Structure-Based Screening of Non-β-Lactam Inhibitors against Class D β-Lactamases: An Approach of Docking and Molecular Dynamics

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ABSTRACT: The manifestation of class D β-lactamases in the community raises significant concern as they can hydrolyze carbapenem antibiotics. Hence, it is exceptionally alluring to design novel inhibitors. Structure-based virtual screening using docking programs and molecular dynamics simulations was employed to identify two novel non-β-lactam compounds that possess the ability to block different OXA variants. Furthermore, the presence of a nonpolar aliphatic amino acid, valine, near the active site serine, was identified in all OXA variants that can be accounted to block the catalytic activity of OXA enzymes.

1. INTRODUCTION

One of the earliest β-lactamases identified was OXA β-lactamases which were considerably more subtle. These ancient enzymes could provide resistance to penicillin as well as oxacillin in contrast to other β-lactamases and hence called oxacillinases and the prefix OXA. These enzymes are expressed by blaOXA genes, which are located in the chromosome, plasmids, or integron, and may show inducible gene expression. OXA β-lactamases are generally present in nosocomial pathogens that are strenuous to cure as these enzymes are resistant to penicillin, cephalosporins, and carbapenems. OXA β-lactamases appear in almost every Acinetobacter baumannii strain, which have been categorized under critical priority pathogen category by WHO. Most of the Gram-negative bacteria such as Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, and A. baumannii demonstrate the presence of class D β-lactamases.

Class D β-lactamases can block ceftazidime, cephalosporins, penicillins, cefotaxime, and more antibiotics. Class D β-lactamases that can hydrolyze the carbapenem drugs like, imipenem, are major obstacles to combat clinically significant infections. The enzymes of this group have the ability to block the currently available drug–inhibitor complexes, such as ampicillin–sulbactam, oxacillin–clavulanic acid, ticarcillin–clavulanic acid, and piperacillin–tazobactam. Moreover, avibactam has very limited spectrum of activity against OXA β-lactamases.

The high resistance and severity of OXA β-lactamases create significant enthusiasm for comprehension of the resistance profile of β-lactamases as well as flourishing novel inhibitor molecules against them. An amount of US$ 2.6 billion dollars has already been accounted as the cost of a new drug discovery process. Application of the computational biology method is one of the best solutions in the drug design and discovery. The aim of this research work was to employ the computational biology method to explore novel non β-lactam inhibitors that possess the ability to block the OXA β-lactamases with much broader spectrum of activity which will further assist in combating the obstinate infections.

2. RESULTS AND DISCUSSION

2.1. Analysis of Active Site of OXA Group Enzymes.

Class D β-lactamase enzymes employ active site serine nucleophile to disintegrate β-lactam antibiotics. However, the close analysis of sequence alignment of all the OXA enzymes revealed the presence of a nonpolar aliphatic amino acid, valine (iso-leucine in case of OXA-51), near the active site serine (Figure 1). There is the presence of a conserved pattern of amino acids SXV
in the active site of selected class D β-lactamases. This was further confirmed by developing a molecular surface model of class D β-lactamases using Pymol visualization software (Figure 2). This valine residue might play an important role in the binding of inhibitors or antibiotics with the OXA enzymes.

2.2. Insilico Docking. Virtual screening has turned out to be one of the most well-known approaches nowadays to minimize the time and cost confinement, which is the difficult issue in drug discovery.18,19 We have employed GOLD software for screening of the drug candidates that are potentially active against selected biological targets. The compounds M1593 (N1-phenyl-N1-[2-(2-pyridyl)ethyl]-5-methyl-2-nitrobenzamide) and M2680 (2-[(4-benzhydrylpiperazino)carbonyl]benzoic acid) were selected for further investigation. The selected compounds have higher GOLD score and binding energies in contrast to commercially available antibiotic and inhibitor were selected for further analysis (Figure 3). The analysis of the docked complex further revealed that Valine along with the two serine residues in the active site play an important role in the binding of antibiotics or inhibitors with the class D β-lactamase enzymes (Figure 4).

Serine, being a polar uncharged amino acid, plays an important role in the hydrogen bonding while valine, being a nonpolar aliphatic amino acid, plays an important role in the hydrophobic interactions of the molecules with the enzyme. If an inhibitor gets bound to serine and valine residues of the active site, then this will successfully block the catalytic activity of enzymes of the OXA group and will eventually rejuvenate the bactericidal effects of antibiotic used.

