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

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ABSTRACT

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 (ΔG = –8.7 kcal/mol), superior to ciprofloxacin (ΔG = –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 instance, a study published in 2023 demonstrated the potential for coumarin derivatives to inhibit DNA gyrase in Escherichia coli and *Proteus mirabilis* strains, resulting in the inhibition of bacterial growth by more than 90% at low doses [5]. Other studies also showed that the application of the integration of the design of a chemical compound and the use of in-silico computational modeling techniques made these compounds more potent and less likely to have side effects compared to traditional antibiotics [6].

Thus, this study aims to contribute to the development and testing of new chemical compounds through the application of molecular modeling techniques and their screening activity as a new antibiotic for *Proteus mirabilis*. Through this approach, new and better approaches for the development of new and better treatments that are less likely to develop 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

Active-site parts that stay the same across species - like tyrosine and serine - were found playing roles in cutting and rejoining DNA. A view of the active site emerged using PyMOL, showing where ligands could fit into available pockets.

D. Ligand Design and Optimization

Out of thin air, fresh coumarin variations took shape on screen through Discovery Studio tools. Lines formed slowly, guided by digital precision and chemical logic behind each twist.

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 trials ran on SwissDock's platform. Before that, the protein plus its binding partner got cleaned up into PDB shape.

Matter most during testing were:

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

Through PyMOL, docking poses came into view, while Discovery Studio Visualizer also offered its own look.

2.4 Statistical analysis

Descriptive statistics were used to report docking scores (mean ± 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 research was carried out using various integrated methodologies, including three-dimensional protein modeling, molecular docking, and computational pharmacological evaluation (ADMET) [11], which was employed to demonstrate the effectiveness of the newly designed compounds and identify potent compounds as alternative drugs to conventional antibiotics. With regard to the target protein, the amino acid sequence of DNA gyrase subunit B was obtained from the UniProt database (Accession ID: A0A7D5W541), where the quality and authenticity of the protein sequence were verified. The structure templates for DNA gyrase subunit B were obtained from PDB, where the templates had high sequence similarities (> 90%) and excellent structural accuracy (< 2.0 Å).

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.

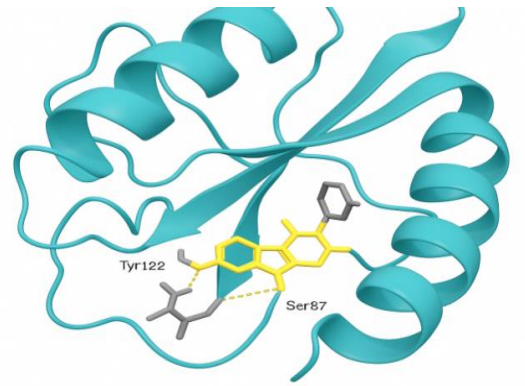

Figure 1. The three-dimensional binding pattern of Coumarin-4 with the active site of DNA Gyrase subunit B

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.

Following 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 ΔG = –8.7 kcal/mol, which is better than the binding energy of standard ciprofloxacin, which was –7.5 kcal/mol. The FullFitness of the compound also recorded high binding energy, with several hydrogen bonds and hydrophobic interactions crucial to enhance the stability of the bindings. Looking at the protein's active spot through PyMOL showed where molecules fit well [13]. Moving beyond shapes, flat maps of connections appeared via Discovery Studio Visualizer. Because of these images, key links - like hydrogen bonds and greasy touches - came into view between coumarins such as Q3, Q4, and Q6 and parts of DNA gyrase, specifically Tyr122 and Ser87. With clarity now on how pieces line up in space, groups like -OH and -NH_2 face just right into vital zones, which lines up neatly with earlier docking numbers (Figure 2).

