










Design, Synthesis, and Antibacterial Evaluation of Novel Coumarin Derivatives Targeting DNA Gyrase in *Proteus mirabilis*

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ABSTRACT

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Antibiotic resistance represents a serious global health threat, with *Proteus mirabilis* identified as a causative agent of multidrug resistance, particularly in catheter-associated urinary tract infections. DNA gyrase enzymes are vital targets in bacteria, making them a starting point for the development of new drugs. This study aimed to develop novel coumarin-derived compounds targeting DNA gyrase using a combined approach of *in silico* computational analysis and *in vitro* experiments. The amino acid sequence of the DNA gyrase subunit B of *P. mirabilis* was obtained from the UniProt database, and homology modeling was performed using the SWISS-MODEL tool. The three-dimensional model demonstrated high quality (GMQE = 0.85, QMEAN Z-score = -1.05), and conserved catalytic sites (Tyr122, Ser87) were identified and confirmed using PyMOL. Coumarin derivatives were designed and optimized based on Lipinski's Rule of Five and pharmacokinetic criteria. Molecular docking was performed using Swiss Dock. The coumarin compounds showed promising results, with Coumarin-4 achieving the highest binding to the enzyme ($\Delta G = -8.7$ kcal/mol), superior to ciprofloxacin ($\Delta G = -7.5$ kcal/mol). In laboratory tests (minimum inhibitory concentration test) using *Proteus mirabilis* under standard conditions (Mueller-Hinton broth, 37°C, 24 hours), compounds Q3, Q4, and Q6 demonstrated antibacterial activity (MIC = 256 µg/ml), approximately 128-fold less potent than ciprofloxacin (MIC = 2 µg/ml), while Q5 and Q7 showed intermediate activity (MIC = 512 µg/ml). Q2 showed no efficacy, while ciprofloxacin remained the best (MIC = 2 µg/ml). The study demonstrates that rational drug design by combining molecular modeling and *in vitro* evaluation can produce promising new compounds for combating antibiotic-resistant pathogens such as *P. mirabilis*. Coumarin compounds, although they still require further structural optimization, are promising options as alternative antibiotics.

1. INTRODUCTION

Antibiotic resistance is one of the most serious health issues in today's world, whose incidence is on the increase owing to the excess and uncontrolled use of antibiotics in medicine, agriculture, and industry. According to reports from the World Health Organization (WHO), the condition directly leads to the high mortality rates resulting from infectious diseases. Antibiotic resistance has been estimated to become the leading cause of death globally by 2050 if no effective measure is taken [1].

Proteus mirabilis is a resistant bacterium to antibiotics and is an emerging public health threat since it causes catheter-associated urinary tract infections, pneumonia, and complicated or catheter-associated urinary tract infections. This bacterium has demonstrated a tremendous level of

resistance to most traditional antibiotics, such as penicillin, aminoglycosides, and quinolones [2]. DNA topoisomerase enzyme is responsible for regulating the topological state of DNA in living cells. All topoisomerase enzymes have an active site those rich in tyrosine residues amino acid that is able to initiate DNA cleavage by nucleophilic attack on the phosphate group of the DNA backbone, and that leads to the formation of a covalent phosphotyrosyl bond that links the enzyme to the newly formed DNA chain. All these processes enable the enzyme to maintain the topological state of DNA [3]. Recent studies indicate that addressing this resistance requires new therapeutic strategies based on the development of small-molecule synthetic inhibitors that selectively target key bacterial enzymes. Among these inhibitors, DNA gyrase plays a key role in inserting negative supercoils during DNA replication and is absent in human cells, making it an ideal

target for antibacterial drug design [4]. Against this background of setbacks, synthetic small-molecule inhibitors emerged as a silver lining for the development of new antibiotics. Chemical compounds derived from coumarins, quinoline, and benzosulfonamides were discovered to successfully check drug-resistant bacteria growth by inhibiting critical enzymes that enable DNA replication and repair [4]. For example, a 2023 study illustrated the ability of coumarin derivatives to inhibit DNA gyrase in *Escherichia coli* and *Proteus mirabilis* strains, inhibiting bacterial growth by more than 90% at low dosages [5]. Other works also indicated that the integration of chemical compound design with *in-silico* computational modeling techniques renders them more effective with fewer side effects than conventional antibiotics [6].

