Synthesis, Identification and Antibacterial Evaluation of Some New Benzothiazole-Based Azo Schiff Bases

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Abstract: We report the synthesis of some novel azo Schiff bases based on benzothiazoles via a multi-stage synthetic approach. Synthesis of the target compounds included an intermediate, followed by an azo coupling reaction and a subsequent condensation reaction with different aromatic aldehydes. The chemical structures of the synthesized compounds were formally confirmed using spectroscopic techniques, including Fourier Transform Infrared Spectroscopy (FT-IR), Proton Nuclear Magnetic Resonance (^{1}H-NMR), and Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR). Furthermore, the antibacterial activity was evaluated using the agar diffusion method against selected Gram-positive and Gram-negative bacterial strains. As a result, several derivatives displayed significant antibacterial activity. The above data strongly indicate that azo Schiff base compounds derived from benzothiazoles will represent a new group of potentially valuable antibacterial agents for further research. D4 and D1 were the most potent derivatives, indicating that both electron-donating and electron-withdrawing groups on the aromatic ring strongly affect antibacterial activity. In conclusion, the results show that benzothiazole azo dye Schiff base derivatives offer molecular scaffolds for new antibacterial drug development. More studies are requested to determine their mode of action and to fully assess their biological potential.

1 INTRODUCTION

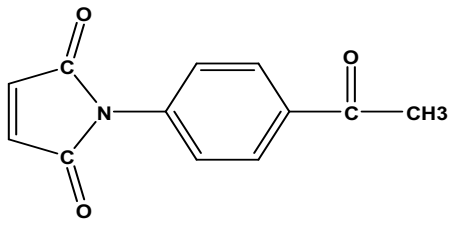
Heterocyclic molecules, also known as heterocycles, have gained a significant importance in organic and medicinal chemistry as heteroatoms like nitrogen, oxygen or sulfur play an essential role in the alteration of electronic and reactional properties of the cyclic system [1]. Owing to their structural diversity and synthetic versatility, such five- and six-membered heterocyclic systems are ubiquitous building blocks in drug discovery [2]. The ability to have selective interactions with the multitude of enzymes, receptors, nucleic acids [3] has made the differences in the heteroatom species and substitution critical in order to modify pharmacological effect, and thus the development of heterocyclic systems of potential therapeutic significance has generated considerable interest [4]. Benzothiazole is an important fused aromatic and represents a common pharmacophoric scaffold with useful electronic properties among sulfur- and nitrogen-containing heterocycles [5]. Different biological activities, involving antibacterial, anticancer, and antioxidant activities, have been ascribed to the benzothiazole derivatives [6]. The extended π-conjugation in the benzothiazole core contributes to molecule stabilization and macromolecular biomolecular binding [7]. The structural modifications of the benzothiazole ring can play a major influence on the biological activity and redox properties, emphasizing the importance of this heterocycle in medicinal chemistry research [8]. Heteropolymers contain the azo (−N=N−) and Schiff base (−C=N−) functions, which are known to exhibit variable coordination properties, extended conjugation, and a wide range of biological activities [9]. The addition of these linkages within heterocyclic frameworks facilitates electronic delocalization and thus improve physicochemical and biological properties [10]. Azo-Schiff bases systems received much attention due to their spectroscopic properties, and a review of the literature revealed a number of reports of the antimicrobial and antioxidant activities of these systems [11]. The incorporation of azo and azomethine groups based on a benzothiazole scaffold should provide an additivity, or even synergy in the biological activity [12]. However, there still remains very scant knowledge of the structure confirmed

biological evaluations of benzothiazole-based azo Schiff bases. The aim was to design and to synthesize new azo Schiff bases based on 2-aminobenzothiazole, through accessible and convenient synthetic methodologies, to characterize the molecular structures with FT-IR and NMR spectroscopies, and to evaluate their antibacterial activity.

2 EXPERIMENTAL

2.1 Materials and General Methods

All starting materials and solvents of analytical grade were used without further purification or preprocessing modifications during this work. Maleic Anhydride (99%) and 4-Aminoacetophenone (99%) were from Merck. All the target azo-Schiff bases (D1-D6) were prepared from the following substituted aromatic aldehydes: 4-Chlorobenzaldehyde (91%) and 2-Hydroxy-1-naphthaldehyde (82% gave appropriate products in the same solvent with Merck; 2,4-Dihydroxybenzaldehyde (98%) purchased from Energy Chemical; 4-Methoxybenzaldehyde (85%) and 2,4-Dimethoxybenzaldehyde (98.6%) from Aladdin Reagent; 2-Methoxybenzaldehyde (87.99%) from (Sigma-Aldrich). Analysis: Ammonium chloride (used as catalyst) and absolute ethanol (99%) (Scharlau) were used as received.



