Abstract:

Title: VIPESTRINAT-DERIVED PEPTIDES AND USES THEREOF

Analogs and derivatives of Viperistatin, a snake venom-derived peptide that inhibits collagen receptors are provided. Further provided are pharmaceutical compositions comprising the analogs and derivatives of Viperistatin, and methods for treating and/or diagnosing and imaging diseases or disorders involving excessive angiogenesis using the same.
VIPERISTATIN-DERIVED PEPTIDES AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to analogs and derivatives of Viperistatin, a snake venom-derived peptide that inhibits the collagen receptor alpha lbeta 1 (αIβ1) integrin. More particularly, the present invention relates to cyclic analogs and derivatives of Viperistatin and their therapeutic uses as anti-angiogenic and anti-adhesive agents.

BACKGROUND OF THE INVENTION

Integrins are a family of various cell surface receptors which play essential roles in cell adhesion and migration. These heterodimeric receptors are composed of non-covalently linked distinct subunits, α and β, each containing a large extracellular binding domain, a transmembrane region, and a short cytoplasmic domain. Upon ligand binding, integrins cluster and recruit via their cytoplasmic domains cytoskeletal, adaptor and signaling proteins, thus eventually forming the focal adhesions, which not only anchors the cell to the subjacent extracellular matrix (ECM) ligands, but also conveys signals into the cell (Hynes, 1992, Cell 69: 11-25). The extracellular domains of both integrin subunits together form the binding pocket, thus determining the specificity for the cognate ECM protein. For example, α5β1 represents the “classical” fibronectin receptor, α4β1 and α4β7 interact with fibronectin and VCAM-1, α8β1 and α9β1 bind tenascin, ανβ7 and ανβ3 are major vitronectin receptors and αIIß3 is the platelet fibrinogen receptor involved in platelet aggregation, whereas integrins α3β1, α6β1, α6β4, and α7ß1 are lammin receptors.

The two major collagen-binding integrins, αIβ1 and α2ß1, preferentially bind to collagen type IV and I, respectively (Hynes, 1992, supra). In contrast to other β1 integrins, a subunits of collagen-binding integrins contain a unique 200 amino acid long A-domain, which is involved in collagen binding (Leitinger & Hogg, 1999, Biochem Soc Trans 27: 826-832). Studies with αl/α2 integrin A-domain chimeras confirmed the preference for different collagen types (Abair et al., 2008, Exp Cell Res 314: 3593-3604). As such, integrin receptors are central to the etiology and pathology of many disease states such as cardiovascular, inflammatory and neurodegenerative, and therefore have been actively targeted for drug discovery (Goodman & Picard, 2012, Trends Pharmacol Sci 33: 405-412). Many approaches have been developed with the aim of identifying integrin inhibitors, ranging from classical small molecules, cyclic peptides and engineered...
antibodies (Goodman & Picard 2012, supra). To date, major drug discovery attempts have been focused on several integrins, such as: gpllbllla, α4β1, αvβ3 and LFA-1 (αLβ2) and have produced several registered drugs, targeting directly gpllbllla (abciximab, tirofiban, intrifiban), and α4β1 (natalizumab) (Millard et al., 2011, Theranostics 1: 154-188; Goodman & Picard 2012, supra).

In the last decade, using knock-out animals and/or neutralizing monoclonal antibodies, the importance of collagen-binding integrins αiβi (Pozzi et al., 2000, P Natl Acad Sci 97: 2202-2207) and α2β1 (Senger et al., 2002, Am. J. Pathol. 160: 195-204) in cardiovascular angiogenesis process was demonstrated. Furthermore, important contributions of αiβi (Chen et al., 2005, Intl J Cancer 116: 52-61) and α2β1 (Yoshimura et al., 2009, Cancer Res 69: 7320-7328; Ibaragi et al., 2011, Anticancer Res 31: 1307-1313) in cancer and metastasis processes were revealed. These findings emphasize the role of αiβi and α2β1 integrins in cancer and angiogenesis and suggested them as important targets in drug discovery and translational medicine (Goswami, 2013, Adv Biol Chem 3: 224-252). However, very little drug discovery regarding targeting αiβi and α2β1 integrins has been reported. In those studies pre-clinical attempts of inhibition of αiβi with antibody (Riikonen et al., 1995, Biochem Biophys Res Co 209: 205-212), and α2β1 with peptides (Ivaska et al., 1999, / Biol Chem 274: 3513-3521; Raynal et al., 2006, / Biol Chem 281: 3821-3831; Lambert et al., 2008, J Biol Chem 283: 16665-16672) and small molecules (Funahashi et al., 2002, Cane Res 62: 6116-6123; Choi et al., 2007, J Med Chem 50: 5457-5462; Miller et al., 2009, P Natl Acad Sci 106: 719-724; Nissinen et al., 2012, / Biol Chem 287: 44694-44702) was performed with consideration of their therapeutic potential for thrombosis or cancer.

Snake venom natural toxins such as disintegrins (Calvete et al., 2005, Toxicon 45: 1063-1074; Marcinkiewicz, 2005, Curr Pharm Des 11: 815-827) and C-type lectins (Arlinghaus et al., 2012, Toxicon 60: 512-519) are important pharmacological tools since they selectively inhibit integrins. The disintegrins Obtustatin, isolated from venom of Vipera lebetina obtusa (Marcinkiewicz et al., 2003, Cancer Research 63: 2020-2023), and its more potent natural analog Viperistatin, isolated from venom of Vipera xantina palestinae (Kisiel et al., 2004, FEBS Letters 577: 478-482), 41-amino acid monomeric polypeptides cross-linked by four conserved disulfide bonds, are the shortest snake venom disintegrins described to date, selectively targeting αiβi integrin (Calvete et al. 2007, / Mass Spectrom 42:1405-1414). They are typical members of disintegrin family...
characterized by the three amino acids, Lys-Thr-Ser (KTS) motif, responsible for the binding to α\(^5\)β\(^3\) integrin (Scarborough et al., 1991, *Journal of Biological Chemistry* 266: 9359-62; Marcinkiewicz et al., 1999, *Biochemistry* 38: 13302-13309; Calvete et al., 2002, *Biochemistry* 41: 2014-2021; Sanz et al., 2006, *Biochem J* 395: 385-392). KTS containing disintegrins are very selective for α\(^5\)β\(^3\) integrin, in contrast to RGD (Arg-Gly-Asp) disintegrins which block α\(^8\)β\(^1\), α\(^5\)β\(^1\), α\(^v\)β\(^1\), α\(^v\)β\(^3\) and α\(^v\)β\(^3\). The potency of KTS disintegrin is strictly dependent on KTS motif since mutation of individual amino acids in this motif, decreased potency by 10 to 80 fold (Brown et al., 2009, *Biochem J All:* 95-101). NMR solution structure followed by computer modeling of KTS disintegrins indicate that the KTS motif, like RGD, is present in the loop conformation required for integrin binding (Moreno-Murciano et al., 2003, *Protein Science* 12: 366-371; Calvete et al., 2007, supra; Brown et al., 2009, supra). Viperistatin and related natural disintegrin peptides have limited value as potential therapeutic molecules for preventing cell adhesion, including potential uses for anti-angiogenesis, due to their relative large size, compared to peptidomimetic drugs, proteolytic degradation upon oral delivery and potential immunogenicity.

WO 2002/022571 discloses compounds which are potent and specific inhibitors of α\(^5\)β\(^3\) integrin. The α\(^5\)β\(^3\) integrin-inhibiting compounds of WO 2002/022571 include the peptide Obtustatin as well as fragments, derivatives, homologs and analogs thereof.

None of the prior art teaches or suggests short analogs and derivatives of Viperistatin, particularly short analogs and derivatives of Viperistatin with dual antagonistic activity towards both α\(^5\)β\(^3\) and α\(^2\)β\(^1\) integrins, with anti-cell adhesion and anti-angiogenic activities.

There is a need for improved compounds with anti-cell adhesion and anti-angiogenic activities, useful, for example, for the treatment or diagnosis of different types of cancer and medical conditions associated with abnormal angiogenesis.

**SUMMARY OF THE INVENTION**

The present invention according to some aspects provides cyclic peptides derived from the disintegrin Viperistatin. The present invention further provides pharmaceutical compositions comprising the peptides and methods for treating diseases involving excessive cell adhesion and/or angiogenesis utilizing the cyclic peptide derivatives.
The present invention discloses for the first time analogs and derivatives of Viperistatin which advantageously show dual selectivity and antagonism towards the two major collagen receptors, $\alpha_\text{i} \beta_1$ and $\alpha_2 \beta_1$ integrins. This is in contrast to the parent compound Viperistatin and the structurally-similar Obtustatin, that each has relative selectivity towards only $\alpha_\text{i} \beta_1$ integrin receptors.

The analogs and derivatives of Viperistatin disclosed herein were found to have lower or intermediate efficacy, meaning lower inhibitory activity, compared to the parent compound. However, such intermediate efficacy is in effect beneficial for therapeutic applications, in that it allows gentle manipulation of the endothelial cell adhesion and angiogenesis. Advantageously, the analogs and derivatives of Viperistatin disclosed herein show increased potency compared to the original compound, meaning that the maximal activity is achieved at lower concentrations. While the natural parent compound Viperistatin and its related compound Obtustatin are snake venom toxins with a narrow therapeutic window, the peptides disclosed herein were found to have an improved therapeutic index inasmuch as they are non-toxic to endothelial cells and safe upon intravenous injection in mice.

The peptides of the present invention have anti-cell adhesion properties. Advantageously, according to some embodiments the anti-cell adhesion activity is capable of inhibiting angiogenesis, as exemplified herein below. In addition, according to additional embodiments the peptides of the present invention were found to have anti-tumor activity, including anti-metastatic activity, as exemplified herein below in a mouse model of melanoma. According to various embodiments, peptides of the present invention are particularly useful for the treatment and/or diagnosis of a variety of medical conditions including, for example, certain types of cancer, retinal disorders involving excessive angiogenesis, e.g. macular degeneration, as well as fibrosis, psoriasis, and other diseases involving $\alpha_\text{i} \beta_1$ and $\alpha_2 \beta_1$ integrins.

The peptides of the present invention comprise at least one cyclization. According to some embodiments, the positions of the cyclization(s) of the peptides of the present invention, namely, the residues involved in the cyclization, are different from the positions of the cyclizations in the parent compound Viperistatin or in Obtustatin.

According to one aspect, the present invention provides a synthetic cyclic peptide of 8-30 amino acids comprising the sequence WKTSXiRTSH (SEQ ID NO: 1), wherein Xi is absent or Cys, the peptide containing said sequence in a cyclic portion thereof.
In some embodiments, there is provided herein a peptide of 9-30 amino acids comprising the sequence WKTSRTSHY (SEQ ID NO: 2) in a cyclic portion thereof. In particular embodiments, a peptide of 11-30 amino acids is provided, comprising the sequence CWKTSRTSHYC (SEQ ID NO: 3) in a cyclic portion thereof.

In some embodiments, the peptide comprises the sequence CWKTSRTSHYC (SEQ ID NO: 3) in a cyclic portion thereof, and is cyclized via a disulfide bridge connecting the side chains of two cysteine residues present in the sequence. According to a particular embodiment the peptide comprising SEQ ID NO: 3 is cyclized via a disulfide bridge between residues one and eleven of said sequence.

In some embodiments, the peptide comprises 15-30 amino acids, for example 15-25 amino acids, or 15-20 amino acids. Each possibility represents a separate embodiment of the invention.

In some embodiments, the peptide is monocyclic, namely, containing a single cyclization.

In some embodiments, the peptide is bi-cyclic, namely, containing two cyclizations.

In additional embodiments, the peptide is tri-cyclic, namely, containing three cyclizations.

In some embodiments, the peptide comprises a plurality of cyclizations, wherein each cyclization is independently selected from the group consisting of side-chain to side-chain; end to end; backbone to backbone and backbone to end. In some embodiments, each cyclization is independently selected from the group consisting of side-chain to side-chain and end to end.

In some embodiments, at least one of said plurality of cyclizations is formed by a disulfide bridge connecting the side chains of two cysteine residues present in the sequence.

In some specific embodiments, all of said plurality of cyclizations are formed by disulfide bridges connecting the side chains of two cysteine residues.

In some alternative embodiments, at least one of said plurality of cyclizations is formed by an amide bond connecting the side chains of two residues in the sequence.

