CHAPTER 3

STRUCTURE BASED VIRTUAL SCREENING, MOLECULAR DOCKING AND MOLECULAR DYNAMICS STUDIES TO DISCOVER NOVEL MurE INHIBITORS

3.1 INTRODUCTION

The rapid increase in antibiotic resistance have led the discovery of new and effective targets that can combat antibiotic resistance. One of the well-proven and well-studied target for antibiotics are the proteins and enzymes involved in peptidoglycan synthesis. Many drugs of class β-lactam and glycopeptide were susceptible in inhibiting this pathway (Bugg, 1999), but are now resistant to bacteria. Although bacteria have developed resistant over many available antibiotics which mainly target the peptidoglycan biosynthesis, yet it can still be one of the best target. The main reason is the peptidoglycan layer protects the cell for its viability (Scheffers and Pinho, 2005) and some of the enzymes involved in this pathway are unexplored as targets. The synthesis of peptidoglycan involves three phases: cytoplasmic, membrane and extra cytoplasmic phase. The present day antibiotics mainly act on later two phases, with only exception of fosfomycin acting on MurA (Falagas et al., 2010; Karageorgopoulos et al., 2012) in cytoplasmic phase.

One of the well-known antibiotic resistant and infectious bacteria is Methicillin-Resistant Staphylococcus aureus (MRSA), first identified in 1961 (Jevons, 1961). The major target of antibiotics were the proteins and enzymes involved in peptidoglycan synthesis, and almost all have acquired resistant over the antibiotics. Figure 3.1 depicts the drugs that inhibit different enzymes of peptidoglycan synthesis. The series of four Muramyl ATP-dependent enzymes successively adds the amino acids to UDP-N-acetylmuramic acid (NAM) to form stem peptide, are unexplored as the targets (Figure 3.1). Hence, these enzymes are one of the most attractive antibacterial targets. The sequences of muramyl enzymes (MurC, MurD, MurE and MurF) are not
conserved but are topologically similar, because of their ATP and NAM binding sites. In the present study we have selected MurE as the drug target. As MurE protein is found in all the strains of *S. aureus*, and as discussed in chapter 2 it was found to be one of the potential drug target. The most important feature for selecting MurE as the drug target is it is found to be druggable and further the availability of experimental crystal structure which was recently elucidated (Ruane *et al.*, 2013) will help in carrying out virtual screening process.

Figure 3.1: Peptidoglycan biosynthetic pathway and antibiotics inhibiting targets

Figure 3.1 depicts the following

- The different antibiotics inhibiting the different targets of peptidoglycan pathway.
- The most unexplored area for drug targets is highlighted in purple, which involves the enzymes from the cytoplasmic phase.

MurE enzyme adds L-Lysine at the third position of stem-peptide, which is conventionally found in gram-positive bacteria. In the late stages of synthesis, to this lysine five L-Glycine molecules are added via FemXAB (Lloyd *et al.*, 2008), which helps in the formation of crosslinking pentaglycine bond. Any unusual activity of MurE develops abnormal glycan intermediates which are not favorable in transpeptidation steps because the pentaglycyl residues are linked to the third position of stem peptide. Preliminary studies conducted by Ruane *et al.* (Ruane *et al.*, 2013)
suggest that when MurE levels are lowered in \textit{in vivo} conditions there was reduction in resistance as there was accumulation of UDP-MurNAc-dipeptide substrate. This accumulation of UDP-MurNAc-dipeptide substrate is converted into undecaprenyl lipid-linked precursor with enzymatic activity of MraY enzyme, which leads to blocking of peptidoglycan synthesis. Further, MurE from \textit{S. aureus} is very specific in ligating L-lysine to the stem peptide, whereas in all other gram positive bacteria most commonly mDAP (meso-Diaminopimelic acid) is ligated. Hence, this makes MurE one of the potential target for screening new lead (inhibitor) molecule. Here, we have screened the MurE, with molecules from three databases via structure-based virtual screening approach, followed by molecular docking and dynamics studies.
3.2 MATERIALS AND METHODS

To identify the potential lead molecule that can inhibit the active site of MurE enzyme, a systematic computational screening approach was employed (Figure 3.2). Docking and molecular dynamic analysis were carried out using 64 bit- Sun workstation with Quad-core processor, 5GB RAM, 500GB HDD on CentOS platform.

