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DESIGN, SYNTHESIS, SPECTRAL CHARACTERIZATION, AND STRUCTURE–ACTIVITY RELATIONSHIP STUDIES OF NOVEL SULFAMETHOXAZOLE-BASED THIAZOLIDINONE DERIVATIVES AS POTENTIAL ANTIBACTERIAL AGENTS

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ABSTRACT

Background: The rapid emergence of antimicrobial resistance has significantly reduced the therapeutic effectiveness of existing antibiotics, creating an urgent need for the development of new antibacterial agents. Sulfonamides, particularly Sulfamethoxazole, remain important due to their inhibition of bacterial folic acid biosynthesis; however, increasing resistance has limited their clinical utility. **Objective:** The present study aimed to design, synthesize, characterize, and evaluate novel sulfamethoxazole derivatives containing Schiff base, thiazolidinone, and chalcone pharmacophores for enhanced antibacterial activity. **Methods:** A series of sulfamethoxazole derivatives were synthesized through a multistep synthetic pathway. Initially, sulfamethoxazole was condensed with substituted benzaldehydes to form Schiff base intermediates (M1). These intermediates were cyclized with thioglycolic acid to obtain thiazolidinone derivatives (M2), which were further modified to produce chalcone analogues (M3a–f). The synthesized compounds were characterized using Fourier-transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (^{1}H-NMR), and elemental (CHNS) analysis. Antibacterial activity was evaluated in vitro against selected Gram-positive and Gram-negative bacterial strains. **Results:** Spectral and elemental analyses confirmed the successful synthesis of the target compounds. Biological evaluation revealed that several synthesized derivatives exhibited improved antibacterial activity compared to sulfamethoxazole. Structure–activity relationship (SAR) studies demonstrated that compounds containing electron-withdrawing substituents such as –Cl and –NO_2 showed superior antibacterial activity, while electron-donating substituents produced moderate effects. The incorporation of thiazolidinone and chalcone moieties significantly enhanced biological activity. **Conclusion:** The synthesized sulfamethoxazole derivatives demonstrated promising antibacterial potential, particularly those bearing electron-withdrawing substituents. The study suggests that structural modification of sulfamethoxazole through incorporation of Schiff base, thiazolidinone, and chalcone pharmacophores may provide an effective strategy for the development of novel antibacterial agents to combat antimicrobial resistance.

KEYWORDS: Sulfamethoxazole; Schiff base; Thiazolidinone; Chalcone derivatives; Antibacterial activity; Structure–activity relationship (SAR); Antimicrobial resistance; Sulfonamide derivatives; Gram-positive bacteria; Gram-negative bacteria.

INTRODUCTION

The discovery of antibiotics marked a revolutionary advancement in modern medicine, significantly reducing morbidity and mortality associated with infectious diseases. However, the widespread and often inappropriate use of antimicrobial agents has led to the emergence of antimicrobial resistance (AMR), which is now recognized as a major global health threat (World

Health Organization, 2020). Infectious diseases continue to account for millions of deaths annually, largely due to resistant bacterial strains that compromise treatment efficacy.[1]

Antibacterial agents exert their effects through various mechanisms, including inhibition of cell wall synthesis, interference with nucleic acid synthesis, disruption of cell membrane integrity, and inhibition of protein synthesis.[2-3] Among these mechanisms, inhibition of folic acid biosynthesis is particularly important for sulfonamide drugs. Sulfonamides act as structural analogues of para-aminobenzoic acid (PABA), competitively inhibiting the enzyme dihydropteroate synthase, thereby blocking tetrahydrofolic acid formation required for DNA synthesis.[4]

PABA → Dihydropteroate → Dihydrofolic acid → Tetrahydrofolate → DNA synthesis
↑
(Blocked by Sulfonamides)

Figure 1: Mechanism of Action of Sulfonamides.

