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Original Article

HOMOLOGY MODELING OF SHORT CHAIN NEUROTOXINS: AN INITIATION TOWARDS UNDERSTANDING THEIR FUNCTIONAL INFERENCE

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ABSTRACT

Objectives: Various types of venoms are being produced by different toxic species which make mild or severe damage to the biological system of target species. The main objective is to disseminate structural information in order to understand the functional importance of the short chain neurotoxins (SCNs).

Methods: Computational homology modeling technique is used to predict the theoretical 3D structure of protein. Structural qualities of all predicted SCNs are analyzed using bioinformatics tools.

Results: Homology modeling was performed for all selected SCNs (62 toxin proteins) which do not have experimental models in structural databases. Structural folding patterns of all constructed models were further analyzed for exploring the functional role of SCNs. Three dimensional structures of SCNs provide a better understanding of molecular mechanisms that underlies the inhibition of neurotransmitter based potassium ion channels.

Conclusion: The retrieved structural information of SCNs will serve as a starting point for designing suitable antidote. Future research is required for analyzing the feasibility of using venomous toxins as a pharmacological agent for several disease targets.

Keywords: Short chain neurotoxins, Neurotoxins, Ion channels, Homology modeling, RMSD, Structural superimposition.

INTRODUCTION

Neuronal acetylcholine receptors (nAChRs) serve as important biological target because it performs a vital role of signal transmission between two adjacent neurons. Most of the pharmacological reviews signify the relation between nAChRs and severe neuronal diseases. Cellular signaling between Neuronal acetylcholine receptors occurs through their binding sites and this mechanism illustrates the basic model of ion transport between neuronal acetylcholine receptors [1]. Among the several ionic channels, potassium (K^+) ion channels play a significant role in shaping the action potentials of nervous system. Intensity of each neurotoxin is species specific and the sequence length of SCNs will be around 60-62 amino acids [2]. All SCNs are stabilized with four disulfide bridges, which provide a reasonable strength to the toxin structure. Most of the SCNs were found in various species of snakes, along with long chain neurotoxins (LCNs) [3, 4]. SCNs targets the calcium activated potassium ($Ca2^+$ activated K^+) ion channels [5, 6]. SCNs block the ligand-binding pocket of nAchR subunits and affect the synaptic activity [4, 7].

According to recent reports of SCNs, toxicity of a species depends on the structural and functional role of toxic proteins in receptor mediated pathway. Apart from toxic activities, SCNs have pharmacological properties for targeting several diseases. SCNs are of low molecular mass and can easily bind with the binding site of certain biological receptors for modulating the action of target proteins, which may produce some adverse effect on targeted species. Based on the mechanism of SCNs, researchers work towards finding diversified disease targets. Some novel SCNs may cause harmful diseases like multiple sclerosis, cancer, neurological diseases and some of the autoimmune diseases [8, 9, 10]. Hence, SCNs with pharmacological activity possess drug like property for most of the diseases. Structural knowledge is important for predicting the functions of SCNs prior to understanding of actual mechanism behind SCNs binding. In this work, we have predicted the three dimensional structures of selected SCNs family proteins (structures which are not available in the macromolecular structural databases) using computational homology modeling method. All predicted structures were allowed for further validation studies to confirm the three dimensional structural quality [11]. The predicted structures of SCNs can be used to predict the activity of a SCN to the specified biological target. This methodology will generate new research outcome in the field of pharmacology.

MATERIALS AND METHODS

Sequence retrieval, secondary structure prediction and template validation

In the family of short chain neurotoxin, around 74 toxin sequences were present in Uniprot database (www.uniprot.org/) however the availability of SCN structures in structural databases are very less in number (only 12 structures in PDB). The rest of the SCNs do not have any experimentally proved models in the protein data bank (http://www.rcsb.org/). In order to predict the structure, all these short chain neurotoxin sequences (62 SCNs) were retrieved from Uniprot database [12]. Secondary structures were predicted for all the retrieved targets using JPred tool (www.compbio.dundee.ac.uk/www-jpred/). After the prediction of structure in secondary level, three dimensional structures were modeled by selecting suitable template protein based on similarity search using BLASTP tool (http://blast.ncbi.nlm.nih.gov/Blast) and template selection was confirmed with PDBSUM database (http://www.ebi.ac.uk/pdbsum/) based on several parameters. The degree of sequence similarity between the template and SCNs sequences were set to greater than 35% with good resolution (>1.0 Å) and Z-score.

