrogen ring that is substituted with a caroxamide.

Also preferred are compounds of Formula IA where R_7 is as described above, provided that R_6 is not the formula:



wherein R_{13} is as described above.

Also preferred are compounds of Formula IA where R_6 is as described above, provided that R_6 is the formula:

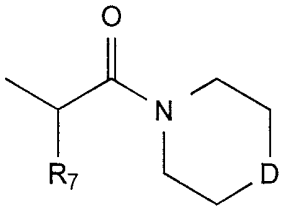


wherein R_{13} is as described above, provided that R_{13} is not carboxamide or lower alkyl carboxamide and, more preferably, provided that R_{13} is not carboxamide.

Also preferred are compounds of Formula IA where R_7 is H.

Also preferred are compounds of Formula IA where R_7 is H.

Especially preferred are compounds of Formula IA where R_2 is lower alkyl, or phenyl lower alkyl and R_6 is



where D is carbon optionally substituted by one or more lower alkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, or alkyl substituted phenyl sulfonamido groups, or

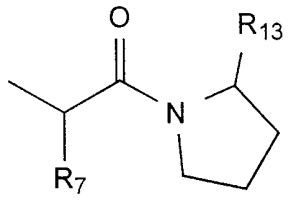
where, D is a nitrogen atom optionally substituted by a lower alkyl, amino carbonyl lower alkyl wherein the nitrogen atom of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, lower alkyl alcohol, lower hydroxy alkyl ether, or lower alkyl carboxylic acid groups, or

where, D is an oxygen atom, or

where, D is a sulfur atom optionally

forming a sulfoxide or sulfone, or

R_6 is



wherein, R_{13} is a lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, carboxamide, or H group.

Also preferred compounds of Formula IA are where R_2 is lower alkyl and, more preferably, methyl, excluding compound numbers 1190.07 and 926.11 listed in Table 1 below.

Most preferred compounds of Formula IA are where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen and R_8 is the ring structure formed between R_8 and R_9 selected from the group consisting of 4-morpholinyl, 4-methylpiperazinyl, and N-piperazinyl.

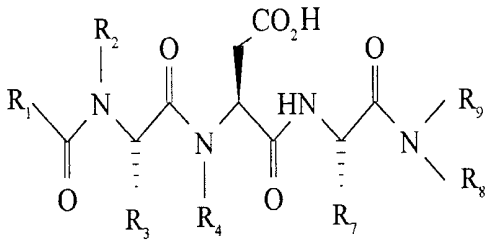
Also most preferred are compounds of Formula IA where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, and R_8 is the 4-morpholinyl ring structure formed between R_8 and R_9.

Also most preferred are compounds of Formula IA where R_1 is benzyl, R_2 is methyl, R_3 is

2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, and R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9.

Also most preferred are compounds of Formula IA where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, and R_8 is the N-piperazinyl ring structure formed between R_8 and R_9.

A contemplated compound can be defined by the following structural formula:



where R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl. The R_1 ring structure can form at R_1, between R_1 and R_2 or between R_1 and R_4 with the proviso that, if the R_1 ring structure forms at R_1, the R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups optionally substituted by an amino group. The R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups. The R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms, and, if the R_1 ring structure is formed between R_1 and R_4, the heteroatoms are 2 nitrogen atoms. The R_1 ring structure can be aromatic, partially saturated, or

saturated. The lower alkyl or lower amino alkyl group can be branched.

R_2 is a H or R_2 and R_1 form the R_1 ring structure group.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_4 is a H or R_4 and R_1 form the R_1 ring structure.

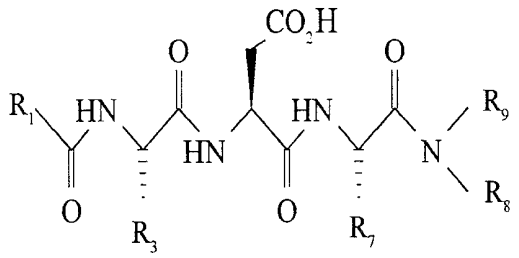
R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long. If the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. If the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein the heteroatoms are 1 or 2 nitrogen atoms. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

R_8 is a ring structure, alkyl alcohol, or thioalkyl amide group. The ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_8 and R_9 and

is an R_8 ring structure. The R_8 ring structure is a 5-, 6- or fused 6,5-membered heterocyclic ring wherein the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms. The R_8 ring structure optionally can be substituted by one or more lower alkyl, lower dialkyl, lower alkyl carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, sulfoxide, or alkyl substituted phenyl sulfonamido groups. The (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups.

R_9 is the R_8 ring structure, a lower alkyl, amine, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group.

A contemplated compound can be defined by the following structural formula:



where R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl. The R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming from one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups optionally substituted by an amino group. The R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein the substituent is one or more alkyl, carbonyl, alcohol, halogen, or

alkyl phenyl groups. The R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms. The R_1 ring structure can be aromatic, partially saturated, or saturated. The lower alkyl or lower amino alkyl group can be branched.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

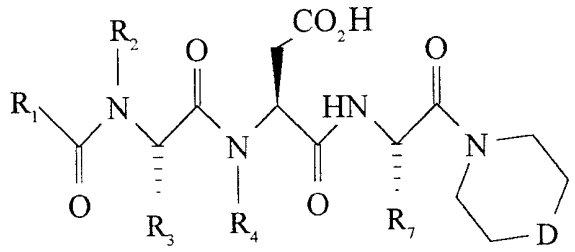
R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long. If the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. If the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein the heteroatoms are 1 or 2 nitrogen atoms. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

R_8 is a ring structure, alkyl alcohol, or thioalkyl amide group. The ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_8 and R_9 and is an R_8 ring structure. The R_8 ring structure is a 5-, 6- or fused 6,5-membered heterocyclic ring

wherein the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms. The R_8 ring structure optionally can be substituted by one or more lower alkyl, amine lower alkyl carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, sulfoxide, or alkyl substituted phenyl sulfonamido groups. The (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups.

R_9 is the R_8 ring structure, a lower alkyl, amine, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group.

A contemplated compound can be defined by the following structural formula:



where D is a carbon, nitrogen, oxygen, or sulfur atom optionally substituted by or forming a lower alkyl, amine lower alkyl carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, sulfoxide, or alkyl substituted phenyl sulfonamido group.

R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl. The R_1 ring structure can form at R_1, between R_1 and R_2 or between R_1 and R_4 with the

proviso that, if the R_1 ring structure forms at R_1, the R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups. The spacer can be optionally substituted by an amino group. The R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups. The R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms, and, if the R_1 ring structure is formed between R_1 and R_4, the heteroatoms are 2 nitrogen atoms. The R_1 ring structure can be aromatic, partially saturated, or saturated. The lower alkyl or lower amino alkyl group can be branched.

R_2 is a H or R_2 and R_1 form the R_1 ring structure group.

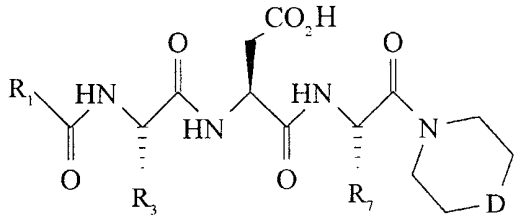
R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_4 is a H or R_4 and R_1 form the R_1 ring structure.

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the

heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

A contemplated compound can be defined by the following structural formula:



where D is a carbon, nitrogen, oxygen, or sulfur atom optionally substituted by or forming a lower alkyl, amine lower alkyl carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, sulfoxide, or alkyl substituted phenyl sulfonamido group.

R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl. The R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming from one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups. The spacer can be optionally substituted by an amino group. The R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups. The R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms. The R_1 ring structure can be aromatic, partially saturated, or saturated. The lower alkyl or lower amino alkyl group can be branched.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

The compound of the formula immediately above wherein D is a nitrogen atom optionally substituted by or forming a lower alkyl, amine lower alkyl, carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid or alkyl substituted phenyl sulfonamido group; R_1 is a lower alkyl or lower amino alkyl group, or 6-membered aromatic ring structure connected by a lower alkyl group; R_3 is a lower alkyl, lower alkyl alcohol, or lower thioalkyl group or cyclohexane connected by an alkyl group 0 to about 3 carbon atoms long; R_3 is a lower alkyl, lower alkyl alcohol, or lower thioalkyl group or 6-membered aromatic ring structure connected by an alkyl group 0 to about 3 carbon atoms long; and the lower alkyl, lower alkyl alcohol or lower thioalkyl group can be branched.

The compound of the formula immediately above wherein D is a carbon atom optionally

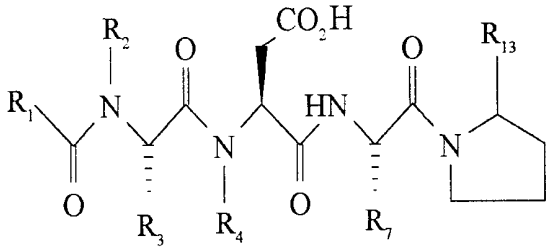
substituted by or forming a lower alkyl, amine lower alkyl, carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid or alkyl substituted phenyl sulfonamido group; R_1 is a lower alkyl or lower amino alkyl group, or 6-membered aromatic ring structure connected by a lower alkyl group; R_3 is a lower alkyl, lower alkyl alcohol, or lower thioalkyl group or cyclohexane connected by an alkyl group 0 to about 3 carbon atoms long; R_3 is a lower alkyl, lower alkyl alcohol, or lower thioalkyl group or 6-membered aromatic ring structure connected by an alkyl group 0 to about 3 carbon atoms long; and the lower alkyl, lower alkyl alcohol or lower thioalkyl group can be branched.

The compound of the formula immediately above wherein D is a oxygen atom; R_1 is a lower alkyl or lower amino alkyl group, or 6-membered aromatic ring structure connected by a lower alkyl group; R_3 is a lower alkyl, lower alkyl alcohol, or lower thioalkyl group or cyclohexane connected by an alkyl group 0 to about 3 carbon atoms long; R_3 is a lower alkyl, lower alkyl alcohol, or lower thioalkyl group or 6-membered aromatic ring structure connected by an alkyl group 0 to about 3 carbon atoms long; and the lower alkyl, lower alkyl alcohol or lower thioalkyl group can be branched.

The compound of the formula immediately above wherein D is a sulfur atom optionally substituted by or forming a lower alkyl, amine lower alkyl, carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid or alkyl substituted phenyl sulfonamido group; R_1 is a lower alkyl or lower amino alkyl group, or 6-membered aromatic ring structure

connected by a lower alkyl group; R_3 is a lower alkyl, lower alkyl alcohol, or lower thioalkyl group or cyclohexane connected by an alkyl group 0 to about 3 carbon atoms long; R_3 is a lower alkyl, lower alkyl alcohol, or lower thioalkyl group or 6-membered aromatic ring structure connected by an alkyl group 0 to about 3 carbon atoms long; and the lower alkyl, lower alkyl alcohol or lower thioalkyl group can be branched.

A contemplated compound can be defined by the following structural formula:



where R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl. The R_1 ring structure can form at R_1, between R_1 and R_2 or between R_1 and R_4 with the proviso that, if the R_1 ring structure forms at R_1, the R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups. The spacer can be optionally substituted by an amino group. The R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups. The R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms, and, if the R_1 ring structure is formed between R_1 and R_4, the heteroatoms are 2 nitrogen atoms. The R_1 ring structure can be

aromatic, partially saturated, or saturated. The lower alkyl or lower amino alkyl group can be branched.

R_2 is a H or R_2 and R_1 form the R_1 ring structure group.

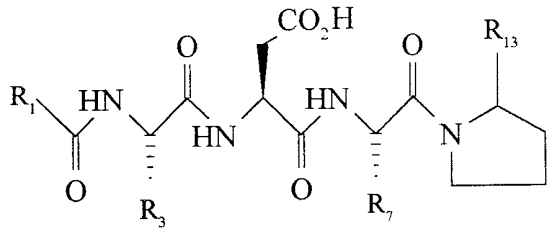
R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_4 is a H or R_4 and R_1 form the R_1 ring structure.

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

R_{13} is a formamide, lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, or H group.

A contemplated compound can be defined by the following structural formula:



where R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl. The R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming from one or more N-amido, N-sulfonimido, N-urea, N-carboxyl groups. The spacer can be optionally substituted by an amino group. The R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups. The R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms. The R_1 ring structure can be aromatic, partially saturated, or saturated. The lower alkyl or lower amino alkyl group can be branched.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

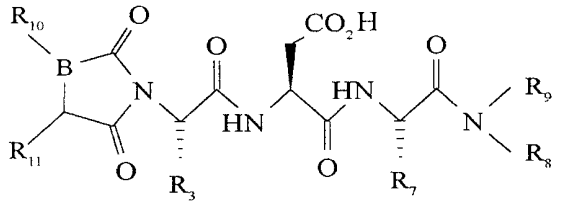
R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is

a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

R_{13} is a lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, or H group.

A contemplated compound is of the formula immediately above wherein R_1 is a lower alkyl or lower amino alkyl group or a 6-membered aromatic ring structure connected by a lower alkyl group; R_3 is a lower alkyl, lower alkyl alcohol or lower thioalkyl group or a cyclohexane connected by an alkyl group 0 to about 3 carbon atoms long; R_7 is a lower alkyl, lower alkyl alcohol, or lower thioalkyl group or a 6-membered aromatic ring structure connected by a lower alkyl group; and R_{13} is a lower alkyl carboxamide.

A contemplated compound can be defined by the following structural formula:



where B is a carbon or nitrogen atom.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms

long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long. If the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. If the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein the heteroatoms are 1 or 2 nitrogen atoms. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

R_8 is a ring structure, alkyl, alkyl alcohol, or thioalkyl amide group. The ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_3 and R_9 and is the R_8 ring structure. The R_8 ring structure is a 5-, 6- or fused 6,5-membered heterocyclic ring wherein the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms. The R_8 ring structure optionally can be substituted by one or more lower alkyl, amine lower alkyl carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, sulfoxide, or alkyl substituted phenyl sulfonamido groups. The (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or

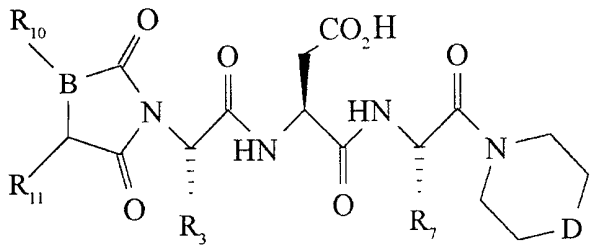
more alcohol, amide, sulfhydryl, or alkyl ester groups.

R_9 is the R_8 ring structure, a lower alkyl, amine, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group.

R_{10} is a H, lower alkyl phenyl group, or R_{10} and R_{11} form a R_{10} ring structure group that is a fused 6- or fused 6,6-membered cyclic or heterocyclic ring wherein the heteroatoms are 1 or 2 nitrogen atoms.

R_{11} is a H, lower alkyl phenyl or the R_{10} ring structure group.

A contemplated compound can be defined by the following structural formula:



where B is a carbon or nitrogen atom and D is a carbon, nitrogen, oxygen, or sulfur atom optionally substituted by or forming a lower alkyl, amine lower alkyl carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, sulfoxide, or alkyl substituted phenyl sulfonamido group.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms

long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

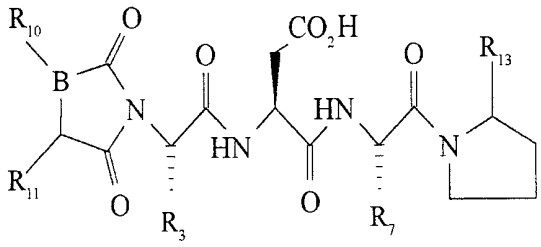
R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

R_{10} is a H, lower alkyl phenyl group, or R_{10} and R_{11} form a R_{10} ring structure group that is a fused 6- or fused 6,6-membered cyclic or heterocyclic ring wherein the heteroatoms are 1 or 2 nitrogen atoms.

R_{11} is a H, lower alkyl phenyl or the R_{10} ring structure group.

A contemplated compound can be defined by the structural formula immediately above wherein B is a carbon atom and the R_{10} ring structure group forms a phthalimido group.

A contemplated compound can be defined by the following structural formula:



where B is a carbon or nitrogen atom.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

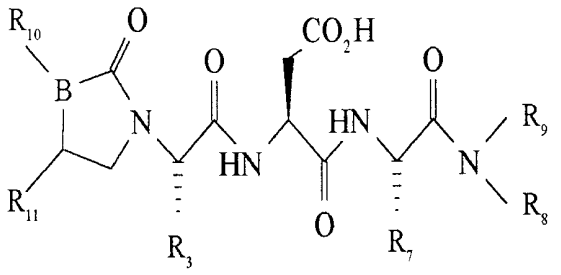
R_{10} is a H, lower alkyl phenyl group, or R_{10} and R_{11} form a R_{10} ring structure group that is a fused 6- or fused 6,6-membered cyclic or heterocyclic ring wherein the heteroatoms are 1 or 2 nitrogen atoms.

R_{11} is a H, lower alkyl phenyl or the R_{10} ring structure group.

R_{13} is a formamide, lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, or H group.

A contemplated compound can be defined by the structural formula immediately above wherein B is a carbon atom and the R_{10} ring structure group forms a phthalimido group.

A contemplated compound can be defined by the following structural formula:



where B is a carbon or nitrogen atom.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long. If the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. If the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein the heteroatoms are 1 or 2 nitrogen atoms. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

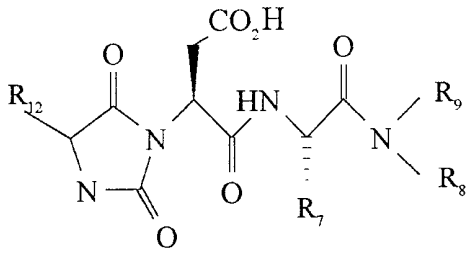
R_8 is a ring structure, alkyl, alkyl alcohol, or thioalkyl amide group. The ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_8 and R_9 and is the R_8 ring structure. The R_8 ring structure is a 5-, 6- or fused 6,5-membered heterocyclic ring wherein the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms. The R_8 ring structure optionally can be substituted by one or more lower alkyl, amine lower alkyl carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, sulfoxide, or alkyl substituted phenyl sulfonamido groups. The (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups.

R_9 is the R_8 ring structure, a lower alkyl, amine, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group.

R_{10} is a H, lower alkyl phenyl group, or R_{10} and R_{11} form a R_{10} ring structure group that is a fused 6- or fused 6,6-membered cyclic or heterocyclic ring wherein the heteroatoms are 1 or 2 nitrogen atoms.

R_{11} is a H, lower alkyl phenyl or the R_{10} ring structure group.

A contemplated compound can be defined by the following structural formula:



where R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long. If the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. If the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein the heteroatoms are 1 or 2 nitrogen atoms. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

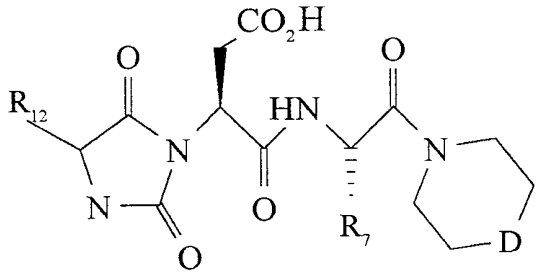
R_8 is a ring structure, alkyl, alkyl alcohol, or thioalkyl amide group. The ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_8 and R_9 and is the R_8 ring structure. The R_8 ring structure is a 5-, 6- or fused 6,5-membered heterocyclic ring wherein the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms. The R_8 ring structure optionally can be substituted by one or more lower alkyl, amine lower alkyl carboxamide, alcohol, lower alkyl alcohol, lower

hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, sulfoxide, or alkyl substituted phenyl sulfonamido groups. The (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups.

R_9 is the R_8 ring structure, a lower alkyl, amine, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group.

R_{12} is a R_{12} ring structure or lower alkyl group. The R_{12} ring structure is a 6-membered cyclic or heterocyclic ring wherein the heteroatoms are one or two nitrogen atoms and can be connected by an alkyl group 0 to 3 atoms long. The lower alkyl group can be branched.

A contemplated compound corresponds to the following structural formula:

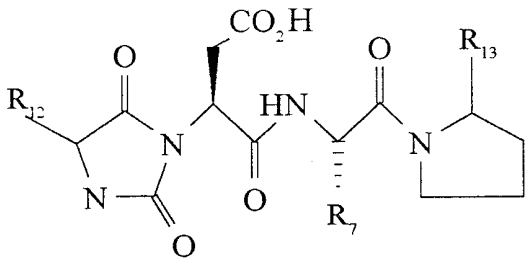


where D is a carbon, nitrogen, oxygen, or sulfur atom optionally substituted by or forming a lower alkyl, amine lower alkyl carboxamide, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, sulfoxide, or alkyl substituted phenyl sulfonamido group.

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

R_{12} is a R_{12} ring structure or lower alkyl group. The R_{12} ring structure is a 6-membered cyclic or heterocyclic ring wherein the heteroatoms are one or two nitrogen atoms and can be connected by an alkyl group 0 to 3 atoms long. The lower alkyl group can be branched.

A contemplated compound corresponds to the following structural formula:

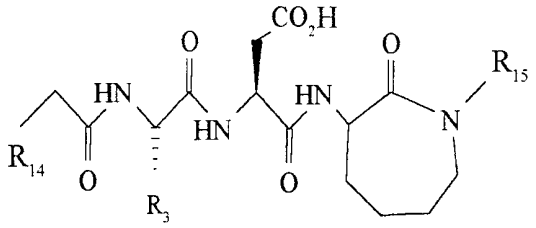


where R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

R_{12} is a R_{12} ring structure or lower alkyl group. The R_{12} ring structure is a 6-membered cyclic or heterocyclic ring wherein the heteroatoms are one or two nitrogen atoms and can be connected by an alkyl group 0 to 3 atoms long. The lower alkyl group can be branched.

R_{13} is a formamide, lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, or H group.

A contemplated compound corresponds to the following structural formula:

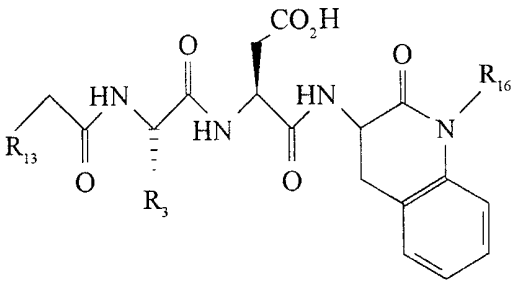


where R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_{14} is a 6-membered aromatic cyclic or heterocyclic ring wherein the heteroatom is a nitrogen atom.

R_{15} is lower alkyl carboxamide or H group.

A contemplated compound corresponds to the following structural formula:



where R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms

long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_{14} is a 6-membered aromatic cyclic or heterocyclic ring wherein the heteroatom is a nitrogen atom.

R_{16} is a lower alkyl, lower alkyl morpholine amide, or H group wherein the lower alkyl can be branched.

Table 1 provides the structural formula of exemplary compounds along with their binding inhibition potencies relative to the standard compound of SEQ ID NO:3, assigned a relative potency of 1. The compound ID number of Table 1 cross-references individual compounds herein.

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1111.06	2600
	1191.17	1140
	1111.03	1010
	1190.03	998

TABLE 1

Compound Structure and Potency

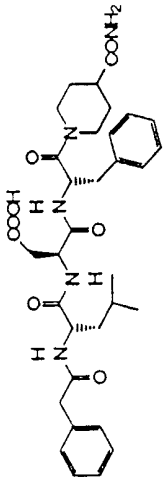
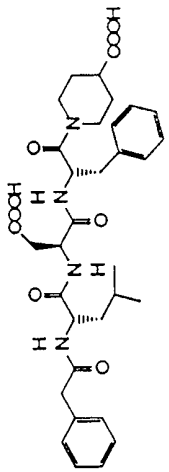
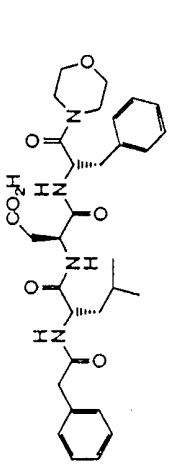
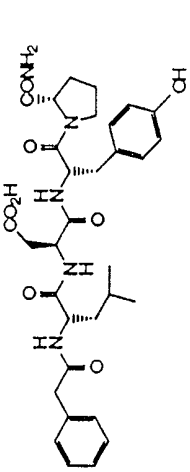
Structure	Compound ID Number	Relative Potency
	1111.05	942
	1111.04	836
	1051.01	709
	896.61	612

TABLE 1

Compound Structure and Potency

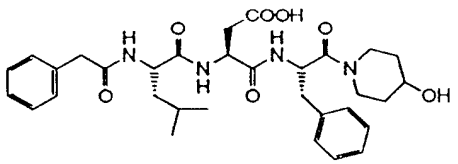
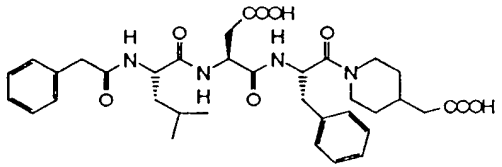
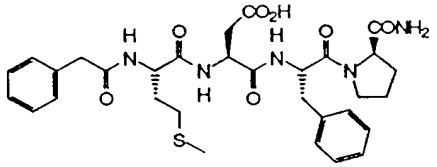
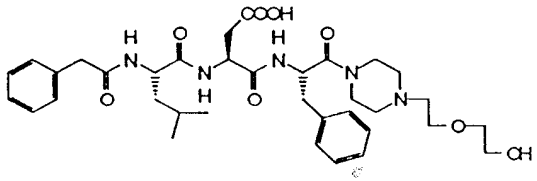
Structure	Compound ID Number	Relative Potency
	1070.02	604
	1190.02	591
	1190.36	561
	1111.07	510

TABLE 1

Compound Structure and Potency

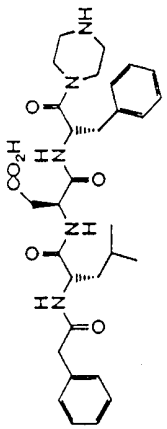
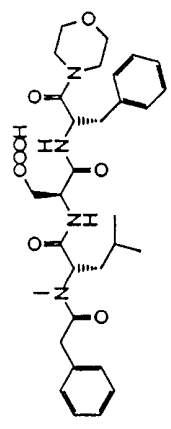
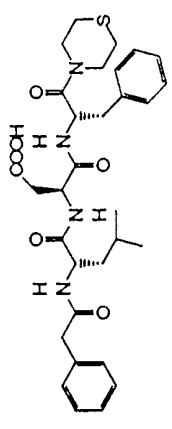
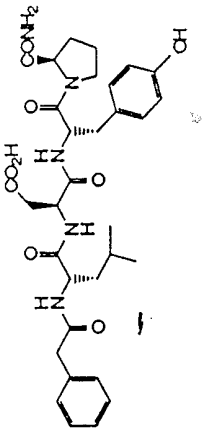
Structure	Compound ID Number	Relative Potency
	1051.02	507
	1190.46	505
	1111.02	450
	1036.01	405

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1190.51	399
	951.22	387
	1111.01	363
	1111.09	324

TABLE 1

Compound Structure and Potency

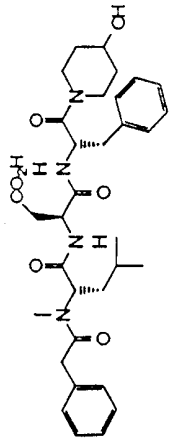
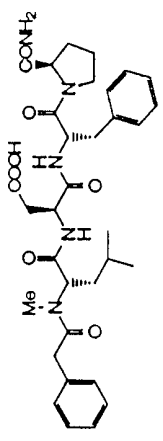
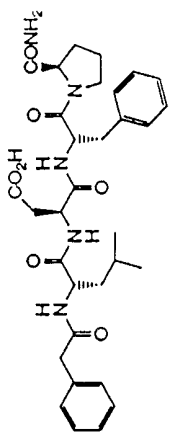
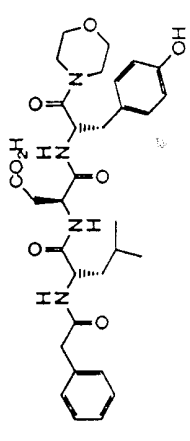
Structure	Compound ID Number	Relative Potency
	1070.08	322
	1190.07	318
	896.52	313
	1045.02	313

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	997.20	291
	896.62	257
	951.14	239
	951.20	228

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1111.08	218
	1160.01	213
	1190.12	210
see footnote	1058.01	210

TABLE 1

Compound Structure and Potency

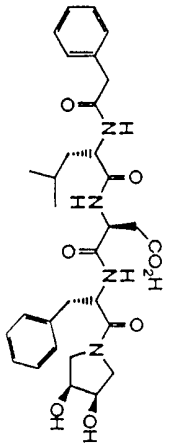
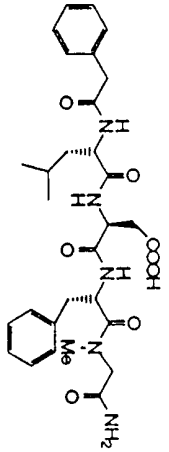
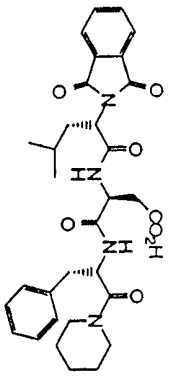
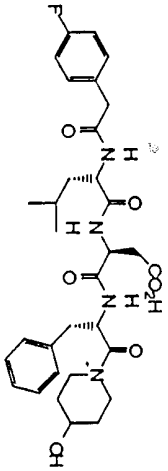
Structure	Compound ID Number	Relative Potency
	1070.07	206
	1190.11	197
	1190.04	196
	1070.01	194

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1070.10	185
	1070.12	179
	951.17	163
	1190.52	156

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1070.17	148
	1190.50	146
	1070.14	137
	896.60	131

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1070.11	127
	951.15	121
see footnote	1092.01	118
	896.51	110

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1111.10	105
	1057.06	103
	896.55	98.9
	1190.29	97

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1190.14	95.7
	1062.03	93.9
	1019.01	81.4
	1190.30	79.3

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1190.48	73.4
	951.12	61.8
	896.69	61
	1190.28	60.4

TABLE 1

Compound Structure and Potency

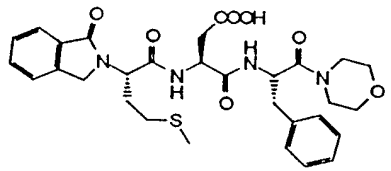
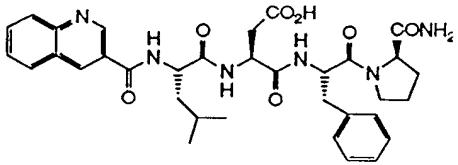
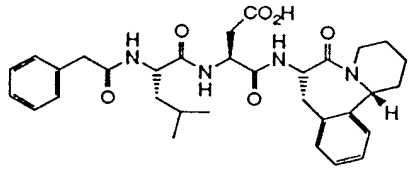
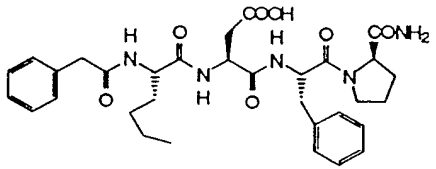
Structure	Compound ID Number	Relative Potency
	1190.49	59.3
	951.05	56.3
	1026.05	50.1
	1160.02	49

TABLE 1

Compound Structure and Potency

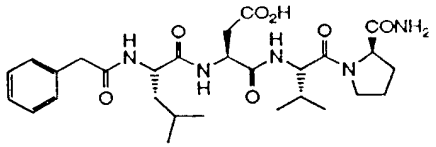
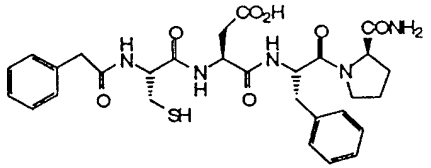
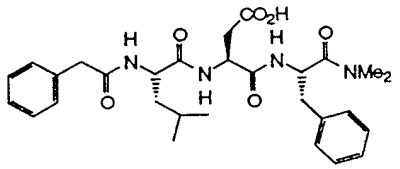
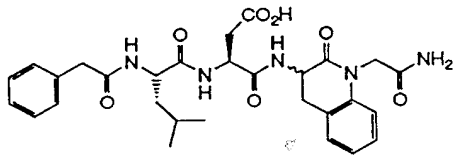
Structure	Compound ID Number	Relative Potency
	896.39	47.5
	1042.22	47
	1190.32	45.4
	997.11	44.8

