

# Identification of Novel Inhibitor Molecules for Choloylglycine Hydrolase of *Enterococcus faecalis*

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**Abstract**— These *Enterococcus faecalis* is a Gram-positive, coccus bacterium which majorly causes nosocomial infections, abdominal infections, wound infections and endocarditis. *E. faecalis* depicts multi drug resistance to antibiotics such as penicillin, ampicillin, vancomycin, gentamycin, streptomycin and daptomycin. The drug resistance of pathogen to the existing drug molecules necessitates the implementation of alternative strategy through *in silico* techniques. sRNAs are non-coding, small RNAs that regulate the metabolic function in the bacteria. Eight sRNA candidates were predicted in *E. faecalis* using sRNAPredict. Choloylglycine hydrolase of linear amide C-N hydrolases cleaves the carbon-nitrogen bonds other than peptide bonds in linear amides. Its critical role in various biological activities such as emulsification, absorption of lipids and glycocholate metabolism leads to growth, multiplication of the pathogens and non-homologous to humans hence, it was selected as putative drug target for *E. faecalis*. Homology model for choloylglycine hydrolase was generated using Modeller 9v13 and validated through Ramachandran plot, ProQ and ProSA. Five existing inhibitors were taken for shape based similarity screening against Asinex database using Phase v3.2 and resulted hits were taken for docking (HTVS, SP and XP) through Maestro v9.6. Further, to validate the docking interactions binding free energy ( $\Delta G$ ) were calculated for each docked complexes. Comparing the leads to the existing inhibitors revealed 15 leads have better binding affinity and molecular interactions. Among that, lead1 has the lowest  $\Delta G$  of -88.90 kcal/mol and found to obey the ADMET properties. Thus the lead1 predicted in the present study is adequate to block the biological activity of choloylglycine hydrolase and in turn decreases the emulsification, absorption of lipids and multiplication of *E. faecalis*.

**Index Terms**— ADMET, Binding free energy, choloylglycine hydrolase, docking, *Enterococcus faecalis*, nosocomial, sRNAPredict

## 1 INTRODUCTION

Small RNAs are regulatory non-coding RNAs which were encoded in both eukaryotic and prokaryotic genomes and most of these RNA transcripts regulate the gene expression by modifying mRNA stability and translation. These RNAs involves in the function by pairing with other RNAs and results in the formation of a part of RNA-RNA complexes, or by adopting the other nucleic acids structures [1]. Some of these RNAs can also control over the virulence gene expression in bacterial pathogens with respect to the host signals [2]. Till date researchers described the role sRNA in the stress response, iron homeostasis, outer membrane protein biogenesis, sugar metabolism and quorum sensing and this is suggesting that sRNAs play an essential and central role in the pathogenicity of many bacteria.

Recently, research has been started to find the role and importance of sRNAs in Gram-negative pathogens such as *Salmonella typhimurium* and *Pseudomonas aeruginosa* [3]. *S. typhimurium* has showed genetic islands showed the host induced expression in macrophages and thus contributed to virulence [4]. 103 sRNA candidates were described in *Listeria monocytogenes* [5], [6], [7], [8]. Only two sRNAs have been identified and studied in *Streptococcus pyogenes* [9], [10] and

five sRNAs in *Streptococcus pneumoniae* [11]. 12 sRNAs were identified in *Staphylococcus aureus*, out of 12; seven were localized on pathogenicity islands. Some sRNAs showed variations in expression levels among pathogenic *S. aureus* strains and it suggest their role in the regulation of virulence factors [12]. In 2010, Mraheil *et al.*, performed comparative global analysis of sRNAs for five major high-risk Gram-positive pathogens on global scale such as *L. monocytogenes*, *S. aureus*, *S. pyogenes*, *E. faecalis* and *C. difficile* by utilizing the Bioinformatics approach [13].

*Enterococcus faecalis* is Gram-positive cocci, facultative anaerobes (Gilmore *et al.*, 2002[14] and they found in soil, water and plants. It is a human commensal and member of the lactic acid producing bacteria. It is also used as an indicator of faecal contamination and to represent causes of the nosocomial infections [15]. Presently, numbers of infections were gradually increasing by cause of *E. faecalis* but virulence mechanism of this organism was poorly understood.

