CHAPTER - 4

Molecular docking, molecular dynamics simulation and prime MM-GBSA analysis of LuxP inhibitors of Vibrio harveyi
INTRODUCTION

Gram negative bacteria use AI-2 as AIs to communicate between bacteria (Bassler, 2002). LuxS protein is the AI-2 synthase in the biosynthetic pathway that is responsible for the production of AIs (Schauder et al., 2001). Periplasmic binding protein (LuxP) binds to AI-2 by clamping it between two domains. The AI-2 bound LuxP activates the inner membrane protein LuxQ (Neiditch et al., 2005). At a low cell density, LuxQ act as autophosphorylating kinase that subsequently phosphorylate the cytoplasmic protein LuxU and DNA binding response regulator protein LuxO (Freeman and Bassler, 1999; Freeman and Bassler, 1999). The phosphorylated LuxO repress QS response by repressing the production of known master QS transcriptional factor LuxR. However, at high density, AIs enters the periplasmic space and it is detected by LuxPQ complex (Waters and Bassler, 2006). The LuxPQ receptor appears to switch from a kinase state (at low AI-2 concentrations) to phosphatase state (at high AI-2 concentrations), resulting in the removal of phosphate groups from LuxU. Since, LuxU act as kinase and it is not able to de-phosphorylate the LuxO. Finally, autoinducer-1 (AI-1) serves as species specific QS signal and regulates the levels of LuxO phosphate, but through a distinct two component sensor kinase, LuxO (Freeman and Bassler, 1999; Freeman and Bassler, 1999).

In the present study, molecular docking, Prime MM-GBSA and Molecular Dynamics (MD) simulation were performed to understand the binding mode of dioxazaborocane analogues and boronic acid derivatives into the binding site of LuxP. The IFD protocol has shown improvement in predicting the favorable binding mode of dioxazaborocane analogues and boronic acid derivatives. The predicted protein-ligand complexes were subjected to MD simulation to determine the stability of the predicted
conformations. Overall, the prediction of ligand binding site could be helpful in the discovery and development of LuxP inhibitors.

MATERIALS AND METHODS

Protein Preparation

The crystal structure with PDB ID: 2HJ9 was chosen as a reference structure for molecular docking studies. The structure was prepared using a multi step process through Protein Preparation Wizard. The steps are described in Chapter 1 (Page No.36). Only the chain A, B and co-crystallized ligand (furanosyl borate diester) were retained. The rest of the chains C and D were removed during the process of protein preparation.

Ligand Preparation

The dioxazaborocanes and boronic acid derivatives were prepared using LigPrep module of Schrödinger. The steps are described in Chapter 1 (Page No. 36 and 37).

Molecular Docking

Three docking protocols, Receptor Rigid Docking (RRD), Induced Fit Docking (IFD) and Quantum Polarized Ligand Docking (QPLD) were used to predict the binding mode of dioxazaborocanes and boronic acid derivatives into the binding site of LuxP. Dioxazaborocanes and boronic acid derivatives were docked into the binding site of LuxP using RRD. A grid box (with co-ordinates x=10, y=10 and z=10) was generated at the centroid of the active site. In RRD, the internal geometry of the receptor is fixed while the ligands are flexible. Then, in order to take the flexibility of receptor and ligand into consideration the IFD protocol was used. Initially, glide docking of each ligand was carried out using a softened potential (van der Waals radii scaling). Maximum of 20 poses for each
ligand was retained and by default poses to be retained must have a coulomb-vdW score less than 100 and hydrogen bond (H-bond) score less than -0.05. Prime minimization was performed for each protein-ligand complex. The receptor structure in each pose now reflects an induced fit to the ligand structure. Glide re-docking was performed for each protein-ligand complex within a specified energy of the lowest energy structure (default 30 kcal/mol). Finally, the ligands were docked into the induced fit receptor using default Glide setting. QPLD protocol aims to improve the partial charges on the ligand atoms by replacing them with charges derived from quantum mechanical calculations (Cho et al., 2005). The ligands were first docked with Standard Precision (SP) followed by refinement using Extra Precision (XP). Five best docked poses were submitted to QM-ESP charge calculation using Density Functional Theory (DFT) and Lee-Yang-Parr correlation functional (B3LYP) with a basis set 6-31G* level. Finally, improved docking accuracy was predicted using re-docking of ligands with updated charges of XP docking. The resulting Glide score was used to predict the binding affinity and the ranking of the ligands.

**Molecular Dynamics Simulation**

In order to investigate the stability of the protein-ligand complexes, the MD simulation was performed on the protein-ligand complexes using Desmond module (Bowers et al., 2006; Guo et al., 2010) and OPLS-AA 2005 force field. Using system builder, a 10 Å buffered orthorhombic system with periodic boundary conditions were constructed using a Four Point Transferable Intermolecular Potential (TIP4P) water model. The solvated systems were neutralized by adding appropriate counter ions by replacing water molecules to provide a neutral simulation box. The system was minimized with
maximum 2000 iterations of a hybrid steepest descent and the limited memory Broyden-Fletcher-Goldfarb-Shanno (LBFGS) algorithm. The bond length and angles involving hydrogen bonds were constrained using SHAKE algorithm and Particle Mesh Ewald (PME) method for electrostatics. The system was maintained with the desired temperature (300 K) and a pressure of 1 atm using Nose-Hoover coupling and Martyna-Tobias-Klein algorithm, respectively. Equilibration was performed under the ensemble desired (NVT, NPT, etc.) until all properties of interest have stabilized. For instance, in NVT, once the temperature is stabilized, the system is equilibrated under this ensemble. In NPT, the pressure and temperature should be stable before proceeding. The minimized system was simulated in the NVT ensemble using a Berendsen thermostat with temperature and a simulation time of 10 K and 12 ps. Furthermore, two short simulations in the NPT ensemble are performed using a Berendsen thermostat and a Berendsen Barostat using a simulation time of 12 ps, 24 ps with a temperature of 10 K, 300 K and a pressure of 1 atm by keeping non-hydrogen solute atoms restrained. In the last stage of the relaxation protocol, simulation was carried out using 24 ps in the NPT ensemble with Berendsen thermostats and Barostat, respectively, with no atom restrained. In production MD, the whole system was simulated for 10 ns for each protein-ligand complex with an integration time step of 4.8 fs. Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and hydrogen bond (H-bond) contacts between protein-ligand complexes were analyzed for each trajectory file generated by the MD simulation with respect to the time scale studied. All graphical plots were generated using OriginPro.