2.3. Molecular Dynamic Simulation. To evaluate the binding stability of inhibitors against the different variants of OXA enzymes (i.e. OXA-1, OXA-10, OXA-23, OXA-24/40, OXA-48, OXA-51, and OXA-58), 50 ns of the molecular dynamics (MD) simulation study was performed for each ligand-bound OXA system. After completion of the 50 ns molecular dynamics (MD), the trajectories were made compact, protein and ligand interactions were analyzed (Figure 3).
complexes were centered to the solvation box, and fit analysis was also done with reference to the starting structure. MD trajectory, after the fit analysis, was used for further analysis of the docked complex structure. All analyses for the complex trajectory were performed using GROMACS inbuilt tools. Root mean square deviation (rmsd) shows the stable trajectory for complexes with minimal fluctuations in the acceptable range.

M1593 and M2680 bound OXA1 have less fluctuation from mean backbone rmsd. M1593- and M2680-bound OXA10 complexes have the most stable rmsd graph while meropenem does not seem to be stably bound to OXA10. All the drug-bound complexes achieved equilibrium during 50,000 ps trajectory and cilastatin seems to form most stable complex with OXA23, OXA24/40, and OXA48 than the other drugs. In case of OXA24/40, M1593 and M2680 are still having better binding than meropenem. Meropenem has highly unstable binding to OXA51 which is evident with rmsd plot, while M2680 seems to have the most stable binding in the case of OXA58 (Figure 5).

M1593 and M2680 bound OXA1 have an optimal number of average hydrogen bond, which proves them as good inhibitor molecules. Higher average number of hydrogen bonds in M1593- and M2680-bound OXA10 complexes to cilastatin- and meropenem-bound OXA10. Cilastatin forms the highest number of hydrogen bonds with OXA23, OXA24, OXA25.

Table 1. GOLD Fitness Score of Selected Compounds, Reference Inhibitor, and Antibiotic

| OXA variants | Cilastatin | Meropenem | M1593 | M2680 |
|--------------|------------|-----------|-------|-------|
| OXA-1        | 57.78      | 59.22     | 71.01 | 71.30 |
| OXA-10       | 55.28      | 54.52     | 58.30 | 62.26 |
| OXA-23       | 73.73      | 75.76     | 82.11 | 82.11 |
| OXA-24/40    | 60.42      | 59.24     | 61.64 | 68.39 |
| OXA-48       | 62.54      | 59.20     | 64.14 | 66.19 |
| OXA-51       | 62.71      | 63.77     | 67.95 | 66.83 |
| OXA-58       | 63.07      | 60.06     | 71.39 | 68.27 |

Figure 2. Molecular surface models of the active-site region of the class D β-lactamases. (A) View down the active-site region of OXA-1 having active-site Ser67 (red), Ser115 (green), and Val117 (cyan); (B) view down the active-site region of OXA-10 having active-site Ser67 (red), Ser115 (green), and Val117 (cyan); (C) view down the active-site region of OXA-23 having active-site Ser79 (red), Ser126 (green) and Val128 (cyan) with the presence of hydrophobic region on the top of the active site, formed by Phe110 and Met221; (D) view down the active-site region of OXA-24/40 having active-site Ser81 (red), Ser128 (green), and Val130 (cyan) with the presence of a hydrophobic bridge (highlighted as yellow mesh) on the top of the active site, formed by Tyr112 and Met223; (E) view down the active-site region of OXA-48 having active-site Ser70 (red), Ser118 (green), and Val120 (cyan); (F) view down the active-site region of OXA-51 having active-site Ser80 (red), Ser127 (green), and Ile129 (blue) with the presence of a hydrophobic bridge (highlighted as pink mesh) on the other side of the active site, formed by Phe111, Trp114, and Trp222; and (G) view down the active-site region of OXA-58 having active-site Ser83 (red), Ser130 (green), and Val132 (cyan) with the presence of the hydrophobic region on the top of the active site, formed by Phe113, Phe114, and Met225.
48, and OXA 51, while M1593 and M2680 have the optimal number of hydrogen bonds. M2680 seems to have the most stable binding with the highest hydrogen bonds with OXA58 (Figure 6).

