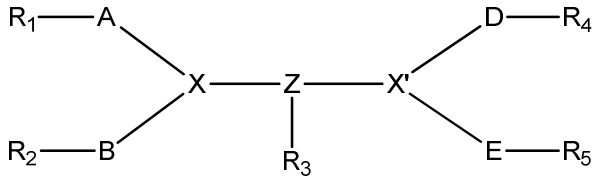
“IMMUNOSUPPRESSION MODULATING COMPOUNDS”

Related application

This application claims the benefit of Indian provisional application number 1805/CHE/2010 filed on June 25, 2010 and US provisional application number 61/381,593 filed on September 10, 2010 all of which hereby incorporated by reference.

FIELD OF INVENTION

The present invention relates to novel peptide compound of formula-I



(I)

or pharmaceutically acceptable salt thereof; wherein; the compound comprises at least 5 amino acids useful as therapeutic agents capable of inhibiting the programmed cell death 1 (PD1) signalling pathway.

The invention also relates to modifications and derivatives of the therapeutic agents.

The invention further relates to pharmaceutical compositions comprising the said novel peptide compound of formula-I or pharmaceutically acceptable salt thereof as therapeutic agents.

The compounds of the present invention are useful in therapy relating to particular diseases or associated disorders where there is an advantage in immuno-potentiation comprising inhibition of an immunosuppressive signal induced due to PD-1, PD-L1, or PD-L2.

The present invention relates to novel peptides as therapeutic agents capable of inhibiting the programmed cell death 1 (PD1) signalling pathway.

The invention also relates to modifications and derivatives of the therapeutic agents.

The invention further relates to pharmaceutical compositions comprising the said novel peptides and their derivatives as therapeutic agents.

The invention also encompasses the use of the said therapeutic agents, modifications and derivatives for treatment of disorders via immuno-potentiation comprising inhibition of an immunosuppressive signal induced due to PD-1, PD-L1, or PD-L2.

BACKGROUND OF THE INVENTION

Programmed Cell Death 1 or PD-1 (also referred to as PDCD1) is a 50 to 55kD type I membrane glycoprotein (Shinohara T et al, Genomics, 1994, Vol. 23, No. 3, pp.704-706). PD-1 is a receptor of the CD28 superfamily that negatively regulates T cell antigen receptor signalling by interacting with the specific ligands and is suggested to play a role in the maintenance of self tolerance.

PD-1 peptide relates to almost every aspect of immune responses including autoimmunity, tumour immunity, infectious immunity, transplantation immunity, allergy and immunological privilege.

The PD-1 protein's structure comprise of –

- an extracellular IgV domain followed by
- a transmembrane region and
- an intracellular tail

The intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, which suggests that PD-1 negatively regulates TCR signals. Also, PD-1 is expressed on the surface of activated T cells, B cells, and macrophages, (Y. Agata et al., *Int Immunol* **8**, 765, May 1996) suggesting that compared to CTLA-4 ((**C**ytotoxic **T**-**L**ymphocyte **A**ntigen **4,** also known as **CD152** (Cluster of differentiation 152) is a protein that also plays an important regulatory role in the immune system), PD-1 more broadly negatively regulates immune responses.

PD-1 has two ligands, PD-L1 (Programmed Death Ligand 1or PDCD1L1 or B7-H1) (Freeman GJ et al, Journal of Experimental Medicine, 2000, Vol. 19, No. 7, pp.1027-1034) and PD-L2 (Programmed Death Ligand 2 or PDCD1L2 or B7-DC) (Latchman Y et al, Nature Immunology, 2001, Vol. 2, No. 3, pp.261-267), which are members of the B7 family. PD-L1 is known to be expressed not only in immune cells, but also in certain kinds of tumour cell lines (such as monocytic leukaemia-derived cell lines, mast cell tumour-derived cell lines, hematoma-

derived cell lines, neuroblastoma-derived cell lines, and various mammary tumour-derived cell lines) and in cancer cells derived from diverse human cancer tissues (Latchman Y et al, Nature Immunology, 2001, Vol. 2, No. 3, pp.261-267) and on almost all murine tumour cell lines, including PA1 myeloma, P815 mastocytoma, and B16 melanoma upon treatment with IFN-γ (Y. Iwai et al., *Proc Natl Acad Sci U S A* **99**, 12293, Sep 17, 2002 and C. Blank et al., *Cancer Res* **64**, 1140, Feb, 2004). Similarly PD-L2 expression is more restricted and is expressed mainly by dendritic cells and a few tumour cell lines. PD-L2 expression has been verified in Hodgkin's lymphoma cell lines and others. There is a hypothesis that some of the cancer or tumour cells take advantage from interaction between PD-1 and PD-L1 or PD-L2, for suppressing or intercepting T-cell immune responses to their own (Iwai Y et al, Proceedings of the National Academy of Science of the United States of America, 2002, Vol. 99, No. 19, pp.12293-12297).

Tumour cells and virus (including HCV and HIV) infected cells are known to express the ligand for PD-1 (to create Immunosuppression) in order to escape immune surveillance by host T cells. It has been reported that the PD-1 gene is one of genes responsible for autoimmune diseases like systemic lupus erythematosis (Prokunina et al, Nature Genetics, 2002, Vol. 32, No. 4, 666-669). It has also been indicated that PD-1 serves as a regulatory factor for the onset of autoimmune diseases, particularly for peripheral self-tolerance, on the ground that PD-1-deficient mice develop lupus autoimmune diseases, such as glomerulonephritis and arthritis (Nishimura H et al, International Immunology, 1998, Vol. 10, No. 10, pp.1563-1572 ; Nishimura H et al, Immunity, 1999, Vol. 11, No. 2, pp.141-151), and dilated cardiomyopathy-like disease (Nishimura H et al, Science, 2001, Vol. 291, No. 5502, pp.319-332).

Hence, in one approach, blocking the interaction of PD-1 with its ligand (PD-L1, PD-L2 or both) may provide an effective way for specific tumour and viral immunotherapy.

Wood et al in US 6,808,710 discloses method for down modulating an immune response comprising contacting an immune cell expressing PD-1 with an antibody that binds to PD-1, in multivalent form, such that a negative signal is transduced via PD-1 to thereby down modulate the immune response. Such an antibody may be a cross-linked antibody to PD-1 or an immobilized antibody to PD-1.

Freeman et al in US6936704 and its divisional patent US7038013 discloses isolated nucleic acids molecules, designated B7-4 nucleic acid molecules, which encode novel B7-4

polypeptides, isolated B7-4 proteins, fusion proteins, antigenic peptides and anti-B7-4 antibodies, which co-stimulates T cell proliferation in vitro when the polypeptide is present on a first surface and an antigen or a polyclonal activator that transmits an activating signal via the T-cell receptor is present on a second, different surface.

There are some reports regarding substances inhibiting immunosuppressive activity of PD-1, or interaction between PD-1 and PD-L1 or PD-L2, as well as the uses thereof. A PD-1 inhibitory antibody or the concept of a PD-1 inhibitory peptide is reported in WO 01/14557, WO 2004/004771, and WO 2004/056875. On the other hand, a PD-L1 inhibitory antibody or a PD-L1 inhibitory peptide is reported in WO 02/079499, WO 03/042402, WO 2002/086083, and WO 2001/039722. A PD-L2 inhibitory antibody or a PD-L2 inhibitory peptide is reported in WO 03/042402 and WO 02/00730.

WO2007005874 describes isolated human monoclonal antibodies that specifically bind to PD-L1 with high affinity. The disclosure provides methods for treating various diseases including cancer using anti-PD-L1 antibodies.

US2009/0305950 describes multimers, particularly tetramers of an extracellular domain of PD-1 or PD-L1. The application describes therapeutic peptides.

Further, the specification mentions that peptides can be used therapeutically to treat disease, e.g., by altering co-stimulation in a patient. An isolated B7-4 or PD-1 protein, or a portion or fragment thereof (or a nucleic acid molecule encoding such a polypeptide), can be used as an immunogen to generate antibodies that bind B7-4 or PD-1 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length B7-4 or PD-1 protein can be used, or alternatively, the invention provides antigenic peptide fragments of B7-4 or PD-1 for use as immunogens. The antigenic peptide of B7-4 or PD-1 comprises at least 8 amino acid residues and encompasses an epitope of B7-4 or PD-1 such that an antibody raised against the peptide forms a specific immune complex with B7-4 or PD-1. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least amino acid residues, and most preferably at least 30 amino acid residues.

Freeman et al in US7432059 appears to disclose and claim methods of identifying compounds that up modulate T cell activation in the presence of a PD-1-mediated signal.

Diagnostic and treatment methods utilizing compositions of the invention are also provided in the patent.

Further, Freeman et al in US 7,709,214 appears to cover methods for up regulating an immune response with agents that inhibit the interactions between PD-L2 and PD-1.

Despite existence of many disclosures as discussed above, however, a significant unmet medical need still exists due to the lack of effective peptides or modified peptides as therapeutic agents as alternatives in the therapeutic area. It is known that synthetic peptides offer certain advantages over antibodies such as ease of production with newer technologies, better purity and lack of contamination by cellular materials, low immunogenicity, improved potency and specificity. Peptides may be more stable and offer better storage properties than antibodies. Moreover, often peptides possess better tissue penetration in comparison with antibodies, which could result in better efficacy. Peptides can also offer definite advantages over small molecule therapeutics counterparts such as lesser degree of toxicity and lower probability of drug-drug interaction.

The present invention therefore may provide the solution for this unmet medical need by offering novel synthetic peptide and its derivatives which are based on the PD1 ectodomain.

BRIEF DESCRIPTION OF ACCOMPANYING DRAWINGS

Figure 1: Potency determination for compound #8 in assay for inhibition of binding of PD1 to PD-L2 using hPDL2 expressing HEK293 cells.

Figure 2: Abrogation of PDL1 mediated inhibition of rat PBMC proliferation with compound 8

Figure 3: EC_{50} determination for compound 8 in rat PBMC proliferation assay in the context of PD-1/PD-L1 pathway.

Figure 4: Restoration of human PBMC proliferation in the context of human PDL1 with various compounds.

Figure 5: Comparison of percentage mouse splenocyte proliferation rescue of compound 8, 13 and 34 with J43 antibody.

Figure 6: Effect of various test compounds on IFN-γ production in Cytotoxic T Lymphocyte assay.

Figure 7: Effect of compound # 8 on tumour growth inhibition of B16F10 cells injected subcutaneously.

Figure 8: Effect of compound #8 on lung metastasis of B16F10 cells injected i.v.

Figure 9: Effect of various compounds on lung metastasis in B16F10 model.

Figure 10: Effect of compound #8 on tumour growth inhibition of 4T1 cells injected orthotopically to mammary fat pad.

Figure 11: Effect of peptides in reducing tumour burden in kidney injected orthotopically with RENCA cells.

Figure 12: In vivo efficacy of compound 8 against E. coli sepsis model.

AMINO ACID SEQUNCE INFORMATION:

SEQ ID NO: 1 shows amino acid sequence of human PD-1 ectodomain

SEQ ID NO: 2 shows amino acid sequence of A strand of human PD-1 ectodomain

SEQ ID NO: 3 shows amino acid sequence of B strand of human PD-1 ectodomain

SEQ ID NO: 4 shows amino acid sequence of BC loop of human PD-1 ectodomain

SEQ ID NO: 5 shows amino acid sequence of C strand of human PD-1 ectodomain

SEQ ID NO: 6 shows amino acid sequence of C-C' loop of human PD-1 ectodomain

SEQ ID NO: 7 shows amino acid sequence of C' strand of human PD-1 ectodomain

SEQ ID NO: 8 shows amino acid sequence of C'-C" loop of human PD-1 ectodomain

SEQ ID NO: 9 shows amino acid sequence of C" strand of human PD-1 ectodomain

SEQ ID NO: 10 shows amino acid sequence of C"-D loop of human PD-1 ectodomain

SEQ ID NO: 11 shows amino acid sequence of D strand of human PD-1 ectodomain

SEQ ID NO: 12 shows amino acid sequence of D-E loop of human PD-1 ectodomain

SEQ ID NO: 13 shows amino acid sequence of E strand of human PD-1 ectodomain

SEQ ID NO: 14 shows amino acid sequence of F strand of human PD-1 ectodomain

SEQ ID NO: 15 shows amino acid sequence of FG loop of human PD-1 ectodomain

SEQ ID NO: 16 shows amino acid sequence of G strand of human PD-1 ectodomain

SEQ ID NO: 17 shows amino acid sequence of mouse PD-1 ectodomain

SEQ ID NO: 18 shows amino acid sequence of rat PD-1 ectodomain

SEQ ID NO: 19 shows amino acid sequence of dog PD-1 ectodomain

SEQ ID NO: 20 shows amino acid sequence of horse PD-1 ectodomain

SEQ ID NO: 21 shows amino acid sequence of C' strand to C'C'' loop of human PD-1 ectodomain

SEQ ID NO: 22 shows amino acid sequence of C' strand to C'' strand of human PD-1 ectodomain

SEQ ID NO: 23 shows amino acid sequence of CC' loop to C' strand of human PD-1 ectodomain

SEQ ID NO: 24 shows amino acid sequence of FG loop to G strand of human PD-1 ectodomain

SEQ ID NO: 25 shows amino acid sequence of D strand to DE loop of human PD-1 ectodomain

SEQ ID NO: 26 shows amino acid sequence of B strand of mouse PD-1 ectodomain

SEQ ID NO: 27 shows amino acid sequence of BC loop of mouse PD-1 ectodomain

SEQ ID NO: 28 shows amino acid sequence of C-Strand of mouse PD-1 ectodomain

SEQ ID NO: 29 shows amino acid sequence of C-C' loop of mouse PD-1 ectodomain

SEQ ID NO: 30 shows amino acid sequence of C'-strand of mouse PD-1 ectodomain

SEQ ID NO: 31 shows amino acid sequence of C" strand of mouse PD-1 ectodomain

SEQ ID NO: 32 shows amino acid sequence of C"-D-loop of mouse PD-1 ectodomain

SEQ ID NO: 33 shows amino acid sequence of D-strand of mouse PD-1 ectodomain

SEQ ID NO: 34 shows amino acid sequence of F-strand of mouse PD-1 ectodomain

SEQ ID NO: 35 shows amino acid sequence of F-G loop of mouse PD-1 ectodomain

SEQ ID NO: 36 shows amino acid sequence of G-strand of mouse PD-1 ectodomain

SEQ ID NO: 37 shows amino acid sequence of C' strand to C'C'' loop of mouse PD-1 ectodomain

SEQ ID NO: 38 shows amino acid sequence of C' strand to C'' strand of mouse PD-1 ectodomain

SEQ ID NO: 39 shows amino acid sequence of CC' loop to C' strand of mouse PD-1 ectodomain

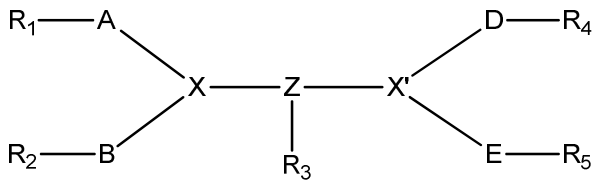
SEQ ID NO: 40 shows amino acid sequence of FG loop to G strand of mouse PD-1 ectodomain

SEQ ID NO: 41 shows amino acid sequence of D strand to DE loop of mouse PD-1 ectodomain

SUMMARY OF INVENTION

In accordance with the present invention, novel modified peptides are provided which are capable of suppressing and/or inhibiting the programmed cell death 1 (PD1) signalling pathway.

In one aspect of the present invention, it provides the structure of compounds as set forth in Formula I.



(I)

or pharmaceutically acceptable salt thereof;

wherein;

the compound comprises at least 5 amino acids,

A is an amino acid sequence of from three amino acids to the full length of a peptide sequence of mammalian PD1 ectodomain fragments selected from FG loop, BC loop, C-C' loop, C strand, C' strand to C'-C'' loop, C C' loop to C' strand, or is Glu-Asp, or is absent;

B is an amino acid sequence of from three amino acids to the full length of a peptide sequence of mammalian PD1 ectodomain fragments selected from BC loop, FG loop, C C' loop to C' strand, C strand, D strand, C' strand to C'-C'' loop, or is Glu-Asp, or is absent;

Z is

(i). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from BC loop, D strand, FG loop, G strand, C strand, F strand, C' strand, C" strand, C"-D loop, C' strand to C'-C'' loop, C' strand to C'' strand or D strand to DE loop;

(ii). G-L-Z'

G is an amino acid sequence of from three amino acids to the full length of a peptide sequence of mammalian PD1 ectodomain fragments from D-strand or is absent;

L is selected from $-CO(CH_2)_n-NH-$, or PEG 2-20 KD;

'n' is an integer selected from 2 to 10, both inclusive; and

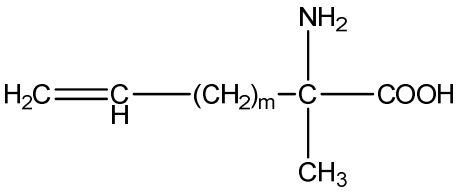
Z' is one to three peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from FG loop and G-strand; or

(iii). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from D-strand, FG loop and G strand, wherein two or more amino acids of the peptide sequence combine together to form a lactam bond between any of the two fragments or within the fragment;

D is up to two peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from BC loop, FG loop, C C' loop to C' strand or is absent;

E is up to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from BC loop, D strand, FG loop, C C' loop to C' strand, G strand , FG loop to G strand or is absent;

X and X' are independently selected from lysine, ornithine, diaminopropionic acid, diaminobutyric acid or olefinic amino acid of formula



which is optionally linked with an additional lysine; or

X and X' combine together forming a ring with olefinic amino acid which is optionally linked with an additional lysine; or

one of the X or X' is absent or both are absent;

'm' is an integer selected from 1 to 6, both inclusive;

R_1 is selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD moiety; or absent.

R_2 and R_3 are independently selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD, absent or R_a-L';

R_a is selected from biotin or maleimido propionic acid;

L' is selected from linkers -$CO(CH_2)_n$-NH-, -$CO(CH_2$-CH_2-O-$)_n$ NH or -$COCH_2$(-OCH_2-$CH_2)_n$NH-; and

'n' is an integer selected from 2 to 10, both inclusive;

R_4 and R_5 are independently NH_2, or one or both of R_4 or R_5 are absent.

with the proviso to the compound of Formula I, that in a compound of Formula I as above defined:

a) up to 5 but not more than 25% of the amino acids may be substituted with other natural or unnatural amino acids;

b) not more than 30% of the amino acids may be omitted;

c) in each said peptide sequence up to 2 amino acids may be added individually at any position;

d) up to 5 but not more than 25% of the peptide bonds may instead be replaced by reduced amide bond (-CH_2NH-);

e) up to 100% of the amino acids may be D-amino acids;

f) up to 100% of the amino acids may be in reverse order.

In another aspect of the present invention, it provides compound of Formula-I useful in the treatment or prevention of disease or disorder, where there is an advantage in inhibition of the programmed cell death 1 (PD1) signalling pathway and ability to reduce PD-L1 or PD-L2 binding to PD-1 and resulting immunosuppressive signalling by PD-1.

DETAILED DESCRIPTION OF THE INVENTION

The term 'peptide' is used herein to designate a sequence of natural or unnatural amino acids bonded in said sequence by peptide bonds or reduced peptide bond.

The term 'compound(s)' as used herein comprises peptides as disclosed in the present invention.

The following common abbreviations of the amino acids are used throughout this specification:

Gly (or G)—glycine	Ala (or A)—alanine	Val (or V)—valine
Leu (or L) — leucine	Ile (or I)—isoleucine	Orn-ornithine
Pro (or P)—proline	Phe (or F)—phenylalanine	Trp (or W)—tryptophan
Met (or M)—methionine	Ser (or S)—serine	Thr (or T)—threonine
Cys (or C)—cysteine	Tyr (or Y)—tyrosine	Asn (or N)—asparagine
Gln (or Q)—glutamine	Asp (or D)—aspartic acid	Glu (or E)—glutamic acid
Lys (or K)—lysine	Arg (or R)—arginine	His (or H)—histidine
DAP —2,3-Diaminopropionic Acid	DAB— 2,4-Diaminobutyric Acid	

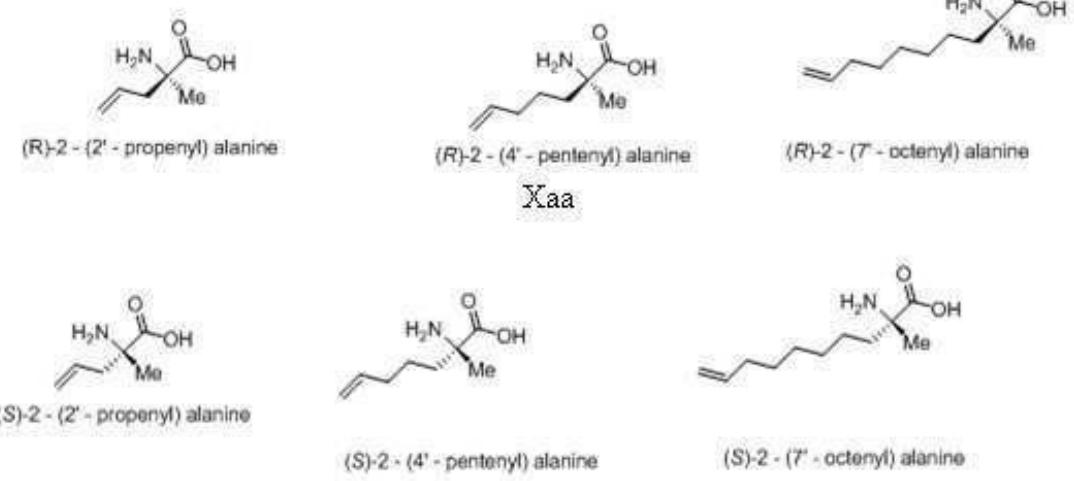
The small letter codes are used to represent the D-amino acids throughout the specification.

Modifications of the peptides discussed hereinafter and wherever relevant include replacements of some or all of the L-amino acids by D-amino acids, bonding of amino acids at other than alpha amino groups, including at side chain amino or carboxylic groups, inclusion of non-peptide linkers between peptide sequences, cross-linking, lipidation, stapling, and PEGylation.

Modifications of the peptides discussed hereinafter and wherever relevant further comprise modified peptide bond between two amino acids, wherein the typical peptide bond (-CONH-) between two amino acids is replaced with reduced amide bond (-CH_2NH-). Using convention method of identification this is characterized as —Ψ[CH_2NH]— with the symbol "Ψ" designating a modified peptide bond.

As used herein, "unnatural amino acid" is an amino acid that is not naturally produced (e.g., encoded by the genetic code or resulting from a posttranslational modification) or naturally found in a mammal. Unnatural amino acids include amino acids that normally do not occur in proteins (e.g., an α-amino acid having the D- configuration, or a (D,L)-isomeric mixture thereof), homologues of naturally occurring amino acids (e.g., a β- or γ- amino acid analogue), an α,α-di-substituted analogue of a naturally occurring amino acid, or an α-amino acid wherein the amino acid side chain has been shortened by methylene groups which includes but are not limited to

diaminopriopionic acid (DAP), diaminobutyric acid (DAB), ornithine (Orn) and the like; or lengthened to up to 10 carbon atoms; or lengthened up to 10 carbon atoms with olefinic groups which includes but are not limited to



Other unnatural amino acids include γ-amino acids that are GABA analogues, such as (S)-3-(aminomethyl)-5-methylhexanoic acid (pregabalin), 2-[l-(aminomethyl)cyclohexyl]acetic acid (gabapentin), or those described in Yogeeswari et al., Recent Patents on CNS Drug Discovery 2006;l:113-118, herein incorporated by reference.

The present invention provides immunosuppression modulating peptides capable of suppressing and/or inhibiting the programmed cell death 1 (PD1) signalling pathway.

The present invention further provides modifications, derivatives of the peptides and pharmaceutical compositions comprising the peptides for treatment of cancer or infection via immuno-potentiation caused by inhibition of immunosuppressive signal induced by PD-1, PD-L1, or PD-L2 and therapies using them, immuno-potentiative substrates included as the active ingredients.

The complete amino acid sequence of human PD-1 is disclosed in US 5629204 (Honjo et. al.) and Finger et al., (Gene, 1997, 197:177-187)**.** Human and mouse PD-1 share around 60% amino acid identity, whereas the extracellular IgV domain shows only 21% and 16% sequence identity with CD28 and CTLA4, respectively.

PD-1 possesses an ectodomain having multiple loop structures and strands between the loops. The amino acid sequence of the human PD-1 ectodomain is as set forth in SEQ ID NO: 1.

SEQ ID NO: 1 human PD-1

PPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDC
RFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVP
TAHPSPSPRSAGQFQTLV

The loop and strand sequences within the human PD-1 ectodomain sequence are as follows:

A strand: PPTFS	SEQ ID NO: 2
B strand: ATFT CSF	SEQ ID NO: 3
BC loop: SNTSESF	SEQ ID NO: 4
C-Strand: VLNWYRM	SEQ ID NO: 5
C-C' loop: SPS NQ	SEQ ID NO: 6
C'-strand: TDKLAAFP	SEQ ID NO: 7
C'-C" loop: ED	SEQ ID NO: 8
C" strand: RSQP	SEQ ID NO: 9
C"-D-loop: GQDCR	SEQ ID NO: 10
D-strand: FRVTQ	SEQ ID NO: 11
DE loop: LPNG R	SEQ ID NO: 12
E strand: DFHMSV	SEQ ID NO: 13
F-strand: GTYLC GAIS	SEQ ID NO: 14
F-G loop: LAPKA	SEQ ID NO: 15
G-strand: QIKE	SEQ ID NO: 16
C' strand to C'C'' loop: FPED	SEQ ID NO: 21
C' strand to C'' strand: TDKLAAFPEDRSQP	SEQ ID NO: 22
CC' loop to C' strand: SPSNQTDKLAAFP	SEQ ID NO: 23
FG loop to G strand: LAPKAQIKE	SEQ ID NO: 24
D strand to DE loop: FRVTQLPNGR	SEQ ID NO: 25

SEQ ID NO: 17 Mouse-PD-1

SLTFYPAWLTVSEGANATFTCSLSNWSEDLMLNWNRLSPSNQTEKQAAFCNGLSQPVQ DARFQIIQLPNRHDFHMNILDTRRNDSGIYLCGAISLHPKAKIEESPGAELVVTERILETST RYPSPSPKPEGRFQGMV

The loop and strand sequences within the mouse PD-1 ectodomain sequence are as follows:

B strand: ATFT CSL	SEQ ID NO: 26
BC loop: SNWSEDL	SEQ ID NO: 27
C-Strand: MLNWNRL	SEQ ID NO: 28
C-C' loop: SPSNQ	SEQ ID NO: 29
C'-strand: TEKQAAFC	SEQ ID NO: 30
C" strand: LSQP	SEQ ID NO: 31
C"-D-loop: VQDAR	SEQ ID NO: 32
D-strand: FQIIQ	SEQ ID NO: 33
F-strand: GIYLC GAIS	SEQ ID NO: 34
F-G loop: LHPKA	SEQ ID NO: 35
G-strand: KIEE	SEQ ID NO: 36
C' strand to C'C'' loop: FCNG	SEQ ID NO: 37
C' strand to C'' strand: TEKQAAFCNGLSQP	SEQ ID NO: 38
CC' loop to C' strand: SPSNQTEKQAAFC	SEQ ID NO: 39
FG loop to G strand: LHPKAKIEE	SEQ ID NO: 40
D strand to DE loop: FQIIQLPNRH	SEQ ID NO: 41

SEQ ID NO: 18 Rat –PD-1

QLSWQSGWLLVSEGANATFTCSFSNWSEDLKLNWYRLSPSNQTEKQAAFCNGYSQPVR DARFQIVQLPNGHDFHMNILDARRNDSGIYLCGAISLPPKAQIKESPGAELVVTERILETP TRYPRPSPKPEGQFQGLV

SEQ ID NO: 19 Dog-PD-1

PLTFSPAQLTVQEGENATFTCSLADIPDSFVLNWYRLSPRNQTDKLAAFQEDRIEPGRDR RFRVMRLPNGRDFHMSIVAARLNDSGIYLCGAIYLPPNTQINESPRAELSVTERTLEPPTQ SPSPPPRLSGQLQGLV

SEQ ID NO: 20 Horse-PD-1

PLTFSPARLMVPEGANATFTCSFSNTSEHFVLNWYRMSPSNQTDKLAAFPEDSSQPGRS GRFRVTRLPNGRDFHMSVLAARRNDSGIYLCGAISLPPKTQINESPRAELTVTERIPEPPT EHPSPPPSPAGQLQGLV

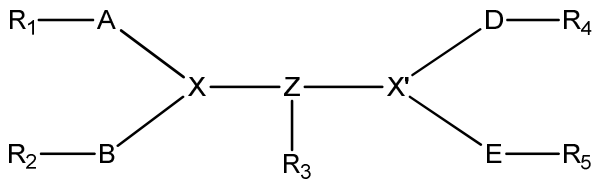
These loop and strand assignments of amino acids are based on the 1.8-Å-resolution structure of the murine PD-1/PD-L2 complex reported in Lazar-Molnar et al, (PNAS, 2008, 105, 30, 10483-10488) taking into account amino acid sequence variations between the human and murine proteins as reported there. Lazar-Molnar et al describes an alignment of the PD-1 ectodomain of mouse, human, rat, dog and horse PD-1 is shown with the β strands in mouse PD-1 denoted with arrow segments above the sequence.

The present invention further provides compounds comprising an ectodomain peptide fragment of murine, rat, dog or horse PD-1 (Lazar-Molnar et al, PNAS, 2008, 105, 30, 10483-10488).

Thus, in a compound comprising branched peptides, one or more branches may contain as a peptide moiety of the said ectodomain portion of human PD-1 (SEQ ID NO: 1) comprising of at least 5 amino acid residues.

In accordance with the present invention, in one of the embodiment there are provided compounds capable of inhibiting ability to inhibit the programmed cell death 1 (PD1) signalling pathway and being capable of reducing PD-L1 or PD-L2 binding to PD-1 and resulting immunosuppressive signalling by PD-1, wherein the compound comprises a peptide moiety comprising no more than 35, preferably no more than 30, amino acid residues.

The structure of compounds according to the invention is as set forth in Formula (I)



(I)

or pharmaceutically acceptable salt thereof;

wherein;

the compound comprises at least 5 amino acids,

A is an amino acid sequence of from three amino acids to the full length of a peptide sequence of mammalian PD1 ectodomain fragments selected from FG loop, BC loop, C-C' loop, C strand, C' strand to C'-C'' loop, C C' loop to C' strand, or is Glu-Asp, or is absent;

B is an amino acid sequence of from three amino acids to the full length of a peptide sequence of mammalian PD1 ectodomain fragments selected from BC loop, FG loop, C C' loop to C' strand, C strand, D strand, C' strand to C'-C'' loop, or is Glu-Asp, or is absent;

Z is

(i). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from BC loop, D strand, FG loop, G strand, C strand, F strand, C' strand, C" strand, C"-D loop, C' strand to C'-C'' loop, C' strand to C'' strand or D strand to DE loop;

(ii). G-L-Z'

G is an amino acid sequence of from three amino acids to the full length of a peptide sequence of mammalian PD1 ectodomain fragments from D-strand or is absent;

L is selected from $-CO(CH_2)_n-NH-$, or PEG 2-20 KD;

'n' is an integer selected from 2 to 10, both inclusive; and

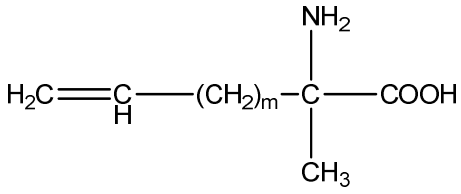
Z' is one to three peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from FG loop and G-strand; or

(iii). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from D-strand, FG loop and G strand, wherein two or more amino acids of the peptide sequence combine together to form a lactam bond between any of the two fragments or within the fragment;

D is up to two peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from BC loop, FG loop, C C' loop to C' strand or is absent;

E is up to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from BC loop, D strand, FG loop, C C' loop to C' strand, G strand , FG loop to G strand or is absent;

X and X' are independently selected from lysine, ornithine, diaminopropionic acid, diaminobutyric acid or olefinic amino acid of formula



which is optionally linked with an additional lysine; or

X and X' combine together forming a ring with olefinic amino acid which is optionally linked with an additional lysine; or

one of the X or X' is absent or both are absent;

'm' is an integer selected from 1 to 6, both inclusive;

R_1 is selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD moiety; or absent.

R_2 and R_3 are independently selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD, absent or R_a-L';

R_a is selected from biotin or maleimido propionic acid;

L' is selected from linkers $-CO(CH_2)_n-NH-$, $-CO(CH_2-CH_2-O-)_n$ NH or $-COCH_2(-OCH_2-CH_2)_nNH-$; and

'n' is an integer selected from 2 to 10, both inclusive;

R_4 and R_5 are independently NH_2, or one or both of R_4 or R_5 are absent.

with the proviso to the compound of Formula I, that in a compound of Formula I as above defined:

a) up to 5 but not more than 25% of the amino acids may be substituted with other natural or unnatural amino acids;

b) not more than 30% of the amino acids may be omitted;

c) in each said peptide sequence up to 2 amino acids may be added individually at any position;

d) up to 5 but not more than 25% of the peptide bonds may instead be replaced by reduced amide bond (-CH_2NH-);

e) up to 100% of the amino acids may be D-amino acids;

f) up to 100% of the amino acids may be in reverse order.

