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Article

Synthesis and Characterization of New Series of Heterocyclic Schiff Bases Derivatives by Cyclization and Studying Their Antibacterial Activity

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Abstract: Thiazolidinone derivatives are known for their broad biological activities, particularly antibacterial properties. This study reports the synthesis of novel thiazolidinone derivatives from Schiff bases via cyclization with thioglycolic acid. The compounds were characterized using FT-IR, ¹H NMR, ¹³C NMR, TLC, and melting point analysis. Antibacterial activity was evaluated against Staphylococcus aureus (S. aureus) and Staphylococcus epidermidis (S. epidermidis) (Grampositive) as well as Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa) (Gramnegative) using the Mueller Hinton agar diffusion method. The results revealed promising antibacterial efficacy, with structural modifications significantly influencing activity. These findings contribute to the development of thiazolidinone-based antibacterial agents and structure-activity insights.

Keywords: Thiazolidinone derivatives, Schiff bases, Cyclization, Antibacterial activity, Structureactivity relationship (SAR).

1. Introduction

A large class of chemicals known as Schiff bases are defined by a carbon-nitrogen double bond that permits the addition of either aryl or alkyl substituents [1]. These substances can be produced in a lab or discovered in nature. Schiff bases are the end products of the reaction of primary amines with carbonyl compounds, and they are named after the German chemist Hugo Schiff, who originally reported them in 1864 [2].

Schiff bases are chemical compounds (imines) that have a hydrocarbyl group on the nitrogen atom R1R2C=NR' ($R' \neq H$), in accordance with IUPAC guidelines. Many people think of them as being interchangeable with azomethines. An aliphatic or aromatic amine and a carbonyl molecule can be combined nucleophilically to create Schiff bases by first generating a hemiaminal, which is then dehydrated to produce an imine. A series of processes involving addition and elimination result in the production of Schiff bases [3].

Schiff bases have the ability to attach to metal ions as ligands. Although they do not go through irreversible changes themselves, they are known as auxiliary ligands because they alter the structure and reactivity of the transition metal ion in the heart of the complex [4]. Schiff bases can be used as reduction, oxidation, or acid catalysts, among other types of catalysts. They are employed in crystal engineering, catalytic reactions, biological systems as photo- or chemodetectors, and, most frequently, medicine [5]. They have

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anticancer, antifungal, antitumor, antipyretic, anesthetic, antibacterial, anti-inflammatory, antimalarial, and antiviral properties. Schiff bases can be used to create thiazolidinone derivatives [6]. An amine and an aldehyde are first used to create Schiff bases, which are subsequently reacted with mercaptoacetic acid to yield thiazolidinone derivatives. Among the many biological activities of these compounds include antibacterial, antifungal, and anticancer qualities [7].

5-amino-1H-imidazole-4-carboxamide thiazolidinone derivatives have been synthesized and their pharmacological properties evaluated [8]. According to the literature, 5-amino-1H-imidazole-4-carboxamide was reacted with aromatic aldehydes to create Schiff bases, which were subsequently transformed into thiazolidinone derivatives [9]. According to tests, the thiazolidinone derivatives were more active than the Schiff bases against harmful microbes. One common technique in chemical synthesis for producing heterocyclic compounds with important biological properties is the cyclization of Schiff bases to yield thiazolidinones. Usually, a Schiff base reacts with a sulfurcontaining substance, most frequently 2-mercaptopropionic acid or thioglycolic acid, in this process [10].

A nucleophilic addition is followed by intramolecular cyclization as the general mechanism for this cyclization. To create a Schiff base, an aldehyde or ketone must first condense with a primary amine [11]. The sulfur-containing molecule and this Schiff base then react, typically with heat and a suitable solvent present. A new C-S bond is created when the sulfur atom targets the imine's carbon (C=N) bond [12]. The distinctive 4-thiazolidinone structure is then formed when the nitrogen and the carboxylic acid group of the mercapto molecule combine, sealing the ring. By altering the amines, initial aldehydes, and sulfur-containing reagents, this synthesis method is very useful for producing a wide variety of thiazolidinone derivatives [13]. Numerous biological actions, such as antibacterial, antifungal, antiviral, anticancer, anti-inflammatory, and analgesic qualities, have been discovered in the resultant thiazolidinones [14].