In case of OXA1, M1593 seems to be making the protein more compact with lesser Rg and SASA values, as compared to other drugs. With lesser Rg and SASA values, M1593 and M2680 are observed to be stably bound to OXA10 in comparison to cilastatin and meropenem. Cilastatin seems to form the most stable complex with OXA23 and OXA24 than the other drugs with the lowest Rg and SASA values. M1593 and M2680 are still having better binding than meropenem, which can be observed with decreasing Rg and SASA values. In OXA24, the meropenem-bound complex has quite SASA and increased Rg, indicating the potential unfolding effects of meropenem on OXA24. Though, cilastatin has the highest number of hydrogen bonds with OXA48 but it has potentially unfolding effect on protein making it unstable. M1593 and M2680 are still having better binding than meropenem and cilastatin, which can be observed with decreasing Rg and SASA values and an optimal number of hydrogen bonds. In OXA51, meropenem have increased Rg as well as SASA values. Other drugs have optimal binding in a manner as M2680 > cilastatin > M1593, which is evident from Rg, SASA, and hydrogen bond plots. In OXA58, M2680 seems to have the most stable binding with decreasing Rg and SASA values. Meropenem has an unfolding effect while M1593 did not impact the average Rg values to a greater extent (Figures 7&8).

### Table 2. Autodock Vina Binding Energy with a Brief Report of Active Site Residues Involved

| OXA variants | binding energy | attributes | cilastatin | meropenem | M1593 | M2680 |
|--------------|----------------|------------|------------|-----------|--------|--------|
| OXA-1        | -5.6           | H bond     | S67, S115, T213, S258 | S67, S115, T213, A215, S258 | S67, A215, S258 | S67, A215, S258 |
|              |                | hydrophobic bond | V117, W102, L161 | V117, W102, L161 | V117, L161, A215, L255 | V117, L161, A215, L255 |
|              |                | other bonds | M99 | M99 | M99 | M99 |
| OXA-10       | -6.0           | H bond     | S67, S112, S115, K205, T206, F208 | S67, K205, S115, T206, F208, R250 | S67, S115, K205, T206, F208 | S67, S115, K205, T206, F208 |
|              |                | hydrophobic bond | M99 | M99 | M99 | M99 |
|              |                | other bonds | M99 | M99 | M99 | M99 |
| OXA-23       | -6.2           | H bond     | S79, S126, K216, T217, M221, D222, R259 | S79, S126, K216, T217, W219, R259 | S79, S126, K216, T217, W219, R259 | S79, S126, K216, T217, W219, R259 |
|              |                | hydrophobic bond | L166, V167 | F110 | F110, W113, V128, L166 | F110, W113, V128, L166 |
|              |                | other bonds | F110 | F110 | F110, V128, L166 | F110, V128, L166 |
| OXA-24/40    | -5.7           | H bond     | S81, T111, S128, W221 | S81, S128, W221 | S81, S128, W221 | S81, S128, W221 |
|              |                | hydrophobic bond | Y112, W221, M223 | W115, V130, M223 | W115, V130, M223 | W115, V130, M223 |
|              |                | other bonds | I102 | I102 | I102 | I102 |
| OXA-48       | -6.2           | H bond     | S70, S118, T209, Y211, T213, R214, R250 | S70, S118, T209, Y211, R214, R250 | S70, T209, Y211, R214, R250 | S70, T209, Y211, R214, R250 |
|              |                | hydrophobic bond | I102 | I102 | I102 | I102 |
| OXA-51       | -6.1           | H bond     | S80, S127, S218, W220, K125, I206 | S80, S127, S218, W220, K125, I206 | S80, S127, S218, W220, K125, I206 | S80, S127, S218, W220, K125, I206 |
|              |                | hydrophobic bond | K125, I206 | F111, W220, K125, I206 | F111, W220, K125, I206 | F111, W220, K125, I206 |
|              |                | other bonds | F111 | F111 | F111, L231, K261 | F111, L231, K261 |
| OXA-58       | -6.3           | H bond     | S83, S221, W223, R263, F114, W117 | S83, S130, S221, R263 | S83, S130, S221, R263 | S83, S130, S221, R263 |
|              |                | hydrophobic bond | F114, W117 | M225 | M225 | M225 |
|              |                | other bonds | F114, W117 | M225 | M225 | M225 |

Figure 3. Comparison of GOLD score and binding energies of selected compounds with the reference antibiotic and inhibitor.
Molecular dynamics results supported the molecular docking studies. It provides mechanistic insights into ligand binding to OXA variants. In conclusion, this study provides a significant understanding of the interaction of ligands to OXA variants at the molecular level that might be useful in drug development.

