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Title: **Enhancement of the Ceftazidime Efficacy through Cutting-Edge Modifications and In Silico Repurposing against Multidrug Resistance in Pseudomonas Aeruginosa**

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Enhancement of the Ceftazidime Efficacy through Cutting-Edge Modifications and *In Silico* Repurposing against Multidrug Resistance in *Pseudomonas Aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa causes acute and chronic infections in patients hospitalized after surgery, heat burns, and other injuries. Different antibiotics are used to treat bacterial infections. A commonly used cephalosporin, namely ceftazidime, shows a significant effect against *P. aeruginosa*. Ceftazidime targets penicillin-binding protein in *P. aeruginosa*. Currently used antibiotics are facing resistance due to different mechanisms. Targeting the mutated penicillin-binding protein 3 of *P. aeruginosa*, this study aims to improve the binding affinities of ceftazidime by adding various functional groups in methylpridinium ring. *In silico* tools were used to modify the structure of ceftazidime to make it effective against the resistant strains of *P. aeruginosa*. The 3D structures of normal and mutated penicillin-binding proteins were retrieved from Protein Data Bank (PDB). The structure of the antibiotic was retrieved from PubChem and EMBL-EBI, which was modified through Chemsketch. Azide and phenyl carbonyl groups were added in the methylpridinium ring of ceftazidime. AutoDock Vina was used to visualize the binding affinities of the engineered ceftazidime with proteins. PyMOL was used for visualization. The binding affinity of the engineered ceftazidime was improved upto -8.9 kcal/mol. Simulation analysis of the complex showed its eigenvalue and covariance. The feasibility of the modified structures was verified using SwissADME. ADMET analysis confirmed that these structures remain feasible for utilization after clinical trials. The above modifications allow a better treatment of infections caused by stronger and resistant *P. aeruginosa* strains. Further analysis needs to be carried out in order to improve other antibiotics and different conjugates for the effective treatment of infections

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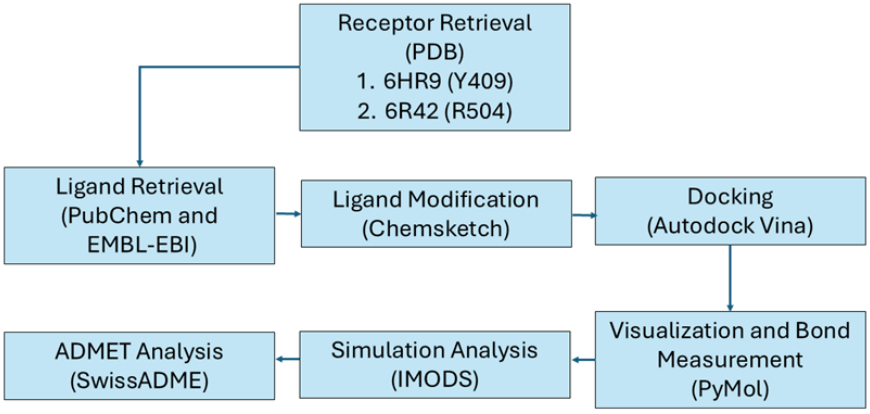
caused by this bacteria.

Keywords: ADMET studies, antibiotic resistance, drug resistance, drug repurposing, drug safety, molecular interaction, *Pseudomonas aeruginosa*

Highlights

- Improved binding affinity of ceftazidime against resistant *P. aeruginosa* strains by adding functional groups.
- *In silico* modification of ceftazidime using molecular modeling techniques.
- Feasibility for clinical trials confirmed, promising treatment for resistant *P. aeruginosa* infections.

GRAPHICAL ABSTRACT



I. INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterium. It inhabits moist surfaces and fresh water and is responsible for many acute infections [1-3]. *P. aeruginosa* is a causative agent for hospital-acquired and ventilator-acquired pneumonia and is also the source of microbial keratitis in patients using contact lenses [4, 5]. It also causes chronic infections in patients with cystic fibrosis, organ transplant, malignant external otitis, endophthalmitis, septicemia, and meningitis. Patients with pseudomonal infections are subject to higher mortality than others. About 7.1-7.3% of all healthcare-associated infections are reportedly caused by *P. aeruginosa* [6, 7].

