Journal of Advanced Bioinformatics Applications and Research
ISSN 0976-2604.Online ISSN 2278 – 6007
Vol 4, Issue3, 2013, pp80-92
http://www.bipublication.com

STUDY OF SOME CALCIUM CHANNEL INHIBITING SPIDER TOXINS THROUGH BIOINFORMATIC TOOLS

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[Received-12/10/2013, Accepted-18/12/2013]

ABSTRACT

In silico comparative study of sixteen selected calcium channel inhibiting spider toxins, were made through different bioinformatics tools. Amino acid composition is predicted through Expasy's Protparam. Secondary structure analysis were done through Expasy's GOR. Hydropathy was predicted through Expasy's Protscale. TMHMM 2.0 was used for predicting disulfide bonds. Signal peptide prediction was done by Signal P 4.1 server. Cysteine disulfide bond prediction was made by DiANNA 1.1 web server. Prdos software was used for predicting protein disordered region. GenomeNet was applied for motif prediction. Phylogenetic tree was constructed by Clustal W. POPI 2.0 was used for immunogenicity prediction of these spider toxins. Results elaborated a total predictive *in silico* picture of various properties of these sixteen spider toxins in a comparative fashion including its immunogenicity, disorder level and evolutionary and phylogenic relationships. Hence such comparative, *in silico* visualization of these spider toxins can be effective in future for *in vitro* research on these toxins and also properties of calcium channel proteins.

Keywords: Spider toxin, calcium channel blocker, bioinformatics.

INTRODUCTION

Spiders are the largest group of venomous invertebrate animals those belong to Phylum Arthropoda, Subphylum Chelicerata, and Class Arachnida, Order Araneae. They produce toxins which are a family of proteins functioning as neurotoxin. These toxins are complex mixture of neurotoxic peptidase and low molecular mass organic molecules. These toxins are important tools for discovering how ions channels operate in the body.

Some of these peptide toxins act on voltage sensitive calcium channel. These channels play important role in cardiac, muscular and neuronal functions. These toxins actually identify a common conserved pattern that is present on the channels. Some peptide spider toxins such as omega-atracotoxin from *Hydronyche* sp.[1,2] and toxins from *Hololena curta* [3,10] operate on insect voltage gated calcium channels, while some peptide toxins operate on mammalian voltage-gated calcium channels.

The toxins (produced by *H. curta*) in addition to its insecticidal activity, also has inhibitor activity (i.e. inhibitor of voltage sensitive calcium channels) and neuro-transmitter release in mammalian brain synaptosomes.Precursors of spider peptide toxins are composed of an N-terminal signal peptide, a highly variable length propeptide region, rich in acidic residues and the mature toxin sequence. Precursors of spider peptide toxin, undergo post-translational modification and release the mature peptide toxins. The mature peptide sequences are evolved through time within toxin superfamilies, but the cysteine sequences remain unchanged [4].

The ion channel spider peptide toxins, in addition to modified N- and C- termini, also have several disulfide bonds, which are adopting a structural motif, designated as Inhibitor Cysteine Knot (ICK).This ICK motif possess a constrained globular conformation to the molecule. [5] This motif consists of triple stranded anti-parallel β sheet with a cysteine knot. This motif represents following amino acid consensus sequences:CX_{3-7} C X_{3-6} CX_{0-5} CX_{1-4} CX_{4-13} C, Where X can be any amino acid[6].Some scientists later discovered that the cysteine knot possess four disulfide bridges.

Various types of voltage gated calcium channels are found, they are L-, N-, P-, Q-, R-, and T-[7]. They possess the same general structures.

The L-, N-, P-, Q-, R- calcium channels are activated at high voltage (HVA) while T- type calcium channel is activated at low voltage (LVA). L-type channel is blocked by a neurotoxin (calciseptine) which is found in black mamba [8]. N-type calcium channels are blocked by omega contoxins and P-/Q- type channels are specifically blocked by omega agatoxin. R- type calcium channels are blocked by SNX-482 (neurotoxin) which is found in the venom of Tarantula [9].

The focus of the present work is based on the *in silico* comparative study of 16 selected calcium channel inhibiting spider toxins. Here, we tried to predict secondary structure, amino acid composition, hydrophobicity, motif, cysteine disulfide, immunogenicity, transmembrane helices, signal peptide, protein family, protein disordered region and phylogenetic tree of 16 different selected calcium channel blocker spider toxins through different bioinformatics tools.

MATERIALS AND METHODS:

1. Sequence retrieval:

Sequence of 16 different calcium channel inhibiting spider toxins were retrived from Arachnoserver database (www. arachnoserver. org). Arachnosever is a data base which contains information on the sequence and biological activity of different spider protein toxins.

2. Amino acid composition prediction:

Amino acid composition and other physico-chemical parameters of the 16 spider toxins were analyzed through Protparam program of Expasy (http:// expasy.org/cgi-bin/protparam).Various physico-chemical parameters for a given toxin protein sequence were analyzed for example; theoretical pI, amino acid composition, extinction coefficient, estimated half life, instability index, aliphatic index and Grand Average of Hydropathicity (GRAVY).

3. **Secondary structure prediction**:

Secondary structure analysis of 16 spider toxins were carried out by using Expasy's GOR software (www.nspa_pbil.ibcp.fr/cgibin/npsa_automat.pI?page npsa_gor4.html).

4. **Hydropathy analysis prediction**:

Hydropathy analysis was carried out using Protscale (http://expasy.org/protscale).

5. **Trans-membrane helices prediction**:

Trans-membrane helices of the spider toxin protein sequences were predicted using TMHMM server version_2.0 (http://agro.vbi.vt.edu/cgi-bin/tmhmm). This is a server through which one can predict trans-membrane helices for a given protein sequence.

6. **Signal sequence prediction** :

Signal sequence of the toxins sequences was carried out by Signal P 4.1 server -CBS.This database (www.cbs.dtu.dk/ services/signalP) helps us to find signal sequences for a given protein sequence.

7. **Cysteine disulfide Bond prediction**:Positions of the cysteine disulphide bond

prediction were made through DiANNA 1.1 web server (www. clarius.bcedu / ~cloetelab /DiANNA). This software helps in disulfide connectivity prediction.

8. **Motif Prediction**:

Motif prediction was made through the use of GenomeNet motif (www.genomejp/toolsmotif).

9. **Protein family prediction**:

This was done through Pfam software (www.pfam.sanger.ac.uk).

