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DESIGN, SYNTHESIS AND *IN VITRO* BIOLOGICAL ASSESSMENT OF NOVEL 2-[(3-ALKYL/ARYL-4,5-DIHYDRO-1*H*-1,2,4-TRIAZOL-5-ONE-4-YL)AZOMETHINE]PHENYL 2,5-DICHLOROBENZENESULFONATES MOLECULES AS ANTIOXIDANT AND ANTIBACTERIAL AGENTS

Fevzi AYTEMİZ [1, *], Haydar YÜKSEK [1]

[1] *Kafkas University, Chemistry Department, Kars, Türkiye*

**** Corresponding Author:*** *fevziaytemiz53@gmail.com*

ABSTRACT

In this study, a total of nine new 2-[(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4a-i**) were synthesized through the reactions of 2-formylphenyl 2,5-dichlorobenzenesulfonate (**3**) with 3-alkyl(aryl)-4-amino-4,5-dihydro-1*H*-1,2,4-triazol-5-ones (**2a-i**). The compounds **4a**, **4e**, **4g** and **4h** were successfully synthesized with yields exceeding 90%. Compounds **4a-i** were elucidated using ^{1}H, ^{13}C NMR and IR spectroscopic methods. The spectroscopic data were compared with the literature, and the results were found to be consistent. The antioxidant effects of compounds **4a–i** were investigated using three different methods, and it was found that compounds **4a-i** showed metal chelation activity close to that of α-tocopherol, which was used as the standard. Additionally, the antimicrobial properties of the compounds **4a–i** were evaluated using the agar well diffusion method against six different bacterial strains. The antimicrobial properties of the new compounds (**4a-i**) were compared with those of standard antibiotics such as neomycin, streptomycin, and ampicillin. It was observed that compound **4a** exhibited better antimicrobial activity than neomycin and streptomycin, and comparable activity to ampicillin.

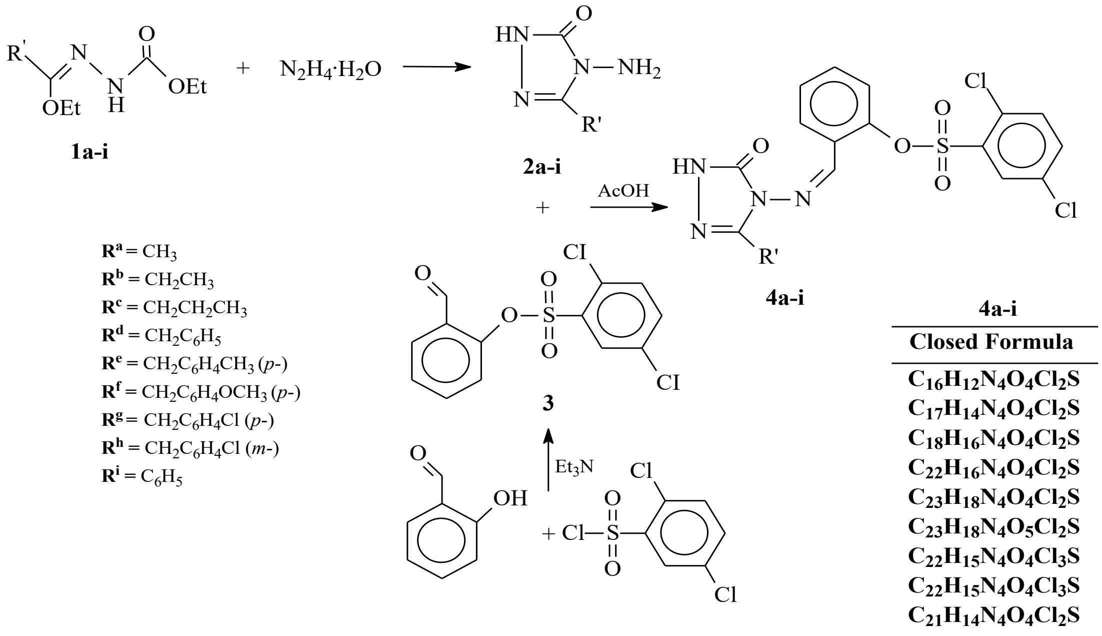
Keywords: 1,2,4-Triazol, Synthesis, Antioxidant, Antibacterial.

1 INTRODUCTION

Schiff bases have been of special interest to organic chemists because they are one of the most frequently encountered ligands in coordination chemistry. These ligands are formed by the condensation reaction of ketones or aldehydes with primary amines [1], [2], [3]. The carbon-nitrogen double bonds (C=N) formed as a result of the reaction are called imines or azomethines [4], [5]. Schiff bases have various biological effects such as anti-inflammatory [6], analgesic [7], anticancer [8], antimicrobial [9], antitumor [10], antiviral [11], antifungal [12], antituberculosis [13] and antioxidant [14]. There are many derivatives of Schiff bases, which were first synthesized by Hugo Schiff in 1864 [15], [16], [17], [18]. One of the most common types of Schiff bases are those containing the 1,2,4-triazole substituent [19], [20]. These derivatives have been reported to possess a wide range of biological properties [21], [22], [23].

Triazoles are heterocyclic compounds with three nitrogen and two carbon atoms and exist in various isomeric forms [24], [25], [26]. These compounds are among the most studied heterocyclic compounds in the field of pharmaceutical chemistry [27], [28]. Additionally, they are used in drug design due to their binding properties and are present in the structures of antifungal drugs such as voriconazole, itraconazole and fluconazole [29], [30], [31], [32], [33].

