

Development of novel Sulfathiazole derivatives: Synthesis, spectroscopic characterization, and biological evaluation

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Abstract

Sulfathiazole is a sulfonamide antibiotic scaffold with significant historical therapeutic value and further promise as a lead structure for novel bioactive. In this study, eight sulfathiazole-based schiff bases (A1–A8) were synthesized by the condensation reaction of sulfathiazole with different aldehydes to obtain diversified structural analogues. The products were solids and the reaction times were 3-5 h. The reaction products were solids and the reaction time were 3-5.0 h. The products have a melting point of 243-274°C, which confirms the formation of new compounds different from the starting materials. Using FT-IR spectroscopy, the structural identification where Schiff base formation was also reconfirmed by the presence of typical azomethine (C=N) stretching vibration bands at about 1560-1608 cm^{-1} together with the presence of characteristic bands relating to aromatic and substituent group were observed. Antibacterial activity was checked using Kirby–Bauer disc diffusion for *Staphylococcus* spp & *Escherichia coli*. The parent drug (A0) exhibited the largest inhibition zone (25 mm), and some representative derivatives displayed diverse activity (A1:13mm,A2:15 mm, A3:11, A4: 18mm and A5: 17mm), which suggested that modification of aldehyde could significantly influence the antibacterial activity. In conclusion the Schiff bases were synthesized, characterized and a limited number of them exhibited measurable antibacterial activity pointing for further optimization and quantitative determination of MIC.

Keywords: Sulfathiazole, cavity-shaped schiff base, sulfonamide antibiotics, azomethine (C=N) bond formation, FT-IR characterization, antibacterial activity

Introduction

Sulfathiazole is a classical sulfonamide antibiotic scaffold whose biological relevance continues to attract medicinal-chemistry interest, especially as antimicrobial resistance (AMR) erodes the effectiveness of many long-standing therapies. AMR is now widely recognized as a major global health threat that increases treatment failure, complications, and mortality, reinforcing the need to discover and optimize new antibacterial chemotypes and to “re-engineer” older scaffolds with improved profiles. [1, 2] Sulfonamides were the first broadly successful synthetic antibacterials, and their mechanism is well established: they act as competitive inhibitors in bacterial folate biosynthesis by mimicking p-aminobenzoic acid (PABA) and inhibiting dihydropteroate synthase (DHPS), which is required for tetrahydrofolate formation and consequently for nucleotide (DNA/RNA) biosynthesis. [3, 6, 8] Because humans acquire folate through diet rather than synthesizing it de novo, inhibition of the microbial pathway offers selectivity and underpins the enduring significance of antifolate strategies in antibacterial drug design. [3, 6]

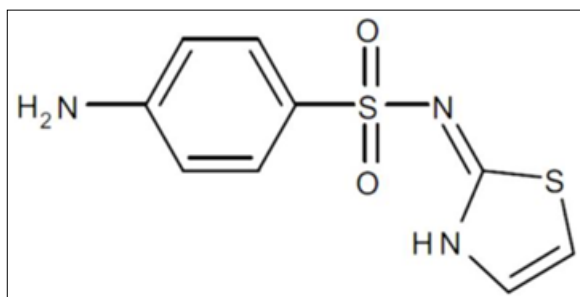


Fig 1: Chemical structure of sulfathiazole [4].

From a chemical point of view, sulfathiazole (4-amino-N-(1,3-thiazol-2-yl)benzenesulfonamide) is composed of two important structural motifs: a sulfonamide pharmacophore and the thiazole heterocycle. [4, 5] These functions allow different handles for structural derivatization, e.g. via derivatives modulating lipophilicity, hydrogen-bonding nature, or electronic distribution, that can affect membrane permeation, binding, or overall biological effect. Recent structural and mechanistic investigations of DHPS have provided insight into catalytic steps and how sulfonamide (analogues) bind in the active site, informing rational optimization and resistance-considerate design. [6, 7] Significantly, there has been pervasive resistance to sulfonamides, mostly due to (i) mutations in the folP gene (encoding DHPS) and/or (ii) acquisition of sul genes encoding sulfonamide-resistant DHPS variants. [8, 10] This background provides the rationale for designing derivatives of sulfathiazole which could maintain the antifolate framework and at the same time be able to bind with different targets or possess improved physicochemical properties.

