JNCS Journal of Nepal Chemical Society July 2025, Vol. 45, No. 2, 45-63
ISSN: 2091-0304 (print)
DOI: https://doi.org/10.3126/jncs.v45i2.83035
RESEARCH ARTICLE

Exploring Aminopenicillin Resistance in Uropathogenic *Staphylococcus species* and Optimized Lead Prediction to Overcome Resistance through In-Silico Modules

Sabin Shrestha[1*], Barsha Adhikari[1*], Rahi Bikram Thapa[2], Pharsuram Adhikari[1], Dharma Prasad Khanal[1], and Prem Paudyal[1]

[1]*Department of Pharmacy, Manmohan Memorial Institute of Health Science, Soalteemode, Kathmandu, Nepal.*

[2]*Hospital Development and Medical Services Division, Ministry of Health, Koshi Province, Nepal.*

[*]*Corresponding E-mail: shresthasabin172@gmail.com, and barshaaadk@gmail.com*

(Received: May 23, 2025, revised: June 20, 2025, accepted: July 21, 2025)

Abstract

Penicillin resistance in uropathogenic *Staphylococcus species*, presents a significant challenge in treating urinary tract infections (UTIs), a prevalent global health concern. With resistance rates on the rise, particularly in developing countries, there is an urgent need to identify novel therapeutic alternatives. This study aims to investigate penicillin resistance in *Staphylococcus species* isolates and identify potential lead compounds using in-silico methods to optimize penicillin derivatives that can overcome resistance. A retrospective observational study of 3,000 UTI cases was conducted at a tertiary care hospital in Nepal, with 246 samples showing significant bacterial growth. Molecular docking using AutoDock 4.2 assessed the binding affinities of 37 novel penicillin derivatives against the penicillin-binding protein of *S. aureus*, PDB ID: 1TVF. Drug-protein interactions were analyzed using Discovery Studio, pharmacokinetics with SwissADME, and biological activity and toxicity predictions with PASS Online and ProTox-II, and molecular dynamics simulations by CABS-flex 2.0. *Staphylococcus aureus* was isolated in 6.5% of cases, with substantial resistance to cefoxitin (56.2%), ampicillin (50%), and amoxicillin (18.75%). Novel derivatives 2-6 Di-Nitro Amoxicillin Derivatives [BA-32] and 3,5 Di-Nitro Amoxicillin Derivatives [BA-33] exhibited superior binding affinities -8.1 and -8.2 kcal/mol compared to standard β-lactam antibiotics, forming stable interactions with key residues like SER27: 2.25, GLN64: 2.16, LEU61: 1.69, GLU368: 3.04 and ARG186: 2.45, ASN141: 2.85, SER75: 2.57, LYS78:2.68, GLU114: 2.75, SER262: 2.16. These derivatives complied with Lipinski's Rule of Five, indicating favorable pharmacokinetics, optimal biological activity, and minor toxicity predictions. Penicillin resistance in *Staphylococcus species* at notable levels, [BA-32] and [BA-33], emerges as a promising candidate for future therapeutic development, while derivatives like [BA-32] require further optimization to address cytotoxicity concerns. Experimental validation and exploration of additional resistance mechanisms are recommended for comprehensive treatment strategies.

Keywords: Penicillin, Antibiotic resistance, *Staphylococcus species,* Molecular docking, UTI

Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases worldwide, significantly impacting public health due to the colonization and infection of the urinary system, which includes the kidneys, ureters, bladder, and urethra [1]. *Staphylococcus spp.*, although less prevalent than *Escherichia coli,* has emerged as a notable uropathogen, especially in specific patient populations such as young women and individuals with indwelling urinary devices [2-4]. *Staphylococcus saprophyticus* is recognized as the second most common cause of UTIs in young women, while *Staphylococcus aureus* is frequently associated with hospital-acquired UTIs [5]. The global prevalence of UTIs is staggering, with an estimated 150–200 million cases annually [6], contributing significantly to patient morbidity, particularly in healthcare settings [7].

The emergence of antimicrobial resistance (AMR) poses a significant challenge in the management of UTIs, especially those caused by uropathogenic *Staphylococcus spp.* Rising resistance rates to penicillin and other antibiotics among these pathogens have been documented across various populations. For instance, a study in Romania highlighted alarming resistance patterns among uropathogens, with *Staphylococcus aureus* exhibiting a high rate of penicillin resistance [8]. This situation is dire in developing countries like Nepal, as antimicrobials are the leading cause of drug therapy-related problems because they are often prescribed in contradiction to local and national antimicrobial treatment guidelines, and the use of medications without valid indications and non-compliance with regulations [9-12]. This practice is the major cause of AMR in Nepal. Research from Nepal found that 95% of S. aureus isolates from pediatric UTIs were resistant to penicillin, underscoring the widespread nature of this issue [13]. Likewise, a recent study revealed *S. aureus* exhibits 100% resistance to amoxicillin and 71.43% resistance to Ampicillin [4]. The mechanisms underlying penicillin resistance in *Staphylococcus spp.* are multifaceted, primarily arising from structural changes in penicillin-binding proteins (PBPs) that reduce their affinity for β-lactam antibiotics, often due to mutations or acquisition of new PBPs like PBP2a [14]. Additional mechanisms include β-lactamase production, efflux pumps, and reduced membrane permeability [15]. Additionally, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in UTIs has been a growing concern, with findings indicating significant proportions of MRSA strains in urine samples from regions like Iraq and Saudi Arabia [16, 17].

Anti-Microbial Resistance in *Staphylococcus spp.* not only limits treatment options but also adds to the economic burden of UTIs, requiring recurrent medical interventions and often extended hospital stays, particularly in drug-resistant cases [18]. Given this, there is an urgent need for innovative strategies to address this public health threat. In silico methods, which utilize computational models to predict the efficacy of potential therapeutic agents, have emerged as promising approaches in combating AMR and cancers [19-21]. Nowadays, in silico methods are being integrated with AI, and AI has great potential in drug discovery and development, as well as in healthcare information like drug-drug interactions [22]. These methods enable the identification of novel lead compounds effective against resistant strains, thereby enhancing treatment options for UTIs [23]. By integrating in silico predictions with empirical data on resistance patterns, it is possible to develop more targeted and effective therapeutic strategies that could ultimately improve patient outcomes and mitigate the impact of AMR in clinical settings [24].

This study aims to investigate penicillin resistance in uropathogenic *Staphylococcus spp.* and apply optimized lead prediction through in silico techniques to identify potential therapeutic candidates. By understanding the molecular mechanisms underlying resistance and utilizing advanced computational methods, this research seeks to contribute to the development of effective treatment options against resistant *Staphylococcus* infections in the urinary tract.

Methods

Ethics approval

The study protocol received ethical clearance from the Institutional Review Committee at Manmohan Memorial Institute of Health Sciences [MMIHS-IRC, Reference No: NEHCO/IRC/080/99]. Additionally, authorization to gather data at Manmohan Memorial Medical College and Teaching Hospital [MMTH] in Kathmandu was granted by the hospital board [Reference No: 241].

Procedure of Penicillin Resistance Prevalence Determination

A retrospective observational study was carried out at Manmohan Memorial Teaching Hospital (MMTH) in Kathmandu. Data were retrospectively collected over six months from both inpatient (IPD) and outpatient (OPD) departments, sourced from the hospital's medical record department. The study concentrated on patients who had undergone urine culture and sensitivity tests related to urinary tract infections (UTIs). Patients without bacterial isolation in their cultures were not included in the analysis. The data were meticulously entered into Excel 2019, and descriptive statistics were used to assess socio-demographic variables. Additionally, the percentage of penicillin-resistant microorganisms among the isolates was calculated.

Procedure of Lead Optimization through in-Silico methods

Molecular Docking

AutoDock 4.2 [25] was employed to predict how small molecules, including penicillin, bind to the penicillin-binding protein of *Staphylococcus aureus* (PDB ID: 1TVF), a known target for urinary tract infections (UTIs). Potential leads from novel penicillin derivatives were identified through in-silico methods.

Preparation of Ligand

Ligands were prepared using various computational tools. PubChem [26] was utilized to obtain SDF files of existing Amoxicillin and Ampicillin, while Marvin Sketch [27] was used to construct novel penicillin derivatives in SDF format. These files were then converted into PDB format using Discovery Studio [28] and subsequently into PDB-QT format via Autodock software [29]. The ligands were composed of amino penicillin acetyl ester derivatives (**Figure 1**) with various scaffolds [R1] (**Figure 2**) and [R2] (**Figure 3**).

