

# Homology Based Inhibitor Design For *Naja Melanoleuca* Short Neurotoxin Using Plant Based Drugs

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## Abstract

Plants are one of the most widely used medicines in the world today due to its less side effects over synthetic drugs. Anabesine derived from *Anabasis sphylla* and Papevarine derived from *Papaver somniferum* were investigated for skeletal muscle relaxant activity against *Naja melanoleuca* short neurotoxin. This neurotoxin produces peripheral paralysis by blocking neuromuscular transmission at the post synaptic site and binds to the muscular nicotinic acetylcholine receptor which was chosen for structure prediction using homology modeling. The sequence was retrieved from snake neurotoxin database and was modeled using MODELLER9v2. The quality of the modeled structure was validated using Structural Analysis and Verification Server. The interaction between the predicted structure of short neurotoxin and the inhibitors were analysed *in silico* by ARGUS LAB. The investigation reveals that Anabesine has maximum inhibitor activity of energy value (-5.98 KJ/Mol) against the targeted protein.

Keywords: Short neurotoxin, *Naja melanoleuca*, MODELLER9v2, ARGUS LAB, Anabesine.

## Introduction

Snake envenomation is a socio-medical problem of considerable magnitude. About 2.5 million people are bitten by snakes annually, more than 100,000 fatally (Koh *et al.*, 2006). Snake venoms are complex mixtures of proteins, nucleotides and inorganic ions. These combinations confer a formidable array of toxic properties on the venom, the peptides and polypeptides being responsible for a variety of toxic properties. The number of venom components in venomous animals like snake, scorpion or cone snail ranges from 50-200 toxins (Tan *et al.*, 2003). Snake venoms are important tools in toxinology, neuroscience, and pharmacology. The venom components are highly variable and functionally complex and they offer many research opportunities (Joyce *et al.*, 2004). *Naja melanoleuca* inhabited mainly primary and secondary forest patches, but also plantations and suburbia (Luiselli *et al.*, 2000).

The main toxins from snake venom that affect the CNS are neurotoxins. Neurotoxins form one of the largest families of proteins with established primary structures. Snake neurotoxin is a toxic agent or substance that inhibits damages or destroys

the tissues of the nervous system and neurons. Neurotoxic proteins isolated from various snake venoms have high affinity for a particular target site are used extensively as pharmacological tools to gain insights into the function of the nervous system. The vast majority of snake venom neurotoxic peptides competitively bind to the nicotinic acetylcholine receptor. The potency of these molecules lies in their affinities towards the biomolecules involved in the functioning of neuromuscular transmission. Nicotinic acetylcholine receptors are prototypes for the pharmaceutically important family of pentameric ligand-gated ion channels (Celie *et al.*, 2004). Among the best studied snake neurotoxins are the - neurotoxins that bind to nicotinic acetylcholine receptors (nAChRs). They are capable of reversibly blocking nerve transmission by competitively binding to the nAChR located at the postsynaptic membranes of skeletal muscles and neurons, preventing neuromuscular transmission and thereby leading to death by asphyxiation (Tsetlin *et al.*, 2004). Short- and long-chain neurotoxins from snake venoms are potent blockers of nicotinic acetylcholine receptors (nAChRs). Short neurotoxin from *Naja melanoleuca* belongs to snake toxin family. It belongs

to the TYPE 1 alpha neurotoxin super family. The neurotoxin produces peripheral paralysis by blocking neuromuscular transmission at the post synaptic site and binds to the muscular nicotinic acetylcholine receptor. Short neurotoxins consist of 60–62 amino acid residues and include 4 disulfide bridges, whereas long-neurotoxins have 66–75 residues and 5 disulfides (Mordvintsev *et al.*, 2006). Sequence annotations, functional and structural data on snake venomneurotoxins (svNTXs) are scattered across multiple databases and literature sources. Sequence annotations and structural data are available in public molecular databases, while functional data are almost exclusively available in published articles (Joyce *et al.*, 2004).

Fossil records revealed that the human use of plants as traditional medicine date back to middle Paleolithic age, approximately 60,000 years ago (Solecki *et al.*, 1975). At present, natural products (and their derivatives and analogs) represent over 50% of all drugs in clinical use, in which natural products derived from higher plants represent ca. 25% of the total. The World Health Organization estimated that over 80% of the people in developing countries rely on traditional remedies such as herbs for their daily needs and about 855 traditional medicines include used crude plant extracts. This means that about 3.5 to 4 billion of the global population rely on plants resources for drugs (Farnsworth, 1988). Medicinal plants have provided a good source of a wide variety of compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other secondary metabolites, which are rich in valuable bioactivities (Maridass *et al.*, 2008). In this study two medicinal plant compound Anabesine derived from *Anabasis sphylla* and Papaverine derived from *Papaver somniferum* were investigated for skeletal muscle relaxant activity against *Naja melanoleuca* short neurotoxin. The minor tobacco alkaloids anabesine have high affinity for nAChRs and act as agonists in most receptor assays (Dwoskin *et al.*, 1995). Papaverine is an opium alkaloid used primarily in the treatment of visceral spasm. Papaverine used as a smooth muscle relaxant in microsurgery where it is applied directly to blood vessels.