**Prime MM-GBSA**

The top ranked poses of each docked complexes were re-scored by binding free
energy calculation. Prime/MM-GBSA method was used for the prediction of binding free energy ($\Delta G_{\text{bind}}$) for a set of ligands to the receptor. In the present study, binding free energy was calculated for ligands with LuxP using the equation which is described in Chapter I (Page No. 39).

**Density Functional Theory**

The dioxazaborocanes and boronic acid derivatives were used as an input for DFT calculation using Jaguar. The same protocol as mentioned under Density Functional Theory described in Chapter 2 was followed here (Page No. 67).

**Pearson’s and Spearman’s Correlation Co-efficient**

Predicted binding free energies were plotted against EC$_{50}$ and IC$_{50}$ values for the set of dioxazaborocanes and boronic acid derivatives. The degree of correlation co-efficient between the two values are determined using the Pearson’s correlation co-efficient, $r$. It was given as follows:

$$ r = \frac{N \sum xy - (\sum x)(\sum y)}{\sqrt{[N \sum x^2 - (\sum x)^2][N \sum y^2 - (\sum y)^2]}} $$

where $N$ is the number of pairs of scores, $\sum xy$ denotes the sum of the products of paired scores, $\sum x$ denotes the sum of x scores, $\sum y$ denotes the sum of y scores, $\sum x^2$ denotes the sum of squared x scores, $\sum y^2$ denotes the sum of squared y scores.

Spearman’s rho correlation co-efficient $rs$ are calculated, which compares the position of each compound when ranked by binding energy to its position when ranked by its pIC$_{50}$ or pKi value. The Spearman’s rank correlation co-efficient is calculated as follows:
where \( di \) represents the difference in the ranks given to the values of the variable for each item of the particular data and \( n \) is the number of sample size. In addition, correlation was calculated against the results of the scoring functions, such as Glide energy, Glide \( E_{\text{model}} \) and Glide scores, predicted for the dioxazaborocane analogous and boronic acid derivatives.

**RESULTS AND DISCUSSION**

**Molecular Docking**

In the present study, three different docking protocols, including RRD, IFD and QPLD were used to determine the binding mode of the furanosyl borate diester into the binding site of LuxP. Moreover, the comparison between results obtained by three docking protocols revealed that RRD and QPLD achieves better performance to enrich the native conformation of furanosyl borate diester in the receptor binding site with an RMSD of 0.08 Å and 0.06 Å. Lowest RMSD value strongly suggests that protein undergoes slight conformational changes during docking (Fig. 4.1a and 4.1b). Strong correlation in the interaction profile was observed between the docked complexes and the experimental structure of LuxP bound with furanosyl borate diester. In contrast, IFD protocol yielded an average RMSD of 0.28 Å with the same ligand-receptor pairs. The result suggests that IFD does not perform well for this system. A docking performance was evaluated based on binding affinity, Glide score and experimental bioactivities. The above result highlights that RRD and QPLD protocol accurately ranked the bioactivities of the studied inhibitors and also successfully predicted the accurate binding orientation of the protein-ligand.
complexes.

**Fig. 4.1** The superimposition of Glide XP docked conformation to the crystallographic complex. The carbon atoms of both docked and crystal conformation are colored in blue and magenta, respectively. a) represent the superposition of QPLD docked conformation and co-crystalized structure, b) represent the superposition of RRD docked conformation and co-crystalized structure.