In this study, we aimed to investigate the in vitro antimicrobial and antioxidant properties of newly synthesized Schiff bases (4a-i) containing 1,2,4-triazole ring.



Scheme 1. Synthesis route of Schiff bases (4a-i).

2 MATERIAL AND METHOD

The required chemicals were purchased from Fluka, Aldrich and Merck. Melting points were measured using a Stuart SMP30 device. All test microorganisms used in the antimicrobial activity studies were obtained from Microbiological Environmental Protection Laboratories (France). ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Ultrashield Plus Biospin NMR spectrotometer. Solutions were prepared in deuterated dimethyl sulfoxide (DMSO-d_6) and tetramethylsilane (TMS) was preferred as standard. IR spectra were recorded on an Alpha-P Bruker FT-IR spectrometer. Antioxidant properties were investigated using a PG Instruments Ltd T80 UV/VIS spectrometer. In the evaluation of antimicrobial activity, concentration measurements were performed using a VELP brand turbidimeter capable of precise determinations within a measurement range of 0-1000 NTU

2.1 Synthesis

2.1.1 Synthesis Method of 2-formylphenyl 2,5-dichlorobenzenesulfonate (3)

A solution of 2,5-dichlorobenzensulfonyl chloride (0.01 mol) in ethyl acetate (20 mL) was prepared, to which 2-hydroxybenzaldehyde (0.01 mol) was added. Triethylamine (0.01 mol) was then gradually added to the mixture at 0-5 °C and the reaction was stirred for 3 hours. The mixture was subsequently refluxed for 4 hours. After cooling, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The resulting product was washed with distilled water and recrystallized from ethanol, yielding pure 2,5-dichlorobenzene sulfonate (**3**) [34], m.p: 108 °C; IR spectrum (υ, cm^{-1}): 781 (1,2-C_6H_4), 1197 and 1387 (SOO), 1696, 1759 (C=O), 2772 and 2896 (CHO). ^{1}H NMR spectrum (δ, ppm):10.17 (s,1H, CHO), 8.00-7.96 (m, 2H, ArH), 7.94-7.93 (m, 1H, H_{arom}), 7.91 (d, 1H, H_{arom}; J=1.60 Hz), 7.77-7.73 (m, 1H, H_{arom}), 7.59 (td, 1H, H_{arom}; J=7.60, 0.80 Hz), 7.18 (dd, 1H, H_{arom}; J=8.40, 0.80 Hz). ^{13}C NMR spectrum (δ, ppm): 187.01 (CHO); 149.32, 136.66, 136.19, 134.46, 133.42, 132.81, 131.29, 130.80, 129.52, 129.04, 128.56, 112.95 (C_{arom}).

2.1.2 General Synthesis Method of Schiff Bases (4a-i)

Firstly, compounds **2a-i** (amino-triazoles) were synthesized according to the literature procedures [35]. The synthesized compounds **2a-i** (0.01 mol) were taken into separate flasks and their solutions were prepared with acetic acid (20 mL). Then, 2-formylphenyl 2,5-dichlorobenzenesulfonate (**3**) (0.01 mol) was added to each flask and the mixtures were refluxed for 3 h. After cooling, distilled water was added to the solution and the product was precipitated and filtered. The precipitates were crystallized twice from ethanol to obtain compounds **4a-i** (2-[(3-methyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4a**), (2-[(3-ethyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-

yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4b**), (2-[(3-propyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4c**), (2-[(3-benzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4d**), 2-[(3-p-methylbenzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4e**), 2-[(3-p-methoxybenzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4f**), 2-[(3-p-chlorobenzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4g**), 2-[(3-m-chlorobenzyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4h**), 2-[(3-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)azomethine]phenyl 2,5-dichlorobenzenesulfonates (**4i**) as pure crystalline solids.

2.2 Antioxidant Activity

The antioxidant activities of compound **4a-i**, along with standard antioxidant agents such as ethylenediaminetetraacetic acid (EDTA), butylated hydroxytoluene (BHT), *α*-tocopherol and butylated hydroxyanisole (BHA), were assessed using various antioxidant assays, including metal chelation activity, reducing power and free radical scavenging activity.

The iron ion chelation activity of compounds **4a-i** was evaluated using the Dinis method [36]. This method is a spectrophotometric assay used to determine the antioxidant capacity of phenolic compounds. It primarily focuses on their ability to inhibit the oxidation of iron (Fe^{2+}) ions, reflecting the metal chelating effect. In this method, a colored complex (purple-pink) is formed between Fe^{2+} ions and ferrozine. If the sample contains chelating (binding) antioxidants, the formation of this complex is inhibited. As a result, the intensity of the color decreases. This decrease is measured spectrophotometrically, typically at 562 nm. A lower absorbance indicates a stronger metal chelating capacity.

The reducing power properties of compounds **4a-i** were investigated using the method described by Oyaizu [37]. This method is used to determine antioxidant capacity and is based on measuring the reducing power of iron ions. This method is commonly referred to as the Ferric Reducing Antioxidant Power (FRAP) assay. Antioxidants facilitate the reduction of Fe^{3+} (ferric ions) to Fe^{2+} (ferrous ions). As a result of this reduction, a colored complex is formed (e.g., with potassium ferricyanide). The absorbance of this colored complex is measured spectrophotometrically, typically at 700 nm. A higher absorbance indicates a greater the antioxidant capacity of the sample.

