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In silico exploration of flavonoids against bacterial TEM-1 beta-lactamase

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Abstract: The increasing prevalence of antibiotic resistance is a major public health concern worldwide, and the discovery of new antibacterial agents is urgently needed. Flavonoids are a diverse group of natural compounds that have been shown to possess antibacterial activity against a range of bacterial strains, including antibiotic-resistant strains. In this study, we used molecular docking to investigate the potential of flavonoids as inhibitors of TEM-1 beta-lactamase, which is a common enzyme responsible for resistance to beta-lactam antibiotics. We screened a library of flavonoids against the crystal structure of TEM-1 beta-lactamase (PDB: 1FQG) using the AutoDockVina software. Among the screened flavonoids, ZINC000013860547 showed the highest negative binding affinity (-9 kcal/mol) to the active site of TEM-1 beta-lactamase. We also analyzed the binding modes of ZINC000013860547 and found that it forms several hydrogen bonds and hydrophobic interactions with key residues in the active site of TEM-1 beta-lactamase. Our results suggest that ZINC000013860547 has the potential to be developed as a novel inhibitor of TEM-1 beta-lactamase after optimization, which may help to overcome beta-lactam resistance in bacterial infections. Further experimental studies are needed to validate the inhibitory activity of ZINC000013860547 against TEM-1 beta-lactamase and its potential as a therapeutic agent for antibiotic-resistant bacterial infections.
Keywords: Antibiotic resistance, Flavonoids, Molecular docking, TEM-1 beta-lactamase

1. Introduction

In the 20th century the invention that made a breakthrough in the field of the therapeutics was the discovery of the antimicrobial drugs and antibiotics as a treatment for the different infections caused by the bacteria. Majorly two events namely isolating the antibiotic activity containing products from the sources containing microbes and second contribution of medicinal chemist in the production of antibiotics [1]. Along with more and more research in the field of antibiotics here raised severe risk of Antibiotic Resistance (AR). The main reason for the growing threat of this AR is Multidrug resistance bacteria also knowing as superbugs which existed with us in animals and the environment [2]. The first case of resistance development in Staphylococci and gonococci for the penicillin was seen after the 2nd world war. Further in 1970s there emerged a Staphylococcus Aurius resistant to the methicillin. In many parts of different geographical areas there were strains of gonococcal species which includes the Neisseria gonorrhoeae having ability to produce the enzyme penicillinase. In coming period, the worldwide use of gentamicin resulted in the Pseudomonas Aeruginosa with having ability to resist the aminoglycosides. P. aeruginosa resistant to the ciprofloxacin and ceftazidime is still a matter of concern till date. From the 1970 the resistance among the respiratory pathogens including Haemophiles influenzae, Moraxella catarrhalis and Streptococcus pneumoniae has grown severely [3]. The main loss suffered from the AR is the loss of the previously cheaper and great treatment options available. This loss has been suffered by the antibiotics like penicillin and oxacillins against the infections caused by the staphylococcus, likewise penicillin as well as fluoroquinolones against the gonorrhoea and the ampicillin and sulphonamides versus UTI (urinary tract

infections)[4]. Fatal infections which are hard or nearly impossible to treat with previously using antimicrobials have been resulted from the multidrug resistant models in gram-negative and gram-positive bacteria. Now-a-days broad spectrum antibiotics are largely being used as prompt identification of microbes which cause infection and susceptibility of them to the patients suffering bacteremia and other deadly infections is failing drastically in numerous healthcare systems [5]. Several factors influence the biological fitness of an antibiotic-resistant bacterium, including the rates at which resistant and sensitive bacteria (i) grow and die inside and outside a host, (ii) are transmitted between hosts and between the host and the environment, and (iii) are cleared from infected hosts[6]. Bacteria are not only just naturally resistant to specific antibiotics but also have ability to adopt a resistance to certain antibiotics due to mutations in genes present in chromosomes and by horizontal gene transfer. The natural innate resistance is due to the specific characteristic of structure, or the function found by inheritance [7]. There are mainly two strategies used by the microbes for the development of the AR first one is the mutation in the genes related with the mechanism of action and second one is acquiring through the DNA coding of foreign microbes by the Horizontal gene transfer (HGT) [8]. The combined amounts of horizontal gene transfer and de novo mutation decides the occurrence of AR in the microbes. The increased amount of drug efflux rates and alteration of the antibiotic targets are most commonly caused by the mutations. The gene amplification, modification of drug modification enzyme and decreased expression of the target is also responsible for the antibiotic resistance. HGT mechanisms include drug modification, target protection, bypass resistance, drug target replacement, and the acquisition of novel efflux pumps [9].