10. **Phylogenetic tree prediction**:

This was done using CLUSTAL W multiple alignment tools (www.genome.jp / tools/clustalW/). This software indicates evolutionary relationship of these 16 selected proteins.

11. **Protein disordered region prediction**:

This was carried out by Prdos (www. prdos.hgc.jp) server through which protein disordered region can be detected.

12. **Immunogenecity of MHC Class-1 and Class-2 predictio**n:

This was carried out through POPI 2.0 server (www. mba.biocuckoo.org).One can compute T-cell immunogenicity through this software.

13. B-cell epitope prediction:

The B-cell epitope prediction was made through the software "Antibody epitope prediction-IEAD Analysis resource"(www.tools. immune epitope.org / main/html/ b.cell_tools.html)

DISCUSSION: In this study, Table.1 represents brief description about 16 spider toxins such as names, sources, peptide sequences and sequence length. Amino acid composition of sixteen spider toxins was predicted and was represented in Table. 2. Among 16 selected toxins some toxins (eg. Actinopoditoxin) are rich in glutamine, aspargine and cysteine, but less in histidine, leucine, lysine, methionine and phenylalanine. Some toxins (eg. Agatoxin) are rich in only glutamine but has less glutamine and isoleucine. Ctenotoxin Cs1a and Pn1a are rich in cysteine and lysine, but are less in tryptophan, valine, tyrosine and histidine respectively. Ctenotoxin Pr1a is rich in cysteine,glycine,lysine, but less in glutamine, histidine, serine, tryptophan. Filistatoxin is rich in arginine and cysteine but scarce in phenylalanine, threonine and aspargine. Hexatoxin is rich in glycine and alanine but less in methionine. Plectoxin is rich in aspargine, cysteine and threonine but less amount of glutamine, glutamic acid, isoleucine, leucine, metionine and phenylalanine. Lycotoxin is rich in cysteine, glutamine and serine but less low level of histidine. Oxotoxin is rich in aspergine, cysteine and lysine but less in methionine and phenylalanine. Segestritoxin is rich in arginine, cysteine, lysine and serine but has less amount of phenylalanine, alanine, tryptophan. Sparatoxin is rich in aspargine and cysteine but less in leucine,alanine,arginine,histdine and leucine. Therapotoxin Asp1a, Bs1a, Hg1a and K-therapotoxin are rich in cysteine, glycine and lysine but these have less percentage of alanine and glutamine. Table-3 shows the predicted physio-chemical properties of these sixteen proteins which represents aliphatic index, instability index and extinction co-efficient. The aliphatic index of a protein is a measure of the relative volume occupied by aliphatic side chain of amino acid [11]. Higher aliphatic index, increases the thermostability of globular proteins (such as in actinopoditoxin, agatoxin, ctenotoxin cs1a, Pn1a, Pr1a,hexatoxin, lycotoxin, oxotoxin, segestritoxin, sparatoxin, filistatoxin, therapotoxin –Asp1a, Bs1a, Hg1a, K-therapotoxin). The low aliphatic index indicates that this protein is not stable for a wide range temperature (eg. plectotoxin). Instability index determines the stability of protein in a test tube [12]. If the index is < 40, then it is probably stable in a test tube (eg. Ctenotoxin- Pr1a, hexatoxin, sparatoxin, plectotoxin, therapotoxin-Asp1a, Bs1a, Hg1a, K-therapotoxin).But actinopoditoxin, agatoxin, filistatoxin, lycotoxin, segestritoxin, ctenitoxin Cs1a, Pn1a, oxotoxin are not stable in a test tube because they have the instability index > 40. Extinction co-efficient of protein is a measurement of how strongly a protein absorbs light at a given wavelength. Among the sixteen toxins, the least extinction co-efficient is of ctenitoxin Cs1a

(500 M^{-1} cm^{-1}), whereas the maximum extinction co-efficient is of Agatoxin (31970 M^{-1} cm^{-1}).

The predicted secondary structure is composed of α-helix, 3_{10} helix, pI helix, extended strand, β-turn, Bend region and random coil. The Table. 4, represents the comparative analysis of secondary structure of sixteen selected calcium channel blocker proteins. From this table, it is clear that random coil is predominantly and frequently present in all 16 toxins, whereas extended strand was found to be less frequent. Out of 16 toxins, only six toxins (agatoxin, ctenitoxin Cs1a, Pn1a, hexatoxin, lycotoxin and oxotoxin) have α-helix, but they are found to be less frequent. Hydrophobicity were predicted for sixteen calcium channel blocker spider toxins characterize its hydrophobic character that may be useful in predicting membrane spanning domains, potential antigenic sites and regions that are likely to expose on protein surface. Kyte-Doolite is widely applied scale for determining hydrophobic character of a protein. If the region is greater than zero, then the region is hydrophobic in character. Table. 5, represents the comparative analysis of predicted hydrophobicity of sixteen selected calcium channel toxins including GRAVY. As the GRAVY value is too low, it was clear that these sixteen toxins were not hydrophobic in nature. The Table.5 shows better interaction of these 16 toxins with water. Folding determinants of a membrane protein, can be better understood by trans-membrane helix [13]. Table. 6 represents that sixteen selected calcium channel blocker toxins have no trans-membrane helix. Thus predicted to be all are soluble proteins.

According to signal hypothesis, proposed by Blobel and Sabatini, proteins synthesized on membrane bound polyribosomes contain a peptide extension i.e. signal peptide at their amino terminals that mediate their attachment to the membranes of the ER. Table. 7, represents the comparative analysis of signal peptide of sixteen selected calcium channels blocker toxins. Ctenitoxin Cs1a and Pn1a both have signal peptides that are present in between 17; 18 and 21; 22 cleavage sites in its sequence. Lycotoxin and oxotoxin both have signal peptides that are present in between 22; 23 and17; 18 cleavage sites in its sequence. Ctenitoxin Cs1a, Ctenitoxin Pn1a, Lycotoxin and oxotoxin have signal peptide in their sequences and their protein synthesis is occurred on membrane bound polyribosomes. Actinopoditoxin, Agatoxin, Hexatoxin, Plectotoxin, Segestritoxin, Sparatoxin, Therapotoxin Asp1a, Bs1a, Hg1a, K-therapotoxin ,Filistatoxin, Ctenitoxin Pr1a do not possess signal peptide in their sequences, so their protein synthesis is occurred on free polyribosomes.

