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A Molecular Docking and Dynamics Study to Screen Potent Anti-Staphylococcal Compounds Against Ceftaroline Resistant MRSA

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ABSTRACT

World Health Organization reports that methicillin-resistant *Staphylococcus aureus* (MRSA) is the origin of higher proportion of hospital acquired infections. In order to combat the effect of MRSA infection, an ideal drug should stimulate the allosteric exposure of active site, prompting penicillin binding proteins (PBP2a) to bind with that particular compound. Ceftaroline shows high binding affinity towards PBP2a and also confers resistance against degrading enzymes. Recently, two amino acid alterations in the allosteric site of PBP2a, asparagine (N) to lysine (K) at position 146 and glutamic acid (E) to lysine at position 150 are reported to confer resistance against ceftaroline resulting in the rise of ceftaroline-resistant MRSA strains. The present study focuses on the identification of potential ligands that can effectively bind with allosteric site of PBP2a, that leads to the access of active site and entry of a β-lactam antibiotic for effective inhibition. The results obtained from our study will be useful for designing effective compounds with potential therapeutic effects against ceftaroline resistant MRSA strains. J. Cell. Biochem. 117: 542–548, 2016.

KEY WORDS: ANTI-MRSA COMPOUNDS; RESISTANCE; LIGANDS; MOLECULAR DYNAMICS

PBPs play a significant role in the peptidoglycan synthesis of bacterial cell wall and act as an attractive target for the development of new antibiotic agents [Jeong et al., 2013]. β-lactam antibiotics exerts their bactericidal effect by acting as a analog of acyl-D-alanyl-D-alanine component, thus interfering the cross linking mechanism [Kohanski et al., 2010]. The transpeptidase forms lethal complex with antibiotics that results in the disruption of bacterial cell wall synthesis. Major resistance to β-lactam antibiotics is acquired by alteration in PBPs resulting in the decrease or loss of binding affinity of β-lactam antibiotics [Malouin and Bryan, 1986]. Despite the adoption of control measures, multi drug resistance (MDR) organisms are found to possess serious threat in recent years [Tanwar et al., 2014]. MRSA is a major cause of hospital acquired infections and it has become difficult to control as it progressively developed resistance against all the classes of antibiotics. Penicillin was the first antibiotic used for the treatment of bacterial infections in early 1950s, by the late 1950s, scientists isolated resistant strains that showed resistance against penicillin by producing a hydrolyzing enzyme, β-lactamase [Kong et al., 2010]. This resistant mechanism was overcome by the synthesis of antibiotics with methoxy group called methicillin; the steric hindrance formed around the amide bond of methicillin reduces its binding affinity for β-lactamases [Stapleton and Taylor, 2002]. Unfortunately, strains which showed resistance against methicillin were found due to the expression of penicillin-binding protein 2a. Selective pressure created by the usage of different antibiotics over the years has lead to the emergence of MRSA strains [Hawkey, 2008]. MRSA has been reported to arise from the expression methicillin resistance gene (*mecA*), which encodes PBP2a with low binding affinity against β-lactam antibiotics [Sakoulas et al., 2001]. Ceftaroline, a novel fifth generation antibiotics exhibits potent antimicrobial activity against MRSA strains as proved in in vitro and in vivo studies [Farrell et al., 2012].

Abbreviations: ADMET, absorption, distribution, metabolism, excretion and toxicity; BBB, blood brain barrier; DScore, dock score; E, glutamic acid; GScore, gold score; K, lysine; MD, molecular dynamics; MRSA, methicillin resistant *Staphylococcus aureus*; N, asparagine; Pa, possibility of active; PBP, penicillin binding protein; Pi, possibility of inactive; PMF, potential mean force; RG, radius of gyration; RMSD, root mean square deviation; RMSF, root mean square fluctuation.