## Materials and methods

### Snake venom neurotoxin database (svNTXdB)

The svNTXdB provides a unique compilation of these toxins collected from public databases and literature sources. Each entry had been analyzed for possible errors and inconsistencies, and annotated with functional information. Short neurotoxin sequence of database Entry ID 6773 was retrieved through querying searchable on-line database of NTX proteins sequences.

### Template identification for the target molecule

The template may be a predefined layout to give an idea about the unknown structure of the query molecule (Brinda *et al.*,

2009). The NCBI BLAST was used to identify the template for modeling the three dimensional structure of short neurotoxin. The sequence of the target molecule in FASTA format was submitted for blastp against pdb database which yields that alpha toxin from *Naja nigricollis* (PDB ID:1NEA) as a suitable template. The target and the template have 53% of residues identical with an E-value of 4e-18.

### Homology modeling

Among all current theoretical approaches, comparative modeling is the only method that can reliably generate a 3D model of a protein from its amino acid sequence. Modeling of protein structures usually requires extensive expertise in structural biology and the use of highly specialized computer programs for each of the individual steps of the modeling process (Tramontano *et al.*, 2001). The method of homology modeling is based on the observation that protein tertiary structure is better conserved than amino acid sequence. (Renom *et al.*, 2000). The three dimensional structure of short neurotoxin has been predicted using MODELLER9v2 (<http://www.salilab.org/modeller/>).

### Model refinements and evaluation

The model generated by MODELLER9v2 was subjected to energy minimization using the steepest descent technique to eliminate bad contacts between protein atoms using swiss pdb viewer. Validation of modeled structure was carried out using Structure Analysis and Validation Server. It performs structure validation calculations using PROCHECK, PROVE, Verify3D, ERRAT and WHAT\_IF programs. The validated result of the modeled protein from the server is an important part of comparative modeling process.

### Active site prediction

After obtaining the final model, the possible binding sites of short neurotoxin were searched using Computed Atlas of Surface Topography of proteins (CASTp). (<http://cast.engr.uic.edu>). These include pockets located on protein surfaces and voids buried in the interior of proteins. CASTp includes a graphical user interface, flexible interactive visualization, as well as on-the-fly calculation for user uploaded structures. (Binkowski *et al.*, 2003)

### Docking the inhibitors against the active site of the Short Neurotoxin

Docking is a computational technique that samples conformations of small molecules in protein binding sites; scoring functions are used to assess which of these conformations best complements the protein binding site (Warren *et al.*, 2006). The inhibitor and target protein was geometrically optimized and docked using docking engine ArgusDock. (<http://www.arguslab.com/>).

## Results and Discussion

The Homology based inhibitor design for the target short neurotoxin involved in paralysis is as follows.

Sequence retrieval of short neurotoxin

The protein sequence was retrieved from snake neurotoxin database and has database entry ID of 6773 was shown in Tab.1

Virulence Factor Sequence Info ID	Sequence Name	Protein or Nucleic	Virulence Factor ID	Reference ID	Database Entry ID
12266	Short neurotoxin 1 (Neurotoxin D)	Protein	10685	1	6773

Table 1: Short neurotoxin protein details

Homology modeling of the target protein

The absence of the three dimensional structure for *Naja melanoleuca* short neurotoxin in PDB prompted us to construct the 3D model of the protein. The three dimensional structure provides information into the function and also helps us to analyses of its interactions with the suitable inhibitors. 1IQ9 alpha toxin from *Naja nigricollis* was chosen as template. The alignment of template and the target protein was performed using the script "align2d.py" which was shown in Fig. 1.

Among the five models generated using the script "model-single.py" the thermodynamically stable model was chosen for further refinement and validation. After the final refinement the modeled protein shows the energy minimization value of -3452.636 KJ/Mol. The energy minimization value suggests that the protein was well refined. The final 3D structure of short neurotoxin obtained after energy minimization was shown in Fig 2.

Validation of the predicted structure

The refined structure was the submitted in Structure validation and analysis server for validation. Ramachandran was used to visualize dihedral angles against of amino acid residues in protein structure (Ramachandran *et al*, 1963). From the Fig. 3 it was concluded that there was no presence of disabled aminoacids.

