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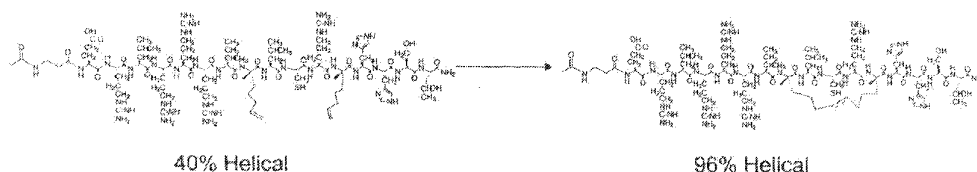
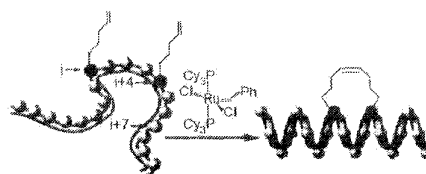
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(57) Abstract: Stably cross-linked a polypeptides related to human MAML are described. These cross-linked polypeptides contain at least two modified amino acids that together form an internal cross-link or tether that can help to stabilize the alpha-helical secondary structure that is thought to be important for binding of MAML peptides to the Notch transcription complex, a complex that includes ICN and CSL.

Stabilized MAML Peptides and Uses Thereof

BACKGROUND

Notch receptors are transmembrane receptors that involved in a variety of important signaling pathways. Mutations in human NOTCH1 are commonly found in human T cell acute lymphoblastic leukemias (T-ALL) and it is thought that abnormalities in Notch signaling are involved in other cancers.

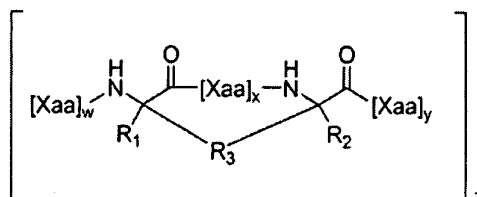
The Notch signaling pathway is complex. When an appropriate ligand binds to Notch a proteolytic event occurs which allows a portion of the Notch receptor called ICN to enter the cell nucleus where it interacts with CSL, a transcription factor that binds DNA, and a protein that is a member of the Mastermind-like (MAML) family. The assembled complex can activate transcription of certain genes. It is known that certain fragments of MAML (e.g., within amino acids 13-74 of human MAML-1) can act to interfere with Notch activation of transcription.

SUMMARY

Described below are stably cross-linked polypeptides related to human MAML. These cross-linked polypeptides contain at least two modified amino acids that together form an internal cross-link (also referred to as a tether) that can help to stabilize the alpha-helical secondary structure that is thought to be important for binding of MAML peptides to the Notch transcription complex, a complex that includes ICN and CSL. It is thought that the constrained secondary structure can increase resistance of the polypeptide to proteolytic cleavage. Accordingly, a cross-linked polypeptide described herein can have improved biological activity relative to a corresponding polypeptide that is not cross-linked. The cross-linked polypeptides described herein can be used therapeutically, e.g., to treat a variety of cancers in a subject. Inhibitors of Notch function may be useful in reducing unwanted immune responses, undesirable angiogenesis, treatment of human T cell acute lymphoblastic leukemias, treatment of mucoepidermoid carcinomas, treatment of breast cancer, treatment of medulloblastoma, and treatment of pancreatic cancer, treatment of lung cancer, treatment of ovarian cancer, treatment of atherosclerosis (e.g., heart disease),

treatment of melanoma, treatment of colon cancer, and treatment of cancers that exhibit resistance to gamma secretase inhibitors.

In one aspect, the invention features a modified polypeptide of Formula (I),



Formula (I)

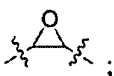
wherein;

each R_1 and R_2 are independently H or a C_1 to C_{10} alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, or heterocyclylalkyl;

R_3 is alkyl, alkenyl, alkynyl; $[\text{R}_4\text{-K-R}_4]_n$; each of which is substituted with 0-6 R_5 ;

R_4 is alkyl, alkenyl, or alkynyl;

R_5 is halo, alkyl, OR_6 , $\text{N}(\text{R}_6)_2$, SR_6 , SOR_6 , SO_2R_6 , CO_2R_6 , R_6 , a fluorescent moiety, or a radioisotope;

K is O, S, SO, SO_2 , CO, CO_2 , CONR_6 , or ;

R_6 is H, alkyl, or a therapeutic agent;

n is 3, 4 or 6;

x is an integer from 2-10;

w and y are independently an integer from 0-100 (e.g., 1, 2, 3, 4, 5, 6 or 7);

z is an integer from 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10); and

each Xaa is independently an amino acid,

wherein the polypeptide comprises at least 8 (e.g., 8, 9, 10, 11, 12, 13 or more) contiguous amino acids of SEQ ID NO:1, 2, 3, 4, 5, 6 or 7 except that: (a)

within the 8 contiguous amino acids of SEQ ID NO:1, 2, 3, 4, 5, 6 or 7 the side

chains of at least one pair of amino acids separated by 3, 4 or 6 amino acids is

replaced by the linking group R_3 which connects the alpha carbons of the pair of

amino acids as depicted in formula I; and (b) the alpha carbon of the first of the pair

of amino acids is substituted with R_1 as depicted in formula I and the alpha carbon

of the second of the pair of amino acids is substituted with R₂ as depicted in formula 1 or a pharmaceutically acceptable salt thereof.

SEQ ID NO:1 is a sequence created from an alignment of human MAML-1, 2, and 3, starting at amino acid 19 of MAML-1 and extending to amino acid 61.

5 SEQ ID NOs:2 - 4 respectively are the amino acid sequences of MAML-1, 2 and 3 over this same region. SEQ ID NOs:5 - 7 respectively are the amino acid sequences of MAML-1, 2 and 3 over a somewhat larger region.

His₁ Ser₂ Xaa₃ Xaa₄ Xaa₅ Glu₆ Arg₇ Leu₈ Arg₉ Xaa₁₀ Xaa₁₁ Ile₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅
 10 Arg₁₆ Xaa₁₇ His₁₈ His₁₉ Xaa₂₀ Xaa₂₁ Cys₂₂ Glu₂₃ Xaa₂₄ Arg₂₅ Tyr₂₆ Xaa₂₇ Xaa₂₈ Xaa₂₉
 Xaa₃₀ Xaa₃₁ Glu₃₂ Xaa₃₃ Xaa₃₄ Xaa₃₅ Xaa₃₆ Glu₃₇ Arg₃₈ Xaa₃₉ Xaa₄₀ Thr₄₁ Xaa₄₂ Xaa₄₃
 Leu₄₄ Xaa₄₅ Xaa₄₆ Xaa₄₇ (SEQ ID NO:1), wherein
 Xaa₃ is Ala or Thr;
 Xaa₄ is Val or Ile;
 15 Xaa₅ is Met or Val;
 Xaa₁₀ is Arg, Ala, or Gln
 Xaa₁₃ is Glu or Ala
 Xaa₁₄ is Leu, Val or Gly
 Xaa₁₇ Arg or Gln
 20 Xaa₂₀ is Ser, Leu or Val
 Xaa₂₁ is Thr, Ser or Asn
 Xaa₂₄ is Arg, Gly or Asn
 Xaa₂₇ is Glu or Gln
 Xaa₂₈ is Ala, Arg or Gln
 25 Xaa₂₉ is Val, Gly or Ala
 Xaa₃₀ is Ser, Arg or Gln
 Xaa₃₁ Pro, Ala or Val
 Xaa₃₃ Arg, Ser or Gln
 Xaa₃₄ Leu or Ser
 30 Xaa₃₅ is Glu or Asp
 Xaa₃₆ is Leu or Arg
 Xaa₃₉ is Gln Glu or Arg

Xaa₄₀ is His, Ser or Asp

Xaa₄₂ is Phe, Leu or Val

Xaa₄₃ is Ala, Gln or Ser

Xaa₄₅ is His, Leu or Tyr

5 Xaa₄₆ is Gln or Ser

Xaa₄₇ is Arg or Leu

Within SEQ ID NO:1, the following pairs of amino acid can be cross-linked:
2/9, 6/13, 13/17, 17/20, 20/27, 20/24, 35/39, 39/46, and 39/43. The corresponding
residues in SEQ ID NOs:2-8 can be cross-linked.

10

SEQ ID NO:2 (MAML-1; amino acids 19-62):

VMERLRRIELCRRHHSTCEARYEAVSPERLELERQHTFALHQR

15

SEQ ID NO:3 (MAML-2):

IVERLRARIAVCRQHHLSCEGRYERGRAESSDRERESTLQLLSL

SEQ ID NO:4 (MAML-3):

VVERLRQRIEGCRRHHVNCENRYQQAQVEQLELERRDTVSLYQR

20

SEQ ID NO:5 (MAML-1; includes predicted domain for binding the transcription
complex):

HSAMERLRRIELCRRHHSTCEARYEAVSPERLELERQHTFALHQRCIAK
AKRAGKH

25

SEQ ID NO:6 (MAML-2; includes predicted domain for binding the transcription
complex):

HSAMERLRARIAVCRQHHLSCEGRYERGRAESSDRERESTLQLLSLVQHGQ
GARKAGKH

30

SEQ ID NO:7 (MAML-3; includes predicted domain for binding the transcription
complex):

AVPKHSTVVERLRQRIEGCRRHHVNCENRYQQAQVEQLELERDRTVSLYQR
TLEQRAKKS

SEQ ID NO:8 (MAML-1 core)

5 ERLRRRIELCRRHHST

SEQ ID NO:9 (MAML-2 core)

ERLRARIAVCRQHHLSC

10 SEQ ID NO:10 (MAML-3 core)

ERLRQRIEGCRRHHVN

In some instances, the modified polypeptide binds a complex of ICN and CSL, e.g., ICN and CSL bound to DNA.

15 In some instances, each y is independently an integer between 3 and 15.

In some instances each y is independently an integer between 1 and 15.

In some instances, R_1 and R_2 are each independently H or C_1 - C_6 alkyl.

In some instances, R_1 and R_2 are each independently C_1 - C_3 alkyl.

In some instances, at least one of R_1 and R_2 are methyl. For example R_1 and

20 R_2 are both methyl.

In some instances R_3 is alkyl (e.g., C_8 alkyl) and x is 3.

In some instances, R_3 is C_{11} alkyl and x is 6.

In some instances, R_3 is alkenyl (e.g., C_8 alkenyl) and x is 3.

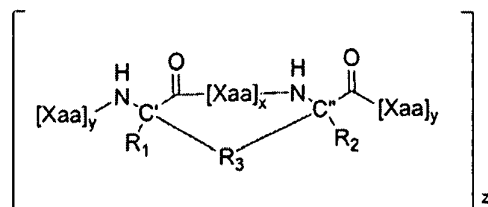
In some instances x is 6 and R_3 is C_{11} alkenyl.

25 In some instances, R_3 is a straight chain alkyl, alkenyl, or alkynyl.

In some instances R_3 is $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-$.

In certain embodiments the two alpha, alpha disubstituted stereocenters (alpha carbons) are both in the R configuration or S configuration (e.g., i, i+4 cross-link), or one stereocenter is R and the other is S (e.g., i, i+7 cross-link). Thus, where

30 Formula I is depicted as



the C' and C'' disubstituted stereocenters can both be in the R configuration or they can both be in the S configuration, for example when x is 3. When x is 6, the C' disubstituted stereocenter is in the R configuration and the C'' disubstituted stereocenter is in the S configuration.

The R₃ double bond may be in the E or Z stereochemical configuration.

In some instances R₃ is [R₄-K-R₄]_n; and R₄ is a straight chain alkyl, alkenyl, or alkynyl.

In some instances, the polypeptide includes an amino acid sequence which is at least about 60% (70%, 80%, 85%, 90%, 95% or 98%) identical to the amino acid sequence of

HSVMERLRRRIELCRRHHSTCEARYEAVSPERLELERQHTFALHQRCIQAK
AKR (SEQ ID NO:8). In some instances the modified polypeptide comprises at

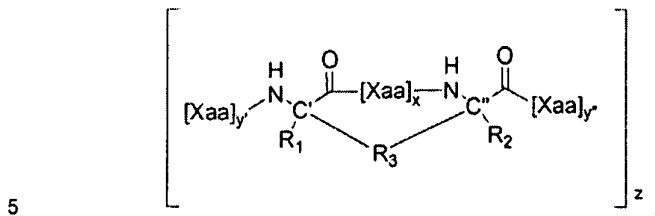
least 8 contiguous amino acids of

HSVMERLRRRIELCRRHHSTCEARYEAVSPERLELERQHTFALHQRCIQAK
AKR (SEQ ID NO:8). except that at least one pair of amino acids within the 8 (e.g., 8, 9, 10, 11, 12, 13 or more) contiguous amino acids are replaced by modified amino acids that can form an internal cross-link.

The tether can include an alkyl, alkenyl, or alkynyl moiety (e.g., C₅, C₈ or C₁₁ alkyl or a C₅, C₈ or C₁₁ alkenyl, or C₅, C₈ or C₁₁ alkynyl). The tethered amino acid can be alpha disubstituted (e.g., C₁-C₃ or methyl). [Xaa]_y and [Xaa]_w are peptides that can independently comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25 or more contiguous amino acids of a MAML polypeptide and [Xaa]_x is a peptide that can comprise 3 or 6 contiguous amino acids of acids of a MAML peptide.

The polypeptide can comprise 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50 amino acids of a MAML polypeptide (e.g., human MAML-1, 2 or 3 or a consensus MAML polypeptide). The amino acids are contiguous except

that one or more pairs of amino acids separated by 3 or 6 amino acids are replaced by amino acid substitutes that form a cross-link, e.g., via R₃. Thus, at least two amino acids can be replaced by tethered amino acids or tethered amino acid substitutes. Thus, where formula I is depicted as



[Xaa]_y and [Xaa]_y' can each comprise contiguous polypeptide sequences from the same or different MAML peptides.

In some instances the polypeptide comprises an amino acid sequence selected from SEQ ID NOs:8, 9 and 10 wherein: (a) the side chains of amino acids 8 and 12 are replaced by the linking group R_3 which connects the alpha carbons of amino acids 8 and 12 as depicted in formula I; and (b) the alpha carbon of amino acid 8 is substituted with R_1 as depicted in formula I and the alpha carbon of amino acid 12 is substituted with R_2 as depicted in formula I.

15 The invention features cross-linked polypeptides comprising at least 8 (e.g.,
8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50
or more) contiguous amino acids of a MAML polypeptide wherein the alpha carbons
of two amino acids that are separated by three amino acids (or six amino acids) are
linked via R₃, one of the two alpha carbons is substituted by R₁ and the other is
20 substituted by R₂ and each is linked via peptide bonds to additional amino acids.

In some embodiments the polypeptide acts as dominant negative inhibitor of Notch.

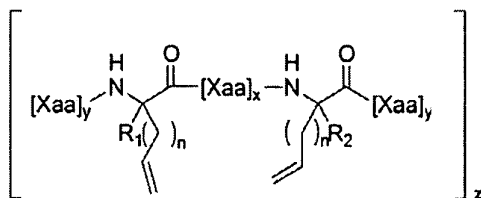
In some instances, the polypeptide also includes a fluorescent moiety or radioisotope.

25 In some instances, R₁ and R₂ are methyl; R₃ is C₈ alkyl, C₁₁ alkyl, C₈ alkenyl, C₁₁ alkenyl, C₈ alkynyl, or C₁₁ alkynyl; and x is 2, 3, or 6.

In some instances, the polypeptide includes an PEG, tat protein, affinity label, a targeting moiety, and/or a biotin moiety.

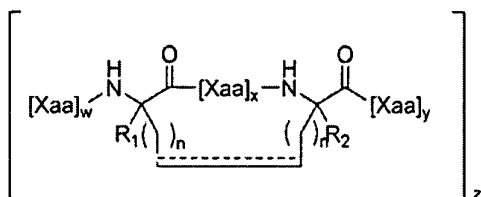
In another aspect, the invention features a method of making a polypeptide of Formula (III), including

providing a polypeptide of Formula (II); and



Formula (II)

treating the compound of Formula (II) with a catalyst to promote a ring closing metathesis, thereby providing a compound of formula (III)



Formula (III)

wherein

each R_1 and R_2 are independently H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl; heteroarylalkyl; or heterocyclalkyl;

each n is independently an integer from 1-15;

x is 2, 3, or 6

w and y are independently an integer from 0-100;

z is an integer from 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10); and

each Xaa is independently an amino acid;

In some instances, the polypeptide binds to a complex of ICN and CSL.

In some instances, the catalyst is a ruthenium catalyst.

In some instances, the method also includes providing a reducing or oxidizing agent subsequent to the ring closing metathesis.

In some instances, the reducing agent is H_2 or the oxidizing agent is osmium tetroxide

In some instances, the invention features a method of treating a subject including administering to the subject any of the compounds described herein. In

some instances, the method also includes administering an additional therapeutic agent.

In some instances, the invention features a method of treating cancer in a subject including administering to the subject any of the compounds described herein. In some instances, the method also includes administering an additional therapeutic agent.

In some instances, the invention features a library of the compounds described herein.

Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., therapeutic administration to a subject or generation of reagents to study or discover a biological pathway either *in vitro* or *in vivo*).

The compounds of this invention may contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers and diastereomeric mixtures. All such isomeric forms of these compounds are expressly included in the present invention. The compounds of this invention may also be represented in multiple tautomeric forms, in such instances, the invention expressly includes all tautomeric forms of the compounds described herein (e.g., alkylation of a ring system may result in alkylation at multiple sites, the invention expressly includes all such reaction products). All such isomeric forms of such compounds are expressly included in the present invention. All crystal forms of the compounds described herein are expressly included in the present invention.