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Ceftaroline acts by stimulating the allosteric binding domain of PBP2a that is located approximately 60 Å distant from the active site. These conformational changes lead to the opening of the active site thus predispose PBP2a to inactivate by second β-lactam antibiotic [Otero et al., 2013]. Many of the published reports have emphasized the significance of ceftaroline against MDR [Iizawa et al., 2004; Sader et al., 2005; Jacobs et al., 2010]. Unfortunately, a recent study that reports two mutations (N146K and E150K) that occurred in the allosteric domain of PBP2a confers resistance against ceftaroline molecules [Long et al., 2014]. Impact of these mutations results in the disruption of allosteric trigger by ceftaroline molecule and leads to the rise of ceftaroline resistant MRSA. As proved in earlier studies allosteric domain of PBP2a plays a crucial mechanistic role in the antibiotic resistance mechanism [Llarrull et al., 2009]. Hence in order to develop effective anti-MRSA agents, we need a clear insight of the structural and molecular basis of resistance mechanism. The aim of our study is to identify effective drug candidates against ceftaroline resistant MRSA. High-throughput screening has increased the possibility of finding new effective ligands in shorter duration than tedious experimental procedures [Coates and Hu, 2007]. To achieve this, molecular docking and molecular simulation studies were employed to investigate the structural and dynamic effects in the protein, specifically at the allosteric site of PBP2a. The wild and mutant structure of PBP2a along with ceftaroline is examined using docking analysis followed by simulation studies. Further a series of antibacterial compounds were docked against the PBP2a of ceftaroline resistance MRSA in order to identify the potent ligands that can effectively bind with the allosteric site of PBP2a. The compound which showed higher binding affinity with the mutant PBP2a proceeded to the subsequent level. The ligands identified can be further validated using in vitro techniques for the development of effective anti-MRSA compounds.

MATERIALS AND METHODS

RETRIEVAL OF TARGET STRUCTURES

The structures of PBP2a used for the present study were retrieved from Protein Data Bank. The corresponding code for the wild structure was 3ZG0 [Otero et al., 2013] and the mutant structure was 4CPK [Fishovitz et al., 2014].

RETRIEVAL OF POTENTIALLY ACTIVE COMPOUNDS BY VIRTUAL SCREENING

The series of drug candidates from ZINC database [Irwin et al., 2012] were docked against the target PBP2a using Dock blaster server [Irwin et al., 2009]. The mutant residues along with the surrounding residues were used to set the grid for calculation. A total of 200 compounds were selected based on the docking energy and the Lipinski rule of five [Lajiness et al., 2004], the rule states that number of donor atom should not be more than five, hydrogen bond acceptor should be less than 10, molecular weight of the compounds should be less than 500 Da and octanol-water partition coefficient should be less than five. The selected compounds were further filtered based on the antibacterial activity using the online pass prediction server [Lagunin et al., 2000]. The selected compounds with higher possibility of active (Pa) in comparison with that of possibility of inactive (Pi) values indicates the compounds have maximum possibilities of activity. The criteria used for selecting compounds with antibacterial activity are as follows:

1) If Pa > Pi, the compounds have higher possibility to exhibit antibacterial activity.
2) If Pa < Pi, the compounds exhibits poor antibacterial activity.

The structures which are filtered using the above said criteria are listed in Table I.

MOLECULAR DOCKING

The selected compounds with antibacterial activity were redocked using the program Surflex-Dock incorporated in SYBYL 2.0 [Jain, 2003]. Surflex dock utilizes consensus score to evaluate the binding affinities of the selected compounds with the protein structure. Prior to docking analysis, the protein structure was optimized by removing the unrelated structures and crystallographic water molecules. The structure of the protein was further optimized by repairing backbone, side chain, and termini treatment. Hydrogen atoms were added to the protein in ideal

