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IDENTIFICATION AND SYNTHESIS OF 4-([1,1'-BIPHENYL]-4-YL)-2-AMINO-5-OXO-5,6,7,8-TETRAHYDRO-4H-CHROMENE-3-CARBONITRILE AND (Z)-3-METHYL-4-((5-NITRO-1H-INDOL-3-YL)METHYLENE)-1-PHENYL-1H-PYRAZOL-5(4H)-ONE AS NOVEL *STAPHYLOCOCCUS AUREUS* SORTASE A INHIBITIONS

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ABSTRACT

The main ground for nosocomial deaths around the world wasGram-positive bacterium. Staphylococcus aureus was one of those sorts. Staphylococcus aureus sortase A was main target which was involved in the virulence of the bacterium through its anchoring mechanism. So computational docking models were developed to screen the in house database consists of 1500 molecules. 4-([1,1'-biphenyl]-4-yl)-2-amino-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (DRC-KS1) and (Z)-3-methyl-4-((5-nitro-1H-indol-3-yl)methylene)-1-phenyl-1H-pyrazol-5(4H)-one (DRC-KS2) were identified as novel molecules that can inhibit Staphylococcus aureus sortase A. Docking score of DRC-KS1 and DRC-KS2 was -4.32 and -4.65. These two molecules were synthesized to test their minimum inhibitory concentration against Staphylococcus aureus sortase A. DRC-KS1 and DRC-KS2 has MIC values of 108.2 and 45.8 µg/mL respectively. These molecules were proved potent and can be used as lead molecules in the drug discovery pipeline.

Keywords *–Docking, Synthesis, Sortase A, Minimum inhibitory concentration*

1. INTRODUCTION

Infectious diseases overwhelmed mankind throughout the times past[1].It was initial revealed by Sir Alexander Ogston from Scotland and can be found over 40% of the human populace. Merely all through the previous decades and in the western regions, infectious diseases ceased to be the major reason of mortality, replaced by cardiovascular and cancer related diseases[2]. Though, infections still stay amongst the foremost reason of death, with microbial sepsis only accountable for almost 10% of deaths in USA[3]. In the prospect, threats caused by microorganisms may be unfortunately once more turn out to be even further serious due to rising bacterial resistance to chemotherapy and to mounting numbers of elderly and immune compromised persons, who are particularly vulnerable to infections.

Sarcastically, doctors now and then succeed the battles with cancer or save apparently hopeless cases in intensive care units, but later on loose the battle with widespread microbes. One of the most important pathogens accountable for infections at the moment was Staphylococcus aureus. In developed countries, it was the most common and frequent species secured from infections of hospital inpatients, and one of the main bacteria isolated from outpatients[4-5]. It was also a chief, though regularly unnoticed and ignored resource of diseases in developing countries[6]. It was the foundation to a wide range of infections: together minor and life-threatening, limited and systemic, sensitive and unceasing.

A typical group of staphylococcal surface proteins were the "surface anchored" proteins[7-8]. A frequent mannerism of most of them was the occurrence of a conserved C-terminal sorting signal, containing an LPXTG sequence. Following the secretion of protein through the cytoplasmic membrane, this sequence was acknowledged by sortase enzymes, and then cleaves the sequence and then covalently attaches the protein to a 5-glycine bridge in the cell wall peptidoglycan. S. aureus has two such sortases: sortase A and B, with sortase B attaching solely IsdC protein, while sortase A is responsible for all other proteins. Many of the surface-anchored proteins had been identified and studied up till 2001. After that, search of sequenced staphylococcal genomes recognized even more supposed surface-anchored proteins carrying LPXTG sequence, named Staphylococcus aureus surface (Sas) proteins: SasA-SasK. So, if these proteins were inhibited there is a chance of reducing the virulence caused by these proteins.

In this context inhibitors against Staphylococcus aureus sortase A were identified using the computationally developed docking models. Molecular dynamic studies proved the stability of the molecules in the binding pocket. So, these molecules were synthesized and elucidated using IR, NMR and MASS spectroscopic techniques. Further these molecules were tested in-vitro and confirmed its activity. In this context, there models can be very much useful for researchers in the future to develop new anti-bacterial molecules.

