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Research Article

IDENTIFICATION OF INHIBITORS FOR RND EFFLUX PUMP OF *Pseudomonas aeruginosa* USING STRUCTURE-BASED PHARMACOPHORE MODELING APPROACH

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ABSTRACT

Objective: Multidrug resistant *Pseudomonas aeruginosa* possess multiple ways of resistance with the expression of efflux pump proteins as one of the major mechanisms. The efflux proteins of *P. aeruginosa* belong to the Resistance Nodulation Division (RND) type pumps. The use of efflux pump inhibitors along with the antibiotics could render the efflux pumps inactive thereby increasing the intracellular concentration of the drug.

Methods: Phase database compounds were screened against the hypothesis generated for MC-207,110, a known inhibitor and the top fitness compounds were subjected for XP docking analysis. The top five compounds with good interaction and scoring were further analyzed for binding energy by MM-GBSA and drug-likeness property.

Results: Based on pharmacophore screening, 500 compounds with good fitness score were shortlisted. By docking the 500 compounds against the protein target, MexB, five best scoring compounds were selected. The compound, ASN05108137 had a maximum dock score of – 8.44 Kcal/mol and -45.12 Kcal/mol docking energy. It exhibited very good binding energy and also a satisfactory drug-likeness.

Conclusion: In this study, a structure-based pharmacophore approach was used to identify new inhibitors targeting the MexB efflux pump protein. The compounds were screened based on their similarity to the pharmacophore feature of MC-207,110 and also docking with MexB protein. The compound ASN05108137 showed very high dock score and interactions compared to MC-207110 and also low binding energy. The compound also had a better drug-likeness property thus could be a very potential inhibitor of the efflux pumps.

Keywords: *Pseudomonas aeruginosa,* Efflux pumps, MC-207,110, e-pharmacophore, Glide, Prime

INTRODUCTION

Treatment of infections caused by Gram-negative organisms is hindered due to the presence of drug resistance and there is an increased incidence of resistance exhibited by pathogens in India [1]. Gram-negative bacteria such as *Pseudomonas aeruginosa* are intrinsically resistant to antimicrobials due to the combined effect of the resistant efflux pumps and lower outer membrane permeability. There are many efflux systems reported in *P. aeruginosa* such as MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM [2].

The MexAB-OprM system contributes mainly for the intrinsic resistance of the organism towards antibiotics that includes quinolones, macrolides, tetracycline, chloramphenicol, novobiocin, and β-lactams but not carbapenem [3, 4]. The MexAB-OprM system consists of three components, MexA, the inner membrane associated fusion protein, MexB the trans-membrane spanning protein and OprM, the outer membrane porin protein. The MexB protein belongs to the Resistance Nodulation Division family and is involved in the extrusion of the substrates with the help of the proton motive force [5].

MexB protein has a very large substrate range and also exports bacterial virulence factors. It has 1046 amino acid residues and is closely related with its counterpart AcrB from *Escherichia coli* with 69.8% identity and 83.2% sequence similarity. They are organized as trimeric units with each monomer consisting of 12 trans-membranes (TMs) and a periplasmic domain composed of 2 loops between TM1 and TM2 and another between TM7 and TM8. There are six sub-domains in the periplasmic domain composed of PN1, PN2, PC1, PC2, DN and DC which is the docking domain to OprM. The crystal structure [Protein Data bank (PDB) entry 2v50] of MexB protein shows the detergent substrate, maltoside bound to the pore domain [3].

The susceptibility of *P. aeruginosa* to antibiotics can be improved with inactivation of the efflux pumps [2]. The use of efflux pump inhibitors (EPIs), helping to increase the accumulation of drugs inside a cell is an efficient approach to overcome drug resistant pathogens [6]. The first RND efflux inhibitor, MC-207,110 was identified by screening 200,000 synthetic compounds and natural product extracts against *P. aeruginosa* overexpressing Mex pumps by Microcide Pharmaceuticals and Daiichi Pharmaceutical Co. MC-207,110 is a substrate that has better affinity than antibiotics thereby acting as a competitive inhibitor. The ability of MC-207,110 to potentiate depends on substrates and since different substrates have unique binding site in the binding cavity of MexB, the action of MC-207,110 is also specific to the binding site [7].

