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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF NEW SERIES OF SULFAMETHOXAZOLE DERIVATIVES

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ABSTRACT

This study reports the synthesis, structural characterization, and antibacterial evaluation of five novel derivatives of sulfamethoxazole, designed through targeted chemical modifications at the para-amino group to enhance antimicrobial potency. The derivatives (SMX-1 to SMX-5) were synthesized via condensation, acylation, substitution, and esterification reactions, and structurally confirmed using Fourier Transform Infrared Spectroscopy *(FTIR), ^{1}H NMR, and mass spectrometry techniques. The antibacterial activity of the synthesised compounds was assessed in vitro using the broth microdilution and agar well diffusion procedures against Staphylococcus aureus and Escherichia coli. Among the derivatives, SMX-3 demonstrated the most potent activity with the largest inhibition zones and lowest minimum inhibitory concentrations (MIC), followed by SMX-4 and SMX-2. Efficiency scores calculated as the ratio of inhibition zone to MIC affirmed the enhanced biological performance of these derivatives compared to the parent drug and standard antibiotic. The results highlight the potential of rational structural derivatization of sulfamethoxazole in overcoming resistance and improving therapeutic efficacy.*

Keywords: *Synthesis, Characterization, Antibacterial Activity, New Series, Sulfamethoxazole Derivatives.*

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1. INTRODUCTION

The continuous emergence of antimicrobial resistance (AMR) has become a critical threat to global health, necessitating the discovery and development of novel antibacterial agents or modifications of existing drugs. Sulfonamides, one of the earliest classes of synthetic antimicrobials, have played a significant role in clinical therapy since the 1930s. Among them, sulfamethoxazole a synthetic bacteriostatic sulphonamide is widely used for the treatment of urinary tract infections, bronchitis, and other bacterial diseases, particularly in combination with trimethoprim (co-trimoxazole), which exhibits synergistic inhibition of the bacterial folic acid pathway.

Sulfamethoxazole functions by inhibiting dihydropteroate synthase (DHPS), an enzyme critical for folate biosynthesis in bacteria. However, extensive and long-term use has led to the development of resistant bacterial strains. Therefore, there is a pressing need to structurally modify this molecule to enhance its antimicrobial spectrum, potency, and resistance profile.

1.1. Need for Derivative Development

Numerous studies have demonstrated that chemical derivatization of parent drugs is an effective strategy for enhancing biological activity and reducing toxicity. Modifications at the para-amino group of sulfamethoxazole offer a favourable site for introducing new pharmacophores via Schiff base formation, acylation, alkylation, and condensationreactions. These transformations can improve the molecule’s **li**pophilicity**,** binding affinity to bacterial enzymes, and overall bioavailability, thereby yielding more potent derivatives.

In addition, structure-activity relationship (SAR) studies suggest that incorporating electron-withdrawing or hydrophilic groups—such as hydroxyl, carbonyl, or unsaturated side chains—can modulate drug-enzyme interactions and cellular uptake. This aligns with modern medicinal chemistry objectives: to optimize molecular scaffolds without altering the pharmacophore responsible for core activity.

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1.2. Scope of the Present Study

This study involves the design, synthesis, and biological evaluation of five novel sulfamethoxazole derivatives, each modified at the para-amino position. The synthesized compounds are:

- **SMX-1**: *N-(5-methylisoxazol-3-yl)-4-[(methylidene)amino]benzenesulfonamide*
- **SMX-2**: *N-(5-methylisoxazol-3-yl)-4-(acetamidomethyl)benzenesulfonamide*
- **SMX-3**: *N-(5-methylisoxazol-3-yl)-4-[(hydroxyacetyl)amino]benzenesulfonamide*
- **SMX-4**: *N-(5-methylisoxazol-3-yl)-4-(prop-2-en-1-ylamino)benzenesulfonamide*
- **SMX-5**: *N-(5-methylisoxazol-3-yl)-4-[(3-hydroxybutanoyl)amino]benzenesulfonamide*

Targeted organic reactions were used to transform the structure of these derivatives and they were checked for structure using FTIR, ^{1}H NMR and mass spectrometry. Their antibacterial potential was evaluated against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), both of which are clinically relevant and known for developing resistance.