2.4. In Silico Pharmacokinetic Study. The pharmacokinetic properties of selected leads, standard antibiotic, and inhibitor were additionally determined after the propitious results of docking and simulation to check the consistence of considered ligands with a standard range. Our data revealed that selected ligands followed Lipinski’s rule five for oral bioavailability (Table 3). Selected ligands showed affirmative binding prediction with plasma-protein binding, in contrast to the reference drug and inhibitor. Besides, selected ligands were found to be noninhibitors of cytochrome P450 2D6, similar to reference drugs and inhibitor and thus may not be metabolized frequently.

The CYP2D6 enzyme is one of the important enzymes involved in drug metabolism (Table 4).

2.5. Covalent Docking. Covalent interactions between proposed inhibitors and the OXA variants were analyzed using Discovery studio software. rmsd underneath 2 Å is normally considered as a rule for effective covalent docking.20 rms values of binding of M2680 with OXA variants were found below 2.0 Å, while rmsd values of binding of M1593 with OXA variants were observed above 2.0 Å (Table 5). The results suggested that M2680 binding could follow biphasic kinetic behavior, which might follow a two-step binding mechanism. First, a noncovalent complex was formed with the conserved residues of the enzyme and later, a covalent complex was formed with the enzymes. This hypothesis can be compared with the binding results of RPX7009 with class A and class C β-lactamases.21 However, M1593 do not show covalent binding with any OXA variants as

Figure 4. Revealing binding site molecular interactions and involved amino acid residues of OXA variants in binding with M1593 and M2680. *Color scheme: M1593 (blue) and M2680 (orange).
rmsd values were found above 2.0 Å. It binds to the entrance of the enzyme active site through noncovalent interactions. This can be compared with the binding of PA-34, a non-β-lactam inhibitor of TEM171 β-lactamases.22 M1593 might be proposed as a competitive reversible inhibitor (Figure 9).

3. CONCLUSIONS

Beta-lactamases hydrolyze the β-lactam ring of the antibiotic, rendering the antibiotic ineffective or clavulanic acid, sulbactam, and tazobactam beta-lactamase inhibitors. This study suggests that M1593 and M2680 are novel non-β-lactam inhibitors that
complement the active site of selected OXA variants and interact with conserved residues involved in \(\beta\)-lactam recognition and hydrolysis. The proposed inhibitors bind to the active site through noncovalent interactions (hydrogen bonding and hydrophobic interactions) and can be used as a reversible competitive inhibitor. This is in contrast to the mechanism-based conventional inhibitors, viz., clavulanic acid, sulbactam, and tazobactam, that bind covalently to the catalytic serine in the active site (suicide inhibitor).

Figure 7. Backbone Rg of OXA variants with cilastatin, M1593, M2680, and meropenem. *Color scheme: OXA24-cilastatin (black), OXA24-M1593(red), OXA24-M2680 (green), and OXA24-meropenem (blue).

Figure 8. SASA of OXA variants in complex with cilastatin, M1593, M2680, and meropenem. *Color scheme: OXA24-cilastatin (black), OXA24-M1593(red), OXA24-M2680 (green), and OXA24-meropenem (blue).
or the inhibitors that bind noncovalently to the allosteric sites, viz., FTA, 3-(4-phenylamino-phenylamino)-2-(1H-tetrazol-5-yl)-acrylonitrile, making them resistive against β-lactamase producers. Several inhibitors have already been discovered like, avibactam, MK7655, and boronic acid derivatives inhibiting class A, C, and D β-lactamase. However, no inhibitors have been proposed yet, which possess ability to block OXA β-lactamase with much broader spectrum of activity. This study further suggests the presence of the valine residue in the active site of most of the OXA variants, which might play an important role in the binding of inhibitors or antibiotics with the OXA enzymes. This may also help to design specific inhibitors to block the action of the enzyme.