The infections caused by *P. aeruginosa* are treated by different classes of antibiotics, mainly including beta-lactams, carbapenems, and aminoglycoside. β-lactams comprise a major class of antibiotics which acts as an inhibitor of penicillin-binding proteins (PBP), mainly PBP3 of *P. aeruginosa* [8, 9]. β-lactams inhibit the transpeptidase and DD-carboxypeptidase activity. *P. aeruginosa* has developed resistance against different β-lactams due to the inhibition of porins, degradation of β-lactams, alteration of PBP3, and increased antibiotic efflux. Due to the high mortality infections caused by *P. aeruginosa*, improved antibiotics need to be developed for their better treatment [10, 11].

Ceftazidime is commonly used, either alone or in combination with other β-lactams, for treating infections caused by *P. aeruginosa* [12]. It is the most common β-lactam antibiotic currently used for the treatment of such infections. Ceftazidime contains a cephem nucleus, with methylpridinium group attached to carbon 3 and an aminoacyl group attached to carbon 7. Alpha carbon of aminoacyl group in ceftazidime attaches a carboxy-propyl-oxyimino chain and an aminothiadizol ring. Methylpridinium group and carboxy-propyl-oxyimino chain show antimicrobial activity against *P. aeruginosa* [13].

6HR9 is the Y409 mutant of PBP3 protein in *P. aeruginosa*. Tyrosine residue at 409 in PBP3 *P. aeruginosa* plays a critical role in the active site which facilitates β-lactam binding and acylation through intramolecular interactions that stabilize key catalytic motifs. Mutations at or near Y409, such as those in the penicillin-binding domain, disrupt these interactions, thus reducing PBP3 affinity for ceftazidime [14]. Whereas, 6R42 is the R504 mutant of PBP3 protein in *P. aeruginosa*. The presence of arginine at position 504 mutations in PBP3 of *P. aeruginosa*, particularly R504C, contribute to β-lactam resistance by altering the active site conformation and reducing binding affinity [14, 15].

Ceftazidime resistance occurs due to the combination of multiple resistance mechanisms including the overexpression of β-lactamase (increasing the hydrolysis of ceftazidime) and efflux pump upregulation [16]. Azide (-N3) and phenyl carbonyl (-C(O)Ph) substitution in ceftazidime analogs targeting *P. aeruginosa* enhances their affinity by modulating polarity, steric bulk, and charge distribution to evade antibiotic hydrolysis and efflux, while improving PBP binding [17]. Azide groups introduce polar, electron-withdrawing moiety with a partial negative charge

density on terminal nitrogen, which increases solubility and reduces efflux susceptibility in *P. aeruginosa* [18]. Phenyloxycarbonyl (Phoc) group acts as an amine protecting group and activates carbamate. Further, it creates stable carbamates resistant to acid/neutral conditions through resonance stabilization [19]. Hence, this can be useful to protect ceftazidime from the action of β-lactamase. It is hypothesized that the addition of electron-withdrawing functional groups to the methylpyridinium ring of ceftazidime would improve binding affinity and electrostatic interactions with the mutated PBP3 active site.

Bacteria undergo modifications, rendering them resistant to particular antibiotics. Successive generations of antibiotics, therefore, have improved their activity against bacteria [20]. This study shows that ceftazidime engineering by the addition of different functional groups can be a successful modification. ADMET analysis proves that these modifications are suitable for use in the body. However, clinical and human testing will remove the biological uncertainty related to modification. Moreover, further studies are required to identify functional groups that increase the binding affinity of antibiotics with corresponding proteins.

2. MATERIALS AND METHODS

2.1. Protein and Ligand Structure Retrieval

3D structures of Y409 mutant of penicillin-binding protein 3 (PBP3) from *P. aeruginosa* (PDB ID; 6HR9) and R504 mutant of PBP3 from *P. aeruginosa* (PDB ID; 6R42) were retrieved from the Protein Data Bank (PDB) in PDB format. PDB (https://www.rcsb.org/) is an authentic and widely used database containing normal and mutated protein structures along with their description. These structures were selected because of their structural relevance and are critical for β-lactam binding. Protein structures were prepared using BIOVIA Discovery studio 2021 (https://discover.3ds.com/). Water molecules and extra ligands were removed to avoid non-specific interactions. 3D structure of ceftazidime ($C_{22}H_{22}N_6O_7S_2$) was retrieved from PubChem in Structure Data Format (SDF). PubChem (https://pubchem.ncbi.nlm.nih.gov/) is an online database containing a wide range of chemical structures in 2-dimensional (2D) and 3-dimensional (3D) formats. BIOVIA Discovery studio was used to convert 3D structures into PDB format. 2D structure of ceftazidime was retrieved in Molfile format from EMBL-EBI (https://www.ebi.ac.uk/).

