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Exploring Resistance of Fluoroquinolones in Uropathogenic E. Coli and Optimized Lead Prediction Through In-Silico

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Abstract

Fluoroquinolone resistance in uropathogenic Escherichia coli (UPEC) poses a significant challenge in the treatment of urinary tract infections (UTIs), a major global health issue. As resistance rates rise, particularly in developing countries, there is an urgent need for novel derivatives. This study aims to explore fluoroquinolone resistance in UPEC isolates and identify potential lead compounds using in-silico methods to optimize fluoroquinolone derivatives that may overcome resistance. A retrospective observational study of 2,306 UTI cases was conducted at a tertiary care hospital in Nepal, with 167 samples showing significant bacterial growth. Molecular docking using AutoDock 4.2 assessed the binding affinities of 42 novel fluoroquinolone derivatives against E. coli DNA gyrase (PDB ID: 4KFG). Drug-protein interactions were analysed with Drug Discovery Studio, pharmacokinetics with SwissADME, and biological activity and toxicity predictions with PASS Online and ProTox-II. E. coli was identified in 64.7% of cases, with significant resistance to fluoroquinolones (levofloxacin: 83.33%, ofloxacin: 57.14%, ciprofloxacin: 32.67%). Novel derivatives SP9, SP12, and SP42 exhibited superior binding affinities (-11.9, -11.7, and -11.6 kcal/mol), and SP25 interacted with the highest number of amino acids compared to standard fluoroquinolones, forming multiple hydrogen bonds with key residues in DNA gyrase. These derivatives complied with Lipinski's Rule of Five, suggesting favourable pharmacokinetics, though toxicity analysis revealed mutagenicity and other toxicities in SP9, SP12, and SP25. Considering fluoroquinolone resistance in E. coli at high levels, SP42 emerges as a promising candidate for future therapeutic development, while SP9, SP12, and SP25 require further optimization due to toxicity concerns.

Keywords: UTI, E-coli, Resistance, Docking, DNA-gyrase, Fluoroquinolones

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Introduction

Urinary tract infections (UTIs) are a significant public health issue, marked by the presence of microbial pathogens in the urinary system, including the kidneys, ureters, bladder, and urethra [1]. UTIs are among the most prevalent infectious diseases globally, with an estimated annual incidence of at least 250 million cases [2]. *Escherichia coli* (E. coli), the primary pathogen responsible for UTIs, accounts for approximately 80-90% of infections in children and is similarly prevalent among adult women [3, 4]. In Nepal, UTI prevalence is particularly concerning, with 20% of women experiencing at least one UTI in their lifetime and 3% experiencing recurrent infections annually [5]. Hospital-acquired UTIs contribute significantly to the burden, accounting for 23%-37% of infections, with a higher incidence rate of 37.84% during pregnancy [6, 7].

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The impact of UTIs extends beyond infection, contributing to substantial morbidity and mortality, particularly among hospitalized patients, where they represent 25-50% of all infections [8]. This high incidence affects patient health and imposes a considerable economic burden on healthcare systems, leading to increased healthcare expenditures and lost productivity due to missed workdays [8].

A wide range of pathogens can cause UTIs, including Gram-negative and Gram-positive bacteria, and even fungi. However, uropathogenic *Escherichia coli* (UPEC) is the most common cause of both uncomplicated and complicated UTIs, responsible for approximately 90% of community-acquired infections and a significant portion hospital-acquired cases [9]. The virulence of UPEC is primarily due to specific adhesin-encoding operons that facilitate adherence to uroepithelial cells, enabling colonization and subsequent infection [10].

Fluoroquinolones have historically been a cornerstone in the empirical treatment of UTIs due to their broad-spectrum activity and favourable pharmacokinetic properties. However, rising resistance rates among uropathogens, particularly *E. coli*, have raised significant concerns regarding their continued efficacy. The emergence of fluoroquinolone resistance is attributed to factors such as over-prescription, inadequate treatment regimens, and the inherent genetic adaptability of bacteria. Globally, fluoroquinolone resistance among uropathogenic E. coli is increasing, with rates as high as 76.2% in Iraq and concerning levels in the United States [11]. This issue is not confined to specific regions but is a global problem, particularly in developing countries where resistance rates often exceed 40% [12]. Numerous studies have documented the high prevalence of fluoroquinolone resistance among *E. coli* isolates from UTIs [13]. For example, a study in Spain reported that 22.9% to 30.8% of *E. coli* isolates were resistant to fluoroquinolones, consistent with other findings indicating a growing concern in healthcare settings [14].

The primary mechanism of action of fluoroquinolones involves the inhibition of two essential bacterial enzymes, DNA gyrase and topoisomerase IV, which are responsible for the supercoiling and relaxation of DNA during replication and transcription [9]. The binding affinities of these drugs to their targets can significantly influence their efficacy and the development of resistance in bacterial populations [15]. Resistance to fluoroquinolones can develop through several mechanisms, including mutations in the genes encoding these target enzymes, alterations in membrane permeability, and the acquisition of efflux pumps that actively transport the drug out of the bacterial cell [15].

In response to the growing threat of antibiotic resistance, there is an urgent need for innovative approaches to predict and mitigate resistance mechanisms. In silico methods, leveraging machine learning and genomic data, have emerged as promising tools for the identification of resistance genes and the prediction of antimicrobial susceptibility profiles [16, 17]. These computational approaches can facilitate the rapid assessment of resistance determinants, enabling clinicians to make informed decisions regarding empirical therapy and potentially guiding the development of novel therapeutic agents [18].

Molecular docking studies can provide insights into the interactions between fluoroquinolones and their targets, aiding in the identification of structural features that enhance or diminish drug efficacy. By utilizing computational methods, researchers can predict how specific mutations in target enzymes affect drug binding and resistance development [19]. These studies can also facilitate the design of novel fluoroquinolone derivatives with improved binding affinities and reduced resistance potential. Tools like AutoDock 4.2 are instrumental in examining drug-receptor interactions and optimizing lead compounds for better therapeutic outcomes.