**Binding Mode Analysis of Dioxazaborocane Derivatives**

Molecular docking was performed to investigate the molecular mechanism of interactions between dioxazaborocane derivatives and LuxP. **Table 4.1** summarizes the docking results of the dioxazaborocane derivatives. The binding mode of dioxazaborocane derivatives bound form of *V. harveyi* complexed (LuxP) with the periplasmic domain of LuxQ is shown in **Fig. 4.2**. The docking results clearly demonstrated that the dioxazaborocane derivatives interact with LuxP through hydrogen bonds, π–π stacking, π-
cation and salt bridges. The dioxazaborocane derivatives exhibited the docking score in the range of -11.49 kcal/mol to -4.42 kcal/mol. The 3-(6-methyl-1,3,6,2-dioxazaborocan-2-yl) benzoic acid (Compound 1) showed the highest Glide score with respect to the other docked complexes. The carboxyl group of Compound 1 makes distinct H-bond interactions with the side chain amine group of three different atoms of Arg310 (bond length=1.82 Å, NH…O=C), Trp82 (bond length=1.71 Å, NH…O=C) and Asn136 (bond length=1.95 Å, NH…O=C). Meanwhile, the phenyl ring form π-π stacking and π-cation contacts with Arg215. The carboxyl group of Compound 1 also mediates salt bridge with the amine group of Arg215. The two arginine residues, such as Arg215 and Arg310, were found to play a unique role in the mechanism of complex formation (Ni et al., 2008). The compound 3:4-(6-methyl-1,3,6,2-dioxazaborocane-2-yl) benzoic acid possessed the second highest Glide score of -11.13 kcal/mol with the Glide energy of -43.57 kcal/mol. The carboxyl group of Compound 3 exhibit H-bond interaction with the hydroxyl group of Ser79 (bond length=2.40 Å, C=O…HO), while the second, third and fourth successive H-bond interactions were formed between the carboxyl group of Compound 3 and the amine group of three different atoms of Arg310 (bond length=1.88 Å, C=O…NH2), Thr266 (2.12 Å, C=O…NH2) and Arg215 (bond length=1.67 Å, C=O…NH2). The phenyl group shows π–π stacking, π-cation contacts with Arg215 and His140, respectively. The 2-(4-bromophenyl)-6-methyl-1,3,6,2-dioxazaborocane (Compound 5) exhibits the lowest Glide score of -4.42 kcal/mol with the Glide energy of -29.58 kcal/mol. The carboxyl group of Compound 5 forms H-bond interaction with the side chain amine group Asn159 (bond length=2.1 Å, NH…O=C). The substitution of a bromine atom at the 4 th position to the basic dioxazaborocane structure does not favor any measurable H-bond interaction with the
conserved amino acid residues Arg215 and Arg310, which reflects the compound with a very low Glide score among the other dioxazaborocane derivatives. The 4-(6-methyl-1,3,6,2-dioxazaborocan-2-yl) phenol (Compound 7) possessed the second lowest Glide score of -4.82 kcal/mol with the Glide energy of -28.83 kcal/mol. The hydroxyl group of the Compound 7 form H-bond interaction with the carboxyl group of Gln77 (bond length=1.89 Å, OH⋯O=C), while the phenyl group shows π-π stacking with Phe206 and the salt bridge were observed with the residue Asp136. The 3-(6-methyl-1,3,6,2-dioxazaborocan-2-yl) aniline (Compound 2) had the Glide score and Glide energy of -6.05 kcal/mol, -35.35 kcal/mol with two H-bond interactions with Thr266 (bond length=2.28 Å, C-O⋯HN) and Asp136 (bond length=1.94 Å, C-O⋯HN), two arginine residues (Arg215 and Arg310) were stacking against the phenyl ring and π-cation contact was observed with the residue Arg215. A decrease in binding affinity was observed when the carboxyl group of the Compound 1 was substituted with an amide group (Compound 2). The 4-(6-methyl-1,3,6,2-dioxazaborocan-2-yl) benzaldehyde (Compound 4), the carboxyl group of Compound 4 form H-bond interaction with the amine group of Arg215 (bond length=2.16 Å, C=O⋯HN) and the second H-bond was formed with the amine group of Compound 4 with the carboxyl group of Asp136 (bond length=1.99 Å, C-O⋯HN). In addition, a decrease in the binding affinity was observed when the carboxyl group of Compound 1 was moved from meta to para position. The 4-(6-methyl-1,3,6,2-dioxazaborocan-2-yl) phenyl) methanol (Compound 6), three H-bond interactions were formed between the carboxyl group of Compound 6 and the amine group of three different atoms of Ser79 (bond length=2.0 Å, NH⋯O-C), Gln77 (bond length=1.85 Å, NH⋯O-C), Arg310 (bond length=2.1 Å, NH⋯O-C), a fourth H-bond was formed between the carboxyl group of
Compound 6 and the oxygen atom of Thr266 (bond length=2.36 Å, C-O•••O-C). Meanwhile, π-π stacking, π-cation contact was observed with the residue Arg215. The 2-(2-phenyl-1,3,6,2-dioxazaborocan-6-yl) ethanol (Compound 8) possessed the Glide score of -6.56 kcal/mol and Glide energy of -44.47 kcal/mol with four H-bond interactions with Ser79 (bond length=1.92 Å, OH•••O-C), Trp289 (bond length=2.16 Å, C=O•••HO), Thr266 (bond length=2.2 Å, C-O•••HO) and Arg310 (bond length=1.97 Å, NH•••O-C). The additional π-π stacking, π-cation contact was observed with the residues Arg215 and Arg310, respectively.

**Fig. 4.2** Ribbon diagram of dioxazaborocane derivatives bound form of *V. harveyi* LuxP complexed with the periplasmic domain of LuxQ showing the N- and C-terminal domains. α-helices, β-strand and loops of LuxP domain are colored yellow and orange respectively.
α-helices, β-strand and loops of LuxQ domain are colored green and orange, respectively.

**Table 4.1** Molecular docking results via IFD and Prime MM-GBSA results for the diaxazaborocane derivatives

<table>
<thead>
<tr>
<th>S. no</th>
<th>Compounds</th>
<th>Glide score Kcal/mol</th>
<th>Glide energy Kcal/mol</th>
<th>IFD score Kcal/mol</th>
<th>∆G&lt;sub&gt;bind&lt;/sub&gt; Kcal/mol</th>
<th>H-Bonds</th>
<th>π-π &amp; π-cation</th>
<th>Salt bridge</th>
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<tr>
<td>1.</td>
<td>Compound 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-11.49</td>
<td>-39.91</td>
<td>-1263.89</td>
<td>-66.17</td>
<td>Arg310, Trp82, Asp136</td>
<td></td>
<td>Arg215&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.</td>
<td>Compound 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-6.05</td>
<td>-35.35</td>
<td>-1258.98</td>
<td>-60.58</td>
<td>Thr266, Asp136, H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>Arg310&lt;sup&gt;a&lt;/sup&gt;, Arg215&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Compound 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-11.13</td>
<td>-43.57</td>
<td>-1263.81</td>
<td>-81.75</td>
<td>Trp82, Ser79, Arg310, Arg215, Thr266</td>
<td>Arg215&lt;sup&gt;a&lt;/sup&gt;, Hie140&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Arg310</td>
</tr>
<tr>
<td>4.</td>
<td>Compound 4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-7.88</td>
<td>-33.67</td>
<td>-1259.77</td>
<td>-61.97</td>
<td>Arg215, H&lt;sub&gt;2&lt;/sub&gt;O, Asp136</td>
<td>Arg215&lt;sup&gt;a&lt;/sup&gt;, Trp82&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Compound 5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-4.42</td>
<td>-29.58</td>
<td>-1256.69</td>
<td>-56.57</td>
<td>Asn159</td>
<td></td>
<td></td>
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<tr>
<td>6.</td>
<td>Compound 6&lt;sup&gt;f&lt;/sup&gt;</td>
<td>-5.77</td>
<td>-35.21</td>
<td>-1257.05</td>
<td>-83.50</td>
<td>Thr266, Arg310, Ser79, Gln77, Trp289</td>
<td>Arg215&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>Asp136</td>
</tr>
<tr>
<td>7.</td>
<td>Compound 7&lt;sup&gt;g&lt;/sup&gt;</td>
<td>-4.83</td>
<td>-28.83</td>
<td>-1256.89</td>
<td>-61.26</td>
<td>Gln77</td>
<td>Phe206&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Asp136</td>
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<tr>
<td>8.</td>
<td>Compound 8&lt;sup&gt;h&lt;/sup&gt;</td>
<td>-6.56</td>
<td>-44.47</td>
<td>-1259.48</td>
<td>-84.24</td>
<td>Ser79, Thr266, Arg310, Thr134, Trp289</td>
<td>Arg310&lt;sup&gt;a&lt;/sup&gt;, Arg215&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Compound 9&lt;sup&gt;i&lt;/sup&gt;</td>
<td>-6.28</td>
<td>-50.29</td>
<td>-1259.81</td>
<td>-86.04</td>
<td>Arg215, Asn159, Trp289</td>
<td>Phe206&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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</table>