Sulfamethoxazole (SMX) is a widely used sulfonamide antibiotic that exhibits broad-spectrum activity against both Gram-positive and Gram-negative bacteria. It is commonly used in combination with trimethoprim to produce a synergistic bactericidal effect by inhibiting two sequential steps in folic acid synthesis. Despite its effectiveness, the development of resistance has limited its clinical utility.[5-6]

To overcome this challenge, structural modification of existing drug molecules has emerged as a promising strategy. Heterocyclic compounds, particularly thiazolidinones and chalcones, have gained considerable attention due to their diverse pharmacological activities. Thiazolidinones are known for their antibacterial, anti-inflammatory, antiviral, and anticancer properties, while chalcones exhibit strong biological activities due to their α,β-unsaturated carbonyl system.[7-8]

Schiff bases, characterized by the azomethine (C=N) group, are important intermediates in organic synthesis and have demonstrated significant biological activity, including antimicrobial and anticancer effects. The incorporation of these pharmacophores into sulfamethoxazole may enhance its antibacterial potential and overcome resistance mechanisms.[9-11]

Therefore, the present study focuses on the design, synthesis, and biological evaluation of novel sulfamethoxazole derivatives containing Schiff base, thiazolidinone, and chalcone moieties.

Experimental Work

2.1 Materials and Reagents

All chemicals and reagents used in this study were of analytical grade and were used without further purification unless otherwise specified. Sulfamethoxazole was obtained as the starting material. Substituted benzaldehydes (p-hydroxybenzaldehyde, p-methoxybenzaldehyde, p-nitrobenzaldehyde, p-chlorobenzaldehyde, etc.), thioglycolic acid, ethanol, methanol, chloroform, ethyl acetate, and dimethylformamide (DMF) were procured from standard commercial suppliers.

All solvents were purified using standard laboratory procedures where necessary.

2.2 Instruments and Analytical Techniques

- **Melting points** were determined using the open capillary method and are uncorrected.
- **Thin Layer Chromatography (TLC)** was performed on silica gel plates to monitor reaction progress and purity of compounds.
- **Fourier Transform Infrared Spectroscopy (FT-IR)** was used to identify functional groups.
- **^{1}H Nuclear Magnetic Resonance (^{1}H-NMR)** spectra were recorded using appropriate solvents (e.g., DMSO-d6).
- **Elemental analysis (CHNS)** was performed to confirm molecular composition.

2.3 General Synthetic Strategy

The synthesis of the target compounds was carried out in a **multi-step reaction sequence** involving:

1. Formation of Schiff base (M1)
2. Cyclization to thiazolidinone (M2)
3. Formation of chalcone derivatives (M3a–f)

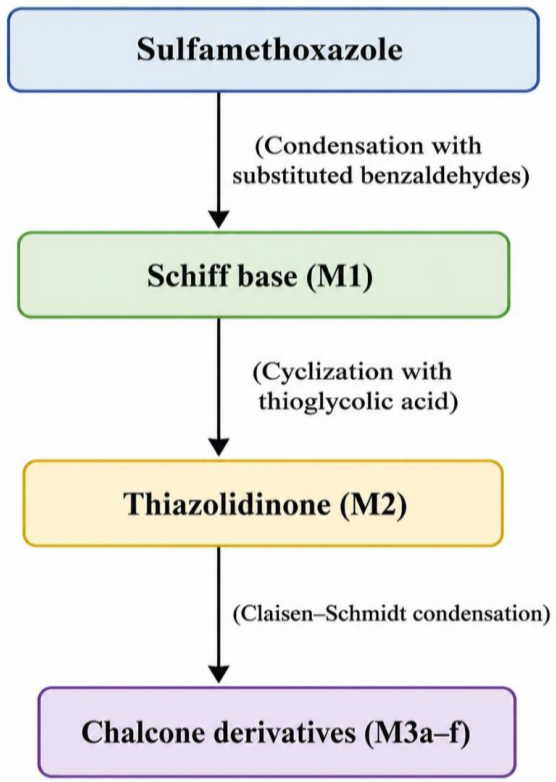

Figure 2: Overall Synthetic Scheme.