2. Material and methods

2.1. Protein and ligand preparation

The 3D crystal structures of TEM-1 Beta-Lactamase (PDB: 1FQG) having 1.70 Å resolution was selected and downloaded from the RCSB Protein data bank [10]. Further, the docwnloaded protein was prepared for docking by cleaning of protein via removal of water molecules and previously bound co-crystalized ligands. Protein structure was then protonated by adding the polar hydrogen atoms [11]. Protein preparation process was done with the help of BIOVIA Discovery Studio [12]. Thereafter prepared protein structure was validated using the PROCHECK and ERRAT tool to determine the quality of protein [13]. The structures of flavonoids were downloaded from Zinc15 database. The downloaded structures of ligands was then prepared using Open Babel by adding hydrogen atoms to make it correct and used for further in silico studies [14].

2.2. Molecular docking

Molecular docking study was done between selected ligands and TEM-1 Beta-Lactamase (PDB: 1FQG). The docking study was conducted via the Auto Dock Vina module of the PyRx 0.8 [15]. The PDB file of TEM-1 Beta-Lactamase and SDF files of ligands structures were imported and selected in Vina wizard module of PyRx program [16]. The structure of flavonoids was energy minimized ad converted to PDBQT format via Open Babel module of the PyRx software. The maximized grid box was selected using Vina workspace to cover the entire protein structure. The exhaustiveness was set to default at 8[17, 18]. Docked poses for each ligands with good binding affinity was saved in SDF file format and then used for visualization of 2D and 3D binding interactions with the targeted protein structure.

3. Result and discussion

The protein and ligand preparation steps were performed before molecular docking as they can significantly affect the accuracy and reliability of the results. Protein preparation was done with the removal of water molecules, non-polar hydrogen atoms, and other extraneous molecules from the protein structure[19]. This step also includes the addition of missing atoms or residues, the correction of any structural errors, and the optimization of the protein's conformation. This step helped to ensure that the protein is in its most stable and biologically relevant form, which is essential for accurately predicting its interaction with a ligand. Prepared protein structure was evaluated for its quality via Ramachandran plot and ERRAT [13, 20, 21]. The generated Ramachandran plot indicated protein structure is of good quality, as 93.9% of the residues are in the most favored regions (A, B, L) of the Ramachandran plot. Additionally, 5.7% of the residues are in the additional allowed regions (a, b, l, p), which are also acceptable regions of the Ramachandran plot. This suggests that the protein

structure has a good backbone conformation. Only 0.4% of the residues are in the generously allowed regions (~a, ~b, ~l, ~p), which are less preferred regions of the Ramachandran plot. This suggests that the protein structure may have some minor deviations from ideal backbone conformation, but these deviations are not significant enough to indicate major structural problems. There are no residues in the disallowed regions of the Ramachandran plot, indicating that there are no major structural problems in the protein structure. The total number of non-glycine and non-proline residues is 228, which represents 100% of the non-Glycine and non-Proline residues in the protein structure. There are 21 Glycine residues and 12 Proline residues, which are shown as triangles on the Ramachandran plot. These residues are known to have restricted conformational space due to their unique side chain structures, and their presence in specific regions of the Ramachandran plot is expected. The total number of residues in the protein structure is 263, including the Glycine and Proline residues. The Ramachandran plot is represented in the **Figure 1**.

The ERRAT program assesses the quality of a protein structure based on its overall quality factor, which is a score ranging from 0 to 100 %. In this case, the given overall quality factor for the prepared protein structure is 97.2549 %, which is a very high score. A score above 50 generally indicates a high-quality protein structure, while a score above 90 is considered excellent. Therefore, based on the overall quality factor of 97.2549 %, we can conclude that the protein structure is of very high quality and has good agreement with the expected stereochemical properties of well-determined protein structures.Ligand preparation process involved the removal of any solvent molecules, energy minimization and the optimization of the ligand's geometry. Molecular docking is a computational tool that plays a crucial role in drug discovery by predicting the binding of a small molecule (ligand) to a target protein. The process involves generating conformations of the ligand and protein and calculating their interaction energies to predict the most favorable binding pose. Molecular docking is important in drug discovery because it allows researchers to predict the binding affinity of a small molecule to a target protein before conducting experimental assays. This information is valuable in optimizing the design of small molecules and selecting potential drug candidates with high binding affinity and selectivity. The overall quality factor plot generated via ERRAT is represented in **Figure 2**.