TABLE 1

Compound Structure and Potency

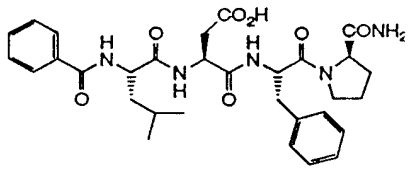
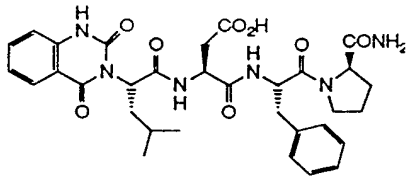
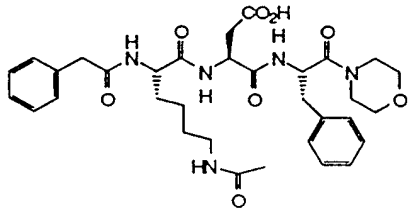
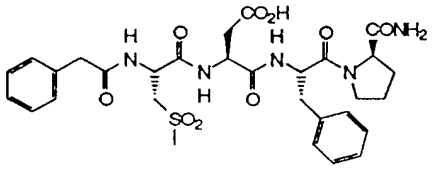
Structure	Compound ID Number	Relative Potency
	896.28	44.6
	896.63	43.7
	1070.19	42.3
	1190.45	41.4

TABLE 1

Compound Structure and Potency

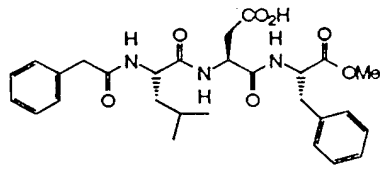
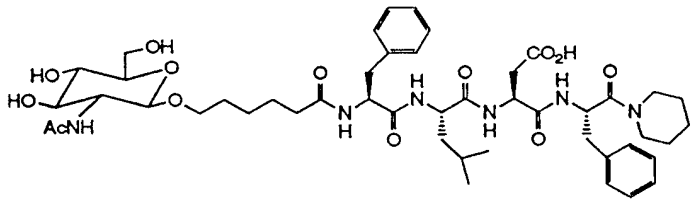
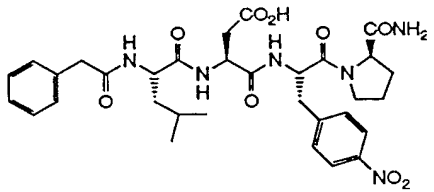
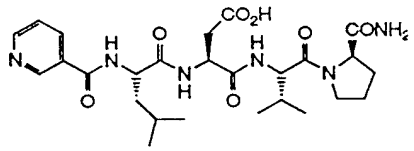
Structure	Compound ID Number	Relative Potency
	1190.31	40.9
	1063.01	40.7
	896.68	39.9
	896.42	38.1

TABLE 1

Compound Structure and Potency

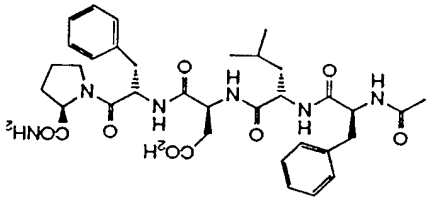
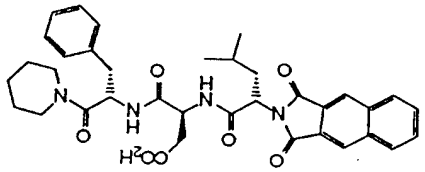
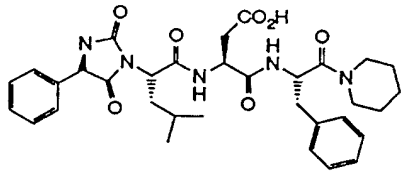
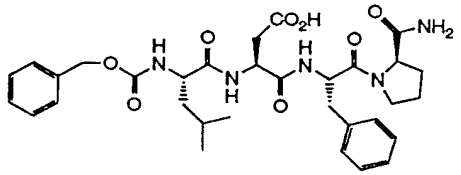
Structure	Compound ID Number	Relative Potency
	1190.15	36.8
	951.42	35.4
	1047.01	34.4
	1056.01	34.4

TABLE 1

Compound Structure and Potency

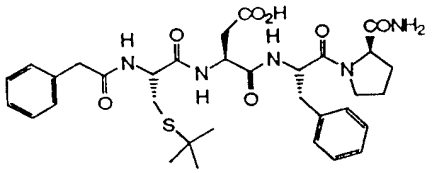
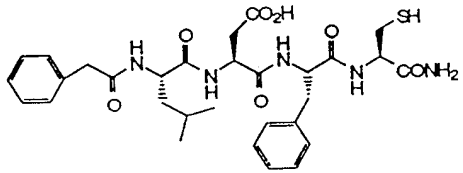
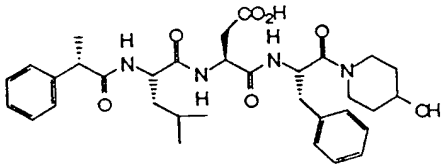
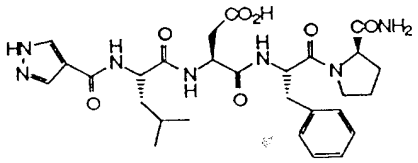
Structure	Compound ID Number	Relative Potency
	1190.37	33.3
	1042.23	33.2
	1070.09	31.4
	951.03	28.2

TABLE 1

Compound Structure and Potency

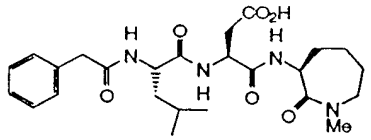
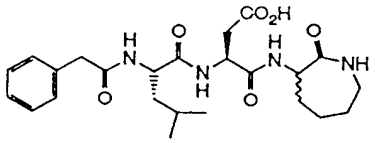
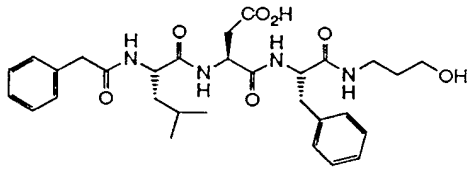
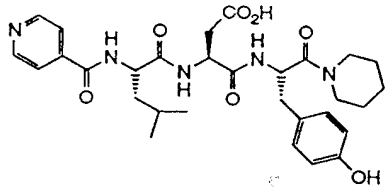
Structure	Compound ID Number	Relative Potency
	1190.34	27.4
	1043.02	26.8
	1051.05	25
	1045.01	23.8

TABLE 1

Compound Structure and Potency

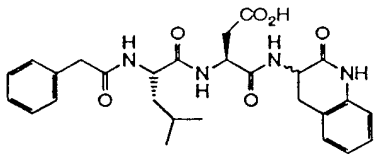
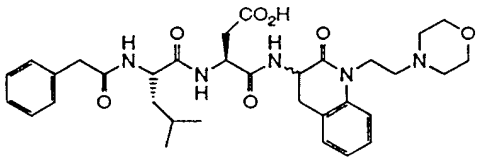
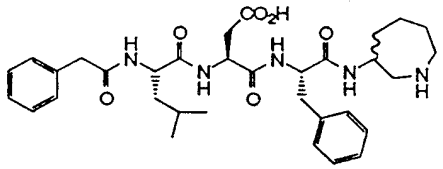
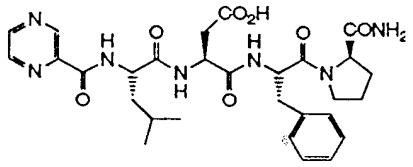
Structure	Compound ID Number	Relative Potency
	997.08	23.5
	997.18	20
	1190.26	19.9
	951.02	19.4

TABLE 1

Compound Structure and Potency

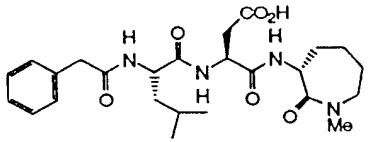
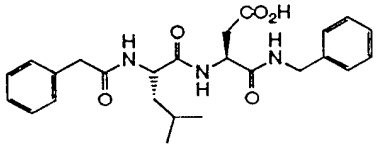
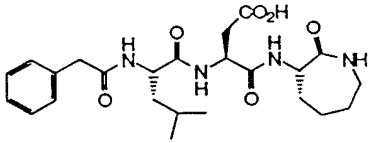
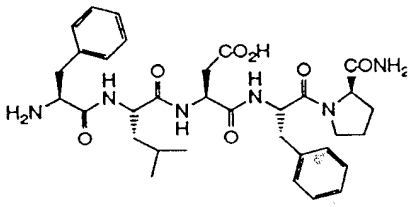
Structure	Compound ID Number	Relative Potency
	1190.33	18.8
	997.02	18.8
	1043.01	17.2
	896.27	16.9

TABLE 1

Compound Structure and Potency

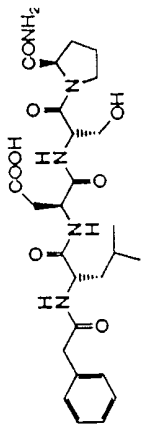
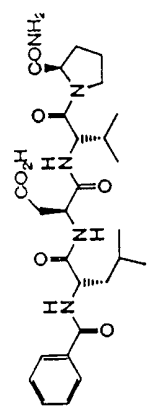
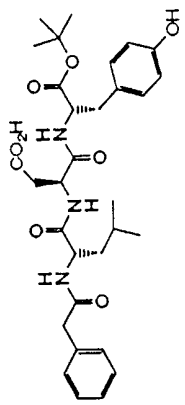
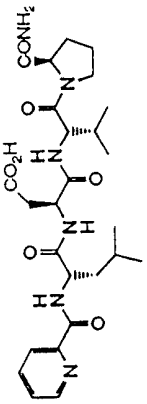
Structure	Compound ID Number	Relative Potency
	896.72	16.9
	896.35	16
	1040.02	14.7
	896.54	13

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	997.03	12.5
	1047.05	12.5
	896.31	11
	926.02	9.6

TABLE 1

Compound Structure and Potency

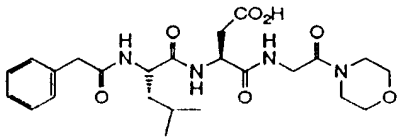
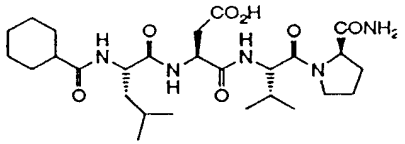
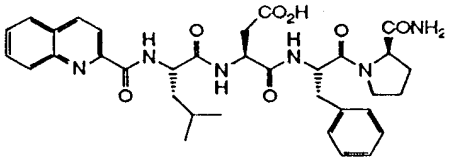
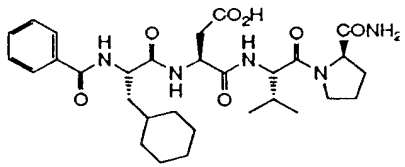
Structure	Compound ID Number	Relative Potency
	997.16	9.23
	896.49	9.08
	951.06	8.97
	896.34	6.73

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1057.02	6.57
	896.38	6.57
	896.46	6.57
	997.10	6.26

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1047.06	6.26
	951.08	6.26
	1033.01	5.84
	1190.27	5.81

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	896.26	5.63
	886.10	5.38
	896.40	5.32
	896.37	5.01

TABLE 1

Compound Structure and Potency

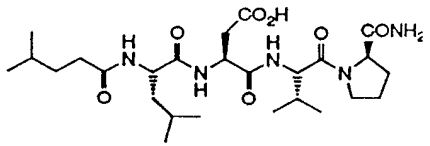
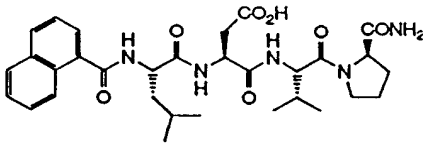
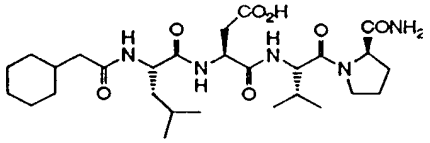
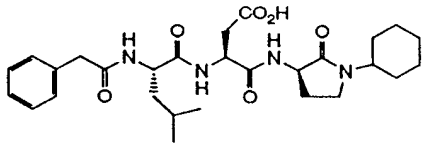
Structure	Compound ID Number	Relative Potency
	896.47	5.01
	896.43	4.38
	896.48	3.76
	1057.03	3.44

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	926.01	3.29
	997.09	3.13
	1047.02	3.13
	1057.04	3.13

TABLE 1

Compound Structure and Potency

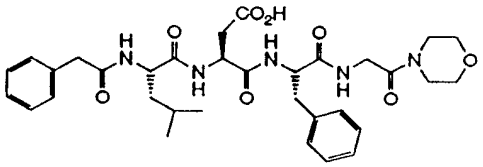
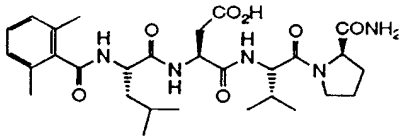
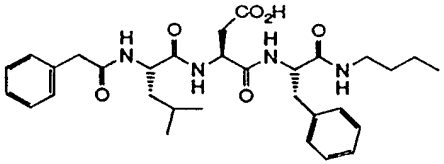
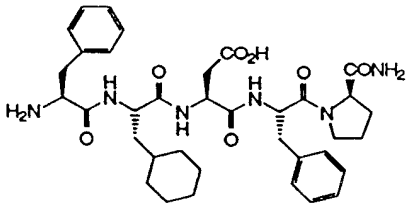
Structure	Compound ID Number	Relative Potency
	1051.04	3.13
	896.36	3.13
	1051.03	2.82
	896.30	2.69

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	997.01	2.66
	1040.01	2.66
	896.33	2.61
	896.44	2.5

TABLE 1

Compound Structure and Potency

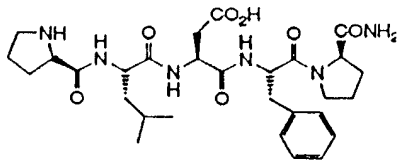
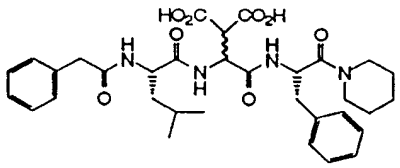
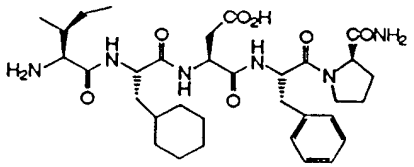
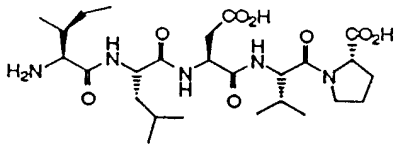
Structure	Compound ID Number	Relative Potency
	951.11	2.5
	1026.04	2.32
	896.29	2.27
	886.05	2

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	896.41	1.97
	926.12	1.99
	1034.01	1.96
	926.04	1.88

TABLE 1

Compound Structure and Potency

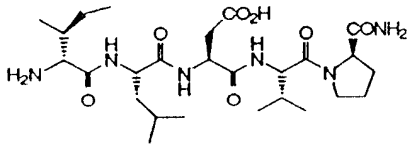
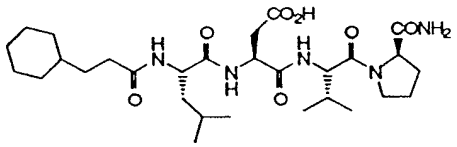
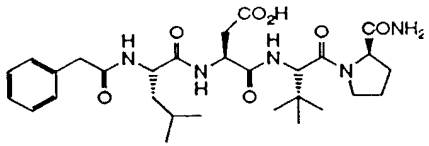
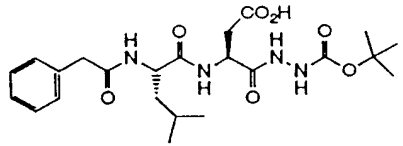
Structure	Compound ID Number	Relative Potency
	926.03	1.83
	896.45	1.75
	896.56	1.72
	997.13	1.57

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	951.07	1.57
	886.03	1.55
	896.32	1.41
	896.50	1.38

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	997.17	1.35
	997.15	1.28
	896.65	1.25
	1014.01	1.22

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1056.09	1.1
	926.05	.94
	1057.01	.88
	951.18	.78

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1032.02	.63
	926.30	.63
	951.10	.63
	1027.01	.5

TABLE 1

Compound Structure and Potency

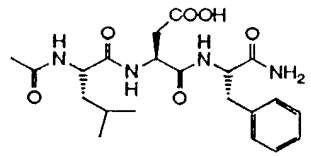
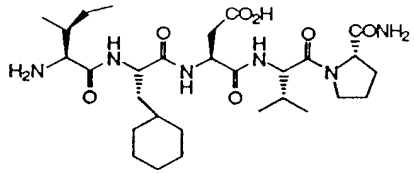
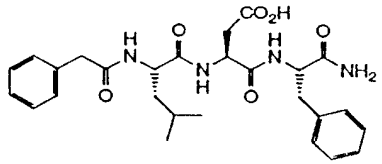
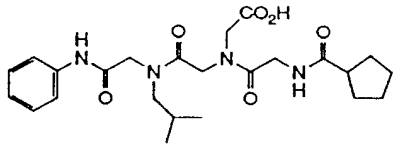
Structure	Compound ID Number	Relative Potency
	926.32	.47
	926.08	.47
	997.12	.45
	1032.01	.42

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	926.09	.41
	1047.07	.38
	1047.08	.38
	926.16	.34

TABLE 1

Compound Structure and Potency

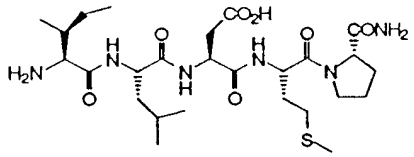
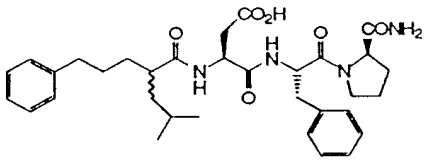
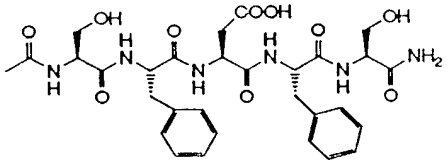
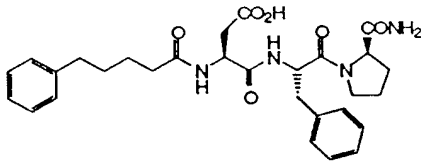
Structure	Compound ID Number	Relative Potency
	926.13	.3
	1056.14	.25
	951.46	.22
	1056.04	.2

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	926.17	.21
	926.10	.13
	926.14	.13
	926.07	.08

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	926.11	0
	926.15	0
	1027.02	80
	1027.03	90

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1026.01	0
	896.59	0
	1026.02	0
	992.01	0

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1027.04	0
	1034.02	0
	1041.01	0
	1047.03	0

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1047.09	0
	1066.01	0
	1026.06	0
	1026.07	0

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1026.08	0
	926.29	0
	926.31	0
	1056.02	0

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1056.03	0
	1056.05	0
	1056.06	0
	1056.07	0

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1056.08	0
	1056.10	0
	1056.12	0
	1056.13	0

TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1056.11	0
	926.18	0
	1190.05	0
	1190.06	0

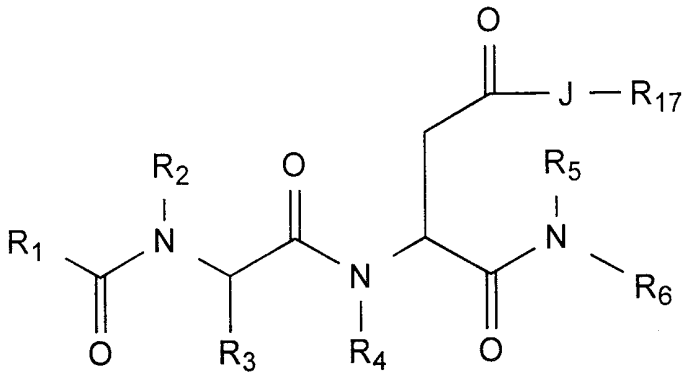
TABLE 1

Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
R_1 =		
R_2 =		

Notes:

The present invention further contemplates a compound that is a prodrug, where a prodrug is a compound that does not necessarily bind the VLA-4 receptor in vitro but is converted in vivo to a compound having such binding activity, e.g., a compound of Formula IIA.



Formula IIA

wherein:

R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl; the R_1 ring structure can form at R_1, between R_1 and R_2 or between R_1 and R_4 with the proviso that, if the R_1 ring structure forms at R_1, the R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups; the spacer can be optionally substituted by an amino group; the R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein, the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups; the R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms, and, if the R_1 ring structure is formed between R_1 and R_4, the heteroatoms are 2 nitrogen atoms; the R_1 ring structure can be conjugated,

partially saturated, or saturated; the lower alkyl or lower amino alkyl group can be branched;

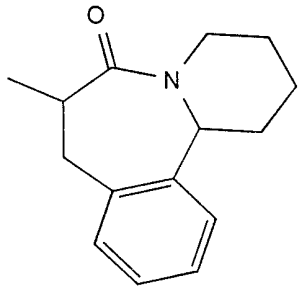
R_2 is a H, lower alkyl, phenyl lower alkyl, or R_2 and R_1 form the R_1 ring structure group;

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl, or di(lower alkyl)thioether; the R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long; the lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched;

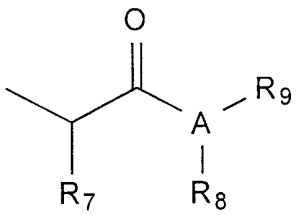
R_4 is a H or R_4 and R_1 form the R_1 ring structure;

R_5 is H or R_5 and R_6 form a R_5 ring structure; the R_5 ring structure is a fused 6,6- ring structure and can be aromatic, partially saturated, or saturated;

R_6 is a benzyl, a 5,6,or 7-membered heterocyclic saturated ring containing 1 or 2 nitrogen atoms optionally substituted by one or more lower alkyl, lower alkyl amide or acyl groups or 1,1 diphenylmethine group, the R_5 ring structure, a group of the formula



or a group of the formula



wherein:

A is nitrogen or oxygen;

when A is nitrogen;

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group; the R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long; if the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein, the heteroatom is a nitrogen atom; if the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein, the heteroatoms are 1 or 2 nitrogen atoms; the R_7 ring structure can optionally be substituted by an alcohol, nitro, or lower alkyl ether group;

R_8 is a ring structure, alkyl, alkyl alcohol, or thioalkyl amide group; the ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R- ring structure, or between R_8 and R_9 and is an R_8 ring structure; the R_8 ring structure is a 5-, 6- 7- or fused 6,5-membered heterocyclic ring wherein, the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms; the R_8 ring structure

optionally can be substituted by one or more lower alkyl, lower dialkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carboxamide, carbonyl, sulfoxide, sulfone or alkyl substituted phenyl sulfonamido groups; the (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups;

R_9 is the R_8 ring structure, a lower alkyl, lower dialkyl, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group;

when A is oxygen:

R_8 is a lower alkyl that can be branched and R_9 is absent;

J is oxygen or sulfur; and

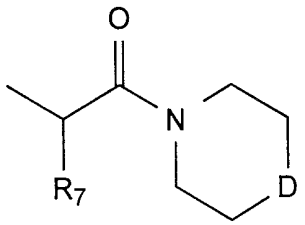
R_{17} is alkyl, alkyl optionally substituted by a hydroxyl, phenyl or phenyl sulfonyl, di(lower alkyl)sulfide, (lower alkoxy)lower alkyl, [(lower alkoxy)lower alkoxy]lower alkyl, (lower alkylcarbonyloxy)lower alkyl, [N-(lower alkyl)amino-carbonyl]lower alkyl, {[(N-(lower alkyl)]-(N-(lower alkoxy)}aminocarbonyl)lower alkyl,(N,N-di(lower alkyl)aminocarbonyl)lower alkyl, (N'-morpholinocarbonyl)lower alkyl, (benzyloxycarbonyl)methyl, 1-((O-((lower alkylcarbonato))eth-1-yl group; 2-oxo-1,3-dioxolen-4-ylmethyl group, cyclopentyl, cyclohexyl, cycloheptyl, phenyl, 1,3- dioxan-2-yl,

3-tetrahydropyranyl, (4-hydroxybutyric)lacton-3-yl, phthalidyl, or fused 6,5-membered aromatic, partially aromatic, or non-aromatic ring, wherein said ring is connected to J either directly by a bond or indirectly by a lower alkyl group; or

a pharmaceutically-acceptable salt thereof.

Preferred compounds of Formula IIA are where R_2 is lower alkyl, or phenyl lower alkyl.

Also preferred are compounds of Formula IIA where R_6 is



wherein D is carbon optionally substituted by one or more lower alkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, or alkyl substituted phenyl sulfonamido groups, or

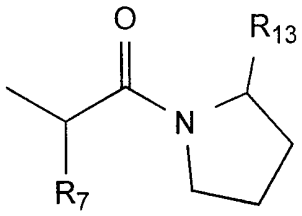
wherein, D is a nitrogen atom optionally substituted by a lower alkyl, amino carbonyl lower alkyl wherein the nitrogen atom of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, lower

alkyl alcohol, lower hydroxy alkyl ether, or lower alkyl carboxylic acid groups, or

wherein, D an oxygen atom, or

wherein, D is a sulfur atom optionally forming a sulfoxide or sulfone.

Also preferred are compounds of Formula IIA where R_6 is



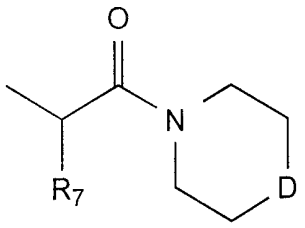
wherein, R_{13} is a lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, carboxamide, or H group.

Also preferred are compounds of Formula IIA where R_4 is H.

Also preferred are compounds of Formula IIA where R_5 is H.

Especially preferred are compounds of Formula IIA where R_2 is lower alkyl, or phenyl lower alkyl and

R_6 is



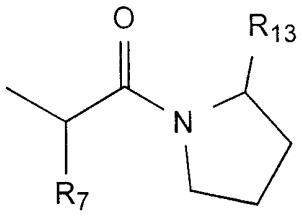
where D is carbon optionally substituted by one or more lower alkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, or alkyl substituted phenyl sulfonamido groups, or

where, D is a nitrogen atom optionally substituted by a lower alkyl, amino carbonyl lower alkyl wherein the nitrogen atom of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, lower alkyl alcohol, lower hydroxy alkyl ether, or lower alkyl carboxylic acid groups, or

where, D is an oxygen atom, or

where, D is a sulfur atom optionally forming a sulfoxide or sulfone, or

R_6 is



wherein, R_{13} is a lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, carboxamide, or H group.

Most preferred compounds of Formula II are where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 is H, R_7 is benzyl, A is nitrogen, R_8 is a ring structure formed between R_8 and R_9 selected from the group consisting of 4-methylpiperazinyl, and 4-morpholinyl, J is oxygen and R_{17} is selected from the group consisting of ethyl, methyl, 2-propyl, cyclohexyl, and neopentyl.

Also most preferred are compounds of Formula IIA where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is ethyl.

Also most preferred are compounds of Formula IIA where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is methyl.

Also most preferred are compounds of Formula IIA where R_1 is benzyl, R_2 is methyl, R_3 is

2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-morpholinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is 2-propyl.

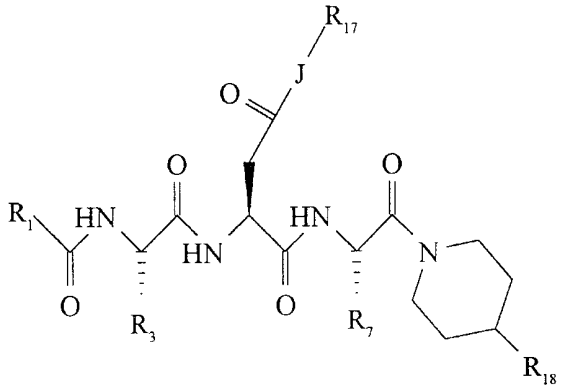
Also most preferred are compounds of Formula IIA where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-morpholinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is ethyl.

Also most preferred are compounds of Formula IIA where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is 2-propyl.

Also most preferred are compounds of Formula IIA where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is cyclohexyl.

Also most preferred are compounds of Formula IIA where R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is neopentyl.

A contemplated prodrug compound corresponds to the following structural formula:



where J is a nitrogen, oxygen, or sulfur atom.

R_{17} forms or is an alkyl ester, alkyl carboxylic ester, alkyl carboxamide carboxylic ester, phenyl alkyl, alkyl carboxamide, alkyl carboxylic acid, alkyl phosphonate, biotin, or H group.

R_{18} is an alkyl ester, biotin or H group with the proviso that R_{17} and R_{18} cannot both be H groups.

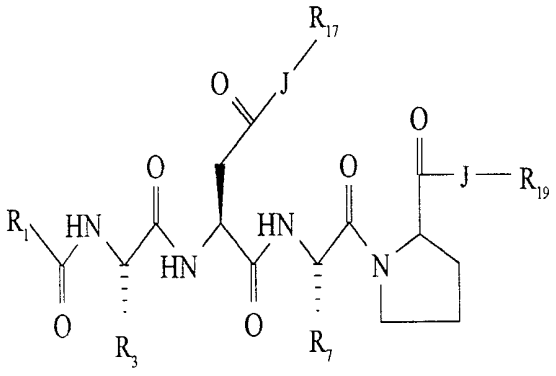
R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl. The R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming from one or more N-amido, N-sulfonimido, N-urea, N-carboxyl groups. The spacer can be optionally substituted by an amino group. The R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups. The R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms. The R_1 ring structure can be aromatic, partially

saturated, or saturated. The lower alkyl or lower amino alkyl group can be branched.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

A contemplated prodrug compound corresponds to the following structural formula:



where J is a nitrogen, oxygen, or sulfur atom.

R_{17} forms or is an alkyl ester, alkyl carboxylic ester, alkyl carboxamide carboxylic ester, phenyl alkyl, alkyl carboxamide, alkyl carboxylic acid, alkyl phosphonate, biotin, or H group.

R_{19} is a phenyl alkyl, alkyl carboxamide, alkyl carboxylic acid, alkyl carboxylic ester, alkyl phosphonate, alkyl carboxamide carboxylic ester, biotin or H group with the proviso that R_{17} and R_{19} cannot both be H groups.

R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl. The R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming from one or more N-amido, N-sulfonimido, N-urea, N-carboxyl groups. The spacer can be optionally substituted by an amino group. The R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups. The R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms. The R_1 ring structure can be aromatic, partially saturated, or saturated. The lower alkyl or lower amino alkyl group can be branched.

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol or lower thioalkyl. The R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long. The lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched.

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group. The R_7 ring structure forms at R_7 and can be connected by an alkyl group 0 to about 3 carbon atoms long and is a 6-, or

fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein the heteroatom is a nitrogen atom. The R_7 ring structure can optionally be substituted by an alcohol, nitro or lower alkyl ether group.

Table 2 provides the structural formula of exemplary prodrug compounds along with their binding inhibition potencies relative to the standard compound of SEQ ID NO:3, assigned a relative potency of 1. The prodrug compound relative potency of Table 2 is the potency before the prodrug compound is enzymatically converted to an active form. The compound ID number of Table 2 cross-references individual compounds herein.

