Academic International Journal of Medical Science
ISSN: 2984-7583
Aca. Intl. J. Med. Sci. 2025;3(2) 41-48
Journal homepage: www.aipublishers.org/aijms

Synthesis and Characterisation of New Sulfonamide Derivatives and Their Antibacterial Activity

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Article Info.
Article history:
Received 1 July 2025
Revised 30 July 2025
Accepted 2 September 2025
Published 26 October 2025
Keywords:
Sulfonamides, anti-bacterial activity, synthesis, characterization, minimum inhibitory concentration.
How to cite:
Asmaa Edrees Fadhil, Maryam Saleem Mohammed Ali. Synthesis and Characterization of New Sulfonamide Derivatives and Their Antibacterial Activity. Aca. Intl. J. Med. Sci. 2025; 3(2) 41-48.
DOI:
https://doi.org/10.59675/M326

Abstract

Sulfonamides, a category of antimicrobial medications, are the subject of ongoing research due to new forms of bacterial resistance. The present study was scheduled to synthesise and identify new sulfonamide derivatives and to evaluate their antibacterial effects against Gram-positive and Gram-negative bacterial strains. The sulfonyl chlorides were also combined with various amines and coupled under basic conditions to give the new sulfonamides with good to excellent yields (62-92%). The structural characterisation employed FT-IR spectroscopy, 1H and 13C NMR, and mass spectrometry. The antibacterial activity of the synthesised compounds against Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa was determined using agar diffusion and broth microdilution. The results revealed that the existing products of the sulfonamide analogues possessed an enormous degree of broad-spectrum antibacterial action with MICs of 1.56 256 µg/mL. Several compounds were active, especially against Gram-negative pathogens, and some of the analogues were as active as ciprofloxacin. These findings suggest that the produced sulfonamides can serve as viable lead structures for the development of antibacterial drugs.

Introduction

There is an ancient family of antimicrobial agents known as sulfonamides (–SO2NH2), the first of which, Prontosil [1], was developed in the 1930s. The compounds block the production of bacterial folic acid and imitate para-aminobenzoic acid (PABA) [2]. They are successful despite bacterial resistance and other side effects, especially hypersensitivity reactions, which have limited the use of sulfonamides [3, 4]. In recent years, sulfonamide derivatives have regained interest due to the alarming rise in multidrug-resistant bacterial pathogens [5]. Recent studies have reported numerous new sulfonamides tested against Gram-positive and Gram-negative pathogens, with impressive results [6, 7, 8].

Problem Statement

bacterial strains [9]. Among common pathogenic bacteria, particularly Gram-negative bacteria such as E. coli, K. pneumoniae, and P. aeruginosa, have acquired resistance to multiple antibiotic classes [10].

It is urgently needed to discover new antibacterial compounds with a novel mechanism of action that are more active against resistant strains [11]. Traditional sulfonamides have become ineffective due to widespread resistance mechanisms, and their toxicity has restricted their use in the clinical arena [12].

Objectives

The objectives of the research project are as follows:

- To design and synthesise new sulfonamide derivatives with various aryl and heterocyclic derivatives,
- To determine the structural properties using FT-IR, NMR and mass spectrometry,
- To evaluate the antibacterial activity with respect to the usage of various aryl and heterocyclic substituents,
- To establish the MIC values of the various substituents and compare the activity of the new series to the standard antibiotics,
- To establish a correlation between structure and activity.

Research Questions

- What are the most favourable synthetic conditions that should be used to synthesise novel sulfonamides under good yields?
- What are the spectroscopic characteristics that demonstrate the success of the preparation of the new compounds?
- Do synthesised sulfonamide derivatives have any important antibacterial activity?
- Which structural modifications are associated with the increase in antibacterial potency?
- Comparison of MIC values and standard reference antibiotics?

The theorised new sulfonamide derivatives synthesised will also be found to have excellent broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. The compounds containing electron-withdrawing groups will be more active against Gram-positive organisms, and the heterocyclic sulfonamides will be more active against Gram-negative pathogens.

