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Research Article

ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF NOVEL S-ALKYLATED AND REDUCED SCHIFF BASE 1,2,4-TRIAZOLE DERIVATIVES

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Abstract: *In this study, four new 1,2,4-triazole derivatives (5–8) were designed, synthesized, and structurally characterized through FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic techniques. Antimicrobial properties were evaluated against standard Gram-positive (S. aureus ATCC 25923, S. pyogenes ATCC 19615) and Gram-negative bacteria (E. coli ATCC 25922, P. aeruginosa ATCC 27853), as well as the fungal strain C. albicans ATCC 10231, using the disk diffusion method. Antioxidant activities were assessed via ferric reducing antioxidant power (FRAP) and DPPH radical-scavenging assays. The results demonstrated that the synthesized compounds exhibited selective antimicrobial efficacy. Schiff base derivatives (5, 6) showed notable inhibitory activity against Gram-positive bacteria, particularly S. aureus and S. Pyogenes, while Gram-negative strains displayed intrinsic resistance. Furthermore, compounds 5 and 6 also presented moderate antifungal activity against C. albicans. Antioxidant analyses revealed that compound 6 possessed remarkable reducing capacity (14.166 mg TE/g) and radical-scavenging activity (IC_{50} = 0.227 mg/mL), outperforming the other derivatives. These findings suggest that structural modifications, particularly the introduction of heptylthio and Schiff base functionalities, play a decisive role in shaping biological activity. Among the tested molecules, compound 6 emerged as the most promising candidate, exhibiting a dual profile of antimicrobial and antioxidant activities. Collectively, this study highlights the potential of 1,2,4-triazole derivatives as versatile scaffolds for the development of new therapeutic agents aimed at addressing infectious diseases and oxidative stress-related pathologies.*

Keywords: *1,2,4-Triazole, Schiff base, Secondary amine, Antimicrobial activity, Antioxidant activity*

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1. Introduction

Antimicrobials are agents designed to eradicate or suppress the proliferation of microorganisms, encompassing bacteria, fungi, viruses, and parasites [1]. The efficacy of antimicrobial agents is increasingly undermined by the escalating global challenge of antimicrobial resistance (AMR). AMR arises when microorganisms acquire the ability to survive exposure to these drugs, thereby complicating treatment regimens and facilitating the transmission of resistant infections [2]. Epidemiological data reveal that a substantial proportion of clinically significant bacterial pathogens, including Escherichia coli and Staphylococcus aureus, have developed resistance mechanisms, resulting in therapeutic failures and heightened morbidity and mortality [3,4].

Also, antioxidants play a pivotal role in safeguarding biological systems against oxidative stress, a condition implicated in the pathogenesis of numerous diseases, including cancer and cardiovascular disorders [5-7]. Their primary mechanism involves neutralizing free radicals and reactive oxygen

species (ROS), thereby mitigating cellular damage and preserving physiological integrity [8,9]. Consequently, the identification and development of novel compounds capable of counteracting intracellular reactive species hold significant therapeutic potential [10,11].

The 1,2,4-triazole nucleus represents a privileged heterocyclic scaffold that has attracted substantial attention in medicinal chemistry and agricultural sciences due to its distinctive structural attributes and wide-ranging biological potential. Structurally, these compounds possess a five-membered triazole ring incorporating three nitrogen atoms and two carbon atoms, a configuration that imparts unique electronic properties and high chemical stability. The ambident nucleophilic character of 1,2,4-triazoles, together with their pronounced ability to participate in strong hydrogen-bonding interactions, enhances their versatility as molecular frameworks for the rational design of novel bioactive agents. Over the past decades, numerous studies have documented their significant pharmacological and agrochemical potential, with demonstrated efficacy against diverse biological targets, including bacterial and fungal pathogens as well as malignant cell lines. This broad spectrum of activity, coupled with their synthetic accessibility and structural modifiability, underscores their importance as a core motif in contemporary drug discovery and development strategies [12-14].

From a therapeutic perspective, 1,2,4-triazoles constitute versatile scaffolds for a broad range of pharmacologically significant compounds, including those exhibiting anti-tubercular, antimicrobial, and anticancer activities. Their inherent structural adaptability permits extensive chemical modifications, enabling fine-tuning of biological properties to selectively target specific disease pathways. Continuous advancements in this area underscore the pivotal role of 1,2,4-triazoles not only as promising drug candidates but also as integral components in the design and development of novel therapeutic agents. This is particularly crucial in addressing the escalating challenges faced by pharmaceutical and agricultural sectors [15,16].

Owing to these distinctive properties, the 1,2,4-triazole moiety constitutes a fundamental structural element in numerous clinically employed pharmaceuticals. These include antifungal agents such as posaconazole, itraconazole, fluconazole, ravuconazole, and voriconazole; hypnotic, anxiolytic, and anticonvulsant drugs including estazolam and alprazolam; skeletal muscle relaxants and anxiolytics like etizolam; antiplatelet agents exemplified by trapidil; antimigraine compounds such as rizatriptan; anticancer therapeutics including anastrozole, letrozole, tucatinib, and talazoparib; as well as antidepressants (trazodone), anticonvulsants (loreclezole), antivirals (ribavirin), and agents targeting multiple myeloma (selinexor KPT-330) [17-19] (Figure 1).

Moreover, Heterocyclic compounds incorporating oxygen, nitrogen, and sulfur atoms have found extensive applications across medical, pharmaceutical, material science, industrial, and agricultural domains [20-23]. Moreover, derivatives of 1,2,4-triazoles functionalized with mercapto (–SH) and thione (C=S) moieties have been extensively reported to exhibit a broad spectrum of biological activities. These include antifungal, antibacterial, antitubercular, and antiviral effects, as well as anti-inflammatory, anticonvulsant, antioxidant, anticancer, anti-tyrosinase, anti-urease, leishmanicidal, and trypanocidal properties [8, 24-27].

