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(54) **Title:** INHIBITING INTERACTION BETWEEN HIF-1 α AND P300/CBP WITH HYDROGEN BOND SURROGATE-BASED HELICES

(57) **Abstract:** The present invention relates to peptidomimetics that mimic helix α B of the C-terminal transactivation domain of HIF-1 α . Methods of using the peptidomimetics to, e.g., inhibit the HIF-1 α -p300/CBP interaction, are also disclosed.



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INHIBITING INTERACTION BETWEEN HIF-1 α AND p300/CBP WITH HYDROGEN BOND SURROGATE-BASED HELICES

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/873,322, filed September 3, 2013, which is hereby incorporated by reference in its entirety.

[0002] This invention was made with U.S. Government support under Grant No. CHE-1161644 awarded by the U.S. National Science Foundation and Grant No. R01GM073943 awarded by the National Institutes of Health. The U.S. Government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] This invention is directed generally to methods of inhibiting the interaction between HIF-1 and p300/CBP using artificially constrained peptides and peptidomimetics that substantially mimic helix α B of the C-terminal transactivation domain of HIF-1 α .

BACKGROUND OF THE INVENTION

The Role of HIF-1 α -Coactivator Interactions in Regulation of VEGF Transcription

[0004] The interaction between the cysteine-histidine rich 1 domain ("CH1") of the coactivator protein p300 (or the homologous CREB binding protein, CBP) and the C-terminal transactivation domain ("C-TAD," aa 786–826 of NCBI accession number NP_001521) of the hypoxia-inducible factor 1 α ("HIF-1 α ") (Freedman et al., "Structural Basis for Recruitment of CBP/p300 by Hypoxia-Inducible Factor-1 α ," *Proc. Nat'l Acad. Sci. USA* 99:5367–72 (2002); Dames et al., "Structural Basis for Hif-1 α /CBP Recognition in the Cellular Hypoxic Response," *Proc. Nat'l Acad. Sci. USA* 99:5271–6 (2002)) mediates transactivation of hypoxia-Inducible genes (Hirota & Semenza, "Regulation of Angiogenesis by Hypoxia-Inducible Factor 1," *Crit. Rev. Oncol. Hematol.* 59:15–26 (2006); Semenza, "Targeting HIF-1 for Cancer Therapy," *Nat. Rev. Cancer* 3:721–32 (2003)). Hypoxia-inducible genes are important contributors in angiogenesis and cancer metastasis, as shown in Figures 1A–C (Orourke et al., "Identification of Hypoxically Inducible mRNAs in HeLa Cells Using Differential-Display PCR," *Eu. J. Biochem.* 241:403–10 (1996); Ivan et al., "HIF α

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Targeted for VHL-Mediated Destruction by Proline Hydroxylation: Implications for O₂ Sensing,” *Science* 292:464–8 (2001)). Under normoxia, the α -subunit of HIF-1 is successively hydroxylated at proline residues 402 and 564 by proline hydroxylases (Ivan et al., “HIF α Targeted for VHL-Mediated Destruction by Proline Hydroxylation: Implications for O₂ Sensing,” *Science* 292:464–8 (2001)), ubiquitinated, and then degraded by the ubiquitin–proteasome system, as shown in Figure 2. This process, mediated by the von Hippel–Lindau tumor suppressor protein (Kaelin, “Molecular Basis of the VHL Hereditary Cancer Syndrome,” *Nat. Rev. Cancer* 2:673–82 (2002)), is responsible for controlling levels of HIF-1 α and, as a result, the transcriptional response to hypoxia (Maxwell et al., “The Tumour Suppressor Protein VHL Targets Hypoxia-Inducible Factors for Oxygen-Dependent Proteolysis,” *Nature* 399:271–5 (1999)). Under hypoxic conditions, HIF-1 α is no longer targeted for destruction and accumulates. Heterodimerization with its constitutively expressed binding partner, aryl hydrocarbon receptor nuclear translocator (“ARNT”) (Wood et al., “The Role of the Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT) in Hypoxic Induction of Gene Expression,” *J. Biol. Chem.* 271:15117–23 (1996)) results in binding to a cognate hypoxia response element (“HRE”) (Forsythe et al., “Activation of Vascular Endothelial Growth Factor Gene Transcription by Hypoxia-Inducible Factor 1,” *Mol. Cell. Biol.* 16:4604–13 (1996)). A third site of regulatory hydroxylation on asparagine 803 is also inhibited under hypoxic conditions (Lando et al., “FIH-1 Is an Asparaginyl Hydroxylase Enzyme That Regulates the Transcriptional Activity of Hypoxia-Inducible Factor,” *Genes & Develop.* 16:1466–71 (2002)), allowing recruitment of the p300/CBP coactivators, which trigger overexpression of hypoxia inducible genes, as shown in Figure 2. Among these are genes encoding angiogenic peptides such as vascular endothelial growth factor (“VEGF”) and VEGF receptors VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), as well as proteins involved in altered energy metabolism, such as the glucose transporters GLUT1 and GLUT3, and hexokinases 1 and 2 (Forsythe et al., “Activation of Vascular Endothelial Growth Factor Gene Transcription by Hypoxia-Inducible Factor 1,” *Mol. Cell. Biol.* 16:4604–13 (1996); Okino et al., “Hypoxia-Inducible Mammalian Gene Expression Analyzed *in Vivo* at a TATA-Driven Promoter and at an Initiator-Driven Promoter,” *J. Biol. Chem.* 273:23837–43 (1998)).

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Epidithiodiketopiperazine Fungal Metabolites as Regulators of Hypoxia-Inducible Transcription

[0005] Because interaction of HIF-1 α C-TAD with transcriptional coactivator p300/CBP is a point of significant amplification in transcriptional response, its disruption with designed protein ligands can be an effective means of suppressing aerobic glycolysis and angiogenesis (*i.e.*, the formation of new blood vessels) in cancers (Hirota & Semenza, "Regulation of Angiogenesis by Hypoxia-Inducible Factor 1," *Crit. Rev. Oncol. Hematol.* 59:15–26 (2006); Rarnanathan et al., "Perturbational Profiling of a Cell-Line Model of Tumorigenesis by Using Metabolic Measurements," *Proc. Nat'l Acad. Sci. USA* 102:5992–7 (2005); Underiner et al., "Development of Vascular Endothelial Growth Factor Receptor (VEGFR) Kinase Inhibitors as Anti-Angiogenic Agents in Cancer Therapy," *Curr. Med. Chem.* 11:731–45 (2004)). Although the contact surface of the HIF-1 α C-TAD with p300/CBP is extensive (3393 Å²), the inhibition of this protein–protein interaction may not require direct interference. Instead, the induction of a structural change to one of the binding partners (p300/CBP) may be sufficient to disrupt the complex (Kung et al., "Small Molecule Blockade of Transcriptional Coactivation of the Hypoxia-Inducible Factor Pathway," *Cancer Cell* 6:33–43 (2004)).

[0006] The present invention is directed to overcoming these and other deficiencies in the art.

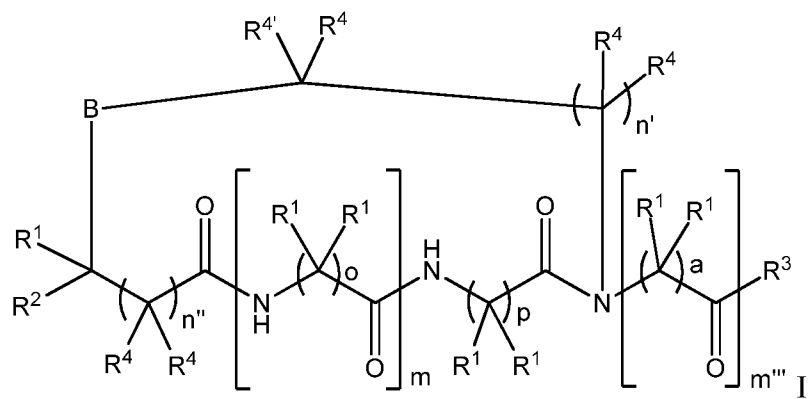
SUMMARY OF THE INVENTION

[0007] A first aspect of the present invention relates to a peptidomimetic, wherein the peptidomimetic:

- (i) mimics a helix having the formula X₁-X₂-X₂-X₃-X₂-X₂-X₁-X₄-X₅, wherein each X₁ is any negatively charged residue, each X₂ is any hydrophobic residue, X₃ is any positively-charged residue, X₄ is any polar residue, and X₅ is absent or any hydrophobic residue; and
- (ii) is selected from the group consisting of:

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(a) a compound of Formula I:

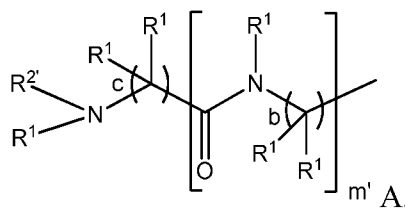


wherein:

B is C(R¹)₂, O, S, or NR¹;

each R¹ is independently hydrogen, an amino acid side chain, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

R² is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; –OR⁵ wherein R⁵ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; –(CH₂)₀₋₁N(R⁵)₂ wherein each R⁵ is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula A:



wherein:

R^{2'} is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; –OR⁵ wherein R⁵

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is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $-(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m' is zero or any number;

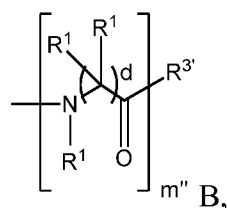
each b is independently one or two; and

c is one or two;

R^3 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a

heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; –

OR^5 wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula B:



wherein:

$R^{3'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl;

a cycloalkyl; a heterocyclyl; an aryl; a

heteroaryl; an arylalkyl; an alpha amino

acid; a beta amino acid; a peptide; a

targeting moiety; a tag; $-OR^5$ wherein R^5

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is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or —N(R⁵)₂ wherein each R⁵ is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m'' is zero or any number; and

each d is independently one or two;

each R⁴ is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

R^{4'} is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, or a double bond between C(R^{4'},R⁴) and B;

a is one or two;

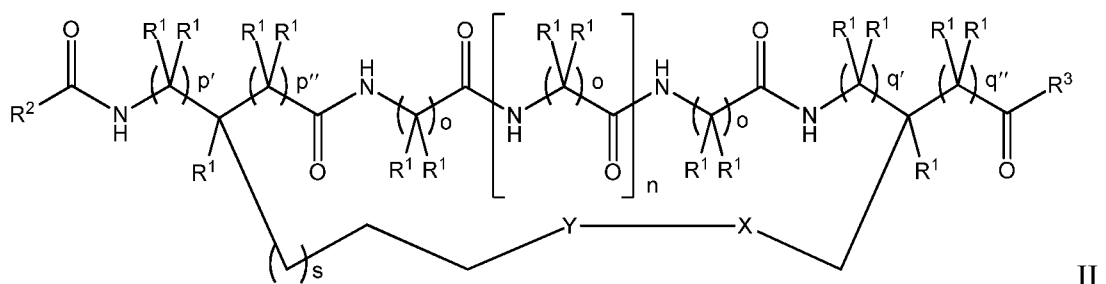
$m, n',$ and n'' are each independently zero, one, two, three, or four;

m''' is zero or one;

each $\mathbf{o}$ is independently one or two; and

p is one or two;

(b) a compound of Formula II:

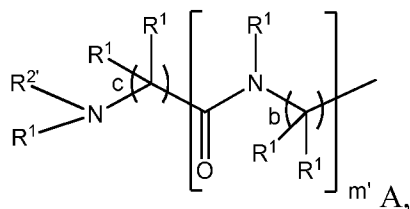


wherein:

each R¹ is independently hydrogen, an amino acid side chain, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

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R^2 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula A:



wherein:

$R^{2'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $-(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

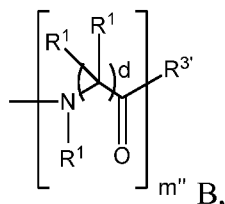
m' is zero or any number;

each b is independently one or two; and

c is one or two;

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R^3 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula B:



wherein:

$R^{3'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m'' is zero or any number; and

each d is independently one or two;

n is one or four;

each o is independently one or two;

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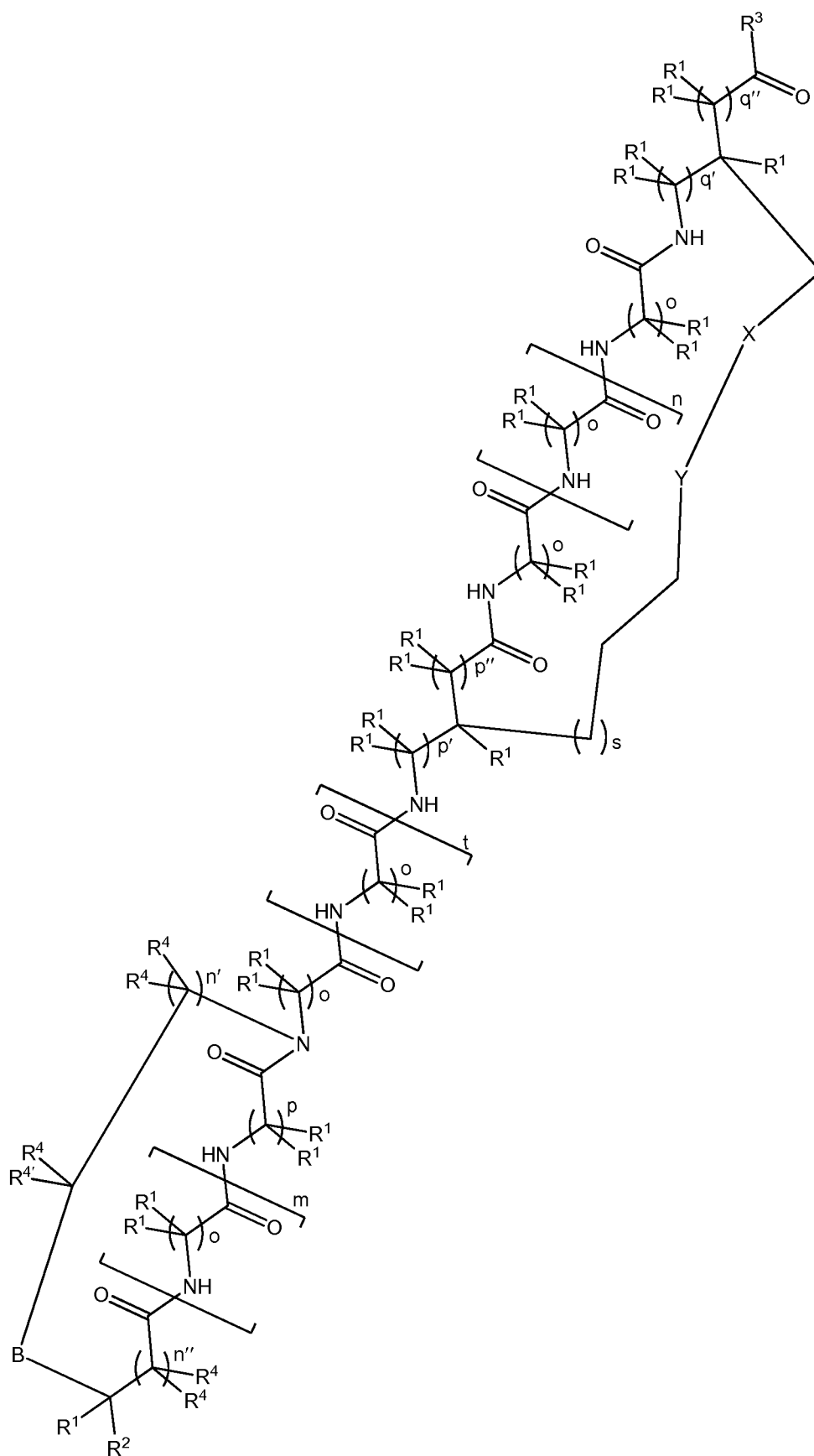
one of p' and p'' is zero and the other is zero or one;

one of q' and q'' is zero and the other is zero or one;

s is one, two, three, four, or five; and

$Y-X$ is a hydrocarbon, an amide bond, an alkane, an alkene, an alkyne,
a triazole, or a disulfide bond; and

(c) a compound of Formula III:

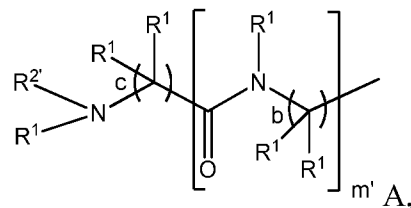


wherein:

B is $C(R^1)_2$, O, S, or NR^1 ;

each R¹ is independently hydrogen, an amino acid side chain, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

R^2 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; —OR⁵ wherein R⁵ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; —(CH₂)₀₋₁N(R⁵)₂ wherein each R⁵ is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula A:



wherein:

R^{2'} is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclcyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; -OR⁵ wherein R⁵ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclcyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or - (CH₂)₀₋₁N(R⁵)₂ wherein each R⁵ is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclcyl, an aryl, a heteroaryl, an

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arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m' is zero or any number;

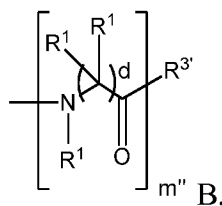
each b is independently one or two; and

c is one or two;

R^3 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a

heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; –

OR^5 wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula B:



wherein:

$R^{3'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl;

a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an

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aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m'' is zero or any number; and

each d is independently one or two;

each R^4 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

$R^{4'}$ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, or a double bond between $C(R^{4'}, R^4)$ and B ;

m , n' , and n'' are each independently zero, one, two, three, or four;

n is one or four;

each o is independently one or two;

p is one or two;

one of p' and p'' is zero and the other is zero or one;

one of q' and q'' is zero and the other is zero or one;

s is one, two, three, four, or five; and

$Y-X$ is a hydrocarbon, an amide bond, an alkane, an alkene, an alkyne, a triazole, or a disulfide bond.

[0008] A second aspect of the present invention relates to a method of modulating transcription of a gene in a cell, where transcription of the gene is mediated by interaction of hypoxia-inducible factor 1α ("HIF- 1α ") with coactivator protein p300 (or the homologous CREB binding protein, CBP). This method involves contacting the cell with a peptidomimetic described herein under conditions effective to modulate transcription of the gene.

[0009] A third aspect of the present invention relates to a method of treating or preventing in a subject a disorder mediated by interaction of HIF- 1α with CBP and/or p300. This method involves administering a peptidomimetic described herein to the subject under conditions effective to treat or prevent the disorder.

[0010] A fourth aspect of the present invention relates to a method of reducing or preventing angiogenesis in a tissue. This method involves contacting the tissue with a peptidomimetic described herein under conditions effective to reduce or prevent angiogenesis in the tissue.

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[0011] A fifth aspect of the present invention relates to a method of decreasing survival and/or proliferation of a cell under hypoxic conditions. This method involves contacting the cell with a peptidomimetic described herein under conditions effective to decrease survival and/or proliferation of the cell.

[0012] A sixth aspect of the present invention relates to a method of identifying a potential ligand of CBP and/or p300. This method involves providing a peptidomimetic described herein, contacting the peptidomimetic with a test agent, and detecting whether the test agent selectively binds to the peptidomimetic. A test agent that selectively binds to the peptidomimetic is identified as a potential ligand of CBP and/or p300.

[0013] Selective blockade of gene expression by designed small molecules is a fundamental challenge at the interface of chemistry, biology, and medicine. Transcription factors have been among the most elusive targets in genetics and drug discovery, but the fields of chemical biology and genetics have evolved to a point where this task can be addressed. The design, synthesis, and *in vivo* efficacy evaluation of a protein domain mimetic targeting the interaction of the p300/CBP coactivator with the transcription factor HIF-1 α is described herein. As indicated herein, disrupting this interaction results in a rapid down-regulation of hypoxia-inducible genes critical for cancer progression. The observed effects were compound-specific and dose-dependent. Gene expression profiling with oligonucleotide microarrays revealed effective inhibition of hypoxia-inducible genes with relatively minimal perturbation of non-targeted signaling pathways. Remarkable efficacy of the compound HBS 1 in suppressing tumor growth was observed in the fully established murine xenograft models of renal cell carcinoma of the clear cell type (RCC). These results suggest that rationally designed synthetic mimics of protein subdomains that target the transcription factor-coactivator interfaces represent a novel approach for *in vivo* modulation of oncogenic signaling and arresting tumor growth.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1A is a schematic diagram illustrating the structure of the complex of the C-terminal transactivation domain ("C-TAD") of the hypoxia-inducible factor 1 α ("HIF-1 α ") with cysteine-histidine rich 1 domain ("CH1") of the coactivator protein p300 (or the homologous CREB binding protein, CBP)

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(Lepourcelet et al., “Small-Molecule Antagonists of the Oncogenic Tcf/ β -Catenin Protein Complex,” *Cancer Cell* 5:91–102 (2004); Vassilev et al., “*In Vivo* Activation of the p53 Pathway by Small-Molecule Antagonists of MDM2,” *Science* 303:844–48 (2004), which are hereby incorporated by reference in their entirety). Figure 1B is the domain map of HIF-1 α showing the basic helix–loop–helix region (“bHLH”), PAS, the N-terminal transactivation domain (“N-TAD”), and the C-TAD. The human HIF-1 α C-TAD sequence (SEQ ID NO: 1) is shown in Figure 1C, along with the location of the α A and α B helices.

[0015] Figure 2 is a schematic diagram illustrating the HIF-1 α pathway. ARNT: aryl hydrocarbon receptor nuclear translocator; VHL: von Hippel-Lindau tumor suppressor; HRE: hypoxia response element; VEGF: vascular endothelial growth factor.

[0016] Figures 3A–C are schematic diagrams relating to the regulation of transcription by HIF-1 α and CBP/p300. As shown in Figure 3A, transcription of hypoxia-inducible genes is controlled by the interaction of HRE-bound HIF-1 α /ARNT heterodimer with transcriptional coactivator CBP/p300. Protein domain mimetics should competitively inhibit the interaction and associated gene expression (see Figure 3B). As shown in Figure 3C, the C-TAD_{793–826} domain of HIF-1 α (SEQ ID NO: 2) utilizes helical motifs to target the cysteine-histidine rich 1 (CH1) region of CBP/p300. HIF-1 α is shown in gold and CBP/p300 in gray (PDB code 1L8C).

[0017] Figures 4A–C are analytical HPLC traces of HBS 1 (Figure 4A), HBS 2 (Figure 4B), and peptide 3 (Figure 4C).

[0018] Figures 5A–C show that HBS 1 targets p300–CH1 with high affinity and inhibits its binding to HIF-1 α C-TAD_{786–826}. Figure 5A is a graph of the affinity of HBS 1, HBS 2, peptide 3, and HIF-1 α C-TAD_{786–826} for the CH1 domain as determined by tryptophan fluorescence spectroscopy. Figure 5B is a molecular model that depicts the results of a ¹H-¹⁵N HSQC NMR titration experiment. The p300-CH1 residues undergoing chemical shift perturbations upon addition of HBS 1 are color-mapped, matching the magnitude of the chemical shift changes. HIF-1 α helix B is shown in gold. The model was refined from the NMR structure of the HIF-1 α /p300 complex (PDB code 1L8C). Figure 5C is a graph of the results of fluorescence anisotropy experiments, showing the ability of HBS 1 to inhibit CH1^{Flu}/HIF C-TAD_{786–826} complex formation.

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[0019] Figures 6A–B show the structures of stabilized helices and linear peptide. HBS 1 (Figure 6A, left panel) mimics the α B domain of HIF-1 α and features four residues that contribute significantly to binding (L818, L822, L823 and L824). HBS 2 (Figure 6A, right panel) was designed to be a specificity control; this compound is identical to HBS 1 with the exception of L822, which was mutated to an alanine group. Peptide 3 (Figure 6B) (SEQ ID NO: 3) is an unconstrained negative control with the amino acid sequence that repeats that of HBS 1.

[0020] Figure 7 is the circular dichroism spectra of HBS 1, HBS 2, and peptide 3. CD studies were performed with 50–100 μ M peptide solutions in 10 mM KF (pH 7.4).

[0021] Figures 8A–D are ^1H – ^{15}N HSQC spectra of the p300-CH1 domain with different concentrations of Zn^{2+} . Figure 8A is the spectra of misfolded p300-CH1: Zn^{2+} (1:< 3). Figure 8B is the spectra of folded p300-CH1: Zn^{2+} (1:3). Figure 8C is the spectra of unfolded p300-CH1: Zn^{2+} with excess Zn^{2+} (1:6). Figure 8D is the spectra of refolded p300-CH1: Zn^{2+} with EDTA to remove the excess of Zn^{2+} (1:3).

[0022] Figure 9 is a schematic diagram of the HIF-1 α /p300-CH1 interaction. Tryptophan-403 resides in the hydrophobic groove targeted by the HIF-1 α α B helix. (PDB code 1L8C.)

[0023] Figure 10 is a graph showing the concentration-dependent changes in the fluorescence spectra of the CH1 domain (1 μ M) upon titration of HBS 1.

[0024] Figure 11 shows the chemical structure of fluorescein-labeled C-TAD (Flu-HIF-1 α C-TAD₇₈₆₋₈₂₆). (Mass $[\text{M}+\text{H}]^+$ calc'd = 4977.1; found = 4976.8.)

[0025] Figure 12 is a graph of the binding of Flu-HIF C-TAD to p300-CH1 as monitored by a fluorescence polarization assay.

[0026] Figure 13 is the overlaid ^1H – ^{15}N HSQC titration spectra of p300–CH1 (blue), CH1:HBS 1 (1:5, red), and CH1:HBS 1 (1:10, green).

[0027] Figure 14 is a mean chemical shift difference ($\Delta\delta\text{NH}$) plot depicting changes in residues of p300-CH1 upon binding with HBS 1.

[0028] Figure 15 is a graph of the results from the luciferase-based promoter activity assay with MDA-MB-231-HRE-Luc cell line treated with HBS 1, HBS 2 (specificity control), or peptide 3. Hypoxia was mimicked with GasPak EZ pouch (300 μ M). Error bars represent $\pm$ s.e.m. of experiments performed in quadruplicate. *

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$P < 0.05$, t -test. The results demonstrate that HBS 1 reduces HIF-1 α inducible promoter activity *in vitro*.

[0029] Figure 16 is a western blot analysis of HIF-1 α levels in the nuclear and cytoplasmic extracts of HeLa cells. Cells were incubated for a total of 24 hours with HBS 1. After 6 hours, hypoxia was mimicked with DFO (300 μ M) for an additional 18 hours. The results demonstrate that HBS 1 does not affect the intracellular levels of HIF-1 α .

[0030] Figures 17A–D show that HBS 1 down-regulates hypoxia-induced transcription in cell culture. As shown in Figures 17A–C, HBS 1 reduced expression levels of *VEGFA* (Figure 17A), *SLC2A1 (GLUT1)* (Figure 17B), and *LOX* (Figure 17C) in a dose-dependent manner in HeLa cells under hypoxia conditions as measured by real-time qRT-PCR. Hypoxia was mimicked with DFO (300 μ M). HBS 2 and peptide 3 show reduced inhibitory activities at the same concentrations. Error bars are $\pm$ s.e.m. of four independent experiments. ** $P < 0.01$, * $P < 0.05$, t -test. Figure 17D is a graph comparing the efficacies of HBS 1 in down-regulating expression levels of *VEGFA* in HeLa cells under two different hypoxia-mimetic conditions (DFO and hypoxia bag) as measured by real-time qRT-PCR. For each experiment under hypoxia-mimetic conditions, mRNA levels were normalized to *VEGFA* mRNA levels found in the vehicle-treated normoxic cells.

[0031] Figure 18 is a graph of VEGF protein levels under hypoxia or normoxia, with or without treatment with varying concentrations of HBS 1. Hypoxia was mimicked with 300 μ M DFO. Error bars represent $\pm$ s.e.m of experiments performed in triplicate. * $P < 0.05$, t -test. The results demonstrate that HBS 1 reduces levels of secreted VEGF protein in HeLa cells in a dose-dependent manner.

[0032] Figure 19 is a graph of the results from MTT assays with HeLa cells treated with HBS 1, HBS 2, or peptide 1 in a concentration range of 1 μ M and 100 μ M for 24 hours. The results demonstrate that HBS 1 shows low cytotoxicity in HeLa cells.

[0033] Figures 20A–C show the results from gene expression profiling obtained with Affymetrix Human Gene ST 1.0 arrays. Figure 20A shows the hierarchical agglomerative clustering of 368 transcripts induced or repressed 2-fold or more (one-way ANOVA, $P < 0.05$) by 300 μ M DFO under the three specified conditions: no treatment (“-”), treatment with 50 μ M HBS 1 (“1”), and treatment with

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50 μ M HBS 2 (“2”). Clustering was based on a Pearson centered correlation of intensity ratios for each treatment compared to DFO-induced cells (controls) using average-linkage as a distance. Of this DFO-induced set, 92 were inhibited and 30 were induced by HBS 1, whereas 81 were inhibited and 70 induced by HBS 2 ($|\text{fold-change}| \geq 1.1$, $P < 0.05$). Figure 20B shows a clustering of expression changes of the 45 transcripts induced or repressed 4-fold or more ($P < 0.05$) by 300 μ M DFO or by the treatments under the designated treatment conditions. Clustering parameters were the same as in Figure 20A. Figure 20C shows Venn diagrams representing transcripts down- and up-regulated ($|\text{fold-change}| \geq 1.1$, $P < 0.05$) by HBS 1 and HBS 2. Numbers inside the intersections represent DFO-induced transcripts affected by both treatments.

[0034] Figure 21 shows the plasma concentration versus time curves for HBS 1 and control peptide 3 in BALB/c mice.

[0035] Figures 22A–C demonstrate that HBS 1 suppresses tumor growth in mouse xenograft models. Figure 22A is a box-whisker diagram of tumor volumes measured throughout the study with boxes representing the upper and lower quartiles and median and error bars showing maximum and minimum volumes. Tumors from mice treated with HBS 1 were smaller (median volume: 138 mm³) than those of the control mice (median: 293 mm³). Figure 22B is a graph showing the results of the weight measurements of control- and HBS 1-treated mice throughout the entire duration of the experiments, showing the absence of toxicity-related weight loss. Figure 22C shows images of mice injected with the tumor-accumulating near-infrared (NIR) contrast agent. Mice from the HBS 1 treated group show significantly lower intensity of the NIR signal as compared to the control group, demonstrating that HBS 1 lowers overall tumor burden in mice.

DETAILED DESCRIPTION OF THE INVENTION

[0036] Transcription factors are among the most challenging, but attractive targets, for drug discovery (Rutledge et al., “Molecular Recognition of Protein Surfaces: High Affinity Ligands for the CBPKIX Domain,” *J. Am. Chem. Soc.* 125(47):14336–47 (2003), which is hereby incorporated by reference in its entirety). High-resolution structures of transcription factors in complex with protein partners offer a foundation for rational drug design strategies. Although many transcription

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factors exhibit significant intrinsic disorder, their complexes with coactivator proteins often feature discrete protein secondary structures (Rutledge et al., “Molecular Recognition of Protein Surfaces: High Affinity Ligands for the CBPKIX Domain,” *J. Am. Chem. Soc.* 125(47):14336–47 (2003), which is hereby incorporated by reference in its entirety), such as α -helices, that contribute significantly to binding and may be used as templates for rational drug design (Semenza, “Targeting HIF-1 for Cancer Therapy,” *Nat. Rev. Cancer* 3(10):721–32 (2003), which is hereby incorporated by reference in its entirety). Described herein is the design of stabilized peptide α -helices that can modulate transcription of hypoxia inducible genes by interfering with interactions of the C-terminal activation domain (“C-TAD”) of hypoxia inducible factor-1 α (“HIF-1 α ”) and the cysteine-histidine rich 1 (“CH1”) domain of the coactivator protein p300 (or the homologous CREB binding protein, CBP) (Figures 3A–C) (O’Rourke et al., “Identification of Hypoxically Inducible mRNAs in HeLa Cells Using Differential-Display PCR: Role of Hypoxia-Inducible Factor-1,” *Eur. J. Biochem.* 241(2):403–10 (1996); Freedman et al., “Structural Basis for Recruitment of CBP/p300 by Hypoxia-Inducible Factor-1 Alpha,” *Proc. Nat’l Acad. Sci. U.S.A.* 99(8):5367–72 (2002), which are hereby incorporated by reference in their entirety). As shown herein, an optimized mimic of HIF-1 α C-TAD, HBS 1, a high affinity ligand of CH1, can downregulate target genes under hypoxic conditions without affecting the endogenous levels of HIF-1 α . HBS 1 does not adversely affect cell growth at high concentrations, which suggests that the compound is generally non-toxic to normoxic cells. This constrained α -helix retains significant activity in mouse plasma as compared to its unconstrained peptide analog (peptide 3) highlighting the ability of stabilized helices to evade serum proteases. The genome-wide effects of HIF-1 α C-TAD mimic 1 and a negative control (HBS 2) were compared using gene expression profiling. The results show that HBS 1 modulates expression of a select set of genes, many of which are of direct relevance to the predicted pathways. Lastly, the ability of HBS 1 to control tumor progression in a mouse tumor xenograft model was examined. The synthetic helix was found to provide rapid and effective regression of tumor growth. These results support the hypothesis that functional mimics of protein subdomains that mediate interactions between partner proteins offer an attractive strategy for inhibitor design. It is predicted that other such peptidomimetics of the α B helix of HIF-1 α would have similar effects.