Given the high resistance rates and the need for more effective treatment strategies, the empirical use of fluoroquinolones in UTI treatment may no longer be justified, especially in light of the high resistance rates reported in various studies [20]. A study conducted in Nepal highlights that irrational use of antimicrobials significantly contributes to drug therapy-related problems [21]. This issue is particularly concerning due to the high rates of antibiotic resistance observed in uropathogenic E. coli, which is the primary causative agent of urinary tract infections (UTIs) [22, 23]. The increasing prevalence of extended-spectrum beta-lactamase (ESBL) producing strains has raised concerns about treatment efficacy, as common antibiotics like ampicillin and ciprofloxacin show reduced effectiveness against these pathogens [22]. This resistance pattern presents a significant challenge for healthcare providers in Nepal, underscoring the need for advanced computational methods to predict and develop optimized treatment strategies against these resistant strains.

This study hypothesizes that newly synthesized fluoroquinolone derivatives can offer improved inhibition of DNA gyrase enzymes, providing a more effective treatment for resistant UTIs. By exploring the resistance of fluoroquinolones in uropathogenic *E. coli* at a Nepali tertiary care hospital and employing in silico approaches to predict optimized lead compounds, this research aims to pave the way for next-generation antimicrobials that are both effective against resistant strains and optimized for safety and efficacy.

Materials and Methods

Ethics approval

The ethical approval for the study protocol was obtained from the Institutional Review Committee of Manmohan Memorial Institute of Health Sciences College (MMIHS-IRC Ref.no.: NEHCO/IRC/080/24). Approval for the data collection from the Manmohan Memorial Teaching Hospital (MMTH), Kathmandu, was obtained from the Hospital board (Ref. No. 241).

Procedure of fluoroquinolones resistance prevalence determination

A retrospective observational study was conducted at Manmohan Memorial Teaching Hospital (MMTH) in Kathmandu. The study involved retrospective collecting data over a six-month period from both inpatient (IPD) and outpatient (OPD) departments, which was obtained from the medical record department of the Hospital. We focused on patients who had undergone urine culture and sensitivity testing in association with urinary tract infections (UTIs). Patients whose cultures did not result in bacterial isolation were excluded from the study. The collected data were then systematically entered into Excel 2019. Descriptive statistics were employed to analyze socio-demographic variables, and the percentage of fluoroquinolone resistance among isolated microorganisms was calculated.

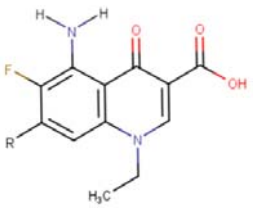
Procedure of lead optimization through in-silico methods

Molecular docking

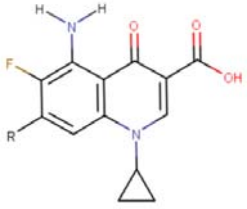
AutoDock 4.2 [24] was employed to predict how small molecules, including fluoroquinolone derivatives, bind to DNA gyrase of Escherichia coli (PDB ID: 4KFG), a known target for urinary tract infections (UTIs) [25]. Potential leads from novel fluroquinolone derivatives were identified through in-silico methods.

Preparation of ligand

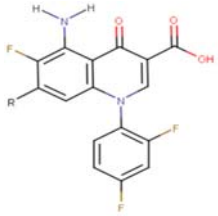
Ligands were prepared using various computational tools. PubChem [26] was utilized to obtain SDF files of existing fluoroquinolones, while Marvin Sketch [27] was used to construct novel fluoroquinolone derivatives in SDF format. These files were then converted into PDB format using Discovery Studio [28] and subsequently into PDBQT format via Autodock software [29]. The ligands were composed of a fused ring system (benzene and pyridine) with various substituents such as ethyl, cyclopropane, and 2-4-difluorobenzene rings at the N-1 position, along with functional groups like carboxylic acid at C-3, a keto group at C-4, an amino group at C-5, a fluoro atom at C-6, and various scaffolds [R] at C-7 as shown in **Figure 1**.



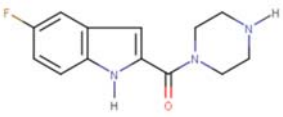
Basic nucleus 1



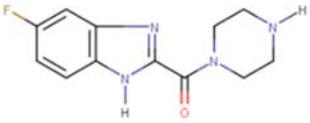
Basic nucleus 2



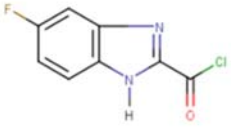
Basic nucleus 3



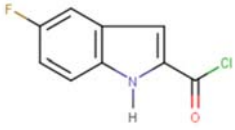
SP-1 SP-2 SP-3



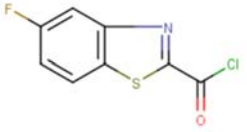
SP-4 SP-5 SP-6



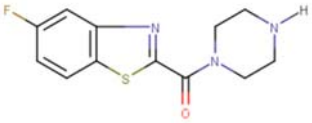
SP-7 SP-8 SP-9



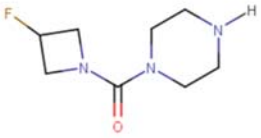
SP-10 SP-11 SP-12



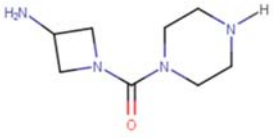
SP-13 SP-14 SP-15



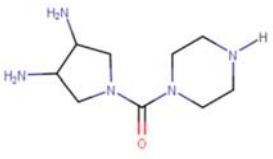
SP-16 SP-17 SP-18



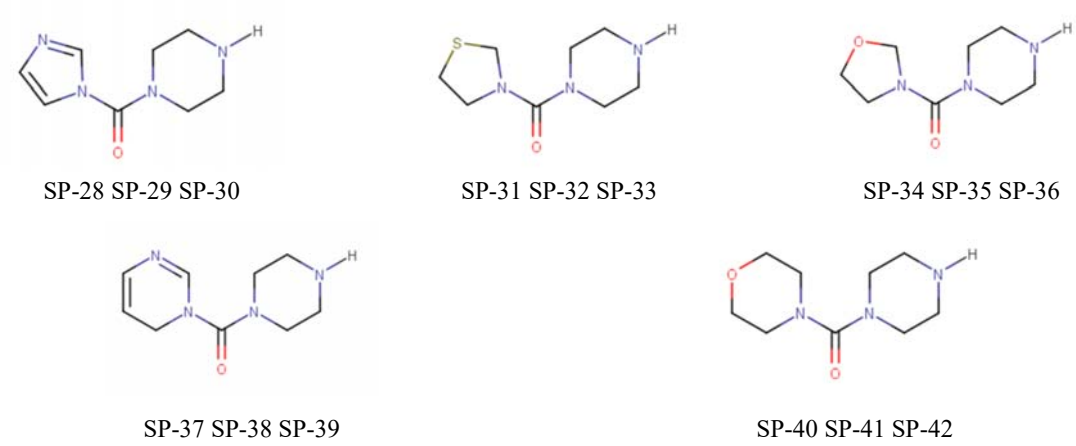
SP-19 SP-20 SP-21



SP-22 SP-23 SP-24



SP-25 SP-26 SP-27



SP-28 SP-29 SP-30 SP-31 SP-32 SP-33 SP-34 SP-35 SP-36

SP-37 SP-38 SP-39 SP-40 SP-41 SP-42

Figure 1. Three different basic nucleus of ligand and different scaffolds of basic nucleus [R]