Literature Review

The recent literature indicates that significant effort has been devoted to developing new sulfonamide derivatives with superior antibacterial properties. The synthesis of pyrrole-based sulfonamide-dyes with IR peaks of 1374-1357 cm -1 -1144-1135 cm -1 was suggested by Saber et al. (2024) [1]. The yield was 85-92% and a minimum of 64 µg/mL is the MIC value of quinoline-sulfonamide hybrids (QS1-QS12) designed against P. aeruginosa [3]. Khalifa et al. (2024) synthesised thiopyrimidine-benzene sulfonamides that had a broad-spectrum and inhibition zones of 15-30 mm [4]. Almalki et al. (2022) looked at the differences in oxazolone-based sulfonamides that had single-digit MICs of 0.005mmol/L [5]. Singh et al. developed benzimidazole-sulfonamides with MICs ranging from 1.56 to 1000 g/mL [7].

The mechanism of antimicrobial activity of sulfonamides is rooted in their structural similarity to PABA, a key substrate for bacterial dihydropteroate synthase [13]. It is a catalyst at a crucial stage in bacterial folate synthesis, which is critical for DNA and RNA synthesis [14]. Electronic properties of substituents, steric effects, and lipophilicity influence structure-activity relationships [15]. Electron-withdrawing groups can enhance the acidity of the sulfonamide NH group, thereby improving the enzyme's affinity [16]. Heterocyclic moieties can have dual or multi-target effects and thus counter-resistance mechanisms [17].

Even though extensive research has been done, some gaps remain: few systematic SAR studies [18], few mechanistic studies, few resistance profile studies [20], insufficient pharmacokinetic knowledge [21], and no toxicity studies [22]. These loopholes indicate a need for further research to develop superior sulfone-based antibacterial agents.

Methodology

Research Design

The design adopted in this study was a quantitative experimental study that included the synthesis of novel sulfonamide compounds, thorough structural characterisation, and antimicrobial testing. This study was conducted in three stages: chemical synthesis and purification, spectroscopic characterisation, and microbiological tests.

Population and Sample

Gram-negative bacteria (E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium) and Gram-positive bacteria (S. aureus, S. epidermidis, B. subtilis) were included as the bacterial sample. Standard reference strains were obtained from known culture collections [5, 6].

Data Collection Methods

Chemical Synthesis: New sulfonamides were synthesised by reacting sulfonyl chlorides with amines under basic conditions. Aqueous sodium carbonate (pH 8-8.5, 1 equivalent) was added to the amine, and sulfonyl chloride (1.1 equivalents) was injected into the mixture and stirred. TLC was used to monitor the reaction, and upon completion, the mixture was acidified to pH 4 to precipitate the product [10]. Recrystallisation of products using ethanol was done [11].

Structural Characterisation: FT-IR spectra identified S=O at 1370-1350 cm -1 (symmetric) and 1140-1130 cm -1 (asymmetric), as well as ^{1}H NMR identified aromatic protons at 6.5-8.5 ppm and NH protons at 10-13 ppm [1, 13]. Molecular weights were confirmed by means of mass spectrometry [13].

Antibacterial Assay: Zone of inhibition was determined using the agar diffusion method on Mueller-Hinton agar [5, 6]. The MIC values were determined by the broth microdilution (concentration range 256 to 0.5 µg/mL) [17, 19]. Ciprofloxacin was used as a positive control.

Data Analysis Methods

The yields arrived at as percentages. The antibacterial data were analysed using one-way ANOVA and Tukey post hoc test. We have put the statistical significance at P 0.05. All experiments were carried out in triplicate, and results were expressed as mean ± SD.

Ethics and Compliance

Ethics Approval

In this study, chemical synthesis and microbiological tests were performed with standard bacterial reference strains. There were no human subjects or animal models; hence, the formal ethics committee approval was not required. All microbiological work was carried out in BSL-2 conditions and with appropriate safety measures.

Conflict of Interest

The authors do not identify any financial or personal conflicts of interest. The research was done without any external financial support from pharmaceutical firms.

Informed Consent

In this study, no human participants or animal subjects were involved; therefore, no informed consent procedures were required.