TABLE 2

Prodrug Compound Structure and Potency

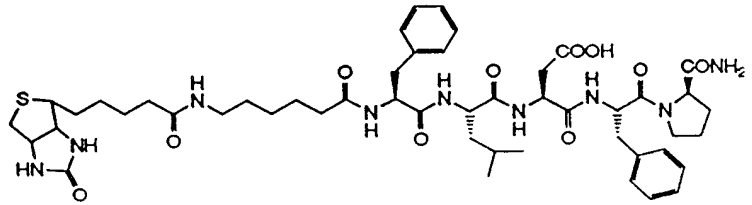
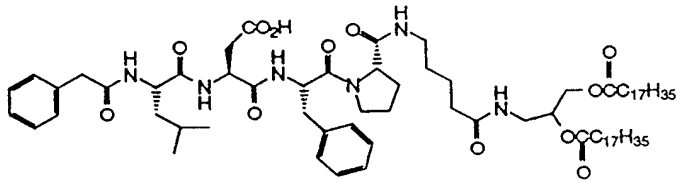
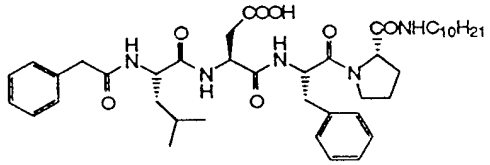
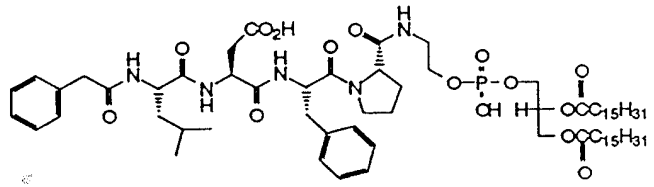
Structure	Compound ID Number	Relative Potency
	951.13	74.5
	1068.03	6.26
	1068.04	2.13
	1068.02	1.1

TABLE 2

Prodrug Compound Structure and Potency

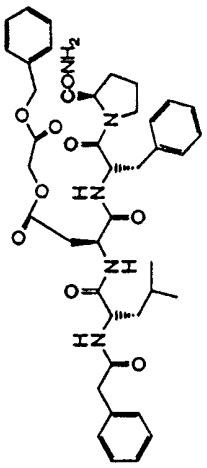
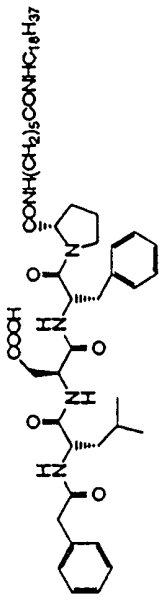
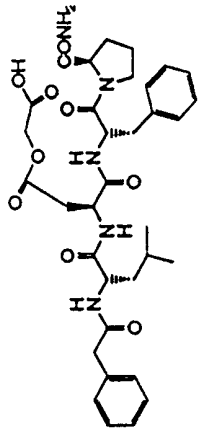
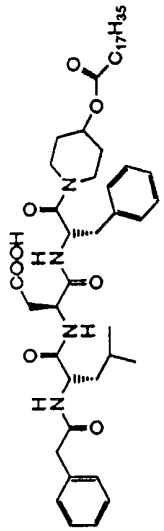
Structure	Compound ID Number	Relative Potency
	1067.01	.97
	1068.01	.91
	1067.02	.81
	1068.05	.63

TABLE 2

Prodrug Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1068.06	.63
	1190.01	0
	1068.07	0
	1068.08	0

TABLE 2

Prodrug Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1068.09	0
	1068.10	0
	1068.11	0
	1068.12	0

TABLE 2

Prodrug Compound Structure and Potency

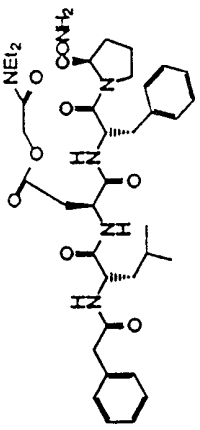
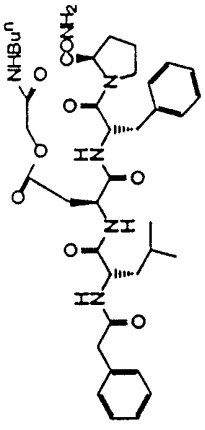
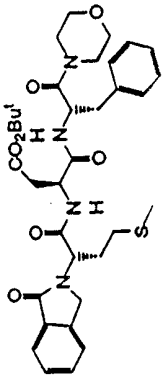
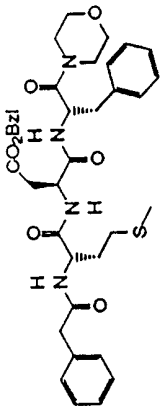
Structure	Compound ID Number	Relative Potency
	1068.13	0
	1068.14	0
	1068.18	0
	1068.17	0

TABLE 2

Prodrug Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1068.32	0
	1068.89	0
	1068.90	0
	1068.92	0

TABLE 2

Prodrug Compound Structure and Potency

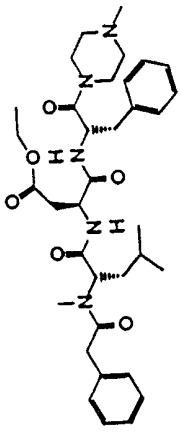
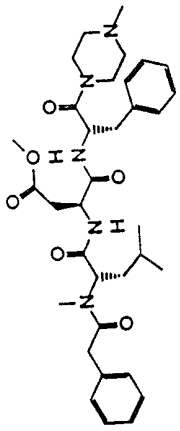
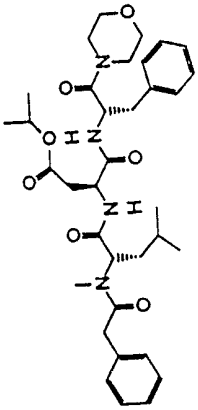
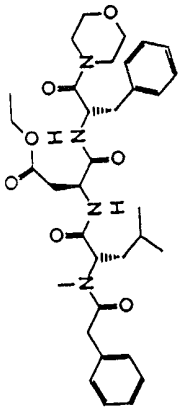
Structure	Compound ID Number	Relative Potency
	1068.94	0
	1068.95	0
	1068.96	0
	1068.97	0

TABLE 2

Prodrug Compound Structure and Potency

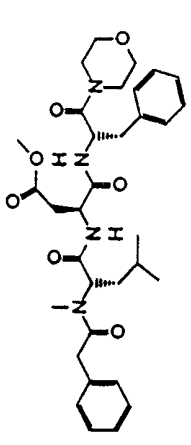
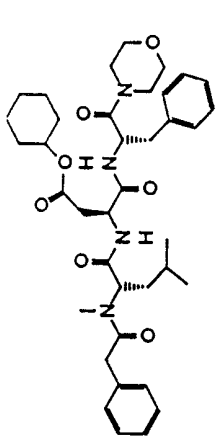
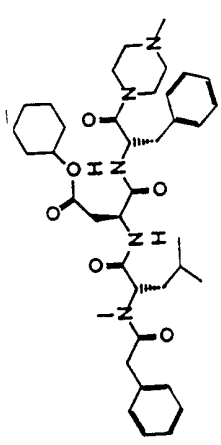
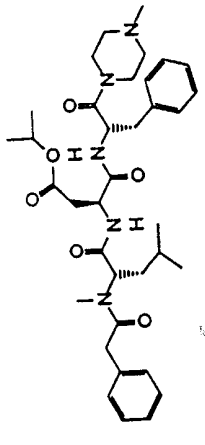
Structure	Compound ID Number	Relative Potency
	1068.98	0
	1068.99	0
	1069.02	0
	1069.01	0

TABLE 2

Prodrug Compound Structure and Potency

Structure	Compound ID Number	Relative Potency
	1069.03	0

A contemplated compound also includes a bioisoster of a disclosed compound. As used herein, the term "bioisoster" refers to a compound differing from a disclosed compound by an one or more atoms expected to produce an equivalent biological effect. An example of a bioisosteric substitution is the interchange of nitrogen and carbon in an aromatic ring. See, for example, Medicinal Chemistry, ed. by Alfred Burger, Interscience Publishers, N.Y. (1960), which is incorporated herein by reference.

A contemplated compound includes a described compound coupled to a fluorescent group, a group that enhances solubility in an aqueous environment, or binding group, such as, for example, an europium epsilon amidocarproyl, N-acetyl glucosamine or biotin group, respectively. A contemplated compound includes a compound containing two or more of the described compounds attached together to form a multi-valent compound by a linking group such as, for example, a tetraethylenepentatamine group.

As used herein, the term "lower alkyl" refers to an alkyl group 1 to about 5 carbon atoms long. The term "alkyl" refers to an alkyl group 1 to about 15 atoms long.

Compounds of the present invention comprise chemical moieties attached to a peptide backbone. For a chemical moiety defining an amino acid, a contemplated inhibitor compound can also be defined using the single or triple letter abbreviation for an amino acid. In this description, the abbreviations used herein for derivatives and residues of the twenty natural amino acids are reproduced in the following Table of Correspondence:

TABLE 3 - TABLE OF CORRESPONDENCE

Abbreviation		Amino Acid
1- Letter	3-Letter	
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
C	Cys	cysteine
X	Xaa	another residue, or one of several residues

In addition, the twenty naturally occurring amino acids can also be categorized according to the chemical structure their respective radicals.

Aliphatic Amino Acids	Radical	3-Letter	1-Letter
glycine	H	Gly	G
alanine	methyl	Ala	A
valine	2-propyl	Val	V

Aliphatic Amino Acids	Radical	3-Letter	1-Letter
leucine	2-methylpropyl	Leu	L
isoleucine	2-butyl	Ile	I

Aromatic Amino Acids	Radical	3-Letter	1-Letter
phenylalanine	benzyl	Phe	F
tyrosine	4-hydroxyphenylmethyl	Tyr	Y
tryptophan	3-indolylmethyl	Trp	W

Acidic Amino Acids	Radical	3-Letter	1-Letter
aspartate	carboxymethyl	Asp	D
glutamate	carboxyethyl	Glu	E
asparagine	aminocarbonylmethyl	Asn	N
glutamine	aminocarbonylethyl	Gln	Q

Basic Amino Acids	Radical	3-Letter	1-Letter
lysine	4-aminobutyl	lys	K
arginine	3-guanylpropyl	arg	R
histidine	4-imidazoylmethyl	his	H

Substituted Amino Acids	Radical	3-Letter	1-Letter
cysteine	thiolmethyl	cys	C
methionine	methylthioethyl	met	M
serine	hydroxymethyl	ser	S
threonine	1-hydroxyethyl	thr	T
proline	--	pro	P

Those of ordinary skill in the art will appreciate that certain physical characteristics are

readily associated with the type of radical on the amino acid, e.g., aliphatic amino acids are hydrophobic; acidic and basic amino acids are hydrophilic. Other groupings of amino acids are not as readily assignable, for example in the case of the aromatic amino acids, phenylalanine is hydrophobic, while tyrosine is hydrophilic.

Compound sequences are written from left to right and in the direction from amino-terminus to carboxyl-terminus. The single letter amino acid abbreviation of Table 3 does not apply to single letter atomic symbol or variable abbreviations used in molecular formulas herein.

A contemplated inhibitor compound defined as an amino acid sequence corresponds to formula A:

X-B-Asp-Z A

wherein

B is an α-hydrophobic amino acid residue.

X is a group amide-linked to the nitrogen atom of the B α-amine. The X group has a ring structure bonded to the carbonyl carbon of the amide-linkage by a spacer having a length of zero to about two methylene groups. The length of X, including the spacer and carbonyl carbon, is about that of a 3-quinoline carbonyl group or smaller. The ring structure is a 5- and 6-membered ring or a fused 6,6- or 6,5-membered ring. Alternatively, the X substituent, including the spacer, cyclic ring structure, the carbonyl group and the α-amino nitrogen atom of B can also together form an aromatic ring-substituted cyclic imido group.

Z is selected from the group consisting of:

(a) Xaa-NCy^1 where Xaa is Val, Ile, Leu or an aromatic amino acid residue; i.e., a residue having a side chain that contains one or two fused aromatic rings, and NCy^1 is a cyclic ring-containing group having a ring nitrogen atom that forms an amide bond with the α-carboxyl group of Xaa, and whose cyclic ring contains 5- or 6-atoms including said ring nitrogen atom; and

(b) NCy^2 where the depicted nitrogen is an amine substituent of a cyclic group whose depicted nitrogen atom forms an amide bond with the α-carboxyl group of the Asp, and which amine substituent is bonded to a 6- or 7-membered ring or to a fused 6,6- or 6,7-membered lactam ring system in which the ring bearing the amine substituent is saturated and contains the amine substituent α to the carbonyl group of the lactam.

A compound of formula A is water-soluble and inhibits the binding of Jurkat cells (ATCC TIB 152) to a solid phase-bound compound of SEQ ID NO:1 in an <u>in vitro</u> assay in an aqueous buffer at a pH value of 7.2-7.4. The binding inhibition exhibited by a compound is measured relative to that of SEQ ID NO:3. Preferably, the binding inhibition of a compound is ten times or more that of SEQ ID NO:3.

The sequence of a compound of formula A appears to require the Asp residue present in the CS-1 (SEQ ID NO:1) and B12 (SEQ ID NO:3) fibronectin compounds. Aside from that Asp, both of whose size and charge appear to be required for binding as Glu and other residues barely inhibit binding, size and relative hydrophobicity appear to be most important in

the selection of the B residue. The requirements of the other residues are discussed hereinafter.

Exemplary B residues as amino acids are selected from the group consisting of leucine (Leu), cyclohexylalanine, norleucine (Nle), Methionine (Met), homoserine, threonine (Thr), phenylalanine (Phe), valine (Val), norvaline (Nva), and isoleucine (Ile). B is most preferably Leu.

Preferably, a contemplated inhibitor compound defined as an amino acid sequence corresponds to formula I:

X-Leu-Asp-Z I

wherein

X is a group amide-linked to the nitrogen atom of Leu, the group having a ring structure bonded to the carbonyl carbon of the amide-linkage by a spacer having a length of zero to about two methylene groups. The length of X, including the spacer and carbonyl carbon, is about that of a 3-quinoline carbonyl group or smaller. The ring structure is a 5- and 6-membered ring or a fused 6,6- or 6,5-membered ring. Alternatively, the X substituent, including the spacer, cyclic ring structure, the carbonyl group and the α-amino nitrogen atom of Leu can also together form an aromatic ring-substituted cyclic imido group.

Z is selected from the group consisting of:

(a) Xaa-NCy^1 where Xaa is Val, Ile, Leu or an amino acid residue having a side chain that contains one or two fused aromatic rings and NCy^1 is a cyclic ring-containing group having a ring nitrogen atom that

forms an amide bond with the α-carboxyl group of Xaa, and whose cyclic ring contains 5- or 6-atoms including said ring nitrogen atom; and

(b) NCy^2, where the depicted nitrogen is an amine substituent of a cyclic group whose depicted nitrogen atom forms an amide bond with the α-carboxyl group of the Asp residue, and which amine substituent is bonded to a 6- or 7-membered ring or to a fused \6,6- or 6,7-membered lactam ring system in which the ring bearing the amine substituent is saturated and contains the amine substituent α to the carbonyl group of the lactam.

A compound of formula I, as well as formulas II and III below, is water-soluble and inhibits the binding of Jurkat cells to a solid phase-bound compound of SEQ ID NO:1 in an in vitro assay in an aqueous buffer at a pH value of 7.2-7.4. The binding inhibition exhibited by a compound is measured relative to that of SEQ ID NO:3. Preferably, the inhibition of a compound is ten times or more that of SEQ ID NO:3.

Examining formula I, it is seen that at least Leu and Asp of the CS-1 (SEQ ID NO:1) and B12 (SEQ ID NO:2) fibronectin compounds are present. Aside from that two residue sequence, the sequence/structure of a contemplated inhibitor compound and the CS-1 or B12 portions are quite different.

Thus, whereas there is an iso-leucine (Ile;I) compound- (amide-) bonded to the α-amine group of the Leu residue in CS-1 and the native protein, a cyclic ring structure-containing group or moiety, X, is \amide-bonded to the nitrogen atom of the B or Leu residue α-amino group (formulas A or I, respectively) via a carboxyl contributed by the cyclic ring

structure-containing group. That amide bond can be present as part of a carboxamide- [-C(O)NH-], urethane- [-O-C(O)NH-] or urea- [-NH-C(O)NH-] containing spacer group that links the cyclic ring structure-containing group to the Leu residue.

The cyclic ring structure broadly can be any 5- or 6-membered ring that is saturated or contains ethylenic unsaturation. The ring structure can contain one or more atoms other than carbon such as nitrogen, oxygen or sulfur. The ring structure can also be a fused ring system where two 6-membered rings are fused (6,6-) or where a 6-membered ring is fused to a 5-membered ring (6,5-membered). The ring of the cyclic ring structure is preferably aromatic.

Exemplary ring structures include tetrahydrofuranyl, tetrahydropyranyl, cyclopentyl, cyclohexyl, phenyl, pyridyl, pyrimidinyl, pyrazinyl, pyrazolyl, pyrrolidyl, furanyl, piperidinyl, naphthyl, quinolinyl, decalinyl, quinazolinyl, imidazyl, thiophenyl, and the like. Of the cyclic ring structures, phenyl and pyridyl are particularly preferred.

A cyclic ring structure can be bonded directly to the carbonyl group [-C(O)-] of the amide bond to the B or Leu residue. That ring can also be spaced away from the carbonyl group by up to about the length of two methylene ($-CH_2-$) groups or an ethylene group ($-CH_2-CH_2-$).

The Van der Waals radius a methylene group (about 2.0 Å) is slightly longer than that of an oxy group (-O-; about 1.40 Å) or an imino group (-NH-; about 1.50 Å). There is sufficient similarity between

the sizes of methylene, oxy and imino so that a spacer group containing a $-CH_2-O-$, $-CH_2-NH-$, $-NH-NH-$, or $-O-NH-$ are of similar lengths and are within the length of an ethylene group, $-CH_2-CH_2-$. A similar result obtains if bond lengths (distances) are used. Contemplated spacers include $-HC(CH_3)-CH_2-$, $-CH_2-CH_2-$, $-NH-O-$, $-HN-NH-$, $-CH_2-O-$ and $-CH_2-NH-$, and are preferably free of unsaturation.

Using a phenyl ring as an exemplary aromatic ring structure, it is seen that the contemplated X groups include 3-methyl-3-phenylpropionyl, 3-phenylpropionyl, phenylhydroxaminocarbonyl [Ph-NH-O-C(O)-], phenylhydrazidecarbonyl [Ph-NH-NH-C(O)-], benzyloxycarbonyl [Ph-CH_2-O-C(O)-], phenoxyacetyl [Ph-O-CH_2-C(O)-], benzylaminocarbonyl [Ph-CH_2-NH-C(O)-], and anilinoacetyl [Ph-NH-CH_2-C(O)-], where "Ph" is a phenyl group.

Thus, it is contemplated that a before-described ring structure be bonded to the carbonyl carbon of the B- or Leu-linked amide group by a spacer having a length of zero methylene groups (a direct bond), one or two methylene groups. Put differently, the spacer has the length of about an ethylene group or less.

A phenylacetyl, phenoxycarbonyl or anilinocarbonyl group bonded to the nitrogen of the B or Leu α-amino group contains a spacer having the length of about one methylene group. Phenyl (benzoyl), 1- or 2-naphthyl (1- or 2-naphthalenecarbonyl), 2-, 3- or 4-pyridyl (2-, 3- or 4-pyridinecarbonyl), 2- or 3-thiophenyl (2- or 3-thienyl; 2- or 3-thiophencarbonyl) and 2- or 3-furanyl (2- or 3-furancarbonyl) ring structures are bonded directly to the amide carbonyl carbon and

therefore define an X group that utilizes a spacer having a length of zero methylene groups. A spacer having a length of about two methylene groups is provided by an X group that is carbobenzyloxy [Ph-CH_2-O-C(O)-], carbobenzylamino [Ph-CH_2-NH-C(O)-], carbophenoxymethylene [Ph-O-CH_2-C(O)-]) and the like groups.

A contemplated 5- or 6-membered ring structure can also be substituted with a C_1-C_2 alkyl or hydroxyl group. Exemplary substituted ring structures using a phenyl ring as illustrative thus include 2-, 3- or 4-ethylphenyl, 2,6-, 3,4- or 2, 3-dimethylphenyl, 2-, 3- or 4-hydroxyphenyl, 2, 6-, 2,4-, 3,4- and 3,5-dihydroxyphenyl, and the like.

The ring structure of the X substituent is thought to act in a contemplated inhibitor in some way to fit the inhibitor compound into the binding pocket of the VLA-4 receptor to position the B or Leu and Asp groups into a proper configuration. Because of that presumed role in fitting the compound into its receptor, there are some size constraints upon the ring structure-containing and spacer portions of X, in addition to those noted before as to the spacer group length. Thus, from the carbonyl-containing carbon of the amide bond to B or Leu, through the end of ring structure or its substituent furthest from the carbonyl group, the total length of the spacer plus ring structure-containing portion of X is about the size of a 3-quinolinecarbonyl group or smaller.

Inasmuch as a 3-quinolinecarbonyl group is the longest contemplated ring structure-containing X substituent, a 3-quinolinecarbonyl group is free from the above-discussed substituents that add to its length.

The length of a given X substituent can be readily determined, as discussed before. For example, one can use space-filling models to build exemplary cyclic ring structure-containing X groups and then compare the relative sizes of the prepared models. One can also use published bond lengths and bond angles to prepare a two-dimensional depiction of the sizes. Computer graphics programs are also well-known and available that can be used to prepare exemplary model X groups for length comparison to 3-quinolinoyl.

The X substituent, including the spacer, cyclic ring structure, the carbonyl group and the α-amino nitrogen atom of B or Leu can also together form an aromatic ring-substituted cyclic imido group. Exemplary of such cyclic imido groups are phthalimido, which is preferred, each of 2,3- and 3, 4-pyridinedicarboximido, homophthalimido and 1,2,3, 4-tetrahydroquinazoline-2,4-dione-3-yl groups in which the aromatic ring and cyclic imido group are fused together.

In another exemplary compound, the B or leucine nitrogen atom is an imido nitrogen atom within the ring of a 5-phenylhydantoin-3-yl group so that the aromatic phenyl ring is a substituent of a cyclic spacer and is spaced about one methylene away from the carbonyl group linked to the Leu residue. A similarly structured imido nitrogen-containing X group is present in a 2-phenylsuccinimido group formed on the B or Leu nitrogen atom.

The cyclic imido- and hydantoin-containing portions of the above-discussed X groups can thus be viewed as specialized spacer groups that limit the conformational degrees of freedom of the ring structures. Thus, for example, whereas the carbonyl,

methylene and phenyl portions of a phenylacetyl group are each free to assume one or more of several conformations, a phthalimido X group can only spin about the axis of the leucine nitrogen-methine bond.

It is noted that although the X substituent must contain a cyclic ring structure that can be substituted as discussed before, that X substituent can also include a further substituent on other than the ring structure. When a further substituent is present, X preferably is an amino acid residue having a cyclic ring side chain that therefore includes a primary or secondary amine. Here, X is preferably a prolyl, phenylalanyl, tyrosinyl or phenylglycyl residue, the nitrogen atom of whose α-amino group is bonded to the further substituent.

That further substituent can be one amino acid residue through the remainder of the CS-1 compound sequence toward the N-terminus thereof, with the sequence of that compound beginning at the isoleucine of position 19 from the N-terminus of SEQ ID NO:1. A single residue or 18 separate amino acid residue substituent sequences are thereby defined.

Another exemplary further substituent linked via an amine group of X is biotin. In a particular example, biotin amide-bonded to ε-aminocaproic acid was amide-bonded to the α-amine of a phenylalanine (Phe) as an X group. The resulting compound contained the biotin fused ring amide-linked to the Phe X group via a chain of twelve atoms.

It should also be understood that an X group amino acid residue having a cyclic ring side chain can also be free of substituent groups. The nitrogen atom of the α-amine of such a residue can also be acylated

as with a C_1-C_6 acyl group such as formyl, acetyl, iso-butyryl, or hexanoyl group. A C_1-C_6 acyl group bonded to the nitrogen of an α-amine group forms an amide bond at that nitrogen atom and provides no ionic charge to the compound at a pH value of 7.2-7.4 as compared to the positive charge provided by an unsubstituted free α-amine.

The Z group of a before-discussed formula can be one of two types of groups. The Z group in one embodiment (A) is a hydrophobic amino acid residue Xaa compound-bonded to the Asp carboxyl and linked to a cyclic ring-containing group NCy^1 that has a ring nitrogen atom (the N of NCy^1) that forms an amide bond with the α-carboxyl group of Xaa. The cyclic ring of NCy^1 contains 5- or 6-atoms, including the depicted nitrogen atom (N of NCy^1). Contemplated hydrophobic amino acid residues are those having aliphatic side chains such as valine, leucine and isoleucine. Xaa more preferably contains a hydrophobic aromatic amino acid residue; i.e., Xaa is an amino acid residue having an aromatic side chain that contains one or two fused aromatic rings. Exemplary of such aromatic amino acids are phenylalanine, tyrosine and tryptophan that are naturally occurring (genetically encoded) as well as phenylglycine, homophenylalanine, P-nitrophenylalanine, thiophenylglycine (thienylglycine), and the like.

Exemplary NCy^1 groups include morpholinyl, thiomorpholinyl, thiomorpholinylsulfone [4-(thiadioxo)piperidinyl], piperidinyl, piperazinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, oxazolidinyl and the like as their respective amides. A NCy^1 cyclic ring can also be substituted with one or two substituent groups selected from the group consisting of carboxyl, carboxamide, C_1-C_4 alkylenecarboxyl C_1-C_4 alkylenecarboxamide, hydroxyl, hydroxymethyl,

$(CH_2CH_2O)_nH$ where n is one, two or three and C_1-C_4 alkyl. Carboxyl substitution at the 2-position of a pyrrolidine provides the amino acid proline, whose D- and L-forms are both contemplated herein. D-Prolyl (sometimes shown in bold face lower case single letter amino acid code as "P" or as D-Pro) is particularly preferred as its amido derivative (D-Pro-NH_2) as are morpholinyl, piperidyl, piperazinyl and 4-hydroxypiperidyl.

Exemplary C_1-C_4 alkyl groups include methyl, ethyl, iso-propyl, n-butyl and t-butyl. A C_1-C_4 alkyl group can also form a quaternary ammonium group with a second nitrogen atom of NCy^1 such as piperazine. Where the C_1-C_4 alkyl group is a methyl group, and iodide is the anion, and exemplary NCy^1 group is a 4,4-N, N-dimethylpiperaziniumyl iodide.

Exemplary C_1-C_4 alkylenecarboxyl and C_1-C_4 alkylenecarboxamide groups include methylenecarboxyl (-CH_2CO_2H; carboxymethyl) and methylenecarboxamido (-CH_2CONH_2; carboxamidomethyl), ethylenecarboxyl (-$CH_2CH_2CO_2H$; carboxyethyl) and ethylenecarboxamido (-$CH_2CH_2CONH_2$; carboxamidoethyl) as well as butylenecarboxyl (-$C_4H_8CO_2H$; carboxybutyl) and butylenecarboxamido (-$C_4H_8CONH_2$; carboxyamidobutyl). Exemplary groups $(CH_2CH_2O)_nH$ where n is one, two or three include 2-hydroxyethyl (n=1), 5-hydroxyethylenoxy-ethylene (ethyleneoxyethanol; 5-hydroxy-3-oxapentyl; n=2) and 8-hydroxy-3,5-dioxa-octyl (n=3).

When NCy^1 includes a piperazinyl group, the second (4-position) nitrogen atom cannot only be quaternized by alkylation, but also amidified. Exemplary acyl portions of the piperazinyl-4-N-amides include C_1-C6 acyl groups such as formyl, acetyl,

propanol, isobutanoyl, hexanoyl and benzoyl, but also sulfonamides such as phenylsulfonamido, toluenesulfonamide (tosyl), methanesulfonamide (mesyl) and trifluoromethylsulfonamido (trifyl).

Thus, in those embodiments where Z is Xaa-NCy^1, Xaa is a specified amino acid residue whose amine group forms an amide (compound) bond with the α-carboxyl of the depicted Asp residue, and whose carboxyl group forms an amide bond with a nitrogen atom present within the 5- or 6-membered ring of NCy^1.

In another embodiment (B) Z is NCy^2 where the depicted nitrogen atom (N of NCy^2) is an amine substituent of a cyclic group (Cy^2) whose substituent nitrogen atom forms an amide bond with the α-carboxyl of the depicted Asp residue. That amine substituent is bonded to a cyclic group that is (I) a 6- or 7-membered ring or (ii) a fused 6,6- or 6,7-membered lactam ring system in which the ring bearing the amine substituent is saturated (free of ethylenic unsaturation) and contains the amine substituent α to the carboxyl group of the lactam.

Here, the nitrogen atom that links the ring system to the remainder of the compound is a substituent of a cyclic ring structure rather than being a ring atom as in NCy^1. In addition, the rings of which that nitrogen can be a substituent are of two types, 6- or 7-membered rings or 6,6- or 6, 7-membered fused ring systems, one of which rings is a lactam. In either situation, there is no Xaa amino acid residue in this embodiment.

Exemplary amine substituent-containing 6- and 7-membered ring NCy^2 groups of this type include benzylamine, phenethylamine,

2-(N-morpholinyl)-ethylamine,
N-[1-(carboxamidomethyl)-caprolactam-3-yl]amine,
N-(caprolactam-3-yl)amine, and
N-(valerolactam-3-yl)amine groups that form the corresponding amides with the α-carboxyl of Asp. Exemplary amino-substituted 6,6- and 6,7-fused ring lactam-containing NCy^2 groups include N-[1-(2-N-morpholinylethyl)-2-oxo-tetrahydroquinolin-3-yl]amine, N-(2-oxo-tetrahydroquinolin-3-yl)amine and the 6,7-fused ring tricyclic compound shown at footnote 7 of Table 1 groups that form corresponding amides with the α-carboxyl of Asp.

It has generally been found that once (I) the X group of a formula discussed herein is occupied by an aromatic ring-containing moiety spaced adjacent to or within about one methylene group's distance from the carbonyl, (ii) Z is an aromatic amino acid, and (iii) NCy^1 is L- or D-proline amide or a 5- or 6-membered nitrogen-containing ring as discussed before, substantially any other substituent can be present linked to either compound terminus without abolishing the inhibitory activity of a contemplated compound, so long as the resulting compound is water-soluble.

Thus, for example, the compound of SEQ ID NO:4 having an N-terminal phenylacetyl group linked to the sequence Leu-Asp-Phe-Pro can further include a substituted tetraethylenediamine group amide-bonded to the Pro residue in which four phenylacetyl-Leu-Asp-Phe-Pro groups were amide-bonded to the tetraethylenediamine nitrogens and still exhibit VLA-4 binding inhibition that was better than the standard 10-mer compound of SEQ ID NO:3.

Similarly, the compound PheLeuAspPhe-D-Pro-NH_2 contained a europium-containing chelate at its N-terminus bonded to the nitrogen atom of the N-terminal Phe. That compound exhibited a binding inhibition better than that of the compound of SEQ ID NO:3.

The compound of SEQ ID NO:5, phenylacetyl-Leu-Asp-Phe-Pro-$NH(CH_2)_5C(O)NHC_{18}H_{37}$, would be predicted to be a good inhibitor. However, that compound is not water-soluble and forms a turbid dispersion rather than a solution. That compound exhibits a binding inhibition similar to that exhibited by the standard 10-mer compound of SEQ ID NO:3.

Any compound having binding activity can be used. However, a preferred contemplated inhibitor compound inhibits the binding of inflammatory cells that contain the VLA-4 receptor [Jurkat cells (American Type Culture Collection, Rockville, MD 20852, ATCC TIB 152)] to the solid phase-bound CS-1 compound (SEQ ID NO:1) in an aqueous buffer at pH 7.2 - 7.4 to an extent that is about 10-fold to about 1000-fold and more preferably 3000-fold better than that binding exhibited by the art standard 10-mer compound of SEQ ID NO:3 [GPEILDVPST in single letter code). More preferably, that binding is inhibited by about 50- to about 3000-fold, and most preferably by about 100- to about 3000-fold. However, an inhibitor compound having less than about 10-fold binding but having <u>in vivo</u> efficacy is also contemplated.

Binding inhibition is measured here as a concentration of compound that inhibits one-half the binding between a standard number of Jurkat cells and a standard amount of CS-1 compound bound to the

surface of a microtiter plate well. Those concentrations are conveniently expressed as IC_{50} values, smaller numbers indicating a lower concentration required to inhibit 50 percent binding and therefore greater potency. Further specifics of this assay are provided hereinafter.