Since alkyl substituents in N-alkyl–derived heterocyclic compounds are known to enhance biological activities [27-30]. In this study, pentyl and heptyl groups were attached to the 3-position of the 1,2,4-triazole-3-thione ring. Also, secondary amines derived from Schiff bases have demonstrated notable biological activities. In particular, secondary amine derivatives of diverse Schiff base ligands have been investigated for their cytotoxic potential against cancer cell lines, indicating their promising application in medicinal chemistry [31]. Also, S-alkylation distinctly increases lipophilicity and promotes better membrane permeation due to the high polarizability of sulfur. Both empirical studies and theoretical models substantiate the pivotal role of such modifications in enhancing the permeability characteristics of diverse molecular entities [32,33]. For this purpose, alkylated 1,2,4-triazoles were

reduced to enhance their biological activity. Considering these factors, the synthesized compounds represent significant pharmacophores for antioxidant and antimicrobial activities.

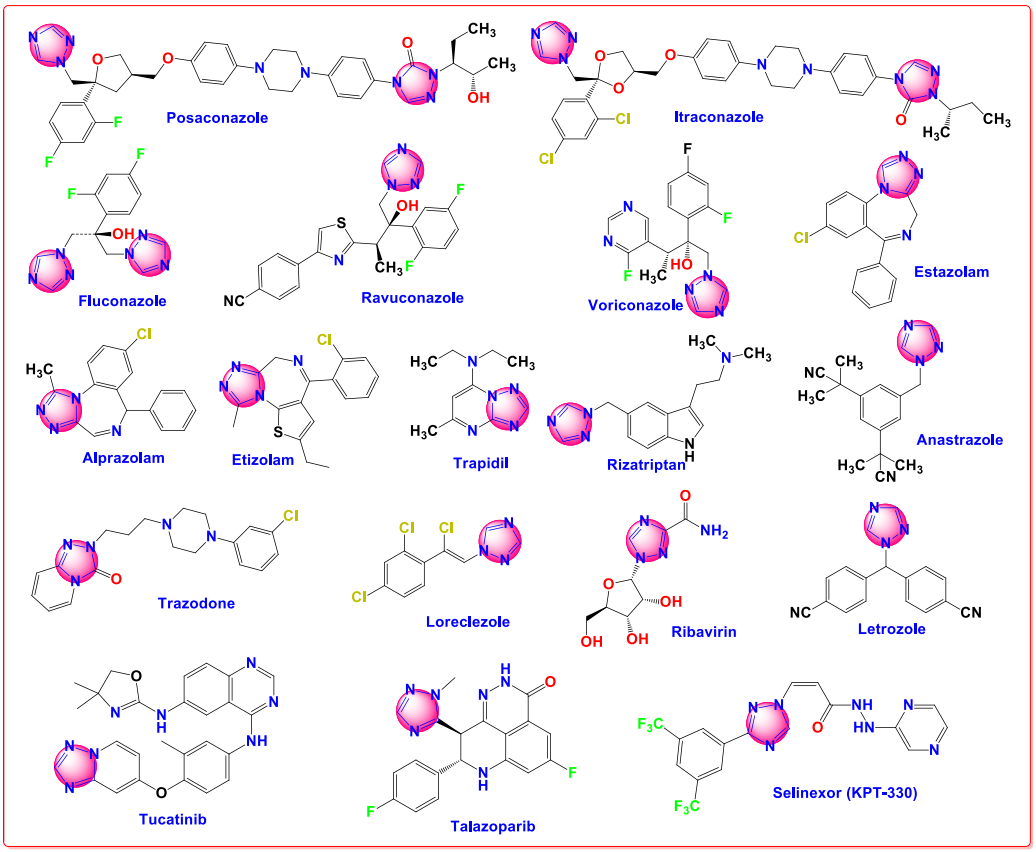


Figure 1. 1,2,4-Triazole-based drugs available for clinical use.

Given that S-alkylation increases lipophilicity and may facilitate membrane permeation while leveraging the high polarizability of sulfur, we selected pentyl- and heptyl-thio substituents to probe chain-length effects on antimicrobial and antioxidant outcomes. Owing to their anticipated biological potential, four novel 1,2,4-triazole derivatives were synthesized, and their biological activities, including antioxidant and antimicrobial properties, were subsequently evaluated.

2. Materials and Methods

The starting materials, reagents, and solvents were procured from Sigma-Aldrich, Merck, or Alfa Aesar. Chemicals of ≥98% purity were used as received without additional purification. Reaction progress was assessed by thin-layer chromatography (TLC) on silica gel HSGF254 plates (thickness 0.20 ± 0.03 mm). A 1:4 ethyl acetate/n-hexane mixture served as the eluent, and spots were visualized under UV light.

A Shimadzu IR Prestige-21 spectrometer was used to record the infrared spectrum (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). Chemical shifts (δ) are given in ppm and referenced to residual solvent resonances; for DMSO-d_6, δH ≈ 2.50 ppm and δC ≈ 40 ppm. Coupling constants (J) are reported in hertz (Hz). The spectrums are given as supplementary information (SI).

2.1. General Method for the Synthesis of S-alkylation (5,6)

A solution of compound **4** (10 mmol) in ethanol was treated with KOH and heated under reflux for 2 h, then allowed to cool to ambient temperature. The corresponding alkyl halide was introduced, and reaction progress was followed by TLC. After completion, the mixture was cooled and the solvent removed in vacuo. The residue was taken up in CH_2Cl_2, transferred to a separatory funnel, and washed with water (30 mL), then extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography furnished compounds **5** and **6**, which were stored in a desiccator until use.

