Pharmacophore mapping based inhibitor selection and molecular interaction studies for identification of potential drugs on calcium activated potassium channel blockers, tamulotoxin

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ABSTRACT

Background: Tamulotoxin (TmTx) from *Buthus tamulus* was found to be a highly venomous toxin which accelerates the neurotransmitter release that directly affects the cardiovascular tissues and the respiratory system leading to death. TmTx from red Indian scorpion is a crucial inhibitor for Ca^{2+} activated K^+ channel in humans. **Objective:** The study is aimed at the identification of potential inhibitors of TmTx through pharmacophore based inhibitor screening and understanding the molecular level interactions. **Materials and Method:** The potential inhibitors for TmTx were identified using pharmacophore model based descriptor information present in existing drugs with the analysis of pharmacokinetic properties. The compounds with good ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) descriptors were subjected to molecular interaction studies. The stability of bound toxin-inhibitor complex was studied using molecular dynamics simulation over a period of one nanosecond. **Results:** From a dataset of 3406 compounds, few compounds were selected as potential inhibitors based on the generated best pharmacophore models, pharmacokinetic analysis, molecular docking and molecular dynamics studies. **Conclusion:** In conclusion, two compounds containing better inhibition properties against TmTx are suggested to be better lead molecules for drug development in future and this study will help us to explore more inhibitors from natural origin against tamulotoxin.

Key words: Absorption, Distribution, Metabolism, Excretion and Toxicity, HipHop, ion channels, molecular docking, pharmacophore, tamulotoxin

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INTRODUCTION

Most of the functions involved in the cellular mechanisms of many human proteins are still unrevealed. Among these proteins, the roles played by ion channel proteins are more crucial as they are responsible for several neurological diseases with severe side effects and high mortality rate. Many researchers have been focusing their work on Ca^{2+} activated K^+ ion channels and their functions because of the blocker toxins, which play a key role in ion channels.[1-3] The origination of neurotoxin from animals become one of the main threats as several short-chain neurotoxins produce a high-level of blockage action in ion channel proteins, which leads to adverse reaction in the host cells.[4,5]

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Tamulotoxin (TmTx) from *Buthus tamulus* was found to be a highly venomous toxin. It accelerates the neurotransmitter release, which directly affects cardiovascular tissues and respiratory system to lead to death.[6,7] This toxin had been classified under short chain toxin having a low molecular mass of 4213 Da. The highly stabilized toxin structure possesses three intra-molecular disulphide (S-S) bonds, which always gives a strong and stable conformation.

Numerous drugs were discovered for sting of TmTx and some of them are showing priceless effects on toxicity. In this category, prazosin occupies an important place and it is a potential drug used for *Buthus* envenomation.[8] Most of the research works on scorpion bites were resolved by prazosin and prazosin was identified as a potential antidote.[9,10] The other three compounds had also occupied the remaining important places like digoxin, digitoxin and dobutamine. It has the significant effect against TmTx

with minimal inhibition concentration (IC50). Even serious effects of scorpion bites were treated with these drugs.[11-13] However, there is a need for developing a better and more potent antidote specific for scorpion bites. This can be achieved by a new and alternate drug designing strategy, i.e. pharmacophore based drug designing.[14]

Several molecular interaction studies and inhibitor designing studies for biological toxins have been carried out using computational biology tools.[15] The earlier computational biology studies on toxins by our group with analog based virtual screening and docking strategies had found new potential inhibitors against various toxins.[16,17] It is worthy to mention that analyzing the toxins with their receptors at molecular level had provided reasonable results and relatively novel findings.[18] In this study, the three-dimensional (3D) structure of TmTx predicted using comparative modeling techniques and stabilized using molecular dynamics (MD) simulation was prepared for further *in silico* analysis. We employed pharmacophore model based compound selection for identification of potential compounds from the compound library. The best pharmacophore model was selected based on the best-fit value obtained from HipHop program in Accelrys Discovery Studio (ADS) and was used to search against the drug like database, Minimaybridge. Molecular interaction studies and MD simulation studies have also been performed. From these studies, we have obtained a series of compounds showing wide range of properties in different levels of screening, and two compounds with good interactions proposed to be the alternative lead molecules for tamulus scorpion sting.