TABLE I. Active Compounds Retrieved From ZINC Database

S.No	ZINC database ID	Pa	Pi
1.	02001740	0.519	0.157
2.	28973441	0.276	0.069
3.	12500934	0.159	0.157
4.	05765126	0.167	0.148
5.	5458899	0.176	0.140
6.	2583773	0.126	0.111
7.	2572652	0.284	0.066
8.	2011625	0.380	0.035
9.	1850623	0.467	0.148
10.	1850396	0.225	0.008
11.	1668943	0.161	0.042
12.	1530701	0.190	0.127
13.	601273	0.171	0.145
14.	404451	0.212	0.107
15.	404450	0.179	0.137
16.	403035	0.284	0.066
17.	402865	0.185	0.133
18.	139370	0.171	0.145
19.	139367	0.192	0.125
20.	1612	0.171	0.032
21.	1370	0.171	0.145
22.	92	0.417	0.026
23.	1175	0.192	0.125
24.	1204	0.171	0.145
25.	896873	0.284	0.066
26.	1850907	0.171	0.14
27.	2011627	0.149	0.058
28.	4098901	0.205	0.113
29.	6021033	0.199	0.119
30.	28973446	0.171	0.145
31.	741	0.284	0.066
32.	1460	0.149	0.058
33.	6427	0.188	0.129
34.	896870	0.284	0.066
35.	2522669	0.387	0.033
36.	01051	0.158	0.046
37.	5459478	0.284	0.066
38	02001152	0.171	0.145
39.	403053	0.182	0.135
40.	601281	0.249	0.084
41.	403036	0.249	0.084
42.	4212674	0.284	0.066
43.	896868	0.194	0.123
44.	441	0.194	0.166
45.	1850715	0.168	0.034

"Pa" indicates the Possibility of active and "Pi" indicates the Possibility of inactive.

geometry and a brief minimization step was performed using the force field AMBER 7 FF99. Finally Surflex-Dock protomol (Pseudo binding site) was generated where the ligands were aligned. Docking analysis of the active compounds was now initiated with the following inputs that is, 3D structure of a receptor protein with hydrogen atoms, protomol and the 3D ligands. The Surflex-Dock uses empirically derived scoring functions that were based on following terms hydrophobic, polar, repulsive, entropic, solvation and crash. Cscore (Consensus score) provides the binding affinity of the compounds by combining Gold score (GScore) [Jones et al., 1995]. Dock score (DScore) [Meng et al., 1992], ChemScore [Eldridge et al., 1997] polar score, potential mean force (PMF), and crash score. The interaction between the ligand and receptor was visualized using the tool Molecular Computer Aided Design.

MOLECULAR SIMULATION ANALYSIS

In order to investigate the structure and dynamics of the protein structure, molecular dynamics were performed for a period of 50 ns using Gromacs 4.5.5 [Hess et al., 2008]. The protein ligand complex was solvated using simple point charge (SPC) [Lee et al., 2014] water molecules followed by the addition of counter ions (Cl− or Na+) in order to neutralize the protein. Gromacs 96 43a1 was used as the force field for the simulation of protein ligand complex. A brief energy minimization was performed in order to remove the van der Waals short contacts. The system was then subjected to two phases of equilibration for a period of 1,000 ps. The first phase included number of particles, volume, and temperature ensemble in which endothermic and exothermic processes were exchanged with thermostat. The second phase contained number of particles, volume, and temperature ensemble at 300 k along with constant pressure. The covalent bonds were constrained using Linear Constraint Solver algorithm. The electrostatic interactions were evaluated using Particle Mesh Ewald method within a charge grid space of 0.30 Å. The Lennard–Jones interactions were analyzed using a 9.0 Å atom-based cut-off. Finally, molecular dynamics (MD) was performed for a period of 50 ns to analyze the stability of each system.

TRAJECTORY ANALYSIS

The convergence of simulation was analyzed in terms of potential energy, root mean square deviation (RMSD), root mean square fluctuation (RMSF), Radius of Gyration (RG), number of H-bonds formed between the ligand, and receptor using the Gromacs utilities [Spoel et al., 2005].

MOLECULAR PROPERTIES OF THE ACTIVE COMPOUND

Finally the molecular properties of the ligand with high binding affinity was analyzed using the server admetSAR [Cheng et al., 2012].