The free radical scavenging activity of compounds **4a-i** was assessed using the Blois method [38]. This method typically measures the radical scavenging capacity of a sample in the presence of free radicals, such as DPPH or ABTS. Antioxidants neutralize these free radicals, resulting in a color change. This color change is measured spectrophotometrically, usually at 517 nm, although the wavelength may vary depending on the radical used. A lower absorbance of the mixture indicates a greater free radical scavenging activity.

2.3 Antibacterial Activity

The antibacterial activity of compounds **4a-i** were analyzed using the well diffusion method. [39], [40]. Gram-positive bacterial strains (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 11778 and *Bacillus subtilis* ATCC11774) and Gram-negative bacterial strains (*Klebsiella pneumoniae* ATCC4352, *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC 25922) were preferred in the study. Streptomycin X3385, Neomycin X3385 and Ampicillin X3261 were used as standard antibiotics to compare the effects of compounds 4a-i.

The microorganisms were suspended in Mueller-Hinton Broth and their turbidity was adjusted to 240 NTU using a Velp brand turbidimeter. Following this, the microorganisms were inoculated onto the surface of Mueller-Hinton Agar using the "flood-inoculation" technique and allowed to dry for 15 minutes. Wells, 5 mm in diameters, were created on the agar surface with a sterile cork-borer, and 250-5000 µg/50 µL of the chemical compounds 4a-i were added into the wells. The plates were incubated at 37 °C for 18 hours. The diameters of the inhibition zones obtained was compared to those of standard antibiotics.

3 RESULTS AND DISCUSSION

3.1 Synthesis

In this study, nine new Schiff bases (**4a-i**) were synthesized from the reaction of compound **3** with compounds **2a-i**. The synthetic pathway for new 2-[(3-alkyl/aryl-4,5-dihydro-1*H*-1,2,4-triazol-5-one-4-yl)-azomethine]-phenyl 2,5-dichlorobenzenesulfonates (**4a-i**) is illustrated in Scheme 1.

The compounds **4a-i** were characterized using IR, ^1H and ^{13}C NMR spectroscopic methods. TMS was used as standard and DMSO-d_6 was used as solvent for both ^1H and ^{13}C NMR spectra. It was seen that the values obtained by spectroscopic methods were compatible with the literature [14], [41]. The obtained results were given in Tables 1-3. The physical properties of the compounds **4a-i** are given in Table 4. In addition, the IR, ^{1}H and ^{13}C NMR spectra of compound **4a** are presented in Figures 1–3. The molecular structure of compound **4a** has been drawn on the spectra with the atoms numbered, and these numbers have been assigned to the corresponding peaks.

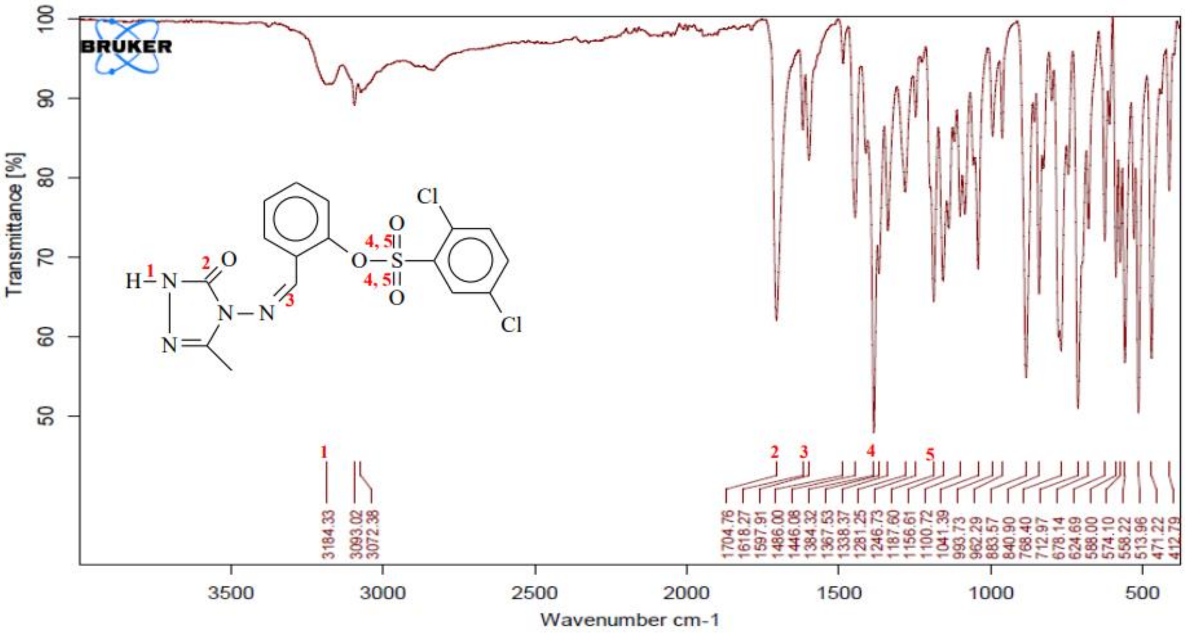


Figure 1. FT-IR spectrometer of Schiff bases 4a.