Preparation of protein molecule

The protein structure of DNA gyrase from Escherichia coli was retrieved from the Protein Data Bank (PDB) ((https://www.rcsb.org/) using the ID 4KFG [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.

Identification of binding pocket

The active binding site of the protein was identified using the Cast-P server ((http://cast.engr.uic.edu), utilizing the amino acid sequence as the binding pocket for blind docking [31]. The amino acid residues involved in the active site were thoroughly analysed and listed for further study.

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:

$$\Delta G(\text{Binding}) = \Delta G(\text{Gauss}) + \Delta G(\text{Repulsion}) + \Delta G(\text{H-Bond}) + \Delta G(\text{Hydrophobic}) + \Delta G(\text{Tors}) \quad (1)$$

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.

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 analysed using Discovery Studio 2023.

Results and Discussion

Out of 2306 patients diagnosed with UTI, 167 samples showed significant growth of microorganisms, and all the samples consisted of a single microorganism. The gender distribution indicated a higher prevalence among females (69.5%) compared to males (30.5%). Age-wise, the majority of participants were between 31-60 years (43.11%), followed by those aged 1-30 years (37.12%), and a smaller portion of the sample aged 61-90 years (19.76%). Regarding bacterial classification, gram-negative bacteria were predominant, accounting for 88.6% of cases, whereas gram-positive bacteria were found in 11.4% of cases.

Twelve Pathogenic bacteria were isolated from 167 patients' urine samples, of which *E. coli* (108, 64.7%) was the most common isolate.

Out of 108 E-coli isolates tested for Ciprofloxacin, 68 were sensitive, while 33 were resistant, yielding a resistance rate of 32.67%. Levofloxacin, however, exhibited a much higher resistance rate. Among the 6 E. coli isolates tested, only 1 was sensitive, and 5 were resistant, leading to an 83.33% resistance rate, the highest among the three antibiotics. Ofloxacin showed intermediate results, with 3 of the 7 isolates being sensitive and 4 being resistant, resulting in a resistance rate of 57.14%. These findings indicate a significant level of resistance of E. coli to fluoroquinolones, particularly to Levofloxacin, suggesting that the effectiveness of these antibiotics may be limited in treating E. coli-related infections.

Molecular docking result

In our study, 42 novel fluoroquinolone ligands were docked with 4KFG (DNA gyrase of *Escherichia coli* protein 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 1**. The ligand showing the lowest binding affinity, a greater number of hydrogen bonds, a shorter bond distance, and a greater number of amino acid interactions was taken as the best ligand for further investigation.

Table 1. Docking result of selected ligands having excellent binding energy with higher number of amino acids interaction.

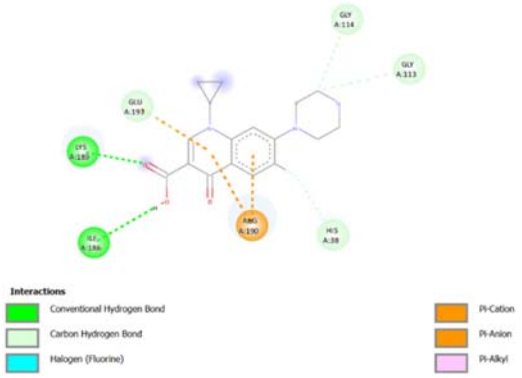
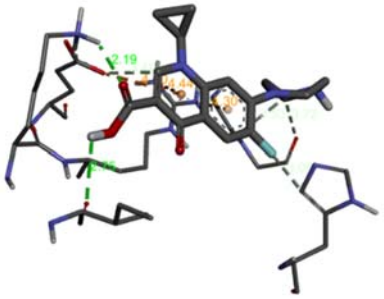
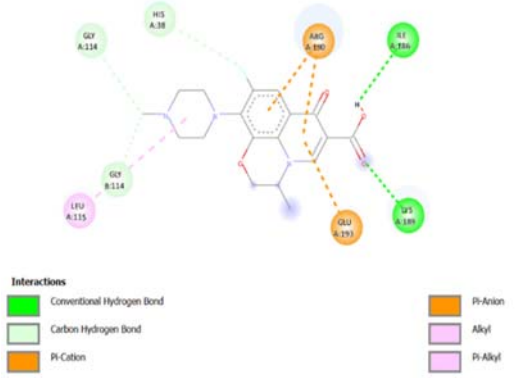
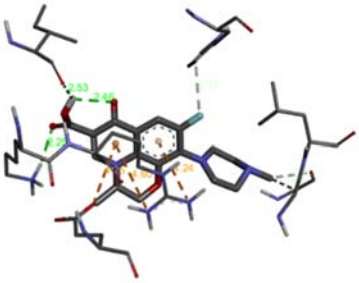
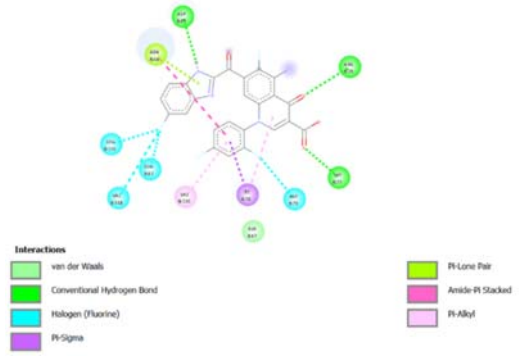
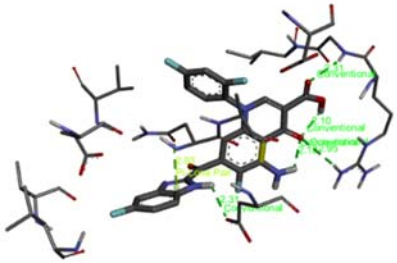
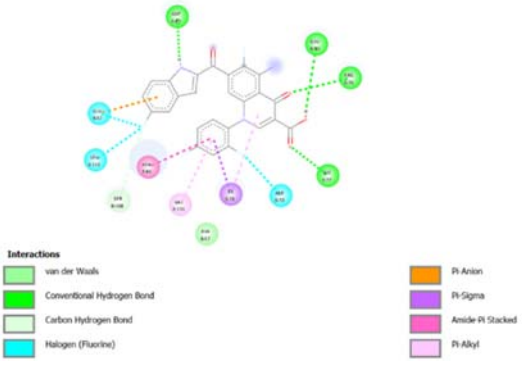
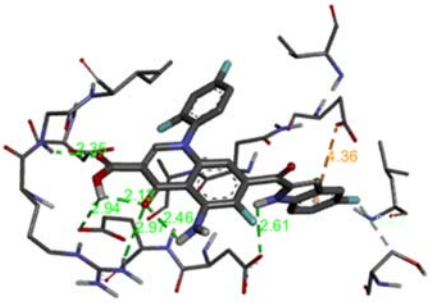
SN	Binding Energy (Kcal/mol)	H-Bond	Amino Acid position and Bond distance [Å]
CIP	**-7.5**	**2**	**LYS-189-2.1, ILE-186-2.7**
OFL	**-7.7**	**2**	**ILE-186-2.2, LYS-189-2.5**
SP1	-9.7	2	ARG-76-3.0, ASP-49-2.1
SP5	-9.6	4	GLY-77-2.3, GLU-42-2.4, VAL-118-2.5, VAL-111-2.3
SP8	-9.9	4	ARG-76-2.5, GLU-50-2.4, GLY-77-2.3, ASP-49-2.5
SP9	**-11.9**	**3**	**ASP-49-2.3, ARG-76-2.1, GLY-77-2.1**
SP12	**-11.7**	**4**	**ASP-49-2.1, GLU-50-2.3, ARG-76-2.1, GLY-77-2.9**
SP15	-11.5	3	ARG-76-2.1, GLU-50-2.9, GLY-77-2.6
SP18	-10.9	3	VAL-111-2.4, GLY-77-2.6, ARG-76-2.8
SP24	-10.5	4	ARG-76-2.7, ILE-90-2.7, VAL-93-2.5, GLY-77-2.3
SP25	**-9.2**	**5**	**ARG-76-2.8, GLY-77-2.2, ILE-90-2.2, VAL-93-2.2, LEU-94-2.3**
SP27	-10.1	3	GLY-77-2.23, ARG-76-2.5, LEU-94-1.9
SP28	-8.5	3	GLY-77-2.1, ASN-46-2.8, ASP-49-2.9
SP30	-10.0	3	ASP-106-2.3, ASN-107-2.1, GLU-50-2.0
SP42	**-11.6**	**2**	**GLY-77-2.7, ARG-76-2.9**

