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ORIGINAL RESEARCH

Exploring the TEM β-Lactamase in *E. coli* From Urinary Tract Infection Patient: Insights From Molecular Docking and Dynamics Simulations

Tina Rostinawati[1,*], Ferlia Pratiwi Riski Aly[1], Imam Adi Wicaksono[2], Ellin Febrina[2,*], Rika Kusumawati[3], Raden Roro Maryana Ulfah[3], Aiyi Asnawi[3], Barolym Tri Pamungkas[4]

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Bandung, West Java, Indonesia; [2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University, Bandung, West Java, Indonesia; [3]Department of Pharmacochemistry, Faculty of Pharmacy, Bhakti Kencana University, Bandung, West Java, Indonesia; [4]Pharmacist Professional Program, Faculty of Pharmacy, Mulawarman University, Samarinda, East Kalimantan, Indonesia

*These authors contributed equally to this work

Correspondence: Tina Rostinawati, Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Bandung, West Java, Indonesia, Email t.rostinawati@unpad.ac.id

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Background: Urinary tract infections (UTIs) caused by *Escherichia coli* (*E. coli*) are becoming increasingly difficult to treat due to rising antibiotic resistance, primarily driven by the production of β-lactamase enzymes such as TEM β-lactamase. Variations in the *TEM* gene can lead to reduced antibiotic efficacy; however, the specific effects of the resulting TEM protein variants on drug binding and enzyme stability remain largely underexplored.

Purpose: In this study, we analyzed a single *E. coli* strain isolated from a UTI patient to characterize the interactions between its TEM β-lactamase and various antibiotics through molecular docking and dynamics simulations, aiming to better understand factors influencing antibiotic resistance and guide future therapeutic strategies.

Methods: The *TEM* β-lactamase gene from clinical isolates in the laboratory collection was amplified, sequenced, and translated into a protein model based on PDB ID 1JWV. This model was then used to analyze interactions with various antibiotics through molecular docking and molecular dynamics simulations. The computational findings were subsequently correlated with published in vitro susceptibility data.

Results: Molecular docking analysis revealed that first-generation cephalosporins, such as cefazolin and cephalothin, exhibited strong binding affinities to TEM-1 β-lactamase ($\Delta G \approx -8$ kcal/mol) yet were resistant in vitro testing, indicating susceptibility to enzymatic hydrolysis. In contrast, second to fourth-generation cephalosporins (eg, cefuroxime, cefotaxime, cefepime) maintained similar binding energies but demonstrated sensitivity, suggesting enhanced structural resistance to β-lactamase. Aminoglycosides and fluoroquinolones showed varying binding affinities but retained in vitro activity, likely due to their distinct mechanisms targeting ribosomes or DNA gyrase, unaffected by TEM-1. Carbapenems, with lower binding energies, remained effective, consistent with their known β-lactamase resistance. These findings highlight the complexity of resistance, where binding energy alone does not determine antibiotic efficacy.

Conclusion: Molecular docking and dynamics simulations revealed that later-generation cephalosporins, carbapenems and aminoglycosides exhibit stable binding and structural resilience against TEM-1 β-lactamase. Despite strong docking and stable interactions observed for first-generation cephalosporins, their clinical inefficacy is due to enzymatic hydrolysis. These findings emphasize that integrating computational binding and stability data with structural and clinical insights is crucial for predicting effective antibiotics against TEM-1-mediated resistance.

Keywords: TEM β-lactamase, *E. coli*, drug resistance, molecular docking, molecular dynamics

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, with *E. coli* being the predominant pathogen responsible for these infections.[1] In recent years, the increase in antibiotic resistance has posed

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significant challenges in the treatment of UTIs, making it difficult to effectively manage these infections.[2] One of the key contributors to this resistance is the TEM β-lactamase enzyme, which belongs to a family of enzymes that degrade β-lactam antibiotics, such as penicillins and cephalosporins.[3] These enzymes allow bacteria to survive despite the presence of these antibiotics, making them particularly problematic in clinical settings. TEM β-lactamase, in particular, has been widely studied due to its prevalence in *E. coli* strains and its role in the development of resistance to common antibiotics.

Several β-lactamase TEM are part of the Extended-Spectrum β-Lactamases (ESBL) group, with its derivatives consisting of TEM-1, TEM-2, and TEM-3. The number of TEM-type β-lactamase has now exceeded 100. TEM-1 β-lactamase is the most commonly found and more than 90% of ampicillin-resistant *E. coli* are resistant due to the presence of the *TEM-1* gene. TEM-1 was first identified in *E. coli* in 1966 from a patient named Temoneira, which is how the enzyme got its name (European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control (ECDC). The TEM type is the largest group of ESBL enzymes and has spread widely. TEM-1 is capable of hydrolyzing penicillin and first-generation cephalosporins.[4]

The presence of TEM-1 β-lactamase among bacterial populations is widespread globally. Research has consistently indicated that *TEM-1* ranks among the most prevalent plasmid-mediated β-lactamase found in Gram-negative organisms. Its distribution is notably high, with some studies estimating that up to 25% of individuals may harbor this gene within their normal gut microbiota. TEM-1 is widely recognized for its role in mediating resistance to penicillins and first-generation cephalosporins. To date, more than 170 distinct variants of the TEM-1 enzyme have been reported across different regions of the world.[5]

The emergence of variations in the *TEM* β-lactamase gene in *E. coli* from UTI patients has raised concerns about the increased resistance to β-lactam antibiotics. Mutations in this gene can lead to the production of enzymes with altered substrate specificity or enhanced activity, further complicating the treatment of infections.[6] These gene variations can result in the failure of standard antibiotic therapies, necessitating the development of novel strategies for drug design and resistance management. Therefore, understanding the structural characteristics and molecular mechanisms underlying these gene variations is critical to addressing the growing issue of antibiotic resistance in *E. coli* and other pathogenic bacteria.

Despite extensive research on antibiotic resistance and β-lactamase enzymes, there remains a significant gap in our understanding of how specific variations in the *TEM* β-lactamase gene affect the structure and function of its encoded enzyme. While many studies have focused on inhibiting the β-lactamase enzyme using specific inhibitors, the detailed molecular mechanisms by which different gene variants of *TEM* β-lactamase influence enzyme-antibiotic interactions in *E. coli* have not been fully elucidated.[7] Moreover, research on how these genetic variations alter the enzyme's interaction with other molecules, such as inhibitors, in clinical settings is limited. Therefore, more comprehensive studies are needed that consider the dynamic effects of gene variations on enzyme behavior, particularly through advanced computational methods like molecular docking and molecular dynamics simulations.

UTIs caused by *E. coli* are commonly treated with beta-lactam antibiotics;[8] however, the rise of beta-lactamase-producing strains has significantly reduced their effectiveness. In response, non-beta-lactam antibiotics, such as aminoglycosides (eg, gentamicin) and fluoroquinolones (eg, ciprofloxacin), have been used as alternative treatments due to their different mechanisms of action, which can remain effective even in the presence of beta-lactam resistance.[9] Despite this, emerging evidence has shown increasing co-resistance to both beta-lactam and non-beta-lactam antibiotic classes, posing a serious threat to current treatment strategies. This growing resistance highlights the urgent need to better understand antibiotic interactions with resistance-related enzymes, such as TEM-1 β-lactamase, and to explore potential inhibitors that can restore antimicrobial efficacy.

In silico screening methods, such as molecular docking and molecular dynamics, have become essential tools in drug discovery and development.[10–12] Molecular docking allows researchers to predict how small molecules, such as ligands, interact with larger macromolecules, such as proteins, by simulating the binding process.[13,14] This method helps identify key binding sites and calculate the binding energy, providing an initial understanding of the potential of the compound as a therapeutic agent. In addition, molecular dynamic offers a more dynamic view by simulating the movements of atoms and molecules over time, giving insights into the stability and flexibility of the ligand-receptor complexes.[13,15] These complementary techniques offer a powerful approach to predict the interactions of natural compounds with their target proteins.

This study aimed to address a gap in existing research by analyzing variations in the *TEM* β-lactamase gene in *E. coli* isolated from a UTI patient, and by using molecular docking and molecular dynamics simulations to predict how the encoded TEM β-lactamase protein interacts with various antibiotics. The *TEM* β-lactamase gene analyzed in this study

was obtained from a bacterial isolate collected from a patient at the Ibrahim Adjie Community Health Center in Bandung, Indonesia. This isolate exhibited resistance to penicillin, penicillin-sulbactam, and first-generation cephalosporins.[16]

This study will provide valuable insights into the complex molecular interactions arising from the *TEM* β-lactamase gene of *E. coli* isolated from UTI patient and their impact on commonly used antibiotics. By employing molecular docking and molecular dynamics simulations, the research aims to predict gene influence the structure and function of the encoded TEM β-lactamase protein, affecting drug binding and stability. Ultimately, this understanding will contribute to the development of more effective therapeutic strategies. Furthermore, the study has the potential to uncover new drug candidates or optimize existing treatments, with significant implications for managing antibiotic resistance and advancing knowledge of bacterial-antibiotic interactions.

