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DIFFERENTIAL STRUCTURAL INTERACTIONS OF THREE-FINGER FAMILY PROTEINS FROM SNAKE VENOMS ON ACETYLCHOLINE RECEPTORS

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ABSTRACT

Three-finger toxin (short and long neurotoxins) belongs to a super-family of non-enzymatic proteins found in all families of snakes. They have common three-dimensional structures of three beta-stranded loops extending from a central core containing all four conserved disulphide bonds. Despite the common scaffold, they bind to neuronal and muscle receptors with varying binding affinities and exhibit a wide variety of biological activities. In the present studies, we have mapped the binding site residues on short and long neurotoxins by analyzing the docking models of the proteins with nicotinic acetylcholine receptors (nAChRs). Moreover, binding modes for tens of small molecules have been studied on both types of neurotoxins and could be classified as either competitive or allosteric antagonists. Two top-ranked lead compounds that may act as competitive antagonists to the neurotoxins were chosen and used to construct a combinatorial library containing 600 small molecules by using LigBuilder. In order to identify high-efficient *de novo* lead compounds from the combinatorial library, the compounds were subjected to high throughput virtual screening by using PyRx and Glide-HTVS docking tools. The top ten *de novo* lead compounds that may be developed as high-efficient anti-venom compounds to neurotoxins from *elapidae* family have been brought into fore and their structural features are also discussed in detail

KEYWORDS: nicotinic acetylcholine receptors, short neurotoxin, long neurotoxin.

I. INTRODUCTION

Short- and long-chain α -neurotoxins from snake venoms are potent blockers of nicotinic acetylcholine receptors (nAChR). Short α -neurotoxins consist of 60–62 amino acid residues and include four disulfide bridges, whereas long α -neurotoxins have 66–75 residues and five disulfides. The spatial structure of these toxins is built by three loops I–III confined by four disulfide bridges, whereas the fifth disulfide bond of long α -neurotoxins is situated close to the tip of the central loop II. This spatial structure known as a "three-finger fold" is characteristic for different proteins recognition, but also as a way for rational design of new nAChR agonists and antagonists for medical purposes [1]. Long-chain toxins, binds with high affinities to both muscular-type and nAChRs. Short-chain toxins binds with high affinity on muscular-type nAChR only [2]. To date 17 nAchR subunits have been identified, these are divided into muscle-type and neuronal-type subunits. Of these 17 subunits α2- α7 and β2-β4 have been cloned in humans, the remaining genes identified in chick and rat genomes [3]. The "muscletype" nAChR, post-synaptically located at the neuromuscular junction, has a uniquely precise stoichiometry of (α1)2β1γδ (fetal form); the adult form is (α1)2β1δε.

Most other nAChRs are located post- or pre-synaptically in autonomic ganglia and cholinergic neurons throughout the CNS; some of the so-called "neuronal" nAChRs, such as the α7, also occur on non-neuronal cells

Neuronal nAChRs have variable stoichiometries formed from various combinations of α and β subunits. This large collection of closely related receptors, current estimates are that as many as 25 nAChR subtypes are active in human presents special challenges to drug discovery efforts. Certainly therapeutics are directed toward specific neurological disorders, which requires selectivity in terms of nAChRs [4]. Hence, the study aims to check the binding affinity of the three-finger family proteins with muscle and neuronal nAChRs and examining structural insights of the putative active sites of the proteins, which may pave the way of designing *de novo* small molecular anti-venom compounds to the toxic proteins

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II. METHODOLOGY

3D structures of short-neurotoxins, and long-neurotoxins from snake venome (*elaphidae* family) are collected from PDB. Neuronal nAChR alpha7 (2WNL, 2WNJ, 2WN9) and beta2 (2KSR, 2K58, 2K59) structures from human were available, but yet they cannot be used for the further studies as the sequences are fragmented. Whereas Neuronal nAChR aplha (2,3,4,5,6,8,9,10) sequences cannot be used for modeling because of their mismatched length with their protein sequences. Based on the availability of matched sequences, Neuronal nAChR alpha7 (P36544) from human was modeled using I-TASSER and used as representative for Neuronal nAChR. 2QC1 alpha1 structure from Muscle nAChR (mouse nAChR) was available[5] and used as representative for MnAchR. To select the representatives of short-neurotoxins and long-neurotoxins, docking studies have been performed with all the available short-neurotoxins with Neuronal nAChR alpha7 and Muscle nAChR alpha1, using patch dock. Based on binding affinities with nAChRs (alpha1 and alpha7), 1V6P was selected as the representative short-neurotoxins. Similarly, IJBD was selected as representative long-neurotoxins.