2.1.1 4-benzylideneamino-3-methyl-5-(pentylthio)-4H-1,2,4-triazole (5)

Liquid substance (86.48% yield); Rf: 0.35 (hexane:ethylacetate 4:1); IR (FTIR-ATR, υ_{max} in cm^{-1}): 3061 (Ar. CH); 2954, 2927, 2856 (Aliph. CH); 1608 (C=N) (SI-1); ^{1}H-NMR (DMSO-d_6, δ ppm): 0.80 (t, 3H, J=6.00 Hz), 1.21-1.32 (m, 4H), 1.57-1.64 (m, 2H), 2.42 (s, 3H), 3.08 (t, 2H, J=6.00 Hz), Ar-H: [7.54-7.65 (m, 3H), 7.92 (d, 2H, J=8.00 Hz)], 8.83 (s, 1H, N=CH) (SI-2); ^{13}C-NMR (DMSO-d_6, δ ppm): 11.42 (CH_2-CH_3), 14.23 (CH_3), 22.03 (CH_2), 29.07 (CH_2), 30.49 (CH_2), 32.81 (S-CH_2), Ar-C: [129.29 (2CH),129.64 (2CH), 132.31, 133.42 (CH)], 146.74 (triazole C-5), 149.45 (triazole C-3), 165.69 (N=CH) (SI-3).

2.1.2 4-benzylideneamino-3-(heptylthio)-5-methyl-4H-1,2,4-triazole (6)

Liquid substance (92.56% yield); Rf: 0.40 (hexane:ethylacetate 3:1); IR (FTIR-ATR, υ_{max} in cm^{-1}): 3061 (Ar. CH); 2953, 2924, 2852 (Aliph. CH); 1608 (C=N) (SI-4); ^{1}H-NMR (DMSO-d_6, δ ppm): 0.80 (t, 3H, J=6.00 Hz), 1.18-1.32 (m, 8H), 1.55-1.62 (m, 2H), 2.42 (s, 3H), 3.07 (t, 2H, J=8.00 Hz), Ar-H: [7.53-7.64 (m, 3H), 7.91 (d, 2H, J=4.00 Hz)], 8.83 (s, 1 H, N=CH) (SI-5); ^{13}C-NMR (DMSO-d_6, δ ppm): 11.41 (CH_2-CH_3), 14.35 (triazole-CH_3), 22.44 (CH_2), 28.26 (CH_2), 28.56 (CH_2), 29.38 (CH_2), 31.55 (CH_2), 32.91 (S-CH_2), Ar-C: [129.29 (2CH), 129.62 (2CH), 132.29, 133.42 (CH)], 146.74 (triazole C-5), 149.46 (triazole C-3), 165.72 (N=CH) (SI-6).

2.2. General Method for the Synthesis of Secondary Amines (7,8)

Schiff base derivatives **5** and **6** (10 mmol) were dissolved in methanol (50 mL), after which $NaBH_4$ (20 mmol) was added portionwise under constant stirring. The suspension was stirred for 3 h, and reaction completion was verified by TLC. The solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2, transferred to a separatory funnel, washed with water (30 mL), and then extracted with CH_2Cl_2 (3 × 50 mL). The combined organic fractions were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified by column chromatography to furnish the corresponding secondary amines (compounds **7** and **8**), which were stored in a desiccator until use [34].

2.2.1 4-benzylamino-3-methyl-5-(pentylthio)-4H-1,2,4-triazole (7)

Liquid substance (81.80% yield); Rf: 0.55 (hexane:ethylacetate 2:1); IR (FTIR-ATR, υ_{max} in cm^{-1}): 3199 (NH), 3028 (Ar. CH); 2954, 2927, 2856 (Aliph. CH) (SI-7); ^{1}H-NMR (DMSO-d_6, δ ppm): 0.85 (t, 3H, J=8.00 Hz), 1.21-1.37 (m, 4H), 1.60-1.67 (m, 2H), 2.09 (s, 3H), 3.07 (t, 2H, J=6.00 Hz), 4.05 (d, 2H, J=4.00 Hz, NH=CH_2), Ar-H: [6.86 (t, 1H, J=4.00 Hz), 7.24-7.33 (m, 4H)] (SI-8); ^{13}C-NMR (DMSO-d_6, δ ppm): 10.22 (CH_2-CH_3), 14.29 (CH_3), 22.11 (CH_2), 29.22 (CH_2), 30.66 (CH_2), 31.73 (S-CH_2), 54.81 (NH-CH_2), Ar-C: [128.19 (CH), 128.75 (2CH), 129.76 (2CH), 136.89], 150.68 (triazole C-5), 152.82 (triazole C-3) (SI-9).

2.2.2 4-benzylamino-3-(heptylthio)-5-methyl-4H-1,2,4-triazole (8)

Liquid substance (79.21% yield); Rf: 0.6 (hexane:ethylacetate 2:1); IR (FTIR-ATR, υ_{max} in cm^{-1}): 3197 (NH), 3028 (Ar. CH); 2953, 2924, 2854 (Aliph. CH) (SI-10); ^{1}H-NMR (DMSO-d_6, δ ppm): 0.84 (t, 3H, J=6.00 Hz), 1.23-1.36 (m, 8H), 1.59-1.67 (m, 2H), 2.08 (s, 3H), 3.07 (t, 2H, J=8.00 Hz), 4.05 (d, 2H, J=8.00 Hz, NH=CH_2), Ar-H: [6.85 (t, 1H, J=6.00 Hz), 7.24-7.32 (m, 4H)] (SI-11); ^{13}C-NMR (DMSO-d_6, δ ppm): 10.22 (CH_2-CH_3), 14.37 (CH_3), 22.47 (CH_2), 28.43 (CH_2), 28.64 (CH_2), 29.53 (CH_2), 31.61 (CH_2), 31.78 (S-CH_2), 54.81 (NH-CH_2), Ar-C: [128.19 (CH), 128.74 (2CH), 129.75 (2CH), 136.90], 150.65 (triazole C-5), 152.81 (triazole C-3) (SI-12).

