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Original article

Computational analysis of bioactive phytochemicals as potential inhibitors for calcium activated potassium channel blocker, tamulotoxin from *Mesobuthus tamulus*

R. Barani Kumar*, M. Xavier Suresh

Department of Bioinformatics, Sathyabama University, Rajiv Gandhi Road, Chennai 600 119, Tamil Nadu, India

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ABSTRACT

Several research works are being carried out on natural medicines because they are cost effective and play a vital role in providing a permanent remedy. Phytochemicals are pharmacologically important source of plant products which targets many diseases especially finding antidote for envenomation. Accidental toxin bite becomes highly lethal due to lack of proper treatment. The red Indian scorpion, *Mesobuthus tamulus* produces a highly toxic protein called tamulotoxin (TmTx). TmTx blocks the $Ca2^{+}$ activated K^{+} ion channels, which is responsible for cellular proliferation and migration. Several antidotes were used for neutralizing the action of scorpion bites because most of the synthetic drugs produce undesirable side effects. In this work, we have found some of the potential lead molecules (chemical compounds and their analogs) obtained from plant source and a series of bioinformatics based studies including computational analogs search, virtual screening, pharmacokinetic profiling and molecular interaction were executed. From this study, we suggest that some of the potential ligands having a therapeutic effect against TmTx protein. This work will help researchers to enhance their analysis toward designing a protocol for antidote based therapy against TmTx protein.

1. Introduction

Ion channels play a significant role in cellular mechanism of *Homo sapiens*. Each ion channel has a different function according to its transport across the membrane proteins.[1] Among several ion channels, calcium activated potassium channels are the fundamental regulators of neuronal excitability.[2,3] Venomous neurotoxins from various toxin species produce a unique toxin for targeting a particular species of biological target, especially ion channels. Malfunctioning of ion channel proteins is inhibited by various toxins like neurotoxin.[4] Neurotoxins mainly target K^{+} channels and collapse the signal transmission between neurons, which cause a series of neurological problems.[5]

According to recent studies among several toxin bites, scorpion bites are reported in maximum numbers in India.[6] Several scorpion species produce various toxins with diverse polypeptides, which modulate the vital functions of ion channels.[5] Scorpion toxins have low molecular mass. Hence they are categorized in several ways based on the targeted action like cardiotoxin, nephrotoxin, hemolytic toxin, etc. Some neurotoxin of scorpion venom is deadlier than neurotoxin of snake venom.[7] The LD50 value of some scorpion neurotoxins has been analyzed to be more potent (>10 fold) than cyanide.[8] Tamulus toxin (or) tamulotoxin (TmTx) is a novel 36 residues protein which is found in Indian scorpion of eastern region (*Mesobuthus tamulus*). It has a potential activity on potassium channel.[9,10] Tamulus toxin is a novel protein and shares no homology with other species of scorpion, although the positions of the six cysteine residues share the same structural folds.[11] In India, several cases of scorpion bites are being reported every year and most of the cases are in highly pathetic condition and we have only few a few antidotes for scorpion sting. But most of them show less precise in their targeted action and also common to its general action.[12–17]

Plants are good source of medication from many centuries and till date they have a maximum therapeutic index. More number of natural products like plant derivatives and analogs represent more than 50% of drugs for clinical purposes.[18,19] In this work, we suggested some of the plant based bioactive antitoxic compounds as a lead molecule for scorpion bites through computational bioinformatics analysis. In our work, we have taken the structural analogs from plant species of *Ocimum sanctum*, *Ocimum basillicum*, *Andrographis paniculata*, *Achyranthes aspera*, *Atropa belladona*, *Argemone*

* Corresponding author. Tel.: +91 44 2450 3245; fax: +91 44 2450 2344.
E-mail address: baranisathyabama@gmail.com (R. Barani Kumar).