2. MATERIALS AND METHODS

2.1 Enzyme refinement

Crystal structure of Staphylococcus aureus sortase A (PDB-ID 2KID)[9] was considered for studies (Figure 1). Protein retrieved was customized for its faults by adding hydrogen atoms and by removing water molecules beyond 5 Å from binding pocket and joined with the changes regarding tautomeric states of residues, orientations of hydroxyl groups, and protonation states of basic and acidic residues. The complex while constraining all heavy atoms (non-hydrogen) to their original positions with optimized hydrogen coordinates was finally minimized using OPLS_2005 (optimized potentials for liquid simulations_2005) force field. Protein with optimized hydrogen coordinates was finally saved as a separate file, to be used for further studies.

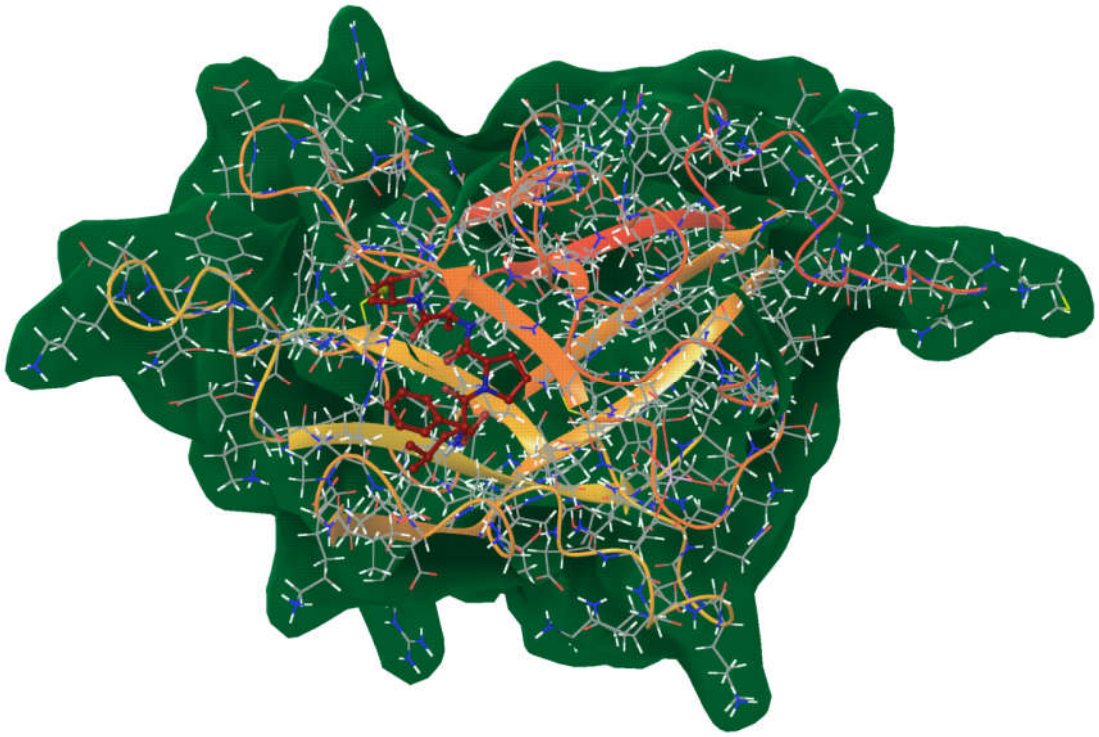


Fig. 1: Three dimensional structure of *Staphylococcus aureus* sortase A. Surface of the protein was shown in green color. Red color molecule was the substrate present in the binding pocket