Table 1. FT-IR spectrum values of Schiff bases (4a-i)

Compounds	ν_{NH}	$\nu_{C=O}$	$\nu_{C=N}$	ν_{SO2}	$\nu_{1,4-}$ aromatic ring	$\nu_{1,3-}$ aromatic ring	$\nu_{1,2-}$ aromatic ring	ν_{mono-} aromatic ring
4a	3184	1705	1618, 1598	1384 and 1188	-	-	768	-
4b	3171	1705	1597	1383 and 1189	-	-	770	-
4c	3183	1701	1587	1377 and 1186	-	-	766	-
4d	3173	1712	1600, 1587	1381 and 1190	-	-	766	776 and 701
4e	3196	1712	1591	1395 and 1189	822	-	772	-
4f	3333	1716	1606	1387 and 1190	820	-	778	-
4g	3175	1702	1587	1357 and 1199	849	-	765	-
4h	3210	1713	1598, 1584	1381 and 1190	-	817 and 686	777	-
4i	3164	1713	1598, 1584	1381 and 1185	-	-	787	765 and 689

In the IR spectra of compounds **4a-i**, N-H stretching vibrations were observed in the range of 3210-3163 cm^{-1}. C=O stretching bands appeared between 1716-1701 cm^{-1}. Furthermore, C=N stretching vibrations were detected in the range of 1618-1584 cm^{-1}. In addition, SO_2 stretching vibration bands were observed in the ranges of 1395-1185 cm^{-1}.

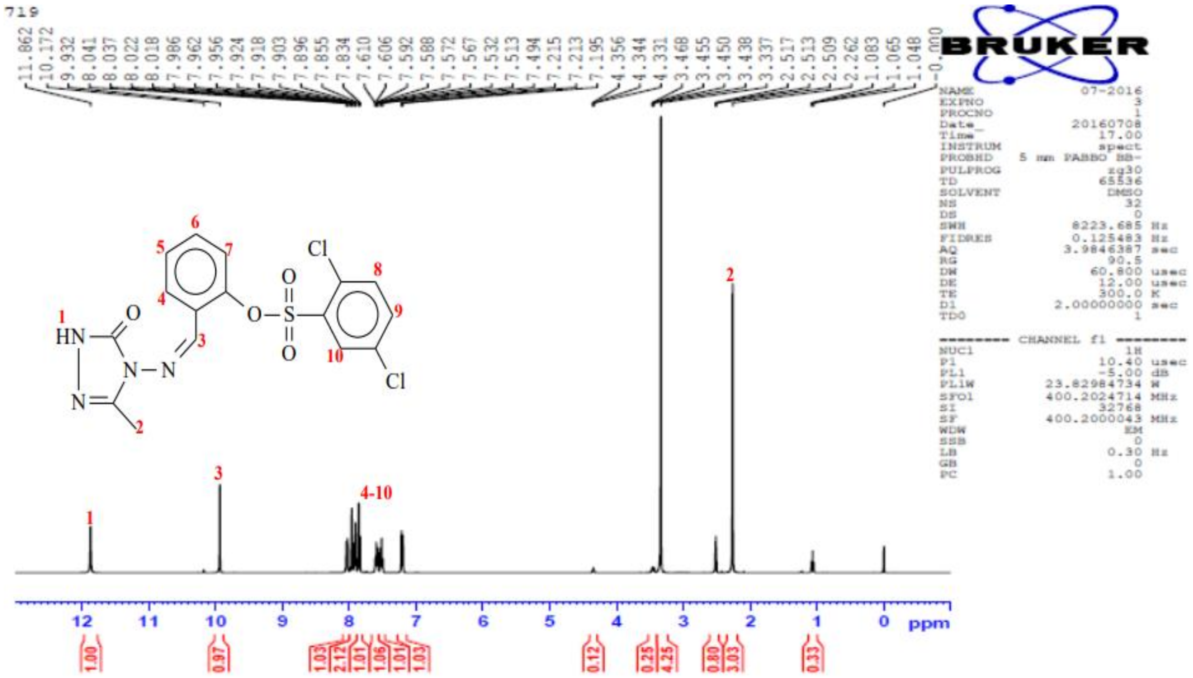


Figure 2. ^{1}H NMR spectrometer of Schiff bases 4a.

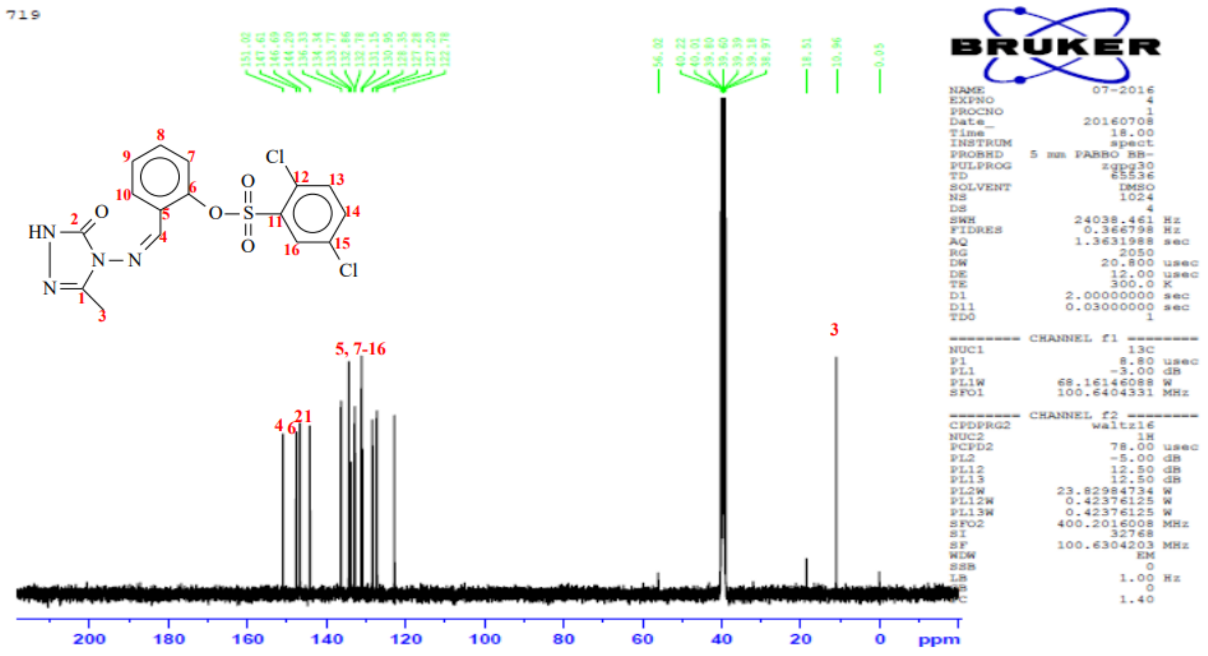
Table 2. ^{1}H NMR spectrum values of Schiff bases (4a-i)