N-(4-acetylphenyl)maleimide

Figure 1: The structure of the maleimide compound.

2.2 Instrumentation and Measurements

The melting points of all synthesized compounds were determined on a Digital Melting Point Advanced SMP3 (Stuart, UK).

FT-IR Spectroscopy: The infrared spectra (400-4000 cm^{-1}), used the KBr disc technique, were performed using a SHIMADZU model FT-IR 8400S University of Diyala) and a IRAffinity-1 system (PBC Center, Baghdad.

NMR spectroscopy: The product identity was determined by recording the ^{1}H-NMR and ^{13}C-NMR spectra of the product in DMSO- d6 solution with Tetramethylsilane (TMS) as an internal standard.

Thin Layer Chromatography (TLC): TLC was used to monitor the purity of the products and the progress of reactions on metal plates coated with silica gel G, which were visualized by UV lamp.

2.3 Synthesis of Intermediate and Target Compounds

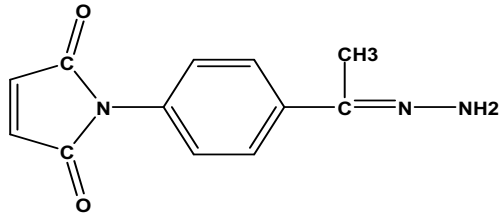
2.3.1 Synthesis of N-(4-acetylphenyl) maleimide (Compound A)

To prepare a homogeneous reaction medium, a mixture of maleic anhydride (2.0 g, 0.0204 mol) was dissolved in 15 mL of absolute ethanol and transferred to a 100 mL round-bottom flask. In parallel, 4-aminoacetophenone (2.75 g, 0.020 mol) was dissolved in 20 mL of the same solvent, to prepare an equimolar solution. After the amine solution was gradually added to the anhydride solution with strong magnetic stirring, the formation of intermediate maleamic acids was allowed to proceed and then incorporated with the formation of the maleimide ring. The mixture was then refluxed at 70-78 °C for 3-4 h. TLC corroborated completion of the reaction, the product's purity, and the full consumption of the starting materials. The mixture was then cooled slowly to room temperature for crystallization after the refluxing was complete. A yellow solid product was formed, and the precipitate was then isolated by vacuum filtration, thoroughly rinsed with distilled water to remove excess acid, and dried. A portion of the crude product was recrystallized from 100% ethanol for purification purposes. The route proceeded to give the desired target compound in 91% yield; Figure 1 [13]. The melting point of the purified product was found to be in the range of 135 to 138 °C, in agreement with its expected molecular formula of $C_{12}O_3H_9N_1$ and molecular weight of 215.21 g/mol (both determined via mass spectrometry).

2.3.2 Synthesis of N-(4-acetylphenyl) maleimide hydrazone (Compound B)

Compound A (2.15 g, 0.01 mol) was dissolved in 30 mL (100% ethanol) in a round-bottom flask under magnetic stirring. Also, hydrazine hydrate (80%,0.5ml) was added to the mixture to initiate a nucleophilic reaction at the carbonyl carbon. TLCs for reaction and purity were performed on the

resulting reaction mixture to determine compound purity and the reaction progress, and the mixture was then refluxed under a condenser at 70-78 °C for 4-5 hours. The resultant mixture was then left to cool to room temperature, then cooled on an ice bath at 0 °C overnight to promote complete crystallization of the product derivative. The light-yellow solid was filtered and washed with cold ethanol to remove the excess precursor. Following a drying step the final product was obtained recrystallized 99,99% pure from ethanol. Figure 2: synthetic pathway using this method [14], a thiosemicarbazone of a functionalized compound B was obtained in a yield of 91%. The compound was isolated as yellow solid (m.p. 175-177 °C), Molecular weight: 259.29 g/mol, and the chemical formula is $C_{12}H_{11}N_3O_2$.