The term "amino acid" refers to a molecule containing both an amino group and a carboxyl group. Suitable amino acids include, without limitation, both the D- and L- isomers of the 20 common naturally occurring amino acids found in peptides (e.g., A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V (as known by the one letter abbreviations)) as well as the naturally occurring and unnaturally occurring amino acids prepared by organic synthesis or other metabolic routes. The

table below provides the structures of the side chains for each of the 20 common naturally-occurring amino acids. In this table the “-” at right side of each structure is the bond to the alpha carbon.

Amino acid	Single Letter	Three Letter	Structure of side chain
Alanine	A	Ala	CH ₃ -
Arginine	R	Arg	HN=C(NH ₂)-NH-(CH ₂) ₃ -
Asparagine	N	Asn	H ₂ N-C(O)-CH ₂ -
Aspartic acid	D	Asp	HO(O)C-CH ₂ -
Cysteine	C	Cys	HS-CH ₂ -
Glutamine	Q	Gln	H ₂ N-C(O)-(CH ₂) ₂ -
Glutamic acid	E	Glu	HO(O)C-(CH ₂) ₂ -
Glycine	G	Gly	H-
Histidine	H	His	N=CH-NH-CH=C-CH ₂ -
Isoleucine	I	Ile	CH ₃ -CH ₂ -CH(CH ₃)-
Leucine	L	Leu	(CH ₃) ₂ -CH-CH ₂ -
Lysine	K	Lys	H ₂ N-(CH ₂) ₄ -
Methionine	M	Met	CH ₃ -S-(CH ₂) ₂ -
Phenylalanine	F	Phe	Phenyl-CH ₂ -
Proline	P	Pro	-N-(CH ₂) ₃ -CH-
Serine	S	Ser	HO-CH ₂ -
Threonine	T	Thr	CH ₃ -CH(OH)-
Tryptophan	W	Trp	Phenyl-NH-CH=C-CH ₂ -
Tyrosine	Y	Tyr	4-OH-Phenyl-CH ₂ -
Valine	V	Val	CH ₃ -CH(CH ₃)-

5


A “non-essential” amino acid residue is a residue that can be altered from the wild-type sequence of a polypeptide (without abolishing or substantially altering its activity. An “essential” amino acid residue is a residue that, when altered from the wild-type sequence of the polypeptide, results in abolishing or substantially

abolishing the polypeptide activity.

10

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families

of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine),
5 nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a MAML polypeptide, for example, is preferably replaced with another amino acid
10 residue from the same side chain family.

The symbol "" when used as part of a molecular structure refers to a single bond or a *trans* or *cis* double bond.

The term "amino acid side chain" refers to a moiety attached to the α -carbon in an amino acids. For example, the amino acid side chain for alanine is methyl, the
15 amino acid side chain for phenylalanine is phenylmethyl, the amino acid side chain for cysteine is thiomethyl, the amino acid side chain for aspartate is carboxymethyl, the amino acid side chain for tyrosine is 4-hydroxyphenylmethyl, etc. Other non-naturally occurring amino acid side chains are also included, for example, those that occur in nature (e.g., an amino acid metabolite) or those that are made synthetically
20 (e.g., an alpha di-substituted amino acid).

The term polypeptide encompasses two or more naturally occurring or synthetic amino acids linked by a covalent bond (e.g., a amide bond). Polypeptides as described herein include full length proteins (e.g., fully processed proteins) as well as shorter amino acids sequences (e.g., fragments of naturally occurring
25 proteins or synthetic polypeptide fragments).

The term "halo" refers to any radical of fluorine, chlorine, bromine or iodine. The term "alkyl" refers to a hydrocarbon chain that may be a straight chain or branched chain, containing the indicated number of carbon atoms. For example, C₁-C₁₀ indicates that the group may have from 1 to 10 (inclusive) carbon atoms in it. In
30 the absence of any numerical designation, "alkyl" is a chain (straight or branched) having 1 to 20 (inclusive) carbon atoms in it. The term "alkylene" refers to a divalent alkyl (i.e., -R-).

The term "alkenyl" refers to a hydrocarbon chain that may be a straight chain or branched chain having one or more carbon-carbon double bonds. The alkenyl moiety contains the indicated number of carbon atoms. For example, C₂-C₁₀ indicates that the group may have from 2 to 10 (inclusive) carbon atoms in it. The
5 term "lower alkenyl" refers to a C₂-C₈ alkenyl chain. In the absence of any numerical designation, "alkenyl" is a chain (straight or branched) having 2 to 20 (inclusive) carbon atoms in it.

The term "alkynyl" refers to a hydrocarbon chain that may be a straight chain or branched chain having one or more carbon-carbon triple bonds. The alkynyl
10 moiety contains the indicated number of carbon atoms. For example, C₂-C₁₀ indicates that the group may have from 2 to 10 (inclusive) carbon atoms in it. The term "lower alkynyl" refers to a C₂-C₈ alkynyl chain. In the absence of any numerical designation, "alkynyl" is a chain (straight or branched) having 2 to 20 (inclusive) carbon atoms in it.

15 The term "aryl" refers to a 6-carbon monocyclic or 10-carbon bicyclic aromatic ring system wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of aryl groups include phenyl, naphthyl and the like. The term "arylalkyl" or the term "aralkyl" refers to alkyl substituted with an aryl. The term "aryloalkoxy" refers to an alkoxy substituted with aryl.

20 The term "cycloalkyl" as employed herein includes saturated and partially unsaturated cyclic hydrocarbon groups having 3 to 12 carbons, preferably 3 to 8 carbons, and more preferably 3 to 6 carbons, wherein the cycloalkyl group additionally may be optionally substituted. Preferred cycloalkyl groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl,
25 cyclohexenyl, cycloheptyl, and cyclooctyl.

The term "heteroaryl" refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if
tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6,
30 or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively), wherein 0, 1, 2, 3, or 4 atoms of each ring may be substituted by a substituent. Examples of heteroaryl groups include pyridyl, furyl or furanyl, imidazolyl,

benzimidazolyl, pyrimidinyl, thiophenyl or thienyl, quinolinyl, indolyl, thiazolyl, and the like. The term "heteroarylalkyl" or the term "heteroaralkyl" refers to an alkyl substituted with a heteroaryl. The term "heteroarylalkoxy" refers to an alkoxy substituted with heteroaryl.

5 The term "heterocyclyl" refers to a nonaromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively),
10 wherein 0, 1, 2 or 3 atoms of each ring may be substituted by a substituent. Examples of heterocyclyl groups include piperazinyl, pyrrolidinyl, dioxanyl, morpholinyl, tetrahydrofuranyl, and the like.

 The term "substituents" refers to a group "substituted" on an alkyl, cycloalkyl, aryl, heterocyclyl, or heteroaryl group at any atom of that group.
15 Suitable substituents include, without limitation, halo, hydroxy, mercapto, oxo, nitro, haloalkyl, alkyl, alkaryl, aryl, aralkyl, alkoxy, thioalkoxy, aryloxy, amino, alkoxycarbonyl, amido, carboxy, alkanesulfonyl, alkylcarbonyl, and cyano groups.

 The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and
20 advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 depicts MAML polypeptides and locations for modification
FIG. 2 depicts a synthetic strategy for the generation of α,α -disubstituted
25 non-natural amino acids containing olefinic side chains.
FIG. 3 depicts certain MAML polypeptides used in studies described herein.
FIG. 4 depicts a stapled MAML polypeptide.
FIG. 5 depicts a CD spectra of a stapled polypeptide.
FIG. 6 depicts stapled MAML polypeptides.
30 FIG. 7 depicts the results of surface plasmon resonance showing that BioSAHN11 binds ICN in a dose dependent manner.

FIG. 7 depicts the results of surface plasmon resonance showing that immobilized ICN associates with CSL in a dose-dependent manner.

FIG. 9 depicts the results of studies examining the binding of immobilized stapled and unstapled SAHN11 to ICN1.

5 FIG. 10 depicts the results of studies on cellular uptake of SAHN1, SAHN2 and SAHN6.

FIG. 11 depicts the results of studies on cellular uptake of stapled and unstapled SAHN1.

10 FIG. 12 depicts the results of a study showing that stapled SAHN11 reduces expression of the CSL-responsive reporter in a dose-dependent manner.

FIG. 13 depicts the results of a study showing that T-ALL1 cells exposed to either SAHN11-FITC or SAHN1-FITC exhibit reduced HES1 expression relative to expression of a housekeeping gene.

15 FIG. 14 depicts the results of a study showing that stapled SAHN2 reduces the viability of MOLT4 cells.

FIG. 15 depicts the results of a study showing that stapled SAHN1, but not unstapled SAHN1, reduces the viability of ALL-SIL cells.

FIG. 16 depicts the results of a study showing that stapled SAHN1, but not unstapled SAHN1, reduces the viability of KOPTK1 cells.

20 FIG. 17 depicts the results of a study showing that stapled SAHN11 reduces the viability of MOLT4 cells.

FIG. 18 depicts the results of a study showing that stapled SAHN11 reduces the viability of TALL1 cells.

25 FIG. 19 depicts the results of a study showing that SAHN1, but not SAHN1-D can bind to ICN1/CSL in T-ALL cellular lysates.

FIG. 20 depicts the results of a study showing that SAHN1 can compete off ICN1 bound to MAML in T-ALL cellular lysates.

FIG. 21 depicts the results of a study showing that SAHN1-D cannot compete off ICN1 bound to MAML in T-ALL cellular lysates

30 FIG. 22 depicts the results of a study showing that SAHN1 and SAHN11 can cause a decrease in transcription from a CSL-responsive reporter in T-ALL cells.

FIG. 23 depicts the results of a study showing that SAHN1, but not SANN1-D n cause a decrease in transcription from a CSL-responsive endogenous genes in T-ALL cells.

FIG. 24 depicts the results of a study showing that SAHN1 can elicit an apoptotic response in T-ALL cells.

FIG. 25 depicts the results of a study showing that SAHN1 decreases the viability of MOLT4 T-ALL cells.

10

DETAILED DESCRIPTION

Described herein are internally cross-linked alpha helical domain polypeptides related to human MAML. The polypeptides include a tether between two non-natural amino acids, which tether significantly enhances the alpha helical secondary structure of the polypeptide. Generally, the tether or cross-link (sometimes referred to as staple) extends across the length of one or two helical turns (i.e., about 3.4 or about 7 amino acids). Accordingly, amino acids positioned at i and $i+3$; i and $i+4$; or i and $i+7$ are ideal candidates for chemical modification and cross-linking. Thus, for example, where a peptide has the sequence ...Xaa₁, Xaa₂, Xaa₃, Xaa₄, Xaa₅, Xaa₆, Xaa₇, Xaa₈, Xaa₉... (wherein "..." indicates the optional presence of additional amino acids), cross-links between Xaa₁ and Xaa₄, or between Xaa₁ and Xaa₅, or between Xaa₁ and Xaa₈ are useful as are cross-links between Xaa₂ and Xaa₅, or between Xaa₂ and Xaa₆, or between Xaa₂ and Xaa₉, etc. The polypeptides can include more than one crosslink within the polypeptide sequence to either further stabilize the sequence or facilitate the stabilization of longer polypeptide stretches. If the polypeptides are too long to be readily synthesized in one part, independently synthesized cross-linked peptides can be conjoined by a technique called native chemical ligation (Bang, et al., *J. Am. Chem Soc.* 126:1377).

The novel cross-linked polypeptides are useful, for example, to mimic or study proteins or polypeptides having one or more alpha-helical domains.

Analysis conserved residues among MAML-1, 2 and 3; analysis of the predicted interaction between MAML and Notch; and analysis of predicted alpha-helical regions led to the identification amino acids that might be replaced to provide a cross-link without significantly inhibiting binding to Notch. Thus, as shown in FIG 2 for MAML-1, residues that might be cross-linked are doubled underlined. Substitutions can be made at discrete locations, namely the "i, and i+4 positions" or the "i, and i+7 positions" shown for each phase (1 to 6) which facilitate cross-linking chemistry by placing reactive residues on the same face of the α -helix. Highly conserved amino acids among MAML polypeptides and those thought to be important in protein-protein interactions based on X-ray crystallographic, are preferably not replaced. In FIG. 2 residues where changes are expected to be tolerated are single underlined. In certain circumstances, conserved amino acids can be replaced by other amino acids (e.g., synthetic non-naturally occurring amino acids).

α,α -Disubstituted non-natural amino acids containing olefinic side chains of varying length can synthesized by known methods (Williams et al. 1991 *J. Am. Chem. Soc.* 113:9276; Schafmeister et al. 2000 *J. Am. Chem Soc.* 122:5891). FIG. 2 is a schematic depiction of the preparation of the non-natural amino acid (Fmoc-S5) used in solid phase peptide synthesis (SPPS) of i linked to i+4 peptides (one turn of the alpha helix is stabilized). For peptides where an i linked to i+7 staple is used (two turns of the helix stabilized) one S5 amino acid is used and one R8 is used. R8 is synthesized using the same route, except that the starting chiral auxiliary confers the R-alkyl-stereoisomer. Also, 8-iodooctene is used in place of 5-iodopentene. Inhibitors are synthesized on a solid support using solid-phase peptide synthesis (SPPS) on MBHA resin. Non-natural amino acids were synthesized by Moellering for incorporation into the final peptide product.

Various internally cross-linked peptides (REM-G1 to REM-G13, also called SAHN1 to SAHN13, respectively) shown in FIG. 3 were produced (X is a modified amino acid forming a cross-link). The underlined portions indicate the extent of

each polypeptide, and the remainder of the MAML-1 sequence in each case is provided for context.

FIG. 4 is a schematic drawing and detailed structural depiction of a modified polypeptide having the sequence of REM-G1 (SAHN1). As seen in the circular
5 dichromism spectra of FIG. 5, the modified polypeptide can be 96% alpha helical as compared to 40% when not cross-linked. FIG. 6 shows the detailed structure of versions of the modified, internally cross-linked polypeptide REM-G11 (SAHN11) that include either biotin or FITC labels.

Surface plasmon resonance was used to demonstrated that the biotin-labeled
10 cross-linker version of REM-G11 (BioSAHN11) binds ICN in a dose dependent manner (FIG. 7).

Biochemical association between stapled peptides and the Notch complex was also investigated using surface plasmon resonance. These studies employed immobilized ICN protein (an anti-GST antibody and a GST-tagged, purified ICN
15 protein comprising the RAM and ANK domains). Other studies employed biotinylated stapled peptides and a streptavidin-functionalized sensor surface.

We demonstrated that immobilized ICN associates with CSL in a dose-dependent manner. The association exhibited a two-phase kinetic association, first with RAM binding and subsequently with a lower-affinity association with the ANK
20 domain (see FIG. 8). Non-specific binding to a reference surface with anti-GST antibody was only minimal.

Binding of immobilized stapled and unstapled SAHN11 to ICN1 demonstrated that stapled (cross-linked) SAHN11 binds ICN with greater affinity ($K_d = 0.96 \mu\text{M}$) than non-stapled SAHN11 ($K_d = 2.63 \mu\text{M}$) (FIG. 9).

25 Immunoprecipitation studies using MOLT4 cell lysates, ALL-SIL cell lysates and KOPK1 cell lysates found that biotin labeled SAHN1 can be used to pull down ICN. A reverse immunoprecipitation assay using FITC labeled SAHN1 found that SAHN1 can be used to pull down ICN in MOLT4 cell lysates.

Automated quantitative immunofluorescence was used to determine the
30 intracellular distribution of fluorophore-labeled stapled alpha helices. Cells were incubated with FITC-conjugated peptides SAHN1, SAHN2, or SAHN6; or control. At 16 hours measurements of cellular fluorescence were taken using epifluorescence

microscopy. The results of these studies are shown in FIG. 10 in which each circle represents an individual cell and each column represents a treatment condition. Both SAHN1 and SAHN2 exhibited significant intracellular passage, whereas SAHN6 did not.

5 Automated quantitative immunofluorescence was also used to determine the intracellular distribution of fluorophore-labeled stapled alpha helices. Cells were incubated with FITC-conjugated peptides SAHN1, or unstapled SAHN1. As shown in FIG. 11, stapling of the peptide did not, in this instance, appear to impact intracellular passage.

10 Epifluorescence microscopy demonstrated that SAHN11 exhibits intracellular distribution. Confocal microscopy analysis suggested that both stapled and unstapled SAHN1 peptides appear to distribute to the intracellular compartment through endocytosis.

MOLT4 cells transfected with a CSL-responsive reporter were used to test
15 whether stapled SAHN11 can interfere with Notch-mediated activation of transcription. As can be seen in FIG. 12, stapled SAHN11 reduced expression of the CSL-responsive reporter in a dose-dependent manner.

HES1 is Notch responsive gene. As shown in FIG. 13, T-ALL1 cells exposed to either SAHN11-FITC or SAHN1-FITC exhibit reduced HES1 expression
20 relative to expression of a housekeeping gene (beta-actin).

As shown in FIG. 14, stapled SAHN2 reduced the viability of MOLT4 cells. As shown in FIG. 15, stapled SAHN1, but not unstapled SAHN1, reduced the viability of ALL-SIL cells. As shown in FIG. 16, stapled SAHN1, but not unstapled SAHN1, reduced the viability of KOPTK1 cells. As shown in FIG. 17, stapled
25 SAHN11 reduced the viability of MOLT4 cells. As shown in FIG. 18, stapled SAHN11 reduced the viability of TALL1 cells.

Immobilized SAHN1 was used to measure the apparent Kd for a pre-assembled ICN-CSL complex by surface plasmon resonance. The result of this analysis revealed an apparent Kd of 98 nM. (I DON'T THINK THERE IS ANY
30 NEED TO SHOW SLIDE 8)

A damaged variant of SAHN1 was created by changing the Glu indicated by * in the SAHN1 depicted in FIG. 6 to an Arg and changing the Arg indicated by +

in the SAHN1 depicted in FIG. 6 to Glu. This damaged variant, which has the same net charge as SANN1, is referred to as SAHN1-D.

