

# Identification of Novel Inhibitors for Inositol Monophosphatase of *Staphylococcus aureus* through Virtual Screening

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**Abstract** — *Staphylococcus aureus* is a Gram-positive bacterium which causes pneumonia, endocarditis, osteomyelitis and meningitis in humans. The growing incidence of bacterial drug resistance of *S. aureus* enforced a new look on existing antimicrobial drugs lead to the development of new drug targets. In view of this genome sequence of *S. aureus* was retrieved from the NCBI to perform sRNA predict. Fifty three sRNA candidates were predicted, among fifteen were identified as enzymes. Among these fifteen enzymes, inositol monophosphatase is essential for the survival of *S. aureus* and non-homologous to the host causes host immunosuppression and disturbs cellular osmoregulation hence was selected as a drug target. The co-crystal structure of inositol monophosphatase (4G60) was retrieved from the PDB. Protein preparation and optimization of the crystal structure was performed and a grid of  $10 \times 10 \times 10 \text{ \AA}$  was generated around  $4 \text{ \AA}$  of the co-crystal ligand by using Schrödinger. Through literature search seven published inhibitors were selected and shape screened against ASINEX 3D ligand database using PHASE module. The obtained shape screened structural analogs and published inhibitors were subjected to molecular docking with 4G60 by Glide virtual screening workflow, which includes high throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP) docking methods. Eighteen leads were obtained and all the eighteen leads showed better binding affinity than the published inhibitor molecules and ranked based on prime MM-GBSA scores. Among which Lead 1 (2-[4,6-Bis-(bis-ethoxycarbonyl-nitro-methyl)-[1,3,5]triazin-2-yl]-2-nitro-maloni) has showed good pharmacological properties, similar binding orientation with that of the co-crystal ligand, better binding affinity, van der Waals interactions and least  $\Delta G$  score of  $-54.86 \text{ kcal/mol}$ . Inhibition of inositol monophosphatase using lead 1 would enable the pathogen to expose to host immune system, cellular osmoregulation and suppresses the disease progression of *Staphylococcus aureus*.

**Index Terms** — Glide, Inositol monophosphatase, Meningitis, Phase, *Staphylococcus aureus*, sRNAPredict, Virtual screening.

## 1 INTRODUCTION

*Staphylococcus aureus* is a Gram-positive bacterium which causes a range of illnesses, from skin infections such as impetigo, boils, cellulitis, carbuncles, scaled skin syndrome and abscesses to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis [1]. *S. aureus* can survive from hours to weeks, or even months on dry surfaces depending on strain [2]. It can infect tissues when the skin or mucosal barriers has been breached. This can lead to many different types of infections including furuncles and carbuncles, a collection of furuncles [1]. Moreover, patient records of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati (Rayalaseema region Andhra Pradesh, India) also reported it is one of the most predominant pathogen causing endocarditis and meningitis [3], [4].

In the mid-1940s as the proportion of infections caused by penicillin-resistant *S. aureus* initiated from European hospitals, United States [5]. These strains produced a plasmid-encoded penicillinase that hydrolyzes the beta-lactam ring of

penicillin essential for its antimicrobial activity. Penicillin-resistant strains were observed to cause both in hospitals and community infections. *S. aureus* infections largely disappeared after the introduction of methicillin [6], but the prevalence of penicillinase-producing strains of other *S. aureus* lineages has remained high ever since. Unlike penicillinase-mediated resistance, which is narrow in its spectrum, methicillin resistance is broad beta-lactam antibiotic class resistance to penicillins, cephalosporins and carbapenems. The ever increasing burden of methicillin resistance *S. aureus* (MRSA) infections in hospitals led to more usage of vancomycin, the last remaining antibiotic to which MRSA strains were reliably susceptible, and under this intensive selective pressure vancomycin intermediate *S. aureus* (VISA, which are not inhibited *in vitro* at vancomycin concentrations below 4 to 8  $\mu\text{g/ml}$ ) [7] and vancomycin-resistant *S. aureus* (VRSA, inhibited only at concentrations of 16  $\mu\text{g/ml}$  or more) [8]. Two molecular markers namely *mecA* and gene encoding Panton-Valentine leukocidin (PVL) found in community associated methicillin *Staphylococcus aureus* (CA-MRSA) regardless of geographical origin: Skin and soft-tissue infections are the most common type of CA-MRSA infection, accounting for approximately 90% of cases, of which 90% are abscesses and/or cellulitis with purulent drainage [9].