To recapitulate, a compound of formulas A or I inhibits binding between the CS-1 compound region of fibronectin and the VLA-4 receptor. Those inhibitors that are at least ten-times better inhibitors than the compound of SEQ ID NO:3 are preferred.

Still more preferred is a compound of formula II, below,

Ar-Y-C(O)-Leu-Asp-Xaa-NCy^1 II

wherein Ar is a pyrazolyl, phenyl, pyridyl (2-, 3- or 4-), or 3-quinolinyl group;

Y is a spacer that is absent, $-CH_2-$, -CH(NH)-, -O- or -NH-;

or Ar-Y-C(O) together with the nitrogen atom of Leu forms a phthalimido, a 1,2,3, 4-tetrahydroquinazoline-2,4-dione-3-yl or 5-phenylhydantoin-3-yl group,

Ar-Y-C(O)- has a length of about 3-quinolinecarbonyl or less;

Xaa is an aromatic amino acid residue; i.e., an amino acid residue having an aromatic side chain, such as phenylalanine, tyrosine, tryptophan,

homophenylalanine, nitrophenylalanine, thienylglycine and phenylglycine; and

NCy^1 is an amine-containing 5- or 6-membered cyclic ring group whose depicted nitrogen atom, N of NCy^1, is within the ring and forms an amide bond with the α-carboxyl of Xaa, as was discussed before.

Of the above combinations, Ar-Y-C(O), a more preferred X group of formula I, is preferably benzoyl, phenylacetyl, 4-pyridinecarbonyl (isonicotinoyl), 3-pyridinecarbonyl (nicotinoyl), 3-pyridinacetyl, anilinocarbonyl, 3-quinolinoyl, pyrazolecarbonyl, tryptophyl and 3,4-dihydroxybenzoyl, with phenylacetyl (benzylcarbonyl) being most preferred, or Ar-Y-C(O) together with the leucine nitrogen atom form a phthalimido group. Xaa is preferably Phe, Tyr or Trp, with Phe being most preferred. NCy^1 is preferably an amide of a morpholinyl, piperidinyl or substituted piperidinyl where the substituent is selected from the group consisting of hydroxyl, carboxyl, carboxamido groups, piperazinyl or 4-substituted piperazinyl in which the 4-substituent is selected from the group consisting of C_1-C_4 alkyl, C_1-C_4 alkylenecarboxyl, C_1-C_4 alkylenecarboxamide, $(CH_2CH_2O)_nH$ where n is 1, 2 or 3, thiomorpholinyl, L- or D-prolinyl amide, pyrrolidinyl, 3,4-dihydroxy-pyrrolidinyl, 2-(hydroxymethyl)-pyrrolidinyl and 4-(thiadioxo)piperidinyl group, with amides of morpholinyl, D-prolinyl amide, piperidinyl, an above-substituted piperidinyl, piperazinyl, an above-substituted piperazinyl and pyrrolidinyl groups being most preferred.

A most preferred compound corresponds in sequence to a compound of formula III, below,

Phenylacetyl-Leu-Asp-Phe-NCy^3 III

wherein NCy^3 is a group of most preferred NCy^1 groups and is selected from the group consisting of morpholinamido, thiomorpholino, 4-(thiadioxo)piperidinamido, D-2-(carboxamido)pyrrolidinamido, piperazinamido, substituted piperazinamido where the substituent is selected from the group consisting of 4-N-carboxymethyl, 4-N-carboxamidomethyl, 4-N-(5-hydroxyethylenoxyethylene) and 4-N-P-toluene-sulfonamido, pyrrolidinamido, piperidinamido and substituted piperidinamido where the substituent is selected from the group consisting of 4-hydroxy, 4-carbamyl, 4-carboxyl groups.

Table 4 below lists exemplary compounds defined by the single letter amino acid sequence abbreviation format. The compound ID number cross-references compounds herein.

TABLE 4 - COMPOUNDS DESCRIBED BY AMINO ACID SEQUENCE AND SUBSTITUENT GROUPS

SEQ ID NO:	FORMULA[1]	X	Z[2]	COMPOUND ID NO:
	X L D F Z	phenylacetyl	4-N(carboxymethyl)-piperazinamide[9]	1111.06
	X L D F Z	phenylacetyl	4-(thiadioxo)piperidinamide[10]	1111.03
	X L D F Z	phenylacetyl	4-(carbamyl)piperidinamide[11]	1111.05
	X L D F Z	phenylacetyl	morpholinamide	1051.01
	X L D F Z	phenylacetyl	4-(carboxyl)piperidinamide	1111.04
	X L D F **p**	pyridine-4-carbonyl	amide	951.20
	X L D Y **p**	phenylacetyl	amide	896.61
	X L D F Z	phenylacetyl	4-hydroxypiperidinamide	1070.02
	X L D F Z	phenylacetyl	piperazinamide	1051.02
	X L D F Z	phenylacetyl	4-N-(5-hydroxyethyloxyethylene)-piperazinamide[12]	1111.07
	X L D F Z	phenylacetyl	thiomorpholinamide	1111.02
	X L D F **p**	pyridine-3-acetyl	amide	951.22
4	X L D Y P	phenylacetyl	amide	1036.01

TABLE 4 - COMPOUNDS DESCRIBED BY AMINO ACID SEQUENCE AND SUBSTITUENT GROUPS

SEQ ID NO:	FORMULA[1]	X	Z[2]	COMPOUND ID NO:
	X L D F P	phenylacetyl	4-N-(p-toluenesulfonamido)-piperidinamide	1111.01
	X L D F Z	phenylacetyl	4-N-(carboxamidomethyl)-piperazinamide	1111.09
	X L D Y Z	phenylacetyl	morpholinamide	1045.02
	X L D F Z	phenylacetyl	4,4-N,N-(dimethyl)-piperaziniumamide iodide	1111.08
	X L D F **p**	phenylacetyl	amide	896.52
	X L D L Z	phenylacetyl	morpholinamide	997.20
	X L D F Z	phenylacetyl	pyrrolidinamide	951.15
	X L D F Z	phenylacetyl	piperidinamide	951.14
	X L D F **p**	anilinocarbonyl	amide	896.62
	X J D F P	phenylacetyl	amide J=cyclohexyL-Ala[5]	1160.01
5	X L D F P Z	phenylacetyl	peptide-subsdtituted tetra-ethylene-pentaamine[3]	1058.01
	X L D F **p**	pyridine-3-carbonyl	amide	896.55
	X L D F Z	phenylacetyl	3,4-dihydroxypryrrolidinamide	1070.01
	X L D F Z	phenylacetyl	2-(hydroxymethyl)prolinamide	951.17
	X L D W **p**	phenylacetyl	amide	896.60

TABLE 4 - COMPOUNDS DESCRIBED BY AMINO ACID SEQUENCE AND SUBSTITUENT GROUPS

SEQ ID NO:	FORMULA[1]	X	Z[2]		COMPOUND ID NO:
	B Z F L D F **p**	B=biotinoyl	Z=ϵ-anudicaoriyl	amide	1019.01
	X L D V **p**	phthalimido	amide		896.51
	Z X F L D F **p**	ϵ-amidocaproyl	Z=Europium label[4]	amide	1092.01
	X L D V **p**	phenylacetyl	amide		896.39
	X L D Z	phenylacetyl	N-[1-(carboxamidomethyl)-caprolactam-3-yl]amide		1057.06
	X L D J **p**	phenylacetyl	amide	J=homo-Phe[5]	1062.03
	X L D F Z	3,4-dihydroxy-phenylacetyl	piperidinamide		1019.01
	X L D F **p**	benzoyl	amide		896.28
	X L D V **p**	pyridine-3-carbonyl	amide		896.42
	X L D J **p**	phenylacetyl	amide	J=Phenyl-Gly[5]	896.69
	X L D F **p**	3-quinolinecarbonyl	amide		951.05
	X L D F **p**	1,2,3,4-tetrahydro-quinazoline-2,4-dione-3-yl	amide		896.63
	X L D Z	phenylacetyl	6,7-fused ring Lactam[7]		1026.05
	X J D F P	phenylacetyl	amide	J=Nle[5]	1160.02
	P L D F **p**	free amine	amide		951.12

TABLE 4 - COMPOUNDS DESCRIBED BY AMINO ACID SEQUENCE AND SUBSTITUENT GROUPS

SEQ ID NO:	FORMULA[1]	X	Z[2]	COMPOUND ID NO:
	X L D Z	phenylacetyl	N-[1-carboxamidomethyl)-2-oxo-tetrahydroquinolin-3-yl]-amide	997.11
6	X F L D L Z	GlcNac-O-$(CH_2)_5$-C(O)	piperidinamide	1063.01
	X L D J **p**	phenylacetyl	amide J=p-nitro-Phe[5]	896.68
7	X L D V P	benzoyl	amide	896.35
	F L D F **p**	acetyl	amide	951.42
	X L D F **p**	benzyloxycarbonyl	amide	1056.01
	X L D F Z	(5-phenyl)hydantoinyl	piperidinamide	1047.01
	X L D F **p**	pyrazolecarbonyl	amide	951.03
	F L D F **p**	acetyl	amide	896.27
	X L D Z	phenylacetyl	N-(2-oxo-tetratrahydroquinolin-3-yl)amide	997.08
	X L D Z	phenylacetyl	N-(caprolactam-3-yl)-amide	1043.02
	X L D F Z	phenylacetyl	propanolamide	1051.05
	X L D Y Z	4-pyridinecarbonyl	piperidinamide	1045.01
	X L D Z	phenylacetyl	N-[1-(2-N-morpholinylethyl)-2-oxo-tetrahydroquinoline-3-yl]amide	997.18
8	X L D V P	pivaloyl	amide	926.01

TABLE 4 - COMPOUNDS DESCRIBED BY AMINO ACID SEQUENCE AND SUBSTITUENT GROUPS

SEQ ID NO:	FORMULA[1]	X	Z[2]	COMPOUND ID NO:
	X L D V **p**	benzoyl	amide	926.02
	X L D Z	phenylacetyl	benzylamide	997.02
	X J D F **p**	benzoyl	amide J=cyclohexyl-Ala[5]	896.31
	X L D Z	phenylacetyl	N-D-(caprolactam-3-yl)amide	1043.01
	X L D S **p**	phenylacetyl	amide	1042.23
	X L D Y Z	phenylacetyl	t-butylester	1040.02
	X L D V **p**	phenylpropionyl	amide	896.40
	X L D Z	phenylacetyl	N-(2-N-morpholinyl)ethylamide	997.03
	X J D V **p**	benzoyl	amide J=cyclohexyl-Ala[5]	896.34
	F L D V **p**	free amine	amide	926.04
	X L D F **p**	2-pyrazinecarbonyl	amide	951.02
	X L D G Z	phenylacetyl	morpholinamide	997.16
	X L D V **p**	2,3-dimethylbenzoyl	amide	896.38
	X L D V **p**	3,4-dimethylbenzoyl	amide	896.37
	X L D V **p**	pyridine-2-carbonyl	amide	896.54
	X L D Z	phenylacetyl	Z=N-[1-(N-cyclohexyl)-butyrolactam-3-yl)amide	1057.02

TABLE 4 - COMPOUNDS DESCRIBED BY AMINO ACID SEQUENCE AND SUBSTITUENT GROUPS

SEQ ID NO:	FORMULA[1]	X	Z[2]	COMPOUND ID NO:
	X L D Z	phenylacetyl	N-(1-iso-butyl-2-oxo-tetra-hydroquinolin-3-yl)amide	997.10
	X L D F Z	benzyl	piperidinamide	1033.01
	f L D V **p**	free amine	amide	926.05
	X L D V **p**	cyclohexanecarbonyl	amide	896.49
	X L D V **p**	2,6-dimethylbenzoyl	amide	896.36
	X L D F **p**	2-quinolinecarbonyl	amide	951.06
	X L D V **p**	3-methylvaleroyl	amide	896.46
	X L D Z	phenylacetyl	N-(tetrahydroisoquinoline-3-yl)amide	997.09
	p L D F **p**	free amine	amide	951.11
	X L D F **p**	8-quinolinesdulfonyl	amide	951.07
	X L D F Z	phenylacetyl	n-butylamide	1051.03
	X L D V **p**	4-methylvaleroyl	amide	896.47
	X L D Y	phenylacetyl	t-butyl ester	1040.01
	X L D Z	phenylacetyl	benzylhydrylamide	
	X L D F **p**	p-bromophenylacetyl	amide	951.08

TABLE 4 - COMPOUNDS DESCRIBED BY AMINO ACID SEQUENCE AND SUBSTITUENT GROUPS

SEQ ID NO:	FORMULA[1]	X	Z[2]	COMPOUND ID NO:
	I L D F **p**	free amine	amide	896.26
9	X L D F P Z	phenylacetyl	decylamide	1068.04
10	I L D V P I L D V P	free amine	amide	926.28
	X J D F **p**	benzylamide	amide J=dicaroboxy-Leu[5]	1034.01
	X L D V **p**	cyclohexaneacetyl	amide	896.48
	X L D Z	phenylacetyl	N'-t-Boc-hydrazide	997.13
11	I L D F P	free amine	amide	926.12
	X L D V **p**	1-naphthoyl	amide	896.43
	X L D V **p**	cyclohexanepropionyl	amide	896.45
	X L D Z	phenylacetyl	N'-benzyl-N'-cyclopenane-carbonylhydrazide	997.15
	F J D F **p**	fee amine	amide cyclohexyl-Ala[5]	896.30
	i L D V **p**	free amine	amide	926.03
	I L D V **p**	free amine	amide	886.10
	I J D F **p**	free amine	amide cyclohexyl-Ala[5]	896.29
	X L D V **p**	cinnamoyl	amide	896.41
3	G P E I L D V P S T	free amine	free acid	872.01

TABLE 4 - COMPOUNDS DESCRIBED BY AMINO ACID SEQUENCE AND SUBSTITUENT GROUPS

SEQ ID NO:	FORMULA[1]	X	Z[2]	COMPOUND ID NO:
12	X L D F P Z	phenylacetyl	$HN(CH_2)_5C(O)NHC_{18}H_{37}$	1068.01
	X L D F	phenylacetyl	amide	997.12
	X L D F Z	phenylacetyl	N-(4-decoyloxyl)piperidinamide	1068.06
	X L D F Z	phenylacetyl	N-(4-stearoyloxy)piperidinamide	1068.05
	L D V	acetyl	amide	926.30
	X L D Z	pyhenylacetyl	hydrazide	
	X L D V **p**	adamantanecarbonyl	amide	896.50
	X L D V **p**	2-naphthoyl	amide	896.44
13	I L D V P	free amine	amide	886.03
14	I L D V P	free amine	free acid	886.05
	L D F	acetyl	amide	926.32
	X D L F **p**	phenylacetyl	amide	1066.02
15	S F D F S	acetyl	amide	951.46
	I J D V **p**	free amine	amide J=cyclohexyl-Ala[5]	896.32
	X L D F **p**	4-bromophenyl-sulfonyl	amide	951.10
	L D F Z	free amine	piperidinamide	1047.09
	J D F Z	J=iso-butyloxy-carbonyl	piperidinamide	1027.04

TABLE 4 - COMPOUNDS DESCRIBED BY AMINO ACID SEQUENCE AND SUBSTITUENT GROUPS

SEQ ID NO:	FORMULA[1]	X	Z[2]		COMPOUND ID NO:
	L D F	free amine	amide		926.31
	L D V	free amine	amide		926.29
	X L D F **p** Z	phenylacetyl	amide	linker arm-biotin[8]	896.61

[1] A lower case letter in bold-faced type is used to designate a D-isomer of the L-amino acid residue designated in single letter code by the same capitol letter. Thus, **p**=D-proline; **f**=D-phenylalanine; **i**=D-isoleucine. The N-terminal α-amine is substituted as shown or indicated to be a "free amine".

[2] The state of the C-terminal carboxyl of Z as an "amide" ($-NH_2$) or "free acid" is noted as appropriate.

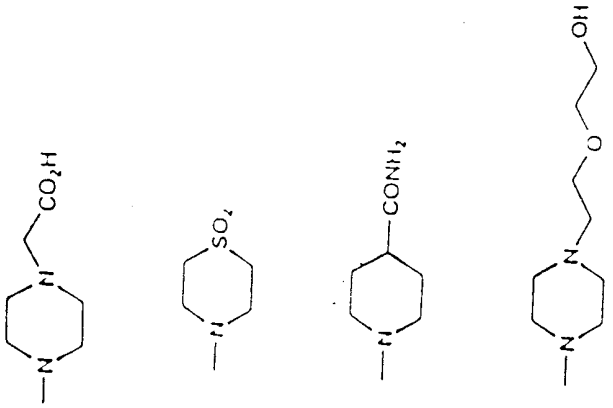
Z is an amide formed between the C-terminal Pro carboxyl and a tetraethylenepentaamine containing four N-phenylacetyl-LDFF peptides amide-bonded thereto.

Diethylenetriaminepentaacetatoeuropium (II) amide-bonded to Z.

"Homo-Phe" = homophenylalanine; "phenyl-Gly" = phenylglycine; "p-nitro-Phe" = p-nitrophenylalanine; β-carboxy-Asp = β-carboxyaspartic acid; "cyclohexyol-Ala" = cyclohexylalanine; and "dicarboxy-Leu" = dicarboxyleucine, Nle = norleucine.

Relative activities of about one-tenth or less than that exhibited by the peptides of SEQ ID NO:3 are assigned a potency activity of zero.

Amide formed from 1,6-hexanediamine amide-bonded to biotin.



The data shown in Figs. 1, 2 and 3 also illustrate the unexpected binding inhibitions exhibited by contemplated compounds relative to other compounds of the art. The compound sequences are shown using single letter code.

For example, Fig. 1 illustrates results of relative in vitro binding inhibition studies carried out using the CS-1 (SEQ ID NO:1) compound, the CS-1 compound B12 portion (CS-1 B12; SEQ ID NO:2), the 10-mer compound used as a standard above, elsewhere herein and in the art (SEQ ID NO:3), and several deletion analogues of the B12 compound, each containing the Leu-Asp sequence. N-Terminal deletion analogues are shown to the left of the standard 10-mer, whereas C-terminal deletions are shown to the right of the 10-mer. As is seen, the CS-1 compound is about three times more potent an inhibitor than is B12, the 10-mer or a 9-mer deletion analogue of the 10-mer. Those latter three compounds were all more potent than the other B12-related compounds.

The similarly obtained data of Fig. 2 illustrate binding inhibition results obtained using deletion analogues of the standard 10-mer compound. Here, deletions made at both N- and C-termini are shown to the left of the standard 10-mer to isolate the Leu-Asp-Val sequence at the C-terminus, whereas those shown to the right of the standard 10-mer isolate the Asp-Val-Pro sequence. These compounds and those of Fig. 1 had free N-terminal amine groups and C-terminal carboxyl groups.

The data of Fig. 3 were similarly obtained, but are shown on a log scale so that all of the data could be accommodated. The data of Fig. 3 are shown in five groups, from left to right.

The first group show data for CS-1 compound and the 10-mer standard. The next three bars shown data for a pentamer C-amide having the sequence including Leu-Asp-Val of the native CS-1 compound, the enhanced effect of using D-proline instead of the native L-proline, and then the enhancement by use of phenylalanine and D-proline in place of valine and D-proline. The next two bars illustrate the further enhancement obtained over the three previous compounds obtained when a cyclic ring-containing X group, here phenylalanine as the free amine, was used to replace the isoleucine of the native sequence. The fourth group of bars illustrates the effects of three X groups of formula I as compared to the phenylalanine group, using the better compound sequence of the two adjacent sequence [XLDFp-NH_2]. Phenylacetyl (ϕAc) was used as an X group in the last three compounds where the D-proline Z group of formula I was varied using three cyclic amines (NCy^1). As is seen, use of a morpholinamide group as Z, along with phenylacetyl as X and phenylalanine as Xaa of formula I, provided the greatest potency in these studies.

Thus, the data of Fig. 3 show inhibitory potencies spanning about three orders of magnitude from the standard 10-mer and compounds of the art, through contemplated compounds that exhibit about a 10-fold enhancement in potency over that standard to those contemplated compounds exhibiting about a 50-fold to about 100-fold enhancement in potency and those exhibiting an enhancement in potency of up to about 1000-fold.

In addition to being more potent than the CS-1 or standard 10-mer compounds, a contemplated inhibitor compound, particularly a compound with non-naturally occurring terminal groups such as N-phenylacetyl and C-morpholinamide or D-Pro-NH_2, is relatively more stable in serum that is the CS-1 compound. Thus, the inhibitor compounds N-phenylacetyl-Leu-Asp-Phe-morpholinamide and N-phenylacetyl-Leu-Asp-Phe-D-Pro-NH_2 exhibited no loss of potency after 24 hours in PBS at 7.2-7.4 that also contained 10 percent mouse or human serum. Contrarily, the CS-1 compound lost its potency in less than one hour under the same conditions.

Syntheses

The contemplated inhibitors are compounds or compound derivatives, and as such, can be readily synthesized using well known synthetic methods. See for example, Stewart, J. M. and J.D. Young, Solid Phase Peptide Synthesis, Pierce Chemical Company, Rockford, Ill., (1984) and M. Bodansky, Peptide Chemistry, A Practical Textbook, 2nd Edition, Springer-Verlag, New York, (1993) which are herein incorporated by reference. Specific synthetic examples are provided hereinafter.

Solid phase synthesis was used for those materials having a C-terminal amino acid amide or free acid residue. Thus, the N-protected, C-terminal residue was linked to a solid support having a benzhydrylamine substituent. Fmoc amine blocking groups were used in these syntheses, although t-Boc, CBZ or other blocking groups can also be used with other solid supports. Upon deblocking the Fmoc group with piperidine, another residue was coupled. That

coupling was followed by further deblocking, coupling, deblocking etc. steps until a solid phase-linked compound of desired sequence was prepared. As appropriate to each compound, an N-terminal X group was added after a final N-deblocking step or sometimes pre-coupled to the N-terminal residue. The desired compound and any accompanying functional group protecting groups were removed from the solid support by reaction with trifluoroacetic acid (TFA). This procedure results in a C-amide-terminated compound when a benzhydrylamine solid support is used.

Contemplated compounds can also be prepared using t-Boc N-protecting groups and another solid support, or a benzylamino-substituted solid support to which a P-hydroxymethylphenylcarboxyl (PAM) group is first reacted with the amine of the support to form a carboxamide. The hydroxyl group is then used to form an ester link to the first compound and standard t-Boc synthetic technology is thereafter followed. Reaction of the completed, deprotected solid phase-linked compound with ammonia provides the C-terminal amide compound discussed before, whereas reaction with another amine such as morpholine or piperidine or other NCy^1 or NCy^2 amine provides a compound whose C-terminal residue is amide-bonded to an NCy^1 or NCy^2 group. Reaction of a deprotected, PAM-linked compound with hydroxide provides the corresponding C-terminal carboxyl group.

In other embodiments, liquid phase compound syntheses were utilized. For example, morpholine or other NCy^1 or NCy^2 group was coupled in solution to a contemplated C-terminal, t-Boc-protected residue using a carbodiimide. The t-Boc protecting group was removed with acid, a further t-Boc-protected residue

added, followed by deblockings and further additions. The N-terminal X group such as phenylacetic acid was added after the last t-Boc removal step and the synthesis was completed, except for deprotecting the Asp residue. That step was carried out by catalytic hydrogenation where a benzyl ester protecting group was used.

Regardless of the synthetic method used, an inhibitor compound is typically recovered and purified prior to use. Recovery and purification techniques are well known and will not be dealt with here.

C. Mass Spectroscopy

Mass spectroscopy data confirmed the expected molecular weight of exemplary compounds. The data was obtained using fast atom bombardment mass spectrometry (FAB), electrospray mass spectrometry (Electrospray), or matrix assisted laser desorption mass spectroscopy (MALDI-TOF-MS).

Briefly, FAB was done using a VG ZAB-VSE double focusing high resolution mass spectrometer equipped with a cesium ion gun. The mass spectrometer was manually tuned to a resolution of 2000 (10% valley definition) with amplifier and multiplier gains of a million (300V). A 35 kev cesium ion beam was used as the fast ion beam and the accelerating voltage of the desorbed ions was 8 kV. The mass spectra were acquired using CSI for calibration; typically ten spectra were accumulated and averaged. Spectra were recorded with a Digital VAX station 3100 and the peaks were automatically centroided. A flat FAB sample holder was used. Standards having 98% or better purity were used. In

a representative experiment, 10.0 micrograms of the sample in methanol was applied to 2.0 microliters of the matrix and the solvent was evaporated. The probe was inserted into the mass spectrometer and spectra accumulated and averaged.

Electrospray mass spectroscopy was conducted on a API III PERKIN ELMER SCIEX triple-quadrupole mass spectrometer and API 100 PERKIN ELMER SCIEX Mass Spectrometer. Samples were introduced into the analyzer at a rate of 4.0 μl/minute. The positive ions generated by the ion evaporation process entered the analyzer through an interface plate and a 100 μm orifice, while the declustering potential was maintained between 50-250 V (typically 100V) to control the collision energy of the entering ions.

MALDI-TOF-MS spectroscopy was performed on a Vesdec Inc. Voyager Biospectrometry workstation. Matrix Assisted ionization of a compound consists of mixing a dilute solution of a compound with a large excess of an appropriate matrix material. The sample is placed in the mass spectrometer and irradiated with a laser. The matrix gives off absorbed light energy which causes vaporization of the compound in the mass spectrometer. In a representative experiment, the compound is prepared in a water and TFA solvent and appropriately diluted. The matrix was the Alpha cyano-4-hydroxy-cinnamic acid, gentisic acid or sinapinic acid. The laser was an N2 laser.

The mass spectroscopy data of exemplary compounds is shown in Table 5. The compound ID number cross-references compounds herein.

TABLE 5

Compound ID Number	Molecular Ion
1111.06	638 (MH^+)
1191.17	596 (MH^+)
1111.03	629 (MH^+)
1190.03	652 (MH^+)
1111.05	622 (MH^+)
1051.01	581 (MH^+)
896.61	624 (MH^+)
1070.02	596 (MH^+)
1190.02	636 (MH^+)
1190.36	727 (MH^+)
1111.07	668 (MH^+)
1051.02	581 (MH^+)
1190.46	617 (MNa^+)
1036.01	624 (MH^+)
1190.51	599 (MH^+)
951.22	610 (MH^+)
1111.01	734 (MH^+)
1111.09	637 (MH^+)
1070.08	609 (MH^+)
1190.07	644 (MNa^+)
896.52	608 (MH^+)
1045.02	597 (MH^+)
997.20	551 (MH^+)
896.62	610 (MH^+)
951.14	579 (MH^+)
951.20	596 (MH^+)
1111.08	608 $(MH\text{-}I)^+$
1160.01	670 (MNa^+)
1190.12	642 (MNa^+)
1070.07	613 (MH^+)
1190.11	591 (MH^+)
1190.04	582 (MH^+)
1070.10	609 (MH^+)
1070.12	608 (MH^+)
951.17	595 ($M+2H^+$)
1190.52	613 (MH^+)
1070.17	594 (MH^+)
1190.50	606 (MH^+)
1070.14	666 (MH^+)

Compound ID Number	Molecular Ion
896.60	647 (MH^+)
1070.11	637 (MH^+)
951.15	565 (M^+)
896.55	596 (MH^+)
1190.29	518 (MH^+)
1111.10	596 (MH^+)
1057.06	532 (MH^+)
1190.14	670 (MH^+)
1062.03	622 (MH^+)
1019.01	611 (MH^+)
1190.30	579 (MH^+)
1190.48	671 (MH^+)
951.12	587 (MH^+)
896.69	594 (MH^+)
1190.28	503 (MH^+)
1190.49	597 (MH^+)
951.05	645 (MH^+)
1026.05	577 (MH^+)
1160.02	608 (MH^+)
896.39	561 (MH^+)
1190.32	539 (MH^+)
997.11	566 (M^+)
896.28	616 (MNa^+)
896.63	636 (MH^+)
1070.19	638 (MH^+)
1190.45	644 (MH^+)
1190.31	526 (MH^+)
1063.01	948 ($MH+Na^+$)
896.68	661 (MH^+)
1190.15	641 (MH^+)
951.42	701 (MNa^+)
1047.01	620 (MH^+)
1056.01	625 (MH^+)
1190.37	755 (MH^+)
1070.09	609 (MH^+)
951.03	585 (MH^+)
1190.34	489 (MH^+)
1043.02	497 (MNa^+)
1051.05	590 $(MNa-H)^+$

Compound ID Number	Molecular Ion
997.08	509 (MH^+)
997.18	644 (MNa^+)
1190.26	608 (MH^+)
951.02	618 (MNa^+)
1190.33	489 (MH^+)
997.02	455 (MH^+)
1043.01	475 (MH^+)
896.27	638 (MH^+)
896.72	570 (MNa^+)
1040.02	607 (MNa^+)
896.54	548 (MH^+)
997.03	477 (M^+)
1047.05	508 (MH^+)
896.31	635 (MH^+)
926.02	547 (MH^+)
997.16	513 (MNa^+)
896.49	538 (MH^+)
951.06	646 (MH^+)
997.10	566 (MH^+)
1047.06	656 (MNa^+)
951.08	709 (MNa^+)
1190.27	461 (MH^+)
896.37	574 (M^+)
896.47	562 $(MNa-H)^+$
997.09	480 (M^+)
1057.04	553 (MH^+)
1051.04	638 (MH^+)
1068.04	770 ($MH+Na^+$)
926.04	590 (MH^+)
926.03	556 (MH^+)
886.03	556 (MH^+)
997.15	566 (MH^+)
1067.01	778 ($MH+Na^+$)
926.05	590 (MH^+)
1068.01	995 (MNa^+)
1067.02	688 ($MH+Na^+$)
1068.05	864 (MH^+)
1068.06	774 (MNa^+)
997.12	512 (MH^+)

Compound ID Number	Molecular Ion
1190.01	636 (MH^+)
1068.07	720 (MNa^+)
1068.08	686 (MNa^+)
1068.09	770 (MNa^+)
1068.10	799 ($MH+Na^+$)
1068.11	805 ($M+2H^+$)
1068.12	883 ($MH+Na^+$)
1068.13	743 (MNa^+)
1068.14	799 (MNa^+)
1190.05	644 (MNa^+)
1190.06	644 (MNa^+)
1068.18	653 (MH^+)
1068.17	690 (MH^+)
1068.32	716 (MH^+)
1068.89	698 (MH^+)
1068.90	685 (MH^+)
1068.92	665 (MH^+)
1068.94	636 (MH^+)
1068.95	622 (MH^+)
1068.96	637 (MH^+)
1068.97	623 (MH^+)
1068.98	609 (MH^+)
1068.99	677 (MH^+)
1069.02	690 (MH^+)
1069.01	650 (MH^+)
1069.03	679 (MH^+)

D. Prodrug Compounds

Prodrug compounds are transformed in vivo from compounds that do not necessarily bind the VLA-4 receptor in vitro to compounds having such binding activity in vivo. Chemical modifications of drugs that make prodrugs are known in the art and include, for example, esters of carboxylic acids or carboxyamide phosphonate groups. Moreover, the synthesis of prodrugs is by well known methods and will not be detailed here. See, for example, Bundraard, Design of Prodrugs, Elsevier Science Pub. Co., N.Y. (1985), and Prodrugs as Novel Drug Delivery Systems Symposium, 168th Annual Meeting, American Chemical Society, Atlantic City, N.J., Eds. T. Higuchi and V. Stella, ACS Symposium Serries 14, 1975, which are herein incorporated by reference.

E. Compositions and Process

As noted elsewhere, immune system leukocyte effector or inflammatory cells such as monocytes, T cells and eosinophils bear the VLA-4 receptor on their cell surfaces. Those cells bind to the CS-1 portion of fibronectin present on the surfaces of vascular endothelial cells at an early step in inflammatory cell emigration (trafficking) from the blood in the tissues. These inflammatory cells immunoreact with monoclonal antibody P4C2 discussed in Wayner et al., J. Cell. Biol., 109:1321-1330 (1989), Wayner WO 98/12809, Hemler et al, J. Biol. Chem., 262(24):11478-11485 (1987) and monoclonal antibody HP1/2 of Lobb WO 93/13798 published July 22, 1993.