1.3. Research Objectives

The research objectives of the study are:

- To synthesize and structurally characterize novel sulfamethoxazole derivatives through selective chemical modifications at the para-amino group.
- To assess the produced derivatives' antibacterial efficacy against E. coli and S. aureus in vitro.
- To compare the antibacterial efficiency of the synthesized compounds with the parent drug (sulfamethoxazole) and standard antibiotic (ampicillin) using efficiency scoring metrics.

2. REVIEWOF LITERATURE

Khorasani et al. (2024) forged new mixes of 1,8-dioxo-decahydroacridine with sulfamethoxazole, explored their unique capabilities and looked at their effectiveness against several microorganisms. They proved that the derivatives can fight against a range of

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microbes and are interesting when exposed to light which may open up new uses for light-based treatments.

Mosleh and Dakhel (2024) studied new sulfonamide compounds with a 1,2,4-triazole-3-thiol ring were made using microwave synthesis. A range of tests and molecular docking were done to see how the compounds might interact with the chosen enzymes. First tests on antimicrobial activity showed that these compounds work against particular bacteria which suggest the process and derivatives are promising.

Sager et al. (2024) studied how new sulfamethoxazole derivatives behave and evaluated their biological responses. The docking method was used in several studies to explain the molecular interactions in this system. They found that the compounds were successfully synthesized, demonstrated effective antimicrobial behavior and scored positively in docking tests to ensure effective attachment to bacteria.

Salem et al. (2024) synthesized hybrids of thiazoles and bis-thiazoles joined to azo-sulfamethoxazole and checked their effectiveness against microbes. The team carried out molecular docking to investigate the way these molecules interact with microbes. The results suggested that bringing several bioactive parts together into one hybrid molecule could improve the effectiveness of the antimicrobial activity.

Vaickelionienė et al. (2023) produced and evaluated many sulfamethoxazole derivatives to investigate their activities against both cancer and infections. They used several approaches to make the compounds and experimented to study their impact on both cancer and dangerous microorganisms. Science found that carbapenem antibiotics might also do more than just kill bacteria.

Wajid et al. (2024) detailed the process for making Schiff bases from sulfamethoxazole and analyzed their main properties. The research team used molecular docking, DFT and ADME (which covers absorption, distribution, metabolism and excretion) to study how the compounds behaved biologically. Schiff bases were developed by linking how they are built to their impacts on living organisms and on medicine, making it simpler to develop them for antimicrobial actions.

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3. METHODOLOGY

The research, novel sulfamethoxazole compounds were synthesized, studied with different forms of spectroscopy and evaluated for their antibacterial activity against laboratory cultures. All steps were performed in a controlled environment so that the results could be repeated with certainty.

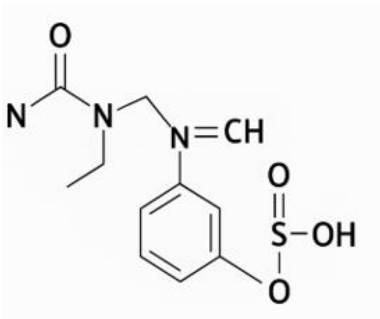
3.1. Chemical Synthesis of Sulfamethoxazole Derivatives

Functional modification of the para-amino group of sulfamethoxazole was done by selectively condensing, acylating and substituting it. Five derivatives were synthesized using different electrophilic agents to introduce distinct pharmacophores. All reactions were carried out in round-bottom flasks under reflux, using ethanol or dimethylformamide (DMF) as solvents, with gentle heating (60–80 °C) and stirring until completion was confirmed by Thin Layer Chromatography (TLC).