MATERIALS AND METHODS

Inhibitor selection and construction of compound database

Strengthening reliable information is the fastest way in drug discovery process. Existing therapeutic agents for scorpion stings were searched and potent antidotes were fetched out from various sources such as literatures, chemical databases, and so on. Prazosin, dobutamine, digoxin, and digitoxin were found to be the best antidotes for red scorpion bites. In order to get reliable pharmacophore models, we have chosen structurally similar drugs of these three compounds. Training set was constructed by considering each compound with high structural similarity and similar pharmacological properties. These compounds were further analyzed for generating common features, which will help in the selection of a suitable inhibitor.

Common feature pharmacophore generation for tamulotoxin

The key features that are responsible for biological function were generated using pharmacophore model generation.[19] The potential ligands with therapeutic background were used for constructing common feature based pharmacophore model. Initially, molecular interaction studies were performed to identify the activities of all compounds. The parameters like principal value, maximum omit feature value and minimum inter feature distances were set to 2.0 and 2.97 Å respectively, and the common feature pharmacophore generation protocol was executed with diverse set conformations, generated using diverse conformation generation protocol. With the result of common feature pharmacophore model generation, desired chemical groups were identified using feature mapping protocol. Best featured model were selected using common feature pharmacophore generation protocol in ADS because this protocol uses HipHop module of catalyst program to derive the best featured model with vital descriptors like hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), hydrophobic (HY), ring aromatic (RA), positive ionizing (PI), and negative ionizing (NI).[20,21] From the generated 10 pharmacophore models, we have selected the best one based on the descriptor set and rank, which is further used to search against MiniMaybridge database in ADS.[22] The obtained compounds were further screened based on the fit value (fit value $\geq$ 2) and this selection parameter, yielded compounds with the specified features for supplementary study.

ADMET based screening

The datasets obtained from MiniMaybridge database are subjected to ADMET screening in ADS 2.0 to derive the drug likeliness. We have analyzed six pharmacokinetic properties, including absorption, solubility, blood brain barrier (BBB), hepatotoxicity, plasma protein binding (PPB) and cytochrome P450 (CYP) D6 enzyme inhibition study.[23] Results based on the above mentioned parameters were analyzed according to the limitation of software. The compounds, which show efficiency in the level of drug likeliness were further allowed to molecular interaction studies.

Molecular interaction study through docking

Compounds with good pharmacokinetic properties were opted for molecular interaction studies. TmTx, the target protein was prepared by adding hydrogen atoms with chemistry at Harvard molecular mechanics (CHARMm) force field. In order to get the stable conformation of target protein, 2000 steps of iterations were performed with Smart minimizer protocol in ADS for energy minimization.[24] Binding site of the protein molecules were identified using Q-site finder and validated using ADS-binding site analysis tool.[25] *In silico* interaction studies were carried out using LigandFit program in ADS. LigandFit is a docking program and it will do three important tasks while docking the ligand

into the active site of the target protein. First, it will dock a set of compounds into the binding site of protein molecule. Then it minimizes the ligand energy when binding to the receptor protein. Finally, it scores the interaction and filters the best scored poses of the ligand. It also provides reliable results among the existing docking programs. Best poses of each ligand was examined with the calculation of Root mean square deviation (RMSD) threshold diversity (1.5 Å). We have used different scoring functions including piecewise linear potentials (PLP1 and PLP2), potential mean force (PMF), Jain Score, Dock Score and ligand internal energy.[26] Compounds showing better scores were taken for further analysis. In addition to this, we have also analyzed properties like H-bonding and non-bonded interactions.

Molecular dynamics simulation studies on toxin-inhibitor complex

Molecular dynamics (MD) simulation studies were carried out to know the strength of the interaction between the toxin and inhibitors and the stability of the complex. For this study, we have used the Standard Dynamics Cascade program present in ADS. This program was executed with 1000 steps of Steepest Descent algorithm followed by 2000 steps of conjugate gradient algorithm.[27] In heating process we have used 1000 steps with the time duration of 0.001 ps and initial temperature was set to 50 K with the target temperature of 300 K. In equilibration, iterations were set to 1000 and the remaining parameters were kept default. The final production step with 1,000,000 cycles of thermodynamics ensemble had been performed with constant volume ensemble (NVT) to control temperature throughout the simulation process.