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ochroleuca and *Martynia annua* through several chemical databases and various drug-likeliness properties are screened. Then all filtered compounds were allowed for pharmacokinetic profiling and molecular docking studies using several bioinformatics tools. Further compounds were retrieved on the basis of their molecular interaction with active site amino acids of tamulotoxin protein.[20,21]

2. Materials and methods

2.1. Bioactive compound selection

Bioactive plants and their phytochemicals having antitoxin properties were studied from several literature. Chemical structures of the extracted compounds and their analogs were searched in chemical databases like PubChem (http://pubchem.ncbi.nlm.nih.gov/), Drug Bank (http://www.drugbank.ca/), KEGG Ligand (http://www.genome.jp/kegg/ligand.html) and Zinc (http://zinc.docking.org/).Some structures were drawn manually in ACD ChemSketch 10.0. Suitable ligands were searched and filtered on the basis of their drug-likeliness.

2.2. Pharmacological based ligand screening

Structural analogs were searched for the selected structures of plant compounds and their derivatives. All selected compounds are further allowed for screening to analyze the level of their drug-likeliness. In this method, we have used Lipinski's rule and tanimoto score as a measure of drug-likeliness. As a result of pharmacological based ligand screening, we have obtained analogs with better drug like properties. Selected structural analogs of compounds were downloaded for further studies.

2.3. Pharmacokinetic profiling

The selected analogs were allowed for further *in silico* based validation studies by analyzing their pharmacokinetic activities. In this study we used, ADMET (Absorption, distribution, metabolism, elimination and toxicity) descriptors of Accelrys Discovery Studio (ADS) 2.0 as an analytical tool. All selected compounds were analyzed based on the default threshold values of each descriptor like blood brain barrier (BBB), solubility, hepatotoxicity, Cytochrome P450 oxidase enzyme (CYP2D6) activity and plasma protein binding (PPB).[22]

Table 1
Bioactive plants and their derivatives and number of retrieved analog compounds.

Bioactive phytochemicals	Name of plant derivatives	No. of analogs retrieved
Achyranthus aspera	Betaine	68
Martynia annua	Chlorogenic acid	507
	Terpenoid	73
	Anthraquinones	254
	Tannins	586
Argemone ochroleuca	Glycosides	80
	Flavanol	3128
	Berberine	1035
	Linoleic acid	1661
	Oleic acid	1653
	Palmitic acid	787
	Chelerytherine	457
	Myristic acid	646
	Optisine	680
Atropa belladonna	Atropine	485
Ocimum sanctum	Eugenol	1217
Ocimum basillicum	Linalool	113
	Eugenol	1217
	1,8-cineole	100
	Bergamotene	93
Andrographis paniculata	Andrographolide	148
	Deoxygrapholide	100
	Neographolide	

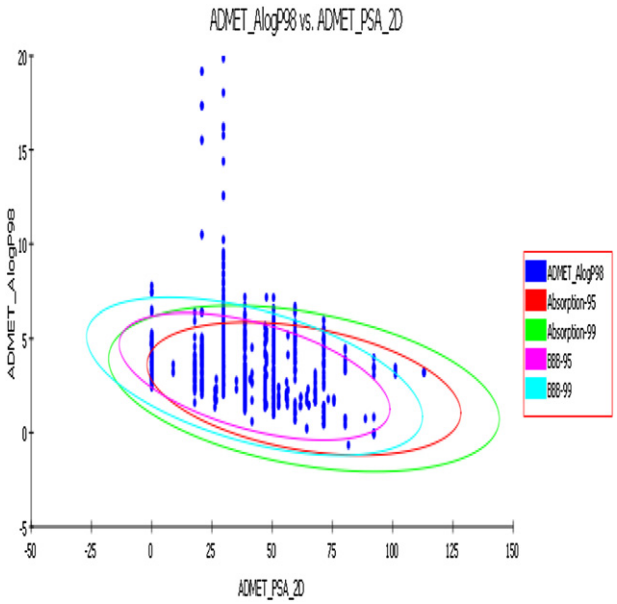

Fig. 1. Analogs passed in all levels of ADMET descriptor analysis.

