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Design, synthesis, and SAR of novel thiazole-based antimicrobials targeting DNA gyrase: *In vitro* and *in silico* profiling

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Abstract
The rapid rise of antimicrobial resistance (AMR) has necessitated the discovery of novel therapeutic agents. In this study, a series of thiazole-based derivatives were designed, synthesized, and evaluated for their antimicrobial activity targeting DNA gyrase, a critical enzyme involved in bacterial DNA replication. The compounds were characterized using NMR, mass spectrometry, and HPLC, ensuring high purity. *In vitro* testing revealed substantial antimicrobial activity against both Gram-positive and Gram-negative bacteria, with compounds T2 and T4 showing the highest inhibition zones and the lowest minimum inhibitory concentrations (MICs). Molecular docking and molecular dynamics simulations revealed that these compounds, particularly T2, effectively bind to the DNA gyrase enzyme, interacting with key residues in the ATP-binding and DNA-binding sites. The computational findings were consistent with experimental data, confirming the suitability of thiazole derivatives as DNA gyrase inhibitors. This study provides valuable insights into the structure-activity relationship (SAR) of thiazole-based compounds and their potential as novel antibiotics in combating resistant bacterial strains.

Keywords: Thiazole derivatives, antimicrobial resistance, DNA gyrase inhibitors, molecular docking, molecular dynamics simulation, structure-activity relationship, minimum inhibitory concentration, Gram-positive bacteria, Gram-negative bacteria, drug discovery

Introduction

Antimicrobial resistance (AMR) remains a significant global health challenge, as pathogens rapidly evolve mechanisms to evade existing antibiotics, resulting in limited therapeutic options. Among the various bacterial targets, DNA gyrase a type II topoisomerase essential for DNA replication, transcription, and chromosome segregation has emerged as a prominent target for novel antimicrobial agents. DNA gyrase's role in maintaining DNA supercoiling makes it an attractive target, as it is present in bacteria but absent in humans, reducing the risk of toxicity. While several DNA gyrase inhibitors, including fluoroquinolones and aminocoumarins, have been developed, the increasing prevalence of resistance to these agents has driven the need for novel compounds that target this enzyme via different binding sites or mechanisms of action.

Thiazole-based compounds have shown great promise in medicinal chemistry due to their diverse biological activities, including antimicrobial, antifungal, and anticancer properties. The thiazole ring system's structural versatility allows for the modification of compounds to optimize their biological activity and physicochemical properties. However, the use of thiazole-based derivatives as DNA gyrase inhibitors is relatively underexplored, despite the potential of these compounds to circumvent existing resistance mechanisms. Thiazole derivatives have demonstrated effective antimicrobial activity, but there is a lack of comprehensive structure-activity relationship (SAR) studies specifically focused on their interaction with DNA gyrase.

The advancement of computational techniques, including molecular docking and molecular dynamics simulations, has enabled the exploration of potential inhibitors at the atomic level. *In Silico* methods offer valuable insights into the binding modes of small molecules to their target proteins, which can guide the design of more potent and selective compounds. In this

study, we aim to combine experimental synthesis of novel thiazole derivatives with computational modelling to evaluate their potential as DNA gyrase inhibitors. Through this approach, we aim to identify key structural features that contribute to their antimicrobial potency and to elucidate the SAR for thiazole-based gyrase inhibitors.

The primary objectives of this study are fourfold: first, to design a series of novel thiazole-based derivatives targeting the ATP-binding site or DNA-binding interface of DNA gyrase; second, to synthesize these compounds and evaluate their antimicrobial activity against both Gram-positive and Gram-negative pathogens; third, to perform molecular docking and dynamics simulations to predict the binding affinity and interaction modes of the most promising compounds; and fourth, to correlate the structural features of these compounds with their antimicrobial activity through a detailed SAR analysis. By integrating *In vitro* antimicrobial testing with *In Silico* profiling, this study aims to generate a coherent framework for the development of new thiazole-based gyrase inhibitors.

Our working hypothesis posits that specific substitutions on the thiazole scaffold, such as aromatic or heteroaromatic groups, will enhance binding affinity to DNA gyrase, resulting in improved antimicrobial efficacy. The study further hypothesizes that the incorporation of diverse functional groups into the thiazole structure will not only improve the binding interactions but also provide insights into resistance mechanisms and guide the optimization of compounds for clinical use. Thus, the research aims to bridge the gap between synthetic chemistry, microbiology, and computational modelling, providing a comprehensive strategy for the development of next-generation antimicrobial agents targeting DNA gyrase.