RESULTS AND DISCUSSION

Compound database and common feature pharmacophore generation for tamulotoxin

Compounds with good antitoxic effects were explored via various resources, from that prazosin, dobutamine, digoxin, and digitoxin were found to be the best drugs which are being used for scorpion bites.[12,13] From this information we have generated 3D database for these four compounds. Initially we have performed interaction and pharmacokinetic studies for these four compounds with TmTx. Initially we made a small database of these four compounds and it was used as input for pharmacophore generation. Common feature pharmacophore generation protocol had been used for this study. These selected inhibitors shows diverse set of pharmacophore features.[28] In order to retrieve the best model, most of the analyzed parameters were HBD, HBA, HY, PI, and NI. As a result, 10 pharmacophore models were generated using HipHop program and the best pharmacophore was analyzed and the consolidated results are given in Tables 1 and 2. From these results, seven Donor acceptor acceptor (DAA) and three hydrophobe Donor acceptor (HDA) descriptors were present in the derived pharmacophore models with scores ranged from 28.340 to 26.338. Hence, best pharmacophore models were selected based on the best fit value and used for further studies.[29] These best models were found to have the most common features with more or less same interfeature distances with different orientation of vectors HBD and HBA. Hypo 1 shows the best features for further studies. The best derived pharmacophore model with the compounds is given in Figure 1.

Database searching

Pharmacophore featured compounds retrieved from MiniMaybridge database using search 3D Database protocol. We have obtained 3406 hits after running the program with fit values ranging from 0.5 to 2.8. Then the selected compounds were filtered based on the fit value ≥2. As a result of filteration, we have obtained a few hundred compounds. In order to analyze the rest of the compounds with less pharmacophore features, we have reduced the fit value to 1. Then the best compounds with all specified properties are selected by removing less featured compounds (fit value ≤1).

Pharmacokinetics profiling

The filtered compounds were further examined by their

Table 1: Common feature pharmacophore results table

Hip Hop hypothesis	Ph. features	Rank	Direct hit	Partial hit	Max. fit value
Hypo 1	DAA	28.340	1111	0000	3
Hypo 2	DAA	28.168	1111	0000	3
Hypo 3	DAA	27.944	1111	0000	3
Hypo 4	DAA	27.944	1111	0000	3
Hypo 5	HDA	27.683	1111	0000	3
Hypo 6	DAA	27.390	1111	0000	3
Hypo 7	DAA	27.317	1111	0000	3
Hypo 8	DAA	27.054	1111	0000	3
Hypo 9	HDA	26.338	1111	0000	3
Hypo 10	HDA	26.338	1111	0000	3

DAA: Donor acceptor acceptor, HDA: Hydrophobe Donor acceptor

Table 2: Training set compounds in pharma cophore models

Compound name	Fit values				
	Hypo 1	Hypo 2	Hypo 3	Hypo 4	Hypo 5
1	3.000	3.000	3.000	3.000	3.000
2	2.648	2.242	2.344	2.186	1.523
3	1.665	1.653	1.784	1.165	1.477
4	1.24	1.026	0.954	0.861	0.527