The standard ligands Ciprofloxacin and Ofloxacin showed the minimum binding energy with the DNA-gyrase of *E-coli* protein 4KFG. The minimum binding energy ΔG = -7.5 kcal/mol was exhibited by ciprofloxacin with two H-bonds LYS-189 and ILE-186 with a bond distances of 2.19 and 2.75 angstroms, whereas minimum binding energy ΔG = -7.7 kcal/mol was exhibited by ofloxacin with two H-bonds LYS-189 and ILE-186 with a bond distance of 2.53 and 2.25 angstroms respectively.

The ligand having Fluro-Indole [SP12], Fluoro-Benzimidazole [SP9], Fluoro-Benzothiazole [SP13, SP15], Morpholino [SP42], Amino-Azetidine [SP23, SP24] and Diamino Pyrrolidine [SP25, SP26, SP27] substituted derivatives showed the maximum binding energy and highest number of hydrogen bond with the protein 4KFG. The maximum binding energy was exhibited by fluoro-benzimidazole, fluoro-indole, and morpholino-substituted derivatives, and the maximum hydrogen bond was exhibited by diamino-pyrrolidine substituted derivatives.

Fluoro-benzimidazole [SP9] exhibited maximum binding affinity ΔG = -11.9 Kcal/mole having three hydrogen bonds ASP-49, ARG-76, GLY-77 with bond length 2.3, 2.1, 2.1 Angstroms. Fluoro-indole [SP12] exhibited maximum binding affinity ΔG = -11.7 Kcal/mole having four hydrogen bonds ASP-49, GLU-50, ARG-76, GLY-77 with bond length 2.1, 2.3, 2.1, 2.9 Angstroms and morpholino [SP42] exhibited maximum binding affinity

ΔG = -11.6 Kcal/mole having two hydrogen bonds GLY-77, ARG-76 with bond length 2.7, 2.9 Angstroms. Diamino pyrrolidine [SP25] exhibited maximum number of hydrogen bonds [five hydrogen bonds] ARG-76, GLY-77, ILE-90, VAL-93, LEU-94 with bond distance 2.8, 2.2, 2.2, 2.2, 2.3 Angstroms. The 2D and 3D interaction of CIP, OFL, SP-9, SP-12, SP-25, SP-42 was represented in **Figure 2**.

