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(54) Title: PARTIAL PEPTIDE MIMETICS AND METHODS

(57) Abstract: Partial peptide mimetics and methods of making and using, wherein the partial peptide mimetics have a first amino acid sequence comprising ANIKLSVQMKL (SEQ ID NO: 8), a homolog thereof, or a segment of SEQ ID NO: 8 or a homolog thereof, a second amino acid sequence comprising IIVKLND (SEQ ID NO:2), a homolog thereof, or a segment of SEQ ID NO:2 or a homolog thereof, and a β -turn inducing scaffold bonded between the first and second amino acid sequences.

Patent

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PARTIAL PEPTIDE MIMETICS AND METHODS

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Cross-Reference to Related Applications

The present application claims priority to U.S. Provisional Patent Application Serial No. 60/359,272, filed on February 20, 2002, which is incorporated herein by reference.

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Background

Angiogenesis, the process of new blood vessel formation, is key to normal organ development, as well as to various pathological disorders like cancer, arthritis, diabetic retinopathy and restenosis (A.W. Griffioen et al., *Biochem. J.*, 354, 233-242 (2001)). β pep-25, which has the sequence ANIKLSVQMKLFKRHLKWKIIVKLNDGRELSLD (SEQ ID NO:1), a designed cytokine-like β -sheet-forming peptide 33mer, is an anti-angiogenic compound being developed to combat these pathological disorders (K.H. Mayo et al., *Angiogenesis*, 4, 45-51 (2001); and S. Liekens et al., *J. Biochem. Pharm.*, 61, 253-270 (2001)).

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The use of agents that can inhibit angiogenesis, particularly in anti-tumor research (e.g., M.S. O'Reilly et al., *Cell*, 88, 277-285 (1997); and T. Boehm et al., *Nature*, 390, 404-407 (1997)), has indicated that anti-angiogenic therapy will be a promising therapeutic modality in the near future. To date, the search for angiogenic inhibitors has been focused on controlling two of the processes that promote angiogenesis: endothelial cell (EC) growth and adhesion (G. Molema et al., *Immunol. Today*, 19, 392-394 (1998); and J. Folkman et al., *Nature Med.*, 1, 27-31 (1995)). Targeting EC as an anti-tumor treatment is attractive primarily because EC are more accessible than are other cells to pharmacologic agents delivered via the blood, and EC are genetically stable and are not easily mutated into drug resistant variants.

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Most anti-angiogenic agents have been discovered by identifying endogenous molecules, primarily proteins, which inhibit EC growth. This traditional approach has produced a number of anti-angiogenics, such as platelet factor-4 (PF4), thrombospondin, tumor necrosis factor- α (TNF- α , depending on its concentration), interferon- γ inducible protein-10, angiostatin, endostatin and vasostatin and bactericidal-permeability increasing (BPI) protein. See, for example, M.S. O'Reilly et al., *Cell*, 88, 277-285 (1997); S.K. Gupta et al., *J. Cell Biol.*, 127, 1121-1127 (1994); S.S. Tolsma et al., *J. Cell Biol.*, 122, 497-511 (1993); N. Sato et al., *Japan Natl. Cancer Inst.*, 76, 1113-1121 (1986); V.J. Palombella et al., *J. Biol. Chem.*, 264, 18128-18136 (1989); B. Robaye et al., *Am. J. Pathol.*, 138, 447-453 (1991); A.D. Luster et al., *J. Exp. Med.*, 182, 219-231 (1995); M.S. O'Reilly et al., *Cell*, 79, 315-328 (1994); S.E. Pike et al., *J. Exp. Med.*, 188, 2349-2356 (1998); and D.W.J. Van der Schaft et al., *Blood*, 96, 176-181 (2000). About forty anti-angiogenic agents are currently known.

Most anti-angiogenic proteins are compositionally similar, having a relatively high incidence of hydrophobic and positively charged residues and are folded primarily as β -sheets (A.R. Mire Sluis et al., *J. Immunol. Methods*, 200, 1-16 (1997)): interleukin-1 (IL-1), tumor necrosis factor (TNF), lymphotoxin (LT or TNF- β), transforming growth factor- β (TGF- β), endostatin. See, for example, J.P. Priestle et al., *Proc. Natl. Acad. Sci. USA*, 86, 9667-9671 (1989); E.Y. Jones et al., *Nature*, 338, 225-228 (1989); M.J. Eck et al., *J. Biol. Chem.*, 267, 2119-2122 (1992); S. Daopin et al., *Science*, 257, 369-373 (1992); and E. Hohenester et al., *EMBO J.*, 17, 1656-1664 (1998).

Recently, a designed amphipathic β -sheet-forming peptide 33mer, β pep-25 was shown to be a potent inhibitor of EC growth and angiogenesis. β pep-25 is more effective at inhibiting EC growth than PF4 and several other well-known angiogenesis inhibitors such as angiostatin, endostatin, AGM-1470 and thrombospondin-1. β pep-25 is believed to act by specifically blocking adhesion and migration of angiogenically-activated EC, leading to apoptosis and ultimately to inhibition of angiogenesis *in vitro* and *in vivo* and inhibits tumor growth by up to 80% in various models. See, for example, D.W.J. Van der

Schaft et al., *Faseb J.*, 16, 1991-1993 (2002); and R.P. Dings et al., *Cancer Res.*, 63, 382-385 (2003).

For the smart design of smaller compounds, the identification of specific amino acid residues and their spatial relationships are used. One of the main goals among structural biologists and pharmaceutical chemists is to develop small molecules and potentially more effective anti-tumor agents. Nevertheless, for anti-angiogenic proteins such structure-activity relationships (SAR), i.e., specific residues and conformations which impart activity, are sorely needed, and even the analysis of high-resolution molecular structures of a number of anti-angiogenic proteins, e.g., endostatin, PF4, and BPI, has yet to provide this information. See, for example, E. Hohenester et al., *EMBO J.*, 17, 1656-1664 (1998); K.H. Mayo et al., *Biochemistry*, 34, 11399-11409 (1995); and L.J. Beamer et al., *Science*, 276, 1861-1864 (1997).

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Summary

From the structures of angiogenesis inhibitors that are known it appears that most anti-angiogenic proteins are compositionally similar. They show a relatively high incidence of hydrophobic and positively charged residues and a β -sheet. β pep-25 also falls into this category as an amphipathic β -sheet-forming peptide 33-mer. It is believed that this β -sheet conformation is necessary for the bioactivity of the 33-mer β pep-25. The present invention is directed to the identification of specific residues and conformations that are responsible for activity, leading to the design of partial peptide mimetics of β pep-25.

As used herein, a peptide mimetic is a compound that mimics a peptide. The use of the adjective "partial" indicates that it is not a full mimetic because it contains some peptide segments, i.e., amino acids in a sequence.

The present invention relates to partial peptide mimetics and methods for using such compounds that include segments (i.e., portions) of the β pep-25 sequence, which is ANIKLSVQMKLFKRHLKWKIIVKLNDGRELSLD (SEQ ID NO:1), or homologs thereof.

In particular, the partial peptide mimetics of the present invention are designed to include all or segments of the sequences ANIKLSVQMKL (SEQ ID

NO:8) or a homolog thereof, and IIVKLND (SEQ ID NO:2) or a homolog thereof, with a β -turn inducing scaffold bonded therebetween (e.g., covalently bonded). Segments of these sequences (SEQ ID NO:8 and SEQ ID NO:2 or homologs thereof) include one or more amino acids. Segments of SEQ ID
5 NO:8 and SEQ ID NO:2 as well as homologs of the segments and the full sequences preferably provide the partial peptide mimetic with at least one of the following activities: inhibition of endothelial cell proliferation *in vitro* at an IC50 level of less than 80 μ M; inhibition of angiogenesis *in vitro* at a level of less than 85% sprouting in a collagen gel-based assay; or reduction in tumor
10 volume *in vivo* (e.g., in an animal model) by at least 25% relative to a control at the end of an administration period.

In certain embodiments, segments of SEQ ID NO:8 or homologs thereof preferably include at least three amino acids of SEQ ID NO:8 or homologs thereof (preferably, contiguous amino acids), and more preferably include at
15 least six amino acids of SEQ ID NO:8 or homologs thereof (preferably, contiguous amino acids). In certain embodiments, segments of SEQ ID NO:8 or homologs thereof include those in which deletions have been made at the N-terminus preferably by 1, 2, 3, 4, 5, 6, 7, or 8 residues, more preferably by 1, 2, 3, 4, or 5 residues. Examples of segments of SEQ ID NO:8 that can be used in
20 forming the partial peptide mimetics of the present invention include MKL, QMKL (SEQ ID NO:4), SVQMKL (SEQ ID NO:5), IKLSVQMKL (SEQ ID NO:6), and NIKLSVQMKL (SEQ ID NO:7). Particularly preferred such segments include, for example, SVQMKL (SEQ ID NO:5), IKLSVQMKL (SEQ ID NO:6), and NIKLSVQMKL (SEQ ID NO:7).

25 In certain embodiments, segments of SEQ ID NO:2 or homologs thereof include at least one amino acid of SEQ ID NO:2 or homologs thereof, and preferably at least four amino acids of SEQ ID NO:2 or homologs thereof (preferably, contiguous amino acids). In certain preferred embodiments, segments of SEQ ID NO:2 or homologs thereof include those in which deletions
30 have been made at the C-terminus by 1, 2, 3, 4, 5, or 6 residues. Particularly preferred segments of SEQ ID NO:2 that can be used in forming the partial

peptide mimetics of the present invention include I, IIVK (SEQ ID NO:3), and IIVKLN (SEQ ID NO:9).

The β -turn inducing scaffold can be a chemical scaffold, such as a dibenzofuran-containing scaffold.

5 Particularly preferred partial peptide mimetics of the present invention include at least the 7 amino acids IIVKLND (SEQ ID NO:2) in combination with at least the 4 amino acids QMKL (SEQ ID NO:4) with a β -turn inducing scaffold, such as a chemical scaffold like a dibenzofuran-containing scaffold, bonded therebetween. Other preferred partial peptide mimetics include at least
10 SEQ ID NO:2 and at least the 6 amino acids SVQMKL (SEQ ID NO:5) with a β -turn inducing scaffold bonded therebetween. Other preferred partial peptide mimetics include at least SEQ ID NO:2 and at least the 9 amino acids IKLSVQMKL (SEQ ID NO:6) with a β -turn inducing scaffold bonded therebetween. Other preferred partial peptide mimetics include at least SEQ ID
15 NO:2 and at least the 10 amino acids NILKSVQMKL (SEQ ID NO:7) with a β -turn inducing scaffold bonded therebetween. Other preferred partial peptide mimetics include at least SEQ ID NO:2 and at least the 11 amino acids ANILKSVQMKL (SEQ ID NO:8) with a β -turn inducing scaffold bonded therebetween.

20 Other preferred partial peptide mimetics include at least ANIKLSVQMKL (SEQ ID NO:8) and at least the amino acid I with a β -turn inducing scaffold bonded therebetween. Other preferred partial peptide mimetics include at least SEQ ID NO:8 and at least the 4 amino acids IIVK (SEQ ID NO:3) with a β -turn inducing scaffold bonded therebetween. Other
25 preferred partial peptide mimetics include at least SEQ ID NO:8 and at least the 6 amino acids IIVKLN (SEQ ID NO:9) with a β -turn inducing scaffold bonded therebetween.

Other preferred partial peptide mimetics include at least SVQMKL (SEQ ID NO:5) and at least the amino acid I with a β -turn inducing scaffold
30 therebetween. Other preferred partial peptide mimetics include at least SEQ ID

NO:5 and at least the 6 amino acids IIVKLN (SEQ ID NO:9) with a β -turn inducing scaffold bonded therebetween.

This invention also relates to methods of using at least one of the partial peptide mimetics disclosed herein. Preferably, the methods are for treatment of various conditions *in vivo*, although *in vitro* methods are also desirable. Typically, such methods involve the use of a composition that includes at least one of the partial peptide mimetics and optionally a carrier, preferably a pharmaceutically acceptable carrier.

Such methods include, for example, treating a bacterial infection or endotoxic shock. In one embodiment, the partial peptide mimetic neutralizes endotoxin, in another the partial peptide mimetic is bactericidal, and in another the partial peptide mimetic is both bactericidal and neutralizes endotoxin. Such partial peptide mimetics can also be used to inhibit bacterial infection or endotoxic shock in a cell culture.