Immobilized SAHN1-D was used to measure the apparent K_d for a pre-assembled ICN-CSL complex by surface plasmon resonance. The result of this analysis revealed an apparent K_d of 1.40 μM . (I DON'T THINK THERE IS ANY
5 NEED TO SHOW SLIDE 10)

Immunoprecipitation studies using T-ALL (KOPTK1) cell lysates demonstrated that SAHN1 and SAHN11, but not SAHN1-D, can pull down both ICN and CSL. The results of the analysis are shown in FIG. 19.

10 A study using T-ALL cellular lysates found that SAHN1 can compete away ICN1 that is bound to immunoprecipitated MAML. The results of this analysis are shown in FIG 20.

A study using T-ALL cellular lysates found that SAHN1-D cannot effectively compete away ICN1 that is bound to immunoprecipitated MAML. The results of this analysis are shown in FIG 21.
15

T-ALL cells (MOLT4) harboring a beta-lactamase gene under the control of a CSL responsive promoter was used to study the effect of SAHN1 and SAHN11 on Notch complex mediated transcription. This study found that both SAHN1 and SAHN11 decreased transcription and that the decrease was similar in magnitude to that caused by an RNAi directed against Notch and an RNAi directed against
20 lactamase. The results of this analysis are shown in FIG. 22.

A study in T-ALL cells (MOLT4) found that SAHN1, but not SAHN1-D, decrease expression of HES1 and HEY1, both Notch-driven genes, in a dose dependent manner. The results of this analysis are show in FIG. 23.

25 A study in gamma secretase resistant T-ALL cells (MOLT4) found that SAHN1, but not SAHN1-D induces an apoptotic response after 24 or 48 hours. The results of this analysis are show in FIG. 24.

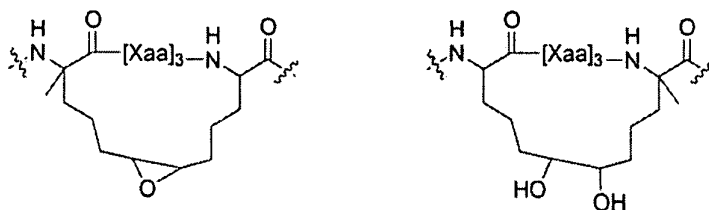
A study in T-ALL cells (KOPTK1) found that SAHN1 decreased cell viability ($\text{IC}_{50} = 8 \mu\text{M}$). SAHN1-D had little effect on cell viability. The results of this analysis are show in FIG. 25.
30

Additional studies found that SAHN1, but not SAHN1-D, is cytotoxic to neoplastic murine lymphocytes derived from transgenic mice harboring the

clinically relevant human Notch mutations (a L to P change at position 1601 and a PEST domain mutation).

Polypeptides

5 In some instances, the hydrocarbon tethers (i.e., cross links) described herein can be further manipulated. In one instance, a double bond of a hydrocarbon alkenyl tether, (e.g., as synthesized using a ruthenium-catalyzed ring closing metathesis (RCM)) can be oxidized (e.g., via epoxidation or dihydroxylation) to provide one of compounds below.



10

Either the epoxide moiety or one of the free hydroxyl moieties can be further functionalized. For example, the epoxide can be treated with a nucleophile, which provides additional functionality that can be used, for example, to attach a tag (e.g., a radioisotope or fluorescent tag). The tag can be used to help direct the compound to a desired location in the body or track the location of the compound in the body. Alternatively, an additional therapeutic agent can be chemically attached to the functionalized tether (e.g., an anti-cancer agent such as rapamycin, vinblastine, taxol, etc.). Such derivitization can alternatively be achieved by synthetic manipulation of the amino or carboxy terminus of the polypeptide or via the amino acid side chain. Other agents can be attached to the functionalized tether, e.g., an agent that facilitates entry of the polypeptide into cells.

15

20

While hydrocarbon tethers have been described, other tethers are also envisioned. For example, the tether can include one or more of an ether, thioether, ester, amine, or amide moiety. In some cases, a naturally occurring amino acid side chain can be incorporated into the tether. For example, a tether can be coupled with a functional group such as the hydroxyl in serine, the thiol in cysteine, the primary amine in lysine, the acid in aspartate or glutamate, or the amide in asparagine or glutamine. Accordingly, it is possible to create a tether using naturally occurring amino acids rather than using a tether that is made by coupling two non-naturally

25

occurring amino acids. It is also possible to use a single non-naturally occurring amino acid together with a naturally occurring amino acid.

It is further envisioned that the length of the tether can be varied. For instance, a shorter length of tether can be used where it is desirable to provide a relatively high degree of constraint on the secondary alpha-helical structure,
5 whereas, in some instances, it is desirable to provide less constraint on the secondary alpha-helical structure, and thus a longer tether may be desired.

Additionally, while examples of tethers spanning from amino acids i to $i+3$, i to $i+4$; and i to $i+7$ have been described in order to provide a tether that is primarily
10 on a single face of the alpha helix, the tethers can be synthesized to span any combinations of numbers of amino acids.

In some instances, alpha disubstituted amino acids are used in the polypeptide to improve the stability of the alpha helical secondary structure. However, alpha disubstituted amino acids are not required, and instances using
15 mono-alpha substituents (e.g., in the tethered amino acids) are also envisioned.

As can be appreciated by the skilled artisan, methods of synthesizing the compounds of the described herein will be evident to those of ordinary skill in the art. Additionally, the various synthetic steps may be performed in an alternate sequence or order to give the desired compounds. Synthetic chemistry
20 transformations and protecting group methodologies (protection and deprotection) useful in synthesizing the compounds described herein are known in the art and include, for example, those such as described in R. Larock, Comprehensive Organic Transformations, VCH Publishers (1989); T.W. Greene and P.G.M. Wuts, Protective Groups in Organic Synthesis, 2d. Ed., John Wiley and Sons (1991); L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis, John
25 Wiley and Sons (1994); and L. Paquette, ed., Encyclopedia of Reagents for Organic Synthesis, John Wiley and Sons (1995), and subsequent editions thereof.

The peptides of this invention can be made by chemical synthesis methods, which are well known to the ordinarily skilled artisan. See, for example, Fields et al.,
30 Chapter 3 in Synthetic Peptides: A User's Guide, ed. Grant, W. H. Freeman & Co., New York, N.Y., 1992, p. 77. Hence, peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with the α -NH₂ protected

by either t-Boc or F-moc chemistry using side chain protected amino acids on, for example, an Applied Biosystems Peptide Synthesizer Model 430A or 431.

One manner of making of the peptides described herein is using solid phase peptide synthesis (SPPS). The C-terminal amino acid is attached to a cross-linked polystyrene resin via an acid labile bond with a linker molecule. This resin is insoluble in the solvents used for synthesis, making it relatively simple and fast to wash away excess reagents and by-products. The N-terminus is protected with the Fmoc group, which is stable in acid, but removable by base. Any side chain functional groups are protected with base stable, acid labile groups.

Longer peptides could be made by conjoining individual synthetic peptides using native chemical ligation. Alternatively, the longer synthetic peptides can be synthesized by well known recombinant DNA techniques. Such techniques are provided in well-known standard manuals with detailed protocols. To construct a gene encoding a peptide of this invention, the amino acid sequence is reverse translated to obtain a nucleic acid sequence encoding the amino acid sequence, preferably with codons that are optimum for the organism in which the gene is to be expressed. Next, a synthetic gene is made, typically by synthesizing oligonucleotides which encode the peptide and any regulatory elements, if necessary. The synthetic gene is inserted in a suitable cloning vector and transfected into a host cell. The peptide is then expressed under suitable conditions appropriate for the selected expression system and host. The peptide is purified and characterized by standard methods.

The peptides can be made in a high-throughput, combinatorial fashion, e.g., using a high-throughput multiple channel combinatorial synthesizer available from Advanced Chemtech. In the modified polypeptides one or more conventional peptide bonds replaced by an a different bond that may increase the stability of the polypeptide in the body. Peptide bonds can be replaced by: a retro-inverso bonds (C(O)-NH); a reduced amide bond (NH-CH₂); a thiomethylene bond (S-CH₂ or CH₂-S); an oxomethylene bond (O-CH₂ or CH₂-O); an ethylene bond (CH₂-CH₂); a thioamide bond (C(S)-NH); a trans-olefine bond (CH=CH); an fluoro substituted trans-olefine bond (CF=CH); a ketomethylene bond (C(O)-CHR) or CHR-C(O) wherein R is H or CH₃; and a fluoro-ketomethylene bond (C(O)-CFR or CFR-C(O) wherein R is H or F or CH₃.

The polypeptides can be further modified by: acetylation, amidation, biotinylation, cinnamoylation, farnesylation, formylation, myristoylation, palmitoylation, phosphorylation (Ser, Tyr or Thr), stearylation, succinylation and sulfurylation. The polypeptides of the invention may also be conjugated to, for
5 example, polyethylene glycol (PEG); alkyl groups (e.g., C1-C20 straight or branched alkyl groups); fatty acid radicals; and combinations thereof.

Methods of Treatment

The present invention provides for both prophylactic and therapeutic
10 methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant (e.g., excessive) Notch activity. This is because the polypeptides are expected to act as dominant negative inhibitors of Notch activity. As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of
15 a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and
20 antisense oligonucleotides.

The polypeptides of the invention can be used to treat, prevent, and/or diagnose cancers and neoplastic conditions. As used herein, the terms "cancer", "hyperproliferative" and "neoplastic" refer to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly
25 proliferating cell growth. Hyperproliferative and neoplastic disease states may be categorized as pathologic, i.e., characterizing or constituting a disease state, or may be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or
30 organs, irrespective of histopathologic type or stage of invasiveness. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor

growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair.

Examples of cellular proliferative and/or differentiative disorders include cancer, e.g., carcinoma, sarcoma, or metastatic disorders. The compounds (i.e., polypeptides) can act as novel therapeutic agents for controlling breast cancer, T cell cancers and B cell cancer. The polypeptides may also be useful for treating mucoepidermoid carcinoma and medulloblastoma.

Examples of proliferative disorders include hematopoietic neoplastic disorders. As used herein, the term "hematopoietic neoplastic disorders" includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Exemplary disorders include: acute leukemias, e.g., erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) Crit Rev. in Oncol./Hematol. 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), multiple myeloma, hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL), Hodgkin's disease and Reed-Sternberg disease.

Examples of cellular proliferative and/or differentiative disorders of the breast include, but are not limited to, proliferative breast disease including, e.g., epithelial hyperplasia, sclerosing adenosis, and small duct papillomas; tumors, e.g., stromal tumors such as fibroadenoma, phyllodes tumor, and sarcomas, and epithelial tumors such as large duct papilloma; carcinoma of the breast including in situ (noninvasive) carcinoma that includes ductal carcinoma in situ (including Paget's disease) and lobular carcinoma in situ, and invasive (infiltrating) carcinoma including, but not limited to, invasive ductal carcinoma, invasive lobular carcinoma,

medullary carcinoma, colloid (mucinous) carcinoma, tubular carcinoma, and invasive papillary carcinoma, and miscellaneous malignant neoplasms. Disorders in the male breast include, but are not limited to, gynecomastia and carcinoma.

5 Pharmaceutical Compositions and Routes of Administration

As used herein, the compounds of this invention, including the compounds of formulae described herein, are defined to include pharmaceutically acceptable derivatives or prodrugs thereof. A “pharmaceutically acceptable derivative or prodrug” means any pharmaceutically acceptable salt, ester, salt of an ester, or other
10 derivative of a compound of this invention which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention. Particularly favored derivatives and prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (e.g., by allowing an orally administered compound to be
15 more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) relative to the parent species. Preferred prodrugs include derivatives where a group which enhances aqueous solubility or active transport through the gut membrane is appended to the structure of formulae described herein.

20 The compounds of this invention may be modified by appending appropriate functionalities to enhance selective biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological compartment (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection,
25 alter metabolism and alter rate of excretion.

Pharmaceutically acceptable salts of the compounds of this invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, benzoate, benzenesulfonate, butyrate, citrate, digluconate, dodecylsulfate, formate, fumarate,
30 glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, palmoate, phosphate, picrate, pivalate, propionate, salicylate,

succinate, sulfate, tartrate, tosylate and undecanoate. Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and N-(alkyl)₄⁺ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or
5 oil-soluble or dispersible products may be obtained by such quaternization.

The compounds of the formulae described herein can, for example, be administered by injection, intravenously, intraarterially, subdermally, intraperitoneally, intramuscularly, or subcutaneously; or orally, buccally, nasally, transmucosally, topically, in an ophthalmic preparation, or by inhalation, with a
10 dosage ranging from about 0.001 to about 100 mg/kg of body weight, or according to the requirements of the particular drug. The methods herein contemplate administration of an effective amount of compound or compound composition to achieve the desired or stated effect. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 6 times per day or
15 alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Alternatively, such
20 preparations contain from about 20% to about 80% active compound.

Lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug
25 combination, the severity and course of the disease, condition or symptoms, the patient's disposition to the disease, condition or symptoms, and the judgment of the treating physician.

Upon improvement of a patient's condition, a maintenance dose of a compound, composition or combination of this invention may be administered, if
30 necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is

retained. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

Pharmaceutical compositions of this invention comprise a compound of the formulae described herein or a pharmaceutically acceptable salt thereof; an
5 additional agent including for example, morphine or codeine; and any pharmaceutically acceptable carrier, adjuvant or vehicle. Alternate compositions of this invention comprise a compound of the formulae described herein or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier, adjuvant or vehicle. The compositions delineated herein include the compounds of
10 the formulae delineated herein, as well as additional therapeutic agents if present, in amounts effective for achieving a modulation of disease or disease symptoms.

The term "pharmaceutically acceptable carrier or adjuvant" refers to a carrier or adjuvant that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof and is
15 nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound.

Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying
20 drug delivery systems (SEDDS) such as d- α -tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes,
25 such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α -, β -, and
30 γ -cyclodextrin, may also be advantageously used to enhance delivery of compounds of the formulae described herein.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir, preferably by oral administration or administration by injection. The pharmaceutical compositions of this invention may
5 contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular,
10 intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using
15 suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic
20 sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in
25 their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions. Other commonly
30 used surfactants such as Tweens or Spans and/or other similar emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The pharmaceutical compositions of this invention may also be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of this invention with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

When the compositions of this invention comprise a combination of a compound of the formulae described herein and one or more additional therapeutic or prophylactic agents, both the compound and the additional agent should be present at dosage levels of between about 1 to 100%, and more preferably between about 5 to 95% of the dosage normally administered in a monotherapy regimen. The additional agents may be administered separately, as part of a multiple dose regimen, from the compounds of this invention. Alternatively, those agents may be part of a single dosage form, mixed together with the compounds of this invention in a single composition.

Screening Assays

The invention provides methods (also referred to herein as “screening assays”) for identifying polypeptides which modulate the activity of one or more Notch complexes.

5 The binding affinity of polypeptides to Notch can be measured using the methods described herein, for example, by using a titration binding assay. Notch complex lacking MAML (i.e., a complex of ICN and CSL) can be exposed to varying concentrations of a candidate compound (i.e., polypeptide) (e.g., 1 nM, 10 nM, 100 nM, 1 μ M, 10 μ M, 100 μ M, 1 mM, and 10 mM) and binding can be measured using
10 surface plasmon resonance to determine the K_d for binding. Candidate compounds could also be screened for biological activity *in vivo*, for example, by measuring expression of a Notch responsive reporter in a suitable cell, e.g., in MOLT-4 cells. Cell permeability screening assays in which fluorescently labeled candidate compounds are applied to intact cells, which are then assayed for cellular
15 fluorescence by microscopy or high-throughput cellular fluorescence detection can also be used.

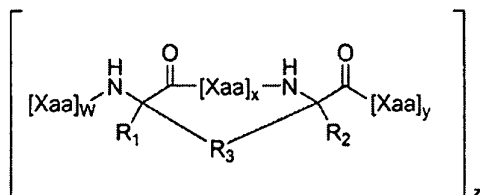
The assays described herein can be performed with individual candidate compounds or can be performed with a plurality of candidate compounds. Where the assays are performed with a plurality of candidate compounds, the assays can be
20 performed using mixtures of candidate compounds or can be run in parallel reactions with each reaction having a single candidate compound. The test compounds or agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art.

Other applications

25 A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

CLAIMS

1. A modified polypeptide of Formula (I),



Formula (I)


Wherein:

each R_1 and R_2 are independently H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, or heterocyclalkyl;

each R_3 is independently alkyl, alkenyl, alkynyl; $[\text{R}_4\text{-K-R}_4]_n$; each of which is substituted with 0-6 R_5 ;

each R_4 is independently alkyl, alkenyl, or alkynyl;

each R_5 is independently is halo, alkyl, OR_6 , $\text{N}(\text{R}_6)_2$, SR_6 , SOR_6 , SO_2R_6 , CO_2R_6 , R_6 , a fluorescent moiety, or a radioisotope;

each K is independently O, S, SO, SO_2 , CO, CO_2 , CONR_6 , or ;

each R_6 is independently H, alkyl, or a therapeutic agent;

n is an integer from 1-4;

x is 3, 4 or 6;

y and w are independently integers from 0-100;

z is an integer from 1-10; and

each Xaa is independently an amino acid;

wherein the modified polypeptide comprises at least 8 contiguous amino acids of a MAML polypeptide except that: (a) within the 8 contiguous amino acids the side chains of at least one pair of amino acids separated by 3, 4 or 6 amino acids is replaced by the linking group R_3 which connects the alpha carbons of the pair of amino acids as depicted in Formula I and (b) the alpha carbon of the first of the pair of amino acids is substituted with R_1 as depicted in formula I and the alpha carbon

of the second of the pair of amino acids is substituted with R_2 as depicted in Formula I and wherein and N or C can be substituted by PEG, spermine, or a carbohydrate.