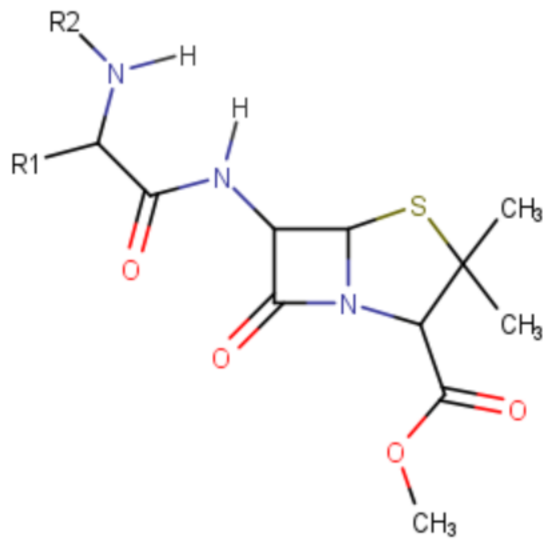
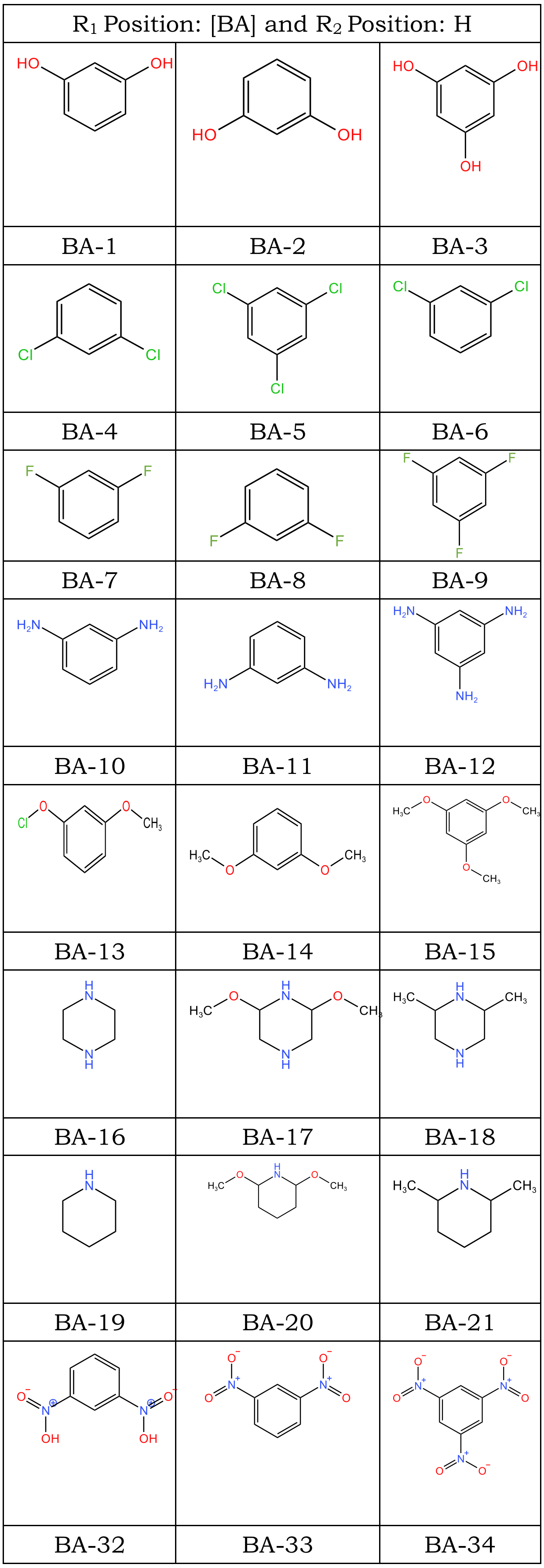


Figure 1: Basic Nucleus of Novel Amino Penicillin Derivatives.

Preparation of Protein Molecule

The protein structure of penicillin-binding protein of *Staphylococcus aureus* (PDB ID:1TVF) was retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/) using the PDB ID:1TVF [30]. The structure, matching required parameters such as x-ray diffraction resolution and no mutations and Ramachandran plot, was optimized by cleaning and removing irrelevant residues, fixing structural errors, and adding polar hydrogen bonds. The final structure was saved in PDB format for subsequent docking studies.



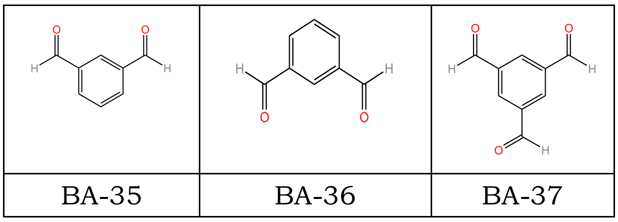


Figure 2: Substituted Scaffolds at R_1 Position

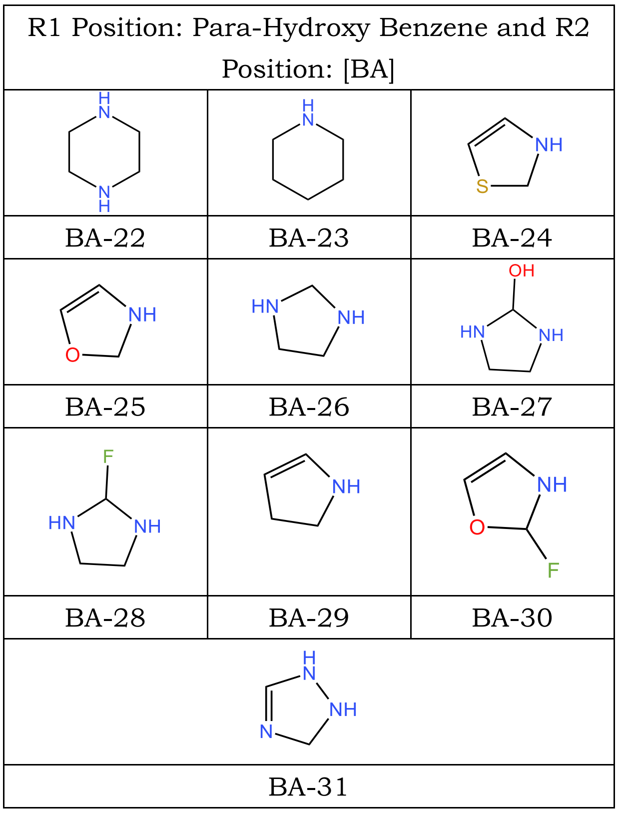


Figure 3: Substituted Scaffolds at R_2 Position.

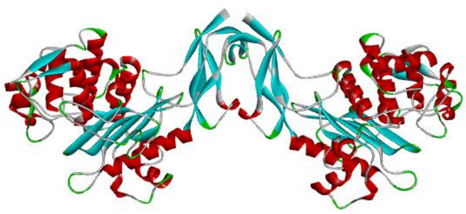


Figure 4: Crystal Structure of penicillin-binding protein of *Staphylococcus aureus*.

Identification of Binding Pocket

The active binding site of the protein was identified using the CASTp server (http://cast.engr.uic.edu), which detects potential binding pockets based on the protein's amino acid sequence. Unlike energy-based scoring methods, CASTp employs a purely geometric approach, applying computational geometry techniques such as alpha-shapes and flow algorithms to locate surface pockets. It then uses analytical calculations to determine each pocket's volume, surface area, and openings, followed by a ranking system to prioritize sites most suitable for ligand binding. The key amino acid residues comprising the active site were thoroughly analyzed and are presented in **Figure 5** and **Table 1** [31].

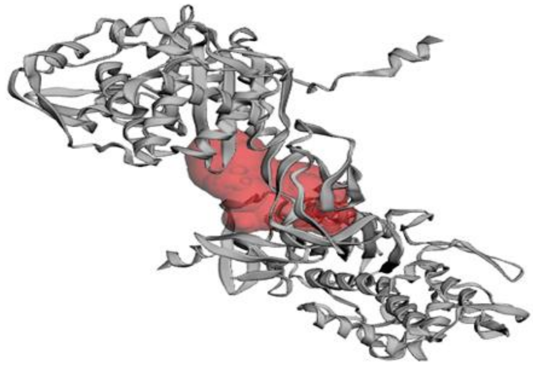


Figure 5: Active binding pockets of 1TVF protein [31].

Table 1: Active Binding pocket of Penicillin-binding protein of *Staphylococcus aureus* on the basis of Computational geometry [31].

ARG-276, GLY-277, LYS-278, PHE-279, GLU-209, PHE-312, ASP-313, TYR-315, LYS-316, VAL-318, LYS-319, ILE-320, LEU-321, SER-322, GLN-326, ARG-327, ILE-328, TYR-340, LEU-343, PHE-347, SER-348, LYS-349, TYR-352, LEU-354, LYS-316, VAL-318, LYS-319, ILE-320, LEU-321, SER-322, LYS-323, GLN-326, ARG-327, ILE-328, LEU-343, PHE-347, SER-348, LYS-349, LYS-350, TYR-352, LEU-354.

Pharmacokinetic and Toxicity Prediction The pharmacokinetic properties, including GI absorption, distribution, metabolism, and excretion (ADME), of all ligands were predicted using the SWISS ADME lab web server (http://www.swissadme.ch/) [32]. Additionally, the toxicity of these compounds was predicted using the ProTox-II web server (https://toxnew.charite.de/) [33], ensuring the identification of safe and effective drug candidates.

Biological Activity Prediction

To validate the docking results, the PASS web server (https://www.way2drug.com/passonline/) [34] was used to predict the biological activity of the bioactive compounds, focusing on antibacterial activity. The results indicated that the probability of these compounds being active (Pa) was greater than the probability of being inactive (Pi), suggesting potential antibacterial properties.