Flavonoids are a diverse class of natural compounds found in many plants, fruits, and vegetables. They have been shown to possess a wide range of biological activities, including anti-inflammatory, anti-cancer, and anti-viral effects. Molecular docking studies have been used to investigate the potential mechanisms of action of flavonoids by predicting their binding modes to various targeted proteins.The results of the molecular docking study conducted between selected flavonoids and the TEM-1 Beta-Lactamase protein (PDB: 1FQG) has unraveled a significant finding. The molecule with ZINC ID ZINC000013860547 showcased a remarkable negative binding affinity with the targeted protein structures, indicating its promising inhibitory potential against TEM-1 Beta-Lactamase. The docked protein-ligand complex of ZINC000013860547-1FQG manifested an intricate arrangement of six hydrogen bonds, comprising one carbon-hydrogen bond and five conventional hydrogen bonds. The formation of these hydrogen bonds involved amino acid residues such as SER235, ASN132, ASN170, and SER70, which were crucial in stabilizing the protein-ligand complex.Moreover, the analysis of the docking pose of ZINC000013860547 revealed the crucial role of hydrophobic interactions in the binding process. Furthermore, the binding affinity of the ZINC000013860547-1FQG complex was estimated to be -9 kcal/mol, indicating its high stability. The 2D and 3D binding interactions are represented in **Figure 3**. The binding affinity, interacting resides and types of interaction between all docked ligands and targeted protein is represented in **Table 1**. These findings suggest that ZINC000013860547 holds immense potential to be developed as a novel inhibitor against TEM-1 Beta-Lactamase, thereby combating the problem of antibiotic resistance. However, further experimental validation is necessary to determine its inhibitory activity and suitability as a therapeutic agent against antibiotic-resistant bacterial infections.