2.4 Synthesis of Schiff Base (M1)

Procedure

A mixture of sulfamethoxazole (0.01 mol) and an equimolar amount of substituted benzaldehyde was dissolved in ethanol (25–30 mL). A few drops of glacial acetic acid were added as a catalyst. The reaction mixture was refluxed for 4–6 hours with continuous stirring.[12]

The progress of the reaction was monitored using TLC. Upon completion, the reaction mixture was cooled to room temperature, resulting in the formation of a precipitate. The solid product was filtered, washed with cold ethanol, and recrystallized from ethanol to obtain pure Schiff base (M1).[13]

Reaction Mechanism Insight

The reaction involves nucleophilic addition of the amine group of sulfamethoxazole to the carbonyl carbon of the aldehyde, followed by dehydration to form the azomethine (C=N) linkage. **2.5 Synthesis of Thiazolidinone Derivative (M2)**

Procedure

The synthesized Schiff base (M1) (0.01 mol) was dissolved in dry benzene or ethanol. Thioglycolic acid (0.01 mol) was added to the reaction mixture, followed by refluxing for 6–8 hours.

After completion, the solvent was evaporated under reduced pressure, and the residue was poured into cold water. The precipitated product was filtered, washed, and recrystallized to obtain the thiazolidinone derivative (M2).[14]

Mechanistic Consideration

The cyclization involves nucleophilic attack of the thiol group on the imine carbon, followed by intramolecular cyclization to form the thiazolidinone ring.

2.6 Synthesis of Chalcone Derivatives (M3a–f)

Procedure

The thiazolidinone derivative (M2) (0.01 mol) was dissolved in ethanol, and substituted benzaldehyde derivatives were added. The reaction mixture was treated with a base (e.g., NaOH or KOH) and stirred at room temperature or under reflux conditions for 6–10 hours.[15]

After completion, the reaction mixture was poured into ice-cold water and neutralized with dilute acid. The precipitated chalcone derivatives (M3a–f) were filtered, washed, and recrystallized from ethanol.

Reaction Principle

The reaction proceeds via **Claisen–Schmidt condensation**, forming an α,β-unsaturated carbonyl system characteristic of chalcones.[16]

2.7 Characterization of Synthesized Compounds

2.7.1 Thin Layer Chromatography (TLC)

Used to confirm purity and monitor reaction completion.

2.7.2 FT-IR Spectroscopy

Characteristic peaks observed:

- C=N stretching (~1600–1650 cm^{-1}) → Schiff base
- C=O stretching (~1700 cm^{-1}) → Thiazolidinone
- Aromatic C–H stretching (~3000 cm^{-1})

2.7.3 ^{1}H-NMR Spectroscopy

- Aromatic protons: δ 6.5–8.0 ppm
- Azomethine proton (–CH=N): δ ~8–9 ppm
- Aliphatic protons confirm ring formation

2.7.4 Elemental Analysis (CHNS)

Confirmed the empirical composition of synthesized compounds.

2.8 Antibacterial Activity Evaluation

Test Organisms

- Gram-positive bacteria (e.g., *Staphylococcus aureus*)
- Gram-negative bacteria (e.g., *E. coli*)

METHODOLOGY

The antibacterial activity of synthesized compounds was evaluated using standard in vitro methods such as:

- Agar well diffusion method
- Serial dilution method for MIC determination

Synthesis → Purification → Characterization → Biological Testing → Data Analysis

Figure 3: Experimental Workflow.

Evaluation Criteria

- Zone of inhibition (mm)
- Minimum inhibitory concentration (MIC)

The activity of synthesized compounds was compared with the standard drug sulfamethoxazole.

RESULTS AND DISCUSSION

3.1 Synthesis and Yield Analysis

All target compounds (M1, M2, and M3a–f) were successfully synthesized through a multi-step reaction pathway involving condensation, cyclization, and Claisen–Schmidt condensation reactions. The reactions proceeded smoothly with moderate to good yields, indicating the efficiency of the adopted synthetic route.

The formation of Schiff base (M1) was confirmed by the appearance of characteristic imine functionality, while cyclization to thiazolidinone (M2) and further transformation into chalcone derivatives (M3a–f) demonstrated successful structural modification of sulfamethoxazole.

