Structure-based Pharmacophore and Virtual Screening for Bacterial MexB Efflux Pump Inhibitors

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Abstract—Pseudomonas aeruginosa shows high degree of intrinsic resistance to a wide variety of antimicrobial agents. Efflux pump is a major mechanism of resistance exhibited by P. aeruginosa. The benefits of broad-spectrum efflux pump inhibitors (EPIs) led to the screening of synthetic and natural product databases for identifying inhibitors of the Mex pumps from P. aeruginosa. In the present study, pharmacophore based virtual screening of databases was used, in an effort to identify compounds that could possess activity against the MexB protein of P. aeruginosa. A pharmacophore hypothesis was created for the known inhibitor MC-207,110. This hypothesis was used to screen a total of 392 natural compounds and 1,95,000 synthetic compounds from Phase database. The compounds shortlisted based on the fitness score were docked against the target protein, MexB using Glide_XP. The compounds shortlisted based on Glide score showed interaction with active site residues. These compounds have to be validated in vitro for their potential efflux inhibitory activity.

Keywords—Pseudomonas aeruginosa; MexAB-OprM efflux pump; e-pharmacophore; efflux pump inhibitors

I. INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen with an intrinsic resistance to a variety of antibiotics. The resistance is recognized due to the result of synergy between broadly specific efflux pumps and low outer membrane permeability [1, 2]. Bacteria adopted efflux mechanism to resist the toxic effects of antibiotics by reducing their intracellular concentration [3]. Efflux pumps which are polyclonal are vital in the defense of P. aeruginosa against antibiotics, antiseptics, and inhibitors. RND (resistance nodulation cell division) family of transporters has been characterized in the pathogen P. aeruginosa [4]. MexAB-OprM, MexXY-OprM, MexCD-OprJ, and MexEF-OprN RND efflux pumps have been characterized [5, 6]. Overexpression of MexAB-OprM leads to resistance to broad variety of structurally diverse compounds.

MC-207110, the known efflux pump inhibitor has resulted from screening from Microcide's library, is a low molecular weight dipeptide amide, with minimal antibacterial activity but potentiated the activity of levofloxacin by 8-fold at 10 µg/ml [7]. However, to date, no efflux pump inhibitor has been licensed for use in clinical treatment.

In silico study of transporter-drug interactions approaches can be of two ways: either from the perspective of the transporter protein or the transported ligand, thus separating the methods into target-based or ligand-based. The target-based approaches calculate the three-dimensional structure of transporters, based on homology to available crystal structures of related transporter proteins [8]. In this study a pharmacophoric approach was carried out in order to virtually screen compounds against MexB protein of P. aeruginosa.

II. METHODS

A. Protein target

The crystal structure of the inner trans-membrane protein MexB of P. aeruginosa MexAB-OprM system (PDB code: 2V50) (Fig.1) was retrieved from Protein Data Bank [9]. The asymmetric unit of MexB consists of two structurally identical trimeric subunits, ABC and DEF respectively [10].

B. Protein and Ligand Preparation

The protein file was prepared using Protein preparation wizard and Impact Energy minimization was performed about 500 cycles of Steepest Descent and 5000 cycles of Conjugate Gradient methods with Optimized Potential for Liquid Simulations (OPLS) 2005 force field using Schrodinger suite version 9 [11]. The active site of the protein was defined and grid files were generated using receptor grid generation panel.

The ligand MC-207,110 structure (Fig.2) was downloaded from PubChem database [12] and minimized using impact energy minimization with 100 cycles of Steepest Descent and 500 cycles of Conjugate Gradient method.

Three hundred and ninety two natural compounds with pre-determined anticancer, antimicrobial and anti-inflammatory activity were identified using Dr. Duke's Phytochemical and Ethnobotanical Database [13]. The three dimensional structures of the compounds were retrieved from PubChem database. A database of all the compounds were generated and then minimized as a single file using LigPrep module of Schrodinger suite.
Apart from natural compounds, small molecules about 1, 95,000 from Phase database (Pharmacophore modeling package) were taken for the virtual screening.

C. Glide XP (Extra-precision) Docking

The generated grid file from the prepared receptor was used for the Glide XP docking calculations. The Minimized conjugate gradient output of the ligand MC-207,110 was used. The “Write XP descriptor information” option and “Compute RMSD” option was enabled and rest of the parameters were kept as default. 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 input RMSD of the ligand was also ascertained.

D. E-Pharmacophore for MC-207,110

The pharmacophore hypothesis was created for the ligand MC-207,110 by using the Xpdes file of the Glide XP output in the Docking Post Processing tool of the Scripts module. The “single ligand” option and “use existing conformers” was specified and the pharmacophore hypothesis was created which denotes the active functional groups in the target compound. The findmatches to hypotheses option of PHASE module was used to screen the natural and PHASE database compounds against the pharmacophore hypothesis of MC-207,110.

E. Docking of pharmacophore screened database compounds against MexB protein

The compounds screened based on their fitness score in the findmatch to hypotheses option were subjected to Glide XP docking with the “Write XP descriptor information” option and “Compute RMSD” option enabled and keeping the rest of the parameters at default.