Molecular modeling, structural comparison and evaluation of SCNs

Homology modeling was performed for all selected SCNs using an automated modeling program called Modeller9v7 [13]. Three dimensional structures of selected SCNs were obtained by satisfying the spatial restraints between template and target proteins. Further, constructed protein models of SCNs were evaluated with PROCHECK program [14] for ensuring the perfect stereo chemical quality of the modeled protein based on the position of amino acids in Ramachandran plot [15]. Accelrys Discovery studio 2.0 software was used for analyzing the selected toxin models of SCNs. In order to

measure the structural errors in constructed models template and target structures were superimposed using superimpose tool of Discovery studio visualizer 3.1. Structural errors were identified and calculated by means of root mean square deviation (RMSD) and were considered for choosing the best 3D models and used for further studies.

RESULTS AND DISCUSSION

SCN secondary structure prediction and template selection

From protein sequence database (Uniprot), unstructured protein sequences of SCNs were retrieved in FASTA format. The suitable template proteins were identified based on the percentage of similarity/ identity between template and target sequences. Sequence similarity threshold were fixed with >35% to obtain an accurate model in homology modeling approach [16]. Selected SCNs (62 templates) were also validated with other parameters like Z-score for finding the folding pattern of target sequence in template structures [17]. Detailed information of template selection methods, % identity, allowed regions in Ramachandran plot and RMSD values are given in the Table 1.