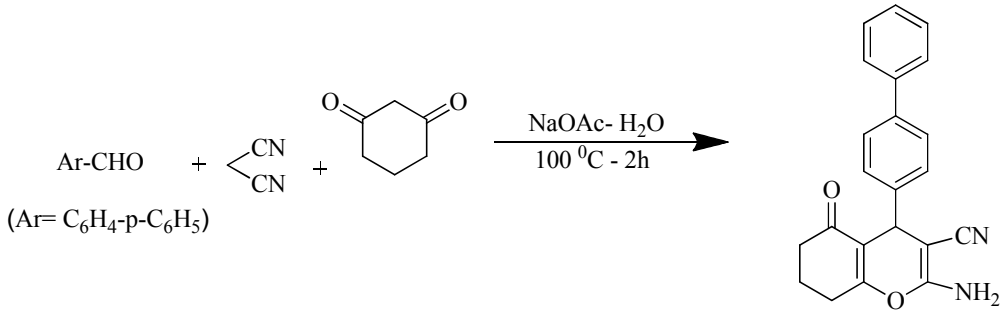
2.2 Extra precision docking protocol

Docking using GLIDE version 3.0 (Schrodinger, Inc.) in 'Extra Precision' mode (Glide XP) was performed, [10, 11] which covers the complete conformational, orientational and positional space by performing a complete systematic search and using scores it even eliminates the unwanted conformers followed by optimization. Monte Carlo sampling of pose conformation further refines the conformation. PDB-ID 2KID was considered in the studies to get ligand binding conformation and binding pattern perception. Protein retrieved was prepared with the addition of hydrogen atoms and the suitable corrections by removing water molecules and coupled with the changes regarding tautomeric states of residues, orientations of hydroxyl groups, and protonation states of basic and acidic residues. The complex while constraining all the heavy atoms (non-hydrogen) to their original positions with optimized hydrogen coordinates was finally minimized using OPLS_2005 force field. The protein with optimized hydrogen coordinates was finally saved for further studies.

2.3 ADME properties

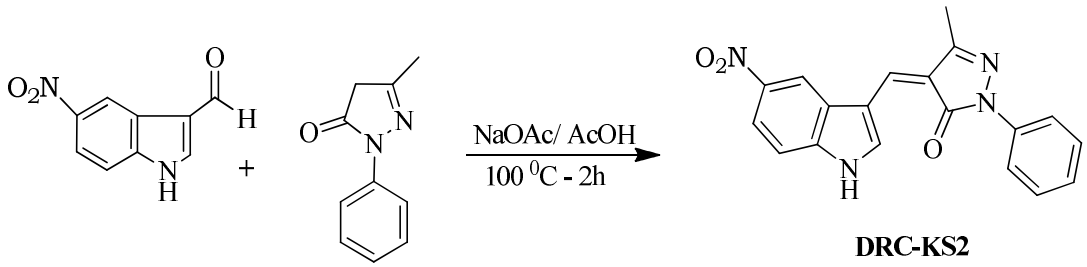
Along with the Lipinski rule of five which was crude filter, many other physiochemical properties were studied. Every molecule should cross this filter to mark itself as druggable. An optimal balance between all these properties is important to obtain good quality lead candidates. Pharmacokinetic data of active leads can be calculated using QikProp 4.4 module[12]. QikProp produces distinct properties provided with a range of values suits with 95% of the known drugs. Octanol/water partition co-efficient, aqueous solubility, IC_{50} value for blockage of HERG K^+ channels, Caco-2 cell permeability, apparent MDCK cell permeability and percentage of human oral absorption were some of the important properties used to observe the ADME of new compounds.

2.4 Synthesis of DRC-KS1 and DRC-KS2



Scheme 1

General procedure for the synthesis of **DRC-KS1**: A mixture of appropriate benzaldehyde (18a, 3.0mmol), malononitrile (10, 3.0mmol), and cyclohexnae-1,3-dione (3, 3.0mmol), piperidinium acetate (catalytic amount- 1omg) and water (25ml) was stirred at R.T for 20-30 mins. At the end of this period, the separated solid was collected by filtration, washed with water (2X25 ml) and dried in a vaccum oven to obtain crude **DRC-KS1**. The latter, were recrystallized from EtOH to get the pure **DRC-KS1**.



Scheme 2

General procedure for the synthesis of **DRC-KS2**:A mixture of appropriate indole-3-aldehyde, 5-Methyl-2-phenyl-2,4-dihydro-pyrazol-3-one (5.0mmol), catalytic amount of sodium acetate (10mg) and acetic acid (25ml) were refluxed for 2hrs at 100^0C . At the end of this period, the reaction mixture was cooled to R.T, the separated solid was collected by filtration, washed with water (5ml) and dried to obtain crude **DRC-KS2.** The latter, were recrystallized from EtOH to get pure **DRC-KS2.**

Melting points of the molecules in this study were recorded in open capillary tube and are uncorrected. Infrared spectra were recorded in KBr pills and values were given in cm^{-1}. Nuclear Magnetic Resonance spectra was run in DMSO at 400 MHz, using tetramethylsilane (TMS) as an internal standard and values were given in parts per million (δ).