The disulfide bond joins two segments of the protein chain. The disulfide bond increases the effective local concentration of protein residues and lowers the effective local concentration of water molecule. As the water molecule attack amide-amide hydrogen bonds and break up secondary structure, so the disulfide bond by excluding the water molecule, stabilize the secondary structure in its vicinity [14]. The maximum score represents the maximum reliability prediction. Table-8, shows the comparative analysis of cysteine disulfide bond prediction of sixteen calcium channel blocker toxins. Result shows Agatoxin has 11 cysteines that form 4 disulfide bonds between cysteine 46 and 53; 53and 64; 55 and 78; 64 and 112. Actinopoditoxin has 6 cysteines that form 1 disulfide bond between cysteine 18 and 19; Ctenitoxin Cs1a and Ctenitoxin Pn1a both have 8 cysteines that form 7 disulfide bonds; Ctenitoxin Pr1a has 8 cysteines that form 1disulfide bond. Filistatoxin has 12 cysteines that form 6 disulfide bonds; Hexatoxin has 6 cysteines that form 3 disulfide bonds. Lycotoxin has 9 cysteines that form 5 disulfide bonds; Oxotoxin and plectotoxin have 10 cysteines that form 5 and 6 disulfide bonds respectively; Segestritoxin has 8 cysteines that form 4 disulfide bonds; Sparatoxin, therapotoxin Asp1a and Hg1a have 6 cysteines that form 4 disulfide bonds; therapotoxin Bs1a and k-therapotoxin have 6 cysteines that form 2 and 1 disulfide bonds; [output of DiANNA server].

After the peptide chain of a protein has been organized into successive stretches of secondary structural elements such as α-helices, β-pleated strand, reverse turns and various other loops, the

combination of such structural elements are arranged first into distinctive groups, called motifs. Table. 9, shows the different types of motifs found in these sixteen toxins. Actinopoditoxin and hexatoxin both have motifs in 4.11 and 52.59 position respectively of its peptide sequence, termed as OMEGA_ACTX_1. Ctenitoxin Cs1a has a motif in 2.28 position of its peptide sequence, termed as SPIDER_CSTX. Plectotoxin has a motif in 23.25 position of its peptide sequence, termed as EGF_2. Therapotoxin Asp1a and Therapotoxin Bs1a both have a motif in 4.36 position of their peptide sequence, termed as HWTX_2.

Every protein belongs to a specific protein family. Table-10 represents the name of protein families in which the calcium channel blocker toxins belong. Many of the toxins in general belonged to certain group of toxin family, for eg. Ctenitoxin Pr1a and Pn1a belonged to Toxin 9 family. Sparatoxin, therapotoxin Hg1a and K-therapotoxin belonged to Toxin 12 family. Actinopoditoxin and hexatoxin belonged to ω toxin family. Therapotoxin Asp1a and Ba1a belonged to Toxin 20 family. Ctenitoxin Cs1a and lycotoxin belonged to toxin 35 family. Agatoxin belonged to Toxin 34 family. The toxin family of filistatoxin, oxotoxin, plectoxin and segestritoxin were not found. The phylogenetic analysis is carried out using CLUSTAL W. Table. 11, shows the evolutionary relationships between sixteen calcium channel blocker toxins. The relationship established by phylogenetic trees, described species evolutionary history [15, 16]. Lycotoxin (seqH) and therapotoxin Hg1a (seqO) are sister toxins as are sparatoxin (seqL) and therapotoxin Ec2c (seqP). Therapotoxin Bs1a (seqN) is the sister toxin to the clade sparatoxin (seqL) – therapotoxin

Ec2c (seqP). That means lycotoxin (seqH) and therapotoxin Hg1a (seqO) are sister toxins of sparatoxin (seqL) – therapotoxin Bs1a (seqN). Sister toxins of course share recent common ancestors at the node that join them together. Actinopoditoxin (seqA) ,plectoxin (seqJ) and segestritoxin (seqK) are distantly related to each other. Again plectoxin (seqJ) and segestritoxin (seqK) are distantly related to the sparatoxin (seqL) – therapotoxin Hg1a (seqO). Hexatoxin (seqG) and lycotoxin (seqH) are sister toxins as are ctenitoxin Pr1a (seqE) and oxotoxin (seqI). Agatoxin (seqB) is the sister toxin to the clade hexatoxin (seqG) – lycotoxin (seqH). Filistatoxin(seqF) is the sister toxin to the clade Ctenitoxin Pr1a (seqE) – 0xotoxin (seqI). Ctenitoxin Cs1a (seqC) and Pn1a (seqD) are the sister toxins to each other, but they are distantly related to atinopoditoxin (seqA), plectoxin (seqJ), segestritoxin (seqK), sparatoxin(seqL), therapotoxin Ec2c (seqP), therapotoxin Bs1a (seqN), therapotoxin Hg1a (seqO).

Table. 12, shows the comparative analysis of predicted protein disordered regions of sixteen calcium channel blocker toxins. The prediction result consists of 2 parts i.e. disordered and ordered. The double underlined residues are predicted to be disordered and black residues are predicted to be ordered. The predicted disordered regions are useful for annotation of proteins. The disordered regions are reportedly involved in many biological processes, such as cell cycle regulation, cell signaling, cell cycle control, molecular recognition of proteins/DNA/RNA and molecular threading.

Disordered proteins undergo transition to more ordered states upon binding to their target. Disordered proteins also help in modulating affinity of protein binding with their receptors regulated by post-translational modification [17]. The % of amino acid disorder is high in Actinopoditoxin (43.58%), Hexatoxin (40%) and Segestritoxin (42.85%) that means they have good binding affinity with their receptors. The % of amino acid disorder is low in Therapotoxin Bs1a (7.69%) that means this toxin has very poor affinity with their receptor. The rest toxins have moderate affinity with their receptors.

Major histocompatability complex (MHC) refers to a cluster of genes responsible for immune responses, transplantation antigens and protein of complement system. MHC Class-I molecules are found on nucleated cells specially lymphocytes and platelets. Theses antigens are responsible for graft rejection.MHC Class-II molecules are present on the

surface of B-cells, macrophages, monocytes, antigen presenting cells and Activated T-cells. These antigens are associated with the regulation of immune responses [18, 19]. The cytotoxic T-cells destroy cancer cell, viruses and bacteria through cytotoxic substances. The helper T-cells release lymphokines that increase the response of B-cells. The B-cells are activated to produce antibodies. These antibodies kill the viruses and bacteria. Table. 13, shows the comparative analysis of immunogenicity of MHC Class-I and II prediction of sixteen toxins. In case of Actinopoditoxin, Agatoxin, Ctenotoxin Cs1a, Pn1a, Pr1a, Filistatoxin, Lycotoxin, Oxotoxin, Plectotoxin, Segestritoxin, Therapotoxin Asp1a, Bs1a and K-therapotoxin, predicted immunogenicity of CTL response is moderate. In case of Agatoxin, Ctenotoxin Cs1a, Pn1a, Pr1a, Filistatoxin, Lycotoxin, Oxotoxin, Therapotoxin Asp1a, Bs1a, predicted immunogenicity of HTL response is little. In case of Hexatoxin and Therapotoxin Hg1a, predicted immunogenicity of CTL and HTL response are not found.