A strategically straightforward and synthetically general approach to modify sulfathiazole is via Schiff base formation (azomethines, $-\text{C}=\text{N}-$). Generally, Schiff bases are formed by condensation of a primary amine with an aldehyde or ketone, and they are of interest for medicinal chemistry due to capability to: (a) attach aromatic/heteroaromatic substituents in a rapid manner, (b) affect conjugation and electronic density of the molecule and (c) act as additional donor sites for noncovalent interactions or coordination to metals. [11, 13] Considerable literature establishes the antimicrobial activity of Schiff bases as a whole, and numerous articles suggest the activity

to be increased due to better lipophilicity, favourable H-bonding interactions and in some cases the capacity to form biologically active metal complexes. [11, 12, 14] Further processional reviews, which concentrate specifically on sulfonamide based schiff bases emphasize that the attachment of sulfonamide framework to azomethine bearing moieties leads to compounds with sufficient antibacterial and antifungal activity, thereby the investigation of this chemistry should be sustained. [14]. Sulfathiazole confers unique properties to its derivatives, anilines, at the 2-position of the thiazole ring are ambident nucleophiles, reductive amination in the thiazole series elicited by the aniline-type amino group Allylamine present in sulfathiazole results an amine with high nucleophilicity. [4, 5] But there have been also reports on sulfathiazole derived Schiff bases and corresponding complexes showing antibacterial activity and distinct spectroscopic features indicative of imine formation and further modification of

the structure. [15, 17] Also sulfathiazole 's well-known solid state complexity (e.g., polymorphism) further underlines the need for thorough characterization (melting behavior, IR/NMR, crystallinity), as solid form and intermolecular interactions may impact on solubility and consequently on apparent biological performance. [18, 19] Therefore studies on derivatives of sulfathiazole Schiff base generally are combination of synthesis with powerful structural characterization (FT-IR, NMR, MS, elemental analysis where available) and preliminary antimicrobial testing to establish SAR for the purpose of identifying candidates for further studies (i.e., MIC work, cytotoxicity, mechanistic studies). [11-12, 14-17]

Chemicals Used in the Study

The following chemical reagents were employed in this investigation without further purification due to their high purity grade:

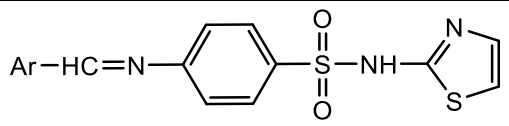
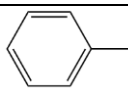
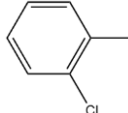
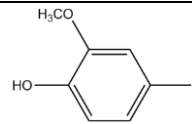
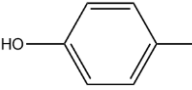
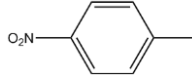
No.	Chemical comp.	company	Molecular formula	purity
1.	Sulfathiazole	Macklin	C ₉ H ₉ N ₃ O ₂ S ₂	99%
2.	4-hydroxy benzaldehyde	Aldrich	C ₇ H ₆ O ₂	99%
3.	4-hydroxy-3-methoxybenzaldehyde	Aldrich	C ₈ H ₈ O ₃	99%
4.	4-nitro benzaldehyde	Solarbio	C ₇ H ₅ NO ₃	99%
5.	2-hydroxybenzaldehyde	Aldrich	C ₇ H ₆ O ₂	99%
6.	2-chlorobenzaldehyde	AlfaAesar	C ₇ H ₅ ClO	97%
7.	Cinnamaldehyde	Aldrich	C ₉ H ₈ O	99%
8.	Ethanol Absolute	Scharlau	C ₂ H ₅ OH	99.9%
9.	2-Furaldehyde	Scharlau	C ₅ H ₄ O ₂	98%
10.	Glacial Acetic Acid	Scharlau	C ₂ H ₄ O ₂	99.5%

