Research Article

Computational Analysis and Designing of Potential Inhibitors against Alpha-Bungarotoxin N3 Using QSAR based Virtual Screening Approach

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Accepted on: 10-03-2016; **Finalized on:** 31-03-2016.

ABSTRACT

Alpha-bungarotoxin N3 is an effective neurotoxin isolated from *Bungarus candidus* which belongs to venomous snake species. Alpha-bungarotoxin N3 usually leads to peripheral paralysis by blocking nicotinic acetylcholine receptor (nAChRs) at the postsynaptic site in the brain. In the current study three dimensional structure of Alpha-bungarotoxin N3 (P85140) was predicted using modeler 9v 12. The antitoxic compounds from plants which possess potential activity against Alpha-bungarotoxin N3 were selected by performing a thorough literature Search. After the identification of antivenomic plant compounds, QSAR studies were performed with them by using software BUILD QSAR. The compounds which lie in best fit line graph of QSAR studies were selected and Molecular docking was performed against Alpha-bungarotoxin N3 (P85140) using Autodock 4.0. From this study two natural plant compounds which possess complete level of inhibition against the Alpha-bungarotoxin N3 were identified. Thus this study can serve as a potential insight to identify new antitoxic drugs against Alpha-bungarotoxin N3.

Keywords: Alpha-bungarotoxin N3, Neurotoxins, P85140, Docking, Nicotinic acetylcholine receptors.

INTRODUCTION

Bungarus candidus (Malayan krait) belongs to Elapidae family and is usually found in Asian countries.[1] Snakes from the *Bungarus* genus are commonly known as kraits. *Bungarus candidus* was reported to be one of the most poisonous snake species in Southeast Asia.[2] Neurotoxicity characterized by Kraits contains two major classes of neurotoxins in the venom, presynaptic & postsynaptic neurotoxins. Presynaptic neurotoxins disrupt the release of the neurotransmitter acetylcholine from the nerve terminal. Postsynaptic neurotoxins inhibit the binding of acetylcholine to the nicotinic receptors on the skeletal muscle. The Postsynaptic neurotoxins known as α-bungarotoxin (α-BTX) was found to be, present in the venom of *Bungarus candidus*.[3] Neurotoxins, in particular α-BTX binds to the nicotinic acetylcholine (ACh) receptor in cholinergic synapses which prevents the normal neurotransmitter induced channel opening and finally result in block of Postsynaptic membrane depolarization.[4-7]

Krait venoms possess acetylcholinesterase, hyalurodinase and L-amino acid oxidase, which leads to toxin diffusion. Respiratory muscle paralysis is the main cause in krait envenoming, which lead to Permanent brain damage due to anoxia, cerebral ataxia, paraplesia, mydriasis.[8] The victims are reported to be around 5,400,000 members per year. *In silico* docking studies of the bioactive compounds against α-BTX protein was found to be very useful in identification of potent inhibitors against Snake venom of *Bungarus candidus*.

Millions of drugs are available for treating the snake bites and the clinical development of these drugs has revealed increased amount of side effects like anaphylactic reactions that are exhibited by some of the victims.[9] Thus an attempt was made in the current study for the development of antivenomic drug from herbal sources, the plant extracts are found to contain snake venom neutralizing ability.[10] In Indian villages, many plants are widely used against snakebites.[11] Each plant contains thousands of bioactive compounds, and each compound possess their own medicinal properties.

The following phytoconstituents are present in plant extracts with good pharmacological effects. Also they are reported to be natural antitoxic agents.[12] Antivenomic herbal drugs are 4-Nerolidylcatechol, Aristolochic Acid, Cabenegrin A2, Cabenegrin A1, Edunol, Tectoridin, Wedelolactone, Ellagic Acid, Curcumin, Atropine, Salireposide, Myricetin, Quercetin, Rosmarinic, Resverotol, Oleanolic acid, Chlorogenic acid, L-dopa. In the present study, we have analyzed the structural and functional role of α-BTX N3 by computational structure prediction method and identified potential inhibitors against α-BTX N3.

MATERIALS AND METHODS

Template identification and Sequence Alignment

The target sequence of the query protein Id: P85140 was retrieved from Uniprot and used as an input in pdbsum. From the results obtained, we identified a best template (PDB ID: 1KFH). Both the target and template sequence were subjected to alignment using T-Coffee.