Several techniques have been developed to maximize this cyclization, such as the one-pot synthesis, which uses efficient multi-component reactions to form the Schiff base and cyclize it to the thiazolidinone in a single step; the microwave-assisted synthesis, a modern approach that uses microwave irradiation to accelerate the reaction, often leading to higher yields and shorter reaction times; and the traditional reflux method, which involves refluxing the Schiff base with thioglycolic acid in dry benzene, frequently with the aid of a water separator [15]. Research on the cyclization of Schiff bases to thiazolidinones is still underway in medicinal chemistry, with the goal of creating novel derivatives with better pharmacological profiles and biological activity [16].

This article focuses on the synthesis of a series of thiazolidinones derivatives made by the cyclization of Schiff bases, along with the characterization utilizing thin-layer chromatography, melting point, FT-IR, and NMR techniques. This was followed by studying the antibacterial effect of the synthesized compounds against different types of bacteria Staphylococcus aureus (S. aureus) and Staphylococcus epidermidis (Staph. E.) Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa).

2. Materials and Methods

2.1. Chemicals

All chemicals were purchased from Fluka and Merck (formerly Sigma-Aldrich); these materials were exceedingly pure and were utilized as is, with no further processing.

2.2. Instruments Used

The synthesized compounds were characterized utilizing Ascend[™] 400 MHz BRUKER for ¹H and ¹³C NMR at the University of Basra, Alpha BRUKER with a DTGS detector for IR spectra (400–4000 cm⁻¹) using KBr disks, SMP40 electromelt-meter for melting points, and Fluka TLC (0.2 mm Silica gel-G) for reaction monitoring and purity

assessment with iodine for spot detection are among the instruments utilized. Other instruments such as autoclave, laboratory incubator, balance, and Vortex shaker were utilized for antibacterial tests

2.3. Procedures

2.3.1. Synthesis of Schiff Bases (A1-A8) [17,18]

In a reaction vessel, (3–4) drops of glacial acetic acid were added to thiazole-2carbaldehyde (2.48 g, 0.022 mol), followed by the addition of different amines (0.022 mol) dissolved in 20 mL of absolute ethanol. The mixture was then heated under reflux for 6 hours, cooled in an ice bath to induce precipitation, and the precipitates were filtered, dried, and recrystallized in absolute ethanol to obtain the desired product.

2.3.2. Synthesis of Thiazolidinones (A9-A16) [19]

Schiff bases (A1-A8) (0.0026 mol) were dissolved in 25 mL of dry benzene, followed by the addition of 2-mercaptoacetic acid (0.23 g, 0.18 mL, 0.0024 mol). The solution was then heated under reflux for 48 hours. After cooling, the reaction mixture was washed three times with a 10% NaHCO₃ solution. Following drying, it was recrystallized from an ethanol-water mixture (1:3).

2.3.3. Procedure for Antibacterial Testing [20,21]

The Mueller Hinton agar medium was made and sterilized in an autoclave at 121°C and 1 atmosphere for 15 minutes. Each bacterial strain had its own inoculum of 1.5 x 10⁸ cells/mL, diluted with sterile physiological saline. The bacterial inoculum was compared to the McFarland standard at a concentration of 0.5, after which three wells were produced for each plate. A cotton swab was used to evenly distribute the bacterial inoculum across the medium's surface. Following that, the test solutions were added to the wells at concentrations of 25%, 50%, and 75%, and incubated at 37°C for 24 hours. The diameter of the inhibitory zones was measured in millimeters with a transparent ruler, and the results were assessed and compared to the antibiotic control, ciprofloxacin. Each test was conducted three times to ensure repeatability.

3. Results

Several thiazolidinone compounds were produced using Schiff bases. Various spectroscopic techniques were used to describe these compounds, including infrared (IR), proton nuclear magnetic resonance (¹H NMR), and carbon nuclear magnetic resonance (¹³C NMR), as well as thin-layer chromatography (TLC) and melting point measurement. The synthesis techniques followed the methodology outlined (Figure 1) below:



a₉-a₁₆



Figure 1. Schematic diagram shows the synthetic routes of compounds (A1-A16).