Epitope means a portion of the antigen, which can bind with the antigen binding site (paratope) of the antibody. They are related to humoral immune responses. Table. 14, shows comparative analysis of B-cells epitope prediction of sixteen toxins. As the number of epitope peptide length increases, the protein would be more effective in humoral immune responses. Actinopoditoxin has 30 epitope peptide lengths, Hexatoxin has 28 epitope peptide length and Lycoyoxin has 22 epitope peptide length, whereas Segestritoxi and sparatoxin both have only 1 epitope peptide length. Oxotoxin (4), Therapotoxin Bs1a (8) and K-Therapotoxin (3) also have lower number of epitope peptide length.

CONCLUSION:

Thus this study could portray and predict a detailed comparative analysis of 16 different calcium channel blocker spider toxins in light of *in silico* protein analysis. Thus this can further open up avenues for *in vitro* research of these spider toxins in future.

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RESULTS:

Table: 1. Brief description about 16 selected spider toxins.

Sl. no.	Toxin name	Sources of spider species	Toxin peptide sequences	Sequence length
1	ω-actinopoditoxin Mb1a (seqA)	*Missulena bradleyi*	SPVCTPSGQPCQPNTQPCCNNAEEEQTINCNGNTVYRCA	39
2	ω-agatoxin Aa1a(seqB)	*Agelenopsis aperta*	MMKFVVFLACLFVAAHSFAVEGEEEYFEAEVPELERAKALPPGSV CDGNESDCKCYGKWHKCRCPWKWHFTGEGPCTCEKGMKHTCIT KLHCPNKAEWGLDWRSEESERSPC	112
3	ω-ctenitoxin Cs1a (seqC)	*Cupiennius salei*	SCIPKHEECTNDKHNCCRKGLFKLKCQCSTFDDESGQPTERCA CGRPMGHQAIETGLNIFRGLFKGKKKNKKTK	74
4	ω-ctenitoxin Pn1a (seqD)	*Phoneutria nigriverter*	MWLKIQVFLLAITLITLGIQAEPNSSPNNPLIEEEARACAGLY KKCGKGASPCCEDRPCKCDLAMGNCICKKKFIEFFGGGK	82
5	ω-ctenitoxin Pr1a (seqE)	*Phoneutria reidyi*	ACAGLYKKCGKGVNTCCENRPCKCDLAMGNCICKKKFVEFFGG	43
6	ω-filistatoxin Kh1a (seqF)	*Kukulcania hibernalis*	AECLMIGDTSCVPRLGRRCCYGAWCYCDQQLSCRRVGRKRECGW VEVNCKCGWSWSQRIDDWRADYSCKCPEDQ	74
7	ω-hexatoxin Ar1a (seqG)	*Atrax robustus*	MNTATGFIVLLVLATVLGAIEAEDAVPDFEGGFASHAREDTVGGK IRRSSVCIPSGQPCPYNEHCCSGSCTYKENENGNTVQRCD	85
8	ω-lycotoxin Gsp261 a (seqH)	*Geolycosa sp.*	MKLSIFFVLFFIAIAYCQPEFLDDEEDEVEETLPVAEEGREKSCI TWRNSCMHNDKGCCFPWSCVCWSQTVSRNSSRKEKKCQCRLW	87
9	ω-oxotoxin OI1a (seqI)	*Oxyopes lineatus*	DWECLPLHSSCDNDCVCCKNHHCHCPYSNVSKLEKWLPEWAKIP D ALKRCSCQRNDKDGKINTCDKYKN	69
10	ω-plectoxin Pt1a (seqJ)	*Plectreurys tristis*	ADCSATGDTCDHTKKCCDDCYTCRCGTPWGANCRCDYYKARCD T	43
11	ω-segestritoxin Sf1a (seqK)	*Segestria florentina*	GSCIESGKSCTHSRSMKNGLCCPKSRCNCRQIQHR HDYLGKRKYSCRCS	49
12	ω-sparatoxin Hv1a (seqL)	*Heteropoda venatoria*	DDDCGWIMDDCTSDSDCCPNWVCSKTGFV KNICKYEM	37
13	ω-therapotoxin Asp1a (seqM)	*Aphonopelma*	LFECVLSCDIKKNGKPCKPKGEKKCSGGWRCKINFCLKV	39
14	ω-therapotoxin Bs1a (seqN)	*Brachypelma smithi*	IFECVFSCDIEKEGKPCKPKGEKKCSGGWKCKIKLCLKI	39
15	ω-therapotoxin Hg1a (seqO)	*Hysterocrates gigas*	GVDKAGCRYMFGGCSVNDDCCPRLGCHSLFSYCAWDLTFSD	41
16	k-therapotoxin Ec2c (seqP)	*Eucratoscelus constrictus*	YCQFKMWTCDSERKCCEDMVCRLWCKLNL	29

Table: 2. Amino acid composition prediction of 16 spider toxins.