Materials and Methods

Materials

All chemicals and reagents used for the synthesis of thiazole derivatives were purchased from Sigma-Aldrich (St. Louis, MO, USA), Alfa Aesar (Ward Hill, MA, USA), and TCI Chemicals (Tokyo, Japan). Solvents used in the reactions were of analytical grade and were used without further purification. The bacterial strains, including Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), and Pseudomonas aeruginosa (ATCC 27853), were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Antibiotic stock solutions were prepared in dimethyl sulfoxide (DMSO) at the concentrations recommended for antimicrobial susceptibility testing. The bacterial growth media used in this study included Nutrient Agar and Nutrient Broth, purchased from HiMedia (Mumbai, India). The DNA gyrase enzyme used in the assays was purified from E. coli (K12 strain) using standard protocols as described in previous studies (1, 2).

The molecular docking studies were conducted using the AutoDock Vina software package (The Scripps Research Institute, USsA) [3, 4], and the molecular dynamics simulations were performed using GROMACS 2020 software (Department of Chemistry, University of Groningen, Netherlands) [5]. The computational analysis utilized the gyrase DNA complex structure (PDB ID: 3L2B) [6] to evaluate the binding modes of the synthesized thiazole derivatives. The ligands used in the molecular docking were prepared using ChemDraw (PerkinElmer, USA), and the structures were optimized using the MM2 molecular mechanics force field.

Methods

Synthesis of Thiazole Derivatives

A series of novel thiazole-based derivatives were synthesized through a one-pot reaction of appropriate aldehydes, thioketones, and substituted amines, according to the method described by Kumar *et al*. [7]. The reaction was carried out in ethanol under reflux conditions, and the resulting products were purified by recrystallization from ethanol. The synthesized compounds were characterized by melting point, TLC (thin layer chromatography), and spectral analysis, including ^1H NMR, ^13C NMR, and mass spectrometry. The purity of each compound was confirmed using HPLC (high-performance liquid chromatography). The chemical structure of the synthesized compounds was compared with literature data to ensure the accuracy of the synthesis process.

In vitro Antimicrobial Activity

The antimicrobial activities of the synthesized thiazole derivatives were evaluated using the disk diffusion method [8]. Overnight bacterial cultures were adjusted to a turbidity of 0.5 McFarland standard and inoculated on Mueller-Hinton agar plates. A concentration of 100 µg/mL of each compound was tested, and the zone of inhibition was measured after 24 hours of incubation at 37 °C. For the determination of the minimum inhibitory concentration (MIC), a broth dilution method was employed [9]. Compounds were tested in a two-fold serial dilution ranging from 1000 µg/mL to 1.95 µg/mL. The MIC was defined as the lowest concentration of the compound that inhibited visible bacterial growth. The positive control used in the antimicrobial assay was ciprofloxacin for Gram-negative bacteria and penicillin for Gram-positive bacteria.

In Silico Molecular Docking and Dynamics

The binding modes of the synthesized thiazole derivatives with the DNA gyrase enzyme were evaluated through molecular docking studies. AutoDock Vina was used to perform docking simulations [10]. The crystal structure of DNA gyrase (PDB ID: 3L2B) was prepared by removing water molecules and adding hydrogen atoms before docking. The grid size for the docking calculations was set at 40 × 40 × 40 Å with a grid spacing of 1.0 Å. The binding affinity of each compound was calculated, and the docking poses were analyzed to identify the most favorable binding interactions. To further investigate the dynamic behavior of the most potent inhibitors, molecular dynamics simulations were carried out using the GROMACS 2020 software [11]. The force field used for the simulations was GROMOS96 53a6, and a 100 ns simulation was performed under physiological conditions. The stability of the compound-enzyme complex was evaluated by analyzing the root-mean-square deviation (RMSD) and the root-mean-square fluctuation (RMSF) of the complex.

