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In silico Identification of Potent Platelet-aggregation Inhibitors for Clumping Factor-A of *Staphylococcus aureus*

Ramadevi Mohan and Subhashree Sankar*

Biomolecules and Genetics division, School of Bio-Sciences and Technology,
VIT University, Vellore - 632007, India.

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Staphylococcus aureus **is known for large repertoire of virulence factors including various enzymes, toxins and cell surface-associated proteins leading to various diseases and infections in host. One among them is Clumping factor A (Clf A), a cell surface-associated protein that causes aggregation of platelets by clumping the bacteria in host blood causing infective endocarditis (IE). In this study, we attempted to identify potent inhibitor for the drug target Clf A of *Staphylococcus aureus* by *in silico*. The Pubchem compound database was used to search platelet aggregation inhibitors resulting in 218 compounds. The structures were drawn in Chemsketch and the ligands were optimized and converted into PDB file format using Open Babel software. The compounds were filtered by using Lipinski's rule by Molinspiration server to predict drug-likeness. Docking analysis was performed using Autodock 4 into the binding sites of the drug target. The analysis revealed that the Pubchem compound 8-((4-chlorophenyl)thio)cyclic-3',5'-GMP could be a potent inhibitor for Clf A based on low negative binding energy value of -8.18kcal/mol that formed 5 hydrogen bond interactions including one of the active site residues (TYR338) and with LYS293 & 389 and ILE387.**

Key words: Clumping factor A, *Staphylococus aureus*, platelet aggregation inhibitors, docking analysis, Autodock 4.0, PyMol.

Aggregation of platelets by bacteria is believed to play an important role in the pathogenesis of infective endocarditis which is involved in a series of complex interactions between the microorganism and a variety of host components including cardiac endothelium, platelets and plasma proteins like fibrinogen[1,2]. Platelets provide an adhesive surface upon damaged endothelium for microbial binding. The microorganisms which are attached to valve surface have the ability to induce localized platelet aggregation thereby resulting in formation of infected vegetation[2-4]. Bacteria that cause platelet aggregation by adhering to damaged cardiac tissue and to platelet-fibrin clots is correlated with the capacity to induce develop infective endocarditis (IE)[5-10]. Infective endocarditis can be defined as a serious form of microbial infection of the endocardial surface, lining of the heart chambers and heart valves with a high mortality rate.

Staphylococcus aureus is a Gram-positive bacterium which is considered to be the most common cause of endovascular infections (intravascular catheter sepsis and hemodialysis access site sepsis) and is the second leading cause of infective endocarditis[11]. *S. aureus* produces several proteins that can specifically bind to fibrinogen of host cell. One among them is Clumping factor A (ClfA) which is a well-established virulence factor and vaccine candidate that belongs to a member of family of bacterial surface proteins known as microbial surface

* To whom all correspondence should be addressed.
Mob.: +91 94869 47377; Fax: +91 416 2242477
E-mail: vsubhashree@vit.ac.in
v_subhashree@hotmail.com

components recognizing adhesive matrix molecules (MSCRAMMs). It contains an N-terminal A domain consisting of 519 aminoacids which comprises three folded sub-domains N1, N2 and N3. The A domain is followed by a Serine-Aspartate dipeptide repeat region and a cell-wall and membrane-spanning region containing LPDTG motif for sortase-promoted anchoring to the cell wall[12]. It functions by binding to the C-terminus of the γ-chain of fibrinogen and induces clumping of bacteria in fibrinogen solution[13-14].

As the protein is attached to the surface of the bacterial cell it mediates direct binding to host fibrinogen and fibrin and promote platelet aggregation. The N-terminal A domain of ClfA binds not only binds to fibrinogen but also to elastin[15] and is found to interact with cytokeratin-8 [16] and Cytokeratin 10 [17-18].

Computational approaches such as molecular docking are routinely used in modern drug design to help understand the drug-receptor interactions which can strongly support and help designing novel and more potent inhibitors[19]. Docking is performed to search for the best conformation of the ligands with lowest binding energy that could mediate biological activity.

MATERIALS AND METHODS

Target protein preparation

The atomic coordinates of the target protein Clumping factor A (PDB code 1N67) of *Staphylococcus aureus* was downloaded from Protein DataBank (PDB) database (http://www.rcsb.org/) using PDB code 1N67. The heteroatoms were removed and the structure was refined by using KOBA[MIN] server which uses energy minimization and geometry correction (http://csb.stanford.edu/kobamin/). The three dimensional structure of 1N67 was shown in the Fig. 1.