2.3. Antimicrobial Activities

2.3.1 Determination of Antimicrobial Activities

The antimicrobial activities of the synthesized compounds were determined using the disk diffusion method, with necessary adaptations, according to Balouiri et al. [35]. In this study, *Staphylococcus pyogenes* ATCC 25923 and *Staphylococcus aureus* ATCC 25923 were selected to represent Gram-positive bacteria, while *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were chosen to represent Gram-negative bacteria. Additionally, *Candida albicans* ATCC 18804 was used as a representative of yeast and molds.

After activation, the passaged test organisms were incubated overnight to prepare fresh cultures. The pathogens with freshly prepared cultures were then diluted in sterile distilled water to a turbidity of 0.5 McFarland standard (1.5×10^8 CFU/mL) using a McFarland densitometer. In the next step, each microorganism was inoculated onto Mueller-Hinton Agar (MHA) plates using the spread plate technique with a sterile swab. Sterile blank disks with a diameter of 6 mm were placed on the plates inoculated with the test microorganisms. Onto these sterile disks, 15 µL of the samples prepared at concentrations of 24 and 12 mg/mL were pipetted. The plates were kept at 4 °C for 2 hours to allow diffusion of the samples into the agar. Commercially prepared imipenem disks at a concentration of 10 µg/disk were used as positive control. Dimethyl sulfoxide (DMSO), the solvent used for the samples, served as the negative control. Each sample was pipetted onto sterile blank disks in a volume of 15 µL. For incubation, bacterial plates were incubated at 37 °C for 24 hours, and yeast plates were incubated at 28 °C for 48 hours. At the end of the incubation period, the diameters of the inhibition zones formed around the disks were measured with a ruler.

2.3.2 Determination of Minimum Inhibitory Concentration (MIC) Values

The MIC values of the compounds were determined using the broth dilution method with necessary modifications according to CLSI [36]. Sterile, lidded 96-well microplates were used for the assay. Mueller-Hinton Broth (MHB) was chosen as the culture medium. During the experiment, 100 µL of double-strength broth was added to the first wells of the microplates, while 100 µL of standard-strength broth was added to the remaining wells. In the next step, the initial concentration of the samples (20 mg/mL) was prepared using DMSO. From these solutions, 100 µL was pipetted into the first wells of the microplates. Ten different concentrations of each sample and the selected antibiotic were obtained through a two-fold serial dilution. Suspensions of the selected pathogens (10 µL, adjusted to 0.5 McFarland turbidity) were then added to the wells. Following inoculation, the plates were incubated at 37 °C for 24 hours for bacteria, and at 28 °C for 48 hours for yeast. After incubation, the MIC values were determined as the lowest concentrations of the samples and Imipenem that inhibited visible growth of the tested pathogenic microorganisms.

2.4. Antioxidant Activities

2.4.1 Determination of Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing power of the synthesized compounds was expressed as mg Trolox equivalent per gram of compound. A modified version of the method developed by Oyaizu was used. In this method, 40 µL of the compound was mixed with 100 µL of 0.2 M phosphate buffer (pH 6.6) and 100 µL of potassium ferricyanide, and the mixture was incubated at 50 °C in the dark for 20 minutes. After incubation, the samples were cooled under water. Then, 100 µL of 10% trichloroacetic acid (TCA) was added to the mixture, which was subsequently centrifuged at 3000×g for 10 minutes. From the upper phase of the centrifuged samples, 100 µL was transferred into wells of a 96-well microplate. To each well, 100 µL of distilled water and 20 µL of ferric (III) chloride were added. The final mixture was incubated at room temperature in the dark for 5 minutes. Following incubation, absorbance was measured at 700 nm using a microplate reader. All analyses were performed in triplicate [37].

2.4.2 DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the synthesized compounds was determined using a modified method developed by Yu et al. [38]. To 125 µL of the compound, 125 µL of a 0.1 mM DPPH radical solution was added, and the mixture was incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance values were measured at 517 nm using a microplate reader. The results were expressed as IC_{50} values. A calibration curve was constructed based on the absorbance values of the samples at 5 different concentrations, and the IC_{50} value was defined as the concentration corresponding to 50% inhibition [6]. The percentage inhibition value was calculated using the formula in Equation 1.