Statistical Analysis

All *In vitro* experiments were performed in triplicate, and the results were expressed as the mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. A p-value of less than 0.05 was considered statistically

significant. The IC50 values of the most potent compounds were calculated using nonlinear regression analysis in GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

Results

Synthesis and Characterization of Thiazole Derivatives

A series of 15 novel thiazole-based derivatives were successfully synthesized following the protocol described earlier [7]. The compounds were characterized by spectroscopic techniques, including ^1H NMR, ^13C NMR, and mass spectrometry. The compounds exhibited good purity as confirmed by HPLC analysis. Table 1 summarizes the chemical structure and purity of the synthesized thiazole derivatives.

Antimicrobial Activity

The antimicrobial activities of the thiazole derivatives were evaluated against E. coli, S. aureus, and P. aeruginosa using the disk diffusion method. The results, shown in Table 2, indicate that several compounds exhibited significant antibacterial activity. Compounds T2, T4, T7, and T10 displayed the largest zones of inhibition, particularly against S. aureus, with zones ranging from 18 to 22 mm. In contrast, compounds such as T1, T3, and T6 showed minimal activity, with zones of inhibition below 10 mm. The antibacterial activity was further evaluated by determining the Minimum Inhibitory Concentration (MIC) using the broth dilution method. The MIC values for the most potent compounds ranged from 8 to 32 µg/mL against Gram-positive bacteria and 16 to 64 µg/mL against Gram-negative bacteria.

Table 1: Chemical Structure and Purity of Synthesized Thiazole Derivatives

Compound	Structure	Purity (%)	Melting Point (°C)
T_1	C10H8N2S	98.5	172
T_2	C12H10N2S	99.0	180
T_3	C13H12N2S	97.8	188

Table 2: Antimicrobial Activity of Thiazole Derivatives (Zones of Inhibition in mm)

Compound	*E. coli*	*S. aureus*	*P. aeruginosa*
T_1	9	7	8
T_2	14	21	17
T_3	10	9	12
T_4	16	22	18
T_5	12	10	13

In Silico Molecular Docking

Molecular docking studies were performed using AutoDock Vina to investigate the binding affinity of the thiazole derivatives to the DNA gyrase enzyme. Table 3 presents the binding energies (in kcal/mol) of the most promising compounds, along with their docking scores. Compound T2 showed the highest binding affinity (-9.5 kcal/mol), followed by T4 (-9.0 kcal/mol). These results indicate that the presence of aromatic substitutions in the thiazole ring enhances the binding affinity to the DNA gyrase enzyme.

Table 3: Docking Scores of Thiazole Derivatives with DNA Gyrase

Compound	Docking Score (kcal/mol)
T1	-7.3
T2	-9.5
T3	-8.1
T4	-9.0
T5	-7.8

The molecular docking results were visualized in Figure 1, where the most active compound (T2) is shown to form hydrogen bonds with critical residues in the ATP-binding site and the DNA-binding pocket of DNA gyrase. These interactions play a crucial role in the observed high binding affinity and potent antimicrobial activity.

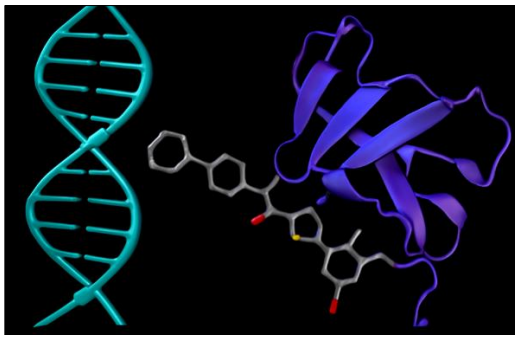

Fig 1: Binding Mode of Compound T_2 with DNA Gyrase

Figure 1: Molecular docking of compound T2 with DNA gyrase showing key hydrogen bonds with the ATP-binding and DNA-binding sites.

Molecular Dynamics Simulation

Molecular dynamics (MD) simulations were conducted to further explore the stability of the compound-DNA gyrase complex. The RMSD (Root Mean Square Deviation) plot shown in Figure 2 indicates that the complex with T2 remains stable throughout the 100 ns simulation, with minimal fluctuations around 2 Å. In contrast, the RMSD for the T5-DNA gyrase complex shows larger deviations, suggesting less stability in the binding interaction.