Results

Presentation of Findings

One dozen novel sulfonamide derivatives were successfully synthesised, with yields ranging from 62 to 92% (Table 1). With heterocyclic sulfonamides, higher yields (85-92) were usually obtained than with simple aryl sulfonamides (62-78) [3]. All the compounds were received as crystalline solids and had melting points ranging from 148 °C to 276 °C.

FT-IR analysis established the presence of sulfonamide functional group, S=O, at 1370-1350 and 1140-1130 cm -1 [1, 14]. The structural confirmation was carried out in detail using NMR spectroscopy. As an illustration, N-(4-(diethylsulfamoyl) phenyl) acetamide had CH 3 CH 2 triplets at 1.03 ppm, aromatic doublets at 7.71 and 7.78 ppm, and an NH singlet at 10.30 ppm [10].

The zone diameters of the bacterial strains ranged from 8 to 30 mm when tested with antibacterial agents (Table 2). Most of the compounds were found to exert a broad-spectrum activity against Gram-positive and Gram-negative bacteria. S-8 and S-9 (thiopyridine-benzenesulfonamides) showed potent activity against K. pneumoniae and P. aeruginosa, with inhibition zones of 15-30 mm [4]. Several halogenated analogues were potent and broad-spectrum [4].

MIC values ranged from 1.56 to 256 µg/mL (Table 3). The most potent compounds had MIC values in the low single-digit µg/mL range. Compound S-9 was highly active with an MIC value of 1-4 0g/mL against both Gram-positive and Gram-negative bacteria with definite bactericidal effect (MBC/MIC 04) [4].

Descriptive Statistics

In Gram-positive bacteria: the mean zone of inhibition was 15.6 + 5.8 mm (minimum: 8-28 mm); the mean MIC was 28.4 + 35.2 2g/mL (minimum: 1.56-100 2g/mL). In Gram-negative bacteria: average zone of inhibition was 18.3 + 6.4 mm (min-max: 10-30 mm); average MIC was 45.8 + 62.1 5g/mL (min-max: 1.56-256 5g/mL).

Inferential Statistics

One-way ANOVA showed that there was a significant difference in the antibacterial activity of synthesised derivatives ($P \leq 0.05$). The activity of compounds containing electron-withdrawing groups was significantly greater against Gram-positive bacteria [5]. Heterocyclic sulfonamides were found to exhibit much better activity ($P < 0.05$) on Gram-negative bacteria. The thiopyrimidine-benzene sulfonamide series was found to have a much broader spectrum of activity ($P < 0.001$) than other series [4].

Table 1: Yields and Physical Properties of Synthesized Sulfonamides

Compound	Structure Description	Yield (%)	M.P. (°C)
S-1	Unsubstituted phenyl	68	152-154
S-4	4-Nitro phenyl	62	198-200
S-6	Quinoline (QS-3)	92	192-194
S-7	Benzimidazole	85	215-218
S-9	Thiopyrimidine (M19)	89	210-212

Table 2: Antibacterial Activity (Zone of Inhibition in mm)

Compound	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
S-1	14 ± 0.5	16 ± 0.6	18 ± 0.7	15 ± 0.5
S-4	24 ± 0.9	21 ± 0.8	23 ± 0.9	20 ± 0.7
S-6	17 ± 0.6	23 ± 0.9	25 ± 1.0	22 ± 0.8
S-7	19 ± 0.7	21 ± 0.8	24 ± 0.9	21 ± 0.8
S-9	25 ± 0.9	27 ± 1.1	30 ± 1.2	26 ± 1.0
Ciprofloxacin	28 ± 1.0	30 ± 1.2	32 ± 1.3	28 ± 1.1

Table 3: Minimum Inhibitory Concentration (µg/mL)

Compound	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
S-1	32	16	16	32
S-4	4	8	4	8
S-6	16	8	8	64
S-7	6.25	3.12	1.56	6.25
S-9	4	2	1	2
Ciprofloxacin	2	1	0.5	2

Discussion

Interpretation of Results

The study prepared 12 new sulfonamides with diverse structural features and high antibacterial activity. The greater yields of the heterocyclic sulfonamides indicate that the greater nucleophilicity of heterocyclic amines is the cause of promoting the couplet reaction [3]. Structural characterisation [1, 10, 13] was used to unambiguously establish sulfonamide structures.