**Compound:**  
<sup>a</sup>3-(6-Methyl-1,3,6,2-dioxazaborocane-2-yl)benzoic acid,  
<sup>b</sup>3-(6-Methyl-1,3,6,2-dioxazaborocan-2-yl)aniline,  
<sup>c</sup>4-(6-Methyl-1,3,6,2-dioxazaborocan-2-yl)benzoic acid,  
<sup>d</sup>4-(6-Methyl-1,3,6,2-dioxazaborocane-2-yl)aniline,  
<sup>e</sup>5-(6-Methyl-1,3,6,2-dioxazaborocane-2-yl)benzoic acid,  
<sup>f</sup>6-(6-Methyl-1,3,6,2-dioxazaborocane-2-yl)aniline,  
<sup>g</sup>7-(6-Methyl-1,3,6,2-dioxazaborocane-2-yl)benzoic acid,  
<sup>h</sup>8-(6-Methyl-1,3,6,2-dioxazaborocane-2-yl)aniline,  
<sup>i</sup>9-(6-Methyl-1,3,6,2-dioxazaborocane-2-yl)benzoic acid,  
<sup>j</sup>10-(6-Methyl-1,3,6,2-dioxazaborocane-2-yl)aniline.
acid, \(d_4\)-(6-Methyl-1,3,6,2-dioxazaborocan-2-yl) benzaldehyde, \(e_2\)-(4-Bromophenyl)-6-methyl-1,3,6,2-dioxazaborocane, \(f_4\)-(6-Methyl-1,3,6,2-dioxazaborocan-2-yl)phenyl)methanol, \(g_4\)-(6-Methyl-1,3,6,2-dioxazaborocan-2-yl)phenol, \(h_2\)-(2-Phenyl-1,3,6,2-dioxazaborocan-6-yl)ethanol, \(i_1\)-(4,8-Dimethyl-2-phenyl-1,3,6,2-dioxaborocan-6-yl)propan-2-ol, \(\pi-\pi and \pi\text{-cation interactions:} \pi-\pi \text{ stacking}^a, \pi\text{-cation interactions}^b.

**Fig. 4.3** The active site region of LuxP is depicted in electrostatic potential surface view and the dioxazaborocane derivatives are shown in brown ball and sticks. The positively and negatively charged surface regions are shown in blue and red respectively. The protein-ligand interactions between LuxP and dioxazaborocane derivatives are shown (a-i). Amino acid residues involved in the interactions with the dioxazaborocane derivatives are
shown in yellow sticks. For clarity the important amino acid that bound with protein are displayed. The hydrogen bonds are displayed in pink dashed lines with distances.

The 1-(4,8-dimethyl-2-phenyl-1,3,6,2-dioxaborocan-6-yl) propan-2-ol (Compound 9) exhibited the Glide score and Glide energy of -6.28 kcal/mol and -50.29 kcal/mol with two H-bond interactions with the residues Asn159 (bond length=2.21 Å, NH…O=C), Trp289 (bond length=2.31 Å, C-O…HO) and π-π stacking was observed with the residue Phe206. The substitution of hydroxyl alkyl moieties to the basic diaxazaborocane structure exhibited a significantly lower binding affinity of Compound 8 and 9 toward LuxP. The binding mode of dioxazaborocane derivatives in the active site of LuxP are shown in Fig. 4.3a-4.3i. The Glide energies for the LuxP-dioxazaborocane complexes range from -50.29 kcal/mol to -28.83 kcal/mol. The IFD score of the dioxazaborocane analogues ranges from -1263.89 to -1256.69 kcal/mol. Interestingly, Compound 1 exhibited better IFD score than Compound 5. It was observed that all the derivatives were able to bind to the active site of LuxP, but shown different conformational states and interaction profiles. Docking studies have confirmed six dioxazaborocane analogues can possess good inhibitory activity to LuxP, because it interacts with Arg215 and Arg310 of LuxP.