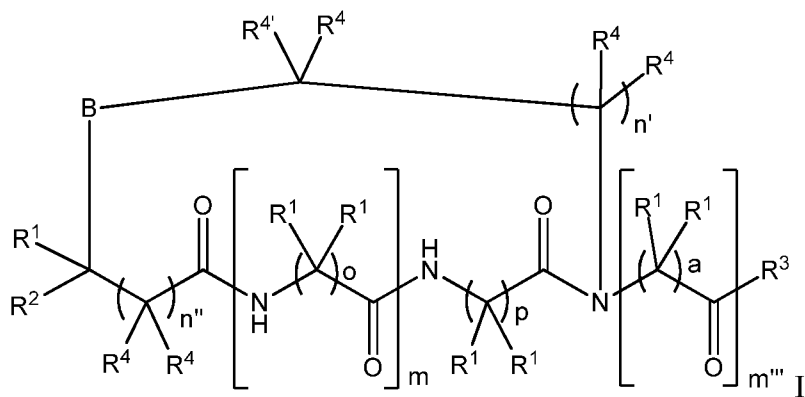
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[0037] The present invention relates to a peptidomimetic, wherein the peptidomimetic:

(i) mimics a helix having the formula $X_1-X_2-X_2-X_3-X_2-X_2-X_1-X_4-X_5$, wherein each X_1 is any negatively charged residue, each X_2 is any hydrophobic residue, X_3 is any positively-charged residue, X_4 is any polar residue, and X_5 is absent or any hydrophobic residue; and

(ii) is selected from the group consisting of:

(a) a compound of Formula I:



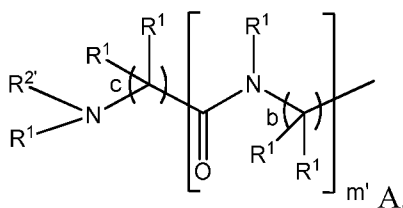
wherein:

B is $C(R^1)_2$, O, S, or NR^1 ;

each R^1 is independently hydrogen, an amino acid side chain, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

R^2 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula A:

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wherein:

$R^{2'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

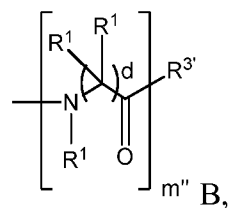
m' is zero or any number;

each b is independently one or two; and

c is one or two;

R^3 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula B:

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wherein:

$\text{R}^{3'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-\text{OR}^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $-\text{N}(\text{R}^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m'' is zero or any number; and

each d is independently one or two;

each R^4 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

$\text{R}^{4'}$ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, or a double bond between $\text{C}(\text{R}^{4'}, \text{R}^4)$ and B;

a is one or two;

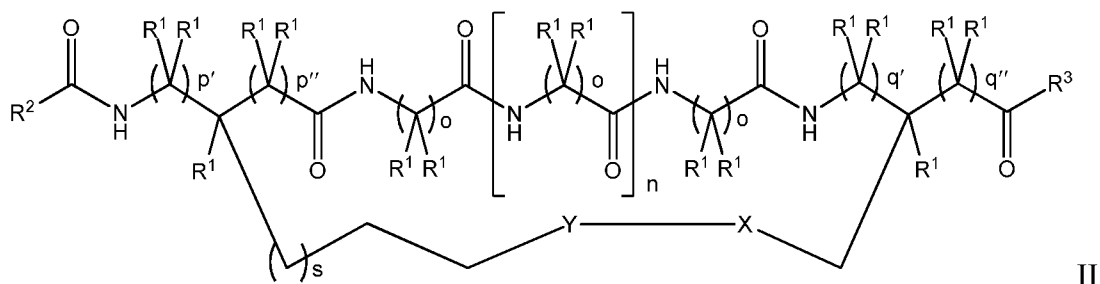
m , n' , and n'' are each independently zero, one, two, three, or four;

m''' is zero or one;

each o is independently one or two; and

p is one or two;

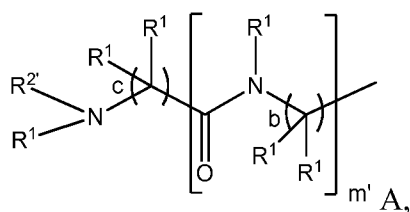
(b) a compound of Formula II:



wherein:

each R¹ is independently hydrogen, an amino acid side chain, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

R² is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; –OR⁵ wherein R⁵ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; –(CH₂)_{0–1}N(R⁵)₂ wherein each R⁵ is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula A:



wherein:

R^{2'} is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; —OR⁵ wherein R⁵ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an

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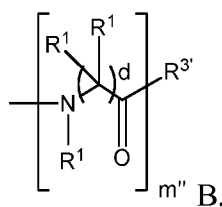
aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or – $(\text{CH}_2)_{0-1}\text{N}(\text{R}^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m' is zero or any number;

each b is independently one or two; and

c is one or two;

R^3 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; – OR^5 wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-\text{N}(\text{R}^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula B:



wherein:

$\text{R}^{3'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-\text{OR}^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an

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aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or – $N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m'' is zero or any number; and

each d is independently one or two;

n is one or four;

each o is independently one or two;

one of p' and p'' is zero and the other is zero or one;

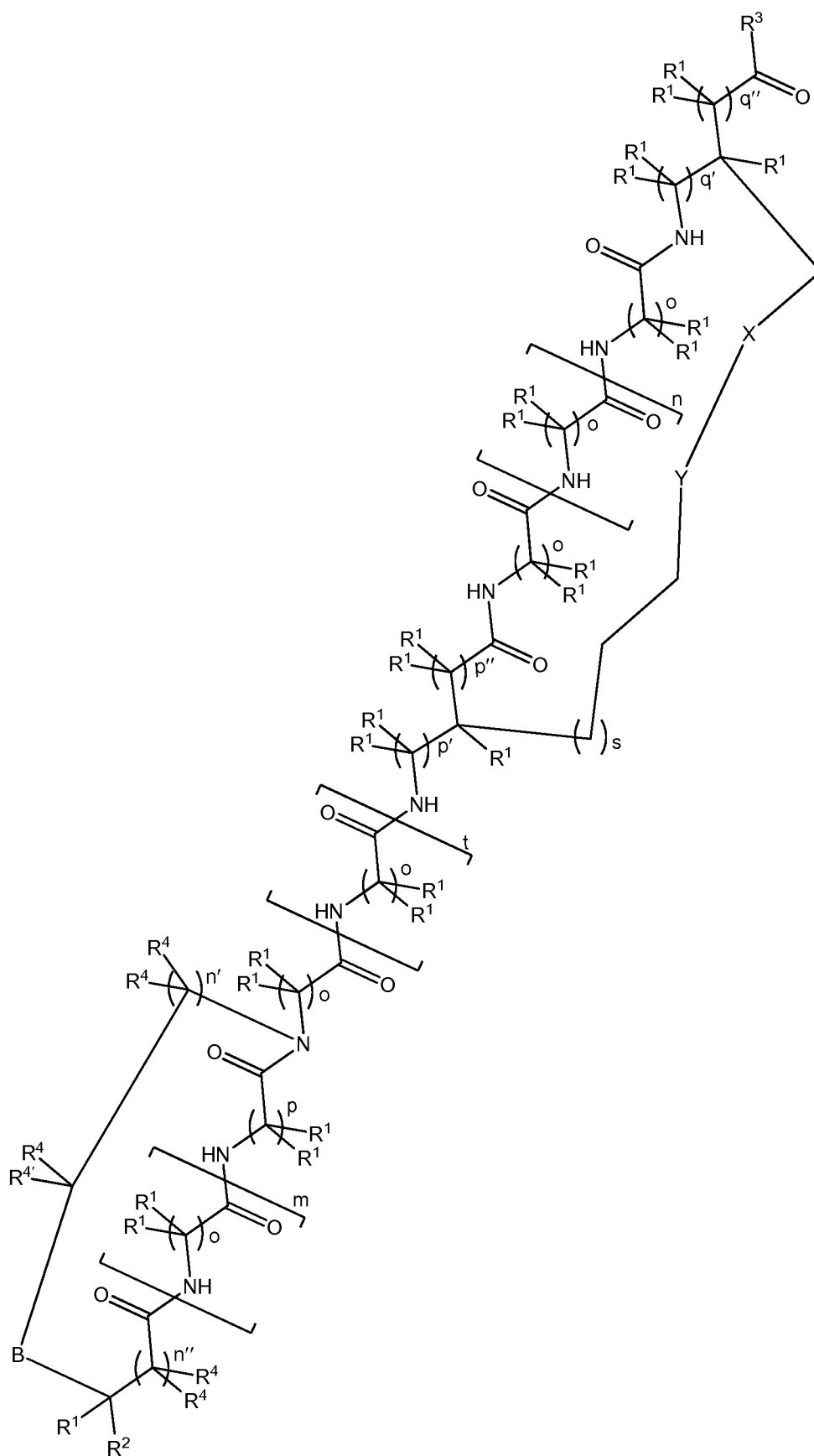
one of q' and q'' is zero and the other is zero or one;

s is one, two, three, four, or five; and

$Y-X$ is a hydrocarbon, an amide bond, an alkane, an alkene, an alkyne, a triazole, or a disulfide bond; and

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(c) a compound of Formula III:



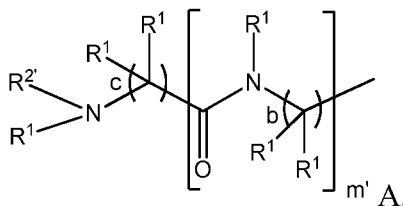
- 27 -

wherein:

B is $C(R^1)_2$, O, S, or NR^1 ;

each R^1 is independently hydrogen, an amino acid side chain, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

R^2 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula A:



wherein:

$R^{2'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $-(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an

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arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m' is zero or any number;

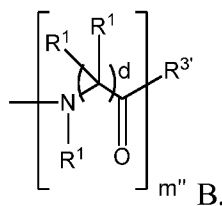
each b is independently one or two; and

c is one or two;

R^3 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a

heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; –

OR^5 wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula B:



wherein:

$R^{3'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl;

a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an

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aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m'' is zero or any number; and

each d is independently one or two;

each R^4 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

$R^{4'}$ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, or a double bond between $C(R^{4'}, R^4)$ and B ;

m , n' , and n'' are each independently zero, one, two, three, or four;

n is one or four;

each o is independently one or two;

p is one or two;

one of p' and p'' is zero and the other is zero or one;

one of q' and q'' is zero and the other is zero or one;

s is one, two, three, four, or five; and

$Y-X$ is a hydrocarbon, an amide bond, an alkane, an alkene, an alkyne, a triazole, or a disulfide bond.

[0038] Amino acid side chains according to this and all aspects of the present invention can be any amino acid side chain from natural or nonnatural amino acids, including from alpha amino acids, beta amino acids, gamma amino acids, L-amino acids, and D-amino acids.

[0039] As used herein, the term “alkyl” means an aliphatic hydrocarbon group which may be straight or branched having about 1 to about 6 carbon atoms in the chain. Branched means that one or more lower alkyl groups such as methyl, ethyl, or propyl are attached to a linear alkyl chain. Exemplary alkyl groups include methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, n-pentyl, and 3-pentyl.

[0040] The term “alkenyl” means an aliphatic hydrocarbon group containing a carbon-carbon double bond and which may be straight or branched having about 2 to about 6 carbon atoms in the chain. Preferred alkenyl groups have 2 to about 4 carbon atoms in the chain. Exemplary alkenyl groups include ethenyl, propenyl, n-butenyl, and i-butenyl.

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[0041] The term “alkynyl” means an aliphatic hydrocarbon group containing a carbon–carbon triple bond and which may be straight or branched having about 2 to about 6 carbon atoms in the chain. Preferred alkynyl groups have 2 to about 4 carbon atoms in the chain. Exemplary alkynyl groups include ethynyl, propynyl, n-butynyl, 2-butynyl, 3-methylbutynyl, and n-pentynyl.

[0042] As used herein, the term “cycloalkyl” refers to a non-aromatic saturated or unsaturated mono- or polycyclic ring system which may contain 3 to 6 carbon atoms, and which may include at least one double bond. Exemplary cycloalkyl groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, *anti*-bicyclopropane, or *syn*-bicyclopropane.

[0043] As used herein, the term “heterocyclyl” refers to a stable 3- to 18-membered ring system that consists of carbon atoms and from one to five heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. The heterocyclyl may be a monocyclic or a polycyclic ring system, which may include fused, bridged, or spiro ring systems; and the nitrogen, carbon, or sulfur atoms in the heterocyclyl may be optionally oxidized; the nitrogen atom may be optionally quaternized; and the ring may be partially or fully saturated. Representative monocyclic heterocyclyls include piperidine, piperazine, pyrimidine, morpholine, thiomorpholine, pyrrolidine, tetrahydrofuran, pyran, tetrahydropyran, oxetane, and the like. Representative polycyclic heterocyclyls include indole, isoindole, indolizine, quinoline, isoquinoline, purine, carbazole, dibenzofuran, chromene, xanthene, and the like.

[0044] As used herein, the term “aryl” refers to an aromatic monocyclic or polycyclic ring system containing from 6 to 19 carbon atoms, where the ring system may be optionally substituted. Aryl groups of the present invention include, but are not limited to, groups such as phenyl, naphthyl, azulenyl, phenanthrenyl, anthracenyl, fluorenyl, pyrenyl, triphenylenyl, chrysenyl, and naphthacenyl.

[0045] As used herein, “heteroaryl” refers to an aromatic ring radical which consists of carbon atoms and from one to five heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. Examples of heteroaryl groups include, without limitation, pyrrolyl, pyrazolyl, imidazolyl, triazolyl, furyl, thiophenyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, thienopyrrolyl, furopyrryl, indolyl,

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azaindolyl, isoindolyl, indolyl, indolizyl, indazolyl, benzimidazolyl, imidazopyridinyl, benzotriazolyl, benzoxazolyl, benzoxadiazolyl, benzothiazolyl, pyrazolopyridinyl, triazolopyridinyl, thienopyridinyl, benzothiadiazolyl, benzofuyl, benzothiophenyl, quinolyl, isoquinolyl, tetrahydroquinolyl, tetrahydroisoquinolyl, cinnolyl, quinazolyl, quinolizilyl, phthalazyl, benzotriazinyl, chromenyl, naphthyridinyl, acrydinyl, phenanzinyl, phenothiazinyl, phenoxazinyl, pteridinyl, and purinyl. Additional heteroaryls are described in COMPREHENSIVE HETEROCYCLIC CHEMISTRY: THE STRUCTURE, REACTIONS, SYNTHESIS AND USE OF HETEROCYCLIC COMPOUNDS (Katritzky et al. eds., 1984), which is hereby incorporated by reference in its entirety.

[0046] The term “arylalkyl” refers to a moiety of the formula $-R^aR^b$ where R^a is an alkyl or cycloalkyl as defined above and R^b is an aryl or heteroaryl as defined above.

[0047] As used herein, the term “acyl” means a moiety of formula $R-$ carbonyl, where R is an alkyl, cycloalkyl, aryl, or heteroaryl as defined above. Exemplary acyl groups include formyl, acetyl, propanoyl, benzoyl, and propenoyl.

[0048] An amino acid according to this and all aspects of the present invention can be any natural or non-natural amino acid.

[0049] A “peptide” as used herein is any oligomer of two or more natural or non-natural amino acids, including alpha amino acids, beta amino acids, gamma amino acids, L-amino acids, D-amino acids, and combinations thereof. In preferred embodiments, the peptide is ~5 to ~30 (*e.g.*, ~5 to ~10, ~5 to ~17, ~10 to ~17, ~10 to ~30, or ~18 to ~30) amino acids in length. Typically, the peptide is 10–17 amino acids in length. In a preferred embodiment, the peptide contains a mixture of alpha and beta amino acids in the pattern $\alpha 3/\beta 1$ (this is particularly preferred for α -helix mimetics).

[0050] A “tag” as used herein includes any labeling moiety that facilitates the detection, quantitation, separation, and/or purification of the compounds of the present invention. Suitable tags include purification tags, radioactive or fluorescent labels, and enzymatic tags.

[0051] Purification tags, such as poly-histidine (His_6), a glutathione-S-transferase (GST), or maltose-binding protein (MBP), can assist in compound purification or separation but can later be removed, *i.e.*, cleaved from the compound

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following recovery. Protease-specific cleavage sites can be used to facilitate the removal of the purification tag. The desired product can be purified further to remove the cleaved purification tags.

[0052] Other suitable tags include radioactive labels, such as, ^{125}I , ^{131}I , ^{111}In , or ^{99}Tc . Methods of radiolabeling compounds are known in the art and described in U.S. Patent No. 5,830,431 to Srinivasan et al., which is hereby incorporated by reference in its entirety. Radioactivity is detected and quantified using a scintillation counter or autoradiography. Alternatively, the compound can be conjugated to a fluorescent tag. Suitable fluorescent tags include, without limitation, chelates (europium chelates), fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin, and Texas Red. The fluorescent labels can be conjugated to the compounds using techniques disclosed in CURRENT PROTOCOLS IN IMMUNOLOGY (Coligen et al. eds., 1991), which is hereby incorporated by reference in its entirety. Fluorescence can be detected and quantified using a fluorometer.

[0053] Enzymatic tags generally catalyze a chemical alteration of a chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Examples of suitable enzymatic tags include luciferases (*e.g.*, firefly luciferase and bacterial luciferase; *see e.g.*, U.S. Patent No. 4,737,456 to Weng et al., which is hereby incorporated by reference in its entirety), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidases (*e.g.*, horseradish peroxidase), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases (*e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (*e.g.*, uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to proteins and peptides are described in O'Sullivan et al., Methods for the Preparation of Enzyme—Antibody Conjugates for Use in Enzyme Immunoassay, in METHODS IN ENZYMOLOGY 147–66 (Langone et al. eds., 1981), which is hereby incorporated by reference in its entirety.

[0054] A targeting moiety according to the present invention functions to (i) promote the cellular uptake of the compound, (ii) target the compound to a particular cell or tissue type (*e.g.*, signaling peptide sequence), or (iii) target the compound to a

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specific sub-cellular localization after cellular uptake (*e.g.*, transport peptide sequence).

[0055] To promote the cellular uptake of a compound of the present invention, the targeting moiety may be a cell penetrating peptide (CPP). CPPs translocate across the plasma membrane of eukaryotic cells by a seemingly energy-independent pathway and have been used successfully for intracellular delivery of macromolecules, including antibodies, peptides, proteins, and nucleic acids, with molecular weights several times greater than their own. Several commonly used CPPs, including polyarginines, transportant, protamine, maurocalcine, and M918, are suitable targeting moieties for use in the present invention and are well known in the art (*see* Stewart et al., “Cell-Penetrating Peptides as Delivery Vehicles for Biology and Medicine,” *Organic Biomolecular Chem.* 6:2242–55 (2008), which is hereby incorporated by reference in its entirety). Additionally, methods of making CPP are described in U.S. Patent Application Publication No. 20080234183 to Hallbrink et al., which is hereby incorporated by reference in its entirety.

[0056] Another suitable targeting moiety useful for enhancing the cellular uptake of a compound is an “importation competent” signal peptide as disclosed by U.S. Patent No. 6,043,339 to Lin et al., which is hereby incorporated by reference in its entirety. An importation competent signal peptide is generally about 10 to about 50 amino acid residues in length—typically hydrophobic residues—that render the compound capable of penetrating through the cell membrane from outside the cell to the interior of the cell. An exemplary importation competent signal peptide includes the signal peptide from Kaposi fibroblast growth factor (*see* U.S. Patent No. 6,043,339 to Lin et al., which is hereby incorporated by reference in its entirety). Other suitable peptide sequences can be selected from the SIGPEP database (*see* von Heijne G., “SIGPEP: A Sequence Database for Secretory Signal Peptides,” *Protein Seq. Data Anal.* 1(1):41–42 (1987), which is hereby incorporated by reference in its entirety).

[0057] Another suitable targeting moiety is a signal peptide sequence capable of targeting the compounds of the present invention to a particular tissue or cell type. The signaling peptide can include at least a portion of a ligand binding protein. Suitable ligand binding proteins include high-affinity antibody fragments (*e.g.*, Fab, Fab' and F(ab')₂, single-chain Fv antibody fragments), nanobodies or nanobody

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fragments, fluorobodies, or aptamers. Other ligand binding proteins include biotin-binding proteins, lipid-binding proteins, periplasmic binding proteins, lectins, serum albumins, enzymes, phosphate and sulfate binding proteins, immunophilins, metallothionein, or various other receptor proteins. For cell specific targeting, the signaling peptide is preferably a ligand binding domain of a cell specific membrane receptor. Thus, when the modified compound is delivered intravenously or otherwise introduced into blood or lymph, the compound will adsorb to the targeted cell, and the targeted cell will internalize the compound. For example, if the target cell is a cancer cell, the compound may be conjugated to an anti-C3B(I) antibody as disclosed by U.S. Patent No. 6,572,856 to Taylor et al., which is hereby incorporated by reference in its entirety. Alternatively, the compound may be conjugated to an alphafeto protein receptor as disclosed by U.S. Patent No. 6,514,685 to Moro, which is hereby incorporated by reference in its entirety, or to a monoclonal GAH antibody as disclosed by U.S. Patent No. 5,837,845 to Hosokawa, which is hereby incorporated by reference in its entirety. For targeting a compound to a cardiac cell, the compound may be conjugated to an antibody recognizing elastin microfibril interfacier (EMILIN2) (Van Hoof et al., "Identification of Cell Surface for Antibody-Based Selection of Human Embryonic Stem Cell-Derived Cardiomyocytes," *J Proteom Res* 9:1610–18 (2010), which is hereby incorporated by reference in its entirety), cardiac troponin I, connexin-43, or any cardiac cell-surface membrane receptor that is known in the art. For targeting a compound to a hepatic cell, the signaling peptide may include a ligand domain specific to the hepatocyte-specific asialoglycoprotein receptor. Methods of preparing such chimeric proteins and peptides are described in U.S. Patent No. 5,817,789 to Heartlein et al., which is hereby incorporated by reference in its entirety.

[0058] Another suitable targeting moiety is a transport peptide that directs intracellular compartmentalization of the compound once it is internalized by a target cell or tissue. For transport to the endoplasmic reticulum (ER), for example, the compound can be conjugated to an ER transport peptide sequence. A number of such signal peptides are known in the art, including the signal peptide MMSFVSLLLVGILFYATEAEQLTKCEVFQ (SEQ ID NO: 4). Other suitable ER signal peptides include the N-terminus endoplasmic reticulum targeting sequence of the enzyme 17 β -hydroxysteroid dehydrogenase type 11 (Horiguchi et al.,

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“Identification and Characterization of the ER/Lipid Droplet-Targeting Sequence in 17 β -hydroxysteroid Dehydrogenase Type 11,” *Arch. Biochem. Biophys.* 479(2):121–30 (2008), which is hereby incorporated by reference in its entirety), or any of the ER signaling peptides (including the nucleic acid sequences encoding the ER signal peptides) disclosed in U.S. Patent Application Publication No. 20080250515 to Reed et al., which is hereby incorporated by reference in its entirety. Additionally, the compound of the present invention can contain an ER retention signal, such as the retention signal KEDL (SEQ ID NO: 5). Methods of modifying the compounds of the present invention to incorporate transport peptides for localization of the compounds to the ER can be carried out as described in U.S. Patent Application Publication No. 20080250515 to Reed et al., which is hereby incorporated by reference in its entirety.

[0059] For transport to the nucleus, the compounds of the present invention can include a nuclear localization transport signal. Suitable nuclear transport peptide sequences are known in the art, including the nuclear transport peptide PPKKKRKV (SEQ ID NO: 6). Other nuclear localization transport signals include, for example, the nuclear localization sequence of acidic fibroblast growth factor and the nuclear localization sequence of the transcription factor NF-KB p50 as disclosed by U.S. Patent No. 6,043,339 to Lin et al., which is hereby incorporated by reference in its entirety. Other nuclear localization peptide sequences known in the art are also suitable for use in the compounds of the present invention.

[0060] Suitable transport peptide sequences for targeting to the mitochondria include MLSLRQSIRFFKPATRTLCSRYLL (SEQ ID NO: 7). Other suitable transport peptide sequences suitable for selectively targeting the compounds of the present invention to the mitochondria are disclosed in U.S. Patent Application Publication No. 20070161544 to Wipf, which is hereby incorporated by reference in its entirety.

[0061] The peptidomimetics of the present invention are designed to mimic a helix having the formula $X_1-X_2-X_2-X_3-X_2-X_2-X_1-X_4-X_5$, wherein each X_1 is any negatively charged residue, each X_2 is any hydrophobic residue, X_3 is any positively-charged residue, X_4 is any polar residue, and X_5 is absent or any hydrophobic residue. In a preferred embodiment, the peptidomimetic mimics a helix having the formula $X_1-X_2-L-X_3-X_2-L-X_1-X_4-X_5$. In a preferred embodiment, the peptidomimetic mimics a helix having the formula $X_1-X_2-L-X_3-X_2-L-D-X_4-X_5$. In a preferred embodiment, the

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peptidomimetic mimics a helix having the formula $X_1-X_2-L-X_3-X_2-L-X_1-Q-X_5$. In a preferred embodiment, the peptidomimetic mimics a helix having the formula $X_1-X_2-L-X_3-X_2-L-D-Q-X_5$ (SEQ ID NO: 8). In a preferred embodiment, the peptidomimetic mimics a helix having the formula XELA*RALDQ (SEQ ID NO: 9), where X is 4-pentenoic acid and A* is *N*-allylalanine.

[0062] As will be apparent to those of ordinary skill in the art, when R^2 and/or R^3 are a moiety of the recited formulae, the overall size of the compounds of Formula I, Formula II, and Formula III can be adjusted by varying the values of m' and/or m'' , which are independently zero or any number. Typically, m' and m'' are independently from zero to about thirty (*e.g.*, 0 to ~18, 0 to ~10, 0 to ~5, ~5 to ~30, ~5 to ~18, ~5 to ~10, ~8 to ~30, ~8 to ~18, ~8 to ~10, ~10 to ~18, or ~10 to ~30). In one embodiment of compounds of Formula I, m' and m'' are independently 4–10. In another embodiment of compounds of Formula I, m' and m'' are independently 5–6.

[0063] As will be apparent to the skilled artisan, compounds of Formula I and Formula III include a diverse range of helical conformation, which depends on the values of m , n' , and n'' . These helical conformations include 3_{10} -helices (*e.g.*, $m = 0$ and $n' + n'' = 2$), α -helices (*e.g.*, $m = 1$ and $n' + n'' = 2$), π -helices (*e.g.*, $m = 2$ and $n' + n'' = 2$), and gramicidin helices (*e.g.*, $m = 4$ and $n' + n'' = 2$). In a preferred embodiment, the number of atoms in the backbone of the helical macrocycle is 12–15, more preferably 13 or 14.

[0064] In at least one embodiment of compounds of Formula I, m''' is one and a is two.

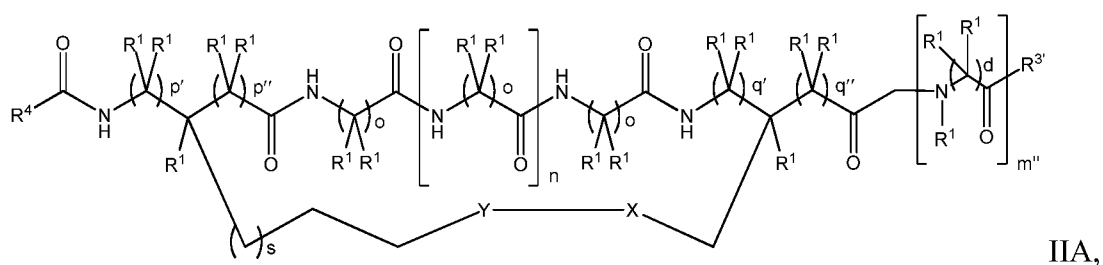
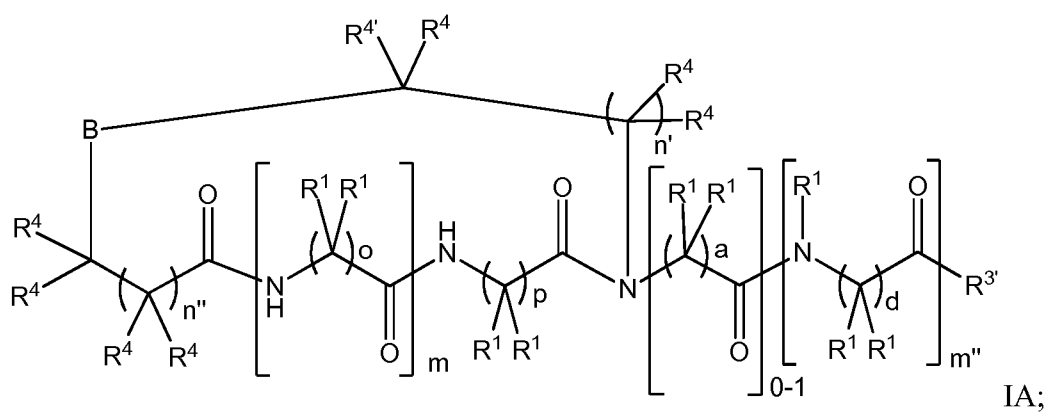
[0065] In at least one embodiment, R^2 is: a beta amino acid, a moiety of Formula A where m' is at least one and at least one b is two, a moiety of Formula A where c is two, or a moiety of Formula A where $R^{2'}$ is a beta amino acid. In at least one embodiment, R^3 is: a beta amino acid, a moiety of Formula B where m'' is at least one and at least one d is two, or a moiety of Formula B where $R^{3'}$ is a beta amino acid. Combinations of these embodiments are also contemplated.

[0066] When R^2 is a moiety of Formula A, m' is preferably any number from one to 19. When R^3 is a moiety of Formula B, m'' is preferably any number from one to nine.

[0067] In preferred embodiments, the compound is a compound of Formula IA, Formula IIA, or Formula IIIA (*i.e.*, a helix cyclized at the N-terminal); Formula

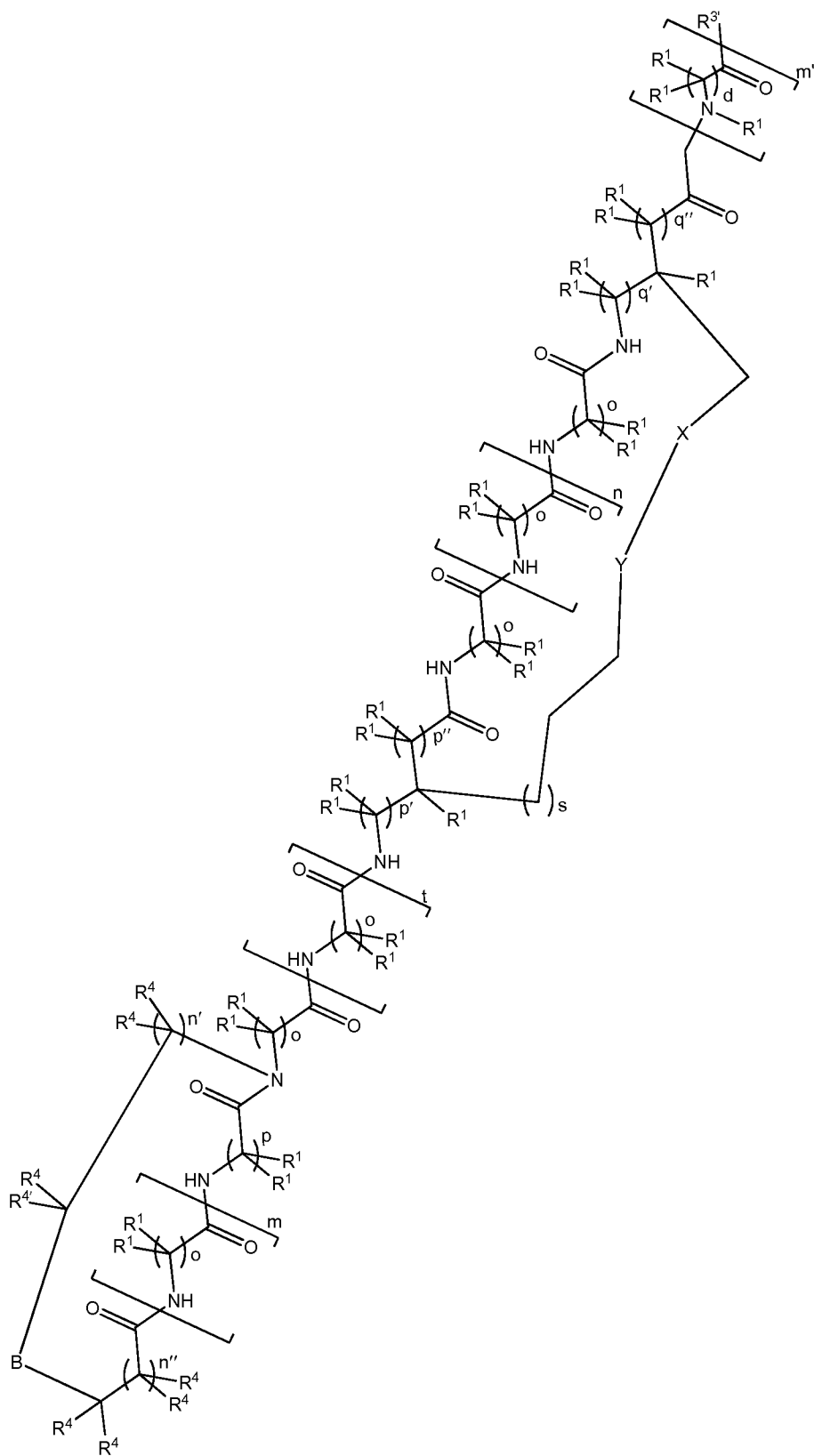
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IB, Formula IIB, or Formula IIIB (*i.e.*, a helix cyclized mid-peptide); or Formula IC, Formula IIC, or Formula IIIC (*i.e.*, a helix cyclized at the C-terminal):

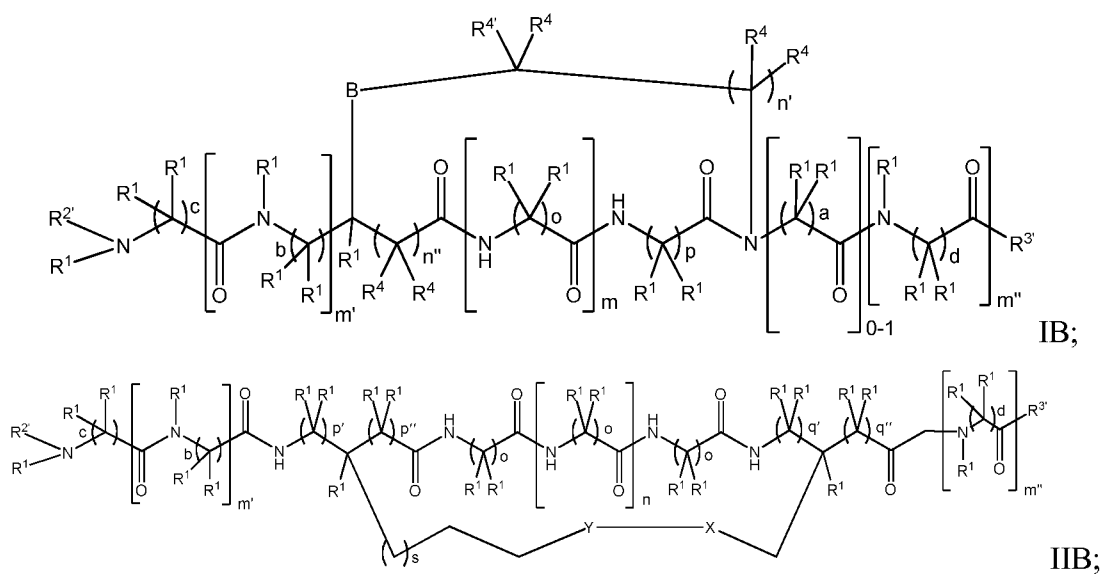


where R^4 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

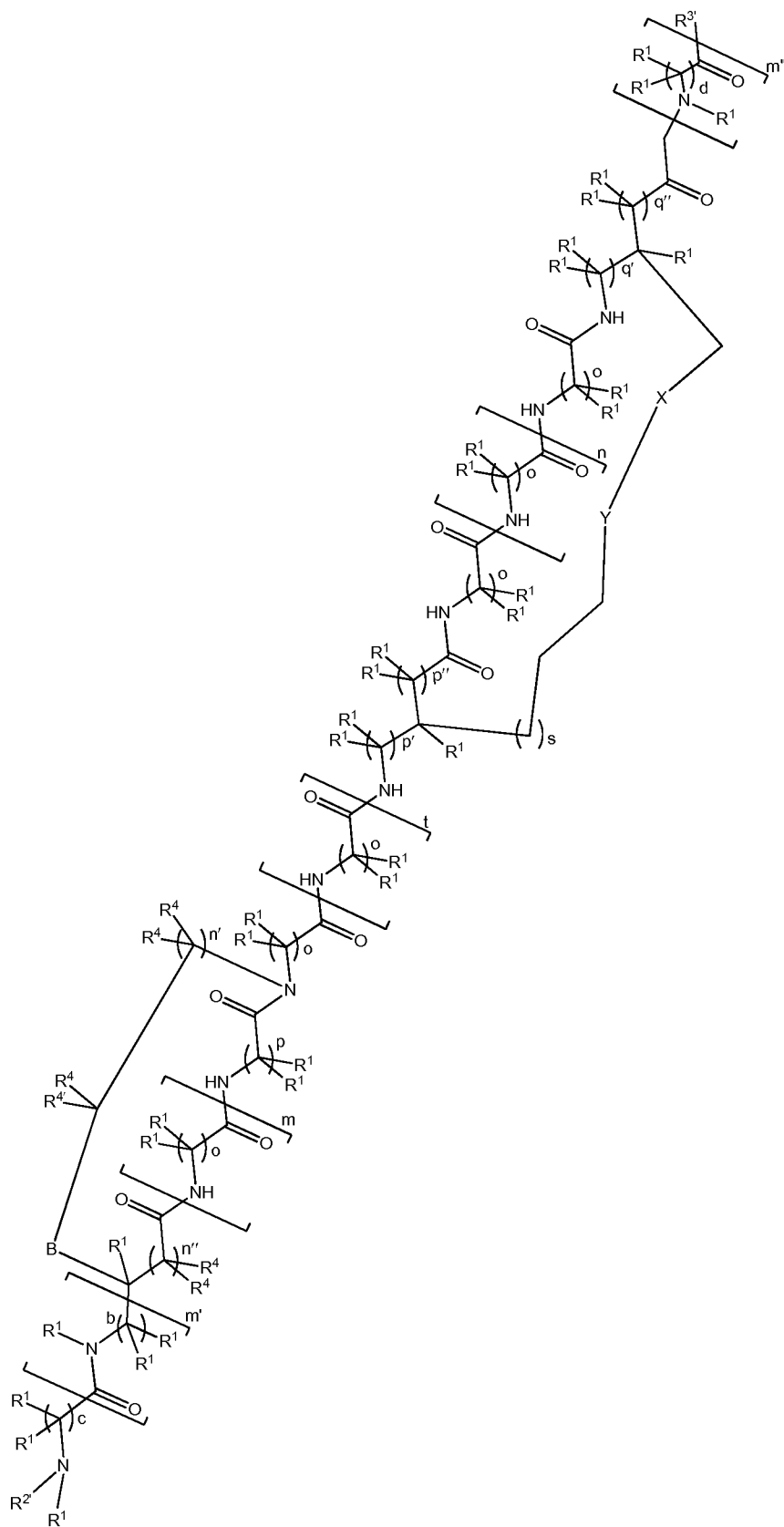
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IIIA;