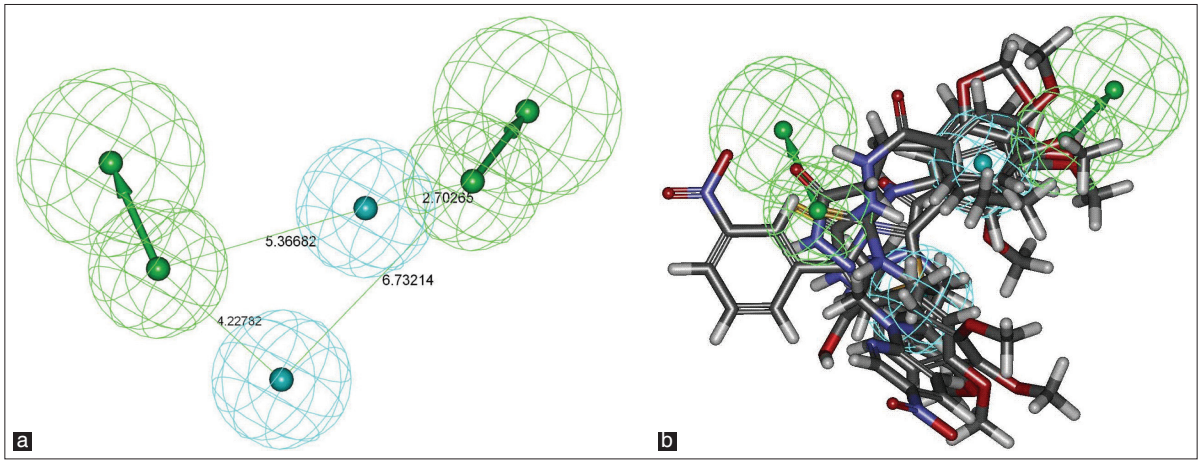

Figure 1: (a) Generated the best pharmacophore model; (b) interfeature distance with the overlaying compounds of similar pharmacophore features

pharmacokinetic and toxicity background using ADMET descriptors analysis protocol in DS. Different levels of parameters were used for this study including BBBs, solubility, bioavailability, hepatotoxicity, etc. to analyze all 3406 compounds. According to the Discovery Studio parameters, standard analysis value like level 0 for human intestinal absorption, level 3 and level 4 for solubility, level 0 for non-inhibitory property with CYP450 2D6, level 3 for BBB penetration and level 0 for non-toxicity were filtered for obtaining drug like compounds. Finally, 217 compounds were obtained and used for further molecular interaction studies.

Molecular interaction studies through docking

Molecular interaction studies were performed for selected compounds with drug likeliness properties using LigandFit protocol of ADS. The target protein (TmTx) with minimized energy and simulated orientation was obtained by applying CHARMm force field. Binding site of the TmTx was predicted using Q-site finder and validated with binding site prediction tool in ADS.[29] As a result of this, we have found only one binding site present in this toxin protein with the volume of 132.750 $Å^3$ with amino acids Asp1, Leu2, Arg4, Cys17, Thr21 and Arg23. Database compounds with good pharmacokinetic background were docked into the binding site of TmTx using space complementarity docking present in Ligand Fit tool of ADS. Five scoring functions have been used to achieve best inhibitors. Ten best poses were retrieved for each ligand with dock score. Other scoring functions were also considered for selection of best compounds.[28] In total 88 compounds have shown better results and dock score >17.5 were taken for analyzing their inhibitory properties. Among the 88 compounds only 12 compounds satisfy all levels of scoring functions then these compounds are screened for H-bonding interaction. At this level, only five compounds were exhibited their interaction with active site residues of toxin protein. The best five compounds were taken based on their pharmacokinetic profile and these potential ligands interact well with the binding site residues are depicted in Figure 2 and the details of interactions are given in Table 3.

The MiniMaybridge database contains five compounds namely HTS03335, CD02928, ML00365, HTS00263, and BTB01034. The detailed chemical structures of the selected compounds are depicted in Figure 3. ML00365 and BTB01034 have reliable dock scores and good interactions with binding site residues among five potential inhibitors. ML00365 has H-bonding, interactions with Asp1 and Thr21. BTB01034 has H-bonding interaction with Leu2. Asp1 with H41 of ML 00365 has formed strong bonding, which modulates the activity of toxin. Another important bonding between Thr21 and ML00365 possesses both acidic/basic and oxidative features. The detailed dock scores with various scoring functions are given in the Table 4.

Molecular dynamics simulation studies of tamulotoxin-inhibitor complex

The stabilization of the docked complex was studied using MD simulation studies. For this study, we have used ADS-Standard Dynamics Cascade program for analyzing the inhibitor bound complex molecules with CHARMm force field. Analysis of the dynamics trajectories revealed that the inhibitor bound toxin complex was well stabilized. It is to note that in addition to the interactions with the binding site residues, there exists a water molecule which provides a strong frame work for the stability through water mediated interactions, which may also implicate in toxin specificity. The detailed studies of dynamics and obtained final energies were given in Figure 4.