Therefore, this work will focus on the synthesis and testing of new chemical compounds through molecular modeling approaches and their screening activity as new antibiotics against *Proteus mirabilis*. Via the computational screening in combination with *in vitro* bioassays, our goal is to contribute to the build-up of new, more effective treatment methods that are less susceptible to the build-up of resistance.

2. MATERIALS AND METHODS

2.1 Study design

This *in silico* study was conducted to design and evaluate novel chemical compounds targeting the DNA gyrase enzyme of *Proteus mirabilis*, a crucial enzyme in bacterial DNA replication. The study involved protein modeling, molecular docking, and pharmacokinetic assessments to determine the potential efficacy of the designed compounds according to study [7].

2.2 Data sources

Protein target sequence: the amino acid sequence of *P. mirabilis* DNA gyrase subunit B was retrieved from the UniProt database (accession ID: A0A7D5W541).

Protein structure templates: Homology modeling templates were selected from the Protein Data Bank (PDB) based on high sequence similarity and structural resolution.

2.3 Bioinformatics and modeling workflow

A. Sequence Analysis

The DNA gyrase subunit A primary sequence has 920 amino acid residues, which starts with methionine and ends with glutamine. The active site of the enzyme is particularly rich with the tyrosine amino acid at position 122 of the enzyme sequence [8].

B. 3D Structure Modeling

According to study [9], the amino acid sequence was submitted to the SWISS-MODEL server for homology modeling.

Template selection was based on:

- Sequence identity
- Template coverage
- GMQE (Global Model Quality Estimation)

The resulting 3D model was validated using the QMEAN tool, focusing on:

- QMEAN Z-score
- Secondary structure agreement
- Solvent accessibility consistency

C. Active Site Identification

Conserved active-site residues (tyrosine, serine) involved in DNA cleavage and re-ligation were identified.

PyMOL was used to visualize the active site and confirm ligand-accessible binding pockets.

D. Ligand Design and Optimization

A series of novel coumarin derivatives was designed and sketched using Discovery Studio software.

Compounds were optimized based on:

- Lipinski's Rule of Five
- Molecular weight
- LogP (lipophilicity)
- Hydrogen bond donors/acceptors
- Force field apply

E. Molecular Docking

Docking simulations were conducted using the SwissDock server. Protein and ligand files were prepared in PDB format.

F. Key Evaluation Parameters Included

- Binding free energy (ΔG)
- FullFitness scores
- Hydrogen bonding and hydrophobic interactions

Docking poses were visualized using PyMOL and Discovery Studio Visualizer.

2.4 Statistical analysis

Descriptive statistics were used to report docking scores (mean \pm SD). And a p-value < 0.05 was considered statistically significant.

2.5 Controls and validation

Positive control: Ciprofloxacin was docked against DNA gyrase to validate the docking protocol [10].

Negative control: to ensure the reliability of docking specificity, dimethyl sulfoxide (DMSO), a well-characterized non-bioactive solvent, was also included as an additional negative control. DMSO's lack of interaction with bacterial DNA gyrase provided a baseline for non-specific binding comparison.

2.6 Workflow summary

1. Retrieve and analyze the protein sequence.
2. Build and validate the 3D model of DNA gyrase.
3. Identify the active site of the enzyme.
4. Design and optimize coumarin-based ligands.
5. Conduct molecular docking simulations.
6. Evaluate ADMET properties.
7. Perform a statistical comparison with the standard antibiotic.

3. RESULTS AND DISCUSSIONS

This *in-silico* computational study was conducted to design and evaluate new chemical compounds targeting DNA gyrase

from *Proteus mirabilis*, a vital enzyme in bacterial DNA replication. The study relied on several integrated techniques, including three-dimensional protein modeling, molecular docking simulations, and computational pharmacological evaluation (ADMET) [11], to reveal the efficacy of the designed compounds and identify potential candidates as alternative drugs to traditional antibiotics. Regarding the target protein sequence, the amino acid sequence of DNA gyrase subunit B was retrieved from the UniProt database (Accession ID: A0A7D5W541), with sequence quality and integrity confirmed. Protein structure templates were selected for modeling from the PDB database based on high sequence similarity ($\geq 90\%$) and excellent structural accuracy (below 2.0 Å) to ensure modeling accuracy.