In some specific embodiments, all of said plurality of cyclizations are formed by amide bonds connecting the side chains of two residues in the sequence.
In some embodiments, the peptide further comprises an additional sequence C-terminal to the above-specified sequences, the additional sequence comprising 1-12 contiguous amino acid residues from residues 30-41 of Viperistatin or Obtustatin. In certain embodiments, the additional sequence comprises 3-6 contiguous amino acid residues derived from residues 30-41 of Viperistatin or Obtustatin.

In some embodiments, the additional sequence C-terminal to the above-specified sequences comprises the sequence TGKS (SEQ ID NO: 4). In some embodiments, the additional sequence is selected from the group consisting of TGKSCG (SEQ ID NO: 5) and TGKSD (SEQ ID NO: 6). Each possibility represents a separate embodiment of the present invention.

In some embodiments, the additional sequence C-terminal to the above-specified sequences comprises PLY. In some embodiments, the additional sequence is selected from the group consisting of PLYPG (SEQ ID NO: 7) and PLYQG (SEQ ID NO: 8).

In some embodiments, the peptide comprises the sequence CWKTSRTSHYC (SEQ ID NO: 3) and an additional sequence of 3-6 contiguous amino acid residues from residues 30-41 of Viperistatin or Obtustatin C-terminal to CWKTSRTSHYC.

In some embodiments, the peptide comprises the sequence CWKTSRTSHYC (SEQ ID NO: 3) and further comprises a sequence selected from the group consisting of TGKSCG (SEQ ID NO: 5) and TGKSD (SEQ ID NO: 6) C-terminal to CWKTSRTSHYC. Each possibility represents a separate embodiment of the present invention.

In some embodiments, the peptide comprises the sequence CWKTSRTSHYC (SEQ ID NO: 3) and further comprises a sequence selected from the group consisting of PLYPG (SEQ ID NO: 7) and PLYQG (SEQ ID NO: 8) C-terminal to CWKTSRTSHYC. Each possibility represents a separate embodiment of the present invention.

In some embodiments, the peptide sequence is selected from the group consisting of CCWKTSRTSHYCTGKSCG (SEQ ID NO: 9), CWKTSRTSHYCPLYPG (SEQ ID NO: 10), CWKTSRTSHYCPLYQG (SEQ ID NO: 11), CWKTSRTSHYCTGKSD (SEQ ID NO: 12) and CWKTSRCSRTSHTGKSD (SEQ ID NO: 13). Each possibility represents a separate embodiment of the invention.

In some particular embodiments, the peptide sequence is selected from the group consisting of CCWKTSRTSHYCTGKSCG (SEQ ID NO: 9) and CWKTSRTSHYCPLYPG (SEQ ID NO: 10).
In some specific embodiments, the cyclic peptide is selected from the group consisting of compounds (10), (8), (7), (6) and (5):

\[
\begin{align*}
H_2N & \quad \text{CCWKTSRTSHYCTGKSCG} \quad OH \\
\end{align*}
\]  
(10)

\[
\begin{align*}
H_2N & \quad \text{CWKTSRTSHYCTGKSCG} \quad \text{NH} \\
\end{align*}
\]  
(8)

\[
\begin{align*}
H_2N & \quad \text{CWKTSRTSHYCTGKSCG} \quad \text{NH} \\
\end{align*}
\]  
(7)
Each possibility represents a separate embodiment of the invention.

According to another aspect, the present invention provides a pharmaceutical composition comprising a peptide of the present invention and optionally a pharmaceutically acceptable excipient.

The pharmaceutical composition of the present invention may be formulated for local or systemic routes of administration.

According to yet another aspect, the present invention provides a method for treating a disease or disorder involving excessive cell adhesion and/or angiogenesis in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition comprising a peptide of the present invention.

According to a further aspect, the present invention provides a pharmaceutical composition comprising a peptide of the invention, for use in the treatment of a disease or disorder involving excessive angiogenesis.

The diseases or disorders amenable to treatment according to the principles of the present invention are those where abnormal, excessive angiogenesis is implicated in their pathogenesis, or facilitates their progression.

In some embodiments, the disease or disorder is cancer. In particular embodiments, the disease or disorder is a cancerous solid tumor. Cancer types particularly suitable for treatment by the method of the present invention are those where angiogenesis is known to underlie their progression. The method may be used for the treatment of primary as well as metastatic cancer. The method is particularly suitable for the treatment of vascularized tumors.
In other embodiment, the disease or disorder is a retinal disorder. For example, proliferative retinopathies may be treated, including retinopathy of prematurity, retrolental fibroplasia, neovascular glaucoma, and age-related macular degeneration (particularly wet age-related macular degeneration (wAMD)). Additional examples include macular edema following retinal vein occlusion (RVO) and diabetic macular edema. Each possibility represents a separate embodiment of the invention.

In addition, other non-neoplastic inflammatory conditions may be treated, including, but are not limited to, rheumatoid arthritis, psoriasis, fibrosis, atherosclerosis and thyroid hyperplasias (including Grave's disease). Each possibility represents a separate embodiment of the invention.

According to a further aspect, the present invention provides a pharmaceutical composition comprising a peptide of the present invention, preferably attached to a detectable label, for use in the diagnosis of a disease or disorder associated with abnormal expression of $\alpha_1 \beta_1$ integrin, $\alpha_2 \beta_1$ integrin or both. Such disease or disorder may include diseases or disorders associated with up regulation, down regulation, or mutation of $\alpha_1 \beta_1$ and/or $\alpha_2 \beta_1$ integrins. Preferably, the diseases or disorders to be diagnosed are those associated with upregulation of $\alpha_1 \beta_1$ and/or $\alpha_2 \beta_1$ integrins.

In some embodiments, the disease or disorder is a disease or disorder involving excessive angiogenesis.

According to yet a further aspect, the present invention provides a pharmaceutical composition comprising a peptide of the present invention, for use in the imaging of angiogenesis.

These and further aspects and features of the present invention will become apparent from the detailed description, examples and claims which follow.

**BRIEF DESCRIPTION OF THE FIGURES**

**Figure 1.** (A) Obtustatin, Viperistatin and (B-D) cyclic peptides derived therefrom in accordance with some embodiments of the present invention (described in Example 1 below).

**Figure 2.** The inhibitory effect of the peptides on $\alpha_1$ and $\alpha_2$ overexpressed cell adhesion. Dose response curves of inhibition of $\alpha_1$ (A-C) (solid symbols) and $\alpha_2$ (D-F) (open symbols) cell adhesion to respective collagens. Viperistatin /Vixapatin (squares)
were used as controls. The number of adherent cells (mean ± SD) is derived from three independent experiments.

**Figure 3.** The inhibitory effect of the peptides on binding GST- α1 and GST- α2 A domains to their respective collagens. Binding of GST- αA domain (A and B) to CB3 (collagen IV fragment); and GST- α2A domain (C and D) to collagen I in the presence of (A and C) 1 µM; (B and D) 2-1000 µM of the peptides. Solid symbols represent Viperistatin (circles), Rhodocetin (squares), whereas the open symbols represent Compound 6 (circles) and Compound 10 (squares).

**Figure 4.** Effect of the peptides on proliferation of endothelial cells. In each HAEC (A) and HUVEC (B) experiment, 50 µg of peptide per sample were used. Obtustatin was used as positive control and proliferation was measured by BrdU assay. */p<0.05 compared to control group. OD, optical density.

**Figure 5.** Inhibitory effect of the peptides on HUVEC migration in the wound healing assay. Representative photos of the wounds at time 0 (left) and 24 hours (right) from wounding in the absence of the peptides (control) (A), in the presence of 5 µg Obtustatin (B), 50 µg Compound 6 (C), and 50 µg Compound 10 (D).

**Figure 6.** The inhibitory effect of the peptides on endothelial cells tube formation in Matrigel assay. The tube formation in Matrigel with HAEC (A) and HUVEC (B) was induced by complete EBM-2 medium in the absence (negative control) or in the presence of 100 µg of each peptide per sample.

**Figure 7.** The inhibitory effect of the peptides on VEGF induced angiogenesis in Japanese quail CAM model. Representative images of mid-arterial end points of CAMs dissected from embryos appear in the panels. Basal level of angiogenesis (Control); angiogenesis induced by 10 µg VEGF /embryo (VEGF); the inhibitory effect of 20 µg Obtustatin/embryo (Obtustatin), 200 µg peptide/embryo (Compounds 3, 6 and 10) on angiogenesis induced by VEGF. */p<0.001 versus Control. **p<0.001 versus VEGF. Df, fractal dimension.

**Figure 8.** The inhibitory effect of the peptides on glioma LN18 cells induced angiogenesis in Japanese quail CAM model. (A) Representative images of mid-arterial endpoints of CAMs dissected from embryos appear in the panels. Basal level of angiogenesis (Control); angiogenesis induced by LN18 cells (lx10 ⁷/embryo) (LN18); the inhibitory effect of 100 µg peptides (Compounds 6 and 10) on angiogenesis induced by
LN18. *p<0.001 versus Control. **p<0.001 versus LN18. (B) Representative micrographs of dissected CAMs. Tumors are framed and marked with an arrow.

**Figure 9.** Inhibitory effect of the peptides on bFGF-induced angiogenesis in corneal micropocket assay. The upper panel shows that newly formed blood vessels are growing toward bFGF pellet (in white); whereas the lower panel shows the quantification of the neovascularization area in the cornea (n=10, mean ± S.D.). *p<0.05 vs control.

**Figure 10.** Serum stability profiles of the peptides. Compound 3 is indicated by squares, Compound 6 by triangles, and Compound 10 by circles. Relative peptide concentrations were determined by integration of the A220 peaks from RP-HPLC chromatograms.

**Figure 11.** Survival of B16 melanoma tumor bearing mice treated with the peptides. Control-melanoma mice model injected with PBS only. All the other groups of mice treated with 10 mg peptides/kg.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is directed according to some aspects to synthetic cyclic peptides derived from the disintegrin Viperistatin and pharmaceutical compositions comprising the peptides. The peptides and compositions of the present invention are useful for treating diseases involving excessive angiogenesis. The peptides and compositions of the present invention are also useful for analyzing in vitro and in vivo anti adhesion and anti-angiogenic effects, applicable for anti-angiogenesis and anti-cancer (including anti-metastatic) therapy. Also, the peptides of the present invention can be used as a diagnostic tool for α5β1 and α2β1 integrins mediated angiogenesis. For example, the peptides can be utilized in the diagnosis of medical conditions associated with abnormal angiogenesis. The peptides can also be used as imaging agents, for imaging of angiogenesis, e.g., in tumors. For diagnostics applications, the peptides are typically labeled, e.g., with fluorescent, radioactive, near infrared tags.

The present invention discloses for the first time non-natural analogs and derivatives of Viperistatin which advantageously show dual selectivity towards the two collagen receptors α5β1 and α2β1 integrins with increased potency. As both of these integrins are known to be involved in angiogenesis, the dual activity of the compounds of the present invention is advantageous in increasing their anti-angiogenesis efficacy.
The peptide compounds disclosed herein are characterized by an intermediate efficacy in inhibition of endothelial cell adhesion, such as inhibition of adhesion of α1/α2 integrin overexpressor cells to respective collagens. An intermediate efficacy as described herein refers to an efficacy of between about 10-60%, for example between about 20-40%.

As exemplified herein below, a very selective natural compound, derived from an Israeli snake *Viperapalestinae* venom and named Viperistatin, belonging to the family of disintegrin proteins, was used as a lead compound to synthesize a platform of linear and cyclic peptides containing the KTS binding motif. The peptides were purified by analytical HPLC and their masses were confirmed by LC-MS. Employing two different *in vitro* adhesion assays, a cellular assay with K562 cells overexpressing α1 or α2 integrin subunit and acellular binding assay, using recombinant α1 and α2 A-domain (extracellular domain of α1β1 and α2β1 integrins, respectively) the potency and efficacy of all peptides for α1 and α2 integrins was determined. Experiments with linear KTS peptides, analogs of Viperistatin and Obtustatin (sequence position 19-29) indicated the importance of Cys19 and Cys29, as well as the presence of Arg at the position 24 for their biological activities. The most active linear peptide, Viperistatin analog containing 11 amino acids, was used as the basic sequence for the synthesis of different cyclic peptides. Single or double cyclizations were performed using intra- or inter-cyclization. All the cyclic peptides were screened for various biological activities. Two of the analogs, designated as Vimocin (Compound 6) and Vidapin (Compound 10), which showed high potency (IC50 0.17 nM) and intermediate efficacy (20 and 40%, respectively) in inhibition of adhesion of α1/α2 integrin overexpressor cells to respective collagens, were particularly effective in various antiangiogenesis assays. Vimocin was more active in inhibition of wound healing (53%) and corneal micropocket (17%) vascularization, whereas Vidapin was more potent in inhibition of migration in the Matrigel tube formation assay (90%). Both compounds similarly inhibited proliferation (50-90%) of endothelial cells, and angiogenesis induced by vascular endothelial growth factor (80%) and glioma (55%) in the chorioallantoic membrane assay. These peptides were not toxic to endothelial cell cultures and caused no acute toxicity upon intravenous injection in mice, and were stable for 10-30 hours in human serum. The antitumor activity of the cyclic peptides was assessed in B16 melanoma mice model, and it was found that Vidapin significantly increased the survival of the mice with melanoma from median survival time of 40 days (control) to 73 days. Compound 6 may be particularly useful for the treatment of eye, such as retinal, disorders.
Compound 10 may be particularly useful for the treatment of cancer, such as solid tumors, primary and/or metastatic.