Ligands preparation

The Pubchem compound database (http://www.ncbi.nlm.nih.gov/pccompound) was searched for platelet aggregation inhibitors and the search resulted in 218 compounds having the particular inhibiting activity. The structures of those compounds were drawn using Chemsketch (http://www.acdlabs.com/), the chemically intelligent drawing interface freeware and saved in .mol file format and the structures were optimized. They were then converted into corresponding PDB files using Open babel software (http://openbabel.org/).

Binding site of the drug target

The detection of ligand-binding sites is important in protein function identification and drug discovery. The residues PRO336 and TYR338 were considered and chosen as binding site residues based on the literature[20].

Lipinski's Rule of five

Lipinski's "Rule of 5" is used to evaluate drug-likeness or drug-like property of a molecule or compounds in study. The rule states that most "drug-like" molecules have log P d" 5, number of hydrogen bond acceptors d" 10, number of hydrogen bond donors d" 5 and molecular weight d" 500 g/mol. The molecules that are violating more than one of these rules may have problems with bioavailability. The rule is called "Rule of 5" because the border values are 5, 500, 2*5, and 5. This filter predicts the Lipinski rule of 5 for the compounds that is based on its 2D structure and provides a pertinent information whether a particular chemical compound contain properties of pharmacological or biological activity that would make it a likely orally active drug for human consumption[21]. Number of rotatable bonds is a simple topological parameter which is a measure of molecular flexibility. It has been shown to be a very good descriptor of oral bioavailability of drugs[22]. Rotatable bond is defined as any single non-ring bond, bounded to non-terminal heavy (i.e., non-hydrogen) atom.

Molecular docking

Docking of small molecules into the receptor binding site and estimation of binding affinity of the complex is a vital part of structure-based drug design 23). We used Autodock 4.0 software for docking purpose. Before docking, the receptor and ligand molecules are prepared. The conversion of PDB files into PDBQT files of molecules for docking is performed as primary step during docking. The receptor molecule was added with polar hydrogens and assigned with Kollman charges and atom types were set. The ligand molecules were added with Gasteiger charges and non-polar hydrogen atoms were merged. The torsions of ligands were made to rotate during docking. A grid box size of 60 x

60 x 60 Å with a grid spacing of 0.375 Å was generated using Autogrid. Docking was done with 10 independent runs with a population size of 150 that were set to terminate after a maximum of 2.5×10^6 energy evaluations with mutation rate of 0.02 and crossover rate of 0.8. We employed Lamarckian Genetic Algorithm (LGA) for ligand conformational searching. It is a hybrid of genetic algorithm and local search algorithm that uses a parameterized free-energy scoring functions to estimate the binding energy. This algorithm first builds a population of individuals (genes), each being a different random conformation of the docked molecule. Each individual is then mutated to acquire a slightly different translation and rotation and the local search algorithm then performs energy minimizations on a user-specified proportion of the population of individuals. The individuals with the low binding energy are transferred to the next generation and the process is then repeated. The algorithm is called Lamarckian because every new generation of individuals is allowed to inherit the local search adaptations of their parents. Cluster analysis was performed on the docked results using an RMS tolerance of 1.0 Å. The cluster with lowest binding energy was considered as the best docked pose of the ligands.

RESULTS AND DISCUSSION

Protein-ligand docking was carried out using Autodock 4.0 version. Most of the drug failures in the drug discovery process in clinical trials were due to poor pharmacokinetic properties and toxicity. Thus, the dataset of 218 compounds obtained from Pubchem compound database having platelet aggregation inhibiting activity were first filtered by Lipinski's rule to predict the oral bioavailability before docking. In these, 36 compounds did not obey the Lipinski's rule of five by violating more than one rule thereby resulting in 182 compounds to be qualified for drug.