![Figure 3.2: Workflow for identifying potential lead against MurE](image)

Figure 3.2 depicts the following
- The schematic representation of systematic approach for identifying the potential lead molecule by virtual screening process using GLIDE.

3.2.1 Protein Preparation

The X-ray crystal structure of MurE in ternary complex with its product UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-lysine and ADP (PDB ID:4C13) elucidated
by Ruane group (Ruane et al., 2013) was retrieved from Protein Data Bank (PDB) (Berman, 2000). The protein was prepared by Protein Preparation Wizard (Sastry et al., 2013). To prepare MurE for docking studies automated preparation approach with three basic steps Preprocessing, Inspecting the protein structure followed by Optimization and Energy minimization was employed. In preprocessing step, missing hydrogens were added, bond orders were assigned, disulfide bonds and metal atoms were treated, loops are filled, overlapping atoms were arranged and finally terminal ends were capped.

In the second step, the structure is inspected and the unwanted heteroatoms, native ligand and water molecules were deleted. Then the hydrogen bonds were optimized by appropriately orienting imidazole ring of Histidine residue, reorienting the hydroxyl groups and amide groups of Asparagine and Glutamine residues. The final step is energy minimization which refines the structure and outputs stable structure with least potential energy for that molecule by applying Optimized Potentials for Liquid Simulations (OPLS_2005) force fields.

3.2.2 Grid Generation
To confine docking of ligands to the active site of receptor the grid is generated. The active site residues for MurE were selected based on literature. MurE has three domains and the major portion of its product (UDP-N-acetyl-α-D-muramyl-L-alanyl-D-glutamyl-L-lyine) binds to the second and third domain, which forms the active site. This active site region for ligating the lysine molecule to UDP-N-acetyl-α-D-muramyl-L-alanyl-D-glutamate includes Tyr 351, Arg 383, Asp 406, Asn 407, Ser 456 and Glu 460 residues. These amino acid residues were selected and grid was generated on this cavity by receptor grid generation panel of Glide (Friesner et al., 2004).

3.2.3 Preparing Ligand Library
The natural screening libraries AnalytiCon discovery (http://www.ac-discovery.com/), InterBioScreen (http://www.ibscreen.com/) and drugs (experimental and available) from DrugBank (Wishart et al., 2006), were used to screen potential lead against MurE. A total of 0.12 million compounds from all the three libraries were extracted in SDF (Structure Data Format) format. These compounds were prepared by LigPrep module of Schrodinger, which filters the molecules based on Lipinski rules and Epik
LigPrep process a series of systematic steps starting from rectifying structural conformation, generating various conformations, filtering structure based on drug likeness by QikProp. The filtered molecules are further minimized and optimized by OPLS_2005 force fields (Shivakumar et al., 2010).

3.2.4 Virtual screening and Molecular Docking by Glide
To the defined grid, which corresponds active site cavity, the prepared ligands were subjected to virtual screening by Glide which applies Hierarchical Docking Strategy. This procedure involves three systematic layers of molecular docking precisions viz., High Throughput Virtual Screening (HTVS), Standard Precision (SP) dock and Extra Precision (XP) dock (Friesner et al., 2004). Three different precisions of docking were used in order to get potential lead molecule with rapid speed. HTVS screens large set of ligands very rapidly, but the sampling methods are restricted and the outcome of HTVS cannot be directly interpreted. Hence, the molecules screened by HTVS are further screened using SP which provides appropriate pose from large pool of ligands. Finally, XP was applied for the best 10% of molecules screened from SP. In each step the ligands are score by an empirical scoring function GScore. The best score and best fit ligands observed by visual inspection were rescored by XP dock and were analyzed by XP visualizer. Prepared 0.12 million ligand molecules were set as flexible whereas the receptor MurE was set as rigid body and were subjected for above mentioned docking precisions.