**Peptides:**

According to an aspect of the present invention, synthetic cyclic peptides are provided.

As used herein "peptide" indicates a sequence of amino acids linked by peptide bonds. Peptides according to some embodiments of the present invention consist of 8-40, for example, 9-40, 10-30, 11-25 amino acids.

The term "amino acid" refers to compounds, which have an amino group and a carboxylic acid group, preferably in a 1,2-, 1,3-, or 1,4- substitution pattern on a carbon backbone. Ot-Amino acids are most preferred, and include the 20 natural amino acids (which are L-amino acids except for glycine) which are found in proteins, the corresponding D-amino acids, the corresponding N-methyl amino acids, side chain modified amino acids, the biosynthetically available amino acids which are not found in proteins (e.g., 4-hydroxy-proline, 5-hydroxy-lysine, citrulline, ornithine, canavanine, djenkolic acid, β-cyanolanine), and synthetically derived α-amino acids, such as amino-isobutyric acid, norleucine, norvaline, homocysteine and homoserine. β-Alanine and γ-amino butyric acid are examples of 1,3 and 1,4-amino acids, respectively, and many others as well known to the art.

Some of the amino acids used in this invention are those which are available commercially or are available by routine synthetic methods. Certain residues may require special methods for incorporation into the peptide, and either sequential, divergent or convergent synthetic approaches to the peptide sequence are useful in this invention. Natural coded amino acids and their derivatives are represented by one-letter codes or three-letter codes according to IUPAC conventions. When there is no indication, the L isomer was used. The D isomers are indicated by "D" or "(D)" before the residue abbreviation.

In some embodiments, the peptide is composed of (or consists of) 8-39 amino acids, 8-38 amino acids, 8-37 amino acids, 8-36 amino acids, 8-35 amino acids, 8-34 amino acids, 8-33 amino acids, 8-32 amino acids, 8-31 amino acids, 8-30 amino acids, 8-29 amino acids, 8-28 amino acids, 8-27 amino acids, 8-26 amino acids, 8-25 amino acids, 8-24 amino acids, 8-23 amino acids, 8-22 amino acids, 8-21 amino acids, 8-20 amino acids, 8-19 amino acids, 8-18 amino acids, 8-17 amino acids, 8-16 amino acids, 8-15
amino acids, 8-14 amino acids, 8-13 amino acids, 8-12 amino acids. Each possibility represents a separate embodiment of the invention.


The peptides of the present invention comprises at least one cyclization, wherein the cyclized sequence, namely, the sequence within a cyclic portion of the peptide, comprises WKTSX_iRTSH (SEQ ID NO: 1), wherein Xi is absent or Cys.

In some embodiments, Xi is absent and the cyclized sequence comprises WKTSRTSH (SEQ ID NO: 26). In other embodiments, Xi is Cys and the cyclized sequence comprises WKTS_CRTSH (SEQ ID NO: 27).

In some embodiments, the peptides comprise a plurality of cyclizations. As used herein, the term "plurality" indicates at least two.

In some embodiments, the cyclized sequence comprises WKTSRTSHY (SEQ ID NO: 2). In particular embodiments, the cyclized sequence comprises CWKTSRTSHYC (SEQ ID NO: 3). In other embodiments, the cyclized sequence comprises CWKTS_CRTSH (SEQ ID NO: 14).

The peptides of the present invention may be monocyclic, bi-cyclic, tri-cyclic or even tetra-cyclic (with four cyclizations). Each possibility represents a separate embodiment of the present invention.

In some embodiments, each cyclization is independently selected from the group consisting of side-chain to side-chain; end to end; backbone to backbone and backbone to end.

According to some embodiments, a peptide of the present invention comprises at least one additional sequence of 1-18 amino acids residues, derived from Viperistatin or Obtustatin. The complete sequences of Viperistatin and Obtustatin are given below. According to some embodiments, the additional sequence is C-terminal to the first sequence noted above, namely, to WKTSX_iRTSH (or WKTSRTSHY, CWKTSRTSHYC
or CWKTSRTSH, according to some embodiments). According to other embodiments, the additional sequence is N-terminal to the first sequence. The additional sequence may be attached to the peptide through a linker or spacer. A linker according to some embodiments is a sequence of 1-4 amino acids.

In some embodiments, the additional sequence is a sequence of 1-12 contiguous amino acids derived from residues 30-41 of Viperistatin or Obtustatin. In some embodiments, the additional sequence is of 3-12, for example, 3-8 or 3-6, contiguous amino acids residues derived from residues 30-41 of Viperistatin or Obtustatin.

In some embodiments, the additional sequence comprises the sequence TGKS (SEQ ID NO: 4). In some embodiments, the additional sequence is TGKSCG (SEQ ID NO: 5). In additional embodiments, the additional sequence is TGKSD (SEQ ID NO: 6). In yet additional embodiments, the additional sequence is TGKSCDCG (SEQ ID NO: 15).

In some embodiments, the additional sequence comprises PLY. In some embodiments, the additional sequence is PLYPG (SEQ ID NO: 7). In additional embodiments, the additional sequence is PLYQG (SEQ ID NO: 8).

In some embodiments, the peptide comprises the sequence CWKTSRTSHYC (SEQ ID NO: 3) and an additional sequence of 1-12, for example 3-8 or 3-6, contiguous amino acid residues derived from residues 30-41 of Viperistatin or Obtustatin, C-terminal to CWKTSRTSHYC.

In some embodiments, the additional sequence is a sequence of 1-18 contiguous amino acid residues derived from residues 1-18 of Viperistatin or Obtustatin.

In some embodiments, the additional sequence is TTGPCCRQKKLPAGTT (SEQ ID NO: 16). In some embodiments, the additional sequence is KTTGPCCRQKKLPAGTTK (SEQ ID NO: 17).

In some embodiments, the peptide comprises the sequence WKTSRTSHYC (SEQ ID NO: 18) and an additional sequence of contiguous amino acid residues derived from residues 1-18 of Viperistatin or Obtustatin, N-terminal to WKTSRTSHYC.

In some embodiments, the peptide sequence is CCWKTSRTSHYCTGKSCG (SEQ ID NO: 9).

In some embodiments, the peptide sequence is CWKTSRTSHYCPLYPG (SEQ ID NO: 10).

In some embodiments, the peptide sequence is CWKTSRTSHYCPLYQG (SEQ ID NO: 11).
In some embodiments, the peptide sequence is CWKTSRTSHYCTGKSD (SEQ ID NO: 12).

In some embodiments, the peptide sequence is CWKTSCRTSHTGKSD (SEQ ID NO: 13).

In some embodiments, the peptide sequence is KTTGPCCRQKKLPAGTTKWKTSSRTSHYCTGKSCDCG (SEQ ID NO: 19).

Non-limiting examples of peptide compounds according to embodiments of the present invention include:

Compound 10:

\[
\text{H}_2\text{N} \xrightarrow{} \text{CCWKTSRTSHYCTGKSCG} \xrightarrow{} \text{OH}
\]

Compound 9:
Compound 8:

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CWKTSRTSHTGKSD} \quad \text{NH} \\
& \quad \text{OH} \\
\text{H}_2\text{N} & \quad \text{CWKTSRTSHTGKSD} \quad \text{NH}
\end{align*}
\]

Compound 7:

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CWKTSRTSHYCTGKSD} \quad \text{NH} \\
& \quad \text{OH} \\
\text{H}_2\text{N} & \quad \text{CWKTSRTSHYCTGKSD} \quad \text{NH}
\end{align*}
\]

Compound 6:

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CWKTSRTSHYCPLYG} \quad \text{OH}
\end{align*}
\]

Compound 5:

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CWKTSRTSHYCPLYQG} \quad \text{OH}
\end{align*}
\]

Each possibility represents a separate embodiment of the invention.
Peptides according to the present invention are constructed with one or more cyclizations, including side-chain to side chain (e.g. disulfide bond between two Cysteine residues), backbone to backbone, terminal to terminal as well as backbone to side-chain or any other peptide cyclization.

The procedures utilized to construct peptide compounds of the present invention generally rely on the known principles of peptide synthesis. However, it will be appreciated that accommodation of the procedures to the specific sequences of the present invention may be required. Examples of several synthetic procedures are given below.

Solid phase peptide synthesis procedures are well known in the art and further described in "Solid-Phase Synthesis: A Practical Guide", Ed. Steven A. Kates and Fernando Albericio, CRC Press; 1st Edition (2000). A skilled artesian may synthesize any of the peptides of the present invention by using an automated peptide synthesizer using standard chemistry such as, for example, t-Boc or Fmoc chemistry. The methods include exclusive solid phase synthesis, partial solid phase synthesis, fragment condensation, classical solution synthesis.

Coupling of the amino acids in solid phase peptide chemistry can be achieved by means of a coupling agent such as but not limited to dicyclohexycarbodiimide (DCC), bis(2-oxo-3-α-oxazolidinyl) phosphinic chloride (BOP-C1), benzotriazolyl-N-oxytrisdimethyl-aminophosphonium hexafluoro phosphate (BOP), 1-oxo-1-chlorophospholane (Cpt-Cl), hydroxybenzotriazole (HOBT), or mixtures thereof.

The use of additional coupling reagents including, but not limited to: coupling reagents such as PyBOP (Benzotriazole-1-yl-α-α-α-α-α-xylo-tris-pyrrolidino-phosphonium hexafluorophosphate), PyBOP (Benzotriazoly-N-oxytrisdimethyl-aminophosphonium hexafluoro phosphate), HBTU (2-(lH-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate), TBTU (2-(lH-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate), may be also utilized for synthesizing the peptide compounds of the present invention.

Additional coupling chemistries may be used, such as pre-formed urethane-protected N-carboxy anhydrides (UNCA’S), pre-formed acyl halides most preferably acyl chlorides.

Such coupling may take place at room temperature and also at elevated temperatures, in solvents such as toluene, DCM (dichloromethane), DMF.
(dimethylformamide), DMA (dimethylacetamide), NMP (N-methyl pyrrolidinone), dioxane, tetrahydrofuran, diglyme and 1,3 dichloropropane, or mixtures of the above.

Synthetic peptides can be purified by preparative high performance liquid chromatography and the composition of which can be confirmed via amino acid sequencing. Some of the peptides of the invention, which include only natural amino acids, may be prepared using recombinant DNA techniques known in the art.

**Cyclic peptides and backbone cyclization**

Cyclization of peptides has been shown to be a useful approach in developing diagnostically and therapeutically useful peptidic and peptidomimetic agents. Cyclization of peptides reduces the conformational freedom of these flexible, linear molecules, and often results in higher receptor binding affinities by reducing unfavorable entropic effects. Because of the more constrained structural framework, these agents are more selective in their affinity to specific receptor cavities. By the same reasoning, structurally constrained cyclic peptides confer greater stability against the action of proteolytic enzymes (Humphrey, et al., 1997, Chem. Rev., 2243-2266).

Methods for cyclization can be classified into cyclization by the formation of an amide bond between the N-terminal and the C-terminal amino acid residues, and cyclizations involving the side chains of individual amino acids. The latter method includes the formation of disulfide bridges between two α-thio amino acid residues (cysteine, homocysteine), the formation of lactam bridges between glutamic/aspartic acid and lysine residues, the formation of lactone or thiolactone bridges between amino acid residues containing carboxyl, hydroxyl or mercapto functional groups, the formation of thioether or ether bridges between the amino acids containing hydroxyl or mercapto functional groups and other special methods. Lambert, et al., reviewed variety of peptide cyclization methodologies (J. Chem. Soc. Perkin Trans., 2001, 1:471-484).