Table 1. Molecular property of 15 potent platelet aggregation inhibitors

S. No.	Compound ID	MW (g/mol)	HBA	HBD	LogP	Rotatable bonds
1	57369743	487.818	12	5	0.258	3
2	24980082	350.455	5	3	1.051	3
3	5281792	360.318	8	5	1.626	7
4	6436588	477.645	5	2	5.779	11
5	5941457	354.431	5	3	3.019	7
6	5486684	328.387	4	1	2.995	7
7	3033963	535.098	7	1	3.556	5
8	123969	487.8181	12	5	0.258	3
9	104794	464.65	5	1	5.408	9
10	65889	455.971	7	0	2.123	4
11	21171	339.391	5	0	2.994	2
12	5631	464.65	5	1	5.408	9
13	3822	395.434	6	1	2.811	5
14	2754	369.469	7	1	3.396	7
15	2403	398.466	6	3	2.676	5

The drug target (1N67) was then docked and each docked complex was analyzed to study the interactions based on the binding energy values and the association of binding and docked poses were visualized using Pymol software. The top fifteen molecules with lowest binding energy was taken and compared. The molecular property of the top 15 compounds was shown in the Table 1 and the results of docking including binding energies, number of hydrogen bond interactions and the position and residues involved in the interactions were also tabulated in Table 2. The free binding energy was calculated from the sum of the intermolecular and the torsional free energies. The lowest-energy solution was accepted as the calculated binding energy and its K_i value was used to define the binding affnity of the inhibitors[24].

The AutoDock scoring function is based on an empirically derived linear free energy model that is designed to reproduce observed binding constants for small organic molecules bound to proteins. AutoDock have terms for van der Waals energy, hydrogen bond energy and Coulombic energy. Scoring function calculates[25] the change in desolvation free energy and[26] the loss of torsional degrees of freedom upon binding. The most favorable ligand binding poses as revealed by clustering histograms along with their corresponding binding energy was obtained from AutoDock Tools. The negative and low value of binding energy indicated strong favorable bonds between protein and the ligands indicating that the ligand is in its most favorable conformation.

Table 2. Interaction of Clf A with 15 compounds analyzed by Autodock and Pymol

S. No.	Compound ID	Binding energy (kcal/mol)	No. of H bond interactions	Residues involved in interactions
1	57369743	-8.18	7	K293, K389, Y338, I387
2	24980082	-7.23	3	G287, E342
3	5281792	-7.21	9	K293, Y338, Q386, I387, K389, N531
4	6436588	-7.99	5	K293, I387, K389, N531
5	5941457	-7.47	4	T334, I387, K389, N531
6	5486684	-7.25	2	V279, N284
7	3033963	-7.29	1	Y338
8	123969	-7.61	6	T291, K293, Y338, Q386, I387, D388
9	104794	-7.48	4	K258, Q386, K293, D388
10	65889	-8.07	1	N531
11	21171	-7.34	2	N284
12	5631	-7.4	2	K258, K293
13	3822	-7.69	7	N286, K293, Y338, I387, K389
14	2754	-7.4	5	Y256, N283, K293
15	2403	-7.57	5	Y338, Q386, I387, D388, K389

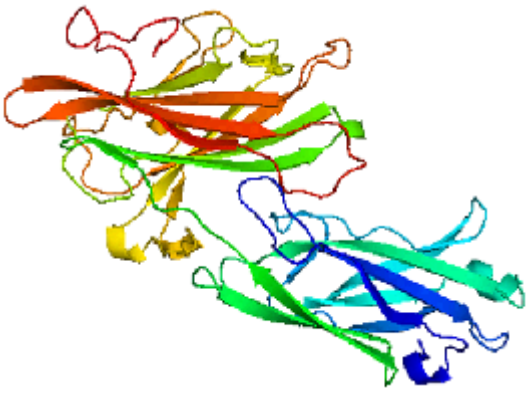

Fig. 1. Structure of Clumping factor A of *Staphylococcus aureus* (1N67) The virulent protein is a cell-surface associated protein that binds to fibrinogen of host causing platelet aggregation. The protein is 359 aminoacids long

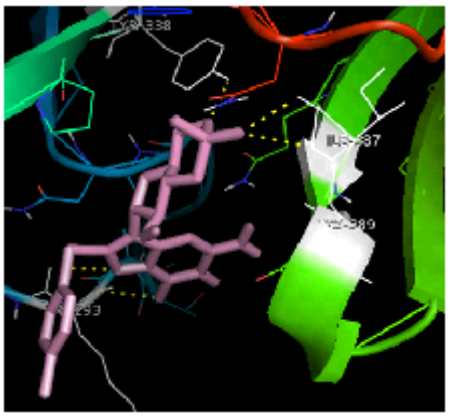

Fig. 2. Binding and interaction pose of the Pubchem compound ID 57369743 with Clf A drug target. The docked complex is analyzed by PyMol in which the compound is represented by sticks in pink and the drug target by cartoon and the aminoacids involved in interactions are colored in white. The compound is shown to interact with K293, Y338, I387 and K389 residues making 5 hydrogen bond interactions. The binding energy value is -8.18kcal/mol which is the best when compared to other compounds in the study.