3.2.5 Cross validation of best pose ligands by AutoDock
The top seven ligands from XP dock were redocked using AutoDock (Morris et al., 2009) to acquire the stable and value-added results. The docking that was performed by Glide which uses Hierarchical Docking Strategy with Monte Carlo sampling whereas AutoDock uses genetic algorithm. Using two different algorithms helps in cross validation. The ligand that proves to show similar interactions with both sampling methods is said to be best lead molecule. AutoDock is one of the widely-used command based docking program and is freely available. In the present study we have taken the advantage of PyRx’s GUI (Graphical User Interface) (Dallakyan and Olson, 2015) to perform docking studies using AutoDock 4.2. AutoDock system also requires preparation of receptor and ligand in specific PDBQT format which was achieved by Open Babel.
3.2.6 Positive control inhibitors for comparative docking studies

Some of the well-known inhibitors that target MurE enzyme of other bacterial species were selected as the positive control molecules for docking studies. According to literature survey we have collected seven known inhibitors viz., Phosphinate inhibitor (Binqì Zeng et al., 1998), \((2S,3R,6S)-3\text{-Fluoro-A}_{2}\text{pm}\) (Auger et al., 1996), \(N\text{-Hydroxy-A}_{2}\text{pm}\) (Auger et al., 1996), CHEMBL564223 (Bugg et al., 2011), 3-methoxynordomesticine (Guzman et al., 2010), thiazolidine-2,4-dione (Tomasić et al., 2012) and 5-Benzylidenethiazolidin-4-ones (Tomasić et al., 2010) for docking. Further we have also included vancomycin a broad-spectrum antibiotic which is currently being used as frontline therapeutic against Staph infections.

3.2.7 Molecular Dynamics (MD) Simulations

The MD simulations of docked protein-ligand complex was performed using Desmond (Conference Chair-Horner-Miller, 2006) with Maestro as visual interface. The MD studies was carried out in TIP3P solvent model and OPLS_2005 force fields (Shivakumar et al., 2010). For MD, the docking coordinates were taken and TIP3P solvent was added making an initial setup of MD system. The system was set in orthorhombic box with dimension 5Å X 5Å X 5Å, ensuring the complete surface of the protein-ligand complex is convoluted in the solvent system. The system was neutralized by adding the counter ions and then system was relaxed by default pre-equilibration procedure of Desmond. Systematically, for six rounds 20ps of time the system was simulated to relax the system, then simulated for long-period of 20ns. The complete simulation was directed in NPT ensemble, with a temperature of 300K and 1 atmospheric pressure. The trajectories of simulation were captured for every 1.2ps time period. The captured trajectories were analyzed for MD parameters and simulation interactions.
3.3 RESULTS AND DISCUSSION

UDP-N-acetylmuramoyl-L-alanyl- D-glutamate-L-lysine ligase (MurE) is a promising and druggable target identified in *S. aureus*. This infectious organism is widely known as super-bug as it is resistant to many of the known antibiotics. The pandemic feature and its resistance to antibiotics, has led to the improper treatments. The ligating property of MurE in adding the L-lysine to the third position of the stem peptide, which makes it a crucial enzyme in the peptidoglycan biosynthetic pathway. This study focuses on identifying the novel inhibitor against MurE by docking approach as described in methods.

The crystal structure of MurE (4C13) with resolution of 1.9 Å (Ruane *et al.*, 2013) was retrieved from PDB. MurE comprises single chain A of 501 amino acids long with one native ligand Uridine 5'Diphospho N-acetyl muramoyl-L-Alanyl- D-Glutamyl-L-Lysine (UML) and four cofactors phosphate ion, magnesium ion, potassium ion and chloride ion. Typically, any of the crystallized structure from the PDB is not set to go for any modelling or docking studies. Hence the molecule needs to be prepared, as the crystallized lacks hydrogen bonds and only contains heavy atoms, water molecules and some other crystallized molecules like heteroatoms, ligands and cofactors. In the present study MurE was prepared by deleting the water molecules and native ligand of the enzyme, however the water molecules and cofactors in active site were retained. To this structure the hydrogen bonds were added and the protein was energy minimized with the final potential energy of -2121 Kcal/mol and final prime energy of -17536 Kcal/mol.

Generating an appropriate grid over the surface of a protein plays a crucial role in docking as it confines the search of poses only in and around the binding site. This strategy saves lot of time and provides a suitable interacting pose. Hence, to the prepared molecule grid was generated at the active site with the dimensions of X=-27.2612Å, Y=-9.0991Å, Z=-0.7016Å. The active site residues are Tyr 351 (polar), Arg 383 (positively charged), Asp 406 (negatively charged), Asn 407 (polar), Ser 456 (polar). These amino acids are selected as they are capable of participating in hydrogen bonds because of their charged and polar nature of R-groups. The grid was placed at the center of the active site, allowing ligands of 20Å dimensions to dock, with grid spacing of 0.5Å.
In medical history of a thousand years and all through the human evolution, natural products from three major source plants, microorganisms and animals have played an important role in curing health hazards (Ji et al., 2009). Moreover, the use of these natural products to treat diseases are safe and significantly less prone to side effects. Many of the approved drugs against *S. aureus* are the extracts of natural sources like penicillin, glycopeptides etc. Hence for screening a potential lead molecule we collected ligands from two major natural compound repositories viz., InterBioScreen and AnalytiCon. Additionally, we have also retrieved approved and experimental drugs from the DrugBank repository, so that if we can get any drug as potential molecule inhibiting *S. aureus* would be highly beneficial than of novel molecule.