Docking Procedure

The docking studies were carried out using Autodock Vina. The binding energy between ligand-receptor interactions was calculated using the formula [35]:

$$\Delta G_{(Binding)} = \Delta G_{(Gauss)} + \Delta G_{(Repulsion)} + \Delta G_{(H\text{-}Bond)} + \Delta G_{(Hydrophobic)} + \Delta G_{(Tors)}$$

Here, ΔG (Gauss) represents the dispersion of two Gaussian functions, ΔG (Repulsion) accounts for repulsion beyond a threshold distance, ΔG (H−Bond) models hydrogen bond interactions, ΔG (Hydrophobic) is a ramp function for hydrophobic interactions, and ΔG (Tors) is proportional to the number of rotatable bonds [35]. The protein structure was loaded into Autodock 4.2, and the format was converted to PDBQT. The ligands were uploaded, their geometrical energies minimized to global maxima, and converted into PDBQT format. The docking grid parameters were set with the following values: x: 16.332, y: 18.653, z: -11.116, with dimensions of x: 69.99, y: 47.320, z: 69.00. The obtained conformations were further analyzed using Discovery Studio 2023. Discovery Studio determines hydrogen bond distances by measuring the straight-line distance between the donor and acceptor atoms, subject to internal cutoffs for both distance and angle that define it as a valid hydrogen bond [36]. The results are presented in the interaction table, but no energetic values are computed by the software [36].

Molecular Dynamic Simulation

The stability of the drug-receptor complex was predicted by calculating the RMSF value using the CABS-flex 2.0 web server [37]. The

CABS-flex server is a web-based tool designed to simulate the flexibility of protein structures using a coarse-grained molecular dynamics (MD) approach. The CABS-flex 2.0 web server calculates Root Mean Square Fluctuation (RMSF) using the standard RMSF formula, which is based on the fluctuation of each residue's position over the simulation trajectory [38].

Results

Socio-demographic and Clinical Characteristics

Out of 3000 patients diagnosed with UTI, 246 samples showed significant growth of microorganisms, and all the samples consisted of a single microorganism. Females comprised a significant portion of 69.5%, and the highest number of specimens was from the age group 31-60 years 110 (44.7%). Detailed socio-demographic and Clinical Characteristics of the Patients are shown in **Table 2.**

Table 2 Socio-demographic and Clinical Characteristics

Variables	Category	Frequency	%
Gender	Male	75	30.5
	Female	171	69.5
Age Group	1-30	81	32.9
	31-60	110	44.7
	61-90	55	22.4
Bacteria Type	Gram Negative	217	88.2
	Gram Positive	29	11.8

Organisms Isolated and their Resistance

Twelve pathogenic bacteria were isolated from 246 urine samples collected from patients. Among these, *E. coli* was the most common isolate, found in 156 samples (63.4%). Other identified bacteria included *Staphylococcus aureus* in 16 samples (6.5%), *Staphylococcus epidermidis* in 4 samples (1.6%), and *Staphylococcus saprophyticus* in 2 samples (0.8%).There were 16 infections caused by *Staphylococcus aureus* and commonly showed resistance to Cefoxitin (56.2%), Ampicillin (50%), Ciprofloxacin (43.75%), Nitrofurantoin (25%), Gentamicin (25%), Cotrimoxazole (25%), Ceftriaxone (25%), Amoxicillin (18.75%), Cefixime (12.5%), Piperacillin Tazobactam (6.25%).

Molecular Docking Result

In our study, 37 novel penicillin derivatives were designed and docked with the 1TVF penicillin-binding protein of *Staphylococcus aureus* to predict the potential anti-bacterial activity. The binding energy, number of hydrogen bonds, bond distance, and amino acid responsible for interaction were presented in **Table 3**. The ligand showing the lowest binding affinity, the greater number of hydrogen bonds, shorter bond distances, and a greater number of amino acid interactions was taken as the best ligand for further investigation.

Molecular docking with 1TVF exhibited binding energy of ΔG = -7 kcal/mol by amoxicillin with three H-bonds: GLU-114 of distance 2.39, 2.48 Å, and TYR-291, GLY-181 of distance 2.99 and 2.90 Å, respectively. Whereas low binding energy of ΔG = -6.4 kcal/mol was exhibited by ampicillin with two H-bonds, SER-27 of distance 2.31 and PRO-366 of distance 2.81Å respectively.

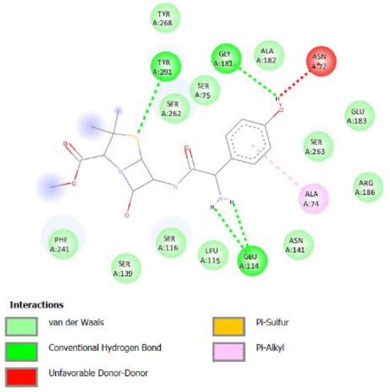
Molecular docking of novel penicillin derivatives with 1TVF protein exhibited best binding energy of ΔG = -8.1 kcal/mol by 2,6 Dinitro derivatives [BA-32] with four hydrogen bonds: SER-27 of bond distance 2.25, and GLN-64 of bond distance 2.16 Å, LEU-61 of distance 1.69, and GLU-368 of bond distance 3.04 Å, respectively. while 3,5 di-nitro derivatives [BA-33] exhibited binding energy of -8.2 Kcal/mol with six hydrogen bonds: SER-262 of bond distance 2.16, LYS-78 of bond distance 2.68, ASN-141 of bond distance 2.85, ARG-186 of bond distance 2.54, SER-75 of bond distance 2.57, and GLU-114 of bond distance 2.75 Å respectively. Other derivatives show the lowest binding energy in the range of

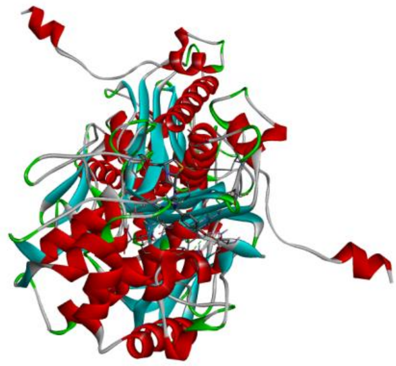
-6 to -7.9 kcal/mol, whereas dimethoxy piperazine derivatives [BA-17] show no hydrogen bond interaction in Table 3.

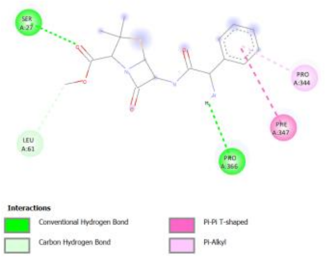
Table 3: Docking result of selected ligands having excellent binding energy with higher number of amino acids interaction.

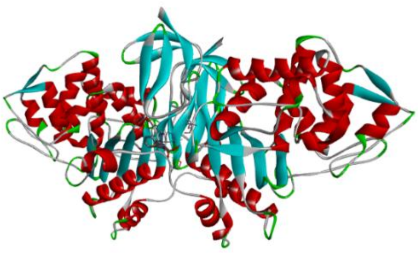
SN	Penicillin-Binding Protein [1TVF]	
	Binding Affinity	Amino Acid with Bond Length
BA-01	-6.5	GLN314: 2.48, 2.69,2.34, SERA27: 1.96, GLN64: 1.89
BA-02	-6.8	GLY375: 2.17, ASP358: 2.10, PRO376: 2.37
BA-03	-7	LEU62: 2.84, LEU61: 2.06, TYR315:2.35, GLN60: 2.9
BA-04	-6.6	SER116: 2.77, TYR: 2.91:3.08
BA-05	-6.5	GLU368: 2.70, SER27: 2.38, PRO366: 2.44
BA-06	-7.2	SER27: 2.23, ARG310: 2.95 GLN314: 2.51,2.61, LEU61: 2.15
BA-07	-6.5	TYR362: 3.09, GLY326: 2.65
BA-08	-6.6	THR237: 2.03, LYS119: 2.15
BA-09	-7.8	ASN72: 2.51, SER262: 3.17, SER116: 1.98
BA-10	-6.5	GLN314: 2.78, SER27: 2.06, TYR315: 2.23, TYR25: 2.55, LEU61: 2.72
BA-11	-7.1	GLU183: 2.30, SER75: 2.24, GLU114: 1.76, 2.85, ASN117: 2.50, SER116: 2.09
BA-12	-6.4	THR 25: 2.77, ASP28: 2.53, TYR315:1.95, ASP28: 2.53, GLU 314: 2.44, 2.51, LEU61: 2.53, 2.55
BA-13	-6.6	GLN314: 2.59, GLN64: 2.60, SER 27: 1.78
BA-14	-6.3	PRO 366: 2.20
BA-15	-5.7	LYS319: 2.63, SER 322: 2.62, GLN 326: 2.36
BA-16	-6.5	LYS 319: 2.63, SER322: 2.62, GLN326: 2.36
BA-17	-6.9	-
BA-18	-6.7	GLN314: 2.02, SER 27: 2.18, GLN64: 2.64, ARG310: 2.13
BA-19	-6	SER322: 2.63, LYS 319: 2.18, SER322: 2.13
BA-20	-6.2	TYR 315: 1.77, 2.38, 2.81
BA-21	-6.4	SER322: 1.95, GLN326: 2.87
BA-22	-7.1	SER322: 2.30, ILE320: 3.07, SER322: 3.03, GLN326: 2.52
BA-23	-7.6	GLN64: 1.85, TYR315: 2.38
BA-24	-7.2	LYS319: 2.11, ILE320: 2.45, LYS323: 2.42, 1.93
BA-25	-7.4	LYS319: 2.33, SER22: 2.74, LYS319: 2.16, SER322: 3.70
BA-26	-7.7	SER27: 2.09, GLY64: 2.14
BA-27	-7.6	SER116: 2.50, SER292: 2.18, ASN138: 2.48, 2.79, 2.12
BA-28	-7.4	TYR315: 2.70, GLN64: 2.28
BA-29	-8	GLN64: 2.20
BA-30	-7.8	GLN375: 2.35, THR378: 2.66, TYR317: 2.17
BA-31	-7.5	ARG300: 1.84, SER27: 2.17
BA-32	**-8.1**	SER27: 2.25, GLN64: 2.16, LEU61: 1.69, GLU368: 3.04