2.4. Target protein selection and active site prediction

The three dimensional structure of target protein (TmTx) was unavailable in macromolecular structural databases. Hence, we

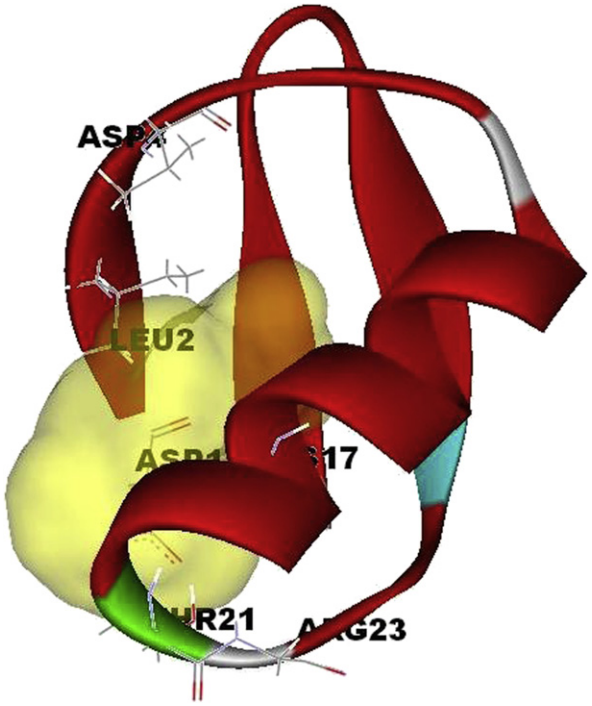

Fig. 2. Predicted active site pocket in surface view along with entire structure of tamulotoxin (TmTx) in ribbon view.

have predicted the three dimensional structure using homology modeling method in our previous work by selecting the suitable template (PDB ID: 1M2S) with a sequence identity of 77.1% between template and target. The final structure was validated in Ramachandran plot with 87.5% of residues in favorable region, 9.4% of residues in additionally allowed regions, 3.1% of residues in generously allowed region and none of the residues were found in disallowed region.[23] Energy minimization and stabilization process were done for overcoming the steric hindrance of the macromolecule by applying CHARMM force field.[24] Active site of target protein, TmTx was predicted using Q-site finder and verified with ADS 2.0.[25]

2.5. Molecular interaction studies of drug like compounds

Molecular interaction studies were done for understanding the strength of interactions like intermolecular bonding and electrostatic forces using AutoDock 4.0[26,27] and confirmation of results were analyzed using ADS-Ligand-Fit 2.0.[28] Except a few set of analogs, most of them produced better interactions with maximum dock score and H-bonding with the active site amino acids of TmTx protein.

3. Results and discussion

3.1. Bioactive plants and their analogs selection and validation

From literature search, we have identified that seven plants (*O. sanctum*, *O. basilicum*, *A. paniculata*, *A. aspera*, *A. belladona*, *A. ochroleuca* and *M. annua*) are having a potential therapeutic value along with devenomic properties against several toxin bites like scorpion and snakes bite.[21,29] Based on this information, we have identified suitable analogs of compounds through similarity based structure search and substructure search in chemical databases. Analogs were used for further virtual screening and the selection was based on the threshold value of tanimoto score. In order to achieve this, we have chosen analog compounds by setting tanimoto score into 0.2. Hence compounds of all selected plants (23 bioactive compounds) and their retrieved analogs (16,755 analogs) obtained from chemical databases are tabulated in Table 1.

Table 2
ADMET descriptors analysis and molecular interactions of selected best analog compounds.

Analogs compound ID	ADMET analysis results						Molecular docking and H-Bonding interactions		
	BBB	ABS	SOL	HEPTOX	CYP2D6	PPB	AutoDock binding energy (Kcal/mol)	DS-LigandFit dock score	H-bonding amino acids and its Distance(Å)
A. paniculata									
44393882	3	0	3	0	0	0	−5.93	39.10	L2, C17 1.99, 3.15
25121240	3	0	3	0	0	0	−4.18	38.61	L2 2.2
10293055	2	0	3	0	0	0	−4.00	37.39	L2, D4 1.43, 2.89
O. basillicum									
10820923	3	0	5	0	0	0	−4.82	61.52	C17, (2)T21 2.89, 2.95, 2.07
18409046	3	0	5	0	0	0	−0.34	54.38	L2, T21 1.92, 2.72
17834762	3	0	5	0	0	0	1.54	54.37	D1, D4 3.13, 2.31
M. annua									
5281400	0	3	1	0	2	1	−4.71	49.06	L2, D4 2.23, 2.31
115721	1	0	3	0	0	0	−1.58	43.78	(2)L2 2.39, 2.23
102235	1	0	3	0	0	0	−3.78	41.50	D1 3.07
A. aspera									
174174	3	1	3	0	0	0	3.21	34.81	T21 2.47
15238096	3	0	3	0	0	1	1.54	25.58	D4, C17 2.75, 2.48
81911	2	0	4	0	0	1	5.74	22.10	C17 2.79
A. ochroleuca									
445639	0	1	3	0	0	2	−2.74	40.03	C17 2.82
240630	0	1	2	0	0	2	−2.54	37.71	D4 2.25
31211	4	2	2	0	0	2	−1.82	37.48	D4 2.32
O. santum									
5280450	3	1	2	0	0	0	2.65	35.66	C17 3.14
53796082	3	0	5	0	0	0	3.52	32.40	D4 2.48
542421	3	0	5	0	0	0	2.35	31.04	T21 2.85