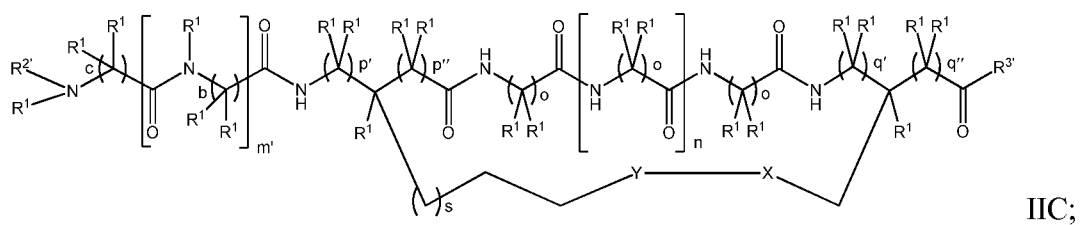
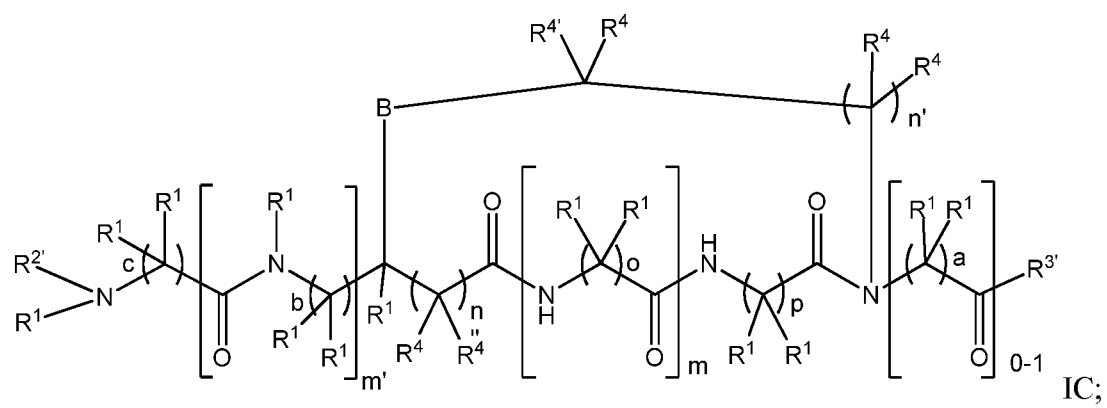


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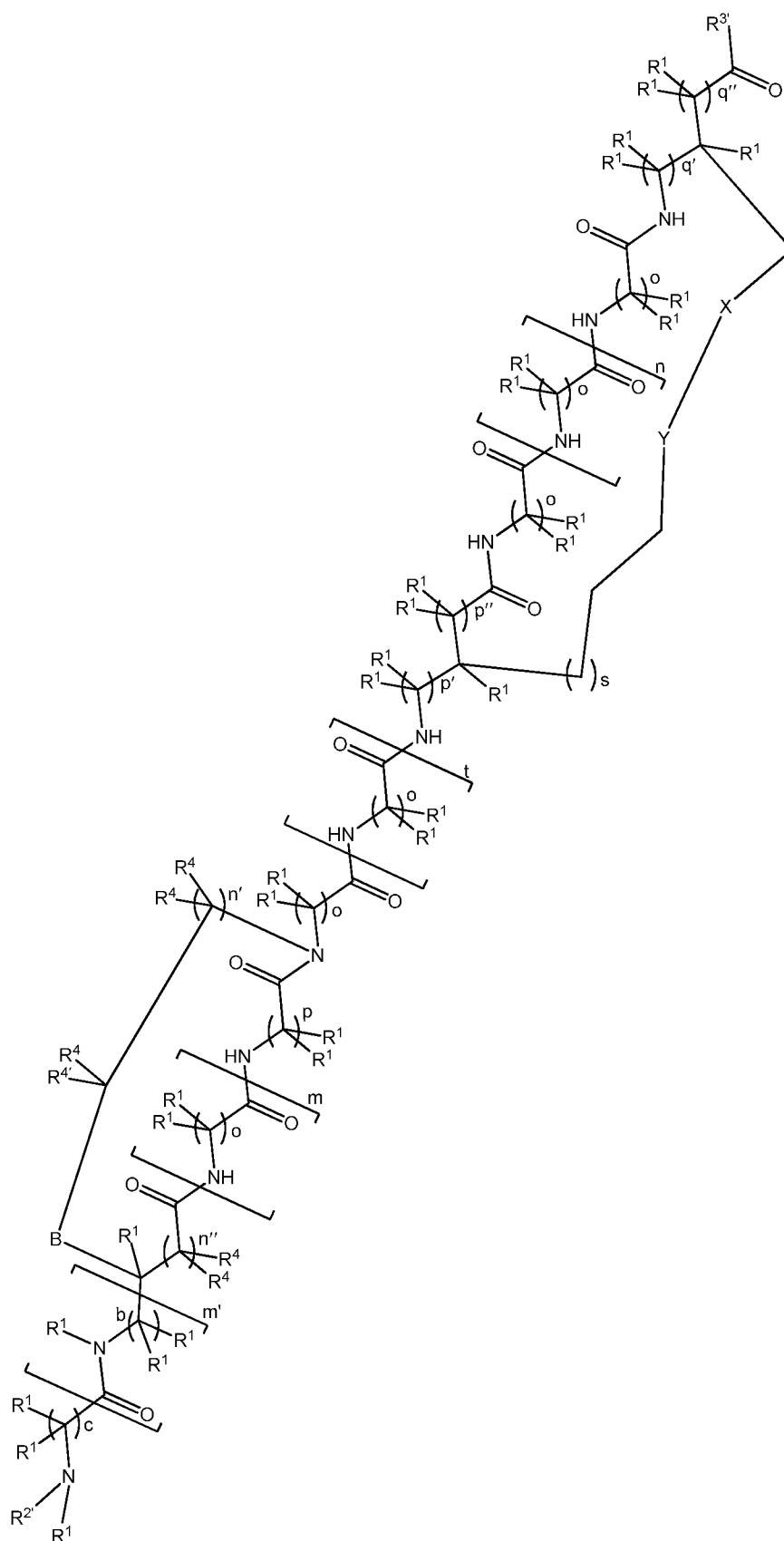


IIIB;

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IIIC.

[0068] As will be apparent to the skilled artisan, the pattern of β substitution in the attached peptides of the peptidomimetics of Formulae I, II, and III can be

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controlled by adjusting the values for m''' and a (when the peptidomimetic is a compound of Formula I), as well as m' , b , and c (when R^2 is a moiety of Formula A), and m'' and d (when R^3 is a moiety of Formula B). Substitution in peptidomimetics of Formulae IA, IIA, IIIA, IB, IIB, IIIB, IC, IIC, and IIIC can further be controlled as will be apparent to the skilled artisan. In a preferred embodiment, the attached peptide has the formula $\alpha 3/\beta 1$. Preferred peptidomimetics containing β -amino acid residues include those that mimic a helix having the formula $X_1\text{-}\mathbf{x}_2\text{-}X_2\text{-}X_3\text{-}X_2\text{-}X_2\text{-}X_1\text{-}X_4\text{-}\mathbf{x}_5$, wherein X_5 is absent or any hydrophobic residue and the beta residues are shown in lower-case bold. Preferred embodiments include, without limitation, XeEGRaLDQ (SEQ ID NO: 10), XeLLRaLDQ (SEQ ID NO: 11), XeLaRaLDQ (SEQ ID NO: 12), and XeEGRaLDQy (SEQ ID NO: 13).

[0069] The peptidomimetics of the present invention may be prepared using methods that are known in the art. By way of example, peptidomimetics of Formula I, which contain a hydrogen bond surrogate, may be prepared using the methods disclosed in, *e.g.*, U.S. Patent Application No. 11/128,722, U.S. Patent Application No. 13/724,887, and Mahon & Arora, "Design, Synthesis, and Protein-Targeting Properties of Thioether-Linked Hydrogen Bond Surrogate Helices," *Chem. Commun.* 48:1416–18 (2012), each of which is hereby incorporated by reference in its entirety. Peptidomimetics of Formula II, which contain a side-chain constraint, may be prepared using the methods disclosed in, *e.g.*, Schafmeister et al., *J. Am. Chem. Soc.* 122:5891 (2000); Sawada & Gellman, *J. Am. Chem. Soc.* 133:7336 (2011); Patgiri et al., *J. Am. Chem. Soc.* 134:11495 (2012); Henchey et al., *Curr. Opin. Chem. Biol.* 12:692 (2008); Harrison et al., *Proc. Nat'l Acad. Sci. U.S.A.* 107:11686 (2010); Shepherd et al., *J. Am. Chem. Soc.* 127:2974 (2005); Phelan et al., *J. Am. Chem. Soc.* 119:455 (1997); Jackson et al., *J. Am. Chem. Soc.* 113:9391 (1991); and Blackwell & Grubbs, *Angew. Chem. Int'l Ed. Engl.* 37:3281 (1998), each of which is hereby incorporated by reference in its entirety. Peptidomimetics of Formula III, which contain both a hydrogen bond surrogate and a side-chain constraint, may be prepared using a combination of the above methods.

[0070] Another aspect of the present invention relates to pharmaceutical formulations comprising any of the above described peptidomimetics of Formula I, Formula II, or Formula III of the present invention (including the peptidomimetics of Formulae IA, IIA, IIIA, IB, IIB, IIIB, IC, IIC, and IIIC) and a pharmaceutically

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acceptable carrier. Acceptable pharmaceutical carriers include solutions, suspensions, emulsions, excipients, powders, or stabilizers. The carrier should be suitable for the desired mode of delivery.

[0071] In addition, the pharmaceutical formulations of the present invention may further comprise one or more pharmaceutically acceptable diluents, adjuvants, excipients, or vehicles, such as preserving agents, fillers, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage forms. Examples of suspending agents include ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. Examples of suitable carriers, diluents, solvents, or vehicles include water, ethanol, polyols, suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Examples of excipients include lactose, milk sugar, sodium citrate, calcium carbonate, and dicalcium phosphate. Examples of disintegrating agents include starch, alginic acids, and certain complex silicates. Examples of lubricants include magnesium stearate, sodium lauryl sulphate, talc, as well as high molecular weight polyethylene glycols.

[0072] The peptidomimetics and pharmaceutical formulations of the present invention may be used, *inter alia*, to inhibit the HIF-1 α -p300/CBP interaction.

[0073] Another aspect of the present invention relates to a method of modulating transcription of a gene in a cell, wherein transcription of the gene is mediated by interaction of HIF-1 α with CBP and/or p300. This method involves contacting the cell with a peptidomimetic of the present invention under conditions effective to modulate transcription of the gene. In a preferred embodiment, the cell is contacted under conditions effective to cause nuclear uptake of the peptide, where the

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peptide disrupts interaction of HIF-1 α and p300/CBP and thereby reduces transcription of the gene.

[0074] Modulating according to this aspect of the present invention refers to up-regulating transcription or down-regulating transcription.

[0075] Genes whose transcription can be modulated according to this aspect of the present invention include *ACADSB*, *ADM*, *AK4*, *ALDOC*, *ALG1*, *ANG*, *ANGPTL4*, *ANKRD37*, *ANKZF1*, *ARHGAP28*, *ARID5A*, *ARNTL*, *ARRDC3*, *ASF1A*, *ASPM*, *AURKA*, *B4GALT4*, *BAMBI*, *BHLHE40*, *BHLHE41*, *BNIP3*, *BNIP3L*, *BOLA1*, *C1orf161*, *C1orf163*, *C3orf58*, *C4orf3*, *C7orf60*, *C7orf68*, *C8orf22*, *C8orf41*, *C14orf126*, *C17orf76*, *C18orf19*, *C1QL1*, *CA12*, *CA5B*, *CA9*, *CASZ1*, *CCDC80*, *CCNB1*, *CCNG2*, *CDC20*, *CDC23*, *CDCP1*, *CDK18*, *CDKN1A*, *CDKN3*, *CENPA*, *CENPE*, *CGGBP1*, *CHAC2*, *CNOT8*, *CPOX*, *CXCL16*, *CXCR4*, *DAPK1*, *DDX10*, *DEPDC1*, *DIS3L*, *DKFZp451A211*, *DLGAP5*, *DUSP5*, *DUSP5P*, *DUSP9*, *E2F5*, *EDN2*, *EFNA3*, *EGLN1*, *EGLN3*, *ELOVL6*, *ENO2*, *ERO1L*, *ERRF1*, *FAM13A*, *FAM72A*, *FAM72B*, *FAM72C*, *FAM72D*, *FAM83D*, *FAM86B1*, *FAM86B2*, *FAM86C*, *FAM115C*, *FAM115C*, *FAM133A*, *FAM162A*, *FARSB*, *FBXO16*, *FBXO32*, *FBXO42*, *FERMT1*, *FLJ23867*, *FLJ35024*, *FLJ44715*, *FN1*, *FOS*, *FOXD1*, *FUT11*, *FXD3*, *FYN*, *G2E3*, *GBE1*, *GDF15*, *GEMIN5*, *GFPT2*, *GOLGA8A*, *GOLGA8B*, *GPATCH4*, *GPR146*, *GPR155*, *GPR160*, *GPRC5A*, *GPT2*, *GTF2IRD2*, *GTF2IRD2B*, *GYS1*, *H1F0*, *H2BFS*, *HAS2*, *HERC3*, *HEY1*, *HIST1H1C*, *HIST1H1E*, *HIST1H2AB*, *HIST1H2AC*, *HIST1H2AD*, *HIST1H2AE*, *HIST1H2AH*, *HIST1H2AI*, *HIST1H2AK*, *HIST1H2AL*, *HIST1H2BC*, *HIST1H2BE*, *HIST1H2BF*, *HIST1H2BG*, *HIST1H2BH*, *HIST1H2BI*, *HIST1H2BJ*, *HIST1H2BK*, *HIST1H2BM*, *HIST1H2BN*, *HIST1H3A*, *HIST1H3D*, *HIST1H3F*, *HIST1H3H*, *HIST1H4B*, *HIST1H4H*, *HIST1H4J*, *HIST1H4K*, *HIST2H2AA3*, *HIST2H2AA4*, *HIST2H2AB*, *HIST2H2AC*, *HIST2H2BA*, *HIST2H2BE*, *HIST2H2BF*, *HIST2H3A*, *HIST2H3C*, *HIST2H3D*, *HIST2H4A*, *HIST2H4B*, *HIST3H2A*, *HIVEP2*, *HK1*, *HK2*, *HMMR*, *HORMAD1*, *HOXD10*, *HPDL*, *HRH1*, *HSPA1A*, *HSPA1B*, *HYMAI*, *ID3*, *IDH2*, *IER3*, *IGFBP3*, *IGSF3*, *IL1RAP*, *IL2RG*, *ING2*, *INSIG1*, *INSIG2*, *IPMK*, *ITGA5*, *JUN*, *KAT2B*, *KCTD11*, *KDM3A*, *KIAA0586*, *KIAA1244*, *KIAA1432*, *KIAA1715*, *KIF14*, *KIF20A*, *KRT17*, *LOC154761*, *LOC645332*, *LOC653113*, *LOC100507405*, *LOX*, *LOXL2*, *LRP1*, *LST-3TM12*, *LTV1*, *MAFB*, *MAFK*, *MAK16*, *MAP2K1*, *MAP3K15*, *METTL7A*, *MLKL*, *MOBK2A*, *MSTO1*, *MSTO2P*, *MUC1*, *MXI1*, *NAMPT*, *NARS2*, *NAV1*, *NDRG1*, *NDUFAF4*,

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NEBL, NFIL3, NLN, NOG, NOL6, NOP2, NOP16, NOTCH3, NRG4, ORAI3, OSMR, OTUD1, P4HA1, P4HA2, PAG1, PAIP2B, PDHA1, PDK1, PDK3, PER1, PER2, PFKFB4, PFKP, PGM2L1, PIAS2, PLA2G4A, PLAGL1, PLIN2, PLK1, PLOD1, PLOD2, PMEPA1, PNO1, POLR1B, PPF1A4, PPL, PPP1R3B, PPP1R3C, PPP2R5B, PPRC1, PRELID2, PRMT3, PTGS2, PTTG1, PYGL, QSOX1, RAB20, RAB40C, RAB8B, RASSF2, RCOR2, RIOK3, RITI, RLF, RNASE4, RNF122, RNF24, RNU4-2, RORA, RPSA, RRAGD, RRS1, RUVBL1, SCARNA5, SCARNA6, SCFD2, SEC14L4, SEC61G, SERPINE1, SERPINI1, SERTAD2, SLC2A1, SLC2A3, SLC6A10P, SLC6A6, SLC6A8, SLC7A11, SLC27A2, SLCO1B3, SLCO4A1, SNAPC5, SNORA1, SNORA2A, SNORA6, SNORA13, SNORA42, SNORA60, SNORA62, SNORA74A, SNORA75, SNORD1A, SNORD14E, SNORD53, SNORD94, SNX33, SPAG4, SPICE1, SPINK5, SPRY1, STAMBPL1, STC2, SYT7, TAF9B, TBC1D30, TCP11L2, TET2, TGFB1, TMCO7, TMEM45A, TMEM45B, TMEM184A, TMOD1, TMPRSS3, TNFRSF10D, TRIM59, TROAP, TSEN2, TSTD2, TTYH3, TWISTNB, UACA, UBASH3B, UFSP2, UPRT, UTP15, UTP20, VEGFA, VLDLR, VTRNA1-1, WDR3, WDR12, WDR35, WDR45L, WDR52, WSB1, XK, YEATS2, ZDBF2, ZNF160, ZNF292, ZNF395, ZNF654, ZSWIM5, adenylate kinase 3, α_{1B} -adrenergic receptor, aldolase A, ceruloplasmin, c-Met protooncogene, CXCL12/SDF-1, endothelin-1, enolase 1, erythropoietin, glucose transporter 1, glucose transporter 3, glyceraldehyde-3-phosphate dehydrogenase, heme oxygenase 1, IGF binding protein 1, insulin-like growth factor 2, lactate dehydrogenase A, nitric oxide synthase 2, p35^{srg}, phosphoglycerate kinase 1, pyruvate kinase M, transferrin, transferrin receptor, transforming growth factor β_3 , vascular endothelial growth factor, vascular endothelial growth factor receptor FLT-1, and vascular endothelial growth factor receptor KDR/Flk-1. Some uses for inhibiting transcription of some of these genes are shown in Table 1. Preferred genes include those identified in Table 5, *infra*.

Table 1. Example Disorders

Gene	Treat/prevent
adrenomedullin	Pheochromocytoma
ceruloplasmin	Lymphoma, acute and chronic inflammation, rheumatoid arthritis
c-Met protooncogene	Tumor Cells Invasion
CXCL12/SDF-1	Cancer Stem Cells Migration
CXCR4	Cancer Stem Cells Migration
endothelin-1	Abnormal vasoconstriction

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endothelin-2	Abnormal vasoconstriction
enolase 1	Hashimoto's encephalopathy, severe asthma
erythropoietin	Abnormal oxygen transport
glucose transporter 1	Aerobic glycolysis (Warburg effect)
glucose transporter 3	Aerobic glycolysis (Warburg effect)
heme oxygenase 1	Abnormal oxygen transport
hexokinase 1	Aerobic glycolysis (Warburg effect)
hexokinase 2	Aerobic glycolysis (Warburg effect)
IGF binding protein 1	Abnormal development and function of organs (brain, liver)
IGF binding protein 3	Abnormal development and function of organs (brain, liver)
insulin-like growth factor 2	Abnormal development and function of organs (brain, liver)
lactate dehydrogenase A	Myocardial infarction
lysyl oxidase	Tumor Cells Invasion
nitric oxide synthase 2	Abnormal vasomotor tone
transferrin receptor	Abnormal iron uptake/metabolism
transferrin	Abnormal iron uptake/metabolism
vascular endothelial growth factor	Angiogenesis (tumor, incl. cancer)
vascular endothelial growth factor receptor FLT-1	Angiogenesis (tumor, incl. cancer)
vascular endothelial growth factor receptor KDR/Flk-1	Angiogenesis (tumor, incl. cancer)

[0076] Yet another aspect of the present invention relates to a method of treating or preventing in a subject a disorder mediated by interaction of HIF-1 α with CBP and/or p300. This method involves administering to the subject a peptidomimetic of the present invention under conditions effective to treat or prevent the disorder.

[0077] Disorders that can be treated or prevented include, for example, abnormal vasoconstriction, retinal ischemia (Zhu et al., "Long-Term Tolerance to Retinal Ischemia by Repetitive Hypoxic Preconditioning: Role of HIF-1 α and Heme Oxygenase-1," *Invest. Ophthalmol. Vis. Sci.* 48:1735–43 (2007); Ding et al., "Retinal Disease in Mice Lacking Hypoxia-Inducible Transcription Factor-2 α ," *Invest. Ophthalmol. Vis. Sci.* 46:1010–16 (2005), each of which is hereby incorporated by reference in its entirety), pulmonary hypertension (Simon et al., "Hypoxia-Induced Signaling in the Cardiovascular System," *Annu. Rev. Physiol.* 70:51–71 (2008); Eul et al., "Impact of HIF-1 α and HIF-2 α on Proliferation and Migration of Human Pulmonary Artery Fibroblasts in Hypoxia," *FASEB J.* 20:163–65 (2006), each of which is hereby incorporated by reference in its entirety), intrauterine growth retardation (Caramelo et al., "Respuesta a la Hipoxia. Un Mecanismo Sistémico Basado en el Control de la Expresión Génica [Response to Hypoxia. A Systemic

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Mechanism Based on the Control of Gene Expression],” *Medicina B. Aires* 66:155–64 (2006); Tazuke et al., “Hypoxia Stimulates Insulin-Like Growth Factor Binding Protein 1 (IGFBP-1) Gene Expression in HepG2 Cells: A Possible Model for IGFBP-1 Expression in Fetal Hypoxia,” *Proc. Nat’l Acad. Sci. USA* 95:10188–93 (1998), each of which is hereby incorporated by reference in its entirety), diabetic retinopathy (Ritter et al., “Myeloid Progenitors Differentiate into Microglia and Promote Vascular Repair in a Model of Ischemic Retinopathy,” *J. Clin. Invest.* 116:3266–76 (2006); Wilkinson-Berka et al., “The Role of Growth Hormone, Insulin-Like Growth Factor and Somatostatin in Diabetic Retinopathy,” *Curr. Med. Chem.* 13:3307–17 (2006); Vinores et al., “Implication of the Hypoxia Response Element of the Vegf Promoter in Mouse Models of Retinal and Choroidal Neovascularization, but not Retinal Vascular Development,” *J. Cell. Physiol.* 206:749–58 (2006); Caldwell et al., “Vascular Endothelial Growth Factor and Diabetic Retinopathy: Role of Oxidative Stress,” *Curr. Drug Targets* 6:511–24 (2005), each of which is hereby incorporated by reference in its entirety), age-Related macular degeneration (Inoue et al., “Expression of Hypoxia-Inducible Factor 1 α and 2 α in Choroidal Neovascular Membranes Associated with Age-Related Macular Degeneration,” *Br. J. Ophthalmol.* 91:1720–21 (2007); Zuluaga et al., “Synergies of VEGF Inhibition and Photodynamic Therapy in the Treatment of Age-Related Macular Degeneration,” *Invest. Ophthalmol. Vis. Sci.* 48:1767–72 (2007); Provis, “Development of the Primate Retinal Vasculature,” *Prog. Retin. Eye Res.* 20:799–821 (2001), each of which is hereby incorporated by reference in its entirety), diabetic macular edema (Vinores et al., “Implication of the Hypoxia Response Element of the Vegf Promoter in Mouse Models of Retinal and Choroidal Neovascularization, but not Retinal Vascular Development,” *J. Cell. Physiol.* 206:749–58 (2006); Forooghian & Das, “Anti-Angiogenic Effects of Ribonucleic Acid Interference Targeting Vascular Endothelial Growth Factor and Hypoxia-Inducible Factor-1 α ,” *Am. J. Ophthalmol.* 144:761–68 (2007), each of which is hereby incorporated by reference in its entirety), and cancer (Marignol et al., “Hypoxia in Prostate Cancer: A Powerful Shield Against Tumour Destruction?” *Cancer Treat. Rev.* 34:313–27 (2008); Galanis et al., “Reactive Oxygen Species and HIF-1 Signalling in Cancer,” *Cancer Lett.* 266:12–20 (2008); Ushio-Fukai & Nakamura, “Reactive Oxygen Species and Angiogenesis: NADPH Oxidase as Target for Cancer Therapy,” *Cancer Lett.* 266:37–52 (2008); Adamski et al., “The

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Cellular Adaptations to Hypoxia as Novel Therapeutic Targets in Childhood Cancer,” *Cancer Treat. Rev.* 34:231–46 (2008); Toffoli & Michiels, “Intermittent Hypoxia Is a Key Regulator of Cancer Cell and Endothelial Cell Interplay in Tumours,” *FEBS J.* 275:2991–3002 (2008), each of which is hereby incorporated by reference in its entirety).

[0078] The subject according to this aspect of the present invention is preferably a human subject.

[0079] The compounds of the present invention can be administered orally, parenterally, for example, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, or by application to mucous membranes, such as, that of the nose, throat, and bronchial tubes. They may be administered alone or with suitable pharmaceutical carriers, and can be in solid or liquid form such as, tablets, capsules, powders, solutions, suspensions, or emulsions.

[0080] The active compounds of the present invention may be orally administered, for example, with an inert diluent, or with an assimilable edible carrier, or they may be enclosed in hard or soft shell capsules, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, these active compounds may be incorporated with excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compound in these compositions may, of course, be varied and may conveniently be between about 2% to about 60% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions according to the present invention are prepared so that an oral dosage unit contains between about 1 and 250 mg of active compound.

[0081] The tablets, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch, or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose, or saccharin. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a fatty oil.

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[0082] Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar, or both. A syrup may contain, in addition to active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye, and flavoring such as cherry or orange flavor.

[0083] These active compounds may also be administered parenterally. Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols such as, propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0084] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

[0085] The compounds of the present invention may also be administered directly to the airways in the form of an aerosol. For use as aerosols, the compounds of the present invention in solution or suspension may be packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The materials of the present invention also may be administered in a non-pressurized form such as in a nebulizer or atomizer.

[0086] Another aspect of the present invention relates to a method of reducing or preventing angiogenesis in a tissue. This method involves contacting the tissue

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with a peptidomimetic of the present invention under conditions effective to reduce or prevent angiogenesis in the tissue.

[0087] Preferred tissues according to this aspect of the present invention include tumors.

[0088] Yet another aspect of the present invention relates to a method of decreasing survival and/or proliferation of a cell under hypoxic conditions. This method involves contacting the cell with a peptidomimetic of the present invention under conditions effective to decrease survival and/or proliferation of the cell.

[0089] Suitable cells according to this and all aspects of the present invention include, without limitation, mammalian cells. Preferably, the cells are human cells. In at least one embodiment, the cells are cancer cells or are contained in the endothelial vasculature of a tissue that contains cancerous cells. Suitable cancer cells include, *e.g.*, sarcoma cells, multiple myeloma cells, prostate cancer cells, melanoma cells, brain cancer cells, ovarian cancer cells, breast cancer cells, renal cancer cells, and eye cancer cells.

[0090] In all aspects of the present invention directed to methods involving contacting a cell with one or more peptidomimetics, contacting can be carried out using methods that will be apparent to the skilled artisan, and can be done *in vitro* or *in vivo*.

[0091] One approach for delivering agents into cells involves the use of liposomes. Basically, this involves providing a liposome which includes agent(s) to be delivered, and then contacting the target cell, tissue, or organ with the liposomes under conditions effective for delivery of the agent into the cell, tissue, or organ.

[0092] This liposome delivery system can also be made to accumulate at a target organ, tissue, or cell via active targeting (*e.g.*, by incorporating an antibody or hormone on the surface of the liposomal vehicle). This can be achieved according to known methods.

[0093] An alternative approach for delivery of protein- or polypeptide-containing agents (*e.g.*, peptidomimetics of the present invention containing one or more protein or polypeptide side chains) involves the conjugation of the desired agent to a polymer that is stabilized to avoid enzymatic degradation of the conjugated protein or polypeptide. Conjugated proteins or polypeptides of this type are described

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in U.S. Patent No. 5,681,811 to Ekwuribe, which is hereby incorporated by reference in its entirety.

[0094] Yet another approach for delivery of agents involves preparation of chimeric agents according to U.S. Patent No. 5,817,789 to Heartlein et al., which is hereby incorporated by reference in its entirety. The chimeric agent can include a ligand domain and the agent (*e.g.*, a peptidomimetic of the invention). The ligand domain is specific for receptors located on a target cell. Thus, when the chimeric agent is delivered intravenously or otherwise introduced into blood or lymph, the chimeric agent will adsorb to the targeted cell, and the targeted cell will internalize the chimeric agent.

[0095] Peptidomimetics of the present invention may be delivered directly to the targeted cell/tissue/organ.

[0096] Additionally and/or alternatively, the peptidomimetics may be administered to a non-targeted area along with one or more agents that facilitate migration of the peptidomimetics to (and/or uptake by) a targeted tissue, organ, or cell. As will be apparent to one of ordinary skill in the art, the peptidomimetic itself can be modified to facilitate its transport to a target tissue, organ, or cell, including its transport across the blood-brain barrier; and/or to facilitate its uptake by a target cell (*e.g.*, its transport across cell membranes). In a preferred embodiment, the peptide of the invention is modified, and/or delivered with an appropriate vehicle, to facilitate its delivery to the nucleus of the target cell (Wender et al., “The Design, Synthesis, and Evaluation of Molecules That Enable or Enhance Cellular Uptake: Peptoid Molecular Transporters,” *Proc. Nat’l Acad. Sci. USA* 97:13003–08 (2000); Roberts, “Buyer’s Guide to Protein Transduction Reagents,” *Scientist* 18:42–43 (2004); Joliot & Prochiantz, “Transduction Peptides: From Technology to Physiology,” *Nat. Cell Biol.* 6:189–96 (2004), each of which is hereby incorporated by reference in its entirety). Some example target cells, tissues, and/or organs for the embodiments described above are shown in Table 2.

Table 2. Example Target Cells/Tissues/Organs

Desired Effect	Example Target(s)
<i>Inhibit transcription of:</i>	
enolase 1	Liver, brain, kidney, spleen, adipose, lung
glucose transporter 1	Tumor, incl. cancer
glucose transporter 3	Tumor, incl. cancer
hexokinase 1	Tumor, incl. cancer

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hexokinase 2	Tumor, incl. cancer
insulin-like growth factor 2	Brain, liver
IGF binding protein 1	Brain, liver
IGF binding protein 3	Brain, liver
lactate dehydrogenase A	Heart
ceruloplasmin	Lymphocytes/lymphatic tissue, inflamed tissue, rheumatoid arthritic tissue
erythropoietin	Liver, kidney
transferrin	Liver
adrenomedullin	Pheochromocytoma
endothelin-1	Endothelium
nitric oxide synthase 2	Vessels, cardiovascular cells/tissue
vascular endothelial growth factor	Tumor cells/tissue, incl. cancer
vascular endothelial growth factor receptor FLT-1	Tumor cells/tissue, incl. cancer
vascular endothelial growth factor receptor KDR/Flk-1	Tumor cells/tissue, incl. cancer
<i>Treat or prevent:</i>	
retinal ischemia	Retina (eye)
pulmonary hypertension	Lungs
intrauterine growth retardation	Uterus
diabetic retinopathy	Retina (eye)
age-related macular degeneration	Retina (eye)
diabetic macular edema	Retina (eye)
Reduce or prevent angiogenesis	Tumor cells/tissue, incl. cancer
Decrease cell survival and/or proliferation	Cancerous cells, cells contained in the endothelial vasculature of a tissue that contains cancerous cells

[0097] *In vivo* administration can be accomplished either via systemic administration to the subject or via targeted administration to affected tissues, organs, and/or cells, as described above. Typically, the therapeutic agent (*i.e.*, a peptidomimetic of the present invention) will be administered to a patient in a vehicle that delivers the therapeutic agent(s) to the target cell, tissue, or organ. Typically, the therapeutic agent will be administered as a pharmaceutical formulation, such as those described above.