Materials and Methods

Bacterial Isolate

The bacterium used in this study was a laboratory collection strain from the Department of Microbiology and Biotechnology, Faculty of Pharmacy, Universitas Padjadjaran. It was originally isolated from a patient at the Ibrahim Adjie Public Health Center (Puskesmas) in Bandung City, Indonesia, and had undergone resistance testing against several antibiotics.[16] The bacterial isolate used in this study was obtained from a patient sample collected as part of routine clinical procedures, following the acquisition of written informed consent from the participant. The use of this clinical sample was approved by the Ethics Committee of Padjadjaran University (ethical approval number: 51/UN6.KEP/EC/2018), and the study was conducted in accordance with the ethical guidelines for human subject research and the principles outlined in the Declaration of Helsinki. The isolated bacterium has been tested for its sensitivity to various antibiotics.[16] These antibiotics, along with amikacin and ciprofloxacin, were also used in the molecular docking and dynamic simulation studies.

Identification of a *TEM* β-Lactamase-Encoding Gene

Isolation of Bacterial Chromosomal DNA

The isolation of bacterial chromosomal DNA was carried out using the PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. Bacterial cells were resuspended in 180 µL of PureLink™ Genomic Digestion Buffer and 20 µL of Proteinase K, then incubated at 55°C for 30 min. After adding RNase A Solution, the mixture was incubated for 2 min, followed by the addition of 200 µL of PureLink™ Genomic Lysis/Binding Solution and 200 µL of ethanol. The lysate was centrifuged, washed with PureLink™ Genomic Wash Buffers I and II, and then DNA was eluted using 200 µL of PureLink™ Genomic Elution Buffer. The isolated chromosomal DNA was then subsequently used as a template for amplifying the *TEM*-encoding gene.

Amplification of the *TEM*-Encoding Gene

PCR consists of three stages: denaturation, annealing, and extension. To perform PCR, 2 µL of DNA was used in a 50 µL mixture containing 1x PCR buffer, 200 µM deoxynucleotide triphosphates, 0.4 pM/µL primers, and 1 U Taq polymerase. Amplification was performed with an initial denaturation at 94°C for 10 min, followed by 30 cycles of 94°C for 40s, 60°C for 40s, and 72°C for 1 min, with a final extension at 72°C for 7 min. The primers used were: MultiTSO-T_for 5'-CATTTCCGTGTCGCCCTTATTC-3' and MultiTSO-T_rev 5'CGTTCATCCATAGTTGCCTGAC-3'.[17] DNA amplification was performed using a Biometra TProfessional thermal cycler (Analytik Jena, Jena, Germany). The amplicons were visualized using agarose gel electrophoresis, and their nucleotide sequences were analyzed at Macrogen, South Korea, using the ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol.

Analysis of Sequencing Results

The sequencing results were aligned with reference sequences available in public databases using the Basic Local Alignment Search Tool (BLAST). Sequence alignment was then performed between the obtained sequence and the reference *TEM*-encoding gene to identify variations or mutations.

Molecular Docking and Dynamics Simulations

Ligand Preparation

The ligand preparation process began with the retrieval of antibiotic chemical structures from the PubChem database in **.sdf* format, which includes essential 3D structural data for accurate molecular docking and dynamics simulations. These structures were then imported into Avogadro for geometry optimization using the MMFF94 force field, ensuring the ligands adopted their most stable conformations. The optimized molecules were subsequently converted to **.pdb* format, making them suitable for further computational studies, including docking and molecular dynamics analyses to assess their interactions with the TEM β-lactamase enzyme in *E. coli*.

These antibiotics—amikacin, ampicillin, nalidixic acid, cefepime, cefotaxime, ceftazidime, cefuroxime, cephalothin, gentamicin, levofloxacin, meropenem, ofloxacin, cefazolin, sulbactam, tobramycin, imipenem, and ciprofloxacin—were used in molecular docking and dynamics simulations to evaluate their potential interactions with target proteins. They represent various clinically important antimicrobial classes. The aminoglycosides included amikacin, gentamicin, and tobramycin. From the beta-lactam group, ampicillin was included as a representative penicillin, while sulbactam was tested independently and not as part of a combination therapy. The cephalosporins tested encompassed multiple generations: cefazolin and cephalothin (first generation), cefuroxime (second generation), cefotaxime and ceftazidime (third generation), and cefepime (fourth generation). The carbapenems, i.e., meropenem and imipenem were also included due to their broad-spectrum activity. From the fluoroquinolone class, ciprofloxacin, levofloxacin, and ofloxacin were tested. In addition, nalidixic acid, a first-generation quinolone, was included in the analysis. The bacterial isolate used in this study was tested for their sensitivity to the listed antibiotics, except for amikacin, sulbactam (as a single agent), and ciprofloxacin.[16]

Macromolecule Preparation

The macromolecule preparation began with selecting PDB ID 1JWV, derived from sequencing UTI patient sample via PCR. This structure showed 100% sequence identity with the target enzyme, making it highly reliable for structural modeling and further analysis. PDB ID 1JWV represents the mutated form of the TEM-1 beta-lactamase enzyme bound to its native ligand, CB4 (Pinacol[[2-amino-alpha-(1-carboxy-1-methylethoxyimino)-4-thiazoleacetyl]amino] methaneboronate).[18] To prepare the structure for docking and molecular dynamics simulations, all water molecules, heteroatoms, and any non-essential protein chains were carefully removed. The protein was then checked for missing atoms or residues, and any gaps were repaired to ensure structural integrity. Finally, the cleaned and optimized macromolecule was saved in PDB format, ready to be used as the receptor in subsequent computational experiments aimed at understanding antibiotic resistance mechanisms in UTIs.[19]

Molecular Docking Procedure

Molecular docking was performed using AutoDock 4.2 to investigate the binding interactions of antibiotics with TEM-1 beta-lactamase (PDB ID 1JWV). Since the native ligand of 1JWV, CB4 (pinacol[[2-amino-alpha-(1-carboxy-1-methylethoxyimino)-4-thiazoleacetyl]amino]methaneboronate), contains a boron atom, which is not supported by AutoDock 4.2's parameter set, a blind docking approach was necessary. To overcome this limitation, the native ligand of the related, non-mutated structure PDB ID 1AXB,[20] [[*N*-(benzyloxycarbonyl) amino]methyl]phosphate (FOS), was used to identify the binding site on 1JWV. The PDB ID 1AXB structure closely resembles 1JWV but lacks the mutation and contains a ligand compatible with AutoDock.

Blind docking was carried out by defining a grid box that encompassed the entire TEM-1 beta-lactamase protein surface to allow unbiased exploration of potential binding sites.[21] Following this, focused docking simulations centered on the identified binding site ensured accurate ligand placement. The docking simulations were run on a high-performance computing system equipped with a Xeon E5 2690 V3 CPU and an RTX 3060 Ti GPU, enabling efficient and reliable prediction of binding modes and interaction energies between the antibiotics and the enzyme.

The docking protocol was validated by comparing the binding mode of FOS docked back into PDB ID 1AXB. The procedure was considered valid because the predicted binding interactions closely matched the known crystal interactions, especially involving key amino acids Ser70, Ser130, and Glu166, which are critical residues in the enzyme's active

site. This validation confirmed that the blind docking approach could reliably identify the binding site and produce meaningful docking results for further analysis with the mutated enzyme 1JWV.

Molecular Dynamics Simulations

Molecular dynamics simulations were conducted using GROMACS 2023 over a 100 ns timescale on the TEM β-lactamase enzyme complexed with the FOS and the two top-scoring docked ligands. Simulations were based on the best docking poses and followed standard NPT ensemble protocols. All simulations were executed on a high-performance computing system equipped with an Intel Xeon E5-2690 v3 CPU, 32 GB RAM, and an NVIDIA RTX 3060 Ti GPU. System stability and ligand interactions were evaluated using Root-Mean-Square Deviation (RMSD), Root-Mean-Square Fluctuation (RMSF), and energy profile analyses to assess binding dynamics and potential resistance mechanisms.

Visualising Data

Data visualization focused on analyzing ligand interactions with the TEM β-lactamase enzyme using results from molecular docking and dynamics simulations. Docking metrics, including binding free energy (ΔG), hydrogen bonding, and hydrophobic interactions, were summarized in a comparative table Key ligand–enzyme interactions were visualized in 3D using Discovery Studio Visualizer. Molecular dynamics results, RMSD, RMSF, and energy components, were analyzed and plotted using Python to evaluate the stability and flexibility of the complexes over the 100 ns simulation period. These visualizations provided insights into ligand binding strength and dynamic behaviour.[21]

Results

This study focused on a patient-derived isolate producing TEM-1 beta-lactamase because TEM-1 remains one of the most commonly encountered beta-lactamase enzymes in clinical infections, particularly in urinary tract infections (UTIs). Although TEM-1 has been extensively studied, the continued rise of antibiotic resistance in clinical settings, especially among *E. coli* isolates, necessitates on going investigation of its interactions with both beta-lactam and non-beta-lactam antibiotics. By analyzing a current, clinically relevant isolate, this study aimed to provide updated insights into potential treatment options and explore whether certain non-beta-lactam antibiotics may still retain effectiveness against TEM-1 producers. This real-world relevance strengthens the translational value of the findings.