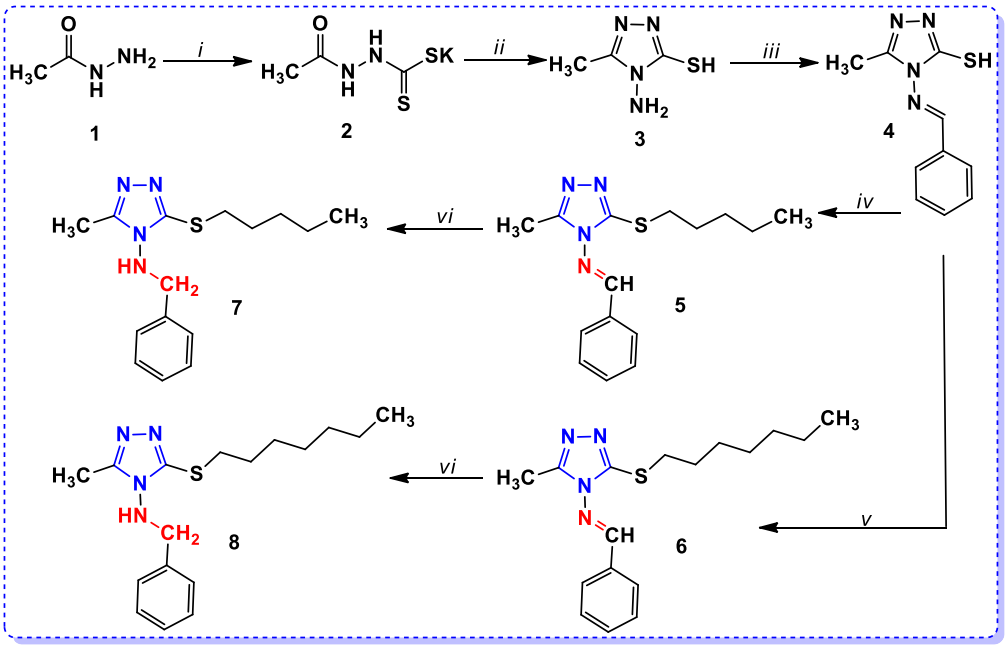
$$\% \text{ Inhibition} = ((A_{control} - A_{sample}) / A_{control}) \times 100 \quad \text{(Equation 1.)}$$

3. Results and Discussion

3.1. Chemistry

In the current study, design, synthesis, antimicrobial and antioxidant activity of 1,2,4-triazole-5-thione molecules (**5-8**) were revealed. A series of novel 1,2,4-triazole-5-thione derivatives (**5-8**) were synthesized in five steps, as shown in *Scheme 1*. The structures of the synthesized triazole- compounds were characterized using Fourier transform infrared-attenuated total reflectance (FTIR-ATR), ^{1}H nuclear magnetic resonance (NMR), and ^{13}C NMR techniques.

In the FTIR spectra, the disappearance of the SH stretching vibration at nearly 2700 cm^{-1} in compound **4**, accompanied by the presence of aliphatic C–H stretching vibrations around 2900 cm^{-1}, confirms the successful progression of the reaction and the formation of the target products (**5**,**6**). The reduction of the Schiff bases to yield secondary amines (compounds **7** and **8**) was confirmed by FTIR spectroscopy. The disappearance of the characteristic imine (C=N) stretching band at approximately 1600 cm^{-1} in compounds **5** and **6**, together with the appearance of N–H stretching vibrations around 3200 cm^{-1}, provides clear evidence for the successful formation of the corresponding secondary amines.



Scheme 1. Synthesis of the target compounds **5-8**. Reagents and conditions: (*i*) CS_2, KOH, absolute C_2H_5OH; (*ii*) $NH_2NH_2.H_2O$, water, reflux; (*iii*) 3-4 drop CH_3COOH, benzaldehyde, absolute C_2H_5OH, reflux; (*iv*) KOH, absolute C_2H_5OH, $C_5H_{11}Br$, reflux; (*v*) KOH, absolute C_2H_5OH, $C_7H_{15}I$, reflux; (*vi*) $NaBH_4$, absolute CH_3OH.

The ^{1}H-NMR spectra of compounds **5** and **6** exhibited signals corresponding to the N=CH protons at 8.83 ppm, confirming the presence of the imine functionality in the Schiff base derivatives. The S-alkylation derivatives (**5**, **6**) showed the disappearance of the –SH proton signals around 14 ppm, while the protons of the pentyl and heptyl substituents resonated between 0.80 and 3.08 ppm, consistent with the successful S-alkylation of the triazole ring. The secondary amine derivatives (**7** and **8**), the signals corresponding to the N=CH protons at 8.83 ppm disappeared, while the protons of the –NH–CH_2 moiety resonated at approximately 4 ppm. However, the NH proton signals were not observed, consistent with the formation of the secondary amine functionality. The appearance of signals corresponding to the –CH_2– group further confirms the successful completion of the reduction reaction and the formation of the secondary amine derivatives.

In the ^{13}C-NMR spectra, the aromatic carbons resonated between 128.19 and 136.90 ppm, whereas the aliphatic carbons appeared in the range of 10.22–32.91 ppm. The C-3 carbon of the triazole ring appeared at approximately 153 ppm in the alkylated Schiff bases and at around 150 ppm in the secondary amines, while the C-5 carbon resonated at about 151 ppm in the alkylated Schiff bases and shifted to approximately 147 ppm in the secondary amines. The N=CH carbons were observed at 165.69 and 165.72 ppm; however, after the reduction of the Schiff bases, the newly formed NH–CH_2 carbons of the secondary amines resonated at 54.81 ppm. The newly synthesized compounds have existed as liquids. These compounds are insoluble in water but soluble in common organic solvents such as ethanol, methanol, dimethyl sulfoxide, acetone, dimethylformamide, dichloromethane, and chloroform. The FT-IR, ^{1}H-NMR, and ^{13}C-NMR results confirm the structures of the synthesized compounds.

3.2. Antimicrobial Activities

In this study, the antimicrobial effects of the synthesized compounds were evaluated using the disk diffusion method with standard microbial strains. The compounds were tested against Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), Gram-

positive bacteria (*Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615), and yeast/mold species (*Candida albicans* ATCC 10231). Dimethyl sulfoxide (DMSO) was used as the solvent control, imipenem (10 µg), and tetracycline (10 µg) served as positive controls (Table 1, Figure 2, SI-13.-SI-16.).