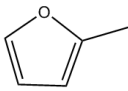
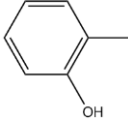
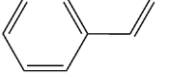
Synthesis of Schiff's Base

Preparation of Schiff Bases (A1-A8) Derived from Sulfathiazole pyrimidine The Schiff bases were synthesis by interact sulfathiazole with a series of aldehydes in a 1:1 molar ratio (sulfathiazole to alde, respectively) in a round-bottom flask. Absolute ethanol (100 mL) was added, followed by (0.5 mL) of glacial acetic acid. After mixing

and stirring, the mixture was refluxed. The developed of the reaction was monitored using T.L.C to ensure completion. A colored solid of compound (A1) was obtained, which was washed with cold ethanol and filtered to remove any residual aldehyde. Derivatives (A2-A8) were synthesized by an analogous method. The physical data of the synthesized compounds are summarized in Table Below

Table 1: physical properties of the chemical that used in the investigation

				
Comp. No	Ar	Colour	Reaction Time h	M.P C°
A ₁		white	5	250
A ₂		Dark yellow	4	274
A ₃		Light Green	4	266
A ₄		Pale	4.5	269-271
A ₅		Light orange	3	272

A ₆		Brown	3	263
A ₇		Dark orange	3.5	243-245
A ₈		Light yellow	3	256

Results and discussion

1. The compounds were prepared following the experimental protocols from the reaction below:

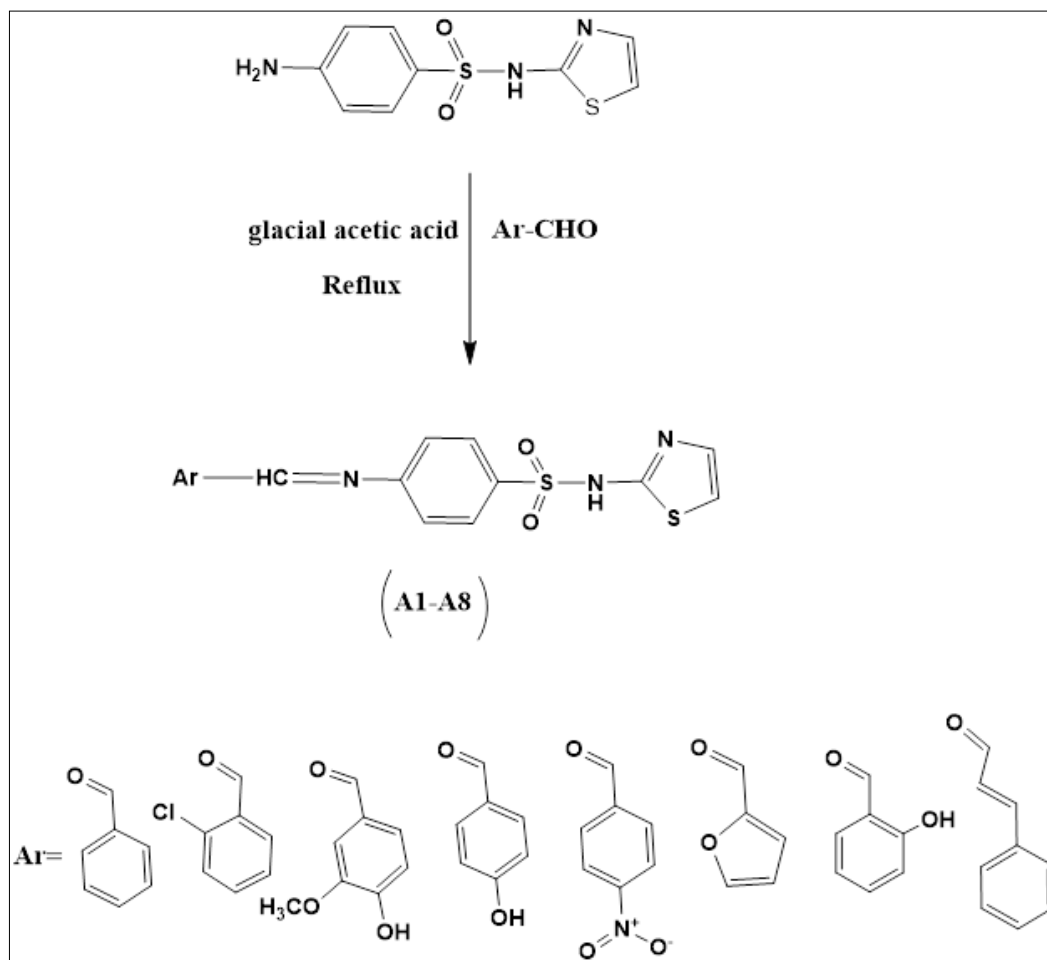