This invention also relates to a method for inhibiting TNF- α levels, inhibiting endothelial cell proliferation, promoting inter-cellular adhesion molecule (ICAM) expression, inhibiting inter-cellular adhesion molecule (ICAM) expression down regulation, inhibiting tumorigenesis, and inhibiting angiogenesis. Such methods can be carried out *in vivo* (e.g., for treating a mammal) or *in vitro* (e.g., in a cell culture). Similarly, such partial peptide mimetics can be used to inhibit pathologic disorders such as atherosclerosis, restenosis, diabetic retinopathy, neovascular glaucoma, rheumatoid arthritis, and endometriosis.

As used herein, "a," "an," "the," "at least one," and "one or more" are used interchangeably.

"Amino acid" is used herein to refer to a chemical compound with the general formula: $\text{NH}_2\text{---CRH---COOH}$, where R, the side chain, is H or an organic group. Where R is organic, R can vary and is either polar or nonpolar (i.e., hydrophobic). The amino acids of this invention can be naturally occurring or synthetic (often referred to as nonproteinogenic). As used herein, an organic group is a hydrocarbon group that is classified as an aliphatic group, a cyclic group or combination of aliphatic and cyclic groups. The term "aliphatic group"

means a saturated or unsaturated linear or branched hydrocarbon group. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example. The term "cyclic group" means a closed ring hydrocarbon group that is classified as an alicyclic group, aromatic group, or heterocyclic group. The term "alicyclic group" means a cyclic hydrocarbon group having properties resembling those of aliphatic groups. The term "aromatic group" refers to mono- or polycyclic aromatic hydrocarbon groups. As used herein, an organic group can be substituted or unsubstituted. One letter and three letter symbols are used herein to designate the naturally occurring amino acids. Such designations including R or Arg, for Arginine, K or Lys, for Lysine, G or Gly, for Glycine, and X for an undetermined amino acid, and the like, are well known to those skilled in the art.

The terms "polypeptide" and "peptide" as used herein are used interchangeably and refer to a compound that includes two or more amino acids. These terms do not connote a specific length of amino acids (e.g., herein, an "amino acid sequence" can include just one amino acid).

The term "partial peptide mimetic" is used herein to refer to a compound that mimics a peptide, and contains some peptide segments, i.e., amino acids in a sequence. At times these compounds are simply referred to as "peptides" or "polypeptides."

The following abbreviations are used throughout the application:

A = Ala = Alanine	T = Thr = Threonine
V = Val = Valine	C = Cys = Cysteine
L = Leu = Leucine	Y = Tyr = Tyrosine
I = Ile = Isoleucine	N = Asn = Asparagine
P = Pro = Proline	Q = Gln = Glutamine
F = Phe = Phenylalanine	D = Asp = Aspartic Acid
W = Trp = Tryptophan	E = Glu = Glutamic Acid
M = Met = Methionine	K = Lys = Lysine
G = Gly = Glycine	R = Arg = Arginine
S = Ser = Serine	H = His = Histidine

Other abbreviations used herein include: DBF, dibenzofuran; EC, endothelial cell; EAM, endothelial adhesion molecule; HUVEC, human umbilical vein EC; PBS, phosphate buffered saline; HPLC, high performance liquid chromatography; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; PF4, platelet factor-4; BPI, bactericidal-permeability increasing protein; NMR, nuclear magnetic resonance; GC-LRMS, gas chromatography-liquid chromatography mass spectrometry; IR, infrared spectroscopy; SPPS, solid phase peptide synthesis; BOP/HOBT, benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate/1-hydroxybenzotriazole; DPC, dodecylphosphocholine.

Brief Description of the Drawings

Figure 1(A). Sequences for the dodecapeptide walk-throughs below the amino acid sequence of β pep-25. Proliferation of bFGF-stimulated (10 ng/ml) human umbilical vascular EC (HUVEC) cultures was measured by quantification of ^3H -thymidine incorporation. Proliferation is expressed as mean

counts per minute (cpm) of quadruplicate cultures in three independent experiments (\pm SEM). EC proliferation results from alanine scanning (tested at 25 μ M dose) are expressed in bar graph format as the percentage of proliferating EC (the arithmetic mean counts per minute (cpm) of triplicate cultures) relative to control cultures. For walk-through peptides, results are given as IC_{50} values from dose response curves; only IC_{50} values for dodecapeptides with significant activity relative to β pep-25 are given.

Figure 1(B). Overall fold for β pep-25 (3 β -strands with 2 turns), shows with functionally key residues boxed-in.

Figure 1(C) shows the parent DBF analog (11DBF7 or CF-8) with the introduction of the scaffold.

Figure 2. The superposition of 6DBF7 derived from NMR analysis is shown in Figure 2(A), with the chemical 3D structure illustrated in Figure 2(B).

Figure 3. Anti-proliferative effects of DBF analogs on bFGF-activated EC are plotted as dose response curves.

Figure 4. MA148 tumor bearing mice were treated with the optimal dose of β pep-25 (10 mg/kg/day) or equivalent doses of 11DBF7 or 6DBF7. In Figure 4(A) treatment was initiated at the time of inoculation with MA148 cells. In Figure 4(B) an intervention study is shown where tumors were allowed to establish to a palpable size before treatment was initiated. In either study, control groups of animals were treated with PBS containing human serum albumin to control for protein content. Tumor volumes (for all groups $n = 11$, \pm SEM) are plotted as mm^3 vs. days post inoculation. The inserts show the body weight development of the mice during the study as a measurement of overall toxicity.

Detailed Description of Illustrative Embodiments of the Invention

The present invention provides partial peptide mimetics and methods of using such partial peptide mimetics. These partial peptide mimetics include segments (i.e., portions) of the β pep-25 sequence, which is ANIKLSVQMKLFRHLKWKIIVKLNDGRELSLD (SEQ ID NO:1), or

homologs thereof. These partial peptide mimetics preferably include at least 7 amino acid residues. Although there is no upper limit to the number of amino acid residues, it is desirable for the partial peptide mimetics to be small and approach the size of conventional therapeutic small organic molecules.

5 In particular, the partial peptide mimetics of the present invention are designed to include all or segments of the sequences ANIKLSVQMKL (SEQ ID NO:8) or a homolog thereof, and IIVKLND (SEQ ID NO:2) or a homolog thereof, with a β -turn inducing scaffold bonded therebetween (e.g., covalently bonded). Segments of these sequences (SEQ ID NO:8 and SEQ ID NO:2 or
10 homologs thereof) include one or more amino acids.

Segments of SEQ ID NO:8 and SEQ ID NO:2 as well as homologs of the segments and the full sequences preferably provide the partial peptide mimetic with at least one of the following activities: inhibition of endothelial cell proliferation *in vitro* at an IC50 level of less than 80 μ M (more preferably
15 less than 50 μ M, and even more preferably less than 25 μ M) as determined using the assay described in the Examples Section; inhibition of angiogenesis *in vitro* at a level of less than 85% sprouting (more preferably less than 75% sprouting, even more preferably 50% sprouting, and even more preferably less than 35% sprouting) as determined using the collagen gel-based assay described
20 in the Examples Section; or reduction in tumor volume *in vivo* by at least 25% (more preferably by at least 50%, and even more preferably by at least 70%) relative to a control at the end of an administration period as determined using the assay with an animal model described in the Examples Section. These values can be determined by one of skill in the art and are typically average
25 values.

In certain embodiments, segments of SEQ ID NO:8 or homologs thereof preferably include at least three amino acids of SEQ ID NO:8 or homologs thereof (preferably, contiguous amino acids), and more preferably include at least six amino acids of SEQ ID NO:8 or homologs thereof (preferably,
30 contiguous amino acids). In certain embodiments, segments of SEQ ID NO:8 or homologs thereof include those in which deletions have been made at the N-terminus preferably by 1, 2, 3, 4, 5, 6, 7, or 8 residues, more preferably by 1, 2,

3, 4, or 5 residues, and even more preferably by 5 residues. Examples of segments of SEQ ID NO:8 that can be used in forming the partial peptide mimetics of the present invention include MKL, QMKL (SEQ ID NO:4), SVQMKL (SEQ ID NO:5), IKLSVQMKL (SEQ ID NO:6), and
5 NIKLSVQMKL (SEQ ID NO:7). Particularly preferred such segments include, for example, SVQMKL (SEQ ID NO:5), IKLSVQMKL (SEQ ID NO:6), and NIKLSVQMKL (SEQ ID NO:7).

In certain embodiments, segments of SEQ ID NO:2 or homologs thereof include at least one amino acid of SEQ ID NO:2 or homologs thereof, and
10 preferably at least four amino acids of SEQ ID NO:2 or homologs thereof (preferably, contiguous amino acids). In certain preferred embodiments, segments of SEQ ID NO:2 include those in which deletions have been made at the C-terminus by 1, 2, 3, 4, 5, or 6 residues. Particularly preferred segments of SEQ ID NO:2 that can be used in forming the partial peptide mimetics of the
15 present invention include I, IIVK (SEQ ID NO:3), and IIVKLN (SEQ ID NO:9).

The β -turn inducing scaffold can be a chemical scaffold, such as a dibenzofuran-containing scaffold or biphenyl-containing scaffold. Preferably, it is a dibenzofuran-containing scaffold. More preferably, the dibenzofuran-containing scaffold is that shown in the Examples Section (Scheme 1) and is
20 referred to herein as "DBF." It should be understood that the length of the hydrocarbon chains off the phenyl rings in the scaffold and attached to the peptides can vary in length.

Such partial peptide mimetics are active with respect to a number of biological activities. This is exemplified by the data shown herein with respect
25 to specific partial peptide mimetics composed of a β -sheet-inducing biphenylfuran scaffold and two short amino acid sequences from β pep-25. They can be as effective as β pep-25 at inhibiting endothelial cell proliferation and angiogenesis *in vitro*. For example, in a mouse xenograft model for ovarian carcinoma, two variations of the partial mimetic are observed to be slightly
30 more effective than β pep-25 by reducing tumor volume by up to 85%. Immunohistochemical staining indicates that antitumor activity is mediated by significant reduction in vessel density and endothelial cell proliferation, as well

as increased cell apoptosis. Overall, the partial peptide mimetic demonstrates improved bioavailability over β pep-25 and the potential for obtaining an orally active, small molecule of an angiogenesis inhibitory protein. Because of this anti-angiogenic behavior it is believed that such partial peptide mimetics can be used to inhibit pathologic disorders such as atherosclerosis, restenosis, diabetic retinopathy, neovascular glaucoma, rheumatoid arthritis, and endometriosis. This can be evaluated readily by one of skill in the art using appropriate *in vitro* and *in vivo* models.

Particularly preferred partial peptide mimetics include 6DBF1, 6DBF7, and 11DBF7, of which even more preferred are 6DBF7 and 11DBF7. The sequences of these DBF analogs (which are partial peptide mimetics) are shown in Table 1.

Such partial peptide mimetics can be in their free acid form or they can be amidated at the C-terminal carboxylate group. The present invention also includes homologs of the peptide sequences listed herein, which typically have structural similarity with such peptides. A "homolog" of a polypeptide includes one or more conservative amino acid substitutions, which are selected from other members of the class to which the amino acid belongs. For example, it is well-known in the art of protein biochemistry that an amino acid belonging to a grouping of amino acids having a particular size or characteristic (such as charge, hydrophobicity and hydrophilicity) can generally be substituted for another amino acid without substantially altering the structure of a polypeptide.

For the purposes of this invention, conservative amino acid substitutions are defined to result from exchange of amino acids residues from within one of the following classes of residues: Class I: Ala, Gly, Ser, Thr, and Pro (representing small aliphatic side chains and hydroxyl group side chains); Class II: Cys, Ser, Thr, and Tyr (representing side chains including an -OH or -SH group); Class III: Glu, Asp, Asn, and Gln (carboxyl group containing side chains); Class IV: His, Arg, and Lys (representing basic side chains); and Class V: Ile, Val, Leu, Phe, Met, Phe, Trp, Tyr, and His (representing hydrophobic side chains). The classes also include related amino acids such as 3Hyp and 4Hyp in Class I; homocysteine in Class II; 2-aminoadipic acid, 2-aminopimelic

acid, γ -carboxyglutamic acid, β -carboxyaspartic acid, and the corresponding amino acid amides in Class III; ornithine, homoarginine, N-methyl lysine, dimethyl lysine, trimethyl lysine, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid, homoarginine, sarcosine and hydroxylysine in Class IV; substituted
5 phenylalanines, norleucine, norvaline, 2-aminooctanoic acid, 2-aminoheptanoic acid, statine and β -valine in Class V; and naphthylalanines, substituted phenylalanines, tetrahydroisoquinoline-3-carboxylic acid, and halogenated tyrosines in Class V.