III. RESULTS AND DISCUSSION

The information of common properties of the binding group is essential for resolving the type of inhibitor binding to the target protein. Major aim of drug design is identification and development of new ligands with high affinity of binding towards protein receptor. A very useful model for achieving this goal is pharmacophore [14]. In the present study, the known inhibitor was docked with the MexB protein and the output file was used to identify the pharmacophore hypothesis which was used to screen compounds from databases.

A. Glide XP docking of MC-207,110

The known ligand MC-207,110 was docked with the active site residues of the MexB protein. The compound exhibited a highest Glide score of -7.84 kcal/mol and glide input RMSD of 70.15661. The compound docked near the vicinity of amino acids Thr98, Gly97, Arg468, Glu861, Phe458 and Glu95, and with hydrogen bonding formation between Phe458 and Glu95 (Table I and Fig. 3). The result of this docking was used to ascertain the pharmacophore features of MC-207,110.

B. E-pharmacophore hypothesis of MC-207,110

The E-pharmacophore hypothesis was generated for MC-207,110 based on its docked conformation and interaction with the active site residues of the protein. The XP results help in defining the pharmacophore features/functional groups in the ligand molecule. The features defined for this molecule included one hydrogen bond donor (D5) and one ring aromatic (R10) group (Table II and Fig. 4). These features were taken for screening the databases using the findmatches to hypothesis option. Of the 392 natural compounds screened, 94 were shortlisted based on the fitness to the hypotheses. Of the 1,95,000 compounds from Phase database screened, 1001 were shortlisted based on fitness score.

C. Docking of pharmacophore screened database compounds against MexB protein

The hits obtained based on top fitness score along with the known inhibitor MC-207,110 were screened against the receptor protein using Glide XP docking. Of the 94 natural compounds and 1001 phase database compounds, the top three compounds from each set were shortlisted based on their high Glide XP dock score (Fig.5a and 5b). The best ligand from the natural compounds database gave a Glide score of -12.96 and fitness of 0.916. The best compound from Phase database gave a Glide score of -9.078 and fitness of 0.9998.

IV. CONCLUSION

Inhibition multidrug resistance efflux pump appears to be a striking approach to the improvement of the clinical efficacies of antibiotics that are substrates of such pumps. MC-207,110, discovered by empiric screening of a small molecule library was the first inhibitor against MexAB-OpRM efflux system of P. aeruginosa. The knowledge of common properties of the binding group is essential for the determination of the type of inhibitor binding the target. A very useful model for achieving this goal is pharmacophore. The e-pharmacophore hypotheses generated for MC-207,110 was used to screen compounds in silico against compound databases and based on their fitness and Glide score. These compounds have to be validated through in vitro analysis for their efflux inhibitory potential.

ACKNOWLEDGMENTS

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REFERENCES

TABLE I. **Glide XP Docking Result Of MC-207,110 With MexB Efflux Protein**

<table>
<thead>
<tr>
<th>Name</th>
<th>Distance (Å)</th>
<th>Glide Score (Kcal/mol)</th>
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<tbody>
<tr>
<td>MC-207,110</td>
<td>N--H---O (Phe458)</td>
<td>1.933</td>
</tr>
<tr>
<td></td>
<td>N--H---O (Glu95)</td>
<td>1.706</td>
</tr>
<tr>
<td></td>
<td>N--H---O (Glu95)</td>
<td>2.100</td>
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TABLE II. **E-pharmacophore Hypothesis Result Of MC-207,110**

<table>
<thead>
<tr>
<th>Glide RMSD (Å)</th>
<th>Glide Score (Kcal/mol)</th>
<th>Pharmacophoric sites</th>
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<tr>
<td>70.15661</td>
<td>-7.84507</td>
<td>D (5) and R (10)</td>
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</tbody>
</table>

TABLE III. **Shortlisted Natural And Phase Database Compounds Based On The Fitness And Match To MC-207,110 Hypotheses**

<table>
<thead>
<tr>
<th>Title</th>
<th>Glide RMSD to input</th>
<th>Glide Score Kcal/mol</th>
<th>Fitness</th>
<th>Matched Ligand sites</th>
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<tr>
<td>30766</td>
<td>14.69728</td>
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<td>0.999896</td>
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<tr>
<td>15147</td>
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<td>0.999985</td>
<td>D (5), R (10)</td>
</tr>
<tr>
<td>17788</td>
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<td>0.999904</td>
<td>D (4), R (10)</td>
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<td>5281771</td>
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<td>0.9164</td>
<td>D(27) R(36)</td>
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<tr>
<td>5481663</td>
<td>11.96775</td>
<td>-10.9759</td>
<td>0.985475</td>
<td>D(18) R(30)</td>
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</tbody>
</table>

Figure 1. Structure of MexB protein from *P.aeruginosa*

Figure 2. Structure of MC-207,110, an inhibitor of MexAB-OprM efflux pump

Figure 3. XP docking showing interactions of MC-207,110 with the active site residues of MexB

Figure 4. E-pharmacophore hypotheses of MC-207,110
Figure 5a. Interactions of the pharmacophore based screened natural compounds with MexB protein

Figure 5b. Interactions of the pharmacophore based screened Phase database compounds with MexB protein