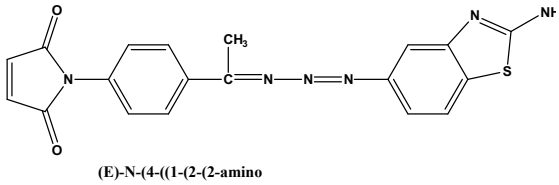


N-(4-acetylphenyl)maleimide hydrazone

Figure 2: The structure of compound B.

2.3.3 Synthesis of N-(4-acetylphenyl) maleimide hydrazone azo-benzothiazole (Compound C)

A reaction flask with Compound B (0.279 g, 1 mmol) and 0.052 mL of 85% phosphoric acid (H_3PO_4) was gently heated and stirred (stirring and continuous heating until everything dissolved). The resulting solution was then cooled to 0°C in an ice bath before a nitrating solution containing 0.0417 mL of concentrated nitric acid (HNO_3) and 0.069 g (10 mmol) of sodium nitrite ($NaNO_2$) was diluted to 2 mL with distilled water and added dropwise. Once the addition was complete, the temperature was stirred using a magnetic stirrer for 10 min, to ensure that the diazonium salt was being synthesized at all times. After diazotization, a mixture of p-amino benzothiazole (0.152 g, 10 mmol) in 0.5 mL distilled water was added to the reaction mixture. TLC confirmed reaction completion. The mixture was diluted to 10 mL of cold water to quench the reaction and precipitate the product. The orange solid was collected, filtered, and washed several times with cold water to eliminate any acidity, and then air-dried. It was further purified by recrystallization from absolute ethanol. The synthesis route and azo-linkage formation mechanism were depicted in Figure 3 [15], [16]. In this step, the azo-intermediate (Compound C) generated was 80% yield. The molecular weight (M.W) of the purified compound is 419.44 g/mol, which indicates the melting point (M.P) of the purified compound is 191-193°C.

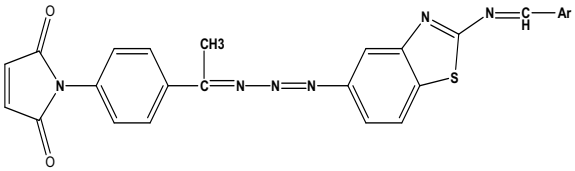


(E)-N-(4-((1-(2-(2-amino

Figure 3: The structure of mechanism of azo-linkage formation and diazotization-coupling sequence for Compound C.

2.3.4 Synthesis of Target Azo-Schiff bases (Compound D, D1-D6)

In a round-bottom flask, compound C (0.570 g, 1 mmol) and the appropriate substituted aromatic aldehyde (1 mmol) were dissolved with sonication in 20 mL of absolute ethanol. About 2-3 drops of glacial acetic acid was added to the mixture with continuous stirring on magnetic stirrer to initiate reaction. This mixture was then allowed to reflux 3-4 h of 70- 78 °C in a heating mantle and the reaction completion along with the purity of each of the components was determined by thin-layer chromatography (TLC). The solution was then cooled to room temperature followed by pouring onto crushed ice to precipitate product after the reflux. This precipitate was subsequently filtrated, collected, washed several times with ice-cold distilled water to remove catalyst traces, and dried. All the compounds (D1-D6) were purified by recrystallization from 100% ethanol, as shown in Figure 4 [17]. This synthetic route provided a range of derivatives D 1 as a creamy yellow solid weighing, yielding 91%, had a melting point of 220-222 °C and a molecular weight of 477.52 mol ($C_{26}H_{17}O_2N_6S_1Cl$). Compound D2 was obtained as a light ocher, yellow solid in 87% yield and observed as a melting point at 188-190 °C (544.58 g/mol) ($C_{30}H_{20}O_3N_6S_1$). D3 appeared as an orange powder 92% yield, m.p 202-204 °C; MW 509.52 g/mol ($C_{26}H_{17}N_6O_4S_1$). D4 was obtained as an off-white/cream solid in 87% yield, mp 234-236 °C, 537.57 g/mol ($C_{28}H_{22}N_6O_4S_1$). D5 was acquired (87%) as a dark orange-brown solid with melting point (m.p) 203-205 C, 557.4 g/mol ($C_{26}H_{17}O_2N_6S_1Br$). D6 was finally obtained as a light orange-brown powder of 79% yield that melted at 199-201 °C and which had a molecular weight of 494.53 g/mol ($C_{26}H_{18}N_6O_3S_1$).