[0098] Exemplary routes of administration include, without limitation, orally, topically, transdermally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intraventricularly, and intralesionally; by intratracheal inoculation, aspiration, airway instillation, aerosolization, nebulization, intranasal instillation, oral or nasogastric instillation, intraperitoneal injection, intravascular injection, intravenous injection, intra-arterial injection (such as via the pulmonary artery), intramuscular injection, and intrapleural instillation; by application to mucous

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membranes (such as that of the nose, throat, bronchial tubes, genitals, and/or anus); and by implantation of a sustained release vehicle.

[0099] For use as aerosols, a peptidomimetic of the present invention in solution or suspension may be packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The peptidomimetics of the present invention also may be administered in a non-pressurized form.

[0100] Exemplary delivery devices include, without limitation, nebulizers, atomizers, liposomes (including both active and passive drug delivery techniques) (Wang & Huang, "pH-Sensitive Immunoliposomes Mediate Target-Cell-Specific Delivery and Controlled Expression of a Foreign Gene in Mouse," *Proc. Nat'l Acad. Sci. USA* 84:7851-55 (1987); Bangham et al., "Diffusion of Univalent Ions Across the Lamellae of Swollen Phospholipids," *J. Mol. Biol.* 13:238-52 (1965); U.S. Patent No. 5,653,996 to Hsu; U.S. Patent No. 5,643,599 to Lee et al.; U.S. Patent No. 5,885,613 to Holland et al.; U.S. Patent No. 5,631,237 to Dzaou & Kaneda; U.S. Patent No. 5,059,421 to Loughrey et al.; Wolff et al., "The Use of Monoclonal Anti-Thy1 IgG1 for the Targeting of Liposomes to AKR-A Cells *in Vitro* and *in Vivo*," *Biochim. Biophys. Acta* 802:259-73 (1984), each of which is hereby incorporated by reference in its entirety), transdermal patches, implants, implantable or injectable protein depot compositions, and syringes. Other delivery systems which are known to those of skill in the art can also be employed to achieve the desired delivery of the peptidomimetic to the desired organ, tissue, or cells *in vivo* to effect this aspect of the present invention.

[0101] Contacting (including *in vivo* administration) can be carried out as frequently as required and for a duration that is suitable to provide the desired effect. For example, contacting can be carried out once or multiple times, and *in vivo* administration can be carried out with a single sustained-release dosage formulation or with multiple (*e.g.*, daily) doses.

[0102] The amount to be administered will, of course, vary depending upon the particular conditions and treatment regimen. The amount/dose required to obtain the desired effect may vary depending on the agent, formulation, cell type, culture conditions (for *ex vivo* embodiments), the duration for which treatment is desired, and, for *in vivo* embodiments, the individual to whom the agent is administered.

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[0103] Effective amounts can be determined empirically by those of skill in the art. For example, this may involve assays in which varying amounts of the peptidomimetic of the invention are administered to cells in culture and the concentration effective for obtaining the desired result is calculated. Determination of effective amounts for *in vivo* administration may also involve *in vitro* assays in which varying doses of agent are administered to cells in culture and the concentration of agent effective for achieving the desired result is determined in order to calculate the concentration required *in vivo*. Effective amounts may also be based on *in vivo* animal studies.

[0104] Another aspect of the present invention relates to a method of identifying an agent that potentially inhibits interaction of HIF-1 α with CBP and/or p300. This method involves providing a peptidomimetic of the present invention, contacting the peptidomimetic with a test agent, and detecting whether the test agent selectively binds to the peptidomimetic, wherein a test agent that selectively binds to the peptidomimetic is identified as a potential inhibitor of interaction between HIF-1 α with CBP and/or p300.

[0105] This aspect of the present invention can be carried out in a variety of ways, that will be apparent to the skilled artisan. For example, the affinity of the test agent for the peptidomimetic of the present invention may be measured using isothermal titration calorimetry analysis (Wiseman et al., "Rapid Measurement of Binding Constants and Heats of Binding Using a New Titration Calorimeter," *Anal. Biochem.* 179:131–37 (1989); Freire et al., "Isothermal Titration Calorimetry," *Anal. Chem.* 62:A950–A959 (1990); Chervenak & Toone, "Calorimetric Analysis of the Binding of Lectins with Overlapping Carbohydrate-Binding Ligand Specificities," *Biochemistry* 34:5685–95 (1995); Aki et al., "Competitive Binding of Drugs to the Multiple Binding Sites on Human Serum Albumin. A Calorimetric Study," *J. Thermal Anal. Calorim.* 57:361–70 (1999); Graziano et al., "Linkage of Proton Binding to the Thermal Unfolding of Sso7d from the Hyperthermophilic Archaeobacterium *Sulfolobus solfataricus*," *Int'l J. Biol. Macromolecules* 26:45–53 (1999); Pluschke & Mutz, "Use of Isothermal Titration Calorimetry in the Development of Molecularly Defined Vaccines," *J. Thermal Anal. Calorim.* 57:377–88 (1999); Corbell et al., "A Comparison of Biological and Calorimetric Analyses of Multivalent Glycodendrimer Ligands for Concanavalin A," *Tetrahedron-Asymmetry* 11:95–111 (2000), which are

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hereby incorporated by reference in their entirety). In one embodiment, a test agent is identified as a potential inhibitor of interaction between HIF-1 α with CBP and/or p300 if the dissociation constant (K_d) for the test agent and the peptidomimetic of the invention is 50 μ M or less. In another embodiment, the K_d is 200 nM or less. In another embodiment, the K_d is 100 nM or less.

[0106] Test agents identified as potential inhibitors of HIF-1 α -p300/CREB interaction may be subjected to further testing to confirm their ability to inhibit interaction between HIF-1 α with CBP and/or p300.

[0107] The present invention may be further illustrated by reference to the following examples.

EXAMPLES

[0108] The following Examples are intended to illustrate, but by no means are intended to limit, the scope of the present invention as set forth in the appended claims.

Example 1 — General Materials and Methods.

[0109] Commercial grade solvents and reagents were used without further purification. Fmoc amino acids and peptide synthesis reagents were purchased from Novabiochem. Hoveyda-Grubbs (second-generation) catalyst was obtained from Sigma. Molecular biology grade salts and buffers were obtained from Sigma. Cell culture media and reagents were purchased from Invitrogen, unless otherwise stated.

Peptide Synthesis

[0110] Peptides were synthesized on a CEM Liberty series microwave peptide synthesizer and purified by reversed-phase HPLC. The identity and purity of the peptides were confirmed by LCMS (see Table 3 below).

Table 3. Mass Spectroscopic Characterization of HBS Helices and Peptide 3

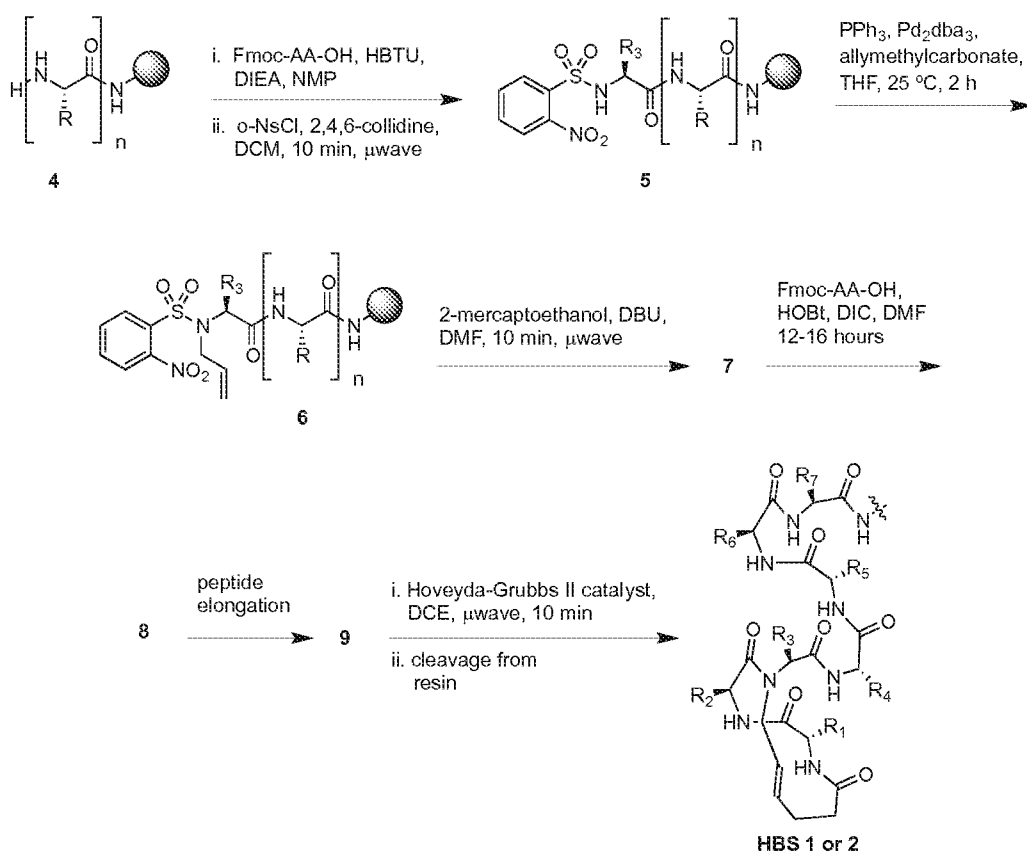
Compound	Sequence ^a	Calculated [M+H] ⁺	Observed [M+H] ⁺
HBS 1	XELA*RALDQ-NH ₂ (SEQ ID NO: 14)	1008.5	1008.5
HBS 2	XELA*RAADQ-NH ₂ (SEQ ID NO: 15)	966.5	966.5
Peptide 3	AcELARALDQ-NH ₂ (SEQ ID NO: 16)	956.5	956.5

^a X denotes 4-pentenoic acid; A* = *N*-allylalanine.

Synthesis of HBS Peptides

[0111] HBS helices containing only α -amino acid residues were synthesized as previously described (Patgiri et al., "Solid-Phase Synthesis of Short α -Helices Stabilized by the Hydrogen Bond Surrogate Approach," *Nat. Protoc.* 5(10):1857–65 (2010), which is hereby incorporated by reference in its entirety) (see Scheme 1 below).

Scheme 1



[0112] Peptide sequences up to the $i+3^{\text{rd}}$ residue of the putative helix (4 in Scheme 1) were synthesized on solid phase on a CEM Liberty Series microwave peptide synthesizer. A solution containing premixed *o*-nitrobenzenesulfonyl chloride (10 eq) and 2,4,6-collidine (10 eq) in DCM was added to Fmoc-deprotected, resin bound 4. Resin was washed sequentially with DCM ($\times 3$), DMF ($\times 3$), DCM ($\times 3$), and diethyl ether. Resin was dried overnight under vacuum. Dried resin, PPh_3 , and $\text{Pd}_2(\text{dba})_3$ were flushed with argon for 30 minutes. Upon addition of THF, allylmethylcarbonate was added to the reaction vessel containing dissolved reactants

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and resin. The solution was agitated at room temperature for 3 to 5 hours under argon to afford **5**.

[0113] Resin was filtered and washed with DCM (×3), DMF (×3), 0.2 M sodium diethylcarbamate trihydrate in NMP, and diethyl ether. The nosyl protecting group was then removed by the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 5eq) and 2-mercaptoethanol (10 eq.) in DMF. Resin was washed with DMF (×3), DCM (×3), and diethyl ether and treated with the desired Fmoc amino acid (20 eq.), DIC (20 eq.), and HOAt (10 eq.) in DMF and was allowed to agitate at room temperature for 12 to 16 hours.

[0114] Resin containing **8** was then washed with DMF (×3), DCM (×3), and DMF (×3), and coupled to the desired Fmoc amino acid residue (5 eq.) and 4-pentenoic acid (5 eq.) with HBTU (5 eq.) and DIEA (10 eq.) in DMF.

[0115] Ring-closing metathesis of bis-olefin **9** was performed with Hoveyda-Grubbs II catalyst (20 mol%) in 1,2-dichloroethane under microwave irradiation at 120°C for 10 minutes as described in Patgiri et al., “Solid-Phase Synthesis of Short α -Helices Stabilized by the Hydrogen Bond Surrogate Approach,” *Nat. Protoc.* 5(10):1857–65 (2010); Chapman & Arora, “Optimized Synthesis of Hydrogen-Bond Surrogate Helices: Surprising Effects of Microwave Heating on the Activity of Grubbs Catalysts,” *Org. Lett.* 8(25):5825–28 (2006); and Patgiri et al., “Solid Phase Synthesis of Hydrogen Bond Surrogate Derived Alpha-Helices: Resolving the Case of a Difficult Amide Coupling,” *Org. Biomol. Chem.* 8:1773–76 (2010), each of which is hereby incorporated by reference in its entirety. Peptides were cleaved from the resin using TFA:TIS:water (95: 2.5: 2.5), and purified by reversed-phase HPLC (C₁₈ column) in 0.1% TFA acetonitrile/ water gradients and characterized by ESI-MS. The computational alanine scanning mutagenesis energies calculated with Rosetta ver. 3.3. are shown in Table 4 below. Scans were performed on the HIF-1 α /CBP complex (PDB codes 1L8C and 1L3E). Peptides were also analyzed by HPLC (see Figures 4A–C).

Table 4. Computational Alanine Scanning Mutagenesis Energies

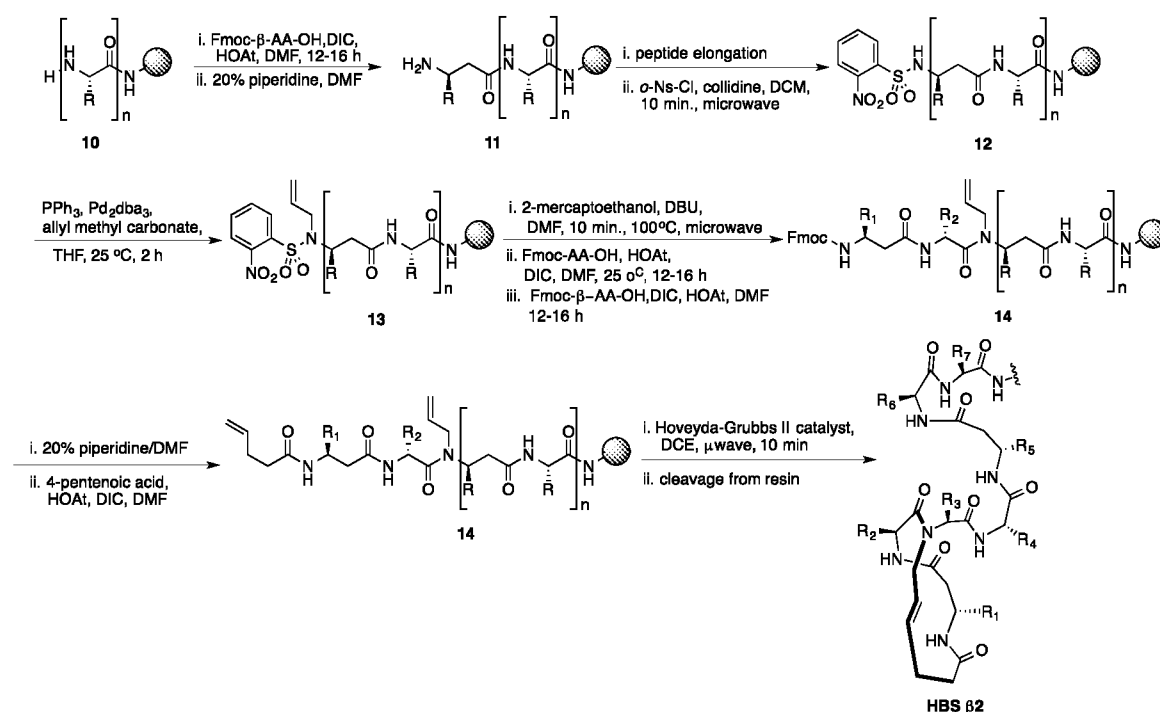
HELIX B (817–824): ELLRALDQ (SEQ ID NO: 17)		
Residue	Helix B residue	$\Delta\Delta G$ (kcal/mol)
Leu	818	1.4
Leu	819	0.5
Arg	820	0.1
Ala	821	0.0

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Leu	822	1.9
Asp	823	1.4
Gln	824	0.3

[0116] An HBS helix containing β -amino acid residues (*i.e.*, XeEG*RaLDQ-NH₂ (SEQ ID NO: 18), bold lower case letters denote β -residues) was synthesized as previously described with the necessary modification (Patgiri et al., “Solid-Phase Synthesis of Short α -Helices Stabilized by the Hydrogen Bond Surrogate Approach,” *Nat. Protoc.* 5(10):1857–65 (2010); Patgiri et al., “Nucleation Effects in Peptide Foldamers,” *J. Am. Chem. Soc.* 134(28):11495–502 (2012), each of which is hereby incorporated by reference in its entirety) (see Scheme 2 below).

Scheme 2



[0117] The peptide sequence up to the putative helix **10** in Scheme 2 was synthesized on solid phase via a CEM Liberty Series microwave peptide synthesizer or by hand. A solution containing premixed β -Fmoc amino acid (20 eq.), DIC (20 eq.), and HOAt (10 eq.) in DMF was added to Fmoc-deprotected resin bound **10** at room temperature for 12 to 16 hours. Resin was washed sequentially with DCM ($\times 3$), DMF ($\times 3$), and MeOH ($\times 3$) to afford **11**.

[0118] After Fmoc-deprotection and two further α -amino acid peptide elongation, a solution containing premixed *o*-nitrobenzenesulfonyl chloride (10 eq) and 2,4,6-collidine (10 eq) in DCM was added to Fmoc-deprotected, resin bound **11**.

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Resin was washed sequentially with DCM (×3), DMF (×3), DCM (×3), and diethyl ether to afford **12**.

[0119] Resin bound **12** was dried overnight under vacuum, then PPh₃, and Pd₂(dba)₃ were added and flushed with argon for 30 minutes. Upon addition of THF, allylmethylcarbonate was added to the reaction vessel containing dissolved reactants and resin. The solution was agitated at room temperature for 3 to 5 hours under argon to afford **13**.

[0120] Resin was filtered and washed with DCM (×3), DMF (×3), 0.2 M sodium diethylcarbamate trihydrate in NMP, and diethyl ether. The nosyl protecting group was then removed by the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 5eq) and 2-mercaptoethanol (10 eq.) in DMF. Resin was washed with DMF (×3), DCM (×3), and diethyl ether and treated with the desired Fmoc amino acid (20 eq.), DIC (20 eq.), and HOAt (10 eq.) in DMF and was allowed to agitate at room temperature for 12 to 16 hours.

[0121] Resin containing **14** was then washed with DMF (×3), DCM (×3), and MeOH (×3), and coupled to the desired β-Fmoc amino acid residue (5 eq.). Use of 4-pentenoic acid (5 eq.) DIC (20 eq.), and HOAt (10 eq.) in DMF afforded **15**.

[0122] Ring-closing metathesis of bis-olefin **15** was performed with Hoveyda-Grubbs II catalyst (20 mol%) in 1,2-dichloroethane under microwave irradiation at 120°C for 10 minutes as described in Patgiri et al., "Solid-Phase Synthesis of Short α-Helices Stabilized by the Hydrogen Bond Surrogate Approach," *Nat. Protoc.* 5(10):1857–65 (2010); Patgiri et al., "Nucleation Effects in Peptide Foldamers," *J. Am. Chem. Soc.* 134(28):11495–502 (2012); Chapman & Arora, "Optimized Synthesis of Hydrogen-Bond Surrogate Helices: Surprising Effects of Microwave Heating on the Activity of Grubbs Catalysts," *Org. Lett.* 8(25):5825–28 (2006); and Patgiri et al., "Solid Phase Synthesis of Hydrogen Bond Surrogate Derived Alpha-Helices: Resolving the Case of a Difficult Amide Coupling," *Org. Biomol. Chem.* 8:1773–76 (2010), each of which is hereby incorporated by reference in its entirety. Peptides were cleaved from the resin using TFA:TIS:water (95: 2.5: 2.5), and purified by reversed-phase HPLC (C₁₈ column) in 0.1% TFA acetonitrile/ water gradients and characterized by ESI-MS.

[0123] Additional mimics containing beta amino acids, including XeLL*RaLDQ-NH₂ (SEQ ID NO: 19), XeLA*RaLDQ-NH₂ (SEQ ID NO: 20),

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XeEG*RaLDQy-NH₂ (SEQ ID NO: 21), will also be synthesized. The β -residue-containing mimics are expected to be more resistant to degradation than their α -amino acid counterparts.

Circular Dichroism Studies

[0124] CD spectra were recorded on an AVIV 202SF CD spectrometer equipped with a temperature controller using 1 mm length cells and a scan speed of 0.5 nm/min at 298K. The spectra were averaged over 10 scans with the baseline subtracted from analogous conditions as those for the samples. The samples were prepared in 10 mM KF with the final peptide concentration of 50 μ M.

Plasmids

[0125] The DNA sequence of human p300 CH1 domain (amino acid residues 323–423) was designed as an insert and subcloned into a pUC57 plasmid by Genscript, Inc. After transformation of the plasmid in JM109 bacteria (Promega), the gene sequence was subcloned into *Bam*HI and *Eco*RI restriction sites of pGEX-4T-2 expression vector (Amersham).

Cloning and Expression of ¹⁵N p300-CH1

[0126] The pGEX 4T-2-p300 fusion vector was transformed into BL21(DE3)-competent *E.coli* (Novagen) in M9 minimal media with ¹⁵NH₄Cl as the main nitrogen source. Protein production was induced with 1 mM IPTG at O.D.600 of 1 for 16 hours at 15°C. Production of the desired p300-CH1-GST fusion product was verified by SDS-PAGE. Bacteria were harvested and resuspended in the lysis buffer with 20 mM Phosphate buffer (Research Products International, Corp.), 100 μ M DTT (Fisher), 100 μ M ZnSO₄ (Sigma), 0.5% TritonX 100 (Sigma), 1 mg/mL Pepstatin A (Research Products International, Corp.), 10 mg/mL Leupeptin A (Research Products International, Corp.), 500 μ M PMSF (Sigma), and 0.5% glycerol at pH 8.0. Pellets were lysed by sonication and centrifuged at 4°C, 20,000 rpm, for 20 minutes. Fusion protein was collected from the bacterial supernatant and purified by affinity chromatography using glutathione Sepharose 4B beads (Amersham) prepared according to the manufacturer's directions. GST-tag was cleaved by thrombin and protein was eluted from resin. Collected fractions were assayed by SDS-PAGE gel; pooled fractions were treated with protease inhibitor cocktail (Sigma) and against a

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buffer containing 10 mM Tris, 50 mM NaCl, 2mM DTT (Fisher), and 3 equivalents ZnSO₄ at pH 8.0 to ensure proper folding (*vide supra*).

Tryptophan Fluorescence Binding Assay

[0127] Spectra were recorded on a QuantaMaster 40 spectrofluorometer (Photon Technology International) in a 10 mm quartz fluorometer cell at 25°C with 4 nm excitation and 4 nm emission slit widths from 200 to 400 nm at intervals of 1 nm/s. Samples were excited at 295 nm and fluorescence emission was measured from 200–400 nm and recorded at 335 nm. Peptide stock solutions were prepared in DMSO. Aliquots containing 1 µL DMSO stocks were added to 400 µL of 1 µM p300-CH1 in 50 mM Tris and 100 mM NaCl (pH 8.0). After each addition, the sample was allowed to equilibrate for 5 minutes before UV analysis. Background absorbance and sample dilution effects were corrected by titrating DMSO into p300-CH1 in an analogous manner. Final fluorescence is reported as the absolute value of $[(F_1 - F_0)/F_1] \times 100$, where F_1 is the final fluorescence upon titration and F_0 is the fluorescence of the blank DMSO titration. EC₅₀ values for each peptide were determined by fitting the experimental data to a sigmoidal dose-response nonlinear regression model on GraphPad Prism 5.0, and the dissociation constants, K_D , were obtained from equation (1)

$$K_D = (EC_{50} \times (1 - F) + P \times F^2) / F - P \quad (1)$$

P = Total concentration of protein

F = Fraction of bound peptide = 0.5

Fluorescence Polarization Assay

[0128] The relative affinity of peptides for ¹⁵N-labeled p300-CH1 was determined using fluorescence polarization binding assay with fluoresceine-tagged HIF-1α C-TAD_{786–826}. The polarization experiments were performed with a DTX 880 Multimode Detector (Beckman) at 25°C, with excitation and emission wavelengths of 485 and 525 nm, respectively. Addition of an increasing concentration (0 nM to 13.5 µM) of p300-CH1 protein to a 15 nM solution of fluorescein labeled HIF peptide in 20 mM Tris pH 8.0, 50 mM NaCl, 2 mM DTT, 3 eq ZnSO₄, and 0.1% pluronic F-68 (Sigma) in 96 well plates afforded the IC₅₀ value, which was fit into equation (2) to calculate the dissociation constant (K_D) for the HIF/p300 complex (Roehrl et al., “A

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General Framework for Development and Data Analysis of Competitive High-Throughput Screens for Small-Molecule Inhibitors of Protein–Protein Interactions by Fluorescence Polarization,” *Biochemistry* 43(51):16056–66 (2004), which is hereby incorporated by reference in its entirety).

$$K_D = (R_T \times (1 - F_{SB}) + L_{ST} \times F_{SB}^2) / F_{SB} - L_{ST} \quad (2)$$

R_T = Total concentration of p300-CH1 protein

L_{ST} = Total concentration of fluorescent peptide

F_{SB} = Fraction of bound fluorescent peptide

[0129] The binding affinity (K_D) reported for each peptide is the average of three individual experiments, and was determined by fitting the experimental data to a sigmoidal dose-response nonlinear regression model on GraphPad Prism 5.0. The K_D of Flu-HIF C-TAD was determined to be 31 ± 3 nM. For competitive inhibition experiments, a solution of 300 nM p300-CH1 and 15 nM Flu-HIF C-TAD in buffer (20 mM Tris (pH 8.0), 50 mM NaCl, 2 mM DTT, and 150 μ M ZnSO₄) and 0.1% pluronic acid was incubated at 25°C in a 96 well plate. After 30 minutes, appropriate concentrations of the HBS or linear peptides were added to the p300-CH1/Flu-HIF C-TAD solution and the resulting mixtures were incubated at 25°C for 30 minutes before measuring the degree of dissociation of Flu-HIF C-TAD by polarization. The EC_{50} was fit into equation (3) to calculate the K_i value of HBS 1. The inhibition curve is shown in Figure 5C.

$$K_i = K_{D1} * F_{SB} * ((L_T / (L_{ST} * F_{SB2} - (K_{D1} + L_{ST} + R_T) * F_{SB} + R_T)) - 1 / (1 - F_{SB})) \quad (3)$$

K_D = K_D of fluorescent probe Flu-HIF C-TAD

R_T = Total concentration of p300-CH1 protein

L_{ST} = Total concentration of HIF fluorescent peptide

F_{SB} = Fraction of bound HBS 1 (at EC_{50})

L_T = Total concentration of HBS 1 (EC_{50})

¹H-¹⁵N HSQC NMR Spectroscopy

[0130] Protein samples were prepared as described above. Uniformly ¹⁵N-labelled p300-CH1 was concentrated to 69 μ M in NMR buffer (10 mM Tris pH 8, 50

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mM NaCl, 2 mM DTT, and 207 μ M ZnSO₄) using a 3 kDa MWCO Amicon Ultra centrifugal filter (Millipore) and supplemented with 5% D₂O. For HSQC titration experiments, data was collected on a 600 MHz Bruker four-channel NMR system at 25°C and analyzed with the TopSpin software (Bruker). For Zn²⁺ experiments, data were collected on Agilent 600 MHz at 25°C and analyzed using Sparky3 (Univ. of California).

[0131] For the HSQC titration experiments, five and ten molar equivalents of HBS **1** in DMSO were added to ¹⁵N-labelled p300-CH1, and the data were collected as described above. Mean chemical shift difference ($\Delta\delta_{\text{NH}}$) observed for ¹H and ¹⁵N nuclei of various resonances were calculated as described in Williamson, "Using Chemical Shift Perturbation to Characterise Ligand Binding," *Prog. Nucl. Mag. Resonance Spectr.* 73(0):1–16 (2013), which is hereby incorporated by reference in its entirety, where α is the range of H ppm shifts divided by the range of NH ppm shifts ($\alpha = 1/8$).

$$d = \sqrt{\frac{1}{2}[\delta_H^2 + (\alpha \cdot \delta_N^2)]}$$

Cell Lines and Cell Culture

[0132] Human cervical epithelial adenocarcinoma (HeLa) and human renal cell carcinoma (786-O) cell lines were obtained from ATCC. Aggressive human breast carcinoma stably transfected with an HRE luciferase construct (MDA-MB-231-HRE-Luc) was a gift of Dr. Robert Gillies. HeLa cells were grown at 37°C in a humidified atmosphere with 5% CO₂ in high glucose Dulbecco's Modified Eagle's Medium (DMEM, Sigma) supplemented with 10%, 2%, or 0.2% of fetal bovine serum (FBS, Irvine Scientific) and 0.5% Pen-Strep (Sigma). MDA-MB-231-HRE-Luc cells were grown in high glucose DMEM supplemented with 10% fetal bovine serum and 0.4 g/L geneticin (RPI). Hypoxia was mimicked with desferrioxamine mesylate (DFO, Sigma) at a concentration of 300 μ M or by GasPak EZ pouch (BD Biosciences). Cell growth and morphology were monitored by phase-contrast microscopy.

Isolation of mRNA

[0133] HeLa cells (~70% confluent) were plated in 6-well dishes (BD Falcon) at a density of 1.5×10^5 cells/mL. After attachment, cells were treated with 1.5 mL of

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fresh media containing HBS **1**, HBS **2**, and peptide **1** at concentrations of 10 μ M and 50 μ M. All samples, including vehicle, contained a final concentration of 0.1% DMSO. After 6 hours, hypoxia was induced with DFO (300 μ M) or GasPak EZ pouch and cells were incubated for another 18 and 42 hours, respectively. Cells were lysed and RNA isolated according to the protocol described in Dubey et al., "Suppression of Tumor Growth by Designed Dimeric Epidithiodiketopiperazine Targeting Hypoxia-Inducible Transcription Factor Complex," *J. Am. Chem. Soc.* 135(11):4537–49 (2013), which is hereby incorporated by reference in its entirety.

Analysis of Gene Expression

[0134] Real-time qRT-PCR was used to determine the effect of HBS **1**, HBS **2**, and peptide **1** on *VEGF*, *LOX*, and *SLC2A1 (GLUT1)* genes in the HeLa cell lines, as described in Dubey et al., "Suppression of Tumor Growth by Designed Dimeric Epidithiodiketopiperazine Targeting Hypoxia-Inducible Transcription Factor Complex," *J. Am. Chem. Soc.* 135(11):4537–49 (2013), which is hereby incorporated by reference in its entirety. Statistical analyses were performed with data from four independent replicates.

Cell Viability Assays

[0135] HeLa cells were plated in a 96-well plate at a density of 6,000 cells/well and allowed to form a monolayer before adding the compounds. After attachment, the media was replaced by 100 μ L of fresh media containing HBS **1**, HBS **2**, or peptide **1** at a concentration ranging from 1 μ M to 100 μ M, and 0.1% DMSO as a vehicle. After 24 hours of incubation with compounds, 11 μ L of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT, Sigma) at a concentration of 5 mg/mL in PBS was added to each well and incubated at 37°C and 5% CO₂ for an additional 3 hours. After 3 hours of incubation, the media was removed and purple crystals were dissolved in 100 μ L of dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm with a correction at 690 nm in order to quantify the amount of formed purple formazan. All experiments were performed in quadruplicate.

Luciferase Assays

[0136] MDA-MB-231-HRE-Luc cells were plated in 24-well plates (BD Falcon) at a seeding density of 35,000 cells/mL. Cells were allowed to adhere and

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form a monolayer before adding the compounds (~70% confluence). After attachment, cells were treated with 1 mL of fresh media containing HBS 1, HBS 2, or peptide 1 at a concentration of 10 μ M and 50 μ M. All samples contained a final concentration of 0.1% DMSO; vehicle samples were treated with cell culture media containing 0.1% DMSO. Cells were incubated for 6 hours at 37°C and 5% CO₂ and then hypoxia was induced by placing the cells into GasPak EZ pouch for another 18 hours. The lysates were isolated by using cell culture lysis reagent (Promega). Prior to collecting cell lysate, halt protease inhibitor cocktail (Thermo Scientific) was added to the cell culture lysis reagent in order to ensure the stability of proteins. Cell lysates were collected into low-adhesion pre-chilled Eppendorf tubes (USA Scientific) and centrifuged at 13,000 rpm for 5 minutes at 4°C. Supernatant was collected into another set of pre-chilled Eppendorf tubes and the pellet was discarded. Luciferase assay reagent (100 μ L, Promega) was added to 20 μ L of cell lysate and luminescence intensity was measured by Turner TD-20e luminometer. The results were normalized to total protein concentration determined by BCA assay. Briefly, 10 μ L of cell lysate was added to 200 μ L of BCA reagent. Absorbance was measured at 562 nm using a BioTek Synergy 2 microplate reader and normalized to BSA solutions at a concentration range of 125 μ g/mL to 2000 μ g/mL as standards.

Determination of Protein Levels with ELISA

[0137] Cells were plated in 24-well dishes (BD-Falcon) at a density of 35,000 cells/mL. Cells were allowed to attach overnight (~70% confluent) before dosing with the compound. After 24 hours, the old media was replaced with fresh media containing 2% FBS, and HBS 1 at concentrations ranging from 1 μ M to 10 μ M. All samples contained a final concentration of 0.1% DMSO; vehicle samples were treated with cell culture media containing 0.1% DMSO. Cells were incubated for 6 hours at 37°C and 5% CO₂ and hypoxia was induced with DFO (300 μ M), and cells were incubated for another 18 hours. The supernatant was collected and the levels of VEGF were measured with the Human Quantikine VEGF kit (R&D Systems) in accordance with the manufacturer's protocol. Absorbance was measured at 450 nm on a BioTek Synergy 2 microplate reader. The readings were normalized to a total protein concentration. Every experiment was performed in quadruplicate.

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Western Blot Analysis of HIF-1 α Levels

[0138] HeLa cells were plated in a 75 cm² culture flask and allowed to reach 70% confluence. Cells were treated with vehicle or HBS 1 at 10 μ M concentration in the cell culture media containing 10% FBS. All samples contained a final concentration of 0.1% DMSO. Cells were incubated for 6 hours and hypoxia was induced with 300 μ M DFO. After incubation for an additional 18 hours, cells were lysed and cytoplasmic and nuclear extracts were collected using a NE-PER kit (Pierce) according to the manufacturer's protocol and blotted as described in Dubey et al., "Suppression of Tumor Growth by Designed Dimeric Epidithiodiketopiperazine Targeting Hypoxia-Inducible Transcription Factor Complex," *J. Am. Chem. Soc.* 135(11):4537–49 (2013), which is hereby incorporated by reference in its entirety.

Plasma Stability and Biodistribution Studies

[0139] Plasma stability and biodistribution studies were performed in 10-week-old female BALB/c mice (Charles River) with 3 mice per time point. Briefly, HBS 1 or peptide 3 was dissolved in 70 μ L of sterile PBS and administered intravenously at a dose of 1 mg/kg. Then, 1 mL of blood was collected by cardiac puncture at euthanasia at the following time points: 30 min., 1 h., 2 h., 4 h., 6 h., 8 h., 12 h., 16 h., and 24 h. after drug administration. The experiments were performed under an approved IACUC protocol at the University of Southern California.