2.5 In-vitro studies

Gram-positive strain namely, S. aureus (ATCC 43300) was used as test organism in screening. For calculating Minimum inhibition concentration prepared molecules were taken in concentration range of 10, 20, 30, 40 and 50 µg/mL and tested in nutrient broth for the determination of bacterial propensity for S. aureus. As mentioned earlier Microorganism suspensions at 2×10^6 CFU/mL (colony forming unit/mL) were used to inoculate the prepared compounds. The culture tubes were incubated at 37 ºC for 24–48 h. At the end the bacterial growth was measured with turbidity measurements.

3. RESULTS AND DISCUSSION

Most extensively used method for identifying active molecules was docking the molecules into the active site of the protein target and subsequent scoring. The in-house database containing fifteen hundred molecules were screened against the putative active site of SrtA protein using virtual screening work flow (Maestro, Schrodinger, 2009). The ligands were prepared at pH 7.0 ± 2.0 using Epik state and the large penalties of high energy ionization or tautomeric states were removed. The protein was kept as scaling Vander Waals radius by 1.0 Å and partial atomic charge that is less than 0.25 Å at default constraint parameters. The Glide extra precision (XP) was performed using selected constraints for each grid in OPLS_2005 force field.

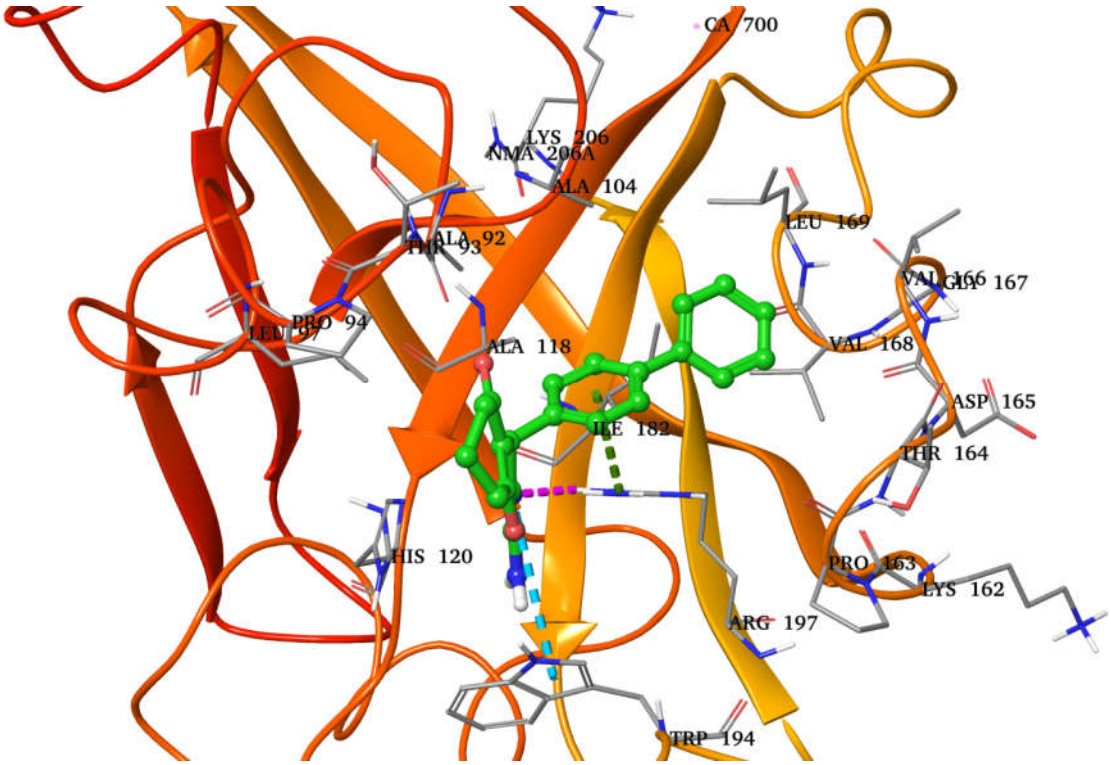


Fig. 2: Binding orientation of DSR-KS1 in the binding pocket of *Staphylococcus aureus* sortase A