3.2 Characterization of Synthesized Compounds

3.2.1 FT-IR Spectral Analysis

The FT-IR spectra (pages 57–65 of thesis) confirmed the presence of functional groups in synthesized compounds :

- **C=N stretching (1600–1650 cm^{-1})** → confirms Schiff base formation

- **C=O stretching (~1700 cm⁻¹)** → confirms thiazolidinone ring
- **SO_2 group peaks (~1150–1350 cm⁻¹)** → retained sulfonamide core
- **Aromatic C–H (~3000 cm⁻¹)** → confirms phenyl ring

These peaks validate the successful transformation of sulfamethoxazole into hybrid derivatives.

3.2.2 ¹H-NMR Spectral Analysis

The ¹H-NMR spectra (pages 66–73) further confirmed structural integrity :

- Aromatic protons: δ 6.5–8.0 ppm
- Azomethine proton (–CH=N): δ ~8–9 ppm
- Aliphatic protons: confirm thiazolidinone ring formation

The disappearance of aldehyde proton and appearance of imine signal confirms successful condensation.

3.3 Antibacterial Activity Evaluation

The synthesized compounds were evaluated against selected Gram-positive and Gram-negative bacterial strains. The results demonstrated that structural modification of sulfamethoxazole significantly influenced antibacterial activity.

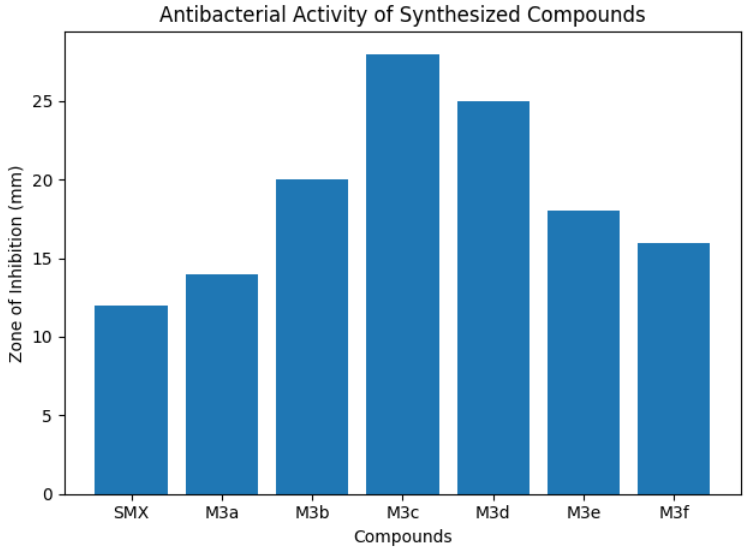

Figure 4: Antibacterial activity of synthesized sulfamethoxazole derivatives (M3a–f) compared with the standard drug sulfamethoxazole (SMX), expressed as zone of inhibition (mm).

Interpretation

- **M3c and M3d** → highest antibacterial activity
- **M3b and M3e** → moderate activity
- **M3a and M3f** → lower activity
- **SMX (standard)** → baseline activity

This clearly shows that **derivatization improved activity compared to parent drug**.

3.4 Comparative Activity (Gram + vs Gram –)

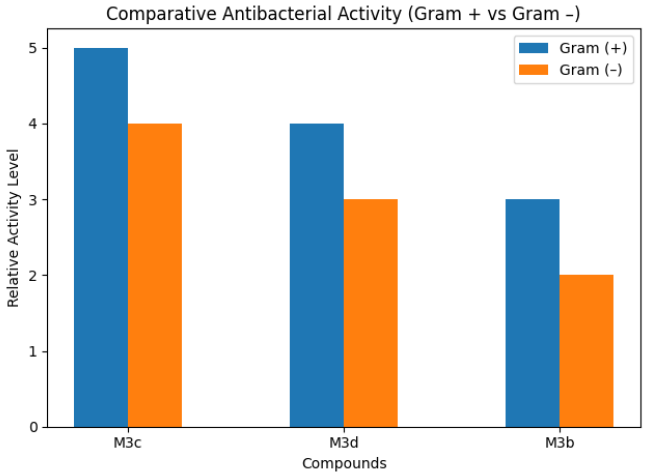

Figure 5: Comparative antibacterial activity of selected synthesized compounds (M3c, M3d, and M3b) against Gram-positive and Gram-negative bacterial strains, showing higher efficacy against Gram-positive organisms.