This study focuses on screening and identifying MexB efflux pump protein inhibitors using structure-based pharmacophore approach. The substrate maltoside binding site was selected for further screening of a possible competitive inhibitor. The pharmacophore hypothesis was generated for the peptidomimetic inhibitor, MC-207,110 and the model created was screened against the Phase database compounds. The shortlisted compounds were docked with the MexB protein target for validation based on the interactions and scoring.

MATERIALS AND METHODS

Preparation of MC-207,110 and Ligand database

MC-207,110, inhibitor of MexB and Phase database (phasedb) compounds were prepared for the screening using the Ligprep module of the Schrodinger Suite v9.2 (http://www.schrodinger.com/). In Ligprep, the 3D ligands structures were subjected to a process that adds hydrogen atoms, removes molecules with inappropriate properties, generates low energy conformers and optimizes geometries. The minimized ligprep output file was used for further analysis.

Preparation of MexB protein target for docking

The crystal structure of MexB protein target was downloaded from protein data bank (ID. 2v50) (Figure 1), image obtained using Pymol (http://www.pymol.org/). The protein preparation was performed using the Protein Preparation Wizard panel of the Schrodinger Suite v9.2. This process was done to refine and hydrogenate the structure that would be used for docking calculations. The wizard removes unwanted water molecules, corrects bond, atom types and charges. The co-crystallized maltoside was taken as the site for receptor grid generation. All parameters were set at default.

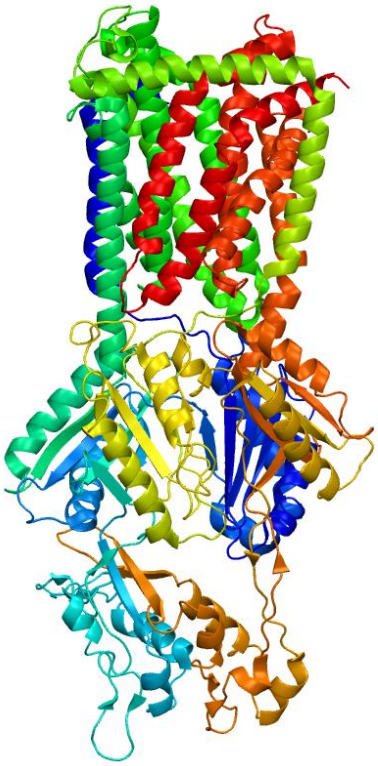

Fig. 1: Chain B monomeric unit of MexB Protein 3D structure retrieved from PDB with ID: 2v50

Glide XP molecular docking of MC-207,110 and MexB protein receptor

The receptor grid file generated was used to dock the prepared MC-207,110 ligand molecule. The Glide extra precision docking (XP) mode of the Schrodinger Suite v9.2 was selected as it is defined for only the best and precise ligand pose with particular conformation. The "write XP descriptor information" option is enabled and this xpdes output file was used for further pharmacophore processing.

e-pharmacophore modeling of MC-207,110

The Scripts e-pharmacophore modeling uses the XP output file generated for the MC-207,110 and the MexB protein docking calculations [8]. The XP docking file gives scoring on the hydrophobic contact, hydrogen-bonding interactions and also gives information on the best conformation of the receptor to which the active ligand binds. Based on the .xpdes output, the Scripts module of the Phase identifies pharmacophore hypothesis which suggests the chemical structures that are essential for binding to receptor. Phase gives six features: hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively charged group (N), positively charged group (P) and aromatic ring (R).