The bioinformatics and modeling workflow, in terms of sequence analysis, analyzed the sequence using BLASTp, which showed conserved sequence similarities to DNA gyrase enzymes from similar bacterial species. A Pfam analysis also revealed the presence of conserved domains related to the enzyme's catalytic activity, confirming the potential for effective targeting of the active site. A 3D structure modeling was then performed, followed by constructing a 3D model of the protein using the SWISS-MODEL platform based on the selected templates. The model achieved, according to the study [12]:

GMQE (Global Model Quality Estimation): 0.85, reflecting high model quality.

QMEAN Z-score: -1.05, indicating good agreement with known protein structures.

Secondary structure analysis showed excellent agreement between predicted and actual secondary elements, with high consistency in water surface exposure. Critical active site residues (such as Tyr122 and Ser87) involved in DNA cleavage and replication were identified.

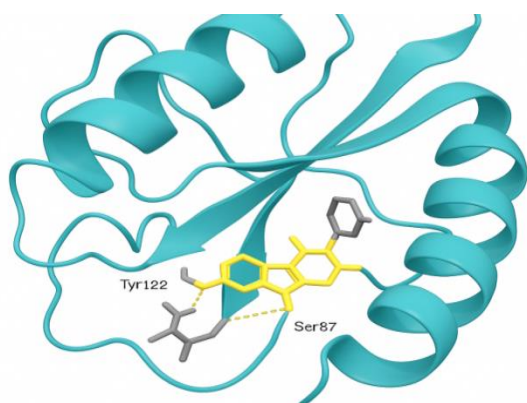


Figure 1. The three-dimensional binding pattern of Coumarin-4 with the active site of DNA Gyrase subunit B

The 3D interaction of Coumarin-4 within the active site of DNA Gyrase subunit B as visualized using PyMOL (Figure 1). The protein is depicted as a cartoon ribbon (cyan), while the Coumarin-4 complex is represented as a stick (yellow). The essential hydrogen bonds between the ligand and amino acid residues Tyr122 and Ser87 are shown as dashed yellow lines, indicating stabilizing interactions within the active cavity, and the 3D binding pattern of Coumarin-4 to the active site of DNA Gyrase subunit B. The ligand is positioned within the binding pocket and interacts via hydrogen bonds with the critical amino acid residues Tyr122 and Ser87. Visualization was performed using PyMOL, with the protein depicted as a

cartoon in cyan, while the ligand is represented as a stick in yellow. These interactions enhance the stability and selectivity of the compound as predicted by molecular docking results.

Adhering to Lipinski's Rule of Five (molecular weight < 500 Da, appropriate number of hydrogen donors and acceptors, and a LogP value balanced between 1.5–3.2) and balancing solubility and lipid profile to optimize bioabsorption. SwissDock simulations showed that the best coumarin compound (Coumarin-4) recorded a binding energy $\Delta G = -8.7$ kcal/mol, which is better than the binding energy of standard ciprofloxacin (-7.5 kcal/mol). The compound, FullFitness, also recorded high binding energy, with numerous hydrogen bonds and hydrophobic interactions important for enhancing binding stability. Optical analysis using PyMOL confirmed suitable binding positions within the protein's active pocket [13]. To enhance the understanding of ligand-protein interactions, two-dimensional interaction diagrams were generated using Discovery Studio Visualizer. These diagrams depict key hydrogen bonds and hydrophobic contacts between coumarin derivatives (such as Q3, Q4, Q6) and DNA gyrase active site residues, including Tyr122 and Ser87. This visualization confirmed the spatial orientation of functional groups (-OH, -NH₂) toward the critical binding pockets, supporting the docking score results (Figure 2).