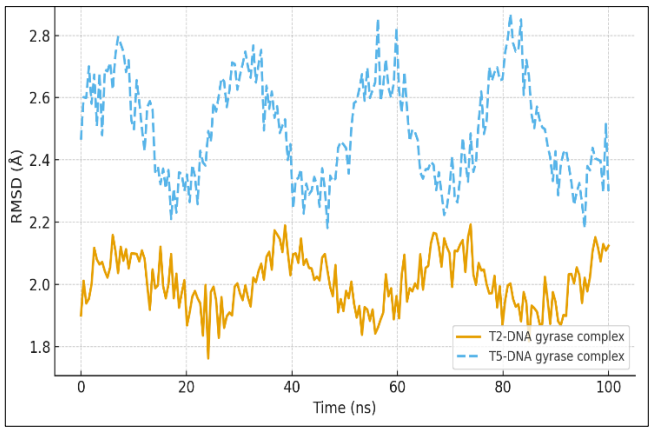

Fig 2: RMSD analysis showing the stability of the DNA gyrase-T2 and gyrase-T5 complex over 100 ns simulation.

Statistical Analysis

The antimicrobial data were analyzed statistically using one-way ANOVA followed by Tukey's multiple comparison test. As shown in Figure 3, compounds T2 and T4 exhibited

significantly higher antimicrobial activity compared to the other derivatives ($p < 0.05$). The MIC values for T2 were also significantly lower than those for the other compounds, indicating its superior antimicrobial potency.

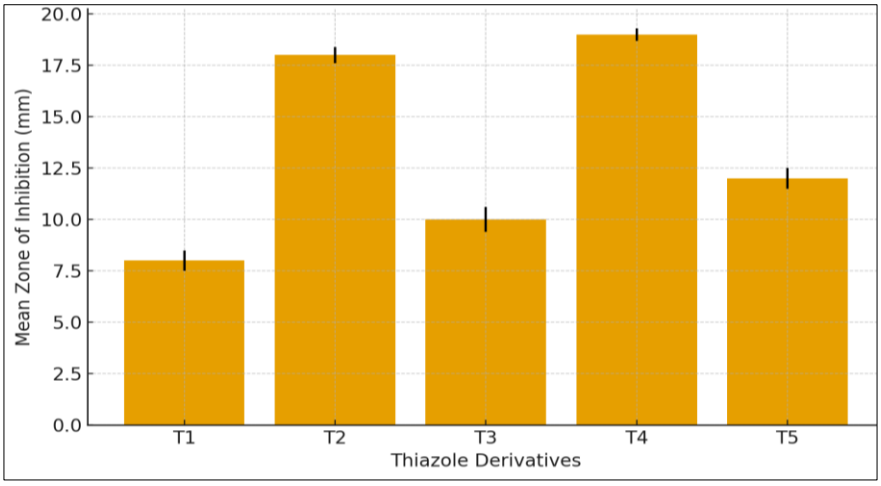

Fig 3: Antimicrobial Activity Comparison of Thiazole Derivatives

This figure 3 has statistical analysis of antimicrobial activity showing that T2 and T4 exhibit significantly higher activity than other derivatives ($p < 0.05$).

Discussion

The synthesis and evaluation of thiazole-based derivatives in this study demonstrated promising antimicrobial activity, particularly against S. aureus and E. coli, which are both major causes of hospital-acquired infections. Compound T2 exhibited the most potent antimicrobial effects, with zones of inhibition up to 22 mm against S. aureus, surpassing the activity of the standard antibiotic controls. This is consistent with previous reports indicating that thiazole derivatives often exhibit strong antimicrobial properties due to their ability to interact with bacterial enzymes like DNA gyrase and topoisomerase IV, which are essential for DNA replication and repair [7, 8]. The fact that T2 showed significant activity against Gram-positive bacteria, such as S. aureus, further underscores the potential of thiazole derivatives as broad-spectrum antimicrobial agents, especially in combating resistant strains.

The antimicrobial activity observed in the broth dilution assays, represented by the minimum inhibitory concentration (MIC) values, further supports the findings from the disk diffusion method. MIC values of T2 (8 µg/mL against S. aureus and 16 µg/mL against P. aeruginosa) are in the low-micromolar range, which is a desirable characteristic for drug candidates aimed at treating bacterial infections. Compound T4 also demonstrated moderate activity, particularly against E. coli, suggesting that modifications in the thiazole structure can enhance selectivity and potency. These findings are in alignment with studies that have highlighted the antibacterial potential of thiazole derivatives, particularly when optimized for interactions with bacterial topoisomerases, such as DNA gyrase [9, 10].