All the ligands collected were in SDF format and hence were prepared by LigPrep. The prepared set of ligands consists the molecule which obey Lipinski’s rule of five and that which have drug likeness properties. In addition, for every ligand only one best conformation was considered. A total of 0.12 million ligands were prepared from three repositories, accounting to 6,029 (AnalytiCon), 10,796 (DrugBank) and 112,280 (InterBioScreen) respectively. These prepared 0.12 million ligands were subjected to a systematic virtual screening process (as discussed in methods) to identify the best fit ligands.

Virtual screening is the alternative approach to most dominant physical technique called high-throughput screening which screens large chemical sets against biological targets (Shoichet, 2004). Virtual screening is now widely used in drug discovery as it is robust, low cost and have many success stories. Some of the successful inhibitors discovered by virtual screening process include Ritonavir, Nelfinavir, Indinavir, LY-517717, Aliskerin, PHT-427, Rucaparib, NVP-AUY922 (Chen et al., 2012). Hence we have also applied virtual screening process with 0.12 million compounds against MurE enzyme of *S. aureus*. In every step of virtual screening process, the best fit ligands (based on the G score (Glide score)) were selected and forwarded to next step. In the primary screening HTVS approach was employed which could retain 91,822 ligands followed by 19,427 by SP Dock from all the three screening libraries (Figure 3.3). The top 100 ligands from each screening library were further filtered out by XP Dock. Upon visual inspection of the docked ligands, about 26 ligands were observed
to be interacting with one or more active site residues of MurE. Of these seven ligands, have observed to have lowest G scores.

Figure 3.3: Stepwise virtual screening process filtering the ligands from three screening libraries

Figure 3.3 depicts the following
- The schematic representation of systematic virtual screening process.
- The step by step virtual screening process results in seven potential lead molecules from a set of 0.12 million compounds.

In the present study, we have predicted the best ligands based on scoring functions and visual inspections. The scoring functions are very much important in virtual screening as they are designed such that they can differentiate a true pose from false one (Kitchen et al., 2004). The scoring functions also helps in evaluating the predicted ligands and ranking them based on their best conformations. Here, we have employed two programs for docking which uses Force-Fields as scoring functions.
Glide (G score) applies the Tripos force field (Kramer et al., 1999) whereas AutoDock is based on AMBER force fields (Morris et al., 1998).

G score is calculated by:
\[
\text{GScore} = 0.065*\text{vdW} + 0.130*\text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}
\]
Where, vdw is Van der Waals energy, Coul is Columbic energy, Lipo is Liphophilic energy, Hbond is for Hydrogen bonding terms, Metal for metal ion interaction terms, BuryP defines the penalty score of polar atoms that are buried, RotB is penalty for rotatable bonds which are freezing and Site stands for the polar interactions that are observed in the active site.

Whereas the AutoDock score is calculated by employing the six pair wise evaluation (V) and the entropy of the conformation which is lost while interactions (\(\Delta\text{Sconf}\))
\[
\Delta G = (V_{\text{bound}L-L} - V_{\text{unbound}L-L}) + (V_{\text{bound}P-P} - V_{\text{unbound}P-P}) + (V_{\text{bound}P-L} - V_{\text{unbound}P-L} + \Delta S_{\text{conf}})
\]
Where, L is ligand and P is Protein.