Polypeptide homologs, as that term is used herein, also include modified
10 polypeptides. Modifications of polypeptides of the invention include chemical and/or enzymatic derivatizations at one or more constituent amino acid, including side chain modifications, backbone modifications, and N- and C-terminal modifications including acetylation, hydroxylation, methylation, amidation, and the attachment of carbohydrate or lipid moieties, cofactors, and
15 the like.

Such homologs provide partial peptide mimetics with one or more of the biological activities described herein.

The partial peptide mimetics, particularly the peptide portions of the partial peptide mimetics of the invention, may be synthesized by the solid phase
20 method using standard methods based on either t-butyloxycarbonyl (BOC) or 9-fluorenylmethoxy-carbonyl (FMOC) protecting groups. This methodology is described by G.B. Fields et al. in *Synthetic Peptides: A User's Guide*, W.M. Freeman & Company, New York, NY, pp. 77-183 (1992).

A preferred partial peptide mimetic is characterized by having at least
25 one of the biological activities described herein. The biological activity of a polypeptide can be determined, for example, as described herein or by methods well known to one of skill in the art.

Compositions comprising one or more of the partial peptide mimetics of this invention with an optional carrier (e.g., a pharmaceutically acceptable
30 carrier) can be added to cells in culture or used to treat patients, such as mammals. Where the partial peptide mimetics are used to treat a patient, the partial peptide mimetic is preferably combined in a pharmaceutical composition

with a pharmaceutically acceptable carrier, such as a larger molecule to promote stability or a pharmaceutically acceptable buffer that serves as a carrier.

Treatment can be prophylactic or therapeutic. Thus, treatment can be initiated before, during, or after the development of the condition (e.g., bacterial infection or endotoxemia). As such, the phrases "inhibition of" or "effective to inhibit" a condition such as bacterial infection and/or endotoxemia, for example, includes both prophylactic and therapeutic treatment (i.e., prevention and/or reversal of the condition).

The partial peptide mimetics of the present invention can be administered alone or in a pharmaceutically acceptable buffer, as an antigen in association with another protein, such as an immunostimulatory protein or with a protein carrier such as, but not limited to, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin, or the like.

Partial peptide mimetics can be conjugated to other proteins using standard methods such as activation of the carrier molecule with a heterobifunctional sulfosuccinimidyl 4-(n-maleimidomethyl) cyclohexane-1-carboxylate reagent. Cross-linking of an activated carrier to a peptide can occur by reaction of the maleimide group of the carrier with the sulfhydryl group of a peptide containing a cysteine residue. Conjugates can be separated from nonconjugated molecules through the use of gel filtration column chromatography or other methods known in the art.

The partial peptide mimetics can be combined with a variety of physiological acceptable carriers for delivery to a patient including a variety of diluents or excipients known to those of ordinary skill in the art. For example, for parenteral administration, isotonic saline is preferred. For topical administration a cream, including a carrier such as dimethylsulfoxide (DMSO), or other agents typically found in topical creams that do not block or inhibit activity of the peptide, can be used. Other suitable carriers include, but are not limited to alcohol, phosphate buffered saline, and other balanced salt solutions.

The partial peptide mimetics of this invention that demonstrate biological activity can be administered in a variety of ways, including intravenously, topically, orally, and intramuscularly to a variety of mammals,

including humans, mice and rabbits. The partial peptide mimetics can be administered as a single dose or in multiple doses. Preferably the dose is an effective amount as determined by the standard methods described herein and includes about 1 microgram to about 1,000 micrograms pretreatment, more preferably about 50 to about 250 micrograms pretreatment. Those skilled in the art of clinical trials will be able to optimize dosages of particular partial peptide mimetics through standard trial studies.

Preferred partial peptide mimetics are anti-angiogenic. Angiogenesis is crucial to numerous biological functions in the body, from normal processes like embryogenesis and wound healing to abnormal processes like tumor growth, arthritis, restenosis, atherosclerosis, diabetic retinopathy, neovascular glaucoma, and endometriosis. The use of agents that can inhibit angiogenesis *in vitro* and *in vivo*, particularly in anti-tumor research, has indicated that anti-angiogenic therapy will be a promising therapeutic modality in the future. The search for angiogenic inhibitors has been focused on controlling two of the processes that promote angiogenesis: endothelial cell (EC) growth and adhesion primarily because ECs are more accessible than are other cells to pharmacologic agents delivered via the blood and ECs are genetically stable and are not easily mutated into drug resistant variants. Most anti-angiogenic agents have been discovered by identifying endogenous molecules, primarily proteins, which inhibit EC growth. This traditional approach has produced a number of anti-angiogenics, such as platelet factor-4 (PF4), thrombospondin, tumor necrosis factor (TNF), interferon- γ inducible protein-10, angiostatin, endostatin, vasostatin, and bactericidal-permeability increasing (BPI) protein. In total, about forty anti-angiogenic agents, identified using various approaches, are currently known.

It has also been postulated that tumor growth can be controlled by deprivation of vascularization (Folkman *J. Natl. Cancer. Inst.*, 82, 4-6 (1990); Folkman et al., *J. Biol. Chem.*, 267, 10931-10934 (1992)). A growing number of endogenous inhibitors of angiogenesis such as platelet factor-4 (PF4), interferon- γ inducible protein-10 (IP-10), thrombospondin-1 (TSP-1), angiostatin, as well as synthetic agents, e.g., thalidomide, TNP-470, and metalloproteinase inhibitors have been described. Some of these agents are

currently being tested in phase I/II clinical trials. Previous research described in Griffioen et al., *Blood*, 88, 667-673 (1996), and Griffioen et al., *Cancer Res.*, 56, 1111-1117 (1996) has shown that pro-angiogenic factors in tumors induce down-regulation of adhesion molecules on endothelial cells in the tumor

5 vasculature and induce energy to inflammatory signals such as tumor necrosis factor α (TNF- α), interleukin-1, and interferon- γ . EC exposed to vascular endothelial cell growth factor (VEGF) (Griffioen et al., *Blood*, 88, 667-673 (1996)) and basic fibroblast growth factor (bFGF) (Griffioen et al., *Blood*, 88, 667-673 (1996); and Melder et al., *Nature Med.*, 2, 992-997 (1996)) have a

10 severely hampered up-regulation of intercellular adhesion molecule-1 (ICAM-1) and induction of vascular cell adhesion molecule-1 (VCAM-1) and E-selectin. This phenomenon, which was named tumor-induced EC anergy, is one way in which tumors with an angiogenic phenotype may escape infiltration by cytotoxic leukocytes.

15 Because angiogenesis-mediated down-regulation of endothelial adhesion molecules (EAM) may promote tumor outgrowth by avoiding the immune response (Griffioen et al., *Blood*, 88, 667-673 (1996); Kitayama et al., *Cancer Res.*, 54 4729-4733 (1994); and Piali et al., *J. Exp. Med.*, 181, 811-816 (1995)), it is believed that inhibition of angiogenesis would overcome the down-

20 regulation of adhesion molecules and the unresponsiveness to inflammatory signals. In support of this hypothesis, a relation between E-selectin up-regulation and the angiostatic agent AGM-1470 has been reported (Budson et al., *Biochem. Biophys. Res. Comm.*, 225, 141-145 (1996)). It has also been shown that inhibition of angiogenesis by PF-4 up-regulates ICAM-1 on bFGF-

25 simulated EC. In addition, inhibition of angiogenesis by PF4 overcomes the angiogenesis-associated EC anergy to inflammatory signals.

Thus, the present invention provides a method for inhibiting endothelial cell proliferation in a patient (e.g., a mammal such as a human). This involves administering to a patient an amount of a composition (typically a

30 pharmaceutical composition) effective to inhibit the growth or endothelial cells, wherein the composition includes one or more partial peptide mimetics described herein. Analogously, the present invention provides a method for

inhibiting endothelial cell proliferation *in vitro* (e.g., in a cell culture). This method involves contacting cells with an amount of a composition effective to prevent and/or reduce the growth of endothelial cells, wherein the composition includes one or more partial peptide mimetics described herein.

5 For determining the amount of endothelial cell proliferation *in vivo*, various methods known to one of skill in the art could be used. For example, for evaluation of endothelial cell growth in tumors, tissue sections can be appropriately stained to quantify vessel density. For determining the amount of endothelial cell proliferation *in vitro*, an EC Proliferation Assay can be used that
10 involves the uptake of tritiated thymidine by cells in cell culture. A partial peptide mimetic that is "active" for inhibiting endothelial cell proliferation is preferably one that causes an at least 10% reduction in endothelial cell proliferation at a concentration lower than 10^{-4} M. Alternatively, inhibition of endothelial cell proliferation for an "active" partial peptide mimetic *in vitro* is
15 preferably at an IC₅₀ level of less than 80 μ M (more preferably less than 50 μ M, and even more preferably less than 25 μ M) as determined using the assay described in the Examples Section.

The present invention also provides a method for inhibiting angiogenic-factor mediated inter-cellular adhesion molecule (ICAM) expression down-
20 regulation (and/or promoting ICAM expression) in a patient (e.g., a mammal such as a human). This involves administering to a patient an amount of a composition effective to prevent and/or reduce the amount of ICAM expression down-regulation, wherein the composition includes one or more partial peptide mimetics described herein. Analogously, the present invention provides a
25 method for inhibiting angiogenic-factor mediated inter-cellular adhesion molecule expression down-regulation (and/or promoting ICAM expression) *in vitro* (e.g., in a cell culture). This method involves contacting cells with an amount of a composition effective to prevent and/or reduce the amount of ICAM expression down-regulation, wherein the composition includes one or
30 more partial peptide mimetics described herein.

The present invention provides a method for inhibiting angiogenesis (i.e., new blood vessel formation) in a patient (e.g., a mammal such as a

human). This involves administering to a patient an amount of a composition effective to prevent and/or reduce angiogenesis, wherein the composition includes one or more partial peptide mimetics described herein. Analogously, the present invention provides a method for inhibiting angiogenesis *in vitro* (e.g., in a cell culture). This method involves contacting cells with an amount of a composition effective to prevent and/or reduce angiogenesis, wherein the composition includes one or more partial peptide mimetics described herein.

For determining the amount of angiogenesis *in vivo*, various methods known to one of skill in the art could be used. For example, for evaluation of angiogenesis in tumors, tissue sections can be appropriately stained to quantify vessel density. For determining the amount of angiogenesis *in vitro*, an Angiogenesis Assay can be used that involves the disappearance of EC sprouting in cell culture. A polypeptide that is "active" for angiogenesis inhibition is preferably one that causes an at least 10% reduction in endothelial cell sprouting at a concentration lower than 10^{-4} M. Alternatively, inhibition of angiogenesis for a partial peptide mimetic *in vitro* is preferably at a level of less than 85% sprouting (more preferably less than 75% sprouting, even more preferably 50% sprouting, and even more preferably less than 35%) as determined using the collagen gel-based assay described in the Examples Section.

Similarly, such anti-angiogenic compositions can be used to control pathologic disorders such as atherosclerosis, restenosis, diabetic retinopathy, neovascular glaucoma, rheumatoid arthritis, and endometriosis. This can be demonstrated using standard techniques and models known to one of skill in the art.

The present invention provides a method for inhibiting tumorigenesis in a patient (e.g., a mammal such as a human). This involves administering to a patient an amount of a composition effective to prevent and/or reduce tumor growth, wherein the composition includes one or more partial peptide mimetics described herein. Methods of determining the inhibition of tumorigenesis are well known to those of skill in the art, including evaluation of tumor shrinkage, survival, etc.

The present invention provides a method for treating bacterial infection and/or endotoxemia in a patient (e.g., a mammal such as a human). This involves administering to a patient an amount of a composition effective to inhibit the bacterial infection and/or to neutralize endotoxin, wherein the
5 pharmaceutical composition includes one or more partial peptide mimetics described herein. Analogously, the present invention provides a method for inhibiting bacterial infection and/or endotoxemia *in vitro* (e.g., in a cell culture). This method involves contacting cells with an amount of a composition effective to inhibit the bacterial infection and/or to neutralize endotoxin,
10 wherein the composition includes one or more partial peptide mimetics described herein.