[0140] Samples were prepared by mixing 30 μ L of plasma with 20 μ L of 50% MeOH and 50% aqueous 1% formic acid. The mixture was vortexed and mixed with an additional 120 μ L of 0.5% formic acid in MeOH/ACN (4:6) and 20 μ L of 2.0 μ g/mL isoproterenol in MeOH/1% aqueous formic acid (1:1) as an internal standard. The mixture was vortexed again for 2 minutes and centrifuged at 13,000 rpm for 4 minutes. Next, 20 μ L of the supernatant was transferred to a new tube and mixed with 180 μ L of 50% MeOH/ACN (4:6) and 50% aqueous 1% formic acid. Standard curves were prepared by mixing the plasma from three untreated mice with 20 μ L of 50% MeOH and 50% aqueous 1% formic acid prepared with HBS 1 or peptide 3 at a concentration range of 0.05–2 μ g/mL. The standard curves, as determined by linear regression, displayed good linearity ($r^2 > 0.98$) over the range tested.

[0141] Samples were analyzed by LC/MS/MS using an Agilent 6210 time-of-flight LC/MS system. HPLC separation was achieved using a Prevail 3u C₁₈ 100 $\times$

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2.1 mm column (Grace Davison, Deerfield, IL, USA). The column temperature was maintained at 20°C. The mobile phase consisted of A (5% acetonitrile and 95% of 0.05% aqueous formic acid) and B (5% of 0.05% aqueous formic acid and 95% acetonitrile). The following gradient program was used: 0% B (0 min, 0.125 ml/min), 100% B (17 min, 0.125 ml/min). The total run time was 35 minutes. The electrospray ionization source of the mass spectrometer was operated in positive ion mode with the capillary voltage set to 4 kV, and the cone and collision cell voltages optimized to 60 and 170 V. The source temperature was 120°C and the desolvation temperature was 300°C. A solvent delay/divert program was used from 0 to 4.0 minutes to minimize the mobile phase to flow to the source. Agilent MassHunter Workstation version B.02.01 software was used for data acquisition and processing.

Gene Expression Profiling

[0142] Experiments were carried out with HeLa cells. The media, time course, DFO, and small molecule treatments were the same as for the qRT-PCR assays. Cultured cells contained vehicle, HBS 1, or HBS 2 at a concentration of 50 µM. RNA was isolated as previously described. Sample preparation and microarray analysis was performed at the Genome Technology Center, New York University School of Medicine. Labeled mRNA was hybridized to Affymetrix Genechip Human Gene 1.0 ST microarrays. Four data sets were collected: normoxic cells with vehicle, hypoxic cells with vehicle, hypoxic cells with HBS 1, and hypoxic cells with HBS 2. Gene expression profiles were analyzed using GeneSpring GX 12.5 software (Agilent). Probe level data have been converted to expression values using a robust multi-array average (RMA) preprocessing procedure on the core probe sets and baseline transformation to median of all samples. A low-level filter removed the lowest 20th percentile of all the intensity values and generated a profile plot of filtered entities. Significance analysis was performed by one-way ANOVA test with Benjamini-Hochberg correction and asymptotic *P*-value computation. Fold change analysis was applied to identify genes with expression ratios above 1.1-fold between treatments and control set (*P* < 0.05). Hierarchical agglomerative clustering was performed using Pearson's centered correlation coefficient and average-linkage as distance and linkage methods. The gene expression profiling data have been

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deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/GEO (accession no. GSE48002).

In Vivo Efficacy Tests of HBS 1 in Mouse Xenograft Tumor Models

[0143] CrTac:NCr-*Foxn1*^{nu} mice (Taconic, Inc.) were used to examine the *in vivo* efficacy of HBS 1. Mice were housed in an A.L.A.C.C. approved barrier facility under the direct supervision of a professional veterinarian. Mice (n=6) were inoculated with 786-O cells (2×10^6 cells) into the right flank and allowed to grow tumors for 21 days. The primary endpoint of efficacy (the rate of increase in tumor volume as compared to control) were evaluated when mice were treated with HBS 1 at 13 mg/kg dissolved in sterile PBS given parenterally on days 4, 7, 11, 25, and 28, a total of 5 injections. In parallel, a control group (n=6) received injections of PBS. Tumor sizes were measured on Days 2, 3, 4, 6, 8, 11, 13, 16, 20, 25, 28, and 33. To address the question of whether tumor growth is affected by treatment with HBS, a comparison of the tumor volumes of the control group and the group treated with HBS 1 was made. At a conclusion of the study, mice were injected intraperitoneally with the near-infrared dye IR-783 contrast agent and the tumors were imaged using Xenogen IVIS 200 small animal imager. Euthanasia was performed as recommended by the American Veterinary Panel (AVMA 202229-249, 1993). The organs and tumors were collected for future histopathology studies.

Example 2 — Design and Synthesis of Stabilized α -Helices.

[0144] HIF-1 α forms a heterodimer with its β subunit, aryl hydrocarbon receptor nuclear translocator (ARNT), to recognize hypoxia response element (HRE) and up-regulate expression of hypoxia-inducible genes, which are important contributors to tumor progression. Pyrrole-imidazole polyamides, which are programmable DNA-binding small molecules, have been shown to regulate transcription of hypoxia-inducible genes by binding to the HRE. Initiation of HIF-mediated transcription also requires complex formation between the CH1 domain of the coactivator protein p300 (or the homologous CREB binding protein, CBP) and the C-TAD₇₈₆₋₈₂₆ of HIF-1 α (Figure 3A). This transcription factor-coactivator interaction represents an alternative target for controlling hypoxia signaling. Structural studies provide a molecular basis for this interaction and identify two short α -helical domains, α A and α B, from HIF-1 α as key determinants for its recognition by p300 (Figure 3C).

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Both α A and α B subdomains of HIF-1 α C-TAD contain residues that contribute significantly to the complex formation, as shown by experimental mutagenesis studies. In earlier work, the α A peptide sequence was stabilized using the hydrogen bond surrogate (HBS) approach, which utilizes a carbon–carbon bond in place of the intramolecular hydrogen bond in α -helices. HBS helices have been shown to disrupt intracellular protein–protein interactions with high affinity and specificity. The α A mimetic was shown to downregulate mRNA levels of *VEGF* and *GLUT1*, two genes under the control of HIF-1 α , while the linear peptide mimic of α A remained inactive. Importantly, the compound did not display significant toxicity as compared to chetomin, a small molecule known to target the same interaction. As described herein, the ability of α B mimics to inhibit the target interaction and control gene expression in cell culture was explored and its efficacy was tested in murine tumor xenograft models.

[0145] A key premise of rational design is that, unlike high throughput screening efforts, a handful of molecules that fit certain criteria need to be designed *de novo*. In an ideal scenario, these predictions would lead to both a potent ligand for the target receptor and a compound serving as a negative control, featuring minor alterations and binding the same protein with reduced affinity. Such a result would confirm the fundamental design principles while allowing the specificity of designed compounds to be evaluated. Accordingly, two stabilized helices based on the wild-type sequence were conceived (Figure 6A), along with the unconstrained control (Figure 6B).

[0146] HBS **1** is a direct mimic of HIF-1 α ₈₁₇₋₈₂₄ with the exception of Leu819, which was changed to an alanine residue to streamline synthetic effort (coupling of an *N*-alkyl alanine to the next residue is more efficient than coupling *N*-alkyl leucine). Computational alanine scanning mutagenesis analysis suggests that Leu819 is not a significant contributor to binding energy as opposed to Leu818, Leu822, Asp823, and Gln824 (see Table 4, *supra*).

[0147] HBS **2** was designed to be a specificity control in which the critical Leu-822 residue is replaced with an alanine; based on computational data, HBS **2** would be expected to bind CH1 with an order of magnitude weaker binding affinity than HBS **1**.

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[0148] Peptide **3** is an unconstrained analog of HBS **1**; allowing the effect of helix stabilization on the activity of the compounds to be evaluated. The HBS helices were synthesized, purified, and characterized by HPLC and circular dichroism spectroscopy, as described above. As shown in Figure 7, The constrained peptides showed characteristic α -helical circular dichroism spectroscopy signatures in aqueous buffers as compared to the unconstrained derivative, which displays no discernible helicity, as expected for a very short peptide.

Example 3 — Designed Ligands Target p300–CH1 in a Predictive Manner.

[0149] The CH1 domain of p300/CBP is stabilized by three zinc ions. Prior NMR structural studies have shown that the purified protein can rapidly aggregate in a buffer with excess or deficiency in Zn^{2+} (Patgiri et al., “A Hydrogen Bond Surrogate Approach for Stabilization of Short Peptide Sequences in Alpha-Helical Conformation,” *Acc. Chem. Res.* 41(10):1289–300 (2008), which is hereby incorporated by reference in its entirety). Attempts to evaluate binding of compounds with this protein have repeatedly resulted in protein aggregation and precipitation, even at low micromolar protein concentrations. The difficulty in working with this protein is directly correlated with its expression protocol, and slight changes in the concentrations of Zn^{2+} in the bacterial growth media, supplemented with ZnSO_4 , could lead to purified protein samples that bind with different binding affinities ($K_d \sim 30 \text{ nM} - 2 \text{ }\mu\text{M}$) to HIF-1 α C-TAD₇₈₆₋₈₂₆. To overcome this variability, ^{15}N labeled protein was prepared and peak dispersion (and protein folding) was monitored by ^1H - ^{15}N HSQC NMR experiments (Figures 8A–D). This ^{15}N -labeled, properly folded protein with the optimal levels of zinc shows a diminished tendency to aggregate and was used for binding assays.

[0150] The affinity of peptides for the ^{15}N -labeled p300 CH1 domain was evaluated using tryptophan fluorescence spectroscopy. The intrinsic fluorescence intensity of Trp403 has been shown to be a sensitive probe for CH1 folding. Significantly, this tryptophan lies in the αB binding pocket of p300/CBP, providing a unique probe for interrogating direct binding of αB mimics (Figure 9). Using this fluorescence method, HBS **1** was calculated to bind to p300-CH1 with a dissociation constant, K_d , of $690 \pm 25 \text{ nM}$ (Figure 5A and Figure 10). For comparison, HIF-1 α C-TAD₇₈₆₋₈₂₆ binds p300-CH1 with a K_d of $38 \pm 0.14 \text{ nM}$ under the same conditions.

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The binding affinity of HIF-1 α C-TAD to CH1 in this assay is consistent with that obtained from a fluorescence polarization assay using fluorescein-labeled HIF-1 α C-TAD (Figure 11 and Figure 12) and those using isothermal titration microcalorimetry. The designed specificity control, HBS 2, targets CH1 with a four-fold weaker binding affinity ($K_d = 2820 \pm 140$ nM), supporting the computational predictions. Peptide 3 is an unconstrained analog of HBS 1 and binds the CH1 domain with a K_d of 6060 ± 320 nM. These results indicate that stabilization of the peptide conformation offers a 9-fold increase in binding affinity.

[0151] To further characterize the interaction of HBS 1 with the CH1 domain, ^1H - ^{15}N HSQC NMR titration experiments were performed with uniformly ^{15}N -labeled CH1. Addition of HBS 1 to 69 μM CH1 in CH1:HBS 1 ratios of 1:1, 1:3, 1:5, and 1:10 resulted in a concentration-dependent shift in the resonances of several CH1 residues (Figure 5B, Figure 13, and Figure 14). Specifically, addition of HBS 1 leads to shifts in the resonances of residues corresponding to the cleft into which the αB helix of HIF binds. This cleft includes Trp403 and chemical shift perturbations observed for this residue support the results of the fluorescence titration experiments. The CH1 domain binds to numerous proteins and has been termed a scaffold for protein folding. Earlier NMR studies have suggested that Zn^{2+} -bound CH1 has a relatively rigid structure, although evidence of plasticity in CH1 has also been discussed. The HSQC titration experiment with HBS 1 described herein supports the view that CH1 has a stable conformation that does not reorganize substantially, at least upon binding of small ligands. Titration of HBS 1 to zinc-bound CH1 led to a relatively large shift in the side chain indole NH of W403 as compared to the backbone amide proton of this residue, suggesting that side chain repacking governs binding of these partners.

Example 4 — HBS 1 Disrupts the HIF-1 α /p300-CH1 Complex in Vitro.

[0152] A fluorescence polarization assay was used to evaluate the ability of HBS 1 to inhibit the binding of fluorescein-labeled HIF-1 α C-TAD_{786–826} domain to p300-CH1. Addition of HBS 1 to the preformed protein complex provided a concentration-dependent decrease in fluorescence polarization with an inhibitory constant, K_i , of 3.5 ± 1.2 μM (Figure 5C). Titration of HBS 2 or peptide 3 did not

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lead to reproducible inhibition of the complex, as expected from their weaker affinity for the CH1 domain.

Example 5 — HBS 1 Downregulates Hypoxia-Inducible Gene Expression and VEGF Protein Levels in Hypoxic Cells.

[0153] Based on the confirmed ability of HBS 1 to bind purified p300-CH1 and disrupt CH1/HIF-1 α C-TAD₇₈₆₋₈₂₆ complex formation, its potential to downregulate the hypoxia-inducible promoter activity was evaluated in a luciferase-based reporter gene system. A construct containing five tandem repeats of the HRE consensus sequence found in the *VEGF* promoter (TACGTGGG (SEQ ID NO: 22)) cloned upstream of the hCMV minimal promoter was used to drive expression of firefly luciferase. This construct was stably transfected into a triple-negative breast cancer (TNBC) cell, MDA-MB-231, that does not express estrogen or progesterone receptors or exhibit HER-2/Neu amplification. The cells were subsequently treated with the peptides. Hypoxia was mimicked by placing cells into a GasPak EZ pouch. Under these conditions, treatment with HBS 1 at a concentration of 50 μ M reduced luciferase expression by 25% (Figure 15). At the same concentrations, specificity control HBS 2 and unconstrained peptide 3 were found to be less effective. Despite the moderate extent of inhibition of the promoter activity, these results are encouraging, because MDA-MB-231 cells are aggressive and under hypoxia conditions exhibit confluence-dependent resistance to some anticancer drugs. The luciferase reporter assays described herein suggest that treatment with HBS 1 results in a statistically significant down-regulation of HIF-1 α -inducible transcription in this cell line.

[0154] To exclude the possibility that the observed down-regulation in the expression of hypoxia-inducible genes was due to a change in the levels of HIF-1 α protein itself, a western blot analysis of HIF-1 α was performed in hypoxic cells treated with HBS 1. HIF-1 α protein was not detectable under normoxia but is strongly induced under hypoxia mimetic conditions. As expected, the levels of induced HIF-1 α protein were unaffected by treatment with HBS 1 (Figure 16).

[0155] The ability of HBS 1 and HBS 2 to inhibit hypoxia-induced transcription of target genes (*VEGFA*, *SLC2A1/GLUT-1*, and *LOX*) was evaluated employing real-time quantitative RT-PCR (qRT-PCR) assays. The data from the qRT-PCR experiments are presented in Figures 17A–D. HBS 1 reduced expression

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levels of *VEGF* by 50% at 10 μ M and greater than 60% at 50 μ M showing marked dose dependence. In contrast, HBS **2** reduced expression levels of this gene by only 10% at 50 μ M and peptide **3** was completely ineffective even at 50 μ M concentration (Figure 17A). Next, it was determined whether this inhibition could be observed for other therapeutically relevant hypoxia-inducible genes. The expression of the *SLC2A1* (*GLUT1*) gene, one of the markers of glycolysis in tumors, and *LOX*, the hypoxia-inducible gene that has been shown to promote metastasis, were examined. In HeLa cells under hypoxia conditions, HBS **1** showed dose-dependent inhibition of *SLC2A1* by 50–60%, comparable to that of *VEGF* gene in the same cell line (Figure 17B). Similarly, HBS **1** significantly downregulated levels of expression of the *LOX* gene in a dose-dependent manner (55% and 70%, respectively, Figure 17C). HBS **2** showed no activity in these assays, while peptide **3** had a reduced activity of 25%. To rule out the possibility that the compounds are only efficacious under DFO mimicked hypoxia, the efficacies of the HBS peptides in downregulating *VEGF* gene expression were compared under two different hypoxia mimetic conditions: DFO and prolonged incubation in an anaerobic pouch. Under both conditions, HBS **1** showed dose-dependent inhibition of *VEGF* expression (Figure 17D). Next, the effect of HBS **1** treatment on the levels of secreted VEGF protein was assessed. An ELISA assay shows that HBS **1** downregulates VEGF protein levels in HeLa cells in a dose-dependent manner (Figure 18).

[0156] HBS **1** is an efficient modulator of contacts between HIF-1 α and p300/CBP. Known inhibitors of this interaction typically function allosterically, by inducing unfolding of p300/CBP through abstraction of zinc ions. This could lead to non-specific abstraction of metal ions from other biomolecules (Block et al., “Direct Inhibition of Hypoxia-Inducible Transcription Factor Complex With Designed Dimeric Epidithiodiketopiperazine,” *J. Am. Chem. Soc.* 131(50):18078–88 (2009), which is hereby incorporated by reference in its entirety). It was predicted that the HIF-1 α mimetics should manifest their function in a more specific manner, and should not be generally cytotoxic. Cell viability assays confirm this hypothesis. It was found that HBS **1** is essentially non-cytotoxic within the entire range of tested concentrations (1 to 100 μ M) (Figure 19). Interestingly, HBS **2** shows higher level of cytotoxicity than HBS **1**, suggesting that this compound may be interacting with a different set of biomolecular targets as seen from gene expression profiling data (*vide*

infra). Thus, HBS 2 may not just be a straightforward lower affinity analog of HBS 1 as designed.

Example 6 — Gene Expression Profiling.

[0157] Proteins p300 and CBP are pleiotropic multi-domain coactivators that directly interact with multiple transcription factors. One potential limitation of the use of coactivator-targeting ligands to control gene expression is that the compounds could lead to inhibition of large numbers of genes that depend on the function of p300 or CBP. Affymetrix Human Gene ST 1.0 arrays containing oligonucleotide sequences representing over 28,000 transcripts were used to evaluate the genome-wide effects of HBS 1 and 2 under hypoxia conditions. Gene expression levels were normalized to DFO-treated cells.

[0158] In hypoxic cells, clustering identified over 5,000 genes that changed in expression levels under one of the specified treatments: DFO, DFO + HBS 1, or DFO + HBS 2 (Figures 20A–C). Treatment with HBS 1 affected the expression of 122 transcripts by at least 1.1-fold ($P < 0.05$), while at the same threshold, control HBS 2 affected expression of 155 transcripts (Figure 20A and Figure 20C) (see Table 5 below). Remarkably, only 33 transcripts were overlapping, indicating that the subtle difference in structure between these two compounds results in a significant difference in genome-wide effects. For comparison, DFO treatment alone affected the expression of 368 transcripts. Clustering analysis was performed to identify similarities in the expression profiles between the different treatments (Figure 20A). The expression profile of cells treated with HBS 1 resembles the profile of cells treated with DFO under the conditions of the analysis and, as mentioned above, is different from the profile of cells treated with HBS 2 despite the structural similarity between the two compounds. As expected, the expression profile of the normoxic cells is significantly different from the other three profiles. Analysis of transcripts affected by both HBS 1 and HBS 2 shows that only 28 and 5 transcripts are commonly down- and up-regulated, respectively, by at least 1.1-fold ($P < 0.05$). It is not surprising that there is some overlap in genes affected by both compounds given the complexity of cellular signaling pathways involved in the hypoxic response. It was found that DFO induced the expression of 45 transcripts by at least 4-fold ($P < 0.05$) (Figure 20B). Within this dataset, multiple genes that belong to the hypoxia-

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inducible pathway were identified. HBS **1** and, to some extent HBS **2**, affected almost all genes in this set.

Table 5. Genes Affected at Least 2-Fold

HBS 1		HBS 2		Control		Gene
Fold Change ^a	Regulation ^b	Fold Change ^c	Regulation ^d	Fold Change ^e	Regulation ^f	
-1.198858	down	1.0873405	up	-2.7937474	down	ACADSB
-1.1193628	down	-1.0069169	down	-5.2134614	down	ADM ^g
-1.0377777	down	1.0465231	up	-2.6769848	down	AK4
1.0583212	up	1.0463357	up	-7.2367773	down	ALDOC
-1.0139477	down	-1.0427985	down	-2.587384	down	ANG/RNASE4
-1.2298646	down	1.1285037	up	-10.575636	down	ANGPTL4 ^h
-1.1789749	down	-1.1638044	down	-4.183105	down	ANKRD37/UFSP2
-1.0263045	down	1.0068687	up	-2.3972414	down	ANKZF1
1.031184	up	1.2810616	up	-2.1560726	down	ARHGAP28
-1.0132513	down	1.0610821	up	-2.1491668	down	ARID5A
-1.129277	down	1.0992572	up	-2.0692933	down	ARNTL
-1.1177615	down	1.0696565	up	-3.0109265	down	ARRDC3
-1.0990012	down	1.1408451	up	2.5837471	up	ASF1A
1.0872076	up	-1.0366824	down	2.6614487	up	ASPM
1.0160675	up	1.1073034	up	2.3719292	up	AURKA
-1.1789101	down	-1.4730334	down	-2.2839973	down	B4GALT4
-1.0872284	down	1.0667108	up	-2.0636718	down	BAMBI
-1.085233	down	1.0502583	up	-5.271643	down	BHLHE40
1.0080966	up	1.1611822	up	-2.6880012	down	BHLHE41
1.0014353	up	1.0378009	up	-4.253393	down	BNIP3
1.0350374	up	1.0081419	up	-5.3825545	down	BNIP3
-1.0603999	down	1.0625899	up	-2.9519002	down	BNIP3L
1.0250388	up	1.2118976	up	2.8752487	up	C14orf126
-1.0156919	down	-1.119514	down	-2.0949163	down	C17orf76
-1.122034	down	1.2555315	up	-2.2554584	down	C18orf19
-1.1636652	down	-1.0204964	down	-6.7090216	down	C1orf161
1.0112627	up	1.055041	up	2.4008303	up	C1orf163
1.0871853	up	1.0450536	up	-2.477235	down	C1QL1

-1.0888706	down	1.094631	up	-2.9811425	down	C3orf58
1.0302335	up	-1.0332097	down	-3.2238207	down	C4orf3
-1.0864575	down	-1.0435097	down	-2.4261425	down	C7orf60
-1.16587	down	-1.019874	down	-5.8869715	down	C7orf68
-1.0614659	down	1.2146437	up	-4.0432177	down	C8orf22
-1.1226574	down	-1.0902045	down	-2.3521466	down	CA12 ^g
-1.108866	down	1.2376684	up	-2.48113	down	CA5B
-1.0285702	down	1.0281031	up	-13.296545	down	CA9 ^g
-1.0158687	down	1.0848932	up	-2.0076687	down	CASZ1
-1.0112811	down	1.0325615	up	-2.894002	down	CASZ1
-1.04192	down	-1.0768336	down	-2.0998538	down	CCDC80
1.0444043	up	-1.1706614	down	3.457119	up	CCNB1
-1.0530787	down	1.0920707	up	-2.177964	down	CCNG2
1.0208882	up	1.2310097	up	2.8682868	up	CDC20
1.0020553	up	-1.0429283	down	-2.8950524	down	CDCP1
1.0366052	up	-1.0903391	down	-2.0103207	down	CDK18
-1.0981873	down	1.118337	up	-2.5513883	down	CDKN1A ^g
1.0392698	up	-1.0248924	down	3.2755635	up	CDKN3
-1.0188439	down	-1.0173886	down	2.3782046	up	CENPA
-1.0183533	down	-1.0308503	down	4.255259	up	CENPE
-1.00388	down	1.118164	up	2.0237246	up	CHAC2
-1.0851383	down	1.1683263	up	-2.276149	down	CNOT8
-1.0972031	down	1.0588787	up	-2.2344368	down	CPOX
1.1451077	up	1.2831794	up	-2.1453977	down	CXCL16
-1.1843526	down	1.1076756	up	-2.5658453	down	CXCR4
-1.0300819	down	1.2587326	up	-2.9216492	down	DAPK1
1.0221276	up	1.0206687	up	2.4073172	up	DDX10
1.0624138	up	-1.0969201	down	2.4726677	up	DEPDC1
1.1574726	up	1.0593747	up	2.51015	up	DIS3L
-1.0776646	down	-1.1640482	down	-2.1677356	down	DKFZp451A211
-1.0658602	down	1.0331173	up	2.4103513	up	DLGAP5
-1.1005502	down	-1.1672455	down	-2.8135295	down	DUSP5

-1.3719523	down	-1.1782583	down	-2.2521324	down	DUSP5P
1.0034794	up	-1.0582042	down	-2.2900689	down	DUSP9
-1.0524148	down	1.2744057	up	2.864903	up	E2F5
-1.0750257	down	-1.0517342	down	-2.33422	down	EDN2 ^g
-1.0114882	down	1.0963054	up	-3.0769978	down	EFNA3 ^h
-1.0063022	down	1.0588189	up	-3.1732266	down	EGLN1 ^g
-1.0551443	down	1.247042	up	-5.1503882	down	EGLN3 ^g
1.0174965	up	1.1876371	up	2.072199	up	ELOVL6
-1.0733106	down	-1.06308	down	-10.603759	down	ENO2 ^g
-1.0835003	down	1.0701011	up	-2.9529157	down	ERO1L
-1.1790224	down	1.2047563	up	-3.7419317	down	ERRFI1
-1.1053089	down	-1.0812296	down	-3.2845836	down	FAM115C
-1.1265318	down	-1.0945897	down	-3.6846316	down	FAM115C/LOC154761
-1.0174642	down	1.0516164	up	2.899864	up	FAM133A
-1.1241008	down	-1.0209429	down	-2.5158167	down	FAM13A
-1.0018321	down	-1.0929846	down	-3.9271286	down	FAM162A
1.0610536	up	-1.0276216	down	3.337046	up	FAM72D/FAM72A/FAM72B/FAM72C
1.0634061	up	-1.0241611	down	3.3964236	up	FAM72D/FAM72A/FAM72B/FAM72C
1.0337092	up	-1.030498	down	3.442782	up	FAM72D/FAM72A/FAM72B/FAM72C
1.0572628	up	-1.0239878	down	3.331725	up	FAM72D/FAM72A/FAM72B/FAM72C
1.0138565	up	-1.1081187	down	2.165065	up	FAM83D
1.043707	up	-1.0308311	down	2.8210485	up	FAM86B1/ALG1/LOC645332/LOC653113
1.068682	up	-1.0393488	down	2.2467046	up	FAM86B1/FAM86B2
1.0729985	up	1.0761985	up	2.617571	up	FAM86B1/FAM86C
1.0354363	up	-1.0027288	down	2.0040247	up	FAM86C
-1.0094354	down	1.3029685	up	2.168605	up	FARSB
1.140963	up	-1.1433238	down	-2.021754	down	FBXO32
-1.0524883	down	1.0493332	up	-2.1420243	down	FBXO42
1.2110548	up	1.0567071	up	2.0648973	up	FERMT1
-1.0951974	down	-1.3137784	down	-2.025113	down	FN1
-1.2603712	down	-1.1467572	down	-2.9039383	down	FOS
1.0239884	up	-1.0724043	down	-2.2796876	down	FOXD1

-1.0264313	down	1.0304923	up	-3.194702	down	FUT11 FLJ44715
-1.038652	down	-1.0402472	down	-2.1154828	down	FXD3
-1.0902228	down	-1.1942844	down	-2.161142	down	FYN
-1.0004762	down	1.0612249	up	2.1665447	up	G2F3
-1.0668463	down	1.0567585	up	-2.9950464	down	GBE1
-1.2743438	down	1.0132078	up	-2.805562	down	GDF15
1.0969177	up	1.0458834	up	2.522929	up	GEMIN5
-1.326227	down	1.1455405	up	-2.8021276	down	GFPT2
-1.2790403	down	-1.3487692	down	-3.023291	down	GOLGA8B GOLGA8A
-1.265511	down	-1.329899	down	-2.905124	down	GOLGA8B GOLGA8A
1.0313923	up	-1.1114029	down	2.2596123	up	GPATCH4
-1.1066624	down	-1.032009	down	-5.079305	down	GPR146
-1.1756448	down	1.1828246	up	-3.0940225	down	GPR155
1.0572137	up	-1.0137892	down	-2.6079721	down	GPR160
-1.0540816	down	1.0293025	up	-2.0090497	down	GPRC5A
-1.0433288	down	1.0330802	up	-2.6747115	down	GPT2
-1.089778	down	1.1071146	up	-2.0776129	down	GTF2IRD2 GTF2IRD2B
-1.0697725	down	1.1319958	up	-2.1862717	down	GTF2IRD2B
-1.0347298	down	-1.0523677	down	-2.2005339	down	GYS1
-1.033671	down	1.2477574	up	3.2375476	up	H1FO
-1.0744232	down	-1.2446554	down	-2.049202	down	HAS2
-1.1388015	down	1.1164491	up	-3.110763	down	HERC3
-1.0328522	down	-1.0706353	down	-2.247471	down	HEY1
-1.0139298	down	-1.1250477	down	2.4303255	up	HIST1H1C
-1.0382714	down	1.2453547	up	2.1910377	up	HIST1H1E
1.1814293	up	-1.0565162	down	4.548209	up	HIST1H2AB
1.028249	up	1.0202644	up	2.5113926	up	HIST1H2AC
1.0760688	up	1.0016093	up	2.7375612	up	HIST1H2AE
1.0772592	up	1.0379401	up	2.6594944	up	HIST1H2AH
1.088042	up	-1.0238472	down	3.1786556	up	HIST1H2AI
1.0760545	up	1.0216632	up	3.3433473	up	HIST1H2AI HIST1H3H
1.0729878	up	1.01679	up	3.328223	up	HIST1H2AK HIST1H2BN

1.0669847	up	1.097534	up	2.094609	up	HIST1H2AL
1.1164097	up	1.0291708	up	2.655007	up	HIST1H2BC
1.0628002	up	1.1290944	up	3.553227	up	HIST1H2BF
1.0641918	up	-1.038236	down	4.369798	up	HIST1H2BG
1.1094822	up	1.0056278	up	3.8479862	up	HIST1H2BH
1.0249968	up	-1.0275244	down	3.34124	up	HIST1H2BI
-1.011945	down	-1.0242423	down	2.3438275	up	HIST1H2BJ
-1.0310844	down	1.5378659	up	2.0198417	up	HIST1H2BK
1.0454845	up	1.0881157	up	2.6407917	up	HIST1H2BK HIST1H2BE H2BFS
1.1658144	up	1.0298574	up	12.502561	up	HIST1H2BM
1.067194	up	1.0149908	up	2.9552643	up	HIST1H3A
1.015362	up	1.0197564	up	2.8443613	up	HIST1H3D HIST1H2AD
1.1092883	up	-1.688728	down	3.1881328	up	HIST1H3F
1.0364317	up	1.0101742	up	2.6978357	up	HIST1H3H
1.1638831	up	-1.3748453	down	3.6814818	up	HIST1H4B
1.0328214	up	-1.316773	down	2.1798692	up	HIST1H4H
1.0930722	up	-1.166608	down	3.6174786	up	HIST1H4J HIST1H4K
1.1102779	up	-1.1824476	down	3.8873343	up	HIST1H4K HIST1H4J
1.0088866	up	1.0189656	up	2.1680818	up	HIST2H2AA3 HIST2H2AA4 HIST2H2AC
1.0085102	up	1.0195583	up	2.1686635	up	HIST2H2AA3 HIST2H2AA4 HIST2H2AC
1.2118632	up	1.1212372	up	2.66082	up	HIST2H2AB
1.0203393	up	-1.1051214	down	2.6113982	up	HIST2H2AC BOLA1
1.0405347	up	1.0846759	up	2.0019317	up	HIST2H2BA HIST2H2BF
1.032434	up	1.0164887	up	2.8465867	up	HIST2H2BE
1.1210366	up	-1.0613894	down	2.0390704	up	HIST2H3D HIST2H3A HIST2H3C
-1.194571	down	1.121211	up	2.1261342	up	HIST2H4A HIST2H4B
-1.1939466	down	1.1216862	up	2.1261613	up	HIST2H4A HIST2H4B
1.0092357	up	1.0444485	up	2.094921	up	HIST3H2A
-1.0752294	down	1.0338196	up	-3.9728498	down	HIVEP2
-1.1303259	down	1.1321235	up	-2.133175	down	HK1 ^g
-1.0533404	down	-1.0264349	down	-5.7125254	down	HK2 ^g
1.1010088	up	1.1335866	up	4.111633	up	HMMR

-1.0138937	down	1.2460188	up	2.145269	up	HORMAD1
-1.0750805	down	-1.0380528	down	-2.1026213	down	HOXD10
1.1196996	up	1.1180418	up	2.7761664	up	HPDL
-1.1325891	down	1.0055424	up	-2.4589784	down	HRH1
-1.1192311	down	1.0154862	up	2.0284126	up	HSPA1A/HSPA1B
-1.1208296	down	1.0085618	up	2.0253496	up	HSPA1A/HSPA1B
-1.1177293	down	-1.0437095	down	2.0822313	up	HSPA1B/HSPA1A
-1.1122378	down	-1.0498594	down	2.0941586	up	HSPA1B/HSPA1A
-1.112368	down	-1.0498853	down	2.0940685	up	HSPA1B/HSPA1A
1.2337483	up	-1.5396951	down	2.4555218	up	ID3
1.1887107	up	-1.1141586	down	-2.5604243	down	IDH2
-1.3824807	down	1.1527516	up	-4.497776	down	IER3
-1.3749976	down	1.1767937	up	-4.707593	down	IER3
-1.3823622	down	1.153014	up	-4.500477	down	IER3
-1.1800597	down	1.0211127	up	-4.437638	down	IGFBP3
-1.121893	down	-1.1431843	down	-2.0187001	down	IGSF3
-1.0634735	down	-1.4005892	down	-2.1043446	down	IL1RAP
-1.3237284	down	1.0446229	up	-5.254703	down	IL2RG
-1.053483	down	-1.0516862	down	-2.0464828	down	ING2
-1.0689135	down	1.3293185	up	-2.1875443	down	INSIG1
-1.2234808	down	1.0493531	up	-7.2295227	down	INSIG2
-1.0548623	down	1.0914948	up	-2.2711992	down	IPMK
-1.104967	down	-1.1261146	down	-5.4967833	down	ITGA5
-1.2723838	down	-1.4516369	down	-4.7319503	down	JUN
-1.0761135	down	-1.0448471	down	-2.08533	down	KAT2B
-1.069219	down	-1.1738038	down	-3.964814	down	KCTD11
-1.0983505	down	-1.0338085	down	-4.5585265	down	KDM3A
1.0428305	up	1.0739981	up	2.0221412	up	KIAA0586
-1.0484446	down	-1.000957	down	-5.1322355	down	KIAA1244
-1.0863793	down	1.0911593	up	-2.0486183	down	KIAA1432
-1.0647811	down	1.1031417	up	-2.3516953	down	KIAA1715
-1.1583545	down	-1.0508852	down	2.1151373	up	KIF14