Result of *TEM* β-Lactamase Encoding Gene Identification

The result of bacterial chromosomal DNA isolation showed a DNA size of approximately 3000–6000 kb, consistent with the expected *E. coli* chromosome size of around 4.6 Mbp (Figure 1). PCR amplification of the *TEM* gene yielded a product of approximately 800 bp, which matches the predicted size based on the primers used (Figure 1). The nucleotide sequences obtained are presented in Table 1. A homology search using BLAST revealed 100% sequence similarity with the plasmid pTA13-1 from *E. coli* strain EA13 (Table 2). Further sequence analysis confirmed 100% similarity with the *TEM*-1 gene variant.

Result of Molecular Docking and Dynamics Simulations

In this study, molecular docking was performed using AutoDock 4.2 to investigate the interactions between various antibiotics and the TEM β-lactamase enzyme, derived from gene sequence of *E. coli* strain isolated from UTI Patient.[20] The docking grid was defined using the gridbox, centered around the native ligand CB4 (pinacol[[2-amino-alpha-(1-carboxy-1-methylethoxyimino)-4-thiazoleacetyl]amino]methaneboronate) from PDB ID 1JWV.[18]

Docking Procedure Validation

The binding energy and inhibition constant of various ligands, including antibiotics commonly used to treat urinary tract infections (UTIs), were evaluated to assess their potential interactions with the TEM-1 β-lactamase enzyme in *E. coli*. The binding energy values (Table 3), denoted as ΔG (kcal/mol), indicate the strength of interaction between each ligand and the target protein—more negative values reflect stronger binding affinity.[22]

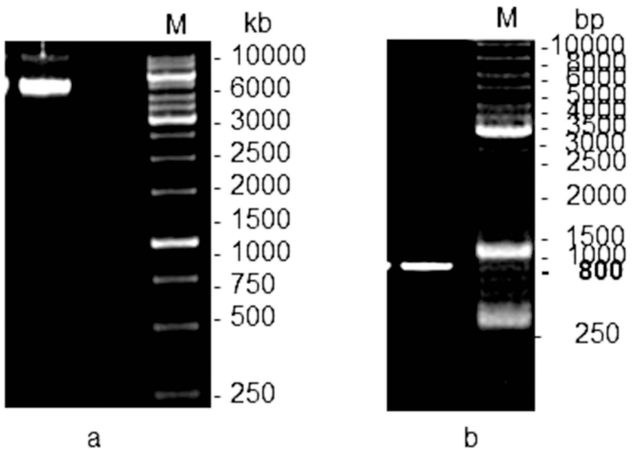

Figure 1 The electropherogram of bacterial chromosomal DNA isolation (**a**) and *TEM* gene amplification (**b**) results.

In this study, the validation of the molecular docking procedure targeting TEM-1 beta-lactamase (PDB ID: 1JWV) was carried out using the AutoDock 4.2 application through a blind docking approach. The need for blind docking arose because the native ligand of 1JWV, CB4 (pinacol[[2-amino-alpha-(1-carboxy-1-methylethoxyimino)-4-thiazoleacetyl]amino]methaneboronate), contains a boron atom, which is not supported by AutoDock 4.2 parameterization. To overcome this limitation, FOS ([[N-

Table 1 Sequencing results of *TEM* Gene

The Primers used for Sequencing	Sequencing Results
MultiTSO-T_for	5'-ATGCTGAAGATCAGT TGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATC CTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAA AGTTCTGCTATGTGGTGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGC AACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCA CCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATG CAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGA CAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGG GATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCAT ACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGT TGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAA TTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTC GGCCCTTCCGGCTGG-3'
MultiTSO-T_rev	5'-CAGTGCTGCAATGATACCGCGAGACCCACG CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCG AGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAAT TGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAA CGTTGTTGCCATTGCTGCAGGCATCGTGGTGTCACGCTCGTCGTTTGGTA TGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCC CCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGT CAGAAGTAAGTTGGCAGCAGTGTTATCACTCATGGTTATGGCAGCACTGC ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGT GAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTG CTCTTGCCCGGCGTCAACACGGGATAATACCGCACCACATAGCAGAACTT TAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGG ATCTTACCGCTGTTGAGATCCAGTTCGATG-3'

Table 2 The Sequence Homology Search Result Using BLAST

Sequence	Gene	% Identities	Gaps	Expect	ID Sequence
Sequencing result using the MultiTSO-T_for primer	*Escherichia coli* strain EA13 plasmid pTA13-1	100	0/580 (0%)	0.0	CP069713.1
Sequencing result using the MultiTSO-T_rev primer		100	0/610 (0%)	0.0	

Table 3 The Docking Data of Ligands Against the TEM β-Lactamase Enzyme (PDB ID 1JWV)

Antibiotics	ID	Binding Affinity, ΔG, (kcal/mol)	Inhibition Constant (μM)	Hydrogen Bond (HB)	Hydrophobic Interaction (HI)	In Vitro Result (P1)*	Correlation & Interpretation
[[*N*-(benzyloxycarbonyl) amino]methyl] phosphate	FOS*	−5.52	89.23	5 HB with Ala2387, Asn132, Ser70, and Ser130	1 HI with Ala238	NP*	-
amikacin	S01	−6.65	13.24	11 HB with Asn132, Asn170, Ala237, Glu166, Glu240, Ser70, and Ser130	0	NP*	-
ampicillin	S02	−7.26	4.76	8 HB with Ala237, Asn132, Lys234, Ser70, Ser235, and Ser130	1 HI with Pro167	R	Easily hydrolyzed by TEM-1 → matches resistance
nalidixic acid	S03	−5.94	44.28	6 HB with Ala237, Arg244, Ser70, Ser130, and Ser235	5 HI with Ala237 and Tyr105	R	Weak binding; resistance likely due to gyrA/parC mutations
cefepime (Cephalosporin Gen IV)	S04	−8.04	1.29	10 HB with Ala237, Ala238, Asn170, Asn132, Glu240, Lys234, Ser130, and Ser235	2 HI with Ala237 and Ala238	S	Broad spectrum, stable against TEM-1 → effective
cefotaxime (Cephalosporin Gen III)	S05	−7.94	1.51	9 HB with Ala237, Arg244, Asn170, Glu240, Lys234, Ser130, and Ser235	3 HI with Ala237, Ala238, and Tyr105	S	Stable against TEM-1 → effective despite high binding
ceftazidime (Cephalosporin Gen III)	S06	−7.22	5.09	10 HB with Ala237, Ala238, Asn170, Asn132, Glu240, Ser70, Ser130, and Ser235	4 HI with Ala237, Ala238, and Met272	S	Anti-Pseudomonas, resistant to β-lactamase → consistent with sensitivity
cefuroxime (Cephalosporin Gen II)	S07	−8.11	1.13	9 HB with Ala237, Arg244, Asn132, Lys234, Ser130, Ser235, and Val216	2 HI with Pro167 and Tyr105	S	Similar ΔG as cefazolin but more stable → remains effective

(*Continued*)

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Table 3 (Continued).

Antibiotics	ID	Binding Affinity, ΔG, (kcal/mol)	Inhibition Constant (μM)	Hydrogen Bond (HB)	Hydrophobic Interaction (HI)	In Vitro Result (P1)*	Correlation & Interpretation
cephalothin (Cephalosporin Gen II)	S08	−7.88	1.67	10 HB with Ala237, Arg244, Asn132, Lys234, Ser70, Ser130, and Ser235	2 HI with Pro167 and Tyr105	R*	Similar to cefazolin → vulnerable to β-lactamase
gentamicin (Aminoglycoside)	S09	−9.04	0.23773	7 HB with Ala237, Asn132, Asn170, Glu240, and Ser70	0	S	High binding to TEM-1 but irrelevant → different target
levofloxacin (Fluoroquinolone Gen III)	S10	−7.07	6.62	7 HB with Arg244, Asn132, Lys73, Ser70, Ser235, and Val216	2 HI with Ala237	S	Ribosome/DNA gyrase target → remains effective
meropenem (Carbapenem)	S11	−7.14	5.85	6 HB with Arg244, Asn132, Lys234, Ser235, and Val216	1 HI with Tyr105	S	Moderate binding but still resistant to TEM-1 degradation → effective
ofloxacin (Fluoroquinolone Gen II)	S12	−7.05	6.79	6 HB with Arg244, Asn170, Ser70, Ser130, Ser235, and Val216	3 HI with Ala237 and Tyr105	S	Same as levofloxacin
cefazolin (Cephalosporin Gen I)	S13	−8.44	0.64564	7 HB with Ala237, Arg244, Asn132, Lys234, Ser130, and Ser235	4 HI with Pro167, Pro219, and Val216	R	High binding → easily hydrolyzed by TEM-1 → consistent with resistance
sulbactam	S14	−5.89	48.26	6 HB with Ala237, Arg244, Lys234, Ser130, and Ser235	0	NP*	-
tobramycin (Aminoglycoside)	S15	−7.43	3.57	9 HB with Ala238, Arg275, Glu104, and Glu240	0	S	Same as gentamicin
imipenem	S16	−6.43	19.37	9 HB with Ala237, Arg244, Asn132, Ser770, Ser130, Ser235, and Val216	0	S	Weak binding → not easily hydrolyzed → consistent with sensitivity
ciprofloxacin (Fluoroquinolone Gen II)	S17	−7.38	3.91	7 HB with Ser70, Ser130, Lys234, Ser235, Ala247, and Glu104	6 HI with Tyr105 and Val216	I	Not a β-lactam; docking to TEM-1 not biologically relevant

Notes: Δ represents the absolute binding energy (ΔG_binding) of each ligand–enzyme complex as calculated from molecular docking. More negative values indicate stronger predicted binding affinities. * The bacterial sensitivity data to several antibiotics were obtained from previously published data using the P1 isolate.[16]
Abbreviations: NP, Not performed; R, Resistant; S, Sensitive; I, Intermediate.