Table 1. Disk diffusion diameters of the synthesized compounds (mm).

		Gram (-)		Gram (+)		Yeast
Samples		***E. coli* ATCC 25922**	***P. aeruginosa* ATCC 27853**	***S. aureus ATCC* 25923**	***S. pyogenes* ATCC 19615**	***C. albicans* ATCC 10231**
5	1 mg/disc	11±0	9.33±0,47	19±0,81	22.33±0,94	17.33±0,47
	0.5 mg/disc	10±0	8.33±0,47	17±0	19.33±0,47	14.33±0,47
6	1 mg/disc	9.33±0,47	--	16±0	22±0	15.33±0,47
	0.5 mg/disc	9±0	--	15±0,81	20.33±0,47	14.66±0,94
7	1 mg/disc	8.66±0,47	--	11±0	15.33±0,47	11±0
	0.5 mg/disc	8.66±0,47	--	10.33±0,47	14±1,41	11.66±0,47
8	1 mg/disc	9±0	--	11±0	14.66±0,47	10.66±1,64
	0.5 mg/disc	9±0	--	10.66±0,47	15±0	9±0
DMSO	--	--	--	--	--	--
Imipenem	10 µg/disc	37.33±0,94	27±0,81	49.33±0,94	39.66±0,47	--
Tetracycline	10 µg/disc	26.66±0,94	10.33±0,47	30.33±0,47	49.33±0,94	--

All analyses were performed in triplicate. Inhibition zones measured after 24 h at 37 °C.

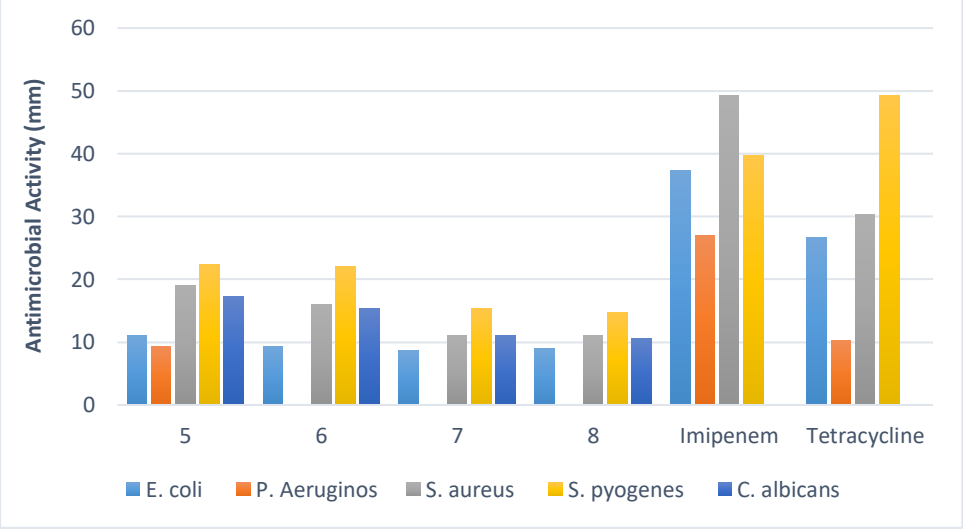


Figure 2. Antimicrobial activity by disk diffusion for compounds **5–8** (1 mg/disc) versus Imipenem and Tetracycline (10 mg/disc).

The antimicrobial activities of the synthesized 1,2,4-triazole derivatives were evaluated using the disk diffusion method, and the results revealed that the tested compounds exhibited distinct activity profiles depending on the microbial group. Overall, the compounds showed limited activity against Gram-negative bacteria, while demonstrating moderate to high antimicrobial effects against Gram-positive bacteria and yeast strains. These findings suggest that structural features-such as the Schiff base

or secondary amine structure and the length of the lipophilic side chain-are critical factors directly influencing biological activity.

The compounds tested against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 exhibited only low levels of inhibition, with inhibition zone diameters ranging from 8.66 to 11 mm. Notably, against the *P. aeruginosa* strain, only 4-benzylideneamino-3-methyl-5-(pentylthio)-4H-1,2,4-triazole (**5**) demonstrated weak activity (9.33 mm), while the other compounds showed no inhibition. This finding is consistent with the structural feature of Gram-negative bacteria, whose outer membrane, surrounded by a lipopolysaccharide layer, acts as a natural barrier that restricts the entry of antibacterial agents into the cell. Such a structural feature represents one of the well-known intrinsic resistance mechanisms of Gram-negative bacteria [39].

The compounds tested against the Gram-positive bacteria *S. aureus* ATCC 25923 and *S. pyogenes* ATCC 19615 demonstrated higher activity. In particular, the Schiff base derivatives 4-benzylideneamino-3-methyl-5-(pentylthio)-4H-1,2,4-triazole (**5**) and 4-benzylideneamino-3-(heptylthio)-5-methyl-4H-1,2,4-triazole (**6**) stood out. Compound **5** exhibited the strongest activity, producing inhibition zones of 19 mm against *S. aureus* and 22.33 mm against *S. pyogenes* at a concentration of 1 mg/disc. Similarly, compound **6** showed a pronounced antibacterial effect against *S. pyogenes*, with an inhibition zone of 22 mm. These findings suggest that the Schiff base framework may confer more selective and potent activity against Gram-positive bacteria. The cell wall of Gram-positive bacteria, consisting of a thick peptidoglycan layer, provides a more permeable structure compared to Gram-negative bacteria, which are characterized by a lipopolysaccharide outer membrane. Consequently, structures such as Schiff bases and triazole derivatives tend to be more effective against Gram-positive strains. Consistent with this observation, previous studies have reported that Schiff base derivatives exhibit significant inhibitory activity, particularly against Gram-positive pathogens such as *S. aureus* and *S. pyogenes* [40].