-1.00198	down	-1.0037445	down	3.4379961	up	KIF20A/CDC23
1.1104406	up	-1.6264613	down	-2.1513863	down	KRT17
1.1725253	up	-1.8421245	down	-2.5044134	down	KRT17
-1.0665327	down	1.024981	up	-7.6152425	down	LOX ^g
-1.0239133	down	-1.0096284	down	-3.1381226	down	LOXL2
-1.0881166	down	-1.1607536	down	-2.4658139	down	LRP1
-1.0412775	down	1.0400462	up	2.0927668	up	LTV1
-1.1852337	down	-1.4142182	down	-2.1554024	down	MAFB
-1.0526593	down	-1.1299944	down	-2.1507175	down	MAFK/TMEM184A
1.0472041	up	-1.0342416	down	2.0437317	up	MAK16/C8orf41
-1.0581415	down	1.0291281	up	-2.0293536	down	MAP2K1/SNAPC5
-1.0998803	down	-1.0539637	down	-2.8863204	down	MAP3K15/PDHA1
-1.1232924	down	1.6498638	up	2.2023304	up	METTL7A
1.0905137	up	1.0289348	up	2.175937	up	MLKL
-1.0716023	down	1.0461466	up	-2.0215554	down	MOBK12A
1.1000898	up	-1.0295926	down	2.5916712	up	MSTO1/MSTO2P
1.1853988	up	-1.0830567	down	2.580263	up	MSTO2P/MSTO1
-1.0930141	down	1.0225625	up	-3.293971	down	MUC1
-1.014464	down	-1.0215149	down	-2.346065	down	MXI1
-1.072508	down	1.1463394	up	-2.0467393	down	NAMPT
-1.0733447	down	1.1515353	up	-2.0046158	down	NAMPT
-1.0171611	down	1.1263885	up	2.024874	up	NARS2
-1.0521922	down	-1.0211507	down	-2.179991	down	NAV1
-1.0180564	down	1.0198303	up	-3.32679	down	NDRG1 ^g
1.0553051	up	1.1875671	up	2.8213596	up	NDUF4F4
1.0536773	up	-1.2928615	down	-2.193419	down	NEBL
-1.0623983	down	-1.1545544	down	-3.262253	down	NFIL3
1.0572549	up	1.0486796	up	2.1284277	up	NLN
-1.3564715	down	1.412258	up	-2.0202577	down	NOG
1.0569912	up	-1.0584995	down	2.6115415	up	NOL6
1.0345374	up	-1.1083401	down	2.9392853	up	NOP16
-1.0246124	down	-1.0321362	down	2.013658	up	NOP2

-1.142908	down	-1.1094075	down	-2.1712103	down	NOTCH3
-1.0493118	down	1.0916766	up	-2.094681	down	NRG4
1.0453973	up	1.0413948	up	-2.9922984	down	ORAI3
-1.2377601	down	-1.0565416	down	-3.1627083	down	OSMR
-1.3154866	down	-1.2208521	down	-2.8162463	down	OTUD1
-1.0067146	down	1.1961997	up	-4.525767	down	P4HA1
-1.0518332	down	-1.0594456	down	-4.467532	down	P4HA2
-1.0599507	down	-1.1152831	down	-2.7483146	down	PAG1
-1.0068985	down	-1.0078048	down	-2.005217	down	PAIP2B
-1.0382358	down	1.0521878	up	-4.557052	down	PDK1
1.0191542	up	1.1393752	up	-3.8439698	down	PDK3
-1.085743	down	1.1143924	up	-2.1830468	down	PER1
-1.0596828	down	-1.0105202	down	-2.0410588	down	PER2
-1.072292	down	-1.0640502	down	-9.719393	down	PFKFB4 ^g
-1.0348089	down	1.0235314	up	-3.3854454	down	PFKP
-1.1552294	down	1.008007	up	-2.0849102	down	PGM2L1
-1.0775337	down	1.1154193	up	-2.0587978	down	PIAS2
-1.0306145	down	1.111947	up	2.663589	up	PLA2G4A
-1.1131518	down	-1.1262151	down	-3.160247	down	PLAGL1 HYMAI
-1.1653019	down	1.0859076	up	-2.9282918	down	PLIN2
-1.0407479	down	1.018729	up	3.1841922	up	PLK1
-1.0310947	down	-1.0200943	down	-2.051884	down	PLOD1
-1.0564666	down	1.0334756	up	-2.6392682	down	PLOD2
-1.0536163	down	-1.0217751	down	-2.9487426	down	PMEPA1
-1.0364561	down	1.1446192	up	2.142106	up	PNO1
1.0623838	up	1.1337379	up	2.3235378	up	POLR1B
-1.1132654	down	-1.2193453	down	-5.72456	down	PPFIA4 LOC100507405
-1.0076138	down	-1.0337875	down	-2.3916671	down	PPL
-1.0528351	down	-1.0763819	down	-2.0917118	down	PPP1R3B
1.0514251	up	-1.0709876	down	-2.185088	down	PPP1R3C
-1.0886943	down	-1.0989375	down	-4.635631	down	PPP2R5B
1.0562048	up	1.0604632	up	2.006964	up	PPRC1

-1.1171283	down	-1.0515006	down	-2.7193942	down	PRELID2
1.0199716	up	-1.006938	down	2.2714365	up	PRMT3
-1.493969	down	1.176382	up	-3.8572378	down	PTGS2 ^h
1.0979699	up	-1.2585038	down	2.1313524	up	PTTG1
-1.084285	down	-1.1066813	down	-2.243677	down	PYGL
1.0216292	up	1.0164917	up	-2.9913483	down	QSOX1/FLJ23867
-1.0165472	down	1.0808368	up	-2.4004233	down	RAB20
-1.0168173	down	-1.0137872	down	-2.0478325	down	RAB40C
-1.091034	down	1.0204805	up	-2.3124177	down	RAB8B
1.0475962	up	1.1967145	up	-3.5479445	down	RASSF2
-1.0217447	down	1.0673434	up	-3.8055146	down	RCOR2
-1.0315185	down	-1.0285182	down	-2.5645835	down	RIOK3
-1.057626	down	1.006583	up	-2.01166	down	RIT1
-1.1474804	down	1.0842069	up	-2.3212476	down	RLF
1.0111393	up	-1.0896454	down	-3.0329592	down	RNF122
-1.0728997	down	1.109433	up	-2.6993163	down	RNF24
1.0031627	up	-2.735376	down	-1.2172973	down	RNU4-2
-1.1661395	down	1.0366671	up	-6.9026365	down	RORA
-1.0086578	down	-1.1610154	down	-4.0498514	down	RRAGD
1.080574	up	1.0721519	up	2.8641217	up	RRS1
1.0885082	up	-1.0089406	down	2.097932	up	RUVBL1
-1.1617795	down	-2.3124695	down	-1.2749236	down	SCARNA5
-1.153035	down	-2.4092975	down	-1.2401854	down	SCARNA6
1.1310145	up	1.0209829	up	2.0414371	up	SCFD2
-1.0548553	down	1.0334789	up	-2.9133189	down	SEC14L4
-1.0628065	down	-1.0827417	down	-2.5861993	down	SEC61G
-1.112961	down	-1.0562894	down	-2.7914515	down	SERPINE1 ^g
-1.163552	down	-1.2216003	down	-2.6976469	down	SERPINI1
-1.0688736	down	1.0619785	up	-2.3279095	down	SERTAD2
1.0343328	up	1.1705835	up	2.0703044	up	SLC27A2
-1.1072197	down	1.1236045	up	-2.8005152	down	SLC2A1 ^g
-1.0290065	down	1.053982	up	-4.183235	down	SLC2A3

-1.248505	down	1.2203912	up	-2.266289	down	SLC6A6
-1.0604588	down	1.0595843	up	-2.7195609	down	SLC6A8 SLC6A10P
-1.0662978	down	1.0684845	up	-2.736111	down	SLC6A8 SLC6A10P
-1.0853571	down	1.2627056	up	2.0000556	up	SLC7A11
-1.2457515	down	-1.0702848	down	-5.947674	down	SLC01B3 LST-3TM12
-1.2625779	down	1.252622	up	-2.0586894	down	SLC04A1
-1.1349522	down	-2.5911605	down	-1.056101	down	SNORA1
1.0827361	up	-1.1856244	down	2.0193295	up	SNORA13
-1.1392936	down	-2.5419006	down	-1.22043	down	SNORA2A
-1.1776297	down	-2.339463	down	-1.0959209	down	SNORA42
-1.0272101	down	-2.0400264	down	1.1371485	up	SNORA6
-1.5449073	down	-2.4887464	down	-1.3515595	down	SNORA60
1.0574348	up	-2.9677162	down	1.0178032	up	SNORA62 RP5A
1.0441421	up	-2.8423023	down	1.2338772	up	SNORA74A
-1.2188872	down	-3.439569	down	1.0985091	up	SNORA75
1.1857955	up	-2.4932704	down	1.1226008	up	SNORD14E
-1.1500319	down	-1.2012677	down	2.0295184	up	SNORD1A
1.1119288	up	-2.5965233	down	-1.0552726	down	SNORD53
1.0002517	up	-2.2845662	down	-1.0211544	down	SNORD94
-1.0808043	down	-1.0452999	down	-2.7743483	down	SNX33
1.0183113	up	-1.0911404	down	-4.066029	down	SPAG4
-1.1739895	down	-1.00757	down	-2.1362014	down	SPICE1
1.0472541	up	-1.1564113	down	3.817899	up	SPINK5
1.0318233	up	-1.0508822	down	-2.477626	down	SPRY1
-1.1322684	down	-1.0328805	down	-2.0766609	down	STAMBPL1
-1.0746213	down	1.0388275	up	-4.0140605	down	STC2
-1.081674	down	1.0557284	up	-2.0054183	down	SYT7
1.1072824	up	-1.0533509	down	2.320572	up	TAF9B
1.1074346	up	-1.0522659	down	2.320647	up	TAF9B
1.0181974	up	1.1829114	up	2.2189808	up	TBC1D30
-1.1135601	down	1.2469167	up	-3.272321	down	TCF11L2
-1.0710702	down	1.0781746	up	-2.2363102	down	TET2

-1.2701089	down	-1.03623	down	-2.419262	down	TGFB1 ^g
1.0019436	up	-1.1744674	down	2.2158334	up	TMC07
-1.0518074	down	1.2325256	up	-7.3965917	down	TMEM45A
-1.1878997	down	1.1371874	up	-2.9050286	down	TMEM45B
-1.1920087	down	1.2894329	up	-2.3936403	down	TMOD1/TSTD2
1.0234529	up	-1.0668982	down	-2.1390693	down	TMPRSS3
-1.0931611	down	1.0688102	up	-2.3202991	down	TNFRSF10D
1.0020133	up	-1.1098602	down	2.4919987	up	TRIM59
-1.0013391	down	-1.1124156	down	2.0286796	up	TROAP
1.0721477	up	1.1957371	up	2.2586193	up	TSEN2
-1.0218694	down	1.0339891	up	-3.1548517	down	TTYH3
-1.0063764	down	-1.0644561	down	2.0020242	up	TWISTNB
-1.1116943	down	-1.1339258	down	-2.000525	down	UACA
-1.1660498	down	-1.0305872	down	-2.3649898	down	UBASH3B
-1.0881262	down	1.0408965	up	-2.4548569	down	UPRT
-1.0898017	down	1.0274819	up	2.0241222	up	UTP15
-1.038064	down	1.0192441	up	2.0523014	up	UTP20
-1.0912127	down	1.030388	up	-2.157118	down	VEGFA ^g
-1.0415335	down	-1.0828103	down	-6.05876	down	VLDLR
-1.0228918	down	1.0196353	up	-2.2292318	down	VLDLR/FLJ35024
-1.1679007	down	-2.2512941	down	1.8234171	up	VTRNA1-1
1.0775124	up	-1.0424249	down	2.056909	up	WDR12
1.183166	up	1.0591956	up	2.5667205	up	WDR3
-1.0831884	down	-1.1266437	down	2.3755276	up	WDR35
-1.0992746	down	1.0805327	up	-2.1783705	down	WDR45L
-1.2116722	down	1.0208603	up	-2.6231897	down	WDR52
-1.2681911	down	-1.0793633	down	-2.2754548	down	WDR52
-1.1009644	down	1.0549855	up	-3.7621908	down	WSB1
1.0491763	up	1.0669556	up	2.080929	up	XK
-1.0340406	down	1.0571353	up	-2.1956234	down	YEATS2
-1.1708703	down	-1.0094752	down	-2.1953986	down	ZDBF2
-1.0179691	down	-1.0534668	down	-2.1707454	down	ZNF160

-1.196344	down	1.0196155	up	-3.2295642	down	ZNF292
1.0262027	up	-1.0581598	down	-2.5864198	down	ZNF395 FBXO16
-1.1907156	down	1.0249686	up	-3.4930103	down	ZNF654 CGGBP1
-1.0689389	down	1.0449277	up	-2.3140717	down	ZSWIM5
1.1991837	up	-1.0838475	down	2.5001824	up	
1.1989849	up	-1.0842861	down	2.501547	up	
-1.0060402	down	-3.5317602	down	1.2592025	up	
-1.0061597	down	-2.4166691	down	1.3725677	up	
1.0993127	up	-2.1863458	down	1.1684631	up	
-1.2011855	down	1.0950639	up	-2.8855257	down	
-1.1395597	down	-2.5428736	down	1.2404431	up	
1.0368	up	-2.245498	down	-1.1451715	down	
1.0456542	up	-2.5189538	down	1.2004068	up	
1.0580661	up	-1.1550292	down	2.0199893	up	
-1.306305	down	-1.7012253	down	-2.5854821	down	
1.0125278	up	-2.0730047	down	1.3469207	up	
-1.0938246	down	-1.0463859	down	-2.0193262	down	
-1.0510107	down	-1.1346707	down	2.074057	up	
1.1218168	up	1.1611335	up	2.0924993	up	
-1.0498437	down	-2.583383	down	1.0472459	up	

^a [HBS1] vs [Induced]^b [HBS 1] vs [Induced]^c [HBS2] vs [Induced]^d [HBS2] vs [Induced]^e [Vehicle] vs [Induced]^f [Vehicle] vs [Induced]^g Hypoxia inducible^h Pro-angiogenic

Example 7 — Antitumor Activity of HBS 1 in Mouse Xenograft Models.

[0159] A mouse xenograft tumor model was used to assess the in vivo efficacy of HBS 1. The relative plasma stabilities of HBS 1 and linear peptide 3 in mice were first measured. In this experiment, female BALB/c mice were injected with either HBS 1 or peptide 3 at a dose of 1 mg/kg and sacrificed at various time points. Blood was collected and the plasma concentration profiles for HBS 1 and peptide 3 were determined, as shown in Figure 21. While both compounds exhibited a bi-exponential pattern of decay, HBS 1 was retained in plasma at much higher concentrations as compared to peptide 3 during the same time intervals, suggesting that the internally constrained structure of HBS 1 favorably impacts its serum stability. This observation is consistent with the fact that proteases largely bind and cleave peptides in extended conformations. The plasma stability of HBS 1 is also consistent with the published stability of hydrocarbon-bridged helices.

[0160] The CrTac:NCr-*Foxn1*^{nu} mouse (Taconic, Inc.) was used for efficacy studies. 786-O renal cell carcinoma of the clear cell type (RCC) cell line was selected, because of its high HIF levels due to a mutation in the *VHL* gene. Measurable tumors (~100 mm³) grew in as little as 2–3 weeks after the inoculation of 2×10^6 cells into the flank of the mice. Mice were then separated into the two experimental groups and one group was treated with HBS 1, whereas the second group was not treated (control). 13 mg/kg was estimated to be an acceptable dose, based on the concentration of HBS 1 required for >50% *VEGF* and *LOX* mRNA downregulation in cell culture and plasma concentrations of the compounds (*vide supra*). Tumor sizes were measured in accordance with literature recommendations. Throughout the course of the treatment and at the experiment endpoint, mice treated with HBS 1 had smaller tumors with median tumor volume reduction of 53% as compared to the mice from the control group (Figure 22A). Both control group and mice treated with HBS 1 under this regimen showed no signs of distress or weight loss (Figure 22B). To rule out the possibility that treatment resulted in a reduction of the size of the main tumor but concurrently resulted in an elevated rate of metastasis, as reported with some anti-VEGF therapeutics, the animals were injected with IR-783, a near-infrared contrast agent that targets tumors, circulating tumor cells, and metastases, and imaged from both sides using a small animal imager. The images

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show no detectable NIR signal within the lymph nodes, brain, or other organs and a significantly reduced signal from the main tumor (Figure 22C).

Discussion of Examples 1–7.

[0161] Synthetic inhibitors that block the transactivation domains of transcription factors from contacting their cognate coactivators and programmable small molecules that sequence-specifically inhibit DNA–transcription factor interactions provide powerful strategies for regulating gene expression. This can be especially attractive in targeting cellular pathways that promote oncogenic transformation and typically involve a large number of signaling proteins that ultimately converge on a much smaller set of oncogenic transcription factors. Given the fact that both CBP and p300 regulate multiple signaling pathways, they provide an intriguing opportunity for an effective modulation of the expression of genes involved in cancer progression and metastasis (Vo & Goodman, “CREB-Binding Protein and p300 in Transcriptional Regulation,” *J. Biol. Chem.* 276(17):13505–08 (2001), which is hereby incorporated by reference in its entirety). The design strategy described herein involves judicious mimicry of transcription factor fragments that contact p300/CBP to rationally develop artificial regulators of transcription.

[0162] The present results indicate that synthetic helices that mimic protein subdomains bind their p300/CBP target with high affinity and disrupt the HIF-1 α C-TAD-p300/CBP complex *in vitro*. Importantly, the designed compounds bound the target protein in a predictable manner; the single residue mutant HBS **2** shows an expected weaker affinity for CH1 as compared to HBS **1**. The CH1 binding site for HBS **1** was confirmed by NMR HSQC titration experiments. As anticipated based on fluorescence experiments, HBS **1** causes a concentration dependent chemical perturbation shift for the side chain ϵ -NH of Trp-403. This result supports the design principle that a locked helix can occupy the binding clefts of individual protein α -helices. The *in vitro* assays showed significant reduction in promoter activity and effective downregulation of the expression of HIF-1 α inducible genes responsible for promoting angiogenesis, invasion, and glycolysis. In addition, the HBS **1**-mediated transcriptional blockade of *VEGF* correlates with decreased levels of its secreted protein product, suggesting that compensatory cellular stress response mechanisms such as internal ribosome entry sites (IRES) or mechanisms enhancing protein

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translation do not affect the observed downregulation in expression. Therefore, reducing the cellular mRNA levels of HIF-1 α target genes with HBS 1 could be an effective means of attenuating hypoxia-inducible signaling in tumors.

[0163] Comparative analysis of the genome-wide effects of HBS 1 and HBS 2 provided additional insights into the ability of the compounds to disrupt transcriptional activity of hypoxia-inducible genes. Despite the similarity in structures, these compounds have a very different impact on the level of expression of hypoxia-inducible genes and show distinct genome-wide effects. Treatment with HBS 1 affects 122 genes (less than 0.5% of the entire transcriptome) at a fixed 1.1-fold threshold, with 92 hypoxia-inducible genes being downregulated. Despite the fact that HBS 2 has a similar genome-wide impact at the same threshold, it does not affect a majority of hypoxia-inducible genes. Because many biological responses are threshold-based, the observed decrease in transcriptional activity of primary hypoxia-inducible genes could have pronounced downstream effects on the levels of protein products of hypoxia-inducible transcription.

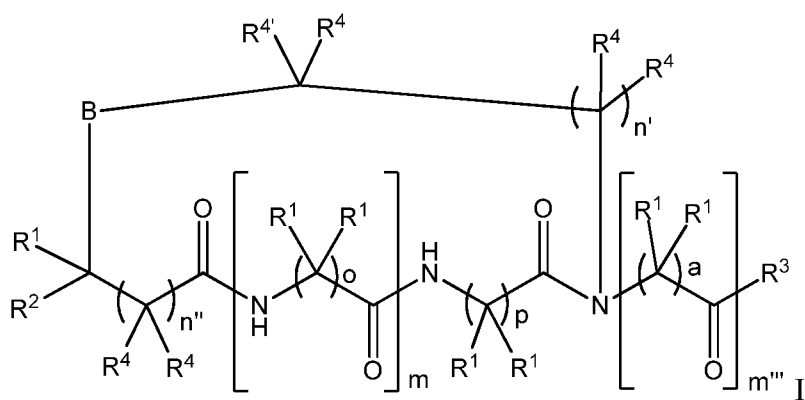
[0164] To assess the in vivo potential of HBS 1, murine tumor xenografts derived from the renal cell carcinoma of the clear cell type (RCC) were treated with the compound. After five injections of HBS 1, the median tumor volume was reduced by 53% in the treated group. Importantly, the HBS 1 treatment did not cause measurable changes in animal body weight or other signs of toxicity in tumor-bearing animals, nor increase the metastasis rate.

[0165] Taken together, the results reported herein support the hypothesis that designed protein domain mimetics can provide valuable tools for probing the mechanisms of transcription. Because the p300/CBP pleiotropic coactivator system interacts with diverse transcription factors, it represents an excellent model system to assess the specificity of designed synthetic ligands in gene regulation. The strategy described herein provides a foundation for the development of novel genomic tools and transcription-based therapies.

[0166] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

WHAT IS CLAIMED:

1. A peptidomimetic, wherein the peptidomimetic:
- (i) mimics a helix having the formula $X_1-X_2-X_2-X_3-X_2-X_2-X_1-X_4-X_5$, wherein each X_1 is any negatively charged residue, each X_2 is any hydrophobic residue, X_3 is any positively-charged residue, X_4 is any polar residue, and X_5 is absent or any hydrophobic residue; and
- (ii) is selected from the group consisting of:
- (a) a compound of Formula I:



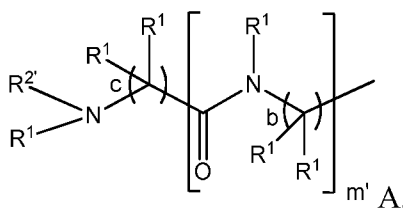
wherein:

B is $C(R^1)_2$, O, S, or NR^1 ;

each R^1 is independently hydrogen, an amino acid side chain, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

R^2 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula A:

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wherein:

$R^{2'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

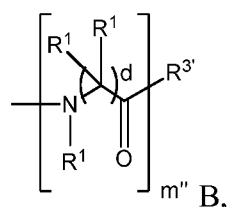
m' is zero or any number;

each b is independently one or two; and

c is one or two;

R^3 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula B:

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wherein:

$\text{R}^{3'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-\text{OR}^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or $-\text{N}(\text{R}^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m'' is zero or any number; and

each d is independently one or two;

each R^4 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

$\text{R}^{4'}$ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, or a double bond between $\text{C}(\text{R}^{4'}, \text{R}^4)$ and B;

a is one or two;

m , n' , and n'' are each independently zero, one, two, three, or four;

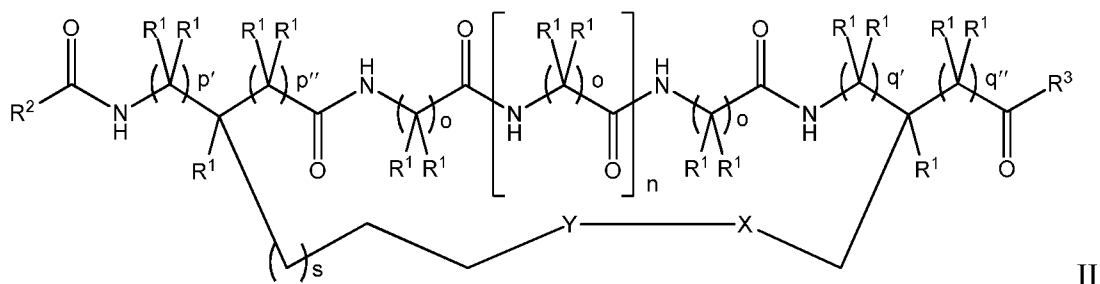
m''' is zero or one;

each o is independently one or two; and

p is one or two;

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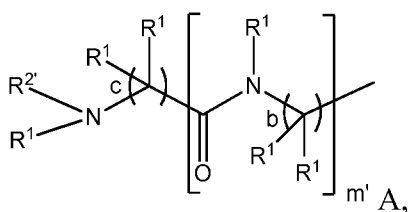
(b) a compound of Formula II:



wherein:

each R^1 is independently hydrogen, an amino acid side chain, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

R^2 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-(CH_2)_{0-1}N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula A:



wherein:

$R^{2'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; $-OR^5$ wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an

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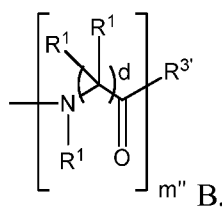
aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or – $(\text{CH}_2)_{0-1}\text{N}(\text{R}^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m' is zero or any number;

each b is independently one or two; and

c is one or two;

R^3 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; – OR^5 wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-\text{N}(\text{R}^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula B:



wherein:

$\text{R}^{3'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; – OR^5 wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an

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aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or – $N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m'' is zero or any number; and

each d is independently one or two;

n is one or four;

each o is independently one or two;

one of p' and p'' is zero and the other is zero or one;

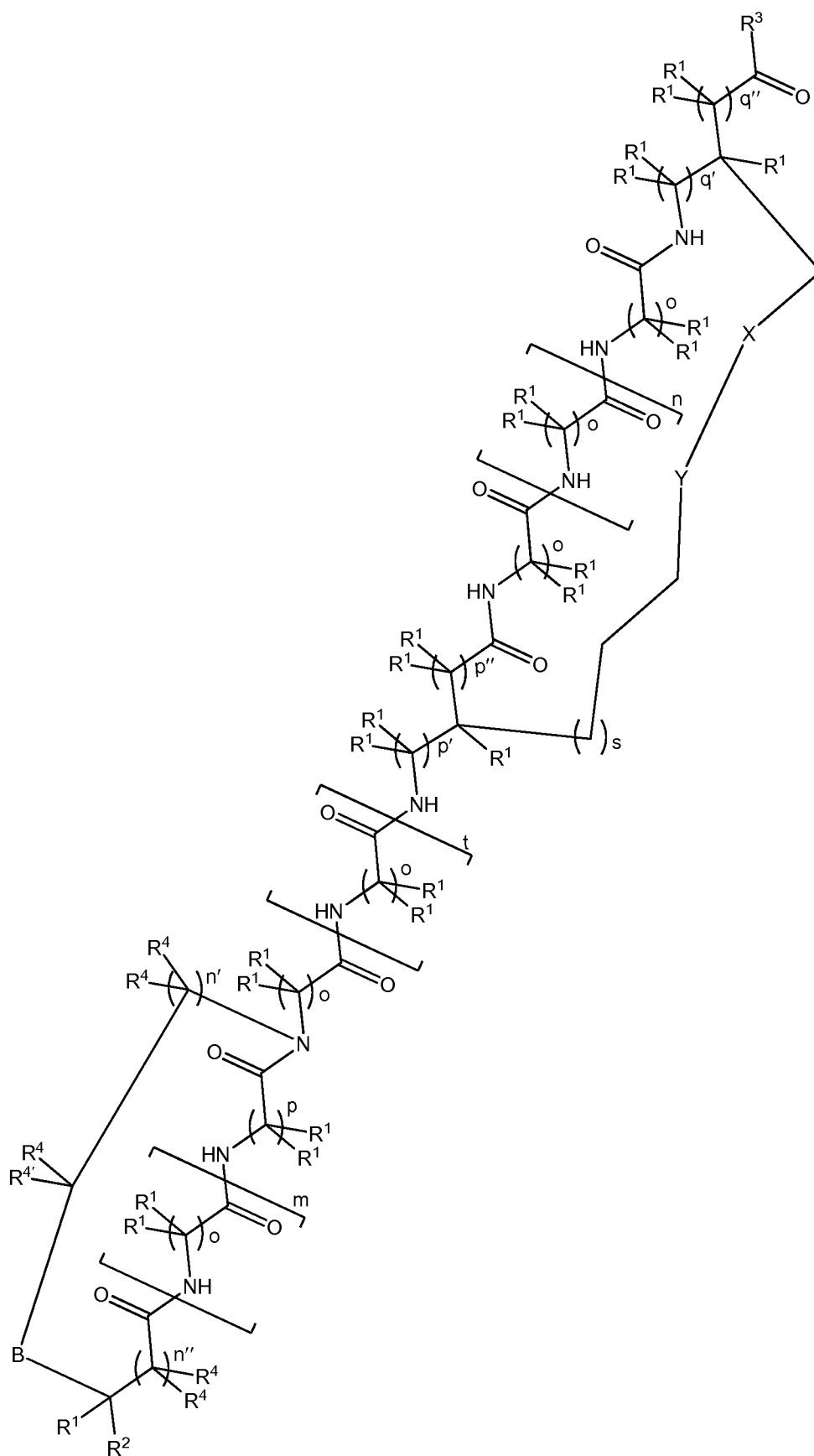
one of q' and q'' is zero and the other is zero or one;

s is one, two, three, four, or five; and

$Y-X$ is a hydrocarbon, an amide bond, an alkane, an alkene, an alkyne, a triazole, or a disulfide bond; and

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(c) a compound of Formula III:



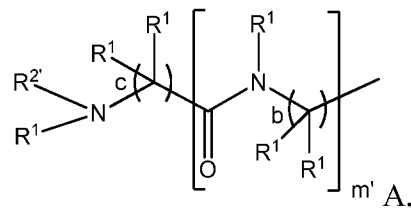
III

wherein:

B is $C(R^1)_2$, O, S, or NR^1 ;

each R¹ is independently hydrogen, an amino acid side chain, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

R^2 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; —OR⁵ wherein R⁵ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; —(CH₂)₀₋₁N(R⁵)₂ wherein each R⁵ is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula A:



wherein:

R^{2'} is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a heterocyclcyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; -OR⁵ wherein R⁵ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclcyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or - (CH₂)₀₋₁N(R⁵)₂ wherein each R⁵ is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclcyl, an aryl, a heteroaryl, an

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arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m' is zero or any number;

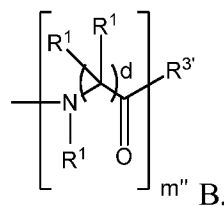
each b is independently one or two; and

c is one or two;

R^3 is hydrogen; an alkyl; an alkenyl; an alkynyl; a cycloalkyl; a

heterocyclyl; an aryl; a heteroaryl; an arylalkyl; an alpha amino acid; a beta amino acid; a peptide; a targeting moiety; a tag; –

OR^5 wherein R^5 is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; $-N(R^5)_2$ wherein each R^5 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag; or a moiety of Formula B:



wherein:

$R^{3'}$ is hydrogen; an alkyl; an alkenyl; an alkynyl;

a cycloalkyl; a heterocyclyl; an aryl; a

heteroaryl; an arylalkyl; an alpha amino

acid; a beta amino acid; a peptide; a

targeting moiety; a tag; $-OR^5$ wherein R^5

is hydrogen, an alkyl, an alkenyl, an

alkynyl, a cycloalkyl, a heterocyclyl, an

aryl, a heteroaryl, an arylalkyl, an acyl, a

peptide, a targeting moiety, or a tag; or –

$N(R^5)_2$ wherein each R^5 is independently

hydrogen, an alkyl, an alkenyl, an

alkynyl, a cycloalkyl, a heterocyclyl, an

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aryl, a heteroaryl, an arylalkyl, an acyl, a peptide, a targeting moiety, or a tag;

m'' is zero or any number; and

each d is independently one or two;

each R^4 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl;

$R^{4'}$ is hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, an arylalkyl, or a double bond between $C(R^{4'}, R^4)$ and B;

m , n' , and n'' are each independently zero, one, two, three, or four;

n is one or four;

each o is independently one or two;

p is one or two;

one of p' and p'' is zero and the other is zero or one;

one of q' and q'' is zero and the other is zero or one;

s is one, two, three, four, or five; and

$Y-X$ is a hydrocarbon, an amide bond, an alkane, an alkene, an alkyne, a triazole, or a disulfide bond.

2. The peptidomimetic according to claim 1, wherein the peptidomimetic mimics a helix having the formula selected from the group consisting of $X_1-X_2-X_2-X_3-X_2-X_2-X_1-X_4-X_5$, $X_1-\mathbf{x}_2-X_2-X_3-X_2-X_2-X_1-X_4-\mathbf{x}_5$, $X_1-X_2-L-X_3-X_2-L-X_1-X_4-X_5$, $X_1-X_2-L-X_3-X_2-L-D-X_4-X_5$, $X_1-X_2-L-X_3-X_2-L-X_1-Q-X_5$, $X_1-X_2-L-X_3-X_2-L-D-Q-X_5$, and XELA*RALDQ, wherein residues in lower case bold are beta residues, X is 4-pentenoic acid, and A^* is *N*-allylalanine.

3. The peptidomimetic according to claim 1, wherein the peptidomimetic is a compound of Formula I.

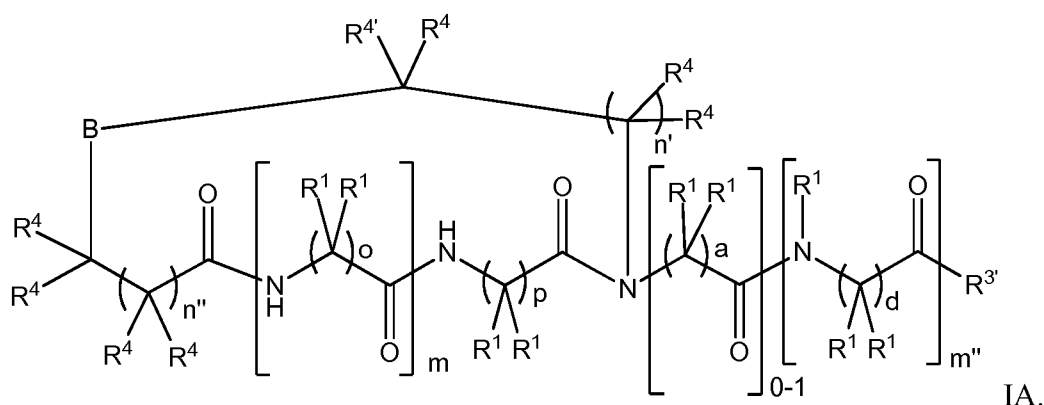
4. The peptidomimetic according to claim 3, wherein B is $C(R^1)_2$.