2. The modified polypeptide of claim 1, wherein the MAML
5 polypeptide comprises any of SEQ ID NOs:1 to 7.

3. The polypeptide of claim 1, wherein the modified polypeptide binds a complex comprising CSL and ICN.

10 4. The modified polypeptide of claim 1, wherein the MAML polypeptide ins selected from human MAML-1, MAML-2 and MAML-3.

5. The polypeptide of claim 1, wherein the polypeptide reduces the viability of at least one of MOLT4 cells, ALL-SIL T-ALL cells, KOPTK1 T-ALL
15 cells and TALL1 cells.

6. The modified polypeptide of claim 1, wherein x is 3.

7. The modified polypeptide of claim 1, wherein x is 4.

20

8. The modified polypeptide of claim 1, wherein x is 6.

9. The modified polypeptide of claim 1, wherein x is 2 or 6; R_3 is an alkenyl containing a single double bond, and both R_1 and R_2 are H.

25

10. The modified polypeptide of claim 1, wherein each y is independently an integer between 3 and 15.

11. The modified polypeptide of claim 1, wherein R_1 and R_2 are each
30 independently H or C_1 - C_6 alkyl.

12. The modified polypeptide of claim 1, wherein R_1 and R_2 are each independently C_1 - C_3 alkyl.
13. The modified polypeptide of claim 11, wherein at least one of R_1 and R_2 are methyl.
14. The modified polypeptide of claim 12, wherein R_1 and R_2 are methyl.
15. The modified polypeptide of claim 1, wherein R_3 is alkyl.
16. The modified polypeptide of claim 14, wherein x is 3.
17. The modified polypeptide of claim 15, wherein R_3 is C_8 alkyl.
18. The modified polypeptide of claim 14, wherein x is 6.
19. The modified polypeptide of claim 17, wherein R_3 is C_{11} alkyl.
20. The modified polypeptide of claim 1, wherein R_3 is alkenyl.
21. The modified polypeptide of claim 18, wherein x is 3.
22. The modified polypeptide of claim 20, wherein R_3 is C_8 alkenyl.
23. The modified polypeptide of claim 19, wherein x is 6.
24. The modified polypeptide of claim 19, wherein R_3 is C_{11} alkenyl.
25. The modified polypeptide of claim 1, wherein R_3 is a straight chain alkyl, alkenyl, or alkynyl.

26. The modified polypeptide of claim 1, wherein R_3 is $[R_4-K-R_4]$; and R_4 is a straight chain alkyl, alkenyl, or alkynyl.

27. The modified polypeptide of claim 1, wherein the polypeptide
5 comprises at least 8 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4 except that within the 8 contiguous amino the side chains of at least one pair of amino acids separated by 3 or 6 amino acids is replaced the linking group R_3 as depicted in Formula I which connects the alpha carbons of the pair of amino acids and the alpha carbon of the first of the pair of amino acids is substituted with R_1 as
10 depicted in formula I and the alpha carbon of the second of the pair of amino acids is substituted with R_2 as depicted in Formula I.

28. The modified polypeptide of claim 1 wherein $[Xaa]_w$ comprises an amino acid sequence selected from: AVMERL, RLRRRI, LCR, LCRRHH, TCE,
15 RYE, TCEARY CEARYE, LERQHT, HTF, and HTFALH.

29. The modified polypeptide of claim 15, wherein at least one of R_1 or R_2 is alkyl.

30. The modified polypeptide of claim 11, wherein each R_1 and R_2 is independently H or C_1 - C_3 alkyl.

31. The modified polypeptide of claim 1, wherein R_1 and R_2 are methyl.

32. The modified polypeptide of claim 1, wherein x is 3 or 6 and z is 1.

33. The modified polypeptide of claim 1, wherein R_3 is C_8 or C_{11} alkyl or alkenyl.

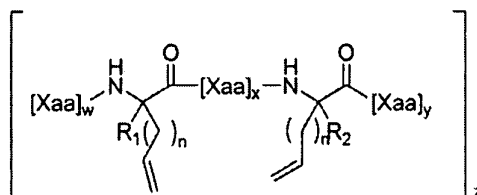
37. The modified polypeptide of claim 1, further comprising a copolymer of lactic and glycolic acid

38. The modified polypeptide of claim 1, further comprising a PEG.

39. The modified polypeptide of claim 1, further comprising an targeting moiety.

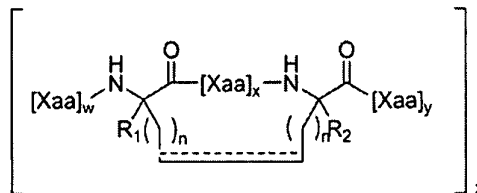
5 40. The modified polypeptide of claim 1, further comprising a biotin moiety.

41. A method of making a polypeptide of Formula (III), comprising providing a polypeptide of Formula (II); and



Formula (II)

treating the compound of Formula (II) with a catalyst to promote a ring closing metathesis, thereby providing a compound of Formula (III)



Formula (III)

wherein

each R_1 and R_2 are independently H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl; heteroarylalkyl; or heterocyclalkyl;

each n is independently an integer from 1-15;

x is 3, 4, or 6

each y is independently an integer from 0-100;

z is an integer from 1-3; and

each Xaa is independently an amino acid; and

wherein the polypeptide comprises an alpha helical structure in aqueous solution.

42. The method of claim 41, wherein the polypeptide binds to a complex comprising ICN and CSL.

5 43. The method of claim 41, wherein the catalyst is a ruthenium catalyst.

44. The method of claim 41, further comprising providing a reducing agent or oxidizing agent subsequent to the ring closing metathesis.

10 45. The method of claim 44, wherein the reducing agent is H₂ or oxidizing agent is osmium tetroxide

46. A method of treating a subject comprising administering to the subject a compound of claim 1.

15

47. The method of claim 46, further comprising administering an additional therapeutic agent.

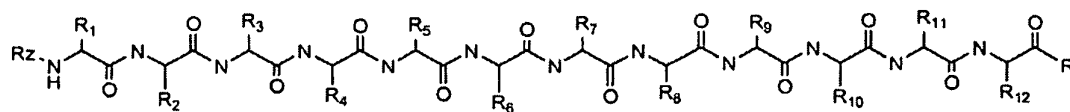
48. A method of treating cancer in a subject comprising administering to
20 the subject a compound of claim 1.

49. The method of claim 48, further comprising administering an additional therapeutic agent.

25 50. A library of compounds of claim 1, formula (I).

51. A compound having the formula

30



5

R_1 is $-\text{CH}_2\text{CH}_2\text{COOH}$ [E] or $-\text{CH}_2\text{COOH}$ [D] or R_1 and R_8 together are R_x ;

R_2 is $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ [L] or $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{H})\text{C}(\text{NH})\text{NH}_2$ [R];

10

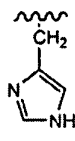
R_3 is $-\text{CH}_2\text{CH}_2\text{COOH}$ [E]

R_4 is $-\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{H})\text{C}(\text{NH})\text{NH}_2$ [R];

15

R_5 is $-\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$ [Q], $-\text{CH}_2\text{CH}_2\text{COOH}$ [E], or $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{H})\text{C}(\text{NH})\text{NH}_2$ [R]; or R_5 and R_9 together are R_y ; or R_5 and R_{12} together are R_x ;

R_6 is $-\text{CH}_2\text{OH}$ [S],



[H] or $-\text{CH}_2\text{COOH}$ [D];

20

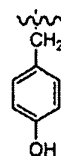
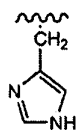
R_7 is $-\text{C}(\text{OH})\text{CH}_3$ [T];

R_8 is benzyl [F], $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ [L], $-\text{CH}(\text{CH}_3)_2$ [V] or R_1 and R_8 together are R_x ;

25

R_9 is selected from: $-\text{CH}_3$ [A] or $-\text{CH}_2\text{CH}_2\text{C}(\text{O})\text{NH}_2$ [Q] and $-\text{CH}_2\text{OH}$ [S]; or R_5 and R_9 together are R_y ;

R_{10} is $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ [L];



R₁₁ is [H], -CH₂CH(CH₃)₂ [L] and

R₁₂ is -CH₂CH₂C(O)NH₂ [Q] and -CH₂OH [S]; or R₅ and R₁₂ together are R_x

5 provided that when R₁ and R₈ together are R_x, R₅ and R₉ are not together R_y and R₅ and R₁₂ are not together R_x;


further provided that when R₅ and R₉ together are R_y, R₁ and R₈ are not together R_x and R₅ and R₁₂ are not together R_x;

10 further provided that when R₅ and R₁₂ are together R_x, R₅ and R₉ are not together R_y and R₁ and R₈ are not together R_x;

R_x and R_y alkyl, alkenyl, alkynyl; [R_{x1}-K-R_{x1}]_n; each of which is substituted with 0-6 R_{x2};

R_{x1} is alkyl, alkenyl, or alkynyl;

15 R_{x2} is halo, alkyl, OR_{x3}, N(R_{x3})₂, SR_{x3}, SOR_{x3}, SO₂R_{x3}, CO₂R_{x3}, R_{x3}, a fluorescent moiety, or a radioisotope;

K is O, S, SO, SO₂, CO, CO₂, CONR_{x3}, or  ;

R_{x3} is H, alkyl or a therapeutic agent; and

R_z and R_w are independently: H, hydroxyl, an amino acid, 2 to 10 amino acids linked by peptide bonds; tat; and PEG.

20

52. The polypeptide of claim 51, wherein each y is independently an integer between 3 and 15.

53. The polypeptide of claim 51, wherein R₁ and R₂ are each
25 independently H or C₁-C₆ alkyl.

54 The polypeptide of claim 51, wherein R₁ and R₂ are each independently C₁-C₃ alkyl.

55. The polypeptide of claim 51, wherein at least one of R_1 and R_2 are methyl.
- 5 56. The polypeptide of claim 51, wherein R_1 and R_2 are methyl.
57. The polypeptide of claim 51, wherein R_3 is alkyl.
58. The polypeptide of claim 51, wherein x is 3.
- 10 59. The polypeptide of claim 51, wherein R_3 is C_8 alkyl.
60. The polypeptide of claim 51, wherein x is 6.
- 15 61. The polypeptide of claim 51, wherein R_3 is C_{11} alkyl.
62. The polypeptide of claim 51, wherein R_3 is alkenyl.
63. The polypeptide of claim 51, wherein R_3 is C_8 alkenyl.
- 20 64. The polypeptide of claim 51, wherein R_3 is C_{11} alkenyl.
65. The polypeptide of claim 51, wherein R_3 is a straight chain alkyl, alkenyl, or alkynyl.
- 25 66. The polypeptide of claim 51, wherein R_3 is $[R_4-K-R_4]$; and R_4 is a straight chain alkyl, alkenyl, or alkynyl.
66. The polypeptide of claim 51, wherein x is 3 and z is 1.
- 30 67. The polypeptide of claim 51, wherein the polypeptide is transported through the cell membrane.

68. The polypeptide of claim 1 wherein polypeptide comprises an amino acid sequence selected from SEQ ID NOs:8, 9 and 10 wherein: (a) the side chains of amino acids 8 and 12 are replaced by the linking group R_3 which connects the alpha carbons of amino acids 8 and 12 as depicted in formula I; and (b) the alpha carbon of amino acid 8 is substituted with R_1 as depicted in formula I and the alpha carbon of amino acid 12 is substituted with R_2 as depicted in formula I.

S5 Amino Acid Synthesis

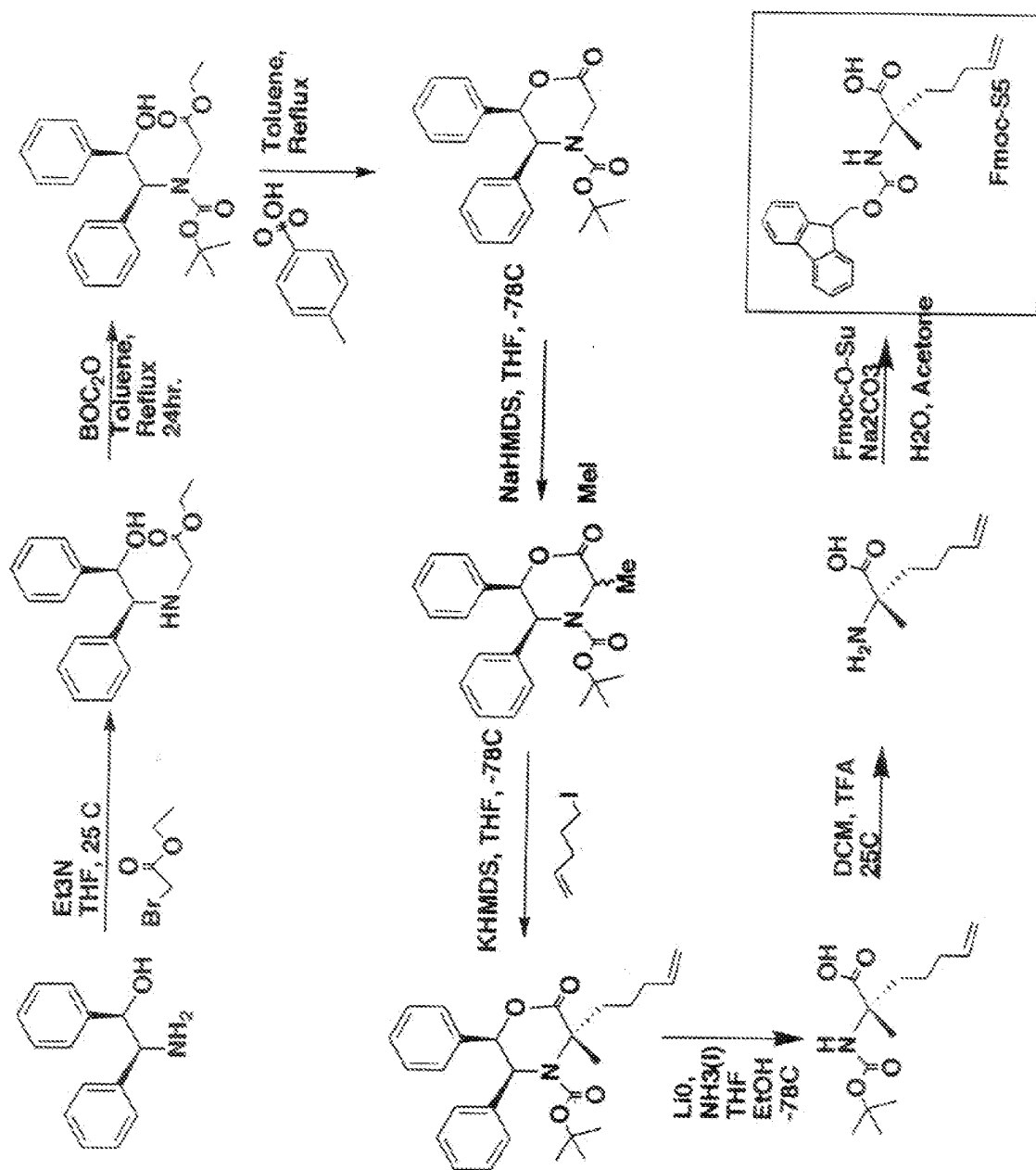
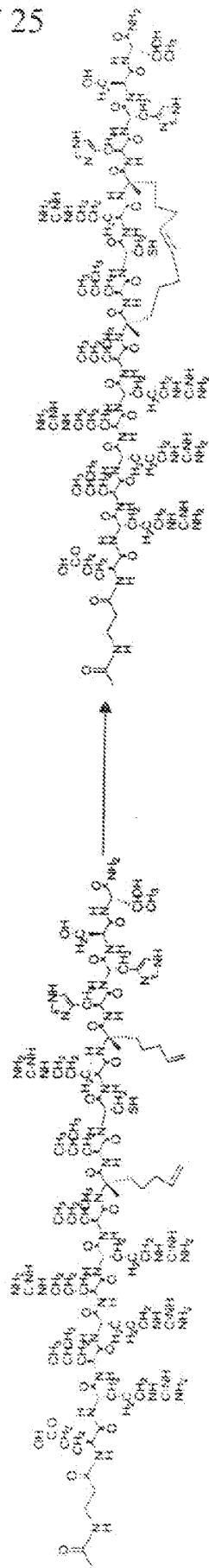
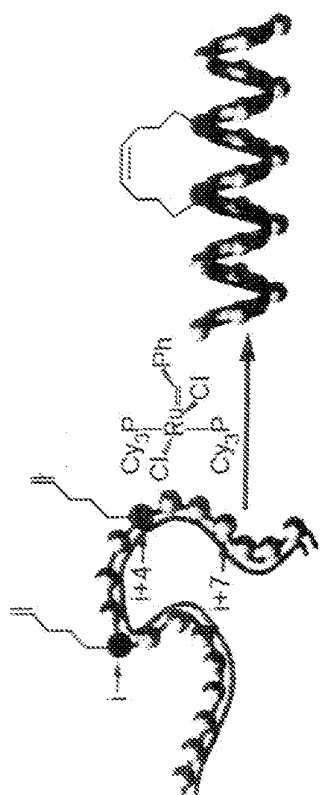