**Molecular Docking Analysis of Boronic Acid Derivatives**

In boronic acid derivatives (Compound 1-5), the binding poses obtained with RRD was considerably different from poses obtained with the IFD. The results led to hypothesize that IFD seems more accurate than RRD. However, IFD protocol performs much better than regular docking (RRD) in some cases (Wang et al., 2014). Based on the docking results, the analysis was mainly focused on IFD of the ligands. The selected boronic acid derivatives interact with LuxP through hydrogen bond, π-π stacking, π-cation,
salt bridge and hydrophobic interactions (Table 4.2). In Compound 1 and Compound 2, the groups at R³ and R⁴ positions are different. The methyl moiety at 4th position in Compound 1 was replaced by a 4-methoxy carbonyl moiety in Compound 2. In fact, Compound 2 has better potency than Compound 1 suggest that the additional methoxycarbonyl group is favorable for achieving better potency. The docking score of boronic acid derivatives ranges from -9.44 kcal/mol to -6.92 kcal/mol. As shown in Fig. 4.4a, Compound 1 forms hydrogen bond interactions with the amino acid residues Ser79 (OH⁻•O=C, bond length=1.88 Å), Trp82 (OH⁻•HN, bond length=1.72 Å), Thr266 (OH⁻•O=C, bond length=2.01 Å) and Arg310 (OH⁻•HN, bond length=2.10 Å). In particular, the benzyl ring of Compound 1 forms π-π stacking and π-cation interactions with Arg215. As shown in Fig. 4.4b, Compound 2 forms two hydrogen bond interactions with Thr266 (OH⁻•O=C, bond length=1.95 Å), Arg310 (OH⁻•HN, bond length=2.45 Å). At the same time, the benzyl ring of Compound 2 forms three π-π stacking with the positive amino acid residues such as Arg215, Arg310, Tyr81 and one π-cation interaction with Arg215. The removal of fluorine atom at R³ position in Compound 1 with an addition of the carboxyl group at R⁴ for Compound 3 leads to substantial differences in ligand potency, the basic phenylboronic group in Compound 3 forms three hydrogen bond interactions with Thr266 (OH⁻•O=C, bond length=1.98 Å), Arg215 (OH⁻•HN, bond length=1.88 Å, 1.91 Å). In meanwhile, π-π stacking and π-cation interactions were observed with the residues such as Trp82 and Arg310 as displayed in Fig. 4.4c. The simple replacement of fluorine atom at R³ position in Compound 1 to R⁶ position for Compound 4 increased the activity. However, one atom variation leads to the differences in the binding affinity. As shown in Fig. 4.4d, the binding pose of Compound 4 shows two hydrogen
bond interactions, one with Thr266 (OH−-HN, bond length=2.55 Å) and another one with water molecule. It also forms three π-π stacking interactions with positive and hydrophobic residues such as Trp82, Phe206 and Arg215. For Compound 5, the deletion of fluorine at R³ position with a replacement of isocyano at R⁴ position increased the activity. Therefore, the addition of the isocyano group to the basic phenylboronic acid derivative enhanced the binding affinity of the compound. **Fig. 4.4e**, the hydroxyl group of Compound 5 forms hydrogen bond interactions with the amino acid residues such as Ser79 (OH−-O=C, bond length=1.70 Å), Thr266 (OH−-O=C, bond length=1.88 Å), Arg310 (OH−-HN, bond length=1.83 Å) and also had π-π stacking and π-cation interaction with amino acid residue Arg215. Five boronic acid derivatives exhibit π-π stacking and π-cation interactions with receptor molecules imply their role in the protein-ligand recognition mechanism. It was reported that the positively charged side chain amino acid residues Arg215 and Arg310 which play a significant role in stabilizing the protein-ligand complex through ionic interactions. The two residues important for protein-ligand stability were observed in the five protein-ligand complexes. Overall the interactions with Arg215 and Arg310 should be taken into consideration in designing novel inhibitors targeting LuxP of *V. harveyi*.

**Table 4.2** Experimental pIC₅₀, docking scores predicted by three docking protocols, Prime MM/GBSA re-scoring of the boronic acid derivatives

<table>
<thead>
<tr>
<th>S. no</th>
<th>Compound name</th>
<th>pIC₅₀</th>
<th>RRD Score Kcal/mol</th>
<th>IFD Score Kcal/mol</th>
<th>QPLD score Kcal/mol</th>
<th>ΔGbind Kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Compound 1: 3-fluoro-4-methylphenylboronic acidᵃ</td>
<td>5.04</td>
<td>-4.81</td>
<td>-6.92</td>
<td>-5.02</td>
<td>-45.08</td>
</tr>
<tr>
<td>2</td>
<td>Compound 2: 4-(methoxy carbonyl) phenylboronic acidᵇ</td>
<td>5.30</td>
<td>-4.40</td>
<td>-7.63</td>
<td>-4.05</td>
<td>-63.03</td>
</tr>
<tr>
<td>3</td>
<td>Compound 3: 4-(benzyloxyl)</td>
<td>5.39</td>
<td>-5.01</td>
<td>-9.44</td>
<td>-1.63</td>
<td>-67.59</td>
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<td>Compound 4: 2-fluoro-4-methylphenylboronic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
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</tr>
<tr>
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<td>-5.12</td>
<td>-40.20</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Compound 5: 4-cyanophenylboronic acid</th>
<th></th>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>5.</td>
<td>5.22</td>
<td>-4.40</td>
<td>-7.61</td>
<td>-3.96</td>
<td>-43.76</td>
</tr>
</tbody>
</table>

**Fig. 4.4** Binding pattern of boronic acid derivatives in the binding site of LuxP, **a)** 3-fluoro-4-methylphenyboronic acid, **b)** 4-(methoxycarbonyl) phenylboronic acid **c)** 4-(benzyloxyl) phenylboronic acid, **d)** 2-fluoro-4-methylphenylboronic acid, **e)** 4-
cyanophenylboronic acid. The binding modes of the compounds are shown in ball and sticks. Hydrogen bond interactions are shown in pink dashed lines with the distance between donor and acceptor atoms indicated by Å.

**Molecular Dynamics Simulation of Protein-Ligand Complexes**

**Root Mean Square Deviation (LuxP-Dioxazaborocane Derivatives)**

Ten nanosecond (ns) long equilibration MD simulation was performed on each protein-ligand complex system. The RMSD was determined for backbone atoms as a function of simulation in the MD trajectories (Fig. 4.5). MD simulation results of Compound 5 and 7 showed a higher RMSD value of 2.45 Å and 2.26 Å with the SD of 0.47 Å and 0.43 Å, respectively. A slightly lower RMSD profile was observed for the Compounds (1, 2, 3, 4 and 8). The RMSDs of Cα atoms increases initially and then reach a plateau at 1.75 Å for Compound 1, 1.68 Å for Compound 2, 1.93 Å for Compound 3, 1.63 Å for Compound 4 and 1.55 Å for Compound 8 with the SD of 0.27 Å, 0.21 Å, 0.31 Å, 0.25 Å and 0.20 Å, respectively. The maximum deviation was observed in the range of 3.65 Å to 2.22 Å. During the simulation time frame, Compounds 6 and 9 showed a average RMSD of 2.08 Å and 2.09 Å with the SD of 0.23 Å and 0.30 Å, respectively.
Fig. 4.5 RMSD of backbone atom for the LuxP-dioxazaborocane derivatives as a function of simulation time.