RESULTS AND DISCUSSION

RETRIEVAL OF POTENTIALLY ACTIVE COMPOUNDS BY VIRTUAL SCREENING

According to the Center for Disease Control and Prevention report [CDC, 2007] among the people infected with *S. aureus*, about 1% of the infections are caused by MRSA. MRSA possess serious threat to public as it exhibits resistance against all the currently available broad spectrum antibiotics. Series of active compounds are screened against MRSA and their effectiveness is compared with ceftaroline. Initially a series of 200 active compounds are selected based on the docking energy from ZINC database. Poor ADMET properties contribute significantly to higher failure rate in the development of effective drugs. Since ADMET studies play a crucial role in the development of effective antibiotics, the physiochemical properties of the selected compounds are also analyzed. Our results suggested that all the molecules have zero violations of the Lipinski's rule of five suggesting the efficiency of these molecules to act as drug candidate. On completion of virtual screening, we employed pass prediction server to filter the compounds with antibacterial activity. Finally we selected 45 compounds from the total of 200 active compounds with antibacterial activity. The compounds selected are then subjected to further analysis using Surflex-Dock software.

MOLECULAR DOCKING

In order to investigate the binding affinity of mutant structure with the active compounds, molecular docking studies are performed. Our result shows that the active compound with zinc id 02001740 shows highest binding energy when compared with other compounds; in terms of binding energy and hydrogen bond interactions. Interestingly the results obtained from docking analysis correlates with the results obtained from pass prediction server. The energy score obtained between the mutant protein structure with active compound and the ceftaroline is tabulated in Table II. To understand the effect of mutation in ceftaroline binding domain of PBP2a, docking energy of the existing drug ceftaroline is analyzed against the wild structure. It is noteworthy that the docking energy of ceftaroline with mutant protein is comparatively low than the docking energy with wild protein. This observation provides a strong clue for the resistance mechanism conferred by the amino acid alteration in the binding site of PBP2a. Our results are supported by a recent study that proves mutations present in the binding pocket of

TABLE II. Binding Affinity of Top Scored Active Compound and Ceftaroline Against the PBP2a of Ceftaroline Resistant MRSA

S. No	Compounds	Mutant protein- zinc id 02001740 complex	Mutant protein-Ceftaroline complex
1	CScore[a]	5.99	3.07
2	Crash score[b]	0.80	−1.57
3	Polar score[c]	4.34	3.38
4	D score[d]	−88.3	50.3
5	PMF score[e]	−51.7	−90.1
6	G score[f]	−170.8	−206.8
7	Chem score[g]	−17.0	−25.8

[a]C-Score is a consensus scoring which uses multiple types of scoring functions to rank the affinity of ligands.
[b]Crash-score revealing the inappropriate penetration into the binding site.
[c]Polar region of the ligand.
[d]D-score for charge and van der Waals interactions between the protein and the ligand.
[e]PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs
[f]G-score showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies.
[g]Chem-score points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.

the PBP2a (N146K and E150K) confers resistance against ceftaroline which leads to the rise of ceftaroline resistant strains [Long et al., 2014]; but they have not reported the mechanism of resistance. Our result supports and provides valuable information on the mechanism of resistance exhibited by N146K and E150K mutants. Interestingly, the mutant protein shows significantly higher binding affinity with the active compound than ceftaroline in terms of docking energy and hydrogen bond interaction. These results highlight the efficiency of active compound to induce conformational changes in allosteric binding domain of PBP2a. The stability of the enzyme ligand complexes are further analyzed using simulation studies. Pictorial representation of hydrogen bond interactions between mutant protein and active compound is shown in Figure 1.

MOLECULAR SIMULATION ANALYSIS

Potential energy. The potential energy values for wild and mutant protein structure with the ceftaroline and active compound is analyzed. It is observed that wild protein shows the lowest energy of −4.52 kJ/mol with ceftaroline compound whereas the mutant structure shows increase in potential energy of −3.80 kJ/mol. These results indicate the effect of mutation in the allosteric binding domain of PBP2a. In order to identify the binding affinity of active compound in the allosteric binding domain of PBP2a, potential energy of mutant protein structure with the active compound is analyzed. It is worth mentioning that mutant structure shows the energy value of −4.32 kJ/mol with the active compound, indicating the best binding affinity of active compound with allosteric domain of PBP2a comparing to the ceftaroline molecule.

