

# A molecular modeling based screening for potential inhibitors to alpha hemolysin from *Staphylococcus aureus*

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## Abstract:

*Staphylococcus aureus*, a Gram-positive bacterium is pathogenic in nature. It is known that secreted toxins remain active after antibiotic treatment. The alpha hemolysin or alpha toxin damages cell membrane and induces apoptosis and degradation of DNA. The titer of alphahemolysin increases and causes hemostasis disturbances, thrombocytopenia, and pulmonary lesions during staphylococcal infection. Therefore, it is of interest to inhibit alpha hemolysin using novel compounds. We used the structure of alpha hemolysin(PDB: 7AHL) to screen structures for 100,000 compounds from the ZINC database using molecular docking with AutoDock VINA. Nine (9) successive hits were then subjected for pharmacokinetic and toxicity properties by PROTOX (a webserver for the prediction of oral toxicities of small molecules) and FAFDrugs (a tool for prediction of ADME and Toxicity). This exercise further identified hit #1 ([3a-(Dihydroxymethyl)-6-hydroxy-2,2-dimethyl-1,3,4-trioxatetrahydro-2H-pentalen-5-yl]methyl)amino(9H-fluoren-9-yl)acetate with binding affinity: -10.3 kcal/mol) and hit #2 (6-(Dihydroxymethyl)-2-[2-[3-(methylamino)propyl]-2-azatricyclo[9.4.0.0<sup>3,8</sup>]pentadeca-1(11),3,5,7,12,14-hexaen-6-yloxy]tetrahydro-2H-pyran-3,4,5-triol with binding affinity: -9.6 kcal/mol) with acceptable toxicity and ADME properties for potential predicted hemolysin inhibition. These compounds should then be evaluated in vitro using inhibitory studies.

**Keywords:** *Staphylococcus aureus*, Alpha toxin, Molecular docking, AutoDock, Virtual screening

## Background:

*Staphylococcus aureus* is a Gram-positive bacterium and a member of the *Firmicutes*. It is frequently found in human skin and repository tracts [1 & 2]. Methicillin-resistant *Staphylococcus aureus* is the most common pathogen among patients with skin and soft tissue infections [3]. Pathogenicity of *Staphylococcus aureus* is closely associated with toxin production. It secretes variety of exotoxins including super antigens [4], toxic shock syndromes [5], enterotoxins [6], exfoliative toxins [7], alpha toxin [8], beta toxin and delta toxin [9]. Moreover, this pathogene laborates immunoevasiveproteins such as protein-A [10]. Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Staphylococcus aureus* (VRSA) strains of this bacterium

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is clinical problem. Anti-biotic resistant Infection-related mortality rate estimated at approximately 13% equal to approximately 2 to 10 deaths annually per 100,000 population [11]. Deactivation of secreted exotoxins will reduce clinical effects. Among exotoxins, alpha hemolysin is the major cytotoxic agent released by *Staphylococcus aureus* and is a member of pore forming beta barrel toxin family [12]. The structure of this protein has been solved by X-ray crystallography. About 68 % of its structure consists of beta sheets. Seven monomers of secreted alpha toxin contribute to build a heptameric hairpin like structure. The heptameric form of alpha toxin tends to enter the hydrophobic cell membrane and make a pore with 14 angstrom diameter which is large



extracted and was used as the template for further study. The monomeric structure was then solved in a water box and neutralized with Na<sup>+</sup> and/or Cl<sup>-</sup> by using Chimera software. The ligand library for virtual screening was constructed based on a subset of drug-like compounds derived from zinc database containing 100000 chemicals [18].

### Virtual screening

For virtual screening purpose, we used PyRX software [19]. PYRX includes AutoDock [20] and AutoDockVina [21] and its scoring function is based on Lamarckian genetic algorithm. More information about Lamarckian scoring function is provided in supplementary data. In this research we used AutoDockVina for molecular docking method. Before initiation of docking operation charge calculated and assigned to protein and ligand structures by AutoDockVina software. Also a big docking radius with a volume of 30 Å and coordinate of X: 31.25, Y: 24.75 and Z: 28.04 was used to cover interacting area of monomers. For simulation of biological condition, docking operation was performed in the presence of water molecules and neutralizing ions.

### Pharmacokinetic analysis

Successive hits of virtual screening data were then analyzed regarding liberation, absorption, distribution and metabolism properties. To do this, FAFDrugs3 web server was used [22]. The ligands were checked for ADME properties in optimal descriptors (hydrogen bonds, charge) in pH=7.4. Also the toxicity properties and probable accessorial targets of successive hits were analyzed by PROTOX web server [23].

### Results & Discussion:

For virtual screening purpose, biological conditions were simulated by adding water box and neutralizing ions. So with the most probability it can be suggested that the provided binding ΔG are close to *in vitro* condition. Among 100.000 drug like chemicals, 9 ligands with highest binding affinity were selected for further study. **Table 1 (see supplementary material)** describes binding avidity of top 9 successive hits. **Figure 1** depicts the structure of top 3 hits in contact with alpha toxin. Although in this study we obtained 9 potential inhibitors we focused on highly specific hits. According to table 1, top 4, hits in comparison with others indicated considerable difference in binding affinity. So we selected top 4 successive hits for further study. Top 4 hits were then analyzed regarding oral toxicity level and the ligand 1 reached the LD50 of 150mg/kg with the toxicity class 3 (1: most toxic and 6: safe). Furthermore, no protein target has been found for this hit. In other words this chemical had not any predicted target in human proteins. This ligand directly interacts with Thr 109, Thr 155 and Ser 106 with hydrogen bond and makes an electrostatic interaction with Lys 154 furthermore it interacts with Pro151 and Val 149 by steric interaction, It is probable that the pharmacophore model of this hit does not match to any vital human proteins. The pharmacokinetic and toxicity analysis indicated that this ligand is an appropriate candidate for further *in vitro* study. But it is clear that the further *in vitro* study of this ligand requires reduction in its toxicity level. The second hit reached the LD50 of 733mg/kg and its toxicity class predicted 4. The LD50 indicates that this ligand has less toxicity than first hit. Interestingly no human protein target has been predicted for this ligand either. The less toxicity enables