Once in the tissues, the inflammatory cells enhance the inflammatory response through one or more

of several mechanisms. In one mechanism, cytokines and chemoattractants reactants such as interleukin-1β (IL-1β), IL-2, tumor necrosis factor α (TNFα) and lymphocyte-derived chemotactic factor are released by the inflammatory cells and cause further inflammatory cells to emigrate to the area. In another mechanism, the inflammatory cells mis-recognize cells of the mammal with the inflammatory disease state as being non-self and attack those cells, killing them. These and other mechanisms of immunoinflammatory response enhancement are well known to skilled workers and need not be further elaborated upon here. The fibronectin CS-1 compound thus mediates inflammatory disease states by assisting emigration of inflammation-enhancing effector cells from the blood into the tissues.

A contemplated inhibitor compound blocks binding between CS-1 and VLA-4, and inhibits the resulting emigration of inflammatory cells bearing VLA-4 receptors into the tissues, and the exacerbation of the inflammatory condition that results. That inhibition of emigration of inflammatory cells results in a reduction of the fibronectin CS-1/VLA-4-mediated inflammatory response caused by those inflammatory cells, and thereby reduces the observed inflammation.

Particular inflammatory disease states that are mediated by CS-1 and VLA-4, and in which a contemplated inhibitor compound can diminish inflammation are quite broad. Illustrative of those types of inflammation are asthma, arthritic conditions such as rheumatoid arthritis and osteoarthritis, allograft rejection, various types of skin inflammation, and demyelinating diseases of the central nervous system.

Specific pathological inflammatory conditions in which expression of CS-1 has been found to be implicated and where no such expression is observed in absence of a pathological condition (i.e., in normal tissue) include: rheumatoid arthritis (synovium), osteoarthritis (synovium), skin psoriasis, kidney transplant, asthmatic lung, and lymph node high endothelial venules (HEV) in humans, as well as in the gut of monkeys infected with SIV and those having inflammatory bowel disease, rabbits having asthmatic lungs and heart transplants, mouse brain in experimental autoimmune encephalomyelitis (EAE) and skin in delayed type hypersensitivity (DTH), and the joints of rats with induced arthritis.

VLA-4 is expressed on mononuclear leukocytes, T cells, B cells and monocytes, as well as eosinophils. Since a contemplated inhibitor compound acts by binding to VLA-4, any inflammatory disease state which involves the aforementioned cells may be treated using the inhibitor compounds of the present invention. For example, inflammatory diseases states such as allergy, arthritis, asthma, atherosclerosis, colitis, diabetes, inflammatory bowel disease, kidney inflammation, skin inflammatory diseases multiple sclerosis, restenosis, and transplantation are VLA-4 dependent inflammatory diseases and can be treated by inhibitor compounds of the present invention.

Although potency is used to screen inhibitor compounds, the compound efficacy is the relevant parameter for clinical applications. Efficacy connotes the property of a drug to achieve a desired response. A compound having relatively low potency but more selectivity can be the clinically

preferred compound. Thus, a compound that binds to the VLA-4 receptor, but does not bind tighter than the CS-1 25-mer compound present in fibronectin, and has efficacy in vivo can be used in a pharmaceutical composition. See, for example, Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA (1985), which is incorporated herein by reference.

A pharmaceutical composition or medicament containing a contemplated inhibitor compound dissolved or dispersed in a pharmaceutically acceptable carrier or diluent that is preferably aqueous is also contemplated for use in treating a CS-1/VLA-4-mediated inflammatory disease state such as those discussed before. Such a composition contains an effective amount of a contemplated compound. In an embodiment, such a composition contains the CS-1/VLA-4 binding-inhibiting (an inflammation-reducing) amount of a before-discussed, contemplated inhibitor compound.

Thus, the present invention also contemplates a pharmaceutical composition that can be used in treating one or more of the aforementioned conditions. A contemplated pharmaceutical composition is comprised of a before-described inhibitor compound that inhibits the binding interaction between VLA-4-containing leukocytes and the fibronectin compound CS-1 portion expressed on endothelial cell surfaces, which compound is dissolved or dispersed in a pharmaceutically acceptable diluent in a binding inhibitory (inflammation-reducing) amount. A contemplated pharmaceutical composition is suitable for use in a variety of drug delivery systems. For a brief review

of present methods for drug delivery, see, Langer, Science, 249:1527-1533 (1990).

For a contemplated pharmaceutical composition, the dose of the compound varies according to, e.g., the particular compound, the manner of administration, the particular disease being treated and its severity, the overall health and condition of the patient, and the judgment of the prescribing physician or veterinarian. A pharmaceutical composition is intended for parenteral, topical, oral or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment. A pharmaceutical compositioncan be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include powder, tablets, pills, capsules and dragees.

A pharmaceutical composition also is administered intravenously. Thus, a composition for intravenous administration is particularly contemplated that comprises a solution of a contemplated inhibitor compound dissolved or dispersed in a pharmaceutically acceptable diluent (carrier), preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.9 percent saline, buffered aqueous ethanol solutions and the like. These compositions can be sterilized by conventional, well known sterilization techniques, or can be sterile filtered. The resulting aqueous solutions can be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. A composition can contain pharmaceutically acceptable auxiliary

substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

The concentration of inhibitor compound utilized is usually at or at least about 0.0001 percent to as much as about 0.1 percent by weight and is selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intravenous infusion can be made up to contain 250 ml of sterile Ringer's solution normal saline or PBS, and about 0.25 mg to about 25 mg of the inhibitor compound. Actual methods for preparing parenterally administrable compounds are known or apparent to those skilled in the art and are described in more detail in for example, Remington's, supra.

For solid compositions, conventional nontoxic solid diluents (carriers) may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95 percent of active ingredient, that is, a before-described inhibitor compound preferably about 20

percent (see, Remington's, supra), preferably using an enteric coating to pass a solid dose through the stomach and into the intestine.

For aerosol administration, a contemplated inhibitor compound is preferably supplied in solution such as aqueous ethanol or DMSO solution along with a surfactant and propellant. Typical percentages of an inhibitor compound are about 0.0001 percent to about 0.1 percent by weight, and preferably about 0.0001 percent to about 0.001 percent. The surfactant must of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride such as, for example, ethylene glycol, glycerol, erythritol, arabitol, mannitol, sorbitol, the hexitol anhydrides derived from sorbitol, and the polyoxyethylene and polyoxypropylene derivatives of these esters. Mixed esters, such as mixed or natural glycerides can be employed. The surfactant can constitute about 0.1 to about 20 percent by weight of the composition, and preferably about 0.25 to about 5 percent. The balance of the composition is ordinarily propellant. Liquefied propellants are typically gases at ambient conditions, and are condensed under pressure. Among suitable liquefied propellants are the lower alkanes containing up to 5 carbons, such as butane and propane; and preferably fluorinated or fluorochlorinated alkanes. Mixtures of the above can also be employed. In producing the aerosol, a container equipped with a suitable valve is filled with the appropriate propellant, containing the finely divided compounds and surfactant. The

ingredients are thus maintained at an elevated pressure until released by action of the valve. A pump-activated spray using air as propellant (atomizer or nebulizer) is also contemplated.

For example, for the treatment of asthma in rabbits, the dose of a contemplated compound is in the range of about 1 to 100 mg/day for a 2-3 kg animal. For a human asthma patient, that dose is in the range of about 1 to about 100 mg/day for a 70 kg patient. Administration for asthma is typically by aerosol from a nebulizer. Ideally, therapeutic administration should begin as soon as possible after the attack begins.

A pharmaceutical composition embodiment is the inhibitor compound of the present invention and a liposome suitable for pharmaceutical use. Examples of suitable liposomes include those disclosed in WO9421281, WO9421235, U.S. Patent 5,225,212, or WO8606959 wheich are herein incorporated by reference.

A pharmaceutical composition containing an inhibitor compound can be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, a composition is administered to a patient already suffering from a disease, as described above, in an amount sufficient to inhibit binding between VLA-4-expressing leukocytes and endothelial cells that express the CS-1 compound portion; i.e., reduce inflammation and thereby at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose", or a "binding-inhibiting amount" or an "inflammation-reducing amount". Amounts effective

for this use depend on the severity of the disease and the weight and general state of the patient, but generally range from about 1 mg/kg to about 500 mg/kg of inhibitor compound per day, with dosages of from about 1 mg/kg to about 10 mg/kg of a compound per day being more commonly used.

In prophylactic applications, a composition containing a contemplated compound is administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be a "prophylactically effective dose" and is also an amount sufficient to inhibit binding of VLA-4-expressing leukocytes to CS-1 compound-expressing endothelial cells. In this use, the precise amounts again depend on the patient's state of health and weight, but generally range from about 1 mg/kg/day to about 500 mg/kg/day, more commonly from about 1 mg/kg/day to about 20 mg/kg/day.

Another way to assess a binding-inhibiting amount of a contemplated inhibitor compound is to compare binding inhibition exhibited by the compound to that provided by CS-1 or the 10-mer standard in an in vitro study. One convenient way to make that comparison is by use of IC_{50} values of the two compared materials, and base the amount used on the amount of CS-1 or standard 10-mer compound and an amount of the inhibitor compound that is a multiple of the IC_{50} value for that reference compound.

Preferably, a compound whose IC_{50} value is at least about one-tenth that of the standard 10-mer (ten-times more potent), when used at one-tenth the molar amount of the 10-mer standard is a useful binding-inhibiting amount. More preferably, the amount is about one-fiftieth the amount of the

10-mer. More preferably still, the amount is equal to about one-hundredth that of the 10-mer. Inasmuch as those amounts inhibit binding by about 50 percent, greater concentrations that inhibit binding still further are preferred.

Thus, for in vitro use, a minimal CS-1/VLA-4-inhibiting amount is the IC_{50} value. For in vivo use, the CS-1/VLA-4-inhibiting amount usually used begins with the IC_{50} value concentration, and can decrease as required or one can increase to the solubility limit of the compound in the utilized aqueous medium; i.e., the aqueous medium at pH 7.2-7.4 used such as normal saline where parenteral administration is used or intestinal fluid where oral administration is used.

Single or multiple administrations of a composition can be carried out with dose levels and pattern being selected by the treating physician or veterinarian. In any event, a pharmaceutical composition is formulated to provide a quantity of an inhibitor compound sufficient to effectively treat the patient.

A pharmaceutical composition embodiment is the inhibitor compound in a pharmaceutically acceptable salt.

Further, a pharmaceutical composition embodiment is the inhibitor compound of the present invention and an antibody to P selectin. Such a composition can treat various conditions, including, for example, restinosis. Another embodiment is the inhibitor compound and an inhibitor of the polylactosamino glycan, sialyl Le^x. Such a

composition can treat various conditions, including, for example, inflammation.

A process for treating fibronectin CS-1/VLA-4-mediated inflammation is also contemplated wherein the inhibitor compound is administered to a mammal in need of such a treatment. This administration is preferably via a before-discussed pharmaceutical composition. The compound is administered in an inflammation-reducing (CS-1/VLA-4 binding inhibiting) amount. The mammal such as mouse, rat, rabbit, monkey or human is maintained until the compound is eliminated by a natural bodily process. Multiple administrations in a single day, over a period of days or weeks, or for the life of the host mammal, where the mammal is the recipient of an allograft, are contemplated, as are single administrations.

Methods for determining an amount sufficient to inhibit binding between CS-1 and VLA-4 have already been discussed, particularly for in vitro studies. For in vivo uses, there are many published assays to determine if inflammation has been reduced by a particular treatment. For example, one can assess the number of painful joints in an arthritic patient or the patient's mobility before and after treatment. Reduction of effects of an asthma attack can be assayed by measurement of dynamic compliance or lung resistance in laboratory animals as is also well known. The amount of edema observed in DTH is also readily measurable, as are the effects of allograft rejection or its absence compared to standard controls.

Compounds of Formula IA and Formula IIA are particularly metabolically stable when R_2 is lower

alkyl or phenyl lower alkyl, more particularly, when R_2 is lower alkyl and, even more particularly, when R_2 is methyl. Many such compounds, for example, are particularly resistant to metabolic degradation and, therefore, are useful for administration, including oral administration, having been shown to possess stability to intestinal proteases (see Example 11, below). Moreover, many such compounds are useful for aerosol administration because they possess stability to lung proteases. In addition, many such compounds are particularly useful for treating allergic conditions, including asthma (see Example 12, below).

Furthermore, many such compounds are particularly permeable across cell membranes, especially the compounds of Formula IIA. Therefore, such compounds have particularly good oral bioavailability.

EXPERIMENTAL

Example 1: Synthesis of X-Leu-Asp-Phe-Z

Protected amino acids, Boc-Phe-OH, Boc-Asp(OBn)-OH and Boc-Leu-OH were purchased from NOVA Biochem Co., La Jolla, CA and were used without further purification. Phenylacetic acid, morpholine, diisopropylethylamine (DIEA) and 1-hydroxybenztriazole (HOBt) were obtained from Aldrich Chemical Co., Milwaukee, WI. Ethyl-3-(3-dimethylamino)-propylcarbodiimide•HCl (EDC) was obtained from Bachem Co., Torrance, CA. 4 Normal HCl in dioxane was obtained from Pierce Co., Rockford, IL used as received.

The ^{1}HNMR spectra were recorded on a GE QE-300, 300 MHZ NMR spectrometer.

A. Preparation of Boc-Phe-Morpholinamide

A 250 ml flask was charged with Boc-Phe-OH (10 g, 38 mmol), morpholine (3.3 g, 38 mmol) and 1-hydroxybenztriazole (5.1 g, 38 mmol) in 100 ml dry dimethylformamide (DMF). To this solution was added diisopropylethylamine (DIEA) at zero degrees C until the pH value reached 8, followed by addition of EDC (8.8 g, 46 mmol). The solution was slowly warmed to room temperature. The mixture was stirred for eight hours at room temperature (about 22°C). The DMF was removed by vacuum evaporator, ethyl acetate and water were added, and the layers were separated. The aqueous layer was extracted with ethyl acetate (50 ml x 2), combined extracts were washed with 1N HCl, saturated $NaHCO_3$, water and brine, dried with $MgSO_4$, filtered and concentrated to give a colorless liquid (12.8 g, 37.7 mmol; 99 percent yield) that was characterized by ^{1}HNMR as Boc-Phe-morpholine.

B. Preparation of HCl-Phe-Morpholinamide Salt

Boc-Phe-morpholinamide (12.8 g, 38 mmol) was placed in a 250 ml flask, then 4N HCl in dioxane (30 ml) was added. The mixture was stirred for six hours at which time TLC (silica gel; $CHCl_3$:MeOH:acetic acid, 90:8:2) indicated that the reaction was completed. Dioxane and excess HCl were removed. A white solid, identified by ^{1}HNMR as HCl-Phe-morpholinamide, was obtained in 100 percent yield (10.3g, 38 mmol).

C. Preparation of N-Phenylacetyl-Leu-Asp(β-O-Benzyl)-Phe-Morpholinamide

Boc-Asp(β-OBn)-OH, Boc-Leu-OH and phenylacetic acid were sequentially added to HCl-Phe-morpholinamide using the coupling and deprotection procedures, described above. The white solid, thus obtained, was crystallized from ethyl acetate and hexane, and identified by ^{1}HNMR as the desired ester in 95 percent yield.

D. Synthesis of N-Phenylacetyl-Leu-Asp(β-O-Bn)-Phe-Morpholinamide

To a solution of the above benzyl ester (10 g, 15 mmol) in methanol (100 ml) was added 10 percent palladium-charcoal (2.0 g). The flask containing this mixture was evacuated and then filled with hydrogen three times. The mixture was then vigorously stirred under a hydrogen atmosphere about five hours until the hydrogenalysis was complete, as indicated by TLC ($CHCL_3$:MeOH:acetic acid, 90:8:2). The reaction mixture was filtered through celite, and the methanol was removed, affording a white solid that was characterized by ^{1}HNMR as XLDFZ (8.4 g, 14.5 mmol) in 97 percent yield.

Compounds having other than carboxamide [C(O)-NH_2] C-terminal amide-linked Z groups were similarly prepared.

Compounds having a 2-H-isoindole substituent at the amino terminus were prepared by coupling 2H-isoindole-2-acetic acid, 1,3 dihydro-1-oxo-methyl, to amino terminus of the a synthesized fragment using standard compound coupling procedures. See, for example, New, J.S. and Yevich, J.P., J. Heterocyclic Chemistry, 21:1355-1360 (1984), which is herein incorporated by reference.

Materials and Methods for Examples 1A to 1E

Protected amino acids, Boc-Phe-OH, Boc-Asp(OBzl)-OH, Boc-Leu-OH and Boc-N-Me-Leu-OH were purchased from Novabiochem., La Jolla, CA. 4-Aminophenylacetic acid, 4-fluorophenylacetic acid, (S)-(+)-2-phenyl propionic acid, (R)-(-)-2-phenyl propionic acid, 4-hydroxypiperidine, morpholine, N-methylpiperazine, Boc-piperazine, phenylacetic acid, allylbromide, 4-dimethylaminopyridine (DMAP), Ethyl-2-methylbenzoate, N-bromosuccinimde, azodiisobutynitrile(AIBN), 4N HCl in dioxane, diisopropylethylamine (DIEA), 10% palladium on carbon, 1-hydroxybenzotriazole(HOBt), dimethylformamide (DMF), tetrahydrofuran (THF), dichloromethane (DCM) and various alcohols were purchased from Aldrich Chemical Co., Milwaukee, WI. Ethyl-3-(3-dimethylamino)-propylcarbodimide.HCl (EDC) and Leu-OBzl.p-Tosylate was obtained from Bachem Co., Torrance, CA. 1-Hydroxy-7-azabenzotriazole (HOAt) was purchased from Perseptive Biosystems. Tetrakis(triphenylphosphine) palladium (0) was purchased from Lancaster. Preparative TLC plates on silica gel (0.25mm or 0.5mm x 20cm x 20cm) were purchased from Aldrich or VWR.

Analytical HPLC was conducted on Beckman System Gold. Beckman System Gold contains 507e autosampler, 125 solvent module and PDA 168 detector. Vydac Protein and Peptide C18 column (0.46cm x 25cm) was used. Flow rate: 1 ml/min, detected at 214 nm. Preparative HPLC was conducted on Waters HPLC with Water's 600E controller, UV detector 441, Gilson's auto sampler 231 and fraction collector FC203B. Vydac protein and peptide C18 column (2.2cm x 25cm) was used. Flow rate: 15 ml/min, detected at 214 nm. HPLC solvents were as follows: solvent A: water with 0.1% trifluoroacetic acid (TFA); solvent B: acetonitrile

with 0.1% trifluoroacetic acid (TFA). Linear gradient conditions were usually used. For example, the gradient condition 5-70%B over 35 min means that the concentration of HPLC solvent B increases from 5% to 70% over 35 min. Water is Milli Q water; acetonitrile was purchased from VWR, HPLC grade EM Science; TFA (HPLC grade) was purchased from Pierce.

The ^{1}H-NMR spectra were recorded on a GE QE-300, 300 MHz NMR spectrometer. The Mass spectrometry experiments were performed at A and A Associates, San Diego, CA. An API 100 Perkin Elmer Sciex mass spectrometer was employed. Electrospray technic was used in positive mode and negtive mode. Thus MH^+ and MH^- were usually obtained. All the compounds in TABLE 1 and TABLE 2 were characterized by MS. TABLE 5 shows the Mass Spectroscopy Data.

Example 1A: Synthesis of Phenylacetyl-N-Me-Leu-Asp-Phe-N-Me-Piperazine

A. Preparation of Boc-Phe-O-Allyl

To a mixture of Boc-Phe-OH (3.0 g, 11.3 mmol) and Na_2CO_3 (1.8 g, 16.95 mmol) in DMF (100 ml) was added ally bromide (3.9 ml, 45.23 mmol). The reaction mixture was stirred at room temperature overnight. The solid was filtered off and DMF was evaporated under reduced pressure. The residue was dissolved in EtOAc, washed with 1N HCl, saturated sodium bicarbonate, brine, and then dried over magnesium sulfate. The solution was concentrated to give Boc-Phe-O-Allyl (3.2 g).

B. Preparation of Boc-N-Me-Leu-Asp(OBzl)-Phe-O-Allyl

Boc-Phe-O-Allyl (6.2 g, 20.4 mmol) was reacted with 4 N HCl in dioxane (30 ml) for 2 hours at room temperature. After removal of the solvent under reduced pressure, the crude product was dried in vacuo. This material was dissolved in DMF (300 ml) and the pH was adjusted to pH 5 by addition of DIEA. HOBt (2.68 g, 19.9 mmol) was added followed by Boc-Asp(OBzl)-OH (6.43 g, 19.9 mmol). The mixture was cooled to 0°C. EDC (3.8 g, 19.9 mmol) was added and the reaction was allowed to warm to room temperature and stirred overnight. After removal of the solvent under reduced pressure the residue was taken up in ethyl acetate (500 ml) and washed with 1N hydrochloric acid, saturated sodium bicarbonate, brine, and dried over magnesium sulfate. The filtrate was concentrated under reduced pressure. Boc-Asp(OBzl)-Phe-O-Allyl (9.7 g) was obtained. In the same manner, Boc-Asp(OBzl)-Phe-O-Allyl was further treated with 1N HCl in dioxane followed by coupling with Boc-N-Me-Leu-OH to give Boc-N-Me-Leu-Asp(OBzl)-Phe-O-Allyl.

C. Preparation of Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-O-Allyl

Boc-N-Me-Leu-Asp(OBzl)-Phe-O-Allyl (4.5 g, 7.1 mmol) was mixed with 4N HCl in dioxane (100 ml) and the reaction mixture was stirred for one hour at room temperature. The solvent was evaporated and the residue was washed with ether to give the HCl salt (4.08g).

To a mixture of HCl.N-Me-Leu-Asp(OBzl)-Phe-O-Allyl (3.8 g, 6.6 mmol) and DIEA (1.22 ml, 6.6 mmol) in DMF at 0°C were added phenyl acetic acid (900 mg, 6.6 mmol), HOAt (900 mg, 6.6 mmol) and EDC (1.3 g, 6.7 mmol). The reaction mixture was stirred at room temperature overnight. The DMF was removed under vacuum

and the residue was dissolved in dichloromethane and washed with 1N HCl followed by saturated $NaHCO_3$, H_2O, brine, and then dried over Na_2SO_4. The filtrate was evaporated and the residue was purified by flash chromatography (30% ethyl acetate/hexane) to give Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-O-Allyl (4.3 g). ^{1}H-NMR (DMSO-d_6) δ 8.17 (d, J = 7.7 Hz), 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.27 - 7.09 (m, 15H), 5.78 - 5.66 (m, 1H), 5.15 (dd, J = 17.2 Hz, 1.5 Hz, 1H), 5.07 (dd, J = 10.6 Hz, 1.5 Hz, 1H), 5.00 - 4.92 (m, 1H), 4.97 (s, 2H), 4.65 - 4.57 (m, 1H), 4.42 (d, J = 5.5 Hz, 2H), 3.66 (d, J = 15.8 Hz, 1H), 5.53 (d, J = 15.4 Hz, 1H), 2.95 - 2.85 (m, 2H), 2.73 - 2.52 (m, 2H), 2.62 (s, 3H), 1.50- 1.33 (m, 2H), 1.25-1.05 (m, 1H), 0.72 (d, J = 6.6 Hz, 6H).

D. Preparation of Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-OH

To a solution of Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-O-Allyl (4.3 g, 6.6 mmol) in THF at 0°C was bubbled through argon. Tetrakis(triphenyl phosphine)palladium (760 mg, 0.66 mmol) and morpholine (5.7 ml, 66 mmol) were added. The reaction mixture was stirred for 5 minutes under argon. The THF was then removed under reduced pressure. The resulting residue was dissolved in dichloromethane and washed with 1N HCl, H_2O, brine, and then dried over Na_2SO_4. The solution was evaporated and the crude Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-OH was washed with hexane and used for next step without further purification. ^{1}H-NMR (DMSO-d_6) δ 7.97 (d, J = 8.1 Hz), 7.87 (d, J = 7.7 Hz), 7.31- 7.08 (m, 15H), 5.00 - 4.95 (m, 1H), 4.97 (s, 2H), 4.64 - 4.56 (m, 1H), 4.33 - 4.26 (m, 1H), 3.66 (d, J = 15.7 Hz, 1H), 3.55 (d, J = 15.4 Hz, 1H), 2.96 - 2.52 (m, 5H), 1.50 - 1.30 (m, 2H), 1.22 - 1.05 (m, 1H), 0.73 (d, J = 6.6 Hz, 6H).

E. Preparation of Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-N-Me-piperazine

To the solution of Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-OH (4.0 g, 6.6 mmol) and N-methylpiperazine in DMF at -40°C (CH_3CN-dry ice bath) were added EDC (1.3 g, 6.6 mmol) and HOAt (850 mg, 6.25 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight. The DMF was evaporated under reduced pressure. The residue was dissolved in dichloromethane and washed with saturated $NaHCO_3$, H_2O, brine, and then dried over H_2O[illegible]Na_2SO_4. The resulting crude product was purified by flash chromatography on silica gel (ethyl acetate:methanol

97:3 to 90:10). The pure Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-N-Me-piperazine (3.5 g) was obtained. ^{1}H-NMR (DMSO-d_6) δ 7.99 (d, J = 7.7 Hz, 1H), 7.94 (d, J = 8.1 Hz,1H), 7.31 - 7.06 (m, 15H), 5.02 -4.95 (m, 1H), 4.97 (s, 2H), 4.91 - 4.74 (m, 1H), 4.59 - 4.52 (m, 1H), 3.66 (d, J = 15.4 Hz, 1H), 3.54 (d, J = 15.8 Hz, 1H), 3.30-3.06 (m, overlaps with H_2O), 2.83 -2.51 (m, 4H), 2.63 (s, 3H), 2.11 - 2.01 (m, 3H), 1.99 (s, 3H), 1.83 - 1.74 (m, 1H), 1.54 - 1.33 (m, 2H), 1.26 - 1.09 (m, 1H), 0.73 (d, J = 6.6 Hz, 6H).

F. Preparation of Phenylacetyl-N-Me-Leu-Asp-Phe-N-Me-piperazine

Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-N-Me-piperazine (2.6 g, 3.7 mmol) was dissolved in EtOH and 10% Pd-C (~200mg) was added. The reaction mixture was hydrogenated under H_2 atmosphere by using a H_2 balloon for 4 hours. The solid was filtered off through celite. The filtrate was evaporated and the remaining solid was further dried under high vacuum for two days to provide Phenylacetyl-N-Me-Leu-Asp-Phe-N-Me-piperazine (2.1g). HPLC retention time: 16.2 min, 5-90% over 25 min. ^{1}H NMR ($CDCl_3$) δ 7.71-7.82 (m, 1H), 7.15-7.35 (m, 9H), 5.15-5.25 (m, 2H), 4.95-5.05 (m, 2H), 4.70-4.80 (m, 2H), 3.70-3.80 (s, 3H), 2.25-3.50 (m, 16H), 1.60-1.70 (m, 3H), 1.30-1.45 (m, 2H), 0.85-1.00 (m, 6H). MS m/z: 608(MH^+), 606 (MH^-).

Phenylacetyl-N-Me-Leu-Asp-Phe-N-Me-piperazine can also be prepared as shown below in Example 1B by using N-Me-piperazine instead of morpholine.

Example 1B: Synthesis of Phenylacetyl-N-Me-Leu-Asp-Phe-Morpholine

A. Preparation of Boc-Asp(OBzl)-Phe-Morpholine

Boc-Phe-OH (50.0 g, 189 mmol) was dissolved in DMF (600 ml), HOBt (25.5 g, 189 mmol) was then added. The solution was cooled with an ice-bath for 10 min, EDC (43.3 g, 226 mmol) was added. After stirring 25 min, morpholine (16.4 g, 189 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred overnight. DMF was then evaporated under vacuum, ethyl acetate (800 ml) was added to the residue. This ethyl acetate solution was washed subsequently with 0.5 N HCl, saturated sodium bicarbonate, brine, and then dried over $MgSO_4$. Boc-Phe-Morpholine (60.0 g) with the purity of 95% was thus obtained as an oily material after filtration and evaporation.
Boc-Phe-morpholine was subjected to Boc protecting group removal by adding 4N HCl in dioxane (200 ml). Before the oil dissolved completely, a white solid was formed. The reaction was completed in 3 hours. The excess HCl and the solvent were evaporated. HCl. Phe-Morpholine was obtained. Boc-Asp(OBzl)-Phe-Morpholine was prepared by reacting Boc-Asp(OBzl)-OH with pre-neutralized HCl.Phe-Morpholine (by DIEA). The coupling procedure was the same as described above. Boc-Asp(OBzl)-Phe-Morpholine (95.0 g, purity 92%) was obtained.

B. Preparation of Boc-N-Me-Leu-Asp(OBzl)-Phe-Morpholine

Boc-Asp(OBzl)-Phe-Morpholine (95.0 g, 176.2 mmol) was treated with 4 N HCl-dioxane for 2 hours. The starting material was completely consumed. The excess HCl and the solvent were evaporated. Et_2O was added to the residue, the solid was filtered and

washed with Et_2O and dried. Thus HCl.Asp(OBzl)-Phe-Morpholine was obtained.

HCl.Asp(OBzl)-Phe-Morpholine (50.0 g, 105.2 mmol) was dissolved in DMF (500 ml) and Boc-N-Me-Leu-OH (26.2 g, 105. 2 mmol) was then added. The mixture was cooled with an ice-bath, HOBt (15.6 g, 115.7 mmol), EDC (24.11 g, 126. 2 mmol), DIEA (18.32 ml, 105.2 mmol) were subsequently added. The reaction mixture was allowed to warm to room temperature and stirred overnight. DMF was removed. Ethyl acetate (500 ml) was added to the residue and washed with 1N HCl, saturated sodium bicarbonate, brine subsequently. The crude Boc-N-Me-Leu-Asp(OBzl)-Phe-morpholine (59.4 g) was obtained.

C. Preparation of Phenylacetyl-N-Me-Leu-Asp-Phe-Morpholine

Compound Boc-N-Me-Leu-Asp(OBzl)-Phe-Morpholine (5.40 g, 6.3 mmol) was treated with 4N HCl, and further coupled with phenylacetic acid (0.85 g, 6.3mmol) by using the same procedure as B. The crude Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-Morpholine (4.10 g) was obtained. The compound was purified by column chromatography on silica gel with ethyl acetate:hexane (9:1) as the eluent. The purity of compound was great than 95% determined by HPLC. Phenylacetyl-N-Me-Leu-Asp(OBzl)-Phe-Morpholine (1.60 g, 2.3 mmol) was dissolved in 200 ml methanol. The reaction mixture was flashed with argon, a catalytic amount of 10% Pd-C was added. The mixture was flashed with hydrogenation while stirring, further stirred for 4 hours under hydrogen atmosphere by using a hydrogen balloon. TLC or HPLC showed the reaction was completed. The mixture was filtered through a

celite-packed funnel. The filtrate was concentrated to give Phenylacetyl-N-Me-Leu-Asp-Phe-Morpholine (1.2 g). HPLC retention time: 22.25 min at 5-90% B over 35 min. ^{1}H NMR (DMSO-d6): δ 7.95 (d, 1H), 7.88 (d, 1H), 7.40-7.05 (m, 10H), 5.06-4.98 (m, 2H), 4.80-4.70 (m, 2H), 4.60-4.35 (m, 3H), 6.50 (dd, 4H), 3.45-2.30 (m, 9H), 1.60-1.10 (m, 3H), 0.75 (dd, 6H). MS m/z: 595(MH^+), 593 (MH^-).

Example 1C: Synthesis of (1-Oxo-2,3-dihydro-isoindol-2-yl)-2-(S)-isobutylacetyl-Asp-Phe-Morpholine

A. Preparation of Ethyl-2-Bromomethylbenzoate:

To a stirred solution of ethyl-2-methylbenzoate (2.0 g, 12.2 mmol) in CCl_4 (100 ml) was added N-bromosuccinimide (2.2 g, 12.2 mmol) and Azodiisobutylnitrile (AIBN) (100 mg). The mixture was refluxed for 3 hours, ^{1}H NMR showed that the reaction was completed. The reaction mixture was filtered and concentrated, an oily material was obtained, which was purified by flash chromatography on silica gel (ethyl acetate/hexane, 1/15). Ethyl-2-bromomethylbenzoate (1.55 g, 6.38 mmol) was obtained. ^{1}H NMR ($CDCl_3$): δ 8.20-7.38 (m, 4H), 4.98 9s, 2H), 4.42 (q, 2H), 1.42 (t, 3H).