Infections caused by the organism mainly affect the young and immunosuppressed subjects in endocarditis, meningitis, pneumonia, peritonitis, visceral abscesses, urinary infections and septicemias [14]. *Enterococci* are now placing the top three nosocomial bacterial pathogens [16], [17], [18] around the globe. They are acquiring resistance to multiple antibiotics thus they become a major health problem to the humans. 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 [19].

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Earlier reports by the US revealed that most patients with vancomycin resistant Enterococci (VRE) were in ICUs and patients with VRE treatment prone to chronic renal failure, cancer, organ transplant recipients and patients who experience prolonged hospitalization. Cell wall active agents such as penicillin, ampicillin or vancomycin plus an aminoglycoside (Gentamycin or Streptomycin), a linezolid and daptomycin were useful for treatment of diseases caused by *E. faecalis* [20]. Other agents such as a chloramphenicol may be used when multi drug-resistant strains are encountered. Ampicillin nitro furation, tetracycline or quinolones may be effective for urinary tracts infections [21].

The burden of human disease caused by *E. faecalis* has grown over recent decades. *E. faecalis* is a leading cause of various diseases. These were showing resistant to the currently available antibiotics pose real therapeutic difficulties [22]. Up to 90% of Enterococcal infections in humans were caused by *Enterococcus faecalis* [23]. In this context, the present study was carried out with the whole genome sequence analysis using the robust bioinformatics tools for sRNA predication, drug target identification and molecular modeling and docking studies to design and identify the potent inhibitors against the *E. faecalis*.

## 2. MATERIALS AND METHODS

### 2.1 sRNA Prediction

Whole genome sequence of *E. faecalis* was retrieved from the NCBI and analyzed. Further, resultant sequence was submitted to sRNAPredict [24] tool to identify sRNA candidates. sRNAPredict tool uses the coordinate-based algorithms to incorporate the particular or relevant positions of individual predictive features of sRNAs and rapidly identify putative intergenic sRNAs.

### 2.2 Prediction of Non-Human-homologous sRNAs

These obtained sRNA candidates of *E. faecalis* were subjected to BLASTP [25], [26] NCBI server against *Homo sapiens* with default parameters to identify the non-homologous sRNA candidates and threshold expectation value with greater than  $10^{-4}$ .

### 2.3 Homology Model Development

Homology modeling or Comparative modeling is the best theoretical method to construct the atomic resolution of a protein from its amino acid sequence (given query sequence or target sequence) and it should be accepted accuracy that is comparable to the best results achieved by experimentally. Generated model quality mainly depends on the identity between the target and template proteins. Choloylglycine hydrolase of *E. faecalis* was queried against the protein databank (PDB) [27] using BLASTP to identify the template structure. An appropriate template was identified based on the sequence identity. ClustalX was used to align the template and target sequences to carry out the homology modeling using Modeller 9v13 [28],

[29]. Twenty models were generated and applied for validation studies.

### 2.4 Model Validation

Based on the discrete optimized protein energy [DOPE] score, the model which was showing the least DOPE score considered as the best model and applied for the validation studies [30], [31]. The best model was validated by inspecting the Ramachandran plot [32], obtained from the PROCHECK analysis [33]. The PROSA [34] analysis was also carried out to the final model to check the potential errors and energy criteria against the potential of mean force derived from known protein structures. ProQ [37] validation was carried out to check the quality of the obtained final model. The root mean square deviation (RMSD) calculations were performed between the obtained final model and the template by superimposing the structure of template on the predicted structure of choloylglycine hydrolase of *E. faecalis* in order to assess the reliability of the model using Modeller 9v13. The final model was refined using Prime [32] and energy of the model was minimized using the OPLS (optimized potentials for liquid simulations) 2005 force-field [37].