CONCLUSION

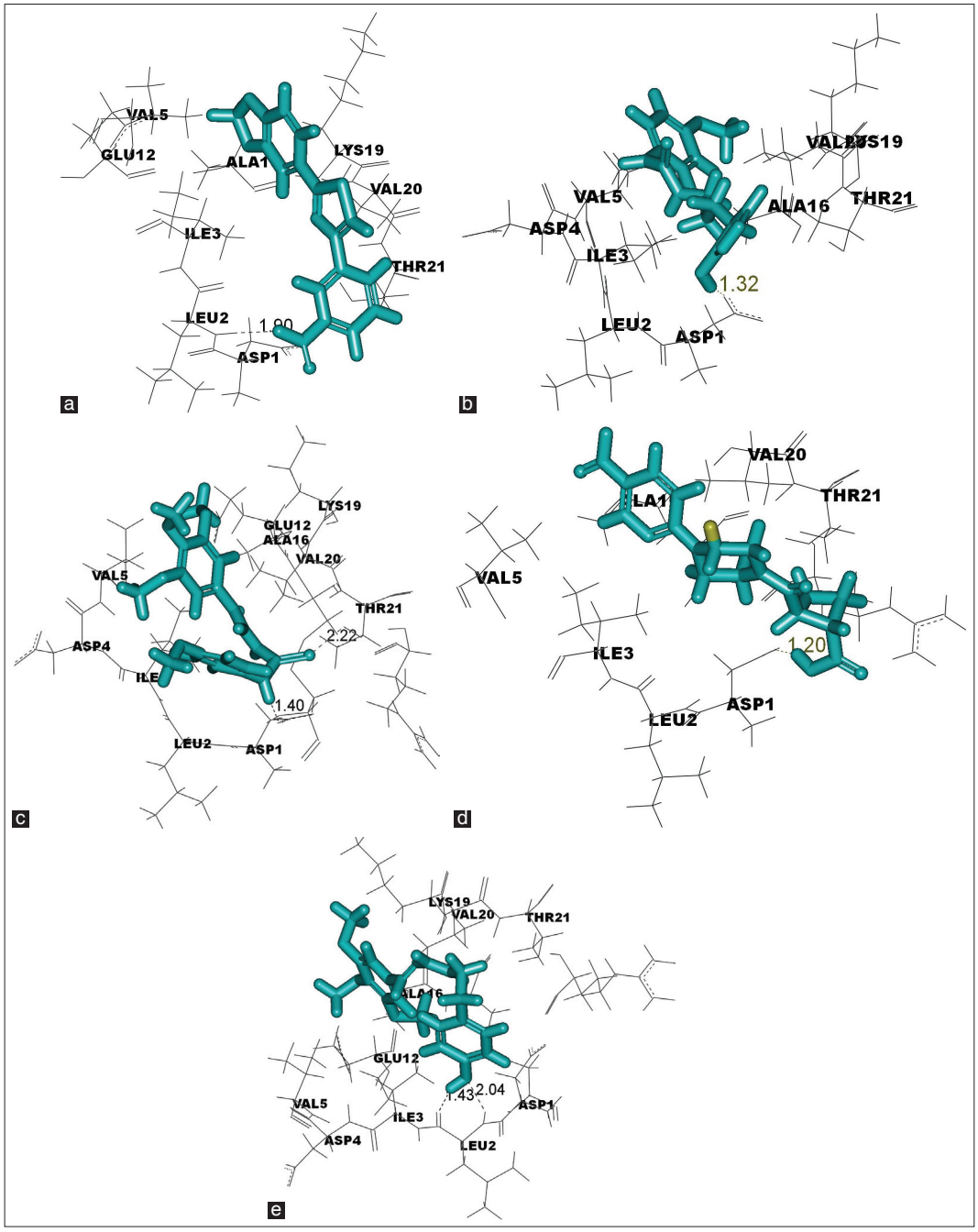

Figure 2: Interactions of selected inhibitors at the binding site (a) HTS03335, (b) CD02928, (c) ML00365, (d) HTS00263, and (e) BTB01034

Table 3: Pharmacokinetics profiling and molecular interactions of the selected inhibitors