Molecular modeling and validation of SCNs

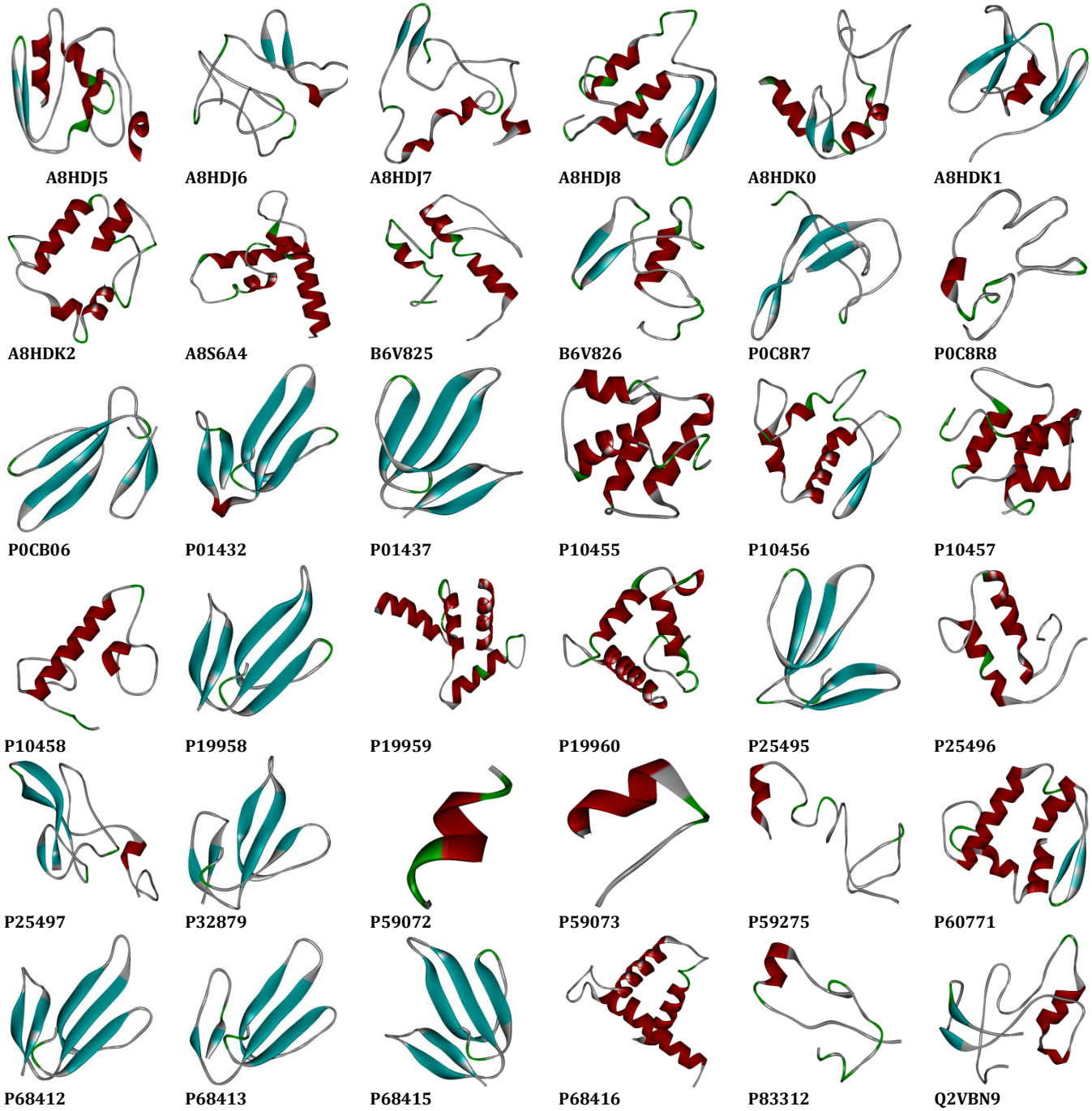
Three dimensional structures of each SCN were predicted by homology modeling technique using Modeller9v7 program. Homology modeling of SCNs would presumably yield highly reliable structures [18]. The obtained models of SCNs are shown in Fig.1. The overall quality of each SCN model was evaluated with structure analysis and verification server (SAVS) using PROCHECK program. The best models were selected based on the presence of amino acid residue in allowed conformations of Ramachandran plot and the detailed distribution of amino acids over Ramachandran plot are depicted as graphical representation in Fig.2. Side chain optimization methodology was followed for obtaining better structures [19]. After validation of selected models, we found that 23 protein structures contain outlier amino acids and those residues were corrected by side chain optimization and energy minimization techniques by using 5000 cycles of steepest descent (SD) and 2000 cycles conjugate gradient (CG) algorithms, prior to that selected protein models were typed with CHARMM force field [20]. The energy minimized models were re-evaluated using PROCHECK program.

Table 1: Details of target-template alignment and 3D protein structure validation of short chain neurotoxins