### 2.5 Ligand Dataset Preparation

Reported five inhibitors of choloylglycine hydrolase (phenyl methyl sulfluoride, benzyl penicillin, cholic acid, deoxycholic acid and phenoxy penicillin) were obtained through the literature search were drawn using 2D sketcher in Schrödinger software. Further these were prepared and converted in to 3D formats. These 3D formats were applied for shape based screening using the prepared 3D platinum database of ASINEX. The obtained conformers were further minimized in the Impact module using the OPLS-2005 force field with distinct dependent dielectric and conjugate gradient algorithm and other all parameters were kept default. All the optimized conformers were prepared using the LigPrep [38] and Epik [39] was applied to remove the conformers which are not obeying the Lipinski's rule of five.

### 2.6 Protein Preparation

The obtained 17<sup>th</sup> best model structure of choloylglycine hydrolase of *E. faecalis* was prepared using the Protein Preparation Wizard workflow of Maestro 9.6 [40]. During the protein preparation process hydrogens were added and water molecules were removed (out of the 5 Å of active sites), partial charges were assigned using OPLS-2005 force field and protonation states were defined and energy minimization with a small number of steps to relax amino acid residue side chains were carried out. Protein minimization was carried out using Impact refinement module in the presence of OPLS-2005 force field and terminated when the root-mean square deviation (RMSD) reached a maximum cutoff of 0.30 Å.

### 2.7 Active Site Prediction

The active site residues of the modeled protein were investigated using the SiteMap [41]. Initially, SiteMap calculation begins with the search step method which identifies or characterizes through the use of grid points that may be suitable for binding ligands to the receptor.

## 2.8 Grid Generation, Docking and Binding Free Energy Calculations

The protein van der Waals radii scaling factors was set as 1.0 (default) and Grid was generated around the centroid of the active site residues in Glide v6.0 [42] and 10 Å X 10 Å X 10 Å grid box was generated.

The prepared ligand data set was docked into the active site residues of choloylglycine hydrolase structure in the high throughput virtual screening (HTVS) protocol [43], [44], [45], [46]. XP docking is the most accurate method, in order to get the best leads we performed docking by applying the HTVS, SP and later with the XP docking mode from lower stringency to higher stringency.

All obtained top docking poses were further applied to the binding free energy analysis and each ligand was re-scored by the molecular mechanics/generalized Born surface area (MM-GBSA) approach in Prime [47].

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

Where,  $\Delta E$  is the minimized energies,  $\Delta G_{sol}$  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 leads with highest negative binding free energy was selected and screened for drug-likeness properties.

## 2.9 ADMET Screening

Absorption, distribution, metabolism, excretion and toxicity (ADMET) screening of obtained the best ranked leads were carried out using QikProp module [48]. QikProp predicts the physically significant descriptors and pharmaceutically relevant properties. QikProp predicted 44 properties for the molecules, consisting of principal descriptors and physicochemical properties, along with a detailed analysis of log P (octanol/water), QP%, and logHERG.

## 3 RESULTS AND DISCUSSION

### 3.1 Prediction of sRNA

The *E. faecalis* genome sequence is of 3.22 (Mb) size and comprising of 3257 genes and 3,112 proteins. By submitting genome sequence to the sRNAPredict tool, eight sRNA candidates were identified.

### 3.2 Prediction of Non-homologous sRNA

Out of eight sRNA candidates, four are hypothetical proteins; two are frame shift candidates, one cobalt transfer protein and

one enzyme, choloylglycine hydrolase. The enzyme choloylglycine hydrolase is a non-homologous to human and which is involving in the glycocholate pathway. Choloylglycine hydrolase enzyme was selected as a drug target.

Present drug discovery and development focused to identify and optimizing the drug candidates that may be act through the inhibition of specific enzyme targets. The importance of enzymes as targets for drug discovery started from the high levels of target validation and target tractability which characterize the protein classes [49]. In this context, Choloylglycine hydrolase enzyme was selected as a drug target; further homology modeling and molecular docking studies were performed.