3.1. Synthesis of Schiff Bases

The synthesis of new Schiff bases (A1-A8) was carried out by the reaction of different aromatic amines with 2-formyl-(1,3)-thiazole in the presence of glacial acetic acid, according to the method described in the experimental part [22] (Figure 2.).



Figure 2. Synthesis of Schiff bases (A1-A8).

It seems that the effect of substituents at the para positions of the aromatic amine ring increases the yield of the products.

The IR data of compound (A3) (Table 1) shows signals in the 3034–3079 cm⁻¹ range, corresponding to the stretching vibrations of the aromatic C-H group. Furthermore, spectral signals within the 2960–2971 cm⁻¹ range were identified as C-H aliphatic group stretching vibrations, while the signal at 1623 cm⁻¹ was indicative of C=N stretching vibrations, and the signal at 1587 cm⁻¹ corresponded to C=C stretching vibrations. The results are consistent with previously published literature [87,88]. Whereas the IR data of compound (A5) showed a signal for aromatic C-H at 3118 cm⁻¹, corresponding to the free CH₃ group of methyl. Additionally, the Schiff base C=N band appeared at 1627 cm⁻¹, while the characteristic peak at 1580 cm⁻¹ was identified for aromatic C=C. A signal was detected at 3089 cm⁻¹, corresponding to the O-H band [22,23].

Comm Codo	٨٣	IR							
Comp. Code	AI	v C-H Ar.	ν C=N	ν C=C Ar.	Others				
A1	N N N	3238	1586	1528					
A2		3107	1624	1594					
A3	ЮН	3111	1623	1587	υ (OH) 3469				
A4	NO ₂	3130	1607	1581	v (N-O) 754 Sym. NO2 (1342) Asy. NO2 (1515)				
A5	€сн₃	3118	1627	1580	v CH3 Sym. CH3 (2867) Asy. CH3 (2929)				
A6	⟨N ^{CH³} CH³	3115	1620	1567	v (CH3) Sym. CH3(2899) Asy. CH3(2991)				
A7	С	3104	1622	1576	υ (C-Cl) 832				
A8	√−осн₃	3127	1621	1534	v (C-O) 1032 Sym. CH3 2846) Asy. CH3 (2971)				

Table 1. IR Spectral Data of Schiff Bases (A1–A8).

¹H NMR spectrum of compound (A3) (Table 2) revealed three singlets at 8.049 ppm for CH-S protons, 8.81 ppm for CH=N protons, and 9.78 ppm for the O-H group, in addition to multiplet bands for the aromatic ring protons at 6.82–7.37 ppm. Similarly, ¹H NMR spectrum of compound (A5) showed singlets at 8.087 ppm for CH-S, 8.821 ppm for CH=N, and 2.33 ppm for C-H, and multiplet bands for aromatic protons at 7.23–7.34 ppm.

Table 2: ¹H NMR Data for Schiff Bases (A1–A8).

Comp. Code	δ CH=N ppm (d,1H)	δ CH- S ppm (d, 1H)	Aromatic protons ppm	Other Bands Ppm
A2	8.824	7.466	7.466- 7.299	
A3	8.81	8.049	7.37 – 6.82	9.78 (OH)
A4	8.86	7.57	8.17 – 6. 60	
A5	8.821	8.087	7.34 – 7.23	2.33(CH ₃)
A6	8.83	7.87	7.42 – 6.73	2.95(CH ₃)

The ¹³C NMR spectrum of compound (A3) (Table 3) exhibited signals at 167.87 ppm for CH=N, 145.01 ppm for CH-S, and 158.06–116.02 ppm for aromatic carbons. Likewise, the ¹³C NMR spectrum of compound (A5) showed signals at 167.33 ppm for CH=N, 145.22 ppm for CH-S, and 112.42–145.22 ppm for aromatic carbons, along with an additional signal at 21.14 ppm corresponding to CH₃.