Homology modeling of protein Alpha-bungarotoxin N3 (P85140)

The 3-Dimensional structure of α-BTX N3 is not predicted so far and hence the structure is not available in protein

data bank, so we made an attempt to model α-BTX N3 by using software MODELLER 9 v12 and the predicted structure was validated using the tool Procheck Server.

Prediction of Active Site for Alpha-bungarotoxin N3

The active site of the target protein was predicted using a program called Difference of Gaussian (DoG) Site Scorer.[13]

Model Evaluation

After modeling the target protein, the model was evaluated by using Ramachandran plot.[14]

Build QSAR

The Build QSAR is used to compare the structural properties of compounds. QSAR helps us to identify inhibitors with high biological activities using regression analysis.[15-17]

Identification of antivenomic plants compounds

Natural antivenomic compounds used for the treatment of snake bites were identified and retrieved from Pubmed Literature. They are 4-Nerolidylcatechol from *Pothomorphe peltata*, Aristolochic Acid from *Aristolochia*, Cabenegrin A2 from *Cabeca-de-negro*, Cabenegrin A1 from *Cabeca-de-negro*, Edunol from *Harpalyce brasiliana*, Tectoridin from *Belamcanda chinensis*, Wedelolactone from *Eclipta alba*, Ellagic Acid from *Casearia sylvestris*, Curcumin from *Curcumin longa*, Atropine from *Atropa Belladonna*, Salireposide from *Symplocos racemosa*, Myricetin from *Mouriri pusa*, Quercetin from *Byrsonima crassa*, Rosmarinic acid from *cordial verbenacea*, Resverotrol from *Cissus Assamica*, Oleanolic acid from *Phytolacca americana*, Chlorogenic acid from *Coffea arabica*, L-dopa from *Mucuna pruriens*. The 3D structure of the above plant compounds was retrieved from PubChem Database. Physicochemical properties were analyzed using Build QSAR.

Molecular Docking Using Auto Dock 4.0

The Molecular docking studies was performed using the docking software AutoDock 4.0. AutoDock performs the docking of the ligand to the target protein.[18] The Binding interaction of natural plant compounds with target protein is important in the drug development process. The potential binding sites of Protein α-BTX N3 were searched using DoG Site Scorer. The plant compounds possessing antivenomic properties were docked into the active site of α-BTX N3 receptor protein. After completion of docking, the docked protein (protein-ligand complex) was analyzed using Discovery Studio 3.0 visualizer. Further the docking poses were saved for each compound and they were ranked according to their binding energy.

RESULTS AND DISCUSSION

3-Dimensional Structure prediction of protein Alpha-bungarotoxin N3

The sequence of α-BTX N3 was retrieved from the uniprot sequence database (www.uniprot.org) with the accession number P85140. The 3D structure of α-BTX N3 was predicted by homology modeling method. The template was chosen based on sequence identity (PDB ID:1KFH 'A' chain).

The sequence alignment was performed for alpha bungarotoxin with the target protein using T-Coffee respectively. **(Fig.1)** The selected template (1KFH) and target protein (α-BTX N3) sequences were aligned perfectly. Based on the alignment, the initial 3D protein model was predicted using Modeler 9 v12. The modelled protein contains 12.33% of alpha helix and 5.48% of beta turn, 27.40% of extended stand and 54.79% of random coil. **(Fig.2)**

The Modeled 3D structure was validated using Ramachandran plot. The predicted model was found to contain, 78.3% of the residues in most favored regions, 18.3% in additional allowed region, 3.3% in generously allowed region, 0% of the residues lying in the disallowed regions. The above results clearly indicate the quality of predicted protein structure is reasonably good. **(Fig.3)**