Compounds	Aliphatic H	Aromatic H	N=CH	NH
4a	2.26 s (3H, C**H**$_3$)	8.03 (dd, 1H, *J*=7.60, 1.60 Hz), 7.96 (d, 1H, *J*=2.40 Hz), 7.91 (dd, 1H, *J*=8.40, 2.40 Hz), 7.84 (d, 1H, *J*=8.40 Hz), 7.59 (td, 1H, *J*=8.20, 1.60 Hz), 7.51 (t, 1H, *J*=7.60, 1.60 Hz), 7.21 (d, 1H, *J*=8.00 Hz)	9.93(s)	11.86(s)
4b	2.66 q (2H, C**H**$_2$CH$_3$, *J*=7.60 Hz), 1.20 t (3H, CH$_2$C**H**$_3$, *J*=7.60 Hz)	8.01 (dd, 1H, *J*=7.60, 1.60 Hz), 7.96 (d, 1H, *J*=2.40 Hz), 7.90 (dd, 1H, *J*=8.40, 2.40 Hz), 7.84 (d, 1H, *J*=8.40 Hz), 7.59 (td, 1H, *J*=8.00, 1.60 Hz), 7.51 (t, 1H, *J*=7.60), 7.21 (d, 1H, *J*=8.00 Hz)	9.92(s)	11.88(s)
4c	2.66 t (2H, C**H**$_2$CH$_2$CH$_3$, *J*=7.60 Hz), 1.68 sext (2H, CH$_2$C**H**$_2$CH$_3$, *J*=7.60 Hz), 0.96 t (3H, CH$_2$CH$_2$C**H**$_3$, *J*=7.60 Hz)	8.01 (dd, 1H, *J*=7.60, 1.60 Hz), 7.96 (d, 1H, *J*=2.40 Hz), 7.90 (dd, 1H, *J*=8.80, 2.40 Hz), 7.84 (d, 1H, *J*=8.80 Hz), 7.59 (td, 1H, *J*=8.00,1.60 Hz), 7.52 (t, 1H, *J*=7.60 Hz), 7.22 (d, 1H, *J*=8.40 Hz)	9.93(s)	11.90(s)
4d	4.05 s (C**H**$_2$Ph)	7.97-7.94 (m, 2H), 7.86 (dd, 1H, *J*=8.80, 2.40 Hz), 7.78 (d, 1H, *J*=8.40 Hz), 7.58 (td, 1H, *J*=8.40, 1.60 Hz), 7.50 (t, 1H, *J*=7.60 Hz), 7.36-7.32 (m, 4H), 7.28-7.22 (m, 2H), 7.19 (d, 1H, *J*=8.00 Hz)	9.92(s)	12.03(s)
4e	3.99 (C**H**$_2$Ph), 2.26 s (C**H**$_3$Ph),	7.98-7.94 (m, 2H), 7.86 (dd, 1H, *J*=8.80, 2.40 Hz), 7.70 (d, 1H, *J*=8.80 Hz), 7.58 (td, 1H, *J*=8.00, 1.60 Hz), 7.50 (t, 1H, *J*=7.60 Hz), 7.21 (d, 1H, *J*=7.60 Hz), 7.19 (d, 2H, *J*=8.00 Hz), 7.13 (d, 2H, *J*=8.00 Hz)	9.92(s)	12.01(s)
4f	3.97 s (C**H**$_2$Ph), 3.72 s (PhOC**H**$_3$),	7.99 (dd, 1H, *J*=8.40, 1.60 Hz), 7.97 (d,1H, *J*=8.40 Hz), 7.87 (dd, 1H, *J*=8.80, 2.40 Hz), 7.79 (d, 1H, *J*=8.80 Hz), 7.58 (td, 1H, *J*=8.40, 1.60 Hz), 7.52 (t, 1H, *J*=7.60 Hz), 7.24 (d, 2H, *J*=8.40 Hz), 7.19 (d, 1H, *J*=8.00 Hz), 6.89 (d, 2H, *J*=8.80 Hz)	9.92(s)	11.99(s)
4g	4.07 s (C**H**$_2$Ph)	7.98-7.96 (m, 2H), 7.89 (dd, 1H, *J*=8.80, 2.40 Hz), 7.19 (d, 1H, *J*=8.40 Hz); 7.81 (d, 1H, *J*=8.80 Hz), 7.58 (td, 1H, *J*=8.00,1.20 Hz), 7.51 (t, 1H, *J*=7.60 Hz), 7.41-7.37 (m, 4H)	9.95(s)	12.08(s)

Table 2 (continued). ^{1}H NMR spectrum values of Schiff bases (4a-i)