The basic principle of regulation in bacteria is gene expression which is controlled by a regulator that interacts with

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a specific sequence or structure in DNA or mRNA at some stage prior to the synthesis of protein. The stage of expression that is controlled can be transcription, when the target for regulation is DNA, or it can be at translation, when the target for regulation is RNA. The regulator can be a protein or RNA. sRNAs are bacterial regulator RNAs, can either bind to protein target and modifies the function of the bound protein or bind to mRNA target and regulates the gene expression. The largest and most extensively studied bacterial regulatory RNAs are non-coding RNAs commonly referred as small RNA (sRNA) due to their small size (50–500 nucleotides). The sRNAs control the expression of genes by base pairing to their target mRNA and modulating the translational activity and/or the stability of mRNA. The sRNAs are essential for the pathogen as they regulate virulence and susceptibility to different stress conditions [10], [11].

Inositol monophosphatase, commonly referred to as IMPase, is an enzyme of the phosphodiesterase family [12]. It is involved in the host immunomodulation and cellular osmoregulation leading pathogenesis and disease progression, which affects wide raiment of cell functions, including cell growth, apoptosis, secretion and information processing. Inositol monophosphatase is a ubiquitous protein with molecular weight ~30 kDa which is abundant in bacteria, unicellular eukaryotes and animal cells.

Phosphatidylinositol (PI) signaling system is absolutely missing in prokaryotes, hence in prokaryotes, the precise role of IMPase orthologs are not merely restricted to hydrolyze D-inositol-1-phosphate; rather they can also dephosphorylate other structurally unrelated group of substrates, such as fructose 1, 6 bisphosphate, NADP(H), 20 PAP, 30 PAP *etc* [13]. Therefore, the exact catalytic role (but not the regulatory role as extragenic suppressor) of prokaryotic IMPase orthologs is solely dependent on the substrate specificity of the corresponding member. The structural function study of SaIMPase presented herewith may indicate its putative *in vivo* role of the missing eubacterial NADP (H) phosphatase. NADP (H) phosphatase is one of the important enzymes that have been suggested to regulate the intracellular balance of NAD (H) / NADP (H) in concert with NAD kinase (ppnK) during oxidative assault and hence is important for disease progression and pathogenesis in humans [13]. The inositol monophosphatase regulates NAD (H) / NADP (H) levels leading to cellular osmoregulation and activates the proteins responsible for biofilm formation. In the present study we made an attempt to identify novel inhibitor molecules for multi drug resistant of *S. aureus* by targeting sRNA molecules which are non-homologous to humans and essential for functionality of the pathogen [13].

## 2 MATERIALS AND METHODS

The genome sequence of *S. aureus* was obtained from the

NCBI (<http://www.ncbi.nlm.nih.gov/>).

### 2.1 sRNA Prediction

Small non-coding bacterial RNAs (sRNAs) play major regulatory roles in a variety of cellular processes. The sRNAPredict is a tool, which uses coordinate-based algorithms to integrate the respective positions of individual predictive features of sRNAs. Major sRNAs act as a post-transcriptional regulators by interacting with the 5' untranslated region of mRNA transcripts, modifying their stability and/or their ability to be translated [14].

The genome sequence of *S. aureus* was submitted to sRNAPredict for the identification of sRNA candidates. The sRNA candidates responsible for enzymatic activity were selected because of their specific biological activity which is important for regulation in growth and proliferation of the pathogen. Non-homologous search of the enzyme against Homo sapiens was performed to overcome cross reactivity of the inhibitor molecule with the host.