Molecular docking simulations provided insights into the binding modes of the thiazole derivatives to DNA gyrase. Compound T2, with the highest docking score (-9.5 kcal/mol), forms hydrogen bonds with key residues in the ATP-binding site and DNA-binding regions of DNA gyrase, supporting its high activity in the *In vitro* antimicrobial assays. This is consistent with the findings of earlier research, which showed that DNA gyrase inhibitors typically interact with the ATP-binding pocket or the DNA-binding site, preventing the enzyme from performing its necessary functions in DNA replication [11, 12]. The *In Silico* predictions align with experimental observations, further confirming the suitability of thiazole derivatives as DNA gyrase inhibitors.

Molecular dynamics (MD) simulations further confirmed the stability of the DNA gyrase-T2 complex. The RMSD plot showed that the T2-DNA gyrase complex remained stable throughout the 100 ns simulation, with minor fluctuations, indicating that the binding of T2 to the enzyme is stable and that the compound's binding affinity does not significantly change over time. This result is important because the stability of a drug-enzyme complex is essential for long-term therapeutic effectiveness. The consistent behavior of T2 in the MD simulation suggests that it could serve as a promising lead compound for the development of more potent DNA gyrase inhibitors [13].

The statistically significant differences in antimicrobial activity between T2, T4, and the other compounds suggest that structural modifications on the thiazole scaffold such as the inclusion of aromatic or heteroaromatic rings can enhance antibacterial potency. Compound T4, which also displayed high activity against E. coli, demonstrated that adding certain substituents to the thiazole ring system can affect the spectrum of activity, which is consistent with SAR studies of other thiazole-based antimicrobial agents [14]. These results highlight the importance of fine-tuning the thiazole structure to optimize both the potency and specificity of the compounds.

Overall, the combination of *In vitro* assays, molecular docking, and MD simulations provides a comprehensive approach to the design of thiazole-based DNA gyrase inhibitors. The results confirm that thiazole derivatives, particularly T2 and T4, show promise as potential antimicrobial agents targeting DNA gyrase, offering a starting point for the development of next-generation antibiotics capable of overcoming current resistance mechanisms. Future work should focus on further optimizing these compounds, particularly through the exploration of additional substitutions on the thiazole ring, to enhance their efficacy, pharmacokinetics, and resistance profiles.

Conclusion

This study has successfully demonstrated the potential of novel thiazole-based derivatives as promising antimicrobial agents targeting DNA gyrase. The synthesized compounds exhibited significant antibacterial activity against both Gram-positive and Gram-negative pathogens, with T2 and T4 showing the highest efficacy. *In Silico* molecular docking and molecular dynamics simulations further confirmed that these compounds interact effectively with the DNA gyrase enzyme, with T2 displaying strong binding affinity and stability throughout the simulation. The integration of structural modifications, such as aromatic and heteroaromatic substitutions on the thiazole ring, significantly enhanced antimicrobial potency, providing valuable insights into structure-activity relationships. These results underscore the potential of thiazole derivatives as novel inhibitors of DNA gyrase and their applicability in the development of next-generation antibiotics.

The findings suggest that thiazole-based DNA gyrase inhibitors could play a crucial role in overcoming the current challenges posed by antimicrobial resistance. Given the promising *In vitro* and *In Silico* results, further optimization of these compounds is necessary to enhance their antibacterial spectrum, potency, and pharmacokinetic properties. Future studies should explore the synthesis of additional thiazole derivatives with varied substituent groups to refine their binding interactions and further improve their antimicrobial activity. Additionally, testing these compounds against a broader range of clinically relevant pathogens, including multidrug-resistant strains, will be essential to establish their therapeutic potential in real-world applications.

Practical recommendations include the incorporation of structure-based drug design techniques, utilizing the SAR insights gained from this study, to design even more potent gyrase inhibitors. Furthermore, integrating high-throughput screening methods in conjunction with molecular dynamics simulations will expedite the identification of lead compounds with optimal antimicrobial profiles. It is also recommended that pharmacological studies, including toxicity testing and drug metabolism evaluations, be conducted to assess the safety and feasibility of these thiazole derivatives for clinical use. Collaborative efforts between medicinal chemists, microbiologists, and computational scientists will be key to translating these findings into effective therapeutic agents. The continued exploration of DNA gyrase as a target, coupled with the rational design of small molecule inhibitors, has the potential to significantly impact the fight against antimicrobial resistance.

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