In both the *in vivo* and *in vitro* methods, "inhibiting" a bacterial infection includes preventing as well as reversing or reducing the growth of bacteria in a patient or a cellular sample, and "neutralizing" endotoxin includes binding LPS
15 and thereby removing it from the system of a patient or a cellular sample. The level of bacterial infection can be determined according to known bactericidal assays. The level of endotoxemia can be determined according to known LPS neutralization assays. These assays can be used to determine the effectiveness of a polypeptide, whether used *in vivo* or *in vitro*. To determine the
20 effectiveness of the treatment of a patient having a bacterial infection, a blood sample can be taken, a culture developed, and the amount of live bacteria determined. To determine the effectiveness of the treatment of a patient having endotoxemia, a blood sample can be taken, a culture developed, and the amount of cytokines (e.g., TNF- α , IL-1) can be determined using methods known to one
25 of skill in the art. For example, the WEHI assay can be used for the detection of TNF- α (Battafarano et al., *Surgery* 118, 318-324 (1995)).

The effective amount of a partial peptide mimetic of the present invention will depend on the condition being treated and on the desired result. For example, treating a bacterial infection will depend on the bacterial infection,
30 the location of the infection, and the partial peptide mimetic. An effective amount of the partial peptide mimetic for treating bacterial infection is that amount that diminishes the number of bacteria in the animal and that diminishes

the symptoms associated with bacterial infection such as fever, pain, and other associated symptoms of the bacterial infection. The effective amount of a peptide can be determined by standard dose response methods.

Alternatively, an effective amount of a partial peptide mimetic for
5 treating a bacterial infection can be determined in an animal system such as a mouse. Acute peritonitis can be induced in mice such as outbred Swiss webster mice by intraperitoneal injection with bacteria such as *P. aeruginosa* as described by Dunn et al. (*Surgery*, 98:283, 1985); Cody et al. (*Int. Surg. Res.*, 52:315, 1992). Bactericidal activity can be evaluated against a variety of
10 bacteria, preferably Gram-negative bacteria, but the types of bacteria can include *Pseudomonas spp* including *P. aeruginosa* and *P. cepacia*, *E. coli* strains, including *E. coli* B, *Salmonella*, *Proteus mirabilis* and *Staphylococcus* strains such as *Staphylococcus aureus*. Partial peptide mimetics with endotoxin neutralizing activity can be used to treat mammals infected with Gram-negative
15 bacteria systemically and that exhibit symptoms of endotoxin shock such as fever, shock, and TNF- α release.

Endotoxin neutralizing activity can be measured by determining the molar concentration at which the peptide completely inhibits the action of lipopolysaccharide in an assay such as the *Limulus* amoebocyte lysate assay
20 (LAL, Sigma Chemicals, St. Louis, MO) or the chromogenic LAL 1000 test (Biowhittacker, Walkersville, MD). Endotoxin neutralizing activity can also be measured by calculating an inhibitory dose 50 (LD₅₀) using standard dose response methods. An inhibitory dose 50 is that amount of peptide that can inhibit 50% of the activity of endotoxin.

25 The present invention also provides a method for inhibiting the amount of TNF- α in a patient (e.g., a mammal such as a human). This involves administering to a patient an amount of a composition effective to inhibit the amount of TNF- α in a patient's system as determined by evaluating serum levels of TNF- α , wherein the composition includes one or more partial peptide
30 mimetics described herein. Analogously, the present invention provides a method for inhibiting the amount of TNF- α *in vitro* (e.g., in a cell culture). This method involves incubating cells with an amount of a composition effective to

decrease TNF- α amounts in the cell culture, wherein the composition includes one or more partial peptide mimetics described herein. For both *in vivo* and *in vitro* methods, the WEHI assay can be used for the detection of TNF- α (Battafarano et al., *Surgery*, 118, 318-324 (1995)) in cell culture or in serum
5 from a patient. Alternatively, the amount of TNF- α in a sample can be assayed using an anti-TNF- α antibody. A partial peptide mimetic "active" for decreasing TNF- α can be evaluated using an *in vitro* test, and preferably shows an at least 10% decrease in the amount of TNF- α .

The invention will be further described by reference to the following
10 detailed examples. These examples are offered to further illustrate the various specific and preferred embodiments and techniques. It should be understood, however, that many variations and modifications may be made while remaining within the scope of the present invention.

15 *Examples*

EXPERIMENTAL PROTOCOL

Peptide Synthesis.

Peptides were synthesized using a Milligen/Biosearch 9600 peptide solid-phase synthesizer using fluorenylmethoxycarbonyl chemistry. Lyophilized
20 crude peptides were purified by preparative reversed-phase HPLC on a C18 column with an elution gradient of 0-60% acetonitrile with 0.1% trifluoroacetic acid in water. The purity and composition of the peptides were verified by HPLC (Beckman Model 6300) analysis of amino acid composition of hydrolysates prepared by treating the peptides under argon in 6 Normal (6 N)
25 HCl for 24 hours at 110°C. The amino acid sequences of peptides were confirmed by N-terminal sequencing and mass spectrometry.

Synthesis of DBF Analogs.

Unexceptional phases of solid-phase peptide synthesis (SPPS) were
30 carried out on a Milligen/Biosearch 9600 peptide synthesizer using fluorenylmethoxy-carbonyl (Fmoc) methodology and BOP/HOBT as coupling reagents. Fmoc-DBF-CO₂H (1) was prepared by slight modification of the

reported method (H. Bekele et al., *J. Org. Chem.*, 62, 2259-2262 (1997)). In this nine-step synthesis, the intermediates and final product were characterized by TLC and ^1H NMR, with GC-LRMS, ^{13}C NMR, melting points (mp), and IR being used as appropriate. Coupling of **1** to L₂₀ (isoleucine at residue 20 of the β pep-25 sequence) as well as of L₁₁ (leucine at residue 11 of the β pep-25 sequence) to the peptide-DBF-NH₂ was performed on the synthesizer. Coupling of Fmoc-K₁₀-CO₂H (wherein K₁₀ refers to the lysine at residue 10 of the β pep-25 sequence) to the peptide-DBF-L₁₁-NH₂ sequence was difficult and required manual SPPS using the more reactive HATU reagent (L.A. Carpino, *J. Am. Chem. Soc.*, 115, 4397-4398 (1993)).

The remaining chemical couplings used for production of the DBF analogs listed in Table 1 were carried out using BOP/HOBT conditions on the peptide synthesizer. After the final Fmoc deprotection, each of the DBF peptide analogs (partial peptide mimetics) was released from the resin with simultaneous removal of all acidolyzable trityl and *tert*-butyl side-chain protecting groups using Reagent K (D.S. King et al., *Int. J. Pept. Protein. Res.*, 36, 255-266 (1990)). A Rink amide or similar resin was used to provide the primary amide form of the C-terminal D24 unit. Lyophilized crude peptides were purified by preparative reversed-phase HPLC on a C18 column using a Hewlett-Packard 1090 system. An elution gradient of 0-60% acetonitrile in water (0.1% trifluoroacetic acid) was used. The purity and composition of the peptides was verified by analytical HPLC, matrix assisted laser desorption ionization (MALDI) mass spectrometry using a Hewlett-Packard G2025A system and sinapinic acid as matrix, and analysis of amino acid composition of hydrolysates (6N HCl, 110°C, 24 hours (h), under argon).

NMR Spectroscopy.

For NMR measurements, freeze-dried DBF analogs were dissolved in water-DMSO-DPC mixture. Peptide concentration was 3 mM. pH was adjusted to pH 5.7 by adding μL quantities of NaOD or HCl to the peptide sample. NMR spectra were acquired on a Varian UNITY Plus-600 NMR spectrometer.

2D-homonuclear TOCSY with DIPSI (M. Piotto et al., *J. Biomol. NMR*, 2, 661-665 (1992)) spinlock (mixing time 80 milliseconds (ms)) was used to identify spin systems. 2D NOESY experiments (G. Wider et al., *J. Magn. Reson.*, 56, 207-234 (1985)) were performed for sequential assignments and conformational analysis. WATERGATE (A.J. Shaka et al., *J. Magn. Reson.*, 77, 274-293 (1988); and S.P. Rucker et al., *Mol. Phys.*, 68, 509-517 (1989)) was used to attenuate the water resonance. All spectra were collected at 25°C as 256 to 512 t1 experiments, each with 2048 complex data points over a spectral width of 6 kHz in both dimensions with the carrier placed on the water resonance. Sixteen scans were time averaged per t1 experiment. Data were processed directly on the spectrometer using VNMR (Varian, Inc., Palo Alto) or NMRPipe (F. Delaglio et al., *J. Biomol. NMR*, 6, 277-293 (1995)) on an SGI workstation.

Structural Modeling.

Analysis of NOE growth curves indicated that backbone to backbone inter-proton NOEs were normally maximum at about 200 ms. Interproton distance constraints were derived from NOEs assigned in ¹H NOESY spectra acquired with mixing times of 100 ms. NOEs were classified as strong, medium, weak, or very weak corresponding to upper bound distance constraints of 2.8, 3.3, 4.0, and 4.5 Å, respectively. The lower bound restraint between non-bonded protons was set to 1.8 Å. Pseudo-atom corrections were added to the upper bound distance constraints where appropriate, and a 0.5 Å correction was added to the upper bound for NOEs involving methyl protons. Hydrogen bond constraints were identified from the pattern of sequential and interstrand NOEs involving NH and CaH protons, together with evidence of slow amide proton-solvent exchange. Each hydrogen bond identified was defined using two distance constraints; $r_{\text{NH-O}} = 1.8$ to 2.5 Å, and $r_{\text{N-O}} = 1.8$ to 2.5 Å.

Derived internuclear distance constraints were used in calculating structures for 6DBF7 by using X-PLOR (A.T. Brunger, *X-plor Manual*, Yale University Press, New Haven (1992)). The molecule was created using paralhdcg.pro force fields. A template coordinate set was generated by using the *Template* routine. The *ab initio* simulated annealing (SA) protocol was then

used. The SA procedure ran high temperature dynamics (3000 K for 120 picoseconds (ps)) and then cooled down to 100 K in 50 K steps with 1.5 ps molecular dynamics at each step. Powell minimization was performed at 100 K for 1000 steps. Structure refinement was done based on simulated annealing starting at 1000 K and ending at 100 K. Final structures were subjected to the X-PLOR Accept routine with the violation threshold for NOEs of 0.5 Å and dihedral angles of 5°. Angles, bond lengths or impropers were not allowed to deviate from ideal geometry more than 5°, 0.05 Å and 5°, respectively. Structures were superimposed using the BIOSYM INSIGHT viewer (Molecular Simulations, Inc.) and were analyzed using X-PLOR analysis routines.

Cells, Cultures, and Reagents.

Human umbilical vein derived EC (HUVEC) were harvested from normal human umbilical cords by perfusion with 0.125% trypsin/EDTA. Harvested HUVECs were cultured in gelatin coated tissue culture flasks and subcultured 1: 3 once a week in culture medium (RPMI-1640 with 20% human serum (HS), supplemented with 2 mM glutamine and 100 U/ml penicillin and 0,1 mg/ml streptomycin). Bovine capillary EC (BCE) were kindly provided by Dr. M. Furie (State University of New York, Stony Brook, USA) and were cultured in fibronectin coated tissue culture flasks in RPMI-1640 medium containing 10% FCS, glutamine and antibiotics.

EC Proliferation Measurement.

EC proliferation was measured using a [³H]- thymidine incorporation assay. Proliferation of bFGF-stimulated (10 nanogram per milliliter (ng/ml)) human umbilical vascular EC (HUVEC) cultures was measured by quantification of ³H-thymidine incorporation. Proliferation is expressed as mean counts per minute (cpm) of quadruplicate cultures in three independent experiments (± SEM). EC were seeded at 5000 cells/well in flat-bottomed tissue culture plates and grown for 3 days in the absence or presence of regulators, in culture medium. During the last 6 hours of the assay, the culture was pulsed with 0.5 μCi [methyl-³H]- thymidine/well. Human umbilical vein

derived EC (HUVEC) were harvested from normal human umbilical cords by perfusion with 0.125% trypsin/EDTA. Harvested HUVECs were cultured in gelatin coated tissue culture flasks and subcultured 1: 3 once a week in culture medium (RPMI-1640 with 20% human serum (HS), supplemented with 2 mM
5 glutamine and 100 U/ml penicillin and 0.1 mg/ml streptomycin).

In vitro Angiogenesis Assay.

Sprouting and tube formation of bovine EC (BCE) were studied using cytodex-3 beads overgrown with BCE in a 3-dimensional collagen gel
10 (vitrogen-100, Collagen Corp., Fremont, CA, USA) as described in D. Van der Schaft et al., *Blood*, 96, 176-181 (2000). Following gelation, culture medium containing 20 ng/mL bFGF, with or without β pep-25 or DBF analogs, was applied on top of the gel. After 24 hours of cell culture at 37°C, photographs were made (not shown). The amount of sprouting in each well (i.e., the total
15 length of the sprouts) was quantified by the computer program NIH image. To quantify differences in sprouting and tube formation, statistical analysis was performed using the Mann-Whitney U test. Using these data the IC₅₀ on EC proliferation and tube formation were quantified and are listed in Table 1.