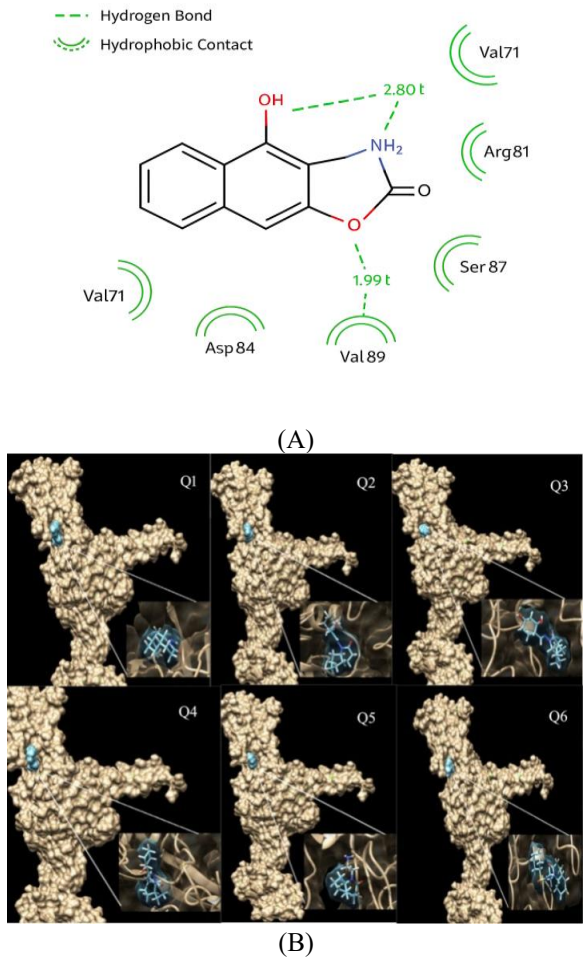

Figure 2. (A) 2D interaction diagram of compound Q3 with DNA gyrase active site residues (generated using Discovery Studio Visualizer), showing hydrogen bonding with Tyr122 and Ser87 and hydrophobic interactions with Val89; (B) Molecular docking interaction between ligands and the active site of DNA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

Pharmacokinetic and toxicological properties were predicted using Chemicalize and an ADME web tool (v2.0), and the predicted properties are displayed in an ADMET radar chart, as shown in Figure 3. The high potential for intestinal absorption (> 85%) and low blood barrier permeability indicate no adverse effects on the system. 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].

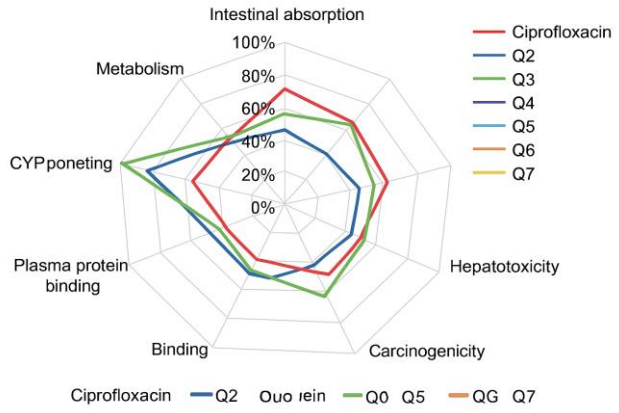

Figure 3. Comparative ADMET radar chart showing the pharmacokinetic and toxicological profiles of the designed coumarin derivatives versus ciprofloxacin

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 µg/ml, reflecting high antibacterial activity.

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

Compound	MIC (µg/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.

Compared to previously studied coumarin-based inhibitors such as Novobiocin, which showed MIC values ranging from 0.5–64 µg/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.

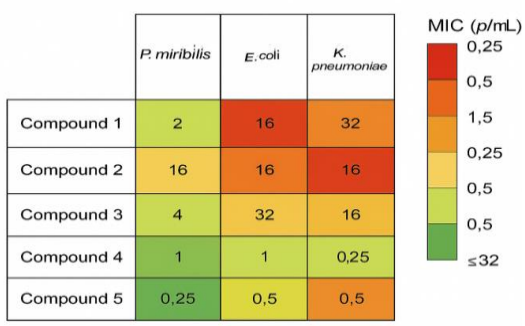

Figure 4. Heatmap illustrating MIC values of synthesized compounds against *P. mirabilis* and possibly other multidrug-resistant bacteria

Compounds: Coumarin-1, Coumarin-2, Coumarin-3, Coumarin-4; Bacteria: *P. mirabilis, E. coli, K. Pneumoniae*; Values in µg/ml/ Color gradient: Red = higher MIC (less effective), Green = lower MIC (more effective)

Compared to the standard antibiotic ciprofloxacin (MIC = 2 µg/ml), compounds Q3, Q4, and Q6 showed MIC values of 256 µg/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 µg/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 the interpretation of the results based on the chemical structure and the active functions, as shown in Table 2, the compound contains multiple active functions, such as (-OH) and ($-NH_2$), which have the ability to form hydrogen bonds with the active site of the DNA gyrase enzyme. The fact that the molecular weight is relatively low (196-294 Daltons) makes the compounds have the ability to diffuse into the bacterial cell and reach the active site.