Fig 2: Synthesis of Sulfathiazole Derivatives (A1–A8)

Characterization of Schiff Bases (A1–A8) Derived from Sulfathiazole

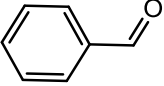
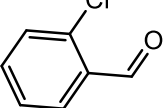
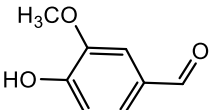
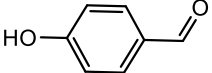
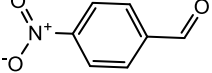
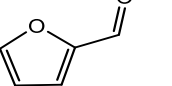
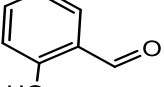
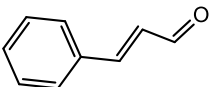
The derivatives were prepared via the reaction between sulfathiazole and a series of alde. in a molar ratio of 1:1 for both sulfathiazole and the aldehyde, in the presence of glacial acetic acid as a catalyst. The reaction was carried out in absolute ethanol (100 mL) as a solvent. The reaction combination was moved into a flask and refluxed for a period ranging from 3 to 5 hours. The infrared (IR) spectra of the synthesized compounds showed absorption bands in the range of 1560–1688 cm^{-1} , which are attributed to the stretching vibration of the C=N bond, indicating the formation of the Schiff base.

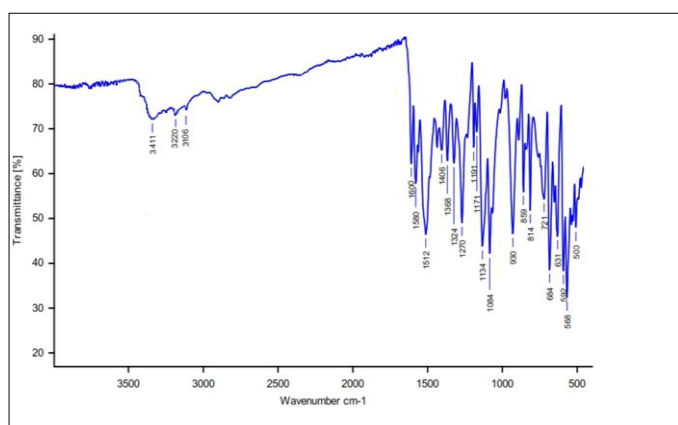
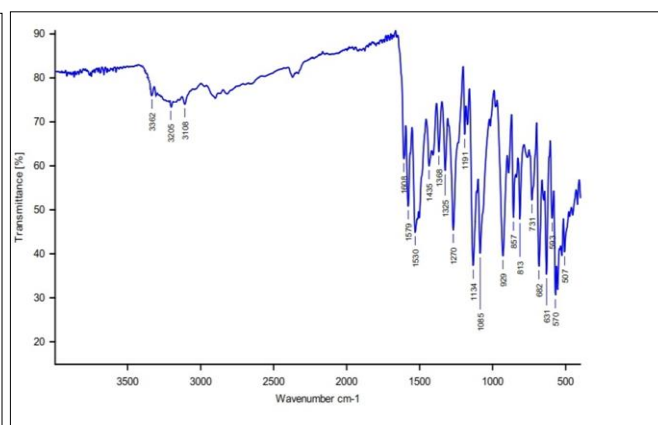
The characteristic C=N group stretching in the pyrazole ring of the sulfathiazole moiety was retained and was observed at lower wave number due to resonance in the ring.