20 Tumor Model Studies.

In all studies, female athymic nude mice (nu/nu, 5-6 weeks old) were used. These mice were purchased from the National Cancer Institute and allowed to acclimatize to local conditions for at least one week. Animals were given water and standard chow *ad libitum*, and were kept on a 12-hour
25 light/dark cycle. All experiments were approved by the University of Minnesota Research Animal Resources ethical committee. Mice were randomized and split into three groups: 1) human serum albumin (10 mg/kg/day), 2) β pep-25 (10 mg/kg/day) and 3) DBF analog (10 mg/kg/day). Compounds were diluted in 100 mM SDS and administered using osmotic mini-pumps (Durect, Cupertino,
30 CA). Exponentially growing MA148 human ovarian carcinoma cells, kindly provided by Prof. Ramakrishnan (R.P. Dings et al., *Cancer Res.*, 63, 382-385 (2003)) were cultured in RPMI 1640 medium (Life Technologies, Grand Island,

NY). This medium was supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Cellgro, Mediatech, Washington, DC) at 37°C and 5% CO₂. One hundred microliters (100 μL) of this tumor cell suspension (2 x 10⁷ cells/ml) was then injected subcutaneously into the right flank of each mouse.

- 5 Pumps were implanted into the left flank of mice for subcutaneous administration of compound over a 28-day treatment span.

Two variants of this model were used: prevention and intervention. For the prevention variant, treatment was initiated at the time of inoculation with MA148 cells. For the intervention variant, tumors were allowed to grow to an average size of 50 mm³ (usually day 7 post inoculation) before treatment was initiated. With either variant, animals were randomized prior to the initiation of treatment. Treatment was administered via osmotic mini-pumps (Durect, Cupertino, CA), which were implanted subcutaneously in the left flank of mice. Concentrated solutions of βpep-25 or DBF analogs were formulated such that the 28-day treatment period would be covered by implantation of a single pump. In each study, control groups of animals were administered either PBS or PBS containing human serum albumin. Tumor growth curves were found to be virtually identical in either of these control cases.

Tumor volume was determined by measuring the diameters of tumors using calipers (Scienceware, Pequannock, NJ) using the equation for the volume of a spheroid: $(a^2 \times b \times \Pi) / 6$, where 'a' is the width and 'b' the length of the tumor. Measurements were performed two or three times per week. At the conclusion of an experiment, tumor weights were also taken following excision of the tumors from euthanized animals. Tumor weights correlated well with tumor volumes calculated in this way.

Immunohistochemistry.

Immunohistochemistry was used to assess microvessel density and the extent of total cell apoptosis. Tumor tissue was embedded in tissue freezing medium (Miles Inc, Elkart, IN) and shock frozen in liquid nitrogen. Ten millimeter (10 mm) thick sections of tissue were prepared for immunohistochemical analysis. For this, tissue sections were brought to room

temperature, air dried overnight, and then fixed in acetone for 10 minutes. Slides were allowed to air dry for at least 30 minutes and were washed three times for 5 minutes each in phosphate-buffered saline (PBS, pH 7.4). Samples were then blocked with PBS containing 0.1% bovine serum albumin and 3%
5 human serum albumin for at least 30 minutes at room temperature in a humidified box. Samples were subsequently incubated with phycoerythrin (PE)-conjugated monoclonal antibody to CD31 (PECAM-1) in a 1:50 dilution (Pharmingen, San Diego, CA) to stain for microvessel density. After 1-hour incubation at room temperature, slides were washed with PBS and immediately
10 imaged using an Olympus BX-60 fluorescence microscope at 200X magnification.

To assess the extent of total cell apoptosis, tissue sections were stained by using the TUNEL (terminal deoxyribonucleotidyl transferase-mediated dUTP-nick-end labeling) assay, which was performed according to the
15 manufacturer's instructions (*in situ* cell death detection kit, fluorescein; TUNEL, Roche). Digital images were stored and processed using Adobe Photoshop (Adobe Inc., Mountain View CA).

Quantification of microvessel density, the rate of proliferation and total cell apoptosis were determined as described earlier (R. Wild et al., *Microvasc.*
20 *Res.*, 59, 368-376 (2000)). Statistical analysis was performed using the Student's *t* test.

Toxicity Assays.

As an indirect measurement of general toxicity, body weights of mice
25 were monitored twice weekly, using a digital balance (Ohaus Florham, NJ). To determine hematocrit and creatinine levels, blood samples were extracted by tail vein bleedings one day after terminating treatment and blood was collected in heparinized micro-hematocrit capillary tubes (Fisher; Pittsburgh, PA). For hematocrit levels, samples were spun down for 10 minutes in a micro-
30 hematocrit centrifuge (Clay-Adams; NY), and the amount of hematocrit was determined using an international microcapillary reader (IEC; Needham, Mass). To obtain creatinine levels, a kit was purchased from Sigma (Sigma

Diagnostics; St Louis, MO) and used according to the manufacturer's instructions.

RESULTS AND DISCUSSION

5 Detailed Structure-Activity Evaluation.

For input into designing the partial peptide mimetics of the present invention, a complete structure-activity analysis with β pep-25 was performed. Working with this relatively small peptide allowed for a thorough assessment of desirable residues using alanine scanning and walk-through variants.

10 Dodecapeptide walk-through variants that walk through the β pep-25 sequence, shifting three residues in each peptide were prepared and are listed in Figure 1A. Their effect on EC proliferation is also indicated in Figure 1A with IC50 values. Only three walk-through peptides demonstrated any significant anti-proliferative activity relative to β pep-25, and, as with results from alanine
15 scanning, these peptides also encompass β -strands 1 and 2. Based on these results, it can be concluded that activity is localized within β -strands 1 and 2. For orientation, the β -strand alignment for β pep-25 is depicted in Figure 1B, with key sequences being boxed-in.

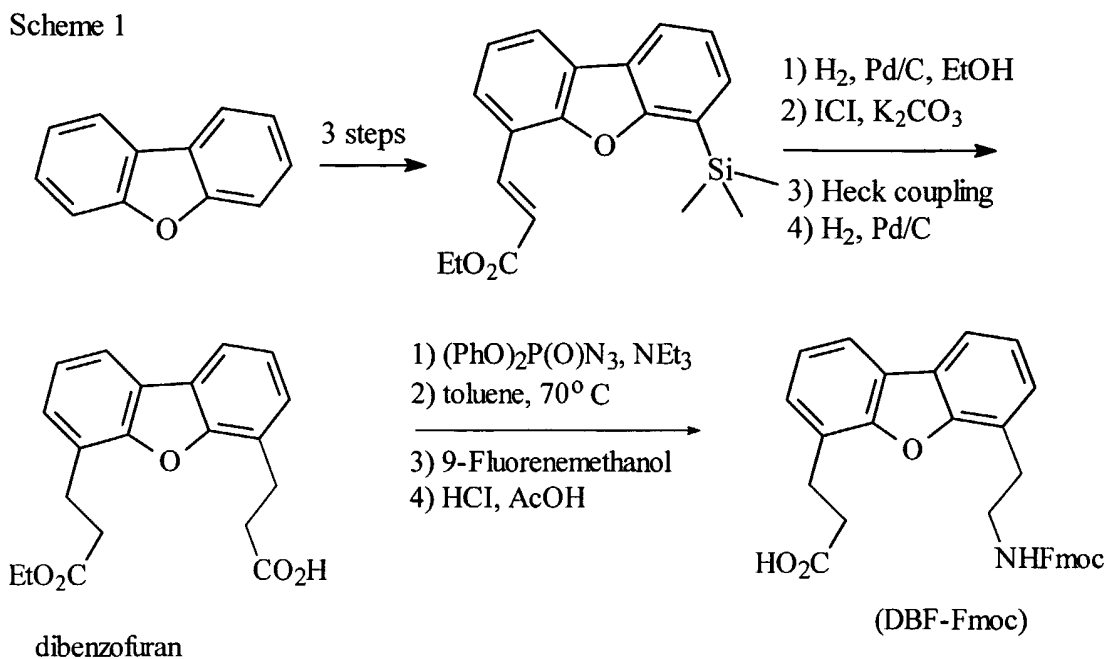
Residues that demonstrated the most significant drop in the ability of
20 β pep-25 to inhibit EC proliferation are hydrophobic residues within the first two β -strands: I₃, L₅, V₇, L₁₁, and I₂₀ (these refer to the specific amino acid residues at the position indicated by the subscript in β pep-25). Conformationally, these hydrophobic residues lie on the same face of the amphipathic anti-parallel β -sheet.

25 Using these structure-activity relationships, a series of new molecules were designed. In these, the β -strand 3 and turn 2 of β pep-25 were omitted and a dibenzofuran (DBF) β -turn mimetic (H. Diaz et al., *J. Am. Chem. Soc.*, 115, 3790-3791 (1993); and K.Y. Tsang et al., *J. Am. Chem. Soc.*, 116, 3988-4005 (1994)) was used in place of turn 1 and the remainder of β -strand 1 (residues 1-
30 11 of β -pep-25 or SEQ ID NO:1) and β -strand 2 (residues 20-26 of β pep-25 or

SEQ ID NO:1). The DBF β -turn mimetic was used in order to maintain the bioactive β -sheet conformation of β pep-25.

A number of β -turn inducing scaffolds are known. The dibenzofuran scaffold developed by Kelly et al. (H. Diaz et al., *J. Am. Chem. Soc.*, 115, 3790-3791 (1993); and K.Y. Tsang et al., *J. Am. Chem. Soc.*, 116, 3988-4005 (1994)) is an α , ω -amino acid that has been incorporated into peptides of varying length by both the Kelly group and others. Notably, replacement of two, β -turn-inducing, natural amino acids in the protein scyllatoxin (a 31-mer) by the DBF scaffold provided an analog whose secondary structure and activity were indistinguishable from the parent toxin. Kelly has demonstrated that when the DBF unit is inserted between a pair of lipophilic amino acid residues, a "hydrophobic cluster" is created wherein the sidechains of those residues are nested within the hydrophobic pocket created by the aromatic rings of the canted DBF subunit. Because of the desire to design peptide analogs of relatively small size, DBF is an excellent choice since it could be inserted between residues I₂₀ and L₁₁ of β pep-25. The lipophilic isoleucine at residue 20 is one of the important, hydrophobic residues associated with β pep-25 anti-angiogenic activity.

The Fmoc-protected version of the Kelly DBF is shown below in Scheme 1 with several subtle improvements made in the synthesis (bolded conditions).



5

The parent DBF-based compound depicted in Figure 1C is called 11DBF7, where numbers at the left and right of DBF refer to the number of amino acid residues in the N- and C-terminal strands from β pep-25, respectively, with the DBF turn mimic replacing amino acid residues 12-19 of β pep-25, SEQ ID NO:1. To identify the shortest sequences required for bioactivity, a series of N- and C-terminal deletion variants of 11DBF7 were made as listed in Table 1. In the provisional application to which this application claims priority, some of these were referred to as CF-1 through CF-8. Furthermore, in the provisional application to which this application claims priority, the segments of SEQ ID NO:1 were written in reverse order (i.e., from the C-terminus to the N-terminus), which upon reading the context of the provisional application would be clear to one of skill in the art. For example, CF-1 was written as DN²⁵LKVII[DBF]L, wherein the N²⁵ indicates the asparagine is residue 25 of β pep-25 or SEQ ID NO:1.

Table 1. N- and C-terminal Deletion Variants of 11DBF7 and Angiostatic Potential.