N-(4-acetyphenyl)maleimide hydrazone azo-benzothiazole Schiff base

Figure 4: The final azo-Schiff base derivatives are prepared using a general synthetic method (D1-D6).

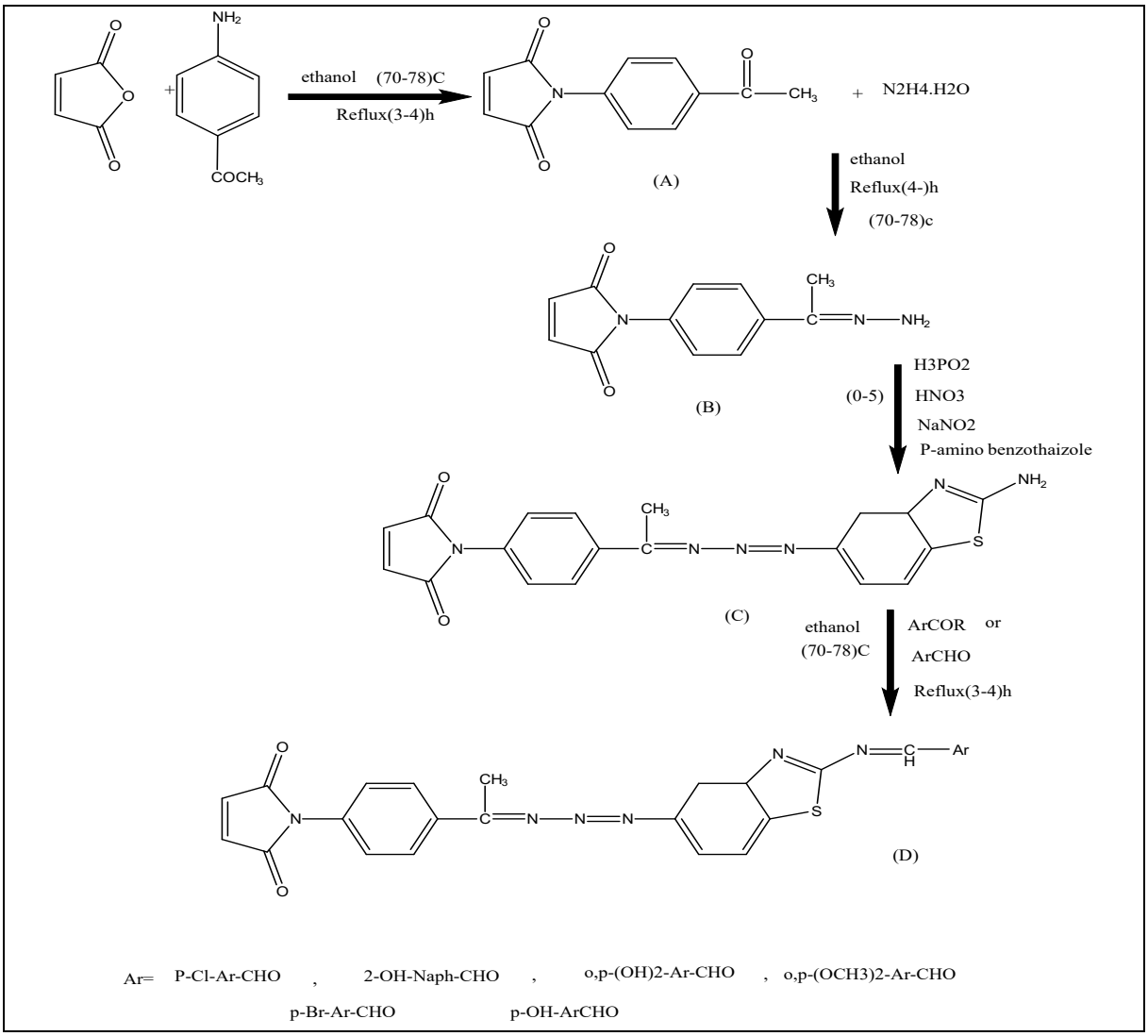
2.4 Assessment of Antibacterial Activity