Amino acid	Amino acid (%)															
	Ω-Actinopoditoxin	ω-Agatoxin	ω-Ctenitoxin Cs1a	ω-Ctenitoxin Pn1a	ω-Ctenitoxin Pr1a	ω-Filistatoxin	ω-Hexatoxin	ω-Lycotoxin	ω-Oxotoxin	ω-Plectoxin	ω-Sgestritoxin	ω-Sparatoxin	ω-Therapotoxin Asp1a	ω-therapotoxin Bs1a	ω-therapotoxin Hg1a	K-therapotoxin Ec2c
Ala	5.1	7.1	2.7	8.5	7.0	4.1	8.2	3.4	2.9	9.1	0.0	0.0	0.0	0.0	4.9	0.0
Arg	2.6	3.6	5.4	2.4	2.3	12.2	4.7	5.7	2.9	6.8	12.2	0.0	2.6	0.0	4.9	6.9
Asn	15.4	1.8	5.4	4.9	7.0	1.4	5.9	3.4	8.7	2.3	4.1	5.4	5.1	0.0	2.4	3.4
Asp	0.0	2.7	4.1	2.4	2.3	8.1	4.7	4.6	10.1	15.9	2.0	18.9	2.6	2.6	12.2	6.9
Cys	15.4	9.8	10.8	9.8	18.6	16.2	7.1	10.3	14.5	22.7	16.3	16.2	15.4	15.4	14.6	20.7
Gln	10.3	0.0	4.1	2.4	0.0	5.4	2.4	3.4	1.4	0.0	4.1	0.0	0.0	0.0	0.0	3.4
Glu	7.7	13.4	6.8	7.3	4.7	5.4	8.2	11.5	4.3	0.0	2.0	2.7	5.1	10.3	0.0	6.9
Gly	5.1	7.1	9.5	9.8	14.0	8.1	10.6	2.3	1.4	6.8	8.2	5.4	10.3	10.3	12.2	0.0
His	0.0	4.5	4.1	0.0	0.0	0.0	2.4	1.1	5.8	2.3	6.1	0.0	0.0	0.0	2.4	0.0
Ile	2.6	0.9	4.1	8.5	2.3	2.7	4.7	4.6	2.9	0.0	4.1	5.4	5.1	10.3	0.0	0.0
Leu	0.0	5.4	5.4	9.8	4.7	4.1	4.7	5.7	7.2	0.0	4.1	0.0	7.7	5.1	7.3	10.3
Lys	0.0	8.9	16.2	11.0	16.3	4.1	2.4	6.9	13.0	6.8	10.2	8.1	23.1	25.6	2.4	10.3
Met	0.0	2.7	1.4	2.4	2.3	1.4	1.2	2.3	0.0	0.0	2.0	5.4	0.0	0.0	2.4	6.9
Phe	0.0	5.4	5.4	4.9	7.0	0.0	3.5	6.9	0.0	0.0	0.0	2.7	5.1	5.1	7.3	3.4
Pro	12.8	6.2	4.1	6.1	2.3	2.7	4.7	3.4	5.8	2.3	2.0	2.7	5.1	5.1	2.4	0.0
Ser	5.1	5.4	4.1	3.7	0.0	6.8	7.1	9.2	7.2	2.3	16.3	8.1	5.1	5.1	9.8	3.4
Thr	10.3	3.6	6.8	2.4	2.3	1.4	7.1	3.4	1.4	13.6	2.0	5.4	0.0	0.0	2.4	3.4
Trp	0.0	4.5	0.0	1.2	0.0	6.8	o.o	4.6	4.3	2.3	0.0	5.4	2.6	2.6	2.4	6.9
Tyr	2.6	1.8	0.0	1.2	2.3	4.1	2.4	1.1	2.9	6.8	4.1	2.7	0.0	0.0	4.9	3.4
Val	5.1	5.4	0.0	1.2	4.7	5.4	8.2	5.7	2.9	0.0	0.0	5.4	5.1	2.6	4.9	3.4

Table: 3. Physico-chemical parameters prediction of 16 spider toxins.

Sl. no.	Toxin name	Extinction coefficient ($M^{-1}cm^{-1}$)	Instability Index	Aliphatic Index
1	ω-actinopoditoxin Mb1a	1490	50.59	30.00
2	ω-agatoxin Aa1a	30480	53.79	47.05
3	ω-ctenitoxin Cs1a	500	47.87	39.59
4	ω-ctenitoxin Pn1a	6990	48.22	83.41
5	ω-ctenitoxin Pr1a	1490	21.49	47.67
6	ω-filistatoxin Kh1a	31970	68.55	46.08
7	ω-hexatoxin Ar1a	2980	39.25	68.82
8	ω-lycotoxin Gsp261a	23490	67.17	60.46
9	ω-oxotoxin OI1a	19480	42.86	50.87
10	ω-plectoxin Pt1a	9970	8.23	9.09
11	ω-segestritoxin Sf1a	2880	77.46	31.84
12	ω-sparatoxin Hv1a	12490	33.95	36.76
13	ω-therapotoxin Asp1a	5500	15.14	64.87
14	ω-therapotoxin Bs1a	5500	31.94	67.44
15	ω-therapotoxin Hg1a	8480	29.73	47.56
16	k-therapotoxin Ec2c	12865	37.13	50.34

Table: 4. Secondary structure prediction of 16 spider toxins.

Sl. no.	Toxin name	α-helix (%)	3_{10} Helix (%)	pI helix (%)	Beta Bridge (%)	Extended strand (%)	Beta turn (%)	Bend region (%)	Randam coil (%)	Ambigous state (%)	Other states (%)
1	ω-actinopoditoxin Mb1a	0.00	0.00	0.00	0.00	20.15	0.00	0.00	79.49	0.00	0.00
2	ω-Agatoxin-Aa1a	18.75	0.00	0.00	0.00	29.46	0.00	0.00	51.79	0.00	0.00
3	ω-ctenitoxin Cs1a	18.72	0.00	0.00	0.00	12.16	0.00	0.00	68.92	0.00	0.00
4	ω-ctenitoxin Pn1a	24.39	0.00	0.00	0.00	18.29	0.00	0.00	57.32	0.00	0.00
5	ω-ctenitoxin Pr1a	0.00	0.00	0.00	0.00	18.16	0.00	0.00	81.40	0.00	0.00
6	ω-filistatoxin Kh1a	0.00	0.00	0.00	0.00	33.78	0.00	0.00	66.22	0.00	0.00

7	ω-hexatoxin Ar1a	14.46	0.00	0.00	0.00	22.89	0.00	0.00	66.22	0.00	0.00
8	ω-lycotoxin Gsp261a	13.79	0.00	0.00	0.00	39.08	0.00	0.00	47.13	0.00	0.00
9	ω-oxotoxin 0I1a	5.80	0.00	0.00	0.00	8.70	0.00	0.00	85.51	0.00	0.00
10	ω-plectoxin Pt1a	0.00	0.00	0.00	0.00	20.45	0.00	0.00	79.55	0.00	0.00
11	ω-segestritoxin Sf1a	0.00	0.00	0.00	0.00	37.44	0.00	0.00	63.27	0.00	0.00
12	ω-sparatoxin Hv1a	0.00	0.00	0.00	0.00	37.44	0.00	0.00	62.16	0.00	0.00
13	ω-therapotoxin Asp1a	0.00	0.00	0.00	0.00	15.38	0.00	0.00	84.62	0.00	0.00
14	ω-therapotoxin Bs1a	0.00	0.00	0.00	0.00	17.95	0.00	0.00	82.05	0.00	0.00
15	ω-therapotoxin Hg1a	0.00	0.00	0.00	0.00	36.59	0.00	0.00	63.41	0.00	0.00
16	k-therapotoxin Ec2c	0.00	0.00	0.00	0.00	31.03	0.00	0.00	68.79	0.00	0.00

Table: 5. Hydrophobicity and Hydropathicity prediction of 16 Spider toxins.