4. MATERIALS AND METHODS

4.1. Enzyme Initial Structure Preparation. The three-dimensional structures of OXA-1, OXA-10, OXA-23, OXA-24/40, OXA-48, OXA-51, and OXA-58 were obtained from protein data bank (PDB ID’s: 1M6K, 1FOF, 4K0X, 3HBR, 4ZDX, 4OH0, and 2JC7, respectively). All water molecules were removed, and hydrogen atoms were added to the enzyme using Discovery Studio 2.5. A minimization procedure was done using MM2 energy minimization followed by the steepest descent method after assigning the CHARMM force field. The surface model of all class D β-lactamases was developed using Pymol software.

4.2. Screening of Chemical Database. Three-dimensional structures of one antibiotic (meropenem) and one β-lactamase inhibitor (cilastatin) were retrieved from PubChem database. Maybridge database was employed for screening of compounds, based on the properties (Lipinski’s Rule of Five: ClogP, molecular weight, rotatable bonds, hydrogen bond donor, and hydrogen bond acceptor) of known inhibitors to retrieve the hit compounds. Finally, almost 7000 compounds (from ~11,000) were selected. Hydrogen atoms were added in all the molecules. All the preparations were done by using the simulation module of the Discovery Studio 2.5 with a conjugate gradient method after assigning the CHARMM force field. The surface model of all class D β-lactamases was developed using Pymol software.

4.3. Multiple Sequence Alignment of OXA Enzymes. FASTA sequence of OXA-1, OXA-10, OXA-23, OXA-24/40, OXA-48, OXA-51, and OXA-58 was retrieved from the NCBI database. Multiple sequence alignment of all OXA enzymes was done using Clustal omega online server using default parameters.

4.4. Docking Studies. Genetic Optimization for Ligand Docking (GOLD) 5.0 version was used for virtual screening of the compound dataset. Docking annealing parameters for van der Waals and hydrogen bonding were set to 5.0 and 2.5, respectively. The parameters used for genetic algorithm were population size 100, selection pressure 1.2, number of operations 1,00,000, number of islands five, niche size 2, migrate 10, mutate 100, and crossover 100. The docked compounds were assessed on the basis of the GOLD fitness score, favorable binding, and molecular interactions with the active site amino acids. Further, AutoDock Vina software was used to validate the results. GOLD fitness score and binding energy from AutoDock Vina were used as a framework for screening of the molecules.

4.5. Molecular Dynamics Simulations. Molecular dynamics simulations of the docked complexes were performed using GROMACS v5.0, assigning GROMOS96 43a1 force field. GROMACS topologies for the ligands were generated using the PRODRG webservers. Each docked complex was solvated in a triclinic water box using spc water molecules and was made electrically neutral using genion tool. All systems were subjected to energy minimization by the steepest descent method for 50,000 ps steps. MD simulations for the complexes were performed in two steps, that is, NVT (isothermal–isochoric) and
then NPT (isothermal–isobaric) equilibration. Both the equilibration steps were performed for 100 ps time. After attaining the constant temperature and pressure, systems were proceeded for MD production run of 50,000 ps/50 ns to attain a stable trajectory of the complex.

4.6. In Silico Pharmacokinetic Study of Selected Molecules. The greater part of drugs comes up at the time of shorting during the discovery procedure to cross human clinical trials on account of poor pharmacokinetic. These essential parameters of the pharmacokinetic study, absorption, distribution, metabolism, excretion, and toxicity (ADMET), are crucial descriptors for human therapeutic utilization of any compound. ADMET modules in Discovery Studio v3.5 software (Accelrys, USA) were used to calculate these parameters. The studied compounds were also evaluated against Lipinski’s rule of five for oral bioavailability because 90% orally active existing drugs/compounds follows Lipinski’s rule.

4.7. Covalent Docking. Covalent docking was performed to determine possible covalent interactions between the inhibitors and the OXA enzymes. It was carried out using Genetic Optimization for Ligand Docking (GOLD) 5.0 version. rmsd value calculation and result analysis were done using Discovery Studio 2.5 software.

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Notes
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