Screening of database compounds for finding matches to hypothesis

The geometric arrangements of the features of the known ligand, MC-207,110, generated using the e-pharmacophore modeling was employed to search the compounds in the phasedb. The structural conformers were aligned to the hypothesis features and scored based on their fitness to the pharmacophore sites. These screened compounds called as hits are arranged in descending order of their fitness score. The top 500 hits were subjected for Glide XP docking mode for further refinement based on the docking calculations and interactions.

MM-GBSA binding free energy calculations and drug-likeness property of hits

The MM-GBSA is an efficient technique that uses the molecular mechanics-Poisson Boltzmann (or Generalized Born) surface area method to calculate the ligand binding affinities [9]. The Prime module was used to calculate the binding energies. The pose viewer file output of the docked ligand-protein complex was used as the input for the calculations. The drug-likeness of the compounds was predicted by checking the Lipinski's rule of 5 using the QikProp tool.

RESULTS

Molecular docking of MC-207,110 with MexB protein

The molecular docking of MC-207,110 in the binding site of MexB protein gave a Glide score of -4.971 Kcal/Mol and Glide energy of -60.221 Kcal/Mol (Table 1). MC-207,110 formed two hydrogen bonds, with the active site residues of MexB protein target; the NH-group of Arg468 formed H-bond with the oxygen of MC-207,110 and the NH-group of Gly461 donated H atom to the NH- group ofMC-207110 (Figure 2). The XP descriptor (xpdes) output file gives the energetically favorable mode of binding of ligand in the active sites residues of MexB.

Table 1: Docking interaction of MC-207,110 with MexB protein

Name	D--H---A	Distance (Å)	XPGlide Score (Kcal/mol)	Glide energy (Kcal/mol)
MC-207,110	(Arg468)N-H---O (Gly461)N-H---O	3.166 2.928	-4.97104	-45.8456

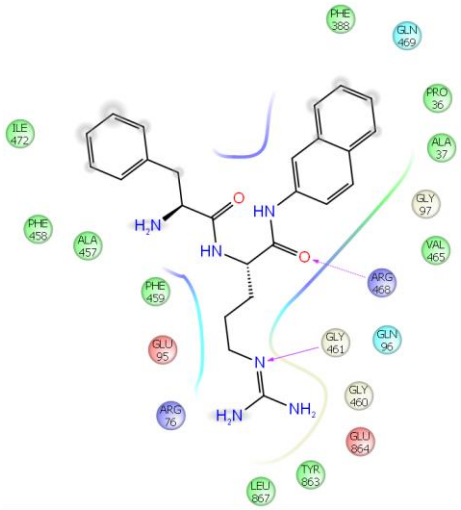

Fig. 2: Molecular interactions (2D) of MC-207110 with active site residues of MexB target protein

E-pharmacophore modeling of MC-207,110 and screening of phase database

The e-pharmacophore maps the energy terms through Glide XP score and assigns the pharmacophoric features. The post-dock processing of the Scripts module sums up the Glide XP score as well as the interaction of MC-207,110 sites with the binding pocket residues of MexB and gives the best features. The pharmacophore features predicted for the MC-207,110-MexB protein complex were two aromatic rings and one H-bond acceptor (Figure 3). The hypothesis thus generated was screened against the Phase database compounds using the Find-Matches to hypothesis option. The database hits were ordered based on their Fitness score, that is how well the compounds are aligned to the MC-207,110 hypothesis.