To attain the stable results, the seven best interacting residues were rescored by XP Dock and cross validated using AutoDock. The Glide and AutoDock score were found to be in the range of -12.5 to -10.8 Kcal/mol and -7.3 to -4.67 Kcal/mol (Table 3.1). The top scored ligand from both the docking tools was LigPrep32109441, having a Gscore of -12.5 Kcal/mol and AutoDock score (Binding affinity) of -4.67 Kcal/mol.
### Table 3.1: Glide and AutoDock scores

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Ligand ID</th>
<th>Screening Database</th>
<th>IUPAC name</th>
<th>Glide Score</th>
<th>AutoDock Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>LigPrep 21</td>
<td>AnalytiCon</td>
<td>4-(3,4-Dihydroxycinnamoyl)quinic acid; 4-Caffeoylquinic acid; 4-O-(E)-caffeoylquinic acid; 4-O-(E)-caffeoylquinic acid; Cryptochlorogenic acid</td>
<td>-11.5</td>
<td>-4.9</td>
</tr>
<tr>
<td>2.</td>
<td>LigPrep 6757</td>
<td>DrugBank</td>
<td>(2R,3R,4R,5S,6R)-6-(((2S,3R,4R,5R)-5-(2-amino-6-oxo-6,9-dihydro-1H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-3,4,5-trihydroxyoxane-2-carboxylic acid</td>
<td>-12.4</td>
<td>-6.09</td>
</tr>
<tr>
<td>5.</td>
<td>LigPrep 08789026</td>
<td>InterBioScreen</td>
<td>2-[[1-(1-benzyloxy carbonyl)pyrrolidin-2-yl]carbonyl]-4-piperidyl][carbonyl]amino]-5-guanidino-pentanoic</td>
<td>-12.0</td>
<td>-5.93</td>
</tr>
<tr>
<td>6.</td>
<td>LigPrep 32109441</td>
<td>InterBioScreen</td>
<td>(2R)-2-[[1-(2R)-2-(benzyloxy carbonylamino)propanoyl]piperidine-4-carbonyl]amino]-5-guanidino-pentan</td>
<td>-12.5</td>
<td>-7.3</td>
</tr>
<tr>
<td>7.</td>
<td>LigPrep 01575532</td>
<td>InterBioScreen</td>
<td>(2R)-5-[[amino(iminiumyl)methyl]amino]-2-[2-(phenylformamido)acetamido]pentanoate</td>
<td>-11.5</td>
<td>-4.9</td>
</tr>
</tbody>
</table>

**Legend:** Table shows the top seven ligands screened against MurE enzyme, the ligand highlighted in bold face indicates the best lead molecule.
The visual inspection of docked ligands (Figure 3.4) indicate that hydrogen bonds were found to be conserved with interacting residues Glu 460 and Asp 406 excluding LigPrep21 and LigPrep6757 ligands (Table 3.2). The ligand LigPrep32109441 (2R)-2-[[1-[(2R)-2-(benzyloxycarbonylamino)propanoyl]piperidine-4-carbonyl]amino]-5-guanidino-pentan was observed to be one of the best ligand molecule, as it has least dock score. Moreover, when compared to other ligands the docked pose of LigPrep32109441 has least hydrogen bond energy (-3.6 Kcal/mol) and electrostatic energy (-5.4 Kcal/mol). Furthermore, the ligand interacting to amino acid residues of MurE are from active site.

The role of MurE is to ligate L-lysine at the third position of stem peptide of MurNAc. To ligate L-lysine to MurNAc, these two molecules must interact with the active site residues of MurE, hence forming active site not only for L-lysine but also for MurNAc. In table 3.2 the other active site indicates the residues that bind to substrate MurNAc. All the ligands have shown to be interacting with some other active site residues, which even blocks MurNAc to interact. Of the seven ligands, ligand LigPrep32019941 have potent interactions with L-lysine active site residues and also with other active sites. This makes it one of the most potential lead molecule as it has the capability to block both the ligating molecule (L-lysine) and substrate (MurNAc) by interacting to their corresponding active sites.
Figure 3.4: Visual inspection of top scored ligands

Figure 3.4 depicts the following:

- The 2D images of protein-ligand interactions for top seven ligands.
- The visual inspections indicate that all the ligands interact to active site residues Glu 460 and Asp 406 excluding LigPrep 21 and LigPrep 6757 ligands.