SN	2D-INTERACTION	3D-INTERACTION
CIP		
OFL		
SP-9		
SP-12		

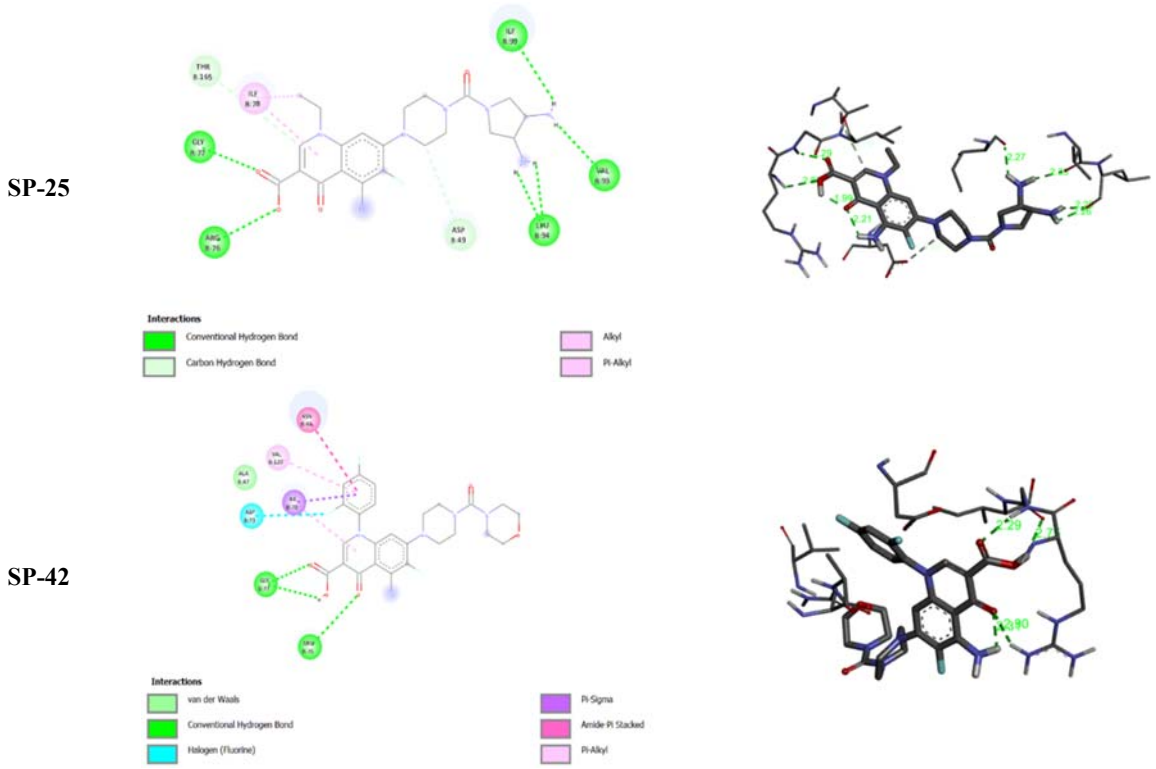


Figure 2. The 2D and 3D interaction standard ligand and potential novel molecules

Biological activity prediction

Docking parameters are further supported by the biological activity prediction tools, which predict the anti-bacterial and DNA-gyrase inhibitors, such as the activity of forty-two novel fluoroquinolones derivatives. The probability to be active (Pa) value of all the bioactive compounds were greater than their probability to be inactive (Pi) values. This finding indicated that all these compounds, including potential molecules SP9, SP12, SP25, and SP42, showed good biological activity against UPEC bacteria, as shown in **Table 2**.

Table 2. Pa and Pi value of standard and novel ligands

Compounds	Biological activity [Pa>Pi]	[Pa] value	[Pi] value
CIP	Anti-bacterial activity	0.588	0.009
	DNA-gyrase inhibitors	0.468	0.001
OFL	Anti-bacterial activity	0.636	0.007
	DNA-gyrase inhibitors	0.387	0.001
SP1	Anti-bacterial activity	0.438	0.023
	DNA-gyrase inhibitors	0.079	0.004
SP5	Anti-bacterial activity	0.480	0.018
	DNA-gyrase inhibitors	0.292	0.002
SP8	Anti-bacterial activity	0.446	0.022
	DNA-gyrase inhibitors	0.126	0.003
SP9	**Anti-bacterial activity**	**0.441**	**0.023**
	DNA-gyrase inhibitors	**0.073**	**0.004**
SP12	**Anti-bacterial activity**	**0.473**	**0.019**
	DNA-gyrase inhibitors	**0.076**	**0.004**
SP15	Anti-bacterial activity	0.570	0.011

SP18	DNA-gyrase inhibitors	0.067	0.004
	Anti-bacterial activity	0.473	0.019
SP24	DNA-gyrase inhibitors	0.133	0.003
	Anti-bacterial activity	0.543	0.013
SP25	DNA-gyrase inhibitors	0.215	0.002
	Anti-bacterial activity	**0.518**	**0.015**
SP27	**DNA-gyrase inhibitors**	**0.180**	**0.002**
	Anti-bacterial activity	0.519	0.015
SP28	DNA-gyrase inhibitors	0.260	0.002
	Anti-bacterial activity	0.405	0.029
SP30	DNA-gyrase inhibitors	0.072	0.004
	Anti-bacterial activity	0.406	0.028
SP42	DNA-gyrase inhibitors	0.100	0.003
	Anti-bacterial activity	**0.456**	**0.021**
	DNA-gyrase inhibitors	**0.193**	**0.002**

Lipinski's rule of five and ADME properties analyses

We analysed all the derivatives and found that none of them violated Lipinski's rule of five, as shown in **Table 3**. This rule helps filter candidates to assess their drug-likeness and durability. Absorption, distribution, metabolism, and excretion (ADME) properties of all derivatives were determined by the swissADME web server, and the result is shown in **Table 4**. Positive findings from the examination of the prospective compounds SP9, SP12, SP25, and SP42's ADME characteristics and Lipinski's rule of five supported their further research and development.

Table 3. Lipinski's rule of five analysis

Lipinski's rule of five					
Compound	**Molecular Weight**	**Rotatable bond**	**Hydrogen bond acceptor**	**Hydrogen bond doner**	**Log-P**
CIP	331	3	5	2	2.24
OFL	361	2	6	1	2.49
SP1	495	5	3	6	2.22
SP5	508	5	3	7	2.08
SP8	424	4	3	7	1.81
SP9	**496**	**4**	**3**	**9**	**2.09**
SP12	**495**	**4**	**3**	**8**	**1.96**
SP15	513	5	2	9	2.49
SP18	597	5	2	9	2.99
SP24	516	5	3	8	2.59
SP25	**461**	**5**	**4**	**7**	**1.59**
SP27	545	5	4	9	2.12
SP28	428	5	2	6	2.1
SP30	512	5	2	8	2.08
SP42	**531**	**5**	**2**	**8**	**2.7**

Table 4. Pharmacokinetics Analysis

ADMET PROPERTIES							
Compound	**Water solubility**	**BBB**	**Intestinal Abs.**	**Skin Per.**	**Drug likeness**	**BA score**	**Synthetic accessibility**