Backbone cyclization is a general method by which conformational constraint is imposed on peptides. In backbone cyclization, atoms in the peptide backbone (N and/or C) are interconnected covalently to form a ring. Backbone cyclized analogs are peptide analogs cyclized via bridging groups attached to the alpha nitrogens or alpha carbonyl of amino acids. In general, the procedures utilized to construct such peptide analogs from their building units rely on the known principles of peptide synthesis; most conveniently, the procedures can be performed according to the known principles of solid phase peptide synthesis. During solid phase synthesis of a backbone cyclized peptide the protected
building unit is coupled to the N-terminus of the peptide chain or to the peptide resin in a similar procedure to the coupling of other amino acids. After completion of the peptide assembly, the protective group is removed from the building unit's functional group and the cyclization is accomplished by coupling the building unit's functional group and a second functional group selected from a second building unit, a side chain of an amino acid residue of the peptide sequence, and an N-terminal amino acid residue.

Backbone cyclization is achieved by covalently connecting at least one amino acid residue in the helix sequence, which was substituted with an Nα-ω-functionalized or an Ca-co-functionalized derivative of amino acid residue, with a moiety selected from the group consisting of: another Nα-ω-functionalized or an Ca-co-functionalized derivative of amino acid residue, with the side chain of an amino acid in the peptide's sequence, or with one of the peptide terminals. Any covalent bond may be used to connect the anchoring positions of the peptide sequence using backbone cyclization.

Some of the methods used for producing backbone cyclized peptides and their building units are disclosed in US Patent Nos.: 5,811,392; 5,874,529; 5,883,293; 6,051,554; 6,117,974; 6,265,375; 6,355613; 6,407059; 6,512092 and international applications WO 95/33765; WO 97/09344; WO 98/04583; WO 99/31121; WO 99/65508; WO 00/02898; WO 00/65467 and WO 02/062819.

The peptides of the invention can be used in the form of pharmaceutically acceptable salts. As used herein the term "salts" refers to both salts of carboxyl groups and to acid addition salts of amino or guanido groups of the peptide molecule. The term "pharmaceutically acceptable" means suitable for administration to a subject, e.g., a human. For example, the term "pharmaceutically acceptable" can mean approved by a regulatory agency of the Federal or a state government or listed in the U. S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Pharmaceutically acceptable salts include those salts formed with free amino groups such as salts derived from non-toxic inorganic or organic acids such as acetic acid, citric acid or oxalic acid and the like, and those salts formed with free carboxyl groups such as salts derived from non-toxic inorganic or organic bases such as sodium, calcium, potassium, ammonium, calcium, ferric or zinc, isopropylamine, triethylamine, procaine, and the like.

Analogs and derivatives of the peptides are also within the scope of the present application.
"Derivatives" of the peptides of the invention as used herein cover derivatives which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e., they do not destroy the activity of the peptide, do not confer toxic properties on compositions containing it, and do not adversely affect the immunogenic properties thereof.

These derivatives may include, for example, aliphatic esters of the carboxyl groups, amides of the carboxyl groups produced by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues, e.g., N-acetyl, formed by reaction with acyl moieties (e.g., alkanoyl or carbocyclic aryl groups), or O-acyl derivatives of free hydroxyl group (e.g., that of seryl or threonyl residues) formed by reaction with acyl moieties.

"Analogs" of the peptides of the invention as used herein cover compounds which have the amino acid sequence according to the invention except for one or more amino acid changes, typically, conservative amino acid substitutions. In order to maintain the activity of the peptide, the core sequence KTSR should remain unchanged. In some embodiments, where the peptide comprises the sequence CWKTSRTSHYC (SEQ ID NO: 3), the two Cys should remain unchanged, or if replaced, should preferably be replaced with two residues capable of forming cyclization.

In some embodiments, an analog has at least about 75% identity to the sequence of the peptide of the invention, for example at least about 80%, at least about 85%, at least about 90%, at least about 99% identity to the sequence of the peptide of the invention.

As used herein, the term "about", when referring to a measurable value is meant to encompass variations of +/-10%, more preferably +/-5%, even more preferably +/-1%, and still more preferably +/-0.1% from the specified value.

Conservative substitutions of amino acids as known to those skilled in the art are within the scope of the present invention. Conservative amino acid substitutions include replacement of one amino acid with another having the same type of functional group or side chain e.g. aliphatic, aromatic, positively charged, negatively charged. One of skill will recognize that individual substitutions, deletions or additions to peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid.
Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

1. Alanine (A), Serine (S), Threonine (T);
2. Aspartic acid (D), Glutamic acid (E);
3. Asparagine (N), Glutamine (Q);
4. Arginine (R), Lysine (K), Histidine (H);
5. Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6. Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Analogs according to the present invention may comprise also peptidomimetics. "Peptidomimetic" means that a peptide according to the invention is modified in such a way that it includes at least one non-coded residue or non-peptidic bond. Such modifications include, e.g., alkylation and more specific methylation of one or more residues, insertion of or replacement of natural amino acid by non-natural amino acids, replacement of an amide bond with another covalent bond. A peptidomimetic according to the present invention may optionally comprise at least one bond which is an amide-replacement bond such as urea bond, carbamate bond, sulfonamide bond, hydrazine bond, or any other covalent bond. The design of appropriate analogs may be computer assisted. Analogas are included in the invention as long as they remain pharmaceutically acceptable and their activity is not damaged.

Pharmaceutical compositions:

The present invention provides pharmaceutical compositions comprising a peptide of the present invention or a salt thereof as the active ingredient.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers, excipients and auxiliaries, which facilitate processing of the active compounds into preparations which can be used pharmaceutically, as known to a person of skill in the art and detailed, for example, in "Handbook of Pharmaceutical Excipients"; Pharmaceutical Press, Ed. Raymond C. Rowe et al., 7th ed., 2012. Proper formulation is dependent upon the route of administration chosen. Techniques for formulation and
administration of drugs may be found in "Remington: The Science and Practice of Pharmacy", Pharmaceutical Press, Editor-in-Chief Loyd V Allen, Jr 22nd ed., 2012.

The term "carrier" refers to a diluent, adjuvant or vehicle with which the therapeutic compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

The term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Non-limiting examples of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils, and polyethylene glycols.

Apart from other considerations, the fact that the active ingredients of the present invention are peptides and peptide analogs dictates that the formulation be suitable for delivery of these types of compounds.

In some embodiments, the pharmaceutical compositions are formulated for parenteral administration. For example, the pharmaceutical compositions may be formulated for injection administration, including but not limited to intravenous, intra-articular, intramuscular, subcutaneous, intradermal or intrathecal. Each possibility represents a separate embodiment of the present invention.

In other embodiments, the pharmaceutical compositions are formulated for oral administration. For oral administration, enteric-coated preparations or dosage forms, microspheres, liposomes and nanoparticles for oral delivery of peptides and proteins may be used.

In some embodiments, the pharmaceutical compositions are formulated for ocular delivery.

Depending on the location of the tissue of interest, the peptides of the present invention can be supplied in any manner suitable for the provision of the peptide to cells within the tissue of interest. Thus, for example, a composition containing the peptides of the present invention can be introduced, for example, into the systemic circulation, which will distribute said peptide to the tissue of interest. Alternatively, a composition can be
applied topically (locally) to the tissue of interest (e.g., injected, or pumped as a continuous infusion, or as a bolus within a tissue, applied to all or a portion of the surface of the skin, etc.).

For administration by injection, the active ingredient may be formulated in aqueous solutions, for example in physiologically compatible buffers including but not limited to Hank's solution, Ringer's solution, or physiological saline buffer. Formulations for injection may be presented in unit dosage forms, for example, in ampoules, or in multi-dose containers with, optionally, an added preservative. The compositions may be in the form of suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing, and/or dispersing agents. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the active ingredients, to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, for example, a sterile, pyrogen-free, water-based solution, before use.

For oral administration, the active ingredient may be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Media such as water, glycols, oils and alcohols can be used in liquid preparations such as suspensions, syrups and solutions. Alternatively, solid carriers such as starches and sugars, lubricants, binders and disintegrating agents can be used.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredient is contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of a compound effective to prevent, alleviate or ameliorate symptoms of a disease of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art.

Toxicity and therapeutic efficacy of the peptides described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the IC50 (the concentration which provides 50% inhibition) and the LD50 (lethal dose causing death in 50% of the tested animals) for a subject compound. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact
formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition.

The combination of a compound of the invention with another agent used for treatment of diseases or disorder involving excessive angiogenesis can be used. Such combination can be used simultaneously or sequentially.

In some embodiments, the composition further comprises at least one more active ingredient. In other embodiments, a pharmaceutical composition is provided, consisting of the peptide of the present invention or a salt thereof as the sole an active ingredient.

The pharmaceutical compositions of the present invention are typically employed for the treatment of a mammal, preferably a human.

Methods and uses:

According to certain aspects of the present invention, there are provided herein methods for treating diseases or disorders involving excessive angiogenesis in a subject in need thereof. The methods disclosed herein comprise administering to the subject a pharmaceutical composition comprising a peptide of the present invention.

According to a further aspect of the present invention, there is provided herein the use of the peptides of the present invention, for the preparation of a medicament for the treatment of diseases or disorders involving excessive angiogenesis.

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels. As used herein, the phrase "diseases or disorders involving excessive angiogenesis" refers to diseases and disorders where abnormally excessive blood vessel development is observed, and/or where abnormal excessive blood vessel development underlies their pathology.

As used herein, the term "treating" or "treatment", refers to reduction, amelioration or even elimination of at least some of the symptoms associated with the relevant disease. The term may also include inhibition of disease progression.

In the case of cancer, the term may include inhibition of tumor growth, reduction in tumor size or even elimination of a tumor, and prevention or reduction of metastasis formation.

In retinal disorders, the term may include, inter alia, inhibition of abnormal formation of blood vessels in the eye.
The diseases or disorders amenable to treatment according to the principles of the present invention are those where abnormal, excessive angiogenesis is implicated in their pathogenesis, or facilitates their progression.

In some embodiments, the disease or disorder is cancer. In particular embodiments, the disease or disorder is a cancerous solid tumor, namely, a malignancy that forms a discrete tumor mass. Tumor types particularly suitable for treatment by the method of the present invention are those where angiogenesis is known to underlie their progression. The method may be used for the treatment of primary as well as metastatic tumors. The method is particularly suitable for the treatment of vascularized solid tumors.

Cancer types that are amenable to treatment include breast carcinomas, lung carcinomas, gastric carcinomas, esophageal carcinomas, colorectal carcinomas, liver carcinomas, ovarian carcinomas, cervical carcinomas, endometrial carcinoma, endometrial hyperplasia, fibrosarcomas, choriocarcinoma, head and neck cancer, nasopharyngeal carcinoma, laryngeal carcinomas, hepatoblastoma, Kaposi's sarcoma, melanoma, skin carcinomas, hemangioma, cavernous hemangioma, hemangioblastoma, pancreas carcinomas, retinoblastoma, astrocytoma, glioblastoma, Schwannoma, oligodendroglioma, medulloblastoma, neuroblastomas, rhabdomyosarcoma, osteogenic sarcoma, leiomyosarcomas, urinary tract carcinomas, thyroid carcinomas, Wilm's tumor, renal cell carcinoma, prostate carcinoma, abnormal vascular proliferation associated with phakomatoses, and Meigs' syndrome. Each possibility represents a separate embodiment of the invention.

More particular examples include squamous cell cancer, lung cancer, colorectal cancer, kidney cancer and glioblastoma. Each possibility represents a separate embodiment of the invention. Lung cancer includes, e.g. small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung. Colorectal cancer includes, e.g. metastatic colorectal cancer. Kidney cancer includes, e.g., metastatic kidney cancer.

In some embodiments, the disease or disorder is a disorder of the eye. In some particular embodiments, the disease or disorder is a retinal disorder.

In some embodiments, the eye or retinal disorder is selected from the group consisting of a proliferative retinopathy (including retinopathy of prematurity), retrolental fibroplasia, neovascular glaucoma, and age-related macular degeneration (particularly wet
age-related macular degeneration). Each possibility represents a separate embodiment of the invention.

In some embodiments, the eye or retinal disorder is selected from the group consisting of macular edema following retinal vein occlusion (RVO), and diabetic macular edema. Each possibility represents a separate embodiment of the invention.