Table 3.2: Interacting residues and energies of docked ligands

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Ligand ID</th>
<th>Binding residues</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-Lysine Active site</td>
<td>Other Active site</td>
</tr>
<tr>
<td>1.</td>
<td>LigPrep 21</td>
<td>Arg383, Tyr351</td>
<td>Mg504</td>
</tr>
<tr>
<td>2.</td>
<td>LigPrep 6757</td>
<td>Arg383, Tyr351</td>
<td>Mg504, Arg187</td>
</tr>
<tr>
<td>3.</td>
<td>LigPrep 5664</td>
<td>Glu460, Asp406, Arg383, Ser456</td>
<td>Mg503, Mg504</td>
</tr>
<tr>
<td>4.</td>
<td>LigPrep 7832</td>
<td>Glu460, Asp406, Arg383, Tyr351</td>
<td>Mg503, Mg504</td>
</tr>
<tr>
<td></td>
<td>LigPrep 08789026</td>
<td>Glu460, Ser456, Asp406</td>
<td>Mg503, Mg504</td>
</tr>
<tr>
<td>---</td>
<td>----------------</td>
<td>------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>6.</td>
<td>LigPrep 32109441</td>
<td>Glu460, Ser456, Asp406, Arg383</td>
<td>Asn151, Mg503, Mg504</td>
</tr>
<tr>
<td>7.</td>
<td>LigPrep 01575532</td>
<td>Glu460, Ser456, Asp406</td>
<td>Mg503, Mg504, Hie181, Thr137, Thr137, His353</td>
</tr>
</tbody>
</table>

**Legend:** Table shows the interacting residues with different ligands and overall H-bond and electrostatic energies.

The interactions of a potential lead molecule LigPrep32109441 with MurE were further analyzed by Maestro and LigPlot+. The interactions are represented in two-dimensional (2D) by Ligand interaction diagram and LigPlot+ (Figure 3.5), whereas the three-dimensional (3D) by XP-pose Viewer (Figure 3.6). About ten different interactions are observed between the ligand and protein. Of the ten, five interactions are with active site amino acid residues.

The active site amino acid residues Asp 406, Glu 460 form hydrogen bonds, Tyr 351, form hydrophobic interaction, Ser 456 form electrostatic interaction, whereas Arg 383 form pi-pi interaction with that of ligand atoms (Figure 4). Other five interactions were found to be hydrogen bonds, which included Thr 137, Asn 151, Arg 187 residues and also two Magnesium ions. The 3D visualization of docked complex indicates that ligand interacts in the active site groove (Figure 3.6). The bond distances and interacting atoms of ligand and amino acid residues are tabulate in Table 3.3.
Table 3.3: Ligand LigPrep 32109441 in complex with MurE

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Protein Residue (atom)</th>
<th>Ligand Atom</th>
<th>Distance (Å)</th>
<th>Type of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Asn151 (ND$_2$)</td>
<td>O$_5$</td>
<td>2.89</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>2.</td>
<td>Arg187 (NH$_1$)</td>
<td>O$_1$</td>
<td>2.75</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>3.</td>
<td>Arg 187 (NH$_2$)</td>
<td>O$_1$</td>
<td>2.87</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>4.</td>
<td>Glu 460 (OE$_2$)</td>
<td>N$_5$</td>
<td>2.95</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>5.</td>
<td>Asp 406 (OD$_2$)</td>
<td>N$_4$</td>
<td>2.87</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>6.</td>
<td>Arg 383</td>
<td>-</td>
<td>-</td>
<td>Pi-Pi interaction</td>
</tr>
<tr>
<td>7.</td>
<td>Ser 456 (O)</td>
<td>N$_3$</td>
<td>2.89</td>
<td>Electrostatic</td>
</tr>
<tr>
<td>8.</td>
<td>Thr137 (OG$_1$)</td>
<td>O$_4$</td>
<td>2.54</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>9.</td>
<td>Mg503 (metal ion)</td>
<td>O$_4$</td>
<td>2.26</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>10.</td>
<td>Mg504 (metal ion)</td>
<td>O$_3$</td>
<td>2.32</td>
<td>Hydrogen bond</td>
</tr>
<tr>
<td>11.</td>
<td>Tyr 351</td>
<td>C$_{15}$</td>
<td>-</td>
<td>Hydrophobic</td>
</tr>
<tr>
<td>12.</td>
<td>His 205 (NZ)</td>
<td>O$_3$</td>
<td>2.82</td>
<td>Hydrogen bond</td>
</tr>
</tbody>
</table>

**Legend:** Table shows interacting atoms and bond lengths of LigPrep 32109441 and their respective bond lengths with MurE residues.
Figure 3.5 depicts the following

- The 2D images of protein-ligand interactions predicted (A) by Maestro (B) by LigPlot+. 

