Virtual Screening of Potential Drug-like Inhibitors against MexB Efflux Protein from Pseudomonas Aeruginosa

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Abstract—Prevalence of multidrug resistant Pseudomonas aeruginosa strains in clinical setup demands the need for developing potent drugs against efflux mechanisms. In this study, drug-like molecules with structural similarity to the known efflux inhibitor were used in order to screen for potential inhibitors. A total of 167 compounds were screened and the Glide XP shortlisted compounds were selected based on Glide score and interaction with active site residues. The virtual screening method was also applied to study the binding modes of substrates. The shortlisted compound could have a better ability to inhibit the MexB protein, hence will provide adjunctive therapy to the antibiotics.

Keywords—Pseudomonas aeruginosa; virtual screening; efflux pump; MC-207,110; Glide

I. INTRODUCTION

Toxic compound efflux from cells is a general mechanism that bacteria have developed to protect themselves against the adverse environments. Efflux pumps are proteins involved in the transfer of toxic compounds from the cell which confers the resistance to drug and a wide variety of structurally unrelated compounds [1]. Pseudomonas aeruginosa is a notoriously difficult organism to control with antibiotics or disinfectants [2]. The organism’s intrinsic resistance has been attributed to the outer membrane, a barrier of limited permeability [3]. While the reduced uptake likely does limit access of antimicrobials to their targets within the cells, additional resistance mechanisms such as drug efflux, helps to reduce the threshold concentration of antibiotics within the cells [4]. The efflux pumps of P. aeruginosa belong to Resistance Nodulation Cell Division (RND) family which forms tripartite system consisting of three components: a transporter (efflux) protein in the inner membrane (MexB), a periplasmic accessory protein, (MexA) and an outer membrane protein (OMP) channel (OprM) [5]. MexAB-OprM is the first identified tripartite system [6], which contributes to both intrinsic and acquired resistance in P. aeruginosa, while the MexCD-OprJ and MexEF-OprM efflux system contributes to acquired resistance. The inner membrane component MexB is responsible for the substrate specificity and uses proton electrochemical gradient to transport its substrates and virulence factors that are important for the colonization and infection of host cells [6]. A widely exploited strategy is the development of inhibitors of efflux pumps [7], which are intended for combination therapy with antibiotics. Inhibition of efflux pumps provides a better way to restore the activity of antibiotics. To date, no efflux pump inhibitors have been approved for the treatment of bacterial infections [6], hence this area affords promising role in drug development. The aim of this study was to elucidate the mode of binding of substrates to the efflux pump and also to identify the efflux inhibitory potential of compounds with structural similarity to the known inhibitor MC-207,110 using virtual screening and molecular docking approaches.

II. METHODS

A. Receptor X-ray structure

The X-ray crystal structure of the inner membrane protein MexB (PDB code: 2V50) (Fig.1), in complex with non-ionic detergent n-dodecyl-D-maltoside resolved at 3.0Å was downloaded from Protein Data Bank (PDB) [8]. The asymmetric unit contained two structurally identical trimers, subunits ABC and DEF. Among the three chains of the MexB structure the B subunit was considered as the binding subunit [9].

B. Protein preparation

PDB files of the protein may have various problems that need to be corrected before proceeding with Virtual Screening studies hence bond orders, ionization states, steric clashes and side chain orientations were fixed and energy minimization was performed with the force field Optimized...
isolates of (MexAB-OprM, MexCD-OprJ and MexEF-OprN) in clinical throughput screening of small molecule libraries as an

D. of Schrodinger suite v 9.

In order to compare the binding modes of the inhibitors with the substrates, docking was performed with the substrates like carbenicillin, erythromycin, levofloxacin, Fig.2 [11]. In order to compare the binding modes of the inhibitors with the substrates, docking was performed with the substrates like carbenicillin, erythromycin, levofloxacin using glide module of Schrodinger suite v 9.

C. Substrates of MexB

The MexB efflux pumps of P. aeruginosa are of particular interest because of their exceptionally broad substrate specificity. The following reporter antibiotics were used as phenotypic markers of the Mex efflux pumps of interest: carbenicillin, erythromycin, levofloxacin, Fig.2 [11]. In order to compare the binding modes of the inhibitors with the substrates, docking was performed with the substrates like carbenicillin, erythromycin, levofloxacin using glide module of Schrodinger suite.

D. Ligand Database

MC-207,110 (Fig.2) has been identified in High-throughput screening of small molecule libraries as an inhibitor of the three multi-drug resistance (MDR) pumps (MexAB-OprM, MexCD-OprJ and MexEF-OprN) in clinical isolates of P. aeruginosa [12]. Similarities in structures are indicative of similarities in bioactivities, therefore, structure based searching of PubChem database (>85% similarity) was carried out. This resulted in 167 compounds. The Structure of these compounds were obtained from PubChem Compound Database [13].

The geometries of these compounds were optimized by adding hydrogens and eliminating unwanted structures using LigPrep module and Database of chemical compounds was created using Schrodinger suite.