### 2.2 Protein Preparation and Active Site Analysis

The co-crystal structure of the inositol monophosphatase (4G60) with D-MYO-inositol-1-phosphate (IPD) possessing 2.50 Å resolution was retrieved from the PDB and prepared using protein preparation wizard in Maestro v9.6 [15]. Protein molecule was optimized at neutral pH. Optimized protein structure was minimized using OPLS-2005 force field [16]. Bond orders and formal chargers were added for hetero groups and hydrogen atoms were added to all atoms in the system. Receptor grid was generated for prepared protein inositol monophosphatase (4G60) using Glide v6.0, keeping the grid as van der Waals scaling factor 1.00 Å and charge cutoff 0.25 Å subjected to units OPLS-2005 force field. A cubic box of specific dimensions centered on the centroid of the active site residues was generated. The grid was set to 10 × 10 × 10 Å for docking analysis.

### 2.3 Shape Screening

Seven published inhibitors of inositol monophosphatase were selected as reference ligands through literature search [17]. These seven published inhibitors were shape screened against prepared ASINEX 3D database using PHASE v3.6. ASINEX database is a collection of more than 600,000 compounds, encompassing great diversity and also incorporating strategic scaffold design. Virtual screening was performed for the identification of leads that are structurally analogous to the existing inhibitors and to speed the rate of discovery in finding the novel lead molecules, while reducing the need of expensive chemicals and reagents usage. Virtual screening uses a high-performance computation to analyze large database of chemical compounds to identify possible drug candidates, which is seen as a complementary approach to experimental strategies [18]. Virtual screening uses computer based methods to dis-

cover new ligand molecules on the basis of biological structure [19].

## 2.4 Docking Studies

Docking and scoring calculations were performed by Glide v6.0 available in Schrödinger 2014. HTVS (high-throughput virtual screening) mode for efficient screening million compound libraries, to the SP (standard precision) mode for reliably docking tends to hundreds from thousands of ligand molecules with high accuracy, to the XP (extra precision) mode where further elimination of false positives was accomplished by more extensive sampling and advanced scoring [20]. Glide approximates a complete systematic search of the conformational, orientation, and positional space of the docked ligand. In this search, an initial rough positioning and scoring phase that dramatically narrows the search space followed by torsional flexible energy optimization on an OPLS-AA non-bonded potential grid for a few hundred surviving candidates were applied for the prepared ligands along with published inhibitors into prepared inositol monophosphatase grid.

## 2.5 Prime/MM-GBSA

Additionally, for calculating  $\Delta G$  by Prime approach, the docked complexes were presented to molecular mechanics/generalized born surface area (MM-GBSA). The binding energy was calculated through MM-GBSA, OPLS-2005 were much accurate than the XP Gscore. The binding energy of the docked complex was calculated by the following equation

$$\Delta G_{bind} = \Delta E + \Delta G_{solv} + \Delta G_{SA}$$

Where,  $\Delta E$  is the minimized energies,  $\Delta G_{solv}$  is solvation free energies,  $\Delta G_{SA}$  is the difference in surface area energy of the inositol monophosphatase-lead complex and sum of the surface energies of inositol monophosphatase and leads.

The lead molecules based on binding free energy were ranked and evaluated for ADMET properties using QikProp3.9. The obtained MM-GBSA  $\Delta G$  score of leads were compared with published inhibitors and proposed as leads for inositol monophosphatase [21].

## 3 RESULTS AND DISCUSSION

### 3.1 Prediction of sRNAs of *S. aureus*

The whole genome sequence of *S. aureus* was retrieved from the NCBI. The genome size is 2.78 Mb, comprising of 2,676 genes and 2,609 proteins. 2,676 genes of *S. aureus* were subjected to sRNAPredict tool resulted 53 sRNA candidates. Out of these 15 were enzymes, 17 were proteins and 13 were hypothetical proteins. As the enzymes mediate crucial metabolic reactions, 15 enzymes were selected and subjected to non-homologous search against Homo sapiens, resulted seven enzymes. Among these, inositol monophosphatase has co-crystal structure and was selected for the further study.