Active site prediction

Among the nineteen binding sites obtained from Castp server site 1 was chosen as best site for the inhibitor binding. The

_aln_pos	10	20	30	40	50	60
1IQ9A	LECHNQSSQPPTTKTCPGETN	CYKQVWRDHRGTII	IERGGCP	TVKPGIKLN	CCTTDKCNN	
NEURO	MECHNQSSQPPTTKTCPGETN	CYKQVWRDHRGTII	IERGGCP	SVKQVGRKIN	CCTTDKCNN	
_consrvd	*****	*****	*****	*****	*****	*****

Fig. 1 Alignment between the target protein and the template

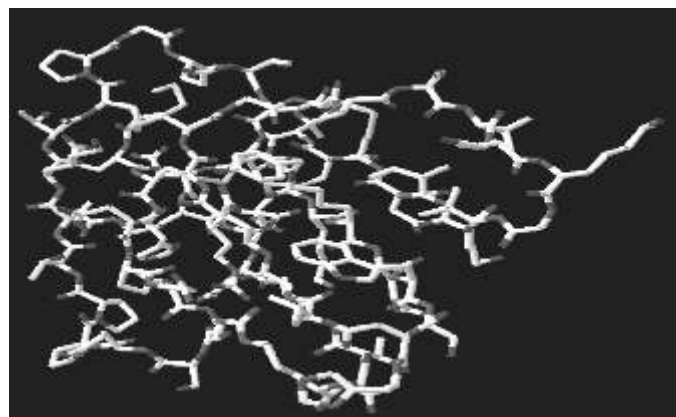


Fig. 2 The final 3D structure of short neurotoxin obtained after energy minimization.

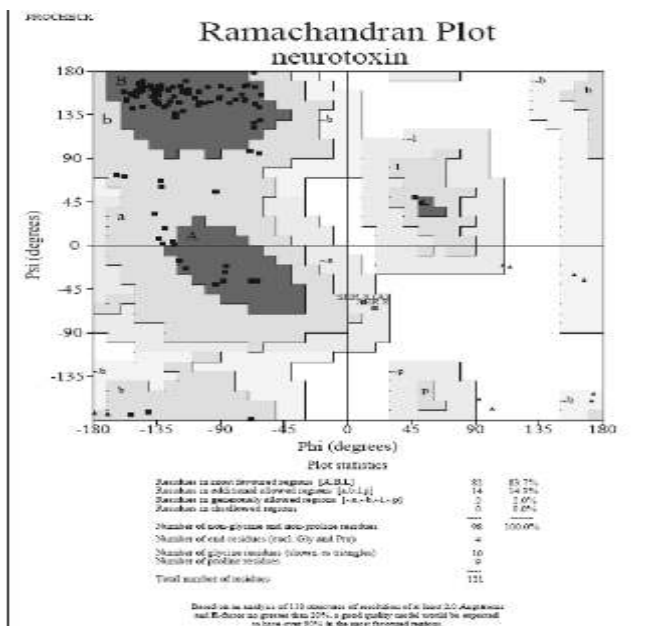


Fig.3 Ramachandran plot showing the position of aminoacids

binding pocket containing the residues Met1, Glu2, Cys3, Cys17, Thr21, Asn22, Cys23, Cys54, Thr55, Thr56 which are shown in Fig. 4 and this was chosen for docking analysis.

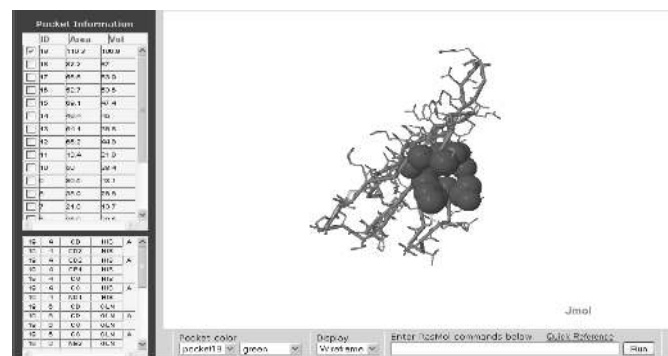


Fig. 4 Green color indicates the active site chosen for docking analysis

Docking between the target protein and the drug molecule:

Owing to the growing number of identified snake venom neurotoxin sequences, it is increasingly difficult to study them by experimentation alone. Detailed bioinformatics analysis offers a convenient methodology for efficient *in silico* preliminary analysis of possible function of new toxins (Joyce *et al.*, 2004). The high specificity of neurotoxins for nAChRs has been utilized as a tool in understanding the structure and function of the nervous system (Koh *et al.*, 2006). The two inhibitors Anabesine derived from *Anabasis sphylla* and Papevarine derived from *Papaver somniferum* were shown in