BBB—Blood brain barrier level; ABS—Absorption level; SOL—Solubility level; HEPTOX—Hepatotoxicity level; CYP2D6—Cytochrome oxidase enzyme activity level; PPB—Plasma protein binding level.

3.2. Pharmacokinetics studies of plant analog compounds

All screened compounds were allowed for the next level pharmacokinetic study to prevent the failures involved in inhibitor analysis. Compounds were removed based on violations in ADMET descriptors. In this study, all descriptors were set as default and resultant compounds were chosen for further study. As a result of this screening, 9062 analogs were eliminated due to its failure in the confidence levels (low solubility, high penetration levels in blood brain barrier level, low binding nature with plasma protein and high toxicity nature of compounds) of ADMET descriptor analysis. Hence, only 7701 analogs were considered by cross validating with all confidence levels of ADMET screening. From the result of screening, we have found that many compounds were poor either in PPB or BBB. As a result of this study, we found only 996 analogs were passed in all levels of ADMET screening and the detailed result of ADMET representation is given in Fig. 1. Analogs with strong pharmacokinetic strength were taken for next level. Finally, the selected compounds were allowed for *in silico* molecular docking analysis.

3.3. Binding site and molecular docking analysis

Active site was identified using Q-Site finder (http://www.modelling.leeds.ac.uk/qsitefinder/) and confirmed using ADS-binding site prediction program. From the active site search, we found ASP 1, LEU 2, ASP 4, CYS 17, THR 21 and ARG 23 were present in an active site and responsible for inhibitory mechanism on ion channel (Ca^{2+} activated K^{+} channel) protein. The detailed active site residues along with the secondary structure of TmTx protein were given in Fig. 2. Identified active site residues were used for molecular docking studies with selected ligand set. Molecular docking was performed in Ligand-Fit program of Discovery Studio 2.0. Using this program, TmTx were docked against a list of selected plant compounds and its analogs. Each compound generates different kinds of interactions with maximum docking score. The docking results were analyzed with five statistical functions (Ligand score 1 & 2, PLP (Piecewise Linear Potential) 1 & 2, PMF (potentials of mean force) and Jain score), dock scores along with H-bonds formed between ligands and active site amino acids were also considered. When we analyzed the docked complexes of natural bioactive compounds for each plant species, we have received some of the amazing results. Most of the bioactive compounds had a maximum binding affinity with TmTx toxin protein.

Among the selected plant species, *A. paniculata* was used as an ancient medicine based on its pharmacological properties against toxin bites.[30–32] *A. paniculata* and their analog compounds showed significant results in *in silico* studies of molecular interaction (both bonded and non-bonded interaction) with target protein. At the same time bioactive compounds and analogs of *O. basillicum* showed good results in pharmacokinetic profiling and non-bonding interactions with vital residues involved in blocking the action of ion channels. Several compounds were selected through a series of studies based on computational biology and we have obtained three top scoring compounds from each plant species through *in silico* analysis and the detailed results of docking analysis along with the details of hydrogen bonding are tabulated in Table 2. Validation studies for selecting best compounds were carried out by analyzing the binding energy of the docked complex. The ligands with best dock score were given in Table 2 and their lead molecules are given in Fig. 3. From the overall interactions studies, most of the selected ligand binds with amino acids (Asp1, Leu2, Asp4, Cys17 and Thr21) which are important for the crucial activity of tamulotoxin. Most of the active site residues of tamulotoxin (Asp1, Leu2, Asp4 and Cys17) are responsible for blocking ionic pores, which are vital for ionic transport located between S5–S6 segments of ion channel. Moreover Thr21 and Arg23 were showed as important with TmTx protein because they give more stability to the toxin structure. Among plant analogs, five compounds showed better activities and the best active compounds are CID 44393882 ([(3S,4E)-4-[2-[(1S,2S,4aS,5R,6R,8aR)-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethylspiro[3,4,4a,6,7,8-hexahydro-1H-naphthalene-2,2′-oxirane]-1-yl] ethylidene]-5-oxooxolan-3-yl] acetate) analog of *A. paniculata*,