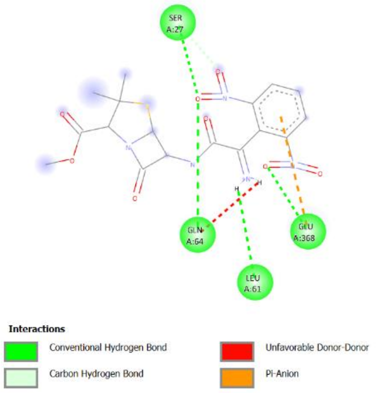
BA-33	**-8.2**	ARG186: 2.45, ASN141: 2.85, SER75: 2.57, LYS78:2.68, GLU114: 2.75, SER262: 2.16
BA-34	-7.4	TYR329: 2.95, SER262: 2.60, ARG300: 1.84, 2.05
BA-35	-6.5	GLN64: 2.40, LEU61: 2.29, TYR315: 2.57
BA-36	-6.5	TYR315: 2.81, LEU61: 2.38, TYR315: 2.32
BA-37	-6.3	SER27: 2.17, LEU61: 2.01, GLN64: 2.48, 2.61
AMX	**-7**	GLU114: 2.39, 2.48, TYR291: 2.99, GLY181: 2.90
AMP	**-6.4**	SER27: 2.31, PRO366: 2.81

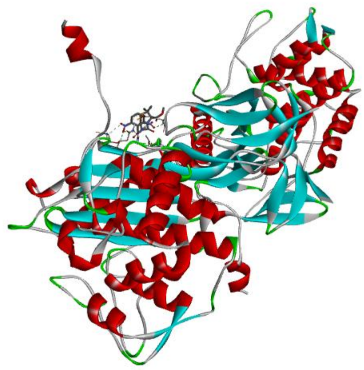












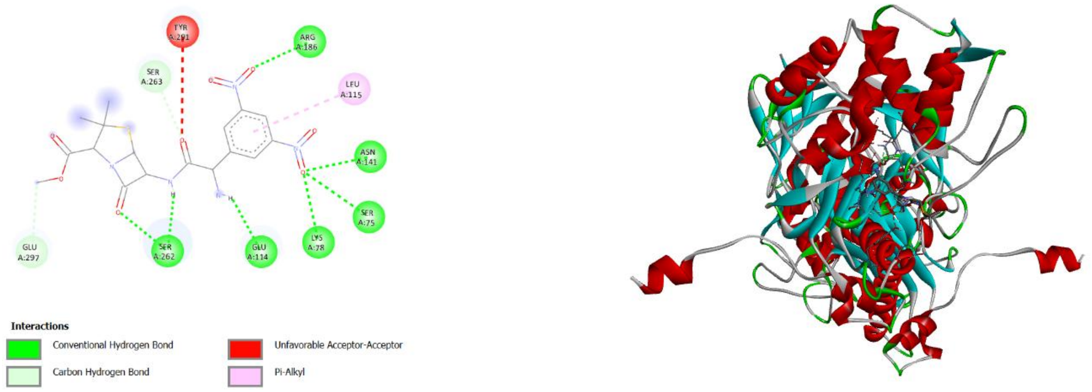


Figure 6: The 2D and 3D interaction of AMX, AMP, BA-32, BA-33 with 1TVF protein

Biological activity prediction:

Docking parameters and pharmacokinetic parameters are further supported by a biological activity prediction tool, which predicted the antibacterial, antibiotic, penicillin-like, and β-lactam-like activity of thirty-seven amoxicillin derivatives. Probability active molecule (Pa) for synthetic derivatives 0.549 < Pa < 0.756 for antibacterial, 0.383 < Pa < 0.575 for antibiotic, 0.301< Pa < 0.680 for antibiotic penicillin-like, and 0.093< Pa < 0.257 for antibiotic ß-lactam like was obtained. The probability to be active (Pa) value of all the bioactive compounds was greater than their probability to be inactive (Pi) values. This finding indicated that all these compounds can show activity against bacteria.

Table 4: Biological Activity Prediction

SN	Pa	Pi	Property
AMX	0,761	0,003	Antibacterial
	0,581	0,003	Antibiotic
	0,694	0,000	Antibiotic Penicillin-like
	0,278	0,001	Antibiotic beta Lactam-like
AMP	0,750	0,003	Antibacterial
	0,570	0,003	Antibiotic
	0,760	0,000	Antibiotic Penicillin-like
	0,381	0,003	Antibiotic beta Lactam-like
BA32	0,737	0,004	Antibacterial
	0,519	0,005	Antibiotic
	0,599	0,000	Antibiotic Penicillin-like
	0,222	0,001	Antibiotic beta Lactam-like
BA33	0,733	0,004	Antibacterial
	0,506	0,005	Antibiotic
	0,617	0,000	Antibiotic Penicillin-like
	0,227	0,001	Antibiotic beta Lactam-like

Lipinski's rule of five and ADME properties analyses

Docking data are further supported by physicochemical properties and absorption, distribution, metabolism, and excretion (ADME) properties of the drug, as shown in the table below. All the derivatives follow Lipinski's rule, i.e, Optimal molecular weight less than 500 Dalton, hydrogen bond acceptor ⩽ 10, and hydrogen bond donor ⩽ 5, have good lipophilic parameters, low skin permeability parameters, and further BA4-BA9 have high intestinal absorption value as compared to the standard.

Table 1: Lipinski's Rule and ADME Analysis

SN	RB	HBA	HBD	LogP	MW	Lipinski's Rule	GI Absorption	BBB	Skin Permeation cm/s	Drug likeliness
AMX	5	6	4	1.4	365	Yes	Low	No	-9.9	Yes
AMP	5	5	3	1.1	349	Yes	Low	No	-9.2	Yes
BA1	6	7	4	1.8	395	Yes	Low	No	-8.1	Yes
BA2	6	7	4	1.6	395	Yes	Low	No	-8.1	Yes
BA3	6	8	5	1.3	411	Yes	Low	No	-8.1	Yes
BA4	6	5	2	2.5	432	Yes	High	No	-7.0	Yes
BA6	6	5	2	2.8	432	Yes	High	No	-6.7	Yes
BA5	6	5	2	3.7	466	Yes	High	No	-7.0	Yes
BA8	6	7	2	2.0	399	Yes	High	No	-7.5	Yes
BA7	6	7	2	2.4	399	Yes	High	No	-7.5	Yes
BA9	6	8	2	2.5	417	Yes	High	No	-7.5	Yes
BA10	6	5	4	1.4	393	Yes	Low	No	-8.2	Yes
BA11	6	5	4	0.9	393	Yes	Low	No	-8.5	Yes
BA12	6	5	5	1.9	408	Yes	Low	No	-8,8	Yes
BA15	9	8	2	2.2	423	Yes	Low	No	-8.0	Yes
BA13	8	7	2	2.9	423	Yes	Low	No	-7.8	Yes
BA14	8	7	2	3.7	453	Yes	Low	No	-7.8	Yes
BA16	6	7	3	2.1	377	Yes	Low	No	-9.0	Yes
BA17	8	9	3	2.8	431	Yes	Low	No	-9.4	Yes
BA18	6	7	3	2.1	399	Yes	Low	No	-6,6	Yes
BA19	6	6	2	2.7	370	Yes	Low	No	-7.9	Yes
BA20	8	8	2	3.2	430	Yes	Low	No	-8.3	Yes
BA21	6	6	2	3.3	398	Yes	High	No	-7.5	Yes
BA22	9	7	4	1.6	491	Yes	Low	No	-9.1	Yes
BA23	9	6	3	3.3	490	Yes	Low	No	-8.0	Yes
BA24	9	6	3	2.5	492	Yes	Low	No	-8.2	Yes
BA25	9	7	3	1.4	476	Yes	Low	No	-8.6	Yes
BA26	9	6	4	2.3	477	Yes	Low	No	-8.5	Yes
BA27	9	8	5	2.2	493	Yes	Low	No	-9.0	Yes
BA28	9	8	4	2.2	495	Yes	Low	No	-8.7	Yes
BA29	9	6	3	2.6	474	Yes	Low	No	-8.2	Yes
BA30	9	8	3	2.0	494	Yes	Low	No	-8.3	Yes
BA31	9	7	4	1.8	476	Yes	Low	No	-9.0	Yes
BA32	8	9	2	1.1	453	Yes	Low	No	-8.2	Yes
BA33	8	9	2	2.0	453	Yes	Low	No	-8.2	Yes
BA34	9	9	2	1.4	498	Yes	Low	No	-8.6	Yes
BA35	8	7	2	0.8	419	Yes	Low	No	-8.5	Yes
BA36	8	7	2	1.3	429	Yes	Low	No	-8.5	Yes
BA37	9	8	2	1.1	447	Yes	Low	No	-9.1	Yes

Toxicity Prediction

The toxicity of all 37 novel penicillin derivatives was predicted. The toxicity profile of the active constituents indicated that none of them showed hepatotoxicity, carcinogenicity, or mutagenicity. However, BA32 shows low cytotoxicity, and BA33 and BA34 show low mutagenicity.