FIG. 2

Inhibitor Design



40% Helical

96% Helical

FIG. 4

Helicity of Stapled Peptides

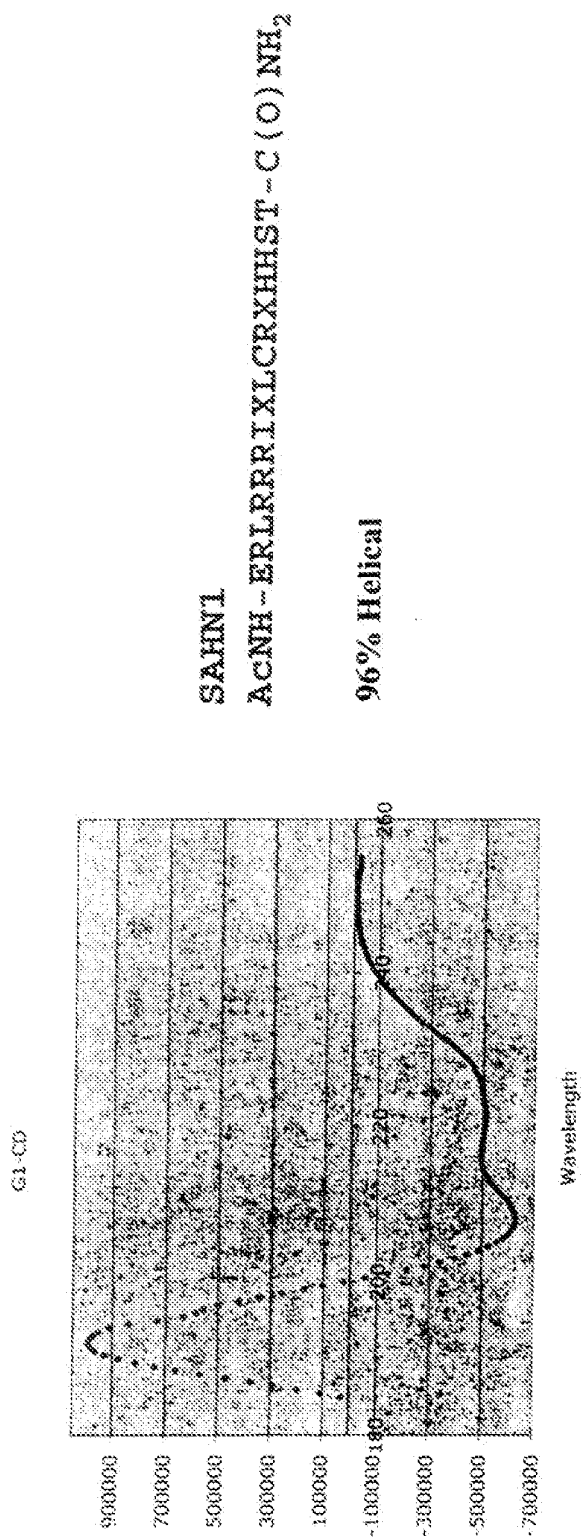


FIG. 5

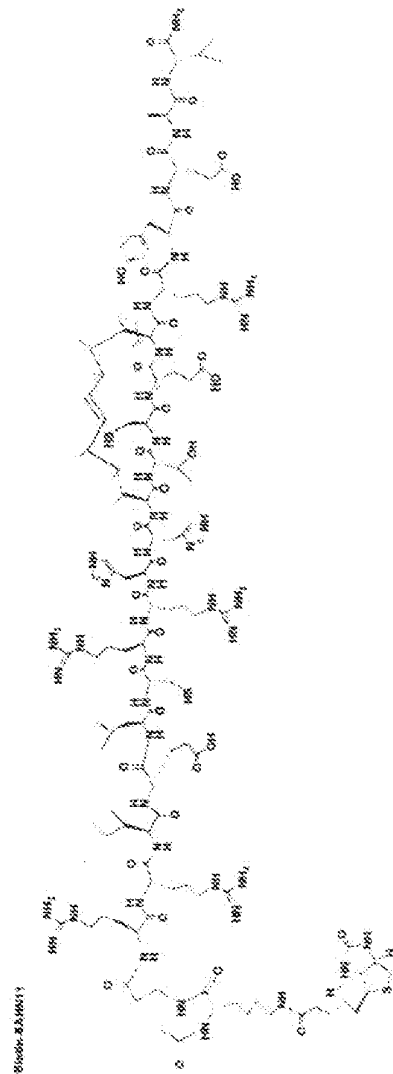
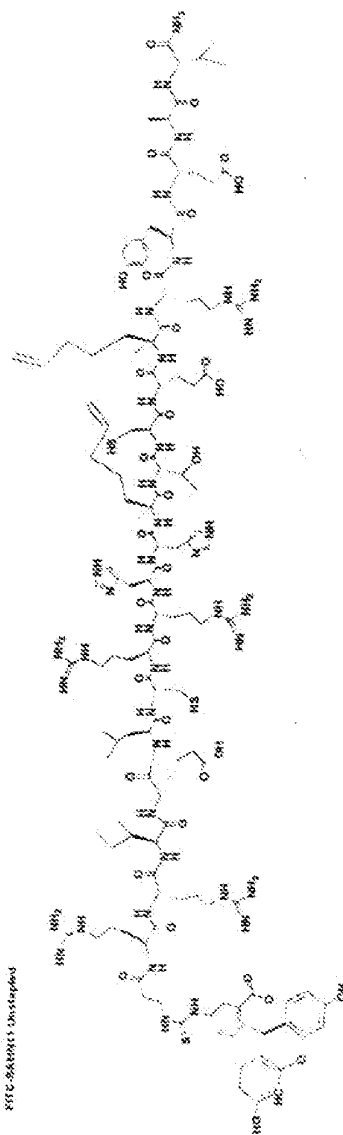
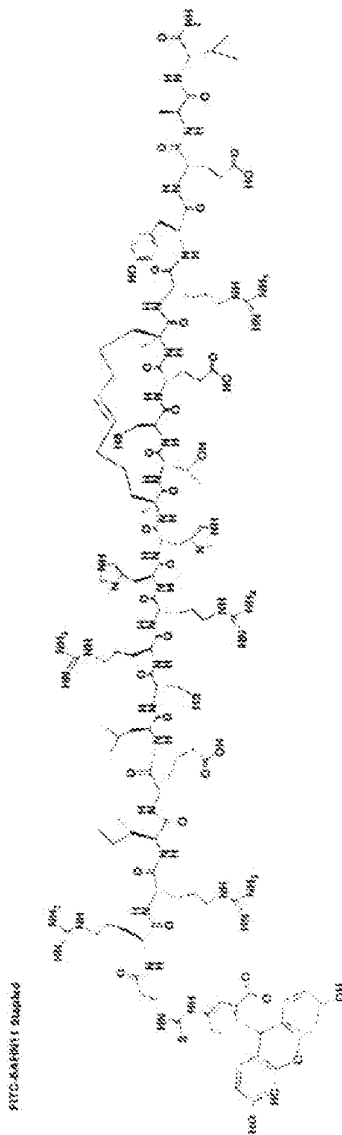
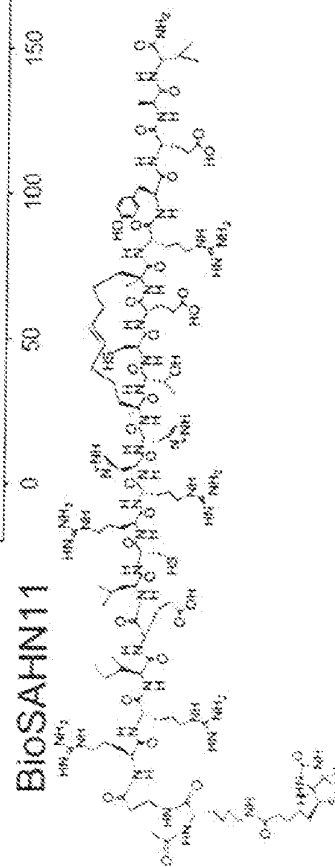
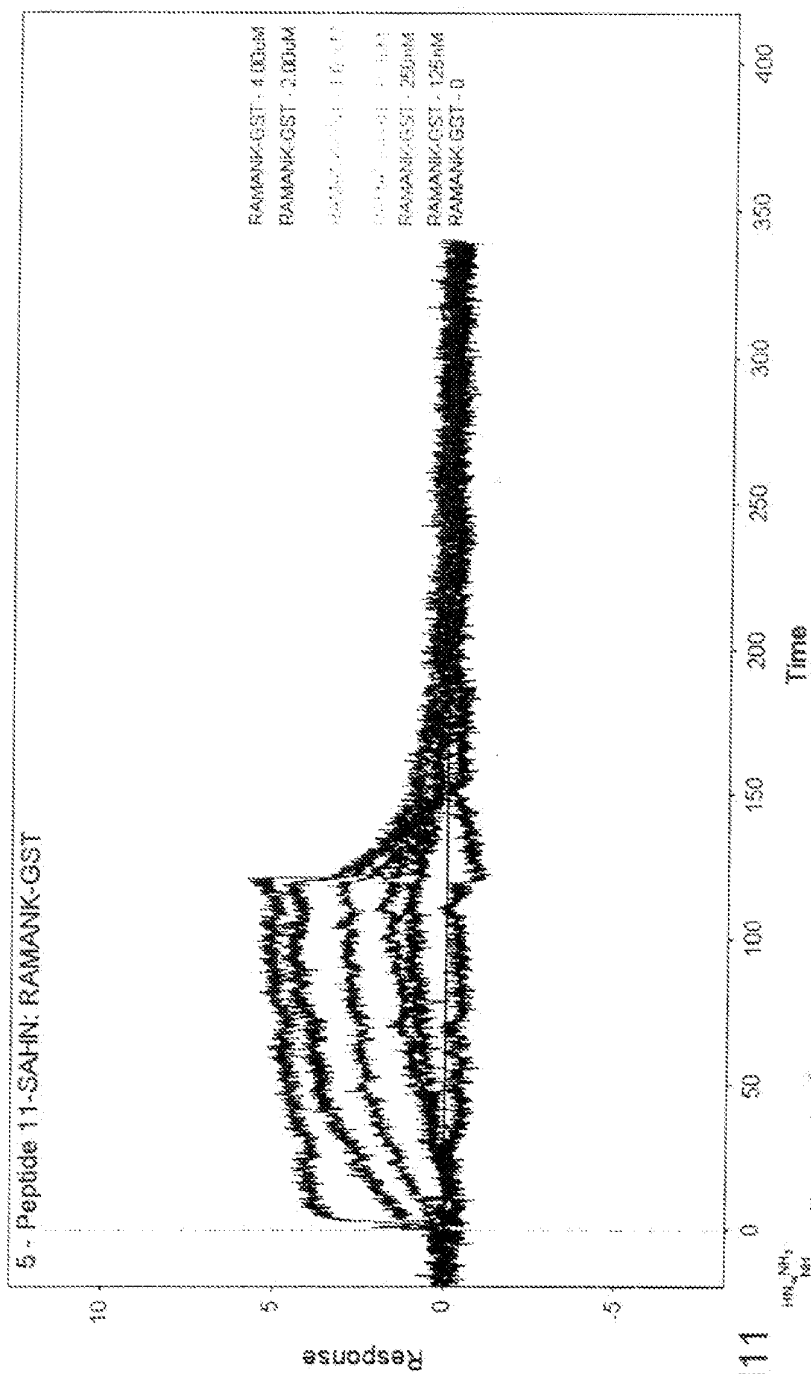


FIG. 6



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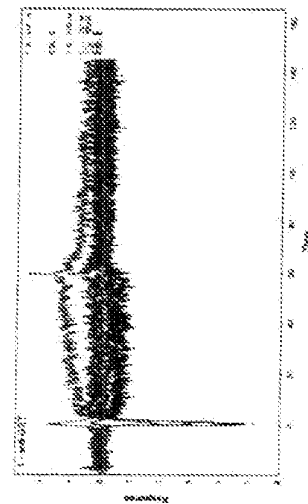
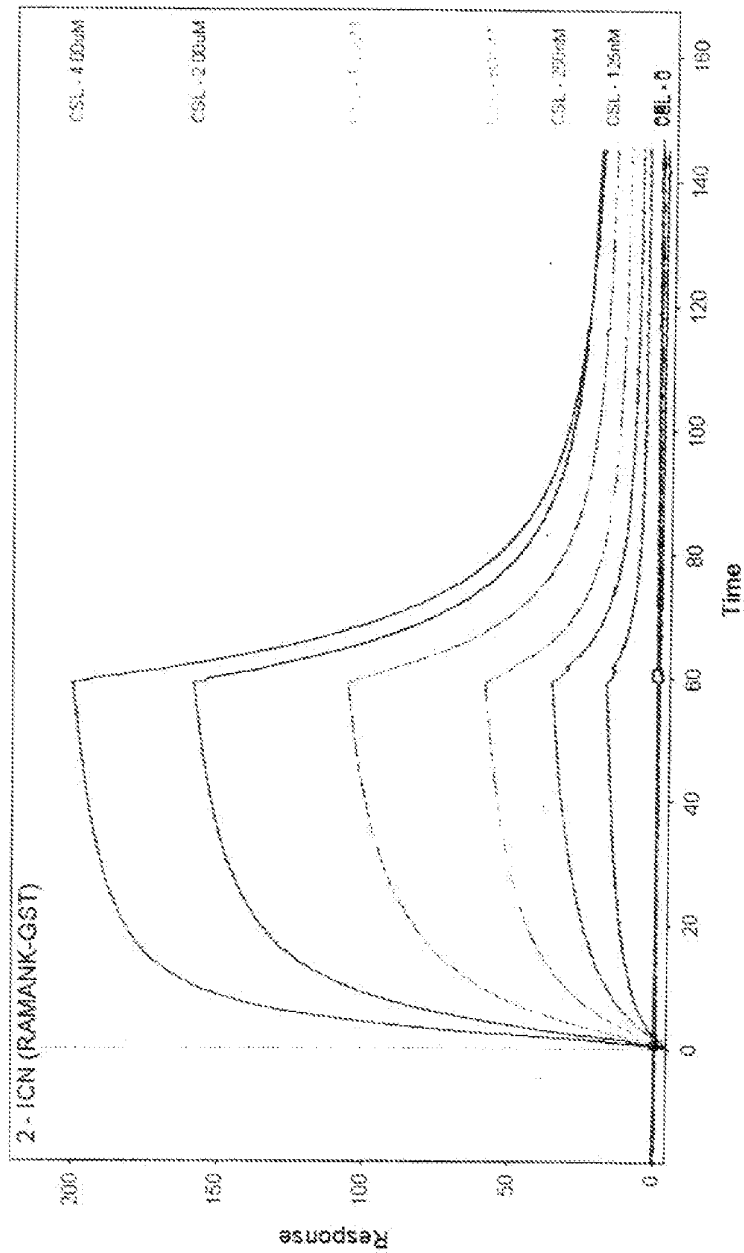