### 3.3 Homology Modeling of Choloylglycine Hydrolase

The main intend of the homology modeling (or) comparative modeling is template selection and sequence similarity between the target and template. Choloylglycine hydrolase of *E. faecalis* protein sequence was queried in the BLAST against the PDB (2BJF) and template hit was found as conjugated bile acid hydrolase from *Clostridium perfringens* in complex with deoxycholate (DXC), it showed 44 % identity, 100% sequence coverage and resolution of 2 Å. This structure was selected as a template for model generation using Modeller 9v13. 20 models were generated and ranked based on the DOPE score. Selection of the best model from the twenty models was scrutinized based on DOPE score [30], [31]. The 17<sup>th</sup> model showed lowest DOPE score of -35607.4 kcal/mol, was selected as the best model, lower DOPE score represents relatively more stable 3D conformation [30], [31] and model was applied to further validation studies (Fig.1).

### 3.4 Validation of the Predicted Structure

The overall geometric and stereochemical quality of the obtained best 17<sup>th</sup> model was assessed by ADIT (PROCHECK) (Fig. 2(A)), ProSA (Fig. 2(B)) and ProQ (Fig. 2(C)). A good quality model would be expected to have more than 90% residues in most favored region [30], [31]. Ramachandran plot showed 95.9% residues in the most favored region was considered to be a valid model with good stereochemical quality [50]. 2% residues were fall under the allowed region, 1.4% in the generously allowed region and 0.7% in the disallowed region. These results revealed that the majority of the amino acid residues were present in the phi-psi distribution that is consistent which gives the the model was reliable with good quality (Fig. 2(A)).

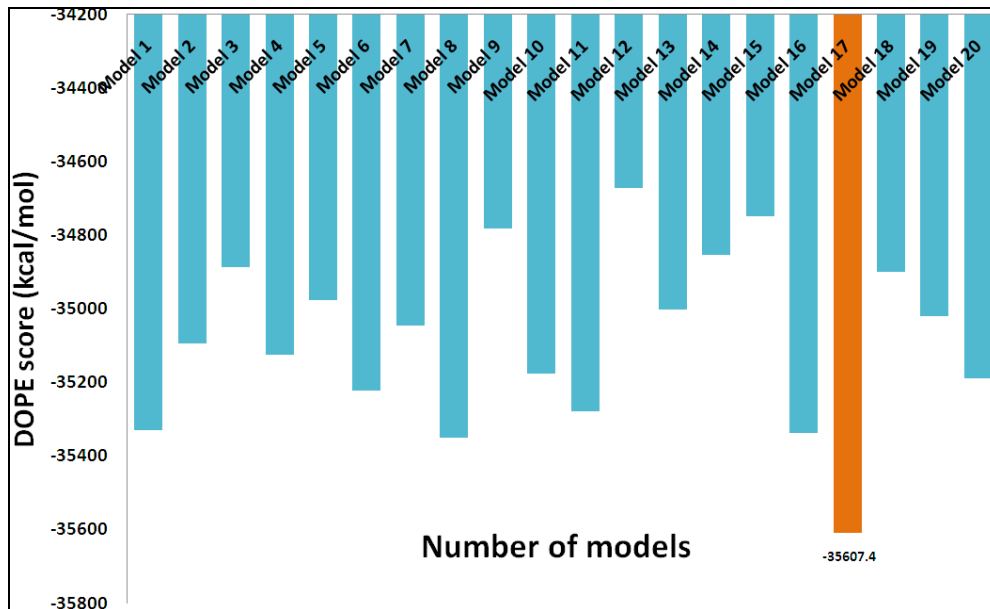


Fig. 1. 20 modeled structures with the DOPE scores

ProSA [36] is a tool widely used to check 3D models of protein structures for potential errors. The overall quality score calculated by ProSA for a specific input structure is displayed in as Z-plot and energy plot (Fig. 2 (B), (C)). The Z-score of -7.41 obtained for choloylglycine hydrolase model reflects the predicted structure correlates well with experimentally determined protein structures of similar length currently available in the protein data bank (PDB). The energy plot reflects overall energy for most part of the protein including active site is negative. The ProSA result affirms the structure is of good quality. The ProQ showed LG score of 4.215 reflects the model is of extremely good quality. The predicted 3D structure was visualized in PyMol (Fig. 2 (D)).