Note: Class of toxicity is given as: Class I: fatal if swallowed, Class II: fatal if swallowed, Class III: toxic if swallowed, Class IV: harmful if swallowed, Class V: may be harmful if swallowed, and Class VI: non-toxic (33).

Table 6: Toxicity prediction of standard and potential novel ligands

SN	Hepato-toxicity	Carcino-Genicity	Respir-Toxicity	Muta-genicity	Cyto-toxicity	Toxicity class
AMX	Inactive	Inactive	Inactive	Inactive	Inactive	6
AMP	Inactive	Inactive	Inactive	Inactive	Inactive	6
BA32	Inactive	Inactive	Inactive	Inactive	Active	5
BA33	Inactive	Inactive	Active	Active	Inactive	5
BA34	Inactive	Inactive	Inactive	Active	Inactive	5

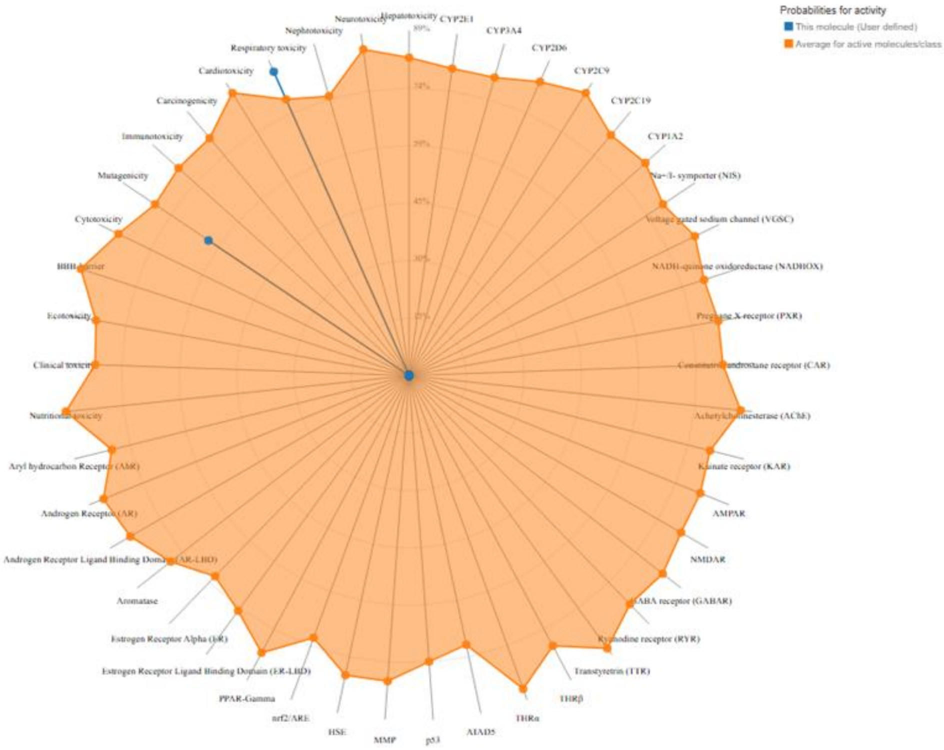


Figure 7 The Toxicity Rader Chart of BA-33

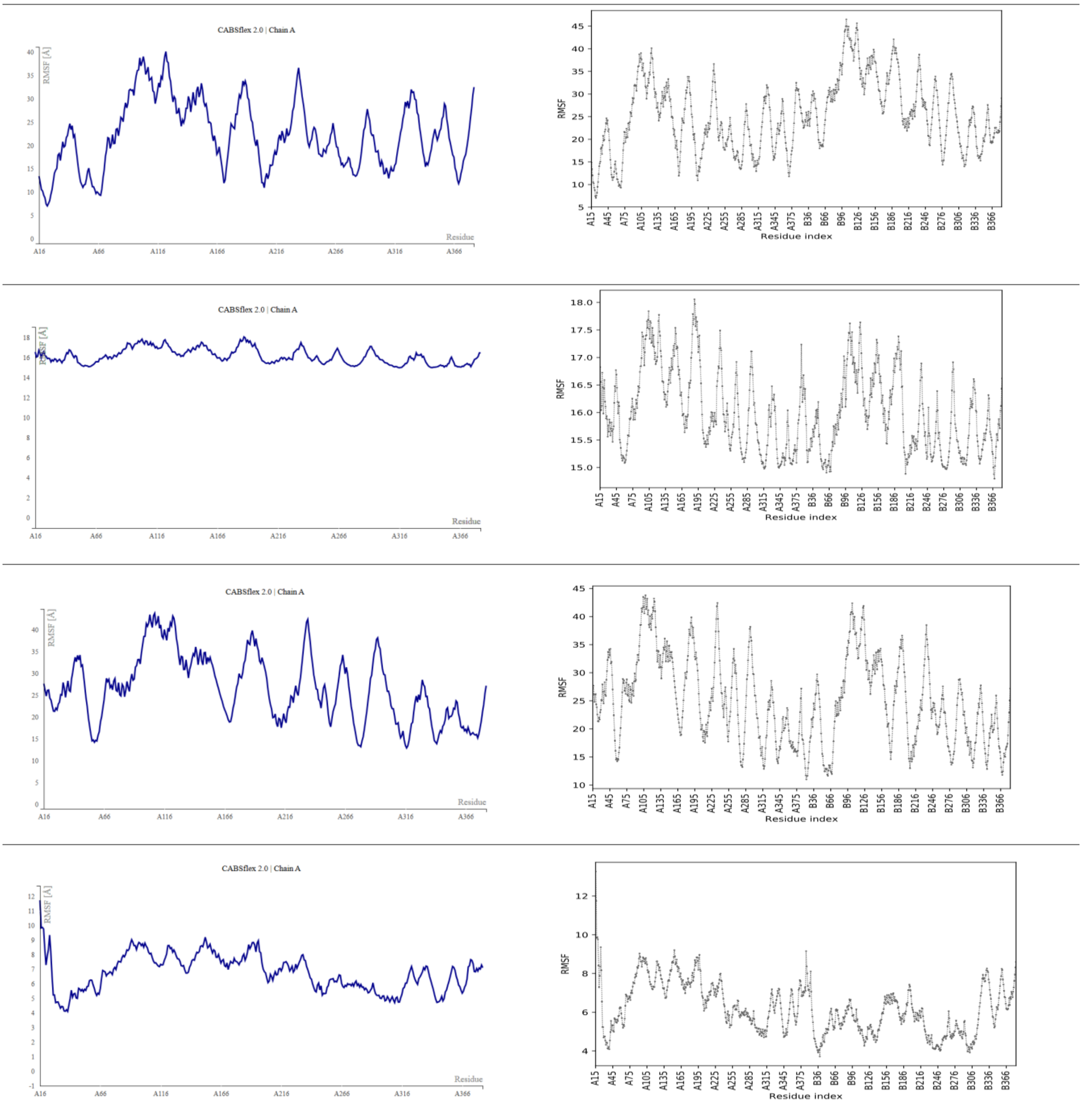
Molecular Dynamics Simulation

RMSF of Adducts

To further support the findings from our molecular docking analysis, we evaluated the stability of four different drug-protein complexes, including protein (BA-32, BA-33, AMX, AMP complex with 1TVF), through a server-based molecular dynamics (MD) simulation. The results of the simulations were analyzed by examining the Root Mean Square Fluctuation (RMSF) values, which reflect the flexibility and movement of the protein residues throughout the simulation. Lower RMSF values suggest limited conformational changes, indicating higher protein stability, while higher values point to increased flexibility [38].

RMSF graph, generated by the CABS-flex 2.0 server, represents X-Axis (Residue Index): Represents the amino acid sequence of the protein, Y-Axis (RMSF in Angstroms): Indicates the average displacement of each residue from its mean position. The RMSF value of 1TVF protein was found to be [46.50 Å-7.02 Å], 1TVF-AMX complex [18.05 Å-14.79 Å], 1TVF-AMP complex [43.79 Å-11.00 Å], 1TVF-BA-32 complex [13.26 Å-3.71 Å], and 1TVF-BA-33 complex [13.34 Å-3.61 Å].