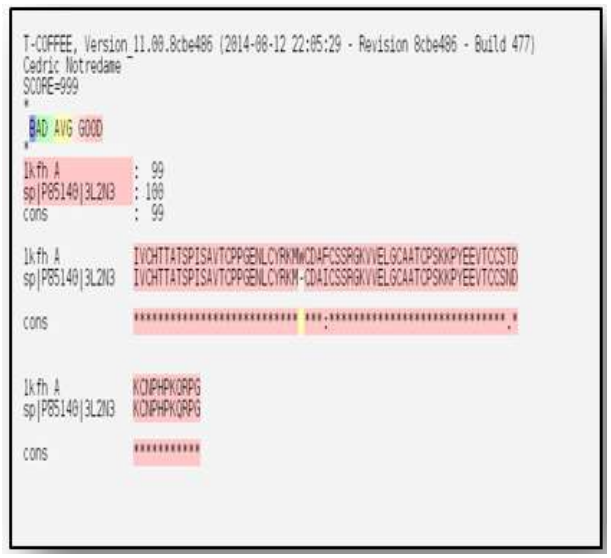


Figure 1: Sequence alignment of α-BTX N3 protein with the template structure 1KFH depicts the conserved region in stars (*) and deleted regions with dashes (-)

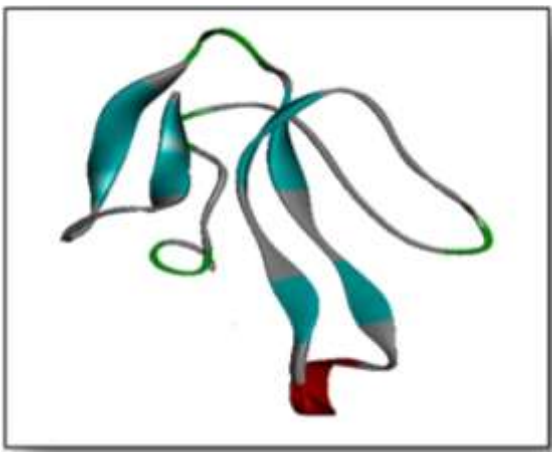


Figure 2: 3D Model of Alpha-bungarotoxin N3

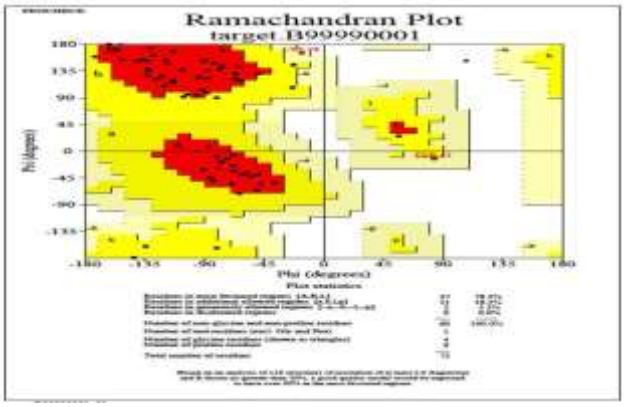


Figure 3: Assessment of the Ramachandran Plot

Active site analysis

Active site of the target protein was identified using Difference of Gaussian (DoG) site scorer. The Difference of Gaussian site scorer predicts the active sites in proteins based on a Difference of Gaussian approach.[19] Difference of Gaussian Site splits predicted pockets into sub-pockets, and identifies binding pockets present in query protein. The active site of modeled protein comprises of 8 binding pockets with the amino acid residues. The pocket one was found to contain the following aminoacid residues TYR24, ARG25, LYS26, MET27, VAL38, VAL39, GLU40, PRO48, SER49, GLU54, GLU55 and VAL56.

Build QSAR

QSAR Model was generated using Build QSAR for 18 antivenomic plant compounds. **(Table.1)** QSAR Model built based on the parameters of ic50, Mi-LogP, TPSA, Natoms, Molecular Weight (MW), Volume, Formula.[20] Antivenomic plants compounds were used as data set to build QSAR model, **(Fig.4)** performed by using Multiple linear regression (MLR).[21] The Best QSAR model of antivenomic plant compounds which lie in Best Fit line of correlation Analysis in the graph were found to be No.2. Aristolochic acid, No.7. Wedelolactone, No.17. Tectoridin shown in **(Fig.5)**.