Using Glide XP, about all the compounds were ranked based on their binding affinities. Glide uses semi-empirical methods for scoring the ligands in the binding pocket and ranks them accordingly. So, top two molecules were taken based on the ranking given by Glide. Top ranked molecules were passed through ADME filter.

DRC-KS1: IR (KBr): 3421, 3332 cm ^{1} (unequal doublet, asymmetric & symmetric stretching of -NH_2), 2215 cm ^{1} (- CN group, sharp), 1710 cm ^{1} (-C=O of chromene moiety, sharp); ^{1}H NMR (400MHz, DMSO-d_6/TMS): δ 1.89 -2.65 (m, 6H –$(CH_2)_3$), 4.23 (s,1H,) 7.04 (S, 2H, -NH_2, D_2O exchangeable), 7.23–7.64 (9 H, m, ArH);Anal. Calcd. for ($C_{22}H_{18}N_2O_2$) requires C,77.17; H,5.30; N,8.18; found C,77.47; H,5.10; N, 8.48; CIMS: *m/z* (M^+ + 1): 343.

DRC-KS2: IR (KBr): 3519 cm^{-1} (broad, medium, -NH), 1648 cm^{-1} (-C=O, amide group), 1562 cm^{-1} (-NO_2 streching); ^{1}H NMR(400 MHz, DMSO-d_6/ TMS): δ 2.46 (s, 3H, -CH_3), 7.17-9.93 (m, 10H, 3H indole aromatic + 1H α-indolyl + 1H vinylic proton + 5H –NPh of Pyrazolone), 12.99 (s, 1H, -NH, D_2O exch; Anal. Calcd. For ($C_{19}H_{14}BrN_3O$) requires, C,60.43; H, 3.42; N, 11.51; O, 4.46; found C, 60.02; H,3.71; N,11.05; O,4.21; CIMS: *m/z* (M^+ + 1): 347