### 3.2 Active Site Prediction

Active site residues of inositol monophosphatase such as Glu 70, Asp 88, Ile 90, Asp 91, Gly 92, Thr 93, Gly 183, Ala 184, Cys 185, Asn 202 and Asp 209 are present within 4 Å region of d-myo-inositol-1-phosphate (IPD). Asp 91, Gly 92, Thr 93, Gly 183, Cys 185, Asn 202, Ca 301 and Ca 302 are involved in hydrogen bond formation with IPD.

### 3.3 Shape Screening

Shape screening of seven published inhibitors of inositol monophosphatase with ASINEX database contributed 350 structural analog molecules. LigPrep was employed to generate multiple conformations from the published inhibitors and structural analog molecules. The obtained 500 conformations were passed through the Lipinski filter, reactive filters and subsequently 387 ligands were obtained.

### 3.4 Docking Studies and MM-GBSA Scoring

The prepared inositol monophosphatase and 387 ligands were applied for three levels of docking (HTVS, SP and XP) approaches. In high throughput virtual screening mode (HTVS) 96 ligands were obtained from 387 ligands. 46 ligands were generated in standard precision (SP) docking. Extra precision mode (XP) which is more accurate docking method generated 48 leads. Further, free energy was calculated for ligand binding through Prime/MM-GBSA analysis revealed 18 lead molecules with better  $\Delta G$  score compared to seven published inhibitors (Fig. 1). Lead 1, the best lead molecule showed the lowest binding free energy score of -54.85 kcal/mol. The results revealed that lead 1 has a better binding free energy when compared to the proposed eighteen lead molecules and seven published inhibitors of inositol monophosphatase.

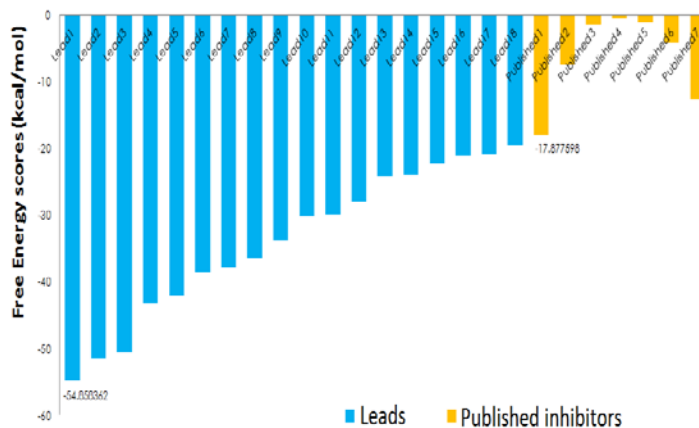


Fig. 1.  $\Delta G$  scores of proposed leads and published inhibitors of IMPase

The co-crystal ligand of inositol monophosphatase (Fig. 2a) is showing six hydrogen bonds with residues Asp 91, Gly 92, Thr 93, Gly 183, Cys 185, Asn 202 and two calcium ions. Whereas, the lead 1 of inositol monophosphatase is showing three hydrogen bonds with active site residues such as Gly 92, Thr 93 and Asn 202. Also lead 1 displayed two salt bridges, one with active site residue, Asp 209 and another with calcium



ion (Fig. 2b). The active site residues of Arg 39, Phe 40, Asp 41, Leu 42, Val 43, Glu 70, Asp 88, Asp 91, Ala 94, Asn 95, Asn 160, Gln 162, Val 163, Leu 181, Gly 183, Ala 184, Cys 185, Thr 203, Asn 204, Pro 205, Lys 206, Trp 208, Ca 301 and Ca 302 also showed additional affinity towards lead 1 in the form of van der Waals interactions. The increased binding affinity and the least binding free energy of lead 1 - inositol monophosphatase complex compared to crystal structure justify lead 1 as a potent inhibitor of inositol monophosphatase. Moreover, the pharmacological properties of lead 1 were well within the range of 95% of existing drug molecules. The two processes namely osmoregulation and biofilm formation by IMPase was inhibited by lead 1 molecule. Therefore, lead 1 would be intriguing as a compound of interest for in vitro and in vivo studies for further analysis to act as a drug molecule against diseases caused by *S. aureus*.