Each compound's synthetic route, structure, and chemical nature are described below:

- **SMX-1**

Synthesized by condensation of sulfamethoxazole with methyl formate, yielding a Schiff base with an imine bond (-N=CH-). This introduces a planar, electron-rich group that may improve conjugation.



- **SMX-2**

Acylation with acetic anhydride resulted in the introduction of an acetamidomethyl group. This enhances steric bulk and electron-withdrawing capacity.

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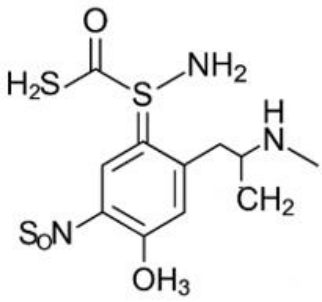
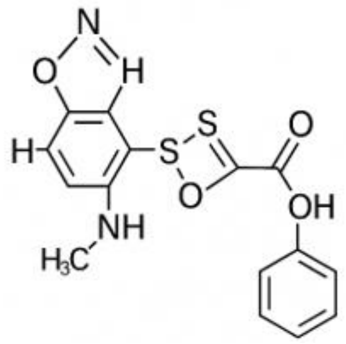


SMX-2

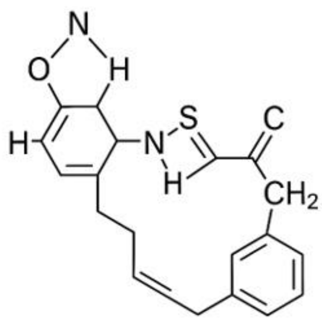
- **SMX-3**

Reaction with glycolic acid formed a hydroxy acetyl derivative. The hydroxyl and carbonyl functional groups contribute to polarity and hydrogen-bonding potential.



- **SMX-4**

Substitution with allyl bromide yielded a prop-2-en-1-ylamino derivative, incorporating an unsaturated side chain that enables potential π–π or hydrophobic interactions.



- **SMX-5**

Esterification with 3-hydroxybutyric acid gave a hydroxybutanoyl substitution, increasing hydrophilicity and molecular flexibility.

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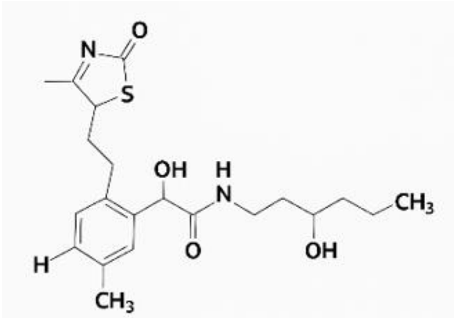

To visually support the synthetic approach, the general synthesis reaction scheme is presented in Figure 1 below.

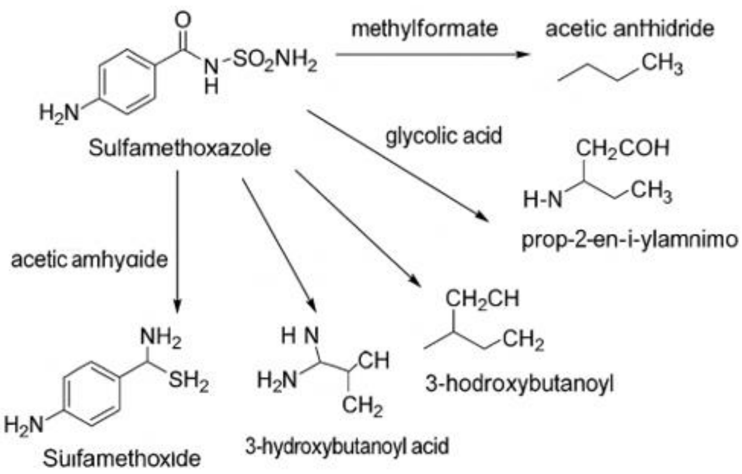

Figure 1: General Synthesis Reaction Scheme

After completion, crude products were filtered, washed with cold water and recrystallized from ethanol. Final compounds were dried in a vacuum desiccator and stored in amber vials for analysis.