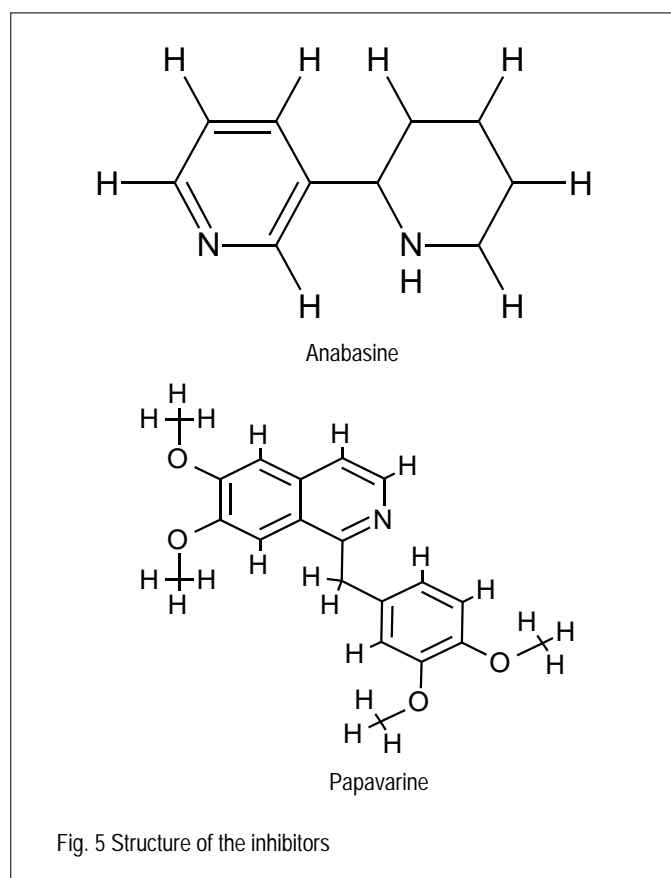


Fig. 5

The scoring function takes a pose as input and returns the number indicating the likelihood that the pose represents a favourable binding interaction. A low (negative) energy indicates a stable system and thus a likely binding interaction (Kitchen *et al.*, 2007). Fig. 6 shows the binding of ligand with the receptor molecule. The docking energy value of Anabesine and papaverine was -5.9846 KJ/Mol and -2.6015 KJ/Mol respectively. The RMS score value is 0.40.

Anabesine has formed one hydrogen bond interaction with Thr 21 and two bonds with Thr 55(2.6,2.8,2.8) of the active site of the target molecule. Papavarine has hydrogen bond interaction with of Glu 2 and Thr 55(2.4,2.7) of the target

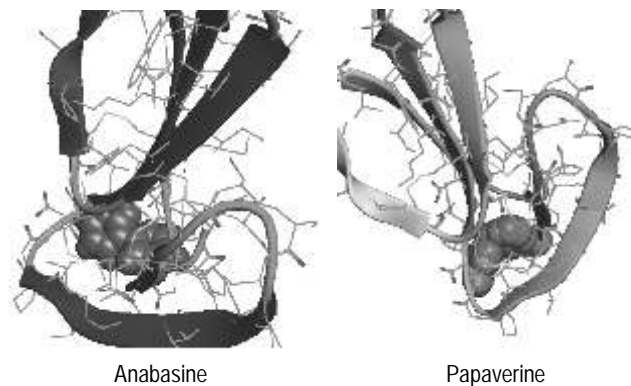
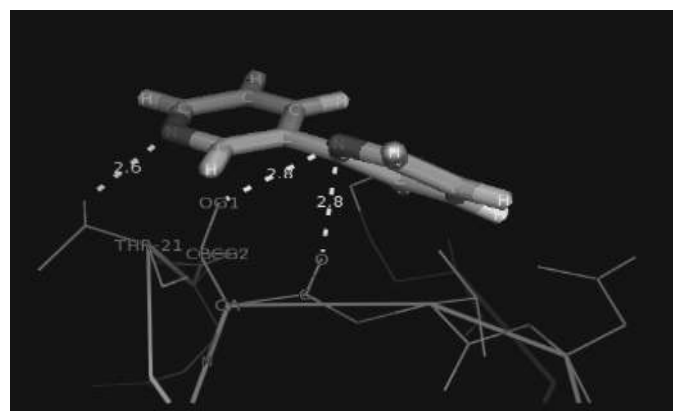


Fig. 6 shows the binding of the ligand with the receptor molecule.



protein, which was shown in Fig. 7.

The specificity of the drug and target protein varies depending upon their binding sites. The docking results show that the compound anabesine has higher specificity and efficiency towards the target protein short neurotoxin, which causes paralysis.

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