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

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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
Bungaroides candidus (Malayan krait) belongs to Elapidae family and is usually found in Asian countries. Snakes from the Bungarus genus are commonly known as kraits. Bungaroides candidus was reported to be one of the most poisonous snake species in Southeast Asia. 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 Bungaroides candidus. 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.

Krait venoms possess acetylcholinesterase, hyaluronidase 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. 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 Bungaroides 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. 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. In Indian villages, many plants are widely used against snakebites. 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. Antivenome herbal drugs are 4-Nerolidol, Cretol, Aristolochic Acid, Cabenigrin A2, Cabenigrin A1, Edunol, Tectoridin, Wedelolactone, Ellagic Acid, Curcumin, Atropine, Salireposide, Myricetin, Quercetin, Rosmarinic, Resveratrol, 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

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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. 

**Model Evaluation**

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

**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.

**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 *Cabeça-de-negro*, Cabenegrin A1 from *Cabeça-de-negro*, Edunol from *Harpylyce brasiliana*, Tectoridin from *Belamcanda chinensis*, Wedelolactone from *Eclipta alba*, Ellagic Acid from *Casearia sylvestris*, Curcumin from *Curcumin longa*, Atropine from *Atropa Belladonna*, Salireposide from *Symlocos racemosa*, Myricetin from *Mouriri pusa*, Quercetin from *Byrsonima crassa*, Rosmarinic acid from *coddial verbenaec*, Resverotrol from *Cissus Assamica*, Oleanolic acid from *Phytolacca americana*, Chlorogenic acid from *Coffee 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. 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
Table 1: Selected antivenomic plants compounds

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

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. 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 amino acid 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 on the parameters of ic50, Mi-LogP, TPSA, Natoms, Molecular Weight (MW), Volume, Formula

Antivenomic plants compounds were used as data set to build QSAR model, (Fig.4) performed by using Multiple linear regression (MLR). 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).

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

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 \( \alpha \)-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 \( \alpha \)-BTX N3 protein. The above antivenomic plant compounds bind to the active site region of the \( \alpha \)-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 GLU4 binds with atom O19 with distance 1.98, amino acid VAL56 binds with atom O19 with distance 3.13. (Fig.6)

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

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

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 \( \alpha \)-BTX N3. The minimum binding energy values obtained between Tectoridin with \( \alpha \)-BTX N3 -7.62 kcal/mol, Wedelolactone with \( \alpha \)-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 \( \alpha \)-BTX N3.
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