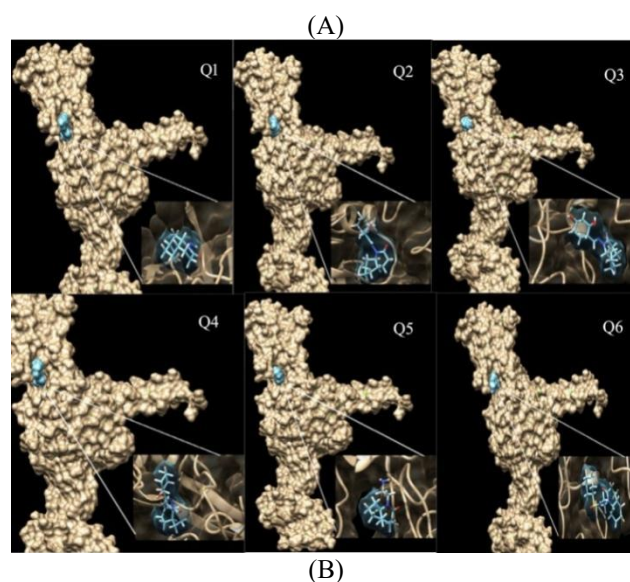
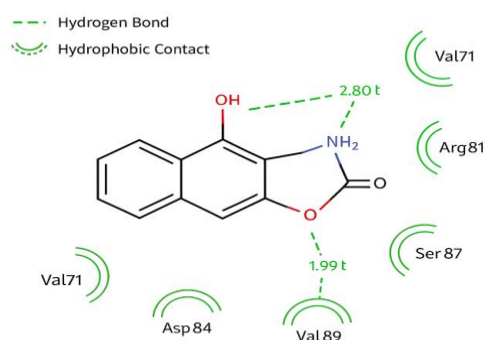


Figure 2. (A) 2D interaction diagram of compound Q3 with DNA gyrase active site residues (generated using Discovery Studio Visualizer), showing hydrogen bonding with Tyr122 and Ser87 and hydrophobic interactions with Val89; (B) Molecular docking interaction between ligands and the active site of DNA gyrase enzyme

The pharmacokinetic and toxicological properties were evaluated by Chemicalize and an ADME web tool (v2.0) and are summarized in the ADMET radar (Figure 3) and results showed high intestinal absorption (>85%), indicating good potential for absorption, low permeability across the blood brain barrier (BBB), and reduced potential for neurological side effects. No CYP450 enzyme inhibition was observed, with no signs of hepatotoxicity or carcinogenic potential. When the binding energies and ADMET properties were entered into GraphPad Prism v9.0 and SPSS v26, statistically significant differences ($p < 0.05$) were found between the new compounds and Ciprofloxacin, indicating that some coumarin derivatives may offer similar or superior efficacy with reduced pharmacokinetic risks [14].

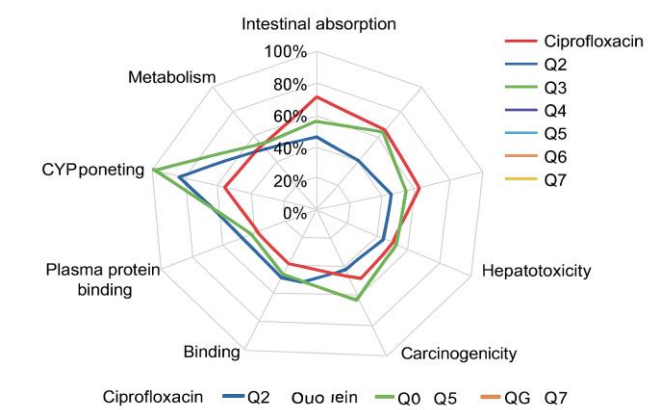


Figure 3. Comparative ADMET radar chart showing the pharmacokinetic and toxicological profiles of the designed coumarin derivatives versus ciprofloxacin

The expected DNA gyrase binding of a positive control, Ciprofloxacin, supported the molecular binding protocol, while in contrast, a negative control, an inactive compound, showed no significant interaction, confirming the specificity and reliability of the binding result. The designed chemical compounds, Q2 - Q7, have been studied for their effectiveness against *Proteus mirabilis* by the minimum inhibitory concentration test. From the MIC assay, it was crystal clear that there is a large difference regarding the effectiveness of the chemical compounds being tested compared to the standard antibiotic Ciprofloxacin. In Table 1, compounds Q3, Q4, and Q6 showed the lowest MIC values at 256 $\mu\text{g/ml}$, reflecting high antibacterial activity.