CIP	-3.5	No	High	-9.0	Yes	0.5	2.5
OFL	-3.1	No	High	-8.7	Yes	0.5	3.6
SP1	-5.1	No	High	-6.9	Yes	0.5	3.3
SP5	-4.9	No	Low	-7.2	Yes	0.5	3.4
SP8	-4.7	No	Low	-6.6	Yes	0.5	2.9
SP9	**-6.0**	**No**	**Low**	**-6.1**	**Yes**	**0.5**	**3.1**
SP12	**-6.3**	**No**	**Low**	**-5.7**	**Yes**	**0.5**	**3.1**
SP15	-6.6	No	Low	-5.6	Yes	0.5	3.3
SP18	-6.8	No	Low	-6.3	Yes	0.5	3.7
SP24	-1.4	No	High	-11.2	Yes	0.5	3.4
SP25	**-0.6**	**No**	**Low**	**-11.3**	**Yes**	**0.5**	**4.2**
SP27	-2.1	No	Low	-10.8	Yes	0.1	4.4
SP28	-3.3	No	High	-7.9	Yes	0.5	3.2
SP30	-4.8	No	High	-7.4	Yes	0.5	3.4
SP42	**-3.4**	**No**	**High**	**-9.1**	**Yes**	**0.5**	**3.6**

Toxicity prediction

The toxicity of all 42-novel fluoroquinolone derivatives was predicted, and the result of selected molecules is shown in **Table 5**. Most of the derivatives exhibit hepatotoxicity and mutagenicity, with fewer cases showing cytotoxicity, carcinogenicity, and immunotoxicity. Compounds SP-9 and SP-12 demonstrate hepatotoxicity, mutagenicity, immunotoxicity, and carcinogenicity. SP-25 shows mutagenicity, while SP-42 is free from all of these toxicities. Different class of toxicity indicates as follows: 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.

Table 5. Toxicity prediction of standard and potential novel ligands

Compounds	Toxicity class	LD (50)	Hepato-toxicity	Cyto-toxicity	Carcino genicity	Muta genicity	Immuno toxicity
CIP	iv	2000	Inactive	Inactive	Inactive	Active	Inactive
OFL	iv	1478	Inactive	Inactive	Inactive	Active	Inactive
SP1	iv	2000	Inactive	Inactive	Inactive	Active	Active
SP5	iv	2000	Active	Inactive	Inactive	Active	Inactive
SP8	v	4000	Active	Inactive	Active	Active	Active
SP9	**v**	**4000**	**Active**	**Inactive**	**Active**	**Active**	**Active**
SP12	**v**	**4000**	**Active**	**Inactive**	**Active**	**Active**	**Active**
SP15	iv	2000	Active	Inactive	Inactive	Active	Inactive
SP18	iv	2000	Active	Inactive	Inactive	Inactive	Inactive
SP24	iv	2000	Inactive	Inactive	Inactive	Inactive	Inactive
SP25	**iv**	**2000**	**Inactive**	**Inactive**	**Inactive**	**Active**	**Inactive**
SP27	iv	2000	Inactive	Inactive	Inactive	Inactive	Inactive
SP28	iv	2000	Inactive	Inactive	Inactive	Active	Inactive
SP30	iv	2000	Inactive	Inactive	Inactive	Inactive	Inactive
SP42	**iv**	**2000**	**Inactive**	**Inactive**	**Inactive**	**Inactive**	**Inactive**

Bacterial resistance is a growing global concern, and the study's dataset comprised 2,306 samples, of which 167 showed significant microbial growth. Escherichia coli (E. coli) was the most frequently isolated bacterium (64.7%), a pattern consistent with similar studies in Iraq and Nepal, which reported E. coli prevalence rates of 75% and 53.5%,

respectively [35, 36]. The incidence of urinary tract infections (UTIs) was higher in females (69.5%) than in males (30.5%), corroborating findings from studies conducted in India [37]. The higher susceptibility in females is linked to anatomical factors, such as the shorter urethral length and proximity to the anus, increasing contamination risk from faecal flora [37]. Most specimens were collected from patients aged 31–60 years (43.11%), a trend also observed in other studies [38].

Fluoroquinolone resistance was particularly high, with resistance rates of 83.33% for levofloxacin, 57.14% for ofloxacin, and 32.67% for ciprofloxacin. These findings are in line with global trends of rising resistance to these antibiotics [39]. The widespread resistance highlights the urgent need for newer, more potent fluoroquinolone derivatives.

Molecular docking studies assessed the binding affinities of fluoroquinolone derivatives to E. coli DNA gyrase (PDB ID: 4KFG). Ciprofloxacin and ofloxacin exhibited binding energies of -7.5 kcal/mol and -7.7 kcal/mol, respectively, which are consistent with other reports [40, 41]. Novel derivatives such as SP9, SP12, and SP42 demonstrated much higher binding affinities (-11.9 kcal/mol, -11.7 kcal/mol, and -11.6 kcal/mol, respectively), with interactions involving key amino acids like ASP-49, ARG-76, and GLY-77. Multiple hydrogen bonds and excellent interactions with active-site residues are responsible for the superior binding of SP9, SP12, SP25, and SP42. These findings were also noted in experiments by Abdel-Aziz *et al*. wherein bulkier fluoroquinolone derivatives showed a better binding affinity to DNA gyrase [41]. These enhanced interactions suggest that structural modifications in the fluoroquinolone core, especially at the C-7 position, contribute significantly to overcoming bacterial resistance [40].

The synthesis of new fluoroquinolone derivatives, as reported by Patel *et al*. highlights the effectiveness of incorporating bulkier moieties to enhance activity against drug-resistant strains [41]. In our study, derivatives such as SP9, SP12, SP25, and SP42, which contain fluoro-benzimidazole, fluoro-indole, Diamino-pyrrolidine, and morpholino substitutions, demonstrated superior binding energies ranging from -11.9 to -9.2 kcal/mol compared to ciprofloxacin (CIP) and ofloxacin (OFL). This aligns with previous research that suggests structural modifications—especially the addition of bulkier arenesulfonyl or benzene-sulfonamido groups—can significantly improve antimicrobial activity [40, 41].

Studies by Abdel-Aziz *et al*. and Patel *et al*. further support the critical role of substitutions at the C-7 position in enhancing the antibacterial efficacy of fluoroquinolones against resistant bacterial strains [40, 41]. Specifically, derivatives with bulkier arenesulfonyl fragments attached to the C-7 piperazine ring demonstrated superior binding affinities compared to standard drugs such as CIP and OFL [40]. This observation is consistent with our findings, where novel derivatives like SP9, SP12, SP25 and SP42 exhibited stronger interactions with DNA gyrase of E. coli, forming multiple hydrogen bonds with key residues such as ASP-49, ARG-76, and GLY-77. These interactions, combined with favourable bond lengths, enhanced the inhibitory effects on bacterial DNA replication.