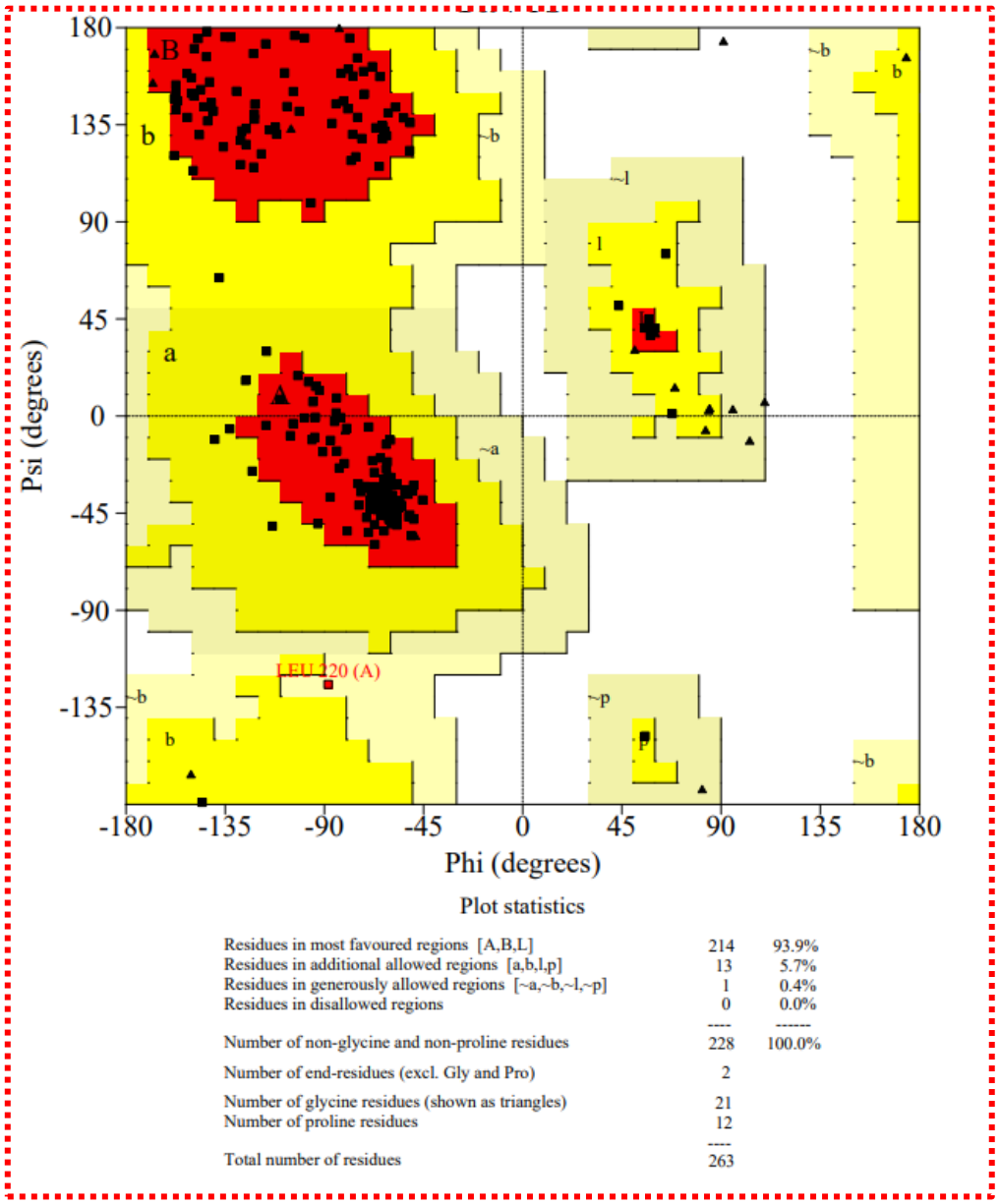

Figure 1. The Ramachandran plot of the prepared protein structure.

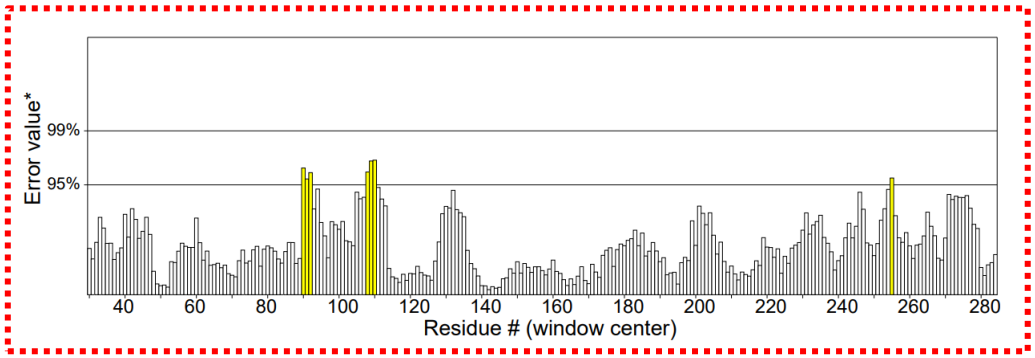

Figure 2. Overall quality factor of prepared protein evaluated via ERRAT.