(benzyloxycarbonyl) amino]methyl]phosphate), the native ligand of PDB ID 1AXB, an unmutated form of 1JWV, was used as a substitute ligand. A grid box of size 48 × 40 × 40 with a grid spacing of 0.375 Å was applied, centered at coordinates X: 14.681, Y: 10.011, and Z: 37.474 to cover the entire binding region during the blind docking.

The main objective of the docking validation was to ensure that FOS, when docked to the structure of 1JWV, could reproduce the same binding mode observed in the native interaction of FOS with 1AXB. This validation approach focused on the ability of the docking protocol to recover key molecular interactions with the critical active site residues Ser70, Ser130, and Glu166, residues known to be essential for the function of TEM-1 beta-lactamase.[20] Since 1JWV is a mutant version of 1AXB, ensuring the conservation of these interactions was crucial for establishing the reliability of the docking setup.

The results of the blind docking demonstrated that FOS could indeed bind in a mode consistent with the native complex in PDB ID 1AXB (Figure 2a). The docking produced a binding affinity of −5.52 kcal/mol and an inhibition constant (Ki) of 89.23 μM, indicating a moderate yet biologically relevant interaction. These findings supported the conclusion that the docking procedure using AutoDock 4.2 was successfully validated and could be used for further docking studies on other antibiotic candidates targeting the TEM-1 beta-lactamase binding site.

Binding Energy

After validating the docking procedure using blind docking with FOS on the TEM-1 beta-lactamase structure (PDB ID: 1JWV), the method was applied to dock a range of antibiotics to evaluate their binding affinities and inhibition constants (Table 3). Binding affinity (ΔG) represents the strength of the interaction between the antibiotic and the target protein,[23] while the inhibition constant (Ki) indicates the concentration needed to inhibit the enzyme's activity by half.[10,24]

These two parameters together provide insight into how effectively each antibiotic could potentially inhibit TEM-1 beta-lactamase, a common resistance factor against β-lactam antibiotics.

Among the antibiotics tested, gentamicin (S09) showed the strongest interaction with TEM-1, with a binding affinity of −9.04 kcal/mol and a remarkably low inhibition constant of 0.24 μM. This suggests that gentamicin may have a high binding efficiency and inhibitory potential toward the enzyme, despite not being a classical β-lactam antibiotic. Similarly, cefazolin (S13), cefuroxime (S07), and cefepime (S04) also demonstrated strong binding, with ΔG values below −8.0 kcal/mol and inhibition constants under 2 μM, highlighting their favorable interactions with the active site of TEM-1 beta-lactamase.

On the other hand, antibiotics such as FOS (used here as a docking validation control), sulbactam (S14), and nalidixic acid (S03) showed relatively weaker interactions, with binding affinities higher than −6.0 kcal/mol and Ki values above 40 μM. These results suggest a lower inhibitory potential, possibly due to weaker or less specific binding to the enzyme's active site. It is worth noting that FOS was not intended as a therapeutic antibiotic in this context but rather served as a reference point for docking accuracy, and its lower binding affinity was consistent with expectations.

Overall, the docking results highlighted several antibiotics, particularly cephalosporins and carbapenems, that exhibited strong binding characteristics toward TEM-1 beta-lactamase. This could indicate their potential as effective inhibitors, or at least their susceptibility to enzymatic degradation by TEM-1, which would be critical to consider in resistance mechanisms. These findings help to identify promising candidates for further experimental validation and structure-based drug design efforts to overcome β-lactam resistance.

Binding Mode

In the molecular docking analysis of TEM-1 β-lactamase using AutoDock 4.2, the interactions of various ligands with key amino acid residues were examined, focussing on hydrogen bonds (HB) and hydrophobic interactions (HI) (Table 3 and Figure 2). Hydrogen bonds are critical in stabilizing ligand–protein complexes, while hydrophobic interactions contribute to the overall binding affinity.[25] The results revealed that different antibiotics exhibited varying numbers and patterns of interactions, which helped in understanding their potential inhibitory effects. FOS, used as the reference ligand, formed 5 hydrogen bonds involving key residues such as Ser70, Ser130, and Asn132, along with 1 hydrophobic interaction with Ala238, validating the docking method's accuracy in identifying critical contacts.

When comparing the antibiotics, amikacin (S01) stood out by forming the highest number of hydrogen bonds (11), including interactions with all three critical residues: Ser70, Ser130, and Glu166. Similarly, ceftazidime (S06), cephalothin (S08), and cefepime (S04) also interacted with both Ser70 and Ser130, along with other stabilizing residues such as Ala237, Asn132, and Glu240. These interactions suggest a strong potential for these antibiotics to bind effectively to

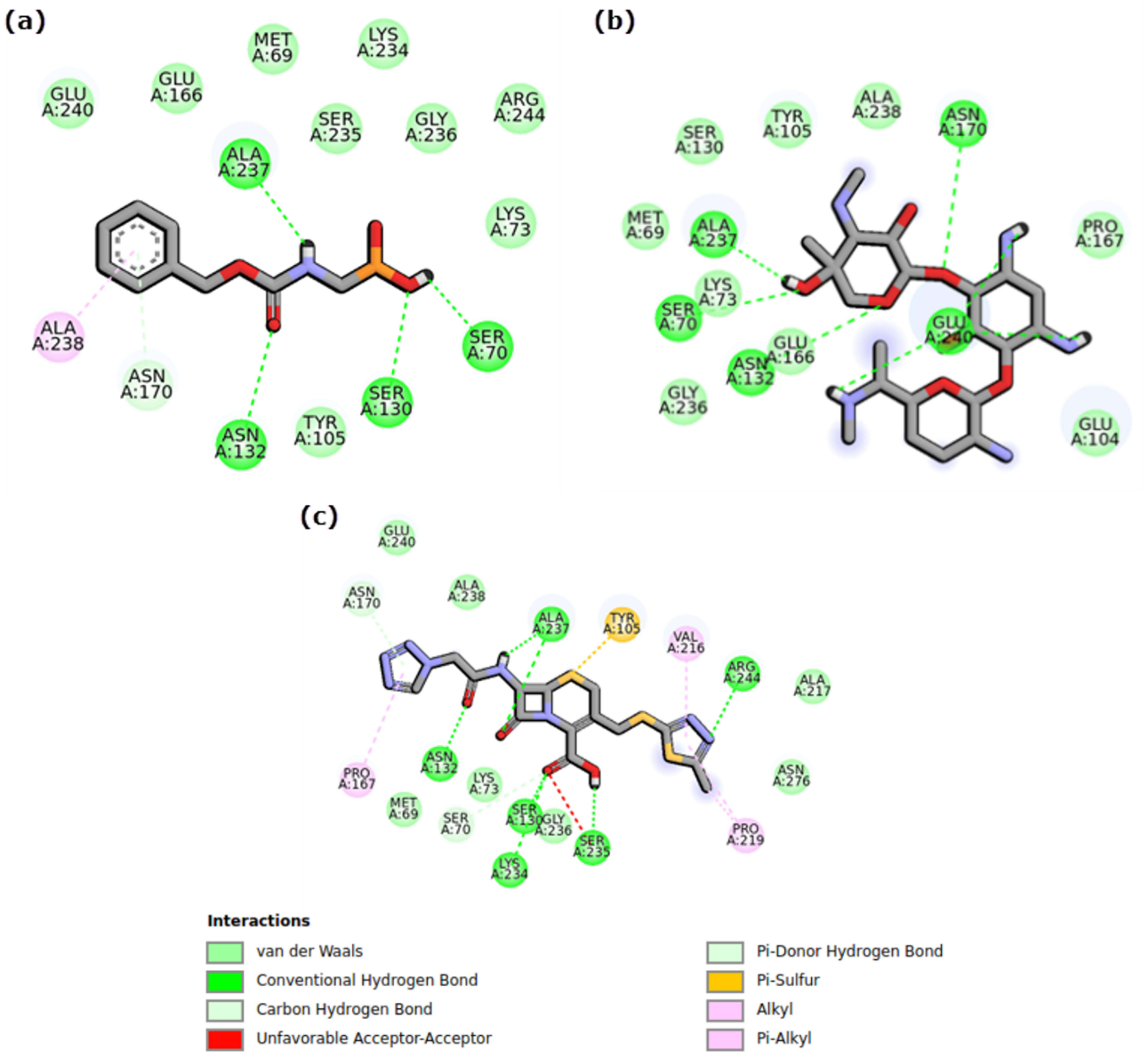

Figure 2 Typical binding modes of the FOS (**a**), S09 (**b**), and S13 (**c**) ligands to the TEM-1 β-lactamase enzyme (PDB ID: 1JWV). Each sub-figure illustrates the interaction of the ligand within the enzyme's active site.

the active site of TEM-1, possibly resisting enzymatic degradation or inhibiting the enzyme's function. On the other hand, gentamicin (S09), despite its highest binding affinity in terms of ΔG, did not show hydrophobic interactions and had fewer hydrogen bonds, although it still included Ser70 and Glu166 in its contact map.