Fig. 8

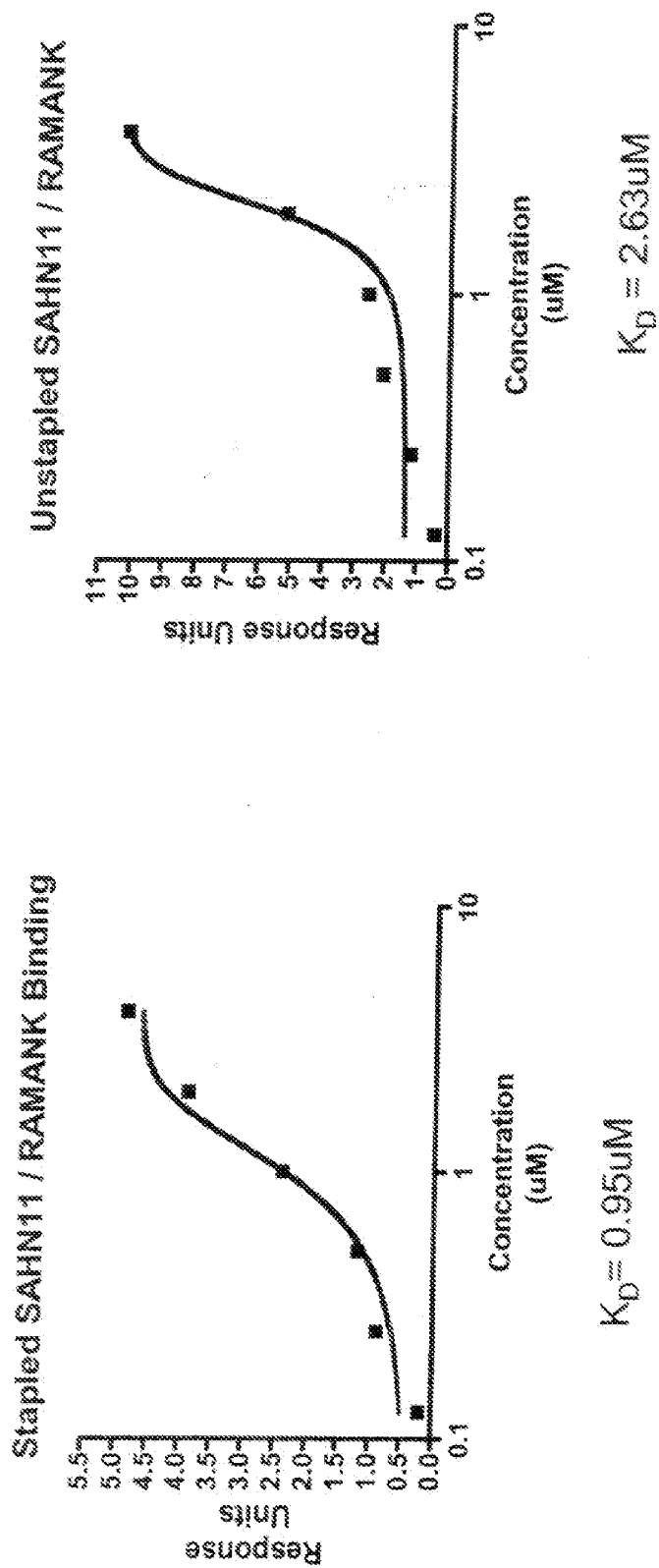


Fig. 9

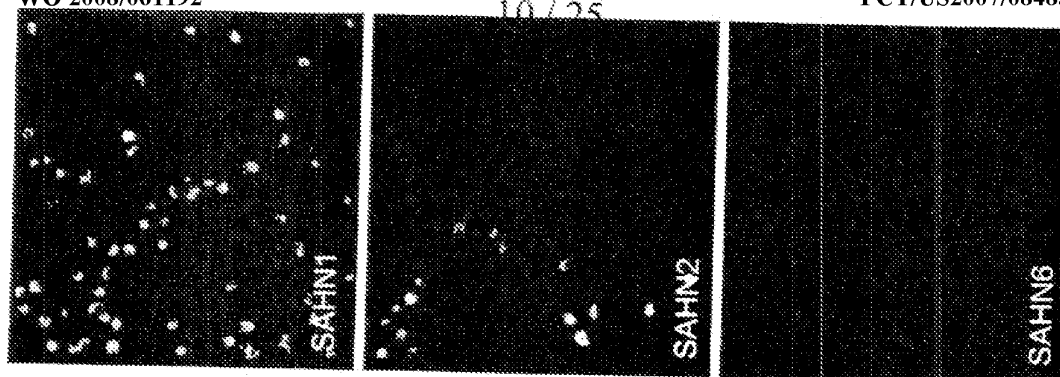
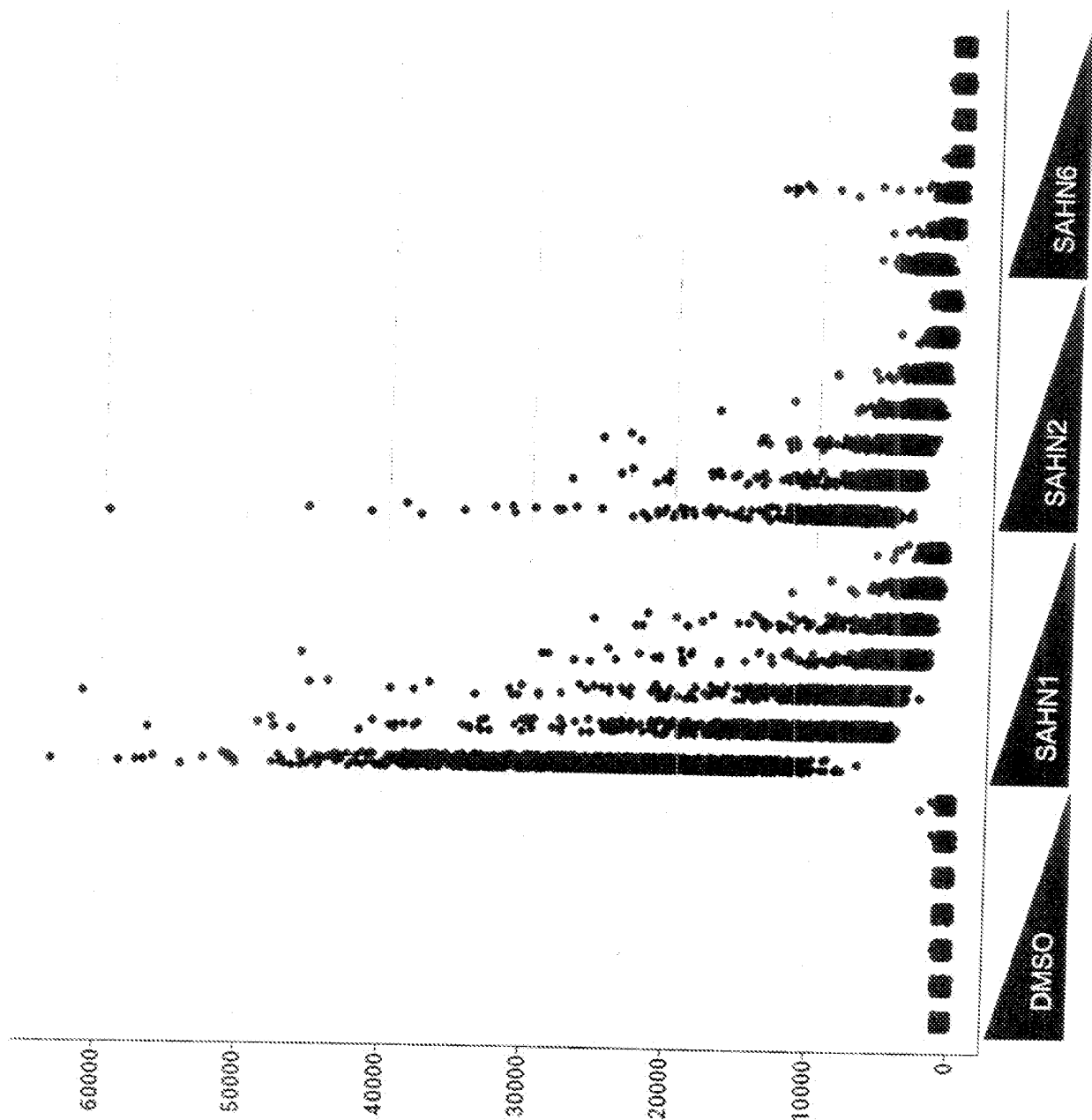