B. Preparation of (1-Oxo-2,3-dihydro-isoindol-2-yl)-2-(S)-isobutylacetic acid

To a stirred solution of H-Leu-OBzl. p-Tosylate (1.3 g, 3.3 mmol) in THF (50 ml) was added DIEA (1.03 g, 8.0 mmol) at 0 °C, followed by adding ethyl-2-bromomethylbenzoate (0.78 g, 3.2 mmol) dropwise, the mixture was slowly warmed to room temperature and stirred overnight. The reaction

mixture was concentrated and the residue was dissolved in ethyl acetate, which was washed with 1N HCl, saturated $NaHCO_3$, water, brine, and dried over $MgSO_4$. The filtrate was concentrated, the residue was purified by flash chromatography (silica gel, ethyl acetate/hexane 1/10 to 2/10). A white solid was obtained (0.8 g, 2.37 mmol) as benzyl (1-Oxo-2,3-dihydro-isoindol-2-yl)-2-(S)-isobutyl acetate. ^1H NMR ($CDCl_3$): δ 7.96-7.21 (m, 9H), 5.38-5.16 (m, 1H), 5.15 (s, 2H), 4.40 (dd, 2H), 2.00-1.81 (m, 2H), 1.55-1.40 (m, 1H), 1.05-0.82 (m, 6H).

This benzyl ester was subject to hydrogenolysis under H_2 atmosphere in the presence of 10% Pd on carbon. A white solid (0.55 g, 2.23 mmol) was obtained as (1-Oxo-2,3-dihydro-isoindol-2-yl)-2-(S)-isobutylacetic acid ^1H NMR (DMSO-d6)): δ 7.72-7.32 (m, 4H), 4.75 (dd, 1H), 4.40 (dd, 2H), 1.91-1.58 (m, 2H), 1.40-1.20 (m, 1H), 0.80 (dd, 6H).

C. Preparation of (1-Oxo-2,3-dihydro-isoindol-2-yl)-2-(S)-isobutylacetyl-Asp-Phe-Morpholine

(1-Oxo-2,3-dihydro-isoindol-2-yl)-2-(S)-isobutylacetic acid (0.55 g, 2.23 mmol) was dissolved in DMF (10 ml), HCl.H-Asp(OBzl)-Phe-Morpholine (1.06 g, 2.23 mmol) and DIEA (0.35 g, 2.67 mmol) were added. The solution was cooled with an ice-bath and EDC (0.51 g, 2.67 mmol), HOBt (0.31 g, 2.23 mmol) were then added. The reaction mixture was allowed to warm to room temperature and stirred overnight. DMF was then evaporated under vacuum, ethyl acetate (150 ml) was added to the residue. This ethyl acetate solution was washed subsequently with 0.5 N HCl, saturated sodium bicarbonate and brine. The ethyl acetate layer was then dried over $MgSO_4$. The product

was purified by flash chromatography on silica gel (EtOAC). A white solid identified as (1-Oxo-2, 3-dihydro-isoindol-2-yl)-2-(S)-isobutylacetyl-Asp(OBzl)-Phe-Morpholine (0.88 g, 1.32 mmol) was obtained. ^{1}H NMR ($CDCl_3$): δ 7.78 (d, 1H), 7.51-7.10 (m, 15H), 5.00-4.81 (m, 4H), 4.78-4.68 (m, 1H), 4.29 (dd, 2H), 3.55-3.25 (m, 4H), 3.22-3.10 (m, 1H), 2.95-2.72 (m, 5H), 2.71-2.60 (m, 1H), 1.88-1.75 (m, 2H), 1.55-1.40 (m, 1H), 0.82 (dd, 6H).

This benzyl ester (0.83 g, 1.24 mmol) was then subjected to hydrogenolysis under H_2 atmosphere in the presence of 10% Pd on carbon. A white solid which was characterized by ^{1}H NMR as (1-Oxo-2,3-dihydro-isoindol-2-yl)-2-(S)-isobutylacetyl-Asp-Phe-Morpholine (0.71 g, 1.23 mmol) was obtained. ^{1}H NMR (DMSO-d6): δ 8.33 (d, 1H), 7.97 (d, 1H), 7.58 (d, 1H), 7.51-7.00 (m, 8H), 4.87-4.68 (m, 2H), 4.62-4.22 (m, 3H), 3.42-2.25 (m, 12H), 1.71-1.48 (m, 2H), 1.29-1.10 (m, 1H), 0.80 (dd, 6H). MS m/z: 579 (MH^+).

Ester prodrugs of the compounds taught in the present application can be prepared by following standard esterification methods of carboxylic acids. For example, condensation of carboxylic acids with alcohols in the pressence of standard coupling reagents, such as, EDC/DMAP. Alternatively, ester prodrugs can be prepared via alkylation of acids with halides, in the presence of base, such as, triethylamine. These esterification methods are equally applicable for the preparation of alkyl, lower alkyl, branched lower alkyl and benzyl esters. In some instances, intermediates that are prepared in the course of synthesizing compounds of the present invention are already in the desired esterified form, e.g., benzyl ester. In these case, the intermediate ester prodrug is purified by column chromatography.

Example 1D: Synthsis of Phenylacetyl-N-Me-Leu-Asp(OCyclohexyl)-Phe-N-Me-Piperazine

To a solution of Phenylacetyl-N-Me-Leu-Asp-Phe-N-Me-Piperazine (150 mg, 0.24 mmol) and cyclohexanol (50 mg, 0.5 mmol) in dichloromethane (20 ml) was added EDC (100 mg, 0.5 mmol) and DMAP (20 mg, 0.16 mmol) and the resulting reaction mixture was stirred overnight. The reaction mixture was then washed with saturated $NaHCO_3$, H_2O, brine, and then dried over Na_2SO_4. The filtrate was evaporated and the residue was purified by prep-TLC plate with 10% $MeOH/CH_2Cl_2$ as the eluent to give Phenylacetyl-N-Me-Leu-Asp(Ocyclohexyl)-Phe-N-Me-Piperazine (90 mg). The purity was 98% determined by analytical HPLC (retention time 23.8 min, 30-65% B over 35 min) MS m/z: 690 (MH^+), 687 (MH^-).

Example 1E: Synthsis of Phenylacetyl-N-Me-Leu-Asp(OIsopropyl)-Phe-N-Me-Piperazine•HCl

To a solution of Phenylacetyl-N-Me-Leu-Asp-Phe-N-Me-Piperazine (1.0 g, 1.65 mmol) and isopropanol (10 ml, 130 mmol) in dichloromethane (50 ml) was added EDC (790 mg, 4.13 mmol) and DMAP (200 mg, 1.6 mmol). The resulting reaction mixture was stirred for two hours. The reaction mixture was then concentrated by evaporation. The residue was purified by flash chromatography on silica gel with 5% methanol in ethyl acetate as the eluent. The obtained pure isopropyl ester prodrug was dissolved in ethyl ether (15 ml). To this solution was added HCl in ethyl ether (1.0 M, 2 ml, 2 mmol) and a white precipitate was formed. The excess HCl and solvent were removed by evaporation. The product was then dried under vacuum for 2 days. Phenylacetyl-N-Me-Leu-

Asp(OIsopropyl)-Phe-N-Me-Piperazine.HCl (517 mg) was obtained. The purity was 98.9% determined by analytical HPLC (retention time 18.5 min, 5-90% B over 25 min). ^{1}H NMR ($CDCl_3$) δ 13.21-12.90 (s, br, 1H), 7.42-6.91 (m, 10H), 5.21-4.80 (m, 4H), 4.70-4.51 (m, 3H), 4.03-3.11 (m, 7H), 3.10-2.11 (m, 12H), 1.81-1.52 (m, 4H), 1.52-1.10 (m, 6H), 1.02-0.72 (m, 6H). MS m/z: 650(MH^+), 684 ($M-Cl^-$).

Example 2: Exemplary Solid Phase Compound Syntheses

Fmoc protected amino acids, hydroxybenzotriazole (HOBt) and Rink amide MBHA resin were obtained from Nova Biochem, La Jolla, CA. Diisopropylcarbodiimide (DIC) was obtained from Chem Impex Inc., Chicago, IL. Piperidine was obtained from Aldrich Chemical Company, St. Louis, MO. Dimethylformamide (DMF), isopropanol (IPA), dichloromethane (DCM), and dimethylacetamide (DMA) were obtained from Burdick and Jackson, Muskegon, MI. All of the above reagents were used as supplied by the manufacturer, with no further purification.

The standard deprotection/coupling cycle iterated during this synthesis is described in terms of the first coupling of Fmoc-Pro to the Rink amide MBHA resin:

The Fmoc-MBHA resin (10.6 g., 5 mmoles) was treated with 20 percent piperidine in DMF (130 ml) for three minutes. The solution was removed by filtration and the resin was again treated with 20 percent piperidine in DMF (130 ml) for 17 minutes. The solution was removed by filtration and the resin was washed five times with DMF (130 ml each), two times with IPA (130 ml) and two times with DMF (130

ml). The HOBt ester of Fmoc-D-proline (formed by reacting a solution of 10 mmoles Fmoc-D-proline and 10 mmoles HOBt in 50 ml DMA with 12 mmoles DIC for 20 minutes at room temperature), in DMA (50 ml), was added to the resin and allowed to react for two hours. The resin was washed five times with DMF (130 ml) and two times with DCM (130 ml). The coupling of amino acid to the resin was checked by standard Kaiser's test.

The above cycle was iterated for each of the subsequent amino acids: Fmoc-Phe, Fmoc-Asp(β-ON), Fmoc-Leu, and phenylacetic acid. The resin was dried in vacuo for 24 hours and then allowed to react with 95 percent TFA/5 percent H_2O (60 ml) for two hours at room temperature. The TFA solution of the compound was separated from the resin by filtration and the TFA was vacuum evaporated. The solid residue was crystallized from anhydrous ethanol to yield 1.8 g of product, Compound ID No. 896.52, N-phenylacetyl-Leu-Asp-Phe-D-Pro-NH_2. The compound was characterized by amino acid analysis on HP Amino Quant 1090 and NMR, and the purity of the compound was checked by HPLC (WATER HPLC Systems).

Example 3: <u>In Vitro Binding Assays</u>

Jurkat cells (ATCC TIB 152), a human T lymphoblastic line, labeled with ^{51}chromium were used to assay <u>in vitro</u> binding inhibition provided by various compounds discussed herein. Costar™ 96 well flat-bottom microtiter plates (catalog No. 9050, Cambridge, MA) were found to provide the best results in these assays.

The plates were prepared as follows: The 25-mer CS-1 compound (SEQ ID NO:1) dissolved at 0.5-1

μg/ml in a buffer of 0.1M $NaHCO_3$ at pH 9.5 that also contained 10 μg/ml of bovine serum albumin (BSA) or a conjugate of the CS-1 compound linked to ovalbumin

(CS-1-OVA) dissolved at 1-2.5 μg/ml in the same buffer was used as the substrate. Each well of the microtiter plates was coated with 50 μl of substrate or buffer alone for controls. The wells were permitted to dry out completely and were then rinsed twice with PBS at pH 7.4. Non-specific binding sites of each well were then blocked using 200 μl per well of RPMI/1 percent BSA for two hours at room temperature. Both solid phase-affixed substrates provided similar results.

Jurkat cells (3-5X10^6 cells) were placed into a 15 ml Falcon™ round-bottom tube with a cap. The tube was centrifuged, and the extra medium was then removed.

Two hundred microliters of a ^{51}Cr labeling solution were added to the centrifuged cells and maintained in contact with the cells for 90-120 minutes in a warm room. This procedure typically provides about 50,000-100,000 cpm/well with about 80-100 percent cell viability. Longer contact times provide a greater amount of labeling but lower cell viability.

The labeled cells are washed with (i) complete medium, (ii) 1mM EDTA/PBS and then (iii) RPM1/1 percent BSA free of serum components. The cells are centrifuged after each washing. The cells are finally resuspended in serum-free RPMI/1 percent BSA at a concentration of 1X10^6 viable cells/ml, which provides a concentration that is diluted by one-half in the assay.

Inhibitor compounds are prepared as stock solutions at 20 mg/ml in DMSO in 1.5 ml cryogenic screwcap vials, and were stored at -70°C. Using Flow™ round-bottom or V-bottom microtiter plates, the inhibitor compounds were prepared at twice the assay concentration in RPMI/1 percent BSA at 60 µl/well.

Four initial dilutions were typically used. For less potent compounds such as the standard 10-mer of SEQ ID NO:3, the initial dilutions were 500 µg/ml, 100 µg/ml, 20 µg/ml and 4 µg/ml. For more potent compounds such as N-phenylacetyl-Leu-Asp-Phe-D-Pro-NH_2, the typical initial concentrations were 10 µg/ml, 2 µg/ml, 0.4 µg/ml and 0.08 µg/ml.

The ^{51}Cr-labeled cells ($1X10^6$ cells at 60 µl/well) were then admixed with the diluted compound solutions. The admixtures were maintained at room temperature (about 22°C) for 30 minutes.

One hundred microliters of each inhibitor compound/cell admixture were transferred to the substrate-coated wells. This was done in triplicate for each dilution. The resulting plates were incubated for 30 minutes at 37°C and then washed gently three times with RPMI/1 percent BSA at 200 µl/well. Binding was observed microscopically, particularly after the second wash.

The bound cells were then lysed by the addition of a 0.5 percent solution of sodium dodecylsulfate in water at 100 µl/well. The resulting solutions were then processed for counting and calculation of IC_{50} values following usual procedures. Appropriate positive and negative controls were used with each plate so that the

results of separate assays could be normalized and compared.

The data of Table 1 are normalized to the binding of SEQ ID NO: 3. The absolute IC_{50} value for the compound ID No. 1051.01, N-phenylacetyl-Leu-Asp-Phe-morpholinamide, is approximately 0.18-0.30 μM. Multiple assays for the same compound were averaged.

Example 4: Delayed Type Hypersensitivity in Mice

A. Initial Study

Chisholm et al., Eur. J. Immunol., 23: 682-688 (1993) reported in vivo results of blocking VLA-4 interactions in a murine contact hypersensitivity model using rat anti-mouse α-4-antibodies. Those workers noted that therapeutics designed to interfere with VLA-4-mediated adhesion should be effective blockers of in vivo inflammatory processes.

An adoptive transfer delayed-type hypersensitivity murine model has been developed using splenic T cells primed to oxazolone. This model is described in Elices et al., Clin. Exp. Rheum., 11(Suppl. 8):577-580 (1993), whose procedures were followed here.

Thus, BALB/c mice were shaved on the belly and painted (50 μl on the belly and 5 μl on each paw) with three percent oxazolone in acetone/olive oil (4:1) at days zero and 1. At day 5, the mice were sacrificed, their spleens removed, and splenic T cells were obtained via nylon wool columns.

Normal saline or saline containing $25X10^6$/animal of the oxazolone-immune T cells were separately injected into naive mice. The mice were then challenged by painting 10 μl of 2 percent oxazolone onto one ear each. All procedures were carried out under sterile conditions and in endotoxin-free buffers.

Prior to challenge or saline injection, the mice were implanted with pumps that subcutaneously administered normal saline, normal saline containing the compound, Compound ID No. 896.52, N-phenylacetyl-Leu-Asp-Phe-D-Pro-NH_2, or normal saline containing a compound with a scrambled sequence continually at 6 mg/kg/day for a 24-hour time period. The swelling diameter at the site of challenge or saline injection was measured with a microcaliper 24 hours thereafter.

The results of this study are shown in the graph of Fig. 5 for the saline and recited inhibitor compound treatments. Although there is a slight overlap in the data possibly due to non-CS-1-mediated inflammation, it is clear that administration of a contemplated inhibitor compound reduced this type of CS-1/VLA-4-mediated immunoinflammation as compared to the untreated controls. Use of the control compound provided no reduction of inflammation.

B. Expanded Study

An expanded study was carried out as described above except that a scrambled compound control was not used as a control for further inhibitor compounds of Table 1 with larger groups of mice. Statistical analyses were carried out versus injection of vehicle alone. As shown below in Table

6, inhibitor compounds resulted in a statistically significant reduction in post-challenge swelling.

Table 6

Compound ID No. Compound n	Percent Inhibition	p Values
896.52 ΦAc-Leu-Asp-Phe-D-Pro-NH_2 30	36	0.0002
1070.02 ΦAc-Leu-Asp-Phe-4-hydroxpiperidinamide 8	29	0.015
1111.06 ΦAc-Leu-Asp-Phe-4-N(carboxymethyl)piperazinamide 24	30	<0.0001

* ΦAc = N-phenylacetyl.

The transferred T cell is the effector cell in the adoptive immune response examined here. T cells express both VLA-4 and VLA-5 that interact with the CS-1- and RGD-containing portions of fibronectin, respectively, during an inflammatory immune response. Ferguson et al., Proc. Natl. Acad. Sci., USA, 88: 8072-8076 (1991) showed that about 50 μg/ml of the standard compound used here (SEQ ID NO:3) when admixed with immune T cells could abrogate a transferred immune response such as the response studied here. Those workers also taught that separate administration of the compound and T cells did not lead to that abrogation.

Here, it is seen that separate administration of 6 μg/ml of a contemplated inhibitor compound provided substantial inhibition of the immune response. It is also seen that the inhibitor compound and T cells need not be premixed here as

they were required to be in the Ferguson et al. results.

Example 5: Treatment of Asthmatic Rabbits

Six New Zealand white rabbits were immunized with house dust mite antigen from birth through four months of age. Upon immunization, three rabbits received a single nebulizer administration of the inhibitor compound, Compound ID No. 1051.01, N-phenylacetyl-Leu-Asp-Phe-morpholinamide in aqueous 50 percent ethanol as diluent in an amount of 100 mg/kg, and the other three received diluent alone. All of the rabbits were challenged with house dust mite antigen about 15-30 minutes after administration of the compound, with those animals not receiving compound serving as controls.

Once immunized and challenged, the inflammatory state subsides to a basal level within about three weeks. The three animals used as controls were thereafter used as subjects for receipt of an inhibitor compound, and the three rabbits that initially received the compound can serve as controls.

Such a crossover study was done here. Thus, the three initial control rabbits were treated with the above inhibitor compound in the above diluent at a time more than three weeks after the above study, and the three previous recipients of the compound were administered the diluent alone. All six were than challenged again.

Initial pulmonary function, measured by dynamic compliance (C_{dyn}) and lung resistance (R_L), and bronchoalveolar lavage (BAL) to obtain an effector

cell count, here eosinophils, were conducted prior to administration of the compound or diluent for both portions of this crossover study. Similar assays were then taken one-half hourly after challenge for six hours (early phase allergic reaction) and at 24 hours after challenge (late stage allergic reaction) for both portions of this study.

These studies were conducted as described by W.J. Metzger in CRC Handbook of Late Phase Reactions, W. Dorsch, ed., Chapter 35, CRC Press, Boca Raton, FL (1990) pages 347-362.

The results of this study for the pulmonary function parameters are shown in Fig. 4A and Fig. 4B, in which data for the challenged, inhibitor compound-treated animals are shown as open circles and data for the challenged, untreated, control animals are shown in blackened circles. These data are averaged values from both portions of the study.

As is seen from Fig. 4A, the C_{dyn} value for the challenged and treated animals stayed at about the initial value for the whole six hours. The C_{dyn} for the challenged, untreated animals quickly fell to about 40 percent of the initial value and then stayed at about that value for the whole six hours.

The data of Fig. 4B show that the R_L values for the challenged, inhibitor compound-treated animals remained between the initial value and about 200 percent of that value for the whole six hours, with a slight rise near the end of that time period. The R_L values for the challenged, but untreated animals rose to about 200-300 percent in the first two hours after challenge and rose to about 400-1200 percent for the last four hours.

A summation of the averaged data for the inhibitor-treated, challenged animals compared to the challenged control animals for early (2-4 hours) and late (24 hours) phases of this inflammatory immune response is provided in Table 7, below.

Table 7

IN VIVO Efficacy of Compound ID No. 1051.01

(ΦAc-Leu-Asp-Phe-Morph*)

Parameter	Phase	% Reduction
C_{dyn}	Early	94.4
	Late	86.6
R_L	Early	80.1
	Late	82.6

*Phenylacetyl-Leu-Asp-Phe-morpholinamide

The BAL count from these studies indicated an 88.1 percent reduction in eosinophils after 24 hours in the inhibitor compound-treated, challenged animals as compared to the untreated, challenged animals in the crossover study.

As can be seen from the above data and those of Figs. 4A and 4B, aerosol administration of an inflammation-reducing amount of a contemplated compound greatly reduced the asthmatic response in the treated animals as compared to those receiving no treatments.

Example 6: Rabbit Cardiac Allograft Model

New Zealand white rabbit SPF hearts were allografted into the necks of similar rabbits to assay a graft-vs-host immunorejection model and the affect of a contemplated compound on that immunoinflammatory response.

Experimental animal model

New Zealand white female rabbits (Charles River Lab., Saint Laurent, Quebec), between 3.5 and 4 kg underwent heterotopic cardiac transplant following an experimental protocol previously described [Alonso et al., Am. J. Pathol., 87:415-442 (1977); Clausell et al., Circulation, 89:2768-2779 (1994)]. The animals were unselected to favor an HLA-mismatch, the host rabbits were Pasteurella-free and the donors, outbred animals. Both host and donor rabbits were fed Purina 5321-0.5 percent cholesterol diet (Research Diets Inc., New Brunswick, NJ), a strategy that has proven useful in accelerating the process of allograft arteriopathy [Alonso et al., Am. J. Pathol., 87:415-442 (1977)]. The diet was commenced four days prior to the transplant and continued in the recipient of the transplant until the completion of the experimental period.

The technique of heterotopic cardiac transplantation has been previously described [Clausell et al., Circulation, 89:2768-2779 (1994)]. Briefly, a vertical incision was performed in the anterior aspect of the neck of the recipient rabbit and the left common carotid artery and the ipsilateral external jugular vein were isolated. The cardiac allograft was placed in the neck by anastomosing the aorta end-to-side to the recipient's carotid artery and the pulmonary end-to-side to the recipient's external jugular vein, following a total period of ischemia for the donor hearts of approximately 30 minutes. Postoperative care was in compliance with the Principles of Laboratory Animal Care formulated by the Canadian National Society for Medical Research.

The treatment consisted of an inhibitor compound, Compound ID No. 896.52, N-phenylacetyl-Leu-Asp-Phe-D-Pro-NH_2 (treated), derived from the leucine-aspartate-valine (LDV) sequence [Komoriya et al., J. Biol. Chem., 266:15075-15079 (1991; Wayner et al. J. Cell Biol., 116:489-497 (1992)], that enhanced inhibition in vitro here, and a scrambled form of the same synthetic compound, N-phenylacetyl-Asp-Leu-Phe-D-Pro-NH_2 (control), resulting in no inhibition of VLA-4. Both compounds were synthesized at Cytel Corporation, San Diego, CA.

Beginning the day of the transplant, the animals were randomized and treated with either scrambled compound (control group) at 1 mg/kg s.c. or the inhibitor compound at 1 mg/kg s.c. The doses of the compounds were empirically extrapolated to the in vivo model based on preliminary in vitro studies, of Table 1. No other immunosuppression therapy was administered. The grafts were monitored daily by palpation and maintained for 7 to 8 days, a previously described endpoint that was associated with myocardial rejection (impaired cardiac contractility) and development of the allograft arteriopathy in this model [Clausell et al., Circulation, 89:2768-2779 (1994)]. A total of fourteen animals were studied in the control (n=7) and treated (n=7) groups.

Preparation of the hearts

The animals were sacrificed using a lethal dose of euthanol (480 mg i.v.) (MTC Pharmaceutical, Cambridge, ON), host and donor hearts were removed, and the coronary arteries were perfused with saline through the aorta, followed by light fixation by perfusion with 2 percent paraformaldehyde (Sigma, St.

Louis, MO). Because of previous descriptions indicating that cardiac allograft arteriopathy in the rabbit was equally distributed throughout the coronary circulation [Foegh et al., Transplant Proc., 21:3674-3676 (1989)] and those of Dr. Rabinovitch and co-workers [Clausell et al., Circulation, 89:2668-2779 (1994)], the hearts were sectioned transversely from base to apex. Different sections of the hearts were either saved in 10 percent formalin (BDH Inc., Toronto, ON) for light microscopy studies or immediately frozen in O.T.C. Compound Tissue Tek (Miles Inc., Elkart, IN) for specific immunohistochemistry studies.

Grading of rejection

Tissue specimens from the donor hearts were stained with hematoxylin:eosin for histological grading of rejection according to a modified Billingham's criteria [Billingham, Hum. Pathol., 10:367-386 (1979)]. The sections were graded by a pathologist without knowledge of whether the donor hearts came from control or inhibitor compound treated animals.

Quantitative assessment of host and donor coronary arteries by light microscopy

Three different paraffin-embedded tissue sections from host and donor hearts from both control and treated rabbits were stained by the Movat pentachrome method for light microscopy. Morphometric analysis was performed using a Zeiss microscope attached to a computer-generated video analysis system (Perceptics Inc., NuVision software), as described in Clausell et al., Circulation, 89:2768-2779 (1994). The number of vessels with intimal lesions were counted in all three heart

sections from each animal studied and are expressed as a percentage of the total vessel number.

In the host hearts, 999 vessels in the control group and 1054 vessels in the treated group were analyzed. In the donor hearts, 827 vessels in the control group and 617 vessels in the treated group were analyzed. To determine the severity of intimal thickening, the diameter of each traceable vessel in all three sections was measured and the coronary arteries were categorized as small (diameter <100μm), medium (diameter >100<500μm) and large (diameter >500μm). The degree of intimal thickening was then quantitatively assessed in each vessel size category as previously described in Eich et al., Circulation, 87:261-269 (1993). The areas encompassed by the outer medial layer (ML), the internal elastic lamina (IEL) and lumen were measured in each affected vessel, and the area of intimal thickening (IT) related to the vessel area was calculated by the formula IT=IEL-lumen area/ML-lumen area X 100.

Immunohistochemistry studies

In all immunohistochemistry analyses, coronary arteries from host and donor hearts were compared, with and without intimal thickening in the different size ranges from control and treated groups. The relative abundance of each specific antigen studied in the sections examined was graded semi-quantitatively as minimal (+/-), little (+), moderately abundant (++) to very abundant (+++) two investigators. The final scoring was based on individual gradings that reached 90 percent agreement.

(1) Characterization of inflammatory cells

To characterize the presence of an immune-inflammatory reaction in the allograft coronary arteries in both groups studied, immnunoperoxidase staining was performed using monoclonal antibodies to rabbit MHC Class II antigens and rabbit T cells (from Dr. Peter Libby, Brigham and Woman's Hospital, Boston, MA) and also to rabbit macrophages (RAM 11, Dako Corp., Carpinteria, CA). The sections were air-dried for two hours, fixed in acetone for 20 minutes, and rinsed with D-PBS (Gibco, Burlington, ON) /0.1 percent BSA (Boehringer-Mannheim, Mannheim, Germany). Endogenous peroxidase activity was blocked by immersing the sections in PBS/0.1 percent BSA + 3 percent hydrogen peroxide (BDH) for 30 minutes. After a non-specific blocking step using 10 percent normal goat serum (Sigma), the antibodies were applied to the sections for 1 hour at a 1:10 dilution at room temperature. The sections were then rinsed, incubated with goat anti-mouse peroxidase-conjugated secondary antibody (Bio-Rad, Richmond, CA) at a 1:50 dilution at room temperature for 45 minutes and developed with 3,3'-diaminobenzidine (DAB) (Sigma) for 10 minutes. Control sections were treated with normal mouse isotypic IgG (Dako Corp.).

(2) Immune-detection of cellular adhesion molecules

To assess the influence of treatment on the expression of adhesion molecules in allograft coronary arteries, immunoperoxidase staining for ICAM-1 and VCAM-1 was performed on frozen sections of both host and donor hearts from the control and the treated groups. Monoclonal antibodies to ICAM-1 (mAb Rb2/3) and to VCAM-1 (mAb Rb1/9) (from Dr. Myron

Cybulsky of Brigham and Women's Hospital, Boston, MA) and were used at a concentration of 1:10 for 1 hour at room temperature. The procedure for immunostaining was essentially the same as described above.

(3) Assessment of fibronectin

Fibronectin expression in coronary arteries of host and donor hearts from both control and treated groups was determined by performing immunoperoxidase staining using frozen sections. A monoclonal antibody anti-cellular fibronectin (Chemicon Int. Inc., Temecula, CA) was used at a dilution of 1:100 for 1 hour at room temperature and the remaining details of the immunohistochemical procedure are essentially the same as described above. This antibody does not recognize plasma fibronectin.

Statistical Analysis

The data were expressed as mean +/-SE. In analyses related to the incidence and severity of lesions from both control and treated groups, the Student's t test was used to test significance. The correlation among categorical variables from the immunohistochemistry studies, considered positive if > + in the two groups (control and treated), was analyzed using Fisher's exact test. Differences were considered significant if $p<0.05$.

The results of the above studies are summarized in Table 8, below.

Table 8

Expression on Coronary Arteries	Inhibitor Compound	Control Compound
MHC Class II	±[c]	++
T cells	±	++
Macrophages	±	+
ICAM-1	±	+
VCAM-1	±	+
Total Fibronectin	±	++
Vessel Intimal Thickening		
Percent of Vessels[a]	35[d]	88
Severity (Percent of Vessel area)[b]	16[e]	36

[a] Baseline (rabbit own host heart) of incidence was 12 percent and 10 percent, respectively, for inhibitor andcontrol compound groups.

[b] Baseline (rabbit own host heart) of severity was 12 percent and 12 percent, respectively, for inhibitor and control compound groups.

[c] Scoring was: -, negative; ±, minimal; +, little; ++, moderately abundant; +++, very abundant.

[d] $p<0.001$.

[e] $p<0.001$.

As is seen from the above results, use of a contemplated inhibitor compound greatly reduced the inflammation-induced damage observed in the allografted hearts. These damage reductions are particularly evident in the vessel intimal thickening results, but are also seen less directly in the

results relating to expressed inflammatory markers shown by the MHC Class II antigen, the increased presence of T cells and macrophages, and the total fibronectin.

Example 7: In Vitro Porcine Allograft Model

A similar study was carried out in vitro using porcine coronary artery endothelial cells (EC; as are present in the IEL) and smooth muscle cells (SMC; as are present in the medial layer of the artery). The two cell types were cultured using a membrane transwell system, with the SMC on the bottom layer in M-199 medium (Gibco Labs.). The SMC were stimulated with 100 ng/ml of interleukin-1β (IL-1β) for 24 hours prior to the start of the assay. Porcine peripheral blood lymphocytes were separated by Ficoll-Hypaque, radiolabeled and incubated overnight (about 18 hours) on the EC.

Transendothelial lymphocyte migration in the IL-1β-stimulated SMC was observed as compared to unstimulated SMC ($p<0.05$). The inhibitor compound of Example 6, Compound ID No. 896.52, phenylacetyl-Leu-Asp-Phe-D-Pro-NH_2, present at 10 μg/ml in the medium reduced lymphocyte migration by about 30 percent ($p<0.05$), whereas the same amount of a control compound whose sequence was scrambled did not reduce migration.

Increased expression of EC and SMC fibronectin and IL-1β are features of an immunoinflammatory response associated with accelerated graft arteriopathy following piglet heterotopic cardiac transplantation. The above results indicate that IL-1β induces fibronectin production in this in vitro model, which in turn

contributes to transendothelial lymphocyte migration. The above results also illustrate that a contemplated inhibitor compound can be used to reduce this immunoinflammatory response.

Example 8: Experimental Autoimmune Encephalomyetlitis in Mice

Experimental autoimmune encephalomyelitis (EAE) is a demyelinating disease of the central nervous system that can be induced in susceptible strains of mice and rats by immunization with myelin basic protein, proteolipid protein (PLP), or their immunodominant T cell determinants, or by injection of CD4-positive T cell clones specific for those determinants. EAE serves as an animal model of human multiple sclerosis. In both diseases, circulating leukocytes such as T cells and monocytes penetrate the blood/brain barrier and damage myelin, resulting in paralysis.