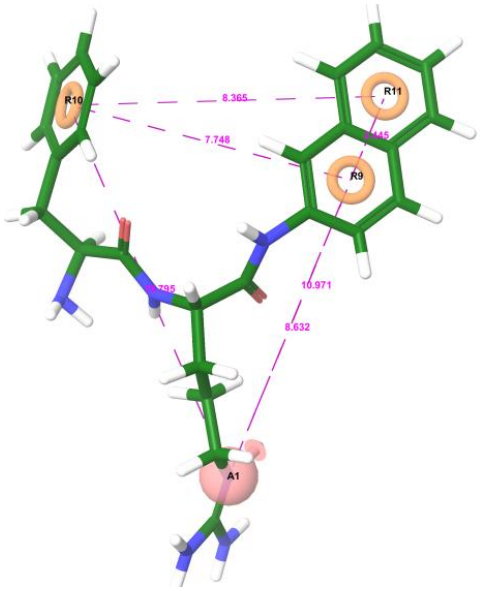

Fig. 3: E-pharmacophore hypothesis generated for MC-207,110 based on the interaction with MexB protein. Acceptor (A) Light red sphere centered on the atom with the lone pair, with arrows pointing in the direction of the lone pairs; Aromatic Ring (R) Orange torus in the plane of the ring

XP docking of the find-match screened hits with MexB protein

The top 500 database hits were shortlisted based on the fitness score with the MC-207,110 hypothesis and were docked at the binding pocket of MexB using Glide XP. The top 5 compounds with docking score more than -7 Kcal/mol and best interaction with the active site residues were shortlisted (Figure 4A, 4B and Table 2).

The best Glide XP score was obtained for compound ASN05108137 that gave a score of -8.44 Kcal/mol and energy of -45.12 Kcal/mol with H-bonds interactions with backbone residues Ser389 and Gly387 and a fitness score of 2.18 with three sites matching the hypothesis. The compound ASN04194191 gave Glide XP score of -8.21 Kcal/mol and energy of -46.26 Kcal/mol with H-bonds interactions with backbone residues Ser389, Gly387 and side chain residue Gln469 and fitness score of 2.24 with three sites matching the hypothesis. A Glide XP score of -7.67 Kcal/mol and energy of -42.80 Kcal/mol was observed with compound ASN04193664 with H-bonds interactions with backbone residues Ser389, Gly97 and side chain residue Gln469 and fitness score of 2.06 with three sites matching the hypothesis. Compound ASN05107178 displayed Glide XP score of -7.33 Kcal/mol and energy of -45.79 Kcal/mol with H-bond interactions with side chain residues Gly96 and bifurcated

interaction with Gln469 and with fitness score of 2.03 with three sites matching the hypothesis. A Glide XP score of -7.18 Kcal/mol and energy of -35.78 Kcal/mol was obtained for compound ASN05303275 with hydrophobic and polar interactions and with fitness score of 2.05 and three sites matching the hypothesis. All these listed compounds showed very good interaction with the active site residues and also better Glide score and energy than the known inhibitor MC-207,110.

Table 2: Top five hits screened through structure-based pharmacophore based on the fitness with MC-207,110 hypothesis and XP glide docking

Phasedb compound ID	Fitness	Matched Ligand Sites	Glide energy (Kcal/mol)	XP Gscore (Kcal/mol)
ASN05108137	2.18	A(1) R(12) R(14)	-45.12	-8.44
ASN04194191	2.24	A(3) R(11) R(12)	-46.26	-8.21
ASN04193664	2.06	A(4) R(13) R(11)	-42.80	-7.67
ASN05107178	2.03	A(2) R(9) R(10)	-45.79	-7.33
ASN05303275	2.05	A(4) R(15) R(14)	-35.78	-7.18