2.2. Structural Engineering of Ligand

Ceftazidime is commonly used to treat patients infected with *P. aeruginosa*, hence it was selected for structural modification. The said modification was carried out using Chemsketch 2023 ((https://www.acdlabs.com/), which is an offline tool used for drawing chemical structures and the modification of existing structures. Azide group was at carbon-6 and a phenyl carbonyl group was added at carbon-5 of ceftazidime.

2.3. Molecular Docking

Normal and modified ceftazidime were docked with 6HR9 and 6R42 (Y409 and R505 mutants, respectively) using AutoDock Vina. AutoDock Vina (version 1.5.7, https://vina.scripps.edu/) is an offline docking tool used to observe the ligand interaction with proteins. Docking analysis was performed to evaluate potential interactions and binding affinities. The grid box in 6HR9 was set at size x, size y, and size z at -5.69, 20.164, and -2.297, respectively. For 6R42, it was set at size x, size y, and size z at 6.104, 21.201, and -1.993, respectively. The center of the grid box in all axis was set at 30. Protein and ligand interaction analysis was performed using PyMOL (https://pymol.org). The bond length was observed to identify the bonds formed between the protein and the modified ligands. The bond length ranging between 2.8 to 3.4 Å represents hydrogen bonding, while bond length ranging between 3.8 to 4.2 Å represents Vander waals forces.

2.4. Molecular Dynamic Simulations

IMODS was used for the simulation analysis of the complex formed after docking. IMODS (https://imods.iqfr.csic.es/) is an online tool for the study of individual and cumulative amino acids in the above complex. It allows for the identification of the interaction of protein with the molecule and the stiffness of the complex. The docked complexes were uploaded in PDB format and simulation analysis was conducted using Normal Mode Analysis (NMA). NMA estimated the eigenvalues and covariance maps. These results were used to evaluate the behavior of such a modified antibiotic in the active sites of PBP3 mutants.

2.5. ADMET Analysis of Engineered Ligands

SwissADME (http://www.swissadme.ch/) is an online *in silico* tool used to test the feasibility of drugs. SwissADME was used for ADMET analysis

and preclinical testing of ceftazidime, modified by the addition of azide group and phenyl carbonyl group. 2D structure of ceftazidime was uploaded and its drug-like qualities were determined.

3. RESULTS

3.1. Ligand Structure Modification and Interaction Studies

Ceftazidime was successfully modified by the addition of azide group at carbon-6 of methylpyridinium ring (Figure 1A). Another modification was done by the addition of phenyl carbonyl group at carbon-5 of methylpyridinium ring (Figure 1B). Improved bond formations were observed in the complexes of 6HR9 and 6R42 with modified ceftazidime (Figure 2). Energy absorption of 6HR9 variant of PBP3 improved from -6.3 to -8.1 kcal/mol by the addition of azide group and upto -7.5 kcal/mol by the addition of phenyl carbonyl group. Whereas, energy absorption of 6R42 improved from -5.6 to -8.9 kcal/mol by azide group and upto -8.1 kcal/mol by the addition of phenyl carbonyl group (Table 1).

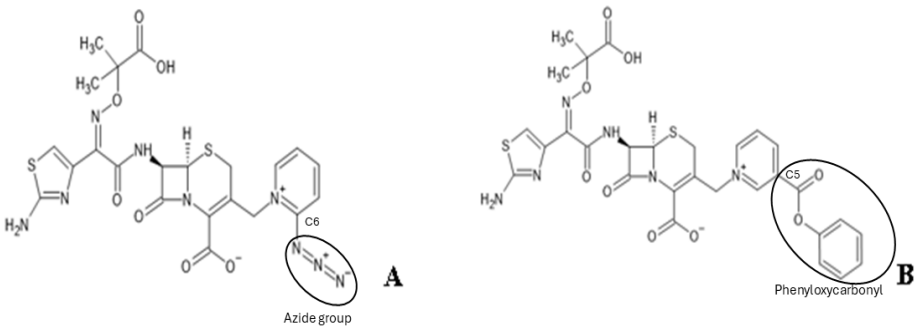


Figure 1. Engineered Structure of Ceftazidime. Addition of Azide Group at Carbon-6 (A). Addition of Phenyloxycarbonyl Group at Carbon-5 (B).

