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Designing of potential inhibitors against *Staphylococcus aureus* sortase A: Combined analogue and structure based approach with *in vitro* validation

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ABSTRACT

Staphylococcus aureus sortase A is an attractive target of Gram-positive bacteria that plays a crucial role in anchoring of surface proteins to peptidoglycan present in bacterial cell wall. Inhibiting sortase A is an elementary and essential effort in preventing the pathogenesis. In this context, *in silico* virtual screening of in-house database was performed using ligand based pharmacophore model as a filter. The developed pharmacophore model AAHR 11 consists of two acceptors, one hydrophobic and one ring aromatic feature. Top ranked molecule **KKR1** was docked into the active site of the target. After profound analysis, it was analyzed and optimized based on the observations from its binding pose orientation. Upgraded version of **KKR1** was **KKR2** and has improved docking score, binding interactions and best fit in the binding pocket. **KKR1** along with **KKR2** were further validated using 100 ns molecular dynamic studies. Both **KKR1** and **KKR2** contain Indole-thiazolidine moiety and were synthesized. The disk diffusion assay has good initial results (ZI of **KKR1**, **KKR2** were 24, 38 mm at 10 μg/mL and ZI of Ampicillin was 22 at 10 μg/mL) and calculated MICs of the molecules (**KKR1** 5.56 ± 0.28 μg/mL, **KKR2** 1.32 ± 0.12 μg/mL, Ampicillin 8 ± 1.1 μg/mL) were in good agreement with standard drug Ampicillin. **KKR1** has shown IC_{50} of 1.23 ± 0.14 μM whereas the optimized lead molecule **KKR2** show IC_{50} of 0.008 ± 0.07 μM. Results from *in silico* were validated by *in vitro* studies and proved that indole-thiazolidine molecules would be useful for future development as lead molecules against *S. aureus* sortase A.

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1. Introduction

Staphylococcus aureus is a member of Firmicutes phylum and Staphylococcaceae family most of which have Gram-positive cell wall structure. The cell wall is accountable for preserving structural steadiness, providing a barrier to osmotic pressures, and assisting interactions with the surrounding environment. *S .aureus* is accountable for community and hospital acquired infections around the globe. It is predominantly responsible for the infections related to soft tissues, skin, respiratory tract and human blood stream, causing diseases impetigo, meningitis and toxic shock syndrome [1]. Amongst *S. aureus* strains, Methicillin resistant *S. aureus* (MRSA) is widespread and lethal, owing to its resistance against broad range of antibiotics [2–4]. Therefore, infections caused by this strain are key concerns of health and substantial economical tension on health care of every country. For any bacterial infection to initiate, the foremost interaction is attachment of bacterial proteins to the peptidoglycan present on the host cell wall. These anchor proteins in bacteria are pivotal for bacterial propagation and pathogenicity. In most of the Gram positive bacteria, including *S. aureus*, sortase enzymes are primarily responsible for arranging the surface proteins on the bacterial peptidoglycan for initiating the docking of bacteria on the host cell wall [5–9], showcasing them as a primary target to inhibit bacterial infections. Generally, many sortases are simple inside the identical genome and will possibly be grouped depending on their particular

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homology into 4 classes. Sortase A is prototypical enzyme belongs to class A family. Class B sortases usually are managed because of the accessibility to iron and anchor the proteins that will take part in heme-iron acquisition. Class C sortases have the effect of your elaboration associated with pili on bacterial surface area. Last but not least, Class D sortases usually are indicated during bacterial sporulation. Relation between different sortase proteins was explained by Thomas et al. [10] showing relationship of sortases among different bacterial species.

Out of all isoforms of sortase enzymes, sortase A is best studied and conserved among all the Gram positive bacteria with the presence of the LPXTG motif at the C-terminal of the protein. *S. aureus* sortase A enzyme (SrtA) is a extracellular cysteine transpeptidase that catalyzes a transpeptidation reaction and allows the anchorage of surface proteins to the peptidoglycan of cell wall as mentioned earlier [11]. The catalytic process or reaction occurs in two steps: (i) cysteine implicated nucleophilic attack occurs at the peptide bond between the threonine and the glycine of a conserved C-terminal motif LPXTG SrtA [12] resulting in the development of the acyl-enzyme transitional state and further covalently attaching to the peptidoglycan of the host cell membrane. The final step is the carboxyl group of the threonine connected to the amino group of the cell wall cross bridge which permits connectivity of the bacteria to host cell [13,14]. Role of cysteine present in the active site of the SrtA is evidently critical for the catalysis reaction whereas the role of other conserved amino acids Histidine and Arginine is highly controversial and imprecise. If the ability of sortase A is blocked, it prevents the anchorage of proteins present on the surface to the peptidoglycan making SrtA an attractive and significant target with superior pharmaceutical importance.