Compound	ADMET descriptors analysis						Molecular interactions			Hydrogen bonding distance (H…A) (Å)
							Tamulotoxin		Inhibitor	
	BBB	Abs	Hep. Tox.	CYP	PPB	Solubility	Residue	Atom	Atom	
HTS03335	2	0	0	0	2	3	Leu2	NH	O1	1.90
CD02928	3	0	0	1	2	3	Asp1	OD1	H31	1.32
ML00365	3	0	0	0	2	3	Asp1	OD1	H41	1.40
							Thr21	O3	HG1	2.22
HTS00263	3	0	1	1	2	2	Asp1	OD1	H32	1.20
BTB01034	3	0	0	0	0	3	Leu2	O	H48	1.42
							Leu2	NH	O26	2.04

BBB: Blood brain barrier level; Abs: Absorption level; Hep.Tox: Hepatotoxicity level; CYP: Cytochrome P450 2D6 (CYP2D6) level; PPB: Plasma protein binding level

Table 4: Ligand Fit scoring functions of selected compounds

Compound	LigScore 1	LigScore 2	PLP 1	PLP 2	Jain	PMF	Dock score	Fit value
HTS03335	1.82	2.81	36.33	38.8	−0.56	28.92	20.869	2.297
CD02928	0.84	2.19	10.95	11.72	−1.25	51.96	26.852	2.001
ML00365	1.88	3.11	28.16	23.39	−1.19	48.36	27.844	1.29
HTS00263	1.9	3.13	23.87	21.58	−2.33	60.36	28.777	1.29
BTB01034	2.05	3.27	23.82	22.09	−0.04	54.68	28.962	1.245

PMF: Potential of mean force; PLP: Piecewise linear potentials

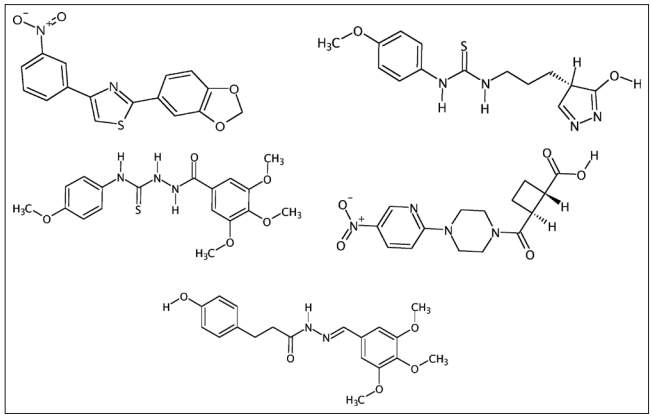

Figure 3: Chemical structure of potential inhibitors revealed through screening and molecular interaction study

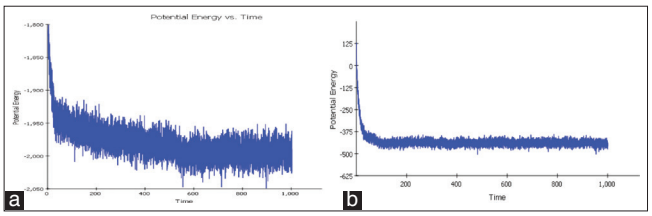

Figure 4: Analysis of the energy profile of the MD simulation trajectory of (a) tamulotoxin–ML00365 complex and (B) tamulotoxin–BTB 01034 complex

Even though there are many drugs available for scorpion stings, the activity and safety of those drugs are always a concern. Common feature pharmacophore models were developed and the best pharmacophore model was used to search the reliable pharmacophore feature for selecting compounds from MiniMaybridge database. The final screening of compounds was carried out based on the pharmacokinetic and interaction studies with the validation of molecular dynamics simulation. As a result of different levels of analysis we have selected five compounds namely HTS03335, CD02928, ML00365, HTS00263 and BTB01034. These five compounds are further sorted based on the LigandFit scoring functions. Among these compounds, ML00365 and BTB 01034 showed better results in all levels of this study. The MD simulation studies were carried out mainly to explore the strength of interaction between the toxin and a ligand. Further *in vitro* studies are needed to confirm the activity of the selected inhibitors to identify the functionality of these compounds as alternative drugs for *tamulus* toxin.

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