These molecules stick well because they fit into a specific spot on the enzyme - tyrosine at position 122 - which stops it from working. However, when scientists took a closer look, they noticed that Q3, Q4, and Q6 were tightly bound through a hydrogen link with significant elements like Tyr122 and Ser87. For instance, Q3 formed three of these links, with the OH part of the molecule binding with Tyr122, and the NH_2 part binding with both Ser87 and Asp81. Other than that, gentle nudges from Val89 and rings that stacked and locked onto nearby aromatic groups further helped hold everything in place within the binding site. This is evident from the good binding, as shown by how well the molecules bind and their low MIC values. Some of them, like Q5 and Q7, show very little binding, though all of them have several chemical groups that, though possibly not aligned properly with the target site, are large enough, with masses of between 312 and 344 Daltons, for them to have trouble getting into the cell and reaching the target 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 -NH_2) that can form strong hydrogen links with the artefacts of the active site DNA -Giiraza (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 -NH_2 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, 25].

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 -NH_2 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.

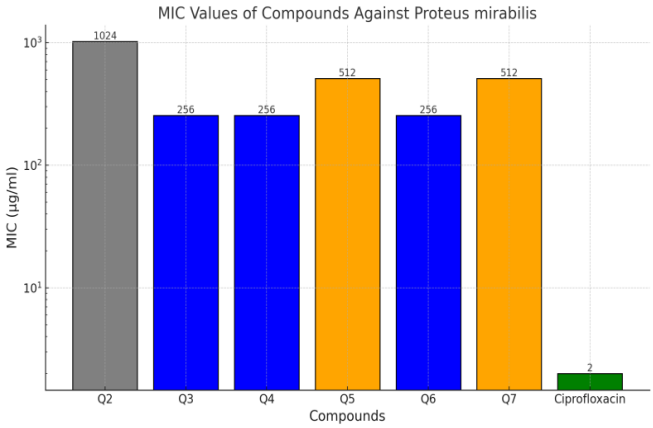

Figure 5. The bars show the MIC values of each compound in µg/ml against *Proteus mirabilis*
A logarithmic scale was used to enhance the display of significant differences between compounds. The compounds Q3, Q4, and Q6, represented in blue, have low MIC values, which in turn signify high activity. Q5 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 Muhammed et al. [28], who noted that there is increasing resistance to fluoroquinolones and that the need to develop other antimicrobial agents cannot be overemphasized, and Gordeev and Yuan [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, -NH_2) 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 structural aspects, such as the molecular weight and the position of the function groups, are important determinants of antibacterial activity. Q3, Q4, and Q6 are potential leads that could be developed into new antimicrobial agents. The data are supported by other studies that promote the development of drugs based on the structural aspects of bacterial enzymes, especially in the face of drug 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-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 (-NH_2), 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. This study was to demonstrate the importance of the use of computer modeling and the design of chemical compounds - a novel approach in the fight against the problem of antibiotic resistance, which is increasingly becoming a global health challenge. The study findings are significant in the pursuit of further research into the discovery of new compounds targeting resistant bacteria like Proteus mirabilis, which emphasizes the significance of the most active compounds (Q3, Q4, Q6) and the need to further improve the structure of these compounds to improve bioavailability and potency; and the need to conduct further in vitro and in vivo biological studies to establish the safety and efficacy of the compounds.

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REFERENCES

[1] Livermore, D.M., Blaser, M., Carrs, O., Cassell, (2011). Discovery research: The scientific challenge of finding new antibiotics. Journal of Antimicrobial Chemotherapy, 66(9): 1941-1944. https://doi.org/10.1093/jac/dkr262

[2] Varela, M.F., Stephen, J., Lekshmi, M., Ojha, M., Wenzel, N., Sanford, L.M., Hernandez, A.J., Parvathi, A., Kumar, S.H. (2021). Bacterial resistance to antimicrobial agents. Antibiotics, 10(5): 593. https://doi.org/10.3390/antibiotics10050593

[3] Xu, Z., Xu, D., Zhou, W., Zhang, X. (2022). Therapeutic potential of naturally occurring benzofuran derivatives and hybrids of benzofurans with other pharmacophores as antibacterial agents. Current Topics in Medicinal Chemistry, 22(1): 64-82. https://doi.org/10.2174/1568026621666211122162439