Table 1. Binding affinities after the modification of ceftazidime.

Ligand	Ligand Type	6HR9	6R42
Ceftazidime	Normal	-6.3	-5.6
Ceftazidime + azide group	modified	-8.1	-8.9
Ceftazidime + phenyl oxycarbonyl	modified	-7.5	-8.1
Ceftazidime + nitro group	modified	-7.1	-7.2
Ceftazidime + tazobactam	Conjugate	-6.9	-6.5

The bond interactions of modified ligands with individual proteins were studied. PyMOL was used for the visualization of protein complexes, along with the interactions and measurement of bond length. The 6R42 complex with azide group modification showed 6 hydrogen bonds and 1 Vander waals force (Figure 2A), while the 6HR9 complex with azide group modification showed 7 hydrogen bonds (Figure 2B). The 6R42 with phenyloxycarbonyl modification formed 7 hydrogen bonds (Figure 2C) and 1 Vander waals interaction force, while the 6HR9 with phenyloxycarbonyl formed 7 hydrogen bonds (Figure 2D).

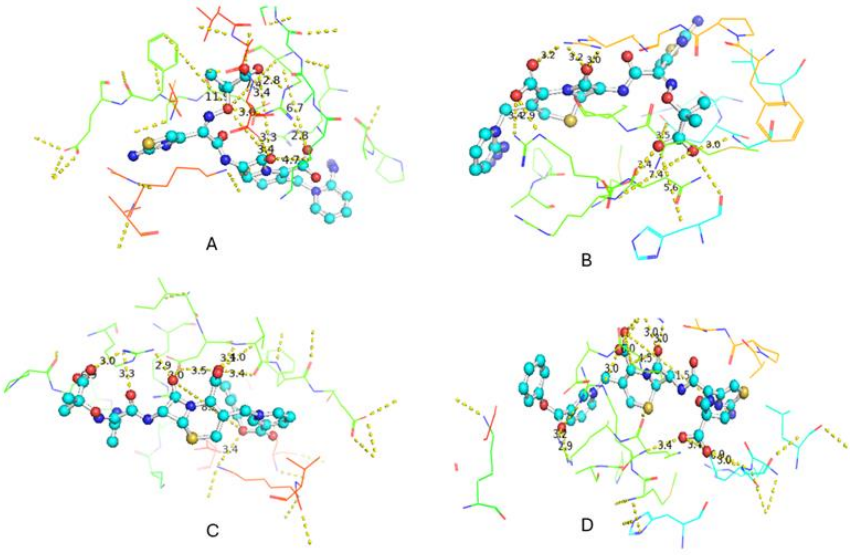


Figure 2. Interaction Analysis of 6R42 with Azide Group (A), 6HR9 with Azide Group (B), 6R42 with Phenyl Carbonyl (C), and 6HR9 with Phenyloxycarbonyl (D).

3.2. Molecular Dynamic Studies

Covariance is the relationship between individual residues. It shows whether the residues experience correlated (red), uncorrelated (white), or anti-correlated (blue) motions. The covariance of residues in 6R42 and 6HR9 complexes, with ceftazidime modified by the addition of azide group and phenyl carbonyl group, was found to be ideal. Figure 3 shows the covariance map of different complexes. The map shows the correlation of amino acids in the protein ligand complexes of mutated proteins with modified antibiotics.