## 4 CONCLUSION

The enzyme inositol monophosphatase performs NAD kinase activity in *S. aureus* and regulates intracellular balance of NAD (H) / NADP (H) thereby regulating osmolarity of the pathogen, it also plays a regulatory role in many cellular processes ranging from protein secretion to biofilm formation, but that activity is not related with its catalytic function because of the absence of inositol derivatives. Therefore, the present study was intended to identify potential inhibitors which resembles as inositol derivatives thereby inhibiting further proliferation of *S. aureus* and its pathogenicity. Co-crystal structure of the inositol monophosphatase with d-myo-inositol-1-phosphate was retrieved from the PDB. The seven published inhibitors including the crystal ligand was shape screened with ASINEX database and applied Lipinski's filter resulted 387 molecules. A grid was generated around the centroid of active site residues of inositol monophosphatase. Virtual screening was performed using virtual screening workflow based on three modes of Glide docking (HTVS, SP and XP) with 387 molecules and Prime MM-GBSA was performed. The lead molecules were ranked based on MM-GBSA scores. 18 leads obtained with better binding free energy than the published inhibitors were proposed as inhibitors of inositol monophosphatase and lead 1 has  $\Delta G$  score of -54.86 kcal/mol. Pharmacological properties of lead 1 molecule correlated favorably with more than 95% approved drug molecule, indicating that they are the potential to inhibit the inositol monophosphatase. Lead 1 will affect the pathogen's NAD (H) / NADP (H) levels leading to dysregulation in osmotic concentration and also inactivates the proteins responsible for biofilm formation. Further in vitro and in vivo evaluations will be carried out to validate lead 1 as a potent drug molecule.

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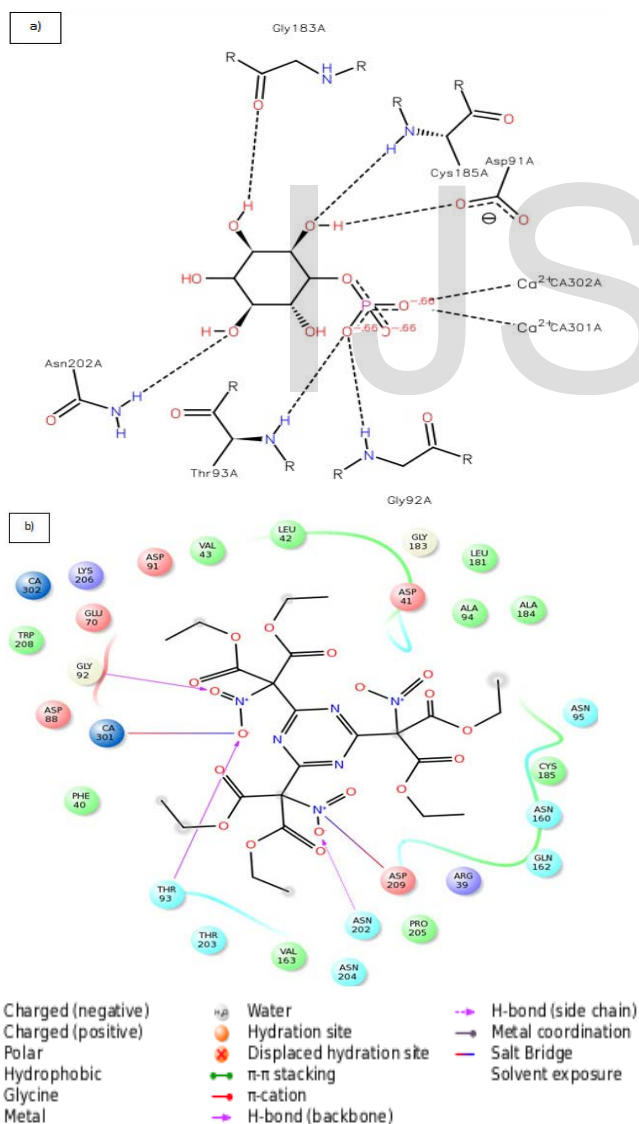


Fig. 2. Molecular interactions of inositol monophosphatase with a) d-myo-inositol-1-phosphate and b) lead 1.

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