Sl. no.	Toxin name	Maximum Value	Minimum Value	Grand average Hydropathicity (GRAVY)
1	ω-actinopoditoxin Mb1a	0.067	-1.933	-0.846
2	ω-agatoxin Aa1a	3.344	-2.833	-0.493
3	ω-ctenitoxin Cs1a	1.033	-3.111	-1.014
4	ω-ctenitoxin Pn1a	3.167	-2.267	0.041
5	ω-ctenitoxin Pr1a	1.067	-1.444	-0.060
6	ω-filistatoxin Kh1a	1.278	-2.122	-0.661
7	ω-hexatoxin Ar1a	3.289	-2.644	-0.192
8	ω-lycotoxin Gsp261a	3.333	-3.256	-0.363
9	ω-oxotoxin OI1a	0.322	-2.689	-0.988
10	ω-plectoxin Pt1a	Nil	-1.922	-0.836
11	ω-segestritoxin Sf1a	0.056	-2.522	-1.051
12	ω-sparatoxin Hv1a	0.778	-1.322	-0.459
13	ω-therapotoxin Asp1a	1.389	-2.244	-0.385
14	ω-therapotoxin Bs1a	1.278	-2.244	-0.344
15	ω-therapotoxin Hg1a	0.911	-0.911	0.015
16	k-therapotoxin Ec2c	1.044	-2.022	-0.314

Table: 6. Trans-membrane helices prediction of 16 spider toxins.

Sl. no.	Toxin name	Sequence length	No. of Trans-membrane helices
1	ω-actinopoditoxin Mb1a	39	No
2	ω-agatoxin Aa1a	112	No
3	ω-ctenitoxin Cs1a	74	No
4	ω-ctenitoxin Pn1a	82	No
5	ω-ctenitoxin Pr1a	43	No
6	ω-filistatoxin Kh1a	74	No
7	ω-hexatoxin Ar1a	85	No
8	ω-lycotoxin Gsp261a	87	No
9	ω-oxotoxin OI1a	69	No
10	ω-plectoxin Pt1a	43	No
11	ω-segestritoxin Sf1a	49	No
12	ω-sparatoxin Hv1a	37	No
13	ω-therapotoxin Asp1a	39	No
14	ω-therapotoxin Bs1a	39	No
15	ω-therapotoxin Hg1a	41	No
16	k-therapotoxin Ec2c	29	No

Table: 7. Signal Peptide prediction of 16 spider toxins.

Sl. no.	Toxin name	Presence of Signal Peptide Sequence	Cleavage site in sequences
1	ω-actinopoditoxin Mb1a	No	----
2	ω-agatoxin Aa1a	No	----
3	ω-ctenitoxin Cs1a	Yes	Between 17 and 18
4	ω-ctenitoxin Pn1a	Yes	Between 21 and 22
5	ω-ctenitoxin Pr1a	No	----
6	ω-filistatoxin Kh1a	No	----
7	ω-hexatoxin Ar1a	No	----
8	ω-lycotoxin Gsp261a	Yes	Between 22 and 23
9	ω-oxotoxin OI1a	Yes	Between 17 and 18
10	ω-plectoxin Pt1a	No	----
11	ω-segestritoxin Sf1a	No	----
12	ω-sparatoxin Hv1a	No	----
13	ω-therapotoxin Asp1a	No	----
14	ω-therapotoxin Bs1a	No	----
15	ω-therapotoxin Hg1a	No	----
16	k-therapotoxin Ec2c	No	----

Table: 8. Cysteine- disulfide bond prediction of 16 spider toxins.

Sl. no.	Toxin name	Sequence length	No. of cysteine in sequence	Cysteine sequence position	Presence of disulfide bond	Scores
1	ω-actinopoditoxin Mb1a	39	6	18-19	1	0.82662
2	ω-agatoxin Aa1a	112	11	46-53,53-64,55-78,64-112	4	0.99894,0.54324,0.80664,0.99984
3	ω-ctenitoxin Cs1a	74	8	9-42,16-42,17-26,17-28,17-42,17-44,28-42	7	0.99692,0.68317,0.99728,0.99745,0.99725,0.95575,0.94318
4	ω-ctenitoxin Pn1a	82	8	46-70	1	0.08146
5	ω-ctenitoxin Pr1a	43	8	2-22,9-24	2	0.99642,0.99734
6	ω-filistatoxin Kh1a	74	12	3-19,20-25,20-33,27-70,49-68,49-70	6	0.8273,0.9980,0.9980,0.98777,0.96216,0.99883
7	ω-hexatoxin Ar1a	85	6	52-66,59-70,65-84	3	0.95838,0.99959,0.98172
8	ω-lycotoxin Gsp261a	87	9	17-58,51-58,51-59,51-84,59-82	5	0.89337,0.99978,0.9399,0.99572,0.91003
9	ω-oxotoxin OI1a	69	10	17-25,17-64,25-64,50-64,52-64	5	0.9969,0.99518,0.95999,0.9967,0.63462
10	ω-plectoxin Pt1a	43	10	3-10,3-17,10-23,10-33,20-33,25-33	6	0.99604,0.93291,0.99318,0.99535,0.77872,0.90908
11	ω-segestritoxin Sf1a	49	8	3-22,3-29,10-22,21-22	4	0.90324,0.97269,0.9873,0.99967
12	ω-sparatoxin Hv1a	37	6	11-23,18-23,23-33	3	0.56643,0.98823,0.99675
13	ω-therapotoxin Asp1a	39	6	4-8,4-25,4-31,8-31	4	0.95783,0.95775,0.96993,0.999038
14	ω-therapotoxin Bs1a	39	6	4-8,4-31	2	0.88885,0.77263
15	ω-therapotoxin Hg1a	41	6	7-20,7-33,14-20,14-26	4	0.99978,0.5029,0.99976,0.99753
16	k-therapotoxin Ec2c	29	6	9-15	1	0.01163

Table : 9. Motif prediction of 16 spider toxins.