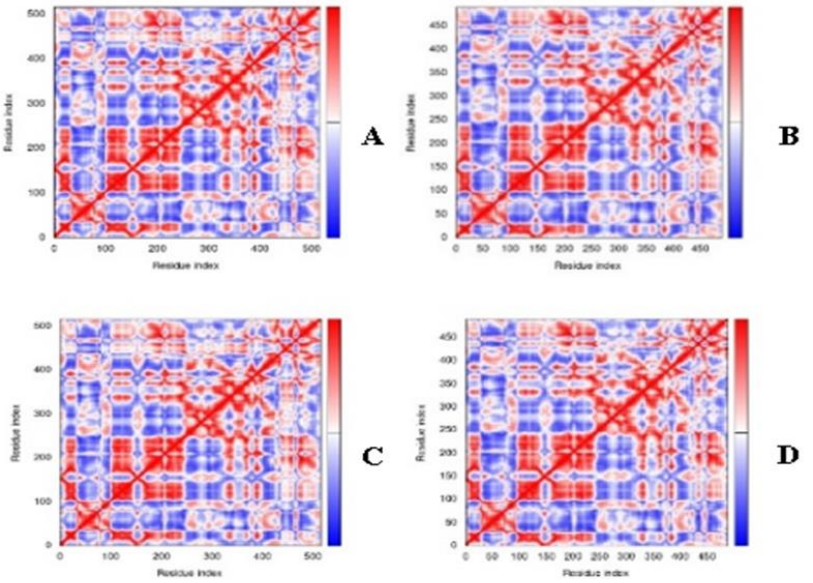


Figure 3. Covariance map of 6R42 with azide group (A), 6HR9 with azide group (B), 6R42 with phenyl carbonyl (C), and 6HR9 with phenyl carbonyl (D).

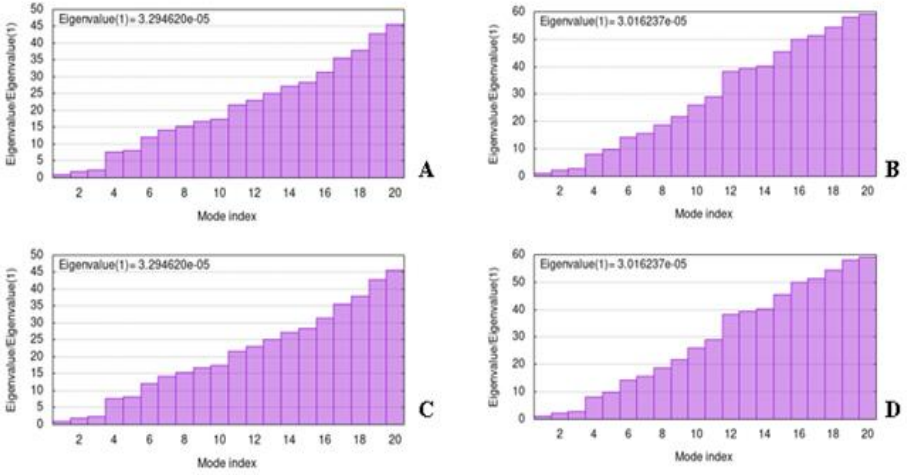


Figure 4. Eigenvalue Graph (A) 6R42 with Azide Group (B) 6HR9 with Azide Group (C) 6R42 with Phenyl Carbonyl (D) 6HR9 with Phenyl Carbonyl

Eigen values represent the mobility of individual residues. The ideal eigen value for a complex should be above 1. Its value is directly related to the energy required to deform the structure. The lower the eigenvalue, the easier the deformation. Eigenvalue of 6R42 and 6HR9 complexes, with ceftazidime modified by the addition of azide group and phenyl carbonyl group, was above 1 in all four complexes. Figure 4 represents the eigenvalues of individual complexes.

3.3. ADMET Analysis

ADMET analysis shows the feasibility of the drug to treat pseudomonal infections. The preclinical testing of modified ceftazidime was done to verify the solubility of the drug and its interaction with other body tissues. ADMET analysis allows for the prediction of the properties and toxicity of the drug. ADMET analysis of ceftazidime modified with azide group and phenyloxycarbonyl group showed that the addition of the functional group did not transform the drug into a toxic metabolite. Modified drugs do not cross the skin and the blood-brain-barrier (BBB), which reduces the probability of possible side effects. The results of ADMET analysis confirmed that the drug containing the azide group and phenyl carbonyl functional group is not an inhibitor of cytochromes. Moreover, the lipophilicity of the drug is optimum according to the Lipinski's rule of 5. Table 2 shows the results obtained from ADMET analysis. The compounds were observed to have a better synthetic accessibility.

Table 2. ADMET Analysis

Drug	Nature	Solubility	Cytochrome inhibitor	BBB permeant	GI absorption	Lipophylicity
Ceftazidime	Original	Soluble	NO	NO	Low	Optimum
Ceftazidime + azide group	Modified	Soluble	NO	NO	Low	Optimum
Ceftazidime + phenyl carbonyl group	modified	Soluble	NO	NO	Low	Optimum

4. DISCUSSION

P. aeruginosa causes severe infections and is treated by various classes of antibiotics. Ceftazidime is one of the common antibiotics used for the treatment of infections caused by *P. aeruginosa* [21]. Ceftazidime, a commonly used β-lactam, interacts with Penicillin Binding Protein 3 or

PBP3, thus interfering with the growth of the organism [DB00438]. Bacteria have become resistant towards particular antibiotics due to different reasons. The modification of ceftazidime by the addition of functional groups aims to improve the binding affinity of the drug in order to improve its efficacy. When the ligand interacts with the receptor protein, bond formation results in the release of energy which is measured in the form of its binding affinity [22, 23].