The analysis revealed that the protein-ligand complexes 1TVF-AMP complex < 1TVF-AMX complex < 1TVF-BA-32 complex < 1TVF-BA-33 complex exhibited greater stability compared to the unbound protein 1TVF. The RMSF plots showed predominantly positive values, suggesting reduced fluctuations in the protein upon ligand binding as demonstrated by the RMSF values of the residues surrounding the ligand within the protein complex, as shown in Figure-8.



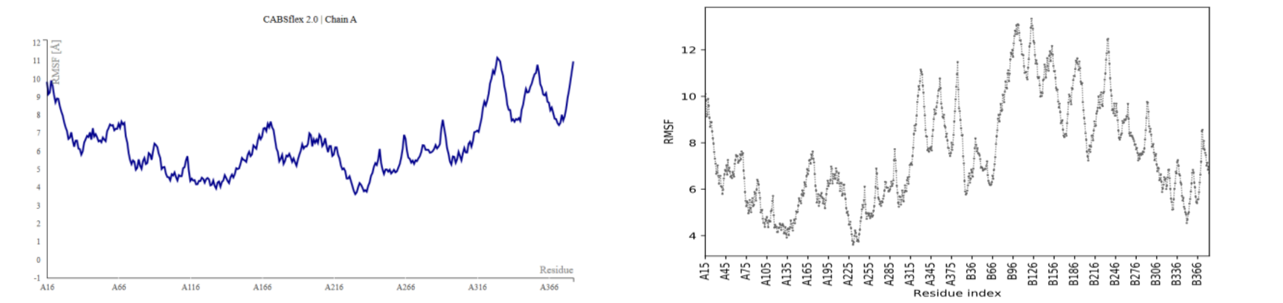


Figure 8: The RMSF graph of 1TVF protein and AMX, AMP, BA-32, BA-33 complex with 1TVF protein

Discussion

In this study, we investigated the resistance patterns and identified potential in-silico leads to address penicillin resistance in Uropathogenic *Staphylococcus spp.*, which are commonly associated with urinary tract infections (UTIs). Analyzing 3000 cases from July 2023 to January 2024, we observed a culture positivity rate of 8.2% (246 cases). This comparatively lower positivity rate, as opposed to earlier studies by Ghimire et al. [39] and Subedi et al. [40], may be attributable to regional variations, differences in population characteristics, or differing screening practices, such as testing asymptomatic individuals or routine pre-surgical screenings [41].The age distribution revealed a higher incidence of UTIs among the young reproductive age group, particularly those aged 31-60. This trend is likely linked to factors such as sexual activity and pregnancy, which facilitate bacterial introduction from the perineum to the bladder. This finding aligns with prior research [39, 42], emphasizing the influence of biological and behavioral factors on infection risk. Consistent with established evidence, UTIs were more prevalent in females in our study than in males. This is due to anatomical factors, such as the shorter female urethra and its proximity to the perineal region [43]. Furthermore, gram-negative bacteria were predominant (88.2%) in this study, with *Escherichia coli* (64.8%) being the most frequent isolate, corroborating existing literature that underscores its colonic origin [40, 41, 44]. Among gram-positive bacteria, *Staphylococcus aureus* accounted for 6.5% of isolates. Our findings are consistent with studies conducted by Shah et al. (2016) and Hp et al. (2012) at Tribhuvan University Teaching Hospital, where *Staphylococcus spp.* was identified as the second most common pathogen [45, 46]. However, in contrast, a study by Acharya et al. at Chitwan Medical College reported *S. aureus* as the least common UTI pathogen, identified in only 1 out of 237 isolates [43].

Antibiotic susceptibility testing revealed substantial resistance in *S. aureus* isolates to β-lactam antibiotics, including ampicillin (50%) and amoxicillin (18.75%). Our results are comparable to findings from Shah et al. (2016) at Tribhuvan University Teaching Hospital, where S. aureus exhibited high resistance rates to cefixime (71.4%), ampicillin (64.7%), and ciprofloxacin (60%) [45]. Similarly, Shakya et al. reported 80% resistance to amoxicillin/ampicillin in their isolates [41]. The increasing resistance to commonly used antibiotics can be attributed to factors such as self-medication, overuse, and the irrational prescription of antibiotics by medical practitioners [9]. This study highlights the persistent challenge posed by penicillin resistance in uropathogens, underscoring the urgent need for alternative therapeutic options.

To address this challenge, our in-silico analysis identified promising lead compounds through molecular docking. Specifically, novel penicillin derivatives such as 2-6 Di-Nitro Amoxicillin Derivatives [BA-32] and 3,5 Di-Nitro Amoxicillin Derivatives [BA-33] demonstrated high binding affinities -8.1 and -8.2 kcal/mol with essential target proteins 1TVF. The interactions involved crucial hydrogen bonds and fluorine-based interactions, consistent with findings by Gullapelli K. [47] and Shamsuddin et al. [48], which suggest that Di-Nitro aromatic rings enhance binding efficiency. Comparative docking studies further supported these results, highlighting that Di-Nitro aromatic compounds frequently exhibit superior binding interactions due to their electronegative properties [49].

Docking analyses of 37 penicillin derivatives against penicillin binding protein of S. aureus identified 2-6 Di-Nitro Amoxicillin Derivatives [BA-32] and 3,5 Di-Nitro Amoxicillin Derivatives [BA-33] as a standout compound, with strong binding interactions involving amino acids such as SER27: 2.25, GLN64: 2.16, LEU61: 1.69, GLU368: 3.04 and ARG186: 2.45, ASN141: 2.85, SER75: 2.57, LYS78:2.68, GLU114: 2.75, SER262: 2.16. These findings align with studies by Pant et al. [50] on hydroxycinnamic acids and Mustafa M. et al. [51] on pyridazine derivatives, demonstrating competitive binding energies for BA-32 and BA-33, which exceed those of traditional inhibitors.

The ADME profiles of these derivatives further confirm their potential as viable drug candidates. All tested compounds adhered to Lipinski's rule of five, indicating favorable drug-likeness. Compounds such as [BA-4–BA-9] exhibited high intestinal absorption, enhancing their bioavailability. While the derivatives showed higher lipophilicity compared to standard aminopenicillins, their limited gastrointestinal absorption and low skin permeability suggest controlled systemic distribution, minimizing risks of central nervous system toxicity [52]. These findings are consistent with prior research, which emphasizes the importance of selecting compounds with optimal LogP values for achieving balanced bioavailability and safety profiles [53].

Biological activity predictions further validated the therapeutic potential of these derivatives, with high Pa/Pi ratios for antibacterial, antibiotic, and β-lactam-like activities. Toxicity assessments classified the derivatives in Class V and VI, indicating minimal risks for hepatotoxicity, carcinogenicity, and mutagenicity. These results are in line with other studies predicting reduced toxicological risks for novel compounds, reinforcing their suitability as drug candidates [54]. Notably, compounds BA-32 and BA-33 exhibited the highest antibacterial potential, characterized by favorable hydrogen bonding interactions.

To validate and extend the molecular docking findings, molecular dynamics (MD) simulations were performed to assess the conformational stability of the ligand-bound complexes via Root Mean Square Fluctuation (RMSF) analysis. The RMSF profiles revealed a significant reduction in residue-level flexibility across all ligand-bound complexes compared to the unbound 1TVF protein (46.50–7.02 Å), confirming enhanced structural stabilization upon ligand binding. Crucially, the novel derivatives BA-33 and BA-32 exhibited the lowest fluctuations (13.34–3.61 Å and 13.26–3.71 Å, respectively), indicating superior complex rigidity. In contrast, conventional antibiotics showed higher flexibility (AMX: 18.05–14.79 Å; AMP: 43.79–11.00 Å), aligning with their weaker docking affinities. This stability gradient—1TVF-BA-33 > 1TVF-BA-32 > 1TVF-AMX > 1TVF-AMP—demonstrates that BA-33 and BA-32 induce the most conformationally stable protein-ligand interfaces. Such reduced mobility signifies persistent target engagement, critical for inhibiting penicillin-binding protein function in resistant *S. aureus*. These dynamic simulations corroborate the docking and ADME results, solidifying BA-33 as the lead candidate with optimal target stabilization for further development.

The combination of resistance profiling, molecular docking, ADME analysis, and MD simulation underscores the potential of these novel compounds to address penicillin resistance, paving the way for further preclinical and clinical evaluations.

Limitations and Future Directions

This study has several limitations that should be addressed in future research. It was based on retrospective data from a single hospital, limiting the generalizability of the findings. Although molecular docking and ADME analyses identified promising leads, the absence of experimental validation, such as in vitro or in vivo testing, limits the clinical applicability of the results. Additionally, while in-silico toxicity predictions indicated low risks, experimental toxicology studies are essential to confirm safety. Future research should expand to multicenter studies across diverse regions, validate identified compounds experimentally, explore alternative targets and combination therapies, and develop derivatives with broad-spectrum activity against both gram-positive and gram-negative pathogens. Comprehensive pharmacokinetic and toxicology evaluations, alongside high-throughput screening of other novel scaffolds, will be crucial for advancing these compounds toward clinical application. Future studies should also investigate the role of efflux pumps and other resistance mechanisms in limiting the efficacy of antibiotics.