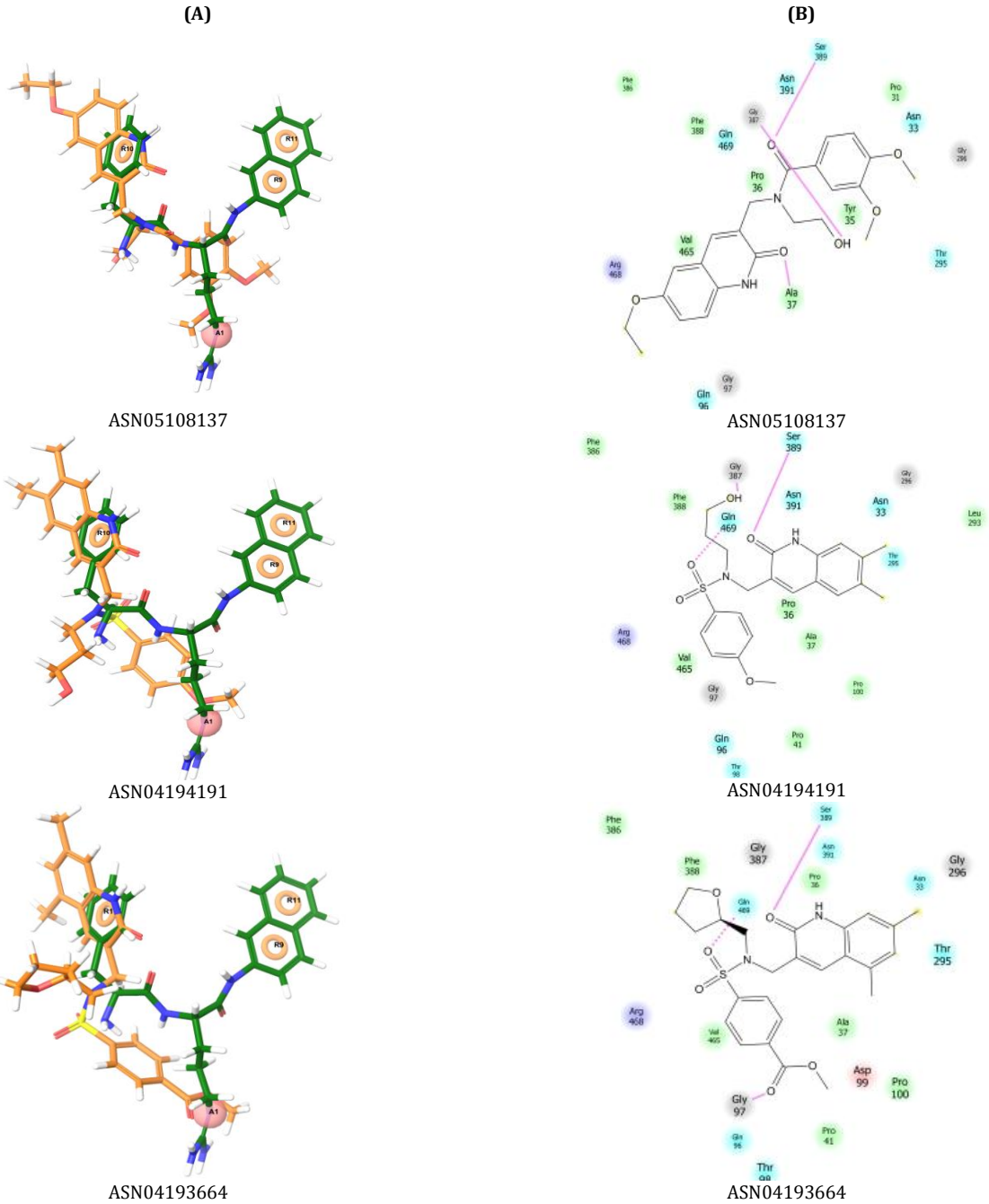

Fig. 4: Continued

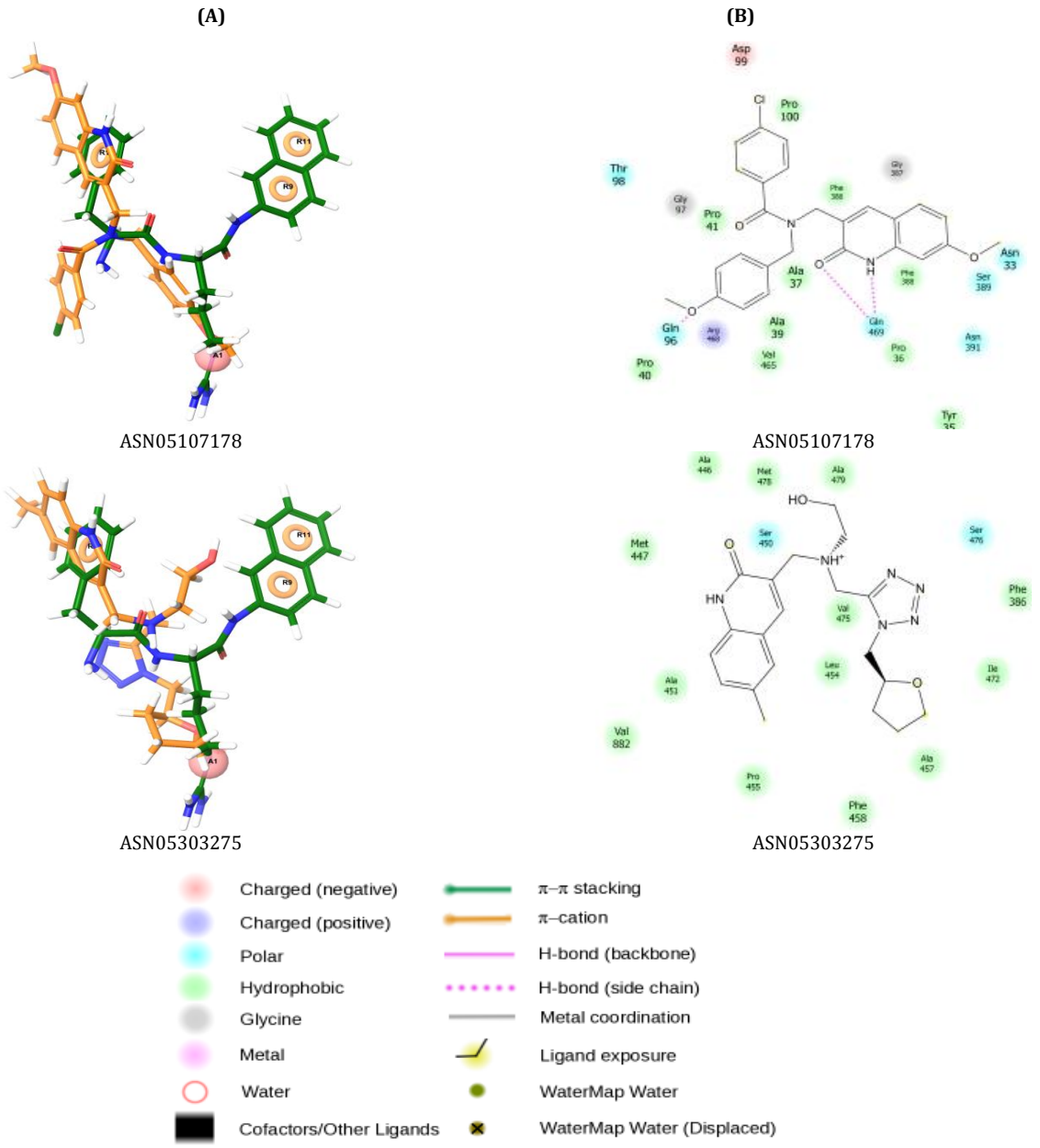

Fig. 4: Docked interaction of the top five pharmacophore hits with the active site of MexB protein.

(A) Superimposition of MC-207,110 hypothesis and the top hits generated using Find-matches to hypothesis and (B) 2D interaction of pharmacophore hits-MexB docked complexes

Ligand binding energy calculations and drug-likeness property predictions

The MM-GBSA analysis showed that compound ASN05108137 showed least ligand binding energy of -202.04 Kcal/mol (Table 3). The H-bond energy for ASN05108137 was -7.379 Kcal/mol and the overall complex binding energy and complex H-bond energy were also significantly very low with -44116.6 and -400.861 Kcal/mol respectively.

The drug-likeness property (Table 4) of the compound ASN05108137 was very satisfactory to the Lipinski's rule of five with the molecular weight of 426.46, H-bond donor of 2, H-bond acceptor of 9.45 and logPo/w of 2.66.