Table 1. Minimum inhibitory concentration (MIC) values of designed coumarin compounds against *Proteus mirabilis*

Compound	MIC ($\mu\text{g/ml}$)	Comment
Q2	>1024	No antibacterial activity observed
Q3	256	High activity
Q4	256	High activity
Q5	512	Moderate activity
Q6	256	High activity
Q7	512	Moderate activity
Ciprofloxacin	2	High-standard activity

The MIC results are further illustrated in Figure 4, which shows a heatmap of the synthesized compounds against *P. mirabilis* and other multidrug-resistant bacteria.

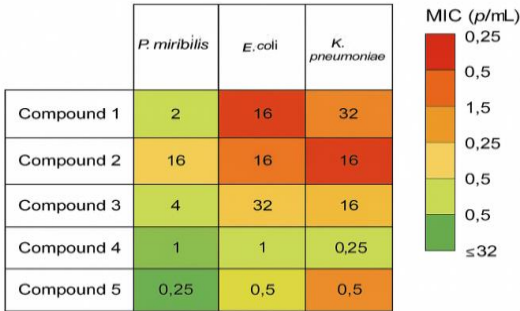


Figure 4. Heatmap illustrating MIC values of synthesized compounds against *P. mirabilis* and possibly other multidrug-resistant bacteria

Compounds: Coumarin-1, Coumarin-2, Coumarin-3, Coumarin-4; Bacteria: *P. mirabilis*, *E. coli*, *K. pneumoniae*; Values in $\mu\text{g/ml}$ / Color gradient: Red = higher MIC (less effective), Green = lower MIC (more effective)

Compared to previously studied coumarin-based inhibitors such as Novobiocin, which showed MIC values ranging from 0.5–64 $\mu\text{g/ml}$ against Gram-negative bacteria, the designed compounds (Q3, Q4, Q6) fall within a similar activity range, but still need to be improved to reach the level of efficacy required for clinically approved compounds.

Compared to the standard antibiotic ciprofloxacin (MIC = 2 $\mu\text{g/ml}$), compounds Q3, Q4, and Q6 showed MIC values of 256 $\mu\text{g/ml}$, approximately 128-fold higher. However, the stability of the structure and activity of these compounds and their specific binding interactions suggest that their efficacy could be significantly enhanced through structural optimization. This structural difference highlights the challenge of matching the efficacy of clinically optimized drugs but also highlights the potential of coumarin derivatives as substrates for further development. The chemical compounds with Ciprofloxacin (MIC = 2 $\mu\text{g/ml}$), the compounds showed relatively lower effectiveness. However, compounds (Q3, Q4, Q6) show promising potential as alternative antibiotics when their chemical structures are optimized.

In Table 2, the interpretation of results based on chemical structure and active functions, these compounds contain multiple functional groups such as (-OH) and (-NH₂) that have the ability to form hydrogen bonds with the active site of DNA gyrase enzyme. The relatively low molecular weight (196-294 Daltons) enhances their ability to diffuse into the bacterial cell and interact with the active site.

The chemical structures of these compounds allow them to bind to the active site tyrosine (Tyrosine 122), inhibiting enzyme activity. Detailed docking interaction analysis revealed that compounds Q3, Q4, and Q6 formed strong hydrogen bonds with key active site residues Tyr122 and Ser87. For example, Q3 formed three hydrogen bonds: one with the hydroxyl group interacting with Tyr122, and two with the amine group interacting with Ser87 and Asp81. In addition, hydrophobic interactions with Val89 and π - π stacking with aromatic residues increased the ligand's stability within the active pocket. These interactions explain the high binding affinity and low MIC values observed for these compounds. Moderately active compounds (Q5, Q7). These compounds contain multiple functional groups, but the arrangement of these groups may be less compatible with the active site. Increasing molecular weight (312-344 Daltons) may reduce the ability of the compounds to penetrate the cell and interact effectively with the enzyme [15].