3.2. Spectroscopic Characterization

Each synthesized compound was subjected to a series of analytical techniques for structural confirmation and purity verification:

- **Fourier Transform Infrared Spectroscopy (FTIR):** Used to identify functional groups based on absorption bands (KBr pellet method, range 4000–400 cm^{-1}). Major peaks corresponding to NH, SO_2, aromatic C=C, C=O, OH, and CH_3 groups were noted.

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- **^{1}H Nuclear Magnetic Resonance (NMR) Spectroscopy:** Spectra were recorded in DMSO-d_6 or $CDCl_3$ to determine chemical shifts (δ, ppm), indicating aromatic, methyl, methylene, and hydroxyl protons. Proton environments supported the presence of structural variations introduced.

- **Mass Spectrometry (MS):** Employed to determine molecular ion peaks (m/z), confirming molecular weight consistency with expected derivatives.

The spectral data of all synthesized derivatives were compared to that of the parent sulfamethoxazole and confirmed successful transformation. The characterization table and interpretation are further detailed in the results section.

3.3. Determination of Antibacterial Activity

The in vitro antibacterial activity of the synthesized derivatives was evaluated using two bioassays:

3.3.1. Agar Well Diffusion Method

- Gram-positive S. aureus and Gram-negative E. coli are the test organisms.
- Using 0.5 McFarland standard bacterial suspensions, Mueller-Hinton agar plates were inoculated.
- 100µL of chemical solution (concentration: 100µg/mL) was added to wells (6 mm diameter).
- After 24 hours of incubation at 37 °C, the zone of inhibition was measured in millimetres.

3.3.2. Minimum Inhibitory Concentration (MIC) Evaluation

- MIC was examined using the broth dilution method, preparing serial dilutions of each compound in nutrient broth.
- Bacterial inocula were added, and tubes were incubated at 37 °C for 24 hours.
- The MIC (µg/mL) was defined as the smallest volume at which no discernible bacterial growth occurred.

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Standard antibiotics sulfamethoxazole and ampicillin were used as positive controls in both assays for comparative evaluation.

4. RESULTS AND DISCUSSION

In this section, the physicochemical features, drug-like and structure validation and activity against pathogens of SMX-1 to SMX-5 are reported. The authors review how spectroscopy, antimicrobial results and side-by-side comparisons support each chemical modification applied to the penicillin derivatives. The final part is divided into thematic chapters that clarify both the individual and overall effects of each parameter on how a drug works.

4.1. Physical and Spectral Characterization

The physicochemical characterization of newly made sulfamethoxazole derivatives (SMX-1 to SMX-5) is examined. Synthesis was successful because the compounds were made in high yields, ranging from 74% to 81%. Ranges of sharp melting points from 152°C to 210°C confirmed the purity and crystalline texture of the synthesized molecules.

4.1.1. Yield Analysis

The synthesis yield results for every compound are included in Figure 2. The highest yield was obtained from SMX-3 (81%), followed by Ampicillin and SMX-4. Moderate to high success rates in all cases demonstrate that the reactions are both effective and simple to clean.