In addition, the disease or disorder is a non-neoplastic inflammatory condition. In some embodiments, the non-neoplastic inflammatory condition is selected from the group consisting of rheumatoid arthritis, psoriasis, fibrosis, atherosclerosis and thyroid hyperplasias (including Grave's disease). Each possibility represents a separate embodiment of the invention.

The methods of the present invention may be combined with one or more known treatments of the above described disorders/diseases.

The present invention further provides methods for diagnosis of diseases or disorders using the peptides of the present invention. The diseases or disorders that can be diagnosed using the peptides of the invention are those associated with abnormal expression of at least one of α1β1 and α2β1 integrins, such as up or down regulation, or mutations, of α1β1 and/or α2β1 integrins. Preferably, the diseases or disorders to be diagnosed are those associated with upregulation of α1β1 and/or α2β1 integrins, for example diseases associated with abnormal endothelial cell growth, abnormal angiogenesis or abnormal cell adhesion. Non-limiting examples include cancer, particularly solid tumors (e.g., vascularized tumors) and collagen bone and ligament disorders.

The present invention further provides methods for imaging angiogenesis in a subject using the peptides of the present invention. In some embodiments, a pharmaceutical composition comprising the peptide is used for imaging angiogenesis.

According to the aforementioned diagnostic applications, the peptides are preferably attached to a detectable label. Non-limiting examples of detectable labels include magnetic, radioactive, fluorescent and near-infrared labels, as known in the art. In some embodiments, the peptides can be used for targeting α1β1 and α2β1 integrins with MRI, near infrared and fluorescent nanoparticles or carriers.

The following examples are presented in order to more fully illustrate certain embodiments of the invention. They should in no way, however, be construed as limiting
the broad scope of the invention. One skilled in the art can readily devise many variations and modifications of the principles disclosed herein without departing from the scope of the invention.

5 EXAMPLES

Materials and Methods

Materials. Collagen IV (from bovine placenta villi) was purchased from Chemicon (Temecula, CA), and collagen I (from rat tail) and Matrigel from BD Biosciences (Bedford, MA). 96-well polystyrene EIA/RIA plates were obtained from Nunc (Roskilde, Denmark), Bovine serum albumin (BSA), Hank’s Balanced Salt Solution (HBSS) sulfate, alkaline phosphatase-conjugated anti-rabbit antibody, p-nitrophenyl phosphate, human recombinant basic fibroblast growth factor (bFGF), and vascular endothelial growth factor 165 kDa (VEGF) were purchased from Sigma-Aldrich (St. Louis, MO). CellTracker™ Green 5-Chloromethylfluorescein Diacetate (CMFDA), was purchased from Invitrogen-Molecular Probes (Eugene, Oregon, USA). Rabbit polyclonal antibodies against GST were purchased from Molecular Probes (Nijmegen, The Netherlands). BrdU kit was purchased from Roche (Mannheim, Germany). Fertilized Japanese quail eggs (Coturnix coturnix japonica) were purchased from Boyd’s Bird Co (Pullman, WA). Lactate Dehydrogenase reagent set was purchased from Pointe Scientific, Inc. (Michigan, USA).

Disintegrins. Viperistatin was obtained from the venom of Vipera xantina palestiniae as previously described (Staniszewska et al., 2009, Cancer Biology & Therapy, 8: 1507-1516). Obtustatin was isolated and purified to homogeneity by two chromatographic steps on HPLC as previously reported (Marcinkiewicz et al., 2003, Cancer Research 63: 2020-2023).

C-type lectin protein. Vixapatin was obtained from the venom of Vipera xantina palestiniae as previously described (Staniszewska et al 2009, supra). Rhodocetin was purified from C. rhodostoma venom as described (Eble et al., 2001, / Biol Chem 276: 12274-12284).

Cell lines. Human aortic endothelial cells (HAECs) were cultured as previously described (Dolle et al., 2005, Journal of Pharmacology and Experimental Therapeutics 315: 1220-1227). Human umbilical vein endothelial cells (HUVECs) were obtained from human umbilical cords from healthy women who underwent normal term pregnancies, as
described elsewhere (Marcinkiewicz et al., 2000) (ref Marcinkiewicz C, Lobb RR, Marcinkiewicz MM, Daniel JL, Smith JB, Dangelmaier C, Weinreb PH, Beacham DA and Niewiarowski S (2000) Isolation and characterization of EMS16, a C-Lectin type protein from Echis multisquamatus venom, a potent and selective inhibitor of the a2al integrin Biochemistry 39:9859-9867). K562 cells transfected with either a1 or a2 integrin subunits (Staniszewska et al 2009, supra). LN18 human glioma cells were prepared as previously described (Walsh et al., 2012, Neuro-Oncology 14: 890-901). Mouse brain capillary cells, bEnd.3, were obtained from ATCC (Manassas, VA) and cultured as previously described (Lecht et al., 2010, Mol Cell Biochem 339:201-213).

Peptide synthesis reagents. All amino acids and Rink resin were purchased from GL Biochem Ltd. (Shanghai, China). N,N-diisopropylethylamine (DIPEA), 2-(1H-benzotriazole-yl)-1,l,3,3-tetramethyllumonium hexafluorophosphate (HBTU), 1-hydroxybenzo-triazole (HOBt) were purchased from BioLab Ltd. (Jerusalem, Israel). All coupling reagents, chemicals and solvents were purchased from Sigma-Aldrich (Rehovot, Israel).

General procedure for peptide preparation and characterization. The peptides were synthesized on solid phase by standard fluorenymethyloxycarbonyl (Fmoc) chemistry (Carpino & Han, 1972, J. Org. Chem. 37: 3404-3409; Chan & P.D. 2000 Oxford University Press, Oxford, UK, pp 303-327). The synthesis was carried out manually on a Rink amide resin using Fmoc-protected amino acids. Coupling was performed for 1h with 4 equivalents of HBTU, 1 equivalents of amino acid in the presence of 4 equivalents of HOBt and 8 equivalents of DIPEA. Fmoc groups were removed with 20% piperidine in N-methyl-2-pyrrolidinone (NMP). Cyclization of the peptides was performed using several approaches, as detailed further for each peptide.

Cleavage from the resin and fully deprotection of peptides was carried out using a mixture of TFA/phenol/H2O/TIS (88:5:5:2 v/v/v v) for 3h at room temperature (Fields & Fields, 1993, Tetrahedron Lett. 34: 6661-6664). The resin was filtrated and the peptide was precipitated by addition of cold diethyl ether to the filtrate. The precipitate was separated by centrifugation at 4000 rcf for 10 min, solubilized in water and lyophilized.

Synthesized peptides were purified by preparative reverse-phase high-performance liquid chromatography (RP-HPLC) with C18 column with an elution gradient of 0-90% acetonitrile with 0.1% trifluoroacetic in water. Peptide purity was verified by Thermo Scientific Dionex UltiMate 3000 analytical HPLC. All peptides showed purity above 95%,
based on the chromatographic peak area revealed at 220 nm. The peptides identities were assessed by electrospray ionization mass spectrometry using a ThermoQuest Finnigan LCQ-Duo in the positive ion mode. The data were processed using ThermoQuest Finnigan’s Xcalibur™ Biomass Calculation and Deconvolution software. Masses found using electrospray ionization mass spectrometry were in the range of standard deviation (0.1% of calculated masses) (Todd JFJ, 1991, Pure Appl Chem 63:1541-1566). In addition, structures of Compounds 6 and 10 were confirmed by high resolution electrospray ionization mass spectrometry using a LTQ Orbitrap, Thermo Scientific in the positive ion mode. Generic names of the peptides were presented with asterisk(s) representing amino acids which are involved in cyclization. Amino acids labeled with the same number of asterisk(s) are interacting and forming the bond.

**Compounds 1, 2, 2a, 3, 4 and 4a.** The peptides were synthesized as described in General procedure. The respective calculated and experimentally found molecular weight values were 1328.54 and 1329.67 for Compound 1 (CWKTSLTSHYC), 1236.35 and 1237.67 for Compound 2 (GWKTSLTSHYG), 1371.56 and 1372.63 for Compound 3 (CWKTSRTSHYC), and 1279.38 and 1280.23 for Compound 4 (GWKTSRTSHYG).

**Compounds 5 and 6.** The peptides were synthesized as described in General procedure. Disulfide bonds within these peptides were formed by oxidation of the two Cys(Trt) amino acids with 0.1 M iodine/MeOH (Kamber et al., 1980, Helv Chim Acta 63: 899-915). The respective calculated and experimentally found molecular weight values were 1928.20 and 1928.01 for Compound 5 (C*WKTSRTSHYC*PLYQG) and 1896.8519 and 1896.8518 for Compound 6 (C*WKTSRTSHYC*PLYPG).

**Compound 7 and Compound 8.** Fmoc-Lys(Fmoc)-OH was coupled to a glycine already bound to the resin. Following cleavage of Fmoc by 20% piperidine, the peptides were assembled on the two free amino groups of Lys, to form two parallel peptide chains of identical sequence. The pair of cysteines inserted along each chain, were protected by trityl (Trt) and acetamidomethyl (Acm). A one-pot reaction for selective formation of two inter-chain disulfide bonds was carried out by adding 2 ml of 0.1 M I₂ in dichloromethane (DCM) for 6h (Bullesbach & Schwabe, 1991, J Biol Chem 266: 10754-10761). This method takes advantage of the difference in the kinetic rates of disulfide bond formation by S-Trt and S-Acm in the presence of iodine, in dichloromethane (DCM). Whereas the Cys(Trt) disulfide bond was formed by oxidation in a few seconds, formation of the Cys(Acm) disulfide bond occurred after about 2 h. The respective calculated and
experimentally found molecular weight values were 3885.36 and 3885.86 for Compound 7 ((C*WKTSRTSHYC**TGKSD,)2K) and 3552.96 and 3553.01 for Compound 8 ((C*WKTSRTSHYC**TGKSD,)2K).

**Compound 9.** Cyclization of the peptide was carried out in a sequential manner during the elongation step. The five cysteines, at positions 6, 7, 29, 34 and 36, were protected by three distinct groups which were introduced as Cys\(^{736}(\text{Trt})\), Cys\(^{6}(\text{Acm})\) and Cys\(^{29}(\text{StBu})\). In addition, the peptide sequence included seven lysines, two of which were Lys\(^{10,19}(\text{Trt})\) and the one at the terminal position was BocLys\(^{\text{Fmoc}}\). After the insertion of Cys\(^{36}(\text{Trt})\), the Trt group was removed with 3% trichloroacetic acid (TCA) and the free SH group formed was allowed to react with bromoacetic acid to form Cys carboxymethyl thioether. The free carboxylic group was then protected by dimethoxytrityl (DMT), resulting in Cys\(^{36}(\text{S-CH2COODMT})\). Following the introduction of Fmoc-Lys\(^{19}(\text{Trt})\) into the assembled sequence, its side chain protecting group and the DMT of Cys\(^{36}(\text{S-CH2COODMT})\) were removed with 3% TCA. The free amino group of the Lys\(^{19}\) and the free carboxylic group linked to the Cys\(^{36}\) were coupled to form an amide bond, as described in the general procedure for peptide synthesis. At this stage Cys\(^{29}(\text{StBu})\) was transformed into Cys\(^{29}(\text{CH2CH2N=Pht})\) by reduction with dithiothreitol (DTT), followed by alkylation with bromoethyl phthalimide. The Cys\(^{34}(\text{Trt})\) was then converted into Cys\(^{34}(\text{S-CH2COODMT})\) by adding 0.1M iodine in methanol, followed by washing, DTT reduction, and alkylation with bromoacetic acid and DMT esterification. The succeeding cyclization was implemented after the insertion of Lys\(^{10}(\text{Trt})\). The Trt and the DMT protecting groups of Lys\(^{10}(\text{Trt})\) and Cys\(^{34}(\text{S-CH2COODMT})\), respectively were removed with 3% TCA and the free amine and the carboxylic groups formed were coupled. The next cyclization took place between Cys\(^{29}(\text{CH2CH2N=Pht})\) and Cys\(^{7}(\text{S-CH2COOH})\). After accessing Cys\(^{7}(\text{Trt})\), the trityl group was replaced by a carboxymethyl group, as previously described. The phthalic acid of Cys\(^{29}(\text{CH2CH2N=Pht})\) was removed by 2M hydrazine hydrate/methanol and the free amine formed was coupled with the free carboxylic group of Cys\(^{7}(\text{S-CH2COOH})\). Subsequent to peptide completion, Cys\(^{6}(\text{Acm})\) was transformed into Cys\(^{6}(\text{S-CH2COOH})\) in the same manner as Cys\(^{34}(\text{Trt})\), while Acm group was cleaved with 0.1 M iodine in methanol, and the Fmoc protecting group of Boc-Lys\(^{\text{Fmoc}}\) was cleaved by 1M tetrabutylammonium fluoride (TBAF). The free amino and carboxylic groups formed were coupled to yield the cyclization. The respective calculated and
experimentally found molecular weight values were 4251.73 and 4251.96 Compound 9
(K*TTGPC*C**RQK***KLKPAGTTK****WKTSRTSHYC**TGKSC***DC****G).