Interestingly, not all antibiotics interacted with the full set of important residues. For instance, sulbactam (S14) formed hydrogen bonds with Ser130 but lacked interaction with Ser70 and Glu166. This may explain its weaker binding affinity compared to others. Similarly, meropenem (S11) and ofloxacin (S12) both interacted with Ser130 but missed Glu166, which could influence their binding stability and efficacy. These variations in residue interaction profiles emphasize that the presence or absence of hydrogen bonding with key residues like Ser70, Ser130, and Glu166 can significantly impact the overall docking performance and predicted inhibitory potential.

Hydrophobic interactions were more prominent in some antibiotics, such as nalidixic acid (S03) and cefazolin (S13), which formed up to 5 and 4 hydrophobic contacts respectively. Although these interactions supported binding, the absence of strong hydrogen bonding with Glu166 or Ser70 in some of these ligands may reduce their inhibitory

efficiency. Overall, antibiotics that combined both strong hydrogen bonding, especially with Ser70, Ser130, and Glu166, and complementary hydrophobic interactions were more likely to show higher binding affinities. These findings help to distinguish which antibiotics may be more resilient against enzymatic hydrolysis by TEM-1 and inform the design of improved β-lactamase inhibitors.

RMSD

The root mean square deviation (RMSD) analysis of the molecular dynamics simulations over 100 nanoseconds (ns) provided valuable insights into the stability of antibiotic binding within the TEM-1 beta-lactamase (PDB ID: 1JWV) binding pocket. RMSD serves as a key indicator of how much a ligand shifts from its original docked position over time, with values greater than 2.0 Å typically suggesting potential detachment or instability in the binding pocket (Figure 3).[26] Three ligands were analyzed: FOS (reference ligand), gentamicin (S09), and cefazolin (S13).

FOS, the reference compound, showed relatively stable interactions during the first half of the simulation, with RMSD values remaining below 1.2 Å up to the 50 ns mark. However, starting at 60 ns, a sharp increase was observed, with RMSD spiking to 2.91 Å, indicating significant displacement. This instability continued through the remainder of the simulation, peaking at 3.65 Å at 85 ns. These findings suggest that FOS may not maintain a stable binding pose within the pocket of TEM-1 over time, possibly due to weaker or less favorable interactions compared to other antibiotics.

In contrast, gentamicin (S09) maintained a remarkably stable RMSD profile throughout the entire 100 ns simulation. The RMSD values consistently hovered between 0.40 and 0.52 Å, with no significant fluctuations or signs of ligand movement away from the binding site. This strong stability aligns with the earlier docking results that indicated high binding affinity and a favorable interaction profile, further supporting gentamicin's robust interaction with TEM-1 beta-lactamase despite being a non-beta-lactam antibiotic.

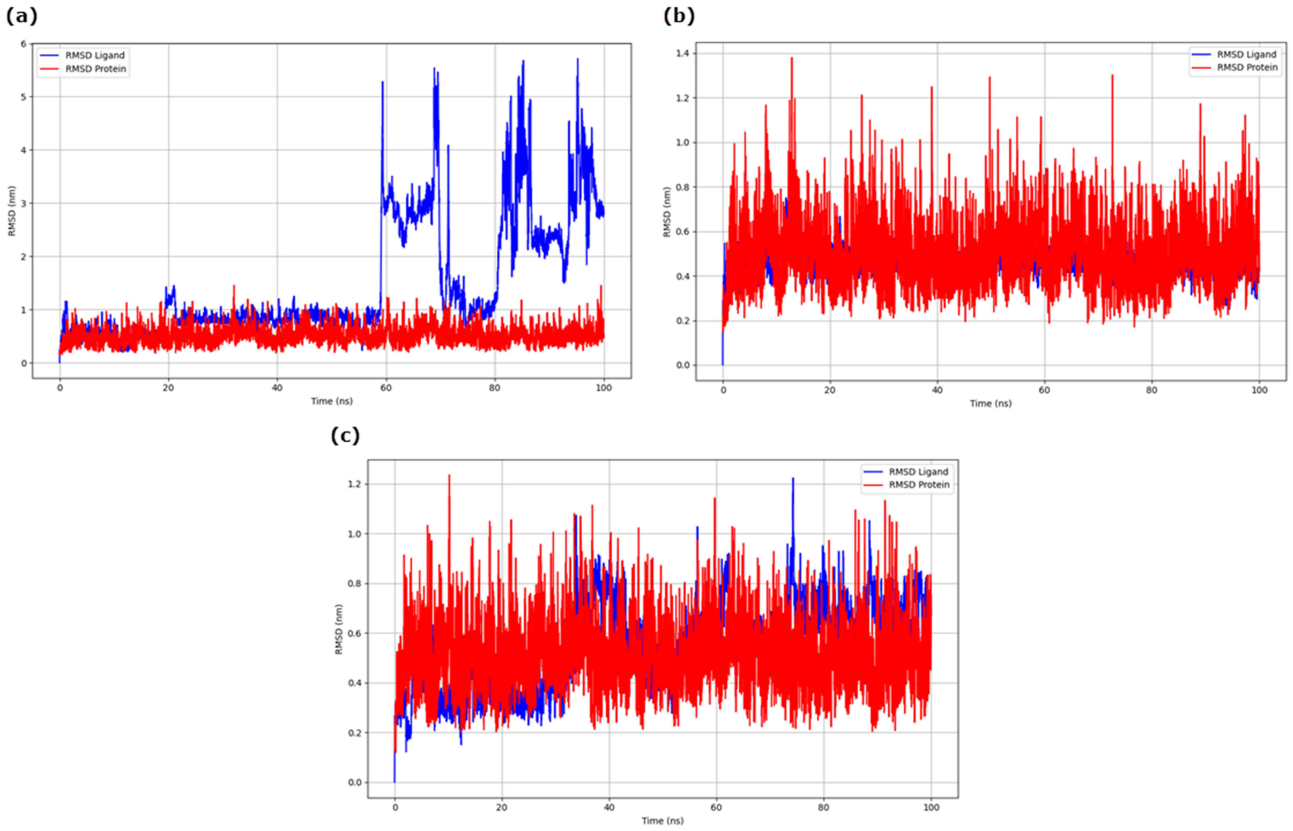

Figure 3 RMSD fluctuations of the FOS (**a**), S09 (**b**), and S13 (**c**) ligands in complex with the TEM-1 β-lactamase enzyme (PDB ID: 1JWV). Each sub-figure illustrates the structural stability of the ligand–enzyme complex over the simulation time.

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Cefazolin (S13) also demonstrated relatively stable binding behavior although with slightly more fluctuation than gentamicin. Its RMSD remained below 0.8 Å until 55 ns, after which some gradual increases were observed, reaching approximately 0.75 Å by the end of the simulation. However, these values remained well below the 2.0 Å threshold, suggesting that cefazolin maintained its binding pose within the pocket with minor adjustments. Compared to FOS, both S09 and S13 exhibited significantly more stable binding modes, highlighting their stronger interactions and potential for effective inhibition of TEM-1. The RMSD data reinforced the conclusion that binding stability, in addition to binding energy, is a critical factor in assessing ligand effectiveness.

RMSF

The Root Mean Square Fluctuation (RMSF) analysis over 100 ns of molecular dynamics simulation revealed notable differences in residue flexibility among the three antibiotics, FOS, gentamicin (S09), and cefazolin (S13), when bound to TEM-1 beta-lactamase (PDB ID: 1JWV).[27] RMSF measures the average deviation of a protein's residues from their mean positions, helping to identify regions of flexibility or instability. A particular focus was placed on critical active-site residues, especially Ser70, Ser130, and Glu166, as fluctuations above 2 Å in these areas may indicate unstable interactions or potential weakening of the binding pocket (Figure 4).

Throughout the simulation, all three antibiotics maintained low RMSF values at the key active-site residues. For Ser70, the RMSF values were 0.0407 (FOS), 0.0431 (S09), and 0.0553 (S13); for Ser130, the values were 0.0477 (FOS), 0.0417 (S09), and 0.0675 (S13); and for Glu166, the fluctuations were 0.0453 (FOS), 0.0406 (S09), and 0.0563 (S13). All values remained well below the 2 Å threshold, suggesting that the binding of these antibiotics did not induce significant instability in the core catalytic residues. This stability indicated the potential for effective and sustained interaction within the binding pocket, especially for gentamicin and cefazolin.