According to one embodiment, specifically provided are compounds of the formula (I) in which

A is an amino acid sequence of from three amino acids to the full length of a peptide sequence of human or murine PD1 ectodomain fragments selected from FG loop, BC loop, C-C' loop, C strand, C' strand to C'-C'' loop, C C' loop to C' strand as in table below, or is Glu-Asp, or is absent;

	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL
C Strand	VLNWYRM	MLNWNRL
C-C' loop	SPSNQ	SPSNQ
C' strand to C'-C'' loop	FPED	FCNG
C C' loop to C' strand	SPSNQTDKLAAFP	SPSNQTEKQAAFC
FG loop	LAPKA	LHPKA

B is an amino acid sequence of from three amino acids to the full length of a peptide sequence of human or murine PD1 ectodomain fragments selected from BC loop, FG loop, C C' loop to C' strand, C strand, D strand, C' strand to C'-C'' loop as in table below, or is Glu-Asp, or is absent;

	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL
C Strand	VLNWYRM	MLNWNRL
C' strand to C'-C'' loop	FPED	FCNG
C C' loop to C' strand	SPSNQTDKLAAFP	SPSNQTEKQAAFC
D Strand	FRVTQ	FQIIQ
FG loop	LAPKA	LHPKA

Z is

(i). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from BC loop, D strand, FG loop, G strand, C strand, F strand, C' strand, C" strand, C"-D loop, C' strand to C'-C'' loop, C' strand to C'' strand or D strand to DE loop;

(ii). G-L-Z'

G is an amino acid sequence of from three amino acids to the full length of a peptide sequence of human or murine PD1 ectodomain fragments from D-strand or is absent;

L is selected from $-CO(CH_2)_n-NH-$, or PEG 2-20 KD;

'n' is an integer selected from 2 to 10, both inclusive; and

Z' is one to three peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from FG loop and G-strand; or

(iii). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from D-strand, FG loop and G strand, wherein two or more amino acids of the peptide sequence combine together to form a lactam bond between any of the two fragments or within the fragment;

wherein said fragments are as defined in table below;

	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL
C Strand	VLNWYRM	MLNWNRL
C' Strand	TDKLAAFP	TEKQAAFC
C' strand to C'-C'' loop	FPED	FCNG
C' strand to C'' strand	TDKLAAFPEDRSQP	TEKQAAFCNGLSQP
C'' Strand	RSQP	LSQP
C''-D loop	GQDCR	VQDAR
D Strand	FRVTQ	FQIIQ
F strand	GTYLCGAIS	GIYLCGAIS
FG loop	LAPKA	LHPKA
G Strand	QIKE	KIEE
D strand to DE loop	FRVTQLPNGR	FQIIQLPNRH

D is up to two peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from BC loop, FG loop, C C' loop to C' strand as in table below or is absent;

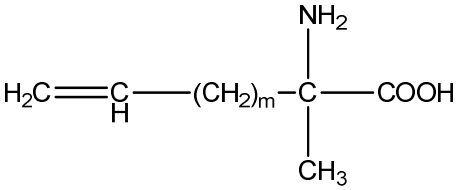
	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL

C C’ loop to C’ strand	SPSNQTDKLAAFP	SPSNQTEKQAAFC
FG loop	LAPKA	LHPKA

E is up to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from BC loop, D strand, FG loop, C C’ loop to C’ strand, G strand , FG loop to G strand or is absent;

	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL
C C’ loop to C’ strand	SPSNQTDKLAAFP	SPSNQTEKQAAFC
D Strand	FRVTQ	FQIIQ
FG loop	LAPKA	LHPKA
G Strand	QIKE	KIEE
FG loop to G strand	LAPKAQIKE	LHPKAKIEE

X and X’ are independently selected from lysine, ornithine, diaminopropionic acid, diaminobutyric acid or olefinic amino acid of formula



which is optionally linked with an additional lysine; or

X and X’ combine together forming a ring with olefinic amino acid which is optionally linked with an additional lysine; or

one of the X or X’ is absent or both are absent;

'm' is an integer selected from 1 to 6, both inclusive;

R_1 is selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD moiety; or absent.

R_2 and R_3 are independently selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD, absent or R_a-L';

R_a is selected from biotin or maleimido propionic acid;

L' is selected from linkers $-CO(CH_2)_n-NH-$, $-CO(CH_2-CH_2-O-)_n$ NH or $-COCH_2(-OCH_2-CH_2)_nNH-$; and

'n' is an integer selected from 2 to 10, both inclusive;

R_4 and R_5 are independently NH_2, or one or both of R_4 or R_5 are absent.

with the proviso to the compound of Formula I, that in a compound of Formula I as above defined:

a) up to 5 but not more than 25% of the amino acids may be substituted with other natural or unnatural amino acids;

b) not more than 30% of the amino acids may be omitted;

c) in each said peptide sequence up to 2 amino acids may be added individually at any position;

d) up to 5 but not more than 25% of the peptide bonds may instead be replaced by reduced amide bond ($-CH_2NH-$);

e) up to 100% of the amino acids may be D-amino acids;

f) up to 100% of the amino acids may be in reverse order.

The embodiment below are illustrative of the present invention and are not intended to limit the claims to the specific embodiments exemplified.

According to one embodiment, specifically provided are compounds of the formula (I) in which Z consists of one said peptide sequence and at least one of A, B, D or E is not absent.

According to another embodiment, specifically provided are compounds of the formula (I) in which A, B, D and E are absent and Z comprises from 2 to 4 same or different peptide sequences.

According to yet another embodiment, specifically provided are compounds of the formula (I) in which Z is a combination of D strand, FG loop and G strand.

According to yet another embodiment, specifically provided are compounds of the formula (I) in which X is lysine and X' is absent.

According to yet another embodiment, specifically provided are compounds of the formula (I) in which both X and X' are lysine.

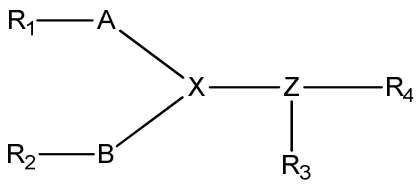
According to yet another embodiment, specifically provided are compounds of the formula (I) in which R_2 and R_3 are selected from C_2-C_{20} acyl, or R_a-L'; wherein,

R_a is maleimido propionic acid;

L' is $-COCH_2(-OCH_2-CH_2)_nNH-$; and

'n' is an integer selected from 2 to 10, both inclusive.

According to yet another embodiment the invention provides a compound of formula (Ia):



(Ia)

or pharmaceutically acceptable salt thereof;

wherein;

A is an amino acid sequence of from three amino acids to the full length of a peptide sequence of human or murine PD1 ectodomain fragments selected from FG loop, BC loop, C' strand, C-C' loop, C strand, C' strand to C'-C'' loop or is absent;

B is an amino acid sequence of from three amino acids to the full length of apeptide sequence of human or murine PD1 ectodomain fragments selected from BC loop, FG loop, C-C' loop to C' strand, C strand, D strand, C' strand to C'-C'' loop or is absent;

Z is from one to three peptide sequences arranged in any order each being of from three amino acids up to the full length of a peptide sequence of human or murine PD1 ectodomain fragments selected from D strand, FG loop, G strand, C strand and F strand;

and X is lysine.

According to yet another embodiment, specifically provided are compounds of the formula (Ia) in which A and B are independently an amino acid sequence of from three amino acids to the full length of said BC loop.

According to yet another embodiment, specifically provided are compounds of the formula (Ia) in which A and B are independently the full length of said BC loop.

According to yet another embodiment specifically provided are compounds of the formula (Ia) in which A is BC loop wherein all the amino acids are in reverse order.

According to yet another embodiment, specifically provided are compounds of the formula (Ia) in which Z is D strand-FG loop-G strand.

According to yet another embodiment, specifically provided are compounds of the formula (Ia) in which R_1, R_2 and R_3 are absent.

According to yet another embodiment, specifically provided are compounds of the formula (Ia) in which R_3 is C16-acyl.

According to yet another embodiment, specifically provided are compounds of the formula (I) or (Ia) in which up to three amino acids are D-amino acids. Optionally up to 10%, alternatively up to 20%, alternatively up to 50%, alternatively up to 80% or up to 90% of the amino acids are D-amino acids, or in which at least 10%, or at least 20%, or at least 50%, or at least 80% or at least 90% are D-amino acids.

According to yet another embodiment, specifically provided are compounds of the formula (I) or (Ia) in which all amino acids are D-amino acids.

According to yet another embodiment, specifically provided are compounds of the formula (I) or (Ia) in which up to 5 but not more than 25% of the peptide bonds may instead be replaced by reduced amide bond (-CH_2NH-). Alternatively, one or up to 2 or 3 or not more than 20% or 10% of the peptide bonds may instead be replaced by reduced amide bond (-CH_2NH-).

According to yet another embodiment, specifically provided are compounds of the formula (I) or (Ia) in which all the amino acids are in reverse order or in which a sequence of two or more amino acids are in reverse order, optionally 5 or more or optionally 10 or more, or in which no more than 10, or no more than 5 are in reverse order.

According to yet another embodiment, specifically provided are compounds of the formula (I) or (Ia) in which up to 5 but not more than 25% of the amino acids may be substituted with other natural or unnatural amino acids.

According to yet another embodiment, specifically provided are compounds of the formula (I) or (Ia) in which not more than 30%, alternatively not more than 20%, or not more than 10% of the amino acids may be omitted.

According to yet another embodiment, specifically provided are compounds of the formula (I) or (Ia) in which in each said peptide sequence up to 3 amino acids may be added individually at any position, alternatively up to 2 amino acids, or alternatively up to only one amino acid.

The compounds of the invention may comprise linear or branched peptides, but compounds comprising branched peptide having more than one peptide moiety are preferred and more than one branch may be present.

One embodiment relates to the compounds of the present invention, said compounds are branched and wherein one or more branches contains a peptide moiety comprising a said ectodomain portion of human or murine PD-1 of at least 5 amino acid residues., wherein the said compound comprises a first N-terminal portion and a second N-terminal portion peptide moiety, both being joined at a branch point to a C-terminal portion peptide moiety.

Another embodiment relates to the compounds of the present invention, said compounds are branched and wherein one or more branches contains a peptide moiety comprising a said ectodomain portion of human or PD-1 of at least 5 amino acid residues., wherein the said compound comprises a first N-terminal portion and a second N-terminal portion peptide moiety, both being joined at a branch point to an intermediate peptide moiety portion which is in turn joined at a further branch point to each of a first C-terminal portion and a second C-terminal portion peptide moiety.

Yet another embodiments of the present invention relates to the compounds as disclosed in the present invention, wherein the compounds are lipidated and/or are glycosylated.

Further embodiment of the present invention relates to the compounds as disclosed in the present invention, wherein the compounds contain a PEG moiety.

Further embodiment of the present invention relates to the compounds as disclosed in the present invention, wherein one or more of the amino acids of the peptide moiety the compounds is substituted with a D-amino acid.

Yet another embodiment of the present invention relates to the compounds as disclosed in the present invention, wherein one or more of the amino acids of the peptide moiety the compounds is substituted with a D-amino acid, wherein D-amino acid is present within 5 amino acids of an N-terminus or the C-terminus of the peptide moiety.

Still yet another embodiment of the present invention relates to the compounds as disclosed in the present invention, wherein one or more of the amino acids of the peptide moiety the compounds is substituted with a D-amino acid, wherein the said D-amino acid is present at the N-terminus or the C-terminus of the peptide moiety.

In one of the embodiment of the present invention there is provided a compound having the ability to inhibit the programmed cell death 1 (PD1) signalling pathway and being capable of reducing PD-L1 or PD-L2 binding to PD-1 and resulting immunosuppressive signalling by PD-1, said compound comprising a peptide moiety of no more than 35 amino acid residues.

In another embodiment of the present invention there is provided a compound having the ability to inhibit the programmed cell death 1 (PD1) signalling pathway and being capable of reducing PD-L1 or PD-L2 binding to PD-1 and resulting immunosuppressive signalling by PD-1, said compound comprising a peptide moiety of no more than 35 amino acid residues, wherein said peptide moiety comprises an ectodomain domain portion of human PD-1 of at least 5 contiguous amino acid residues comprising one of the following three amino acid residue sequences:

<u>from the A strand:</u>	<u>from the A'-B loop and B strand:</u>
PPT	NAT
PTF	ATF
TFS	TFT
<u>from the BC loop:</u>	FTC

SNT
NTS
TSE
SES
ESF
from the C-C' loop
SPS
PSN
SNQ
from the C' strand:
TDK
DKL
KLA
LAA
AFP
from the C'' strand:
RSQ
SQP
from the D strand:
FRV
RVT
VTQ
from the E strand:
DFH
FHM
HMS
MSV
from the F-G loop:
LAP
APK
PKA
from the F-G loop and G strand
KAQ
AQI
C'strand to C'' strand
TDK
DKL
KLA
LAA
AAF
AFP
FPE
PED
EDR
DRS
TCS
CSF
from the C strand:
VLN
LNW
NWY
WYR
YRM
from the C-C' loop to C' strand:
SNQ
NQT
QTD
TDK
from the C' strand to C'-C'' loop:
PED
EDR
from the C'' strand to C'' D-loop
QPG
PGQ
GQD
QDC
from the D-E loop:
LPN
PNG
NGR
from the F strand:
GTY
TYL
YLC
LCG
CGA
GAI
AIS
from the G strand:
QIK
IKE
D strand to DE loop
FRV
RVT
VTQ
TQL
LPN
PNG
NGR

RSQ
SQP

or containing a variant of such a ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues, wherein not more than two of said residues are deleted, substituted or modified or not more than two additional amino acid residues are added.

Further embodiment of the present invention provides a compound as disclosed in the present invention, wherein the compound comprising at least one of the sequences:

PPTFS	EDRSQ
NATFT	GQDCR
FTCSP	FRVTQ
SNTSESF	LPNGR
VLNWY	DFHMSV
NWYRM	GTYLC
SNQTDK	CGAIS
TDKLAA	LAPKA
KLAAFP	QIKE
PEDSQP	

Another embodiment relates to the compounds of the present invention, wherein no more than one of the amino acid residues included within a said three amino acid residue sequence is deleted, substituted or modified.

Yet another embodiment relates to the compounds of the present invention, wherein said compounds are branched and wherein one or more branches contains a peptide moiety comprising a said ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues.

Yet another embodiment relates to the compounds of the present invention, said compounds are branched and wherein one or more branches contains a peptide moiety comprising a said ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues., wherein the said compound comprises a first N-terminal portion and a second N-terminal portion peptide moiety, both being joined at a branch point to a C-terminal portion peptide moiety.

Yet another embodiment relates to the compounds of the present invention, said compounds are branched and wherein one or more branches contains a peptide moiety

comprising a said ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues., wherein the said compound comprises a first N-terminal portion and a second N-terminal portion peptide moiety, both being joined at a branch point to an intermediate peptide moiety portion which is in turn joined at a further branch point to each of a first C-terminal portion and a second C-terminal portion peptide moiety.

Yet another embodiment of the present invention relates to the branched compounds of the present invention comprising the first N-terminal portion and the second N-terminal portion peptide moiety, wherein said first and/or said second N-terminal portion peptide moiety comprises an ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues comprising one of the following three amino acid residue sequences:

<u>from the BC loop:</u>	<u>from the C-C' loop</u>
SNT	SPS
NTS	PSN
TSE	SNQ
SES	<u>from the C 'strand to C'-C'' loop:</u>
ESF	PED
<u>from the C-C' loop to C' strand:</u>	EDR
SNQ	<u>from the F-G loop:</u>
NQT	LAP
QTD	APK
TDK	PKA
<u>from the D-E loop:</u>	<u>D strand to DE loop</u>
LPN	FRV
PNG	RVT
NGR	VTQ
	TQL
	QLP
	LPN
	PNG
	NGR

Yet another embodiment of the present invention relates to the branched compounds of the present invention comprising the first N-terminal portion and the second N-terminal portion peptide moiety, wherein said first and/or said second N-terminal portion peptide moiety comprises an ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues comprising one of the following three amino acid residue sequences:

<u>from the A strand:</u>	<u>from the A'-B loop and B strand:</u>
PPT	NAT
PTF	ATF

TFS
from the C strand:
VLN
LNW
NWY
WYR
YRM
from the C'' strand:
RSQ
SQP
from the E strand:
DFH
FHM
HMS
MSV
from the F-G loop to G strand
KAQ
AQI
from the G strand:
QIK
IKE

TFT
FTC
TCS
CSF
from the C' strand:
TDK
DKL
KLA
LAA
AFP
from the D strand:
FRV
RVT
VTQ
from the F strand:
GTY
TYL
YLC
LCG
CGA
GAI
AIS

Yet another embodiment relates to the compounds of the present invention, said compounds are branched and wherein one or more branches contains a peptide moiety comprising a said ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues, wherein the said compound comprises a first N-terminal portion and a second N-terminal portion peptide moiety, both being joined at a branch point to a C-terminal portion peptide moiety, wherein said C-terminal portion or the first and/or said second C-terminal portion comprises an ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues comprising one of the following three amino acid residue sequences

from the A strand:
PPT
PTF
TFS
from the C strand:
VLN
LNW
NWY
WYR
YRM
from the C'' strand:
RSQ

from the A'-B loop and B strand:
NAT
ATF
TFT
FTC
TCS
CSF
from the C' strand:
TDK
DKL
KLA
LAA

SQP	AFP
<u>from the E strand:</u>	<u>from the D strand:</u>
DFH	FRV
FHM	RVT
HMS	VTQ
MSV	<u>from the F strand:</u>
<u>from the F-G loop to G strand</u>	GTY
KAQ	TYL
AQI	YLC
<u>from the G strand:</u>	LCG
QIK	CGA
IKE	GAI
	AIS

Yet another embodiment relates to the compounds of the present invention, said compounds are branched and wherein one or more branches contains a peptide moiety comprising a said ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues, wherein the said compound comprises a first N-terminal portion and a second N-terminal portion peptide moiety, both being joined at a branch point to an intermediate peptide moiety portion which is in turn joined at a further branch point to each of a first C-terminal portion and a second C-terminal portion peptide moiety, wherein one of said first and said second C-terminal portions comprises an ectodomain portion of human PD-1 of at least 5 contiguous amino acid residues comprising one of the following three amino acid residue sequences:

<u>from the BC loop:</u>	<u>from the C-C' loop</u>
SNT	SPS
NTS	PSN
TSE	SNQ
SES	<u>from the C'-C'' loop:</u>
ESF	PED
<u>from the C-C' loop to C' strand:</u>	EDR
SNQ	<u>from the F-G loop:</u>
NQT	LAP
QTD	APK
TDK	PKA
<u>from the D-E loop:</u>	<u>D strand to DE loop</u>
LPN	FRV
PNG	RVT
NGR	VTQ
<u>from the F-G loop to G strand</u>	TQL
KAQ	LPN
AQI	PNG

C'strand to C'' strand	NGR
TDK	
DKL	
KLA	
LAA	
AAF	
AFP	
FPE	
PED	
EDR	
DRS	
RSQ	
SQP	

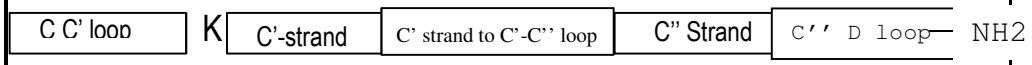
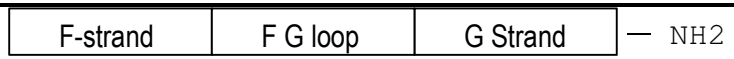
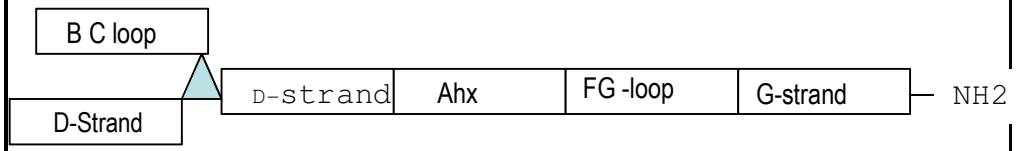
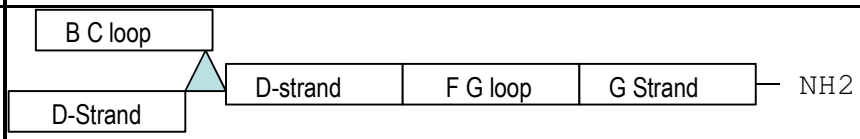
In one of the embodiment of the present invention there is provided a compound having the ability to inhibit the programmed cell death 1 (PD1) signalling pathway and being capable of reducing PD-L1 or PD-L2 binding to PD-1 and resulting immunosuppressive signalling by PD-1, said compound comprising a peptide moiety of no more than 35 amino acid residues.

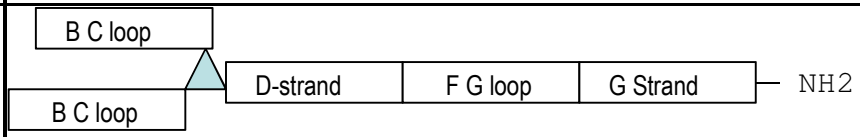
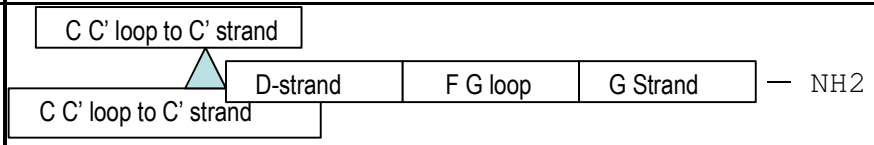
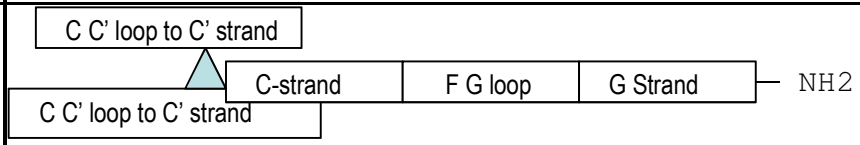
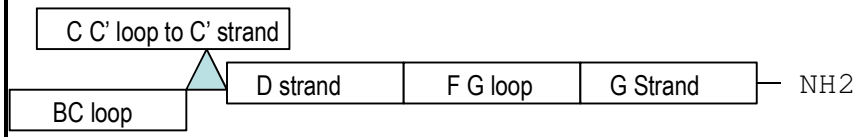
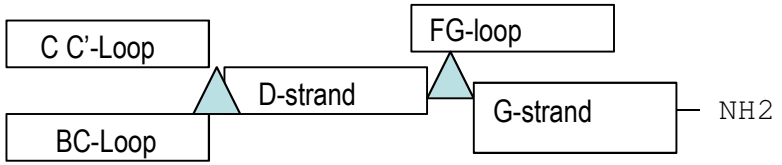
Further embodiment of the present invention provides a compound as disclosed in the present invention, wherein the compound comprising at least one of the sequences selected from individual species as in table below:

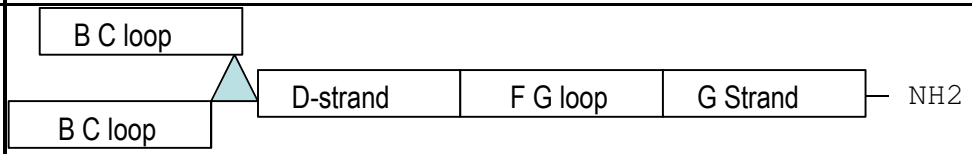
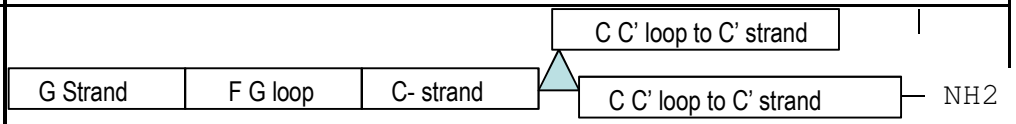
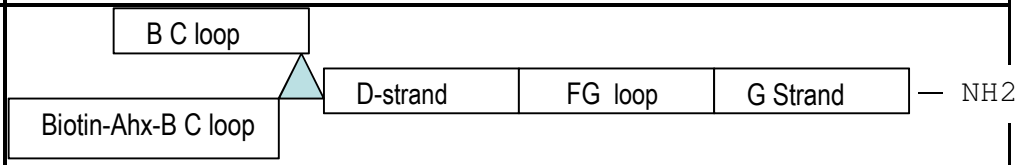
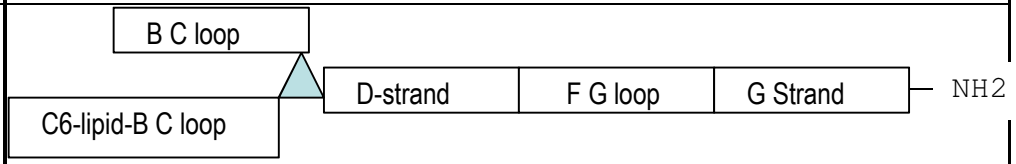
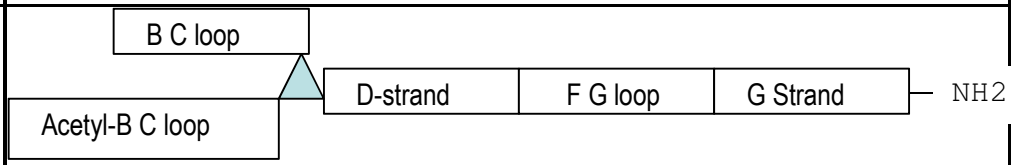
	hPD-1	mPD-1	rat PD-1	Dog PD-1	Horse PD-1
A Strand	PPTFS	SLTFY	QLSWQ	PLTFS	PLTFS
B strand	ATFTCSF	ATFTCSL	ATFTCSF	ATFTCSL	ATFTCSF
BC Loop	SNTSESF	SNWSEDL	SNWSEDL	ADIPDSF	SNTSEHF
C Strand	VLNWYRM	MLNWNRL	KLNWYRL	VLNWYRL	VLNWYRM
C-C' loop	SPSNQ	SPSNQ	SPSNQ	SPRNQ	SPSNQ
C' Strand	TDKLAAFP	TEKQAAFC	TEKQAAFC	TDKLAAFQ	TDKLAAFP
C'-C" loop	ED	NG	NG	ED	ED
C" Strand	RSQP	LSQP	YSQP	RIEP	SSQP
C"-D loop	GQDCR	VQDAR	VRDAR	GRDRR	GRSGR
D Strand	FRVTQ	FQIIQ	FQIVQ	FRVMR	FRVTR
D-E loop	LPNGR	LPNRH	LPNGH	LPNGR	LPNGR
E strand	DFHMSV	DFHMNI	DFHMNI	DFHMSI	DFHMSV
F strand	GTYLCGAIS	GIYLCGAIS	GIYLCGAIS	GIYLCGAIY	GIYLCGAIS
FG loop	LAPKA	LHPKA	LPPKA	LPPNT	LPPKT
G Strand	QIKE	KIEE	QIKE	QINE	QINE

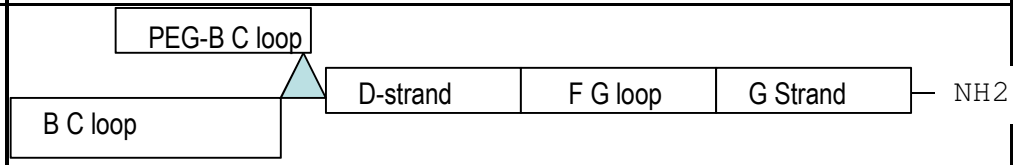
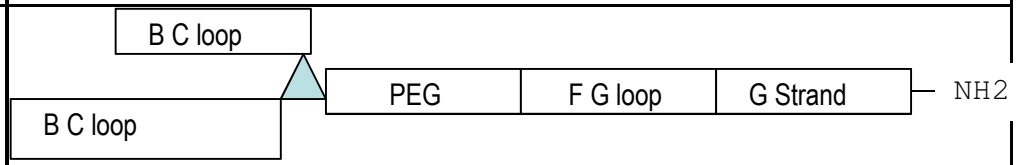
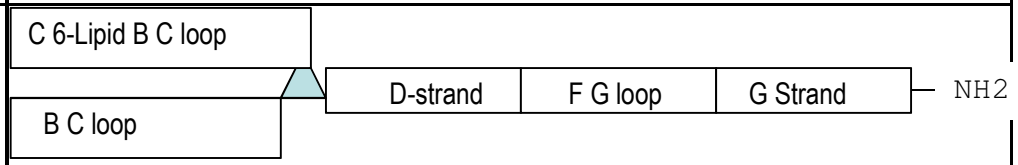
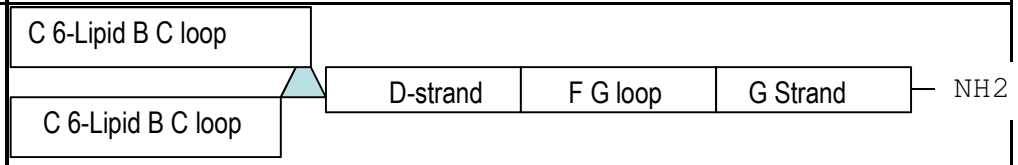
C’ strand to C’-C’’ loop	FPED	FCNG	FCNG	FQED	FPED
C C’ loop to C’ strand	SPSNQTDKLAAFP	SPSNQTEKQAAFC	SPSNQTEKQAAFC	SPRNQTDKLAAFQ	SNQTDKLAAFP
C’ strand to C’’ strand	TDKLAAFPEDRSQP	TEKQAAFCNGLSQP	TEKQAAFCNGYSQP	TDKLAAFQEDRIEP	TDKLAAFPEDSSQP
D strand to DE loop	FRVTQLPNGR	FQIIQLPNRH	FQIVQLPNGH	FRVMRLPNGR	FRVTRLPNGR
FG loop to G strand	LAPKAQIKE	LHPKAKIEE	LPPKAQIKE	LPPNTQINE	LPPKTQINE

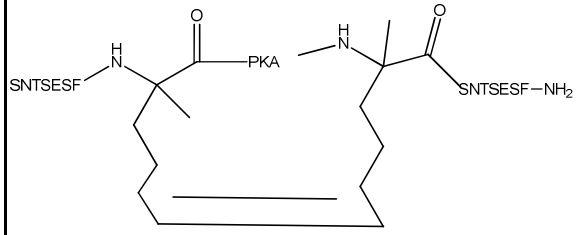
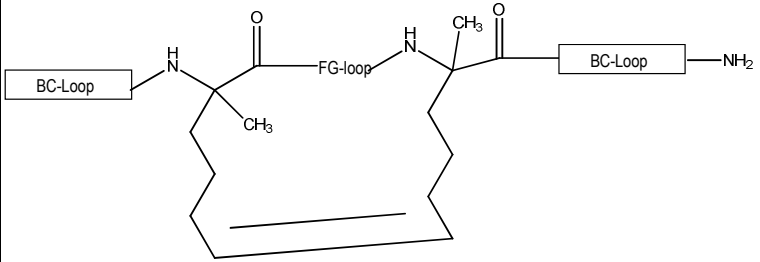
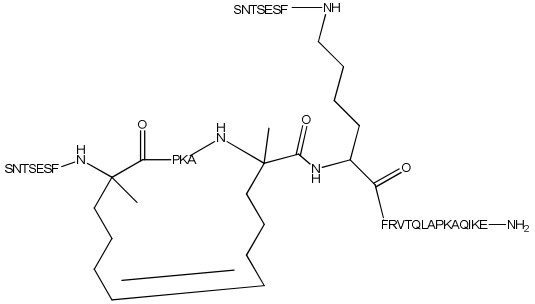
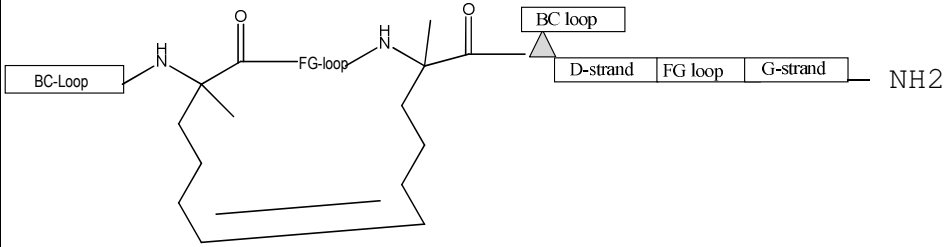
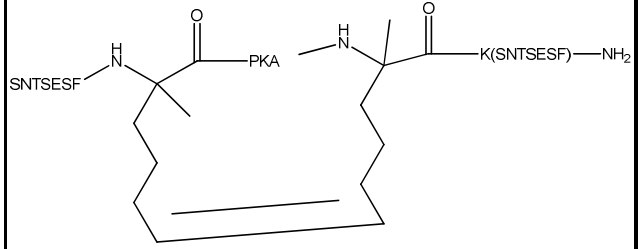
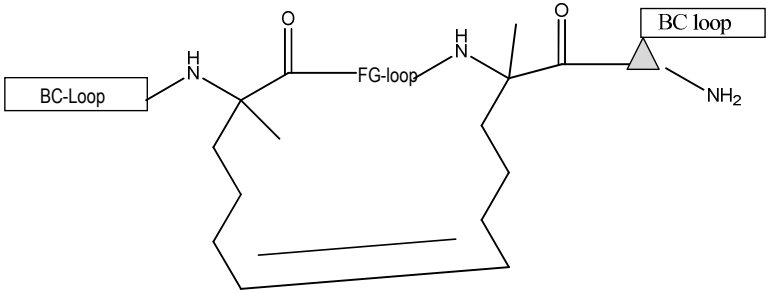
Below are the representative compounds, which are illustrative in nature only and are not intended to limit to the scope of the invention.