Sl. no.	Toxin name	Sequence length	Position	Found Motif	Name
1	ω-actinopoditoxin Mb1a	39	4.11	CTPSGQPC	OMEGA_ACTX_1
2	ω-agatoxin Aa1a	112	nil	Nil	Nil
3	ω-ctenitoxin Cs1a	74	2.28	CIPKHEECTNDKWNCCRKGLFKLKCQC	SPIDER_CSTX
4	ω-ctenitoxin Pn1a	82	nil	Nil	Nil
5	ω-ctenitoxin Pr1a	43	nil	Nil	Nil
6	ω-filistatoxin Kh1a	74	Nil	Nil	Nil
7	ω-hexatoxin Ar1a	85	52.59	CIPSGQPC	OMEGA_ACTX_1

8	ω-lycotoxin Gsp261a	87	Nil	Nil	Nil
9	ω-oxotoxin OI1a	69	Nil	Nil	Nil
10	ω-plectoxin Pt1a	43	23.35	CRCGTPWGAANCRC	EGF_2
11	ω-segestritoxin Sf1a	49	Nil	Nil	Nil
12	ω-sparatoxin Hv1a	37	Nil	Nil	Nil
13	ω-therapotoxin Asp1a	39	4.36	CVLSCDIKKNGKPCKPKGEKKCSGGWRC KINFC	HWTX_2
14	ω-therapotoxin Bs1a	39	4.36	CVFSCDIEKEGKPCKPKGEKKCSGGWKC KIKLC	HWTX_2
15	ω-therapotoxin Hg1a	41	Nil	Nil	Nil
16	k-therapotoxin Ec2c	29	Nil	Nil	Nil

Table: 10. Protein family prediction of 16 spider toxins.

Sl. no.	Toxin name	Protein Family
1	ω-actinopoditoxin Mb1a	ω Toxin
2	ω-agatoxin Aa1a	Toxin 34
3	ω-ctenitoxin Cs1a	Toxin 35
4	ω-ctenitoxin Pn1a	Toxin 9
5	ω-ctenitoxin Pr1a	Toxin 9
6	ω-filistatoxin Kh1a	None Found
7	ω-hexatoxin Ar1a	ω Toxin
8	ω-lycotoxin Gsp261a	Toxin 35
9	ω-oxotoxin OI1a	None Found
10	ω-plectoxin Pt1a	None Found
11	ω-segestritoxin Sf1a	None Found
12	ω-sparatoxin Hv1a	Toxin 12
13	ω-therapotoxin Asp1a	Toxin 20
14	ω-therapotoxin Bs1a	Toxin 20
15	ω-therapotoxin Hg1a	Toxin 12
16	k-therapotoxin Ec2c	Toxin 12

Table:11. Phylogenetic tree prediction of spider toxins:

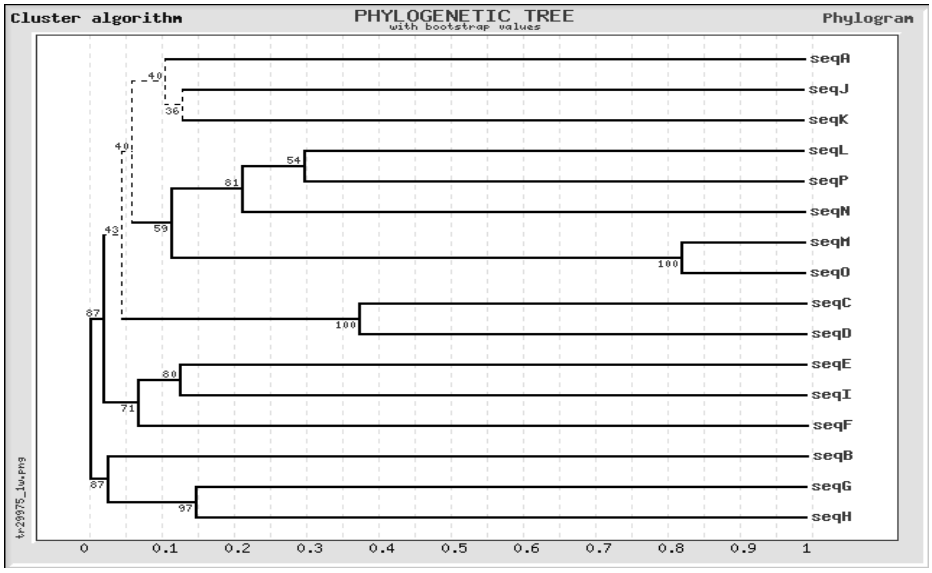

Table: 12. Protein disordered regions prediction of 16 spider toxins.

Sl. no.	Toxin name	Protein disordered sequences (double-underlined portions)	% of amino acid in disordered state
1	ω-actinopoditon Mb1a	SPVCTPSGQPCQPNTQPCCNNAEEEQTINCNGNTVYRCA	43.58
2	ω-agatoxin Aa1a	MMKFVVFLACLFVAAHSFAVEGEEEYFEAEVPELERAKALPPGSVCDGNESDCKCYGKWHKCRCPWKWHFTGEGPCTCEKGMKHTCITKLHCPNKAEWGLDWRSEESERSPC	15.17
3	ω-ctenitoxin Cs1a	SCIPKHEECTNDKHNCCRKGLFKLKCQCSTFDDESGQPTERCACGRPMGHQAIETGLNIFRGLFKGKKKNKKTK	22.97
4	ω-ctenitoxin Pn1a	MWLKIQVFLLAITLITLGIQAEPNSSPNNPLIEEEARACAGLYKKCGKGASPCCEDRPCKCDLAMGNCICKKKFIEFFGGGK	21.95
5	ω-ctenitoxin Pr1a	ACAGLYKKCGKGVNTCCENRPCKCDLAMGNCICKKKFVEFFGG	16.27
6	ω-filistatoxin Kh1a	AECLMIGDTSCVPRLGRRCCYGAWCYCDQQLSCRRVGRKRECGWVEVNCKCGWSWSQRIDDWRADYSCKCPEDQ	13.51
7	ω-hexatoxin Ar1a	MNTATGFIVLLVLATVLGAIEAEDAVPDFEGGFASHAREDTVGGKIRRSSVCIPSGQPCPYNEHCCSGSCTYKENENGNTVQRCD	40
8	ω-lycotoxin Gsp261a	MKLSIFFVLFFIAIAYCQPEFLDDEEDEVEETLPVAEEGREKSCITWRNSCMHNDKGCCFPWSCVCWSQTVSRNSSRKEKKCQCRLW	17.24
9	ω-oxotoxin OI1a	DWECLPLHSSCDNDCVCCKNHHCHCPYSNVSKLEKWLPEWAKIPDALKRCSCQRNDKDGKINTCDKYKN	17.39
10	ω-plectoxin Pt1a	ADCSATGDTCDHTKKCCDDCYTCRCGTPWGANCRCDYYKARCDT	27.90
11	ω-segestritoxin Sf1a	GSCIESGKSCTHSRSMKNGLCCPKSRCNCRQIQHRHDYLGKRKYSCRCS	42.85
12	ω-sparatoxin Hv1a	DDDCGWIMDDCTSDSDCCPNWVCSKTGFVKNICKYEM	13.51
13	ω-therapotoxin Asp1a	LFECVLSCDIKKNGKPCKPKGEKKCSGGWRCKINFCLKV	15.38
14	ω-therapotoxin Bs1a	IFECVFSCDIEKEGKPCKPKGEKKCSGGWKCKIKLCLKI	7.69
15	ω-therapotoxin Hg1a	GVDKAGCRYMFGGCSVNDDCCPRLGCHSLFSYCAWDLTFSD	19.51
16	k-therapotoxin Ec2c	YCQFKMWTCDSERKCCEDMVCRLWCKLNL	10.34