### 3.5 Prediction of Ligand Binding Site Residues Using SiteMap

Using the SiteMap, Cys2, Arg18, Met20, Ile22, Tyr24, Phe26, Phe61, Thr66, Phe67, Ala68, Gly80, Leu81, Asn82, Val102, Tyr103, Ile133, Ile137, Pro138, Asn139, Thr140, Leu142, Trp144 and Trp144 were predicted as active site residues which were also correlated with the interactions shown by the template with co-crystal ligand.

### 3.6 Protein Preparation

The validated model was optimized; energy minimization was carried out to improve favorable steric contacts. The prepared choloylglycine hydrolase was directed towards molecular docking for virtual screening with the prepared ligand dataset.

### 3.7 Compiling Ligand Dataset

Shape based screening of five published inhibitors were carried against the prepared ASINEX 3D database [51] using PHASE and obtained hits were applied to LigPrep and Epik tools. 2550 ligand conformers were obtained after the Post LigPrep and Epik analysis. The prepared in house library of

2550 conformers was applied for docking.

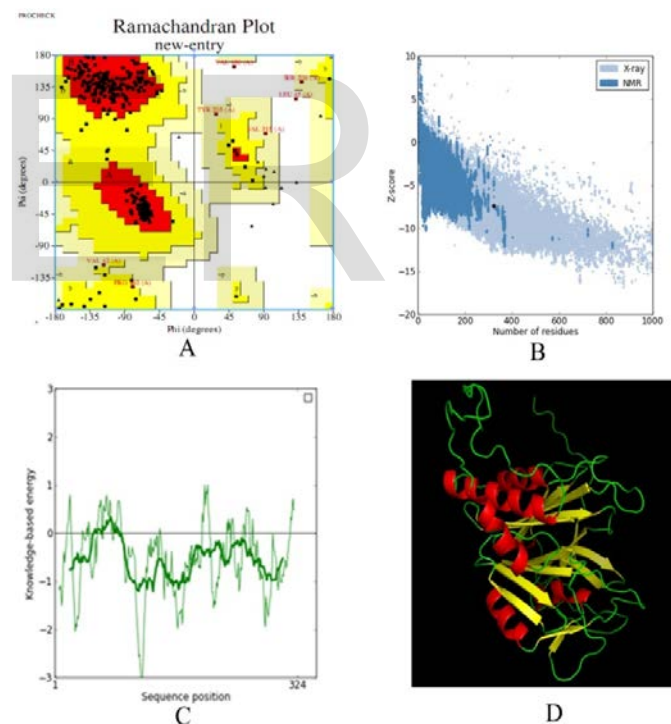


Fig. 2. Validation of choloylglycine hydrolase of *E. faecalis* (A) Ramachandran plot of modeled choloylglycine hydrolase of *E. faecalis*. (B) Z-score plot ProSA analysis, (C) Energy plot obtained through ProSA web analysis (D). 3D-Structure visualization of choloylglycine hydrolase by PyMOL.

### 3.8 Glide Molecular Docking and Binding Free Energy Analysis

Molecular docking is a computational method that gives

binding interactions between the small molecules and with the binding site/active site residues of the protein; scoring functions were used to assess which of these small molecule conformations reveals the best complement of the protein-binding site. Two types of procedures were present to assess the quality of docking methods: (i) Docking accuracy, which deals with the true or exact binding mode of the ligand to the target protein, and (ii) Screening enrichment, which gives how much better a docking method is identifying true binding ligands than random screening. In this we followed screening procedure through HTVS, SP and XP docking [43], [44], [45], [46].

TABLE 1

MM-GBSA binding scores of five published inhibitors

S. No.	Inhibitors	MM-GBSA score(kcal/mol)
1	Phenyl methyl sulfoxide	-33.854
2	Benzyl penicillin	-56.111
3	Cholic acid	-31.010
4	Deoxy cholic acid	-44.934
5	Phenoxy penicillin	-30.734