**Compound 10.** The peptide was synthesized as described in General procedure. The two intra-chain disulfide bonds of Compound 10 were prepared in the same manner as Compounds 7 and 8. The respective calculated and experimentally found molecular weight values were 2003.7979 and 2003.7977 for Compound 10 (C*C**WKTSRTSHYC**TGKSC*G).

**Cell adhesion assay.** The assay was carried out as described previously with minor changes (Bazan-Socha et al 2004, *Biochemistry* 43: 1639-1647). The day before the experiment each well of a 96-well plate was coated with 10 µg/ml collagen I or 1 µg/ml collagen IV in 0.02 M acetic acid and incubated overnight at 4°C. Thereafter non-specific binding was blocked by incubating the wells with 1% (w/v) bovine serum albumin (BSA) in Hank's Balanced Salt Solution (HBSS) containing 5 mM MgCb, at room temperature for 1 h prior to use. The cells were labeled by incubation with 12.5 µM 5-chloromethylfluorescein diacetate (CMFDA) in HBSS without 1% BSA at 37°C for 30 min. The labeled cells were then centrifuged at 1,000 rpm and washed twice with HBSS containing 1% BSA to remove excess CMFDA. Labeled cells (1 x 10^5 cells/well) were added to each well in the absence or presence of inhibitor and incubated at 37°C for 60 min. In the presence of peptide, cells were added to the well after prior incubation with peptide for 30 min at 37°C. Unbound cells were removed by washing the wells three times with 1% (w/v) BSA in HBSS, and bound cells were lysed by the addition of 0.5% Triton X-100 (diluted in DDW). The fluorescence in each well was quantified with a SPECTRAFluor Plus plate reader (Tecan, San Jose, CA), at λ_{ex} = 485 nm and λ_{em} = 530 nm. To determine the number of adhered cells from the fluorescence values, a standard curve was generated by serial dilutions of known numbers of CMFDA-labeled cells.

**Inhibition of GST-a1A/GST-a2A binding to type IV/I collagens by peptides.**
Inhibition ELISA was performed as published previously (Eble & Tuckwell, 2003, *Biochem. J.* 376: 77-85), with the following modifications: CB3 (collagen IV fragment) or collagen I was immobilized overnight at 4°C on a microtiter plate at 10 µg/ml in TBS/MgCb (50 mM Tris-HCl, 150 mM NaCl, and 2 mM MgCb, pH 7.4) and 0.1 M acetic acid, respectively. After blocking the plate with 1% BSA in TBS/MgCb, the GST-tagged a1A/GST-tagged a2A domain was allowed to bind to type IV collagen/collagen I in the presence or absence of different peptides for 2 h at room temperature. The bound GST-
αIA/GST-α2A domain was fixed for 10 min with 2.5% glutaraldehyde in HEPES buffer (50 mM HEPES, 150 mM NaCl, and 2 mM MgCl$_2$, and 1 mM MnCh pH 7.4). The bound GST-αIA/GST-α2A was quantified with rabbit polyclonal antibodies against GST, followed by alkaline phosphatase-conjugated anti-rabbit antibody, which served as the primary and secondary antibodies, respectively, each diluted in 1% BSA in TBS/MgCb.

The conversion of p-nitrophenyl phosphate was measured at 405 nm in an ELISA reader Bio Tek (Bad Friedrichshall, Germany). Nonspecific binding was assessed by binding of GST-αIA/GST-α2A to BSA or of α6β1/α2β1 integrin to collagen IV/collagen I in the presence of 10 mM EDTA.

**Cell proliferation assay.** HAEC and HUVEC proliferation assay was performed using (BrdU) kit according to the manufacturer's instructions (Staniszewska et al., 2007, *Circ Res* 100: 1308-1316).

**Cell migration assay.** HUVEC migration was measured using a wound healing assay (Lecht et al. 2010, *Mol Cell Biochem* 339:201-213). In brief, 1 x 10$^6$ cells/well were added to a 24-well culture plate. Upon formation of a confluent monolayer, cell migration was initiated by scratching the HUVEC monolayer with a small sterile pipette tip, thus generating a cell-free area (wound) of approximately 1 mm in width. The wounded cells were washed three times with 1% FCS supplemented endothelial cell growth medium 2 and the photos of the wounds at time 0 hours were taken. Thereafter, the culture medium was changed to 2% fetal bovine serum medium as the cell migration stimulator, in the presence or absence of synthetic peptides (50 μg/ml) or obustatin (5 μg/ml) and the cultures were allowed to migrate for 24 hours. At the end of the migration experiment, another set of photos was taken of the same regions. Images were analyzed using ImageJ software (NIH, Bethesda MD). To assess cell migration at the wound edge and to calculate the area covered by migrating cells, the cell-free areas of the wounds at 24 hours after wounding were subtracted from the area of the wounds at the 0-hour time point and calculated as a percentage of untreated (control) cultures (Lecht et al., 2010, supra).

**Human aortic endothelial cells tube formation in Matrigel assay.** The assay was performed using 96-well plate coated with growth factor reduced Matrigel. Briefly, 1x10$^4$ human aortic endothelial cells (HAEC) per well (in complete EMB-2 media or 2% FBS) were added in the presence or absence of peptides, and the plate was incubated overnight at 37°C in 5% CO$_2$. Images were captured under inverted microscope (Olympus 1X81) with 35x magnification. The images were transferred to ImageJ software and the...
number of branching points was counted per observation field (Momic et al., 2012, *Toxins 4*: 862-877).

**Angiogenesis in chorioallantoic membrane (CAM) quail embryonic model.**

The assay of VEGF induced and tumor induced angiogenesis in the quail embryonic system was performed as described previously (Parsons-Wingerter et al., 1998, *Microvasc Res* 55: 201-214; Lazarovici et al., 2006, *Current Pharmaceutical Design* 12: 2609-2622; Brown et al., 2008, *Int J Cancer* 123: 2195-2203). Fertilized Japanese quail eggs (*Coturnix coturnix japonica*) were cleaned with ethanol, and maintained at 37°C until embryonic day 3 in incubator without CO2. The shells were then opened with a razor blade. Using minced sterile scissor, the contents transferred into 6-well tissue culture plates and returned to the 37°C incubator. At embryonic day 7, the compounds, were applied under sterile conditions to the surface of the CAM and after 24 h the effect on the aortic tree was evaluated. The embryos were divided into experimental groups, each containing at least 10 embryos. The control group received a vehicle (PBS) treatment. In the experiments using LN18 induced tumors under the CAM, LN18 glioma cells (1×10^7/50μl) were injected at day 7 under the CAM and tumor induced angiogenesis was measured at day 12. At the end of the experiment the embryos were fixed with 5 ml of pre-warmed 2% gluteraldehyde, 4% paraformaldehyde in PBS for 48 hours at room temperature. Membranes with and without tumor were dissected and mounted onto the glass slide. The membranes were mounted onto glass slides for evaluation of the fractal dimension (Df). The area of CAM selected as a square for analysis of the vascularization ratio, was localized as previously described (Parsons-Wingerter et al 1998, supra; Lazarovici et al 2006, supra) or in the case of the tumor induced angiogenesis, in the site opposite to the tumor on the membrane. For example, if the tumor developed in the right corner of the CAM, the vascularization tree for analysis was framed in the left corner of membrane (Lazarovici et al 2006, supra).

**Corneal micropocket assay.** The corneal micropocket assay was performed as previously detailed (Benny et al., 2008, *Nat Biotechnol*, 26:799-807). In brief, pellets containing 80 ng carrier-free recombinant human bFGF or 160 ng VEGF (R&D Systems, Minneapolis, MN) were implanted into micropockets that were created in the cornea of anesthetized mice. Mice were treated daily by eye drops (8 mg/ml) for 5 days, and the vascular growth area was then measured using a slit lamp. The area of neovascularization was calculated as the vessel area calculated as the vessel length measured from the limbus
and clock hours around the cornea, using the following equation: Vessel area (in millimeters squared) = \[\pi \times \text{clock hours} \times \text{vessel length (in millimeters)} \times 0.2 \text{ mm}\].

**Stability of the peptides in human serum.** One milliliter of RPMI media supplemented with 25% (v/v) of human serum was introduced into a 1.5-ml Eppendorf tube and was temperature equilibrated at 37°C for 15 minutes before adding 5 µl peptide stock solution to make a final peptide concentration of 50 µg/ml. The initial time was recorded and at known intervals, 100 µl of the reaction solution was removed and added to 200 µl of 96% ethanol to precipitate serum proteins. The sample was cooled at 4°C for 15 minutes and then centrifuged at 18,000g for 2 minutes to precipitate serum proteins. The supernatant was then applied to a C-18 column for separation by RP-HPLC. A linear gradient from 100% buffer A (0.1% TFA in water), to 50-50% buffer A and buffer B (0.1% TFA in acetonitrile), was applied for over 30 minutes. The flow rate was 1 ml/min and absorbance was detected at 220 nm (Jenssen and Aspmo 2008) Serum stability of peptides., in Peptide-based drug design (Laszlo O ed) pp 177-186, Humana Press, New York, NY.).

**Cell death assay.** Cell death was assessed by measuring the release of lactate dehydrogenase (LDH) into the medium using Lactate Dehydrogenase reagent set as previously described (Tabakman et al., 2002, J Neurosci Res 68: 463-469). LDH activity was determined spectrophotometrically at 340 nm by following the rate of conversion of oxidized nicotinamide adenine dinucleotide (NAD) to the reduced form of (NADH). LDH release was expressed as the optical density (OD) units.

**Toxicity to mice.** Experiments with animals and animal care were approved by the Committee of Ethics of The Hebrew University and were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. A 50 mg/kg quantity of Compounds 6 and 10, in 0.2 ml were injected intravenously (iv) to male C57BL/6 mice and SABRA-M mice (n=3) and they were monitored for three consecutive weeks. The animals were examined for autonomic symptoms by measuring salivation, urinary delivery, pupillary constriction, hart rate, blood pressure, and hair contraction. Neurotoxicity was evaluated by general locomotors activity of the animals in the cage and the ability of the peptide-injected mice to maintain balance and motor coordination by crossing 3 cm, 2 cm and 1 cm width balance beam. Occurrence of either flaccid or spastic paralysis of the legs were also measured. Blood
samples were taken from control and peptide-injected mice after 10 hours from injection and submitted from hematocrit and biochemical analysis.

**Metastasis model of B16F10 melanoma in mice.** B16 melanoma cells were incubated with the peptides in PBS for 2 hours (at 37°C) or left untreated (control). Thereafter, male C57BL/6 mice were injected intravenously (iv) with 1x10⁵ B16 melanoma cells in 0.2 ml injection volume (2.5 mg/mL of peptide) (Staniszewska et al 2009, supra). The final concentration of the peptides was 10 mg/kg of mouse. Mice survival was followed-up every day for 10 weeks after cells inoculation and death was recorded. In any case that a mouse showed clinical signs of discomfort or pain, it was euthanized and necropsy was performed. The analysis of survival was done by GraphPad Prism 5, performing both the log-rank (Mantel-Cox) test and the Gehan-Breslow-Wilcoxon test. Experiments were performed under double-blind testing.

**Statistics.** The Student's and ANOVA were used to determine the significance of the differences between different treatments compared to control groups, and p value ≤ than 0.05 was considered significant.