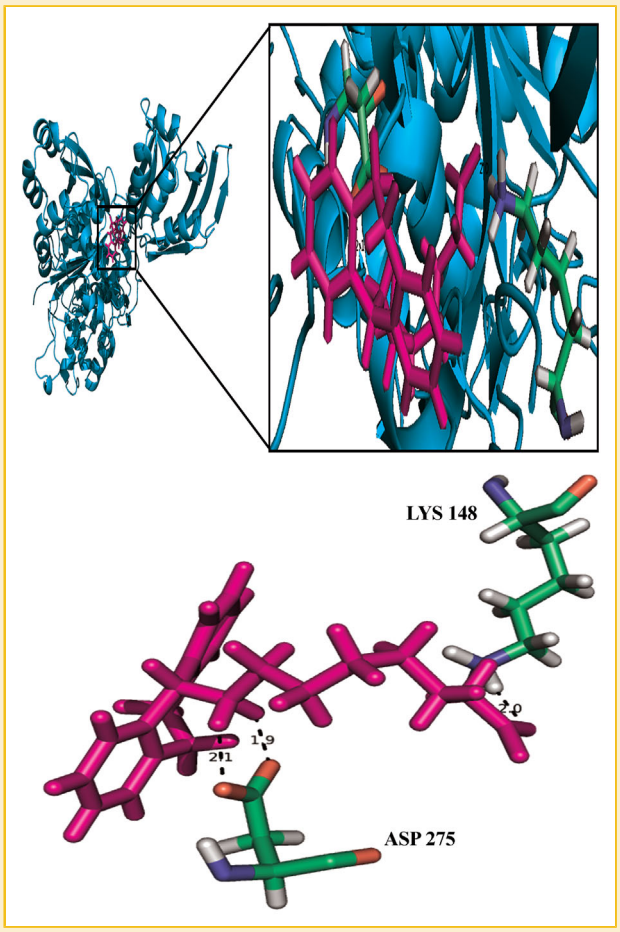

Fig. 1. Pictorial representation of hydrogen bond interactions between mutant protein and the active compound.

RMSD analysis. The RMSD analysis is a key parameter to analyse the equilibration of MD trajectories. Measurement of RMSD value for each complex provides complete insight into the conformational stability of each system. RMSD analysis is carried out for the mutant as well as the wild structures. Considerable structural changes are observed in all the systems analyzed. Our result demonstrates that wild protein with ceftaroline compound shows higher stability with the allosteric binding domain of PBP2a, whereas the mutations that occurred in the ceftaroline binding site of PBP2a reduces the binding affinity of ceftaroline with the mutant structure that is clearly evident by the higher RMSD values. Further the stability of mutant protein with the active compound is also analyzed. Pictorial representation of RMSD values of the complexes are illustrated in Figure 2. From the RMSD analysis, it is observed that constant range of deviation and higher RMSD value in ceftaroline complex reflects that mutation affects the dynamics behavior of ceftaroline complex, whereas wild structure shows minimum deviation and attains the RMSD value at 0.75 nm. Final RMSD values for all the three trajectories is analyzed and it is observed that ceftaroline complex shows maximum deviation till end and attains the value of 1 nm at 50 ns whereas active compound shows the value of 0.75 nm at 50 ns. The deviation of ceftaroline complex is higher than other two complexes. Higher RMSD value of ceftaroline complex clearly indicated lesser stability of the ceftaroline complex that of the active compound complex.

RMSF analysis. Differences in flexibility among the residues are analyzed using the parameter root mean square deviation. RMSF value of the mutant protein structure is analyzed with the active compound and the ceftaroline compound, and the results are shown in Figure 3. From the figure it is observed that mutant protein with ceftaroline shows higher fluctuation than wild protein, which clearly