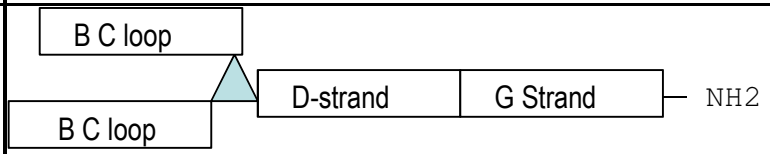
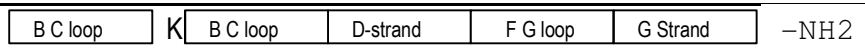
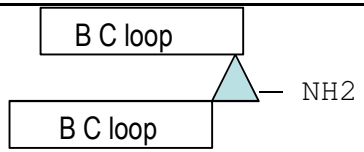
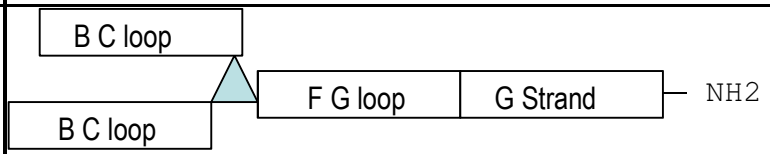
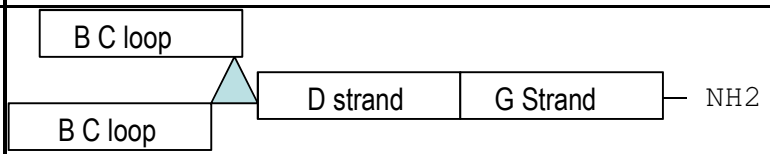
Comp. No.	Sequence	Structure
1.	SNTSESF–NH2 (SEQ ID NO: 42)	
2.	CH3CO–SNTSESF–NH2 (SEQ ID NO: 43)	
3.	SNQTDKLAAFPEDSQPGQD–NH2 (SEQ ID NO: 44)	
4.	EDRSQPGQDCR–NH2 (SEQ ID NO: 45)	
5.	CGAISLAPKAQIKE–NH2 (SEQ ID NO: 46)	
6.	FRVTQK(SNTSESF)FRVTQAhxLAPKAQIKE–NH2 Ahx = 6–aminohexanoic acid (SEQ ID NO: 47)	
7.	FRVTQK(SNTSESF)FRVTQLAPKAQIKE–NH2 (SEQ ID NO: 48)	

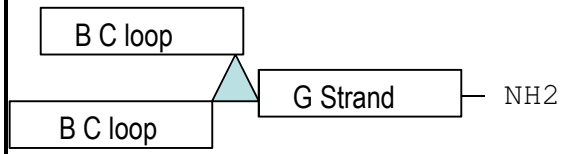
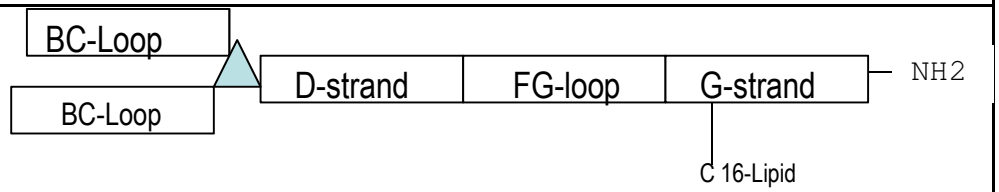
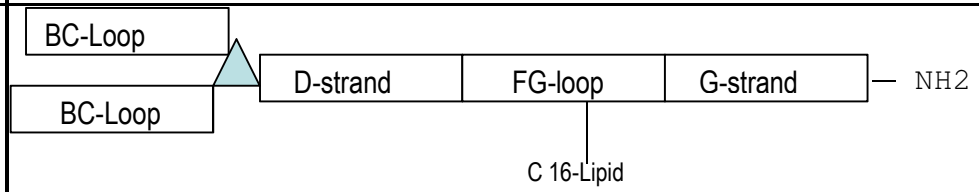
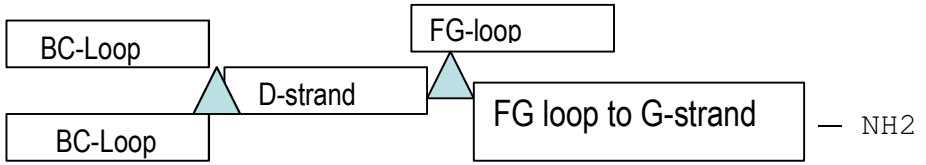
Comp. No.	Sequence	Structure
8.	SNTSESFK(SNTSESF)FRVTQLAPKAQIKE–NH2 (SEQ ID NO: 49)	
9.	SNQTDK (SNQTDK) FRVTQ LAPKAQIKE–NH2 (SEQ ID NO: 50)	
10.	SNQTDK (SNQTDK) VLNWYRM LAPKAQIKE–NH2 (SEQ ID NO: 51)	
11.	SNTSESFK(SNQTDK)FRVTQLAPKAQIKE–NH2 (SEQ ID NO: 52)	
12.	SNTSESFK(SNQTDK)FRVTQK(LAP)AQIKE–NH2 (SEQ ID NO: 53)	

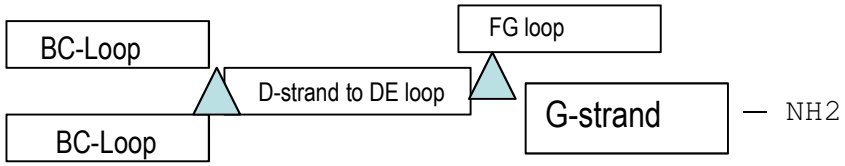
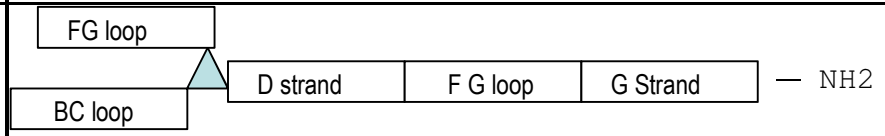
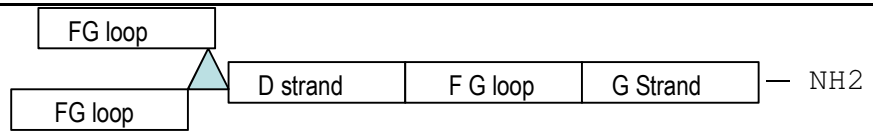
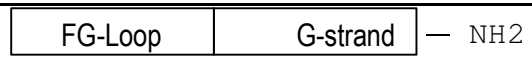
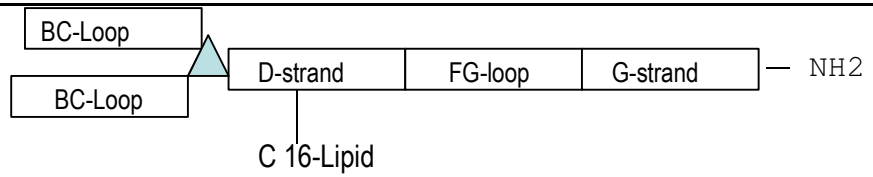
Comp. No.	Sequence	Structure
13.	sntsesfk(sntsesf)frvtqlapkaqike-NH2 All D-amino acids (SEQ ID NO: 54)	
14.	EKIQAKPAYWNLVK(KDTQNS)DTQNS (SEQ ID NO: 55)	
15.	Biotin-Ahx-SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 56)	
16.	C6 lipid-SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 57)	
17.	Ac-SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 58)	

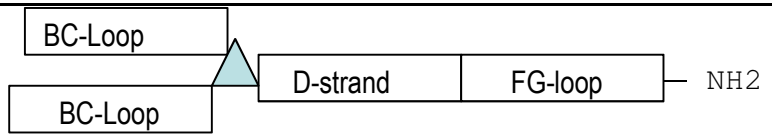
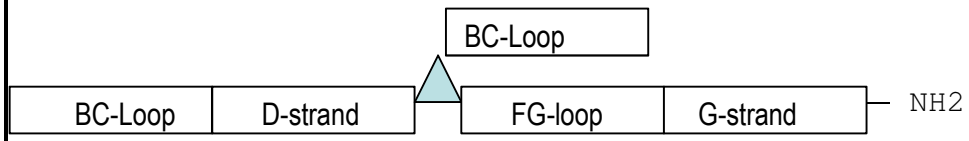
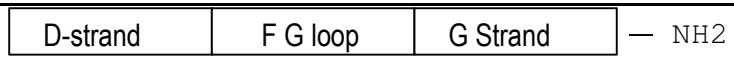
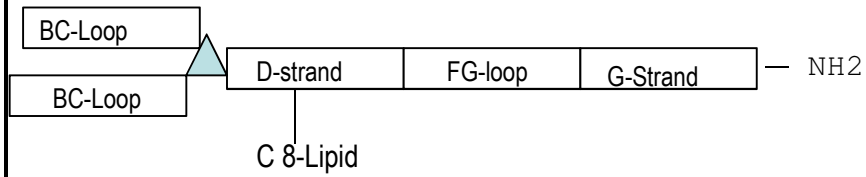
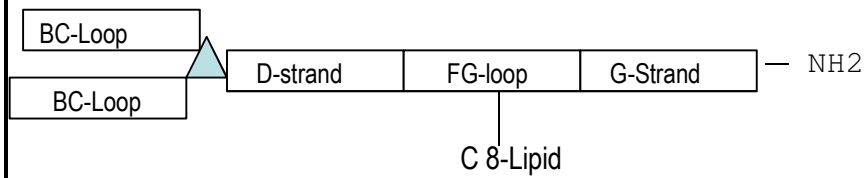
Comp. No.	Sequence	Structure
18.	SNTSESFK(NH2-[PEG]$_{11}$-CO-SNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 59)	
19.	SNTSESFK(SNTSESF)-NH-[PEG]$_{11}$-CO-)LAPKAQIKE-NH2 (SEQ ID NO: 60)	
20.	SNTSESFK($CH_3(CH_2)_4CO$-SNTSESF)FRVTQLAPKAQIKE-NH2 Lipid in branch (SEQ ID NO: 61)	
21.	CH3(CH2)4CO-SNTSESFK(CH3(CH2)4CO-SNTSESF)FRVTQLAPKAQIKE-NH2 Lipid on both Branch & N-termini (SEQ ID NO: 62)	

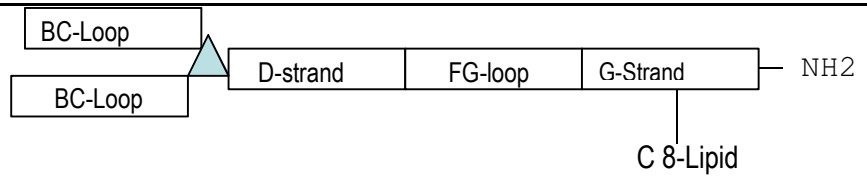
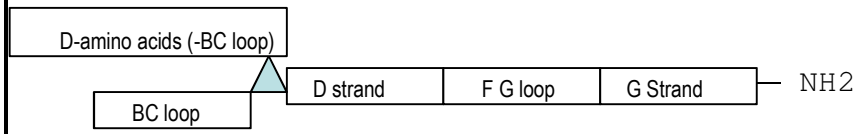
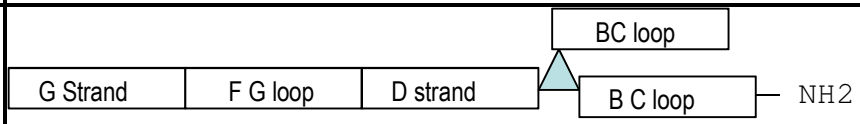
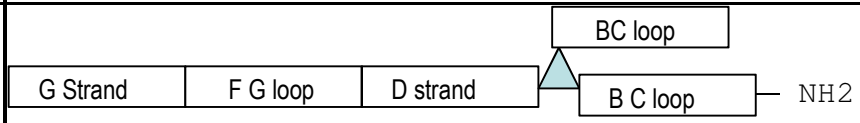
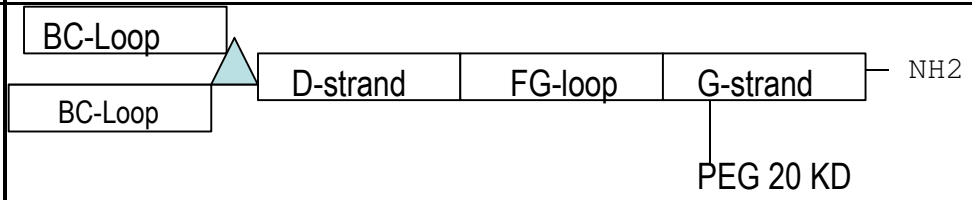
Comp. No.	Sequence	Structure
22.	 (SEQ ID NO: 63)	
23.	 (SEQ ID NO: 64)	
24.	 (SEQ ID NO: 65)	

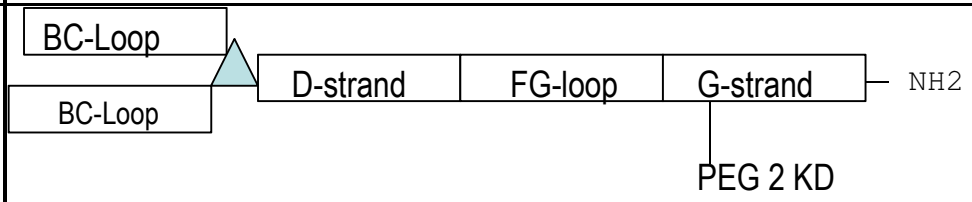
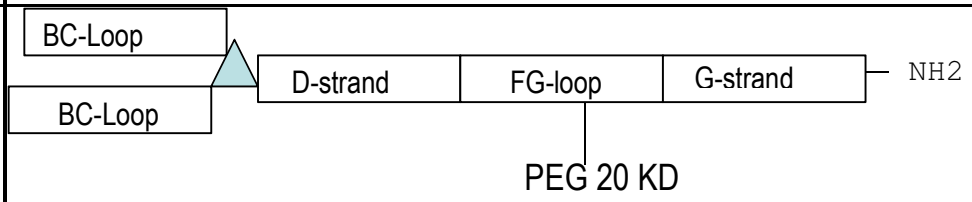
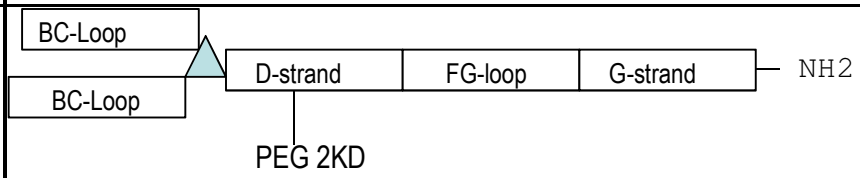
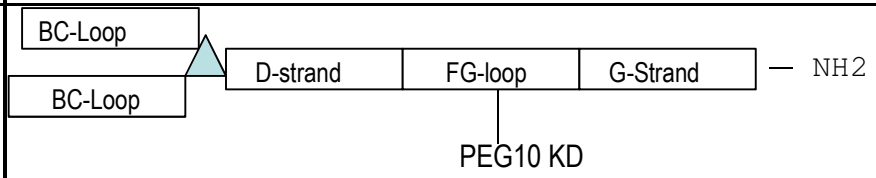
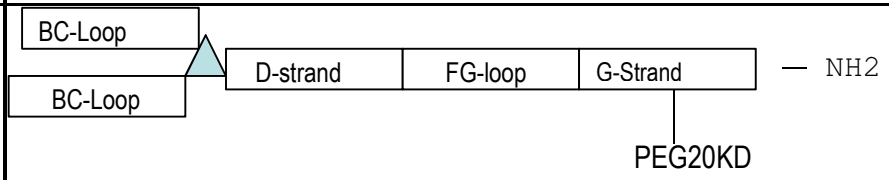
Comp. No.	Sequence	Structure
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26.	SNTSESFFRVTQLAPKAQIKE-NH2 (SEQ ID NO: 67)	
27.	SNTSESFKFRVTQLAPKAQIKE-NH2 (SEQ ID NO: 68)	
28.	SNTSESFKSNTSESFFRVTQLAPKAQIKE-NH2 (SEQ ID NO: 69)	
29.	SNTSESFK(SNTSESF)-NH2 (SEQ ID NO: 70)	
30.	SNTSESFK(SNTSESF)LAPKAQIKE-NH2 (SEQ ID NO: 71)	
31.	SNTSESFK(SNTSESF)FRVTQKAQIKE-NH2 (SEQ ID NO: 72)	

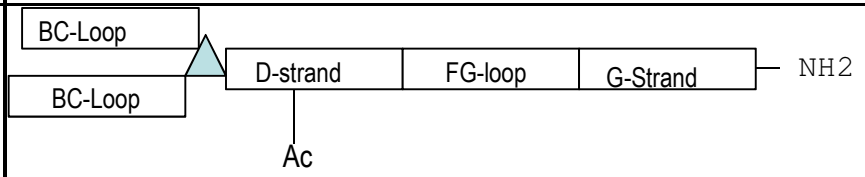
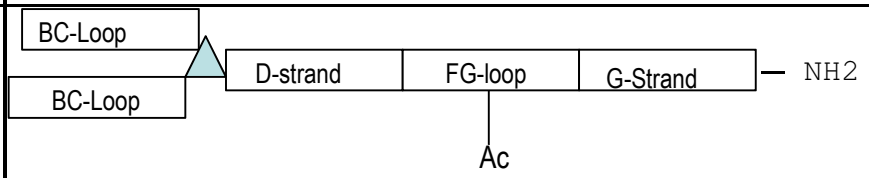
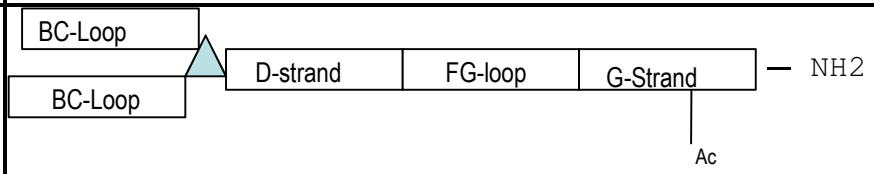
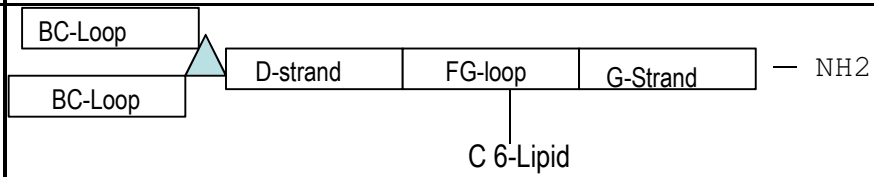
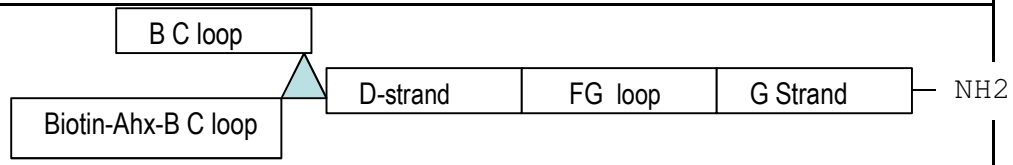
Comp. No.	Sequence	Structure
32.	SNTSESFK(SNTSESF)KAQIKE-NH2 (SEQ ID NO: 73)	
33.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{14}CO$)E-NH2 (SEQ ID NO: 74)	
34.	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_{14}CO$)AQIKE-NH2 (SEQ ID NO: 75)	
35.	SNTSESFK(SNTSESF)FRVTQK(LAP)KAQIKE-NH2 (SEQ ID NO: 76)	

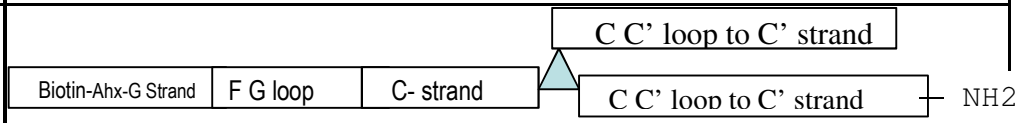
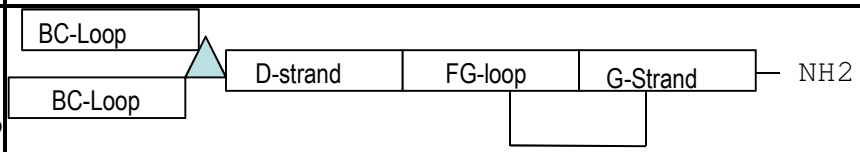
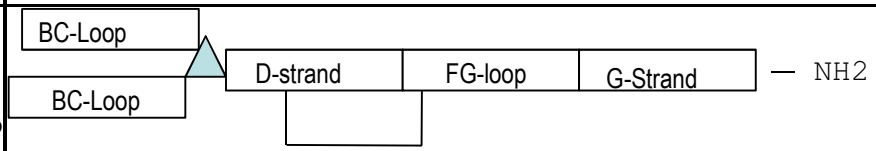
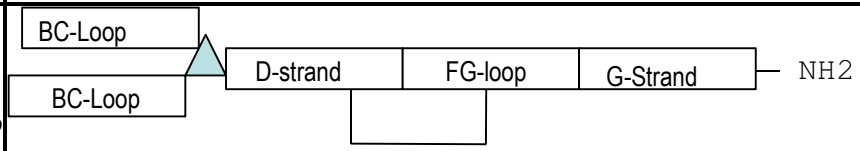
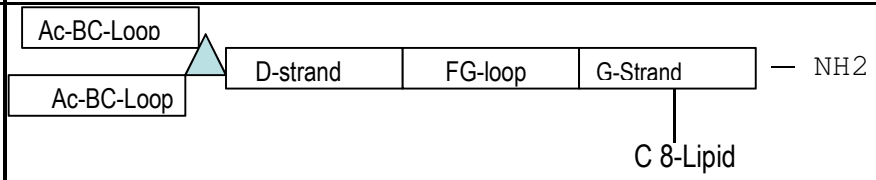
Comp. No.	Sequence	Structure
36.	SNTSESFK(SNTSESF)FRVTQLAK(PKA)QIKE-NH2 (SEQ ID NO: 77)	
37.	SNTSESFK(LAP)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 78)	
38.	LAPK(LAP)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 79)	
39.	LAPKAQIKE-NH2 (SEQ ID NO: 80)	
40.	SNTSESFK(SNTSESF)FK($CH_3(CH_2)_{14}CO$))VTQLAPKAQIKE-NH2 Arg in D strand replaced by Lys (SEQ ID NO: 81)	

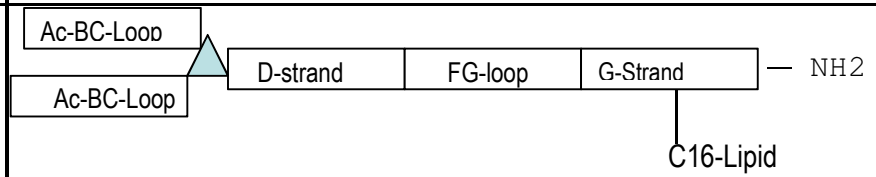
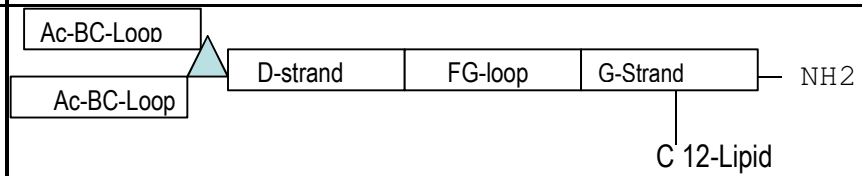
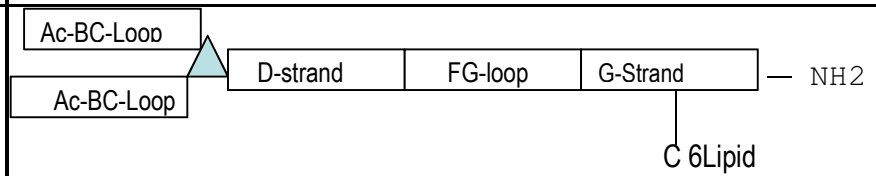
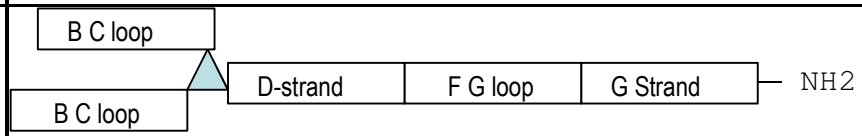
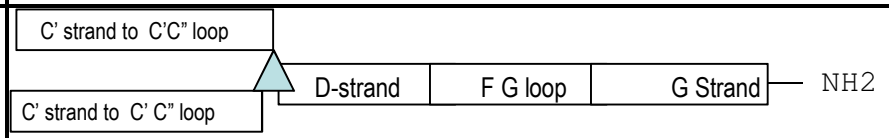
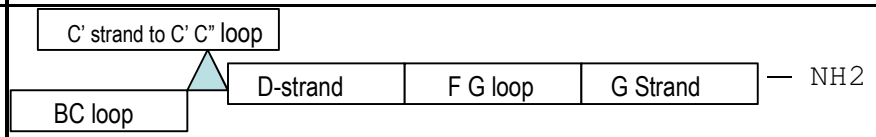
Comp. No.	Sequence	Structure
41.	SNTSESFK(SNTSESF)FRVTQLAP–NH2 (SEQ ID NO: 82)	
42.	SNTSESFFRVTQK(SNTSESF)LAPKAQIKE–NH2 (SEQ ID NO: 83)	
43.	FRVTQLAPKAQIKE–NH2 (SEQ ID NO: 84)	
44.	SNTSESFK(SNTSESF)FK($CH_3(CH_2)_6CO$)VTQLAPKAQIKE–NH2 Arg in D strand replaced by Lys (SEQ ID NO: 85)	
45.	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_6CO$)AQIKE–NH2 (SEQ ID NO: 86)	

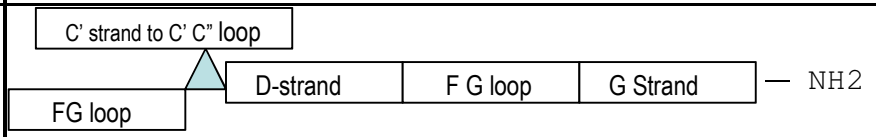
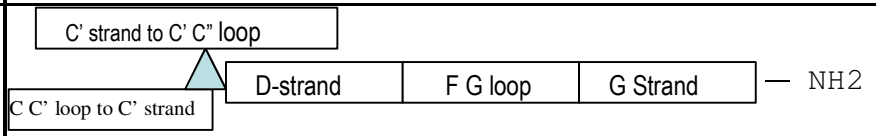
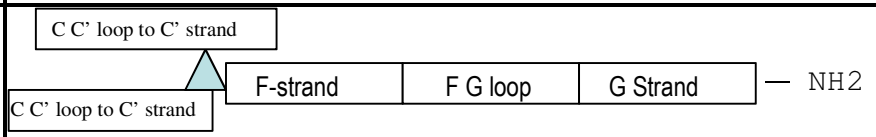
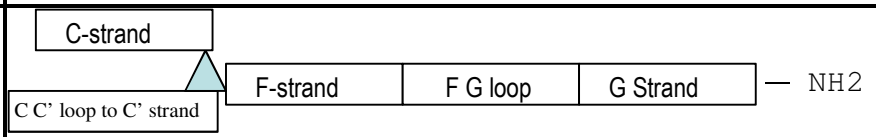
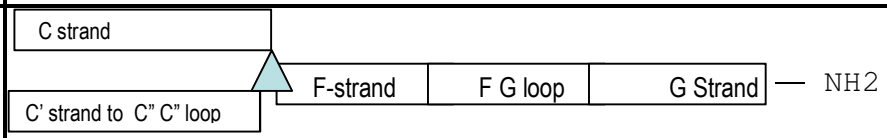
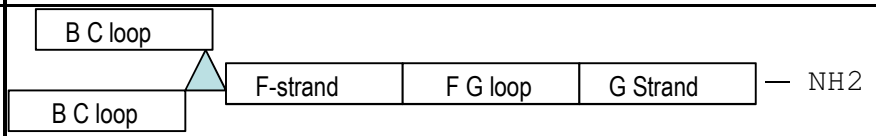
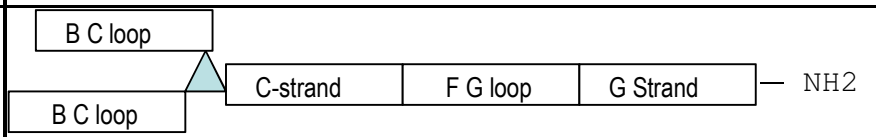
Comp. No.	Sequence	Structure
46.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(CH$_3$(CH$_2$)$_6$CO)E (SEQ ID NO: 87)	
47.	SNTSESFK(sntsesf)FRVTQLAPKAQIKE D-amino acids in the branch (SEQ ID NO: 88)	
48.	EKIQAKPALQTVRFK(FSESTNS)FSESTNS-NH2 (SEQ ID NO: 89)	
49.	ekiqakpalqtvrfk(fsestns)fsestns-NH$_2$ Retro inverso (All D-amino acid) (SEQ ID NO: 90)	
50.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(PEG-20KD)E-NH2 (SEQ ID NO: 91)	

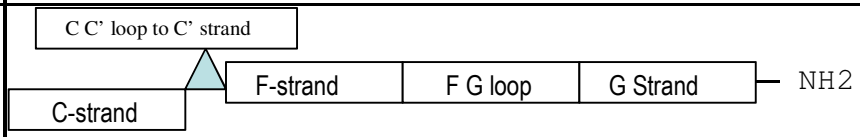
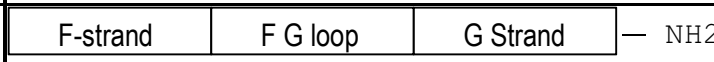
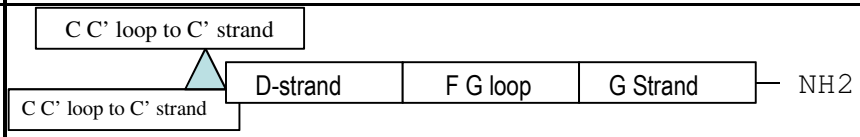
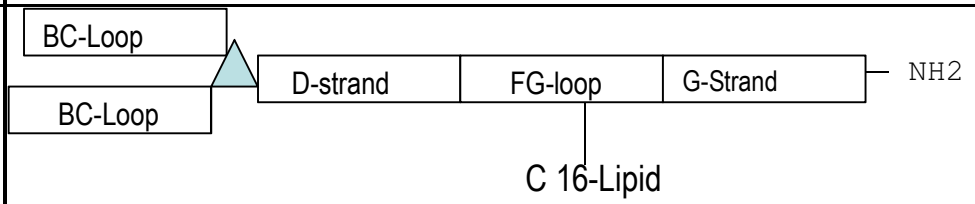
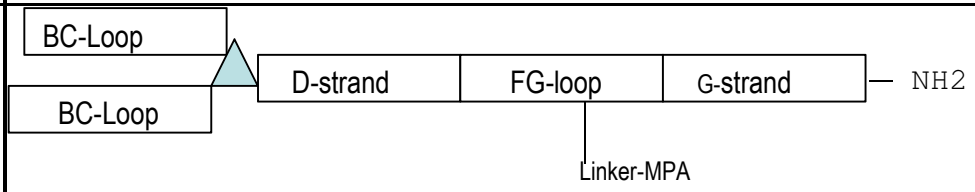
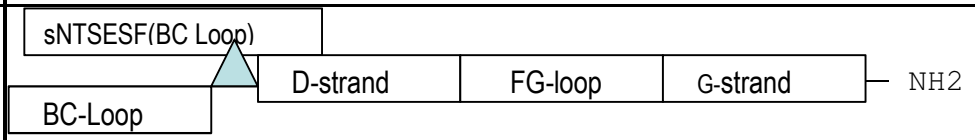
Comp. No.	Sequence	Structure
51.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(PEG-20KD)E-NH2 (SEQ ID NO: 92)	
52.	SNTSESFK(SNTSESF)FRVTQLAPK(PEG 20 KD)AQIKE-NH2 (SEQ ID NO: 93)	
53.	SNTSESFK(SNTSESF)FK(mini PEG)VTQLAPKAQIKE-NH2 Arg in D strand replaced by Lys (SEQ ID NO: 94)	
54.	SNTSESFK(SNTSESF)FRVTQLAPK(PEG 10KD)AQIKE-NH2 (SEQ ID NO: 95)	
55.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(PEG20KD)E-NH2 (SEQ ID NO: 96)	

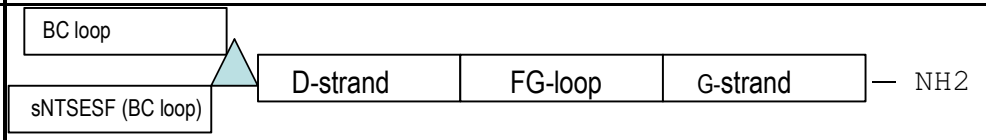
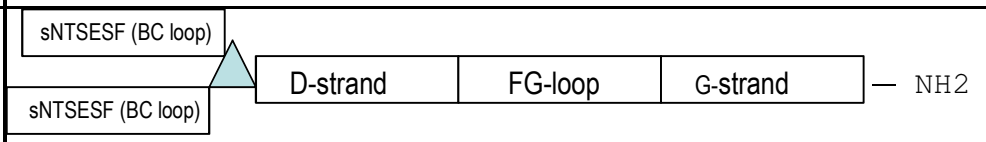
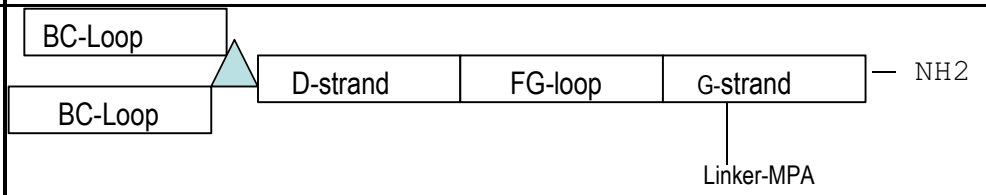
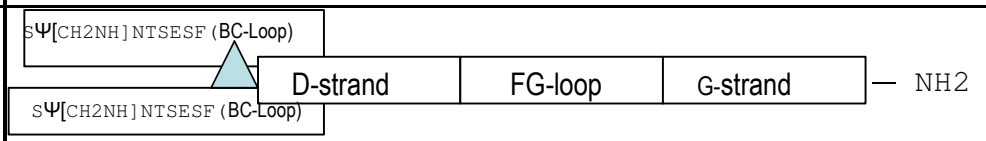
Comp. No.	Sequence	Structure
56.	SNTSESFK(SNTSESF)FK(CH3CO)VTQLAPK AQIKE-NH2 (SEQ ID NO: 97)	
57.	SNTSESFK(SNTSESF)FRVTQLAPK(CH3CO) AQIKE-NH2 (SEQ ID NO: 98)	
58.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(CH 3CO)E-NH2 (SEQ ID NO: 99)	
59.	SNTSESFK(SNTSESF)FRVTQLAPK(CH3- (CH2)4-CO)AQIKE-NH2 (SEQ ID NO: 100)	
60.	Biotin-Ahx- sntsesfk(sntsesf)frvtqlapkaqike- NH2 All D Amino acids (SEQ ID NO: 101)	