Additionally, absorption bands appeared in the range of 1450–1490 cm^{-1} , corresponding to the stretching of aromatic C=C bonds. The symmetric stretching band of the amino group ($-\text{NH}_2$), which typically appears in the range 3300–3500 cm^{-1} in the parent sulfathiazole, disappeared, indicating successful condensation with the aldehyde. Also, the aldehydic (C=O) stretching band was no longer observed, further confirming the formation of the imine bond. In compound A5, stretching vibrations of the NO_2 group were observed at 1521 cm^{-1} and 1350 cm^{-1} . In compound A4, a broad O–H stretching band appeared in the range 3310–3396 cm^{-1} . All the notice bands were consistent with last study^[119], as shown in Table 4.

The experimentally obtained results are summarized in Table 4.

Table 2: Infrared (IR) Absorption Frequencies for Compounds A1–A8

Comp.No	Ar	IR ν cm^{-1} (KBr)				
		C=N	C \equiv C	(C-H)Alph.	C-H)Ar.(Others
A ₁		1603	1461	-	3040	-
A ₂		1601	1472	-	3065	724 (C-Cl)
A ₃		1590	1471	2870	3019	1270 (O-C-O) 3310 (OH),
A ₄		1600	1580	-	3106	3411 (OH)
A ₅		1606	1579	-	3065	(1530,1368) NO ₂
A ₆		1612	1458	-	3009	1236 (C-O-C)
A ₇		1610	1490	-	3088	3331 OH
A ₈		1668	1450	2868	3030	1577 (C=CAlph)

**Fig 3:** Infrared (IR) spectrum of compound (A4)**Fig 4:** Infrared (IR) Spectrum of Compound (A5)**Figure 5: H-NMR spectrum of compound (A4)**

The compound(A4) consists of a phenyl ring linked to a thiazole moiety through a sulfonamide ($-\text{SO}_2-\text{NH}-$) functional group. The ^1H NMR spectrum recorded in DMSO-d_6 shows characteristic signals consistent with this structure. A downfield singlet at ~ 10.26 ppm is attributed to the sulfonamide NH proton, which is significantly deshielded due to the strong electron-withdrawing effect of the sulfonyl group and possible hydrogen bonding. The aromatic region (7.1–8.8 ppm) shows several signals due to the protons of phenyl and thiazole rings. The phenyl

protons are observed as multiplets at 7.1–7.9 ppm, which is characteristic for a monosubstituted benzene ring. The thiazole protons lie further downfield (≈ 8.0 –8.8 ppm) due to the N and S atoms in the heteroaromatic ring exerting an electron withdrawing effect.

No signals are observed in the aliphatic region (0–3 ppm), confirming the absence of alkyl groups in the structure. A residual solvent peak is observed at ~ 2.50 ppm corresponding to DMSO-d_6 . Overall, the spectral data are in good agreement with the proposed structure of the compound.