Compounds	Aliphatic H	Aromatic H	N=CH	NH
4h	4.09 s (C**H**$_2$Ph)	7.98-7.95 (m, 2H), 7.89-7.86 (m, 1H), 7.80 (d, 1H, *J*=8.40 Hz), 7.60-7.56 (m, 1H), 7.50 (t, 1H, *J*=7.60 Hz), 7.39-7.30 (m, 4H), 7.18 (d, 1H, *J*=8.40 Hz)	9.93(s)	12.06(s)
4i		7.96-7.92 (m, 2H), 7.88-7.80 (m, 4H), 7.61(td, 1H, *J*=8.00, *J*=1.60 Hz), 7.55-7.49 (m, 4H), 7.26 (d, 1H, *J*=8.00 Hz)	9.93(s)	12.44(s)

Upon analysis of the ^{1}H NMR spectra of compounds **4a–h**, signals corresponding to N–H protons were observed in the δ 12.08–11.86 ppm range. However, for compound **4i**, the signal of the N–H proton attached to the phenyl group at the C$_3$ position of the triazoles ring, which is a characteristic feature, was observed at δ 12.44 ppm. Additionally, peaks corresponding to N=CH protons were detected in the δ 9.95–9.92 ppm range, while aromatic protons appeared in the δ 8.03–6.89 ppm range. The peaks of the CH$_3$ proton of compound **4a** appeared at δ 2.26 ppm. The peaks of the CH$_2$C**H**$_3$ proton of compound **4b** appeared at δ 1.20 ppm and the peaks of the C**H**$_2$CH$_3$ proton appeared at δ 2.66 ppm. In compound **4c**, peaks were detected at δ 0.96 ppm for CH$_2$CH$_2$C**H**$_3$ protons, at δ 1.68 ppm for CH$_2$C**H**$_2$CH$_3$ protons and at δ 2.66 ppm for C**H**$_2$CH$_2$CH$_3$ protons. The peaks of C**H**$_2$Ph protons of compounds **4d-h** appeared at δ 4.05 ppm, δ 3.99 ppm, δ 3.97 ppm, δ 4.07 ppm and δ 4.09 ppm, respectively. Also, the peaks of PhC**H**$_3$ protons of compound **4e** appeared at δ 2.26 ppm and the peaks of PhOC**H**$_3$ protons of compound **4f** appeared at δ 3.72 ppm.



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B                        0
C                     1.40
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Figure 3. ^{13}C NMR spectrometer of Schiff bases 4a.

Table 3. ^{13}C NMR spectrum values of Schiff bases (4a-i)

Compounds	N=CH	Triazole C_5	Triazole C_3	Aromatik C	Alifatik C
4a	151.02	147.61	144.20	122.78, 127.20, 127.28, 128.35, 130.35, 131.15, 132.78, 132.86, 133.37, 134.34, 136.33, 146.69,	10.96 ($\mathbf{C}H_3$)
4b	151.17	147.97	146.68	122.82, 127.10, 127.30, 128.40, 130.95, 131.16, 132.78, 132.87, 133.75, 134.34, 136.34, 147.62	10.02 ($CH_2\mathbf{C}H_3$), 18.39 ($\mathbf{C}H_2CH_3$)
4c	151.11	147.61	146.66	122.82, 127.05, 127.29, 128.40, 130.96, 131.15, 132.77, 132.84, 133.75, 134.32, 136.32, 146.83	13.43 ($CH_2CH_2\mathbf{C}H_3$), 18.88 ($CH_2\mathbf{C}H_2CH_3$), 26.56 ($\mathbf{C}H_2CH_2CH_3$)
4d	151.03	147.65	146.12	122.71, 126.95, 127.24, 128.44, 130.91, 131.18, 132.78, 132.90, 133.69, 134.30, 136.32, 146.35 C_3; 126.72, 128.35 (2C), 128.76 (2C), 135.65	31.94 ($\mathbf{C}H_2Ph$)
4e	151.04	147.64	146.26	122.70, 126.94, 127.26, 128.34, 130.91, 131.16, 132.77, 132.88, 133.70, 134.29, 136.30, 147.08 C_3; 128.58 (2C), 128.99 (2C), 132.53, 135.80	20.57($Ph\mathbf{C}H_3$), 30.54($\mathbf{C}H_2Ph$)
4f	151.05	147.69	146.34	122.71, 126.98, 127.26, 128.39, 130.91, 131.17, 132.78, 132.90, 133.71, 134.30, 136.33, 146.42 C_3; 113.91 (2C), 127.42, 129.84 (2C), 158.14	30.08($\mathbf{C}H_2Ph$), 55.04($PhO\mathbf{C}H_3$)
4g	151.08	147.65	145.77	122.68, 126.96, 127.20, 128.36, 130.92, 131.17, 132.77, 132.91, 133.71, 134.28, 136.31, 146.46 C_3; 128.36 (2C), 130.69 (2C), 131.48, 134.59	30.28($\mathbf{C}H_2Ph$)
4h	151.00	147.66	145.62	122.71, 126.99, 127.20, 128.32, 130.92, 131.17, 132.78, 132.98, 133.69, 134.30, 136.32, 146.45 C_3; 126.83, 127.55, 128.95, 130.25, 133.69, 138.02	30.57($\mathbf{C}H_2Ph$)
4i	151.15	148,75	144.51	122.92, 127.07, 127.12, 128.04, 130.92, 131.16, 132.79, 133.10, 133.73, 134.28, 136.35, 147.75 C_3; 126.38, 128.04 (2C), 128.48 (2C), 130.13	