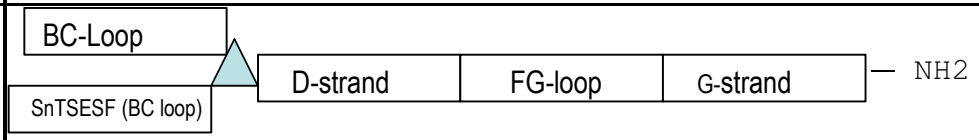
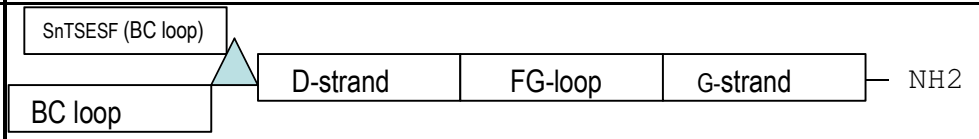
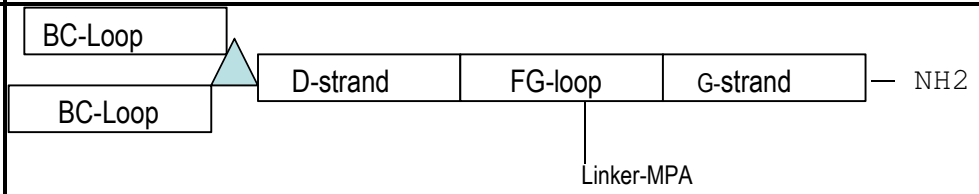
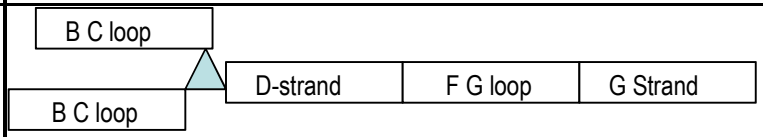
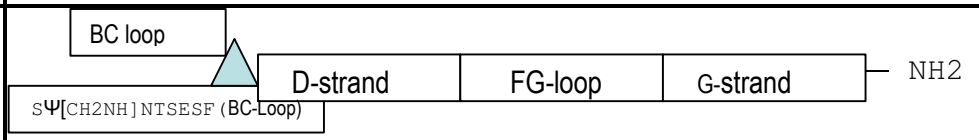
Comp. No.	Sequence	Structure
61.	Biotin-Ahx-EKIQAKPAYWNLVK(KDTQNS)DTQNS-NH2 (SEQ ID NO: 102)	
62.	SNTSESFK(SNTSESF)FRVTQLAPE*AQIK*E-NH2 (SEQ ID NO: 103) Lactam bond formed between asterisked(*) amino acids	
63.	SNTSESFK(SNTSESF)FK*VTQE*APKAQIKE-NH2 (SEQ ID NO: 104) Lactam bond formed between asterisked(*) amino acids	
64.	SNTSESFK(SNTSESF)FRVTE*LAPK*AQIKE (SEQ ID NO: 105) Lactam bond formed between asterisked(*) amino acids	
65.	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_6CO$)E (SEQ ID NO: 106)	

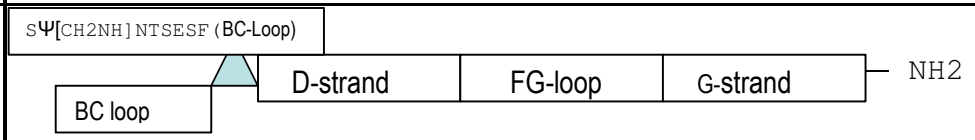
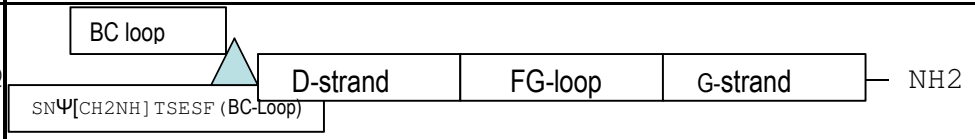
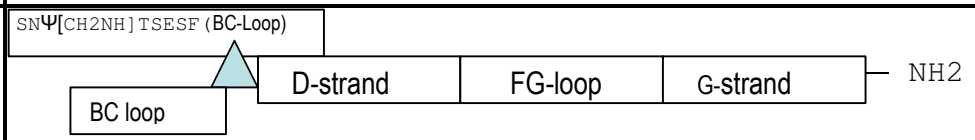
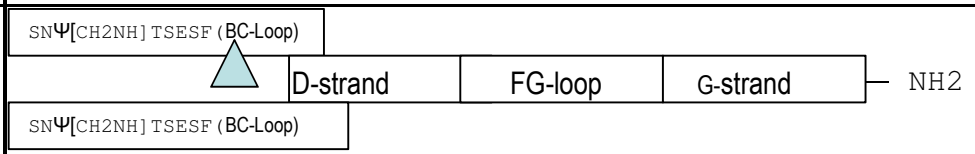
Comp. No.	Sequence	Structure
66.	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{14}CO$)E (SEQ ID NO: 107)	
67.	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{10}CO$)E at Lys (SEQ ID NO: 108)	
68.	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_4CO$)E (SEQ ID NO: 109)	
69.	SNWSEDLK(SNWSEDL)FQIIQLHPKAKIEE-NH2 (SEQ ID NO: 110)	
70.	EDK(ED)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 111)	
71.	SNTSESFK(ED)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 112)	

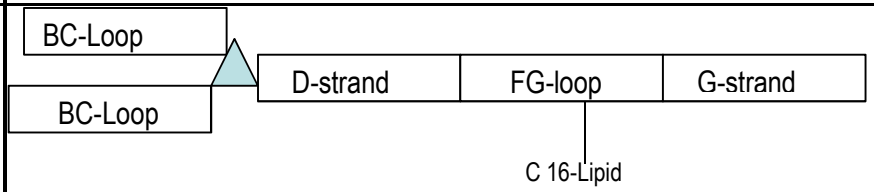
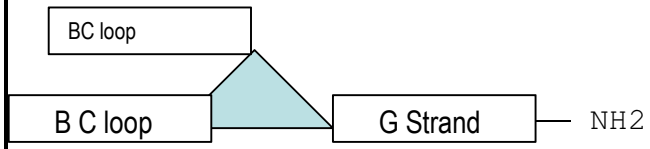
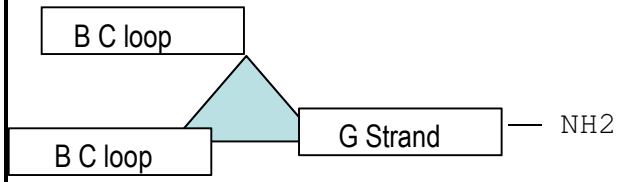
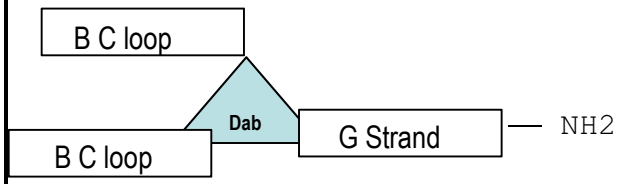
Comp. No.	Sequence	Structure
72.	LAPKAK(ED)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 113)	
73.	SNQTDKK(ED)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 114)	
74.	SNQTDK(SNQTDK)GIYLCGAISLAPKAQIKE-NH2 (SEQ ID NO: 115)	
75.	SNQTDKK(VLNWYRM)GIYLCGAISLAPKAQIKE-NH2 (SEQ ID NO: 116)	
76.	EDK(VLNWYRM)GIYLCGAISLAPKAQIKE-NH2 (SEQ ID NO: 117)	
77.	SNTSESFK(SNTSESF)GIYLCGAISLAPKAQIKE-NH2 (SEQ ID NO: 118)	
78.	SNTSESFK(SNTSESF)VLNWYRMLAPKAQIKE-NH2 (SEQ ID NO: 119)	

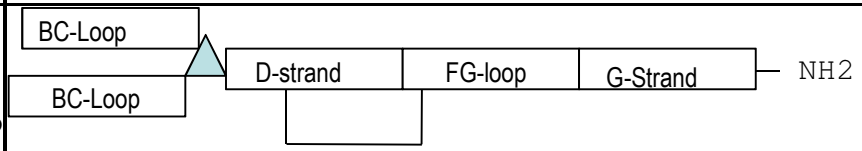
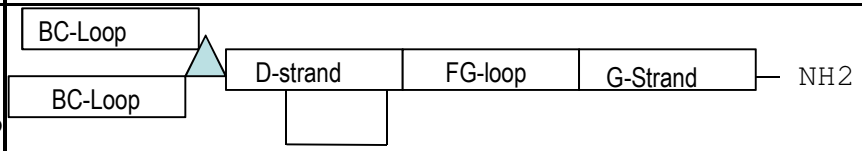
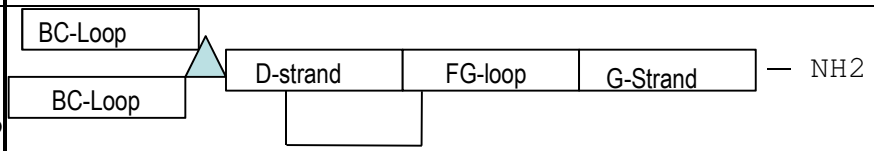
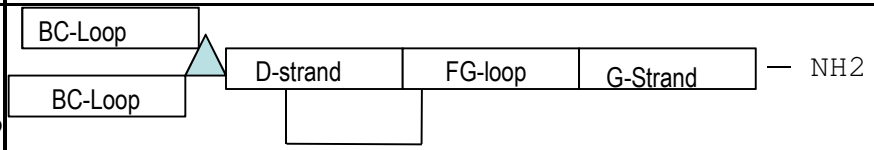
Comp. No.	Sequence	Structure
79.	VLNWYRMK(SNQTDK)GIYLCGAISLAPKAQIKE-NH2 (SEQ ID NO: 120)	
80.	GIYLCGAISLAPKAQIKE-NH2 (SEQ ID NO: 121)	
81.	SNQTDKK (SNQTDK) FRVTQ LAPKAQIKE-NH2 (SEQ ID NO: 122)	
82.	SNWSEDLK(SNWSEDL)FQIIQLHPK($CH_3(CH_2)_{14}CO$)AKIEE -NH2 (SEQ ID NO: 123)	
83.	SNTSESFK(SNTSESF)FRVTQLAPK(MPA-NH-CH_2-CH_2-O-CH_2-CH_2-O-CO)AQIKE-NH2 (SEQ ID NO: 124)	
84.	SNTSESFK(sNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 125)	

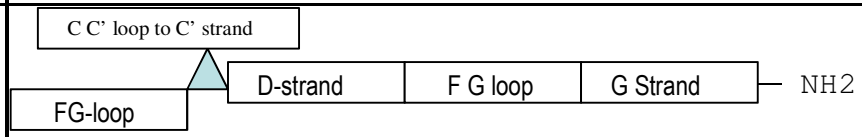
Comp. No.	Sequence	Structure
85.	sNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 126)	
86.	sNTSESFK(sNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 127)	
87.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(MPA-NH-CH_2-CH_2-O-CH_2-CH_2-O-CO)E-NH2 (SEQ ID NO: 128)	
88.	SΨ[CH2NH]NTSESFK(SΨ[CH2NH]NTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 129)	

Comp. No.	Sequence	Structure
89.	SnTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2 D-Asn at N-terminus (SEQ ID NO: 130)	
90.	SNTSESFK(SnTSESF)FRVTQLAPKAQIKE-NH2 D-Asn in the branch (SEQ ID NO: 131)	
91.	SNWSEDLK(SNWSEDL)FQIIQLHPK(MPA-NH-CH_2-CH_2-O-CH_2-CH_2-O-CO)AKIEE-NH2 (SEQ ID NO: 132)	
92.	SNTSESFK(SNTSESF)FRVTQLAPKAQIKE (SEQ ID NO: 133)	
93.	SΨ[CH2NH]NTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 134)	

Comp. No.	Sequence	Structure
94.	SNTSESFK(SΨ[CH2NH]NTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 135)	
95.	SNΨ[CH2NH]TSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 136)	
96.	SNTSESFK(SNΨ[CH2NH]TSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 137)	
97.	SNΨ[CH2NH]TSESFK(SNΨ[CH2NH]TSESF)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 138)	

Comp. No.	Sequence	Structure
98.	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_{14}CO$)AQIKE C-terminus acid (SEQ ID NO: 139)	
99.	SNTSESF-Orn-(SNTSESF)KAQIKE-NH2 (SEQ ID NO: 140)	
100.	SNTSESF-Dap-(SNTSESF)KAQIKE-NH2 (SEQ ID NO: 141)	
101.	SNTSESF-Dab-(SNTSESF)KAQIKE-NH2 (SEQ ID NO: 142)	

Comp. No.	Sequence	Structure
102.	SNTSESFK(SNTSESF)FRVTQLAPK*IAQE*KE-NH2 (SEQ ID NO: 143) Lactam bond formed between asterisked(*) amino acids	
103.	SNTSESFK(SNTSESF)K*RVTE*LAPKAQIKE-NH2 (SEQ ID NO: 144) Lactam bond formed between asterisked(*) amino acids	
104.	SNTSESFK(SNTSESF)FRK*TQLE*PKAQIKE-NH2 (SEQ ID NO: 145) Lactam bond formed between asterisked(*) amino acids	
105.	SNTSESFK(SNTSESF)FRVE*QLAK*PAQIKE-NH2 (SEQ ID NO: 146) Lactam bond formed between asterisked(*) amino acids	
106.	LAPKA-NH2 (SEQ ID NO: 147)	

Comp. No.	Sequence	Structure
107.	LAPKA(SNQTDK)FRVTQLAPKAQIKE-NH2 (SEQ ID NO: 148)	

Corresponds to Lysine (K)

Compounds of the invention may comprise peptide moieties that are lipidated, PEGylated and/or are glycosylated. One or more of the amino acids of the peptide may be a D-amino acid with a view to increasing stability *in vivo*.

The invention includes compounds as described above, formulated for pharmaceutical administration, typically by combination with a pharmaceutically acceptable carrier or diluent.

The invention includes compounds as described above for use in a method of medical treatment, e.g. in the treatment of cancer, treatment of bacterial and viral infections

The invention further includes a method of screening compounds for ability to block interaction between PD-1 and a PD-1 ligand, comprising contacting candidate compounds of the kind described above with PD-1 or a PD-1 ligand binding portion of PD-1 and with a PD-1 ligand or a PD-1 binding portion of a PD-1 ligand, and measuring the extent of PD-1/PD-1 ligand binding.

As indicated above, compounds of the invention may be peptides modified by PEGylation. Polymers have been used extensively to improve the pharmacokinetics and pharmacodynamics and hence, drug performance of drugs such as peptides, proteins, and small molecules. The most widely used polymer for pharmaceutical applications is polyethylene glycol ("PEG"). "PEGylation" is the process by which the drug is chemically modified to result in the covalent attachment ("coupling") of one or more PEG molecules to the drug (depending on how many sites are available on the drug to interact with, and be conjugated to PEG). The improved pharmacological and biological properties associated with PEGylation of drugs are well known in the pharmaceutical art. For example, PEGylation can increase therapeutic efficacy by means including, but not limited to, reducing degradation by proteolytic enzymes and thereby increasing drug concentration; increasing the size of the drug to which it is attached, thereby improving drug biodistribution; and shielding antigenic epitopes in reducing immunogenicity where desired. By increasing the therapeutic efficacy, the frequency of dosing and/or the amount of drug need to achieve a therapeutic effect may be reduced.

PEG, as a linear polyether, has the general structure: HO-(CH_2-CH_2O) n-CH_2CH_2-OH where n can typically range from about 10 to about 2000.

PEG may be modified which includes H2N-(CH_2-CH_2O) n-CH_2CH_2-COOH where n can typically range from about 10 to about 2000.

Many of the PEG modifications, in forming PEG derivatives (PEG and PEG derivatives are known in the art as "PEG"), are directed to the end groups ("functionalities") in adding or varying their chemically reactive functionalities to be used to covalently attach the PEG molecule to a drug. Various PEG derivatives are well known in the art. To couple PEG to a drug, typically a functionality of the PEG molecule needs to be activated so as to be chemically reactive. The type and specificity of functionality is based upon the choice of chemically reactive group on the drug to which the PEG molecule is to be coupled. Most commonly for proteins and peptides, the chemically reactive group is present on an amino acid which may be an internal amino acid having a side chain with a free chemically reactive group (e.g. including, but not limited to, lysine, cysteine, glutamic acid, serine, threonine, and the like), the N-terminal amino acid (having a N-terminal amine group, or a side chain amine group, as a free chemically reactive group), a C-terminal amino acid (having a C-terminal carboxylic acid, or side chain amine group as a free chemically reactive group) or a combination thereof. Of the sites of a peptide to be coupled to PEG, most frequently chosen is the N-terminal amine group ("alpha amine") of the peptide's N-terminal amino acid, and the epsilon amine group ("epsilon amine") of a lysine (a lysine found within the amino acid sequence which is not the N-terminal amino acid or the C-terminal amino acid of the peptide) or an epsilon amine group of lysine when the lysine is present in a peptide as a N-terminal amino acid or as a C-terminal amino acid.

Where there are multiple lysine groups in the peptide and multiple binding of PEG molecules is not desired, one can incorporate into its amino acid sequence at selected amino acid positions during synthesis, one or more amine groups (e.g. one or more of an alpha amine or an epsilon amine(s)) which is blocked with a chemical protecting agent from chemically reactive with amine-reactive functionality of PEG during PEGylation, thereby leaving available for PEGylation only the free amine group (s) in selected amino acid positions (through chemical modification) of the synthetic peptide to be covalently coupled to PEG.

Preferred polyols for use in PEGylation comprise a water-soluble poly (alkylen oxide) polymer, and can have a linear or branched chain. The term "polyol" preferably refers to a water-soluble, polyalcohol which may include, but is not limited to, polyethylene glycol ("PEG"), polypropylene glycol ("PPG"), diethylene glycol, triethylene glycol, ethylene glycol, dipropylene glycol, copolymers comprising PPG (e. g., ethylene glycol/PPG), copolymers comprising PEG (e. g. , PEG/PPG), mPEG (monomethoxy-poly (ethylene) glycol), and the like. Suitable polyols

encompass both homopolymers and copolymers, and further may have a structure comprising a branched structure or linear structure as known to those skilled in the art.

Preferably, the polymer is substantially non-toxic when used for in vivo applications in individuals. In a preferred embodiment, the polymer has a molecular weight in the range between about 200 daltons to about 40,000 daltons; and in a more preferred embodiment, the polymer has a molecular weight range between about 400 daltons to about 10,000 daltons. A preferred polymer for application in the present invention comprises a polyethylene glycol ("PEG"), and a more preferred polymer for application in the present invention comprises a polyethylene glycol having a molecular weight range, wherein the molecular weight range is no less than about 400 Daltons and is no more than about 20,000 daltons.

As described previously herein, there are various forms of PEG that typically differ in the end groups or chemically reactive functional groups to be used to covalently attach the PEG molecule to a drug. Various PEGs are well known in the art. A preferred PEG, for use in coupling to one or more unprotected amine groups of the synthetic peptide in accordance with the present invention, has a chemically reactive group (e. g. "functionality") which can be used covalently couple PEG to the to one or more unprotected amine groups. PEG may include but are not limited to, PEG-tresylate, heterobifunctional PEG, PEG dichlorotriazine, PEG succinimidyl carbonate, PEG benzotriazole carbonate, PEG p-nitrophenyl carbonate, PEG trichlorophenyl carbonate, PEG carbonylimidazole, PEG succinimidyl succinate, mPEG succinimidyl propionate, mPEG succinimidyl butanoate, PEG butyraldehyde, mPEG-propionaldehyde, PEG aldehyde, PEG-acetaldehyde, PEG acetaldehyde diethyl acetal, PEG carboxylic acid, mPEG phenyl ether succinimidyl carbonates, mPEG benzamide succinimidyl carbonates, PEG thioester, linear PEG, branched PEG, and linear forked PEG.

In addition, compounds of the invention may be combined with carrier molecules such as dendrimers, e.g. pAMAM dendrimers, liposomes, micro-particles and nanoparticles such as polycyanoacrylate nanoparticles, and these also may be PEGylated.

Compounds similar to those of the invention but where modifications are made that render the compound less active in blocking PD-1 signalling pathway may be useful, for instance as controls in screening protocols. Amongst such compounds are expected to be analogues of any of the compounds of the invention described herein where all or most of the amino acids are

D-amino acids, analogues of any of the compounds of the invention described herein where all or most of the amino acids are reversed in sequence (retro-compounds) and analogues of such retro-compounds where all or most of the amino acids are D-amino acids, retroinverso-compounds.

Further embodiment of the present invention relates to the compounds as disclosed in the present invention, wherein one or more of the amino acids of the peptide moiety the compounds is substituted with a D-amino acid.

The compounds as disclosed in the present invention are formulated for pharmaceutical administration.

Another embodiment of the present invention provided a pharmaceutical composition comprising the compound as disclosed, and a pharmaceutically acceptable carrier or diluent.

Yet another embodiment of the present invention provides use of the compounds as disclosed in the present invention for the preparation of a medicament for the treatment of cancer.

Yet another embodiment of the present invention provides use of the compounds as disclosed in the present invention for the preparation of a medicament for the treatment of bacterial and viral infection.

Yet another embodiment of the present invention provides a method of treatment of cancer, wherein the method comprises administration of an effective amount of the compound and/or peptides of the present invention to the subject in need thereof. Yet another embodiment of the present invention provides a method for inhibiting growth of tumour cells and/or metastasis by administering an effective amount of the compound of the present invention to the subject in need thereof.

The said tumour cells include cancer such as but not limited to melanoma, renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine

system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumours of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumour angiogenesis, spinal axis tumour, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

Still yet another embodiment of the present invention provides a method of treatment of infectious disease via immunopotentiation caused by inhibition of immunosuppressive signal induced by PD-1, PD-L1, or PD-L2, wherein the method comprises administration of an effective amount of the compound and/or peptides of the present invention to the subject in need thereof.

The infectious disease includes but not limited to HIV, Influenza, Herpes, Giardia, Malaria, Leishmania, the pathogenic infection by the virus Hepatitis (A, B, & C), herpes virus (e.g., VZV, HSV-I, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus, pathogenic infection by the bacteria chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, E. coli, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lyme's disease bacteria, pathogenic infection by the fungi Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum, and pathogenic infection by the parasites Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma

brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, Nippostrongylus brasiliensis.

The compounds of the present invention are for use in a method of medical treatment.

The compounds of the present invention may be used as single drugs or as a pharmaceutical composition in which the compound is mixed with various pharmacologically acceptable materials.

The pharmaceutical composition is usually administered by a parenteral administration route, but can be administered by oral or inhalation routes. Examples of the parenteral administration include administration by injection, and percutaneous, transmucosal, transnasal and transpulmonary administrations.

The injectable materials include a solution, a suspension, and a solid injection that is dissolved or suspended in a solvent before use.

The injection is used after one or more active ingredients are dissolved, suspended or emulsified in a solvent. Examples of the solvent include water-soluble solvents (e.g., distilled water, physiological saline and Ringer's solution), oil solvents (e.g., vegetable oils such as olive oil, sesame oil, cotton oil and corn oil, and alcohols such as propylene glycol, polyethylene glycol and ethanol), and combinations thereof.

Further, the injection may contain a stabilizer (e.g., human serum albumin), solubilizing agent (e.g., polyethylene glycol, propylene glycol, D-mannitol, trehalose, benzyl benzoate, ethanol, trisaminomethane, cholesterol, triethanolamine, sodium carbonate, sodium citrate, sodium salicylate and sodium acetate), suspending agent (e.g., surfactants such as stearyl triethanolamine, sodium lauryl sulfate, lauryl aminopropionic acid, lecithin, benzalkonium chloride, benzethonium chloride and glyceryl monostearate; hydrophilic polymers such as polyvinyl alcohol, polyvinyl pyrrolidone, sodium carboxymethyl cellulose, methyl cellulose, hydroxymethyl cellulose, hydroxyethyl cellulose and hydroxypropyl cellulose; polysorbates; and polyoxyethylene hardened castor oil), emulsifier, soothing agent (e.g., benzyl alcohol), tonicity agent (e.g., sodium chloride, glycerin, D-mannitol, D-sorbitol and glucose), buffer, preservative (e.g., methylparaben, propylparaben, benzyl alcohol, chlorobutanol and phenol), antiseptic (e.g., paraoxybenzoic esters, chlorobutanol, benzyl alcohol, phenethyl alcohol, dehydroacetic acid and

sorbic acid), antioxidant (e.g., sulfite and ascorbate) and dispersant (e.g., Polysorbate 80, Polyoxyethylene hardened castor oil 60, ethylene glycol, carboxymethyl cellulose and sodium alginate).

These injections may be prepared by known methods in the formulation technology field, such as by a method described in various Pharmacopoeia. They are prepared, for example, through a sterilization process at the final stage, or by aseptic manipulation. It is also possible to use an aseptic solid formulation, such as a freeze dried product, wherein the aseptic solid formulation is prepared and dissolved in aseptic or sterilized distilled water for injection or other solvents before use.

These parenteral solutions may be supplied in a vessel with a standard capacity, such as a plastic or glass vial, ampoule, syringe and injector, or in a vessel with a large capacity, such as a bottle.

The dosage of the compounds of the present invention varies depending on age, weight, symptom, therapeutic efficacy, dosing regimen and/or treatment time. Generally, they may be administered by a parenteral route (preferably intravenous administration) in an amount of 1 ng to 100 mg per time, from once a couple of days, once 3 days, once 2 days, once a day to a couple of times a day, in the case of an adult, or continuously administered by intravenous administration from 1 to 24 hours a day. Since the dosage is affected by various conditions, an amount less than the above dosage may sometimes work well enough, or higher dosage may be required in some cases.

Parenteral administration by injection includes all forms of injections, and also includes intravenous fluids. For example, it includes intramuscular injections, subcutaneous injections, intradermal injections, intraarterial injections, intravenous injections, intraperitoneal injections, injections to spinal cavity, and intravenous drops.

The compounds of the present invention may be administered in combination with other drugs for (1) complementation and/or enhancement of prevention and/or therapeutic efficacy of the preventive and/or therapeutic drug of the present invention, (2) dynamics, absorption improvement, dosage reduction of the preventive and/or therapeutic drug of the present invention, and/or (3) reduction of the side effects of the preventive and/or therapeutic drug of the present invention.

A concomitant medicine comprising the peptide of the present invention and other drug may be administered as a combination preparation in which both components are contained in a single formulation, or administered as separate formulations. The administration by separate formulations includes simultaneous administration and administration with some time intervals. In the case of the administration with some time intervals, the compound of the present invention can be administered first, followed by another drug or another drug can be administered first, followed by the compound of the present invention. The administration method of the respective drugs may be the same or different.

The dosage of the other drug can be properly selected, based on a dosage that has been clinically used. The compounding ratio of the compound of the present invention and the other drug can be properly selected according to age and weight of a subject to be administered, administration method, administration time, disorder to be treated, symptom and combination thereof. For example, the other drug may be used in an amount of 0.01 to 100 parts by mass, based on 1 part by mass of the compound of the present invention. The other drug may be a combination of two or more kind of arbitrary drugs in a proper proportion. The other drug that complements and/or enhances the preventive and/or therapeutic efficacy of the compound of the present invention includes not only those that have already been discovered, but those that will be discovered in future, based on the above mechanism.

Diseases on which this concomitant use exerts a preventive and/or therapeutic effect are not particularly limited. The concomitant medicine can be used for any diseases, as long as it complements and/or enhances the preventive and/or therapeutic efficacy of the compound of the present invention.

Particularly, since the compound of the present invention exhibits an effect of stimulating or proliferating lymphoid cells, the concomitant use is able to reduce a dosage of chemotherapeutics commonly used or an irradiation dosage in radio therapy. This results in suppression of side effects that accompany with chemotherapy and radio therapy.

The compound of the present invention can be used with an existing chemotherapeutic concomitantly or in a mixture form. Examples of the chemotherapeutic include an alkylation agent, nitrosourea agent, antimetabolite, anticancer antibiotics, vegetable-origin alkaloid, topoisomerase inhibitor, hormone drug, hormone antagonist, aromatase inhibitor, P-glycoprotein

inhibitor, platinum complex derivative, other immunotherapeutic drugs and other anticancer drugs. Further, it can be used with a cancer treatment adjunct, such as a leucopenia (neutrophenia) treatment drug, thrombocytopenia treatment drug, antiemetic and cancer pain intervention drug, concomitantly or in a mixture form.

The compound of the present invention can be used with other immunomodulators concomitantly or in a mixture form. Examples of the immunomodulator include various cytokines. Examples of the cytokines that stimulates immune responses include GM-CSF, M-CSF, G-CSF, interferon-α, β, or γ, IL-1, IL-2, IL-3 and IL-12.

The concomitant use of the compound of the present invention and a cancer antigen is able to give an additive or synergetic enhancement effect. Examples of the cancer antigen include HLA-A1 and HLA-A2 derived peptides derived from MAGE-1 or MAGE-3 of malignant melanoma, MART-1 and gp100, HER2/neu peptide of breast cancer and ovarian cancer, MUC-1 peptide of adenocarcinoma and NY-ESO-1 of metastatic cancer.

Since a compound of the present invention binds to its ligand molecule strongly and specifically, the labelling agent thereof is able to be used as a test or diagnostic drug or a research reagent for diseases in which the cell surface functional molecule or the ligand molecule is involved.

Examples of the labelling agent that can label the compounds of the present invention include radioisotopes, enzymes, fluorescent materials, luminous materials, ultraviolet absorption materials.

When a compound of the present invention is used in an enzyme-linked immunosorbent assay (EIA) method, it can be used by labelling it with enzymes, such as alkali phosphatase, β-galactosidase, peroxidase, microperoxidase, glucose oxidase, glucose-6-phosphate dehydrogenase, acetylcholinesterase, malate dehydrogenase and luciferase.

When a compound of the present invention is used in a radioimmunoassay (RIA) method, it can be used by labelling it with a radioisotope, such as ^{131}I, ^{125}I, ^{99m}Tc, ^{355}S, ^{32}P, ^{14}C and ^{3}H.

When a compound of the present invention is used in a fluorescence immunoassay (FIA) method, it can be used by labelling it with a fluorescent material, such as fluorescein, dansyl, fluorescamine, coumarin, naphthylamine, fluorescein isothiocyanate, rhodamine, rhodamine X

isothiocyanate, sulforhodamine 101, Lucifer yellow, acridine, acridine isothiocyanate, riboflavin and the derivatives, and europium (Eu).

When the compound of the present invention is used in a chemiluminescent immunoassay (CLIA) method, it can be used by labelling it with a luminous material, such as luminol derivatives, e.g., luciferin, isoluminol, luminol, aminoethyl isoluminol, aminoethylethyl isoluminol, aminopropyl isoluminol, aminobutyl isoluminol and aminohexylethyl isoluminol; luciferin; lucigenin; and bis(2,4,6-trifluorophenyl) oxalate.

When a compound of the present invention is used in an ultraviolet absorption method, it can be used by labelling it with a substance that has absorption in a wavelength of ultraviolet rays, such as phenol, naphthol, anthracene and derivatives thereof.

When a compound of the present invention is used in an electron spin resonance (ESR) method, it can be used by labeling it with a spin labelling agent represented by a compound having an oxyl group, such as 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl, 3-amino-2,2,5,5-tetramethylpyrrolidine-1-oxyl, 2,6-di-t-butyl-α-(3,5-di-t-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-p-tolyl oxyl.

Further, the compound of the present invention can be labelled with any labelling agents that are commonly used in the field.

In order to bind the above labelling agent to the peptide moiety of the compound of the present invention, known labelling methods, which are commonly performed in EIA, RIA, FIA and the like, are applied, such as those described in "Medical Chemistry Experimental Course, 1st Ed., Vol. 8, Edited by U. Yamamura, published by Nakayama Bookstore, 1971; Illustrated Fluorescent Antibody, 1st Ed., written by A. Kawao, published by Softscience, 1983; and Enzyme-linked immunoassay, 2nd Ed., compiled by E. Ishikawa, T. Kawai and K. Muroi, published by Igakushoin, 1982".

Of such labelling methods, a preferred method includes that exploits a reaction between avidin (or sreptavidin) and biotin. In the case of exploiting the reaction between avidin (or streptavidin) and biotin, a method for binding biotin with the peptide moiety of the compound of the present invention includes a method for reacting a commercially available biotinylating agent (e.g., an agent prepared by binding biotin to which a succineimide group (e.g., NHS-biotin) is

introduced or N-hydroxysuccinic acid imide (NHS) with biotin through a spacer) with an amino group in protein (Journal of Biological Chemistry, 1989, Vol. 264, pp.272-279); a method for reacting a commercially available biotin-HPDP (N-[6-(biotinamide)hexyl]-3'-(2'-pyridylthio)propione amide) or N-iodoacetyl-N-biotinylhexylenediamine with a thiol group in protein (Ann. New York Acad. Sci., 1975, Vol. 254, No. 203); or a method for reacting biotin, to which a hydrazine group is introduced, with an aldehyde group in aldehyded protein (Biotech. Apple. Biochem., 1987, Vol. 9, pp.488-496).

The invention will be further described and illustrated in the Examples below.

Automated peptide synthesis was carried out in Symphony parallel synthesizer from Protein Technologies Inc.