FIG. 10



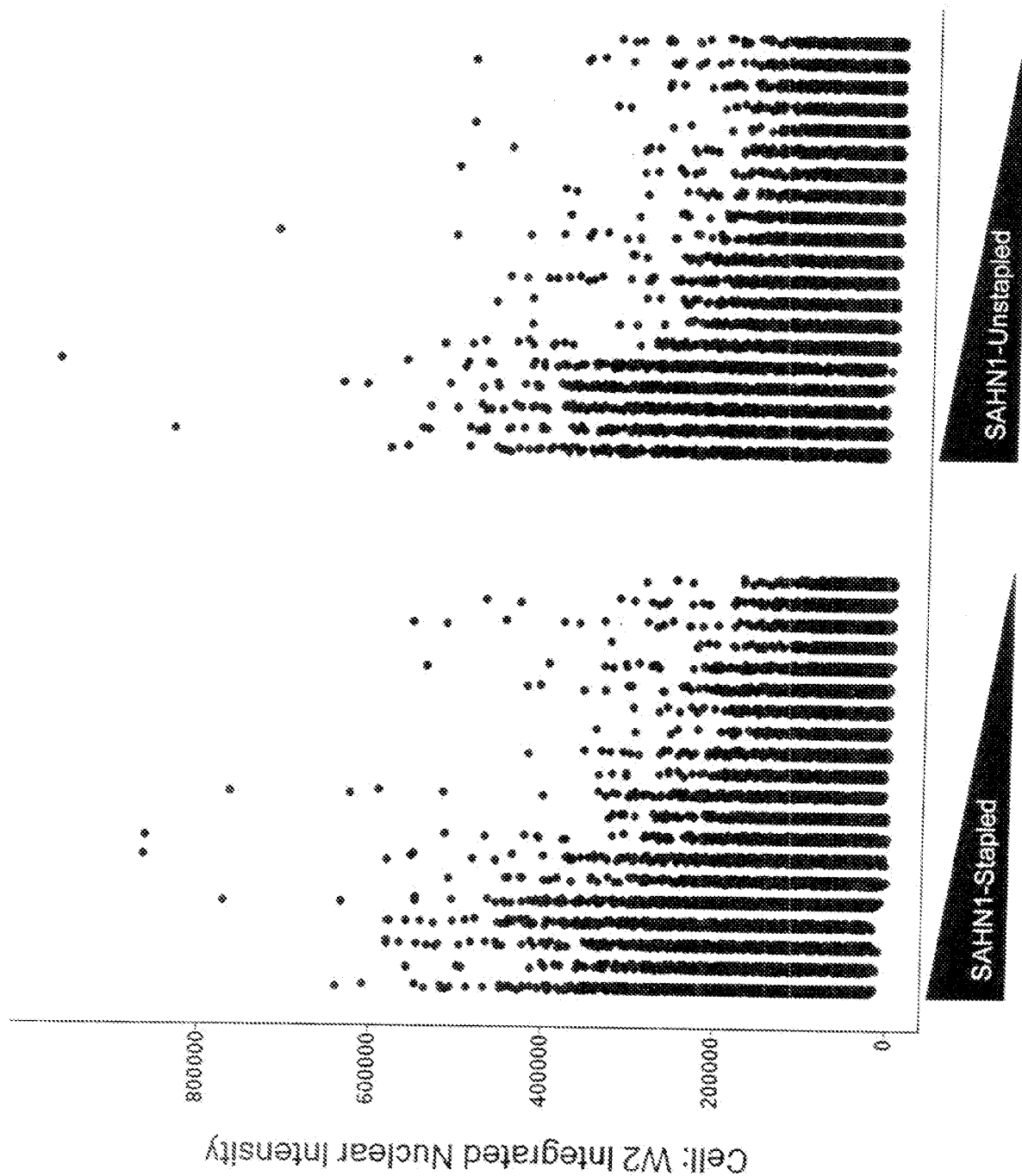


FIG. 11

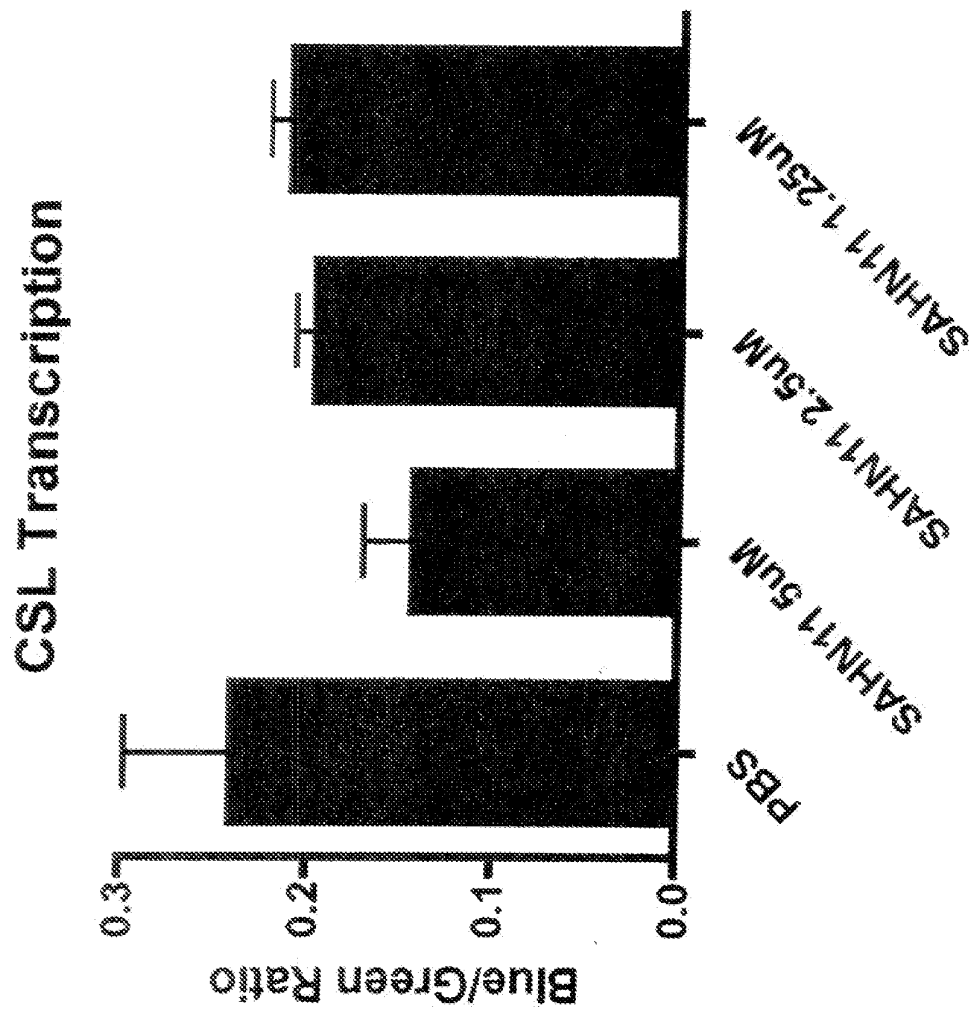


FIG. 12

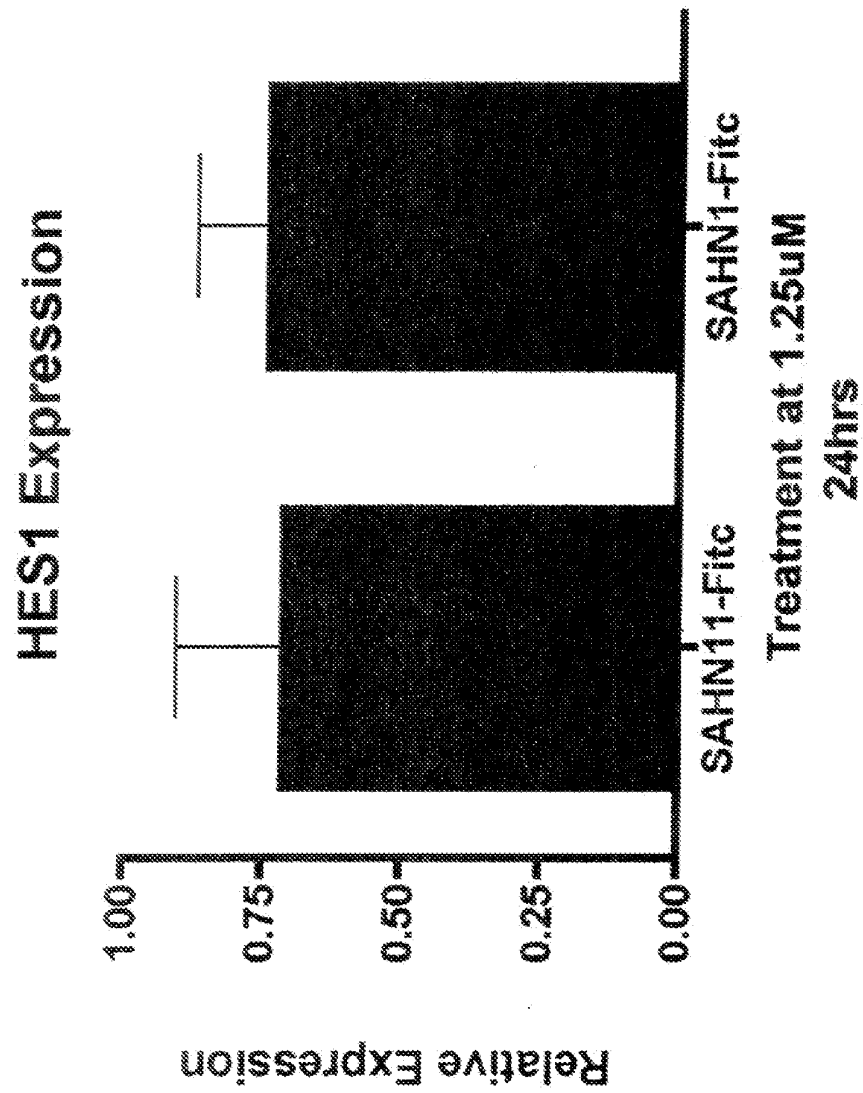


FIG. 13

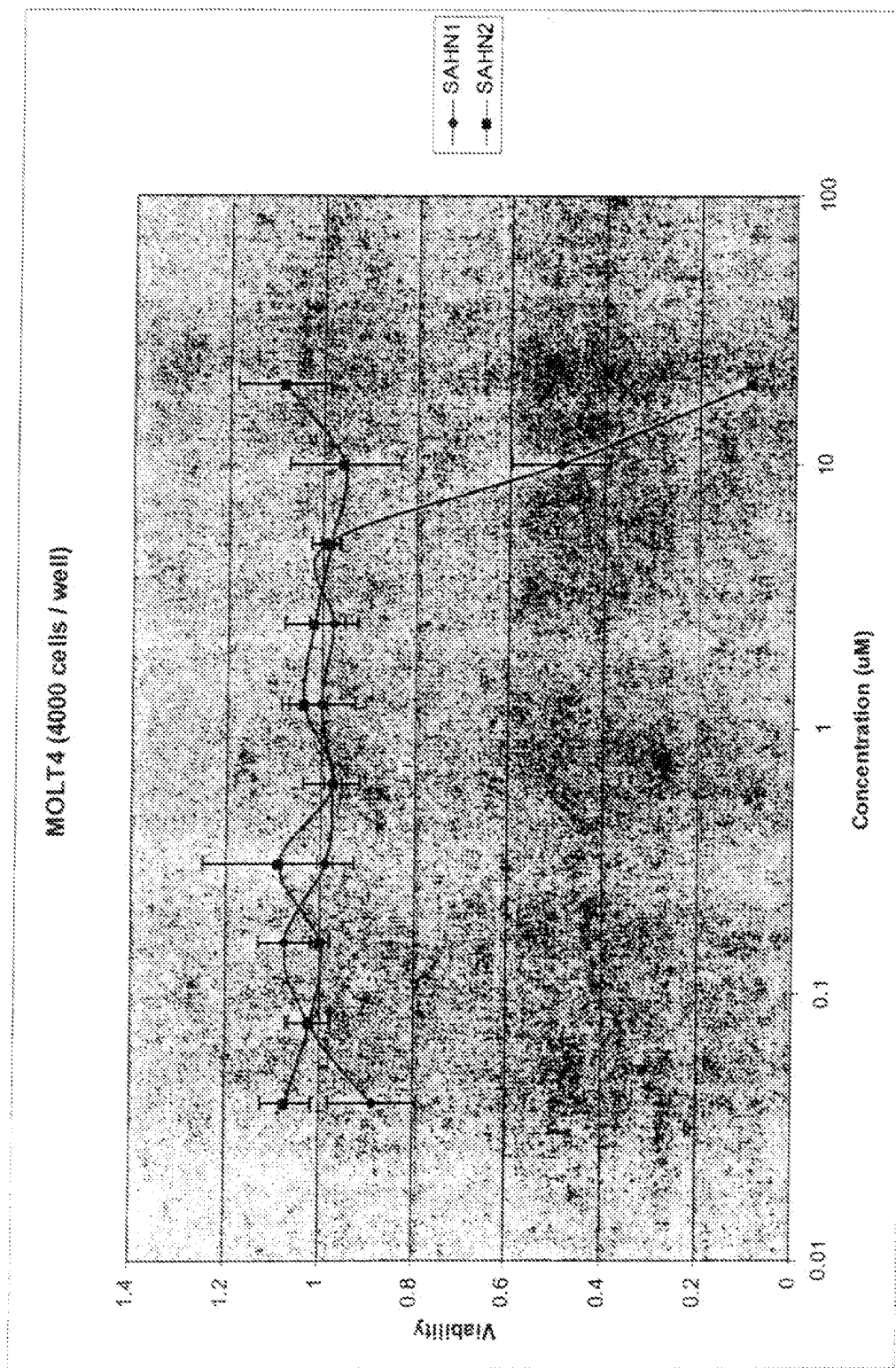


FIG 14

ALL-SIL SAHN1 vs Un

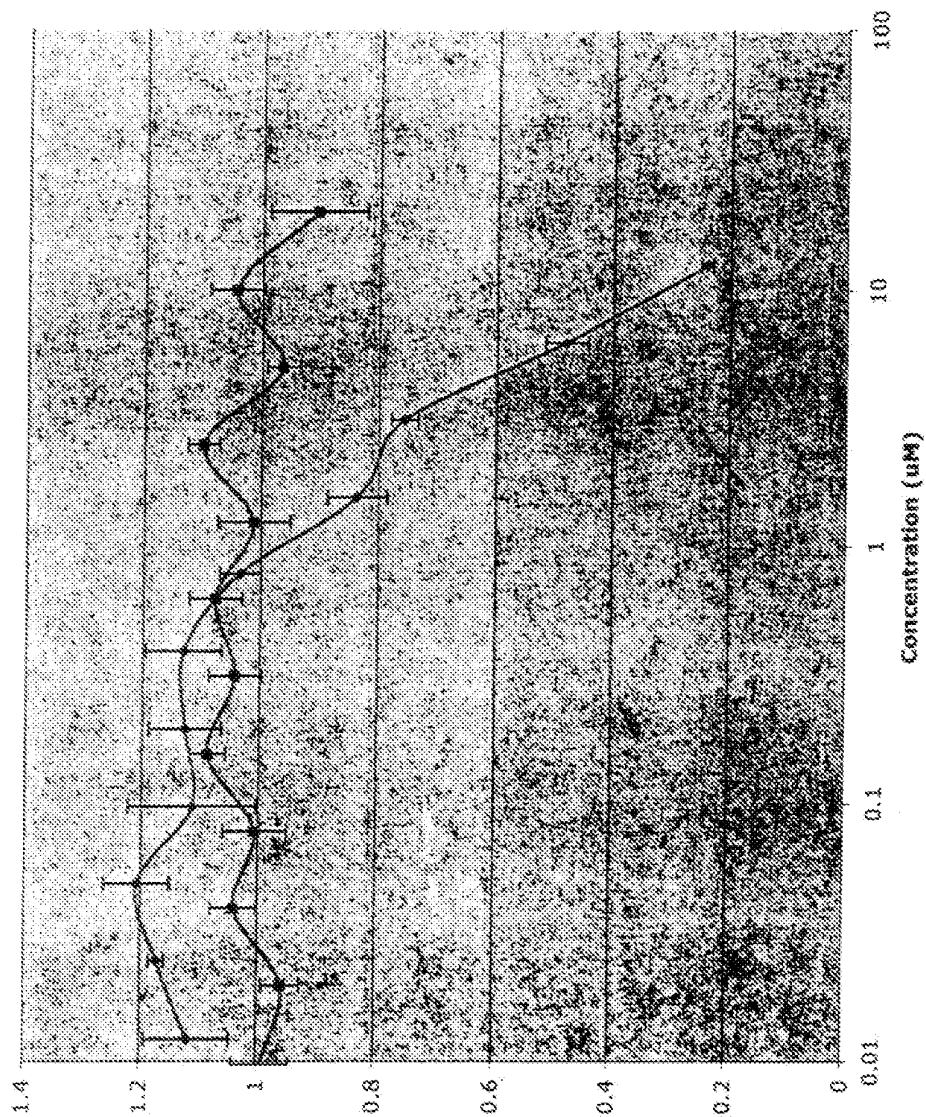


FIG 15

KOPTK1 SAHN1 vs. Unstapled

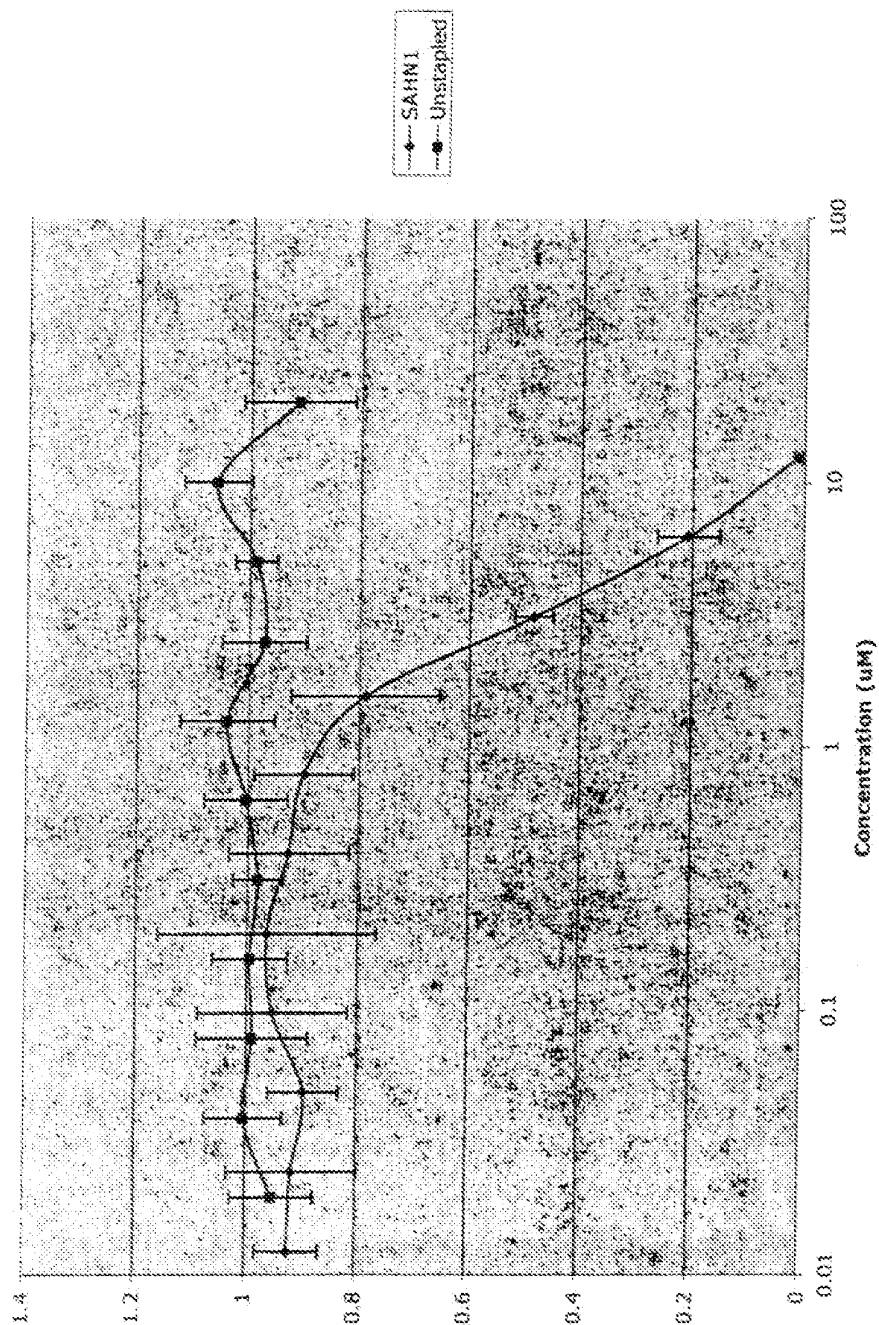


FIG 16

MOLT4 Cytotoxicity

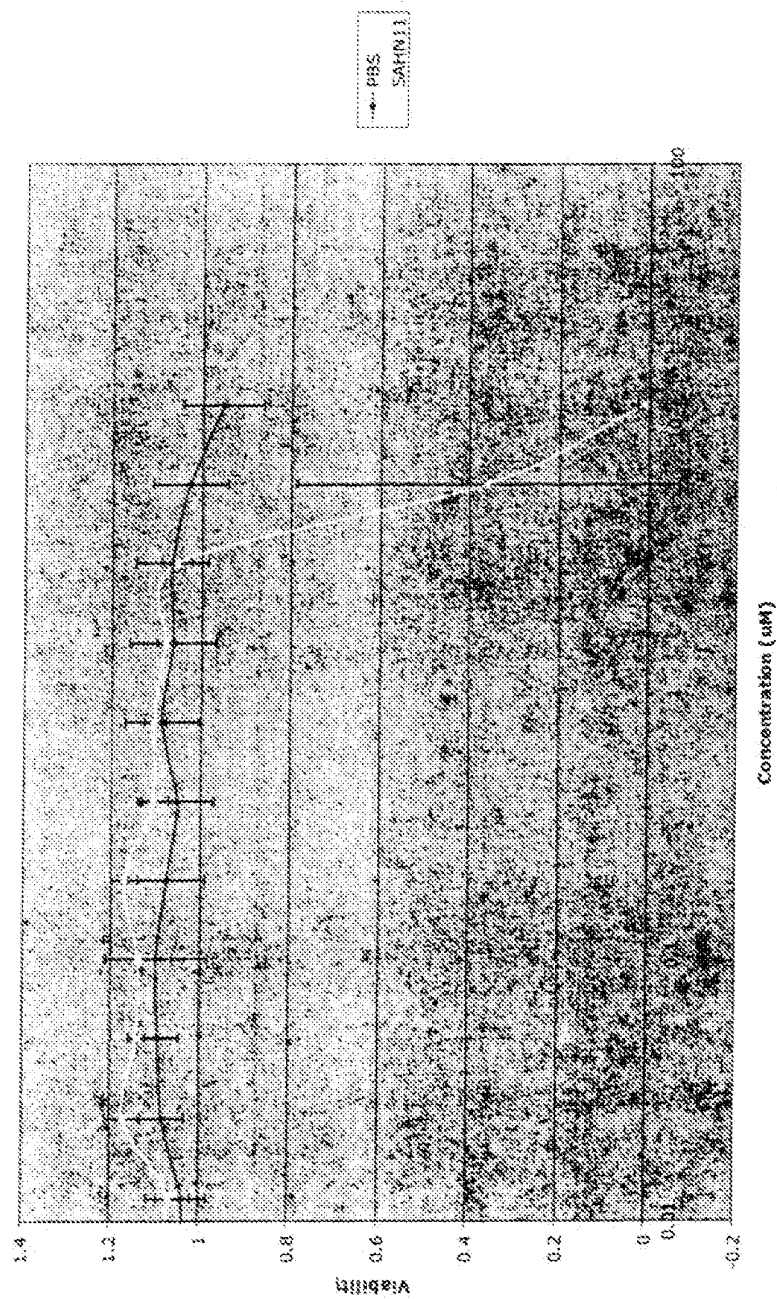


FIG 17

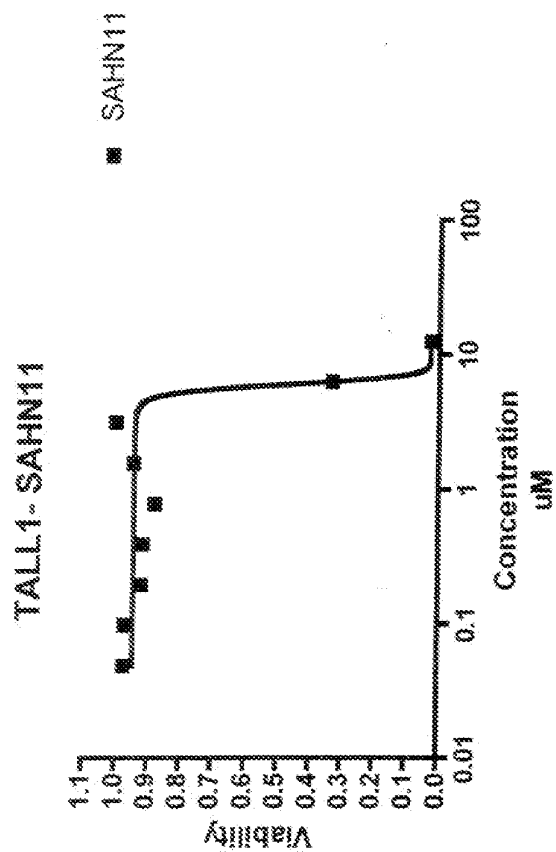