S. No.	SCNs- Uniprot Id	Template Pdb id	(%) Identity	% of amino acids in ramachandran plot				RMSD (Å)
				F.R*	A.R*	G.A.R*	D.A.R*	
1.	A8HDJ4	2PZX	36.4	91.4	6.9	1.7	0	1.6
2.	A8HDJ5	1OWS(A)	40.8	85	15	0	0	1.7
3.	A8HDJ6	1FON(A)	42.9	87.1	12.9	0	0	1.9
4.	A8HDJ7	3JQL(A)	65.3	87.5	8.3	4.2	0	1.7
5.	A8HDJ8	1LN8(A)	48.7	84.2	14	0	1.8	1.6
6.	A8HDJ9	1F81(A)	42.9	83.9	12.9	0	3.2	1.4
7.	A8HDK0	3QWP(A)	47.4	87.5	12.5	0	0	1.8
8.	A8HDK1	1U27(A)	60	90.9	9.1	0	0	1.6
9.	A8HDK2	1GH4(A)	32.4	75.8	17.7	6.5	0	1.9
10.	A8S6A4	2KJE(A)	35.3	77.8	17.5	1.6	3.2	1.5
11.	B6V825	2OSN(A)	58.3	91.8	6.1	0	2.0	1.7
12.	B6V826	1G2X(A)	84.2	89.3	10.7	0	0	1.7
13.	P0C8R7	3DKU(A)	63.9	93.1	3.4	0	3.4	1.7
14.	P0C8R8	2VHK(A)	58.8	81.5	16.7	1.9	0	1.7
15.	P0CB06	1JGK(A)	45.5	81.8	12.7	3.6	1.8	1.9
16.	P01432	1KFH(A)	85.7	81.3	14.3	4.8	0	1.7
17.	P01437	2QC1(A)	90	80	19.2	0	0	1.6
18.	P10455	1BFA(A)	89.5	90	10	0	0	1.7
19.	P10456	1TC8(A)	39	81.2	15.6	3.1	0	1.7
20.	P10457	1BEA(A)	82.1	80.8	19.2	0	0	1.7
21.	P10458	1MH2(A)	83.8	86.7	10	3.3	0	1.6
22.	P19958	1HC9(A)	45.5	70.9	25.5	3.6	0	1.6
23.	P19959	3IO2(A)	41.7	83	16.7	0	0	1.6
24.	P19960	2KA6(A)	50	75	25	0	0	1.7
25.	P25495	2H8U(A)	77.8	84.4	12.5	3.1	0	1.7
26.	P25496	3NJU(A)	69.2	75	16.7	8.3	0	1.5
27.	P25497	1FJR(A)	58.3	83.7	16.3	0	0	1.7
28.	P32879	1BXP(A)	82.3	90.3	90.7	0	0	1.7
29.	P59072	2OEX(A)	91	86.2	13.8	0	0	1.7
30.	P59073	1RKI(A)	38.1	92.9	3.6	0	3.6	1.3
31.	P59275	3KWE(A)	54.5	62.2	37.9	0	0	1.7
32.	P60771	1U4J(A)	47.6	85.7	8.9	1.8	3.6	1.7
33.	P62376	3BDW(A)	69.2	79.5	20.5	0	0	1.7
34.	P68412	1HC9(A)	60	89.7	10.3	0	0	1.6
35.	P68413	1KC4(A)	54.5	100	0	0	0	1.5
36.	P68415	2QC1(A)	44.4	81.6	16.3	2	0	1.7
37.	P68416	1F81(A)	41	81.2	15.6	3.1	0	1.0
38.	P83312	1ZUB(A)	67.6	83.3	13.3	3.3	0	1.5
39.	Q2VBN9	2QJ9(B)	81.1	85.7	14.3	0	0	1.7
40.	Q2VBP0	2QJ9 (B)	89.7	90	10	0	0	1.6
41.	Q2VBP1	1GAO(A)	67.6	83.3	13.3	0	3.3	1.7
42.	Q2VBP2	1PCO(A)	96	4	0	0	0	1.6
43.	Q8UW26	3102 (A)	55	70.4	21.6	0	0	1.7
44.	Q8UW27	2K8F (A)	92	88.8	11.2	0	0	1.7
45.	Q9UBD1	3NJU(A)	90	80	20	0	0	1.5
46.	Q9W7J6	2HLQ(A)	47.1	88.9	11.1	0	0	1.7
47.	Q9W7J7	2D8Y(A)	40.7	84	8	8	0	1.7
48.	Q9W7J9	2JTK(A)	40.7	80	20	0	0	1.7