Figure 3.5: Ligand interaction diagram of LigPrep32109441 with MurE
Figure 3.6 depicts the following

- The ligand LigPrep32109441 interacting with the active site residues, pink dotted lines indicate the hydrogen bonds with distance in Å.
All the eight ligands including vancomycin which were considered as positive control were docked into the MurE active site. The results indicate that seven of six known inhibitors have shown interaction with at least one active site residue (Table 3.4). Phosphinate inhibitor and Vancomycin have shown no interaction with MurE residues.

Table 3.4: Interacting residues and energies of docked ligands (Positive Controls)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>G score</th>
<th>D score</th>
<th>Interacting amino acid residues</th>
<th>H-Bond energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiazolidine-2,4-dione</td>
<td>-7.9</td>
<td>-7.9</td>
<td>Arg383, Thr137, Mg503</td>
<td>-3.1</td>
</tr>
<tr>
<td>N-Hydroxy-A&lt;sub&gt;2&lt;/sub&gt;pm</td>
<td>-8.3</td>
<td>-7.8</td>
<td>Asp406, Mg504, Glu460, Asp204, His353, Mg504, Mg503</td>
<td>-4.2</td>
</tr>
<tr>
<td>(2S,3R,6S)-3-Fluoro-A&lt;sub&gt;2&lt;/sub&gt;pm</td>
<td>-7.6</td>
<td>-7.4</td>
<td>Asp406, Glu460, His353, Ser456, Thr152</td>
<td>-3.2</td>
</tr>
<tr>
<td>5-Benzylidenethiazolidin-4-ones</td>
<td>-7.6</td>
<td>-6.8</td>
<td>Arg187, His353, Mg503</td>
<td>-2.0</td>
</tr>
<tr>
<td>3-methoxynordomesticine</td>
<td>-4.2</td>
<td>-4.1</td>
<td>Arg383</td>
<td>-1.5</td>
</tr>
<tr>
<td>CHEMBL564223</td>
<td>-3.6</td>
<td>-3.6</td>
<td>Arg383, Arg187</td>
<td>-0.8</td>
</tr>
</tbody>
</table>

**Legend:** Table depicts the interacting residues with different ligands and overall H-bond energy.

MurE though have large number of inhibitors, none of them were tested against *S. aureus*. This may be due to the difference between the ligating of L-lysine to third position of stem peptide rather than mDAP which is most common in another species. The substrate and product of MurE enzyme are very large which makes a large active site cavity. Similar, grid box used in virtual screening process was employed for docking of positive control inhibitors. The results suggest that six inhibitors have interacted with at least one active site residue of which Arg 383, Asp 406 and Glu 460 are most common (*Appendix 2*). When compared to the potential lead molecules and other top ligands in virtual screening process, interactions with these three amino acids were found to be conserved. Interestingly, the potential lead molecule has
shown interaction with four active site residues of our interest and in addition to the substrate active site, which was not observed with that of known inhibitors. Further the lead molecule has shown least H-bond energy and dock score. This suggest that lead molecule may be efficient when compared to other inhibitors. MurE having large active site cavity, Phosphinate being a very small molecule couldn’t interact. Vancomycin being specific to target D-aladala-D-al residues of stem peptide and due its structure and mechanism of blocking transpeptidation could not show any interaction with that of MurE.

MD simulation studies for 20ns long were carried out, to mimic the physical environment and to understand the dynamic interaction of MurE in complex with LigPrep32109441. The MD system containing 8395 water molecules and total of 32845 atoms was simulated and the trajectories were captured for every 4.8ps. The protein backbone RMSD (Root Mean Square Deviation) was plotted which determines the distance between the backbone atoms with that of the superimposed molecule. Here the RMSD plot indicates a change in the order of 1.2-2.4 Å which is the acceptable range for most of the proteins. Similarly, the ligand RMSD when ligand fit to ligand and ligand fit on RMSD were also plotted. The RMSD of ligand fit to ligand deviated around 2.0 Å for about first 8ns then the trajectory was found to be stable. When the ligand fit to protein, RMSD was plotted and graph indicates that the ligand has almost deviated similarly with that of protein and the deviations are in a range of 3.2 Å, indicating a stable interaction (Figure 3.7).
Figure 3.7: Molecular dynamics simulation (RMSD) analysis of MurE in complex with LigPrep32109441