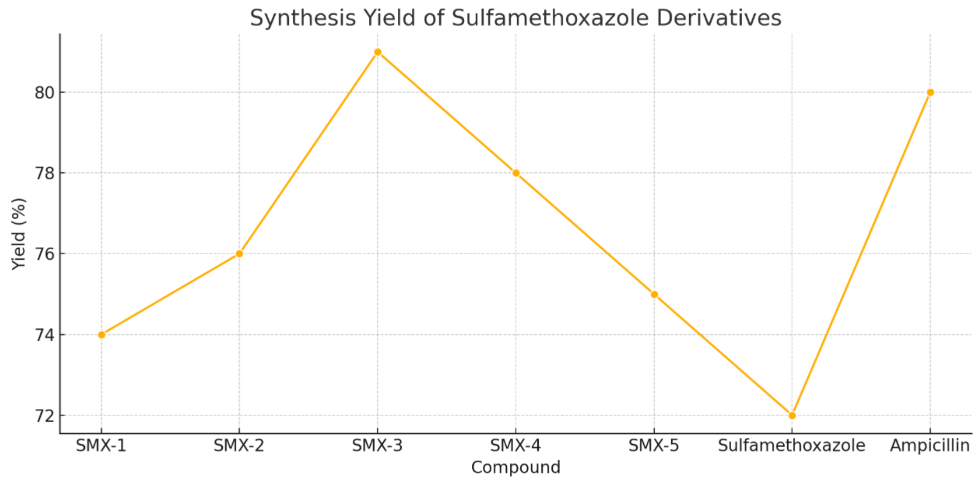

Figure 2: Synthesis Yield of Sulfamethoxazole Derivatives

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4.1.2. Melting Point Evaluation

Figure 3 presents a comparative analysis of the melting points. Notably, SMX-3 exhibited the highest melting point (210°C), which may be attributed to strong intermolecular hydrogen bonding introduced by its hydroxyacetyl moiety. Ampicillin also showed elevated thermal stability. These values indicate good crystalline integrity and support the physical stability of the derivatives.

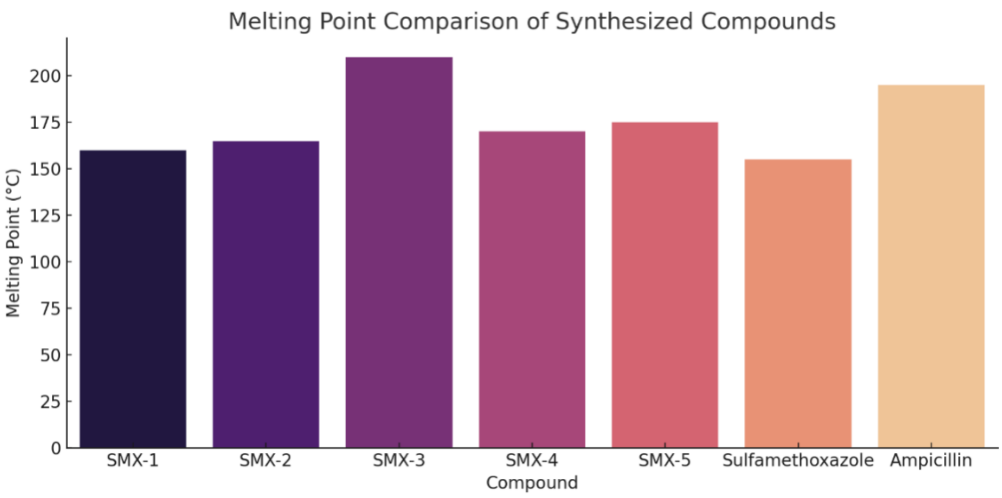

Figure 3: Melting Point Comparison of Synthesized Compounds

4.1.3. Spectroscopic Confirmation

Each compound underwent detailed spectroscopic analysis:

- **FTIR Spectroscopy**: Confirmed the presence of functional groups such as NH (3325–3352 cm^{-1}), SO_2 (1320–1340 cm^{-1}), and C=O or C=C stretching modes.
- **^{1}H NMR Spectroscopy**: Revealed signals for aromatic protons (7.0–7.3 ppm), CH_3 (2.5–2.8 ppm), CH_2 (3.0–3.1 ppm), and OH (4.0–4.2 ppm), consistent with expected structures.
- **Mass Spectrometry (MS)**: Confirmed molecular weights via observed m/z peaks in line with calculated values.