E. Virtual Screening studies for substrates

The virtual screening of substrate database with prepared protein was performed with OPLS2001 force field using virtual screening workflow (VSW) module of the Schrodinger Suite. VSW uses glide docking to rank the best compound which utilizes the scoring functions, High throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP). HTVS and SP modes are used for large set of ligands and XP docking is more accurate than the above two methods. It uses the ligand poses that have a high score from SP docking. The XP GlideScore scoring function was used to order the best ranked compounds and the specific interactions like pi-cation and pi-pi stacking were analyzed using XP visualizer in Glide module. The binding mode and interactions of the substrates were ascertained to compare with the inhibitors.

F. Virtual Screening studies for inhibitors

The ligand based virtual screening of inhibitor compound database with prepared protein was performed with OPLS2001 force field using virtual screening workflow (VSW) module of the Schrodinger Suite. The same protocol used for the substrates was followed. The interactions and bonding mode of the inhibitors and their structurally similar compounds were compared with those of the substrates.

III. RESULT AND DISCUSSION

Computer based strategies for structure based drug discovery presents a valuable alternative to the costly and time-consuming process of random screening. In the present study, two different virtual screening approaches were used. First, MC-207,110 analogues were docked into the active site of MexB protein. For the validation of docking process, the substrates of the MexB were introduced as a control with the purpose of screening the compounds with docking score greater than the substrate.

The docking of the substrates to the active site is shown in Fig 3a-c, the substrates carbenicillin, erythromycin and levofloxacin exhibited molecular docking with Glide score value of -4.271, -3.672, -3.507 and -3.025 (kcal/mol) respectively. The substrates docked near the vicinity of the amino acids Thr495, Gly461, Arg468 and Gly97 and were observed be form hydrogen bond (Table I).

Out of the 167 compounds virtually screened, the shortlisted molecule ([2R]-2,5-diamino-N-[(2S)-3-(4-methylphenyl)-1-(naphthalen-2-ylamino)-1-oxopropan-2-yl]pentanamide (Pubchem ID: 21970537)) was obtained from the XP output based on its good interaction, forming hydrogen bonds with the active site residues Arg468, Ala37, Gln96 and Glide score of -5.344 (Fig. 4 and Table II). Hydrogen bonding measures the intermolecular interaction between the protein and ligands. The compound possessed 1.78 score in the hydrophobic map prediction, hppmap tool of Schrodinger suite (Fig. 5) proved its strong binding and interaction. The shortlisted inhibitor docked with better Glide score than the substrates. However hydrogen bonding with the active site residue Arg468 was observed dominant among both the inhibitor and substrate. It is known that MC-207,110 is a competitive efflux inhibitor which competes for the binding sites in the receptor with the substrates [14].

IV. CONCLUSION

Combination therapy is a significant approach to combat drug resistance in bacteria. Efflux pump inhibitors can serve as an additional drug to alleviate the activity of the existing antimicrobials. In this study, molecular docking approach was imparted to study the binding modes and to correlate the docking score between substrates and inhibitors of efflux pump MexAB-OprM. The docking studies provide better insights onto the binding mechanism of substrate and inhibitor at the molecular level. The virtually screened and shortlisted compound could act as a potential competitive inhibitor with the substrate for MexAB-OprM efflux pump.

ACKNOWLEDGMENT

The authors are thankful to Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India for their financial assistance and SRM University for their support.

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REFERENCES


TABLE I. Interaction of Antibiotic Substrates of MexAB-OprM Efflux Pump with MexB Protein

<table>
<thead>
<tr>
<th>Substrates</th>
<th>D—H—A</th>
<th>Distance (Å)</th>
<th>Glide Score (Kcal/mol)</th>
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<tbody>
<tr>
<td>Carbenicillin</td>
<td>O—H—O(Thr495)</td>
<td>2.629</td>
<td>-4.271</td>
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<tr>
<td></td>
<td>(Gly461)N—H—O</td>
<td>2.620</td>
<td></td>
</tr>
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<td>Erythromycin</td>
<td>(Arg468)N—H—O</td>
<td>2.877</td>
<td>-3.672</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>(Gly97)N—H—O</td>
<td>3.081</td>
<td>-3.507</td>
</tr>
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TABLE II. Interaction of Xp Shortlisted Compound (PubChem ID-21970537) with MexB Protein

<table>
<thead>
<tr>
<th>Inhibitor</th>
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<th>Distance (Å)</th>
<th>Glide Score (Kcal/mol)</th>
</tr>
</thead>
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<tr>
<td>Pubchem ID:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21970537</td>
<td>(Arg468)N—H—O</td>
<td>2.883</td>
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<tr>
<td></td>
<td>(Ala37)N—H—O</td>
<td>3.128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N—H—O (Ala37)</td>
<td>2.891</td>
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</tr>
<tr>
<td></td>
<td>N—H—O (Gln96)</td>
<td>2.733</td>
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</table>

Figure 1. Structure of MexB protein from P. aeruginosa

Figure 2. Structure of MC-207,110, an inhibitor of MexB efflux pump and substrates
Figure 3. a. Interaction of MexB (B-chain) with carbenicillin (The substrate is in ball and stick model and the amino acids in lines)

Figure 4. b. Interaction of MexB (B-chain) with erythromycin

Figure 3. c. Interaction of MexB (B-chain) with levofloxacin

Figure 4. Interaction of MexB (B-chain) with XP docked inhibitor PubChem ID: 21970537 (in ball and sticks)

Figure 5. Hydrophobic map of XP output for the inhibitor showing the strong hydrophobic patches (blue)