Conclusions

This study highlights the critical challenge of penicillin resistance in uropathogenic *Staphylococcus spp*, particularly in UTIs. In-silico docking revealed that novel penicillin derivatives, such as 2-6 Di-Nitro Amoxicillin Derivatives [BA-32] and 3,5 Di-Nitro Amoxicillin Derivatives [BA-33], exhibited stronger binding affinities as well as stable ligand-protein complexes to penicillin-binding proteins compared to traditional β-lactam antibiotics, demonstrating significant potential as next-generation treatments. While most compounds passed ADME and toxicity analyses, among them derivatives like [BA-32] showed minor cytotoxicity, and [BA-33] and [BA-34] showed minor mutagenicity, requiring further optimization. 3,5 Di-Nitro Amoxicillin Derivatives [BA-33], in contrast, exhibited favorable binding energy, minimal toxicity, and promising pharmacokinetic properties, making it a strong candidate for further development. Future research should focus on in vitro and in vivo evaluations, investigate the role of efflux pumps in resistance, and explore combination therapies to address resistance mechanisms comprehensively.

Acknowledgements

The authors are grateful to the Department of Pharmacy, Manmohan Memorial Institute of Health Sciences, and Manmohan Memorial Teaching Hospital's staff and administration team for supporting and motivating the research work.

Author's contribution statement

S. Shrestha, B. Adhikari: Conceptualization, Supervision, Investigation, Methodology, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **R. B. Thapa, D. P. Khanal:** Conceptualization, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **P. Adhikari, P. Paudyal:** Conceptualization, Methodology, Data Curation, Formal Analysis Validation, Visualization, Funding acquisition, Writing – Review & Editing.

Conflict of Interest

No conflicts of interest are to be declared.

Data Availability Statement

Data will be made available from the corresponding author on reasonable request.

References

1. K. Gupta and W. E. Stamm, Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections, *Annals of Internal Medicine*, 2001, 135, 41-50.

(DOI:10.7326/0003-4819-135-1-200107030-00012)

2. D. De Backer and G. Verschraegen, Evolution of bacterial susceptibility pattern of Escherichia coli and Staphylococcus saprophyticus in uncomplicated urinary tract infections in women, *International Journal of Antimicrobial Agents*, 2008, 31, 492-497. (DOI: https://doi.org/10.1093/jac/dkn197)
3. R. Raksha and R. S. Macaden, Occurrence and characterization of uropathogenic isolates of Staphylococcus saprophyticus in urinary tract infections, *Indian Journal of Medical Microbiology*, 2003, 21, 112-116.
4. R. B. Thapa, S. Shrestha, P. Adhikari and R. Shrestha, Antibiotic resistance patterns in uropathogens: insights from a Nepalese tertiary care setting, *Therapeutic Advances in Infectious Disease*, 2025, 12, 20499361251339383.(DOI: https://doi.org/10.1177/20499361251339383)
5. R. C. Beier and S. D. Pillai, Escherichia coli and Staphylococcus aureus in human infections. In Food Microbiology: Fundamentals and Frontiers, *American Society of Microbiology*, 2003.
6. B. Foxman, Epidemiology of urinary tract infections: incidence, morbidity, and economic costs, *The American Journal of Medicine*, 2002, 113, 5-13. (DOI: https://doi.org/10.1067/mda.2003.7)
7. A. Flores-Mireles and S. J. Hultgren, Urinary tract infections: epidemiology, mechanisms of infection and treatment options, *Nature Reviews Microbiology*, 2015, 13, 269-284. (DOI: https://doi.org/10.1038/nrmicro3432)
8. A. Rusu and A. M. Cazan, Antibiotic resistance in urinary tract infections: A comparative study of Staphylococcus aureus and Escherichia coli in Romanian clinical settings, *Journal of Antimicrobial Chemotherapy*, 2023, 78, 679-686.
9. R. B. Thapa, P. Dahal, S. Karki and U. K. Mainali, Exploration of drug therapy related problems in a general medicine ward of a tertiary care hospital of Eastern Nepal, *Exploratory Research in Clinical and Social Pharmacy*, 2024, 16, 100528. (DOI: https://doi.org/10.1016/j.rcsop.2024.100528)
10. D. K. Neupane, R. B. Thapa, S. Shrestha, R. Basnet and N. Upreti, Knowledge and compliance with drug legislation in the community pharmacy of Dharan, *International Journal of Novel Research and Development*, 2024, 9.
11. K. Sapkota, A. K. Sah, R. B. Thapa, Y. Thapa, S. Dangi and R. K. Adhikari, Morbidity and Drug Prescribing Pattern in Pediatric Outpatient Department of Kankai Nagar Hospital of Eastern Nepal, *Sage Open Pediatrics*, 2025, 12, 30502225251319878. (DOI: 10.1177/30502225251319878)
12. S. Karki, R. B. Thapa and R. Shrestha, Exploring Potentially Inappropriate Medication Use on Elderly Patients in a General Medicine Ward Using 2023 AGS Beers Criteria, *Aging Medicine*, 2025, 8, 238-248. (DOI: https://doi.org/10.1002/agm2.70025)
13. B. T. R. Shrestha and R. Shrestha, High prevalence of antibiotic resistance in urinary pathogens isolated from pediatric UTIs in Nepal, *Infectious Diseases in Clinical Practice*, 2019, 27, 74-79.
14. R. Urban-Chmiel, A. Marek, D. Stępień-Pyśniak, K. Wieczorek, M. Dec, A. Nowaczek and J. Osek, Antibiotic Resistance in Bacteria—A Review, *Antibiotics*, 2022, 11, 1079. (DOI: https://doi.org/10.3390/antibiotics11081079)
15. S. Deyno and A. Astatkie, Resistance of Staphylococcus aureus to antimicrobial agents in Ethiopia: A meta-analysis, *Antimicrobial Resistance & Infection Control*, 2017, 6, 1-9. (DOI: https://doi.org/10.1186/s13756-017-0243-7)
16. A. S. Bazaid and et al., High prevalence of multidrug-resistant Staphylococcus aureus in Saudi Arabia: A cross-sectional study in urinary tract infections, *Saudi Medical Journal*, 2021, 42, 523-529.
17. I. A. Naqid and I. S. Ali, Methicillin-resistant Staphylococcus aureus in urinary tract infections in Iraq: Prevalence and susceptibility

patterns, *Journal of Infection and Public Health*, 2020, 13, 1210-1215. (DOI: https://doi.org/10.5812/mejrh.103529.)

18. M. Micoladze and T. Gotsadze, Economic burden of drug-resistant urinary tract infections, *Journal of Public Health Policy*, 2021, 42, 321-335.
19. S. Shrestha, D. P. Khanal, P. Adhikari, R. B. Thapa and R. Maji, Molecular Docking Studies on The Anti-Cancer Activity of Novel Tri-Substituted Fluoro Indole Derivatives Against Human Topoisomerase-Ii Enzyme using In-Silico Methods, *Journal of Manmohan Memorial Institute of Health Sciences*, 2024, 9, 35-42. (DOI: 10.3126/jmmihs.v9i1.68639)
20. S. Shrestha, R. B. Thapa, P. Adhikari and D. P. Khanal, Exploring Resistance of Fluoroquinolones in Uropathogenic E. Coli and Optimized Lead Prediction Through In-Silico, *Bulletin of Pioneering Researches of Medical and Clinical Science*, 2024, 3, 56-68. (DOI: https://doi.org/10.51847/bCkw7UO27g)
21. S. Shrestha, B. Adhikari, R. B. Thapa, P. Adhikari, M. Bajracharya, U. Giri and D. P. Khanal, Exploring Aminopenicillin Resistance in Uropathogenic Escherichia Coli and Optimized Lead Prediction to Overcome Resistance Through New Generation Tools, *Journal of Manmohan Memorial Institute of Health Sciences*, 2025, 10, 22-30. (DOI: https://doi.org/10.3126/jmmihs.v10i1.77714)
22. R. B. Thapa, S. Karki and S. Shrestha, Exploring potential drug-drug interactions in discharge prescriptions: ChatGPT's effectiveness in assessing those interactions, *Exploratory Research in Clinical and Social Pharmacy*, 2025, 17, 100564. (DOI: https://doi.org/10.1016/j.rcsop.2025.100564)
23. J. Roberts and J. H. Martin, Therapeutic drug monitoring of antimicrobials, *British Journal of Clinical Pharmacology*, 2019, 85, 402-415. (DOI: 10.1111/j.1365-2125.2011.04080.x)
24. D. Subedi and R. Gautam, Computational drug discovery: In silico methods in antibiotic resistance, *Journal of Pharmacological and Toxicological Methods*, 2019, 98, 106589.
25. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, *J Comput Chem*, 2009, 30, 2785-2791. (DOI: https://doi.org/10.1002/jcc.21256)
26. S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B. A. Shoemaker, P. A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang and E. E. Bolton, PubChem 2023 update, *Nucleic Acids Research*, 2022, 51, D1373-D1380. (DOI: https://doi.org/10.1093/nar/gkac956)
27. B. Cherinka, B. H. Andrews, J. Sánchez-Gallego, J. Brownstein, M. Argudo-Fernández, M. Blanton, K. Bundy, A. Jones, K. Masters, D. R. Law, K. Rowlands, A.-M. Weijmans, K. Westfall and R. Yan, Marvin: A Tool Kit for Streamlined Access and Visualization of the SDSS-IV MaNGA Data Set, *The Astronomical Journal*, 2019, 158, 74. (DOI: 10.3847/1538-3881/ab2634)
28. B. L. Jejurikar and S. H. Rohane, Drug designing in discovery studio, 2021, DOI: 10.5958/0974-4150.2021.00025.0 (DOI: 10.5958/0974-4150.2021.00025.0)
29. S. Dallakyan and A. J. Olson, Small-molecule library screening by docking with PyRx, *Methods Mol Biol*, 2015, 1263, 243-250. (DOI: 10.1007/978-1-4939-2269-7_19)
30. H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov and P. E. Bourne, The Protein Data Bank, *Nucleic Acids Res*, 2000, 28, 235-242. (DOI: https://doi.org/10.1093/nar/28.1.235)
31. W. Tian, C. Chen, X. Lei, J. Zhao and J. Liang, CASTp 3.0: computed atlas of surface topography of proteins, *Nucleic acids research*, 2018, 46, W363-W367. (DOI: https://doi.org/10.1093/nar/gky473)
32. A. Daina, O. Michielin and V. Zoete, SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules, *Nucleic acids research*, 2019, 47, W357-W364. (DOI: https://doi.org/10.1093/nar/gkz382)