Till date, most of the inhibitors against sortase A were obtained by traditional methods, channeling the need for discovery of much effective small molecule inhibitors [15–21] by employing sophisticated computational drug discovery methods. Usage of pharmacophore modeling as a tool for the discovery of novel drugs has turned out to be progressively more popular since this method fine-tunes by considering all the aspects, leading to the discovery of novel and potent inhibitor [22]. Also, analog based pharmacophore as a filter in virtual screening is the most reliable and is comparable to its biological activity. Although, some of the researchers developed sortase A inhibitors using pharmacophore and 3D-QSAR models with only one prototype of inhibitors but, they overlook the wide range of small molecule inhibitors in the literature.

Therefore in the present study, pharmacophoric features of the inhibitors of SrtA were developed for screening Phase CAC (Commercially Available Compounds) database along with some other collection of inhibitors from our in-house database. Docking study was performed to find the right orientation of the molecule and further designed a new molecule with improved activity based on the observations. Molecular dynamics (MD) simulations were performed to find the orientation and stability of the selected molecules in the binding pocket. Molecules studied theoretically were synthesized and validated *in vitro*, which proved that the lead and fine-tuned molecules from *in silico* study were active and potent when compared to the standard drug Ampicillin.

2. Materials and methods

2.1. In silico *approaches*

2.1.1. *Ligand preparation*

The entire work was performed using MAESTRO 9.3 (Schrodinger, Inc., LLC, New York, USA), in Dell precision T3500 workstation. A dataset of 149 compounds collected from the literature [15,16,23,24]. Every compound in the data set underwent the same cell growth suppression analysis, which makes the results uniform. The dataset consists of binding data (IC_{50}) spanning over three orders of magnitude from 0.30 to 1000 μM, covering all ranges of activity from active to moderately active to inactive. All sketched compounds were imported to the project table and refined using LigPrep module [25]. OPLS_2005 (optimized potentials for liquid simulations_2005) force field was assigned to the compounds and possible states of ionization were generated using Epik [26]. Tautomers were generated and chiralities of the compound were retained. Only one low energy ring conformation was generated for each ligand. All original GI50 values of each inhibitor were converted into pGI50 (−log GI50) in order to use the data as a dependent variable.

2.1.2. *Pharmacophore model generation and database screening*

Common feature pharmacophore generation was carried out using Phase 3.1 [27] in the Maestro, a versatile product for pharmacophore perception, structure alignment and activity prediction. The workflow, building a pharmacophore model involves conformations generation with maximum 100 conformers per rotatable bond and 1000 conformers per each structure. A thorough sampling was accomplished with 100 minimization steps using OPLS_2005 force field. Distance dependent dielectric solvent treatment was considered and the maximum relative energy difference was taken as 10 kcal/mol. Redundant conformers were eliminated by setting the RMSD to 1 Å. A set of pharmacophore sites was created using pharmacophore features for all the ligands. The pharmacophore model selected was then validated by Güner-Henry (GH) scoring method, which quantifies the hits by recalling the actives from inactives from the database containing 2548 molecules. Of these 2548 molecules, 48 molecules are known inhibitors of SrtA with diverse activities. While the other 2500 are decoy molecules downloaded from the DUD decoys database (http://dude.docking.org/targets/mmp13). The decoys were used in the 1:50 ratio to find the enrichment of the developed pharmacophore model. This database with known actives and inactives was screened with the ligand and protein based pharmacophore models to calculate the GH score. The GH score has been successfully applied to quantify model selectivity, accuracy of hits and the recall of actives from a molecule dataset consisting of known actives and inactives. The GH score ranges from 0 to 1 indicating null model and ideal model, respectively.

Following is the formulae to analyze the hit lists based on ligand based pharmacophore model [28]

$$E = \frac{H_a/H_t}{A/D} \tag{1}$$

$$\mathrm{GH} = \left[\frac{H_a(3A + H_t)}{4H_tA}\right]\left[1 - \frac{H_t - H_a}{D - A}\right] \tag{2}$$

where E is the enrichment factor, GH is the goodness of hit, D is the total compounds in the dataset, A is the total number of actives in the dataset, H_t is the total hits and H_a is the active hits.

A virtual screening strategy was followed for the identification of novel SrtA inhibitors that contains a series of filters. Ligand based pharmacophore was used as filter and screening was performed against the database using the Tanimoto metric to assess similarity. Database compounds were ranked based on their similarity. Physiochemical screening (ADME) was also used as a filter.

2.1.3. *Glide ligand docking*

A GLIDE version 3.0 (Schrodinger, Inc.) in 'Extra Precision' mode (Glide XP) was used for docking [29–31] which covers all the conformations, orientations and positional space by performing a complete systematic search and eliminating unwanted conformers

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