The compounds tested against *C. albicans* ATCC 10231 exhibited moderate antifungal activity. In particular, compound **5** showed the highest antifungal effect, with an inhibition zone of 17.33 mm, while compound **6** also demonstrated significant activity with a zone diameter of 15.33 mm. These findings are consistent with clinical evidence indicating that triazole derivatives are effective against fungal pathogens. Indeed, commonly used antifungal drugs such as fluconazole, itraconazole, and voriconazole also contain a triazole ring and act by inhibiting ergosterol biosynthesis, thereby disrupting fungal cell membranes [41-43]. From this perspective, the activity displayed by the synthesized compounds against *C. albicans* is biologically meaningful and suggests potential for further pharmacological evaluation.

Compared with literature, Schiff base-triazole chemotypes, e.g., 6-fluoroquinazolinyl-substituted triazole thioethers (Ding et al.), amide-linked triazole thioethers (Shao et al.), and thiazolyl–triazole Schiff bases (Mohamed et al.), our hybrids distinctively integrate a reduced Schiff base with systematic S-alkyl chain-length variation to disentangle lipophilicity-driven contributions to antimicrobial and antioxidant activity. Unlike architectures that rely on extended π-chromophores for free-radical scavenging (e.g., coumarin-decorated triazole Schiff bases), our antioxidant effect emerges without such aromatic amplifiers [44-46].

3.3. Antioxidant Activities

The antioxidant activities of the synthesized 1,2,4-triazole derivatives were evaluated using the FRAP and DPPH methods, and the results revealed that structural features play a decisive role in their biological efficacy. In particular, when comparing the Schiff base derivatives (**5** and **6**) with the secondary amine derivatives (**7** and **8**), it became evident that the secondary amine nature and the characteristics of the side chains are the key factors shaping the antioxidant capacity (Table 2).

Table 2. Antioxidant results of the synthesized compounds.

Samples	FRAP (mg TE/g örnek)	DPPH (IC_{50} mg/mL)
5	0.505±0,016	>12
6	14.166±0,160	0.227±0,028
7	0.117±0,008	>12
8	0.187±0,016	>12
Trolox	--	0.0069±0,0001

All analyses were performed in triplicate.

The FRAP results demonstrated that, among the compounds, 4-benzylideneamino-3-(heptylthio)-5-methyl-4H-1,2,4-triazole (**6**) exhibited the highest reducing capacity, with a value of 14.166 mg TE/g. In contrast, the FRAP values of the other compounds were found to be considerably lower, ranging between 0.117 and 0.505 mg TE/g. These findings highlight the critical role of structural factors in the antioxidant effects of the compounds through electron transfer mechanisms. Consistently, previous studies have reported that the presence of electron-rich functional groups in Schiff base and amine derivatives can enhance redox capacity [47,48].

The DPPH radical-scavenging analyses showed a trend parallel to the FRAP results. 4-benzylideneamino-3-(heptylthio)-5-methyl-4H-1,2,4-triazole (**6**) demonstrated strong radical-scavenging activity with an IC_{50} value of 0.2737 mg/mL. In contrast, compounds **5**, **7**, and **8** exhibited IC_{50} values >12 mg/mL, indicating no significant radical-scavenging capacity. The weak activity of compounds **7** and **8**, despite their secondary amine structures, suggests that the pentyl/heptyl-thio side chain alone is insufficient, and that the presence of the N–H group, along with electron density and conjugation, is also crucial. Conversely, the high activity of compound **6** may be attributed to the heptylthio chain, which could enhance lipophilicity and facilitate interactions within the radical environment. Indeed, previous studies have also reported that long-chain lipophilic substituents can enhance antioxidant capacity [48].

The Schiff base derivatives **5** and **6**, in theory, could provide an advantage in electron transfer due to their conjugated systems. However, the results demonstrated that conjugation alone is not sufficient for strong antioxidant activity. The absence of the N–H group limited the hydrogen-donating capacity of these compounds, leading to weak effects on the DPPH radical. Consistent with this, previous studies have reported that Schiff bases exhibit low antioxidant activity when not supported by additional donor functional groups such as phenolic or hydroxyl moieties [49,50]. The consistent and significant activity of 4-benzylideneamino-3-(heptylthio)-5-methyl-4H-1,2,4-triazole (**6**) across both assays highlights its potential as a promising candidate with notable antioxidant properties.

Recent studies show that the triazolic scaffold and sulfur-containing functionalities can meaningfully augment antioxidant responses. The 2024 review by Rana et al. summarizes HAT/SET pathways and structural determinants of DPPH quenching in Schiff bases, while Temüz et al. (2024) report strong free-radical scavenging of triazole-3-thiol derivatives validated by DPPH/ABTS, and Quy Huong et al. (2024) document experimentally/computationally supported antioxidant capacities across triazole series. Consistent with these trends, our findings indicate that the reduced Schiff bond plus S-alkyl motif enhances FRAP/DPPH performance [48,51,52].