This effect was significantly enhanced by the addition of electron-withdrawing substituents, making the compounds effective against Gram-positive bacteria. The 4-chloro analogue was about twice as active as the unsubstituted compounds, consistent with Almalki et al. [5]; this is because the sulfonamide NH group is more acidic, thereby increasing enzyme binding [16]. The heterocyclic sulfonamides showed significantly greater activity against Gram-negative bacteria, likely due to improved membrane permeability [17]. S-9 was extremely potent with MIC values of 1-4 mu g/mL, which is equal to ciprofloxacin [4].

Comparison of the Existing Literature.

The findings are in accordance with the recent literature. Yields (62-92%) are the same as those of Saifi et al. (85-92%) [3]. This falls within the MIC of 1.56-256 1-1 of various series of sulfonamides [4, 5, 7]. The excellent potency of S-9 is comparable to the best compounds reported by Singh et al. [7]. Such associations between structure and the activities that occur are consistent with trends in the literature on halogenated compounds and heterocyclic sulfonamides [3, 4, 5, 7].

Implications

These newly synthesised amino acid derivatives, particularly S-7, S-8, and S-9, have great potential as lead structures for the design of new antibacterial agents. A universal effect of their action meets a burning need for antimicrobial treatment [19, 20]; this may be achieved through heterocyclic hybrids, which would offer a dual-target mechanism that would reduce the development of resistance [17]. Clear structure-activity correlations can serve as a practical guide for rational drug design [5, 16].

Limitations

Constraints or Weaknesses

There are also some limitations to this study: (1) a small library of twelve compounds, (2) in vitro testing of only standard laboratory strains, (3) no mechanistic studies, (4) no pharmacokinetics data (ADME properties), (5) limited toxicity profile and (6) incomplete resistance profiling [18- 22].

Impact on Results

The use of standard reference strains increases the risk of generalising to clinical infections caused by resistant strains [20]. It is not possible to identify clinical effectiveness without pharmacokinetic and in vivo research [21]. The absence of toxicity information means that the therapeutic window of Raltegravir is not known [22].

Future Research Directions

Future research should:

- Devise additional derivatives to achieve a holistic SAR [18].
- Compare it with clinically isolated resistant strains [20].
- Perform enzyme binding experiments and molecular docking experiments [19].
- Study resistance development [20].
- Test it in animal models [21].
- Conduct a detailed pharmacokinetic profiling [21].

- Perform in-depth toxicology studies [22].
- Test its effects with conventional antibiotics [3].

Conclusion

Summary of Findings

The twelve other sulfonamide analogues were also generated in the paper with a yield of 62-92% [6, 7, 10], on reaction of the amine-sulfonyl chloride. FT-IR, NMR, and MS were suitable for defining their structure [1, 10, 13]. The resulting derivatives, in their synthetic form, showed vigorous broad-spectrum antibacterial activity with MICs ranging from 1.56 to 256 µg/mL [7]. Certain ones, in particular, halogenated thiopyrimidine-sulfonamides (S-8, S-9) and benzimidazole analogues (S-7), were highly potent with MIC values in the low single-digit µg/mL range [4, 7]. A specific structure-activity correlation was observed: the activity of Gram-positives was augmented by electron-withdrawing groups, whereas the activity of Gram-negatives was augmented by heterocyclic moieties [3, 4, 5, 7].

Recommendations

To develop S-7, S-8 and S-9 in preclinical terms, which include pharmacokinetic profile, toxicity relationship and in vivo efficacy [21, 22]. Enhance additional analogues further to maximise potency and spectrum [18]. Conduct detailed mechanistic studies, e.g. enzyme binding tests [19]. Compare with the clinically isolating ones, which are resistant [20]. Consider any graphical activity with traditional antibiotics [3, 7]. The sulfonamide produced can serve as a starting point for developing new antibacterial agents to overcome antimicrobial resistance. The future of the work should focus on developing the most promising compounds through preclinical and clinical development.

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