III. RESULTS

The active site residues for both the short-neurotoxin and long-neurotoxin were analyzed and studied. T5, V14, Y24, K26, V39, E41, L42, G43, C44, A45 are the important amino acid residues which were identified in the binding site of long-neurotoxin (1JBD). N5, T15, Y25, E38, R39, G40, C41 are the important amino acid residues which were identified in the binding site of short-neurotoxin (1V6P). In the following figures 1-4, protein is displayed in surface and white colour and receptor is displayed in cartoon and red colour. The interacting residues between protein and receptors are shown in blue colour. Receptor binds to protein on concave side.

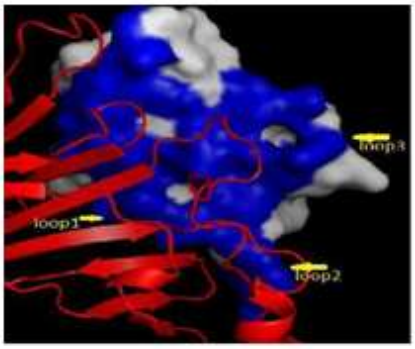

Figure 1 Complex of Short-Neurotoxin(1V6P) with 2QC1

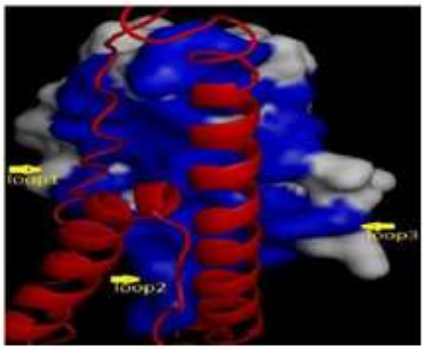

Figure 2 Complex of Short-Neurotoxin(1V6P) with alpha7

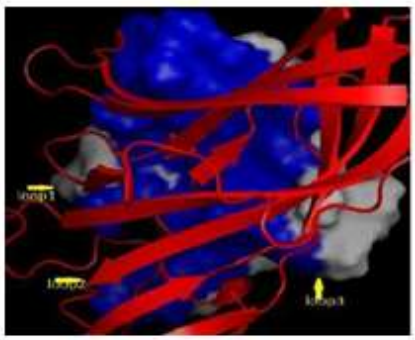

Figure 3 Complex of Long-Neurotoxin(1JBD) with 2QC1

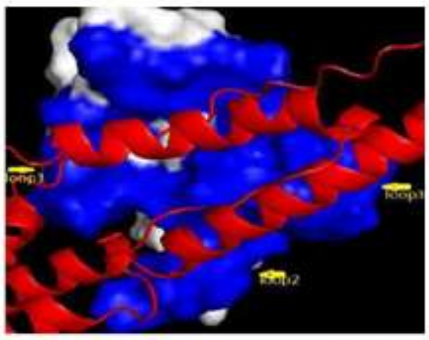

Figure 4 Complex of Long-Neurotoxin(1JBD) with alpha7

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Table 1 Binding energies of Short-and Long-chain neurotoxins with receptors

Sl.No	Complexes	BindingEnergy(kcal/mol)
1	1V6P, 2QC1	-28.66
2	1V6P, alpha7	-3.49
3	1JBD, 2QC1	-18.19
4	1JBD, alpha7	-22.99

Ligands

Lead 01, 02, 03 from reference [6], lead 04, 05 from reference[7], lead 06 from reference [8], lead 07, 08 from reference [9], lead 09, 10 from reference [10], lead 11 from reference [11] were chosen. The complexes of short neurotoxin and long neurotoxin with all the selected ligands and finding competitive and allosteric ligands are shown in figure 6-9.

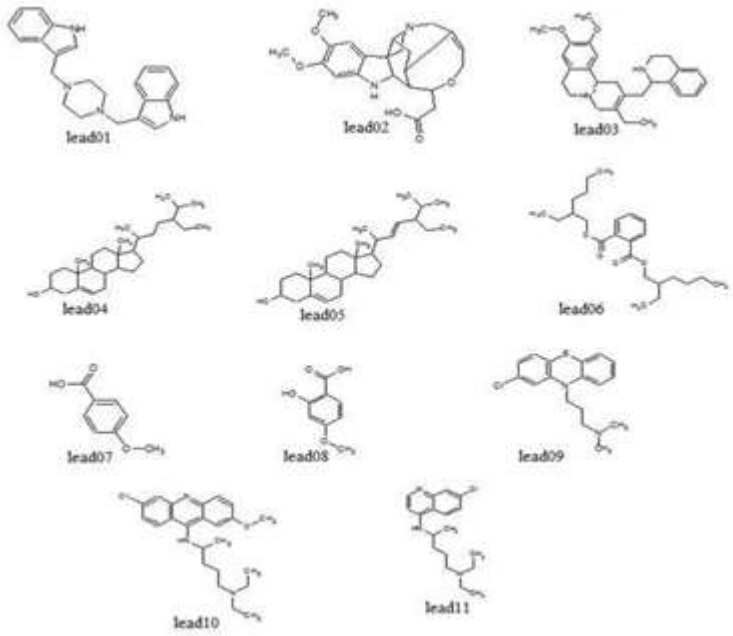

Figure 5 Eleven ligands which have function against neurotoxicity are collected from literature