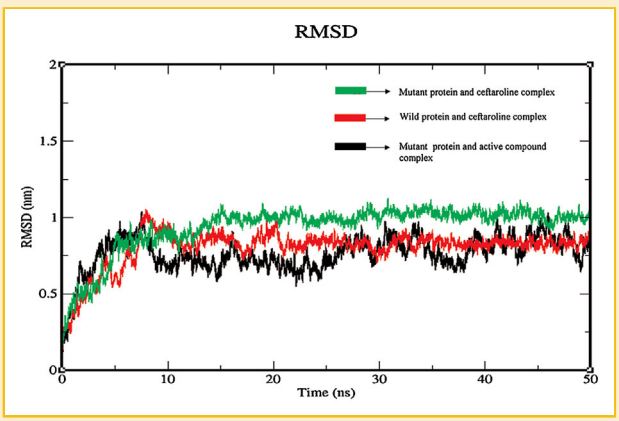

Fig. 2. Root mean square deviations correspond to MD simulation at 300 k.

indicates the impact of mutation in allosteric binding domain of PBP2a. On analyzing the results from mutant protein and active compound complex, we find that mutant protein with active compound shows lesser fluctuation than mutant structure with ceftaroline complex, indicating the restricted movements during simulation.

H-bond analysis. Hydrogen bond plays a crucial role in the overall stability of the protein structures. Intermolecular hydrogen bonds are analyzed for mutants well as the wild structures and the results are shown in Figure 4. Our analysis illustrated that wild protein with ceftaroline complexes showed a maximum of seven intermolecular hydrogen bonds throughout the simulation whereas hydrogen bond interaction is gradually decreased in mutant protein. Mutant protein structure and ceftaroline complex shows a maximum of five hydrogen bonds, showing decrease in the binding affinity of ceftaroline with the mutant structure. The binding affinity of mutant protein structure with the active compound is analyzed and a maximum of seven hydrogen bonds are observed, showing the higher binding affinity of active compound with the allosteric binding site of PBP2a.

Rg analysis. In order to analyze the overall compactness of protein structure in the presence of ceftaroline, we determined the Rg values for wild and mutant structure. The calculated Rg values for wild protein with ceftaroline compound, mutant protein structure with ceftaroline compound and mutant protein with the active compound complex is shown in Figure 5. It is observed that Rg values of wild and mutant structures fluctuate between 3.2 nm and 3.7 nm. As depicted in Figure 5, Rg value of mutant protein structure with ceftaroline complex fluctuates near 3.6 nm and decreased to a minimum value of 3.4 nm, whereas the mutant protein structure with active compound fluctuates near 3.6 nm and decreased to a minimum value of 3.2 nm. From the graph it is clear that mutant protein structure with active compound shows lower Rg values than mutant protein structure with ceftaroline compound that indicates little conformational changes throughout the simulation.

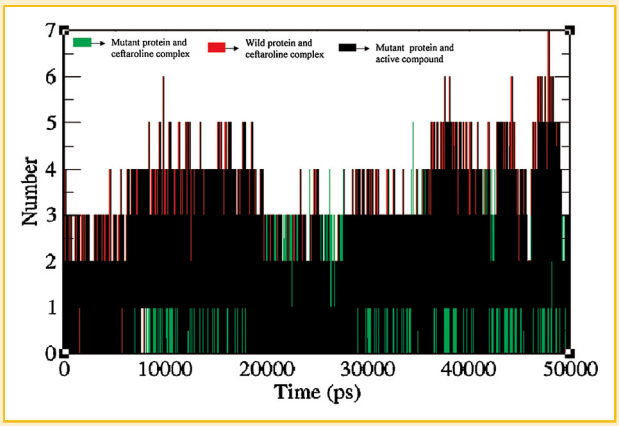

Fig. 4. H bonds observed between wild protein and ceftaroline complex; mutant protein and ceftaroline complex and mutant protein and active compound complex.

MOLECULAR PROPERTIES OF THE ACTIVE COMPOUND

The pharmacokinetic properties of the top scored active compound is analyzed and the results are tabulated in Table III. ADMET profiling of drug candidates include parameters such as blood-brain barrier (BBB), Human intestinal absorption, Caco-2 Permeability, P-glycoprotein inhibitor, renal organic cation transporters, and AMES Toxicity. Blood brain barrier impedes the entry of most of the drugs into the brain and makes it difficult for the development of effective drug candidates. As reported in recent studies 98% of the drug fails in clinical trials because of the inability to cross BBB and reach the specific target [Geldenshuys et al., 2012]. In vivo studies have demonstrated the significance of P-glycoprotein as biological barrier by rate limiting the process of cellular uptake and excretion of drugs [Lin and Yamazaki, 2003]. Caco-2 permeability is analyzed to measure the intestinal absorption of drugs as it shows physiological and functional similarities with human intestinal enterocytes [Bohets et al., 2001]. In order to exert its pharmacological effects, drug has to fulfil the criteria associated with ADMET