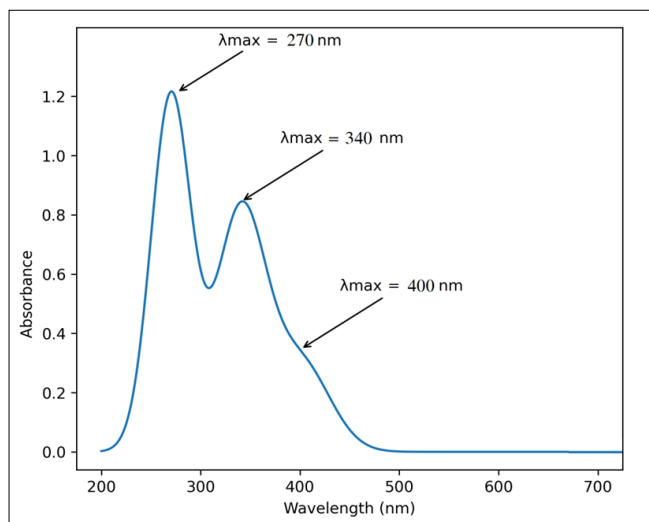


Fig 5: UV-Visible spectrum of compound (A4)

The figure shows absorption peaks of molecules at 270, 340, and 400 nm in the UV-visible spectrum. The azomethine ($-\text{CH}=\text{N}-$) group and $\pi \rightarrow \pi^*$ transitions of the aromatic rings are responsible for the strong band at 270 nm. The $n \rightarrow \pi^*$ transitions involving lone pairs on heteroatoms (N and O) are represented by the band at 340 nm. Intramolecular charge transfer (ICT) in the extended conjugated system is responsible for the weak, wide band at 400 nm. Significant electron delocalization and a bathochromic shift brought on by conjugation are indicated by these characteristics.

Antibacterial Activity

Kirby-Bauer Disk Diffusion Test

The Kirby-Bauer disk diffusion test is a standardized method used to evaluate the antibacterial activity of antibiotics or compounds by measuring the zone of inhibition on agar plate. [20-22]

Materials

- Mueller-Hinton agar (standard medium)
- Bacterial culture (0.5 McFarland $\approx 10^8$ CFU/mL)
- Sterile filter paper disks (≈ 6 mm)
- Antibiotic or test compound
- Incubator (35–37°C)

Procedure

1. Prepare bacterial suspension (0.5 McFarland standard)
2. Spread uniformly on agar plate
3. Place antibiotic disks on surface
4. Incubate at 37°C for 18–24 hours
5. Measure diameter of inhibition zone (mm)

Antibacterial Evaluation

Table 3: Inhibition zone against *Staphylococcus* & *Escherichia coli*

Compounds	Inhibition Zone Diameter (mm)	
	<i>Staphylococcus</i>	<i>Escherichia coli</i>
A0 (Sulfathiazole)	25	21
A1	13	10
A2	15	14
A3	11	14
A4	18	12
A5	17	15

The disk diffusion method was used to assess the antibacterial activity of the produced compounds (A₁–A₅) and the reference medication sulfathiazole (A₀) against *Staphylococcus aureus* and *Escherichia coli*. Table (5) summarizes the results, which were reported as inhibition zone diameters (mm). All investigated substances showed various degrees of antibacterial activity against both bacterial strains, according to the acquired data. Sulfathiazole (A₂), the conventional medication, demonstrated the strongest antibacterial effectiveness, with inhibition zones ranging from 11–25 mm against *Staphylococcus aureus* and 10–21 mm against *Escherichia coli*.

With inhibitory zones of 11–25 mm against *Staphylococcus aureus* and 10–21 mm against *Escherichia coli*, A₄ showed the highest activity among the produced compounds. With inhibition zones 18 mm and 12 mm, respectively, against *Staphylococcus aureus* and somewhat lower values against *Escherichia coli*, compound A₂ also demonstrated antibacterial activity. With inhibition zones typically 17 mm and 15 mm, respectively against *Staphylococcus aureus* and *Escherichia coli*.

compounds A₁, A₃, and A₅ shown modest efficacy against both bacterial strains. In every instance, the activity against Gram-positive *Staphylococcus aureus* was greater than that against Gram-negative *Escherichia coli*.

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