EAE was induced in female SJL/J mice (8 to 14 weeks old) by immunization on day zero with 50 μg of a compound corresponding to positions 139-151 of PLP emulsified in a 1:1 mixture of PBS and complete Freund's adjuvant (CFA). Each mouse was injected with 0.2 ml of the adjuvant emulsion subcutaneously (s.c.) at two sites in the hind flank. All mice received 10^7 killed Bordetella pertussis units in 100 μl were injected intravenously 24 to 72 hours later.

Mice were observed daily, beginning at day 8 for clinical signs of EAE, and disease was scored on a scale of 0-5 as: 0 = no disease; 1 = floppy tail; 2 = moderate hind limb weakness; 3 = paraparesis; 4 = paraplegis with moderate forelimb weakness; 5 = qualdriplegis or premoribund state.

The inhibitor compound, Compound ID No. 896.52, N-phenylacetyl-Leu-Asp-Phe-D-Pro-NH_2 was administered intraperitoneally at 1 mg/mouse in 0.2 ml of incomplete Freund's adjuvant at days 8 and 9. A compound having a scrambled sequence [N-phenylacetyl-Asp-Leu-Phe-D-Pro-NH_2] was similarly administered to serve as a control. The relative potency of this control compound is shown in Table 1 to be 0.

Summed or averaged scores for clinical signs were plotted vs. time. The area under the resulting curves was calculated between day 8 and day 35 to calculate percentage inhibition of EAE by an inhibitor compound. The percent inhibition was calculated as follows:

$$\% \text{ Inhibition} = 100 - \frac{(\text{Area of inhibitor compound})}{(\text{control area})} \times 100$$

Two exemplary plots through day 31 are shown in the graph of Fig. 6 in which the darkened circles are averaged scores for six mice treated with the inhibitor compound and darkened squares are averaged scores for six mice that received the scrambled sequence control compound. As can be seen, animals treated with an inhibitor compound contemplated herein exhibited marked improvement in clinical signs as compared to those animals treated with the control compound.

Example 9: CS-1 Expression in Human Rheumatoid Arthritis

Surgically-obtained synovial specimens from human rheumatoid arthritis (RA) patients were examined microscopically for the expression of the CS-1 compound portion of fibronectin. Ultrathin

sections of tissue were stained by the immunoperoxidase technique using anti-CS-1 antibodies, and were studied using transmission electron microscopy. These studies showed that CS-1 was expressed on the lumenal aspect of blood vessel endothelium, on the lumenal plasma membrane. The plasma membrane of synoviocytes in the synovial intimal lining at the interface with the joint space was also stained. The CS-1 compound portion was not found to be expressed in normal synovium.

Binding studies were carried out using the Jurkat T cell line and frozen RA synovial sections. Jurkat cell adhesion could be inhibited by anti-VLA-4 antibodies or the 10-mer CS-1 compound portion (500 μg/ml) used as standard here (SEQ ID NO:3), but not with antibodies to VLA-5, VCAM-1-A or VCAM-1-B or a compound in which the 10-mer sequence was scrambled. Stimulated MOLT-4 cells behaved similarly. These results are reported in Elices et al., J. Clin. Invest., 93:405-416 (January 1994).

A similar inhibition of binding of Jurkat cells to human RA synovial sections and not to normal synovial sections was observed using the inhibitor compound, Compound ID No. 896.52, N-phenylacetyl-Leu-Asp-Phe-D-Pro-NH_2. That compound was used at its IC_{50} value shown in Table 1 to be about 312 times less than the IC_{50} value for the standard 10-mer. The absolute value of that IC_{50} value is about 0.5 μmolar.

These results illustrate the importance of the CS-1 compound portion and VLA-4 in a human chronic immunoinflammatory disease state, rheumatoid arthritis. These results also show that a contemplated inhibitor cell can inhibit the binding

of inflammatory cells in this human immunoinflammatory disease state.

Example 10: Treatment of Asthmatic Sheep

Six asthmatic sheep were treated in a double blind cross-over study. The sheep had been shown to develop both early and late bronchial responses to inhaled Ascaris suum antigen. This study was carried out generally as described in Abraham et al., J. Clin. Invest., 93:776-787 (1994).

The inhibitor compound used here was Compound ID No. 896.52, N-phenylacetyl-Leu-Asp-Phe-D-Pro-NH_2, with a control compound that comprised the previously described compound having the same residues in a scrambled sequence. The vehicle for the nebulized compounds was phosphate-buffered saline. The compounds were administered at a dose of 1 mg/kg each, twice a day for three days prior to challenge, as well as 0.5 hours prior to and four hours post challenge on day 4. Because this was a crossover study, each animal received one or the other treatment, followed by a rest and then the other treatment. Each animal therefore served as its own control.

As can be seen from the graph of Fig. 7, animals treated with the inhibitor compound exhibited less of a change from baseline specific lung resistance (SR_L) on challenge and then more rapidly returned to their baseline pulmonary functions than did animals treated with the control compound. In addition, pulmonary function remained at about baseline values from about 3-4 hours after challenge through the end of the study (8 hours post challenge), whereas the control compound-treated

animals had an increase in that pulmonary function (SR_L).

Post challenge airway responsiveness was also assayed. Here, specific lung resistance, SR_L, returned to baseline values 24 hours after challenge, however, the sheep were hyperresponsive to inhaled carbachol at that time. A comparison of PC_{400} values on carbachol inhalation prior to and 24 hours after challenge indicated that a much smaller dosage of carbachol [about 12 breath units (BU)] was required to increase the SR_L value for control compound-treated animals 4-fold over a saline control value, as compared to the amount required for the inhibitor compound-treated group (about 27 BU). The pre-challenge values here were about 20-23 BU, so that the inhibitor compound caused the animals' SR_L to be greater than pre-challenge values, indicating a lessened response to carbacol than the pre-challenge response.

Example 11: Determination of Proteolytic Cleavage

Examples 1A, 1B, 1D, and 1E are directed to the preparation of representative compounds of the present invention where R_2 is methyl. Methyl substituted at R_2 is of particular interest, because it inhibits the metabolic degradation of compounds of the present invention, and it was found that methyl substitution at the other two possible amino functionalities (i.e., R_4 and R_5) resulted in compounds exhibiting lesser potency.

Preparation of Intestinal Homogenates

An *in vitro* assay was developed using isolated mice intestinal homogenates to measure proteolysis.

Female BALB/cByJ (20-25 grams, ca.10 weeks old) were euthanized by cervical dislocation. The inferior vena cava was cut to allow blood drainage. For liver and intestinal tissues, an incision was made through the left ventricle and cannulated and the circulatory system was flushed with 20mL of PBS containing 40µl/mL heparin. All organs were visually monitored for bleaching to ensure the removal of contaminating blood. Mouse lungs were collected from separate animals which were cannulated through an incision in the right ventricle and perfused with 5mL of PBS/heparin. Livers, lungs, and intestines (from the gut to the caecum) were dissected out and away from as much non-target tissue as possible. The tissue samples were placed in clean tubes, suspended in ice cold PBS to 100mg tissue/mL PBS, minced with scissors, and homogenized at 4°C in Tenbroeck tissue grinders (Kimble-Kontes #885000). The ground suspensions were then filtered through 70µm nylon cell strainers (Becton Dickinson #2350), divided into 1 mL aliquots and frozen at -70°C. Just prior to assay, the liver and lung homogenates were thawed out and prepared by diluting 1:4 PBS:homogenate. Final tissue concentration was 80mg/mL in PBS. For Intestinal experiments, the diluent was 500mM MES pH6, (final concentration 100mM). pH6 was chosen for the intestinal system in order to more closely mimic the analogous *in vivo* environment. To estimate acid

stability for prodrugs intended for oral gavage, test articles were incubated in 10mM HCL.

Stability/metabolism assays:

The assays are formatted such that the VLA-4 inhibitor of interest, either active or prodrug form, is dissolved in DMSO to a stock 100x concentration of 20mM, and then spiked and vortexed into the prepared tissue homogenates at time zero (C_0=200μM). A t=0 timepoint is immediately taken by removing 100μL of the assay mixture and quenching into an equal volume of 64% acetonitrile in water and freezing on dry ice. Subsequent timepoints are sampled to establish a kinetic profile of decomposition or metabolism. Optimal timecourses for the various assays depend on the compound tested. For active principal analysis, timepoints are collected in a 0 to 24 hours window in plasma, liver and lung homogenates, and at shortened (6-8 hr.) times for intestinal proteolysis determination. Prodrugs are tested on hourly time scales for acid and intestinal exposures in order to simulate expected residence times in the digestive tract, but require minute timescale sampling for liver and plasma metabolism in order to calculate a linear initial esterase activity rate for the conversion of the prodrug to the active form. Stability for the resultant active principle can then be inferred from the 24 hour liver and plasma assays run on the appropriate parent compound.

In order to differentiate tissue-dependent metabolic events from spontaneous degradation of the autosampler-vialed HPLC samples, previously

chromatogramed samples were re-injected to compare peak signals to the original analyses. The first experiment showed that after being stored in the autosampler (15°C) for ca. 48hr., there was no change in the sample composition. A similar determination after 4 days storage at 10°C also showed that the metabolism samples were stable in the autosampler for a time period significantly longer than the duration of the analysis period. These experiments also demonstrate the effectiveness of acetonitrile quenching of the metabolism samles in that no further esterase cleavage of the prodrugs tested occurred after the samples were quenched and prepared for the HPLC.

Assaying representative compounds of the invention where R_2 is H or methyl demonstrated that the presence of a methyl group at R_2 inhibited proteolysis.

Example 12: Mouse Eosinophilia Model

BALB/c mice were sensitized with ovalbumin complexed with aluminum hydroxide in saline by intraperitoneal injection on days 0, 7 and 14. On day 21, the mice were challenged with ovalbumin administered via an aerosol in saline. Five minutes prior to this challenge, the mice were dosed intranasally either with (a) compound ID no. 1070.12, as listed in Table 1 above (Phenylacetyl-N-Me-Leu-Asp-Phe-N-Me-piperazine); or with (b) phosphate-buffered saline (vehicle) as control.

Bronchoalveolar lavage fluid collection.

After exposing and cannulating mice tracheae following euthanasia, lungs were lavaged twice with 0.5 ml of Hanks' buffered salt solution containing 0.1% EDTA (HBSS/EDTA). Bronchoalveolar lavage fluid was recovered after 30 seconds, pooled for each mouse and centrifuged at 2,000 rpm for 15 minutes at 5°C. The resulting cell pellets were resuspended in 0.5 ml HBSS/EDTA to determine white cell count, while the cell supernatants were frozen in aliquots to determine mediator concentration.

Mediator concentration was measured at 24 hours and 72 hours post-challenge using ELISA kits containing antibodies specific to murine IL-4, IL-5 and IL-12 (all from Endogen, Woburn, MA), as well as eotaxin (R&D Systems, Minneapolis, MN). The limit of detection was about 5-10 pg/ml.

Table 9 below lists the concentration of cytokine (IL-4, IL-5 and IL-12) and chemokine (eotaxin) mediators in bronchoalveolar lavage fluid, as well as eosinophil (EOS) and total white blood cell (WBC) count following ovalbumin challenge.

Table 9:

	24 hours		72 hours	
	Vehicle	CY-9701	Vehicle	CY-9701
IL-4(pg/ml)	1197±71	724±175*	134±13.6	33.8±2.3*
IL-5 (pg/ml)	290±49	144±35*	28.7±1.9	7.6±0.46*
IL-12 (pg/ml)	367±61	400±11.7	131±10.7	185±14.3*
Eotaxin (pg/ml)	63.4±7.4	21.0±7.4*	ND#	ND#
EOS (10^6/ml)	0.07±0.01	0.03±0.01*	0.24±0.05	0.01±0.002*
WBC (10^6/ml)	0.65±0.09	0.079±0.09	1.03±0.10	0.81±0.05

* Statistically significant difference with respect to vehicle control ($p<0.05$).
\# Not detectable

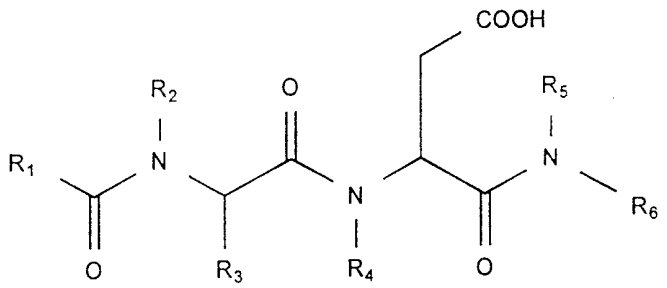
As shown in Table 9 above, for mice treated with compound ID no. 1070.12, concentrations of IL-4 and IL-5 (i.e., T_H2 cytokines) are decreased at both the 24 hour and 72 hour marks compared to the concentrations in mice treated with vehicle. Similarly, the eosinophil chemoattractant eotaxin also decreased at the 24 hour mark in mice treated with compound ID no. 1070.12 compared to the concentration in mice treated with vehicle. In contrast, IL-12 (i.e., a T_H1 cytokine) was elevated at 72 hours compared to the concentration in mice treated with vehicle, indicating a shift in the T_H1/T_H2 balance in the lung at this time.

Thus, the above example demonstrates that administration of compound ID no. 1070.12 decreases the amount of T_H2 allergic mediators in bronchoalveolar lavage fluid and causes a profound reduction of lung eosinophilia in murine model of allergic asthma.

Although the present invention has now been described in terms of certain preferred embodiments, and exemplified with respect thereto, one skilled in the art will readily appreciate that various modifications, changes, omissions and substitutions may be made without departing from the spirit thereof.

WE CLAIM:

1. A compound of the formula,



wherein:

R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl; the R_1 ring structure can form at R_1, between R_1 and R_2 or between R_1 and R_4 with the proviso that, if the R_1 ring structure forms at R_1, the R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups; the spacer can be optionally substituted by an amino group; the R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein, the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups; the R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms, and, if the R_1 ring structure is formed between R_1 and R_4, the heteroatoms are 2 nitrogen atoms; the R_1 ring structure can be conjugated, partially saturated, or saturated; the

lower alkyl or lower amino alkyl group can be branched;

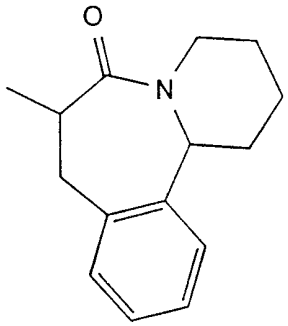
R_2 is a H, lower alkyl, phenyl lower alkyl, or R_2 and R_1 form the R_1 ring structure group;

R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl, or di(lower alkyl)thioether; the R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long; the lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched;

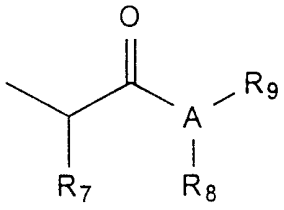
R_4 is a H or R_4 and R_1 form the R_1 ring structure;

R_5 is H or R_5 and R_6 form a R_5 ring structure; the R_5 ring structure is a fused 6,6- ring structure and can be aromatic, partially saturated, or saturated;

R_6 is a benzyl, a 5,6,or 7-membered heterocyclic saturated ring containing 1 or 2 nitrogen atoms optionally substituted by one or more lower alkyl, lower alkyl amide or acyl groups or 1,1 diphenylmethine group, the R_5 ring structure, a group of the formula



or a group of the formula



wherein:

A is nitrogen or oxygen;

when A is nitrogen;

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group; the R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long; if the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused 6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein, the heteroatom is a nitrogen atom; if the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein, the heteroatoms are 1 or 2 nitrogen atoms; the R_7 ring structure can optionally be substituted by an alcohol, nitro, or lower alkyl ether group;

R_8 is a ring structure, alkyl, alkyl alcohol, or thioalkyl amide group;

the ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_8 and R_9 and is an R_8 ring structure; the R_8 ring structure is a 5-, 6- 7- or fused 6,5-membered heterocyclic ring wherein, the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms; the R_8 ring structure optionally can be substituted by one or more lower alkyl, lower dialkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, carboxamide, lower alkyl carboxylic acid, carbonyl, sulfoxide, sulfone or alkyl substituted phenyl sulfonamido groups; the (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups;

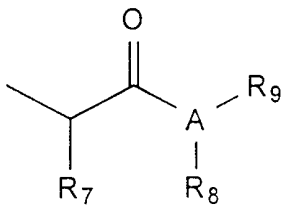
R_9 is the R_8 ring structure, a lower alkyl, lower dialkyl, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group; and

when A is oxygen:

R_8 is a lower alkyl that can be branched and R_9 is absent.

2. The compound of claim 1, wherein R_2 is lower alkyl, or phenyl lower alkyl.

3. The compound of claim 2, wherein R_6 is



4. The compound of claim 3, wherein A is nitrogen.

5. The compound of claim 4, wherein R_2 is lower alkyl.

6. The compound of claim 4, wherein R_2 is phenyl lower alkyl.

7. The compound of claim 4, wherein R_5 is H.

8. The compound of claim 4, wherein R_4 is H.

9. The compound of claim 5, wherein R_5 is H.

10. The compound of claim 5, wherein R_4 is H.

11. The compound of claim 6, wherein R_5 is H.

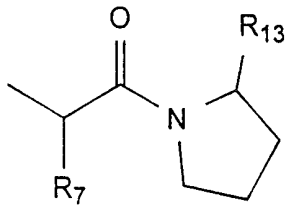
12. The compound of claim 6, wherein R_4 is H.

13. The compound of claim 4, wherein R_8 is the ring structure between R_8 and R_9, wherein said R_8 ring structure is a 5-, 6- 7- or fused 6,5-membered heterocyclic ring wherein, the heteroatoms are 1 or 2 nitrogen atoms, and 0 or 1 oxygen or sulfur atoms.

14. The compound of claim 13, wherein R_8 is the 5-membered heterocyclic ring wherein, the heteroatoms are 1 or 2 nitrogen atoms.

15. The compound of claim 13, wherein the R_8 ring structure is the 6-membered heterocyclic ring.

16. The compound of claim 14, wherein R_6 is of the formula:



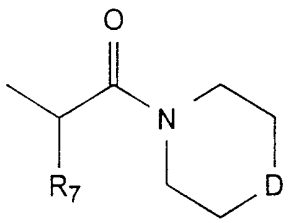
wherein R_{13} is a lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, carboxamide, or H group.

17. The compound of claim 16, wherein R_2 is lower alkyl, or phenyl lower alkyl.

18. The compound of claim 17, wherein R_4 is H.

19. The compound of claim 17, wherein R_5 is H.

20. The compound of claim 15, wherein R_6 is of the formula:



wherein, D is selected from the group consisting of carbon, nitrogen and oxygen.

21. The compound of claim 20, wherein R_2 is lower alkyl, or phenyl lower alkyl.

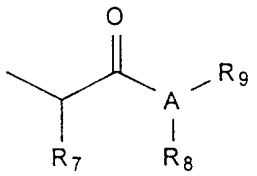
22. The compound of claim 20, wherein D is carbon optionally substituted by one or more lower alkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid, carbonyl, or alkyl substituted phenyl sulfonamido groups.

23. The compound of claim 20, wherein D is a nitrogen atom optionally substituted by a lower alkyl, amino carbonyl lower alkyl wherein the nitrogen atom of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, lower alkyl alcohol, lower hydroxy alkyl ether, or lower alkyl carboxylic acid groups.

24. The compound of claim 20, wherein D is an oxygen atom.

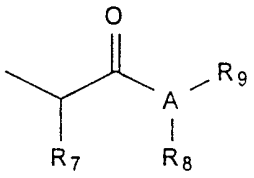
25. The compound of claim 20, wherein D is a sulfur atom optionally forming a sulfoxide or sulfone.

26. The compound of claim 1, wherein when R_6 is of the formula:



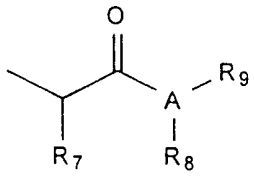
the R_6 ring structure is not a 5-membered heterocyclic ring where the heteroatom is 1 nitrogen atom and the ring structure is substituted with a carboxamide.

27. The compound of claim 26, wherein when R_6 is of the formula:



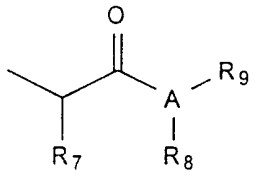
the R_8 ring structure is not a 5-membered heterocyclic ring where the heteroatom is 1 nitrogen atom and the ring structure is substituted.

28. The compound of claim 27, wherein when R_6 is of the formula:



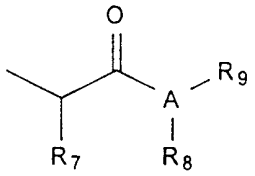
the R_6 ring structure is not a 5-membered heterocyclic ring where the heteroatom is 1 nitrogen atom and the ring structure is substituted or unsubstituted.

29. The compound of claim 28, wherein when R_6 is of the formula:



the R_6 ring structure is not a 5-membered heterocyclic ring where the heteroatoms are 1 or 2 nitrogen atoms and the ring structure is substituted or unsubstituted.

30. The compound of claim 29, wherein when R_6 is of the formula:

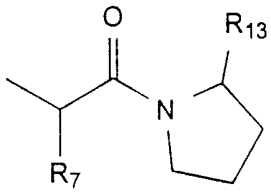


the R_6 ring structure is not a 5-membered heterocyclic ring where the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms and the ring structure is substituted or unsubstituted.

31. The compound of claim 26, wherein R_2 is lower alkyl.

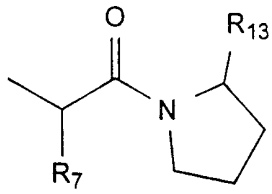
32. The compound of claim 31, wherein R_2 is methyl.

33. The compound of claim 1, provided that when R_6 is of the formula:



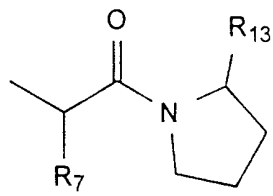
R_{13} is not carboxamide.

34. The compound of claim 33, provided that when R_6 is of the formula:



R_{13} is not carboxamide or lower alkyl carboxamide.

35. The compound of claim 34, provided that R_6 is not of the formula:



36. The compound of claim 33, wherein R_2 is lower alkyl.

37. The compound of claim 33, wherein R_2 is methyl.

38. The compound of claim 1, wherein R_2 is lower alkyl, excluding compounds numbers 1190.07 and 926.11 listed in Table 1.

39. The compound of claim 38, wherein R_2 is methyl.

40. The compound of claim 21, wherein R_2 is methyl.

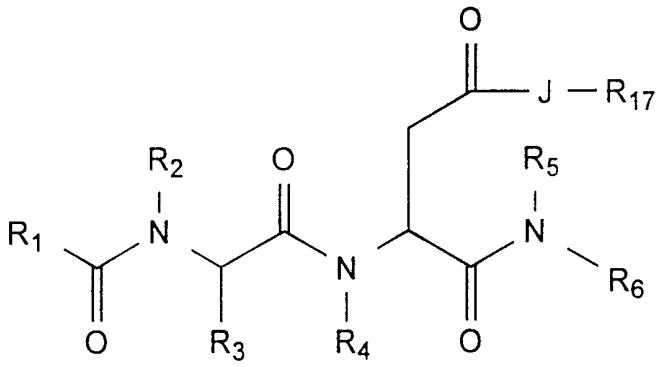
41. The compound of claim 1 wherein, R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen and R_8 is the ring structure formed between R_8 and R_9 selected from the group consisting of 4-morpholinyl, 4-methylpiperazinyl, and N-piperazinyl.

42. The compound of claim 1 wherein, R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, and R_8 is the 4-morpholinyl ring structure formed between R_8 and R_9.

43. The compound of claim 1 wherein, R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, and R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9.

44. The compound of claim 1 wherein, R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, and R_8 is the N-piperazinyl ring structure formed between R_8 and R_9.

45. A compound of the formula



wherein:

R_1 is a R_1 ring structure, lower alkyl, or lower amino alkyl; the R_1 ring structure can form at R_1, between R_1 and R_2 or between R_1 and R_4 with the proviso that, if the R_1 ring structure forms at R_1, the R_1 ring structure is connected by a spacer 0 to about 5 atoms long forming

one or more alkyl, N-amido, N-sulfonimido, N-urea, N-carboxyl groups; the spacer can be optionally substituted by an amino group; the R_1 ring structure is a substituted or unsubstituted 5-, 6-, fused 6,6- or fused 6,5-membered ring wherein, the substituent is one or more alkyl, carbonyl, alcohol, halogen, or alkyl phenyl groups; the R_1 ring structure is cyclic or heterocyclic with the proviso that the heteroatoms are 1 or 2 nitrogen atoms, and, if the R_1 ring structure is formed between R_2 and R_4, the heteroatoms are 2 nitrogen atoms; the R_1 ring structure can be conjugated, partially saturated, or saturated; the lower alkyl or lower amino alkyl group can be branched;

R_2 is a H, lower alkyl, phenyl lower alkyl, or R_2 and R_1 form the R_1 ring structure group;

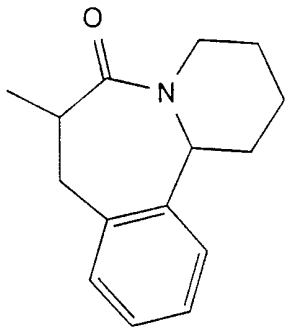
R_3 is a R_3 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl, or di(lower alkyl)thioether; the R_3 ring structure group is a 6- membered ring that is connected by an alkyl group 0 to about 3 carbon atoms long; the lower alkyl, lower alkyl alcohol, or lower thioalkyl group can be branched;

R_4 is a H or R_4 and R_1 form the R_1 ring structure;

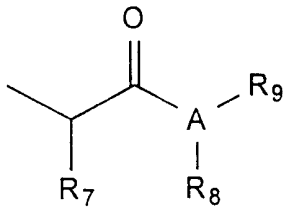
R_5 is H or R_5 and R_6 form a R_5 ring structure; the R_5 ring structure is a fused 6,6-ring structure and can be aromatic, partially saturated, or saturated;

R_6 is a benzyl, a 5,6,or 7-membered heterocyclic saturated ring containing

1 or 2 nitrogen atoms optionally substituted by one or more lower alkyl, lower alkyl amide or acyl groups or 1,1 diphenylmethine group, the R_5 ring structure, a group of the formula



or a group of the formula



wherein:

A is nitrogen or oxygen;

when A is nitrogen;

R_7 is a R_7 ring structure, lower alkyl, lower alkyl alcohol, lower thioalkyl or H group; the R_7 ring structure can form at R_7 or between R_7 and R_8 with the proviso that, if the R_7 ring structure forms at R_7, the R_7 ring structure is connected by an alkyl group 0 to about 3 carbon atoms long; if the R_7 ring structure is formed at R_7, the R_7 ring structure is a 6-, or fused

6,5-membered aromatic or non-aromatic cyclic or heterocyclic ring group wherein, the heteroatom is a nitrogen atom; if the R_7 ring forms between R_7 and R_8, the R_7 ring structure is a 5-, fused 6,6-, fused 6,5-, or 7- membered heterocyclic ring group wherein, the heteroatoms are 1 or 2 nitrogen atoms; the R_7 ring structure can optionally be substituted by an alcohol, nitro, or lower alkyl ether group;

R_8 is a ring structure, alkyl, alkyl alcohol, or thioalkyl amide group; the ring structure can form at R_8 and is (N-morpholino) amino, between R_7 and R_8 and is the R_7 ring structure, or between R_8 and R_9 and is an R_8 ring structure; the R_8 ring structure is a 5-, 6- 7- or fused 6,5-membered heterocyclic ring wherein, the heteroatoms are 1 or 2 nitrogen atoms and 0 or 1 oxygen or sulfur atoms; the R_8 ring structure optionally can be substituted by one or more lower alkyl, lower dialkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether, carboxylic acid, lower alkyl carboxylic acid,

carboxamide, carbonyl, sulfoxide, sulfone or alkyl substituted phenyl sulfonamido groups; the (N-morpholino) amino, alkyl, alkyl alcohol, or thioalkyl amide group can optionally contain one or more alcohol, amide, sulfhydryl, or alkyl ester groups;

R_9 is the R_8 ring structure, a lower alkyl, lower dialkyl, lower alkyl carboxamide, lower alkyl morpholine amide, cyclohexane or H group;

when A is oxygen:

R_8 is a lower alkyl that can be branched and R_9 is absent;

J is oxygen or sulfur; and

R_{17} is alkyl, alkyl optionally substituted by a hydroxyl, phenyl or phenyl sulfonyl, di(lower alkyl)sulfide, (lower alkoxy)lower alkyl, [(lower alkoxy)lower alkoxy]lower alkyl, (lower alkylcarbonyloxy)lower alkyl, [N-(lower alkyl)aminocarbonyl]lower alkyl, {[(N-(lower alkyl)](N-(lower alkoxy)}amino-carbonyl)lower alkyl,(N,N-di(lower alkyl)aminocarbonyl)lower alkyl, (N'-morpholinocarbonyl)lower alkyl, (benzyloxycarbonyl)methyl, 1-((O-((lower alkylcarbonato))eth-1-yl group; 2-oxo-1,3-dioxolen-4-ylmethyl group, cyclopentyl, cyclohexyl, cycloheptyl, phenyl, 1,3- dioxan-2-yl, 3-tetrahydropyranyl, (4-hydroxybutyric)lacton-3-yl, phthalidyl, or fused 6,5-membered aromatic, partially aromatic, or non-

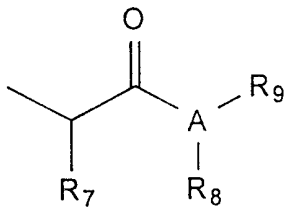
aromatic ring, wherein said ring is connected to J either directly by a bond or indirectly by a lower alkyl group; or

a pharmaceutically-acceptable salt thereof.

46. The compound of claim 45, wherein R_2 is lower alkyl, or phenyl lower alkyl.

47. The compound of claim 46, wherein R_2 is methyl.

48. The compound of claim 46, wherein R_6 is



49. The compound of claim 48, wherein A is nitrogen.

50. The compound of claim 49, wherein R_2 is lower alkyl.

51. The compound of claim 49, wherein R_2 is phenyl lower alkyl.

52. The compound of claim 49, wherein R_5 is H.

53. The compound of claim 49, wherein R_4 is H.

54. The compound of claim 50, wherein R_5 is H.

55. The compound of claim 50, wherein R_4 is H.

56. The compound of claim 51, wherein R_5 is H.

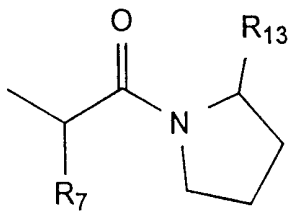
57. The compound of claim 51, wherein R_4 is H.

58. The compound of claim 49, wherein R_8 is the ring structure between R_8 and R_9, wherein said R_8 ring structure is a 5-, 6- 7- or fused 6,5-membered heterocyclic ring wherein, the heteroatoms are 1 or 2 nitrogen atoms, and 0 or 1 oxygen or sulfur atoms.

59. The compound of claim 58, wherein R_8 is the 5-membered heterocyclic ring, wherein the heteroatoms are 1 or 2 nitrogen atoms.

60. The compound of claim 58, wherein the R_8 ring structure is the 6-membered heterocyclic ring.

61. The compound of claim 59, wherein R_6 is of the formula:



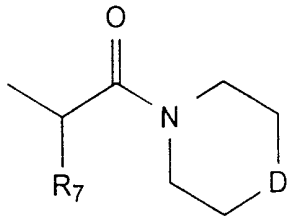
wherein, R_{13} is a lower alkyl carboxamide, lower alkyl alcohol, carboxylic acid, carboxamide, or H group.

62. The compound of claim 61, wherein R_2 is lower alkyl or phenyl lower alkyl.

63. The compound of claim 62, wherein R_4 is H.

64. The compound of claim 63, wherein R_5 is H.

65. The compound of claim 59, wherein R_6 is of the formula:



wherein D is selected from the group consisting of carbon, nitrogen and oxygen.

66. The compound of claim 65, wherein R_2 is lower alkyl, or phenyl lower alkyl.

67. The compound of claim 65, wherein D is carbon optionally substituted by one or more lower alkyl, lower alkyl carbonyloxy, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, alcohol, lower alkyl alcohol, lower hydroxy alkyl ether,

carboxylic acid, lower alkyl carboxylic acid, carbonyl, or alkyl substituted phenyl sulfonamido groups.