A receptor grid of  $10\text{\AA} \times 10\text{\AA} \times 10\text{\AA}$  was generated around the active site residues of choloylglycine hydrolase. Initial docking of 2550 compounds into choloylglycine hydrolase grid showed 241 compounds docked with significant Gscore in HTVS. The 75 top ranked compounds were re-docked using standard precision (SP) docking. Sixty compounds were observed to show significant Gscore in SP docking, hence re-docked using extra precision mode (XP). The top 25 compounds ranked based on XP Gscore were evaluated through Prime/MM-GBSA free energy calculations. Out of 25 lead molecules, five lead molecules showed highest negative  $\Delta G$  bind score compared to all published inhibitors and among all lead 1 showed the lowest binding energy of -88.9062.4 kcal/mol. Among the five existing inhibitors (Table 1), benzyls penicillin showed  $\Delta G$  score of -56.11 kcal/mol were shown in Tab.1.

In the co-crystal experimental findings showed that Arg18 was involving in the hydrogen bond interaction with the ligand DXC. The binding affinity of lead 1 was observed to be better compared to existing five inhibitors and well collaborated with the template co-crystal active site residues. The binding orientation of lead 1 also collaborated well with existing inhibitors. Importantly, lead 1 is interacting with active site

residues through hydrogen bonding, van der Waals interactions and  $\pi$ - $\pi$  stacking. Lead 1 is interacting with Arg18  $\pi$ - $\pi$  stacking and Cys2, Met20, Ile22, Tyr24, Phe26, Phe61, Thr66, Phe67, Ala68, Gly80, Leu81, Asn82, Val102, Tyr103, Ile133, Ile137, Pro138, Asn139, Thr140, Leu142, Trp144 residues were involved in the van der Waals interactions (Fig.3). Lead 2 is forming one hydrogen bond with the Thr66; Lead 3 was formed one hydrogen bond with the Thr140; Lead 4 was formed two hydrogen bonds with the Tyr24 and Thr140 and Lead 5 was formed two hydrogen bonds with the Val102 and Leu106. All the five lead molecules exactly binding in the active site region which were well collaborated with the template 2BJF active site region. Residues involved in hydrogen bonding and van der Waals interactions of five lead molecules were analyzed and showed in the Fig.3.

### 3.9 Predicted ADME Properties

The drug like properties of five lead compounds were analyzed using the QikProp [48] and 44 physically significant descriptors and pharmaceutically relevant properties were reported (Tab. 2). The properties which includes molecular weight <500 Daltons, <5 H-bond donors, <10 H-bond acceptors,  $\log P < 5$  (octanol/water) these properties all are well collaborated within the acceptable range of Lipinski's rule of five. The rule is to evaluate the drug likeness of compounds that explains the chemical compound is that may be active drug by following the pharmacological and biological properties. It also describes the drug molecular properties that are very important in the pharmacokinetics in the human body by following the ADMET properties. Further, ADMET properties,  $\log P$  MDCK,  $\log K_p$  (skin permeability), humoral absorption, partition coefficient (QP  $\log P(o/w)$ ) and the water solubility (QP  $\log S$ ), the cell permeability (QP PCaco) and all other pharmacokinetic properties were within the acceptable range, which defines the proposed five lead molecules would act as a potential drug-like molecules reinforce these findings.

Choloylglycine hydrolase enzyme is mainly involving in the glycocholate, carbohydrate, amino acid and lipid metabolisms. These pathways were key energy sources for the the organism. By inhibiting the choloylglycine hydrolase leads to the starvation of *E. faecalis*. Infections caused by this organism are gradually increasing in the present world and a virulence mechanism was unknown. Hence five lead molecules were proposed as potent inhibitor molecules would be intriguing for rational drug design against Enterococcal infections and could be highly encouraging for future endocarditis therapy if tested in animal models.