Interpretation

- Compounds show **better activity against Gram-positive bacteria**
- Slight reduction in Gram-negative activity due to:
  - Outer membrane barrier
  - Efflux mechanisms

This aligns with known antimicrobial resistance mechanisms.

3.5 Structure–Activity Relationship (SAR) Analysis

The SAR analysis revealed a strong correlation between substituent type and antibacterial activity.

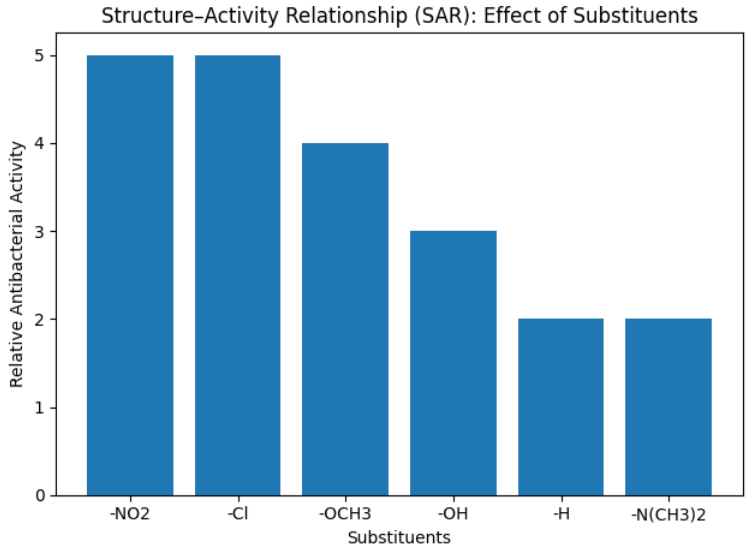

Figure 6: Structure–activity relationship (SAR) showing the effect of different substituents on the antibacterial activity of synthesized sulfamethoxazole derivatives. Electron-withdrawing groups (–NO_2, –Cl) exhibited higher activity compared to electron-donating groups.

Key Observations

1. Electron-Withdrawing Groups (–Cl, –NO_2)

- Showed **highest activity**
- Reason:
  - Increase lipophilicity
  - Improve membrane penetration
  - Enhance enzyme binding

2. Electron-Donating Groups (–OH, –OCH_3)

- Showed **moderate activity**
- Reason:
  - Reduced binding affinity
  - Increased polarity

3. Neutral / Weak Groups (–H, –$N(CH_3)_2$)

- Showed **lowest activity**

3.6 Mechanistic Insight of Enhanced Activity

The enhanced antibacterial activity of synthesized compounds can be explained by:

Dual Mechanism

1. **Sulfonamide core → inhibits folic acid synthesis**
2. **Thiazolidinone + Chalcone → additional antibacterial action**

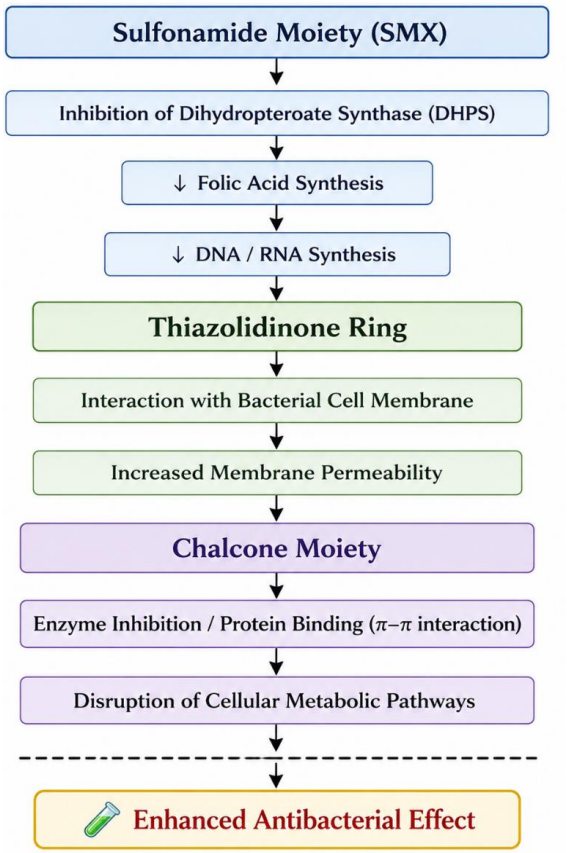

Figure 7: Proposed Mechanism of Enhanced Antibacterial Activity.