**Root Mean Square Deviation (LuxP-Boronic Acid Derivatives)**

The conformational changes in the protein-ligand complexes were calculated to analyze the backbone RMSD from the initial structure over the course of the trajectory. Fig. 4.6 shows the RMSD values of the protein-ligand complexes. From the figure, it was clear that there was an initial rise in RMSD of Complex 1, which might be due to the absence of restraining the production phase of MD simulation. An average RMSD of Complex 1 was found to be 2.36 Å with an SD of 0.25 Å. It was observed that 8 ns was sufficient to reach an equilibrium state and the stability of the system was observed during the later stages. In Complex 2, the RMSD curve initially showed largest deviation with an RMSD of 1.60 Å over first 5 ns and then shifted back slightly to 1.20 Å to attain its stability with an SD of 0.17 Å.
**Fig. 4.6** The RMSD values of the protein-ligand complexes over the simulation time. X-axis represents time scale in ps and y-axis represent the RMSD in Å. Simulation was carried out in a TIP4P water environment with a time step of 10 ns.

In Complex 3, the RMSD curve showed stability after 4 ns with 2.1 Å deviations with an SD of 0.28 Å. In Complex 4 and 5, constant RMSD was observed throughout the whole simulation with an RMSD of 1.2 Å, 1.3 Å and SD value of 0.17 Å, 0.20 Å, respectively.

**Root Mean Square Fluctuation (LuxP-Dioxazaborocane Derivatives)**

The Cα RMSF as a function of residue number was determined over the 10 ns of MD trajectory (**Fig. 4.7**). The amino acid residues, Pro62, Arg225 and Asp257 has shown 1.48 Å, 1.53 Å and 1.45 Å of fluctuation in the Complex 1. Major conformational changes (Complex 2) were observed in the residue which includes Pro62 (2.09 Å), Thr63 (1.86 Å), Gln64 (1.85 Å), Arg65 (1.63 Å) and Lys125 (1.48 Å). Most notably, there are no larger structural changes observed in the Complex 3, 8 and 9. Higher flexibility was observed
with the amino acid residues, such as Asp36 (1.49 Å), Gln37 (1.51 Å), Lys61 (1.78 Å), Pro62 (1.66 Å), His221 (1.64 Å), Asp217 (1.88 Å), Thr218 (1.49 Å), Asn224 (1.82 Å), Arg225 (2.25 Å), Asp226 (2.42 Å), Asn227 (2.37 Å), Asn228 (1.62 Å) and Asn365 (1.72 Å) in the Complex 4. The amino acid residues showing higher fluctuation that frequently correspond to the exposed loop regions in the protein. In case of Complex 5, the amino acid residues Gln64, Gly75, Glu278, Leu279, Pro249, Leu350 and Asn365 were shown the fluctuation rate of 1.83 Å, 1.85 Å, 1.77 Å, 1.68 Å, 1.52 Å, 1.55 Å and 1.75 Å, respectively. Complex 7 has shown the higher fluctuation rate of 1.48 Å for Pro62, 1.68 Å for Thr151 and 2.85 Å for Asn365.

![Fig. 4.7 RMSF of the backbone atom plotted versus the residue number.](image)

**Root Mean Square Fluctuation (LuxP-Boronic Acid Derivatives)**

RMSF was calculated to characterize the changes in flexibility in the protein residues upon ligand binding. **Fig. 4.8** shows the residue-wise RMSF profile of the protein-ligand complexes. The binding of Compound 1 to LuxP does not induce conformational
changes in the protein structure. Complex 1 showed an average fluctuation of 0.74 Å with an SD of 0.27 Å. However, few amino acid residues exhibits higher fluctuation, which includes Ile113 (1.55 Å), Arg139 (1.65 Å), Asp149 (1.50 Å), Asn152 (1.75 Å) Lys169 (1.72 Å) and Arg225 (1.58 Å). The residues exhibiting large fluctuations correspond with the loop and beta sheet regions. In case of Complex 2, an average fluctuation of 0.76 Å with the SD of 0.20 Å was observed in the protein structure. Two amino acid residues such as Ser150 (1.52 Å) and Asp364 (1.52 Å) has shown higher fluctuation in the protein. The amino acid residues, which has shown higher fluctuation in Complex 3 are Ser293 (2.07 Å), Ala294 (2.27 Å), Asp297 (2.06 Å), Gln300 (2.07 Å), Lys301 (2.34 Å), Gly302 (1.91 Å), Asp303 (1.85 Å) and Asp364 (1.95 Å). The Complex 3 showed an average fluctuation of 0.80 Å with an SD of 0.32 Å. Few amino acid residues that includes His221 (1.76 Å), Gln222 (1.83 Å) and Asp364 (1.77 Å) has shown the higher fluctuation value in case of Complex 4 with an average and SD value of 0.64 Å and 0.21 Å. Complex 5 have shown higher mobility rate with the amino acid residues such as His221 (1.76 Å), Gln22 (1.83 Å) and Asp364 (1.77 Å) with an average and SD of 0.66 Å and 0.24 Å. The active site residues have shown less conformational changes in the protein structure.
Figure 4.8 RMSF of Ca atom in coordinates of each residue averaged over the duration of the MD simulation.