The parameters such as hydrogen bond interactions, π - π interactions, binding energy, RMSD of active site residues and orientation of the docked compound within the active site was used to analyze the docked complexes[27]. PyMOL helps in elucidating the type of interactions (e.g. hydrogen-bond, π-π interaction and cation-π interaction) contributing to ligand binding.

The binding energy values of the top 15 molecules ranged between -7.21 to -8.18kcal/mol. The least negative binding energy value was seen with Pubchem compound ID 57369743

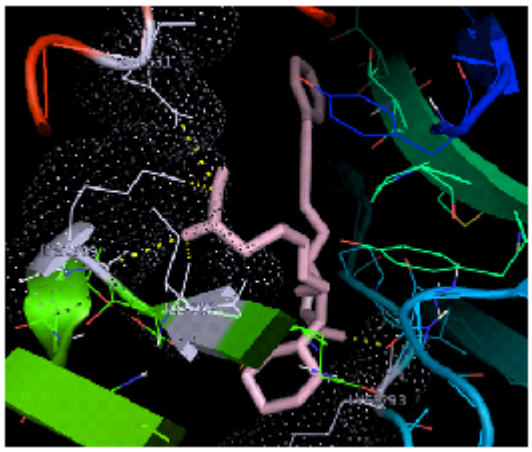

Fig. 3. Pymol view of the compound 6436588 with Clf A receptor of *S. aureus*. The compound showed binding energy value of -7.99 kcal/mol by making stable complex with 4 aminoacids at K293, I387, K389 and N531. The receptor is shown in cartoon with dots and the compound is represented by sticks

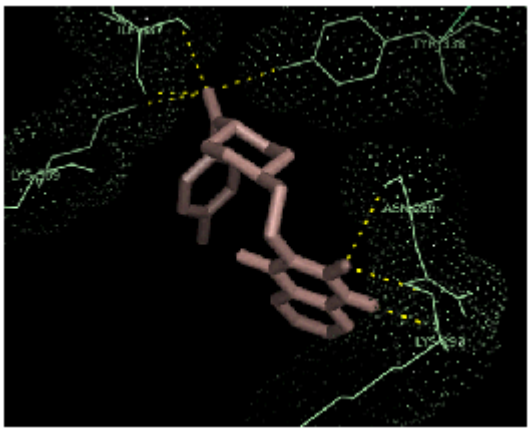

Fig. 4. Analysis of 1N67 complexed with compound ID 3822. On analyzing the docked complex resulted by Autodock 4.0 by PyMol showed 7 hydrogen bond interactions interacting with aminoacids N286, K293, Y338, I387 and K389 in which Y338 is binding site residue. The complex is stabilized by binding energy value of -7.69 kcal/mol. The compound is represented by sticks in pink and the residues of receptor involved in bond interactions is shown in lines with dots in green color

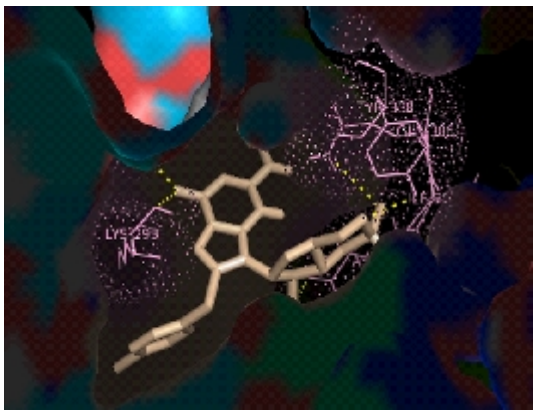

Fig. 5. Binding pose and pattern of Pubchem compound ID 123969 with Clumping factor A. The complex is formed by binding at aminoacid positions T291, K293, Y338, Q386, I387 and D388 with energy value of -7.61kcal/mol. The compound is represented in sticks and the receptor in surfaces by differentiating the interacting residues in lines with dots