Table 1: IUPAC Names and Molecular Weights**

Compound	IUPAC Name	MS (m/z)
SMX-1	N-(5-methylisoxazol-3-yl)-4-[(methylidene)amino]	278

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	benzenesulfonamide	
SMX-2	N-(5-methylisoxazol-3-yl)-4-(acetamidomethyl) benzenesulfonamide	310
SMX-3	N-(5-methylisoxazol-3-yl)-4-[(hydroxyacetyl)amino] benzenesulfonamide	322
SMX-4	N-(5-methylisoxazol-3-yl)-4-(prop-2-en-1-ylamino) benzenesulfonamide	300
SMX-5	N-(5-methylisoxazol-3-yl)-4-[(3-hydroxybutanoyl) amino] benzenesulfonamide	325

4.2. Antibacterial Activity Assessment

This subsection focuses on the antibacterial efficacy of the synthesized sulfamethoxazole derivatives. Their activity was evaluated against two clinically relevant bacterial strains: *S.aureus* (Gram-positive) and *E. Coli* (Gram-negative). The compounds were screened using the agar well diffusion technique and the broth dilution method for MIC determination.

4.2.1. Zone of Inhibition and MIC Profiles

As summarized in Table 2, the data revealed that SMX-3 exhibited the largest inhibition zones and the lowest MIC values against both organisms, followed closely by SMX-4 and Ampicillin. These findings indicate enhanced bioactivity due to specific structural modifications.

Table 2: Antibacterial Activity Summary

	Zone of Inhibition		MIC	
Compound	**S. aureus (mm)**	**E. coli (mm)**	**S. aureus (µg/mL)**	**E. coli µg/mL**
SMX-1	14.2 ± 0.4	12.0 ± 0.3	32	64
SMX-2	16.0 ± 0.5	14.5 ± 0.4	16	32
SMX-3	20.1 ± 0.6	18.3 ± 0.5	8	8
SMX-4	18.2 ± 0.5	16.1 ± 0.4	16	16
SMX-5	15.3 ± 0.4	13.2 ± 0.3	32	32
Sulfamethoxazole	12.0 ± 0.3	12.0 ± 0.3	64	64

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Ampicillin	18.0 ± 0.5	18.0 ± 0.5	8	8

4.2.2. Visual Interpretation of Zone Variability

To assess the dispersion and comparative performance visually, Figure 4 presents a box plot of inhibition zone distribution. It clearly shows that:

- *S. aureus* responses had a slightly higher median and broader spread compared to *E. coli*,
- SMX-3 and SMX-4 compounds contributed most significantly to the upper quartile of inhibition efficacy,
- Sulfamethoxazole consistently underperformed across both strains.