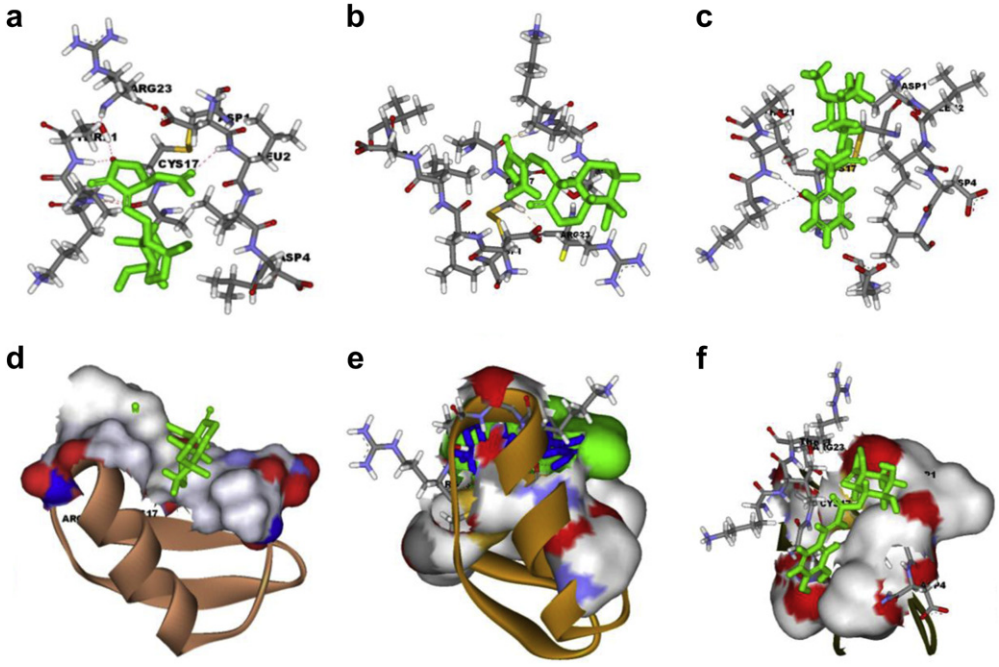

Fig. 3. Atom level interactions of selected best analogs of (a) *A. paniculata*; (b) *O. basillicum;* (c) *A. aspera* and the corresponding analogs bound at the binding site of TmTx protein represented in surface view (d) CID 44393882 (*A. paniculata*); (e) CID10820923 (*O. basillicum*); (f) CID174174 (*A. aspera*).

CID10820923 ((1R,5R)-6,6-dimethyl-4-propylbicyclo[3.1.1]hept-3-ene) from *O. basillicum*, CID174174 ([(1R,5S)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl] 3-hydroxy-2-phenylpropanoate) from *A. aspera*.

4. Conclusion

Phytochemicals of plant source with pharmacological importance is an alternative approach for the modern medication against several toxic envenomations. According to recent studies, usage of phytochemicals are reported as the most reliable and efficient compounds with therapeutic significance. *In silico* studies provide an insight for developing novel inhibitors against tamulotoxin using plant based bioactive chemicals with their analogs. The inhibitory efficiency of selected compounds were computed and analyzed through number of hydrogen bonds formed between the target protein and the ligand molecule, electrostatic interactions and dock score. In this study we found the selected analogs of *A. paniculata* and *O. basillicum* were efficient against TmTx protein. Computational virtual screening, pharmacokinetic and molecular interaction studies will help researchers to design novel inhibitors for neutralizing the action of tamulotoxin.

Conflicts of interest

All authors have none to declare.

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