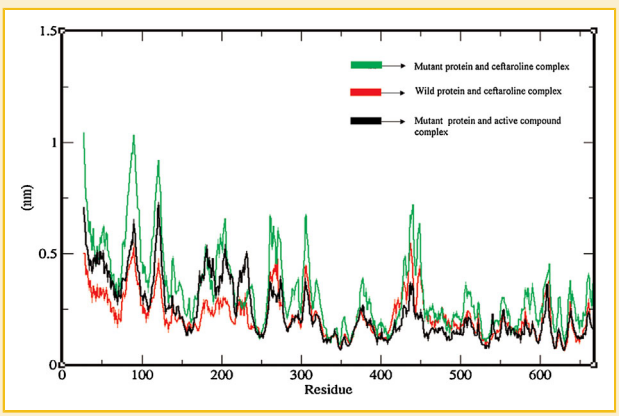

Fig. 3. Root mean square fluctuation corresponds to MD simulation at 300 k.

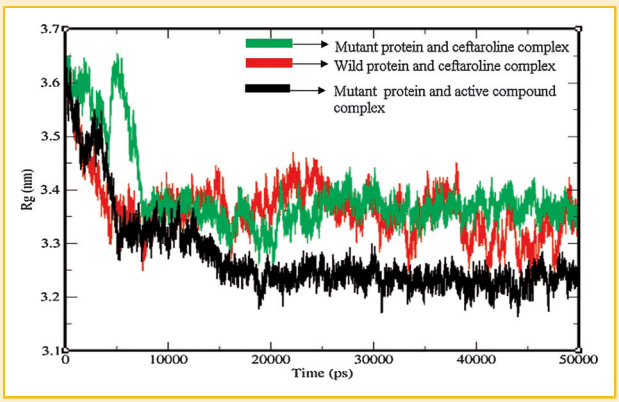

Fig. 5. Radius of gyration corresponds to MD simulation at 300 k.

TABLE III. Molecular Properties of Top Scored Active Compound (Zinc id 02001740)

Absorption	
Models	
Blood-brain barrier	BBB+
Human intestinal absorption	HIA+
Caco-2 permeability	Caco2+
P-glycoprotein inhibitor	NI
Renal organic cation transporter	NI
Metabolism	
CYP450 2C9 substrate	NS
CYP450 2D6 substrate	NS
CYP450 3A4 substrate	NS
CYP450 1A2 inhibitor	NI
CYP450 2C9 inhibitor	NI
CYP450 2D6 inhibitor	NI
CYP450 2C19 inhibitor	NI
CYP450 3A4 inhibitor	NI
Toxicity	
AMES toxicity	NT
Carcinogens	NC

NI, non-inhibitor; NS, non-substrate; NT, non-toxic; NC, non-carcinogen.

profiling. The top scored active compound retrieved from ZINC database shows good correlation with all the parameters analyzed in ADMET profiling proving the capability of the compound to act as efficient drug candidate against ceftaroline resistant MRSA.

CONCLUSION

Among the compounds screened from ZINC database, the active compound with zinc id 02001740, showed highest binding affinity with the PBP2a of ceftaroline resistant MRSA. The results obtained from pass prediction server reveals the capability of active compound to act as an ideal antibacterial agent. Furthermore, the consensus score obtained from Surflex-dock reveals the effective binding of active compound with the allosteric binding domain of PBP2a. It is noteworthy to mention that molecular simulation studies also validate the stability of active compound with the PBP2a from ceftaroline resistant MRSA. The ADMET analysis and physiochemical properties of the compound also show positive results revealing the capability of active compound that can be developed as a potent antibiotic in future. We believe that the results obtained from the present study will be helpful for the development of effective molecules against ceftaroline resistant MRSA.

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