Protocol using Symphony Parallel Synthesizer

Program for swelling, first residue attachment and capping, (Table I)

#	Step	Time
1	DCM (7.5 mL)	30 min X 2
2	DMF (7.5 mL)	30 min X 2
3	Deprotection: 20 % Piperdine /DMF (5 mL)	7.30 min X 2
4	Washing: DMF, DCM, DMF (10 mL)	30 sec X 3 each
5	Coupling: 0.25 M Fmoc AA (2.5 mL)/ 0.25 M HBTU/ 1 M NMM/DMF (2.5 mL)	2h X 1
6	DMF, DCM, DMF (10 mL)	30 sec X 3 each
7	Acylation (Acetic anhydride:Pyridine:DCM; 1:8:8) (5 mL)	7.30 min X 2
8	DCM, DMF (10 mL)	30 sec X 3 each

Program for coupling of amino acids (Table II)

#	Step	Time
1	Deprotection: 20 % Piperidine /DMF (5 mL)	7.30 min X 2

2	Washing: DMF, DCM, DMF (10 mL)	30 sec X 3 each
3	Coupling : 0.25 M Fmoc AA (2.5 m L)/ 0.25 M HBTU/ 1 M NMM/DMF (2.5 m L)	2h X 1
4	DMF, DCM, DMF (10 mL)	30 sec X 3 each

Program for coupling of N-terminal amino acid (Table III)

#	Step	Time
1	Deprotection: 20 % Piperidine /DMF (5 mL)	7.30 min X 2
2	Washing: DMF, DCM, DMF (10 mL)	30 sec X 3 each
3	Coupling : 0.25 M Fmoc AA (2.5 m L)/ 0.25 M HBTU/ 1 M NMM/DMF (2.5 m L)	2h X 1
4	DMF, DCM, DMF (10 mL)	30 sec X 3 each
5	Deprotection: 20 % Piperidine /DMF (5 mL)	7.30 min X 2
6	Washing: DMF, DCM, DMF (10 mL)	30 sec X 3 each

Procedure for cleavage of peptidyl resin and global deprotection

The peptidyl Resin was washed with MeOH (6 X 15 ml) and solvent ether (3 X 15 ml) and dried under vacuum. The cleavage of the peptides from the solid support is achieved by treating the peptide-resin with cleavage cocktail as specified for each peptide at room temperature for 2.5 h. Cleavage mixture was collected by filtration and the resin was washed with TFA and DCM. The excess TFA and DCM was concentrated to small volume under nitrogen and DCM was added to the residue and evaporated under nitrogen. The process was repeated 3-4 times to remove most of the volatile impurities. The residue was cooled to 0^0C and anhydrous ether was added to precipitate the peptide. The precipitated peptide was centrifuged and the supernatant ether was removed and fresh ether was added to the peptide and re-centrifuged. The residue was dissolved in Millipore water and lyophilized to obtain the crude peptide.

Cleavage cocktail A= 82.5% TFA / 5% phenol / 5% thioanisole / 2.5% 1,2 ethanedithiol / 5% water

Cleavage cocktail B= 80% TFA / 5% phenol / 5% thioanisole / 2.5% 1,2 ethanedithiol / 5% DCM/ 2.5 % DMS

Cleavage Cocktail C= 90% TFA / 5% TIPS / 5% water

Purification and characterization of peptide

The Reverse phase analytical HPLC was performed using on Zorbax Eclipse XDB-C18 silica column (4.6 mm X 250mm, 5µm).

The elution conditions used are

Method -1: Buffer A: 0.1% TFA/Water, Buffer B: 0.1% TFA in 9:1 acetonitrile /water.

Equilibration of the column with 2 % buffer B and elution by a gradient of 2% to 70% buffer B during 15 min.

Method -2: Buffer A: 0.1% TFA/Water, Buffer B: 0.1% TFA in 9:1 acetonitrile /water.

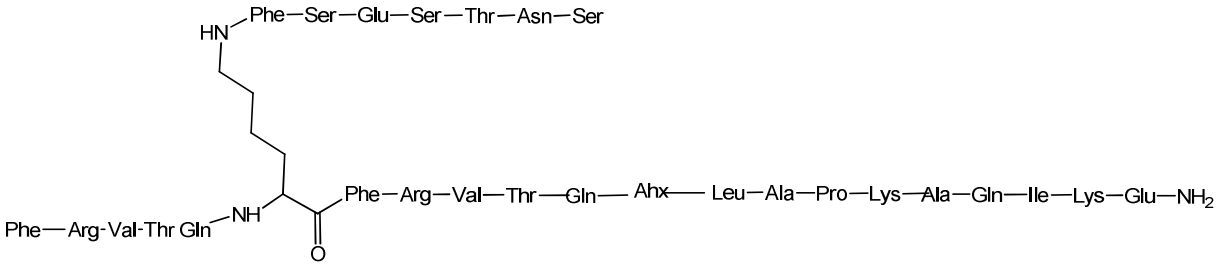
Equilibration of the column with 2 % buffer B and elution by a gradient of 2% to 25% buffer B in 5 min and from 25% to 40% buffer B in total run time of 20 min

Method -3: Buffer A: 0.1% TFA/Water, Buffer B: 0.1% TFA in 9:1 acetonitrile /water.

Equilibration of the column with 2 % buffer B and elution by a gradient of 0-15 min=2-70% buffer B, 15-20 min = 70-95 % buffer B

LCMS was performed on AP1 2000 LC/MS/MS triple quad (Applied biosystems) with Agilent 1100 series HPLC with G1315 B DAD diode array detector, using Mercury MS column

Example 1; Synthesis of



(SEQUENCE ID NO 47)

Synthesis of linear fragment: FRVTQKFRVTQ[Ahx]LAPKAQIKE-NH2

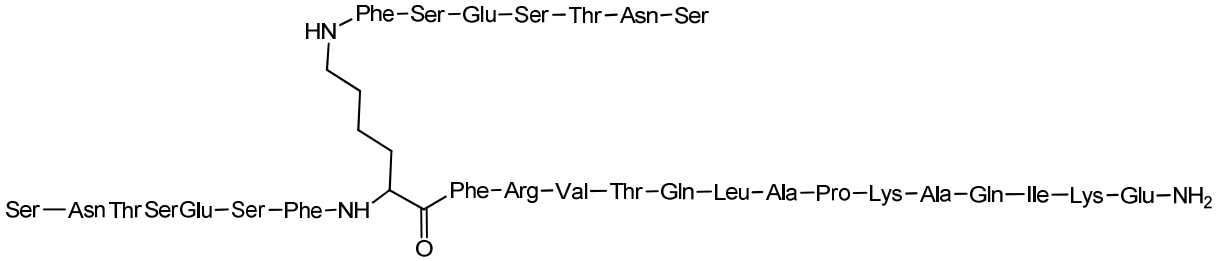
Desiccated CLEAR-Amide resin ((100-200 mesh) 0.4 mmol/g, 0.5 g) was distributed in 2 polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent amino acid coupling was carried out as mentioned in Table II. The amino acids used in the synthesis were Fmoc Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ahx-OH (Fmoc-6-aminohexanoic acid), Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH. After the completion of linear synthesis fragment in automated peptide synthesiser, the resin was taken out, pooled together and synthesis was carried out manually.

The N-terminal amino acid phenylalanine in the linear chain of the peptide was coupled as N – Boc amino acid in the same procedure as employed for N-Fmoc amino acid. The ε-amino group of sixth lysine (from N terminus) used as branching point was protected with Dde group. After completion of the linear peptide chain Dde protection of ε-amino group of the lysine was orthogonally deprotected by treating the resin with 2.5% (v/v) Hydrazine hydrate solution in DMF for 5 and 15 minutes. The resin is then filtered and washed with DMF (6 X 10 mL), DCM (6 X 10 mL) and DMF (6 X 10 mL). Deprotection is confirmed by positive ninhydrin test. **Fmoc-Phe-OH** (0.39 g; 5 equiv 1 m mol) in dry DMF was added to the deprotected resin and coupling was initiated with DIC (0.16 m L ;5 equiv) and HOBT (0.14 g ;5 equiv) in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of coupling was negative. The Fmoc group of the peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (10 m L). The resin was washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. Next amino acid in the peptide sequence **Fmoc-Ser (OtBu)-OH** (0.39 g; 5 equiv 1 m mol) in dry DMF was added to the deprotected resin and coupling was initiated with DIC (0.16 m L; 5

equiv) and HOBT (0.14 g; 5 equiv) in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of coupling was negative. On completion of Serine coupling Fmoc group on the Serine was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (10 m L). The resin was washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. Next amino acid **Fmoc-Glu (OtBu)-OH** (0.43 g; 5 equiv 1 m mol) in dry DMF was added to the deprotected resin and coupling was initiated with DIC (0.16 m L ;5 equiv) and HOBT (0.14 g ;5 equiv) in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of coupling was negative. The Fmoc group on the peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (10 mL). The resin was washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. Next amino acid **Fmoc-Ser (OtBu)-OH** (0.39 g; 5 equiv 1 m mol) in dry DMF was added to the deprotected resin and coupling was initiated with DIC (0.16 m L ;5 equiv) and HOBT (0.14 g ;5 equiv) in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of coupling was negative. The Fmoc group on the peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (10 mL). The resin was washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. Next amino acid **Fmoc-Thr (OtBu)-OH** (0.4 g; 5 equiv 1 m mol) in dry DMF was added to the deprotected resin and coupling was initiated with DIC (0.16 m L ;5 equiv) and HOBT (0.14 g ;5 equiv) in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L).

Kaiser test on peptide resin aliquot upon completion of coupling was negative. The Fmoc group on the peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (10 mL). The resin was washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. Next amino acid **Fmoc-Asn (Trt)-OH** (0.6 g; 5 equiv 1 m mol) in dry DMF was added to the deprotected resin and coupling was initiated with DIC (0.16 m L ;5 equiv) and HOBT (0.14 g ;5 equiv) in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of coupling was negative. The Fmoc group on the peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (10 mL). The resin was washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. Next amino acid **Fmoc-Ser (OtBu)-OH** (0.39 g; 5 equiv 1 m mol) in dry DMF was added to the deprotected resin and coupling was initiated with DIC (0.16 m L ;5 equiv) and HOBT (0.14 g ;5 equiv) in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of coupling was negative. The Fmoc group on the peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (10 mL). The resin was washed with DMF (6 X 10 m L), DCM (6 X 10 m L) and DMF (6 X 10 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail A to yield (450 mg), 69 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column (9.4 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: 0.1% TFA in 9:1 acetonitrile./water. The peptide was eluted by gradient elution 0-5 min = 5-20 % buffer B, 5-40 min = 20-35 % buffer B with a flow rate of 5 mL/min. HPLC: (method 2): RT -14.6 min (94.4%); LCMS Calculated Mass: 3237.83, Observed Mass: 1619.9 $[M/2+H]^+$;1080.2 $[M/3+H]^+$

Example 2: Synthesis of



(SEQUENCE ID NO 49)

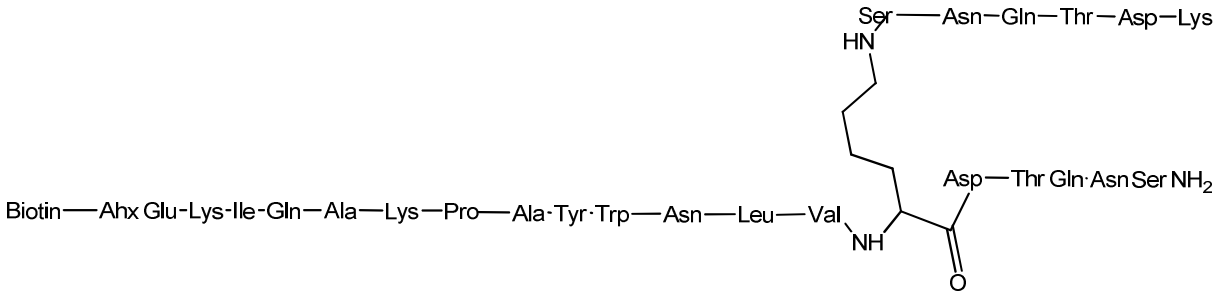
Synthesis of linear fragment- Fmoc-FRVTQLAPKAQIKE

Desiccated CLEAR-Amide resin ((100-200 mesh) 0.4 mmol/g, 0.5 g) was distributed in 2 polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent amino acid coupling was carried out as mentioned in Table II. The amino acids used in the synthesis were Fmoc Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH. After the completion of Fmoc-Phe-OH coupling the resin was taken out form peptide synthesiser and manual coupling was carried out as follows

Fmoc-Phe-OH peptidyl resin from automated synthesiser was pooled in to a glass vessel with frit. The Fmoc group of the peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (10 m L). The resin was washed with DMF (6 X 15 m L), DCM (6 X 15 m L) and DMF (6 X 15 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. **Fmoc-Lys (Fmoc)-OH** (0.48 g; 4 equiv. 0.8 m mol) in dry DMF was added to the deprotected resin and coupling was initiated with DIC (0.15 m L; 5 equiv, 1 m mol) and HOBT (0.08 g; 5 equiv, 0.6 m mol) in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 3h. Resin was filtered and washed with DMF (6 X 15 mL), DCM (6 X 15 mL) and DMF (6 X 15 mL). Kaiser test on peptide resin aliquot upon completion of coupling was negative. The Fmoc group on the peptidyl resin is deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (15 mL). The resin was washed with DMF (6 X 15 mL), DCM (6 X 15 mL) and DMF (6 X 15 mL). Kaiser test on peptide resin

aliquot upon completion of Fmoc-deprotection was positive. After the deprotection of Fmoc group on Fmoc-Lys(Fmoc)-attached peptidyl resin the peptide chain growth was carried out from both the free amino terminus suing 8 equivalent excess of amino acid (1.6 m mol, 8 equivalent excess of HOBt (0.22 g, 1.6 m mol) and 10 equivalent excess of DIC (0.32 m L, 2 m mol) relative to resin loading. The coupling was carried out at room temperature for 3 h. The amino acids coupled to the peptidyl resin were; Fmoc-Phe-OH (0.62 g; 8 equiv,1.6 m mol), Fmoc-Ser (OtBu)-OH (0.62 g; 8 equiv, 1.6 m mol), Fmoc-Glu (OtBu)-OH (0.68 g; 8 equiv, 1.6 m mol) , Fmoc-Ser (OtBu)-OH (0.62 g; 8 equiv,1.6 m mol), Fmoc-Thr (OtBu)-OH (0.64 g; 8 equiv, 1.6 m mol), Fmoc-Asn (Trt)-OH (0.95 g; 8 equiv, 1.6 m mol) and N-terminus amino acids as Boc-Ser (OtBu)-OH (0.41 g; 8 equiv, 1.6 m mol) The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail A to yield (565 mg), 70 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column (9.4 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: Acetonitrile. The peptide was eluted by gradient elution 0-5 min = 5-10 % buffer B, 10-20 min = 29 % buffer B with a flow rate of 7 mL/min. HPLC: (method 1): RT -12 min (96 %); LCMS Calculated Mass: 3261.62, Observed Mass: 1631.6 $[M/2+H]^+$; 1088 $[M/3+H]^+$;); 816.2$[M/4+H]^+$;

Example 3: Synthesis of



(SEQUENCE ID NO 102)

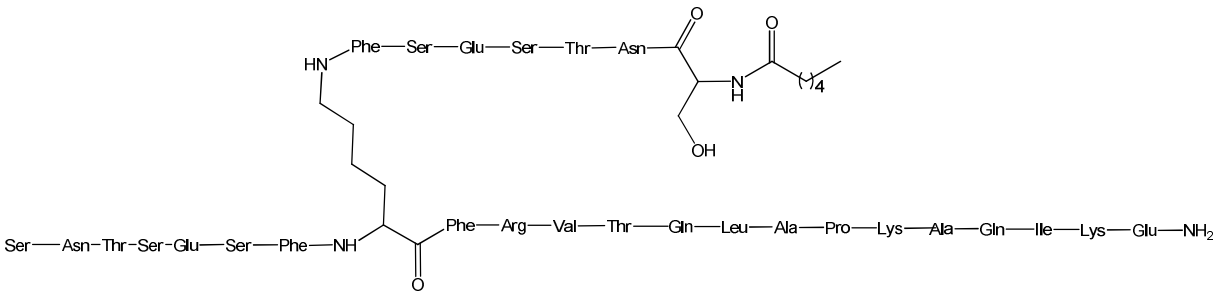
Desiccated CLEAR-Amide resin ((100-200 mesh) 0.49 mmol/g, 0.5 g) was distributed in 2 polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Ser(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent Fmoc-Amino acids are coupled as mentioned in Table II. The amino acids used in the synthesis were Fmoc-Glu(OtBu)-OH, Fmoc-Tyr(OtBu)-OH, Fmoc-Trp(Boc)-OH,

Fmoc-Asp(OtBu)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH, Fmoc-Ahx-OH. In the case of Biotin coupling the Biotin (0.15g in 2.5 m L DMF) was dissolved in DMF/DMSO (1:1) and added manually to the reaction vessel in automated peptide synthesiser and coupling reaction was continued as in step 3 and 4 table II (Amino acid is replaced with biotin).

Orthogonal deprotection of Dde group

The ε-amino group of sixteenth residue lysine (from N-terminus) used as branching point was protected with Dde group. After completion of the linear peptide chain Dde protection of ε-amino group of the lysine was orthogonally deprotected by treating the resin with 2.5% (v/v) Hydrazine hydrate solution in DMF (2 X 7.30 min) was carried out in automated synthesizer via manual addition of hydrazine hydrate solution. The resin was further washed on a manual program in parallel synthesizer as in step 4, table II. The coupling of amino acid in the branch was carried out by the incorporation of subsequent amino acids using program for amino acid coupling as mentioned in table II starting with Fmoc-Ser (OtBu)-OH. The N-terminus acid in the branch peptidyl resin; Fmoc-Lys(Boc)-OH was coupled using the program as mentioned in Table III. . The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail B to yield (516 mg), 65 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column (9.4 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: acetonitrile. The peptide was eluted by gradient elution 0-4 min = 10 % buffer B, 4-25 min = 10-28 % buffer B with a flow rate of 7 mL/min. HPLC: (method 1): RT -11.94 min (96 %); LCMS Calculated Mass: 3245.69, Observed Mass: 1623 $[M/2+H]^+$; 1082.3 $[M/3+H]^+$; 811.8 $[M/4+H]^+$;

Example 4: Synthesis of



(SEQUENCE ID NO 61)

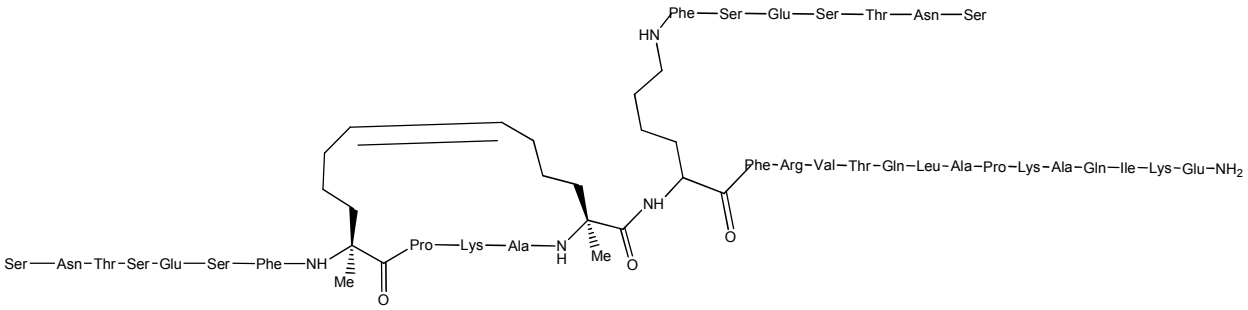
Desiccated CLEAR-Amide resin ((100-200 mesh) 0.4 mmol/g, 0.3 g) was taken in polyethylene vessel equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent Fmoc-Amino acids are coupled as mentioned in Table II. The amino acids used in the synthesis were Fmoc Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ile-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Glu(OtBu)-OH.

Orthogonal deprotection of Dde group

The N-terminal amino acid Ser(OtBu)-OH in the linear chain of the peptide was coupled as N – Boc amino acid in the same procedure as employed for N-Fmoc amino acid. The ε-amino group of fifteenth residue lysine (from C-terminus) used as branching point was protected with Dde group. After completion of the linear peptide chain Dde protection of ε-amino group of the lysine was orthogonally deprotected by treating the resin with 2.5% (v/v) Hydrazine hydrate solution in DMF (2 X 7.30 min) was carried out in automated synthesizer via manual addition of hydrazine hydrate solution. The resin was further washed on a manual program in parallel synthesizer as in step 4, table II. The coupling of amino acid in the branch was carried out by the incorporation of subsequent amino acids using program for amino acid coupling as mentioned in table II starting with Fmoc-Phe-OH. The amino acids used in the synthesis were Fmoc-Thr(OtBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Glu(OtBu)-OH. The N-terminus amino acid coupling was carried out using hexanoic acid (0.22 g in 2.5 m L DMF) was added manually to the reaction vessel in automated peptide synthesiser and coupling reaction was continued as in step 3 and 4 table 2 (Amino acid is replaced with hexanoic acid). . The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail A to yield (320 mg), 74 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column (9.4 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: 0.1% TFA in 9:1 acetonitrile/water. The peptide was eluted by gradient elution 0-25 min = 5-50 % buffer B with a

flow rate of 7 mL/min. HPLC: (Method 1): RT -12.8 min (94.4%); LCMS Calculated Mass: 3359.62, Observed Mass: 1680.3 $[M/2+H]^+$; 1120.4 $[M/3+H]^+$; 840.5 $[M/4+H]^+$

Example 5: Synthesis of



(SEQUENCE ID NO 64)

The synthesis of Example 5 was performed on Clear amide resin (0.46 mmol/g; 500 mg) from peptide international using Symphony parallel synthesizer in two reaction vessels. First amino acid attachment and capping of the peptidyl resin was carried out as per the protocol in Table 1. Subsequent amino acid coupling was carried out as mentioned in Table 2. During the automated synthesis for the coupling of position 8 and 12, the sequence was paused after wash step (step 2) in table II followed by manual addition of Fmoc-(R)-2-(4'-pentenyl) Alanine and subsequent manual program addition of reagent as mentioned in step 3, 4 in Table 2 and the sequence is resumed for subsequent amino acid coupling. The N-terminus amino acids are attached as Boc amino acid using similar procedures used for Fmoc-amino acid.

Orthogonal deprotection of Dde group

The ε-amino group of fifteenth residue lysine from C-terminus was used as branching point and was protected with Dde group. After completion of the linear peptide chain Dde protection of ε-amino group of the lysine was orthogonally deprotected by treating the resin with 2.5% (v/v) Hydrazine hydrate solution in DMF (2 X 7.30 min) was carried out in automated synthesizer via manual addition of hydrazine hydrate solution. The resin was further washed as manual program as in step 4, table II. The coupling of amino acid in the branch was carried out by the incorporation of subsequent amino acids using program for amino acid coupling as mentioned in table II starting with Fmoc-Phe-OH. The amino acids used in the synthesis were Fmoc-Thr(OtBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Glu(OtBu)-OH. The N-

terminus amino acids are attached as Boc amino acid using similar procedures used for Fmoc-amino acid.

Stitching of olefinic amino acids

Stitching of the two olefinic amino acids was carried out in customized solid phase peptide vessel of 20 mL capacity with a sintered disc. After the completion of the linear peptide sequence, ring closure metathesis of resin bound N-terminal capped peptides was performed using 20 mol% Grubbs catalyst (1.7 g in 15 ml) in degassed 1, 2 dichloroethane (DCE) for 2 hours at room temperature. After 2h the resin was washed with DCE, DCM, methanol and then dried in Vacuum over night. . The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail C to yield (530 mg), 63 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column (9.4 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: acetonitrile. The peptide was eluted by gradient elution 0-30 min = 10-80% buffer B, with a flow rate of 7 mL/min. HPLC: (method 1): RT - 12.9 min (95.4%); LCMS Calculated Mass: 3697, Observed Mass: 1232.5 $[M/3+H]^+$; ;924.9 $[M/4+H]^+$; 740.2$[M/5+H]^+$

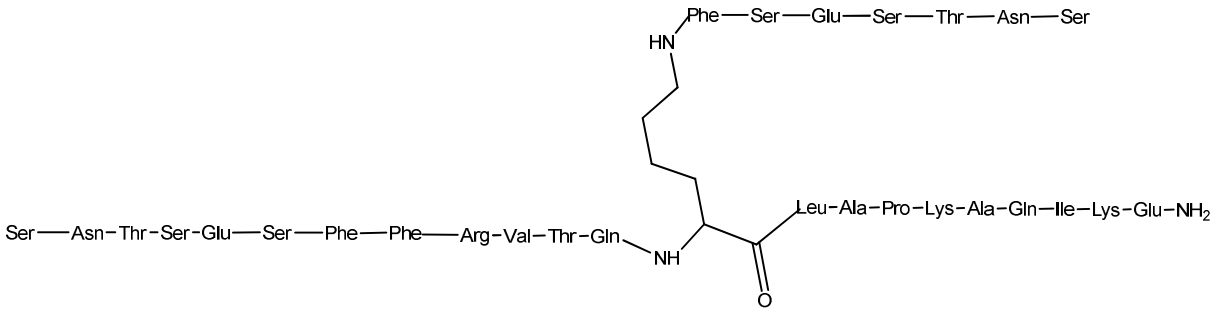
Example 6: Synthesis of

Ser-Asn-Thr-Ser-Glu-Ser-Phe-Lys-Ser-Asn-Thr-Ser-Glu-Ser-Phe-Phe-Arg-Val-Thr-Gln-Leu-Ala-Pro-Lys-Ala-Gln-Ile-Lys-Glu-NH2 (SEQUENCE ID NO 69)

Desiccated CLEAR-Amide resin ((100-200 mesh) 0.46 mmol/g, 0.25 g) was taken in polyethylene vessel equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent Fmoc-Amino acids are coupled as mentioned in Table II. The amino acids used in the synthesis were Fmoc Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH, Fmoc-Ser(OtBu)-OH. The N-terminus amino acid Fmoc-Ser(OtBu) was coupled as per the program in table III.. The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail A to yield (265 mg), 70 % yield.

The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column (9.4 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: acetonitrile. The peptide was eluted by gradient elution 0-30 min = 10-80 % buffer B, with a flow rate of 7 mL/min. HPLC: (method 1): RT -12.04 min (95.2%); LCMS Calculated Mass: 3261.8, Observed Mass: 1630.7 $[M/2+H]^+$; 1087.5 $[M/3+H]^+$;

Example 7: Synthesis of



(SEQUENCE ID NO 83)

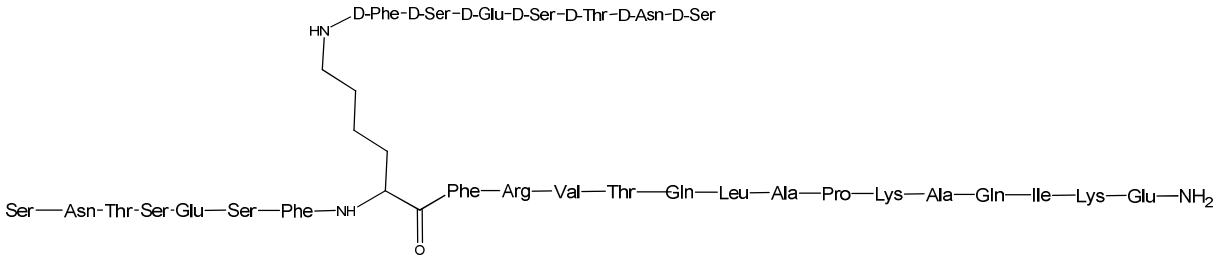
Desiccated CLEAR-Amide resin ((100-200 mesh) 0.4 mmol/g, 0.3 g) was taken in polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent amino acid coupling was carried out as mentioned in Table II. The amino acids used in the synthesis were Fmoc Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH.

Orthogonal deprotection of Dde group

The N-terminal amino acid Ser(OtBu)-OH in the linear chain of the peptide was coupled as N – Boc amino acid in the same procedure as employed for N-Fmoc amino acid. The ε-amino group of thirteenth residue lysine from N-terminus used as branching point was protected with Dde group. After completion of the linear peptide chain Dde protection of ε-amino group of the lysine was orthogonally deprotected by treating the resin with 2.5% (v/v) Hydrazine hydrate solution in

DMF (2 X 7.30 min) was carried out in automated synthesizer via manual addition of hydrazine hydrate solution. The resin was further washed as in step 4, table II. The coupling of amino acid in the branch was carried out by the incorporation of subsequent amino acids using program for amino acid coupling as mentioned in table II starting with Fmoc-Phe-OH. The amino acids used in the synthesis were Fmoc-Thr(OtBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Glu(OtBu)-OH. The N-terminus amino acid Fmoc-Ser(OtBu) was coupled as per the program in table III. . The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail A to yield (320 mg), 74 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 Prep-HT column (21.2 mm X 150mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: 0.1% TFA in acetonitrile. The peptide was eluted by gradient elution 0-5 min = 5-20 % buffer B, 5-22 min = 20-30 % buffer B with a flow rate of 18 mL/min. HPLC: (method 1): RT -12.5 min (97.6 %); LCMS Calculated Mass: 3261.62, Observed Mass: 1631.6 $[M/2+H]^+$; 1088 $[M/3+H]^+$; 816.2 $[M/4+H]^+$;

Example 8: Synthesis of



(SEQUENCE ID NO 88)

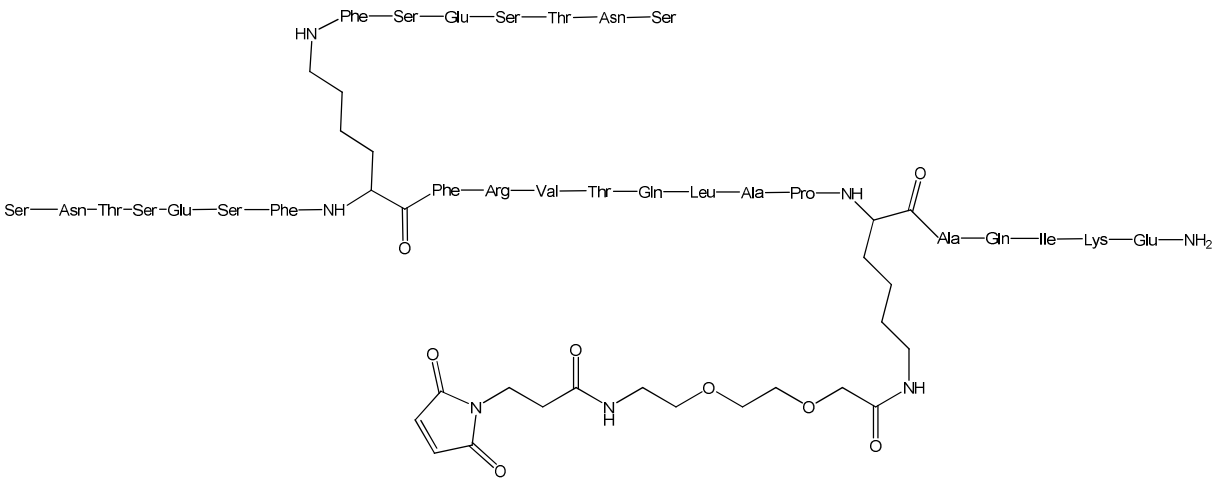
Desiccated CLEAR-Amide resin ((100-200 mesh) 0.4 mmol/g, 0.3 g) was taken in polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent amino acid coupling was carried out as mentioned in Table II. 5 equivalent excess of amino acid and coupling reagent relative to resin loading was used for coupling. The amino acids used in the synthesis were Fmoc Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-

Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH.

Orthogonal deprotection of Dde group

The N-terminal amino acid Ser(OtBu)-OH in the linear chain of the peptide was coupled as N – Boc amino acid in the same procedure as employed for N-Fmoc amino acid. The ε-amino group of eighth residue lysine used as branching point was protected with Dde group. After completion of the linear peptide chain Dde protection of ε-amino group of the lysine was orthogonally deprotected by treating the resin with 2.5% (v/v) Hydrazine hydrate solution in DMF (2 X 7.30 min) was carried out in automated synthesizer via manual addition of hydrazine hydrate solution. The resin was further washed as in step 4, table II. The coupling of amino acid in the branch was carried out by the incorporation of subsequent D-amino acids using program for amino acid coupling as mentioned in table II starting with Fmoc-D-Phe-OH. The amino acids used in the synthesis were Fmoc-D-Thr(OtBu)-OH, Fmoc-D-Asn(Trt)-OH, Fmoc-D-Ser(OtBu)-OH, Fmoc-D-Glu(OtBu)-OH. The N-terminus amino acid Fmoc-D-Ser(OtBu) was coupled as per the program in table III. . The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail A to yield (350 mg), 89 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 Prep-HT column (21.2 mm X 150mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: 0.1% TFA in acetonitrile. The peptide was eluted by gradient elution 0-5 min = 5-20 % buffer B, 5-18 min = 20-27 % buffer B with a flow rate of 18 mL/min. HPLC: (method 1): RT -12.06 min (96.6%); LCMS Calculated Mass: 3261.6, Observed Mass: 1631.6 $[M/2+H]^+$; 1088 $[M/3+H]^+$; 816.2 $[M/4+H]^+$;

Example 9: Synthesis of



(SEQUENCE ID NO 124)

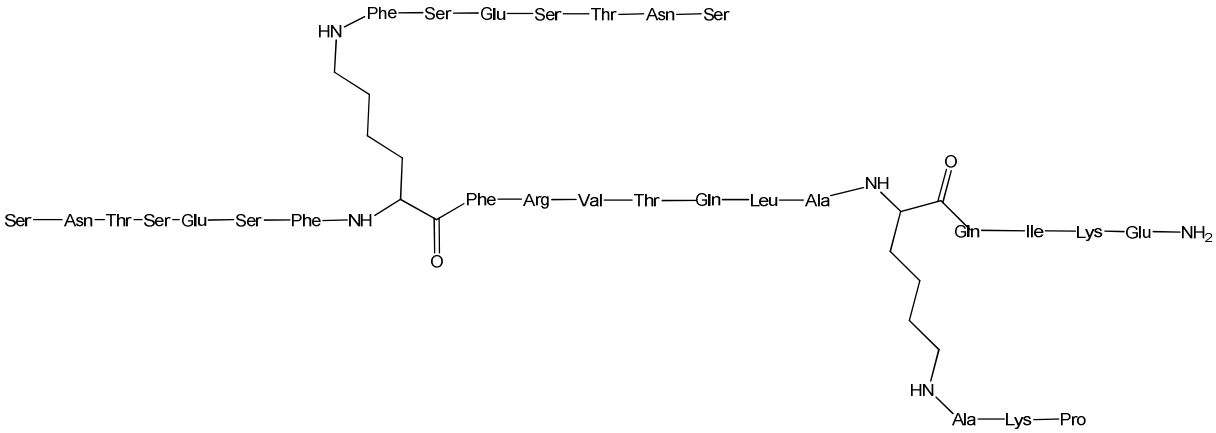
Desiccated Rink-Amide MBHA resin (100-200 mesh) 0.66 mmol/g, 1 g) was distributed in 4 polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent amino acid coupling was carried out as mentioned in Table II. The amino acids used in the synthesis were Fmoc Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH. In the linear sequence 8th amino acid from the N-terminus, Lys, was coupled as Fmoc-Lys(Fmoc)-OH. From the next residue onwards (Fmoc-Phe-OH) the coupling of subsequent amino acids was carried out as in Table II with 10 equivalents excess of amino acid (5 m L each of 0.25 M amino acid, 0.25 M HBTU in 1 M NMM/DMF) coupling reagent relative to resin loading was used for coupling. The N-terminal amino acid Ser(OtBu)-OH at the both N-terminus of the peptide was coupled as N –Boc amino acid in the same procedure as employed for N-Fmoc amino acid.