Table 3: Free binding energy for the pharmacophore hits-MexB protein complex

Compound	MMGBSA dG Binding Energy	MMGBSA dG Binding Hbond	Complex Energy	Complex Hbond
ASN05108137	-202.04	-7.37	-44116.6	-400.86
ASN04194191	-0.54	0	0	-9.66
ASN04193664	-10.97	0	-12.92	0
ASN05107178	0.15	0	26.05	0
ASN05303275	-8.89	0.27	0.27	-1

Table 4: Prediction of drug-likeness of pharmacophore hits based on Lipinski's rule of 5

Compound	Molecular weight	Donor HB	Acceptor HB	QPlogPo/w
ASN05108137	426.468	2	9.45	2.66
ASN04194191	430.518	2	9.45	2.32
ASN04193664	484.566	1	10.7	2.883
ASN05107178	462.932	1	7	4.605
ASN05303275	398.464	2	10.9	0.873

DISCUSSION

With an increase in the incidence of drug resistant bacteria, there is an imperative need for the development of new drugs that can synergize the effect of the available drugs. Efflux pump inhibitors can enhance the bio-efficacy of the drugs. Chen *et al*., 2012 have analyzed the efficiency of identification of P-glycoprotein inhibitors using computational methods. They have reported that scoring by the molecular docking alone cannot be used to differentiate whether the screened compound could be an inhibitor or substrate of P-glycoprotein [10]. The structure-based pharmacophore approach employs the features generated from the known inhibitor as a template to search against a database of compounds to identify more efficient molecules.

The MC-207,110 is a peptidomimetic compound that has an inhibitory effect against the *P. aeruginosa* MexAB-OprM efflux pumps. This compound had a docking score of -4.97 Kcal/mol and interacted with the active site residues of the MexB. This docked complex was used to predict the structure based hypothesis of the pharmacophore features of MC-207110. The hypothesis model generated consisted of three aromatic groups and one H-acceptor. The phase database of six lakh compounds were screened against this hypothesis of MC-207110 generated. The first 500 hits were selected based on their fitness score of more than 1.5. These compounds were docked by the Glide XP mode to ascertain the docking score and interactions. The compound ASN05108137 showed the highest docking score and docking energy of -8.44, -45.12 Kcal/mol respectively.

The compound ASN05108137 exhibited maximum similarity to the pharmacophore hypothesis of MC-207110 with a fitness score of 2.18. This compound also showed the drug-likeness property satisfying the Lipinski's rule of five.

A study on *Staphylococcus aureus* NorA pump, using ligand based pharmacophore approach, three compounds were identified as effective inhibitors in the NorA over expressing strains [11]. Based on our present structure-based approach, compound ASN05108137 with good scoring and interactions with the active site residues of MexB than the known inhibitor MC-207110 was identified. This compound could be much efficient as an inhibitor against the drug-resistant *P. aeruginosa* strains expressing efflux pump.

CONCLUSIONS

The continuous increase in the advent of multidrug resistant organisms has lead to a major problem for the treatment of infectious diseases. Many of the multidrug resistant organisms express the efflux pumps that extrude out antibiotics. The combination of the antibiotics with efflux pump inhibitors can reduce the minimal inhibitory concentration of the antibiotics. The structure-based drug design is a useful method for identifying active inhibitors with better refinement. The pharmacophore features identified using the interactions of MC-207,110 with the MexB target protein was used to screen active compounds. The shortlisted phase database compounds displayed better interactions with MexB with high Glide score and Glide energy. The compound ASN05108137 showed good docking interaction, Glide score and excellent fitness score. This compound showed a very low binding energy calculated by MM-GBSA and also had good drug-likeness property. Hence, this compound identified using refined pharmacophore-based analysis and docking calculations, could be a better inhibitor of the efflux pumps than MC-207,110.

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Conflict of interest: Conflict of interest declared none.

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