In the ^{13}C NMR spectra of compounds **4a-i**, peaks corresponding to N=CH carbons were observed in the δ 151.17-151.00 ppm range, peaks for triazole $\mathbf{C}_5$ (aromatic carbon atoms located at position 5 of the triazole ring) carbons appeared between δ 148.75-147.761 ppm, and peaks for triazole $\mathbf{C}_3$ (aromatic carbon atoms located at position 3 of the triazole ring) carbons were detected in the δ 146.68-144.20 ppm range. For compound **4a**, peaks corresponding to the $\mathbf{C}H_3$ carbon was observed at δ 10.96 ppm. The peaks of the $CH_2\mathbf{C}H_3$ carbon of compound **4b** appeared at δ 10.02 ppm and the peak of the $\mathbf{C}H_2CH_3$ carbon appeared at δ 18.39 ppm. The peaks of $CH_2CH_2CH_3$ carbons of compound **4c** appeared as $CH_2CH_2\mathbf{C}H_3$ carbon peak at δ 13.43

ppm, $CH_2\mathbf{C}H_2CH_3$ carbon peak at δ 18.88 ppm and $\mathbf{C}H_2CH_2CH_3$ carbon peak at δ 26.56 ppm. The peaks of CH_2Ph carbons of compounds **4d-h** appeared at δ 31.94 ppm, δ 30.54 ppm, δ 30.08 ppm, δ 30.28 ppm and δ 30.57 ppm, respectively. The peak of $\mathbf{C}H_3Ph$ carbon of compound **4e** appeared at δ 20.57 ppm and the peak belonging to $PhOCH_3$ carbon of compound **4f** appeared at δ 55.04 ppm.

Table 4. Physical properties of Schiff bases (4a-i)

Compounds	Melting Point (°C)	Yield (%)	Crystallization Solvent	Closed Formula
4a	217	90.14	Ethanol	$C_{16}H_{12}N_4O_4Cl_2S$
4b	191	86.48	Ethanol	$C_{17}H_{14}N_4O_4Cl_2S$
4c	171	81.25	Ethanol	$C_{18}H_{16}N_4O_4Cl_2S$
4d	148	77.24	Ethanol	$C_{22}H_{16}N_4O_4Cl_2S$
4e	175	90.27	Ethanol	$C_{23}H_{18}N_4O_4Cl_2S$
4f	204	86.66	Ethanol	$C_{23}H_{18}N_4O_5Cl_2S$
4g	205	92.30	Ethanol	$C_{22}H_{15}N_4O_4Cl_3S$
4h	166	94.36	Ethanol	$C_{22}H_{15}N_4O_4Cl_3S$
4i	223	77.71	Ethanol	$C_{21}H_{14}N_4O_4Cl_2S$

Compounds **4a-i** were crystallized in ethyl alcohol. The melting points of the compounds **4a-i** were found to be between 223 ^{0}C and 148 ^{0}C. As a result of the reactions, the highest yield was obtained for compound **4h**, while the lowest yield was obtained for compound **4d.**

3.2 Antioxidant Activity

The increase in absorbance of the reaction mixture is directly proportional to the reducing power of the sample. The decrease in absorbance of the reaction mixture is directly proportional to the free radical scavenging activity of the sample. The antioxidant properties of compounds **4a-i** were examined and it was observed that they have no reducing power and free radical scavenging activity. However, the compounds **4a-i** have chelating activity, albeit low. Figure 4 shows that the antioxidant properties of compounds **4a-i** are close to those *α*-tocopherol, which was used as a standard antioxidant. It was observed that the compounds have higher activity at lower concentrations.

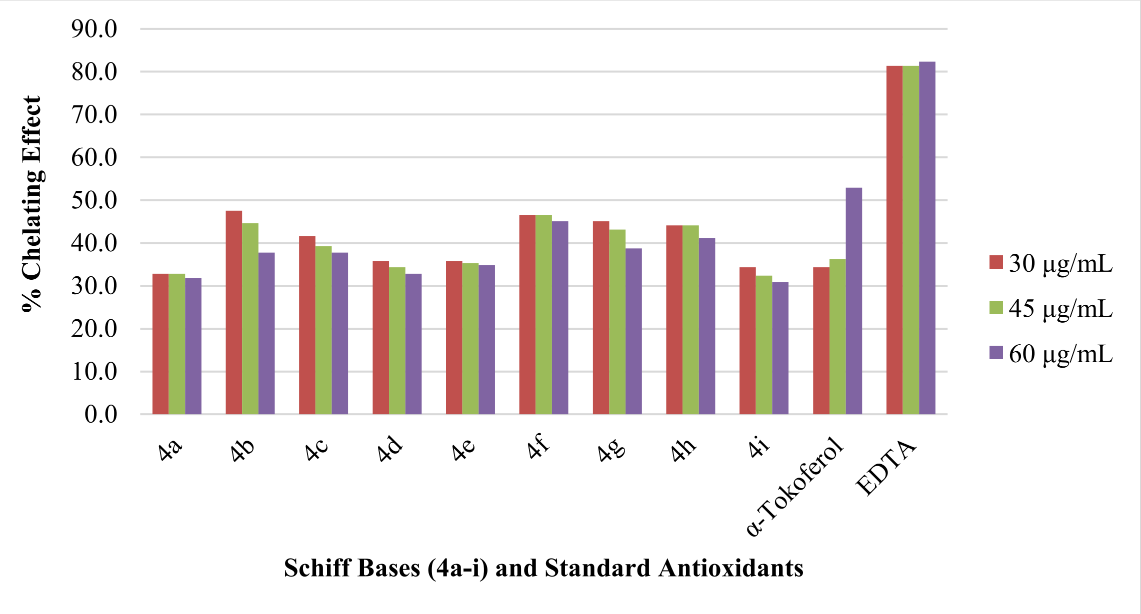

Figure 4. Metal chelating activities of Schiff bases (4a-i).