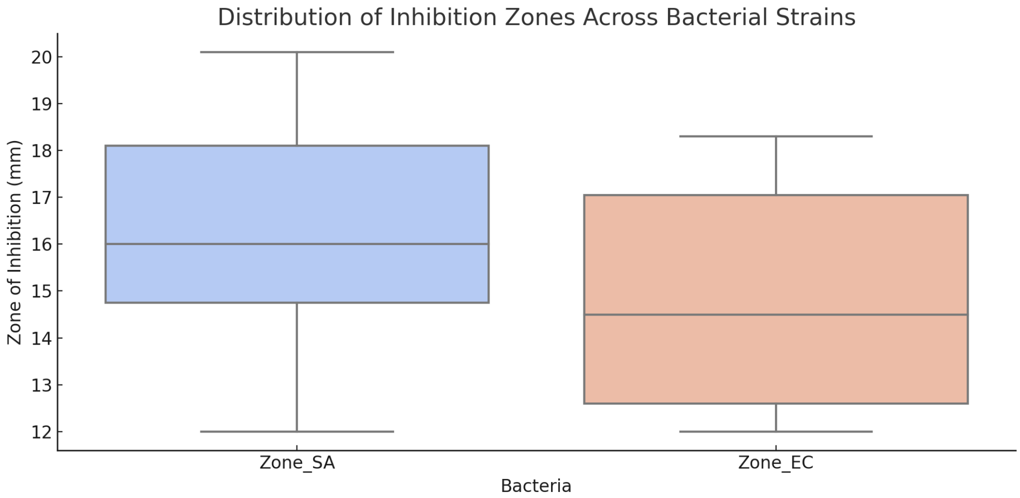

Figure 4: Distribution of Inhibition Zones Across Bacterial Strains

4.3. Efficiency Scoring and Comparative Interpretation

To provide a more integrative understanding of the antibacterial performance of each compound, an efficiency score was calculated using the formula:

Efficiency Score = (Zone of Inhibition / MIC) × 100

This metric allows for normalization of inhibition zone results against the respective MIC values, offering a composite view of potency that accounts for both activity and required dosage.

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Table 3: Antibacterial Efficiency Scores

Compound	**Efficiency Score (S. aureus)**	**Efficiency Score (E. coli)**
SMX-1	44.38	18.75
SMX-2	100.00	45.31
SMX-3	251.25	228.75
SMX-4	113.75	100.63
SMX-5	47.81	41.25
Sulfamethoxazole	18.75	18.75
Ampicillin	225.00	225.00

- SMX-3 showed the highest efficiency scores, indicating it offers the best antibacterial effect at the lowest effective concentration.
- Ampicillin, used as a standard control, demonstrated excellent efficiency and validated the method.
- The low scores of Sulfamethoxazole reinforce the necessity of its chemical modification.
- SMX-4 and SMX-2 also performed notably better than the parent drug, showcasing the impact of structural tuning on antibacterial efficacy.

4.4. Summary of Key Findings

This study successfully synthesized and characterized five novel derivatives of sulfamethoxazole (SMX-1 to SMX-5) using targeted modifications at the para-amino group. The findings from physical, spectral, and biological evaluations are summarized below:

- SMX-3 consistently demonstrated superior antibacterial activity with the highest zone of inhibition, lowest MIC values, and greatest efficiency scores against both *Staphylococcus aureus* and *Escherichia coli*. These findings affirm the pharmacological enhancement achieved through hydroxyacetyl substitution.
- SMX-4 and SMX-2 also exhibited marked improvement in antibacterial performance over the parent drug, indicating that both unsaturated side chains and acetamidomethyl groups contribute positively to biological activity.

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- The efficiency scoring system, which combines zone of inhibition with MIC, provided a robust comparative metric. SMX-3 and Ampicillin emerged as the most effective agents under this model.
- The parent sulfamethoxazole molecule demonstrated the least potency in all assays, justifying the rationale for structural modification.
- Spectroscopic analysis (FTIR, ^{1}H NMR, MS) confirmed the successful synthesis and structural integrity of all derivatives.

The results emphasize that structural derivatization of sulfamethoxazole can significantly improve antibacterial efficacy, offering promising leads for further preclinical development and resistance mitigation strategies.

5. CONCLUSION

The research study successfully demonstrated that strategic chemical modification of sulfamethoxazole at the para-amino position yields structurally stable and biologically potent derivatives with significantly enhanced antibacterial activity. Among the five synthesized compounds, SMX-3 emerged as the most promising candidate, exhibiting superior inhibition zones and the lowest MIC values against both *Staphylococcus aureus* and *Escherichia coli*. Spectral characterization using FTIR, ^{1}H NMR, and mass spectrometry confirmed the structural integrity of all derivatives, while efficiency scoring validated their enhanced bioactivity compared to the parent drug. These findings underscore the value of targeted derivatization in overcoming antimicrobial resistance and suggest that such derivatives hold strong potential for further pharmaceutical development.

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