49.	Q9W7K0	2D8Y(A)	84.2	89.3	10.7	0	0	1.8
50.	Q9W7K1	1U27(A)	42.1	83.3	13.3	0	3.3	1.7
51.	Q9YGC2	1MH2(A)	47.1	77.6	16.3	6.1	0	0.9
52.	Q9YGC4	3FPU(A)	45.5	81.8	10.9	3.6	3.6	1.6
53.	Q9YGC7	1TC8 (A)	54.5	79.4	17.6	0	2.9	1.6
54.	Q9YGI0	2IOW	43.6	88	12	0	0	1.7
55.	Q9YGW8	3FPR(A)	45.8	86.7	6.7	6.7	0	1.8
56.	Q9YGX0	1PWO	50	81	19	0	0	1.7
57.	Q9W7K2	1U27	93.1	88	12	0	0	1.7
58.	Q45Z11	2HLQ(A)	93.1	96	4	0	0	1.6
59.	Q53B47	1GAO(A)	94.1	92.6	7.4	0	0	1.7
60.	Q53B48	2DIT(A)	47.1	90.3	9.7	0	0	1.7
61.	Q53B49	2TGI(A)	35.3	96.3	0	3.7	0	1.8
62.	Q53B50	2WG8(A)	91.8	85.2	11.1	0	3.7	1.7

*F.R-Favored region; A.R-Allowed region; G.A.R-Generously allowed region; D.A.R-Disallowed region