<u>Compound</u>	<u>Amino Acid Sequence</u>	<u>EC Proliferation (IC50)</u> IC50	<u>% Inhibition</u> at 25 μ M	<u>% Sprouting</u>
5	β -pep-25 (parent)	3 μ M	90%	8 \pm 5%*
	ANIKLSVQMMLFKRHLKWKIIVKLNDRGRLSLD (SEQ ID NO:1)			
10	<u>DBF Analogs</u>			
	11DBF7 (CF-8)	12 μ M	92%	23 \pm 10%*
	ANIKLSVQMML-[DBF]-IIVKLND (SEQ ID NO:8)-[DBF]-(SEQ ID NO:2)			
	10DBF7 (CF-7)	12 μ M	69%	57 \pm 17.5%*
	NIKLSVQMML-[DBF]-IIVKLND (SEQ ID NO:7)-[DBF]-(SEQ ID NO:2)			
15	9DBF7 (CF-6)	20 μ M	57%	76 \pm 40%
	IKLSVQMML-[DBF]-IIVKLND (SEQ ID NO:6)-[DBF]-(SEQ ID NO:2)			
	6DBF7 (CF-4)	15 μ M	68%	60 \pm 12%*
	SVQMML-[DBF]-IIVKLND (SEQ ID NO:5)-[DBF]-(SEQ ID NO:2)			
20	4DBF7 (CF-3)	25 μ M	46%	99 \pm 5%
	QMKL-[DBF]-IIVKLND (SEQ ID NO:4)-[DBF]-(SEQ ID NO:2)			
	3DBF7 (CF-2)	----- ^a	0%	84 \pm 30%
	MKL-[DBF]-IIVKLND [DBF]-(SEQ ID NO:2)			
25	1DBF7 (CF-1)	-----	0%	-----
	L-[DBF]-IIVKLND [DBF]-(SEQ ID NO:2)			

11DBF6	ANIKLSVQMKL-[DBF]-IIVKLN (SEQ ID NO:8)-[DBF]-(SEQ ID NO:9)	22 µM	ND	
11DBF4	ANIKLSVQMKL-[DBF]-IIVK (SEQ ID NO:8)-[DBF]-(SEQ ID NO:3)	35 µM	ND	
5	ANIKLSVQMKL-[DBF]-I (SEQ ID NO:8)-[DBF]	23 ± 5µM	ND	
10	6DBF6	SVQMKL-[DBF]-IIVKLN (SEQ ID NO:5)-[DBF]-(SEQ ID NO:9)	42 µM	ND
	6DBF4	SVQMKL-[DBF]-IIVK (SEQ ID NO:5)-[DBF]-(SEQ ID NO:3)	----	ND
	6DBF3	SVQMKL-[DBF]-IIV (SEQ ID NO:5)-[DBF]	----	ND
15	6DBF2	SVQMKL-[DBF]-II (SEQ ID NO:5)-[DBF]	----	ND
	6DBF1	SVQMKL-[DBF]-I (SEQ ID NO:5)-[DBF]	76.1 ± 10.9µM	ND

* Significant inhibition (p < 0.03; Mann-Whitney U test).

^a No effect on the assay; ND = Not Done.

β -Sheet Structure is Preserved in DBF Analogs.

NMR spectroscopy was used to investigate whether β -sheet conformation was preserved in DBF analogs. Analogs 11DBF7 and 6DBF7 that have more equivalent β -strand lengths, were the focus of this structural study.

5 Due to limited water solubility and the desire to mimic a membrane-like environment, these compounds were investigated in dodecylphosphocholine (DPC) micelles. At the millimolar concentrations required for NMR work, 11DBF7 gave overlapping resonances that made spectral analysis ambiguous. 6DBF7 gave excellent NMR spectra that allowed complete structure analysis.

10 NOEs and coupling constants diagnostic of anti-parallel β -sheet conformation were readily identified and used in computational modeling. Superposition of the resulting 28 structures is shown in Figure 2A, along with a simplified illustration of this folding pattern in Figure 2B, which highlights residues on both hydrophobic and hydrophilic surfaces of the β -sheet. Notice in this

15 structure that the two aliphatic hydrophobic residues Leu and Ile in 6DBF7 are packed against the phenyls of the DBF group. In effect, this sets up and helps stabilize the β -sheet fold. Based on this structural information on 6DBF7, it can be concluded that other DBF analogs would fold similarly, albeit to various extents depending on the lengths of the two strands, i.e., strands of equal length

20 are expected to be better able to form β -sheets.

DBF Analogs Inhibit EC Proliferation and Retain Anti-angiogenic Activity.

In endothelial cell (EC) proliferation assays, it was demonstrated that 11DBF7, as well as a number of shorter analogs, are effective at inhibiting EC

25 growth. Dose response curves for all xDBF7 analogs are exemplified in Figure 3, and IC_{50} values for all DBF analogs (i.e., partial peptide mimetics) and percent inhibition of EC growth at 25 μ M are given in Table 1. Compared to parent β -pep-25 (IC_{50} , 3 μ M), most DBF analogs, although slightly less active (IC_{50} about 10 μ M), do perform relatively well in this assay. This may, in part,

30 be explained by their decreased size. In addition, some of the analogs are missing residues identified by alanine scanning as being functionally important

(e.g., L₅ and I₃). Nonetheless, a number of these shorter analogs remain reasonably active, and it appears that the N-terminal hexapeptide SVQMKL (SEQ ID NO:5) and C-terminal tetrapeptide IIVK (SEQ ID NO:3) enhance anti-angiogenic activity.

5 Angiostatic potential was further demonstrated *in vitro* in the collagen gel-based sprout formation assay (R.P. Dings et al., *Cancer Res.*, 63, 382-385 (2003)). Whereas the control showed numerous sprouts, treatment with β pep-25, 11DBF7 and 6DBF7 all demonstrated highly reduced sprouting (data not shown). Quantitative results for all analogs are given in Table 1. In general,
10 reducing the number of amino acid residues in the β -strands led to reduced inhibition of sprout formation, comparable with the proliferation assay. However, some analogs were active and even one of the shortest analogs, 6DBF7, had a significant inhibitory effect on tube formation. The kinetics of inhibition using DBF analogs, moreover, were the same as those for β pep-25
15 (data not shown).

DBF Analogs Inhibit Tumor Growth in Mice.

 Because specific use of an anti-angiogenic agent is in the area of tumor biology, the *in vivo* efficacy of two of the most *in vitro* active DBF analogs,
20 11DBF7 and 6DBF7, were assessed along with β pep-25 as a positive control, in the MA148 xenograft ovarian carcinoma tumor model in athymic mice as previously described in R.P. Dings et al., *Cancer Res.*, 63, 382-385 (2003). Initial experiments using this model administered the DBF analog, 11DBF7, subcutaneously via mini-pumps implanted at the time of inoculation with the
25 tumor cell line. This prevention model demonstrated that treatment of tumor forming animals with 11DBF7 resulted in inhibition of tumor growth (Figure 4A). Surprisingly, 11DBF7 functioned, on average, slightly better than β pep-25 by reducing tumor volume by up to 90% relative to control animals.

 In further experiments, treatment was initiated seven days following
30 inoculation with tumor cells to allow establishment of small tumors prior to the start of treatment. Using this protocol, β pep-25, 11DBF7, as well as 6DBF7,

were tested and were found to inhibit tumor growth by up to 70% during the course of treatment and by about 50% at the end of the four-week administration period (day 35) (Figure 4B). The rate of tumor growth then began to increase, but remained at about 50% ten days post-treatment when animals were sacrificed for analysis of tumor tissue. Interestingly, the smaller DBF analog, 6DBF7, was observed to be slightly more effective at inhibiting tumor growth than β pep-25 or 11DBF7. On day 35, for example, 6DBF7 sustained tumor growth inhibition to more than 70% relative to tumors from control animals (Figure 4B).

In vivo anti-angiogenic potential was demonstrated by staining tumor cross-sections from treated animals with fluorescently-labeled anti-CD31 antibody used immunohistochemically to identify blood vessels. Vessel density, relative to control, was significantly reduced by treatment with β pep-25, 11DBF7, or 6DBF7. In addition, these anti-angiogenic compounds had a significant effect as well on vessel architecture, demonstrating a drop in the number of end points, branch points and vessel length (Table 2). In addition, anti-angiogenic treatment also reduced the rate of proliferation of tumor cells as determined by immunohistochemical staining of PCNA in cryosections of tumors (Table 2). As a result of angiogenic inhibition, the number of apoptotic tumor cells increased from 311 ± 103 in the control, to 620 ± 146 and 851 ± 162 in β pep-25 and 6DBF7 treated animals, respectively.

Table 2.

Microvessel density and proliferation rate in tumors of treated mice

	Proliferation ^a	Vessel Density ^b	End Points ^c	Branch Points ^d	Vessel Length ^e
Vehicle	848 ± 104	5858 ± 656	26.2 ± 2.2	7.6 ± 1.4	5.9 ± 0.7
β pep-25	414 ± 44	2245 ± 329	22.9 ± 2.2	2.1 ± 0.6	0.9 ± 0.3
11DBF7	553 ± 75	2879 ± 385	21.2 ± 3.3	3.1 ± 0.9	3.0 ± 0.6
6DBF7	501 ± 68	2213 ± 256	21.7 ± 2.6	2.3 ± 1.1	2.5 ± 0.4

^a After binarization of the images of the PCNA-staining, proliferation was estimated by scoring the total number of white pixels per field.

^b After binarization of the images of the CD31-staining, microvessel density was estimated by scoring the total number of white pixels per field.

5 ^c Mean number of vessel end points as determined after skeletonization of the images (R. Wild et al., *Microvasc. Res.*, 59, 368-376 (2000)).

^d Mean number of vessel branch points/nodes per image.

^e Mean total vessel length per image.

All results are expressed as mean pixel counts per image (\pm standard error).

10

In all *in vivo* experiments, treatment with β pep-25, 11DBF7, and 6DBF7 did not show any sign of toxicity as assessed by unaltered behavior and normal weight gain during experiments (data not shown). Moreover, hematocrit and
15 creatinine levels in treated animals were normal relative to control, indicating the absence of toxicity to bone marrow and kidney. Macro- and microscopic morphology of internal organs on autopsy were also observed to be normal within all experimental groups of animals.

20

Conclusion

In summary, amino acid residues that promote β pep-25's angiostatic activity have been identified, and partial peptide mimetics of β pep-25 have been designed and synthesized. The more effective mimetics carried only 13 of its 33 amino acid residues and showed that the partial peptide mimetic is an effective
25 anti-angiogenic agent both *in vitro* and *in vivo*. Moreover, whereas *in vitro* activity is somewhat less than that of β pep-25, *in vivo* activity appears to be improved, suggesting increased bioavailability with DBF analogs. Both the significant reduction in the number of residues required to promote angiostatic activity and the use of an organic β -sheet-inducing scaffold allows for the
30 development of an orally-active, small molecule mimetic of an anti-angiogenic protein.

The DBF analogs not tested in these analyses would be expected by one of skill in the art to provide some activity in at least one of the assays due to the homology of their structures. Also, although not shown, DBF analogs of the type described herein demonstrate some bacteriocidal activity and some
5 endotoxin neutralizing activity. Furthermore, because of the results shown herein, DBF analogs of the type described herein would be expected by one of skill in the art to inhibit TNF- α levels, promote inter-cellular adhesion molecule (ICAM) expression, and inhibit inter-cellular adhesion molecule (ICAM) expression down regulation. Also, particularly because of the results with
10 respect to the inhibition of angiogenesis, DBF analogs of the type described herein would be expected by one of skill in the art to inhibit pathologic disorders such as atherosclerosis, restenosis, diabetic retinopathy, neovascular glaucoma, rheumatoid arthritis, and endometriosis.

15 All references cited herein are incorporated by reference, in their entirety, into this text. Although the invention has been described in the context of particular embodiments, it is intended that the scope of coverage of the patent be limited only by reference to the following claims.

20

Sequence Listing Free Text
Amino Acid Sequence of Peptides

5 βpep-25 (SEQ ID NO:1)
 ANIKLSVQMKLFKRHLKWKIIVKLNDGRELSLD

 (SEQ ID NO:2)
 IIVKLND

10 (SEQ ID NO:3)
 IIVK

 (SEQ ID NO:4)
 QMKL

15 (SEQ ID NO:5)
 SVQMKL

 (SEQ ID NO:6)
20 IKLSVQMKL

 (SEQ ID NO:7)
 NIKLSVQMKL

25 (SEQ ID NO:8)
 ANIKLSVQMKL

 (SEQ ID NO:9)
 IIVKLN

30

WHAT IS CLAIMED IS:

1. A partial peptide mimetic comprising a first amino acid sequence comprising: ANIKLSVQMKL (SEQ ID NO:8), a homolog thereof, or a
5 segment of SEQ ID NO:8 or a homolog thereof; a second amino acid sequence comprising IIVKLND (SEQ ID NO:2), a homolog thereof, or a segment of SEQ ID NO:2 or a homolog thereof; and a β -turn inducing scaffold bonded between the first and second amino acid sequences; wherein the homologs or segments provide the partial peptide mimetic with at least one of the following activities:
10 inhibition of endothelial cell proliferation *in vitro* at an IC50 level of less than 80 μ M; inhibition of angiogenesis *in vitro* at a level of less than 85% sprouting in a collagen gel-based assay; or reduction in tumor volume *in vivo* by at least 25% relative to a control at the end of an administration period.
- 15 2. The partial peptide mimetic of claim 1 wherein the first amino acid sequence comprises at least three amino acids of SEQ ID NO:8 or a homolog thereof.
3. The partial peptide mimetic of claim 2 wherein the first amino acid
20 sequence comprises SEQ ID NO:8, or a homolog thereof, from which a deletion has been made at the N-terminus by 1, 2, 3, 4, 5, 6, 7, or 8 residues.
4. The partial peptide mimetic of claim 3 wherein the first amino acid
25 sequence comprises at least one sequence selected from the group consisting of MKL, QMKL (SEQ ID NO:4), SVQMKL (SEQ ID NO:5), IKLSVQMKL (SEQ ID NO:6), and NIKLSVQMKL (SEQ ID NO:7).
5. The partial peptide mimetic of claim 1 wherein the first amino acid
30 sequence comprises ANIKLSVQMKL (SEQ ID NO:8) or a homolog thereof and the second amino acid sequence comprises IIVKLND (SEQ ID NO:2) or a homolog thereof.