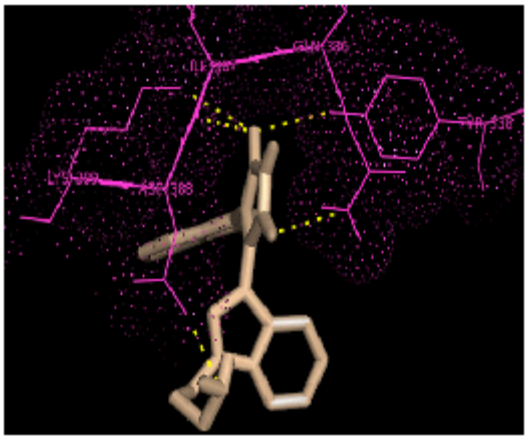

Fig. 6 Interaction between the Pubchem compound 2403. The receptor is shown in lines with dots and the compound in sticks. The complex is formed with binding energy value of -7.57kcal/mol by making 5 interactions at Y338, Q386, I387, D388 and K389

which is known as 8-((4-chlorophenyl) thio) cyclic-3',5'-GMP. The compound formed stable complex with the target protein with binding energy value of -8.18 kcal/mol making 7 hydrogen bond interactions with residues Lys293 & 389, Tyr338, Ile387. It was interesting to note that the compound was found to have interaction with binding site residue (TYR338) which further confirmed its interaction with the drug target aiding inhibitory action of the compound towards Clumping factor A protein. The next stable complex was identified as Pubchem compound ID 65889 by its binding energy value of -8.07 kcal/mol. The compound interacted with the drug target with one hydrogen bond interaction with amino acid Arg531. The binding pose of the drug target with the compounds whose IDs were 57369743, 6436588, 3822, 123969, 2403, 5281792 was shown (Figure 2-7).

The maximum number of 9 hydrogen bond interactions was seen with the compound 5281792 interacting with 6 residues at Lys293, Tyr338, Gln386, Ile387, Lys389 and Asn531 and its binding energy was found to be -7.21 kcal/mol. In the top 15 compounds ranked with low negative binding energy, 6 compounds were found to interact and form stable complex with the active site residue Tyr338 of the protein drug target.

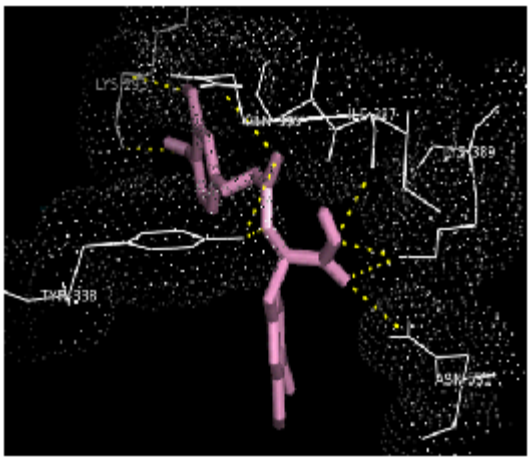

Fig. 7. Visualization of docked complex 1N67- 5281792 by Pymol. The analysis of the complex by PyMol revealed that there are 9 hydrogen bond interactions binding with K293, Y338, Q386, I387, K389 and N531. The complex has been stabilized by binding energy value of -7.21 kcal/mol. The compound is shown in sticks and the interacting residues in lines with dots

CONCLUSION

Molecular docking was performed to explore the binding mechanism of the drug target with the ligands in order to find potent inhibitors. The molecular docking study was done using Autodock 4 with 218 molecular compounds identified from Pubchem compound database. The filtering of molecules based on Lipinski's rule of 5 resulted in 182 compounds. The docking analysis using Autodock 4.0 revealed that the Pubchem compound as 8-((4-chlorophenyl) thio)cyclic-3',5'-GMP whose ID was 57369743 interacted with the drug target by its least negative binding energy value of -8.18kcal/mol. The compound was found to interact with 4 amino acid residues including one binding site residue TYR338 and LYS293, LYS389 and ILE387 forming 7 hydrogen bond interactions. This inferred that the compound could be a potent inhibitor of ClfA protein of *Staphylococcus aureus* which is involved in platelet aggregation.

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