5. The peptidomimetic according to claim 3, wherein B is O.

6. The peptidomimetic according to claim 3, wherein B is S.

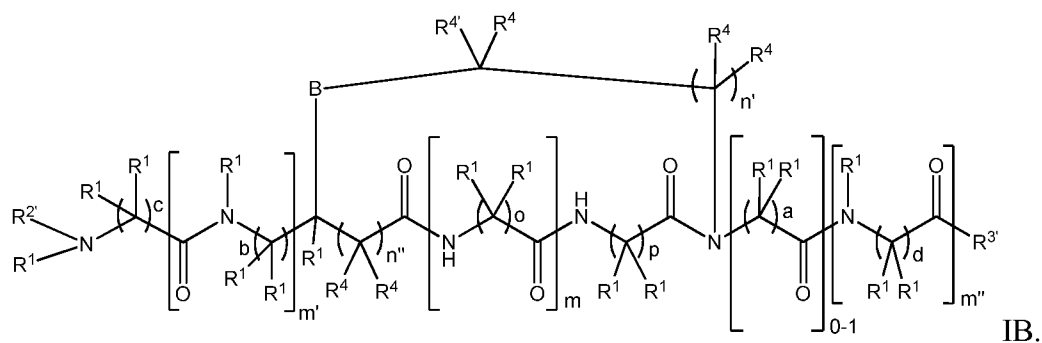
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7. The peptidomimetic according to claim 3, wherein B is NR^1 .
8. The peptidomimetic according to claim 3, wherein there are 9 to 12 atoms in the macrocycle portion of the compound.
9. The peptidomimetic according to claim 8, wherein there are 11 atoms in the macrocycle portion of the compound.
10. The peptidomimetic according to claim 3, wherein there are 12 to 15 atoms in the macrocycle portion of the compound.
11. The peptidomimetic according to claim 10, wherein there are 14 atoms in the macrocycle portion of the compound.
12. The peptidomimetic according to claim 3, wherein there are 15 to 18 atoms in the macrocycle portion of the compound.
13. The peptidomimetic according to claim 12, wherein there are 17 atoms in the macrocycle portion of the compound.
14. The peptidomimetic according to claim 3, wherein there are 20 to 24 atoms in the macrocycle portion of the compound.
15. The peptidomimetic according to claim 14, wherein there are 22 atoms in the macrocycle portion of the compound.
16. The peptidomimetic according to claim 3, wherein the peptidomimetic is a compound of Formula IA:

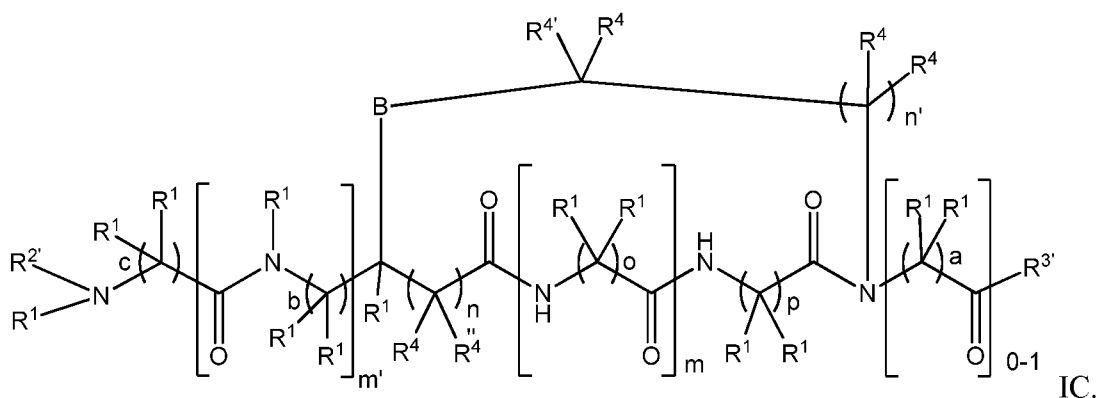


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17. The peptidomimetic according to claim 3, wherein the peptidomimetic is a compound of Formula IB:

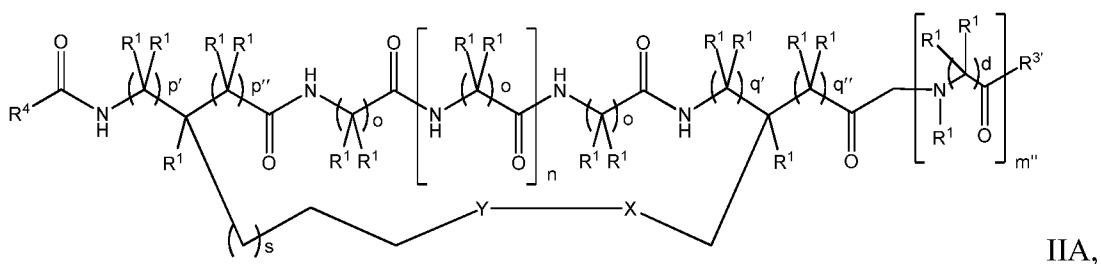


18. The peptidomimetic according to claim 3, wherein the peptidomimetic is a compound of Formula IC:



19. The peptidomimetic according to claim 1, wherein the peptidomimetic is a compound of Formula II.

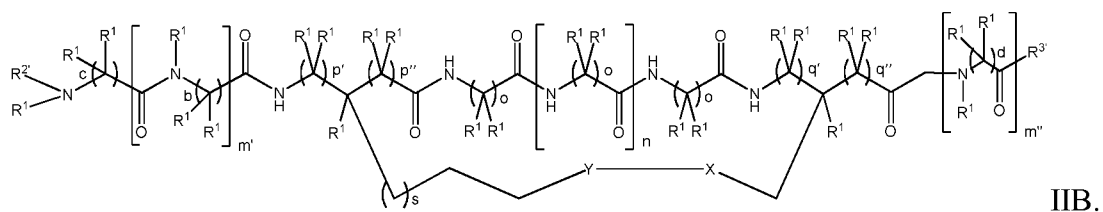
20. The peptidomimetic according to claim 19, wherein the peptidomimetic is a compound of Formula IIA:



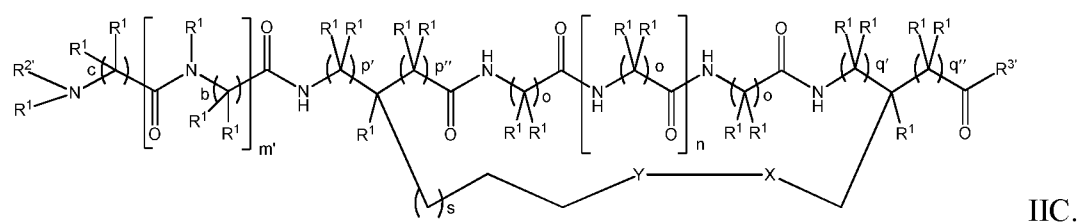
wherein R^4 is independently hydrogen, an alkyl, an alkenyl, an alkynyl, a cycloalkyl, a heterocyclyl, an aryl, a heteroaryl, or an arylalkyl.

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21. The peptidomimetic according to claim 19, wherein the peptidomimetic is a compound of Formula IIB:



22. The peptidomimetic according to claim 19, wherein the peptidomimetic is a compound of Formula IIC:

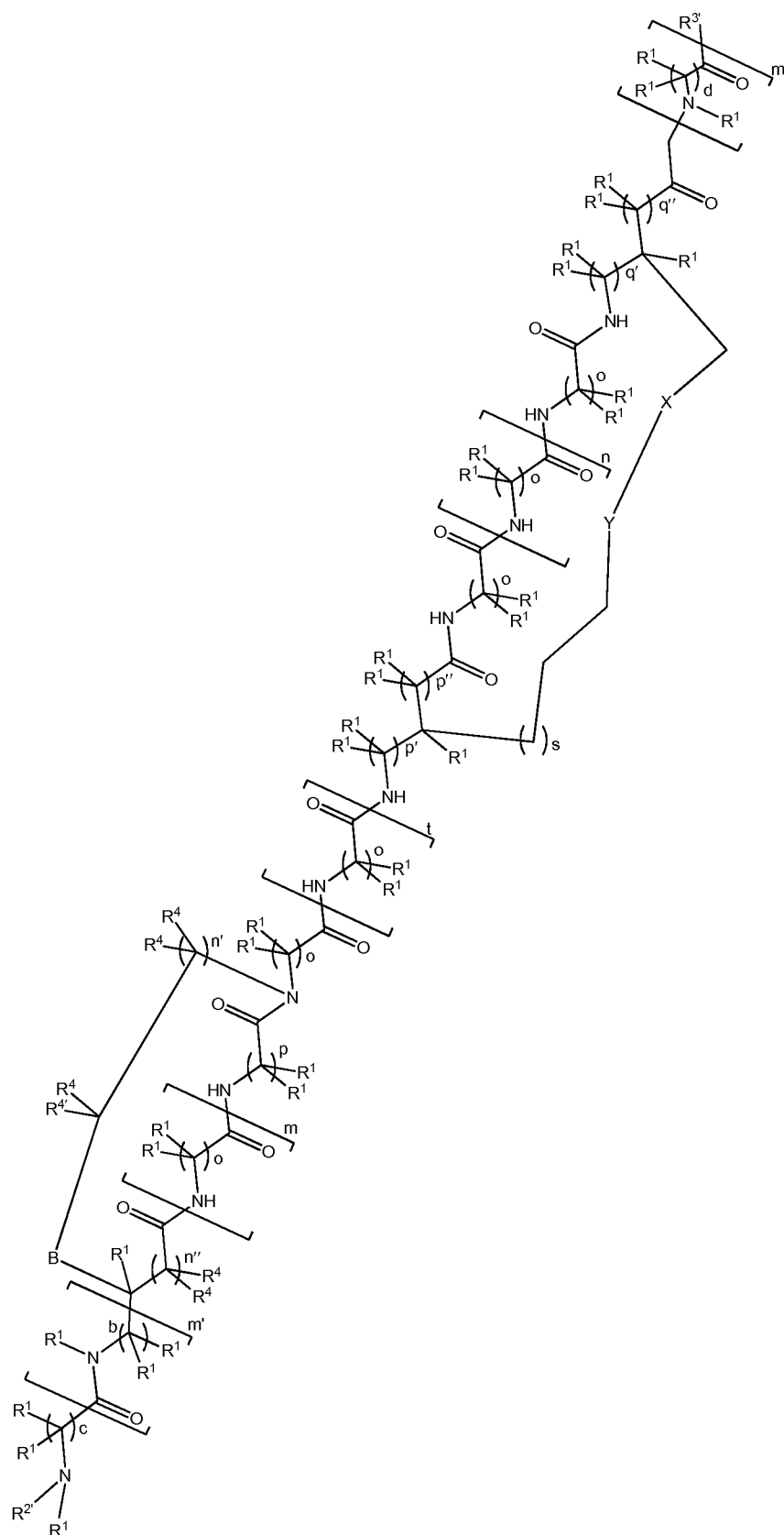


23. The peptidomimetic according to claim 1, wherein the peptidomimetic is a compound of Formula III.



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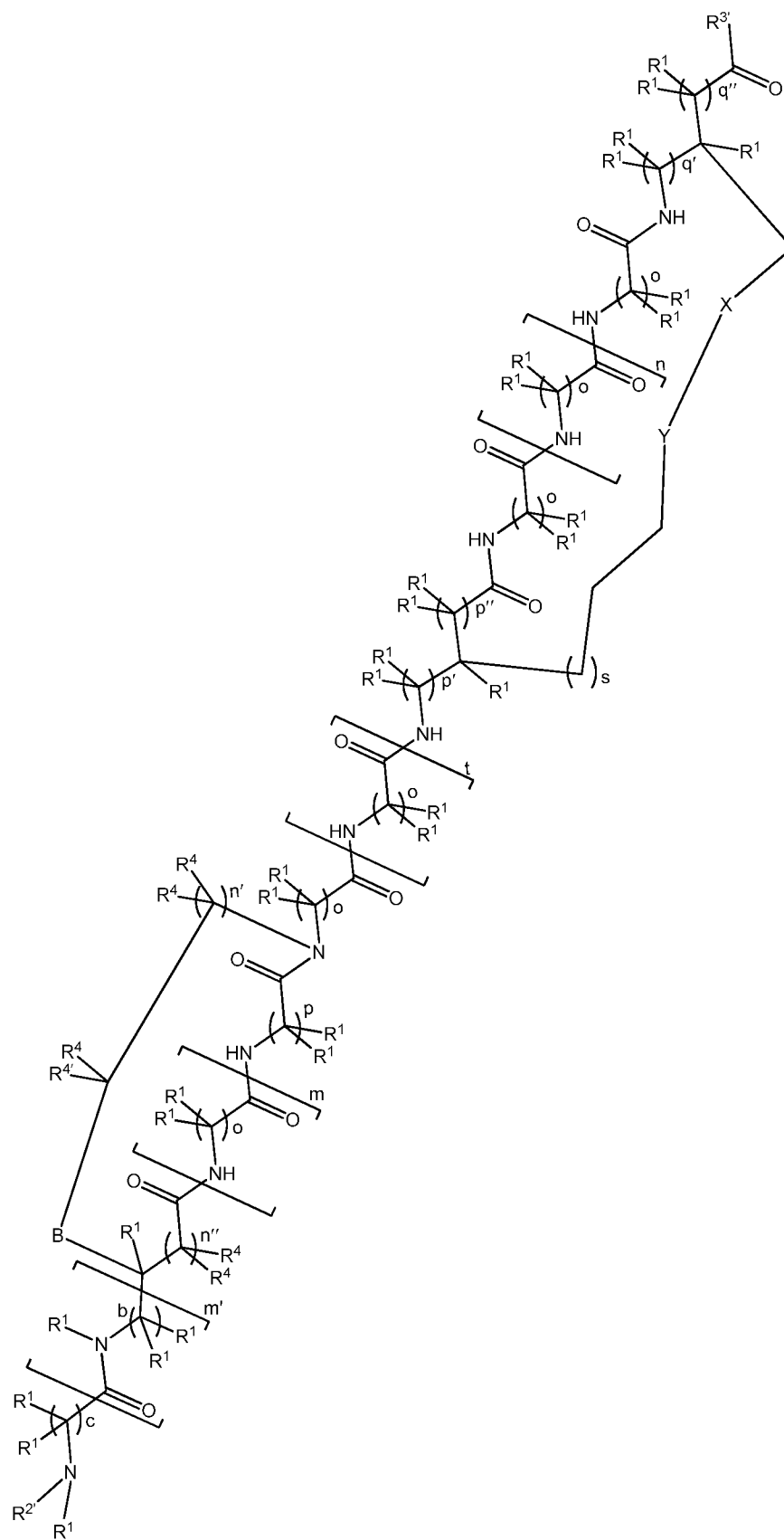
25. The peptidomimetic according to claim 23, wherein the peptidomimetic is a compound of Formula IIIB:



IIIB.

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26. The peptidomimetic according to claim 23, wherein the peptidomimetic is a compound of Formula IIIC:



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27. A pharmaceutical composition comprising a peptidomimetic according to claim 1 and a pharmaceutically acceptable vehicle.

28. The pharmaceutical composition according to claim 27, wherein the peptidomimetic is a compound of Formula I.

29. The pharmaceutical composition according to claim 28, wherein the peptidomimetic is a compound of Formula IA.

30. The pharmaceutical composition according to claim 28, wherein the peptidomimetic is a compound of Formula IB.

31. The pharmaceutical composition according to claim 28, wherein the peptidomimetic is a compound of Formula IC.

32. The pharmaceutical composition according to claim 27, wherein the peptidomimetic is a compound of Formula II.

33. The pharmaceutical composition according to claim 32, wherein the peptidomimetic is a compound of Formula IIA.

34. The pharmaceutical composition according to claim 32, wherein the peptidomimetic is a compound of Formula IIB.

35. The pharmaceutical composition according to claim 32, wherein the peptidomimetic is a compound of Formula IIC.

36. The pharmaceutical composition according to claim 27, wherein the peptidomimetic is a compound of Formula III.

37. The pharmaceutical composition according to claim 36, wherein the peptidomimetic is a compound of Formula IIIA.

38. The pharmaceutical composition according to claim 36, wherein the peptidomimetic is a compound of Formula IIIB.

39. The pharmaceutical composition according to claim 36, wherein the peptidomimetic is a compound of Formula IIIC.

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40. A method of modulating transcription of a gene in a cell, wherein transcription of the gene is mediated by interaction of Hypoxia-Inducible Factor 1 α with CREB-binding protein and/or p300, said method comprising:

contacting the cell with a peptidomimetic according to claim 1 under conditions effective to modulate transcription of the gene.

41. The method according to claim 40, wherein transcription is up-regulated.

42. The method according to claim 40, wherein transcription is down-regulated.

43. The method according to claim 40, wherein the gene is selected from the group of *ACADSB*, *ADM*, *AK4*, *ALDOC*, *ALG1*, *ANG*, *ANGPTL4*, *ANKRD37*, *ANKZF1*, *ARHGAP28*, *ARID5A*, *ARNTL*, *ARRDC3*, *ASF1A*, *ASPM*, *AURKA*, *B4GALT4*, *BAMBI*, *BHLHE40*, *BHLHE41*, *BNIP3*, *BNIP3L*, *BOLA1*, *C1orf161*, *C1orf163*, *C3orf58*, *C4orf3*, *C7orf60*, *C7orf68*, *C8orf22*, *C8orf41*, *C14orf126*, *C17orf76*, *C18orf19*, *C1QL1*, *CA12*, *CA5B*, *CA9*, *CASZ1*, *CCDC80*, *CCNB1*, *CCNG2*, *CDC20*, *CDC23*, *CDCP1*, *CDK18*, *CDKN1A*, *CDKN3*, *CENPA*, *CENPE*, *CGGBP1*, *CHAC2*, *CNOT8*, *CPOX*, *CXCL16*, *CXCR4*, *DAPK1*, *DDX10*, *DEPDC1*, *DIS3L*, *DKFZp451A211*, *DLGAP5*, *DUSP5*, *DUSP5P*, *DUSP9*, *E2F5*, *EDN2*, *EFNA3*, *EGLN1*, *EGLN3*, *ELOVL6*, *ENO2*, *ERO1L*, *ERRF1*, *FAM13A*, *FAM72A*, *FAM72B*, *FAM72C*, *FAM72D*, *FAM83D*, *FAM86B1*, *FAM86B2*, *FAM86C*, *FAM115C*, *FAM115C*, *FAM133A*, *FAM162A*, *FARSB*, *FBXO16*, *FBXO32*, *FBXO42*, *FERMT1*, *FLJ23867*, *FLJ35024*, *FLJ44715*, *FN1*, *FOS*, *FOXD1*, *FUT11*, *FXD3*, *FYN*, *G2E3*, *GBE1*, *GDF15*, *GEMIN5*, *GFPT2*, *GOLGA8A*, *GOLGA8B*, *GPATCH4*, *GPR146*, *GPR155*, *GPR160*, *GPRC5A*, *GPT2*, *GTF2IRD2*, *GTF2IRD2B*, *GYS1*, *H1F0*, *H2BFS*, *HAS2*, *HERC3*, *HEY1*, *HIST1H1C*, *HIST1H1E*, *HIST1H2AB*, *HIST1H2AC*, *HIST1H2AD*, *HIST1H2AE*, *HIST1H2AH*, *HIST1H2AI*, *HIST1H2AK*, *HIST1H2AL*, *HIST1H2BC*, *HIST1H2BE*, *HIST1H2BF*, *HIST1H2BG*, *HIST1H2BH*, *HIST1H2BI*, *HIST1H2BJ*, *HIST1H2BK*, *HIST1H2BM*, *HIST1H2BN*, *HIST1H3A*, *HIST1H3D*, *HIST1H3F*, *HIST1H3H*, *HIST1H4B*, *HIST1H4H*, *HIST1H4J*, *HIST1H4K*, *HIST2H2AA3*, *HIST2H2AA4*, *HIST2H2AB*, *HIST2H2AC*, *HIST2H2BA*, *HIST2H2BE*, *HIST2H2BF*, *HIST2H3A*, *HIST2H3C*, *HIST2H3D*, *HIST2H4A*, *HIST2H4B*,

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HIST3H2A, HIVEP2, HK1, HK2, HMMR, HORMAD1, HOXD10, HPDL, HRH1, HSPA1A, HSPA1B, HYMAI, ID3, IDH2, IER3, IGFBP3, IGSF3, IL1RAP, IL2RG, ING2, INSIG1, INSIG2, IPMK, ITGA5, JUN, KAT2B, KCTD11, KDM3A, KIAA0586, KIAA1244, KIAA1432, KIAA1715, KIF14, KIF20A, KRT17, LOC154761, LOC645332, LOC653113, LOC100507405, LOX, LOXL2, LRP1, LST-3TM12, LTV1, MAFB, MAFK, MAK16, MAP2K1, MAP3K15, METTL7A, MLKL, MOBKL2A, MSTO1, MSTO2P, MUC1, MXI1, NAMPT, NARS2, NAV1, NDRG1, NDUFAF4, NEBL, NFIL3, NLN, NOG, NOL6, NOP2, NOP16, NOTCH3, NRG4, ORAI3, OSMR, OTUD1, P4HA1, P4HA2, PAG1, PAIP2B, PDHA1, PDK1, PDK3, PER1, PER2, PFKFB4, PFKP, PGM2L1, PIAS2, PLA2G4A, PLAGL1, PLIN2, PLK1, PLOD1, PLOD2, PMPA1, PNO1, POLR1B, PPFA4, PPL, PPP1R3B, PPP1R3C, PPP2R5B, PPRC1, PRELID2, PRMT3, PTGS2, PTTG1, PYGL, QSOX1, RAB20, RAB40C, RAB8B, RASSF2, RCOR2, RIOK3, RITI, RLF, RNASE4, RNF122, RNF24, RNU4-2, RORA, RPSA, RRAGD, RRS1, RUVBL1, SCARNA5, SCARNA6, SCFD2, SEC14L4, SEC61G, SERPINE1, SERPINI1, SERTAD2, SLC2A1, SLC2A3, SLC6A10P, SLC6A6, SLC6A8, SLC7A11, SLC27A2, SLCO1B3, SLCO4A1, SNAPC5, SNORA1, SNORA2A, SNORA6, SNORA13, SNORA42, SNORA60, SNORA62, SNORA74A, SNORA75, SNORD1A, SNORD14E, SNORD53, SNORD94, SNX33, SPAG4, SPICE1, SPINK5, SPRY1, STAMBPL1, STC2, SYT7, TAF9B, TBC1D30, TCP11L2, TET2, TGFB1, TMC07, TMEM45A, TMEM45B, TMEM184A, TMOD1, TMPRSS3, TNFRSF10D, TRIM59, TROAP, TSEN2, TSTD2, TTYH3, TWISTNB, UACA, UBASH3B, UFSP2, UPRT, UTP15, UTP20, VEGFA, VLDLR, VTRNA1-1, WDR3, WDR12, WDR35, WDR45L, WDR52, WSB1, XK, YEATS2, ZDBF2, ZNF160, ZNF292, ZNF395, ZNF654, ZSWIM5, adenylate kinase 3, α_{1B} -adrenergic receptor, aldolase A, ceruloplasmin, c-Met protooncogene, CXCL12/SDF-1, endothelin-1, enolase 1, erythropoietin, glucose transporter 1, glucose transporter 3, glyceraldehyde-3-phosphate dehydrogenase, heme oxygenase 1, IGF binding protein 1, insulin-like growth factor 2, lactate dehydrogenase A, nitric oxide synthase 2, p35^{srg}, phosphoglycerate kinase 1, pyruvate kinase M, transferrin, transferrin receptor, transforming growth factor β_3 , vascular endothelial growth factor, vascular endothelial growth factor receptor FLT-1, and vascular endothelial growth factor receptor KDR/Flk-1.

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44. A method of treating or preventing in a subject a disorder mediated by interaction of Hypoxia-Inducible Factor 1 α with CREB-binding protein and/or p300, said method comprising:

administering to the subject a peptidomimetic according to claim 1 under conditions effective to treat or prevent the disorder.

45. The method according to claim 44, wherein the disorder is selected from the group of abnormal vasoconstriction, retinal ischemia, pulmonary hypertension, intrauterine growth retardation, diabetic retinopathy, age-related macular degeneration, diabetic macular edema, and cancer.

46. A method of reducing or preventing angiogenesis in a tissue, said method comprising:

contacting the tissue with a peptidomimetic according to claim 1 under conditions effective to reduce or prevent angiogenesis in the tissue.

47. The method according to claim 46, wherein the method is carried out *in vivo*.

48. The method according to claim 46, wherein the tissue is a tumor.

49. A method of decreasing survival and/or proliferation of a cell under hypoxic conditions, said method comprising:

contacting the cell with a peptidomimetic according to claim 1 under conditions effective to decrease survival and/or proliferation of the cell.

50. The method according to claim 49, wherein the cell is cancerous or is contained in the endothelial vasculature of a tissue that contains cancerous cells.

51. A method of identifying a potential ligand of CREB-binding protein and/or p300, said method comprising:

providing a peptidomimetic according to claim 1,

contacting the peptidomimetic with a test agent, and

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detecting whether the test agent selectively binds to the peptidomimetic, wherein a test agent that selectively binds to the peptidomimetic is identified as a potential ligand of CREB-binding protein and/or p300.

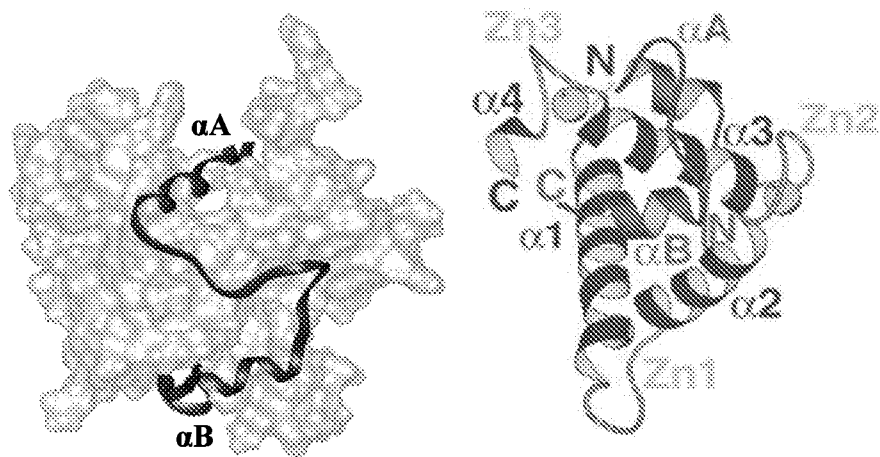


Figure 1A



Figure 1B

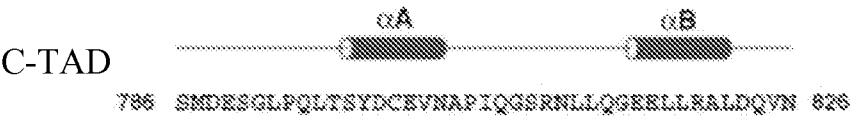


Figure 1C

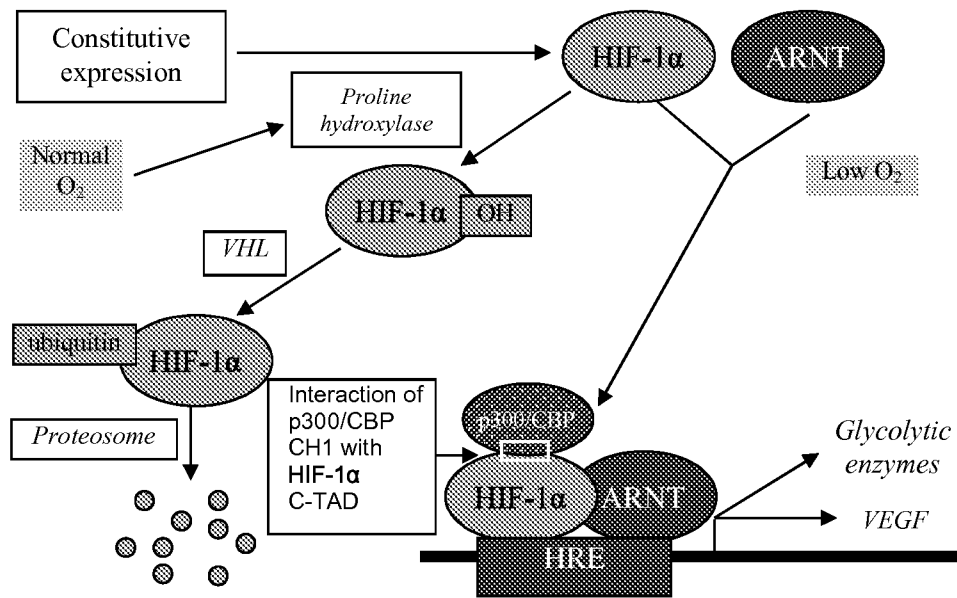
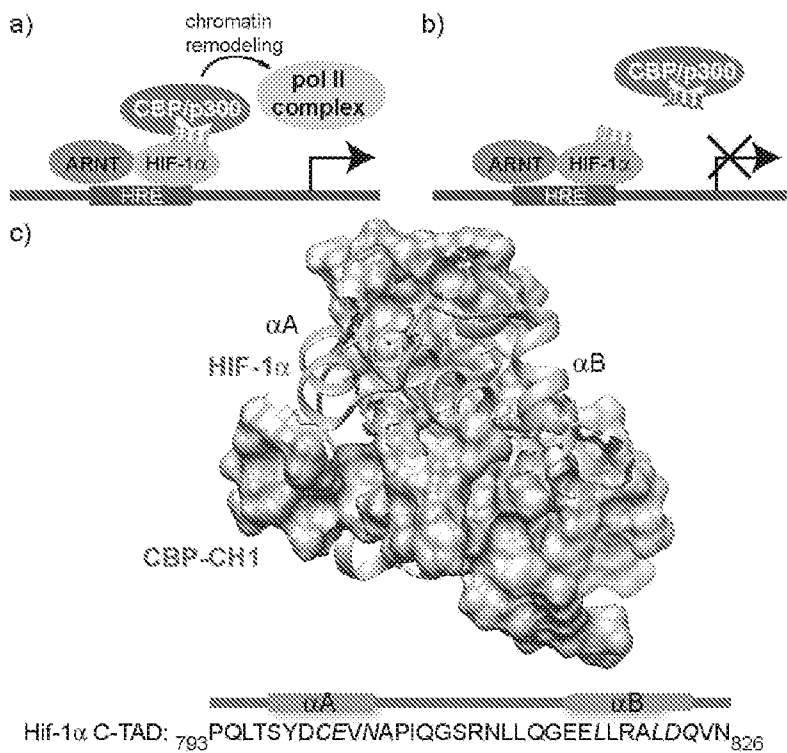


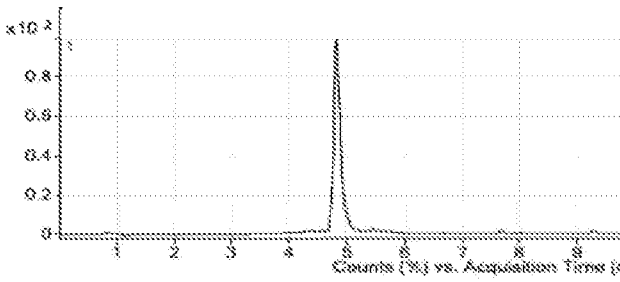
Figure 2



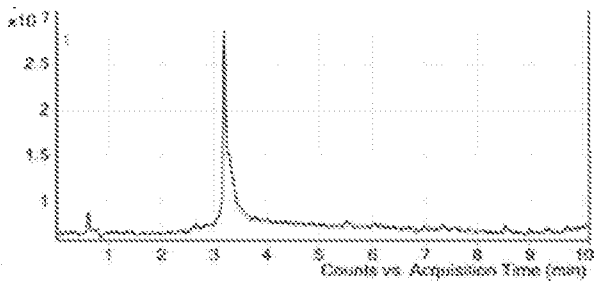
Figures 3A–C

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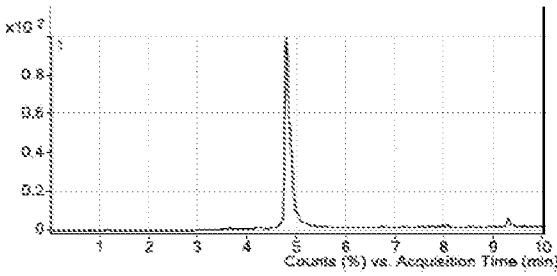
HBS 1



HBS 2

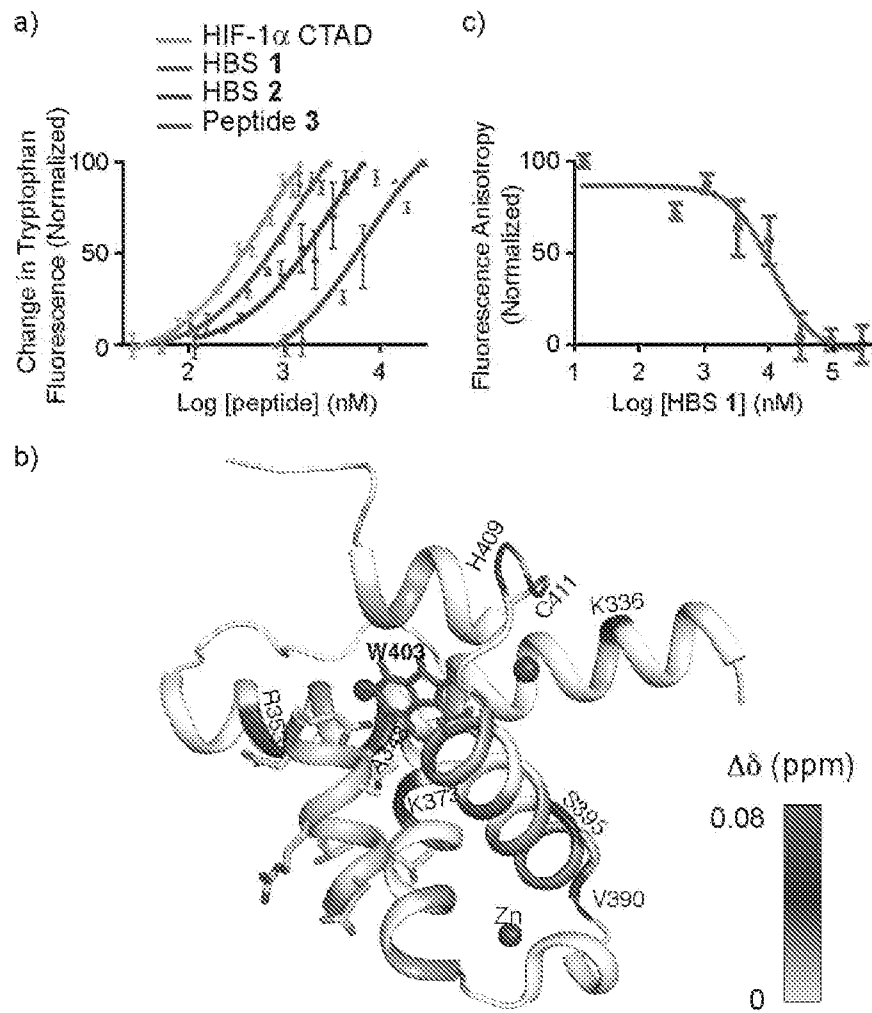


Peptide 3



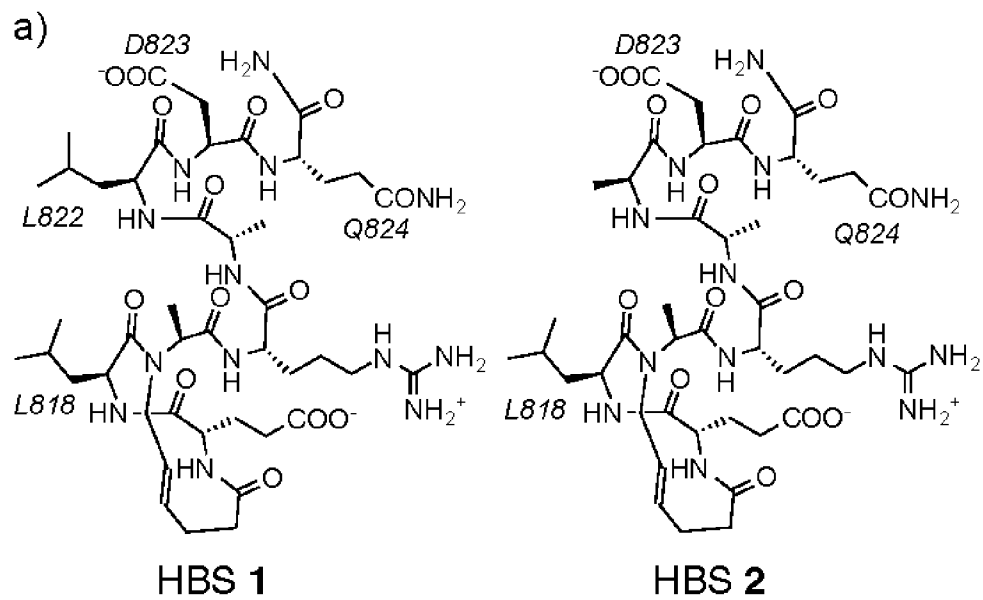
Figures 4A–C

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Figures 5A–C

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b) Peptide 3: Ac-ELARALDQ-NH₂