The ADME (absorption, distribution, metabolism, and excretion) properties of the novel derivatives demonstrated strong compliance with Lipinski's Rule of Five, indicating their drug-likeness and potential for oral bioavailability [42]. SP9, SP12, SP25 and SP42 displayed favourable pharmacokinetic characteristics, including low skin permeability, minimal penetration of the blood-brain barrier, high drug likeliness and Synthetic accessibility parameters. These findings are in line with earlier research in which new fluoroquinolone compounds showed comparable pharmacokinetic characteristics, including poor skin permeability [40]. Our results further support the development of SP9, SP12, SP25, and SP42 as viable therapeutic candidates by confirming their potential for further in vivo studies [41].

However, toxicity assessments revealed hepatotoxicity and mutagenicity in some derivatives, notably SP9 and SP12, which raises concerns about their clinical applicability. This result aligns with findings from Patel *et al*. who observed that bulky derivatives like 8g could induce similar toxicities yet with no carcinogenicity [41]; structural optimization is needed to mitigate these toxic effects without compromising antibacterial efficacy.

Limitations and future directions

While our study highlights the potential of novel fluoroquinolone derivatives to overcome resistance in uropathogenic E. coli, it is important to recognize the limitations. The in-silico docking and ADME analyses provide valuable insights, but in-vitro and in-vivo validation are necessary to confirm the efficacy and safety of these compounds. Moreover, structural optimization is essential to reduce the observed toxicity profiles. Future studies should also investigate the role of efflux pumps and other resistance mechanisms in limiting the efficacy of fluoroquinolone derivatives.

Conclusion

This study highlights the growing issue of fluoroquinolone resistance in E. coli strains, particularly in UTIs. In-silico docking revealed that novel derivatives, such as SP9, SP12, SP25, and SP42, exhibited stronger binding to bacterial DNA gyrase compared to traditional fluoroquinolones, showing potential as next-generation treatments. Further optimisation is necessary,

nevertheless, due to worries about mutagenicity and other toxicities in SP9, SP12, and SP25. Conversely, 2-4 Difluoro Benzene with morpholino scaffolds [SP42] was a perfect candidate for continued development because it passed all analyses, including the toxicity prediction. In order to successfully address resistance mechanisms, future research should concentrate on in-vitro and in-vivo evaluations and investigate combination therapy.

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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.
2. Czajkowski K, Broś-Konopielko M, Teliga-Czajkowska J. Urinary tract infection in women. Menopause Rev. 2021;20(1):40-7. doi:10.5114/pm.2021.105382
3. Muntean C, Săsăran MO. Vitamin D status and its role in first-time and recurrent urinary tract infections in children: A case-control study. Children. 2021;8(5):419. doi:10.3390/children8050419
4. Bargotya M, Kumar L, Kachhap P, Das P, Sachdeva V, Bhattar S. A flow cytometric and cytochemistric analysis of urine to detect early urinary tract infection. Ann Pathol Lab Med. 2020;7(1):A7-12. doi:10.21276/apalm.2659
5. Thapa P, Sunar A, Lamichanne D, Apeksha KC, Dhungana A, Paudel R, et al. Uropathogenic bacteria and antimicrobial sensitivity pattern among diabetic patients with urinary tract infection. Microbiol Res J Int. 2020;30(10):85-92. doi:10.9734/mrji/2020/v30i1030276
6. Sharma D, Preston S, Hage R. Emerging antibiotic resistance to bacterial isolates from human urinary tract infections in grenada. Cureus. 2019;11(9). doi:10.7759/cureus.5752
7. Kayastha B, Tamrakar SR. Maternal and perinatal outcome of urinary tract infection in pregnancy at Dhulikhel hospital, Kathmandu university hospital. Kathmandu Univ Med J. 2022;20(1):82-6. doi:10.3126/kumj.v20i1.49944
8. Alhomayani F, Alazwari NM, Alshhrani MS, Alkhudaydi AS, Basaba AS, Alharthi T, et al. The prevalence of multiple drug resistant urinary tract infections. Saudi Med J. 2022;43(8):927-32. doi:10.15537/smj.2022.43.8.20220238
9. Zeind CS, Carvalho MG, Cheng JW, Zaiken K, LaPointe T. Applied therapeutics: The clinical use of drugs: Lippincott Williams & Wilkins; 2023.
10. Kim B, Kim JH, Lee Y. Virulence factors associated with Escherichia coli bacteremia and urinary tract infection. Ann Lab Med. 2022;42(2):203-12. doi:10.3343/alm.2022.42.2.203
11. Choudhury D, Alanbari R, Saveliev P, Sokurenko E, Fuzi M, Tchesnokova V. Clonal and resistance profiles of fluoroquinolone-resistant uropathogenic Escherichia coli in countries with different practices of antibiotic prescription. Front Microbiol. 2024;15:1446818. doi:10.3389/fmicb.2024.1446818
12. Akgoz M, Akman I, Ates AB, Celik C, Keskin B, Ozmen Capin BB, et al. Plasmidic fluoroquinolone resistance genes in fluoroquinolone-resistant and/or extended spectrum beta-lactamase-producing Escherichia coli strains isolated from pediatric and adult patients diagnosed with urinary tract infection. Microb Drug Resist. 2020;26(11):1334-41. doi:10.1089/mdr.2020.0007
13. Faine B, Rech MA, Vakkalanka P, Talan DA. 1419. High prevalence of fluoroquinolone-resistant urinary tract infection among US emergency department patients diagnosed with UTI, 2018-2020. Open Forum Infect Dis. 2021;8(Supplement_1):S793-S4. doi:10.1093/ofid/ofab466.1611
14. Stapleton AE, Wagenlehner FME, Mulgirigama A, Twynholm M. Escherichia coli resistance to fluoroquinolones in community-acquired uncomplicated urinary tract infection in women: A systematic review. Antimicrob Agents Chemother. 2020;64(10):e00862-20. doi:10.1128/AAC.00862-20
15. Bhatt S, Chatterjee S. Fluoroquinolone antibiotics: Occurrence, mode of action, resistance, environmental detection, and remediation – A comprehensive review. Environ Pollut.