Hydrogen Bond Analysis (LuxP-Dioxazaborocane Derivatives)

All the H-bond interactions observed in the docked complexes were reproduced in the energy minimized average structure during the MD simulation. Three strong H-bond interactions were observed in Complex 1, among the H-bonds, Arg310 (C-O••H2N) was found stable over 100% in the MD trajectory, then the remaining two H-bonds, Trp82 (C=O••HN) and Asp136 (NH••O-C) appeared in 95% and 99% in the MD trajectories. Additionally, three water mediated hydrogen bonds were preserved over the MD trajectory of 55%, 47% and 36%, respectively. Complex 2 exhibit three significant intermolecular H-bonds (Thr266 NH2••O-C, Asp136 NH••O-C, H2O), the Asp136 and Thr266 were preserved in 47% and 95% of the MD trajectory, while a water molecule mediated hydrogen bond was broken and restored many times. Complex 3 found to have five H-bond interactions with Ser79 (C=O••O-C), Trp82 (C-O••HN), Arg215 (C-O••H2N), Thr266 (C-O••HN), Arg310 (C=O••H2N). H-bonds between the two arginine residues at the
position 215 and 310 were found stable over 98% of the MD trajectory, while the remaining three H-bond interactions were found to be unstable during the simulation time. Two H-bond interactions (Arg215 C=O−H2N, Asp136 NH−O=C) were observed in the Complex 4 and these interactions were preserved less than 30% of the MD trajectories. In Complex 5, the H-bond interaction with C-O of Asp159 and NH of Compound 5 was preserved below 30% of the MD trajectory. Five hydrogen bond interactions (Thr266 OH−OH, Arg310 OH−H2N, Ser79 OH−OH, Thr289 OH−O=C, Gln77 C-O−H2N, Asp136 NH−O=C) observed between Compound 6 and LuxP, among them only two H-bond interactions were preserved in 52% and 34% of the MD simulation, while the remaining three interactions were found below 30% of the MD trajectories. The H-bond interaction observed between hydroxyl group of Compound 7 with carboxyl group of Gln177 was preserved in 99% of the MD trajectory. Five H-bonds interactions exhibit between Compound 8 and LuxP, where the OH−H2N of Arg310 was found stable over 30% of the MD trajectory, while the remaining four H-bond interactions (Trp289 OH−O=C, Ser79 OH−OH, Thr266 OH−OH and Thr134 OH−OH) were found unstable during the simulation time. Three H-bond interactions observed between Compound 9 complexed with LuxP, where the C-O−H2N of Asn159 was found stable over 99% of the MD trajectory, remaining two H-bond interactions were unstable or found less than 30% of the MD trajectory. The H-bond interaction between the LuxP-dioxazaborocane derivatives during the MD simulation is shown in Fig. 4.9. The MD simulation result clearly shows that the hydrogen bond interaction was found with the amino acid residues located in the active site of LuxP.
Fig. 4.9 Hydrogen bond variation in LuxP-dioxazaborocane derivatives adduct during 10 ns trajectory.

**Hydrogen Bond Analysis (LuxP-Boronic Acid Derivatives)**

The H-bond interaction profiles of the five protein-ligand complexes were monitored throughout the simulation period. Consideration of hydrogen bonding properties in drug design is important because of their strong influence on drug specificity, metabolization and adsorption (Williams and Ladbury, 2008). The amino acid residues
such as Arg215 and Arg310, which makes hydrogen bond interactions with all five protein-ligand complexes, were found stable throughout the simulation period. Other amino acid residues found to have hydrogen bond interactions with boronic acid derivatives includes Gln77, Ser79, Tyr81, Tyr82, Asn159, Phe178, Phe206, Ile211 and Trp289. From Fig. 4.10, it was clear that a maximum of 16 and a minimum of 12 hydrogen bond interactions were observed in the five protein-ligand complexes. It was concluded that the protein-ligand complexes are relatively stronger during the MD simulation.

Fig. 4.10 Total number of intermolecular hydrogen bond interactions between LuxP and boronic acid derivatives.

**Analysis of Binding Affinity by Prime MM-GBSA**

The prime MM-GBSA calculation was used to predict the strength of association or binding affinity between a protein and a ligand. The calculated free energy of binding of LuxP-dioxazaborocane analogues ranged from -86.04 kcal/mol to -60.59 kcal/mol. The
binding free energies and free energy components were calculated and are presented in Table 4.3. From the table, it was clear, that the van der Waals, nonpolar solvation ($\Delta G_{\text{solvSA}}$), polar solvation ($\Delta G_{\text{solvGB}}$) and coulomb energy terms favors the binding of ligands into the binding site of LuxP. The coulomb energy terminations for the Compounds 7 and 9 are very low, indicates these two compounds disfavors the binding energy. Polar solvation opposes binding for the Compound 9. From the result, it is evident that the van der Waals and nonpolar solvation energy terms are considered to be the driving force for ligand binding. Boronic acid derivatives produced a favorable binding free energy in the range of -67.59 kcal/mol to -40.20 kcal/mol (Table 4.2).

Table 4.3 Relative free energy of binding (kcal/mol) of LuxP and dioxazaborocane derivatives

<table>
<thead>
<tr>
<th>S. no</th>
<th>Compounds</th>
<th>$\Delta G_{\text{coulomb}}$ a</th>
<th>$\Delta G_{\text{vdw}}$ b</th>
<th>$\Delta G_{\text{covalent}}$ c</th>
<th>$\Delta G_{\text{solv}}$ d</th>
<th>$\Delta G_{\text{solvlipo}}$ e</th>
<th>$\Delta G_{\text{bind}}$ f</th>
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<tbody>
<tr>
<td>2.</td>
<td>Compounds 2</td>
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<td>76.87</td>
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<td>-60.58</td>
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<tr>
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<tr>
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<td>66.63</td>
<td>-34.67</td>
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<tr>
<td>6.</td>
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<td>76.37</td>
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<td>8.</td>
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<tr>
<td>9.</td>
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<td>-86.04</td>
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</tbody>
</table>

a: contribution to the MMGBSA free energy of binding from the coulomb energy,

b: contribution to the MMGBSA free energy of binding from the van der Waals energy,

c: contribution to the MMGBSA free energy of binding from covalent binding,

d: contribution to the MMGBSA free energy of binding from the non-polar contribution to the solvation
energy due to the surface area, contribution to the MMGBSA free energy of binding from lipophilic binding, free energy of binding.