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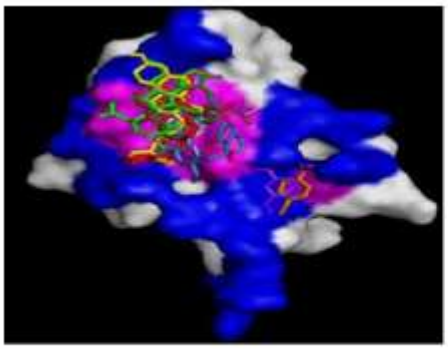

Figure 6 Complex of short-neurotoxin with all ligands

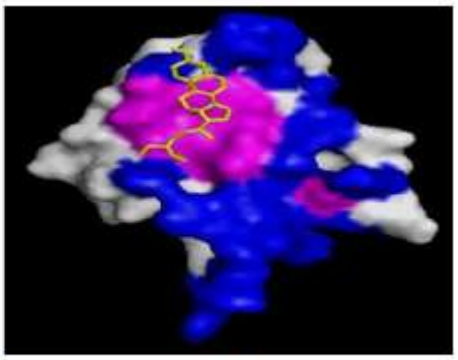

Figure 7 Complex of short-neurotoxin with representative ligand

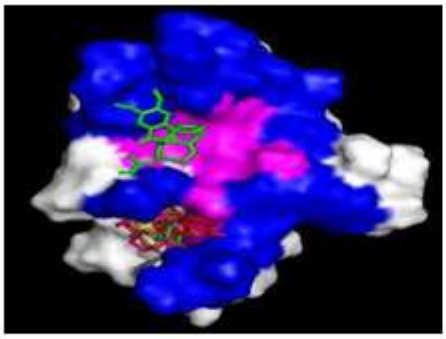

Figure 8 Complex of Long-neurotoxin with all ligands

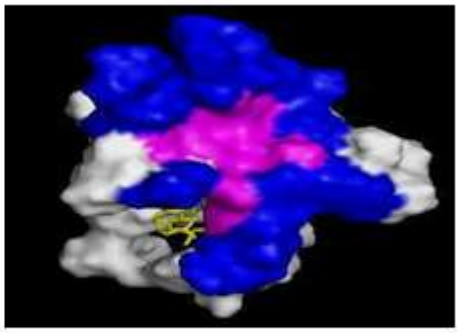

Figure 9 Complex of Long neurotoxin with representative ligand

Table 2 Binding energies of leads with short neurotoxin (1V6P)

Leads	BindingEnergy (kcal/mol)
Lead01	-6.89
Lead02	-6.73
Lead03	-7.32
Lead04	**-8.73**
Lead05	-8.70
Lead06	-5.37
Lead07	-5.74
Lead08	-6.33
Lead09	-5.90
Lead10	-5.66
Lead11	-5.50

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Table 3 Binding energies of leads with long neurotoxin (1JBD)

Leads	BindingEnergy (kcal/mol)
Lead01	-5.09
Lead02	-6.26
Lead03	-5.95
Lead04	**-7.08**
Lead05	-6.90
Lead06	-4.49
Lead07	-5.09
Lead08	-4.88
Lead09	-5.63
Lead10	-4.65
Lead11	-4.45

Ligands that are reported as anti-venom compounds in the literature [8,9,10,11,12,13] at molecular levels and studied on both short and long chain types of neurotoxins and could be classified as either competitive or allosteric antagonists by docking studies using Auto dock. If the ligands bind to the concave side (where the critical binding residues are located) of the protein, it is considered as competitve antagonist and if ligand binds to convex side (opposite side) of the protein it is considered as allosteric antagonists. For short-neurotoxin (1V6P), all the 11 representative ligands were competitive. whereas, for long-neurotoxin lead02 was competitive and all the rest 10 representative ligands were allosteric. Surprisingly, for both short and long-neurotoxins lead04 showed highest binding affinity but it was allosteric to long-neurotoxin (1JBD) as shown in Table 2 and 3.