Orthogonal deprotection of Dde group

The ε-amino group of seventeenth residue lysine from N-terminus used as branching point was protected with Dde group. After completion of the linear peptide chain Dde protection of ε-amino group of the lysine was orthogonally deprotected by treating the resin with 2.5% (v/v)

Hydrazine hydrate solution in DMF (2 X 7.30 min) was carried out in automated synthesizer via manual addition of hydrazine hydrate solution. The resin was further washed using manual program in synthesizer as in step 4, table II. The coupling of amino acid in the branch was carried out by the incorporation of subsequent amino acids using program for amino acid coupling as mentioned in table II starting with Fmoc-8-amino 3,6-dioxaoctanoic acid (0.24 g in 2.5 m L DMF) and for maleimdo propanoic acid (0.1 g in 2.5 m L DMF). The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail C to yield (1.6 g), 70 % yield. The crude material was purified by preparative HPLC on Phenomenex -C18 silica column (10 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: acetonitrile. The peptide was eluted by gradient elution 0-9 min = 10-15 % buffer B with a flow rate of 6 mL/min. HPLC: (method 1): RT -11.7 min (95 %); LCMS Calculated Mass: 3557.09, Observed Mass: 1779.8$[M/2+H]^+$ 1187.1$[M/3+H]^+$

Example 10: Synthesis of



(SEQUENCE ID NO 77)

Desiccated CLEAR amide (100-200 mesh) 0.4 mmol/g, 0.3 g) was taken in polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent amino acid coupling was carried out as mentioned in Table II. In the linear sequence 8^{th} amino acid from the N-terminus, Lys, was coupled as Fmoc-Lys (Dde)-OH. The Sixteenth

Lysine residue in the linear chain was coupled as Fmoc-Lys (Alloc)-OH. The N-terminal amino acid Ser(OtBu)-OH in the linear chain of the peptide was coupled as N –Boc amino acid in the same procedure as employed for N-Fmoc amino acid.

Orthogonal deprotection of Dde group

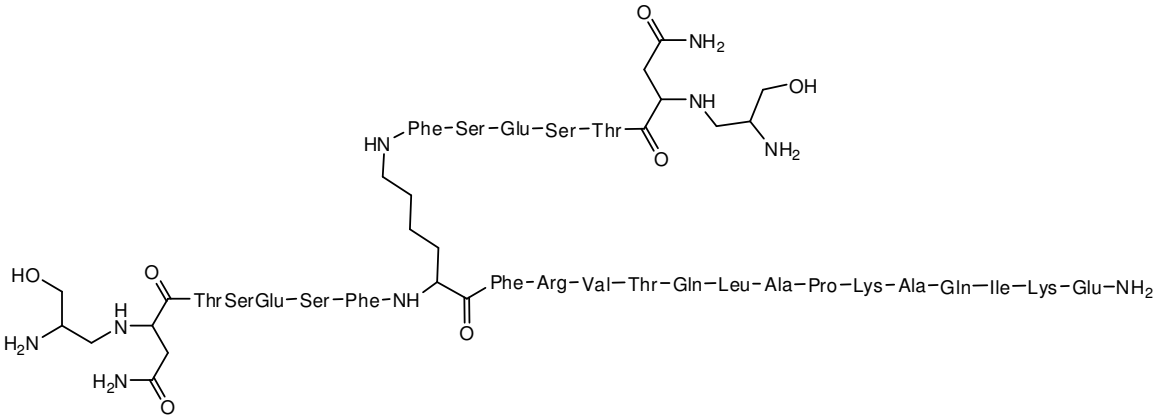
The ε-amino group of eighth residue lysine from N-terminus used as branching point was protected with Dde group. After completion of the linear peptide chain Dde protection of ε-amino group of the lysine was orthogonally deprotected by treating the resin with 2.5% (v/v) Hydrazine hydrate solution in DMF (2 X 7.30 min) was carried out in automated synthesizer via manual addition of hydrazine hydrate solution. The resin was further washed as in step 4, table II. The coupling of amino acid in the branch was carried out by the incorporation of subsequent amino acids using program for amino acid coupling as mentioned in table II. The amino acids used were Fmoc-Phe-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Glu(OtBu)-OH. OH. The N-terminal amino acid in the branch, Ser(OtBu)-OH in the linear chain of the peptide was coupled as N –Boc amino acid in the same procedure as employed for N-Fmoc amino acid.

Deprotection of Alloc protecting group

The ε-amino group sixteenth residue Lysine from N-terminus was coupled as Lys(Alloc) to initiate an additional branching point in the linear sequence. Alloc protecting group from Lys (Alloc) was removed from the peptidyl resin by treating with tetrakistriphenylphosphine palladium (0) (5 Equiv; 2.8 g) and Phenylsilane (10 eqv 0.65 m L) in a solution of chloroform/N-methylpyrrolidine (95/5 v/v) for 6 h under argon. The resin was washed with a solution of 10% NMP in chloroform (6 X 10 m L), 1% DIEPA in DMF (6 X 10 m L), DCM (6 X 10 m L), DMF (6 X 10 m L). After the completion of Alloc deprotection the resin was loaded back to automated peptide synthesiser. The free amino group was used as second branch point in the sequence. The coupling of amino acid in the branch was carried out by the incorporation of subsequent amino acids using program for amino acid coupling as mentioned in table II. The amino acids used were Fmoc-Lys(Boc)-OH, Fmoc-Ala-OH, Boc-Pro-OH. The N-terminal amino acid in the branch, Proline was coupled as N –Boc amino acid in the same procedure as employed for N-Fmoc amino acid. The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail A to yield (200 mg), 50 % yield. The crude material was purified by

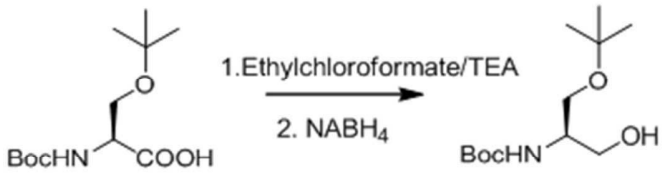
preparative HPLC on Zorbax Eclipse XDB-C18 column (9.4 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: 0.1% TFA in acetonitrile. The peptide was eluted by gradient elution 0-2 min = 10-20 % buffer B, 2-8 min = 20-25 % buffer B, 8-19 min = 25-29 % buffer B with a flow rate of 7 mL/min. HPLC: (method 1): RT -12.3 min (94 %); LCMS Calculated Mass: 3389.8, Observed Mass: 1695.2 $[M/2+H]^+$;1130.5 $[M/3+H]^+$ 848.3 $[M/4+H]^+$

Example 11: Synthesis of



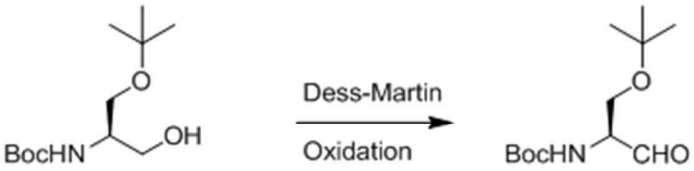
(SEQUENCE ID NO 129)

Synthesis of building block



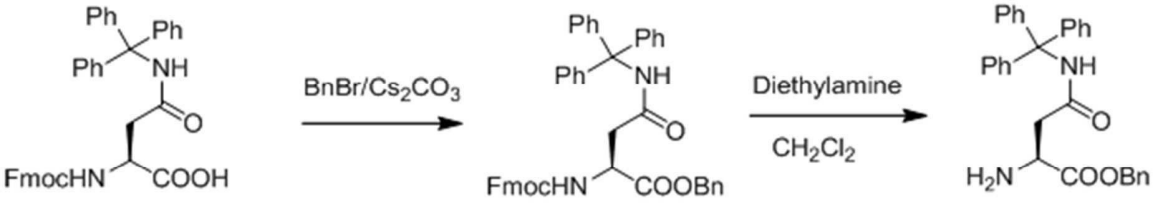
To a solution of *N*-Boc amino acid (10.0 g, 38.28 mmol) in THF (100 mL) at -20 °C, was added N-methyl morpholine (NMM, 4.25 g, 42.11 mmol) and ethylchloroformate (4.57 g, 42.11 mmol) and the resultant mixture was stirred at same temperature for 20 min. The inorganic salts were filtered off and the filtrate was treated with moist $NaBH_4$ (2.9 g, 76.56 mmol) for 10-15 min. The reaction mixture was then partitioned between water and EtOAc. Organic layer was washed with water, 10% $NaHCO_3$ solution (100 mL x 2) and brine, dried over Na_2SO_4 and evaporated under

reduced pressure to yield *N*-Boc aminol, which was further purified by silica gel column chromatography (eluent: 0-50% EtOAc in Hexane) to yield 8.2 g of product [yield: 85.4%, Mass: Cal.247.3, Obs:248.2 (M+1), 270.2 (M+Na)].



1

To the solution of *N*-Boc-aminol (3.0 g, 12.13 mmol) in distilled DCM (30.0 mL) was added Dess-Martin periodinane (10.3 g, 24.27 mmol) in a portion wise manner at 0 °C and stirred at rt under N_2 atmosphere for 30 min until the reactants were consumed as determined by TLC analysis. The reaction mixture was quenched by adding 1.0M $Na_2S_2O_3$ solution, and the product was extracted with DCM. The organic extract was washed with (5%, 1:1) $Na_2S_2O_3$/$NaHCO_3$ solution (20 mL x 2), brine (20 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give the crude product, which was further purified by silica gel column chromatography (eluent: 0-20% EtOAc in Hexane) to yield 2.4 g pure product **1** (yield-82%, Mass-Cal.245.32, Obs-247.9 (M+1), 265.1 (M+Na)).

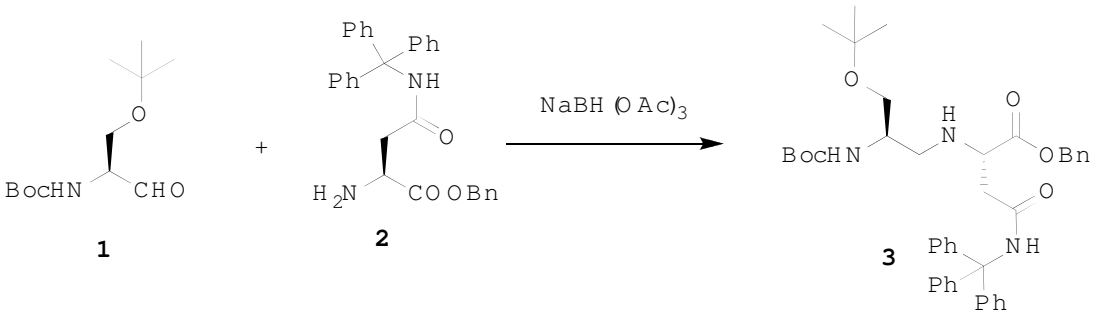


2

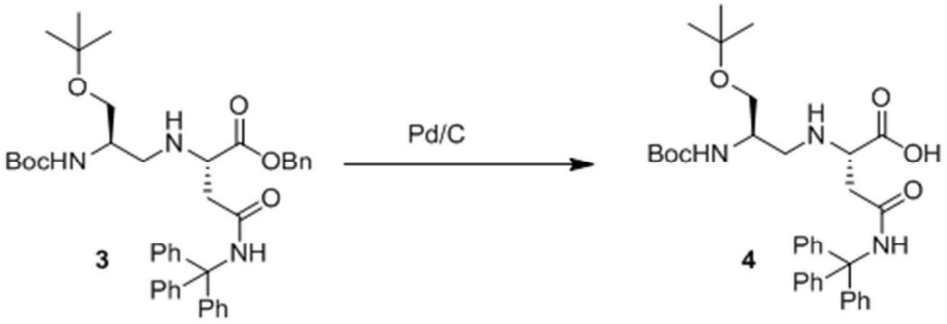
To a solution of Fmoc-Asn(trt)-OH (4.0 g, 6.7 mmol) in 30.0 mL of DMF was added Cs_2CO_3 (2.62 g, 8.0 mmol). The mixture was then cooled to 0 ^oC and benzyl bromide (1.37 g, 8.0 mmol) was added and the resultant solution was stirred for 30 min at 0 ^oC and then at rt for 12 h. The reaction mixture was concentrated under reduced pressure and diluted with EtOAc (50 mL), the organic layer was washed with $NaHCO_3$ (2 x 50 m L) and brine (1 x 50 m L), dried over Na_2SO_4, filtered and concentrated *in vacuuo* and purified by silica gel column chromatogrophy (eluent: 0-

30% EtOAc in Hexane) to furnish Fmoc-Asn(trt)-OBn as white solid [yield: 4.5 g, 98.0%;, Mass: Cal.686.28, Obs-687.3 (M+1), 709.1 (M+Na]

To a solution of Fmoc-Asn(Trt)-OBn (3.5 g, 5.1 mmol) in DCM (14.0 mL), diethylamine (14.0 m L) was added and stirred for 1 h at rt. The resulting solution was concentrated *in vacuuo* and the thick-residue was purified by neutral alumina column chromatography (eluent: 0-50% EtOAc in Hexane then 0-5% MeOH in $CHCl_3$) to yield H-Asn(trt)-Obn **2** (yield: 1.75 g, 73.0%; Mass: Cal. 464.21, Obs-465.3 (M+1), 487.2 (M+Na]).



H-Asn(Trt)-OBn (4.5 g, 9.7 mmol), DIPEA (2.5 g, 19.4 mmol) and Boc-Ser(OtBu)-CHO (2.4 g, 9.7 mmol) were mixed in DCM (45 mL) at 0 °C and then allowed to stir at room temperature for 1 h. Again the reaction mixture was cooled to 0 °C and treated with sodium triacetoxyborohydride (4.1g, 19.4 mmol) and then mixture was allowed to stir at room temeperature under N_2 atmosphere for 6 h until the reactants were consumed as determined by TLC analysis. The reaction mixture was quenched by adding water, and the product was extracted with DCM. The organic extract was washed with 5% $NaHCO_3$ solution (50 mL x 2), 5% citric acid solution (50 mL x 2), brine (50 mL) and dried over Na_2SO_4. The solvent was evaporated to give the crude product which was further purified by silica gel column chromatography (eluent: 5-40 % EtOAc in Hexane) to furnish the desired product **3** (yield: 4.2 g, 62.0%; Mass: Cal.693.27, Obs-694.4 (M+1), 716.0 (M+Na)).

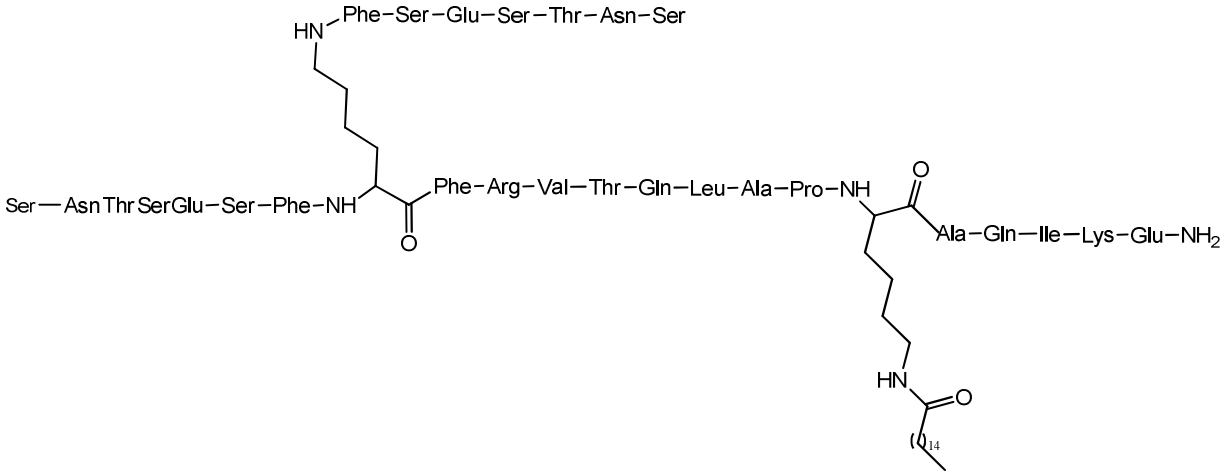


To a solution of compound **3** (4.0 g) in methanol (70.0 m L) under inert atmosphere, was added 10% Pd-C (1.0 g) and the mixture was stirred for 4 h under H_2 atmosphere. The completion of the reaction was confirmed by TLC analysis. The catalyst was then removed by filtration through a celite pad, which was then washed with 50 mL of methanol. The combined organic filtrate, on evaporation under reduced pressure resulted in the isolation of the product **4**, (Yield: 3.3 g, 96.0 %; Mass: Cal.603.25, Obs-604.4 (M+1), 626.4 (M+Na).

Peptide synthesis: Desiccated Rink-Amide MBHA resin (100-200 mesh) 0.66 mmol/g, 0.1 g) was taken in polyethylene vessel equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent amino acid coupling was carried out as mentioned in Table II. 5 equivalent excess of amino acid and coupling reagent relative to resin loading was used for coupling. The amino acids used in the synthesis were Fmoc Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH.In the linear sequence 8^{th} amino acid from the N-terminus, Lys, was coupled as Fmoc-Lys(Fmoc)-OH. From the next residue onwards (Fmoc-Phe-OH) the coupling of subsequent amino acids was carried out as in Table II with 10 equivalents excess of amino acid (5 m L each of 0.25 M amino acid, 0.25 M HBTU in 1 M NMM/DMF) coupling reagent relative to resin loading was used for coupling. The amino acids used in the synthesis were Fmoc-Thr(OtBu)-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Glu(OtBu)-OH The N-terminal amino acid Boc-SerΨ[CH2NH]Asn(Trt)-OH (0.4 g in 5 m L) at the both N-terminus was coupled as N –Boc amino acid in the same procedure as employed for N-Fmoc amino acid. . The peptidyl resin was cleaved as mentioned in procedure for cleavage using

cleavage cocktail C to yield (150 mg), 70 % yield. The crude sample was preparative HPLC purified and Lyophilised. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column (9.4 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: acetonitrile. The peptide was eluted by gradient elution 0-25 min = 2-30% buffer B, with a flow rate of 7 mL/min. HPLC: (method 1): RT -12 min (93 %); LCMS Calculated Mass: 3232.8, Observed Mass: 1617.4$[M/2+H]^+$ 809.6$[M/4+H]^+$

Example 12: Synthesis of



(SEQUENCE ID NO 75)

Desiccated Rink Amide MBHA-Amide resin (100-200 mesh, 0.66 mmol/g, 15 g) was distributed equally in to 5 customized solid phase peptide vessel of capacity with a sintered disc glass reactor vessel with a frit. Resin in each of the reactor was swelled in DCM (30 m L) for 1 h and DMF (30 m L) for 1 h. The Fmoc group of the Rink Amide MBHA-Amide was deprotected by treating it twice with 20% (v/v) piperidine/DMF solution for 5 and 15 min (30 m L). The resin was washed with DMF (6 X 30 m L), DCM (6 X 30 m L) and DMF (6 X 30 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. The C-terminal amino acid, **Fmoc-Glu(OtBu)-OH** (21 g ;5 equiv. 49.5 mmol) dissolved in dry DMF was distributed in equal volume to all the five glass reactor. The coupling was initiated by addition of DIC (7.6 m L; 5 equiv, 49.5 m mol) and HOBT (6.7 g ; 49.5 m mol) dissolved DMF distributed equally to the 5 glass reactor vessel. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF (6 X 30 m L), DCM (6 X 30 m L) and DMF (6 X 30 m L).

Kaiser test on peptide resin aliquot upon completion of coupling was negative After the first amino acid attachment, the unreacted amino group, if any, in the resin is capped used acetic anhydride/pyridine/DCM (1:8:8) for 20 minutes to avoid any deletion of the sequence. After capping, resin in individual reactor was washed with DCM (6 X 30 m L), DMF (6 X 30 m L), DCM (6 X 30 m L) and DMF (6 X 30 m L). The Fmoc group on the C-terminal amino acid attached peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (30 m L). The resin was washed with DMF (6 X 30 m L), DCM (6 X 30 m L) and DMF (6 X 30 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. The subsequent amino acids Fmoc-Lys(Boc)-OH, Fmoc-Ile-OH, Fmoc-Gln(Trt)-OH and Fmoc-Ala-OH was coupled using 5 equivalent excess each of amino acid, DIC and HOBt relative to resin loading. The Sixth residue from the C-terminus Lys was coupled as Fmoc-Lys(Dde)-OH. Fmoc-Lys(Dde)-OH (16 g; 3 equiv. 29.7 mmol) dissolved in dry DMF was distributed in equal volume to all the five glass reactor. The coupling was initiated by addition of DIC (4.5 m L; 3 equiv, 29.7 m mol) and HOBT (4 g ; 3 equiv, 29.7 m mol) dissolved in DMF distributed equally to the 5 glass reactor vessel. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 3 h. Resin was filtered and washed with DMF (6 X 30 m L), DCM (6 X 30 m L) and DMF (6 X 30 m L). Kaiser test on peptide resin aliquot upon completion of coupling was negative. The Fmoc group of the peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (30 m L). The resin was washed with DMF (6 X 30 m L), DCM (6 X 30 m L) and DMF (6 X 30 m L). Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive. The subsequent amino acids Fmoc-Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln (Trt)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH was coupled to the peptidyl resin using 5 equivalent excess of amino acid,HOBt and DIC method. The reaction was carried out for 3 h at room temperature. The fifteenth residue from the C-terminus, Lys was coupled as Fmoc-Lys(Fmoc)-OH. Fmoc-Lys(Fmoc)-OH (17.5 g; 3 equiv. 29.7 mmol) dissolved in dry DMF was distributed in equal volume to all the five glass reactor. The coupling was initiated by addition of DIC (4.5 m L; 3 equiv, 29.7 m mol) and HOBT (4 g ; 3 equiv, 29.7 m mol) dissolved DMF distributed equally to the 5 glass reactor vessel. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 3 h. Resin was

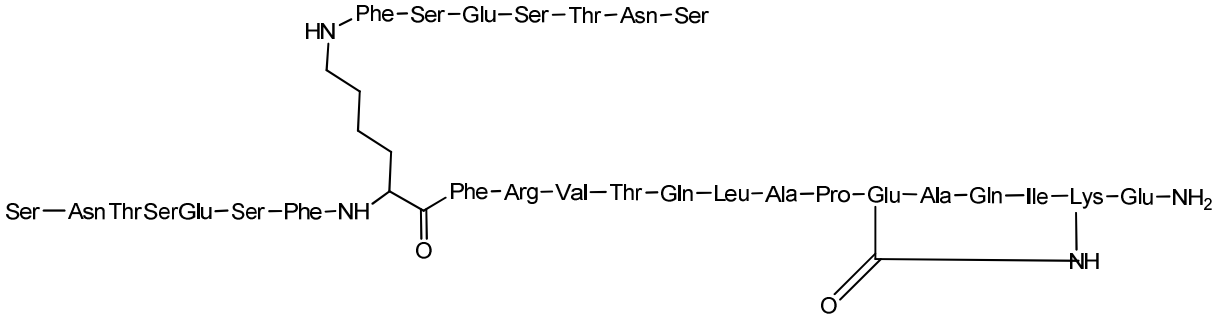
filtered and washed with DMF (6 X 30 m L), DCM (6 X 30 m L) and DMF (6 X 30 m L). Kaiser test on peptide resin aliquot upon completion of coupling was negative. The Fmoc group of the peptidyl resin was deprotected by treating it twice with 20% (v/v) piperdine/DMF solution for 5 and 15 min (30 m L). The resin was washed with DMF (6 X 30 m L), DCM (6 X 30 m L) and DMF (6 X 30 m L). After the deprotection of Fmoc-Lys(Fmoc)-attached peptidyl resin the peptide chain growth was carried out from both the free amino terminus suing 8 equivalent excess of amino acid (79 m mol), 8 equivalent excess of HOBt (79 m mol) and 10 equivalent excess of DIC (99 m mol) relative to resin loading. The reaction was carried out for 2 h at room temperature. The amino acids used in the synthesis were Fmoc-Phe-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Asn (Trt)-OH, Fmoc-Ser(OtBu)-OH and Fmoc-Glu(OtBu)-OH. The N-terminal amino acid Serine was coupled as Boc-Ser(OtBu)-OH using DIC/ HOBT method (8 equivalent excess relative to resin loading). **Orthogonal deprotection of Dde group :** The ε-amino group of seventeenth residue lysine from N-terminus used as branching point was protected with Dde group. After completion of the linear peptide chain Dde protection of ε-amino group of the lysine was orthogonally deprotected by treating the resin with 2.5% (v/v) Hydrazine hydrate solution in DMF as explained in example 1.

Coupling of palmitic acid

To the free ε-amino group of lysine, palmitic acid (12 g,5 equiv, 49.5 m mol) dissolved in DMF was added in equal proportion to five reactor vessel followed by the addition of DIC (7.6 m L; 5 equiv, 49.5 m mol) and HOBT (6.7 g ; 49.5 m mol) in DMF in similar manner.

The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail C to yield (22 g), 65 % yield. The crude sample was preparative HPLC purified and Lyophilised. The crude material was purified by preparative HPLC on Waters X-Bridge Prep C18-OBD column (19 mm X 150mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: 0.1% TFA/ acetonitrile. The peptide was eluted by gradient elution 0-3 min = 15-25% buffer B, 3-5 min = 25-38% buffer B, 5-15 min = 38-50% buffer B, 15-19 min = 50 % buffer B with a flow rate of 15 m L/min. HPLC: (method 3): RT -17.5 min (95.4 %); LCMS Calculated Mass: 3499.62, Observed Mass: 1750.3 $[M/2+H]^+$;1167.2 $[M/3+H]^+$

Example 13: Synthesis of



(SEQUENCE ID NO 103)

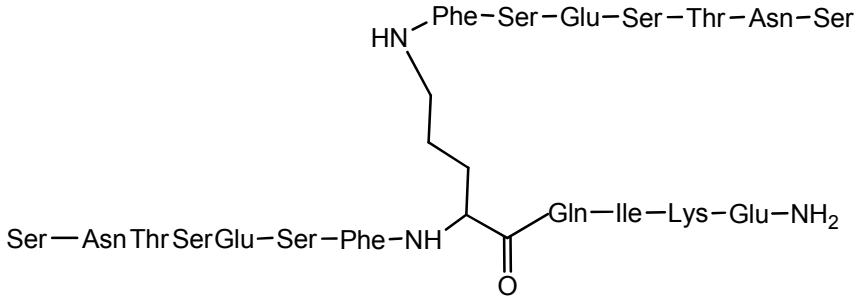
Desiccated Rink-Amide MBHA resin ((100-200 mesh) 0.6 mmol/g, 0.28 g) was taken in polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent amino acid coupling was carried out as mentioned in Table II. The amino acids used in the synthesis were Fmoc Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH. In the linear fragment the second amino acid from the C-terminus was coupled as Fmoc-Lys(Alloc)-OH and sixth amino acid from C-terminus was coupled as Fmoc-Glu(OAll)-OH. In the linear sequence 8th amino acid from the N-terminus, Lys, was coupled as Fmoc-Lys(Fmoc)-OH. From the next residue onwards (Fmoc-Phe-OH) the coupling of subsequent amino acids was carried out as in Table II using 5 m L each of 0.25 M amino acid, 0.25 M HBTU in 1 M NMM/DMF as coupling reagent. The amino acids used in the synthesis were Fmoc-Thr(OtBu)-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Glu(OtBu)-OH. The N-terminus amino acids at both the ends are coupled as Boc-Ser(OtBu)-OH.

Deprotection of Alloc protecting group

After completion of the entire peptide sequence, the orthogonal Alloc and Allyl protecting group present in Lysine and Glutamic acid was removed from the peptidyl resin by treating with tetrakistriphenylphosphine palladium (0) (10 Equiv; 2.1 g) and Phenylsilane (20 eqv 0.32 m L) in a solution of chloroform/N-methylpyrrolidine (95/5 v/v) for 6 h under argon. The resin was washed with a solution of 10% NMP in chloroform (6 X 10 m L), 1% DIEPA in DMF (6 X 10 m

L), DCM (6 X 10 m L), DMF (6 X 10 m L).The ε-free amino group of lysine and γ-free carboxyl group of Glutamic acid was kept for lactam bridging using HOBt (0.12 g; 0.92 m mol) and DIC(0.18 m L; 1.1 m mol) dissolved in NMP. After 17 h the resin was filtered and washed with DMF (6 X 10 m L), DCM (6 X 10 m L), DMF (6 X 10 m L). The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail C to yield (407 mg), 68 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column (9.4 mm X 250mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: Acetonitrile. The peptide was eluted by gradient elution 0-4 min = 10 % buffer B, 4-21.5 min = 10-30.9 % buffer B, 21.5-30.9 min = 30.9-35 % buffer B with a flow rate of 7 mL/min. HPLC: (method 1): RT -12.5 min LCMS Calculated Mass: 3244.12, Observed Mass: 1622.9 $[M/2+H]^+$; 1082.1 $[M/3+H]^+$

Example 14: Synthesis of



(SEQUENCE ID NO 140)

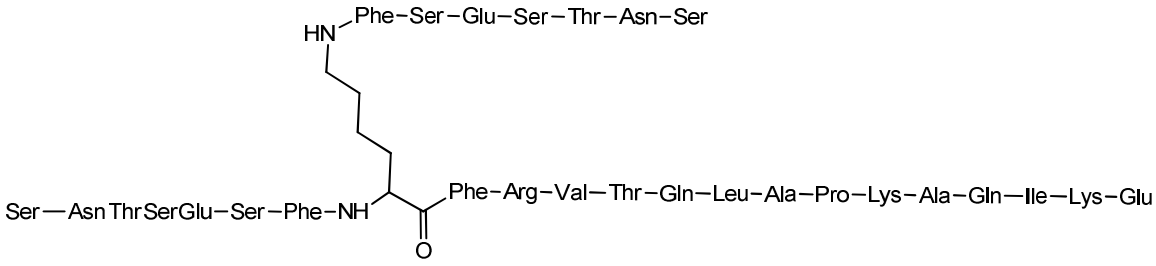
Desiccated Rink amide MBHA (100-200 mesh) 0.66 mmol/g, 0.3 g) was taken in polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Swelling, C-terminal amino acid [Fmoc-Glu(OtBu)-OH] attachment and capping of the peptidyl resin was carried out as per the protocol in Table I. Subsequent amino acid coupling was carried out as mentioned in Table II. In the linear sequence 8^{th} amino acid from the N-terminus, Orn, was coupled as Fmoc-Orn- (Dde)-OH. The N-terminal amino acid Ser(OtBu)-OH in the linear chain of the peptide was coupled as N –Boc amino acid in the same procedure as employed for N-Fmoc amino acid.

Orthogonal deprotection of Dde group

The δ-amino group of eighth residue ornithine used as branching point was protected with Dde group. After completion of the linear peptide chain Dde protection of δ-amino group of the ornithine was orthogonally deprotected by treating the resin with 2.5% (v/v) Hydrazine hydrate solution in DMF (2 X 7.30 min) was carried out in automated synthesizer via manual addition of hydrazine hydrate solution. The resin was further washed using manual programme in synthesizer as in step 4, table II. The coupling of amino acid in the branch was carried out by the incorporation of subsequent amino acids using program for amino acid coupling as mentioned in table II. The amino acids used were Fmoc-Phe-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Glu(OtBu)-OH. OH. The N-terminal amino acid in the branch, Ser(OtBu)-OH in the linear chain of the peptide was coupled as N –Boc amino acid in the same procedure as employed for N-Fmoc amino acid.