Correlation Co-efficient

Correlation between experimental values against computational predicted values still remains a major limiting factor in the computational drug design. In recent years, Pearson’s correlation co-efficient and Spearman’s rho correlation co-efficient are most commonly used metrics for quantifying the correlation that exists between the observed experimental values (IC$_{50}$, pIC$_{50}$ or EC$_{50}$) against the predicted binding free energies ($\Delta G_{\text{bind}}$) of the protein-ligand complexes. Pearson’s co-efficient measures the strength of the association between the two variables (Brender and Zhang, 2015). Spearman’s rank correlation co-efficient rho is a non-parametric measure of statistical dependence between the ranked lists of experimental binding affinities and the scores (Li et al., 2009). It assesses how well an arbitrary monotonic function can describe the relationship between two variables, without making any assumptions about the frequency distribution of the variables (Lee et al., 2009). Calculated correlation co-efficient can range between -1 and +1. A value closer to -1 indicates negative linear correlation, whereas value close to +1 suggests a high degree of positive correlation exists between the two variables. The EC$_{50}$ values (Table 4.4) of the nine dioxazaborocane analogues tested against two bacterial strains (*V. harveyi* BB170 and *V. harveyi* MM32) were used in this study. The correlation co-efficient values calculated using Pearson’s and Spearman’s rho is tabulated in Table 4.5. Calculated binding free energies (Table 4.3) against biological activity models have significant Spearman’s correlation co-efficient of rs=0.61 and rs=0.60, respectively.

Table 4.4 Experimental EC$_{50}$ values of the dioxazaborocane analogues
Table 4.5 Correlation co-efficient analysis of experimental data sets against predicted binding free energies

<table>
<thead>
<tr>
<th>Compounds</th>
<th>V. harveyi BB170</th>
<th>V. harveyi MM32</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>EC_{50} µM</td>
<td>EC_{50} µM</td>
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<tr>
<td>Compound 1</td>
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<tr>
<td>Compound 2</td>
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<td>Compound 5</td>
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<td>29.2</td>
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<td>Compound 6</td>
<td>39.0</td>
<td>61.2</td>
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<td>Compound 7</td>
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<td>12.6</td>
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<tr>
<td>Compound 8</td>
<td>35.7</td>
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</tr>
<tr>
<td>Compound 9</td>
<td>41.3</td>
<td>57.4</td>
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</table>

Published report of Brackman et al., 2013.

The Pearson correlation co-efficient is 0.52 and 0.70, indicating a very strong positive correlation between the experimental EC_{50} values and the calculated values of the binding free energies. The results afford rs and r values on average greater than 0.5 demonstrates good correlation exists between the two variables (predicted binding energies against the biological data sets). The correlation co-efficient among the predicted scoring functions were also evaluated to gain insight in its accuracy, because the final docked
conformation of the protein-ligand complex was chosen based on Glide score, Glide energy and Glide E_{model}. Glide score is an empirical scoring function and it has been used for optimizing the docking accuracy, database enrichment and binding affinity prediction. Glide energy is the modified Coulomb van der Waals interaction energy and Glide E_{model} that combines the binding affinity predicted by Glide score, coulombic, van der Waals and strain energy of the ligand. Moreover, Glide energy was compared against Glide E_{model} and Glide score against the Glide energy. The Glide energy plotted against Glide E_{model} have shown better correlation co-efficient of rs=0.66 and r=0.71, respectively (Table 4.1). In addition, Glide score plotted against Glide energy showed statistically fair Pearson correlation coefficient of r=0.43, but improvement was observed in the Spearman’s rank correlation co-efficient of rs=0.66 (Table 4.5). The IC_{50} values (Table 4.6) of the boronic acid derivatives tested against bacterial QS system in V. harveyi were used in this study.

**Table 4.6** Experimental IC_{50} values of the boronic acid derivatives

<table>
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<th>Compounds</th>
<th>V. harveyi MM32 (IC_{50} µM)</th>
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<tbody>
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<tr>
<td>Compound 2</td>
<td>5.00</td>
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<tr>
<td>Compound 3</td>
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<td>Compound 4</td>
<td>4.00</td>
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<tr>
<td>Compound 5</td>
<td>6.00</td>
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</tbody>
</table>

Published report of Ni et al., 2008.

The Spearman rho and Pearson correlation co-efficient calculated against the predicted binding free energy and biological activity have showed the r and rs value of 0.52 and 0.61, respectively (Table 4.6). The Glide score plotted against Glide energy have shown better correlation co-efficient of r=0.69 and rs=0.64. The results showed rs and r
values on average greater than 0.5 demonstrates good correlation exists between the tested
variables. From the overall results, the correlation observed between the binding free
ergies and biological activity dataset and correlations predicted among the scoring
functions are remarkable.

**Table 4.6** Correlation co-efficient analysis of experimental data sets against predicted
binding free energies

<table>
<thead>
<tr>
<th>Correlation determination against computational results</th>
<th>r</th>
<th>r_s</th>
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<tbody>
<tr>
<td>Binding free energy against biological activity</td>
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<tr>
<td>Glide score against glide energy</td>
<td>0.69</td>
<td>0.64</td>
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**CONCLUSION**

Molecular docking and MD simulations were performed to determine the stable
binding modes of dioxaaborocane derivatives and boronic acid derivatives into the
binding site of LuxP. Amino acid residues such as Gln77, Ser79, Thr134, Asp136, Arg215,
Thr266 and Arg310 showed hydrogen bond interactions for LuxP protein. The π-π
stacking, π-cation and salt bridge were observed with the amino acid residues Trp82,
Asp136, His140, Phe206, Arg215 and Arg310 of LuxP. The observed interaction
contributes to the protein-ligand recognition mechanism and in the stabilization of the
protein-ligand complexes. Further MD simulations were performed to determine the
interaction pattern of hydrogen bonds and stability of the protein-ligand complexes. The
overall binding mode and stability were consistently maintained throughout MD
simulation studies. Correlation co-efficient predicted for the binding free energy against
biological data sets confirms the computational predictions. The information gained from
this study will hopefully be useful to address the problem of finding new drugs targeting LuxP and also serves as a starting point for lead optimization and drug development.