33. P. Banerjee, A. O. Eckert, A. K. Schrey and R. Preissner, ProTox-II: a webserver for the prediction of toxicity of chemicals, *Nucleic acids research*, 2018, 46, W257-W263. (DOI: https://doi.org/10.1093/nar/gky318)
34. D. A. Filimonov, A. A. Lagunin, T. A. Gloriozova, A. V. Rudik, D. S. Druzhilovskii, P. V. Pogodin and V. V. Poroikov, Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource, *Chemistry of Heterocyclic Compounds*, 2014, 50, 444-457. (DOI: https://doi.org/10.1007/s10593-014-1496-1)
35. V. Ahmad, M. I. Khan, Q. M. S. Jamal, F. A. Alzahrani and R. Albiheyri, Computational Molecular Docking and Simulation-Based Assessment of Anti-Inflammatory Properties of Nyctanthes arbor-tristis Linn Phytochemicals, *Pharmaceuticals*, 2024, 17, 18. (DOI: https://doi.org/10.3390/ph17010018)
36. A. Madushanka, R. T. Moura, N. Verma and E. Kraka, Quantum Mechanical Assessment of Protein–Ligand Hydrogen Bond Strength Patterns: Insights from Semiempirical Tight-Binding and Local Vibrational Mode Theory, *International Journal of Molecular Sciences*, 2023, 24, 6311.
37. A. Kuriata, A. M. Gierut, T. Oleniecki, Maciej P. Ciemny, A. Kolinski, M. Kurcinski and S. Kmiecik, CABS-flex 2.0: a web server for fast simulations of flexibility of protein structures, *Nucleic Acids Research*, 2018, 46, W338-W343. (DOI: https://doi.org/10.1093/nar/gky356)
38. S. Singh, Q. Bani Baker and D. B. Singh, in *Bioinformatics*, eds. D. B. Singh and R. K. Pathak, Academic Press, 2022, DOI: 10.1016/B978-0-323-89775-4.00014-6,
39. S. Ghimire, S. Paudel, S. Kc, P. Gautam and V. Paudel, Epidemiological and Bacteriological Profile of Urinary Tract Infection and the Antibiotic Sensitivity Pattern of Causative Organisms in National Medical College and Teaching Hospital - A Retrospective Cross-Sectional Study, *Med Phoenix*, 2020, 5, 40-45. (DOI: 10.3126/medphoenix.v5i1.31398)
40. N. Subedi and S. Pudasaini, Bacteriological profile and antibiotic sensitivity pattern in patients with Urinary tract infection, *Journal of Pathology of Nepal*, 2017, 7, 1066-1069. (DOI: https://doi.org/10.3126/jpn.v7i1.16910)
41. S. Shakya, J. Edwards, H. A. Gupte, S. Shrestha, B. M. Shakya, K. Parajuli, H. P. Kattel, P. S. Shrestha, R. Ghimire and P. Thekkur, High multidrug resistance in urinary tract infections in a tertiary hospital, Kathmandu, Nepal, *Public Health Action*, 2021, 11, 24-31. (DOI: https://doi.org/10.5588/pha.21.0035)
42. P. Adhikari and D. P. Khanal, Antibiotic Resistance Patters and Evaluation of Treatment of Inpatient with Urinary Tract Infections in Two Tertiary Care Hospitals at Kathmandu Valley, *Journal of Pharmacy Practice and Community Medicine*, 2020, 6. (DOI: https://doi.org/10.5530/jppcm.2020.1.4)
43. A. Acharya, R. Gautam and L. Subedee, Uropathogens and their antimicrobial susceptibility pattern in Bharatpur, Nepal, *Nepal Med Coll J*, 2011, 13, 30-33. (DOI:
44. J. R. Dhungana, A. Budhathoki, G. Poudel, J. Basnet and R. Shah, Urinary Tract Infection among Patients Visiting Ganesh Man Singh Memorial Hospital and Research Center, Lalitpur, Nepal, *Tribhuvan University Journal of Microbiology*, 2019, 6, 108-112. (DOI: https://doi.org/10.3126/tujm.v6i0.26593)
45. G. Shah, B. Pokhrel, A. K. Shah, P. B. Bista and A. Bhattarai, Bacterial pathogens and antibiotic resistance patterns in children with urinary tract infection admitted at tertiary hospital in Nepal, *Journal of Patan Academy of Health Sciences*, 2016, 3, 32-35. (DOI: https://doi.org/10.3126/jpahs.v3i2.20274)
46. H. P. Kattel, S. K. Mishra, J. Acharya, M. R. Sigdel, N. Prasad, A. S. S. Shah, B. P. Rijal, J. B. Sherchand and B. M. Pokhrel, Antibiotic sensitivity profile of different uropathogens in a tertiary care center in Nepal, *Journal of Nepal association of medical laboratory science*, 2012, 11, 19-33.
47. K. Gullapelli, G. Brahmeshwari, M. Ravichander and U. Kusuma, Synthesis,

antibacterial and molecular docking studies of new benzimidazole derivatives, *Egyptian Journal of Basic and Applied Sciences*, 2017, 4, 303-309.
(DOI: 10.1016/j.ejbas.2017.09.002)

48. T. Shamsuddin, M. A. Hosen, M. S. Alam, T. B. Emran and S. M. A. Kawsar, Uridine derivatives: Antifungal, PASS outcomes, ADME/T, drug-likeliness, molecular docking and binding energy calculations, *Med. Sci. Int. Med. J*, 2021, 10, 1373-1386.
(DOI: 10.5455/medscience.2021.05.175)
49. N. Korol, O. Holovko-Kamoshenkova, R. Mariychuk and M. Slivka, Insights into bacterial interactions: Comparing fluorine-containing 1,2,4-triazoles to antibiotics using molecular docking and molecular dynamics approaches, *Heliyon*, 2024, 10, e37538. (DOI: https://doi.org/10.1016/j.heliyon.2024.e37538)
50. L. Pant, S. Thapa, B. Dahal, R. Khadka and M. S. Biradar, In Silico and In Vitro Studies of Antibacterial Activity of Cow Urine Distillate (CUD), *Evid Based Complement Alternat Med*, 2024, 2024, 1904763.
(DOI: https://doi.org/10.1155/2024/1904763)
51. M. Mustafa and Y. A. Mostafa, Antimicrobial Pyridazines: Synthesis, Characterization, Cytotoxicity, Substrate Promiscuity, and Molecular Docking, *Chemistry & Biodiversity*, 2020, 17, e2000100.
(DOI: 10.1002/cbdv.202000100)
52. A. Rauf, H. Khan, M. Khan, A. Abusharha, G. Serdaroğlu and M. Daglia, In Silico, SwissADME, and DFT Studies of Newly Synthesized Oxindole Derivatives Followed by Antioxidant Studies, *Journal of Chemistry*, 2023, 2023, 1-16.
(DOI: https://doi.org/10.1155/2023/5553913)
53. A. Daina, O. Michielin and V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, *Scientific Reports*, 2017, 7, 42717. (DOI: https://doi.org/10.1038/srep42717)
54. K. J. Downes and J. L. Goldman, Too much of a good thing: defining antimicrobial therapeutic targets to minimize toxicity, *Clinical Pharmacology & Therapeutics*, 2021, 109, 905-917. (DOI: https://doi.org/10.1002/cpt.2190