**Example 1 - Synthesis of linear and cyclic peptides**

Fig. 1A shows the sequence and structure of Obtustatin and Viperistatin (SEQ ID NOs: 24 and 25, respectively). First, linear Obtustatin and Viperistatin peptide analogs containing the KTS motif were synthesized and their biological activity was investigated by an *in vitro* adhesion assay with K562 cells overexpressing α1 integrin subunit, using the ligand collagen IV. The peptides with the highest anti-adhesive activity are presented in Table 1. The most active peptide was Compound 3 Viperistatin analog. Compound 1 Obtustatin analog, the sequence of which differs from Compound 3 only by one amino acid at position 24 where Leu²⁴→Arg²⁴, had six fold decreased activity. This suggests that the positively charged side chain of Arg form a better productive interaction with residues within the ligand-binding pocket of the αβ integrin (Brown et al., 2009, *Biochem J All*: 95-101) than the alkyl chain of Leu. In addition, it was found that the two cysteins of Compound 3, at the carboxy and amino termini of the sequence had a strong impact on its potency. Upon exchanging the two terminal Cys with two Gly, the αl-mediated anti-adhesive activity of Compounds 4 and 2 decreased significantly by 35-fold and 7-fold, respectively. The activity was further decreased upon exchanging the Trp at position 20
with Val (Compounds 2a and 4a), in addition to the Cys→Gly exchange in the two termini.

Table 1. Synthesized linear peptide

<table>
<thead>
<tr>
<th>Compound</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
<th>IC₅₀ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (SEQ ID NO: 20)</td>
<td>C</td>
<td>W</td>
<td>K</td>
<td>T</td>
<td>S</td>
<td>L</td>
<td>T</td>
<td>S</td>
<td>H</td>
<td>Y</td>
<td>C</td>
<td>0.645</td>
</tr>
<tr>
<td>2 (SEQ ID NO: 21)</td>
<td>G</td>
<td>W</td>
<td>K</td>
<td>T</td>
<td>S</td>
<td>L</td>
<td>T</td>
<td>S</td>
<td>H</td>
<td>Y</td>
<td>G</td>
<td>&gt; 4 (not active)</td>
</tr>
<tr>
<td>2a (SEQ ID NO: 21)</td>
<td>G</td>
<td>V</td>
<td>K</td>
<td>T</td>
<td>S</td>
<td>L</td>
<td>T</td>
<td>S</td>
<td>H</td>
<td>Y</td>
<td>G</td>
<td>&gt; 4 (not active)</td>
</tr>
<tr>
<td>3 (SEQ ID NO: 3)</td>
<td>C</td>
<td>W</td>
<td>K</td>
<td>T</td>
<td>S</td>
<td>R</td>
<td>T</td>
<td>S</td>
<td>H</td>
<td>Y</td>
<td>C</td>
<td>0.105</td>
</tr>
<tr>
<td>4 (SEQ ID NO: 22)</td>
<td>G</td>
<td>W</td>
<td>K</td>
<td>T</td>
<td>S</td>
<td>R</td>
<td>T</td>
<td>S</td>
<td>H</td>
<td>Y</td>
<td>G</td>
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<tr>
<td>4a (SEQ ID NO: 23)</td>
<td>G</td>
<td>V</td>
<td>K</td>
<td>T</td>
<td>S</td>
<td>R</td>
<td>T</td>
<td>S</td>
<td>H</td>
<td>Y</td>
<td>G</td>
<td>&gt; 4 (not active)</td>
</tr>
</tbody>
</table>

Next, conformational constrained cyclic sequences containing the KTS motif were prepared. It is assumed that the three-dimensional structure of these peptides will provide an improved binding model to the integrin-binding loop of Viperistatin compared to the linear peptide, Compound 3. In the first group (Fig. 1B) two cyclic peptides were synthesized with one intradisulfide bridge between Cys¹⁹ and Cys²⁹ i.e., Compounds 5 and 6, the structure of which differs by only one amino acid Gln³³→Pro³³, respectively. To generate new analogs, a second group was synthesized (Compounds 7 and 8) (Fig. 1C), represented by two peptides with double intercyclization. The cyclization was achieved by the formation of two disulfide bonds between two identical strand sequences containing modified 19-35 Viperistatin sequence. In both peptides Cys³⁴ was omitted. In Compound 8, an additional modification was done by exchanging the position of Cys from position 29, to position 24. In both peptides interbinding was implemented by linking Cys of
identical protecting groups residing on two identical peptide strands. Two interdisulfide bonds were formed between Cys\textsuperscript{19,19} and Cys\textsuperscript{29,29} of Compound 7, and between Cys\textsuperscript{19,19} and Cys\textsuperscript{24,24} in Compound 8 (Fig. 1C). The third group of peptides is presented by Compounds 9 and 10 (Fig. ID). The structure of Compound 9 is a modified Viperistatin sequence, where the amino acids at positions 37-40 are eliminated. The peptide is characterized by four intra-strand bonds between Lys\textsuperscript{1} and Cys\textsuperscript{6}, Lys\textsuperscript{10} and Cys\textsuperscript{34}, Lys\textsuperscript{19} and Cys\textsuperscript{36}, and Cys\textsuperscript{7} and Cys\textsuperscript{29}. Compound 10 includes the original Viperistatin 19-34 sequence and one additional Cys at position 29. Its two intradisulfide bonds involved are between Cys\textsuperscript{18} and Cys\textsuperscript{34} and Cys\textsuperscript{19} and Cys\textsuperscript{29}.

**Example 2 - Potency and efficacy of Viperistatin analogs in cell adhesion assays**

In the cellular adhesion assay, inhibitory effect of all synthesized peptides was first tested using al-K562 transfectants. To measure the potency and efficacy of Viperistatin analogs, dose-response experiments were performed in a range of physiological concentrations between 0.1 nM-100 nM using collagen IV coated plates. As expected, Viperistatin blocked a1 mediated cell adhesion to collagen IV (Fig. 2A) with an apparent IC\textsubscript{50} of 0.6 nM and 100% efficacy (Table 2). Compounds 5 and 6 with one intracyclization were characterized by very similar IC\textsubscript{50} values of 0.20 and 0.17 nM, respectively, and an equal efficacy of 20% (Fig. 2A, Table 2). Apparently, the substitution at position 33, of Gin in Compound 5 by Pro in Compound 6 did not affect the activity. Compounds 7 and 8, with inter-cyclization, were characterized by an identical IC\textsubscript{50} of 0.15 nM and an efficacy of 25% (Fig. 2B, Table 2). Compounds 9 and 10 with intracyclization were characterized by an identical IC\textsubscript{50} of 0.17 nM and efficacies of 40% (Fig. 2C, Table 2). All the synthesized peptides were characterized by increased potency compared with Viperistatin (Figure 2A-C, Table 2). By contrast, inter- or intracyclization of the peptides resulted in reduced efficacy, to a level of 20% for Compounds 5 and 6, 25% for Compounds 7 and 8 and 40% for Compounds 9 and 10, vs Viperistatin (Figure 2A-C, Table 2). In conclusion, cyclization significantly increased the potency but reduced the efficacy of the synthesized peptides, in comparison with Viperistatin.

To test the specificity of the Viperistatin analogs towards the α(β\textsuperscript{1}) and α2β\textsuperscript{1} integrins, a2-K562 transfectants were used, and potency and efficacy of the analogs were measured in the same range of concentrations, using collagen I-coated plates and
Vixapatin, an α2 selective C-type lectin protein (Arlinghaus et al., 2012, *Toxicon* 60: 512-519), as positive control. Vixapatin generated a dose-response inhibitory effect on α2 mediated adhesion, with an apparent IC50 of 3 nM and an efficacy of 100%. The Viperistatin analogs showed a high potency of inhibition, with an apparent IC50 in the range of 0.12-0.25 nM and efficacy in the range of 10-30% (Fig. 2 D-F, Table 2). These findings indicate dual functional antagonism of synthesized peptides towards both α1β1 and α2β1 integrin mediated adhesion.

Table 2. Potency and efficacy of the peptides toward α-l- and α2-K562 cells adhesion.

<table>
<thead>
<tr>
<th>Peptides</th>
<th>α1 overexpressor cells</th>
<th>α2 overexpressor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Potency IC50 (nM)</td>
<td>Efficacy (%)</td>
</tr>
<tr>
<td>Viperistatin</td>
<td>0.60</td>
<td>100</td>
</tr>
<tr>
<td>Vixapatin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Compound 5</td>
<td>0.20</td>
<td>20</td>
</tr>
<tr>
<td>Compound 6</td>
<td>0.17</td>
<td>20</td>
</tr>
<tr>
<td>Compound 7</td>
<td>0.15</td>
<td>25</td>
</tr>
<tr>
<td>Compound 8</td>
<td>0.15</td>
<td>25</td>
</tr>
<tr>
<td>Compound 9</td>
<td>0.17</td>
<td>40</td>
</tr>
<tr>
<td>Compound 10</td>
<td>0.17</td>
<td>40</td>
</tr>
</tbody>
</table>

A cell-free assay was performed to assess whether the synthesized peptides directly interact with A domains of α1 or α2, thus providing a possible mechanism for their inhibitory effect in the cellular adhesion assay. For this purpose Viperistatin and Rhodocetin, another C-type lectin protein selective for α2β1 integrin (Eble & Tuckwell 2003, supra), as well as Compounds 3, 5-10 were incubated together with the GST-linked A domains, and allowed to bind to the immobilized collagen I or CB3 (collagen IV fragment). The amount of bound recombinant A domain provided information on the inhibitory potential and biochemical recognition ability of the peptides (Fig. 3). Similar to Obtustatin (Marcinkiewicz et al 2003, supra), Viperistatin did not inhibit binding of the GST- αlA domain to collagen IV in a physiological range of concentrations (Fig. 3A). However, at high concentrations (100-1000 µM), Compounds 6 and 10 dose-dependently
inhibited binding of GST- a1A by 50% (Fig. 3B). Without being bound by any theory of a mechanism of action, this finding suggests that Viperistatin affects a1 integrin outside of the A domain, most probably by conformational change, whereas Compounds 6 and 10 recognized with low potency a motif in the A domain. In the experiments with GST- a2A domain at physiological concentrations, a similar lack of inhibition was observed (Fig. 3C). However, in the range of the concentration between 0.01 and 1.0 µM, as previously reported (Eble & Tuckwell 2003, supra) Rhodocetin inhibited binding of the GST- a2A domain to collagen I in dose-dependent manner (Fig. 3D). Surprisingly, Compounds 6 and 10 increased the binding of GST- a2A to collagen I in a dose-dependent manner. These results may suggest that Viperistatin analogs affect a1A and a2A integrin domains at non-physiological concentrations, but the mechanism requires further investigation.

**Example 3 - Viperistatin analogs inhibit proliferation and migration of endothelial cells**

To further characterize the effect of the peptides on endothelial function, their influence on HUVEC and HAEC proliferation was screened, using the BrdU assay (Fig. 4). Similarly to Obtustatin, 50 µg of Compounds 6 and 10 inhibited proliferation of HAEC by 90%. Compounds 3, 7, 8 and 9 had no effect (Fig. 4A). The effect of Compound 6 on the inhibition of HUVEC and HAEC proliferation was 50-90% (Fig. 4A,B). Compound 10, like Obtustatin, completely inhibited the proliferation of both cell lines. These results indicate the angiostatic effect of Viperistatin analog Compounds 6 and 10 on endothelial cells.

In another approach, the effect of 50 µg of Compounds 6 and 10, which were the most active in inhibition of endothelial cells proliferation, on HUVEC migration using a wound healing assay was characterized (Fig. 5). In untreated cells (control), 100% wound closing was measured, and Obtustatin completely blocked wound closing (100%). Interestingly, Compound 10 blocked by 26% and Compound 6 to a higher extent, inhibited by 53% wound closing. These results also indicate antiangiogenic effects of these compounds on HUVECs.

**Example 4 - Viperistatin analogs inhibit tube formation in Matrigel assay**

To investigate the ability of the peptides to inhibit tube formation by HAEC and HUVEC, the Matrigel angiogenesis assay was performed. Treatment for 14 h with 100 µg
of Obtustatin, as well as with Compounds 6 and 10 completely inhibited tube formation (Fig. 6). These results indicated the angiostatic effect on endothelial cells of Viperistatin analogs, Compounds 6 and 10.

**Example 5 - Viperistatin analogs anti-angiogenic effect in CAM assay**

First, the effect of Viperistatin analogs on VEGF-induced angiogenesis in the CAM quail embryonic model was measured. The binary images of the mid-arterial endpoint CAM fragments showed a significant overgrowth of small capillaries after 24 h of treatment with VEGF compared with control vehicle-treated (PBS) embryos (Fig. 7, VEGF, Control). At 200 µg per embryo, Compounds 6 and 10 significantly (80%) inhibited VEGF-induced capillary formation, as found with 20 µg Obtustatin per embryo (Fig. 7).