[4] Arandjelovic, P., Doerflinger, M., Pellegrini, M. (2019). Current and emerging therapies to combat persistent intracellular pathogens. Current Opinion in Pharmacology, 48: 33-39. https://doi.org/10.1016/j.coph.2019.03.013

[5] Lande, L., George, J., Plush, T. (2018). Mycobacterium avium complex pulmonary disease. Current Opinion in Infectious Diseases, 31(2): 199-207. https://doi.org/10.1097/qco.0000000000000437

[6] Grosdidier, A., Zoete, V., Michielin, O. (2011). SwissDock, a protein-small molecule docking web service. Nucleic Acids Research, 39(2): W270-W273. https://doi.org/10.1093/nar/gkr366

[7] Yilmaz, S., Yalcin, I., Okten, S., Onurdag, F.K., Aki-Yalcin, E. (2017). Synthesis and investigation of binding interactions of 1, 4-benzoxazine derivatives on topoisomerase IV in *Acinetobacter baumannii*. SAR and QSAR in Environmental Research, 28(11): 941-956. https://doi.org/10.1080/1062936X.2017.1404490

[8] Ebenezer, O., Singh-Pillay, A., Koorbanally, N.A., Singh, P. (2021). Antibacterial evaluation and molecular docking studies of pyrazole–thiosemicarbazones and their pyrazole–thiazolidinone conjugates. Molecular Diversity, 25(1): 191-204. https://doi.org/10.1007/s11030-020-10046-w

[9] Pacios, O., Blasco, L., Bleriot, I., Fernandez-Garcia, L., (2020). Strategies to combat multidrug-resistant and persistent infectious diseases. Antibiotics, 9(2): 65. https://doi.org/10.3390/antibiotics9020065

[10] Qin, Y., Xu, L., Teng, Y., Wang, Y., Ma, P. (2021). Discovery of novel antibacterial agents: Recent developments in D-alanyl-D-alanine ligase inhibitors. Chemical Biology & Drug Design, 98(3): 305-322. https://doi.org/10.1111/cbdd.13899

[11] Petrella, S., Capton, E., Raynal, B., Giffard, C., Thureau, A., Bonneté, F., Alzari, P.M., Aubry, A., Mayer, C. (2019). Overall structures of Mycobacterium tuberculosis DNA gyrase reveal the role of a corynebacteriales GyrB-specific insert in ATPase activity. Structure, 27(4): 579-589. https://doi.org/10.1016/j.str.2019.01.004

[12] Smith, P.A., Koehler, M.F., Girgis, H.S., Yan, D., et al. (2018). Optimized arylomycins are a new class of Gram-negative antibiotics. Nature, 561(7722): 189-194. https://doi.org/10.1038/s41586-018-0483-6

[13] Newman, D.J., Cragg, G.M. (2016). Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products, 79(3): 629-661. https://doi.org/10.1021/acs.jnatprod.5b01055

[14] Bush, N.G., Diez-Santos, I., Abbott, L.R., Maxwell, A., (2020). Quinolones: Mechanism, lethality and their contributions to antibiotic resistance. Molecules, 25(23): 5662. https://doi.org/10.3390/molecules25235662

[15] Levin-Reisman, I., Ronin, I., Gefen, O., Braniss, I., Shoresh, N., Balaban, N.Q. (2017). Antibiotic tolerance facilitates the evolution of resistance. Science, 355(6327): 826-830. https://doi.org/10.1126/science.aaj2191

[16] Blower, T.R., Williamson, B.H., Kerns, R.J., Berger, J.M. (2016). Crystal structure and stability of gyrase–fluoroquinolone cleaved complexes from Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences, 113(7): 1706-1713.