6. The partial peptide mimetic of claim 1 wherein the second amino acid sequence comprises at least one amino acid of SEQ ID NO:2 or a homolog thereof.
- 5 7. The partial peptide mimetic of claim 6 wherein the second amino acid sequence comprises SEQ ID NO:2, or a homolog thereof, from which a deletion has been made at the C-terminus by 1, 2, 3, 4, 5, or 6 residues.
8. The partial peptide mimetic of claim 7 wherein the second amino acid
10 sequence comprises at least one sequence selected from the group consisting of I, IIVK (SEQ ID NO:3), and IIVKLN (SEQ ID NO:9).
9. The partial peptide mimetic of claim 8 wherein the first amino acid
15 sequence comprises at least one sequence selected from the group consisting of MKL, QMKL (SEQ ID NO:4), SVQMKL (SEQ ID NO:5), IKLSVQMKL (SEQ ID NO:6), and NIKLSVQMKL (SEQ ID NO:7).
10. The partial peptide mimetic of claim 1 wherein the β -turn inducing scaffold is a dibenzofuran-containing scaffold.
- 20
11. A partial peptide mimetic comprising a first amino acid sequence consisting of ANIKLSVQMKL (SEQ ID NO:8) or a segment thereof, a second amino acid sequence consisting of IIVKLND (SEQ ID NO:2) or a segment thereof, and a β -turn inducing scaffold bonded between the first and second
25 amino acid sequences, wherein the segment of SEQ ID NO:8 is one in which a deletion has been made at the N-terminus by 1, 2, 3, 4, 5, 6, 7, or 8 residues, and wherein the segment of SEQ ID NO:2 is one in which a deletion has been made at the C-terminus by 1, 2, 3, 4, 5, or 6 residues.
- 30 12. A partial peptide mimetic comprising a first amino acid sequence having at least 4 amino acids of the sequence QMKL (SEQ ID NO:4), a second sequence having at least the amino acid I, and a β -turn inducing scaffold bonded

therebetween, wherein the partial peptide mimetic has at least one of the following activities: inhibition of endothelial cell proliferation *in vitro* at an IC50 level of less than 80 μ M; inhibition of angiogenesis *in vitro* at a level of less than 85% sprouting in a collag-en gel-based assay; or reduction in tumor volume *in vivo* by at least 25% relative to a control at the end of an administration period.

13. The partial peptide mimetic of claim 12 wherein the first amino acid sequence has at least one of the following:
- 10 6 amino acids of the sequence SVQMKL (SEQ ID NO:5);
 9 amino acids of the sequence IKLSVQMKL (SEQ ID NO:6);
 10 amino acids of the sequence NIKLSVQMKL (SEQ ID NO:7); or
 11 amino acids of the sequence ANIKLSVQMKL (SEQ ID NO:8).
- 15 14. The partial peptide mimetic of claim 12 wherein the second amino acid sequence has at least one of the following:
- 4 amino acids of the sequence IIVK (SEQ ID NO:3);
 6 amino acids of the sequence IIVKLN (SEQ ID NO:9); or
 9 amino acids of the sequence IIVKLND (SEQ ID NO:2).
- 20 15. A partial peptide mimetic selected from the group consisting of:
- ANIKLSVQMKL-[DBF]-IIVKLND;
 NIKLSVQMKL-[DBF]-IIVKLND;
 IKLSVQMKL-[DBF]-IIVKLND;
 25 SVQMKL-[DBF]-IIVKLND;
 QMKL-[DBF]-IIVKLND;
 MKL-[DBF]-IIVKLND;
 L-[DBF]-IIVKLND;
 ANIKLSVQMKL-[DBF]-IIVKLN;
 30 ANIKLSVQMKL-[DBF]-IIVK;
 ANIKLSVQMKL-[DBF]-I;
 SVQMKL-[DBF]-IIVKLN;

SVQMKL-[DBF]-IIVK;
SVQMKL-[DBF]-IIV;
SVQMKL-[DBF]-II; and
SVQMKL-[DBF]-I;

5 wherein DBF is a dibenzofuran β -turn mimetic.

16. A method for inhibiting bacterial infection and/or endotoxemia, the
method comprising contacting cells with an amount of a composition effective
to inhibit the bacterial infection and/or to neutralize endotoxin, wherein the
10 composition comprises a partial peptide mimetic of claim 1.

17. The method of claim 16 wherein the contacting step occurs *in vitro*.

18. The method of claim 16 wherein the contacting step occurs *in vivo*.

15

19. The method of claim 16 wherein the cells are present in a cell culture, a
tissue, an organ, or an organism.

20. The method of claim 16 wherein the cells are mammalian cells.

20

21. The method of claim 20 wherein the cells are human cells.

22. The method of claim 16 wherein the partial peptide mimetic neutralizes
endotoxin, is bactericidal, or is both bactericidal and neutralizes endotoxin.

25

23. A method for decreasing the amount of TNF- α , the method comprising
contacting cells with an amount of a composition effective to decrease the
amount of TNF- α , wherein the composition comprises a partial peptide mimetic
of claim 1.

30

24. The method of claim 23 wherein the contacting step occurs *in vitro*.

25. The method of claim 23 wherein the contacting step occurs *in vivo*.
26. The method of claim 23 wherein the cells are present in a cell culture, a tissue, an organ, or an organism.
- 5
27. The method of claim 23 wherein the cells are mammalian cells.
28. The method of claim 27 wherein the cells are human cells.
- 10 29. A method for inhibiting endothelial cell proliferation, the method comprising contacting cells with an amount of a composition effective to inhibit endothelial cell proliferation, wherein the composition comprises a partial peptide mimetic of claim 1.
- 15 30. The method of claim 29 wherein the contacting step occurs *in vitro*.
31. The method of claim 29 wherein the contacting step occurs *in vivo*.
32. The method of claim 29 wherein the cells are present in a cell culture, a
20 tissue, an organ, or an organism.
33. The method of claim 29 wherein the cells are mammalian cells.
34. The method of claim 29 wherein the cells are human cells.
- 25
35. A method for inhibiting angiogenic-factor mediated inter-cellular adhesion molecule expression down-regulation, the method comprising contacting cells with an amount of a composition effective to inhibit angiogenic-factor mediated inter-cellular adhesion molecule expression down-
30 regulation, wherein the composition comprises a partial peptide mimetic of claim 1.

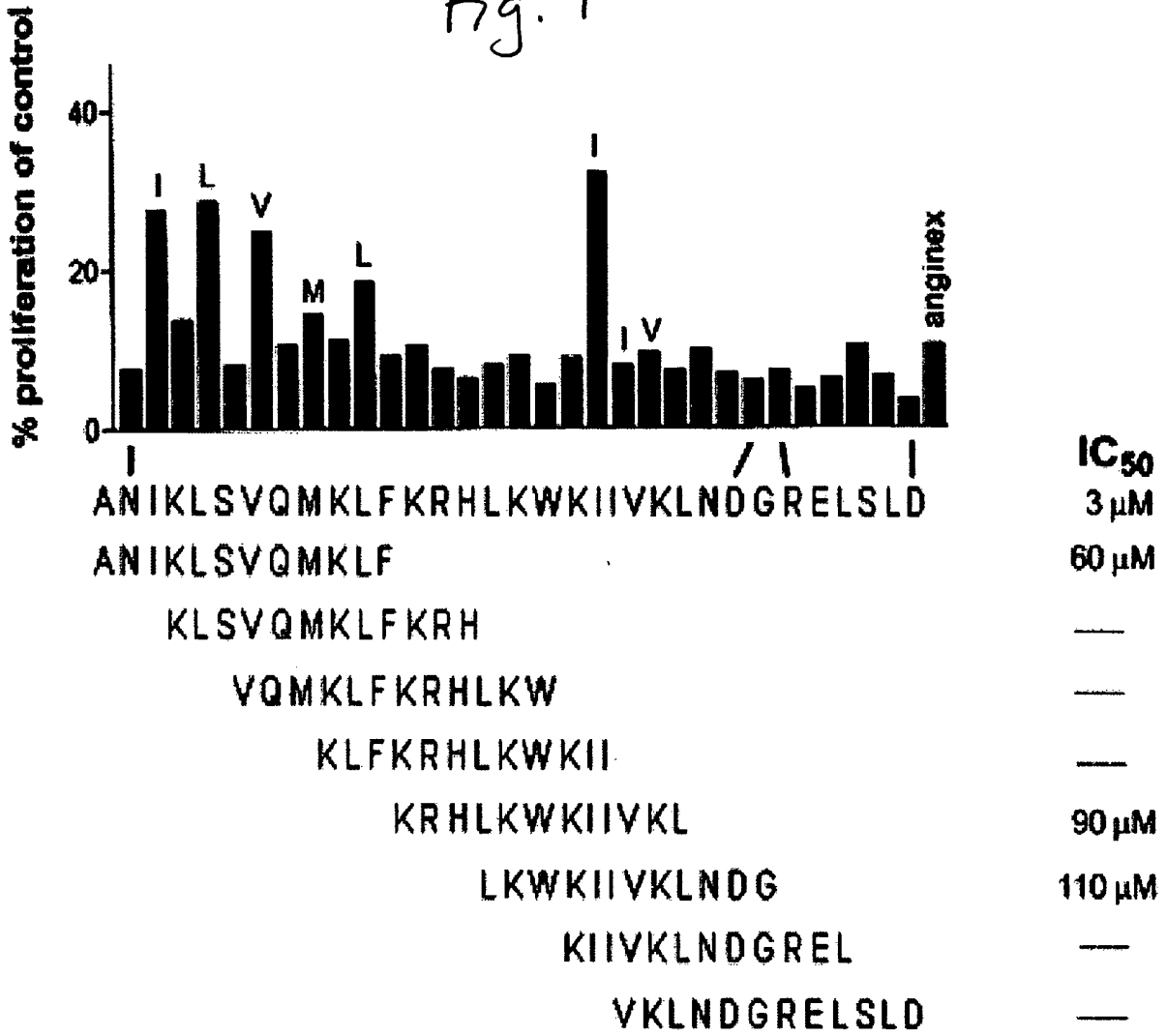
36. The method of claim 35 wherein the contacting step occurs *in vitro*.
37. The method of claim 35 wherein the contacting step occurs *in vivo*.
- 5 38. The method of claim 35 wherein the cells are present in a cell culture, a tissue, an organ, or an organism.
39. The method of claim 35 wherein the cells are mammalian cells.
- 10 40. The method of claim 35 wherein the cells are human cells.
41. A method for promoting angiogenic-factor mediated inter-cellular adhesion molecule expression, the method comprising contacting cells with an amount of a composition effective to promote angiogenic-factor mediated inter-cellular adhesion molecule expression, wherein the composition comprises a partial peptide mimetic of claim 1.
- 15 42. The method of claim 41 wherein the contacting step occurs *in vitro*.
- 20 43. The method of claim 41 wherein the contacting step occurs *in vivo*.
44. The method of claim 41 wherein the cells are present in a cell culture, a tissue, an organ, or an organism.
- 25 45. The method of claim 41 wherein the cells are mammalian cells.
46. The method of claim 41 wherein the cells are human cells.
47. A method for inhibiting angiogenesis, the method comprising contacting cells with an amount of a composition effective to inhibit angiogenesis, the composition comprising a partial peptide mimetic of claim 1.
- 30

48. The method of claim 47 wherein the contacting step occurs *in vitro*.
49. The method of claim 47 wherein the contacting step occurs *in vivo*.
- 5 50. The method of claim 47 wherein the cells are present in a cell culture, a tissue, an organ, or an organism.
51. The method of claim 47 wherein the cells are mammalian cells.
- 10 52. The method of claim 47 wherein the cells are human cells.
53. A method for inhibiting tumorigenesis in a patient, the method comprising administering to the patient a therapeutically effective amount of a composition comprising a partial peptide mimetic of claim 1.
- 15 54. A method for inhibiting atherosclerosis in a patient, the method comprising administering to the patient a therapeutically effective amount of a composition comprising a partial peptide mimetic of claim 1.
- 20 55. A method for inhibiting restenosis in a patient, the method comprising administering to the patient a therapeutically effective amount of a composition comprising a partial peptide mimetic of claim 1.
56. A method for inhibiting diabetic retinopathy in a patient, the method
25 comprising administering to the patient a therapeutically effective amount of a composition comprising a partial peptide mimetic of claim 1.
57. A method for inhibiting neovascular glaucoma in a patient, the method
30 comprising administering to the patient a therapeutically effective amount of a composition comprising a partial peptide mimetic of claim 1.