Beyond the active site, broader RMSF comparisons showed that FOS induced slightly lower fluctuations across the protein overall, maintaining consistent values even in flexible loop regions. In contrast, cefazolin exhibited higher RMSF values in multiple peripheral regions, particularly around residues 215–220 and 252–256, which showed fluctuations

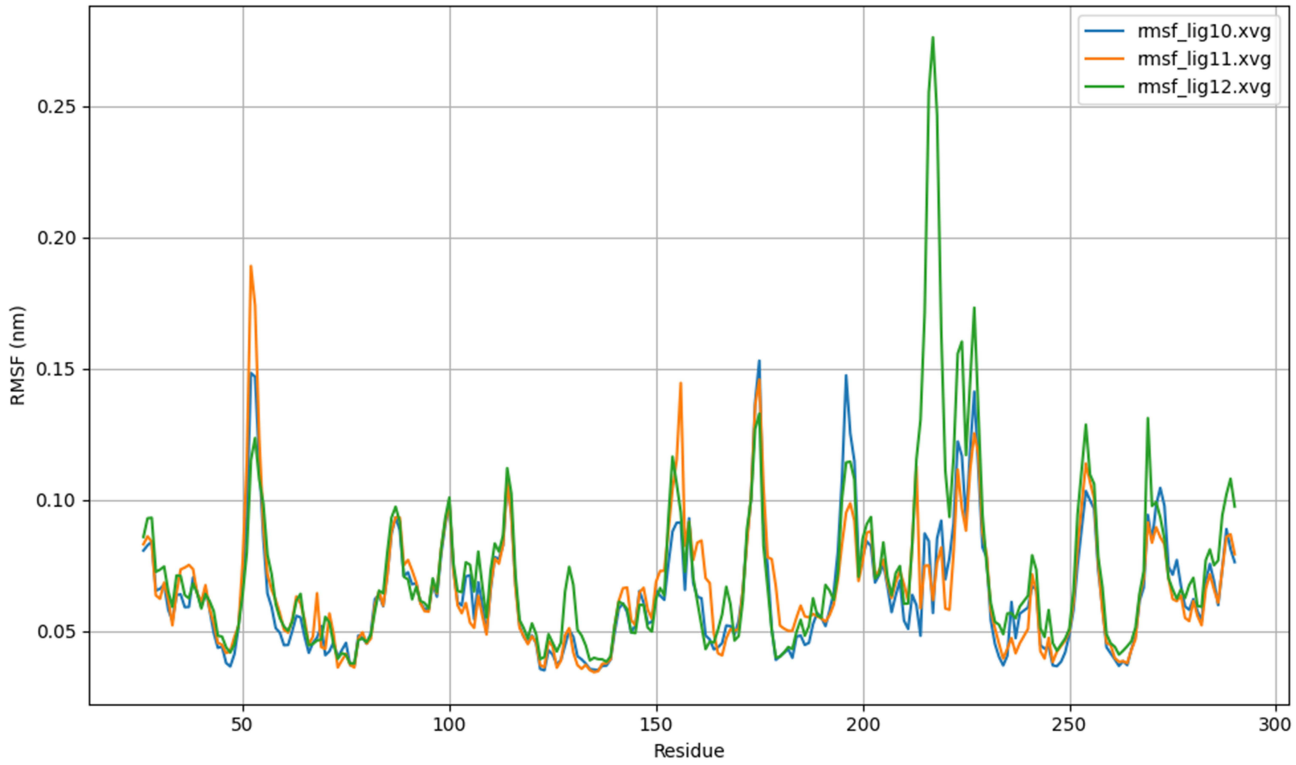

Figure 4 RMSF fluctuations of the FOS, S09, and S13 ligands in complex with the TEM-1 β-lactamase enzyme (PDB ID: 1JWV). FOS, S09, and S13 are represented in blue, orange, and green, respectively. Each sub-figure shows the residue flexibility profile of the enzyme when bound to the respective ligand.

approaching or exceeding 0.25 nm (~2.5 Å). This could suggest that while S13 remained stable at the core binding site, it may have introduced localized flexibility or subtle conformational shifts elsewhere in the protein structure.

Gentamicin, on the other hand, demonstrated both strong binding (as supported by its low RMSD and high binding affinity) and minimal fluctuation across the protein, including at both active and peripheral sites. This reinforced the hypothesis that gentamicin formed a highly stable complex with TEM-1 beta-lactamase, potentially contributing to its resistance-evasion capabilities. Overall, RMSF analysis confirmed that all three ligands preserved the structural integrity of the active site, but gentamicin stood out by maintaining overall protein rigidity, while cefazolin's influence extended more toward surface-level flexibility.

Energy Component

Analysis of the energy components associated with the TEM-1 B-Lactamase protein, encoded by *TEM-1* gene in *E. coli* isolate from urinary tract infection (UTI) patient revealed significant differences in molecular dynamics simulation results (Figure 5). The energy decomposition analysis provided deeper insights into the nature of the interactions between the antibiotics, FOS, gentamicin (S09), and cefazolin (S13), and the TEM-1 beta-lactamase (PDB ID: 1JWV) during the 100 ns molecular dynamics simulations using GROMACS. Each energy component reflected a specific contribution to the binding stability, allowing for a more refined interpretation of how and why certain ligands demonstrated stronger binding than others.

Gentamicin (S09) and cefazolin (S13) exhibited significantly more favorable van der Waals interactions (ΔVDWAALS: −35.54 and −34.9 kcal/mol, respectively) compared to FOS (−12.82 kcal/mol). These strong non-covalent contacts suggested deeper accommodation of both S09 and S13 into the binding pocket, likely due to their better complementarity in size and shape with the enzyme's active site. FOS, in contrast, showed weaker van der Waals interactions, indicating a less optimal fit or more superficial engagement with the binding cavity.

Electrostatic energy (ΔEEL) was also more favorable for S09 and S13, with values of −6.5 and −7.99 kcal/mol, respectively, as opposed to just −1.47 kcal/mol for FOS. This highlighted that both S09 and S13 formed more stable charged interactions or salt bridges with residues in the protein. However, these gains were partially offset by higher polar solvation penalties (ΔEGB), particularly for S09 (25.03 kcal/mol) and S13 (22.07 kcal/mol), as polar solvation tends to counteract strong electrostatics. FOS showed a much smaller ΔEGB (6.77 kcal/mol), consistent with its lower polar interaction potential.

When combining gas-phase interactions (ΔGGAS) and solvation energy (ΔGSOLV), the total binding free energies (ΔTOTAL) further emphasized the superiority of gentamicin (−21.8 kcal/mol) and cefazolin (−25.15 kcal/mol) over FOS (−9.49 kcal/mol). The more negative ΔTOTAL values for S09 and S13 demonstrated stronger overall binding stability,

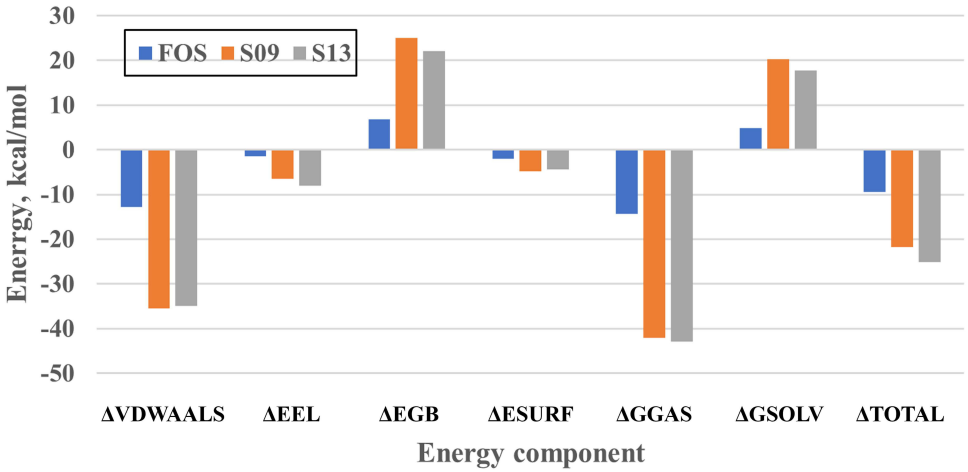

Figure 5 Energy components of FOS, S09, and S13 in interaction with the TEM-1 β-lactamase enzyme (PDB ID: 1JWV). Bold text indicates the compound names for emphasis. Δ denotes the difference in energy values between van der Waals and electrostatic interactions for each compound. Only energy components relevant to the binding interactions in this figure are shown.

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confirming earlier findings from RMSD, RMSF, and docking data. These results not only reinforced the greater affinity of S09 and S13 but also underscored the importance of balanced electrostatic and van der Waals interactions in determining the robustness of ligand binding to TEM-1 beta-lactamase.

Discussion

When the energies obtained from molecular docking and molecular dynamics for the TEM-1 B-Lactamase protein which is encoded by the *TEM-1* gene in E. coli isolate from urinary tract infection (UTI) patient were compared, a clear distinction emerged between the two methods in their energy profiles. The comparison between the binding energies obtained from molecular docking and molecular dynamics (MD) simulations for FOS, gentamicin (S09), and cefazolin (S13) to TEM-1 beta-lactamase (PDB ID: 1JWV) revealed important differences in how each method captured the ligand–protein interaction landscape. While docking provided a quick, static estimate of binding affinity based on optimal pose fitting, MD offered a dynamic and more comprehensive view by accounting for atomic fluctuations, solvent effects, and long-term stability.[28,29]

For all three antibiotics, the binding energies calculated from MD simulations were significantly more negative than those from docking. FOS showed an increase in binding energy from −5.52 kcal/mol (docking) to −9.49 kcal/mol (MD), indicating a moderate improvement in interaction stability once the system underwent equilibration and conformational flexibility was considered. This suggested that FOS, despite its relatively weak initial docking score, may still form a moderately stable complex under dynamic conditions.