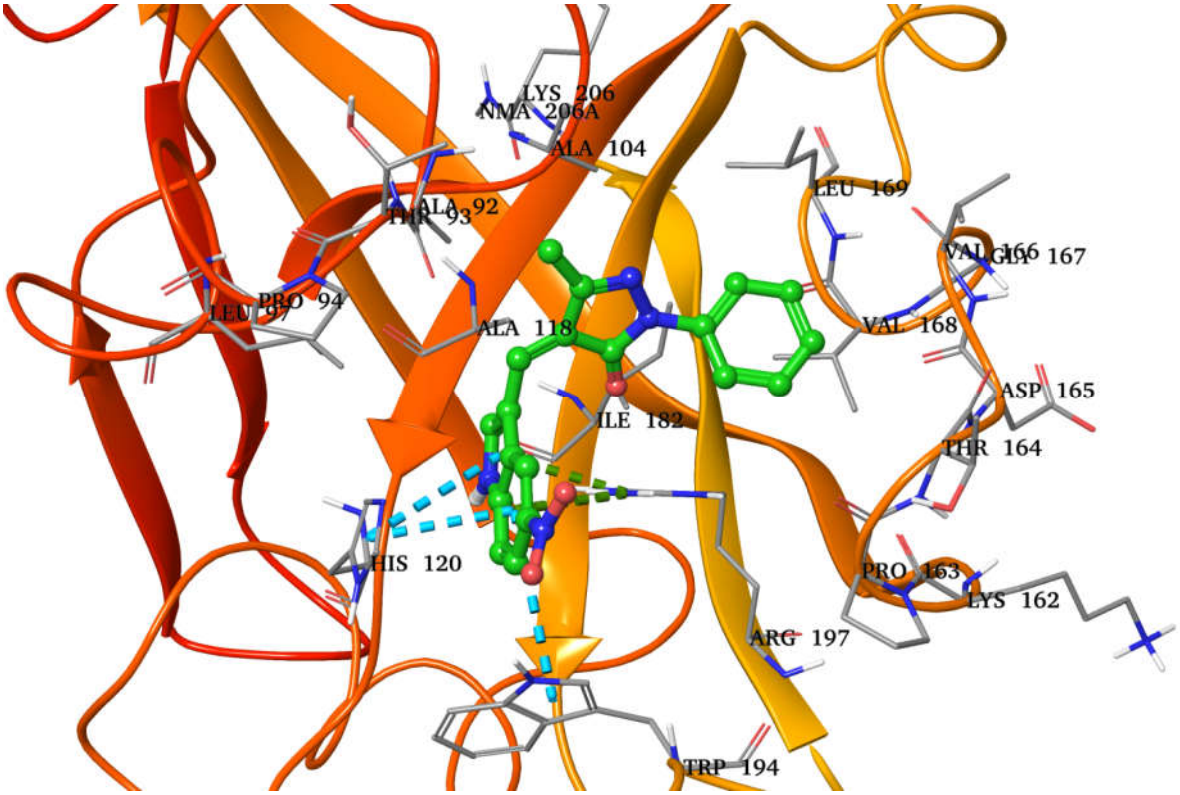


Fig. 3: Binding orientation of DSR-KS2 in the binding pocket of *Staphylococcus aureus* sortase A

Two molecules finalized and synthesized were **DRC-KS1** and **DRC-KS2. DRC-KS1** was 4-([1,1'-biphenyl]-4-yl)-2-amino-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile and **DRC-KS2** was (Z)-3-methyl-4-((5-nitro-1H-indol-3-yl)methylene)-1-phenyl-1H-pyrazol-5(4H)-one. Table 1 shows the docking and ADME scores of both the identified molecules. Both **DRC-KS1** and **DRC-KS2** molecules has same type of binding pattern in the binding pocket of SrtA. These molecules were surrounded by the amino acids His 120, Arg 197, Pro 163, Lys 162, Thr 164, Asp 165, Val 168, Leu 169, Ala 104, Ala 92, Pro 94 and Leu 97. **DRC-KS1** has shown interactions with Arg 197 and Trp 194 which was depicted in figure 2. **DRC-KS2** has shown interactions with His 120 and Arg 197 which was depicted in figure 3. Docking scores of **DRC-KS1** and **DRC-KS2** were -4.32 and -4.65.

Table 1:*In-silico* results of **DRC-SK1**, **DRC-SK2** along with their ADME properties

Compound Name	DSR-KS1	DSR-KS2
Hydrophobic network (within 5 Å)	P 94, L 97, H 120, A 104, I 182, A 118, V 166, L 169, K 162, T 164	P 91, L 97, H 120, R197, I 182, W 194, V 166, H 120, K 162, T 164,
Glide score (XP)	-4.32	-4.65
QPlogPo/w[1]	2.56	3.29
QPlogS[2]	-4.49	-5.36
QPPCaco[3]	804	232
QPPMDCK[4]	704	102
Percent human oral absorption[5]	94	89
MIC (µg/mL)	108.2	45.8

[1]*Predicted octanol/water partition coefficient (recommended range -2.0 to 6.5).*

[2] *Predicted aqueous solubility, log S. S in mol/dm3 in the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid (recommended range -2.0 to 6.5).*

[3] *Predicted apparent Caco-2 cell permeability in nm/s (recommended range <25 poor to >500 great).*

[4] *Predicted apparent MDCK cell permeability in nm/s (recommended range <25 poor to >500 great).*

[5] *Percentage of human oral absorption (recommended range <25% poor to >80% high).*

These molecules can be advanced to optimize interaction network with additional important amino acid residues in the binding pocket. Increased interaction network has every chance of improved effectiveness of the molecules. These outcomes indicate that the molecules can be used as lead molecules in the future drug discovery process.

4. CONCLUSION

Due to the necessitate of the new and potent sortase A inhibitors. Here, we demonstrate the binding of **DRC-KS1** and **DRC-KS2**. These lead molecules obtained from docking were synthesized and tested for its activity biologically and they were proved to act as inhibitors of Staphylococcus aureus sortase A.

5. ACKNOWLEDGMENTS

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