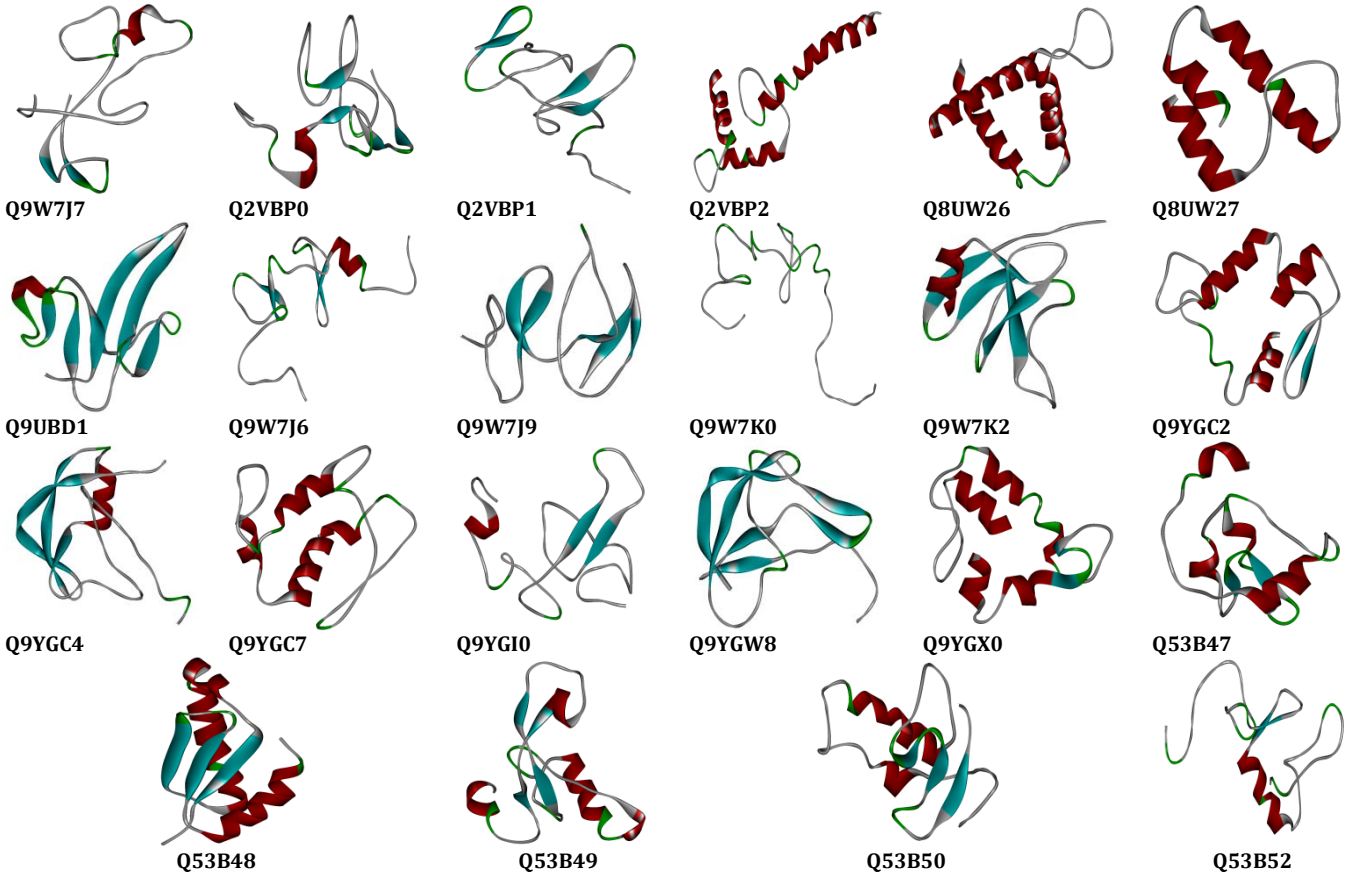


Fig. 1: Modeled 3D structures of selected short chain neurotoxins

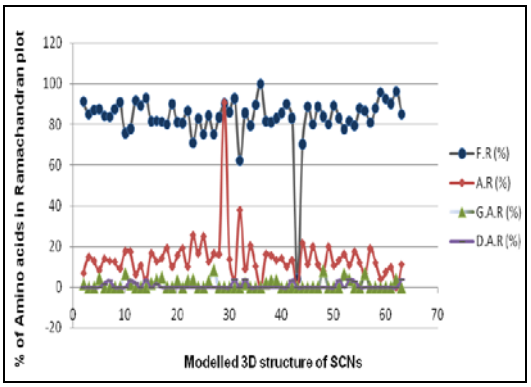


Fig. 2: Amino acids distribution of modeled SCN structures in Ramachandran plot validation

Deposition of all predicted structures to the NEUROTOX database

Predicted three dimensional structures of 62 SCNs were deposited in unique online resource of animal neurotoxins called NEUROTOX which is a comprehensive resource developed by our group. In NEUROTOX, separate sections were created for visualizing and downloading the predicted structures of SCNs along with the detailed validation (Ramachandran plot) of each protein. Then all predicted structures were allowed for further quality check by cross checking their secondary structure with predicted structure. Another important evaluation of protein models were done by using superimposition method. All predicted structures were superimposed with their corresponding template for finding the overall folding pattern. The results obtained from structural superimposition showed considerable RMSD values for each protein pair and it states that the modeled protein has considerable structural homology with their corresponding templates. Predicted homology models of SCNs showed RMSD values less than 1.9Å. The detailed RMSD values of all predicted structures of SCNs were given as a graphical representation in Fig. 3.

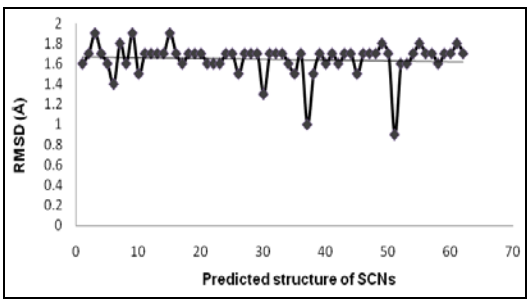


Fig. 3: Overall RMSD based quality assessment of 62 SCN models

CONCLUSION

Several computational analyses are being performed for understanding biological problems in gene or protein level. Computational homology modeling approach has been proved to be the most reliable technique for predicting the 3D structure of protein. Determining the three dimensional structure of SCNs has provided a better understanding of molecular mechanisms that underlie inhibition of K^+ ion channel, the modeled structures of short chain neurotoxins will help us to predict the mode of binding and the amino acids which are responsible for blocking the ligand-binding pockets. Further structural analysis is required for predicting the common domain or folds which are responsible for causing toxicity in the catalytic region of SCNs. All homology

modeled structures of SCNs is an initiation towards predicting suitable inhibitors for highly venomous toxins.

CONFLICT OF INTERESTS

Declared None

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