ligand 2 to be considered as a drug candidate for further study. This ligand makes 3 hydrogen bonds with Thr 109, Thr 155 and Ser106 and directly engages in interaction with Asp 108 and Lys 154 by electrostatic bond. Moreover it interacts with Val 149, Pro 151 and Lys 154 by steric, interaction. The ligand 3 which passed the pharmacokinetic tests of FAFdrugs3, indicated high toxicity value in PROTOX. Its toxicity level was predicted in level 1 with the LD50 of 5 mg/kg. The high toxicity value of this ligand was due to its pharmacophore properties which were 44.72% fit with Amine Oxidase A and 40.26% fit with Prostaglandin G/H Synthase 1. This ligand makes 3 hydrogen bonds with Thr 155, Asp108 and Lys 110 and also 3 steric interactions with Ser 106, Asp 108 and Thr 155. Because of high toxicity value, this ligand is not a good candidate in order to be used as a base structure for rational drug design purposes. Moreover the toxicity analysis results of fourth successive hit indicated LD50 of 3750 mg/kg with the toxicity level 5. Among all 100.000 virtual screening candidate chemicals, this ligand could rank as a successive hit and pass pharmacokinetic test and interestingly reached the least toxicity level. But it is remarkable that the pharmacophore model of ligand 4 fits 28.76% to its human target Amine Oxidase A. This ligand contacts with Ser 106, Thr 109 and Val 149 by hydrogen bond and makes steric interactions with Thr 155, Val 149 and Tyr 148. The overall properties of 4 described ligands are available in **Table 2 (see supplementary material)**.

Although previously some inhibitors of alpha toxin were reported the demand of new specific inhibitors are still perceptible, Before a clinical study a wide range of primary structure is needed to be tested in *in silico* and *in vivo* conditions regarding cyto-toxicity. Variation in inhibitor structures helps to reach proper lead compounds. CD-cholesterol which has been previously reported as the potent inhibitor, has a hydrophobic structure by nature. So it is mostly probable that it binds to hydrophobic transmembrane, region of toxin structure by hydrophobic interactions. Apigenin (4', 5, 7-trihydroxyflavone) also has a hydrophobic sterol like structure and it prevents production of Alpha hemolysin. So the application of Apigenin after toxin production by bacteria, would not be helpful for inactivation of heptamer toxin molecules. The found inhibitors of this study bind to the structure of alpha toxin not only by hydrophobic interactions and as depicted in figure1, hydrogen bonds as well as steric interactions plus electrostatic interactions are tightly engaged in interaction with alpha toxin structure. It leads to more specific interaction with the target exactly in monomers interacting area. In comparison with Apigenin and CD-cholesterol, the presented successive hits theoretically prevents active form (heptamer) assembly by a more specific pharmacophore fit. So if the further required *in vivo* experiments validates these hits as a drug, they can be used after invasion and toxin production by bacteria to inhibit hemolysin process. Also the top found inhibitors passed *in silico* tests, but further modifications will help to reach a better fit to the target.

### Conclusion:

The circulating secreted alpha hemolysin toxin from Staphylococcus aureus causes side effects even after antibiotic treatments. Therefore, there is a need to inhibit alpha hemolysin using novel compounds. A molecular docking

based screening of compounds from the ZINC database against the known hemolysin toxin structure identified two hits with predicted binding values -10.3 kcal/mol (hit #1) and -9.6 kcal/mol (hit #2) having acceptable toxicity and ADME properties for further consideration.

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## Supplementary material:

**Table 1:** The binding affinity of top 9 successive hits of virtual screening among a database containing 100,000 drug like chemicals.

Hit number	Binding affinity ( $\Delta G$ )
Ligand 1	-10.3 kcal/mol
Ligand 2	-9.6 kcal/mol
Ligand 3	-9.1 kcal/mol
Ligand 4	-8.8 kcal/mol
Ligand 5	-7.9 kcal/mol
Ligand 6	-7.8 kcal/mol
Ligand 7	-7.8 kcal/mol
Ligand 8	-7.4 kcal/mol
Ligand 9	-7.3 kcal/mol

**Table 2:** The properties of top 4 successive ligands which are derived from virtual screening data.

Ligand	Molecular weight	Rotatable bonds	flexibility	H acceptor	H donor	Ring	Carbon atom	Hetero atom	Oral bioavailability	LD50 Mg/Kg	Toxicity class*
Hit 1	455.46	6	0.18	9	3	2	42	9	good	150	3
Hit 2	458.50	7	0.23	9	5	2	24	9	good	733	4
Hit 3	480.59	7	0.23	8	4	3	24	8	good	5	1
Hit 4	425.45	6	0.23	9	5	3	19	10	good	3750	5

- 1: most toxic and 6: safe