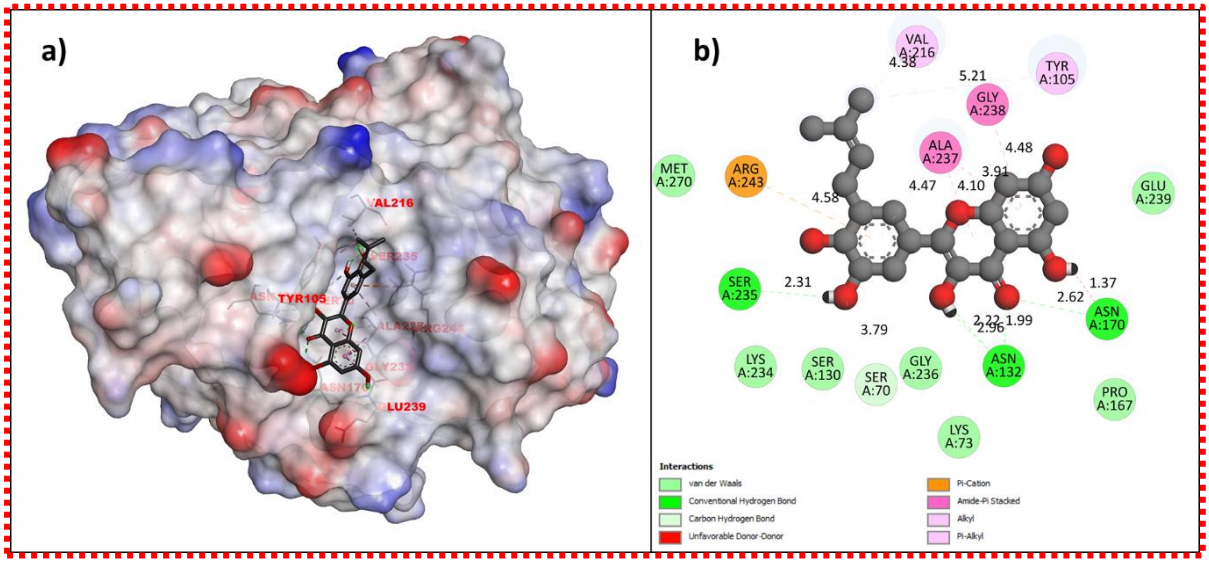

Figure 3. a) 3D interaction in the binding pocket; b) 2D interaction between ZINC000013860547 and targeted protein.

Table 1. Binding affinity along with the binding interactions of docked flavonoids with targeted protein.

Zinc ID	Binding affinity (kcal/mol)	Interacting residues	Type of interaction
ZINC000013860547	-9	MET270, LYS234, SER130, GLY236, LYS73, PRO167, GLU239	VDW
		SER235, ASN132, ASN170	Conventional H Bond
		SER70	Carbon H Bond
		ARG243	Pi-Cation
		ALA237, GLY238	Amide-Pi Stacked
		VAL216, TYR105	Alkyl, Pi-Alkyl
ZINC000140867960	-8.9	GLY238, MET69, LYS234, SER130, GLY236, SER70, ASN132, PRO167, GLU104, TYR105, MET270, ALA217	VDW
		ASN170, ALA237, VAL216	Conventional H Bond
		ARG243	Pi-Cation
ZINC000006520226	-8.8	TYR105, LYS73, SER70, GLY236, SER130, LYS234, VAL216, LEU220, ALA217, MET270, GLU239, PRO167	VDW
		ASN132, SER235, ASN170	Conventional H Bond
		ALA237, GLY238	Amide-Pi Stacked
ZINC000033833929	-8.8	TYR105, LYS73, SER70, GLY236, SER130, VAL216, ALA217, MET270, GLU239, PRO167, ASN170	VDW
		ASN132, LYS234, SER235	Conventional H Bond
		ARG243	Pi-Cation

		GLY238, ALA237	Amide-Pi Stacked
ZINC000140338495	-8.8	VAL216, GLY236, SER235, ARG243, SER70, LYS73, ASN166, GLU239, GLU104, GLY238, ARG273	VDW
		SER130, ALA237, ASN132	Conventional H Bond
		TYR105	Pi-Pi Stacked, Amide-Pi Stacked
		PRO167, MET270	Alkyl, Pi-Alkyl
ZINC000001750388	-8.7	GLU104, SER70, LYS73, ALA237, SER130, TYR105, MET270, LYS234, GLY236, GLY238, GLU239	VDW
		ASN132, SER235, ARG243, ASN170	Conventional H Bond
		PRO167, VAL216	Alkyl, Pi-Alkyl
ZINC000002151148	-8.7	ASP176	VDW
		ALA172, THR264, ARG43	Conventional H Bond
		PRO67	Carbon H Bond
		ASN175	Pi-Donor H Bond
		ARG65, PRO174	Pi-Sigma
		ARG240	Amide-Pi Stacked
ZINC000004731234	-8.7	MET270, LYS73, TYR105, PRO167, GLU239, GLY236, LYS234, SER130	VDW
		SER235, VAL216, SER70, ASN132, ASN170	Conventional H Bond
		ARG243	Pi-Cation
		ALA237, GLY238	Amide-Pi Stacked
ZINC000014643615	-8.7	PRO167, LYS73, TYR105, GLY236, SER130, MET270, GLU239	VDW
		ASN170, VAL216, ASN132, SER70, LYS234, SER235	Conventional H Bond
		ARG243	Pi-Cation
		GLY238, ALA237	Amide-Pi Stacked
ZINC000014727455	-8.7	SER70, GLY236, SER130, LYS234, ALA217, MET270, GLU239, PRO167	VDW
		ASN132, VAL216, ASN170	Conventional H Bond
		GLY238, ALA237	Amide-Pi Stacked
ZINC000033833930	-8.7	PRO167, GLU239, TYR105, SER70, GLY236, SER130, LYS234, ALA217, MET270	VDW