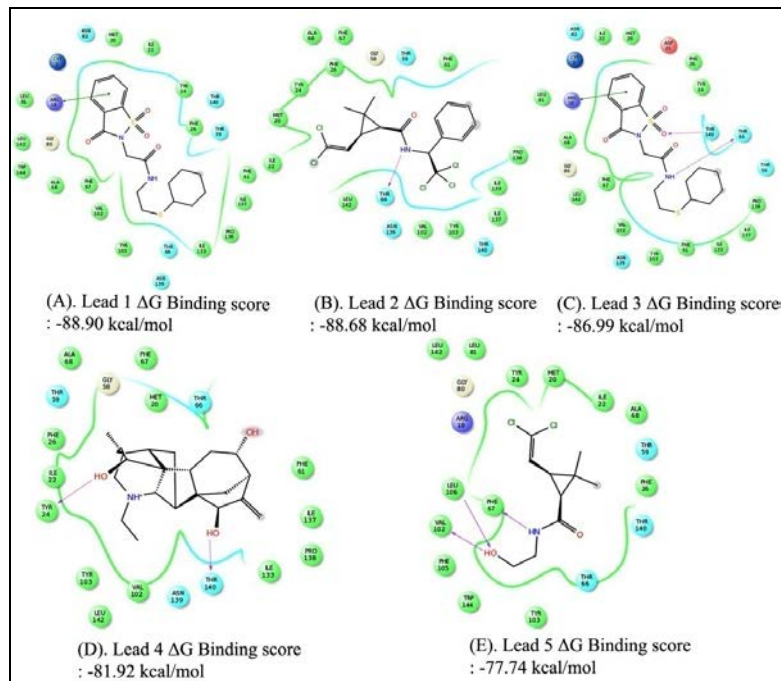


Fig. 3. Docking interactions of five lead molecules with  $\Delta G$  bind score

TABLE 2  
QikProp properties of top five lead compounds

QP polrz	QPlog PC16	QPlogP w	QPlog Po/w	QPlogS	QPlog Poct	CIQ PlogS	QPlog HERG	QP PCaco	QP logBB	QPP MDCK	QP logKp	QPlog Khsa	% Oral Absorp- tion	PSA	Rule Of Five	Rule Of Three
37.46	11.33	12.06	1.89	-3.73	18.07	-3.59	-3.64	460.6	-1.00	361.27	-2.80	-0.40	85.68	102.3	0	0
35.26	11.35	5.71	5.36	-6.38	15.72	-6.20	-3.15	685.0	0.84	10000	-0.84	0.54	100	24.7	1	1
35.46	10.75	12.46	1.68	-2.59	17.41	-3.59	-3.00	589.4	-0.76	469.37	-2.44	-0.51	86.39	100.4	0	0
34.71	9.97	12.41	1.80	-2.26	19.26	-2.62	-3.95	326.9	-0.03	163.46	-4.79	0.07	82.53	61.5	0	0
23.14	7.70	9.20	1.67	-2.66	12.78	-1.81	-2.45	881.6	-0.27	3016.4	-2.70	-0.53	89.47	57.4	0	0

## 4 CONCLUSION

*Enterococcus faecalis* is a multifaceted lactic acid bacterium with an intimate relationship to human health and disease. It also colonizes the gastrointestinal tracts of new borns and adult people. *E. faecalis* is also a prominent cause of multi-resistant nosocomial infections. Small RNAs (sRNAs) are playing important roles in the wide variety of cellular processes and regulatory roles in a variety of cellular processes and also control over the virulence gene expression with respect to the host signals.

In the present study, sRNAPredict analysis was performed against the whole genome sequence of *Enterococcus faecalis*. Eight sRNA candidates were obtained and choloylglycine hydrolase which is non-homologous to the *Homo sapiens* and it was proposed as drug target. Three-dimensional structure was generated using Modeller 9v13 and structural analysis for generated model revealed that the model is reliable and a good quality model with stable lowest energy. We performed shape based screening towards the inhibitors of choloylglycine hydrolase of *E. faecalis* from using the ASINEX platinum subset database and prepared in-house library of dataset and import-

ed to Maestro v9.6. We proposed five lead molecules from the docking studies and free energy calculations which are having better binding affinity, than the five published inhibitors.

Further, binding conformation analysis of the five leads revealed that active site residues of choloylglycine hydrolase Arg18, Tyr24, Thr66, Val102, Leu106 and Thr140 were interacting with the leads. All the five lead molecules were obeying ADMET and other pharmacological properties. Hence, these five lead molecules blocks the enzyme activity leads to starvation of *E. faecalis* and its pathogenicity.

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