The in vitro antibacterial activity of the prepared benzothiazole-based azo Schiff bases (designated as D1-D6) was evaluated against four pathogenic bacterial strains consisting of Gram-positive bacteria including *S. aureus and S. epidermidis* and Gram-negative bacteria including *E. coli and K. pneumoniae*. The Agar Well Diffusion Method [18] was used to assess the effectiveness. After activation, the bacterial isolates were adjusted to 0.5 McFarland standard, approximately 1.5×10^8 CFU/mL. Wells of 6 mm in diameter were made on Mueller-Hinton Agar (MHA) plates with sterile corn borer. Dissolve the compounds (D1-D6) in DMSO (e.g., these are prepared at dosages with complete solubility as 10 mg/mL). The volumes of each of the solutions added to the respective wells was 100 µL, with ceftriaxone and erythromycin being used as positive controls and pure DMSO as a negative control for comparison of potency of the synthesized compounds. Plate incubation was done at 37°C for 24-48 h. The diameter of insect, parasite, or fungus-induced ZOI was measured in millimeters (mm) with hand-held calipers to assess antibacterial activity. The results indicate the susceptibility of tested microorganisms to synthesized compounds. Differences in activity between the compounds (D1-D6) were therefore rationalised based on the electronic properties of the substituents, their influence on the lipophilicity of the molecules likely influencing penetration through bacterial cell walls.

3 RESULTS AND DISCUSSION

3.1 Characterization and Synthesis

The synthesis of the new series of benzothiazole-based azo-Schiff bases (D1-D6) was successfully achieved through a clear three-step process as depicted in Figure 5. The first step for this reaction involved the formation of a cyclic imide intermediate (Compound A) from the reaction of maleic anhydride and 4-aminoacetophenone in absolute ethanol, this led to a stable heterocyclic building block for further elaboration. The second step was the introduction of the azo linkage (-N=N-) by diazotization of the amino-intermediate at 0-5 ℃ and coupling with p-amino benzothiazole to give the required core azo structure in the target compounds. In the step 3, the azo intermediate was then condensed with different substituted aromatic aldehydes in absolute ethanol at room temperature, in the presence of glacial acetic acid as mild and green and efficient catalyst. Through this step, the most pure and highest yield azo-Schiff base derivatives (D1-D6) were obtained. The derivative is confirmed through various spectroscopic analysis (FT-IR, ^{1}H-NMR & ^{13}C-NMR) data of the chemical structures. Azo and imine constituents were confirmed in the benzothiazole framework through comparison of observed spectra with spectra predicted from proposed molecular structures, in the synthesis of target compounds.



Scheme 1: Synthetic pathway for preparing the target azo-Schiff bases (D1-D6). (Reagents and conditions: (a) Acetic acid, reflux for 4 hours; (b) $NaNO_2$, HNO_3, 0-5 °C, then coupling; (c) Substituted aromatic aldehydes, ethanol, NH_4Cl, reflux for 3-4 hours).

3.2 Spectral Analysis

All the synthesized benzothiazole based azo-Schiff bases (D1-D6) were then fully characterized using FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic techniques. Synthesis and FT-IR Spectrum of Benzothiazole-Based azo-Schiff Base Derivatives D1-D6. The benzothiazole-based azo-Schiff base derivatives D1-D6 were synthesized, and their FT-IR spectra were screened to confirm the formation of the desired compounds and to study the influence of different substituents on the vibrational properties. The complete disappearance of the characteristic bands of the primary amine (-NH_2) and the aldehyde (C=O) stretching suggested the condensation reaction was complete and that the characteristic Schiff base linkage had been formed. A conspicuous band of absorption in the range of 1635-1678cm^{-1} was attributed to the stretching vibration of the azomethine (C=N) bond, supposedly due to the formation of the imine bond and π-electron delocalization along the conjugated system. Confirmation of the heterocyclic framework stability and integrity was detected by the presence of cyclic imide carbonyl (C=O) vibrations in the range of 1712-1793 cm^{-1}. Particular effects pertain to the substituent D2: broad phenolic (−OH) bands at 3344-3352 cm^{-1} corroborating hydrogen bonding

interactions stabilizing molecular structure; D4 and D6: methoxy (C-O-C) stretching bands consistent with electron-donating resonance effects at the aromatic ring). Similarly, carbon halogen vibrations (C-Cl in D1 and C-Br in D5) showed the effect of electron withdrawing functionalities in bond polarities; as has been previously established for benzothiazoles-derived Schiff bases, substituent electronics substantially influence the position and intensity of IR bands [19]. Summaries of the absorption frequencies of all derivatives are given in detail in Table 1 and Figure 6.