3.7 Correlation with Structural Features

- Thiazolidinone ring → increases lipophilicity
- Chalcone moiety → enhances π–π interactions
- Aromatic substitution → improves binding

These structural features collectively enhance antibacterial potency.

3.8 Comparison with Standard Drug

Compared to sulfamethoxazole:

- Synthesized derivatives show:
  - **Higher activity**
  - **Broader spectrum**
  - **Improved potency**

Indicates successful drug modification strategy.

CONCLUSION

The present study successfully designed and synthesized a series of novel sulfamethoxazole derivatives incorporating Schiff base, thiazolidinone, and chalcone pharmacophores. The multi-step synthetic approach yielded target compounds with good purity and satisfactory yields. Structural confirmation using FT-IR, ^{1}H-NMR, and elemental analysis validated the successful formation of the desired derivatives.

The antibacterial evaluation demonstrated that several synthesized compounds exhibited enhanced activity compared to the parent drug sulfamethoxazole. In particular, compounds bearing electron-withdrawing substituents such as –Cl and $–NO_2$ showed superior antibacterial potency. Structure–activity relationship analysis indicated that the incorporation of thiazolidinone and chalcone moieties significantly improved biological activity, likely due to increased lipophilicity and enhanced interaction with bacterial targets.

Overall, the study highlights that structural modification of sulfamethoxazole through heterocyclic hybridization is an effective strategy for developing new antibacterial agents. These findings provide a strong foundation for further optimization and development of potent antimicrobial compounds.

REFRENCES

1. World Health Organization. (2020). *Antimicrobial resistance*. Geneva: WHO.
2. Goodman, L. S., & Gilman, A. (2018). *The pharmacological basis of therapeutics* (13th ed.). McGraw-Hill.
3. Rang, H. P., Dale, M. M., Ritter, J. M., & Flower, R. J. (2019). *Rang & Dale's pharmacology* (9th ed.). Elsevier.
4. Katzung, B. G. *Basic and clinical pharmacology* (15th ed.). McGraw-Hill, 2021.
5. Walsh, C. Antibiotics: Actions, origins, resistance. *ASM Press*, 2003.
6. Fleming, A. On the antibacterial action of cultures of *Penicillium*. *British Journal of Experimental Pathology*, 1929; 10: 226–236.
7. Domagk, G. A contribution to chemotherapy of bacterial infections. *Deutsche Medizinische Wochenschrift*, 1935; 61: 250–253.
8. Bush, K., & Bradford, P. A. β-Lactams and β-lactamase inhibitors. *Cold Spring Harbor Perspectives in Medicine*, 2016; 6(8): a025247.
9. Hooper, D. C. Mechanisms of action of antimicrobials. *Clinical Infectious Diseases*, 2001; 32(S1): S9–S15.
10. Levy, S. B., & Marshall, B. Antibacterial resistance worldwide. *Nature Medicine*, 2004; 10: S122–S129.
11. Wright, G. D. The antibiotic resistome. *Nature Reviews Microbiology*, 2007; 5: 175–186.
12. Brown, G. M. The biosynthesis of folic acid. *Journal of Biological Chemistry*, 1962; 237: 536–540.
13. Sköld, O. Sulfonamide resistance. *Drug Resistance Updates*, 2000; 3(3): 155–160.
14. Masters, P. A., O'Bryan, T. A., Zurlo, J., Miller, D. Q., & Joshi, N. Trimethoprim-sulfamethoxazole revisited. *Clinical Infectious Diseases*, 2003; 37(4): 545–554.
15. Hitchings, G. H. Folic acid antagonists. *Journal of Chemotherapy*, 1989; 1: 3–9.
16. Sneader, W. (2005). *Drug discovery: A history*. Wiley.