In the case of gentamicin (S09), the shift was more pronounced, with the docking energy of −9.04 kcal/mol improving to −21.8 kcal/mol in MD. This large difference highlighted that gentamicin's interactions were significantly strengthened during simulation, likely due to its extensive hydrogen bonding and favorable accommodation in the active site, which may not have been fully captured in the rigid docking setup. The dynamic environment allowed for better optimization of both ligand orientation and residue interactions, contributing to stronger binding.

Cefazolin (S13) followed a similar trend, with its docking energy of −8.44 kcal/mol deepening to −25.15 kcal/mol during MD. This suggested that S13 maintained a highly stable and energetically favorable binding throughout the simulation.

The docking study revealed that certain non-beta-lactam antibiotics, such as gentamicin (an aminoglycoside) and ciprofloxacin (a fluoroquinolone), exhibited notable binding affinities to TEM-1 beta-lactamase, a protein typically associated with resistance to beta-lactam antibiotics. Gentamicin demonstrated the strongest binding affinity among all tested compounds, with a ΔG of −9.04 kcal/mol and a very low inhibition constant of 0.24 μM. This suggests a strong and potentially effective binding to the enzyme. Ciprofloxacin also showed a respectable binding affinity of −7.38 kcal/mol with a Ki of 3.91 μM, indicating a moderate but biologically meaningful interaction with TEM-1.

Despite these strong binding interactions, especially in the case of gentamicin, neither gentamicin nor ciprofloxacin are traditional targets of beta-lactamase enzymes. Their observed binding modes suggest an off-target or non-classical interaction with TEM-1. Gentamicin formed seven hydrogen bonds with residues including Ser70, a key catalytic residue, along with Asn132 and Glu240. Ciprofloxacin, similarly, engaged Ser70 and Ser130, along with other residues such as Glu104 and Ala247, and established multiple hydrophobic interactions with Tyr105 and Val216. These binding modes suggest that these non-beta-lactam antibiotics may still interact meaningfully with the beta-lactamase active site, possibly influencing resistance behavior indirectly.

The findings raise important implications in the context of co-resistance. There is growing evidence that bacteria may develop resistance mechanisms that affect both beta-lactam and non-beta-lactam antibiotic classes, possibly through overlapping or adjacent binding interactions with enzymes like TEM-1. The ability of gentamicin and ciprofloxacin to bind within the TEM-1 active site, despite not being direct substrates, could contribute to complex resistance phenotypes. This underlines the need to consider cross-class resistance mechanisms when designing antibiotic therapies or inhibitors and highlights the potential role of beta-lactamases in broader resistance beyond beta-lactams alone.

The differences in chemical structure between [[*N*-(benzyloxycarbonyl) amino]methyl]phosphate (FOS), Gentamicin (S09), and Cefazolin (S13) played a notable role in influencing their binding energies during both molecular docking and

molecular dynamics (MD) simulations with TEM-1 beta-lactamase (PDB ID 1JWV). Each compound's unique size, functional groups, and flexibility directly impacted how it behaved in the enzyme's binding pocket over time.

FOS, with its relatively simple and small structure, showed modest binding affinity during docking (−5.52 kcal/mol). However, its total energy improved significantly during MD simulations (−9.49 kcal/mol). This suggested that while its initial interaction was limited, FOS adapted its position more favorably within the binding pocket over time, possibly due to flexibility and reorientation of its phosphate and benzyloxycarbonyl groups during the dynamic environment.

Gentamicin (S09), a bulkier aminoglycoside with multiple hydroxyl and amino groups, displayed strong binding during docking (−9.04 kcal/mol), and this affinity deepened significantly during MD (−21.8 kcal/mol). Its rigid, multi-ringed structure likely allowed for more stable hydrogen bonding and electrostatic interactions, which were maintained and optimized throughout the simulation. The stability of these interactions reflected gentamicin's ability to fit tightly and stably into the binding pocket over time.

Cefazolin (S13), a β-lactam antibiotic, showed one of the strongest docking energies (−8.44 kcal/mol) and an even more pronounced stabilization during MD (−25.15 kcal/mol). The fused β-lactam and dihydrothiazine rings, along with functional side chains, contributed to a highly specific and complementary fit within the active site. Its ability to interact with both hydrophilic and hydrophobic regions likely enabled this consistent increase in binding energy, emphasizing its strong and stable inhibition of TEM-1 beta-lactamase during longer timeframes.

Overall, the structure of each antibiotic strongly influenced the observed energy changes. Simple molecules like FOS gained better positioning during simulation, while larger, more functionally diverse structures like gentamicin and cefazolin maintained or improved their interactions, highlighting the importance of structural complexity in long-term enzyme binding stability.

Our previous study about in vitro findings demonstrated that clinical *E. coli* isolate was resistant to penicillins, β-lactamase inhibitor combinations, first–fourth generation cephalosporins, and fluoroquinolones while remaining susceptible to aminoglycosides and carbapenems. This resistance pattern strongly suggests the presence of β-lactamase enzymes, particularly TEM-1, which are well known for hydrolyzing penicillins and early-generation cephalosporins.[16]

The correlation between the binding energy of antibiotics to TEM-1 β-lactamase and their in vitro susceptibility results reveals important insights into resistance mechanisms. Cephalosporins of the first generation, such as cefazolin and cephalothin, demonstrated high binding affinity to TEM-1 ($\Delta G \approx -8$ kcal/mol) yet were classified as resistant in in vitro testing. This suggests that despite strong interactions, these antibiotics are easily hydrolyzed by TEM-1 due to their structural vulnerability. In contrast, second to fourth-generation cephalosporins, including cefuroxime, cefotaxime, ceftazidime, and cefepime, exhibited similar or slightly lower binding energies but remained effective, likely due to structural modifications that confer stability against β-lactamase degradation. Non-β-lactam antibiotics such as aminoglycosides and fluoroquinolones showed varying binding affinities to TEM-1, though their antimicrobial activity is unaffected by this enzyme, as they target different cellular mechanisms (ribosomes or DNA gyrase). Carbapenems like imipenem and meropenem showed weak to moderate binding but remained clinically effective, consistent with their resistance to β-lactamase hydrolysis. These findings highlight that binding energy alone does not predict antimicrobial efficacy, especially without considering structural resistance and target specificity.

This study investigated these findings by employing molecular docking and molecular dynamics simulations on the TEM-1 β-lactamase protein obtained from the same isolates. The results suggest significant binding interactions between TEM-1 and β-lactam antibiotics, such as cefazolin, which seems to correlate with their limited effectiveness observed in vitro. On the other hand, gentamicin appears to exhibit a notably stable binding affinity with TEM-1 (ΔG –21.8 kcal/mol), which may help explain its sustained antibacterial activity in vitro. These findings highlight how structural studies can support phenotypic resistance results.

Thus, the in vitro phenotypic resistance correlates with the in silico molecular mechanism, highlighting TEM-1 β-lactamase as the key driver of β-lactam resistance. Aminoglycosides and carbapenems remain effective because their mechanisms bypass TEM-mediated hydrolysis. The findings presented are consistent with existing literature. The high prevalence of *E. coli* and its antibiotic resistance in urinary tract infections (UTIs) has been well documented.[30] Furthermore, extended-spectrum beta-lactamases (ESBLs), particularly TEM variants, have been shown to compromise the efficacy of β-lactam antibiotics.[31]

The *TEM* β-lactamase gene from clinical isolate in the laboratory collection was successfully amplified, sequenced, and translated into a protein model based on the crystal structure with PDB ID 1JWV, showing 100% homology.

Structural analysis of this protein revealed the presence of the G238A mutation, which involves the substitution of glycine at position 238 with alanine.

This specific mutation has been studied alongside other substitutions at the same position, such as G238S, G238C, and G238T, to evaluate their impact on hydrolytic activity against extended-spectrum β-lactam antibiotics, including cefotaxime and ceftazidime. Among these, the G238S mutation is known to significantly enhance the hydrolysis of extended-spectrum antibiotics due to its optimal side chain volume and its ability to form hydrogen bonds with the substrate. In contrast, the G238A mutation does not confer the same advantage. Alanine lacks a polar side chain and therefore cannot engage in hydrogen bonding. Additionally, its small size fails to induce the necessary steric changes to open the active site pocket of the enzyme. As a result, the G238A mutation does not significantly broaden the substrate spectrum of TEM-1 and is not considered a major contributor to resistance against extended-spectrum β-lactam antibiotics. This finding is consistent with previously published in vitro studies.[31]

These findings emphasize the importance of bridging in vitro resistance data with in silico structural insights. Such an approach provides a more comprehensive understanding of resistance mechanisms and informs clinical decision-making and the design of novel β-lactamase inhibitors. Researchers can better predict resistance trends and optimize antibiotic use in urinary tract infection management by combining phenotypic assays with molecular modeling.