The ^{1}H-NMR spectra of the synthesized derivatives clearly indicated their formulation to the proposed structures of azo Schiff base. The corresponding one-dimensional signal characteristic of each compound, located at δ 1.80-2.50 ppm, was ascribed to the methyl protons (CH_3) attached to an azomethine group. The signal distribution depends on the electronic properties of the substituents that were present in the aromatic ring. A striking downfield shift for compound D5 (δ 2.29-2.50 ppm) was observed in comparison to D3 (δ 1.80-2.00 ppm). The reason for this deshielding effect is the electron-withdrawing bromine substituent, which lowers the electron density around the nearby protons by a perturbation called an inductive effect. The longer π-conjugation of the benzothiazole increases the extent of electron delocalization to result in the further deshielding of protons and shifting the resonance to the upper chemical shift region. Downfield shifts depend on substituents which have been previously reported where similar substituent-dependent downfield shifts have been observed for substituted benzothiazole azo derivatives where electron-withdrawing groups significantly lowered ^{1}H-NMR chemical shifts due to decreased shielding effects [20]. Characteristic signals appeared in expected areas of derivatives with electron releasing groups (D3, D4 and D6). The methoxy group (-OCH_3) yielded a separate singlet at δ 3.7-3.9 ppm (C) in compound D4, corresponding to methyl protons connected to oxygen. However, the phenolic proton (-OH) in D3 appeared at δ 6.4 ppm, probably due to intramolecular hydrogen bonding and resonance stabilization in the conjugated aromatic system. Multiplet signals within the δ 6.30-8.20 ppm range for all derivatives' aromatic protons showed the expected patterns of substituted aromatic rings. The high correspondence of the spectrum data for those isolated compounds confirm that the synthesized compounds are consistent with those proposed from the isolated sample. Proton assignments are listed in Table 2 and Figure 7.

Their structures were further supported by comparison of ^{13}C-NMR spectra of compounds D1-D6 with that of the Downfield signals (δ 163.0-190.0 ppm) were assigned to carbonyl (C=O) and thiocarbonyl (C=S) carbons, both of which are deshielded due to the presence of the electronegative heteroatoms and due to possible conjugation. The Schiff base linkage was thus confirmed by the presence of the azomethine carbon (C≡N) signal at δ 160.0-170.0 ppm. The effects of the substituents can be seen in D5: the carbon bound to bromine (C-Br) resonates at δ 137.0 ppm as a result of bromine's inductive withdrawing effect, whereas the carbons on the oxygen containing substituents (C-O) appear at δ 143.0-155.0 ppm (D3, D4, D6), consistent with resonance donation from oxygen containing C1 substituents. Methyl carbons (CH_3) of aliphatic represented the up field around δ 20.0-30.0 ppm. Residual solvent signal of DMSO-d_6 appeared at δ 39.5 ppm such observations are in good agreement with previously reported benzothiazole Schiff base derivatives observing the influence of conjugation and substituent electronic effects on carbon chemical shifts [21]. Table 3 and Figure 8 provide a comprehensive summary of all ^{13}C-NMR assignments.

Table 1: Diagnostic FT-IR Data (cm^{-1}) for Synthetic azo-Schiff Bases (D1-D6).

No.	ν(O-H)	ν(C-H) $_{Ar}$	ν(C-H) $_{Aliph}$	ν(C=O) $_{Imide}$	ν(C=N) $_{Schiff}$	ν(C=C) $_{Ar}$	Diagnostic Bands
D1	---	3084	2889-2985	1712	1654	1624	ν(C-Cl): 725, Skeletal: 1408
D2	3344	3078	2831-2881	1721	1635-1658	1600	ν(C-O): 1342, ν(C-N): 1006
D3	---	3078	2900	1712	1635	1597	Skeletal: 1161, 1269
D4	---	3082	2985	1716	1654	1624	ν(C-O): 1211, 1281
D5	---	3016-3086	---	1712	1635-1678	1597	ν(C-Br): 500-650, Para-sub: 800
D6	3352	3078	---	1793	1654	1581	ν(C-O-C): 1215- 1257

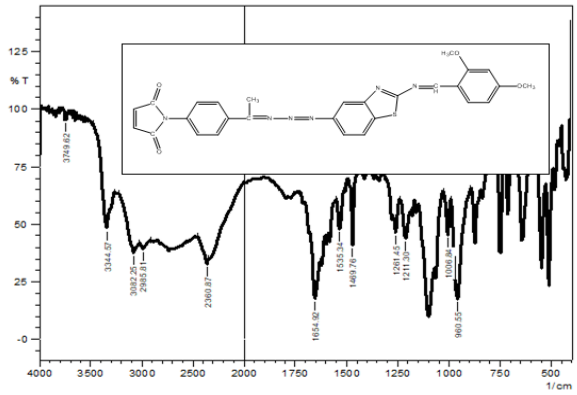


Figure 6: FT-IR spectra of compound D4.