The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail C to yield (295 mg), 70 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column 21.2 mm X 150mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: Acetonitrile. The peptide was eluted by gradient elution 0-5 min = 5-20 % buffer B, 5-15 min = 20-25 % buffer B with a flow rate of 18 mL/min. HPLC: (method 1): RT -11.1 min LCMS Calculated Mass: 2135.77, Observed Mass: 1067.9 $[M/2]^+$; 711.9 $[M/3]^+$

Example 15: synthesis of



(SEQUENCE ID NO 133)

Desiccated H-Glu (OtBu)-2-chlorotrityl chloride resin (100-200 mesh) 0.5 mmol/g, 0.35 g was taken in polyethylene vessels equipped with a polypropylene filter. The linear peptide synthesis on solid phase were carried out automatically, using Symphony parallel synthesizer (PTI) using the synthesis programs mentioned in the table below. Subsequent amino acid coupling was carried out as mentioned in Table II. The amino acids used in the synthesis were Fmoc Phe-OH,

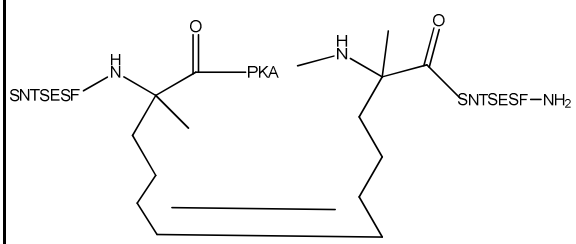
Fmoc-Arg(Pbf)-OH, Fmoc-Val-OH, Fmoc-Thr(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-Pro-OH, Fmoc-Ile-OH. In the linear sequence 8th amino acid from the N-terminus, Lys, was coupled as Fmoc-Lys(Fmoc)-OH. From the next residue onwards (Fmoc-Phe-OH) the coupling of subsequent amino acids was carried out as in Table II using 5 m L each of 0.25 M amino acid, 0.25 M HBTU in 1 M NMM/DMF as coupling reagent. The N-terminal amino acid Ser(OtBu)-OH at the both N-terminus of the peptide was coupled as N –Boc amino acid in the same procedure as employed for N-Fmoc amino acid. The peptidyl resin was cleaved as mentioned in procedure for cleavage using cleavage cocktail C to yield (400 mg), 66 % yield. The crude material was purified by preparative HPLC on Zorbax Eclipse XDB-C18 column 21.2 mm X 150mm, 5µm) with buffer A: 0.1% TFA/Water, buffer B: Acetonitrile. The peptide was eluted by gradient elution 0-5 min = 5-20 % buffer B, 5-18 min = 20-27 % buffer B with a flow rate of 18 mL/min. HPLC: (method 1): RT -12.1 min LCMS Calculated Mass: 3262.62, Observed Mass: 1088.6 $[M/3+H]^+$; 813.4 $[M/4+H]^+$

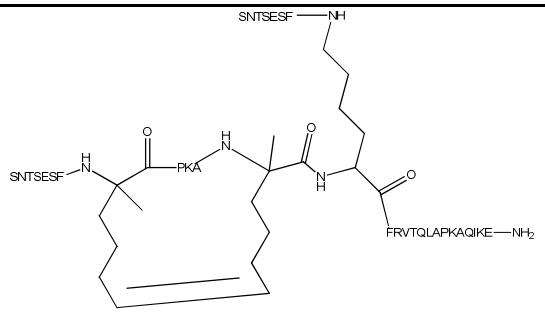
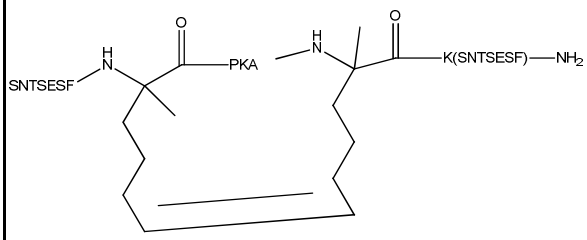
The other compounds of the invention were prepared by following similar procedure as described above with suitable modification known to the one ordinary skilled in the art. The identity of peptide was confirmed by LCMS (Table IV).

Table IV:

Comp No	**Sequence**	**LCMS** **Calculated**	**observed**
1	SNTSESF-NH2	769.7	770.0$[M]^+$
2	CH3-CO-SNTSESF-NH2	811.81	812.3 $[M]^+$
3	SNQTDKLAAFPEDSQPGQD-NH2	2047.14	1024.3 $[M/2+H]^+$
4	EDRSQPGQDCR-NH2	1288.35	1289.8$[M+H]^+$; 645.6$[M/2+H]^+$
5	CGAISLAPKAQIKES-NH2	1513.81	1515.2$[M+H]^+$; 758.2$[M/2+H]^+$

6	FRVTQK(SNTSESF)FRVTQAhxLAPKAQIKE-NH2 Ahx = 6-aminohexanoic acid	3237.83	1619.9 $[M/2+H]^+$;1080.2 $[M/3+H]^+$
7	FRVTQK(SNTSESF)FRVTQLAPKAQIKE-NH2	3140.62	1571.2 $[M/2+H]^+$; 1047.8 $[M/3+H]^+$
8	SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2	3261.62	1631.6 $[M/2+H]^+$; 1088 $[M/3+H]^+$;); 816.2$[M/4+H]^+$;
9	SNQTDK (SNQTDK) FRVTQ LAPKAQIKE-NH2	2975.34	1487.7 $[M/2+H]^+$; 992.2 $[M/3+H]^+$; ;744.5 $[M/4+H]^+$;
10	SNQTDK (SNQTDK) VLNWYRM LAPKAQIKE-NH2	3306.78	1653.8 $[M/2+H]^+$; 1102.7 $[M/3+H]^+$; 827.4$[M/42+H]^+$
11	SNTSESFK(SNQTDK)FRVTQLAPKAQIKE-NH2	3182.57	1591.8 $[M/2+H]^+$; 1061.7 $[M/3+H]^+$; 796.6$[M/4+H]^+$;
12	SNTSESFK(SNQTDK)FRVTQK(LAP)AQIKE-NH2	3182.57	1591.7 $[M/2+H]^+$; 1061.2$[M/3+H]^+$;796.3 $[M/4+H]^+$;
13	sntsesfk(sntsesf)frvtqlapkaqike-NH2	3261.62	1630.8 $[M/2+H]^+$; 1087.5 $[M/3+H]^+$; 815.8 $[M/4+H]^+$;
14	EKIQAKPAYWNLVK(KDTQNS)DTQNS	2906	1454.2 $[M/2+H]^+$ 969.5 $[M/3+H]^+$; 727.6 $[M/4+H]^+$
15	Biotin-Ahx-SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2	3601.22	1200.9 $[M/3+H]^+$; 901.0 $[M/4+H]^+$

16	C6 lipid-SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2	3359.62	1680.4 $[M/2+H]^+$; 1120.5$[M/3+H]^+$;;840.7$[M/4+H]^+$;
17	Ac-SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2	3303.66	1653.2 $[M/2+H]^+$; 1101.9 $[M/3+H]^+$; 826.9 $[M/4+H]^+$;
18	SNTSESFK(NH-(PEG)11-CO-SNTSESF)FRVTQLAPKAQIKE-NH2	3861.42	773.6 $[M/5+H]^+$;
19	SNTSESFK(SNTSESF)(PEG)LAPKAQIKE-NH2	3229.89	1615.17$[M/2+H]^+$; 1077.1 $[M/3+H]^+$; 808.3 $[M/4+H]^+$
20	SNTSESFK(CH3(CH2)4CO-SNTSESF)FRVTQLAPKAQIKE-NH2 C6 lipid in branch	3359.62	1680.3 $[M/2+H]^+$; 1120.4 $[M/3+H]^+$; 840.5 $[M/4+H]^+$
21	CH3(CH2)4CO-SNTSESFK(CH3(CH2)4CO-SNTSESF)FRVTQLAPKAQIKE-NH2 Lipidation on both Branch & N-termini	3457.62	1729.1 $[M/2+H]^+$; 1152 $[M/3+H]^+$;
22		2070.6	1035.5 $[M/2+H]^+$;

23		3697	1232.5 $[M/3+H]^+$; ;924.9 $[M/4+H]^+$; 740.2$[M/5+H]^+$
24		2198.8	1099.4 $[M/2+H]^+$;
25	SNTSESFK(SNTSESF)FRVTQLAQIKE-NH2	2965.25	1483 $[M/2+H]^+$; 988.9 $[M/2+H]^+$
26	SNTSESFFRVTQLAPKAQIKE-NH2	2380.71	1191.0 $[M/2+H]^+$; 794.3 $[M/3+H]^+$;
27	SNTSESFKFRVTQLAPKAQIKE-NH2	2508.88	1254.9 $[M/2+H]^+$; 837.0 $[M/3+H]^+$;
28	SNTSESFKSNTSESFFRVTQLAPKAQIKE-NH2	3261.8	1630.7 $[M/2+H]^+$; 1087.5 $[M/3+H]^+$; ;
29	SNTSESFK(SNTSESF)-NH2	1650.69	1650.5 $[M]^+$ 825.9 $[M/2+H]^+$
30	SNTSESFK(SNTSESF)LAPKAQIKE-NH2	2629.89	1315.1 $[M/2+H]^+$; 877.0 $[M/3+H]^+$;
31	SNTSESFK(SNTSESF)FRVTQKAQIKE-NH2	2980.27	1489.9 $[M/2+H]^+$; 993.9 $[M/3+H]^+$; 745.7 $[M/4+H]^+$;
32	SNTSESFK(SNTSESF)KAQIKE-NH2	2348.53	1174.5 $[M/2+H]^+$; 783.2 $[M/3+H]^+$

33	SNTSESFK(SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{14}CO$)E-NH2	3499.62	1166.8 $[M/3+H]^+$
34	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_{14}CO$)AQIKE-NH2	3499.62	1750.3 $[M/2+H]^+$;1167.2 $[M/3+H]^+$
35	SNTSESFK(SNTSESF)FRVTQK(LAP)KAQIKE-NH2	3389.8	1695 $[M/2+H]^+$;1130.2 $[M/3+H]^+$;848.3 $[M/4+H]^+$
36	SNTSESFK(SNTSESF)FRVTQLAK(PKA)QIKE-NH2	3389.8	1695.2 $[M/2+H]^+$;1130.5 $[M/3+H]^+$;848.3 $[M/4+H]^+$
37	SNTSESFK(LAP)FRVTQLAPKAQIKE-NH2	2790.24	1395.2 $[M/2+H]^+$; 930.6 $[M/3+H]^+$; 698.4 $[M/4+H]^+$
38	LAPK(LAP)FRVTQLAPKAQIKE-NH2	2318.86	1159.7 $[M/2+H]^+$; 773.6 $[M/3+H]^+$; 580.6 $[M/4+H]^+$
39	LAPKAQIKE-NH2	996.23	996.7 $[M]^+$
40	SNTSESFK(SNTSESF)FK($CH_3(CH_2)_{14}CO$))VTQLAPKAQIKE-NH2 Arg in D strand replaced by Lys	3471.61	1736.5 $[M/2+H]^+$; 1158.2 $[M/3+H]^+$; 869.1 $[M/4+H]^+$
41	SNTSESFK(SNTSESF)FRVTQLAP-NH2	2563.78	1282.1 $[M/2+H]^+$; 855.1 $[M/3+H]^+$;
42	SNTSESFFRVTQK(SNTSESF)LAPKAQIKE-NH2	3261.62	1631.6 $[M/2+H]^+$; 1088 $[M/3+H]^+$; 816.2 $[M/4+H]^+$;

43	FRVTQLAPKAQIKE-NH2	1627.97	1627.7 $[M]^+$; 814.3 $[M/2+H]^+$; 543.3 $[M/3+H]^+$;
44	SNTSESFK(SNTSESF)FK($CH_3(CH_2)_6CO$)VTQLAPKAQIKE-NH2 Arg in D strand replaced by Lys	3359.61	1680.2 $[M/2+H]^+$; 1121.1 $[M/3+H]^+$
45	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_6CO$)AQIKE-NH2	3387.62	1129.9 $[M/3+H]^+$; 847.3 $[M/4+H]^+$;
46	SNTSESFK(SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_6CO$)E-NH2	3387.62	1693.8 $[M/2+H]^+$; 1129.7 $[M/3+H]^+$; 847.5 $[M/4+H]^+$;
47	SNTSESFK(sntsesf)FRVTQLAPKAQIKE-NH2	3261.62	1631.6 $[M/2+H]^+$; 1088 $[M/3+H]^+$; 816.2 $[M/4+H]^+$;
48	EKIQAKPALQTVRFK(FSESTNS)FSESTNS-NH2	3261.62	1631.6 $[M/2+H]^+$; 1088 $[M/3+H]^+$; 816.2 $[M/2+H]^+$;
49	ekiqakpalqtvrfk(fsestns)fsestns-NH_2	3261.62	1630.6 $[M/2+H]^+$; 1087.5 $[M/3+H]^+$; 815.7 $[M/4+H]^+$;
57	SNTSESFK(SNTSESF)FRVTQLAPK(CH3CO)AQIKE-NH2	3304.61	1653.3 $[M/2+H]^+$; 1102.5 $[M/3+H]^+$;
59	SNTSESFK(SNTSESF)FRVTQLAPK(CH3-(CH2)4-CO)AQIKE-NH2	3359.62	1681.3 $[M/2+H]^+$; 1121.2 $[M/3+H]^+$;
60	Biotin-Ahx- sntsesfk(sntsesf)frvtqlapkaqike-NH2	3601.22	1201.1 $[M/3+H]^+$ 901.3$[M/4+H]^+$

61	Biotin-Ahx-EKIQAKPAYWNLVK(KDTQNS)DTQNS-NH2	3245.69	1623 $[M/2+H]^+$; 1082.3 $[M/3+H]^+$; 811.8 $[M/4+H]^+$;
62	SNTSESFK(SNTSESF)FRVTQLAPE*AQIK*E-NH2 Lactam bond formed between asterisked(*) amino acids	3244.12	1622.9 $[M/2+H]^+$; 1082 $[M/2+H]^+$;
63	SNTSESFK(SNTSESF)FK*VTQE*APKAQIKE-NH2 Lactam bond formed between asterisked(*) amino acids	3232.57	1615.4 $[M/2+H]^+$; 1078 $[M/3+H]^+$;
64	SNTSESFK(SNTSESF)FRVTE*LAPK*AQIKE-NH2 Lactam bond formed between asterisked(*) amino acids	3244.12	1622.4 $[M/2+H]^+$; 1082.4 $[M/3+H]^+$;
65	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_6CO$)E-NH2	3471	1736 $[M/2+H]^+$;1158 $[M/3+H]^+$
66	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{14}CO$)E-NH2	3583	1792.6 $[M/2+H]^+$;1195.4 $[M/3+H]^+$
67	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{10}$ CO)E-NH2	3529.2	1764.7 $[M/2]^+$;1176.8 $[M/3]^+$
68	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_4$ CO)E-NH2	3442.1	1722.1 $[M/2+H]^+$;1148.1 $[M/3+H]^+$
69	SNWSEDLK(SNWSEDL)FQIIQLHPKAKIEE-NH2	3484.09	1742.9 $[M/2+H]^+$;1162.3 $[M/3+H]^+$

70	EDK(ED)FRVTQLAPKAQIKE-NH2	2245.54	1122.8 $[M/2+H]^+$;748.9 $[M/3+H]^+$
71	SNTSESFK(ED)FRVTQLAPKAQIKE-NH2	2754.07	918.4 $[M/3+H]^+$;689.3 $[M/4+H]^+$
72	LAPKAK(ED)FRVTQLAPKAQIKE-NH2	2481.0	828 $[M/3+H]^+$; 621.1$[M/4+H]^+$
73	SNQTDKK(ED)FRVTQLAPKAQIKE-NH2	2674.03	892.0 $[M/3+H]^+$;669.2 $[M/4+H]^+$
74	SNQTDK(SNQTDK)GIYLCGAISLAPKAQIKE-NH2	3227.66	1077.5 $[M/3+H]^+$;
75	SNQTDKK(VLNWYRM)GIYLCGAISLAPKAQIKE-NH2	3639.22	1214.5 $[M/3+H]^+$; 727.8 $[M/5+H]^+$
76	EDK(VLNWYRM)GIYLCGAISLAPKAQIKE-NH2	3208.44	1605.8 $[M/2+H]^+$; 1070.8$[M/4+H]^+$
77	SNTSESFK(SNTSESF)GIYLCGAISLAPKAQIKE-NH2	3506.74	874.0 $[M/4+H]^+$
79	VLNWYRMK(SNQTDK)GIYLCGAISLAPKAQIKE-NH2	3638.32	1214.1 $[M/3+H]^+$; 727.7 $[M/5+H]^+$
80	GIYLCGAISLAPKAQIKE-NH2	1873.29	937.6$[M/2+H]^+$
81	SNQTDKK (SNQTDK) FRVTQ LAPKAQIKE-NH2	3103.51	1552.4 $[M/2+H]^+$; 1035.7 $[M/3+H]^+$; 777.0 $[M/4+H]^+$;
82	SNWSEDLK(SNWSEDL)FQIIQLHPK($CH_3(CH_2)_{14}CO$)AKIEE-NH2	3727.8	1241.9$[M/3]^+$

83	SNTSESFK(SNTSESF)FRVTQLAPK(MPA-NH-CH_2-CH_2-O-CH_2-CH_2-O-CO)AQIKE-NH2	3557.09	1779.8$[M/2+H]^+$ 1187.1$[M/3+H]^+$
84	SNTSESFK(sNTSESF)FRVTQLAPKAQIKE-NH2 (D-ser in the branch)	3261.62	1631 $[M/2+H]^+$; 1088.2 $[M/3+H]^+$; 816.2 $[M/4+H]^+$;
88	SΨ[CH2NH]NTSESFK(SΨ[CH2NH]NTSESF)FRVTQLAPKAQIKE-NH2	3232.8	1617.4$[M/2+H]^+$ 809.6$[M/4+H]^+$
89	SnTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2 D-Asn at N-terminus	3261.62	1631.6 $[M/2+H]^+$; 1087.8 $[M/3+H]^+$; 816.2 $[M/4+H]^+$;
90	SNTSESFK(SnTSESF)FRVTQLAPKAQIKE-NH2 D-Asn in the branch	3261.62	1631.5 $[M/2+H]^+$; 1088.1 $[M/3+H]^+$; 816.1 $[M/4+H]^+$;
91	SNWSEDLK(SNWSEDL)FQIIQLHPK(MPA-NH-CH_2-CH_2-O-CH_2-CH_2-O-CO)AKIEE-NH2	3779.2	1261.1$[M/2+H]^+$ 946.0$[M/4+H]^+$
92	SNTSESFK(SNTSESF)FRVTQLAPKAQIKE	3262.62	1088.6 $[M/3+H]^+$; 813.4 $[M/4]^+$;
98	SNTSESFK(SNTSESF)FRVTQLAPK(CH_3(CH_2)$_{14}$CO)AQIKE	3500.62	1167.4 $[M/3+H]^+$
99	SNTSESF-Orn-(SNTSESF) KAQIKE-NH2	2135.77	1067.9 $[M/2]^+$; 711.9 $[M/3]^+$
100	SNTSESF-Dap-(SNTSESF) KAQIKE-NH2	2107.8	1053.9 $[M/2]^+$; 703.0 $[M/3]^+$
101	SNTSESF-Dab-(SNTSESF) KAQIKE-NH2	2121.22	1060.5 $[M/2]^+$;
106	LAPKA-NH2	497.64	498.3 [M+H]+;

107	LAPKA(SNQTDK)FRVTQLAPKAQIKE-NH2	2910.44	971.2[M/3+H]+ 728.5[M/4+H]+

Example 16: Binding Inhibition Analysis by Flow Cytometry

Reagents:

Expression of PD1 fusion with murine Fc region in mammalian cells: Extracellular domain of human PD1 (1-167 amino acid residues) was cloned in pFUSE-Fc (Invivogen, CA). This construct was transfected into HEK293 cells (CRL 1573, ATCC) using Lipofectamine 2000 (Invitrogen) and expressed transiently. The secreted recombinant protein containing extracellular PD1 protein fused with Fc region of murine IgG was purified from the culture media in which transfected cells were grown.

Cell lines as source of ligand for PD1: MDA-MB-231 cells were found to express PD-L1 by RT-PCR and therefore used as a source of PD-L1 in the assays. Recombinant PDL2 expressing HEK293 cells were used as a source of PDL2 in the cellular context.

Flow cytometry based determination of binding of PD1 with its ligands:

This assay was done to determine the effect of compounds on binding of PD1 with its ligands PD-L1 and PD-L2. Briefly, HEK 293 cells (ATCC:CRL-1573) overexpressing recombinant hPDL-2 in a stable manner and pcDNA Myc HisA transfected HEK 293 (negative control) cell lines were grown upto 60-70% confluency using complete DMEM media (Sigma D5648-1L). Cells were dissociated with cell dissociation buffer (Invitrogen-13151-014) and counted following which 2x 10^5 cells were re-suspended in 50µl of complete DMEM media in FACS tube.

Before dissociating cells, soluble PD1 tagged with Fc (derived from concentrated supernatant of transiently transfected cells) was incubated with test compound #8; in a total volume of 10µl for one hour. PD1-Neutralizing antibody (mouse monoclonal IgG2B clone 192106, Cat. No MAB1086; R&D Labs, USA) was also pre-incubated with soluble PD1 to be used as a positive control for inhibition of binding of PD1 to its ligand. The mixture of PD1/pFuse pre-incubated with Compound #8 at varying doses; Compound was added to the cells and allowed to bind.

To detect the association of PD1 to ligand expressing cells, anti-mouse secondary antibody conjugated to Alexafluor 488 (Invitrogen, Cat. No.11001) at 1:1000 dilution was added to the cells and incubated for half hour at 4°C. Five hundred microliters of FACS sheath fluid (BD, Catalog No.336524) was added to these tubes after half hour and analysis was done in BD FACS Calibur machine. Compound #8 was able to inhibit binding of PD1 to PD-L2 expressed in HEK 293 cells in a potent manner as shown in Figure 1.

Example 17:Measurement of Restoration of Rat PBMC Proliferation

This assay was done to test the effect of peptides on the function of PBMC. As a consequence of the effect of peptide on the interaction between PD1 (expressed on PBMC) and its ligands (expressed on tumour cells) PBMC proliferation is affected. PBMC proliferation is expected to increase upon abrogation of binding between PD1 and its ligands in the presence of a disrupting peptide. In order to determine the effect of test compounds on this interaction, hPDL1 expressing MDA-MB231 cells (ATCC:CRL HTB-26) were seeded in a 24-well plate containing complete DMEM media (Sigma catalog no. D5648) where Geneticin at 100µg/ml (Sigma, catalog no. G8168) was added for hPDL2 cells.

One milliliter of rat blood diluted with 5X DMEM medium along with PMA (Phorbol 12-myristate 13-acetate) at 6.25ng/ml (Sigma, Cat. No.8139) and Ionomycin at 0.5µg/ml (Sigma, Cat. No.I0634) was overlaid on the top of the cells. Test compounds were added at appropriate concentration in a volume not exceeding 10µl/well along with the blood to the respective wells and the plate was incubated in CO_2 incubator at 37ºC.

After forty eight hours, rat PBMC's were isolated using Histopaque1077 (Sigma, Cat. No.10771) and the samples were fixed in 70% ethanol followed by treatment with RNase (Sigma, R6513) and stained with Propidium iodide (Sigma Cat. No. 81845). These samples were analyzed in BD FACS Calibur flow cytometer. As shown in Figure 2, while minimal proliferation was observed for PMA stimulated PBMC cells in the presence of ligand expressing MDA-MB231 cells, the introduction of Compound #8 was able to restore proliferation effectively.

Example 18: Determination of potency of compound 8 in rat PBMC proliferation rescue assay

This study was completed using the protocol described earlier in example 17. Various concentrations of compound 8 were tested in this assay and potency of peptide compound 8 was determined in restoration of proliferation of PMA stimulated PBMCs. This assay was done in the context of PD-L1 expressing MDA-MB231 as shown in Figure 3.

Example 19: Measurement of Restoration of Human PBMC Proliferation

Human blood was collected with heparin and was diluted with phosphate buffered saline and peripheral blood mononuclear cells (PBMC) were isolated using Histopaque. A 24-well plate was pre-coated with anti-Cd3 and anti-Cd28 (5ug/ml) for 3 hours. Another plate was pre-coated with Isotype control antibodies (IgG1/IgG2a) for use as negative control for proliferation induction. The PBMCs were counted and seeded at 0.6 million cells/ml per well along with recombinant hPDL1 at 1 µg/ml per well followed by test compounds at the appropriate concentrations. The plate was incubated for 48 hours and PBMCs were collected and fixed with ethanol for analysis of cell cycle profile by propidium iodide staining. Data for restoration of human PBMC proliferation for tested compounds is shown in Figure 4.

Example 20: The effect of peptides on mouse splenocyte proliferation inhibited by MDMBA231 tumour cells expressing PDL1

Use of MDA-MB-231 cells as a source of PD-L1:

MDA-MB-231 cells were found to express PD-L1 by RT-PCR and therefore used as a source of PD-L1 in the assays.

Requirement:

Mouse splenocytes harvested from 6-8 weeks old C57 BL6 mice.; RPMI 1640 (GIBCO, Cat # 11875);DMEM with high glucose (GIBCO, Cat # D6429);Fetal Bovine Serum [Hyclone, Cat # SH30071.03];Pencilin (10000unit/ml)-Streptomycin(10,000µg/ml) Liquid (GIBCO, Cat # 15140-122);MEM Sodium Pyruvate solution 100mM (100x), Liquid (GIBCO, Cat # 11360);Nonessential amino acid (GIBCO, Cat # 11140);L-Glutamine (GIBCO, Cat # 25030);Anti-CD3 antibody (eBiosciences – 16-0032); Anti-CD28 antibody (eBiosciences – 16-0281);ACK lysis buffer (1mL) (GIBCO, Cat # -A10492);Histopaque (density-1.083 gm/ml) (SIGMA 10831);Trypan blue solution (SIGMA-T8154);Hemacytometer (Bright line-SIGMA

Z359629);FACS Buffer (PBS/0.1% BSA): Phosphate Buffered Saline (PBS) pH 7.2 (HiMedia TS1006) with 0.1% Bovine Serum Albumin (BSA) (SIGMA A7050) and sodium azide (SIGMA 08591);5 mM stock solution of CFSE: CFSE stock solution was prepared by diluting lyophilized CFSE with 180µL of Di methyl Sulfoxide (DMSO C_2H_6SO, SIGMA-D-5879) and aliquoted in to tubes for further use. Working concentrations were titrated from 10µM to 1 µM. (eBioscience-650850-85); 96-well format ELISA plates (Corning CLS3390);BD FACS caliber (E6016).

Protocol

Splenocyte preparation:

Splenocytes harvested in a 50ml falcon tube by mashing spleen in a 40µm cell strainer were further treated with 1ml ACK lysis buffer for 5mins at RT. After washing with 9ml of RPMI complete media, cells re-suspended in 3ml of 1xPBS in a 15ml tube. 3ml of histopaque was added very carefully to the bottom of the tube without disturbing overlaying splenocyte suspension. Spin the tube at 800xg for 20mins at RT. Opaque layer of lymphocytes is collected carefully without disturbing/mixing any of the layers. Cells washed twice with cold 1xPBS followed by total cell counting using trypan blue exclusion method and used further for cell based assays.

CFSE Proliferation assay:

Tumour cells (MDMBA231) are cultured and maintained in high glucose complete DMEM media. $1x10^5$ tumour cells were plated in 96 well plates along with required conc. of PD1 derived peptide and allowed to adhere at 37°C for 4 hrs. $1x10^6$ cells / ml of harvested splenocytes are treated with 5µM of CFSE in pre warmed 1xPBS/0.1%BSA solution for 10mins at 37°C. Excess CFSE was quenched using 5 volumes of ice-cold culture media to the cells and incubated on ice for 5mins. CFSE labeled splenocytes were further given three washes with ice cold complete DMEM media. CFSE labeled $1x10^5$ splenocytes added to above wells containing tumours cells and test compounds. Splenocytes were stimulated with anti-CD3 and anti-CD28 antibody (1µg/ml each) and the co-culture was further incubated for 72hrs at 37°C with 5% CO_2. Cells were harvested and washed thrice with ice cold FACS buffer and % proliferation was analyzed using a FACS caliber with 488nm excitation and 521nm emission filters. Each experimental condition was carried out in triplicates and each experiment at least carried out

three times. Splenocyte proliferation was analyzed using cell quest FACS program and fold induction was calculated by normalizing individual values to percent background proliferation

Fold induction = Percent splenocyte proliferation / percent background proliferation

Stimulated splenocytes: Splenocytes + anti-CD3/CD28 stimulation

Background proliferation: Splenocytes + anti-CD3/CD28 + Tumour

Percent Proliferation rescue = {(compound percent proliferation – Background percent proliferation) / (Stimulated percent proliferation – Background percent proliferation)}*100.

(a) Comparison of percentage proliferation rescue of compound 8, 13 and 34 with mAb J43 antibody. mAb J43 (Int. Immunol. 1996 -8(5):765-72) and test peptides were analysed for mouse splenocyte proliferation rescue studies using CFSE method as described protocol above and results are summarized in Figure 5.

(b) Effect of compounds on CFSE based mouse splenocyte proliferation rescue studies

The test compounds were analysed for mouse splenocyte proliferation rescue studies using the method as mentioned in protocol above and data is presented as percent activity in comparison with compound 8 peptide as in Table V.

Table V: Percent of activity for various compounds via Mouse splenocyte proliferation assay using CFSE method

Comp No.	Sequence	Percent rescue with various compounds (screened at 100nM concentration and compared with 100nM Comp 8) in mouse splenocyte proliferation CFSE based assay
1	SNTSESF-NH2	96.5
3	SNQTDKLAAFPEDSQPGQD-NH2	37.3
4	EDRSQPGQDCR-NH2	74.7
5	CGAISLAPKAQIKES-NH2	68
7	FRVTQK(SNTSESF)FRVTQLAPKAQIKE-NH2	61.5

8	SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2	100
9	SNQTDK (SNQTDK) FRVTQ LAPKAQIKE-NH2	11.3
10	SNQTDK (SNQTDK) VLNWYRM LAPKAQIKE-NH2	10.6
11	SNTSESFK(SNQTDK)FRVTQLAPKAQIKE-NH2	12.4
13	sntsesfk(sntsesf)frvtqlapkaqike-NH2	0-12 nM-control peptide
14	EKIQAKPAYWNLVK(KDTQNS)DTQNS-NH2	18
16	C6 lipid-SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2	45.8
17	Ac-SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2	32.9
20	SNTSESFK(CH3(CH2)4CO-SNTSESF)FRVTQLAPKAQIKE-NH2	27.8
21	CH3(CH2)4CO-SNTSESFK(CH3(CH2)4CO-SNTSESF)FRVTQLAPKAQIKE-NH2	76.7
22	SNTSESFR5PKAR5SNTSESF	71.4
25	SNTSESFK(SNTSESF)FRVTQLAQIKE	27.6
26	SNTSESFFRVTQLAPKAQIKE-NH2	86.6
27	SNTSESFKFRVTQLAPKAQIKE-NH2	73.6
28	SNTSESFKSNTSESFFRVTQLAPKAQIKE-NH2	56.5
29	SNTSESFK(SNTSESF)-NH2	31.1
30	SNTSESFK(SNTSESF)LAPKAQIKE-NH2	58.8

31	SNTSESFK(SNTSESF)FRVTQKAQIKE-NH2	11.3
32	SNTSESFK(SNTSESF)KAQIKE-NH2	14.8
33	SNTSESFK(SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{14}CO$)E-NH2	98
34	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_{14}CO$)AQIKE-NH2	106
35	SNTSESFK(SNTSESF)FRVTQK(LAP)KAQIKE-NH2	81.2
36	SNTSESFK(SNTSESF)FRVTQLAK(PKA)QIKE-NH2	89.7
37	SNTSESFK(LAP)FRVTQLAPKAQIKE-NH2	89.60
38	LAPK(LAP)FRVTQLAPKAQIKE-NH2	51.5
40	SNTSESFK(SNTSESF)FK($CH_3(CH_2)_{14}CO$))VTQLAPKAQIKE-NH2 Arg in D strand replaced by Lys	82
41	SNTSESFK(SNTSESF)FRVTQLAP-NH2	5.1
42	SNTSESFFRVTQK(SNTSESF)LAPKAQIKE-NH2	52.9
43	FRVTQLAPKAQIKE-NH2	66
45	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_6CO$)AQIKE-NH2	68
46	SNTSESFK(SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_6CO$)E-NH2	67
48	EKIQAKPALQTVRFK(FSESTNS)FSESTNS-NH2	25
49	ekiqakpalqtvrfk(fsestns)fsestns-NH_2	73
60	Biotin-Ahx- sntsesfk(sntsesf)frvtqlapkaqike-NH2	14.9

61	Biotin-Ahx-EKIQAKPAYWNLVK(KDTQNS)DTQNS-NH2	67
62	SNTSESFK(SNTSESF)FRVTQLAPE*AQIK*E-NH2	37
63	SNTSESFK(SNTSESF)FK*VTQE*APKAQIKE	68
64	SNTSESFK(SNTSESF)FRVTE*LAPK*AQIKE	69.1
69	SNWSEDLK(SNWSEDL)FQIIQLHPKAKIEE-NH2	48
82	SNWSEDLK(SNWSEDL)FQIIQLHPK($CH_3(CH_2)_{14}CO$)AKIEE-NH2	58.3
84	SNTSESFK(SNTSESF)FRVTQLAPK(MPA-NH-CH_2-CH_2-O-CH_2-CH_2-O-CO)AQIKE-NH2	80.5
91	SNWSEDLK(SNWSEDL)FQIIQLHPK(MPA-NH-CH_2-CH_2-O-CH_2-CH_2-O-CO)AKIEE-NH2	69.4

Example 21: Cytotoxic T Lymphocyte IFN-γ assay

Tumour cells are cultured and maintained in high glucose complete DMEM media. $1x10^5$ tumour cells were plated in 96 well plates along with required concentration of test compounds and allowed to adhere at 37°C for 4 hrs. $1x10^5$ of harvested splenocytes was added to wells containing tumours cells and test compounds. Splenocytes were stimulated with anti-CD3 and anti-CD28 antibody (1µg/ml each) and the co-culture was further incubated for 72hrs at 37°C with 5% CO_2. After 72 hours of incubation the cell culture supernatants were collected and processed for mouse IFN gamma measurement by ELISA following manufacturer's protocol (e Biosciences; 88-7314).