Figures 6A–B

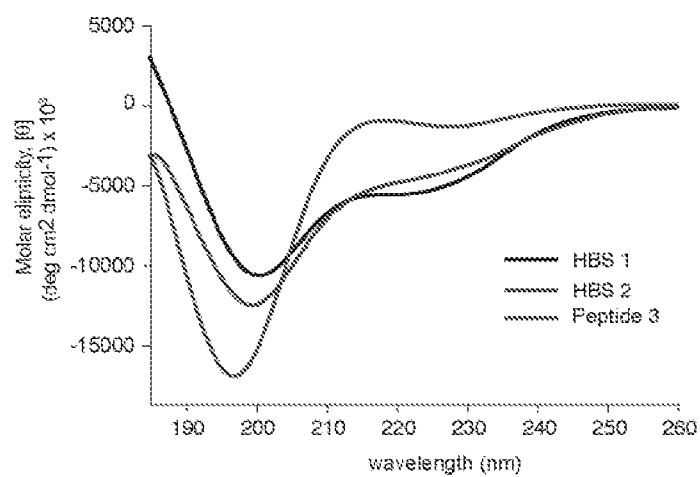
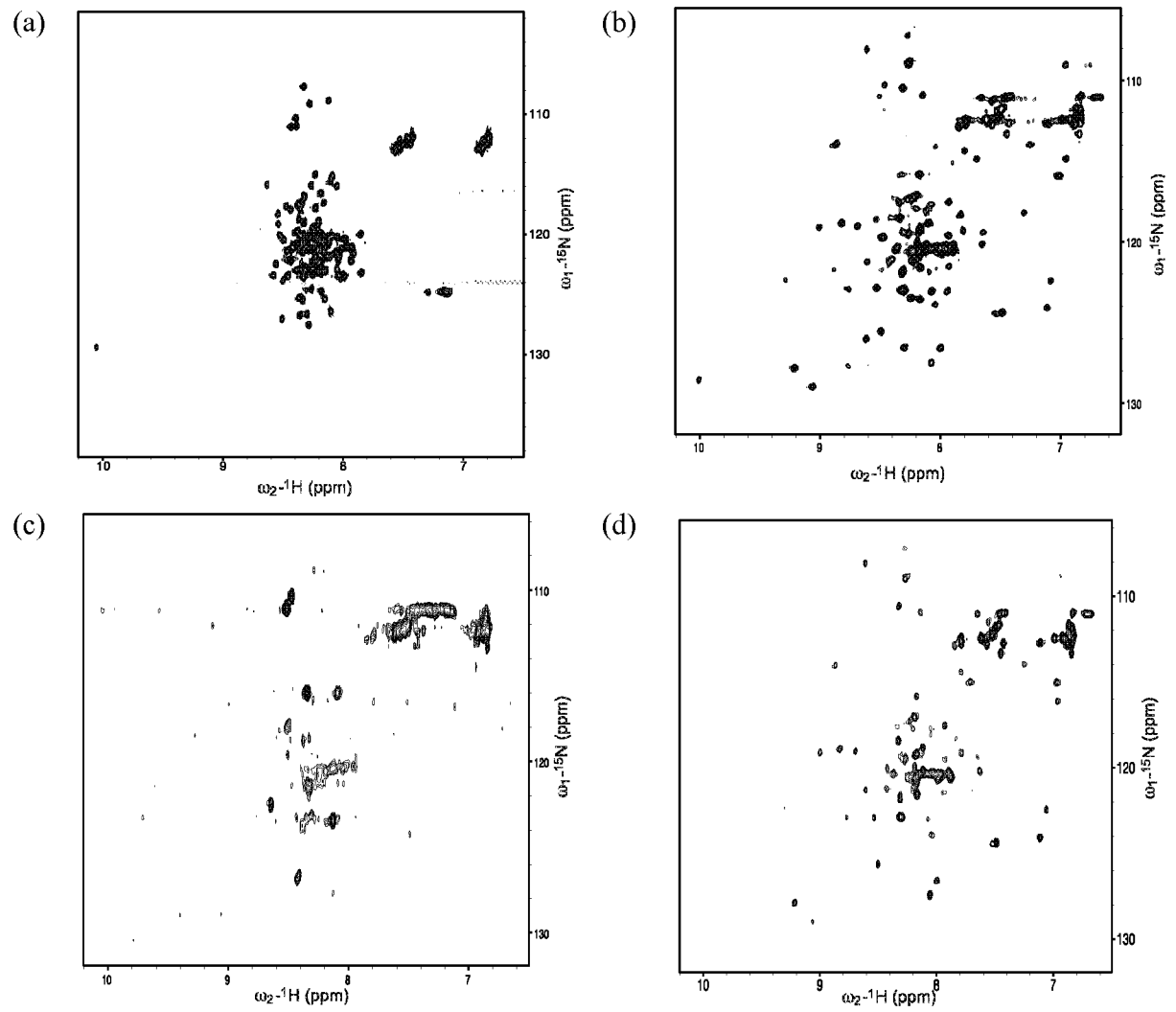


Figure 7

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Figures 8A–D

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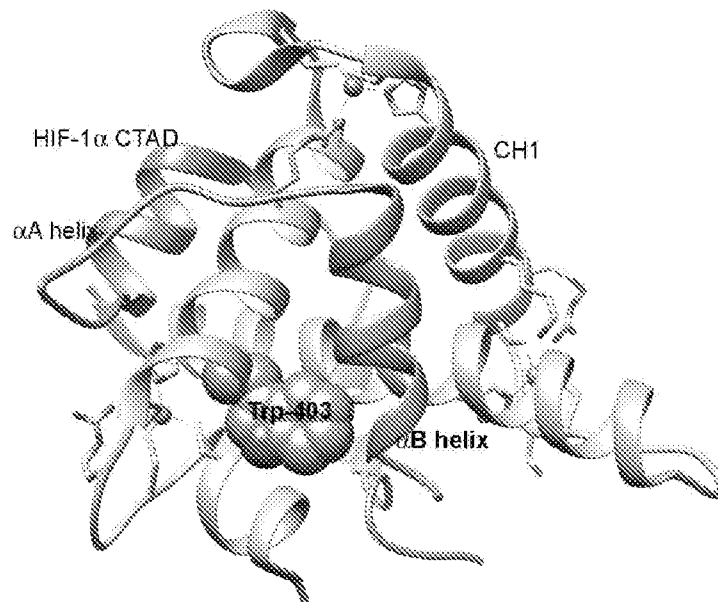


Figure 9

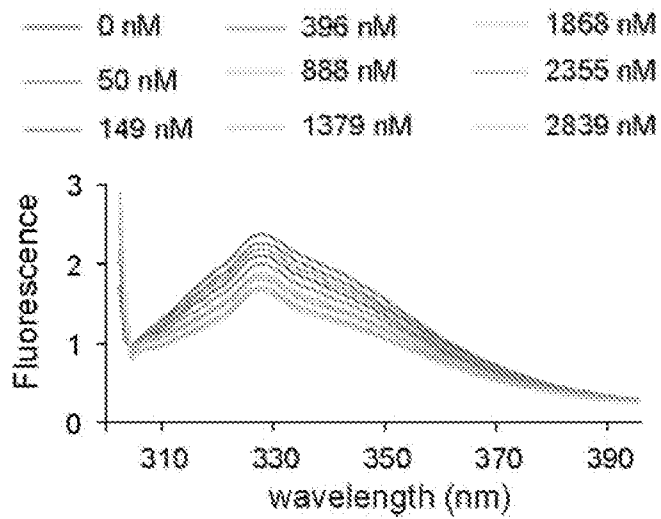


Figure 10

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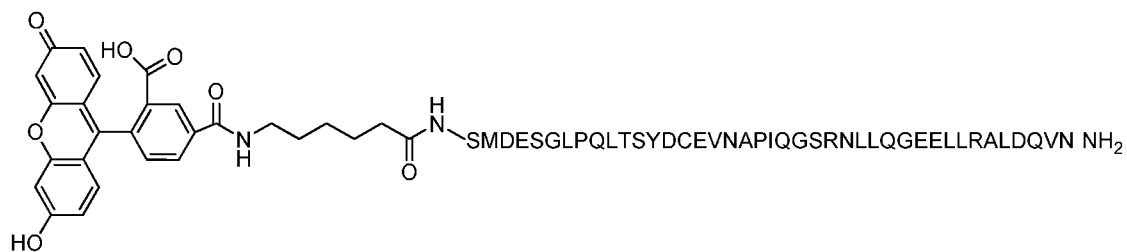


Figure 11

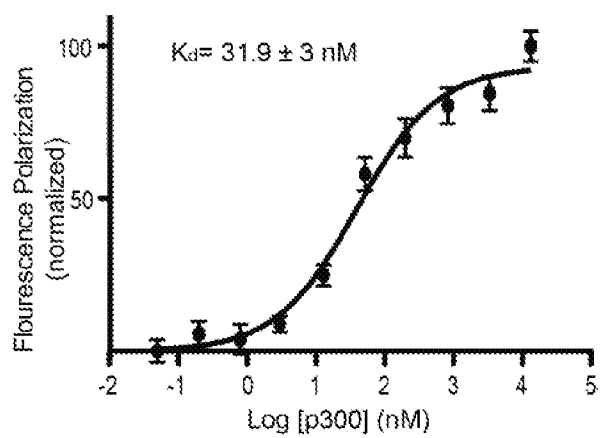


Figure 12

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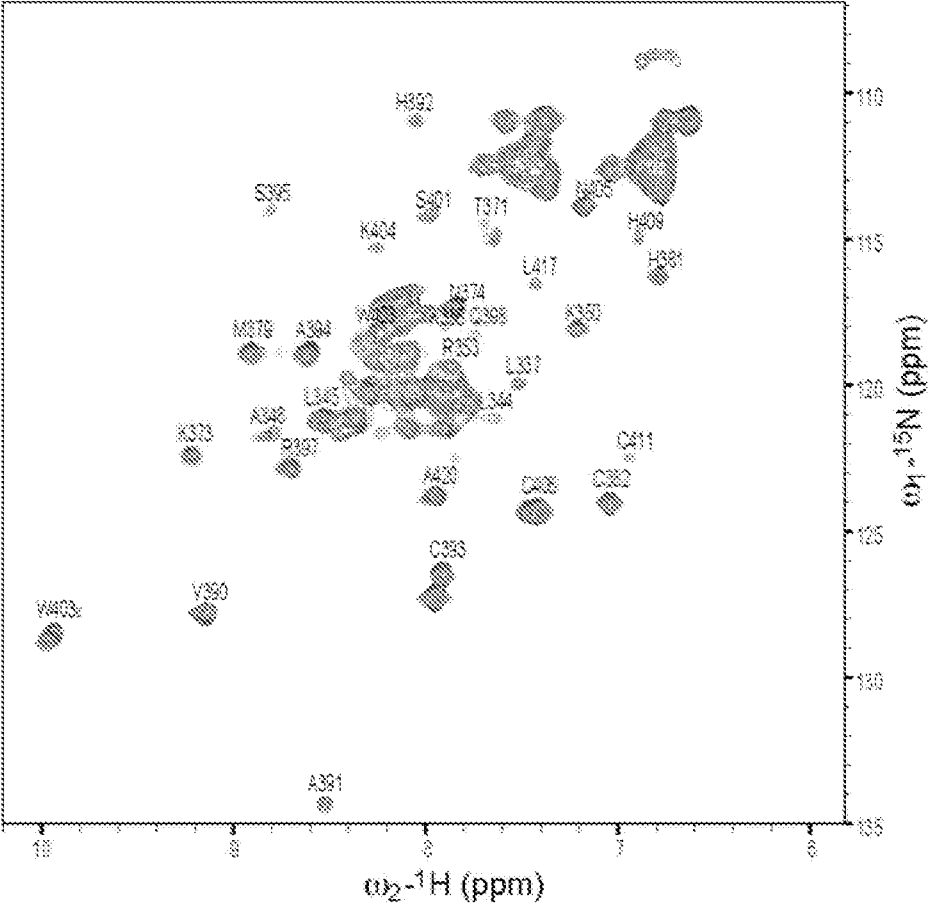


Figure 13

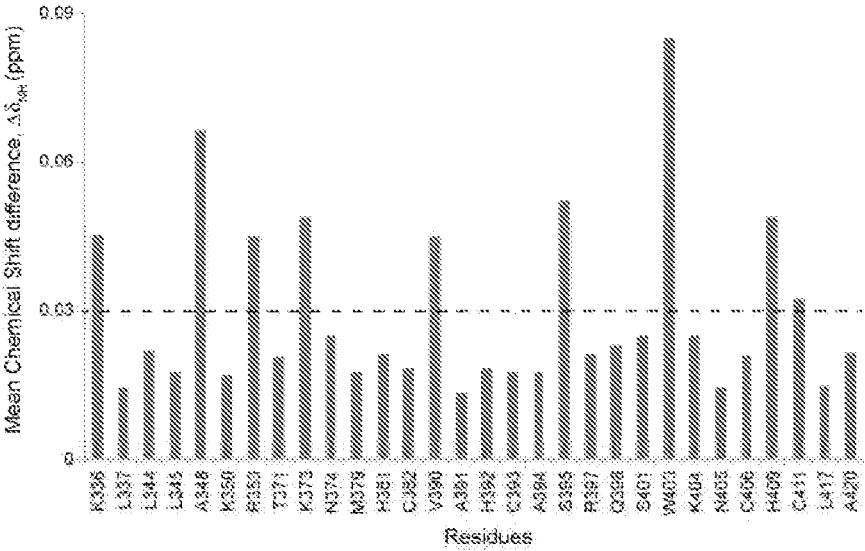


Figure 14

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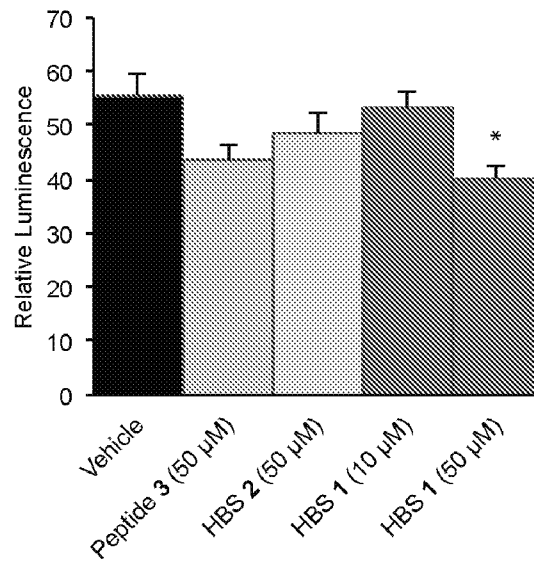


Figure 15

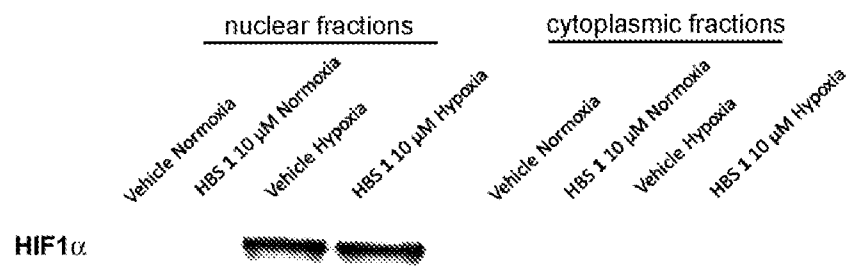
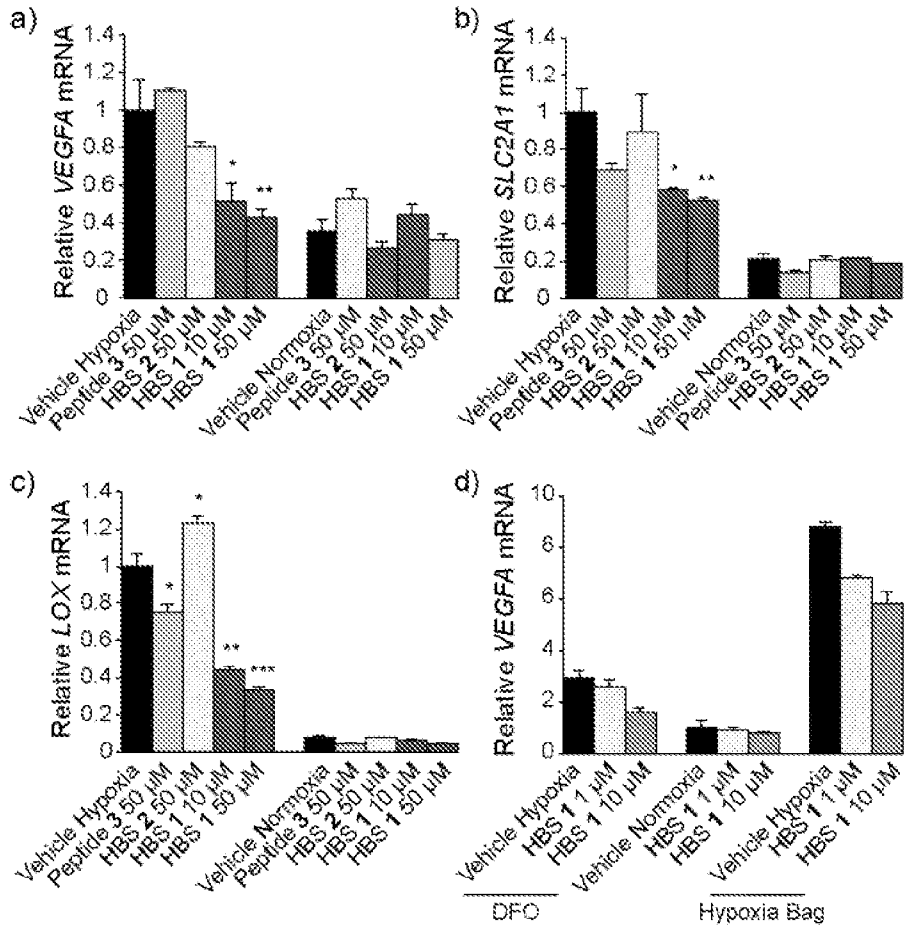


Figure 16



Figures 17A-D

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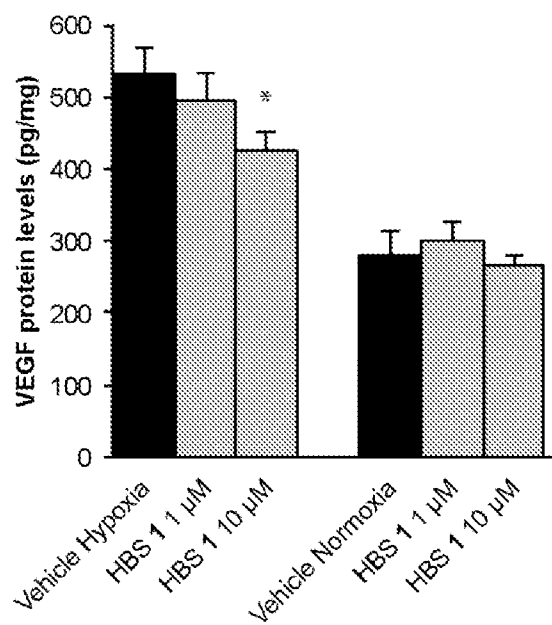


Figure 18

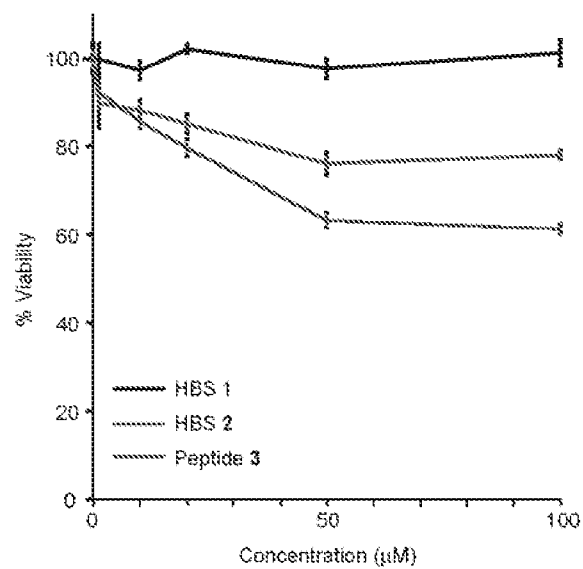
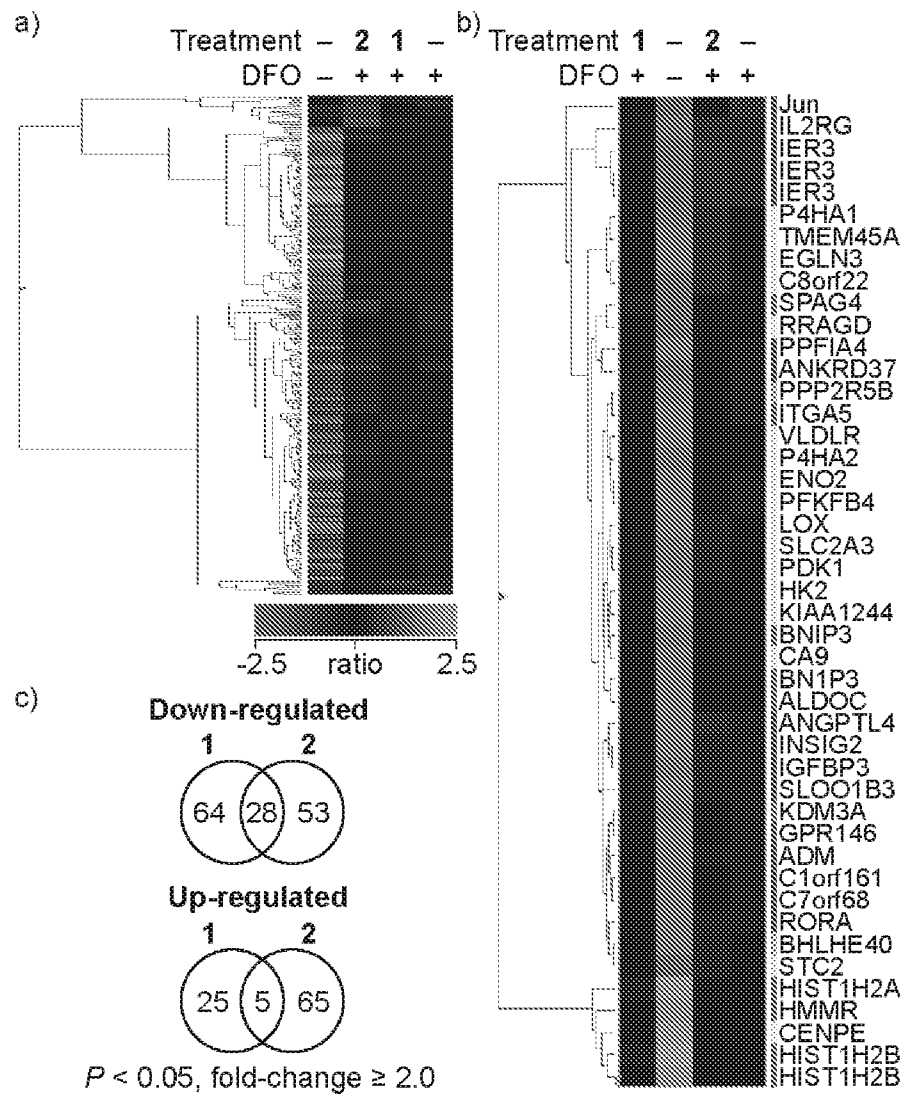


Figure 19



Figures 20A–C

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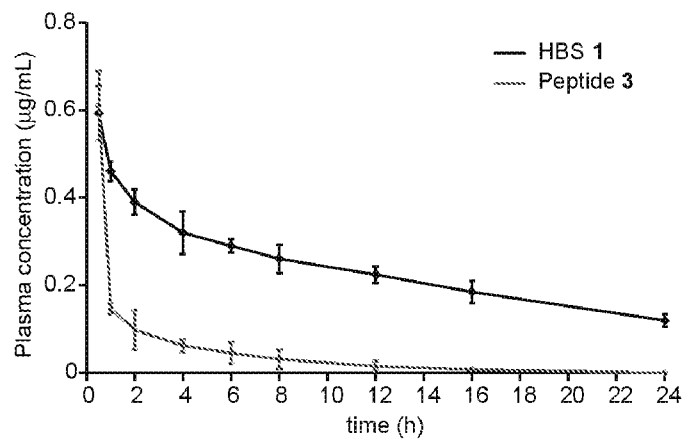
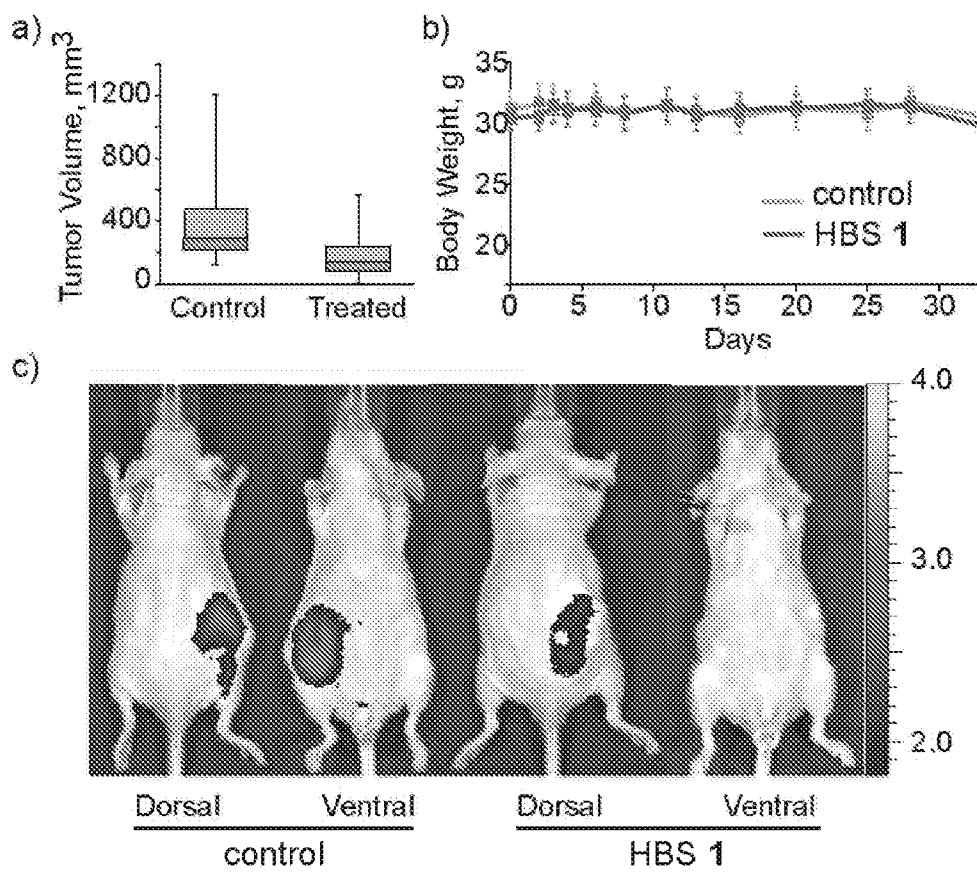


Figure 21



Figures 22A-C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/53911

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/02; A61P 35/00; C12N 5/071 (2014.01)

CPC - A61K 38/00, 39/00; C07K 14/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): A61K 38/02; A61P 35/00; C07K 2/00; C12N 5/071 (2014.01)

CPC: A61K 38/00, 39/00; C07K 14/00; USPC: 514/13.3; 530/300; 530/328

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

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

MicroPatent; Google; Google Scholar; IP.com; PubMed; New York University, University of Southern California, Arora, Lao, Henchey, peptidomimetic, peptidomimetic mimics, peptide, cyclic, negatively charged, hydrophobic, positively-charged, polar, 4-pentenoic acid, Hypoxia-Inducible Factor I alpha, CREB-binding protein, p300, cancer, retinal ischemia, pulmonary hypertension

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/0245175 A1 (ARORA, PS et al.) October 6, 2011; figure 1C; paragraphs [0018]-[0021], [0057], [0097]-[0098]; claims 1-2, 6, 17	1-4, 10-12, 16-18, 27-31, 40, 42-51
---		---
Y		5-9, 13-15, 41
Y	US 2013/0123196 A1 (ARORA, PS et al.) May 16, 2013; paragraphs [0005], [0121]; claim 15	5-9, 13-15
Y	REEVES, R et al. Architectural Transcription Factor HMGI(Y) Promotes Tumor Progression and Mesenchymal Transition of Human Epithelial Cells. MOLECULAR AND CELLULAR BIOLOGY, Vol. 21, No 2, 2001, pp. 575-594 [online], [retrieved on 2014-10-16]. Retrieved from the Internet <URL: http://mcb.asm.org/content/21/2/575.full.pdf+html >; abstract; page 582, column 1, paragraph 2	41

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

16 October 2014 (16.10.2014)

Date of mailing of the international search report

29 JAN 2015

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

international application no.

PCT/US14/53911

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group I+: Claims 1-51, are directed toward a peptidomimetic, wherein the peptidomimetic: (i) mimics a helix having the formula XI-X2-X2-X3-X2-X2-XI-X4-Xs, wherein each XI is any negatively charged residue, each X2 is any hydrophobic residue, X3 is any positively-charged residue, X4 is any polar residue, and X5 is absent or any hydrophobic residue; and (ii) is selected from the group consisting of: a compound of formula I, II, or III.

Group I+ will be searched to the extent that it encompasses the first named invention, which is limited to a peptidomimetic comprising a compound of Formula I, without fee. All values of X1-X5, as recited in claim 1(i) are also included in the scope of the first named invention (and thus the original search fee). It is believed that Claims 1-18, 27-31, and 40-51 are encompassed in the scope of the first named invention. An Exemplary Election for the further search of Group I+ would be to elect a peptidomimetic comprising a compound of Formula II. Another Exemplary Election would be to elect Formula III.

-Continued Within the Next Extra Page-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Group I+: Claims 1-51, Formula I (Claims 1-18, 27-31, 40-51)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

-Continued from Box No. III: Observations where unity of invention is lacking-

Applicant must indicate, if applicable, the claims which encompass the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of the first named invention include a peptidomimetic comprising a compound of Formula I, which are not present in the additional "+" groups; the special technical features of the additional "+" groups, include a peptidomimetic comprising compounds of Formula II or Formula III, which are not present in the first named invention.

The common technical features of the compounds of Group I+ are a peptidomimetic, wherein the peptidomimetic: (i) mimics a helix having the formula $X1-X2-X2-X3-X2-X2-X1-X4-Xs$, wherein each $X1$ is any negatively charged residue, each $X2$ is any hydrophobic residue, $X3$ is any positively-charged residue, $X4$ is any polar residue, and $X5$ is absent or any hydrophobic residue.

These common technical features, and a compound of Formula I, are previously disclosed by US 2011/0245175 A1 to Arora, et al. (hereinafter "Arora'175"). Arora'175 discloses a peptidomimetic (the compound may be reacted to promote formation of a carbon-carbon bond in the preparation of peptidomimetics; paragraph [0057]), wherein the peptidomimetic (preparation of peptidomimetics; paragraph [0057]): (i) mimics a helix having the formula $X1-X2-X2-X3-X2-X2-X1-X4-X5$, wherein each $X1$ is any negatively charged residue, each $X2$ is any hydrophobic residue, $X3$ is any positively-charged residue, $X4$ is any polar residue, and $X5$ is absent or any hydrophobic residue (the peptide comprises a sequence that mimics helix alpha B of the C-terminal transactivation domain of Hypoxia-Inducible Factor 1 alpha, where the helix alpha B contains sequence ELLRALDQV, i.e., Glutamic acid, negatively charged $X1$ - Leucine, hydrophobic $X2$ - Leucine, hydrophobic $X2$ - Arginine, positively-charged $X3$ - Alanine, hydrophobic $X2$ - Leucine, hydrophobic $X2$ - Aspartic Acid, negatively charged $X1$ - Glutamine, polar $X4$ - Valine, hydrophobic $X5$; figure 1C; claim 1); and (ii) is (a) a compound of Formula I, as shown (peptide of formula I; claim 2) wherein: B is $C(R1)_2$, where $R1$ is hydrogen (the carbon atom connecting the carbon atom linked to the $R1$ moiety and the carbon atom in the Cn moiety linked to the A3 group; claim 2); $R1$ is an amino acid, where $R1$ is linked to the carbon atom, which is connected to B ($R1$ is an amino acid; claim 2), $R1$ linked to the carbon atom of the amino acid chain are hydrogens ($R8$ is hydrogen; claim 2); $R2$ is hydrogen, where $R2$ is linked to the carbon atom, which is connected to B (hydrogen is connected to the carbon atom, where the carbon atom is linked to the $R1$; claim 2); $R3$ is hydrogen ($R3$ is hydrogen; claim 2); $R4$ are hydrogens (hydrogens are attached to the carbon atoms in Cn moieties; claim 2); a is one ($A4$ is an amino acid moiety, as shown; claim 2); m is one ($A1$ is an amino acid moiety, as shown; claim 2), n' is one (n is 2 in Cn moiety linked to the A3 group; claim 2), n'' is two (n is 1 in Cn moiety linked to the carbon atom attached to the $R2$ moiety; claim 2); m''' is one (m is any positive integer; claim 2); o is one ($A1$ is an amino acid moiety, as shown; claim 2); p is one ($A2$ is an amino acid moiety, as shown; claim 2).

Since the common technical features, and a compound of Formula I, are previously disclosed by Arora'175, these common structural elements are not special and thus, unity of invention is lacking.