Comp. Code	CH=N ppm	CH–S ppm	Aromatic carbons ppm	Other Bands Ppm
A2	167.11	143.14	147.68 - 122.14	
A3	167.87	145.01	158.06 - 116.02	
A4	166.22	145.78	156.17 – 125.67	
A5	167.33	145.22	145.22- 122.00	21.14 (CH ₃)
A6	168.42	144.88	147.47- 112.42	40.61 (CH ₃)

Table 3: ¹3C NMR Data for Schiff Bases (A1–A6).

3.2. Thiazolidinones Characterization

The second part of this work involves the synthesis of a new series of thiazolidinone derivatives (A9–A16) by reacting the prepared Schiff bases with 2-mercaptoacetic acid in benzene, as described in the experimental section. (Figure 3).



Figure 3. Schiff bases cyclization reaction.

The proposed reaction mechanism suggests the attack of the sulfur atom in 2-mercaptoacetic acid on the carbon atom of the carbon-nitrogen double bond, followed by a cyclization step through dehydration after the protonation of the hydroxyl group, as shown below [24]. (Figure 4)



Figure 4. Suggested mechanism of Schiff base cyclization.

The reaction was carefully monitored by observing changes in physical properties, including melting point, thin-layer chromatography (TLC) patterns, and color. Infrared (IR) spectroscopy was used for spectral analysis, revealing several key peaks. The IR spectrum of compound (A10) showed C=O stretching vibrations at 1677 cm⁻¹.The stretching vibration of the aliphatic C-H group appeared at 2946 cm⁻¹, while aromatic C-H stretching vibrations were detected at 3126 cm⁻¹. These findings were consistent with expected values and aligned with literature reports. Detailed results are provided in Table 4 [25].

The IR data of compound (A16) (Table 4), showed a band at 1031 cm⁻¹ for C-O. The peak at 3114 cm⁻¹ corresponded to typical aromatic C-H stretching, while aliphatic C-H stretching was identified at 2922 cm⁻¹. The hydrogen-bonded C=O group appeared at a comparatively longer wavelength of 1663 cm⁻¹. The peak at 1433 cm⁻¹ was detected for aromatic C=C [25].

Table 4. FTIR data of compounds (A9-A16).

				IR	
Comp. Code	Ar	ν C- H Ar.	v C=O	v C=C Ar.	Others
A9		3095	1590	1431	
A10	$\langle \rangle$	3126	1677	1498	
A11	-он	3094	1602	1432	υ (O-H) 1316
A12		3128	1690	1393	υ (N-O) 791 Sym.NO2 (1393) Asy. NO2 (1564
A13	√−сн₃	3123	1675	1427	υ (CH3) 3123 Sym.CH3 (2943) Asy.CH3 (2991)
A14	N ^{CH3} CH3	3112	1668	1368	υ (CH3)2 3112 Sym.CH3 (2820) Asy.CH3 (2904)
A15	С_сі	3100	1678	1499	υ (C-Cl) 752
A16	ОСН3	3114	1663	1433	υ (C-O)1031 Sym.CH3 (2850) Asy.CH3 (2922)

Table 5 shows the 1H NMR spectra for some thiazolidinone derivatives (A9–A16). (Fig. 5) For compound (A10), the protons of the CH2 group in the thiazolidinone ring are observed as two doublet signals at (3.86–4.06) ppm, which is good evidence of the successful synthesis of the product. Additionally, the proton of the (S–CH–N) group in the thiazolidinone ring appears at (6.91) ppm, while the protons of the other groups appear at (7.36–7.71) ppm [26].

The 1H NMR spectrum of compound (A16) (Fig. 6, Table 5) shows the protons of the CH2 group in the thiazolidinone ring as a singlet signal at (3.84–4.03) ppm, indicating successful product formation. The proton of the S–CH–N group in the thiazolidinone ring appears at (6.79) ppm, while the protons of the CH-N groups appear at (7.74) ppm. Additionally, a multiplet band appears at (7.70–7.73) ppm for the protons of the aromatic ring [27].