Next, the effect of Viperistatin analogs on angiogenesis induced by glioblastoma tumor cells was estimated. LN18 glioma cells were injected into the shell-less embryonic CAM system and the effect of Viperistatin analogs on mid-arterial capillary sprouting was measured (Fig. 8A). LN18 glioma tumor induced a significant increase in angiogenesis (Fig. 8B), expressed by an increase in fractal dimension (Df) values from 1.116 to 1.162, 50% greater than that of the control. Treatment with 100 µg of Compound 6 reduced the LN18 induced-angiogenic effect by 50%, very similar to the effect caused by the same amount of Compound 10 (Fig. 8A,B). Those results characterized Compounds 6 and 10 as anti-angiogenic compounds.

**Example 6 - Viperistatin analogs effects on corneal micropocket angiogenesis**

The antiangiogenic properties of Compounds 6 and 10 were evaluated *in vivo* with the corneal micropocket assay (Benny et al., 2008, supra). After 5 days of treatment with 8 mg/kg of Compounds 6 and 10, the antiangiogenic effect was measured with 80 ng bFGF-induced angiogenesis in the eyes of treated mice compared with untreated mice (Control) (Fig. 9, upper panel). Quantification of the angiogenesis area (Fig. 9, lower panel) showed 20% ± 5% inhibition of angiogenesis with Compound 6 compared with control (P = 0.05, n = 10). Compound 10 did not show statistically significant inhibition in this assay. Similar peptide inhibitory results were obtained with 200 ng VEGF-induced angiogenesis in the cornea.
**Example 7 - Stability of the peptides in human serum**

To investigate the effects of the peptide cyclization on proteolytic susceptibility, the degradation of the intact peptides incubated in human serum at 37°C was followed by RP-HPLC. Incubations were done for different periods of time and the results are presented in **Fig. 10**. The linear peptide (Compound 3, squares) was degraded with a half-life of 3 hours. In contrast with the linear peptide, cyclization of peptides prolonged their stability. Compound 6 (triangles) containing one cyclization was completely degraded after 24 hours with a half-life of 10 hours. Compound 10 (circles) with double cyclization showed a more complex degradation behavior, and 50% of the starting peptide amount was preserved for 30 hours in human serum.

**Example 8 - Safety of Compound 6 (designated Vimocin) and Compound 10 (designated Vidapin)**

First, to verify that the antiangiogenic effect of viperistatin analogs is not due to toxicity toward endothelial cells, Compounds 6 and 10 (100 µM) were incubated with HUVECs and HAECs for 7 days and the amount of LDH release in the medium was estimated. No significant release of LDH over the control (10% ± 5%) was measured for all cultures up to 7 days of treatment, indicating a lack of necrotic cell death. Considering that the IC50 value of these compounds for inhibition of cell adhesion is 0.17 nM, it is possible to estimate a therapeutic index in vitro of 1000, indicating high safety for endothelial cells.

Second, to investigate the safety of Viperistatin analogs in mice, male mice were injected intravenously with a dose of 50 mg/kg of Compounds 6 and 10 for 3 consecutive weeks. Acute tolerability was observed. At injection of this high dose of 50 mg/kg body weight, the mice did not suffer from visible weakness and/or exhaustion. No paralysis, altered motor activity, or irregular behavior was observed in mice treated with Compounds 6 and 10, suggesting a lack of neurotoxicity. Cutaneous hematomas around the injection or at distant locations site within 24 hours after injection have not been observed. Furthermore, no mice sudden deaths occurred within 24 hours after the injection or during the 3 weeks of observation. After 10 hours, the blood of mice injected with Compounds 6 and 10 was submitted for hematologic and biochemical analysis. The values for white blood cells, red blood cells, and platelet counts were in the normal range of 6-15 x 10^3/µl, 7-12 x 10^3/µl, and 200-450 x 10^3/µl, respectively, for mice injected with Compounds 6
and 10 similarly with the values obtained for control mice. Additional evidence on the lack of hemorrhage or anemic conditions was indicated by the similar hematocrit value in the range of 35-45% and the mean corpuscular hemoglobin of 11.1-12.7 pg/mice, between mice injected with Compounds 6 and 10 compared with control mice. Lack of lymphopenia, monocytopenia, and granulocytopenia was indicated by similar values in the range of 20-40%, 3-5%, and 7-13%, respectively, between mice injected with Compounds 6 and 10 compared with control mice. Alkaline phosphatase and LDH values were in the range of 100-214 units per liter and 1000-2400 units per liter, respectively, suggesting no toxic effects to liver and other tissues.

Example 9- Survival of B16 melanoma mice treated with Viperistatin analogs

C57BL/6 mice were injected intravenously (iv) with B16 melanoma cells ($10^5$ cells/mouse). Cells were incubated with the peptides in PBS for 2 hours (in 37°C) or left untreated (control), and thereafter the cells were injected into the mice. Survival of mice treated with different peptides is shown in Fig. 11. It is evident that Compound 10 (Vidapin) significantly increased the survival of the mice with melanoma from median survival time of 40 days (control) to 73 days. All mice treated with Vidapin did not show any clinical signs at the end point of 73 days.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed chemical structures and functions may take a variety of alternative forms without departing from the invention.
CLAIMS

1. A cyclic peptide of 8-30 amino acids comprising the sequence WKTSXiRTSH (SEQ ID NO: 1), wherein Xi is absent or Cys, the peptide containing said sequence in a cyclic portion thereof.

2. The peptide of claim 1, comprising the sequence WKTSRTSHY (SEQ ID NO: 2) in a cyclic portion thereof.

3. The peptide of claim 2, comprising the sequence CWKTSRTSHYC (SEQ ID NO: 3) in a cyclic portion thereof.

4. The peptide of claim 3, cyclized via a disulfide bridge connecting the side chains of two cysteine residues present in the sequence.

5. The peptide of claim 1, wherein the peptide comprises 15-25 amino acids.

6. The peptide of claim 1, wherein the peptide is monocyclic.

7. The peptide of claim 1, wherein the peptide is bi-cyclic.

8. The peptide of claim 1, wherein the peptide is tri-cyclic.

9. The peptide of claim 1, comprising a plurality of cyclizations, wherein each cyclization is independently selected from the group consisting of side-chain to side-chain; end to end, backbone to backbone and backbone to end.

10. The peptide of claim 9, wherein each cyclization is independently selected from the group consisting of side-chain to side-chain and end to end.

11. The peptide of claim 10, wherein at least one of said plurality of cyclizations is formed by a disulfide bridge connecting the side chains of two cysteine residues present in the sequence.

12. The peptide of claim 10, wherein all of said plurality of cyclizations are formed by disulfide bridges connecting the side chains of two cysteine residues.
13. The peptide of claim 10, wherein at least one of said plurality of cyclizations is formed by an amide bond connecting the side chains of two residues in the sequence.

14. The peptide of claim 10, wherein all of said plurality of cyclizations are formed by amide bonds connecting the side chains of two residues in the sequence.

15. The peptide of claim 1, further comprising an additional sequence C-terminal to the sequence WKTSxRTSH, the additional sequence comprising 1-12 contiguous amino acid residues from residues 30-41 of Viperistatin (SEQ ID NO: 25) or Obtustatin (SEQ ID NO: 24).

16. The peptide of claim 15, wherein the additional sequence comprises 3-6 contiguous amino acid residues from residues 30-41 of Viperistatin or Obtustatin.

17. The peptide of claim 16, wherein the additional sequence comprises the sequence TGKS (SEQ ID NO: 4).

18. The peptide of claim 17, wherein the additional sequence is selected from the group consisting of TGKSCG (SEQ ID NO: 5) and TGKSD (SEQ ID NO: 6).

19. The peptide of claim 18, wherein the peptide comprises the sequence CWKTSRTSHYC (SEQ ID NO: 3) and further comprises a sequence selected from the group consisting of TGKSCG (SEQ ID NO: 5) and TGKSD (SEQ ID NO: 6).

20. The peptide of claim 16, wherein the additional sequence comprises the sequence PLY.

21. The peptide of claim 20, wherein the additional sequence is selected from the group consisting of PLYPG (SEQ ID NO: 7) and PLYQG (SEQ ID NO: 8).

22. The peptide of claim 21, wherein the peptide comprises the sequence CWKTSRTSHYC (SEQ ID NO: 3) and further comprises a sequence selected from the group consisting of PLYPG (SEQ ID NO: 7) and PLYQG (SEQ ID NO: 8).

23. The peptide of claim 1, wherein the peptide sequence is selected from the group consisting of:
   CCWKTSRTSHYCTGKSCG (SEQ ID NO: 9);
24. The peptide of claim 23, wherein the peptide sequence is selected from the group consisting of:

CCWKTSRTSHYCTGKSCG (SEQ ID NO: 9); and

CWKTSRTSHYCPYQG (SEQ ID NO: 10).

25. The peptide of claim 1, wherein the peptide is a compound selected from the group consisting of:

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CCWKTSRTSHYCTGKSCG} \quad \text{OH} \\
(\text{10}) & \quad \\
\text{H}_2\text{N} & \quad \text{CWKTSRTSHYCTGKSD} \quad \text{NH} \\
(\text{8}) & \quad \\
\text{H}_2\text{N} & \quad \text{CWKTSRTSHYCTGKSD} \quad \text{NH}
\end{align*}
\]
26. A pharmaceutical composition comprising a peptide according to any one of claims 1-25 and optionally a pharmaceutically acceptable excipient.

27. A method for treating a disease or disorder involving excessive angiogenesis in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition according to claim 26.

28. The method of claim 27, wherein the disease or disorder is a cancer involving angiogenesis.
29. The method of claim 28, wherein the disease or disorder is a cancerous solid tumor.

30. The method of claim 27, wherein the disease or disorder is a retinal disorder.

31. The method of claim 30, wherein the retinal disorder is selected from the group consisting of a proliferative retinopathy, retrolental fibroplasia, neovascular glaucoma, age-related macular degeneration, macular edema following retinal vein occlusion (RVO), and diabetic macular edema.

32. The method of claim 27, wherein the disease or disorder is a non-neoplastic inflammatory condition.

33. The method of claim 32, wherein the non-neoplastic inflammatory condition is selected from the group consisting of rheumatoid arthritis, psoriasis, fibrosis, atherosclerosis and thyroid hyperplasias.

34. A pharmaceutical composition comprising a peptide according to any one of claims 1-25 for use in the treatment of a disease or disorder involving excessive angiogenesis.

35. A pharmaceutical composition comprising a peptide according to any one of claims 1-25 for use in the diagnosis of a disease or disorder associated with abnormal expression of $\alpha 1\beta 1$ integrin, $\alpha 2\beta 1$ integrin or both.

36. The pharmaceutical composition of claim 35, wherein the disease or disorder is a disease or disorder involving excessive angiogenesis.

37. A pharmaceutical composition comprising a peptide according to any one of claims 1-25 for use in the imaging of angiogenesis.

38. A pharmaceutical composition comprising a peptide according to any one of claims 1-25 for use in treating cancer.
Obtustatin

Viperistatin

Figure 1A
Figure 3A

![Binding of GST-α1A to CB3](image)

Figure 3B

![Binding of GST-α1A to CB3](image)
Figure 3C

Binding of GST-α2A to collagen I (% of control)

Control  Rhodocetin  Compound 6  Compound 10

Figure 3D

Binding of GST-α2 to collagen I (% of non-inhibited binding)

Log [Peptide] (µM)

Rhodocetin  Compound 6  Compound 10
Figure 7
Figure 8A

Figure 8B
Figure 9
Figure 10
Figure 11

- Control
- Comp 9
- Comp 10
- Comp 3
- Comp 7
- Viperistatin
- Comp 6
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K38/00 C07K7/64 C07K14/46

According to International Patent Classification (IPC) and/or both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEMABS Data, Sequence Search, EMBASE, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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Date of the actual completion of the international search

7 April 2015

Date of mailing of the international search report

21/04/2015

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Fax: (+31-70) 340-3016

Authorized officer

Petri, Bernhard
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<td>MOMIC T ET AL: &quot;Medical chemistry approach for solid phase synthesis of peptide mimetics of vepi statins and integrin as lead compounds for alpha 1/alpha 2 integrin receptors&quot;, JOURNAL OF PEPTIDE SCIENCE, vol. 18, no. Suppl. 1, September 2012 (2012-09), page S106, XP002738132, &amp; 32ND EUROPEAN PEPTIDE SYMPOSIUM; ATHENS, GREECE; SEPTEMBER 02-07, 2012</td>
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