68. The compound of claim 65, wherein D is a nitrogen atom optionally substituted by a lower alkyl, aminocarbonyl lower alkyl wherein the nitrogen of the amino group is bound to any combination of two groups selected from the group consisting of alkyl, aryl and H groups, lower alkyl alcohol, lower hydroxy alkyl ether, or, lower alkyl carboxylic acid groups.

69. The compound of claim 65, wherein D is an oxygen atom.

70. The compound of claim 65, wherein D is a sulfur atom optionally forming a sulfoxide or sulfone.

71. The compound of claim 45, wherein R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 is H, R_7 is benzyl, A is nitrogen, R_8 is a ring structure formed between R_8 and R_9 selected from the group consisting of 4-methylpiperazinyl, and 4-morpholinyl, J is oxygen and R_{17} is selected from the group consisting of ethyl, methyl, 2-propyl, cyclohexyl, and neopentyl.

72. The compound of claim 45, wherein R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is ethyl.

73. The compound of claim 45, wherein R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the

4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is methyl.

74. The compound of claim 45, wherein R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-morpholinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is 2-propyl.

75. The compound of claim 45, wherein R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-morpholinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is ethyl.

76. The compound of claim 45, wherein R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is 2-propyl.

77. The compound of claim 45, wherein R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is cyclohexyl.

78. The compound of claim 45, wherein R_1 is benzyl, R_2 is methyl, R_3 is 2-methylpropyl, R_4 and R_5 are H, R_7 is benzyl, A is nitrogen, R_8 is the 4-methylpiperazinyl ring structure formed between R_8 and R_9, J is oxygen, and R_{17} is neopentyl.

79. A pharmaceutical composition comprising the compound of claim 1 and a pharmaceutically acceptable carrier.

80. A pharmaceutical composition comprising the compound of claim 2 and a pharmaceutically acceptable carrier.

81. A pharmaceutical composition comprising the compound of claim 17 and a pharmaceutically acceptable carrier.

82. A pharmaceutical composition comprising the compound of claim 21 and a pharmaceutically acceptable carrier.

83. A pharmaceutical composition comprising the compound of claim 26 and a pharmaceutically acceptable carrier.

84. A pharmaceutical composition comprising the compound of claim 33 and a pharmaceutically acceptable carrier.

85. A pharmaceutical composition comprising the compound of claim 38 and a pharmaceutically acceptable carrier.

86. A pharmaceutical composition comprising the compound of claim 41 and a pharmaceutically acceptable carrier.

87. A pharmaceutical composition comprising the compound of claim 45 and a pharmaceutically acceptable carrier.

88. A pharmaceutical composition comprising the compound of claim 46 and a pharmaceutically acceptable carrier.

89. A pharmaceutical composition comprising the compound of claim 62 and a pharmaceutically acceptable carrier.

90. A pharmaceutical composition comprising the compound of claim 66 and a pharmaceutically acceptable carrier.

91. A pharmaceutical composition comprising the compound of claim 71 and a pharmaceutically acceptable carrier.

92. A method of treating inflammation, comprising administering the composition of claim 79.

93. A method of treating inflammation, comprising administering the composition of claim 80.

94. A method of treating inflammation, comprising administering the composition of claim 81.

95. A method of treating inflammation, comprising administering the composition of claim 82.

96. A method of treating inflammation, comprising administering the composition of claim 83.

97. A method of treating inflammation, comprising administering the composition of claim 84.

98. A method of treating inflammation, comprising administering the composition of claim 85.

99. A method of treating inflammation, comprising administering the composition of claim 86.

100. A method of treating inflammation, comprising administering the composition of claim 87.

101. A method of treating inflammation, comprising administering the composition of claim 88.

102. A method of treating inflammation, comprising administering the composition of claim 89.

103. A method of treating inflammation, comprising administering the composition of claim 90.

104. A method of treating inflammation, comprising administering the composition of claim 91.

105. The method of claims 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103 or 104, wherein the compound is resistant to proteolysis.

Fig. 1

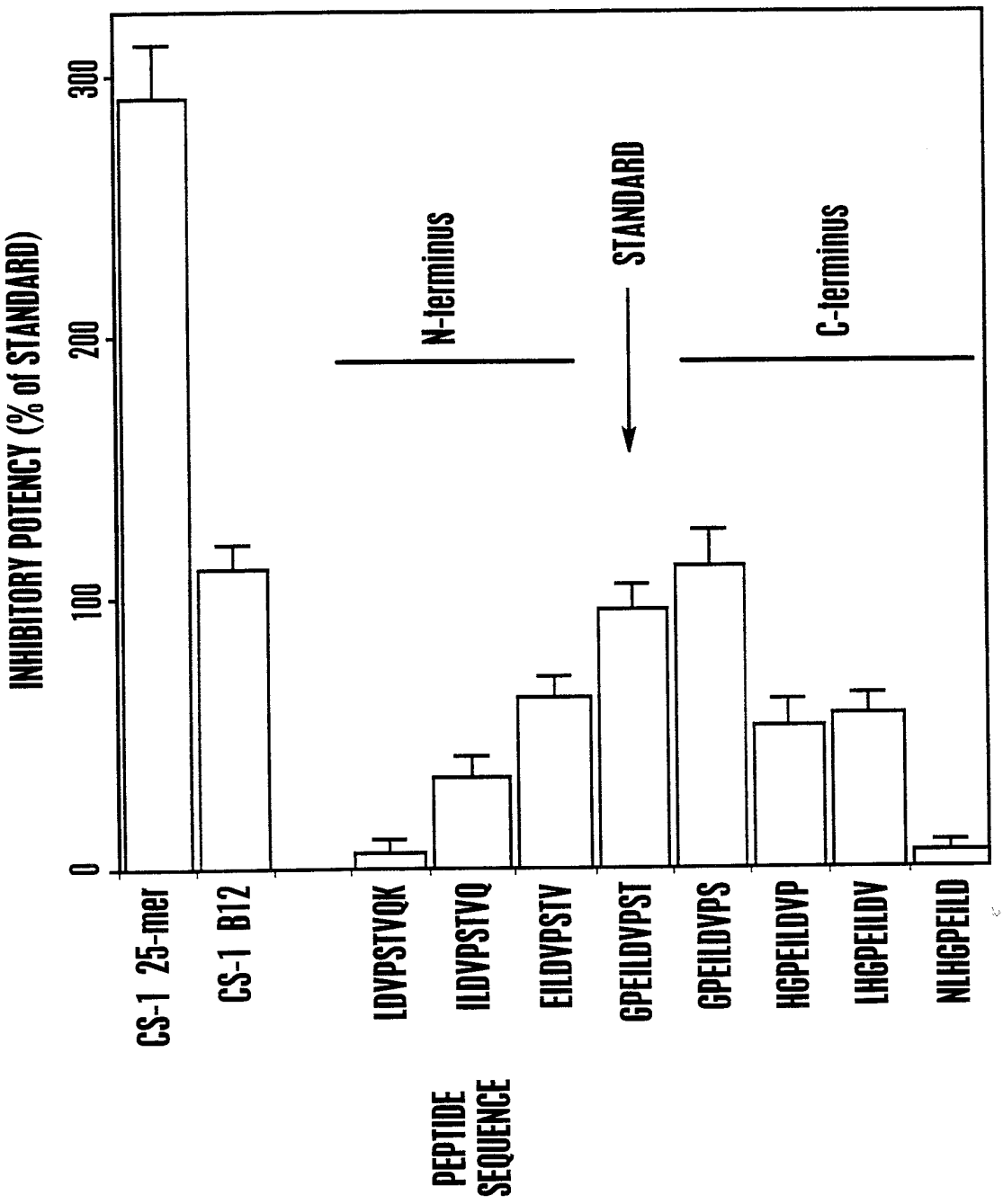

INHIBITORY POTENCY (% of STANDARD)
0
100
200
300
N-terminus
STANDARD
C-terminus
CS-1 25-mer
CS-1 B12
LDVPSTVQK
ILDVPSTVQ
EILDVPSTV
GPEILDVPST
GPEILDVPS
HGPEILDVP
LHGPEILDV
NLHGPEILD
PEPTIDE SEQUENCE

Fig. 2

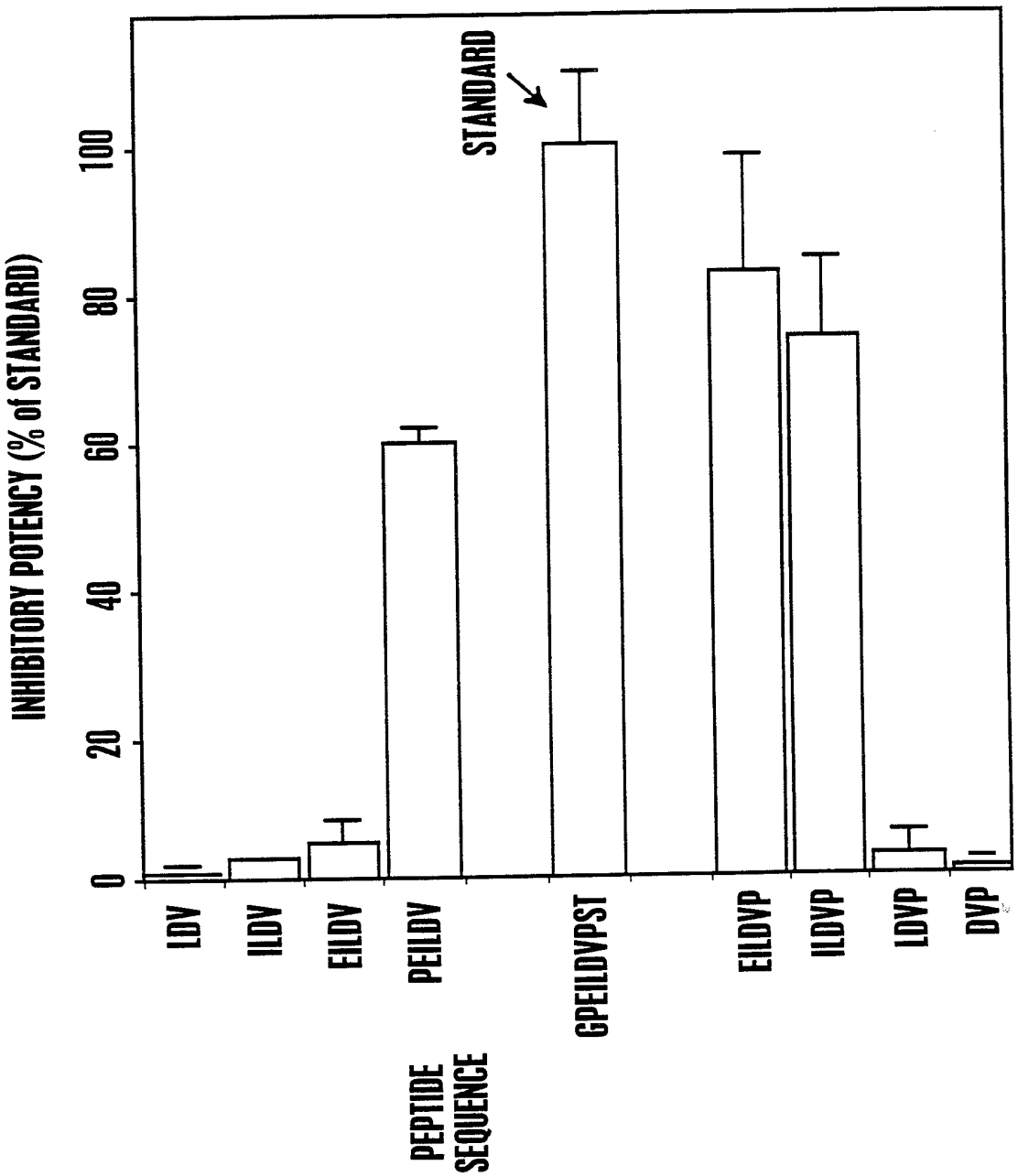

INHIBITORY POTENCY (% of STANDARD)
0
20
40
60
80
100
STANDARD
LDV
ILDV
EILDV
PEILDV
GPEILDVPST
EILDVP
ILDVP
LDVP
DVP
PEPTIDE SEQUENCE

Fig. 3

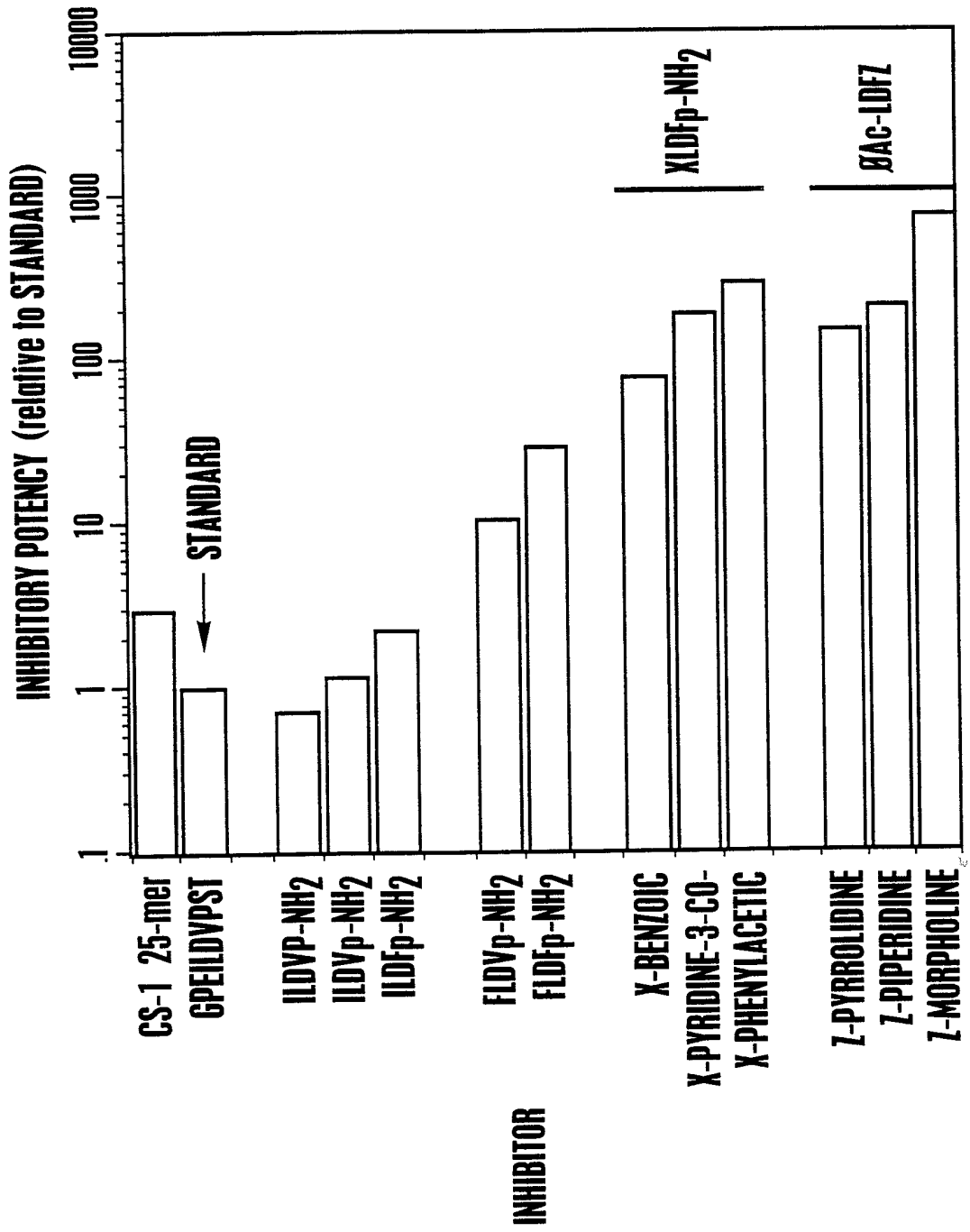

INHIBITORY POTENCY (relative to STANDARD)
1
1
10
100
1000
10000
STANDARD
XLDfp-NH2
ØAc-LDfZ
CS-1 25-mer
GPEILDVPST
ILDVP-NH2
ILDVp-NH2
ILDfp-NH2
FLDVp-NH2
FLDfp-NH2
X-BENZOIC
X-PYRIDINE-3-CO-
X-PHENYLACETIC
Z-PYRROLIDINE
Z-PIPERIDINE
Z-MORPHOLINE
INHIBITOR

Fig. 4A

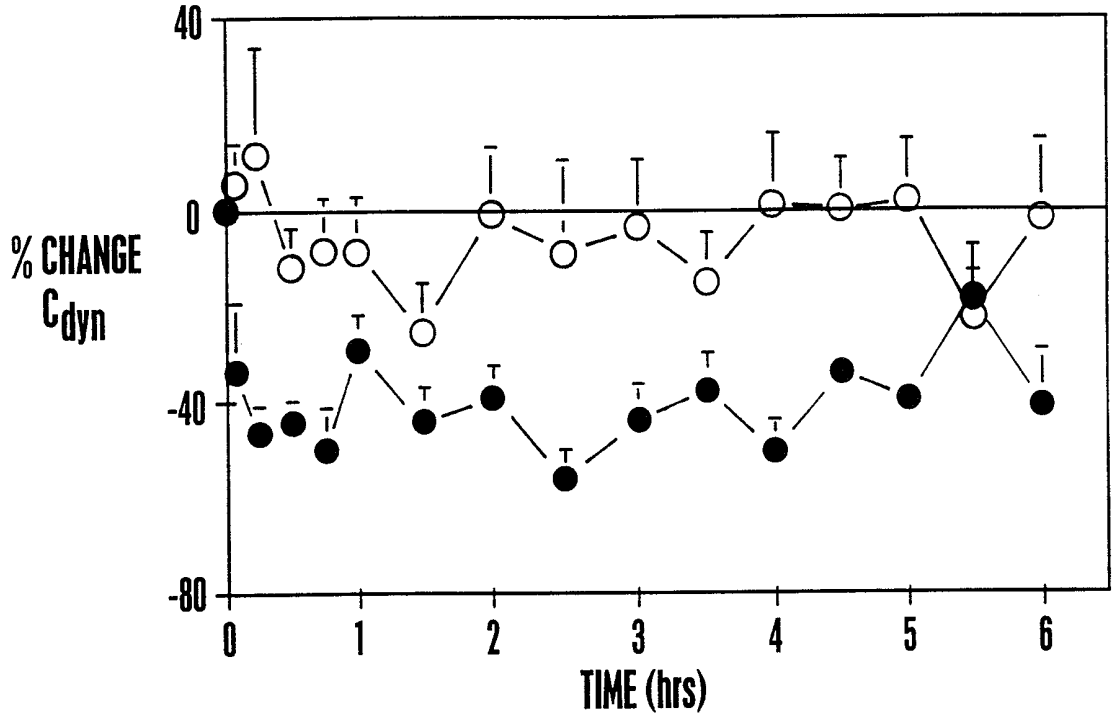

40
0
-40
-80
% CHANGE
C_{dyn}
0
1
2
3
4
5
6
TIME (hrs)

Fig. 4B

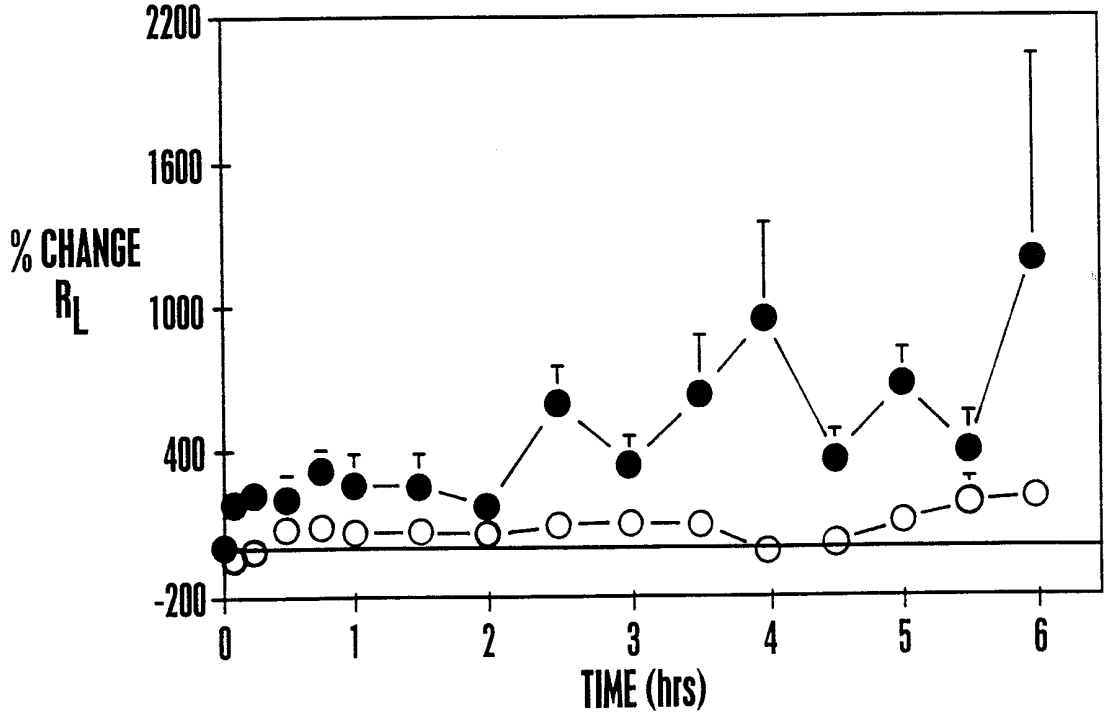

2200
1600
1000
400
-200
% CHANGE
R_L
0
1
2
3
4
5
6
TIME (hrs)

Fig. 5

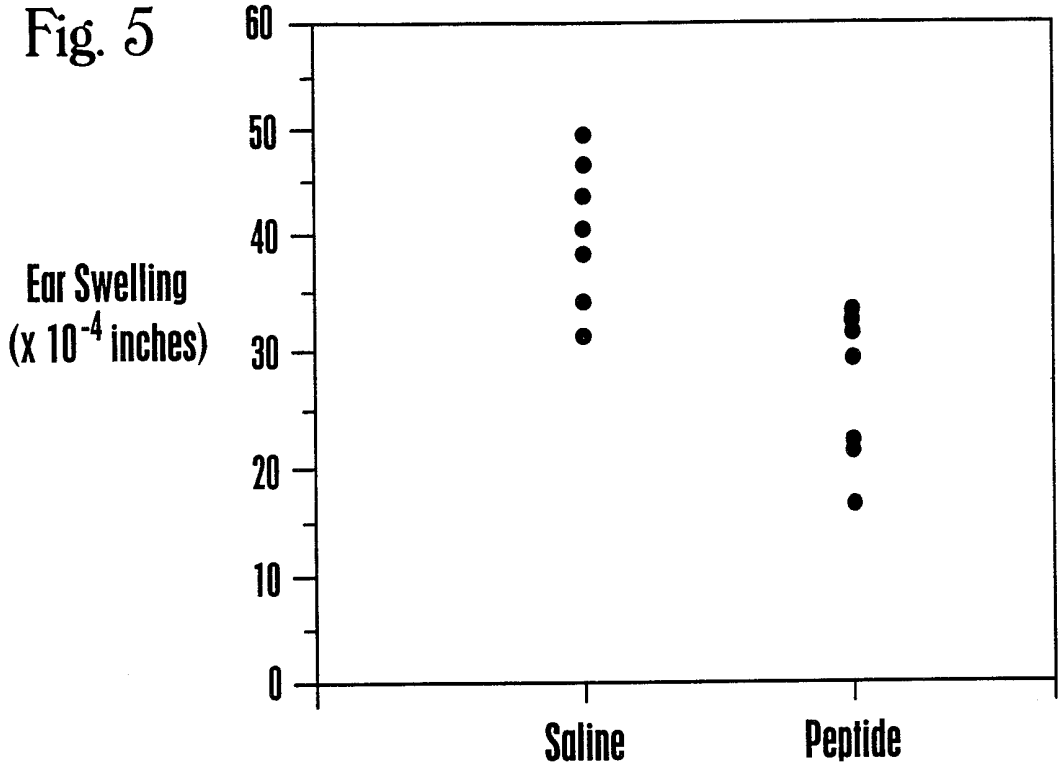

Ear Swelling
(x 10-4 inches)
60
50
40
30
20
10
0
Saline
Peptide

Fig. 6

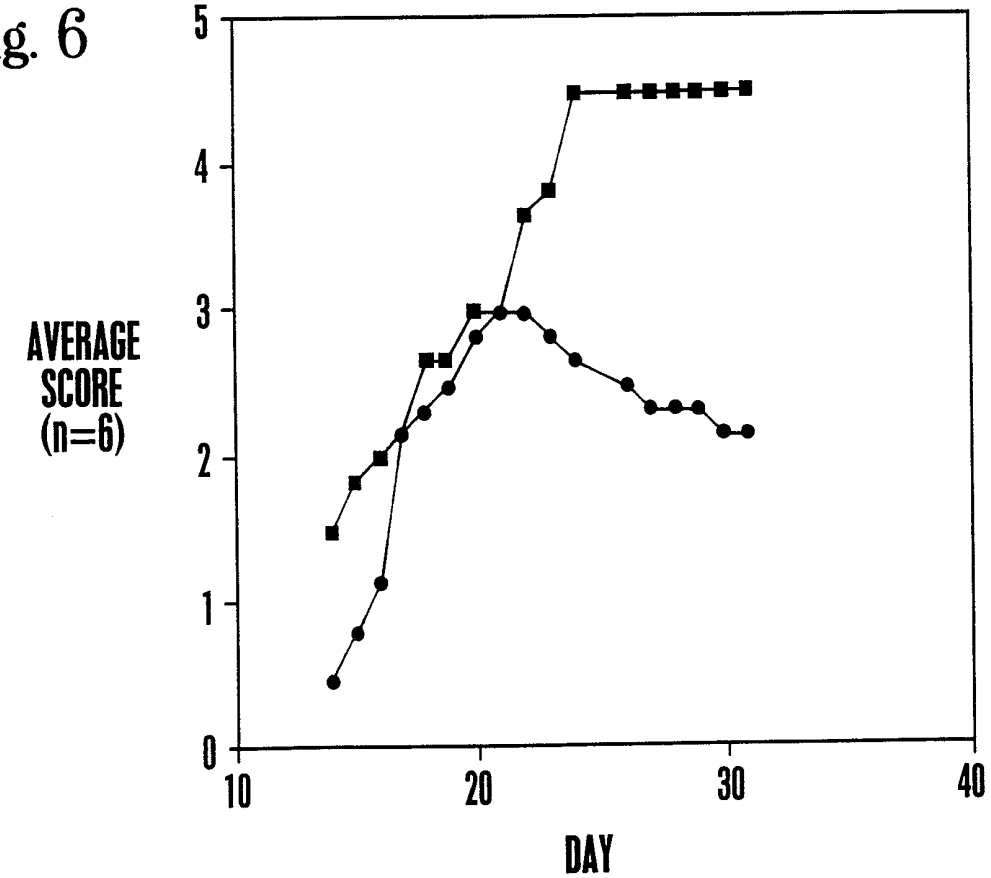
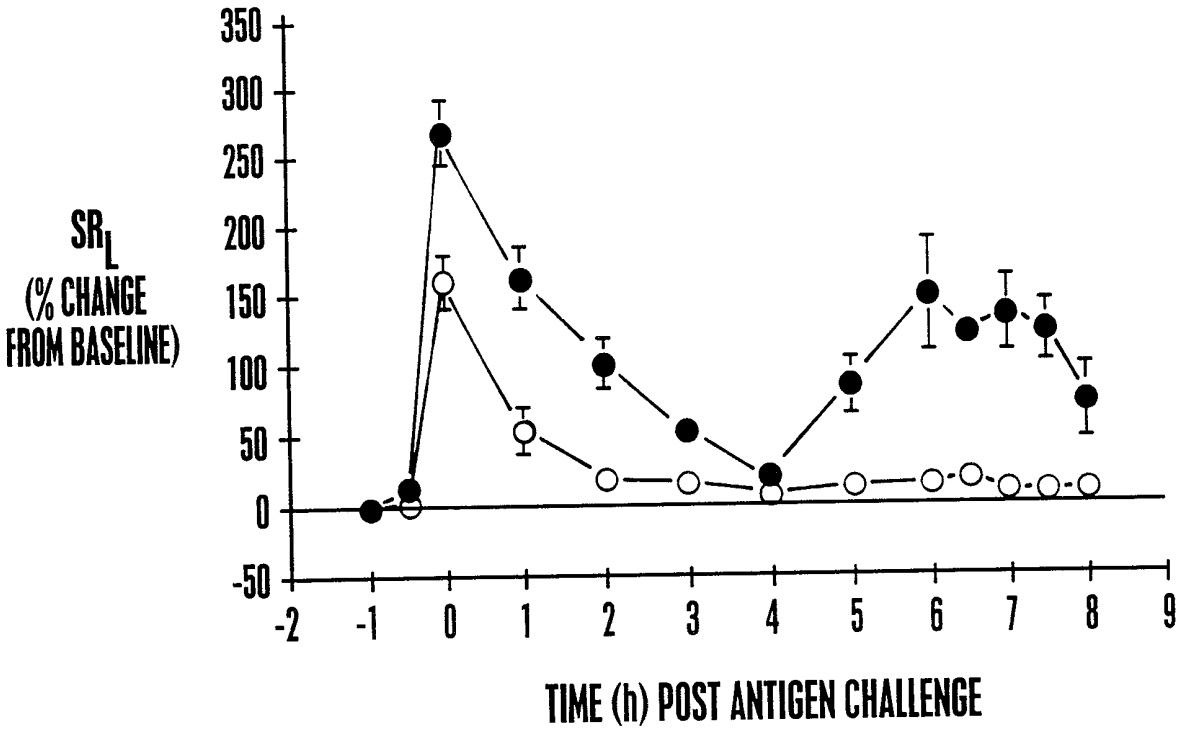

AVERAGE
SCORE
(n=6)
5
4
3
2
1
0
10
20
30
40
DAY

Fig. 7


SRL
(% CHANGE
FROM BASELINE)
350
300
250
200
150
100
50
0
-50
-2
-1
0
1
2
3
4
5
6
7
8
9
TIME (h) POST ANTIGEN CHALLENGE

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Cytel Corporation

(ii) TITLE OF INVENTION: CS-1 PEPTIDOMIMETICS, COMPOSITIONS AND METHODS OF USING THE SAME

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Campbell & Flores LLP
(B) STREET: 4370 La Jolla Village Drive, Suite 700
(C) CITY: San Diego
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 92122

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/461,056
(B) FILING DATE: 05-JUN-1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/349,024
(B) FILING DATE: 02-DEC-1994

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/164,101
(B) FILING DATE: 06-DEC-1993

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Campbell, Cathryn A.
(B) REGISTRATION NUMBER: 31,815
(C) REFERENCE/DOCKET NUMBER: FP-CY 3356

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (619) 535-9001
(B) TELEFAX: (619) 535-8949

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu His Gly
1 5 10 15

Pro Glu Ile Leu Asp Val Pro Ser Thr
20 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Leu His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = phenylacetyl-Leu."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = an amide formed between the C-terminal Pro carboxyl and a substituted tetraethylenediamine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Xaa Asp Phe Xaa
1

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
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(B) LOCATION: 1
(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = phenylacetyl-Leu."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /label= Xaa
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Xaa Asp Phe Xaa
1

INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US 98/26605

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/02 C07K5/08 C07K5/10 C07K7/06 C07K5/06
C07K14/78 A61K38/04

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 770 573 A (ARRHENIUS ET AL.) 23 June 1998 see the whole document	1-105
X	WO 98 04913 A (BIOGEN INC.) 5 February 1998 see the whole document	1-105
X	WO 97 03094 A (BIOGEN INC.) 30 January 1997 see the whole document	1-105
X	WO 95 15973 A (CYTEL CORPORATION) 15 June 1995 see the whole document	1-105

-/--

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 May 1999	01/06/1999

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

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Ir ıtional Application No
PCT/US 98/26605

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 98 42656 A (CYTEL CORPORATION) 1 October 1998 see the whole document -----	1-105

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/26605

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 92-105
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 92-105 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In .tional Application No
PCT/US 98/26605

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