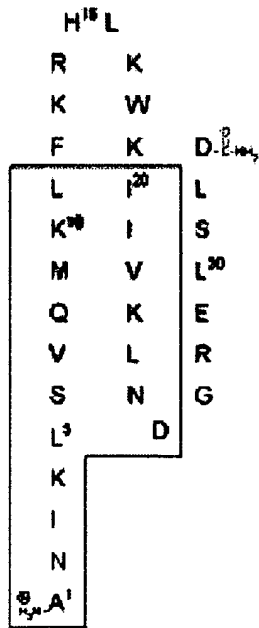
58. A method for inhibiting rheumatoid arthritis in a patient, the method comprising administering to the patient a therapeutically effective amount of a composition comprising a partial peptide mimetic of claim 1.
- 5 59. A method for inhibiting endometriosis in a patient, the method comprising administering to the patient a therapeutically effective amount of a composition comprising a partial peptide mimetic of claim 1.

Fig. 1

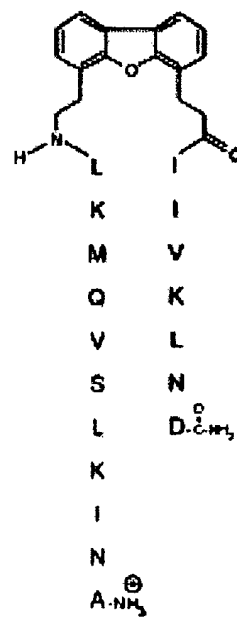
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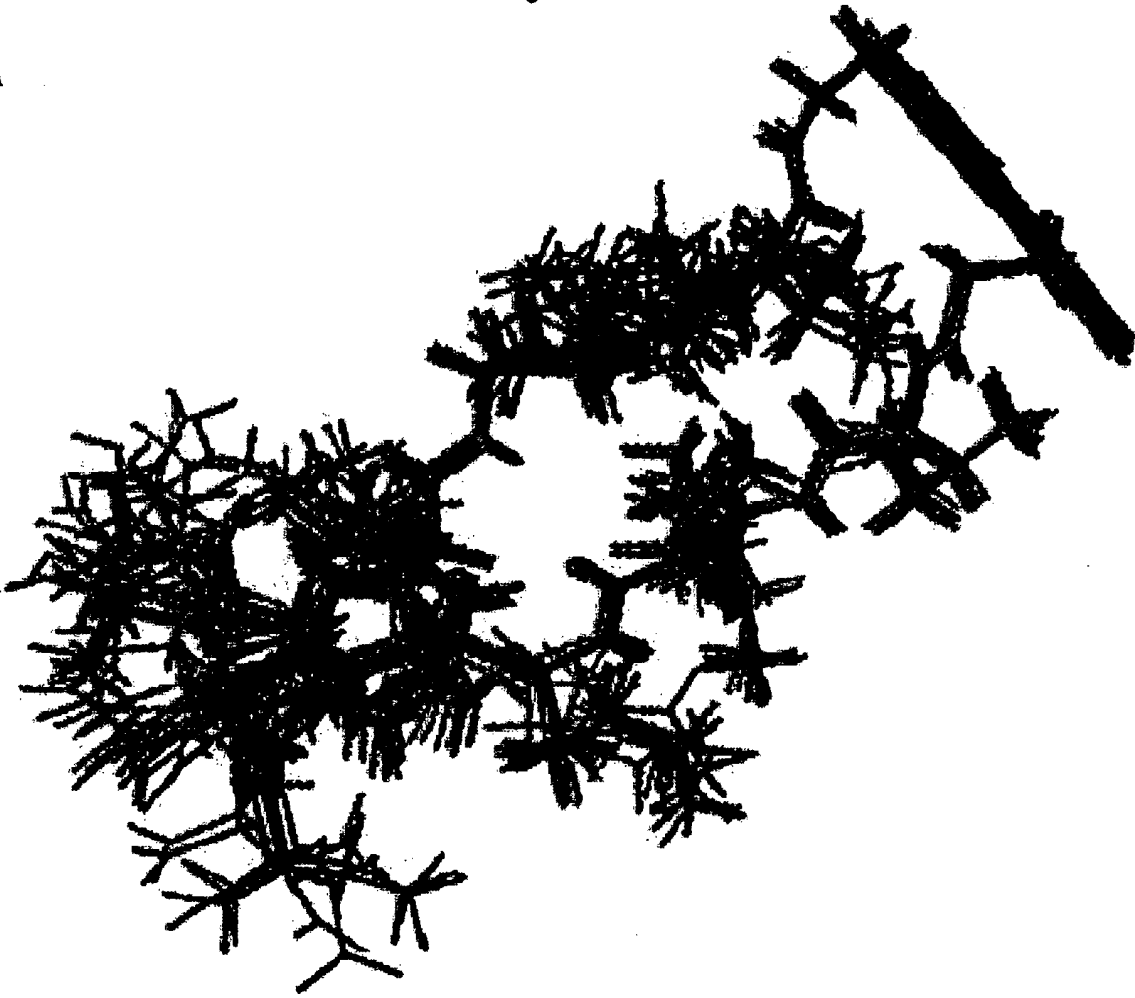
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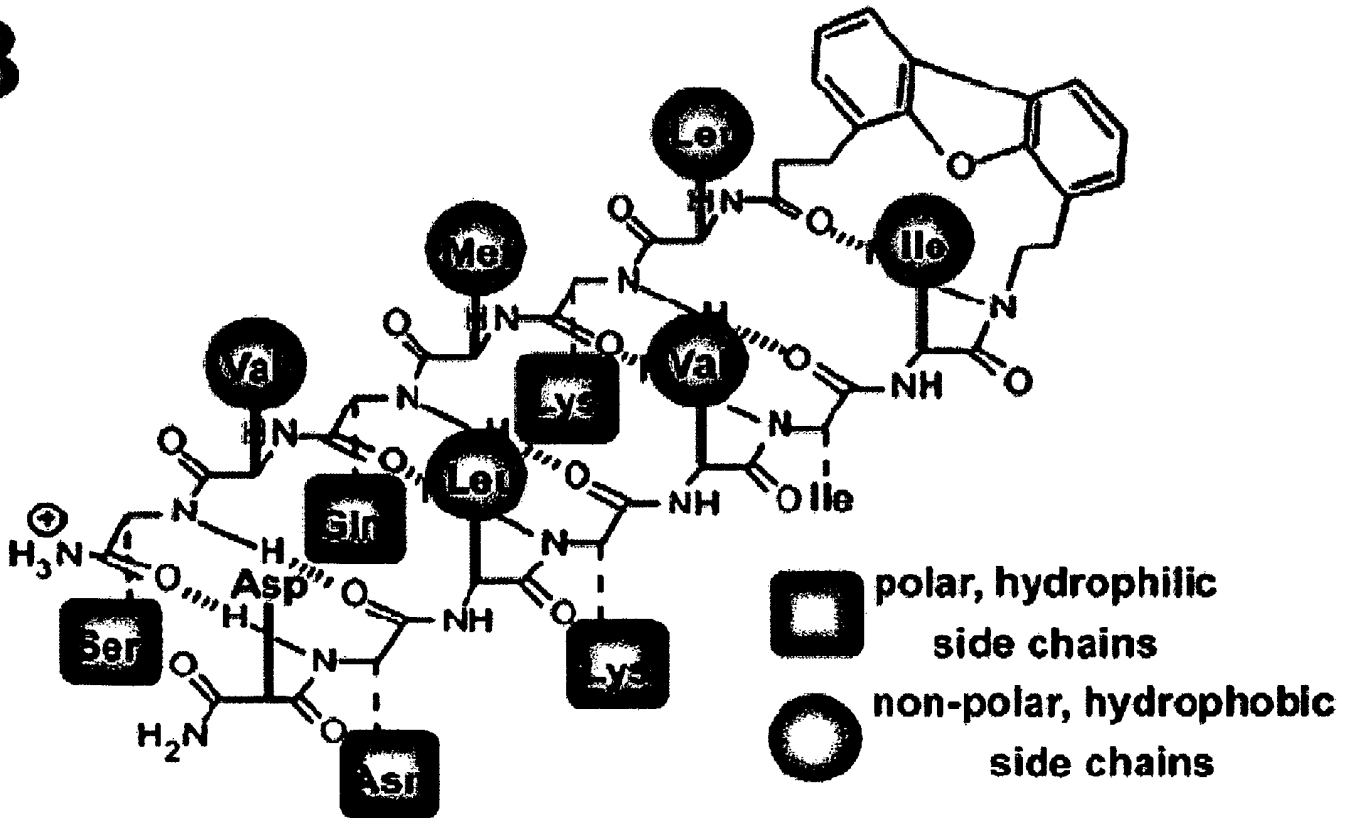
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B



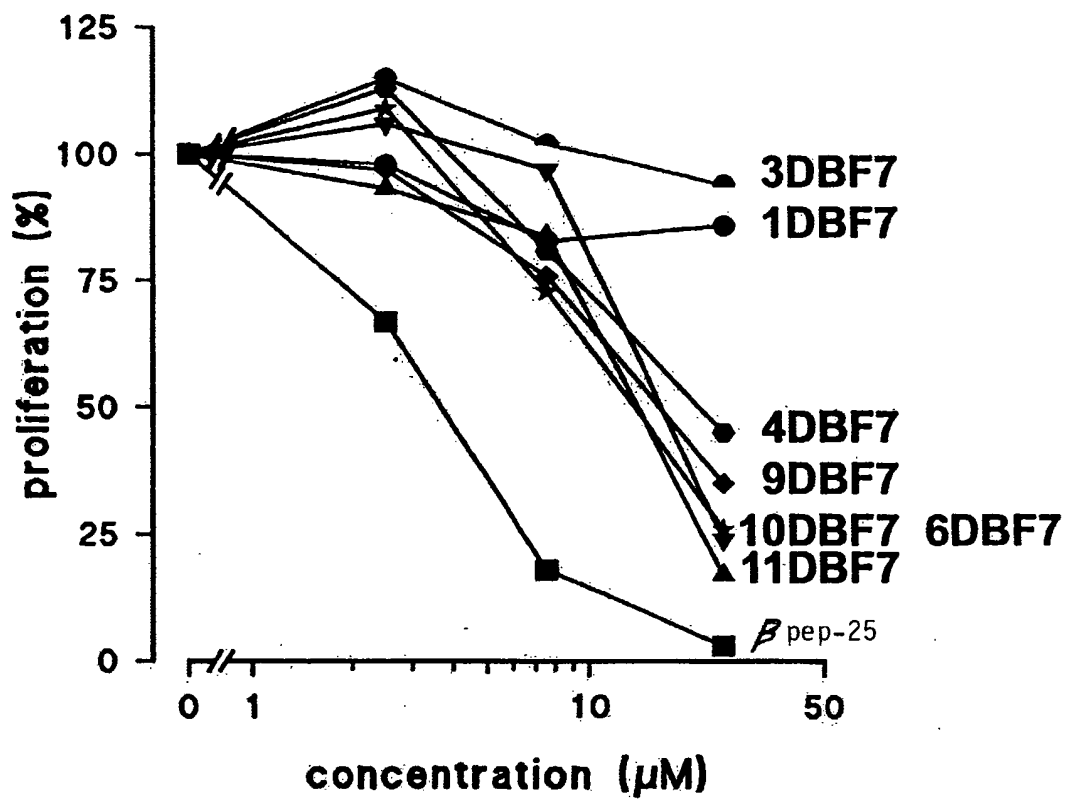


FIGURE 3

FIGURE 4