	COMPOUND NAME	Mi-LogP	TPSA	MOLECULAR WEIGHT	IC50 VALUE	Natoms	VOLUME	FORMULA
1	4-NEROLIDYLCATECHOL	6.505	40.456	314.469	0.4	23	332.599	C21H30O2
2	ARISTOLOCHIC ACID	3.566	110.825	341.275	100	25	271.838	C17H11NO7
3	CABENEGRIN_A_2	3.709	77.392	370.401	-5.9	27	325.954	C21H22O6
4	CABENEGRIN_A_1	3.602	77.392	368.385	-1.5	27	319.742	C21H20O6
5	EDUNOL	4.843	57.164	352.386	25	26	311.483	C21H20O5
6	SALIREPOSIDE	0.885	145.913	406.387	543.979	29	348.379	C20H22O9
7	WEDELOLACTONE	2.299	113.269	314.249	46.6	23	247.737	C16H10O7
8	ELLAGIC ACID	0.943	141.334	302.194	71.5	22	221.776	C14H6O8
9	CURCUMIN	2.303	93.066	368.385	0.15	27	332.182	C21H20O6
10	ATROPINE	1.767	49.771	289.375	685	21	279.031	C17H23NO3
11	MYRICETIN	1.392	151.579	318.237	-30	23	248.102	C15H10O8
12	QUERCETIN	1.683	131.351	302.236	5.25	22	240.084	C15H10O7
13	ROSMARINIC ACID	1.626	144.516	360.319	27	26	303.539	C18H16O8
14	RESVEROTROL	2.986	60.684	228.247	60	17	206.922	C14H12O3
15	OLEANOLIC ACID	6.725	57.527	456.711	53.5	33	471.139	C30H48O3
16	CHLOROGENIC ACID	0.453	164.744	354.311	0.75	25	296.267	C16H18O9
17	TECTORIDIN	0.268	179.281	462.407	0.193	33	381.716	C22H22O11
18	L-DOPA	-2.199	103.779	197.19	11	14	171.999	C9H11NO4

Figure 4: Selected antivenomic plants compounds were used in data set to build QSAR model

Table 1: Selected antivenomic plants compounds

S. No	Compounds Name	Plant Name
1.	4-Nerolidylcatechol	*Pothomorphe peltata*
2.	Aristolochic Acid	*Aristolochia indica*
3.	Cabenegrin_A_2	*Cabeca-de-negro*
4.	Cabenegrin_A_1	*Cabeca-de-negro*
5.	Edunol	*Harpalyce brasiliana*
6.	Salireposide	*Belamcanda chinensis*
7.	Wedelolactone	*Ecliptaalba*
8.	Ellagic Acid	*Casearia sylvestris*
9.	Curcumin	*Curcumin longa*
10.	Atropine	*Atropa Belladonna*
11.	Myricetin	*Mouriri pusa*
12.	Quercetin	*Byrsonima crassa*
13.	Rosmarinic Acid	*cordial verbenacea*
14.	Resverotol	*Cissus Assamica*
15.	Oleanolic Acid	*Phytolacca americana*
16.	Chlorogenic Acid	*Coffea arabica*
17.	Tectoridin	*Belamcanda chinensis*
18.	L-Dopa	*Mucuna pruriens*

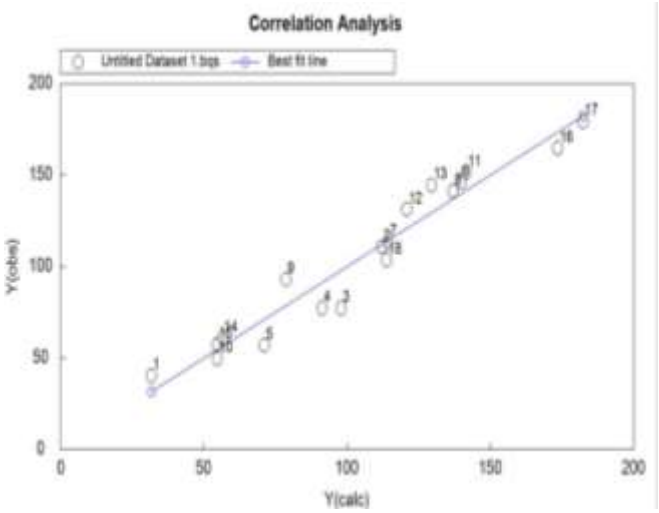


Figure 5: Antivenomic plants compounds which lie in Best Fit line of correlation Analysis