3.3 Antibacterial Activity

In this study, conducted with six different bacterial strains, including three Gram-positive strains (*S. aureus* ATCC 6538, *B. cereus* ATCC 11778 and *B. subtilis* ATCC 11774) and three Gram-negative strains (*K. pneumoniae* ATCC 4352*, P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922). Highly effective results were obtained. All of the new compounds **(4a-i**) were effective against several bacterial species. Most of the compounds exhibited activity comparable to the standard antibiotic neomycin and were more effective than streptomycin. The results were evaluated according to the inhibition zone diameters as follows: ≥17 mm high effect (+++); 11-16 mm medium effect (++); 5.5-10 mm low effect (+); <5.5 mm no effect (-) [42].

Table 5. In vitro antimicrobial activity of Schiff bases (4a-i)

Compounds and Standarts	**Level of the antibacterial effects and diameter of inhibition zone (mm)**					
	S. aureus	***B. cereus***	***B. subtilis***	***K. pneumoniae***	***P. aeruginosa***	***E. coli***
4a	24 (+++)	25 (+++)	23 (+++)	27 (+++)	20 (+++)	34 (+++)
4b	18 (+++)	16 (++)	14 (++)	20 (+++)	-	30 (+++)
4c	11 (++)	20 (+++)	12 (++)	27 (+++)	-	17 (+++)
4d	-	21 (+++)	12 (++)	-	-	14 (++)
4e	-	15 (++)	10 (+)	-	-	16 (++)

Table 5 (continued). In vitro antimicrobial activity of Schiff bases (4a-i)

Compounds and Standarts	**Level of the antibacterial effects and diameter of inhibition zone (mm)**					
	S. aureus	***B. cereus***	***B. subtilis***	***K. pneumoniae***	***P. aeruginosa***	***E. coli***
4f	12 (++)	-	10 (+)	-	-	-
4g	10 (+)	14 (++)	11 (++)	-	-	-
4h	12 (++)	13 (++)	23 (+++)	20 (+++)	12 (++)	14 (++)
4i	13 (++)	12 (++)	16 (++)	-	-	16 (++)
Streptomycin	21 (+++)	12 (++)	12 (++)	11 (++)	12 (++)	10 (+)
Neomycin	13 (++)	17 (+++)	17 (+++)	16 (++)	17 (+++)	16 (++)
Ampicillin	37 (+++)	36 (+++)	33 (+++)	35 (+++)	36 (+++)	34 (+++)

Compounds **4a** and **4b** showed high activity against *S. aureus*, while effects of **4c**, **4f**, **4h** and **4i** exhibited activity and **4g** was low activity. However, compounds **4d** and **4e** showed no effect. Only compound 4f was not effective against *B. cereus*, while **4a**, **4c** and **4d** showed high activity and **4b**, **4e**, **4g-4i** were moderate activity. Compounds **4a**, **4c** and **4d** showed high activity against *B. cereus* bacteria. This value is higher than neomycin, which is used as a standard antibiotic. While compound **4f** showed no activity, the other compounds exhibited moderate antibacterial effects. Compounds **4a** and **4h** exhibited strong antibacterial activity against *B. subtilis*, whereas compounds **4e** and **4f** demonstrated weak activity and the remaining compounds exhibited moderate antibacterial effects.

While compounds **4a-c** and **4h** showed high levels of activity against *K. pneumoniae*, one of the agents responsible for lung infections, other compounds showed no effect. The new compounds showed the least effect against *P. aeruginosa*. Compounds **4a-c** showed high levels of activity against *E. coli* bacteria. In particular, the effect of compound **4a** was at the same level as that of ampicillin, which is used as a standard antibiotic. While compounds **4d**, **4e**, **4h** and **4i** showed moderate effects, compounds **4f** and **4g** showed no effect.

4 CONCLUSION AND SUGGESTIONS

Nine new heterocyclic Schiff bases containing 1,2,4-triazole rings were synthesized and characterized by ^1H and ^{13}C NMR spectroscopic methods. The metal chelating activities of the Schiff bases showed antioxidant activity close to α-tocopherol used as standard, however, they did not exhibit significant free radical scavenging or chelating activity. In addition, radical scavenging activities were not at the desired level in the study using BHT and BHA as standard antioxidants [43]. Compounds **4a-i** were more effective against Gram-positive bacteria than Gram-negative bacteria. Compound **4a** showed the highest activity against all bacteria, and these effects were similar to ampicillin, the most effective of the standard antibiotics. All compounds showed activity against *B. subtilis*, but two compounds (**4a and 4h**) were able to show activity against *P. aeruginosa*. Most of the compounds were close to the standard antibiotic neomycin and more effective than streptomycin. In recent years, the increase in antibiotic resistance has highlighted the need for the development of new antibiotics. The positive antibacterial results encourage the synthesis of more Schiff bases containing triazole.

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Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of any artificial intelligence (AI) tools. All content, including text, data analysis, and figures, was solely generated by the authors.

Contributions of the Authors

H.Y. designed the study and performed the characterization of compounds 4a-i. H.Y. and F.A. synthesized the compounds 4a-i. F.A. performed the antioxidant and antimicrobial studies and wrote the article.

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