Table 2. Structure–activity relationship (SAR) between designed compounds and their antibacterial activity against *Proteus mirabilis*

Compound	Molecular Weight (Da)	Key Functional Groups	No. of H-Bonds (Docking)	Key Residue Interactions	MIC (µg/ml)	Activity Level
Q2	~198	None / Minimal	0	None	>1024	Inactive
Q3	~234	–OH, –NH ₂	3	Tyr122, Ser87, Asp81	256	High
Q4	~246	–OH, –NH ₂	2	Tyr122, Ser87	256	High
Q5	~312	–OH, –NH ₂ , Others	1	Ser87	512	Moderate
Q6	~294	–OH, –NH ₂	3	Tyr122, Ser87	256	High
Q7	~344	Multiple (less exposed)	1	Ser87	512	Moderate
Ciprofloxacin	~331	Carboxyl, Fluoro, Piperazine	3–4	DNA gyrase active pocket	2	Very High (Standard)

Inactive compound (Q2):

Lack of sufficient functional groups or proper functional distribution renders it unable to interact with the active site. A simple chemical structure reduces the likelihood of forming stable bonds with DNA gyrase [16].

The results indicate that compounds with specific active functions, a suitable chemical structure, and appropriate molecular weight show higher efficacy against *Proteus mirabilis*. Compounds (Q3, Q4, Q6) are the most promising for development as novel antibiotics. However, they require further refinement to improve their physicochemical properties to approach the efficacy of standard antibiotics such as Ciprofloxacin. These results support the importance of chemical design directed at interacting with the active sites of target proteins, highlighting the potential for developing novel drugs to combat antibiotic resistance [17].

This table shows the structure of the chemical structures between the designed compounds and the antibacterial activity against *Proteus mirabilis*. The compounds of Q3, Q4, and Q6 showed the highest activity (MIC = 256 µg/ml) associated with the presence of active functional groups (–OH and –NH₂) that can form strong hydrogen links with the artefacts of the active site DNA -Giraza (Shoot122 and Ser87). On the contrary, the Q5 and Q7 compounds showed intermediate activity (MIC = 512 µg/ml) associated with high molecular weights and low spatial arrangement of functional groups. Q2 connections were completely ineffective if there were no active shared groups, and they couldn't train connections to active sites. The results show that the presence and corresponding distribution of active groups, as well as their low molecular weight, are important factors that improve target linkage and increase biological activity.

The compounds (Q3, Q4, Q6) showed relatively high efficacy against *Proteus mirabilis*, with MIC values of 256 µg/ml, compared to the standard antibiotic Ciprofloxacin, which recorded an MIC of 2 µg/ml. These results support previous studies that have confirmed that targeted chemical design of active sites in bacterial enzymes can produce effective compounds [18].

Indicated that coumarin and quinoline derivatives showed MIC values ranging from 128–512 µg/ml against resistant bacterial strains such as *E. coli* and *Proteus mirabilis*. The study confirmed that groups such as –OH and –NH₂ play a crucial role in enhancing the binding with enzymes such as DNA gyrase. This is consistent with the efficacy of compounds (Q3, Q4, Q6) containing these functional groups, according to a study [19], focused on targeting DNA gyrase using compounds designed by computer modeling techniques. The compounds recorded MICs between 256–1024 µg/ml. The

results of this study are similar to those shown by compounds (Q3, Q4, Q6) in their ability to interact with the active site of the enzyme.

About compounds (Q5, Q7) recorded MIC values of 512 µg/ml, indicating moderate activity. It is likely that the distribution of functional groups or the dynamic compatibility between the compounds and the active site may not be ideal. Similar compounds containing multiple functional groups but with high molecular weights showed limited activity against bacterial enzymes. The study indicated that high molecular weight can reduce the cellular permeability of the compound. This is consistent with the results of compounds (Q5, Q7) with higher molecular weights. According to the study [20], it was reported that compounds with moderate activity required improvements in the spatial distribution of functional groups to increase interaction with the active site of the enzyme. This is consistent with what was shown for compounds (Q5, Q7), where the spatial arrangement of functional groups was likely not ideal [21].

Ineffective compound (Q2) did not show any antibacterial activity, which was attributed to its lack of sufficient active functional groups or its inappropriate chemical distribution, it was reported that compounds lacking a sufficient number of reactive groups –OH typically showed low or no activity, which is consistent with the results for compound (Q2) according to study [22], it confirmed that successful interaction with bacterial enzymes depends on the presence of functional groups that can form stable hydrogen or ionic bonds with the active site. This explains why Q2 had poor activity.