https://doi.org/10.1073/pnas.1525047113
[17] Muteeb, G., Rehman, M.T., Shahwan, M., Aatif, M. (2023). Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. Pharmaceuticals, 16(11): 1615. https://doi.org/10.3390/ph16111615
[18] Aragaw, W.W., Cotroneo, N., Stokes, S., Pucci, M., Critchley, I., Gengenbacher, M., Dick, T. (2022). In vitro resistance against DNA gyrase inhibitor SPR719 in *Mycobacterium avium* and *Mycobacterium abscessus*. Microbiology Spectrum, 10(1): e01321-21. https://doi.org/10.1128/spectrum.01321-21
[19] Bradford, P.A., Miller, A.A., O'Donnell, J., Mueller, J.P. (2020). Zoliflodacin: An oral spiropyrimidinetrione antibiotic for the treatment of *Neisseria gonorrhoeae*, including multi-drug-resistant isolates. ACS Infectious Diseases, 6(6): 1332-1345. https://doi.org/10.1021/acsinfecdis.0c00021
[20] Baldwin, S.L., Larsen, S.E., Ordway, D., Cassell, G. (2019). The complexities and challenges of preventing and treating nontuberculous mycobacterial diseases. PLoS Neglected Tropical Diseases, 13(2): e0007083. https://doi.org/10.1371/journal.pntd.0007083
[21] Muhammed, M.T., Aki-Yalcin, E. (2021). Computational insight into the mechanism of action of DNA gyrase inhibitors; revealing a new mechanism. Current Computational Chemistry, 20(3): 224-235. https://doi.org/10.2174/1573409919666230419094700
[22] Muhammed, M.T., Aki-Yalcin, E. (2021). Pharmacophore modeling in drug discovery: Methodology and current status. Journal of the Turkish Chemical Society, Section A: Chemistry, 8(3): 749-762. https://doi.org/10.18596/jotcsa.927426
[23] Qidwai, T. (2017). QSAR modeling, docking and ADMET studies for exploration of potential anti-malarial compounds against Plasmodium falciparum. In Silico Pharmacology, 5(1): 6. https://doi.org/10.1007/s40203-017-0026-0
[24] Waterhouse, A., Bertoni, M., Bienert, S., Studer, (2018). SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Research, 46(W1): W296-W303. https://doi.org/10.1093/nar/gky427
[25] Latif, A.S., Magtooph, M.G., Alubadi, A.E.M. (2020). *In silico* and *In vitro* evaluation of some synthesized quinoline derivatives into MexB protein of *Pseudomonas aeruginosa*. International Journal of Drug Delivery Technology, 10(2): 195-199.
[26] Dahlgren, D., Lennernäs, H. (2019). Intestinal permeability and drug absorption: Predictive experimental, computational and in vivo approaches. Pharmaceutics, 11(8): 411. https://doi.org/10.3390/pharmaceutics11080411
[27] Martin, Y.C. (2005). A bioavailability score. Journal of Medicinal Chemistry, 48(9): 3164-3170. https://doi.org/10.1021/jm0492002
[28] Muhammed, M.T., Kuyucuklu, G., Kaynak-Onurdag, F., Aki-Yalcin, E. (2022). Synthesis, antimicrobial activity, and molecular modeling studies of some benzoxazole derivatives. Letters in Drug Design & Discovery, 19(8): 757-768. https://doi.org/10.2174/1570180819666220408133643
[29] Gordeev, M.F., Yuan, Z.Y. (2014). New potent antibacterial oxazolidinone (MRX-I) with an improved class safety profile. Journal of Medicinal Chemistry, 57(11): 4487-4497. https://doi.org/10.1021/jm401931e
[30] Christensen, S.B. (2021). Drugs that changed society: History and current status of the early antibiotics: Salvarsan, sulfonamides, and β-lactams. Molecules, 26(19): 6057. https://doi.org/10.3390/molecules26196057
[31] Tian, W., Chen, C., Lei, X., Zhao, J., Liang, J. (2018). CASTp 3.0: Computed atlas of surface topography of proteins. Nucleic Acids Research, 46(W1): W363-W367. https://doi.org/10.1093/nar/gky473
[32] Taylor, S.N., Marrazzo, J., Batteiger, B.E., Hook, E.W., et al. (2018). Single-dose zoliflodacin (ETX0914) for treatment of urogenital gonorrhea. The New England Journal of Medicine, 379: 1835-1845. https://doi.org/10.1056/NEJMoa1706988
[33] Basarab, G.S., Kern, G.H., McNulty, J., Mueller, J.P (2015). Responding to the challenge of untreatable gonorrhea: ETX0914, a first-in-class agent with a distinct mechanism-of-action against bacterial Type II topoisomerases. Scientific Reports, 5(1): 11827. https://doi.org/10.1038/srep11827
[34] Latif, A.S., Fadel, Z.A. (2022). Evaluation study of the effectiveness for some antibacterial agent against dna gyrase enzyme of Staphylococcus aureus. European Chemical Bulletin, 11(7): 29-32.