Table 2: ^{1}H-NMR spectral results (ppm) for Compounds D1-D6.

Compound	CH_3 (Singlet)	Aromatic Protons (Ar-H)	Other Signals
D1	2.1 - 2.4	6.7 - 8.1	—
D2	2.1 - 2.4	6.8 - 8.2 (7.5-8.0 near azo)	—
D3	1.8 - 2.0	6.4 - 7.2 (Ar-OH)	6.4 (OH ortho), 7.7-8.0 (Ar near azo)
D4	2.1 - 2.4	6.7 - 8.1	3.7 - 3.9 (-OCH_3)
D5	2.29 & 2.3 - 2.5	7.0 - 8.2 (7.8 specifically)	—
D6	2.0	6.7 - 8.0 (6.7 ortho/para to OH)	2.5 - 3.3 (DMSO)

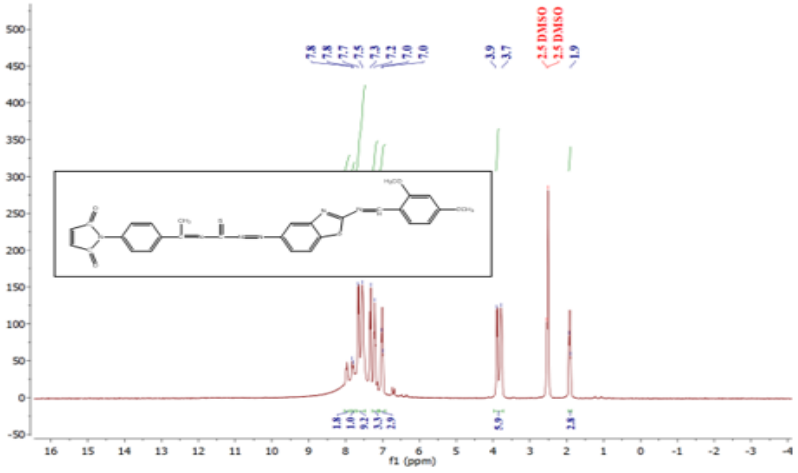


Figure 7: ^{1}H-NMR spectrum of compound D4.

Table 3: ^{13}C-NMR Spectral Results (ppm) for Compounds D1-D6.

Compound	C=O (Imide/Thio) δ ppm	C=N (Azomethine) δ ppm	Aromatic Carbons δ ppm	Aliphatic / Other δ ppm
D1	165-190	160-170	110-150	20-30 (CH_3)
D2	180-185	160-170	118-143 & 150-153 (C-N=N)	27 (CH_3), 39 (DMSO)
D3	176 (Thioamide)	163	110-143 & 143-155 (C-OH)	27 (CH_3)
D4	165-190	160-170	110-150 & 150-155 (Ar-OCH_3)	55-60 (-OCH_3), 20-30 (CH_3)
D5	167	167	119-143 & 137 (C-Br)	153 (Ipso), 39 (DMSO)
D6	163-167	163	110-143 & 143 (C-OH / C-N=N)	27 (CH_3)

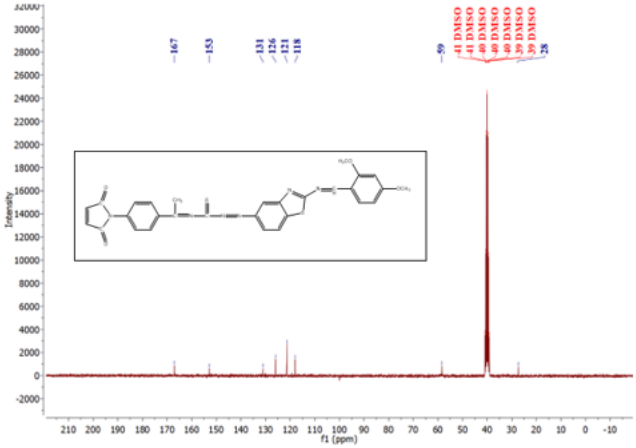


Figure 8: ^{13}C-NMR spectrum of synthesized compound D4.