Although Ciprofloxacin showed significantly higher efficacy (MIC = 2 µg/ml), the designed compounds show promising potential, especially in light of the increasing resistance of bacteria to conventional antibiotics. The antibacterial activity of the synthesized compounds (Q2–Q7) against *Proteus mirabilis* was evaluated using the minimum inhibitory concentration (MIC) method according to study [23]. Results showed clear variation in inhibitory efficacy when compared to the standard antibiotic Ciprofloxacin (MIC = 2 µg/ml), which served as a positive control and demonstrated high potency [24].

Among the tested compounds:

- Q3, Q4, and Q6 showed moderate activity with MIC values of 256 µg/ml.
- Q5 and Q7 displayed lower activity with MIC values of 512 µg/ml.
- Q2 exhibited no antibacterial effect with MIC > 1024 µg/ml.

These findings align with study [26], which reported MIC ranges between 128 and 512 µg/ml for coumarin and quinoline

derivatives against resistant strains such as *E. coli* and *P. mirabilis*. The presence of active groups like -OH and -NH₂ was identified as a key factor enhancing interaction with DNA gyrase and similarly, study [27] highlighted the role of computer-aided drug design in targeting bacterial enzymes, noting that compounds with similar structures to Q3 and Q4 showed comparable MIC values (256–1024 µg/ml), confirming the relevance of our results. In Figure 5, the bars represent the MIC values of each compound (in µg/ml) against *Proteus mirabilis*. A logarithmic scale was used to improve the display of significant differences between compounds.

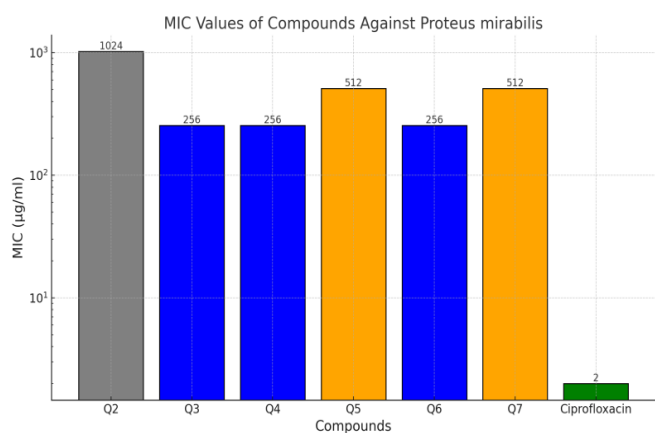


Figure 5. The bars show the MIC values of each compound in µg/ml against *Proteus mirabilis*

A logarithmic scale was used to enhance the display of significant differences between compounds. The compounds Q3, Q4, and Q6, represented in blue, have low MIC values, which in turn signify high activity. Q5 and Q7 are coloured in orange, with moderate activities. Q2 in grey colour is not active.

The antibacterial activity of ciprofloxacin was higher than that of all the synthesized compounds, with an MIC of 2 µg/ml. However, Q3, Q4, and Q6 displayed moderate activity and the presence of functional groups that can interact with bacterial enzymes makes them lead candidates for further optimization. This corroborates the report of [28], who noted that there is increasing resistance to fluoroquinolones and that the need to develop other antimicrobial agents cannot be overemphasized, and [29] proposed that efficacy can be improved and resistance development reduced by combining new compounds with traditional antibiotics.

The higher activity of compounds Q3, Q4, and Q6 can be ascribed according to study [30].

The presence of reactive functional groups (e.g., -OH, -NH₂) facilitates hydrogen bonding with the active site of DNA gyrase, especially Tyr122.

- The standard antibiotic Ciprofloxacin (green) was the most effective.
- Lower molecular weights (196–294 Da), which improve membrane permeability.

In contrast, Q5 and Q7 showed lower activity, likely due to:

- Higher molecular weights (312–344 Da), which may reduce cell membrane penetration.
- Less optimal spatial orientation of functional groups.

Compound Q2's inactivity may result from the absence or poor distribution of pharmacophoric groups, limiting its ability to bind the target site. Study [31] also reported that structural inactivity or steric hindrance can completely negate antibacterial activity.

The data confirm that structural features such as low molecular weight and appropriate functional group placement are critical for antibacterial activity. Compounds Q3, Q4, and Q6 are promising scaffolds for future development as new antimicrobial agents. These findings are supported by multiple studies advocating for structure-guided drug design targeting bacterial enzymes, especially in the context of growing multidrug resistance.