Table : 13. B-cell epitope prediction of 16 spider toxins.

Sl. no.	Toxin name	Start position	End position	Epitope Peptide	Peptide length
1	ω-actinopoditoxin Mb1a	1	30	SPVCTPSGQPCQPNTQPCCNNAEEEQTINC	30
	ω-agatoxin Aa1a	20	29	VEGEEEYFEA	10
		31	54	VPELERAKALPPGSVCDGNESDCK	24
3	ω-ctenitoxin Cs1a	3	13	IPKHEECTNDK	11
		30	43	TFDDESGQPTERCA	14
		47	52	PMGHQA	6
4	ω-ctenitoxin Pn1a	20	33	QAEPNSSPNNPLIE	14
		37	37	R	1
5	ω-ctenitoxin Pr1a	11	11	K	1
		16	16	C	1
		21	21	P	1
6	ω-filistatoxin Kh1a	11	12	CV	2
		58	58	R	1
7	ω-hexatoxin Ar1a	1	2	MN	2
		21	46	EAEDAVPDFEGGFASHAREDTVGGKI	26

8	ω-lycotoxin Gsp261a	21	42	FLDDEEDEVEETLPVAEEGREK	22
9	ω-oxotoxin OI1a	10	10	S	1
		41	43	AKI	3
10	ω-plectoxin Pt1a	1	12	ADCSATGDTCDH	12
		26	32	GTPWGAN	7
11	ω-segestritoxin Sf1a	3	13	CIESGKSCTHS	11
		15	15	S	1
12	ω-sparatoxin Hv1a	1	3	DDD	3
		11	18	CTSDSDCC	8
13	ω-therapotoxin Asp1a	11	27	KKNGKPCKPKGEKKCSG	17
14	ω-therapotoxin Bs1a	11	28	EKEGKPCKPKGEKKCSGG	18
15	ω-therapotoxin Hg1a	1	4	GVDK	4
		16	19	VNDD	4
16	k-therapotoxin Ec2c	12	14	ERK	3

Table: 14. Immunogenicity of MHC Class-I and Class-II prediction of 16 spider toxins.

Sl. no.	**Toxin name**	**Epitope sequences**	**Predicted immunogenicity CTL response**	**Predicted immunogenicity HTL response**
1	ω-actinopoditoxin Mb1a	SPVCTPSGQPCQPNTQPCCNNAEEEQTINCNGNTVYRCA	Moderate	None
2	ω-agatoxin Aa1a	MMKFVVFLACLFVAAHSFAVEGEEEYFEAEVPELERAKALPPGS VCDGNESDCKCYGKWH	Moderate	Little
3	ω-ctenitoxin Cs1a	SCIPKHEECTNDKHNCCRKGLFKLKCQCSTFDDESGQPTERCACG RPMGHQAIETGLNIF	Moderate	Little
4	ω-ctenitoxin Pn1a	MWLKIQVFLLAITLITLGIQAEPNSSPNNPLIEEEARACAGLYKKC GKGASPCCEDRPCK	Moderate	Little
5	ω-ctenitoxin Pr1a	ACAGLYKKCGKGVNTCCENRPCKCDLAMGNCICKKKFVEFFGG	Moderate	Little
6	ω-filistatoxin Kh1a	AECLMIGDTSCVPRLGRRCCYGAWCYCDQQLSCRRVGRKRECG WVEVNCKCGWSWSQRID	Moderate	Little
7	ω-hexatoxin Ar1a	MNTATGFIVLLVLATVLGAIEAEDAVPDFEGGFASHAREDTVGG KIRRSSVCIPSGQPCP	None	None
8	ω-lycotoxin Gsp261a	MKLSIFFVLFFIAIAYCQPEFLDDEEDEVEETLPVAEEGREKSCITW RNSCMHNDKGCCF	Moderate	Little
9	ω-oxotoxin OI1a	DWECLPLHSSCDNDCVCCKNHHCHCPYSNVSKLEKWLPEWAKIP DALKRCSCQRNDKDGK	Moderate	Little
10	ω-plectoxin Pt1a	ADCSATGDTCDHTKKCCDDCYTCRCGTPWGANCRCDYYKARC DT	Moderate	None
11	ω-segestritoxin Sf1a	GSCIESGKSCTHSRSMKNGLCCPKSRCNCRQIQHRHDYLGKRKYS CRCS	Moderate	None
12	ω-spararoxin Hv1a	DDDCGWIMDDCTSDSDCCPNWVCSKTGFVKNICKYEM	None	Little
13	ω-therapotoxin Asp1a	LFECVLSCDIKKNGKPCKPKGEKKCSGGWRCKINFCLKV	Moderate	Little
14	ω-therapotoxin Bs1a	IFECVFSCDIEKEGKPCKPKGEKKCSGGWKCKIKLCLK	Moderate	Little
15	ω-therapotoxin Hg1a	GVDKAGCRYMFGGCSVNDDCCPRLGCHSLFSYCAWDLTFSD	None	None
Mb1a	Mb1a	Mb1a	Moderate	None
HTL: Helper T-Lymphocyte and CTL: Cytotoxic T-Lymphocyte				