Table 4: In vitro antibacterial activity of synthesized Benzothiazole-based azo-Schiff base (D1-D6) against pathogenic bacteria.

Sample code	Con. (mg/ml)	Gram- positive		Gram- negative	
		S.aureus	*S.epidermidis*	*E.coli*	*K.Pneumonia*
D1	100	14	16	13	19
	50	10	12	9	14
	25	0	9	8	8
D2	100	0	0	11	0
	50	0	0	10	0
	25	0	0	10	8
D3	100	0	0	10	0
	50	0	0	10	0
	25	0	0	7	0
D4	100	18	20	15	19
	50	14	16	11	15
	25	11	12	9	10
D5	100	12	14	11	12
	50	9	11	8	8
	25	0	8	8	8
D6	100	11	8	0	0
	50	8	15	10	13
	25	0	11	7	8
Ceftriaxone		30	0	20	20
Erythromycin		0	0	30	0
Azithromycin		0	30	0	0
DMSO		0	0	0	0

3.3 Biological Antibacterial Efficacy

The resulting azo derivatives of benzothiazole Schiff base (D1-D6) were tested in terms of antibacterial activity against the Gram-positive and Gram-negative bacteria representative, and their results are presented in Table 4. The concentration of the substituents employed on the benzene ring and the nature of the substituents had a major effect on the biological activity. The structure-activity Relationship (SAR) showed that the derivatives were highly diversified in their action, with D4 (2,4- dimethoxy) and D1 (p-Cl) being the most active ones. In particular, D4 had a maximum inhibition zone of 20 mm with S. epidermidis at 100 mg/mL. The addition of a methoxy group (-OCH_3) to a compound with several aromatic and/or heterocyclic rings may substantially modify various important physicochemical and biological properties. These are its lipophilicity, polarity and distribution of charges, the capability of the molecule

to enter the bacterial membrane, and its affinity to intracellular biological targets. Therefore, the methoxy group can be more effective in the antibacterial effect due to the better hydrophilicity-lipophilicity ratio, which is expected to increase membrane permeability and biological contact [22]. Conversely, the electron-withdrawing group (EWG) of D5 (p -Br) was weaker, particularly in lower concentrations. This implies that its dimethoxy substitution of D4 has made it electronically stable, in the sense that the ratio between the dimethoxy and the other parts of the molecule is more favorable to the interaction with the bacterial cell envelope than the halogenated analogs. Furthermore, all derivatives were clearly dose-dependent with respect to their antibacterial effect, the zone of inhibition growing with the concentration of the derivatives between 25 and 100mg/mL. This is an indication of successful molecular diffusion in the agar media with high dosage. It is interesting to note that the negative control (DMSO) was also inhibited (0 mm) in all the strains which means that it is possible to rule out the solvent as the origin of the observed bioactivity, but not the synthesized compounds. Comparatively, derivatives D1 and D4 had a strong degree of inhibitory activity, yet was weaker than the clinical standards like Ceftriaxone, Erythromycin, and Azithromycin which had a zone of 30 mm. It implies that, despite the fact that the benzothiazole-based azo Schiff base scaffold is a potentially useful lead structure in the development of antimicrobials, additional molecular optimization is necessary in order to match the commercial antibiotics in terms of their therapeutic efficacy levels.

4 CONCLUSIONS

In this work, a new series of benzothiazole-based azo Schiff bases (D1-D6) was synthesized stepwise, as characterized by (FT-IR, ^{1}H-NMR, and ^{13}C-NMR). The spectra obtained were confirmed to support chemical structures and to confirm the formation of the azo and azomethine bonds. Based on biological tests, the chemicals exhibited consistent antibacterial activity against various strains of Gram-positive and Gram-negative bacteria. D4 and D1 were the most potent derivatives, indicating that both electron-donating and electron-withdrawing groups on the aromatic ring strongly affect antibacterial activity. In conclusion, the results show that benzothiazole azo dye Schiff base derivatives offer molecular scaffolds for new antibacterial drug development. More studies are requested to determine their mode of action and to fully assess their biological potential.

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