		ASN132, ASN170, VAL216, SER235	Conventional H Bond
		ARG243	Pi-Cation
		GLY238, ALA237	Amide-Pi Stacked
ZINC000261492354	-8.7	ALA217, PRO219, MET270, GLY236, ASN166, SER70, GLY238, LYS73, SER130	VDW
		ASN132, VAL216, ARG243, ALA237, ASN170	Conventional H Bond
		TYR105	Pi-Sigma
ZINC000000044208	-8.6	GLU239, MET270, VAL216, GLY236, TYR105, LYS234, SER70	VDW
		ASN170, ARG243, SER235, LYS73, SER130, ASN132	Conventional H Bond
		GLY238, ALA237	Amide-Pi Stacked
ZINC000000044209	-8.6	VAL216, ALA217, LEU220, MET270, SER235, GLY236, TYR105, SER70, GLU239	VDW
		LYS73, SER130, ASN132, ASN170	Conventional H Bond
		GLY238	Amide-Pi Stacked
		ALA237	Pi-Alkyl
ZINC000001573789	-8.6	GLY238, ASN132, ALA237, SER70, LYS73, LYS234, GLY236, SER235, LEU220, ALA217, GLU104	VDW
		ASN170, SER130, ARG243, VAL216	Conventional H Bond
		TYR105	Pi-Pi Stacked
ZINC000002105774	-8.6	GLY238, GLY236, SER70, GLU239, GLU104, LYS73, SER130	VDW
		ARG243, SER235, ASN170, ASN132	Conventional H Bond
		TYR105	Pi-Pi Stacked
		VAL216, PRO167, ALA237	Alkyl, Pi-Alkyl
ZINC000003875620	-8.6	PRO167, GLU239, TYR105, LYS73, SER70, LYS234, GLY236, MET270	VDW
		ASN170, SER235, VAL216, SER130, ASN132	Conventional H Bond
		ARG243	Pi-Cation
		ALA237, GLY238	Amide-Pi Stacked
ZINC000005998785	-8.6	GLU239, MET270, ALA217, LYS234, GLY236, SER130, TYR105, SER70, LYS73	VDW
		VAL216, SER235, ASN132, GLY238, ASN170	Conventional H Bond
		ARG243	Pi-Cation

		ALA237	Amide-Pi Stacked
		PRO167	Alkyl, Pi-Alkyl
ZINC000014757232	-8.6	PRO167, GLU104, LYS73, SER70, TYR105, SER235, VAL216, MET270, GLU239, GLY241	VDW
		ARG243, SER130, ASN132, ARG273, ASN170	Conventional H Bond
		ALA237, GLY238	Amide-Pi Stacked
ZINC000033833444	-8.6	GLU64, GLY265, SER242, ILE173, PHE66, THR180, ASP176, GLU177	VDW
		ALA172, THR264	Conventional H Bond
		PRO67	Carbon H Bond
		ARG43	Pi-Cation
		ASN175	Pi-Donor H Bond
		ARG65	Pi-Sigma
		ARG240, PRO174	Alkyl, Pi-Alkyl

4. Conclusions

In conclusion, our study highlights the potential of flavonoids as promising inhibitors against antibiotic resistance. The preformed molecular docking of selected flavonoids against TEM-1 Beta-Lactamase (PDB: 1FQG) demonstrated the binding potential of ZINC000013860547, which exhibited the highest negative binding affinity with the targeted protein structures. The docked protein-ligand complex of ZINC000013860547-1FQG formed six hydrogen bonds, involving crucial amino acid residues such as SER235, ASN132, ASN170, and SER70. Moreover, the analysis of the docking pose revealed the crucial role of hydrophobic interactions in stabilizing the protein-ligand complex. The estimated binding free energy of the ZINC000013860547-1FQG complex (-9 kcal/mol) indicated high stability. These findings demonstrate the potential of flavonoids as a source of novel inhibitors against antibiotic resistance, particularly against TEM-1 Beta-Lactamase. However, further experimental validation is required to confirm the inhibitory potential of ZINC000013860547 against TEM-1 Beta-Lactamase and its suitability as a therapeutic agent against antibiotic-resistant bacterial infections.

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