This study has several limitations. The resistance mechanisms that likely involve gyrA, parC, or efflux were not considered due to the focus on TEM-1 β-lactamase. Additionally, molecular dynamics and docking remain unproven computer predictions that require biochemical validation. The 100 ns simulation may also be too short to capture long-term dynamics. Future research should investigate multiple isolates, target a wider range of factors, include experimental validation, and utilize longer simulations for more robust insights.

Conclusion

Molecular docking and dynamics simulations provided valuable insights into antibiotic interactions with TEM-1 β-lactamase; however, computational binding strength and stability alone did not fully correlate with clinical susceptibility. First-generation cephalosporins, while showing strong and stable binding in silico, remained clinically ineffective due to structural vulnerability to enzymatic hydrolysis. In contrast, later-generation cephalosporins, carbapenems, and aminoglycosides retained clinical efficacy through enhanced stability or different mechanisms of action. These results underscore the importance of integrating computational predictions with structural and clinical data to better inform antimicrobial therapy and resistance management.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C. Urinary tract infections: the current scenario and future prospects. *Pathogens*. 2023;12(4):623. doi:10.3390/pathogens12040623
2. Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, et al. Antibiotic resistance: the challenges and some emerging strategies for tackling a global menace. *J Clin Lab Anal*. 2022;36(9):1–10. doi:10.1002/jcla.24655
3. Lima LM, da SBNM, Barbosa G, Barreiro EJ. β-lactam antibiotics: an overview from a medicinal chemistry perspective. *Eur J Med Chem*. 2020;208:112829. doi:10.1016/j.ejmech.2020.112829
4. Ghafourian S, Sadeghifard N, Soheili S, Sekawi Z. Extended spectrum beta-lactamases: definition, classification and epidemiology. *Curr Issues Mol Biol*. 2014;17(1):11–22. doi:10.21775/cimb.017.011
5. Salverda ML, De Visser JABM, Barlow M. Natural evolution of TEM-1 β-lactamase: experimental reconstruction and clinical relevance. *FEMS Microbiol Rev*. 2010;34(6):1015–1036. doi:10.1111/j.1574-6976.2010.00222.x
6. Ur Rahman S, Ali T, Ali I, Khan NA, Han B, Gao J. The growing genetic and functional diversity of extended spectrum beta-lactamases. *Biomed Res Int*. 2018;2018:1–14. doi:10.1155/2018/9519718

7. Davies DT, Everett M. Designing inhibitors of β-lactamase enzymes to overcome carbapenem resistance in gram-negative bacteria. *Acc Chem Res*. 2021;54(9):2055–2064. doi:10.1021/acs.accounts.0c00863
8. Larramendy S, Deglaire V, Dusollier P, et al. Risk factors of extended-spectrum beta-lactamases-producing escherichia coli community acquired urinary tract infections: a systematic review. *Infect Drug Resist*. 2020;13:3945–3955. doi:10.2147/IDR.S269033
9. Balsalobre L, Blanco A, Alarcón T. Beta-lactams. In: Capelo-Martínez JL, Igrejas G, editors. *Antibiotic Drug Resistance*. Hoboken, NJ: Wiley; 2019:57–72. doi:10.1002/9781119282549
10. Febrina E, Asnawi A. Lead compound discovery using pharmacophore-based models of small-molecule metabolites from human blood as inhibitor cellular entry of SARS-CoV-2. *J Pharm Pharmacogn Res*. 2023;11(5):810–822. doi:10.56499/jppres23.1688_11.5.810
11. Nursamsiar NS, Febrina E, Asnawi A, Syafiie S, Syafiie S. Synthesis and inhibitory activity of curculigoside a derivatives as potential anti-diabetic agents with β-cell apoptosis. *J Mol Struct*. 2022;1265:133292. doi:10.1016/j.molstruc.2022.133292
12. Yuliantini A, Ocktavyanie S, Febrina E, Asnawi A. Virtual screening using a ligand-based pharmacophore model from ashitaba (Angelica keiskei K.) isolates and molecular docking to obtained new candidates as α-glucosidase inhibitors. *Trop J Nat Prod Res*. 2024;8(1):5811–5819. doi:10.26538/tjnpr/v8i1.15
13. Asnawi A, Febrina E, La Ode A, Razi F. Exploring binding affinities of acetoacetate in acrylamide-based polymers (Pam) for molecularly imprinted polymers (Mips): a molecular docking and molecular dynamics study. *Int J Appl Pharm*. 2023;15(special issue 2):101–108. doi:10.26538/tjnpr/v8i1.15.
14. Putra PP, Fitria N, Asnawi A, Djamaan A. Metalloprotein parameters in molecular dynamics simulation for amber, charmm, gromacs, and namd: a systematic review. *Int J Appl Pharm*. 2024;8:90–98. doi:10.22159/ijap.2024v16i5.51513
15. Ischak NI, Ode LA, Hasan H, La Kilo A, Asnawi A. In silico screening of andrographis paniculata secondary metabolites as anti-diabetes mellitus through PDE9 inhibition. *Res Pharm Sci*. 2023;18(1):100–111. doi:10.4103/1735-5362.363616
16. Rostinawati T, Pamungkas TB, Moektiwardojo M, Subarnas A. Pola Resistensi Antibiotik Bakteri Penyebab Infeksi Saluran Kemih di Puskesmas Ibrahim Adjie Kota Bandung. *J Sains Farm Klin*. 2021;8(1):27–34. doi:10.25077/jsfk.8.1.27-34.2021
17. Dallenne C, Da Costa A, Decré D, Favier CAG, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in enterobacteriaceae. *J Antimicrob Chemother*. 2010;65(3):490–495. doi:10.1093/jac/dkp498
18. Wang X, Minasov G, Shoichet BK. Evolution of an antibiotic resistance enzyme constrained by stability and activity trade-offs. *J Mol Biol*. 2002;320(1):85–95. doi:10.1016/S0022-2836(02)00400-X
19. Xiang Z. Advances in homology protein structure modeling. *Curr Protein Pept Sci*. 2006;7(3):217–227. doi:10.2174/138920306777452312
20. Maveyraud L, Pratt RF, Samama JP. Crystal structure of an acylation transition-state analog of the TEM-1 β-lactamase: mechanistic implications for class A β-lactamases. *Biochemistry*. 1998;37(8):2622–2628. doi:10.1021/bi972501b
21. Hetényi C, Van Der Spoel D. Blind docking of drug-sized compounds to proteins with up to a thousand residues. *FEBS Lett*. 2006;580(5):1447–1450. doi:10.1016/j.febslet.2006.01.074
22. Susilawati Y, Indradi RB, Asnawi A, Febrina E. Molecular docking and dynamics studies of 8,9-dimethoxy ellagic acid contained in Peperomia pellucida (L.) Kunth against various diabetes mellitus receptors. *J Pharm Pharmacogn Res*. 2024;12(5):929–942. doi:10.56499/jppres23.1936_12.5.929
23. Nursamsiar M, Khairuddin FJS, Henny Kasmawati WA, Asnawi A. Isolation, characterization, and in vitro inhibitory activity of a new alpha-glucosidase inhibitor from schleichera oleosa (Lour.) Oken leaves. *J Pharm Pharmacogn Res*. 2025;13(1):140–151. doi:10.56499/jppres24.1960_13.1.140
24. Aligita W, Saputri D, Mariska R, et al. Exploring the therapeutic potential of Leea indica leaf extract in diabetic mice: in vivo, phytochemical analysis, and molecular interaction studies. *Trends Sci*. 2025;22(8):10130. doi:10.48048/tis.2025.10130
25. Yao H, Ke H, Zhang X, et al. Molecular recognition of hydrophilic molecules in water by combining the hydrophobic effect with hydrogen bonding. *J Am Chem Soc*. 2018;140(41):13466–13477. doi:10.1021/jacs.8b09157
26. Liu K, Kokubo H. Exploring the stability of ligand binding modes to proteins by molecular dynamics simulations: a cross-docking study. *J Chem Inf Model*. 2017;57(10):2514–2522. doi:10.1021/acs.jcim.7b00412
27. Martínez L. Automatic identification of mobile and rigid substructures in molecular dynamics simulations and fractional structural fluctuation analysis. *PLoS One*. 2015;10(3):e0119264. doi:10.1371/journal.pone.0119264
28. Badar MS, Shamsi S, Ahmed J, Alam MA. Molecular dynamics simulations: concept, methods, and applications. In: Rezaei N, editor. *Transdisciplinarity*. Cham: Springer; 2022:131–151. doi:10.1007/978-3-030-94651-7_7
29. Pantsar T, Poso A. Binding affinity via docking: fact and fiction. *Molecules*. 2018;23(8):1899. doi:10.3390/molecules23081899
30. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol*. 2015;13(5):269–284. doi:10.1038/nrmicro3432
31. Cantu C, Palzkill T. The role of residue 238 of TEM-1 β-lactamase in the hydrolysis of extended-spectrum antibiotics. *J Biol Chem*. 1998;273(41):26603–26609. doi:10.1074/jbc.273.41.26603

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