ELISA: As per manufacturer's protocol

In brief, 96 well ELISA plates were coated with 100µl/well of capture antibody in coating buffer and incubated overnight at 4°C. Plates washed five times with wash buffer and further blocked with 200µl of 1x assay diluents for 1hr at RT. Following wash step, 100µl of cell culture supernatants were added to wells and further incubated for 2hrs at RT. Appropriate standards

were also included. After the wash step, plates were incubated for one hour with 100µl/well of detection antibody. Wash steps were repeated and the plates were incubated for 30minutes with 100µl/well of Avidin-HRP. The plates were washed again several times with wash buffer and incubated for 15 minutes with 100µl/well of substrate solution. 50µl of stop solution was added to each well, and the plates were read at 450nm. The absorbance values were then plotted against the standards and the concentration of IFN-γ was determined using Graph Pad Prism software (Figure 6). Each experimental condition was carried out in triplicates and each experiment at least carried out three times.

Fold induction = IFN-γ released by stimulated splenocyte in absence or presence of compound / Background IFN-γ release

Stimulated splenocytes: Splenocytes + anti-CD3/CD28 stimulation in absence or presence of test compound

Background IFN-γ release: Splenocytes + anti-CD3/CD28 + Tumour

Example 22: In vivo efficacy of compound #8 on primary tumour growth and lung metastasis in B16F10 subcutaneous melanoma model

Animals:

C57/Black6 female mice (Aurigene, Bangalore, India) aged 6 to 8 weeks were used for the experiment. Animals were acclimatized for a week in the experimental room before conducting the experiment.

On day 0, B16F10 cells grown in DMEM containing 10% FBS at 70 to75% confluency were harvested and 1 X 10^6 cells per animal were injected to mice subcutaneously on the right flank region. On day 1, peptide (Compound #8) at 5mg/kg dose dissolved in PBS, pH 7.4 was dosed subcutaneously at the rate of 10ml/kg volume for fourteen days once daily. Vehicle control group of mice received only saline. Each group consisted of ten animals. Body weight and clinical signs were recorded daily. Tumour volume was measured by slide callipers. Compound #8 dosed at 5 mg/kg, inhibited the tumour growth to the extent of 44 percent ($p < 0.001$, 2-way ANOVA). There was no body weight reduction during the period of dosing and no clinical signs observed. Mean tumour volume of vehicle treated animals was 496 mm3, whereas Compound #8 treated animals showed 276mm^3 at the end of 14 days dosing period (Figure 7). At the end of dosing period lung was harvested and analysed for metastasis by counting the black nodules. It was observed that Compound 8 showed greater than 60 percent

reduction in metastasis. The picture of lung treated with vehicle and Compound 8 is shown as (Figure-8)

Example 23 In vivo efficacy of compounds # 8, 34, 69, 82, 83, 91 on inhibition of metastasis in B16F10 metastasis model

In the case of metastasis model, $0.1X10^6$ B16F10 cells were injected i.v. to C57/Black6 mice. Test compounds dissolved in PBS, pH 7.4 was dosed subcutaneously at 5 mg/kg once daily. Vehicle control group of mice received only saline. Each group consisted of ten animals. Body weight and clinical signs were recorded daily. After 11 days of treatment, lung metastasis was quantitated by counting number of nodules under dissection microscope. Figure 9 shows that at the end of 11 days of dosing.

Example 24 Effect of Compound #8 in 4T1 orthotopic mammary carcinoma model

On day 0, 4T1 cells grown in RPMI 1640 containing 10% FBS at 70 to75% confluency were harvested and 0.1 X 10^6 cells per animal were injected along with matrigel orthotopically to mammary fat pad. On day 1, peptide (Compound #8) at 3 mg/kg dose dissolved in PBS, pH 7.4 was dosed subcutaneously at the rate of 10ml/kg volume for 5.5 weeks once daily. Vehicle control group of mice received only saline. Each group consisted of ten animals. Body weight and clinical signs were recorded daily. Tumour volume was measured by slide callipers. At the end of the study, metastasis to lungs was quantitated by counting the number of tumour nodules on the lung using dissection microscope. Figure 10 shows that at the end of 40 days of dosing, Compound #8 dosed at 3 mg/kg inhibited the primary tumour volume to the extent of 44 percent. There was no body weight reduction during dosing period. Ten percent of the animals in the Compound #8 treated group showed complete regression and another ten percent showed partial regression of tumour growth. On the 40^{th} day, animals were euthanized and their lungs were observed for tumour nodules under the dissection microscope. One animal though the tumour volume was similar to vehicle treated group, there was complete inhibition of metastasis (no tumour nodules were seen). The animal that showed complete regression in primary tumour volume also showed no visible tumour nodules. Compound #8 treated animals showed mean reduction in metastasis to the extent of >60 percent.

Example 25 Effect of Compound 34 & 83 in renal cell carcinoma model

Female 6- to 8-week-old BALB/c mice were kept in a temperature-controlled room on a 12/12 h light/dark schedule with food and water ad libitum. RENCA cells harvested from non confluent monolayer were injected under the renal capsule on day1 without opening the peritoneum. The skin incision was closed with auto suture clips. Renca cells (15 X 10^4) in 40 µl of medium were injected under the kidney capsule of mice on day 1. Peptides were dosed subcutaneously from day 2 at 5mpk, qd for 21 days. At the end of dosing (21 days) animals were euthanized and tumour weight of the left and right kidney (control) were measured and plotted as percent inhibition as shown in Figure 11.

Example 26: Procedure for minimum inhibitory concentration (MIC) determination by broth microdilution method

Required quantity of the compound was weighed and dissolved in water / dimethyl sulphoxide / suitable solvent to yield 1mg/ml stock solution. The stock solution was diluted in MHB / CAMHB to yield 8 µg/ml or 64µg/ml or 512 µg/ml solution as per requirement. Serial two fold dilutions of the compound was prepared using MHB / CAMHB in 96 well microtitre trays using a multichannel pipette. Organisms were grown in MHB / CAMHB for 4–5 hrs at 35±2°C, 110±10 rpm. Optical density (OD) of the cultures is adjusted to 625 nm which corresponds to 0.5 Mc Farland standard (1–2 x 10^8 cfu/ml) and further diluted to achieve a final inoculum of (5±5) x 10^4 cfu/well. Broth, compound and organism controls were set up. Microdilution trays were incubated at 35±2°C for 16-20 hours in an ambient air incubator. After the incubation period, growth of organism in the wells was detected by unaided eye facilitated by a viewing device. The lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye was taken as MIC. MIC value indicates that test compound 8 (MIC (µg/ml)- >32) do not exhibit any antibacterial activity, whereas the standards viz, levofloxacin and ciprofloxacin shows an MIC ((µg/ml) values of 0.015 and 0.007 in the same experiment.

Example 27: In vivo efficacy of compound # in Mouse model of *E. coli* sepsis

Materials and Methods

Bacterial strain used was *E. coli* ATCC 25922 and bacterial suspension was prepared in normal saline to obtain an inoculum of ~5 x 10^8 CFU/ml. BALB/c or Swiss albino mice weighing 18-22

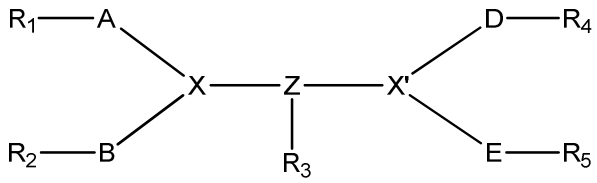
gms were used for the study. Animals were quarantined for a period of 5 days in individually ventilated cages (IVCs). Food and water was provided *ad libitum*. Temperature was maintained at 22-26º C with a light dark cycle of 12hrs.

Procedure

The final inoculum containing ~ 1-2 x 10^8 CFU/ml was administered to the animals by intravenous route. Treatment was initiated after 24 hrs of infection and the compound was administered thrice a day at an interval of 3 hours for 2 days. One group of animals was kept untreated to serve as infection control. After completion of treatment for two days, all the animals (treated and untreated) were sacrificed 3 hrs after the last dose and various organs (kidneys, spleen & lungs) were collected aseptically and processed for enumeration of bacterial counts. Organ samples collected were homogenized, serially diluted in normal saline and plated on TSA plates. The plates were incubated at 35 $\pm$ 2º C for 18-24 hrs and bacterial counts enumerated. Results were analyzed by comparing treated Vs control animals & suitable statistical method was applied to calculate the statistical significance as shown in Figure 12.

WE CLAIM:

1. A compound of formula (I)



(I)

or pharmaceutically acceptable salt thereof;

wherein;
the compound comprises at least 5 amino acids,

A is an amino acid sequence of from three amino acids to the full length of a peptide sequence of mammalian PD1 ectodomain fragments selected from FG loop, BC loop, C-C' loop, C strand, C' strand to C'-C'' loop, or is Glu-Asp;

B is an amino acid sequence of from three amino acids to the full length of a peptide sequence of mammalian PD1 ectodomain fragments selected from BC loop, FG loop, C C' loop to C' strand, C strand, D strand, C' strand to C'-C'' loop, or is Glu-Asp, or is absent;

Z is

(i). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from BC loop, D strand, FG loop, G strand, C strand, F strand, C' strand, C" strand, C"-D loop, C' strand to C'-C'' loop, C' strand to C'' strand or D strand to DE loop;

(ii). G-L-Z'

G is an amino acid sequence of from three amino acids to the full length of a peptide sequence of mammalian PD1 ectodomain fragments from D-strand or is absent;

L is selected from $-CO(CH_2)_n-NH-$, or PEG 2-20 KD;

'n' is an integer selected from 2 to 10, both inclusive; and

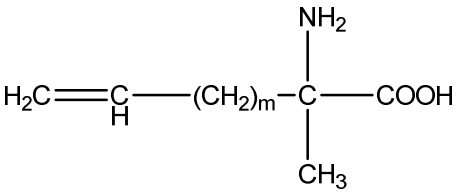
Z' is one to three peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from FG loop and G-strand; or

(iii). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from D-strand, FG loop and G strand, wherein two or more amino acids of the peptide sequence combine together to form a lactam bond between any of the two fragments or within the fragment;

D is up to two peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from BC loop, FG loop, C C' loop to C' strand or is absent;

E is up to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a mammalian PD1 ectodomain fragment selected from BC loop, D strand, FG loop, C C' loop to C' strand, G strand , FG loop to G strand or is absent;

X and X' are independently selected from lysine, ornithine, diaminopropionic acid, diaminobutyric acid or olefinic amino acid of formula



which is optionally linked with an additional lysine; or

X and X' combine together forming a ring with olefinic amino acid which is optionally linked with an additional lysine; or

X' is absent;

'm' is an integer selected from 1 to 6, both inclusive;

R_1 is selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD moiety; or absent.

R_2 and R_3 are independently selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD, absent or R_a-L';

R_a is selected from biotin or maleimido propionic acid;

L' is selected from linkers -$CO(CH_2)_n$-NH-, -$CO(CH_2$-CH_2-O-$)_n$ NH or -$COCH_2(-OCH_2$-$CH_2)_n$NH-; and

'n' is an integer selected from 2 to 10, both inclusive;

R_4 and R_5 are independently NH_2, or one or both of R_4 or R_5 are absent.

with the proviso to the compound of Formula I, that in a compound of Formula I as above defined:

a) up to 5 but not more than 25% of the amino acids may be substituted with other natural or unnatural amino acids;

b) not more than 30% of the amino acids may be omitted;

c) in each said peptide sequence up to 2 amino acids may be added individually at any position;

d) up to 5 but not more than 25% of the peptide bonds may instead be replaced by reduced amide bond (-CH2NH-);

e) up to 100% of the amino acids may be D-amino acids;

f) up to 100% of the amino acids may be in reverse order.

2. The compound according to claim 1, wherein said mammalian PD1 ectodomain fragments are from human, murine, dog, horse or rat PD1.

3. The compound according to claim 1, wherein;
A is an amino acid sequence of from three amino acids to the full length of a peptide sequence of human or murine PD1 ectodomain fragments selected from FG loop, BC loop, C-C' loop, C strand, C' strand to C'-C'' loop, as in table below, or is Glu-Asp;

	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL
C Strand	VLNWYRM	MLNWNRL

C-C' loop	SPSNQ	SPSNQ
C' strand to C'-C'' loop	FPED	FCNG
FG loop	LAPKA	LHPKA

B is an amino acid sequence of from three amino acids to the full length of a peptide sequence of human or murine PD1 ectodomain fragments selected from BC loop, FG loop, C C' loop to C' strand, C strand, D strand, C' strand to C'-C'' loop as in table below, or is Glu-Asp, or is absent;

	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL
C Strand	VLNWYRM	MLNWNRL
C' strand to C'-C'' loop	FPED	FCNG
C C' loop to C' strand	SPSNQTDKLAAFP	SPSNQTEKQAAFC
D Strand	FRVTQ	FQIIQ
FG loop	LAPKA	LHPKA

Z is

(i). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from BC loop, D strand, FG loop, G strand, C strand, F strand, C' strand, C" strand, C"-D loop, C' strand to C'-C'' loop, C' strand to C'' strand or D strand to DE loop;

(ii). G-L-Z'

G is an amino acid sequence of from three amino acids to the full length of a peptide sequence of human or murine PD1 ectodomain fragments from D-strand or is absent;

L is selected from $-CO(CH_2)_n-NH-$, or PEG 2-20 KD;

'n' is an integer selected from 2 to 10, both inclusive; and

Z' is one to three peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from FG loop and G-strand; or

(iii). from one to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from D-strand, FG loop and G strand, wherein two or more amino acids of the peptide sequence combine together to form a lactam bond between any of the two fragments or within the fragment;

wherein said fragments are as defined in table below;

	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL
C Strand	VLNWYRM	MLNWNRL
C' Strand	TDKLAAFP	TEKQAAFC
C' strand to C'-C'' loop	FPED	FCNG
C' strand to C'' strand	TDKLAAFPEDRSQP	TEKQAAFCNGLSQP
C'' Strand	RSQP	LSQP
C''-D loop	GQDCR	VQDAR
D Strand	FRVTQ	FQIIQ
F strand	GTYLCGAIS	GIYLCGAIS
FG loop	LAPKA	LHPKA
G Strand	QIKE	KIEE

D strand to DE loop	FRVTQLPNGR	FQIIQLPNRH

D is up to two peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from BC loop, FG loop, C C' loop to C' strand as in table below or is absent;

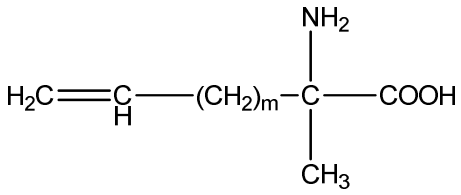
	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL
C C' loop to C' strand	SPSNQTDKLAAFP	SPSNQTEKQAAFC
FG loop	LAPKA	LHPKA

5

E is up to four peptide sequences arranged in any order each being of from three amino acids up to the full length of a human or murine PD1 ectodomain fragment selected from BC loop, D strand, FG loop, C C' loop to C' strand, G strand , FG loop to G strand or is absent;

	hPD-1	mPD-1
BC Loop	SNTSESF	SNWSEDL
C C' loop to C' strand	SPSNQTDKLAAFP	SPSNQTEKQAAFC
D Strand	FRVTQ	FQIIQ
FG loop	LAPKA	LHPKA
G Strand	QIKE	KIEE
FG loop to G strand	LAPKAQIKE	LHPKAKIEE

X and X' are independently selected from lysine, ornithine, diaminopropionic acid, diaminobutyric acid or olefinic amino acid of formula



which is optionally linked with an additional lysine; or

X and X' combine together forming a ring with olefinic amino acid which is optionally linked with an additional lysine; or

X' is absent;

'm' is an integer selected from 1 to 6, both inclusive;

R_1 is selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD moiety; or absent.

R_2 and R_3 are independently selected from group consisting of C_2-C_{20} acyl, PEG 2-20 KD, absent or R_a-L';

R_a is selected from biotin or maleimido propionic acid;

L' is selected from linkers $-CO(CH_2)_n-NH-$, $-CO(CH_2-CH_2-O-)_n$ NH or $-COCH_2(-OCH_2-CH_2)_nNH-$; and

'n' is an integer selected from 2 to 10, both inclusive;

R_4 and R_5 are independently NH_2, or one or both of R_4 or R_5 are absent.

with the proviso to the compound of Formula I, that in a compound of Formula I as above defined:

a) up to 5 but not more than 25% of the amino acids may be substituted with other natural or unnatural amino acids;

b) not more than 30% of the amino acids may be omitted;

c) in each said peptide sequence up to 2 amino acids may be added individually at any position;

d) up to 5 but not more than 25% of the peptide bonds may instead be replaced by reduced amide bond (-CH2NH-);

e) up to 100% of the amino acids may be D-amino acids;

f) up to 100% of the amino acids may be in reverse order.

4. The compound according to any of claims 1 to 3, wherein Z consists of one said peptide sequence and at least one of A, B, D or E is not absent.

5. The compound according to any of claims 1 to 3, wherein if A, B, D and E are absent then Z comprises from 2 to 4 said peptide sequences.

6. The compound according to any of claims 1 to 3 wherein Z is a combination of D strand, FG loop and G strand.

7. The compound according to any of claims 1 to 6, wherein X is lysine and X' is absent.

8. The compound according to any of claims 1 to 6, wherein both X and X' are lysine.

9. The compound according to any of claims 1 to 8, wherein R_2 and R_3 are selected from C_2-C_{20} acyl, or R_a-L'; wherein,

R_a is maleimido propionic acid;

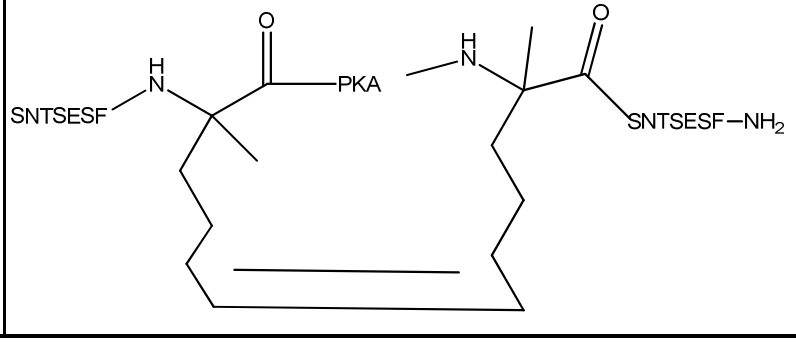
L' is $-COCH_2(-OCH_2-CH_2)_nNH-$; and

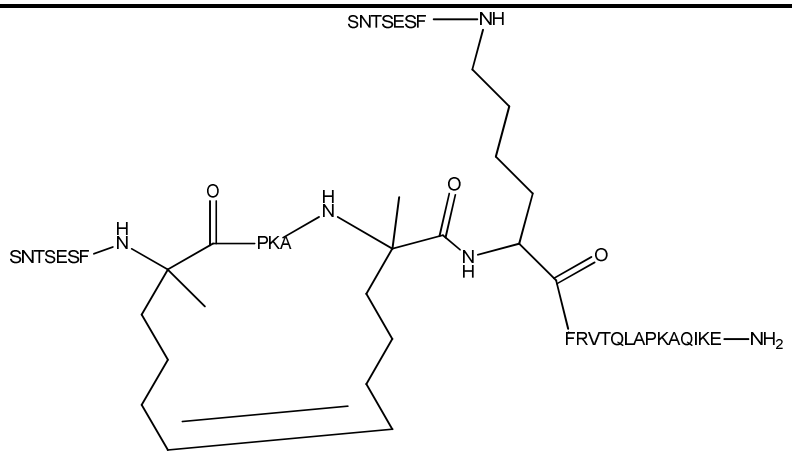
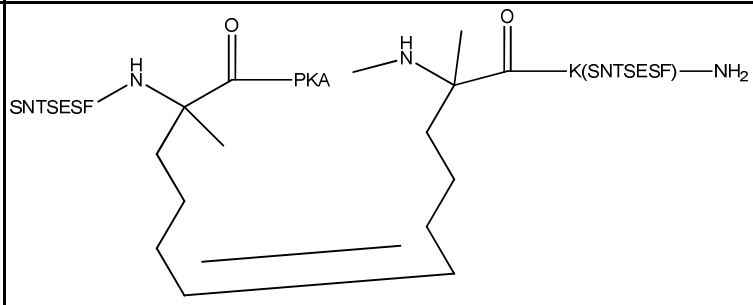
'n' is an integer selected from 2 to 10, both inclusive.

10. The compound according to any of claims 1 to 6, wherein X', D, E and R_5 are absent and R_4 is NH_2.

11. The compound selected from:

Comp. No.	**Compound**	**Sequence ID**
6.	FRVTQK(SNTSESF)FRVTQAhxLAPKAQIKE-NH2	47.
7.	FRVTQK(SNTSESF)FRVTQLAPKAQIKE-NH2	48.
8.	SNTSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2	49.
11.	SNTSESFK(SNQTDK)FRVTQLAPKAQIKE-NH2	52.
12.	SNTSESFK(SNQTDK)FRVTQK(LAP)AQIKE-NH2	53.
13.	sntsesfk(sntsesf)frvtqlapkaqike-NH2 All D-amino acids	54.

Comp. No.	Compound	Sequence ID
15.	Biotin–Ahx–SNTSESFK(SNTSESF)FRVTQLAPKAQIKE–NH2 Ahx = 6–aminohexanoic acid	56.
16.	C6 lipid–SNTSESFK(SNTSESF)FRVTQLAPKAQIKE–NH2	57.
17.	Ac–SNTSESFK(SNTSESF)FRVTQLAPKAQIKE–NH2	58.
18.	SNTSESFK(NH2–$[PEG]_{11}$–CO–SNTSESF)FRVTQLAPKAQIKE–NH2	59.
19.	SNTSESFK(SNTSESF)–NH–$[PEG]_{11}$–CO–)LAPKAQIKE–NH2	60.
20.	SNTSESFK(CH3(CH2)4CO–SNTSESF)FRVTQLAPKAQIKE–NH2 Lipid in branch	61.
21.	CH3(CH2)4CO–SNTSESFK(CH3(CH2)4CO–SNTSESF)FRVTQLAPKAQIKE–NH2 Lipid on both Branch & N–termini	62.
22.		63.

Comp. No.	Compound	Sequence ID
23.		64.
24.		65.
25.	SNTSESFK(SNTSESF)FRVTQLAQIKE-NH2	66.
27.	SNTSESFKFRVTQLAPKAQIKE-NH2	68.
28.	SNTSESFKSNTSESFFRVTQLAPKAQIKE-NH2	69.
29.	SNTSESFK(SNTSESF)-NH2	70.
30.	SNTSESFK(SNTSESF)LAPKAQIKE-NH2	71.
31.	SNTSESFK(SNTSESF)FRVTQKAQIKE-NH2	72.
32.	SNTSESFK(SNTSESF)KAQIKE-NH2	73.
33.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{14}CO$)E-NH2	74.
34.	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_{14}CO$)AQIKE-NH2	75.
35.	SNTSESFK(SNTSESF)FRVTQK(LAP)KAQIKE-NH2	76.

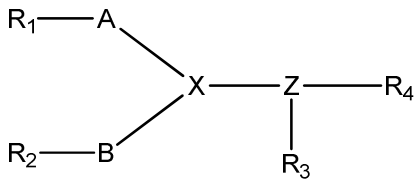
Comp. No.	Compound	Sequence ID
36.	SNTSESFK(SNTSESF)FRVTQLAK(PKA)QIKE-NH2	77.
37.	SNTSESFK(LAP)FRVTQLAPKAQIKE-NH2	78.
38.	LAPK(LAP)FRVTQLAPKAQIKE-NH2	79.
40.	SNTSESFK(SNTSESF)FK($CH_3(CH_2)_{14}CO$))VTQLAPKAQIKE-NH2 Arg in D strand replaced by Lys	81.
41.	SNTSESFK(SNTSESF)FRVTQLAP-NH2	82.
44.	SNTSESFK(SNTSESF)FK($CH_3(CH_2)_6CO$)VTQLAPKAQIKE-NH2 Arg in D strand replaced by Lys	85.
45.	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_6CO$)AQIKE-NH2	86.
46.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_6CO$)E	87.
47.	SNTSESFK(sntsesf)FRVTQLAPKAQIKE D-amino acids in the branch	88.
48.	EKIQAKPALQTVRFK(FSESTNS)FSESTNS-NH2	89.
49.	ekiqakpalqtvrfk(fsestns)fsestns-NH_2 Retro inverso (All D-amino acid)	90.
50.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(PEG-20KD)E-NH2	91.
51.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(PEG-20KD)E-NH2	92.
52.	SNTSESFK(SNTSESF)FRVTQLAPK(PEG 20 KD)AQIKE-NH2	93.
53.	SNTSESFK(SNTSESF)FK(mini PEG)VTQLAPKAQIKE-NH2 Arg in D strand replaced by Lys	94.
54.	SNTSESFK(SNTSESF)FRVTQLAPK(PEG 10KD)AQIKE-NH2	95.
55.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(PEG20KD)E-NH2	96.

Comp. No.	Compound	Sequence ID
56.	SNTSESFK(SNTSESF)FK(CH3CO)VTQLAPKAQIKE-NH2	97.
57.	SNTSESFK(SNTSESF)FRVTQLAPK(CH3CO)AQIKE-NH2	98.
58.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(CH3CO)E-NH2	99.
59.	SNTSESFK(SNTSESF)FRVTQLAPK(CH3-(CH2)4-CO)AQIKE-NH2	100.
60.	Biotin-Ahx- sntsesfk(sntsesf)frvtqlapkaqike-NH2 All D Amino acids	101.
61.	Biotin-Ahx-EKIQAKPAYWNLVK(KDTQNS)DTQNS-NH2	102.
62.	SNTSESFK(SNTSESF)FRVTQLAPE*AQIK*E-NH2 Lactam bond formed between asterisked(*) amino acids	103.
63.	SNTSESFK(SNTSESF)FK*VTQE*APKAQIKE-NH2 Lactam bond formed between asterisked(*) amino acids	104.
64.	SNTSESFK(SNTSESF)FRVTE*LAPK*AQIKE Lactam bond formed between asterisked(*) amino acids	105.
65.	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_6CO$)E	106.
66.	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{14}CO$)E	107.
67.	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_{10}CO$)E at Lys	108.
68.	CH3CO-SNTSESFK(CH3CO-SNTSESF)FRVTQLAPKAQIK($CH_3(CH_2)_4CO$)E	109.
69.	SNWSEDLK(SNWSEDL)FQIIQLHPKAKIEE-NH2	110.
70.	EDK(ED)FRVTQLAPKAQIKE-NH2	111.
71.	SNTSESFK(ED)FRVTQLAPKAQIKE-NH2	112.
72.	LAPKAK(ED)FRVTQLAPKAQIKE-NH2	113.

Comp. No.	Compound	Sequence ID
73.	SNQTDKK(ED)FRVTQLAPKAQIKE-NH2	114.
75.	SNQTDKK(VLNWYRM)GIYLCGAISLAPKAQIKE-NH2	116.
76.	EDK(VLNWYRM)GIYLCGAISLAPKAQIKE-NH2	117.
77.	SNTSESFK(SNTSESF)GIYLCGAISLAPKAQIKE-NH2	118.
78.	SNTSESFK(SNTSESF)VLNWYRMLAPKAQIKE-NH2	119.
79.	VLNWYRMK(SNQTDK)GIYLCGAISLAPKAQIKE-NH2	120.
82.	SNWSEDLK(SNWSEDL)FQIIQLHPK($CH_3(CH_2)_{14}CO$)AKIEE-NH2	123.
83.	SNTSESFK(SNTSESF)FRVTQLAPK(MPA–NH–CH_2–CH_2–O–CH_2–CH_2–O–CO)AQIKE–NH2	124.
84.	SNTSESFK(sNTSESF)FRVTQLAPKAQIKE–NH2	125.
85.	sNTSESFK(SNTSESF)FRVTQLAPKAQIKE–NH2	126.
86.	sNTSESFK(sNTSESF)FRVTQLAPKAQIKE–NH2	127.
87.	SNTSESFK(SNTSESF)FRVTQLAPKAQIK(MPA–NH–CH_2–CH_2–O–CH_2–CH_2–O–CO)E–NH2	128.
88.	SΨ[CH2NH]NTSESFK(SΨ[CH2NH]NTSESF)FRVTQLAPKAQIKE–NH2	129.
89.	SnTSESFK(SNTSESF)FRVTQLAPKAQIKE–NH2 D–Asn at N–terminus	130.
90.	SNTSESFK(SnTSESF)FRVTQLAPKAQIKE–NH2 D–Asn in the branch	131.
91.	SNWSEDLK(SNWSEDL)FQIIQLHPK(MPA–NH–CH_2–CH_2–O–CH_2–CH_2–O–CO)AKIEE-NH2	132.
92.	SNTSESFK(SNTSESF)FRVTQLAPKAQIKE	133.
93.	SΨ[CH2NH]NTSESFK(SNTSESF)FRVTQLAPKAQIKE–NH2	134.
94.	SNTSESFK(SΨ[CH2NH]NTSESF)FRVTQLAPKAQIKE–NH2	135.

Comp. No.	Compound	Sequence ID
95.	SN Ψ[CH2NH]TSESFK(SNTSESF)FRVTQLAPKAQIKE-NH2	136.
96.	SNTSESFK(SN Ψ[CH2NH]TSESF)FRVTQLAPKAQIKE-NH2	137.
97.	SN Ψ[CH2NH]TSESFK(SN Ψ[CH2NH]TSESF)FRVTQLAPKAQIKE-NH2	138.
98.	SNTSESFK(SNTSESF)FRVTQLAPK($CH_3(CH_2)_{14}CO$)AQIKE	139.
99.	SNTSESF-Orn-(SNTSESF)KAQIKE-NH2	140.
100.	SNTSESF-Dap-(SNTSESF)KAQIKE-NH2	141.
101.	SNTSESF-Dab-(SNTSESF)KAQIKE-NH2	142.
102.	SNTSESFK(SNTSESF)FRVTQLAPK*IAQE*KE-NH2 Lactam bond formed between asterisked(*) amino acids	143.
103.	SNTSESFK(SNTSESF)K*RVTE*LAPKAQIKE-NH2 Lactam bond formed between asterisked(*) amino acids	144.
104.	SNTSESFK(SNTSESF)FRK*TQLE*PKAQIKE-NH2 Lactam bond formed between asterisked(*) amino acids	145.
105.	SNTSESFK(SNTSESF)FRVE*QLAK*PAQIKE-NH2 Lactam bond formed between asterisked(*) amino acids	146.
107.	LAPKA(SNQTDK)FRVTQLAPKAQIKE-NH2	148.

12. A compound according to claim 3 having formula (Ia)



(Ia)

or pharmaceutically acceptable salt thereof;

wherein;

A is an amino acid sequence of from three amino acids to the full length of a peptide sequence of human or murine PD1 ectodomain fragments selected from FG loop, BC loop, C' strand, C-C' loop, C strand, C' strand to C'-C'' loop;

B is an amino acid sequence of from three amino acids to the full length of apeptide sequence of human or murine PD1 ectodomain fragments selected from BC loop, FG loop, C-C' loop to C' strand, C strand, D strand, C' strand to C'-C'' loop or is absent;

Z is from one to three peptide sequences arranged in any order each being of from three amino acids up to the full length of a peptide sequence of human or murine PD1 ectodomain fragments selected from D strand, FG loop, G strand, C strand and F strand;

and X is lysine.

13. The compound according to claim 12, wherein A and B are independently an amino acid sequence of from three amino acids to the full length of said BC loop.
14. The compound according to claim 13, wherein A and B are independently the full length of said BC loop.
15. The compound according to claim 12, wherein Z is D strand-FG loop-G strand.
16. The compound according to claim 12, wherein R_1, R_2 and R_3 are absent.
17. The compound according to claim 12, wherein R_3 is C16-acyl.
18. The compound according to claim 12, wherein up to three amino acids are D-amino acids.
19. The compound according to claim 12, wherein all amino acids are D-amino acids.
20. The compound according to claim 12, comprising up to three reduced amide bonds (-CH_2NH-).
21. A compound of Formula I or a pharmaceutically acceptable salt thereof as claimed in claim 1, for use as a medicament.
22. A compound of Formula I or a pharmaceutically acceptable salt thereof as claimed in claim 1, for use as a medicament for the treatment of cancer or infectious disease.
23. A pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof as claimed in claim 1, and a pharmaceutically acceptable diluent or carrier.

24. Use of a compound according to any one of claims 1 to 20 in the manufacture of a medicament for modulating an immune response mediated by PD-1 signaling pathway in a subject.

25. The use of claim 24, wherein the medicament comprises a therapeutically effective amount of the compound.

26. Use of a compound according to any one of claims 1 to 20 in the manufacture of a medicament for inhibiting growth of tumour cells and/or metastasis in a subject.

27. The use of claim 26, wherein the medicament comprises a therapeutically effective amount of the compound.

28. The use of claim 26 or claim 27, wherein the tumour cells are of a cancer selected from the group consisting of melanoma, renal cancer, prostate cancer, breast cancer, colon cancer and lung cancer.

29. The use of claim 26 or claim 27, wherein the tumour cells are of a cancer selected from the list consisting of bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumours of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumour angiogenesis, spinal axis tumour, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

30. Use of a compound according to any one of claims 1 to 20 in the manufacture of a medicament for treating an infectious disease in a subject.

31. The use of claim 30, wherein the medicament comprises a therapeutically effective amount of the compound.
32. Use of a compound according to any one of claims 1 to 20 in the manufacture of a medicament for treating bacterial and viral infections in a subject.
33. The use of claim 32, wherein the medicament comprises a therapeutically effective amount of the compound.

ABSTRACT

"IMMUNOSUPPRESSION MODULATING COMPOUNDS"

The present invention provides immunosuppression compounds capable of inhibiting the programmed cell death 1 (PD1) signalling pathway. The present invention further provides peptide based compositions for treatment of cancer or treatment of infections via immunopotentiation caused by inhibition of immunosuppressive signaling induced by PD-1, PD-L1, or PD-L2 and therapies using them, immunopotentiative substrates included as the active ingredient. Further, the invention provides an application of the compositions containing the peptide moieties for preventive and/or therapeutic agents for cancer, cancer metastasis, immunodeficiency, an infectious disease or the like and an application of peptide moieties as a testing or diagnostic agent or a research agent for such a disease.

Figure 4