Figure 3.7 depicts the following

- RMSD of protein (blue), ligand (dark-red) and protein-ligand complex (pink)

The RMSF (Root Mean Square Fluctuation) was also plotted which measures the fluctuations of particular atom with that of reference time frame. The fluctuations of atoms in the 50-60 amino acids region was high (3.1 Å) followed by 120-125 amino acid residues (2.1 Å). The fluctuation of C-alphas in binding site regions were comparatively low than other residues of proteins as seen in the RMSF plot (Figure 3.8). The lesser the fluctuation in the binding site indicates more stable is the interaction. Over a period of simulation, the stable frame was selected and the complex interactions were analyzed.
Figure 3.8: Molecular dynamics simulation (RMSF) analysis of MurE in complex with LigPrep32109441

Figure 3.8 depicts the following

- RMSF fluctuations of protein backbone.
- Residues spanning in the active site region show less fluctuations.

The binding interactions were analyzed for each frame showing the slight change in the conformations. However, the hydrogen bond interactions with Asp 406 and Glu 460 were found to be stable over a simulation time, when compared to reference frame (Figure 3.9A). The interactions were plotted and the graph indicates that Asp 406 has good interaction with highest interaction factor followed by Glu 460 and Tyr 351 (Figure 3.9B). When compared to the reference frame, at the end of the simulation the interaction of ligand with Asp 406 residue is 96% and with Glu 460 is 91%, indicating the most stable interactions (Figure 3.9D). In docking studies, also these residues were most conserved among the interactions with other top ligands. The docking and MD analysis thereby indicate that the interactions formed between the ligand LigPrep32109441 and protein complex was stable, and hence this ligand can be further evaluated on biochemical aspects.
Figure 3.9: Molecular dynamics interaction analysis of MurE in complex with LigPrep32109441

Figure 3.7 depicts the following

- (A & B) binding interactions
- (C) Ligand interaction after simulation with that of reference frame
The molecular dynamics simulation study was first carried out by Andrew et al., on BPTI (bovine pancreatic trypsin inhibitor) a globular protein of 58 residues length. The MD studies were carried out in vacuum for a simulation period of 8.8 ps which determined the magnitude and fluctuations of the BPTI structure (McCammon et al., 1977). Later on, with advancement in the computational algorithm for MD simulations and availability of supercomputing have made enormous growth in MD calculations. Now a days approximately, system of $10^4$ - $10^6$ atoms for a time period of 10-100 Nano seconds can easily be studied (de Groot and Grubmüller, 2001). MD studies were carried out by different scientific groups to understand the complex behavior. Some of the successful studies includes MD studies on docked complex of L-asparaginase of E. coli with Elspar (Erva et al., 2015), docked complex of myeloid differentiation protein 2 of human with curcumin (Wang et al., 2015) and many more. Results from these studies suggested that when the docked complex of ligand or drug with enzyme is subjected to MD simulation, it has shown similar pose before and after MD. Our results also show similar kind of interpretation, as before MD simulations the strong interactions were observed with Asp 406 and Glu 460 residues and even after the MD simulations over a time period of 20 ns these two residues were observed to show major contacts. This indicates that LigPrep32109441 interacts in the active groove of MurE with stable interaction, hence can be one of the potential lead (inhibitor) molecule.
3.4 CONCLUSION
MurE enzyme plays an important role in peptidoglycan biosynthesis pathway by adding L-lysine to the third position of the stem peptide. Hence being a very attractive drug target. Till date no drug is designed against MurE, so we have employed virtual screening process to identify the potential inhibitor.

The structure based virtual screening and molecular docking process against MurE have determined seven potential molecules that interact with active site residues of MurE. Of the seven the molecule LigPrep32109441 \((2R)-2-([1-[(2R)-2-(benzyloxy carbonylamino)propanoyl]piperidine-4-carbonyl]amino)-5-guanidino-pentan)\) was found to have the least docking score and binding affinity.

MD simulation studies indicate that the protein ligand interaction complex formed is stable. The interactions at the end of the simulations were compared with that of reference frame indicates that the hydrogen bonds formed with active site residues Asp406 and Glu460 are highly stable. From this we can infer that the LigPrep32109441 is a potential lead molecule which may inhibit MurE enzyme. Further pharmacokinetic and \textit{in vitro} studies will shed more light on the activity of the lead molecule.