In this study, ceftazidime interactions were investigated against the mutated PBP3 protein through docking which showed its decreased binding affinity. Poor binding affinity represents poor interaction between the ligand and the receptor, hence reduced efficacy. The modification of ligand through the addition of effective functional groups has been a useful approach for improved treatment strategies [24]. Hence, ceftazidime was modified by the addition of azide group at carbon-6 and phenyloxycarbonyl group at carbon-5 of methylpridinium ring (Figure 1). The study was performed by investigating the effect of the modification of the antibiotic on two mutant PBP3, namely Y409 (PDB ID: 6HR9) and R504 (PDB ID: 6R42) (Table 1).

Multiple modifications were done by the addition of functional groups including nitro group, azide group, phenyloxycarbonyl group, and conjugation of ceftazidime and tazobactam. Docking analysis indicated that the addition of azide and phenyloxycarbonyl group improved the interaction between ceftazidime and receptor. Azide group is a stable high-energy functional group and has been previously used for the modification of ligand structures for improved results [25].

IMODS analysis of the modified ceftazidime was performed for simulation analysis. The analysis confirmed that the movements of most amino acids in modified ceftazidime-PBP3 complex are correlated, as represented in the red region of Figure 3. The better correlation of amino acids indicates that the ligand is an appropriate drug to target the desired mutated protein [26]. Simulation analysis validated the conjugates formed after the modification of the drug, while high eigenvalues ensured the stability of the conjugates as a high amount of energy is required to deform the complexes [24]. An increased energy requirement for the deformability of the complexes ensures the stability of the modified antibiotics [27].

ADMET analysis of modified ceftazidime showed that it is water-

soluble, validating it as an appropriate candidate for treatment. The lipophilicity of the modified drug is also optimum, indicating better membrane permeability and absorption. Drug absorption in GI tract should be low in order to reduce the accumulation of the drug in the body, which can cause toxicity. The drug should not be permeant to the blood-brain-barrier (BBB) because it can lead towards adverse effects including tumor cell growth [28]. If the drug crosses the BBB, it affects the central nervous system which can lead towards possible side effects. ADMET analysis of modified ceftazidime indicated low GI absorption and no BBB permeation, which makes it a suitable candidate for the treatment of *P. aeruginosa* infection. ADMET analysis also validated that the modified drug is not an inhibitor of cytochromes. Cytochromes play an important role in the body as they are key factors in electron transport chain and cell cycle regulation [29].

This *in silico* study showed that the modification of commonly used ceftazidime by the addition of azide group and phenyloxycarbonyl can improve the treatment of patients. Since, the modified drug has better synthetic accessibility, the production cost for chemical synthesis can be readily reduced while increasing its efficacy. However, the results of this study need to be validated by moving towards preclinical testing on mouse models to assess the risks. The study can also be extended by investigating the effects of other functional groups on ceftazidime against mutated protein.

4.1. Conclusion

The modification of ceftazidime by the addition of azide and phenyl carbonyl groups showed an effective response against *P. aeruginosa*. The engineering of ceftazidime improved the absorption of energy by the Y409 mutant of PBP3 from *P. aeruginosa* (6HR9) and R504 mutant of PBP3 from *P. aeruginosa* (6R42). It was observed that ceftazidime could serve as an effective candidate for the treatment of infections caused by stronger and resistant *P. aeruginosa*. However, preclinical and clinical trials are required before making it available for human use. Further studies are also required to improve the efficiency of the antibiotic through the identification of functional groups, which increases the binding affinity of antibiotics with their corresponding proteins.

Author Contribution

Muhammad Aqib Shabbir: conceptualization, investigation, supervision. **Emmania Abid:** formal analysis, software. **Laiba Tanveer:** formal analysis, software. **Mahnoor Absar:** visualization, writing - original draft. **Mahnoor Tariq:** visualization, writing - original draft. **Areesha Alvi:** writing – review & editing.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

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Generative AI Disclosure Statement

The authors did not use any type of generative artificial intelligence software for this research.

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