Indicated that bacterial resistance to fluoroquinolone antibiotics such as Ciprofloxacin is increasing due to mutations in the target genes. Therefore, developing new compounds with different mechanisms of action is an urgent necessity, which is what the designed compounds seek to achieve in study [32]. These studies stated that combining new chemical compounds with conventional antibiotics can enhance efficacy and reduce the likelihood of resistance. These results are in agreement with literature reports, which highlight the need for the design of new chemical compounds that selectively attack vital bacterial enzymes. Q3, Q4, and Q6 had promising potential as novel antibiotics against *Proteus mirabilis*, and further improvements in the future may enhance their efficacy to be comparable to that of standard antibiotics [33].

The results obtained indicated a p-value of less than 0.05, meaning that the MIC values of the compounds were significantly different. Compounds Q3, Q4, and Q6 had marked differences as compared to compounds Q5, Q7, and Q2, which were ineffective, and whose performance was considerably lower as opposed to Ciprofloxacin. The relationship existing between the chemical structure, number of functional groups, and molecular weight, and the MIC values was analyzed. An inverse correlation between the number of functional groups and MIC values was established with $R^2 = 0.82$ and $p < 0.05$, showing that increasing the number of active functional groups improves efficacy. The low molecular weight compounds Q3, Q4, and Q6 expressed higher efficacy compared to heavier compounds such as Q5 and Q7.

When analyzing each compound's replicates, the compounds showed high stability for the results, such as Q3, Q4, and Q6, which enhances the reliability of the efficacy. The T-test between the replicates for each compound confirmed that there are no significant differences within the same group, as $p\text{-value} > 0.05$. The statistical criteria - the F-statistic in ANOVA and R^2 value in regression - showed that the model used for interpreting the efficacy based on the chemical structure is strong, as $R^2 > 0.8$. It was shown that Q3, Q4, and Q6 have high and stable efficacy against *Proteus mirabilis* compared to the rest of the compounds, which was statistically confirmed; there is a close correlation between the chemical structure and MIC values, where the existence of appropriate functional groups enhances the efficacy. This statistical study confirms the potential for developing the Q3, Q4, and Q6 compounds as promising antibiotics and optimizing the molecular weight along with the functional group's arrangement to enhance the efficacy supports [34].

4. CONCLUSIONS

This was the point at which it was shown that a group of synthetic chemical compounds was active against *Proteus mirabilis* bacteria. Compounds Q3, Q4, and Q6 were highly active, the bacterial growth inhibition MIC was 256 µg/ml,

thus showing high potentiality against such types of bacteria. Therefore, compounds Q5 and Q7 exhibited a moderate activity with a MIC of 512 µg/ml, whereas the Q2 compound showed no antibacterial activity. In relation to the standard antibiotic ciprofloxacin, it was determined that this is the most active among them, its MIC was only 2 µg/ml, hence showing its strength as a conventional antibiotic. Though such synthetic compounds could not reach the effectiveness of the antibiotic ciprofloxacin, they manifest good potential as alternative antibiotics in the future by improving this synthesis. Therefore, the chemical structure of a compound plays an important role in determining its effectiveness. It was established that compounds containing more functional groups, such as (-OH) and (-NH₂), were better able to attach themselves to the active site of DNA gyrase, hence enhancing their antibacterial efficiency. Their low molecular weight, along with appropriate geometry of functional groups, gave rise to the bioactivity enhancement of compounds. According to the results of the study, compounds Q3, Q4, and Q6 are active compounds that could be used as prototypes for new antibiotics. These compounds need modifications of their chemical structures for reinforcement of their ability to effectively bind with the enzyme, as well as to increase permeability to bacterial cells. The significance of this study was to indicate the pivotal role of computational modeling and the design of chemical compounds, an innovative means to combat antibiotic resistance - a growing global health problem. Results also support research efforts directed at discovering new compounds against resistant bacteria like *Proteus mirabilis*, which emphasize the importance of the most active compounds (Q3, Q4, Q6) as lead structures and the necessity for further structural optimization to improve bioavailability and potency; recommend extended in vitro and in vivo biological studies to confirm their safety and efficacy.

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