FIG 18

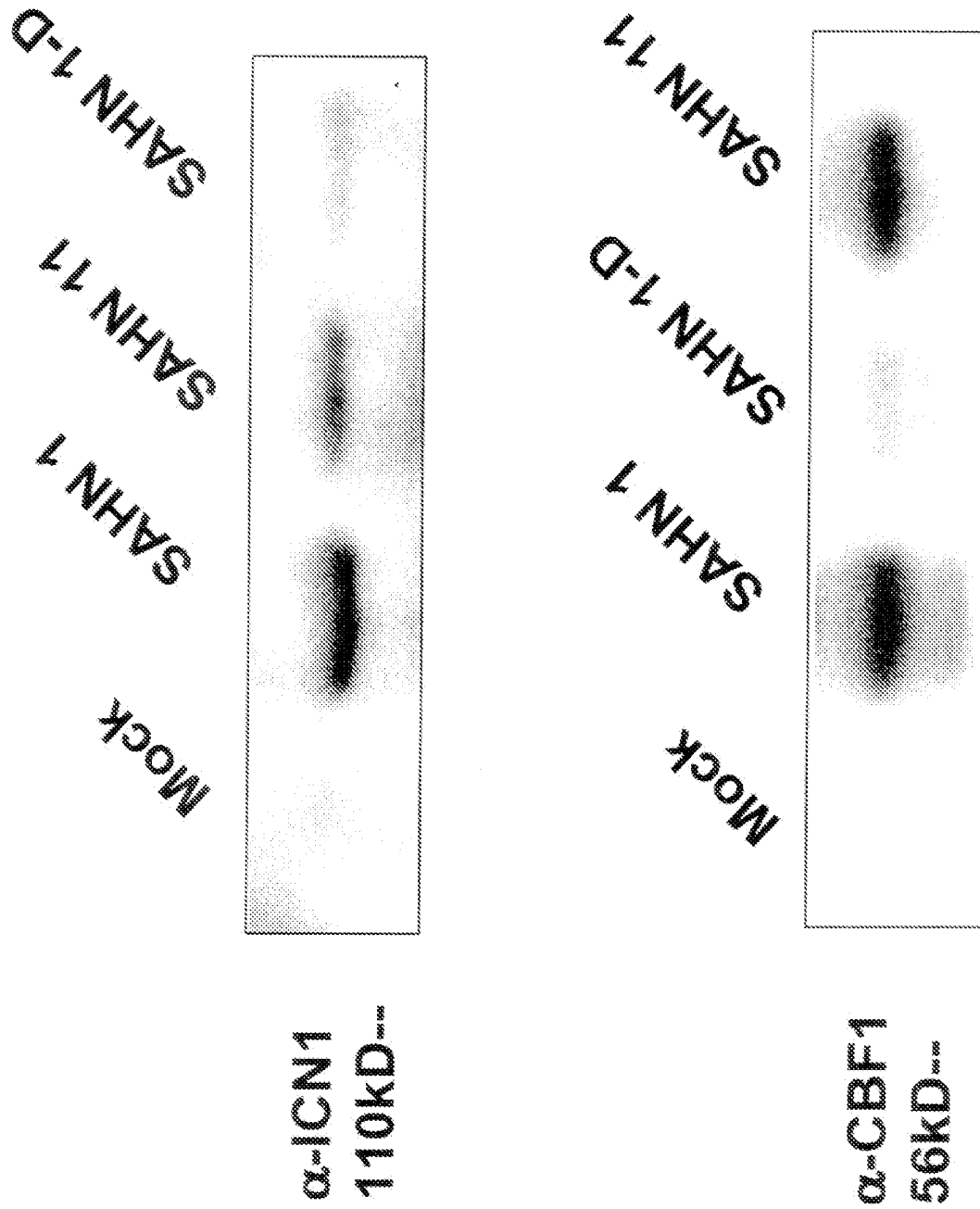


FIG. 19

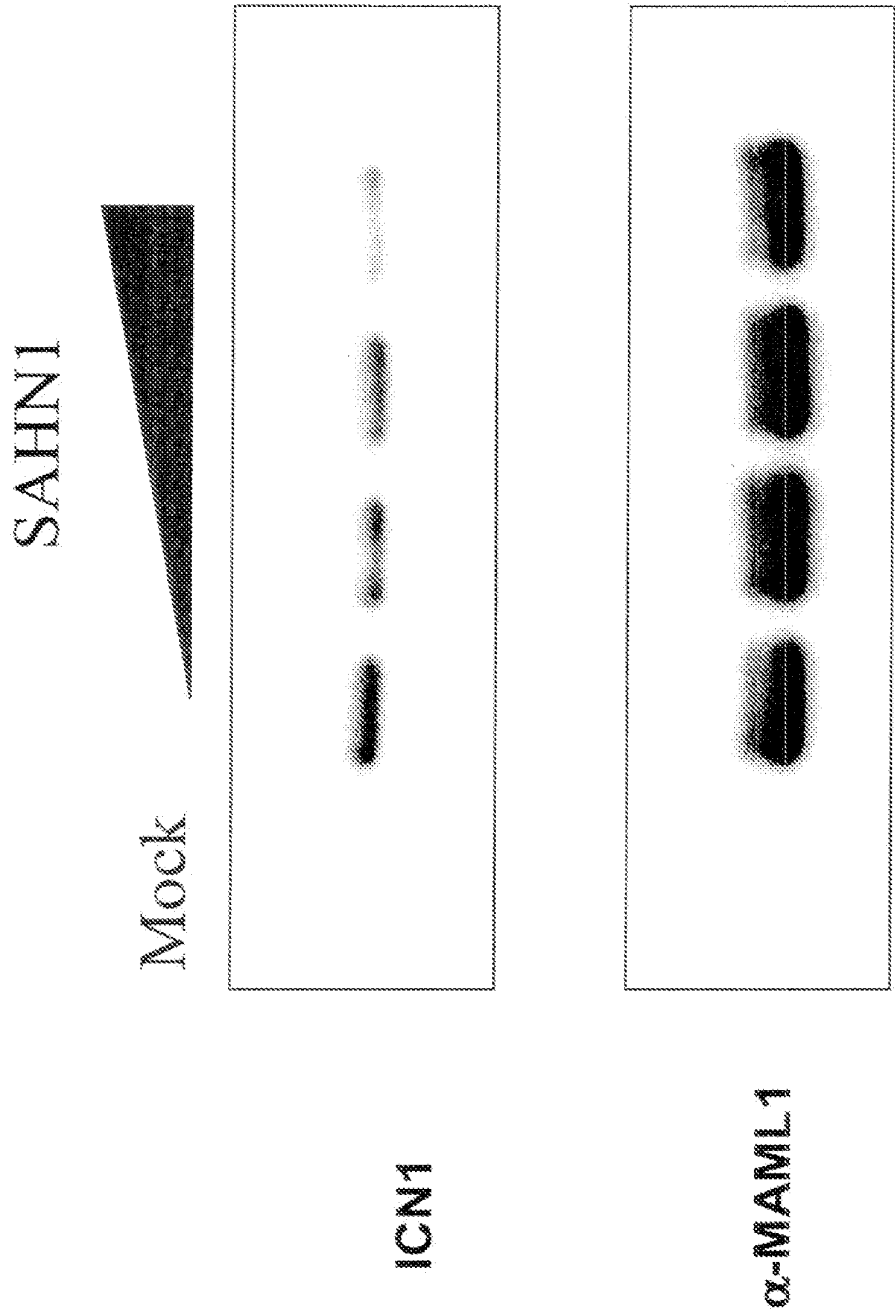


FIG. 20

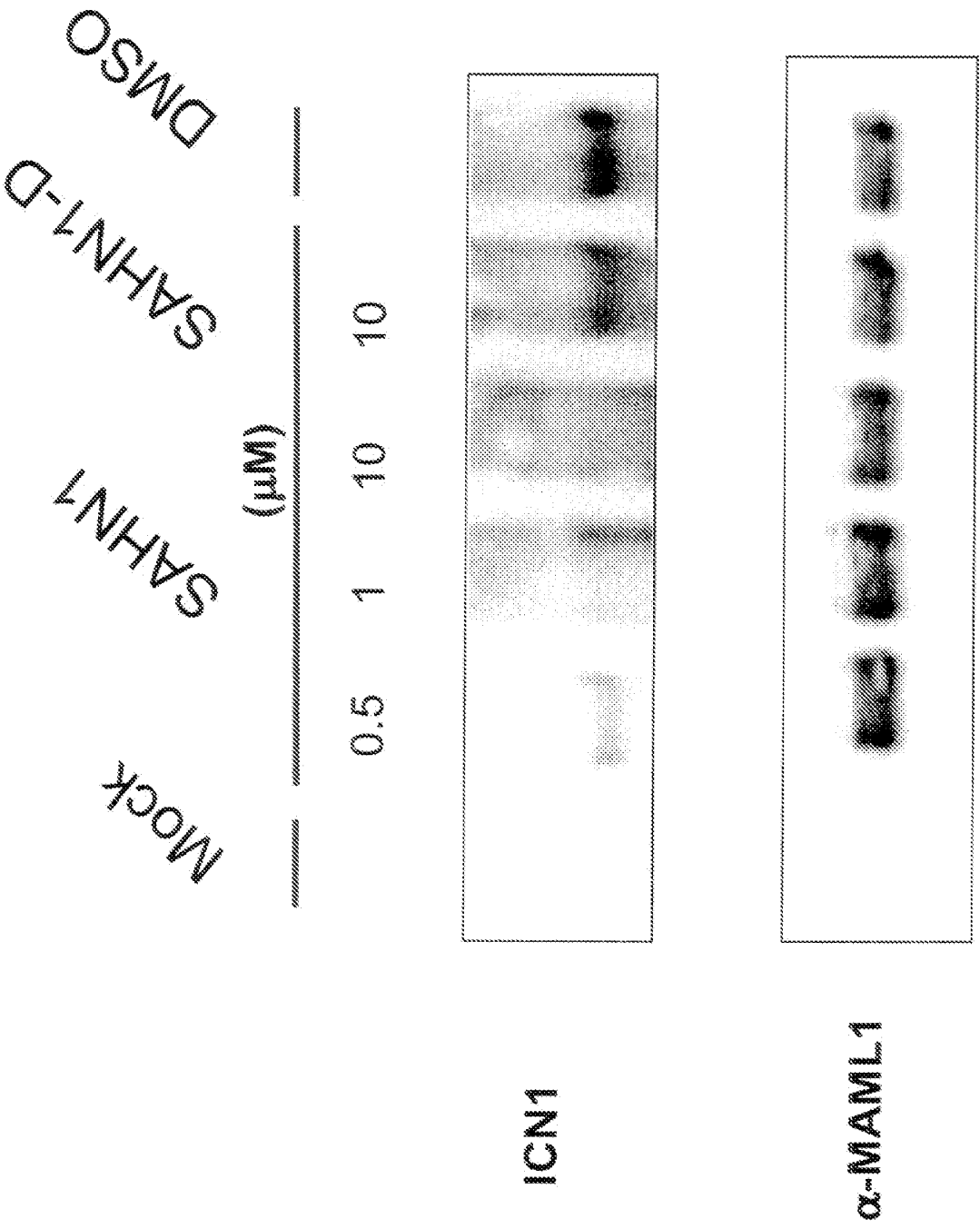


FIG. 21

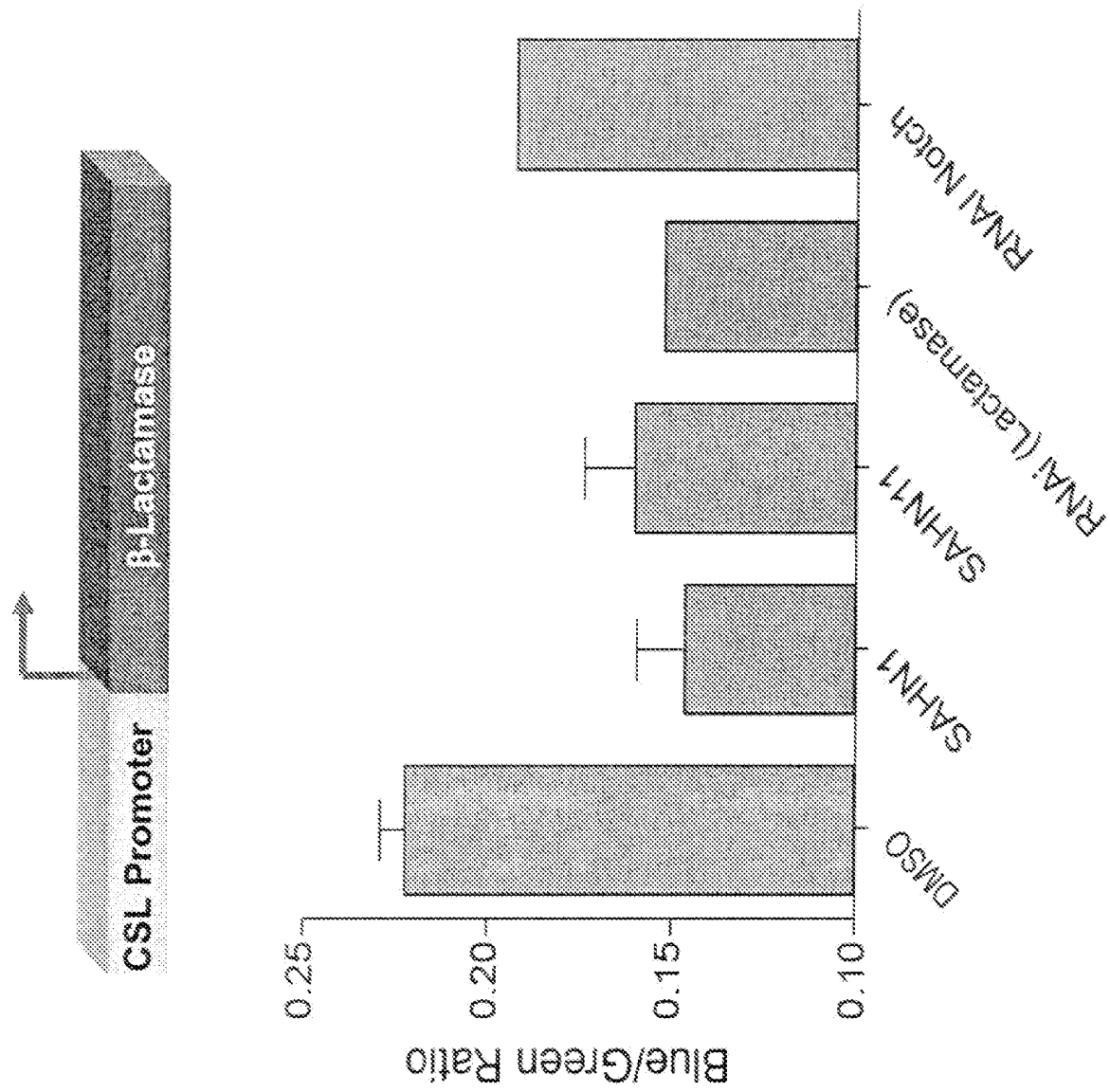


FIG. 22

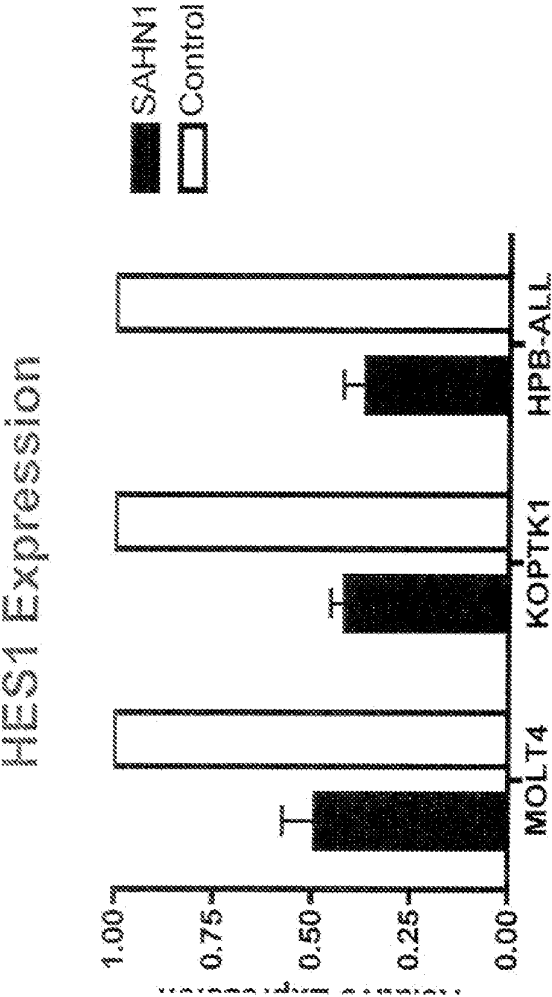
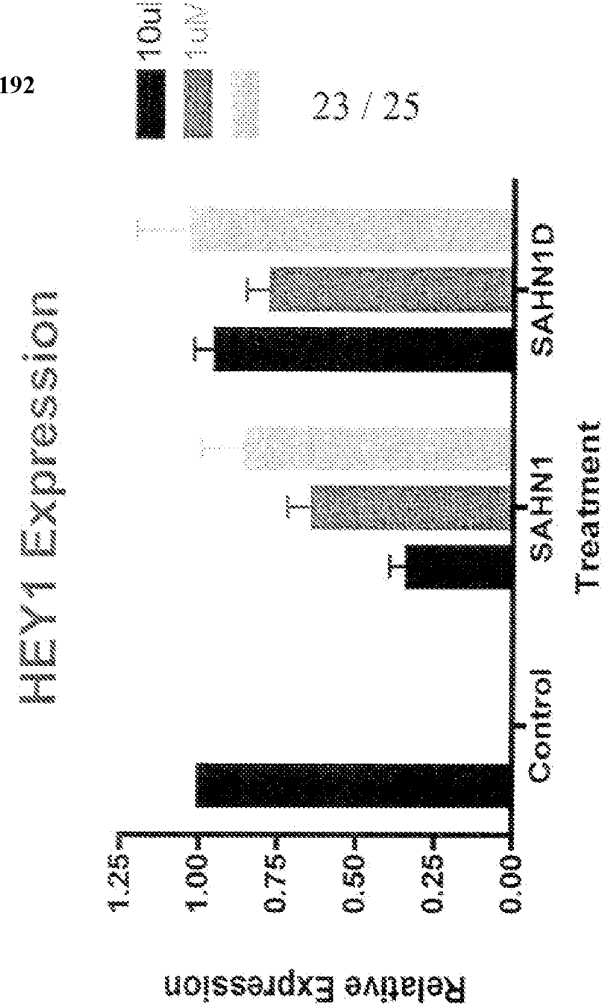


FIG. 23

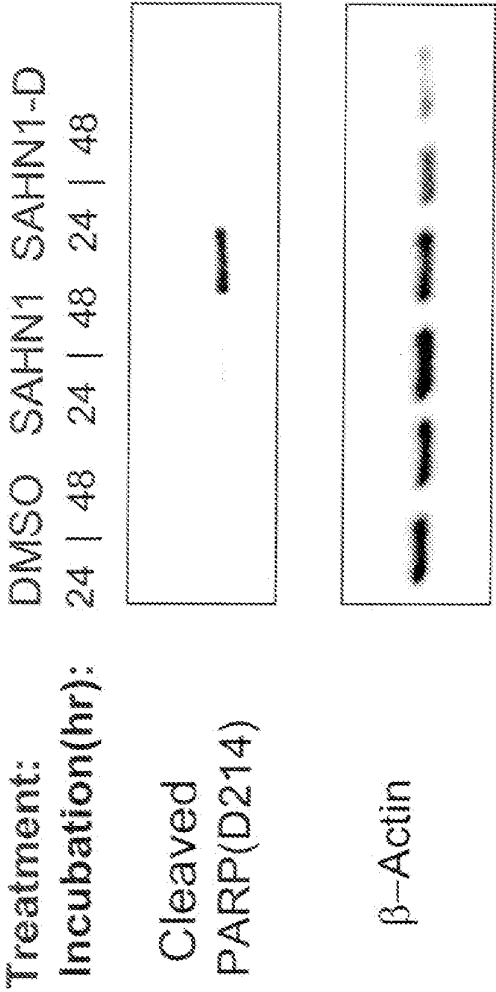


FIG. 24

Cytotoxicity in KOPTK1 Cells

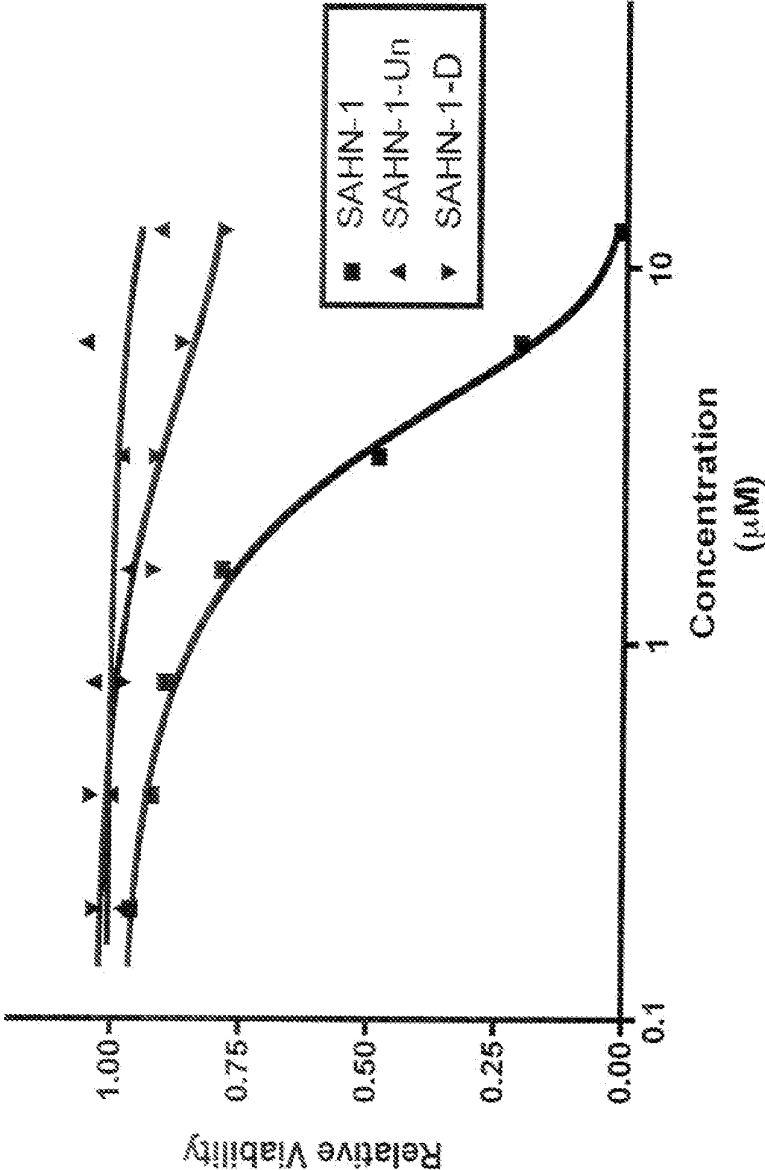


FIG. 25