Constructing Combinatorial library

1JBD, lead04 (allosteric), 1JBD, lead02 (competitive), 1V6P, lead04 (competitive), top-ranked lead compounds that may act as competitive antagonists to the neurotoxins were chosen and used to construct a combinatorial library containing 600 small molecules by using LigBuilder. In order to identify high-efficient *de novo* lead compounds from the combinatorial library, the compounds were subjected to high throughput virtual screening by using PyRx. The PyRx results top 10 compounds has been selected for long-neurotoxins and short-neurotoxins and all the 30 compounds (table4-6) constructed, were found to be *denovo.*

Table 4 Long-neurotoxin (1JBD) from Bangarus Multicinctus lead02 (competitive) denovo molecules binding energies

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Lead molecules	Denovo/Existing	Binding Energy (kcal/mol)
Parent(lead04)	Existing	-5.5
CL1	**Denovo**	**-7.2**
CL2	Denovo	-6.9
CL3	Denovo	-6.9
CL4	Denovo	-6.8
CL5	Denovo	-6.8
CL6	Denovo	-6.7
CL7	Denovo	-6.6
CL8	Denovo	-6.5
CL9	Denovo	-6.5
CL10	Denovo	-6.5

Table 5 Long-neurotoxin (1JBD) from Bangarus Multicinctus lead04 (allosteric) denovo molecules binding energies

Lead Molecules	Denovo/Existing	Binding Energy (kcal/mol)
Parent(lead02)	Existing	-6.3
AL1	**denovo**	**-9.3**
AL2	denovo	-9
AL3	denovo	-8.8
AL4	denovo	-8.6
AL5	denovo	-8.5
AL6	denovo	-8.4
AL7	denovo	-8.4
AL8	denovo	-8.4
AL9	denovo	-8.4
AL10	denovo	-8.5

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Table 6 Short-neurotoxin (1V6P) from Naja Atra lead04 (competitive) denovo molecules binding energies

Lead molecules	Denovo/Existing	Binding Energy (kcal/mol)
Parent(lead04)	Existing	-4.4
CS1	**Denovo**	**-5.7**
CS2	Denovo	-5.6
CS3	Denovo	-5.6
CS4	Denovo	-5.6
CS5	Denovo	-5.6
CS6	Denovo	-5.6
CS7	Denovo	-5.6
CS8	Denovo	-5.6
CS9	Denovo	-5.6
CS10	Denovo	-5.6

From the comprehensive analyses of the docking data, the denovo small molecular anti-venom compounds identified for short neurotoxin (1V6P) and long neurotoxin (1JBD) are listed in Table 4,5 and 6. Lead CL1, Lead AL1 and Lead CS1 with their respective binding affinities of -7.2 kcal/mol, -9.3 kcal/mol and -5.7 kcal/mol are found to have higher binding affinity than the parent lead molecules. Since, the allosteric long neurotoxin compound (Lead AL1) found to have higher binding energy than the competitive long neurotoxin lead (Lead CL1), Lead AL1 was taken further as the potent and promising lead for long neurotoxins (Figure 10). And for short neurotoxin Lead CS1 was considered as a potent lead for short neurotoxins (Figure 11).

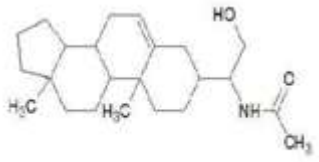

Figure 10 Denovo small molecular anti-venom Long-neurotoxin (1JBD)

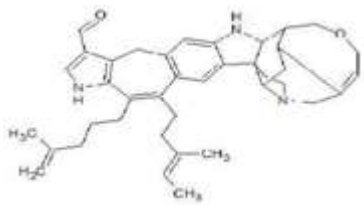

Figure 11 Denovo small molecular anti-venom compound for Short-Neurotoxin (1V6P)

IV. CONCLUSION

Molecular docking studies of the three-finger family proteins with muscle and neuronal nicotinic acetylcholine receptors have been performed using an array of molecular docking tools. Structural insights of all the docking models have been probed using structural analyzing tools such as PyMol, DS and Glide. Anti-venom compounds reported in the literature were retrieved and used for generating docking models on short- and long-neurotoxins. Based on the binding modes, the compounds could be classified either as competitive or as allosteric ligands. Two top-ranked lead compounds that are found to be competitive antagonists as rationalized

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by the present studies to the neurotoxins, were chosen and used to construct a combinatorial library containing 600 small molecules by using LigBuilder. In order to identify high-efficient de novo lead compounds from the combinatorial library, the compounds were subjected to high throughput virtual screening by using PyRx. From the comprehensive analyses of the docking data, the potent denovo small molecular anti-venom compound identified for short neurotoxin and long neurotoxin were Lead CS1 and Lead AL1. These two leads found to have higher binding affinity than the previously published lead molecules (parent lead molecules). Therefore, Lead CS1 and Lead AL1 could be considered as the potent leads for their anti-venom property and could be taken further for invitro and invivio studies

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