Docking studies

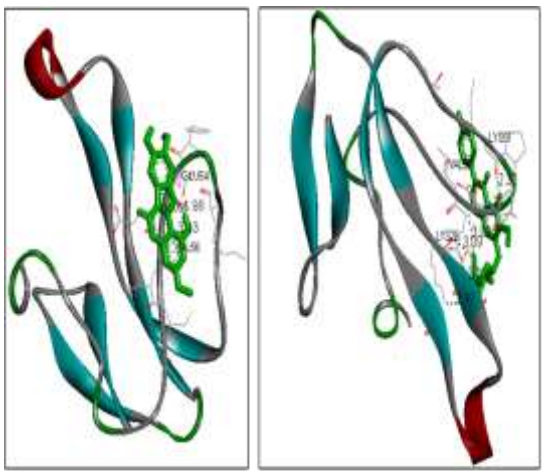
To study the binding interactions between the ligands and α-BTX N3 protein and to investigate their binding mode, Molecular docking study was performed using AutoDock 4.0.

The binding interactions obtained from the docking studies for the protein α-BTX N3 with various compounds were visualized using Accelrys Discovery Studio Visualizer 3.5. The binding pocket of the protein includes amino acid residues TYR24, ARG25, LYS26, MET27, VAL38, VAL39, GLU40, PRO48, SER49, GLU54, GLU55 and VAL56. In docking study only 2 compounds exhibited better binding

interactions with the binding pocket of modelled protein α-BTX N3 namely, Wedelolactone and Tectoridin. Antivenomic compound Wedelolactone having a binding energy value of -6.18 kcal/mol, Tectoridin having a binding energy value of -7.62 kcal/mol. Hence, the above two compounds were chosen as probable drug candidates that inhibit the activity of α-BTX N3 protein. The above antivenomic plant compounds bind to the active site region of the α-BTX N3 protein and their hydrogen bond interaction profiles are presented in **(Table.2)**.

Interaction profile

The interaction of Tectoridin with the amino acid LYS51 binds with atom O9 with distance 2.32, the amino acid TYR53 binds with atom O7 with distance 3.28, amino acid GLU54 binds with atom O9 with distance 3.07, the amino acid GLU55 binds with atom O31 with distance 3.09, the amino acid LYS26 binds with atom O31 with distance 3.09, amino acid LYS26 binds with atom O26 with distance 3.10, amino acid GLU55 binds with atom O27 with distance 3.09. The interaction of Wedelolactone with the amino acid LYS51 binds with atom O19 with distance 3.2, the amino acid GLU54 binds with atom O19 with distance 1.98, amino acid VAL56 binds with atom O19 with distance 3.13. **(Fig.6)**



a) Wedelolactone docked with Alpha-bungarotoxin N3 b) Tectoridin docked with Alpha-bungarotoxin N3

Figure 6: Docking complex of Alpha-bungarotoxin N3 with Wedelolactone, Tectoridin

Table 2: Binding energy and H-bonds distance of antivenomic plant compounds with Alpha-bungarotoxin N3

S. No	Compounds Name	Binding Energy	Hydrogen Bond Interaction (Amino acid – Atom)	H-Bonding distance (Å)
1	Tectoridin	-7.62 kcal/mol	LYS51-O9	2.32
			TYR53-O7	3.28
			GLU54-O9	3.07
			GLU55-O31	3.09
			LYS26-O31	3.09
			LYS26-O26	3.10
			GLU55-O27	3.09
2	Wedelolactone	-6.18 kcal/mol	LYS51-O19	3.2
			GLU54-O19	1.98
			VAL56-O19	3.13

CONCLUSION

Bungarus candidus belongs to a most poisonous snake species and it remains as a painful reality in the daily life of millions of people in world-wide. Hence there is an urgent need for the development of a new antivenomic drug. In this study, we performed various approaches like molecular modeling of Alpha-bungarotoxin N3, Build QSAR approach was performed for eighteen antivenomic compounds, and finally two compounds with good biological and structural activity were selected such as Wedelolactone and Tectoridin. Molecular interaction studies were performed to help us to find out the suitable antivenomic inhibitors. Docking study was carried out between two antivenomic compounds namely; Tectoridin and Wedelolactone with protein α-BTX N3. The minimum binding energy values obtained between Tectoridin with α-BTX N3 -7.62 kcal/mol, Wedelolactone with α-BTX N3 -6.18 kcal/mol respectively.

Hence, we conclude that identified compounds can be considered as potential drug candidates, an alternative as well as a good antidote for *Bungarus candidus* toxic protein α-BTX N3.

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Source of Support: Nil, **Conflict of Interest:** None.