Comp. Code	δCH2 (S,2H) For thiazolidinone ring	δ-S-CH-N (S, 1H) For thiazolidinone ring	Ar. H. ppm	Others	
A9	2.99-3.12	6.63	7.61-6.75		
A10	3.86-4.06	6.91	7.36-7.71		
A11	3.87-4.08	6.85	7.30-7.73	δ (O-H) 12.48	
A13	3.85-4.08	6.86	7.70 -7.72	δ (CH3) 2.24	
A14	4.02-4.14	7.06	7.73 - 7.74	δ (CH3)2	

Table 5. 1H NMR data for thiazolidinone derivatives (A9-A16).

				3.14
A15	3.88-4.06	6.94	7.31 - 7.72	
A16	384403	670		δ (O-CH3)
Alo	5.64-4.05	0.79	1.10-1.15	3.36

In the 13C NMR spectrum of compound (A10) (Fig. 7, Table 6), the signals for the C=O group appear at 170.81 ppm, the CH-S group appears at 122.39 ppm, and the CH-N group appears at 143.04 ppm. The signals for the aromatic carbons appear at (7.36–7.71) ppm.

Similarly, the 13C NMR spectrum for compound (A16) (Fig. 8, Table 6) shows the signals for the C=O group at 170.71 ppm, the CH-N group at 143.02 ppm, and the CH-S group at 122.39 ppm. The signals for the aromatic carbons appear at (127.57–130.33) ppm, and another peak appears at 3.36 ppm, which corresponds to the O-CH3 group (see Table 6).

Table 6. Chemical shifts of 13C NMR data for thiazolidinones (A9-A16).

The figure presents the ¹H NMR spectrum of compound A10, displaying characteristic proton chemical shifts. The spectrum includes expanded regions highlighting specific proton environments. Peak assignments indicate the presence of aromatic, aliphatic, and solvent signals, confirming the structural integrity of the compound. The data support the molecular characterization and structural elucidation of compound A10.



Figure 5.1H NMR spectrum of compound A10.

The figure presents the ¹H NMR spectrum of compound A16, displaying distinct proton chemical shifts. Expanded regions highlight key spectral features, including aromatic, aliphatic, and solvent signals. The spectrum confirms the molecular structure and functional group environments, supporting the structural characterization and verification of compound A16's chemical identity using NMR spectroscopy.



Figure 6. ¹H NMR spectrum of compound A16.

The figure presents the ¹³C NMR spectrum of compound A10, displaying characteristic carbon chemical shifts. The spectrum highlights distinct signals corresponding to aromatic, carbonyl, and other functional group environments. The data confirm the molecular framework, supporting the structural elucidation and verification of compound A10's chemical composition through ¹³C NMR spectroscopy.



200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 f1 (ppm)

Figure 7.13C NMR spectrum of compound A10.

The figure presents the ¹³C NMR spectrum of compound A13, displaying distinct carbon chemical shifts. The spectrum identifies characteristic peaks corresponding to aromatic, carbonyl, and alkyl functional groups. The observed chemical shifts support



the structural confirmation and molecular characterization of compound A13, reinforcing its chemical identity through ¹³C NMR spectroscopy analysis.

Figure 8. 13C NMR spectrum of compound A13.

The figure presents the ¹H NMR spectrum of compound A14, displaying characteristic proton chemical shifts. Expanded regions highlight key spectral features corresponding to aromatic, aliphatic, and solvent peaks. The observed chemical shifts confirm the structural integrity of compound A14, supporting its molecular characterization and verification through ¹H NMR spectroscopy analysis.



Figure 9. 1H NMR spectrum of compound A14.

The figure presents the ¹³C NMR spectrum of compound A13, highlighting distinct carbon chemical shifts. The spectrum includes peaks corresponding to aromatic, carbonyl, and alkyl functional groups. The observed chemical shifts confirm the structural integrity of compound A13, supporting its molecular characterization and verification through ¹³C NMR spectroscopy analysis.



Figure 10.13C NMR spectrum of compound A13.

3.3. Results of Antibacterial Activity

The antibacterial activity of some of the prepared compounds against Gram-positive and Gram-negative bacteria was evaluated using four different types of bacteria: Staphylococcus aureus (S. a), Staphylococcus epidermidis (S. epidermidis), Escherichia coli (E. Coli), and Pseudomonas aeruginosa (P. Aeruginosa). These bacteria were selected for their varying resistance to antibiotics. The disk diffusion method was used, and the inhibition zones were measured in cm. The results showed that the prepared compounds were able to inhibit the growth of the tested bacteria, both Gram-positive and Gram-negative, at varying rates, as shown in (Table 7).