2022;315:120440. doi:10.1016/j.envpol.2022.120440
16. Kim JW, Greenberg DE, Pfifer R, Jiang S, Xiao G, Xie Y, et al. VAMPr: VAriant mapping and prediction of antibiotic resistance via explainable features and machine learning. 2019. doi:10.1101/537381
17. Pandey D, Kumari B, Singhal N, Kumar M. Protocol for detection of bacterial proteins involved in efflux mediated antibiotic resistance (ARE) and their sub-families. 2021. doi:10.21203/rs.3.pex-1371/v1
18. Melo MCR, Maasch JRMA, de la Fuente-Nunez C. Accelerating antibiotic discovery through artificial intelligence. Commun Biol. 2021;4(1):1050. doi:10.1038/s42003-021-02586-0
19. Boolchandani M, D'Souza AW, Dantas G. Sequencing-based methods and resources to study antimicrobial resistance. Nat Rev Genet. 2019;20(6):356-70. doi:10.1038/s41576-019-0108-4
20. Machalek DA, Tao Y, Shilling H, Jensen JS, Unemo M, Murray G, et al. Prevalence of mutations associated with resistance to macrolides and fluoroquinolones in Mycoplasma genitalium: A systematic review and meta-analysis. Lancet Infect Dis. 2020;20(11):1302-14. doi:10.1016/S1473-3099(20)30154-7
21. Thapa RB, Dahal P, Karki S, Mainali UK. Exploration of drug therapy related problems in a general medicine ward of a tertiary care hospital of Eastern Nepal. Explor Res Clin Soc Pharm. 2024:100528. doi:10.1016/j.rcsop.2024.100528
22. Parajuli NP, Maharjan P, Parajuli H, Joshi G, Paudel D, Sayami S, et al. High rates of multidrug resistance among uropathogenic escherichia coli in children and analyses of ESBL producers from Nepal. Antimicrob Resist Infect Control. 2017;6(1):1-7. doi:10.1186/s13756-016-0168-6
23. Baral P, Neupane S, Marasini BP, Ghimire KR, Lekhak B, Shrestha B. High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. BMC Res Notes. 2012;5(1):1-9. doi:10.1186/1756-0500-5-38
24. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009;30(16):2785-91. doi:10.1002/jcc.21256
25. Velankar S, Burley SK, Kurisu G, Hoch JC, Markley JL. The protein data bank archive. Methods Mol Biol. 2021;2305:3-21.
26. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2023 update. Nucleic Acids Res. 2022;51(D1):D1373-80. doi:10.1093/nar/gkac956
27. Cherinka B, Andrews BH, Sánchez-Gallego J, Brownstein J, Argudo-Fernández M, Blanton M, et al. Marvin: A tool kit for streamlined access and visualization of the SDSS-IV MaNGA data set. Astron J. 2019;158(2):74. doi:10.3847/1538-3881/ab2634
28. Jejurikar BL, Rohane SH. Drug designing in discovery studio. Asian J Res Chem. 2021;14(2):135-8. doi:10.5958/0974-4150.2021.00025.0
29. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2015;1263:243-50. doi:10.1007/978-1-4939-2269-7_19
30. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The protein data bank. Nucleic Acids Res. 2000;28(1):235-42. doi:10.1093/nar/28.1.235
31. Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: Computed atlas of surface topography of proteins. Nucleic Acids Res. 2018;46(W1):W363-7. doi:10.1093/nar/gky473
32. Daina A, Michielin O, Zoete V. Swiss target prediction: Updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019;47(W1):W357-64. doi:10.1093/nar/gkz382
33. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Res. 2018;46(W1):W257-63. doi:10.1093/nar/gky318
34. Filimonov DA, Lagunin AA, Gloriozova TA, Rudik AV, Druzhilovskii DS, Pogodin PV, et al. Prediction of the biological activity spectra of organic compounds using the pass online web resource. Chem Heterocycl Compd. 2014;50(3):444-57. doi:10.1007/s10593-014-1496-1
35. Khudiar H, Yaseen W, Alezerjawi N, Al-fahham A. Pathogenic bacteria associated with urinary tract infections. Int J Health Med Res. 2024;03(06):346-51. doi:10.58806/ijhmr.2024.v3i06n16
36. Shrestha L, Adhikari S, Palikhey A, Pokhrel BR, Shrivastava A, Joshi B, et al. Prescription pattern of antibiotics in urinary tract infection based on antimicrobial susceptibility test at a tertiary care hospital. J Gandaki Med Coll Nepal. 2023;16(2):89-94. doi:10.3126/jgmcn.v16i2.60725
37. Marepalli NR, Nadipelli AR, Manohar Kumar Jain RJ, Parnam LS, Vashyani A. Patterns of antibiotic resistance in urinary tract infections: A retrospective observational study. Cureus. 2024;16(6):e62771. doi:10.7759/cureus.62771
38. Pandey B, Pandit M, Jaiswal S, Sah AK, Chand RS, Shrestha R. Antimicrobial susceptibility pattern of

pathogenic bacteria causing urinary tract infection in tertiary care hospital in Kathmandu, Nepal. Int J Pharm Sci Res. 2020;11:6448-55. doi:10.13040/IJPSR.0975-8232.11(12).6448-55

39. Muhammad A, Khan SN, Ali N, Rehman MU, Ali I. Prevalence and antibiotic susceptibility pattern of uropathogens in outpatients at a tertiary care hospital. New Microbes New Infect. 2020;36:100716. doi:10.1016/j.nmni.2020.100716
40. Alaa A-M, Asiri YA, Al-Agamy MH. Design, synthesis and antibacterial activity of fluoroquinolones containing bulky arenesulfonyl fragment: 2D-QSAR and docking study. Eur J Med Chem. 2011;46(11):5487-97. doi:10.1016/j.ejmech.2011.09.011
41. Patel MM, Patel LJ. Design, synthesis, molecular docking, and antibacterial evaluation of some novel flouroquinolone derivatives as potent antibacterial agent. Sci World J. 2014;2014(1):897187. doi:10.1155/2014/897187
42. Lungu IA, Moldovan OL, Biriș V, Rusu A. Fluoroquinolones hybrid molecules as promising antibacterial agents in the fight against antibacterial resistance. Pharmaceutics. 2022;14(8):1749. doi:10.3390/pharmaceutics14081749