	Gram-positive Bacteria						Gram-negative Bacteria					
Comp. Code	S. aureus		S. e	epidermidis			E. coli		P. aeruginosa			
	25%	50%	75%	25%	50%	75%	25%	50%	75%	25%	50%	75%
A9	0	14	22	25	26	31	9	11	16	25	26	31
A10	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
A11	11	12	14	9	10	11	10	12	13	9	10	11
A12	15	17	19	10	16	20	16	18	22	10	16	20
A13	10	13	14	13	18	20	9	10	14	13	18	20
A14	10	11	13	10	11	13	15	16	18	10	11	13
A15	15	16	20	16	17	18	12	13	16	16	17	18
A16	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a
	23	31	43	21	29	38	0	23	35	18	31	37
Ciprofloxacine												

Table 7. Shows the inhibition zone diameters around the prepared compounds.

4. Discussion

Results in (Table 7) evaluates the antibacterial efficacy of chemical compounds (A9 to A16) and Ciprofloxacin against Gram-positive (S. aureus, S. epidermidis) and Gramnegative (E. coli, P. aeruginosa) bacteria. Inhibition activity was assessed at 25%, 50%, and 75% concentrations, measured as inhibition zone diameters (mm). (n.a.) indicates that bacteria are inactive towards this compound . Inhibition generally increases with concentration, as expected. Effectiveness varies among compounds, with (A13, A14 and A15) showing notable activity compared to the literature [28]. Gram-positive bacteria are generally more susceptible, likely due to structural differences in their cell walls. Ciprofloxacin serves as a control, exhibiting strong inhibition against Gram-positive bacteria and increasing inhibition against Gram-negative bacteria at higher concentrations.

Among the tested compounds, (A9) shows some Gram-positive inhibition, increasing with concentration, whereas (A10) exhibits no measurable activity. (A11) moderately inhibits both Gram-positive and Gram-negative bacteria, with a marginal concentration-dependent increase. (A12) displays moderate to strong inhibition across bacterial types, suggesting promising antibacterial potential. When compared to Ciprofloxacin, the antibiotic consistently outperforms most tested compounds against Gram-positive bacteria, particularly at higher concentrations. Against Gram-negative bacteria, Ciprofloxacin shows minimal inhibition at 25% but increases at 50% and 75%, matching or exceeding the tested compounds [29].

Mechanistic studies are required to determine how these compounds inhibit bacterial growth. Overall, compounds (A11, A12, A13, A14 and A15) exhibit antibacterial activity, though Ciprofloxacin remains superior, particularly against Gram-positive bacteria. Further studies should explore their mechanisms and optimize their antibacterial properties [30]. (Figure 11)



Figure 11. Control test results for antibacterial test.

The figure illustrates the antibacterial inhibition of different bacterial strains using various concentrations (25%, 50%, and 75%) of thiazolidinone derivatives. The inhibition zones indicate dose-dependent bacterial susceptibility. The observed results suggest potential antibacterial activity, supporting the efficacy of thiazolidinone derivatives as promising candidates for antimicrobial applications in pharmaceutical research.



Figure 12. Inhibition of studied bacteria using different concentration of thiazolidinone derivatives.

5. Conclusion

In this study, a series of thiazolidinone derivatives were successfully synthesized from Schiff bases through a cyclization reaction with thioglycolic acid. The structural characterization using FT-IR, ¹H NMR, and ¹³C NMR confirmed the formation of the expected products, with TLC and melting point measurements supporting their purity. The antibacterial screening demonstrated that certain derivatives exhibit notable activity, highlighting the potential of thiazolidinones as antibacterial agents. Furthermore, the study established a correlation between structural modifications and antibacterial potency, suggesting that electron-donating and electron-withdrawing groups at specific positions can enhance activity. These findings pave the way for further optimization and investigation into the pharmacological applications of thiazolidinone derivatives.

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