Also, the SAR results demonstrated that S-alkylated Schiff base derivatives, particularly **5** and **6**, displayed more pronounced activity against Gram-positive strains. Compound **5** showed the strongest profile with zone diameters of 19 mm against *S. aureus* and 22.33 mm against *S. pyogenes*, while **6** was likewise active against *S. pyogenes* (22 mm) at 1 mg/disc. In contrast, all compounds were weak against Gram-negative strains; only **5** exhibited limited activity toward *P. aeruginosa* (9.33 mm). This pattern

suggests that (i) the Schiff-base functionality in **5** and **6** aligns with greater Gram-positive permeability, (ii) extending the S-alkyl chain (pentyl → heptyl) maintains activity particularly in *S. pyogenes*, and (iii) the outer-membrane barrier of Gram-negative bacteria restricts uptake and limits activity. The FRAP and DPPH results further clarify chain-length and functional-group effects. The heptylthio Schiff base **6** outperformed the series (FRAP = 14.166 mg TE/g; DPPH IC_{50} = 0.2737 mg/mL), whereas **5**, **7**, and **8** afforded FRAP values of 0.117–0.505 mg TE/g and DPPH IC_{50} > 12 mg/mL. These findings indicate that (i) a longer S-alkyl chain (heptyl) enhances lipophilicity and interactions within radical media, thereby strengthening electron-transfer–based reducing capacity, and (ii) although reduction to secondary amines introduces N–H (**7**, **8**), the pentyl/heptylthio chain alone is insufficient; an effective combination of conjugation and appropriate electron density is required for strong antioxidant response.

4. Conclusion

In this study, four novel 1,2,4-triazole derivatives (**5**–**8**) were successfully synthesized and comprehensively characterized using FT-IR, ^{1}H-NMR, and ^{13}C-NMR analyses. The structural confirmations demonstrated the successful progression of both S-alkylation and Schiff base reduction reactions, yielding compounds with distinct chemical properties. The biological evaluations revealed that these structural variations directly influenced the antimicrobial and antioxidant activities of the synthesized molecules.

The antimicrobial assays indicated that the synthesized triazole derivatives were more effective against Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and the fungal strain *Candida albicans* compared to Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). This selectivity is consistent with the intrinsic permeability barriers of Gram-negative pathogens and highlights the potential of Schiff base derivatives, particularly compounds **5** and **6**, as promising scaffolds for targeting Gram-positive microorganisms. The observed antifungal activities further reinforce the relevance of the triazole moiety, given its established role in clinically used antifungal agents.

The antioxidant assays demonstrated that compound **6** (4-benzylideneamino-3-(heptylthio)-5-methyl-4H-1,2,4-triazole) possessed superior reducing power and radical-scavenging capacity compared to the other synthesized molecules. The strong performance of this compound suggests that the presence of a heptylthio chain significantly enhances lipophilicity and facilitates interactions with radical species, thereby contributing to improved antioxidant efficacy. Conversely, the limited activities of compounds **5**, **7**, and **8** indicate that conjugation alone is insufficient to confer strong antioxidant effects in the absence of additional donor functionalities.

Overall, these findings highlight the importance of structural modifications in shaping the bioactivity of 1,2,4-triazole derivatives. The Schiff base derivative **6**, in particular, emerged as a promising candidate with notable antioxidant and antimicrobial properties, underscoring its potential as a lead compound for further pharmacological optimization.

Ethical Statement

The ethical committee or legal/special permission is not required for this article.

Conflict of Interest

The authors declare no conflict of interest.

Supporting information summary

The FT-IR, ^{1}H-NMR, ^{13}C-NMR, and antimicrobial analysis photos of the synthesized compounds are included as support.

Authors' Contributions

E. G: Conceptualization, Methodology, Investigation, Material, Data Curation, Original Draft Writing, Review and Editing, Visualization, Observation.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

References

[1] Leal, JR., Conly, J., Henderson, EA., & Manns, BJ., "How externalities impact an evaluation of strategies to prevent antimicrobial resistance in health care organizations", *Antimicrobial Resistance & Infection Control*, 6(1), 53, 2017. https://doi.org/10.1186/s13756-017-0211-2

[2] Economou, V., Gousia, P., "Agriculture and food animals as a source of antimicrobial-resistant bacteria", *Infection and drug resistance*, 8, 49-61, 2015. https://doi.org/10.2147/idr.s55778

[3] Zhang, N., Liu, E., Tang, A., Ye, MC., Wang, K., Jia, Q., Huang, Z. "Data-driven analysis of antimicrobial resistance in foodborne pathogens from six states within the US", *International Journal of Environmental Research and Public Health*, 16(10), 1811, 2019. https://doi.org/10.3390/ijerph16101811

[4] Nyenye, L., Nwako, AB., "Antimicrobial Resistance: Unexplained Failure to Antibiotic Therapy in a Case at Maluti Adventist Hospital Berea Lesotho", *Journal of Advances in Medical and Pharmaceutical Sciences*, 24(6), 1-4, 2022. https://doi.org/10.9734/jamps/2022/v24i630305

[5] Liu, T., Xiao, B., Xiang, F., Tan, J., Chen, Z., Zhang, X., ... & Deng, J., "Ultrasmall copper-based nanoparticles for reactive oxygen species scavenging and alleviation of inflammation related diseases", *Nature communications*, 11(1), 2788, 2020. https://doi.org/10.1038/s41467-020-16544-7

[6] Canbolat, D., Turan, İ., Küpeli, YE., Kılınç, S., Piliç, S., "Artvin Şavşat Yöresi Propolisinin Farklı Sıcaklıklarda Hazırlanan PBS'li Ekstraktlarının Antioksidan Özelliklerinin ve Eritrosit Hemoliz İnhibisyonu Üzerine Etkisinin Araştırılması", *KSÜ Tarım ve Doğa Dergisi*, 24, 464-472, 2021. https://doi.org/10.18016/ksutarimdoga.vi.765838

[7] Turan, I., Canbolat, D., Demir, S., Kerimoğlu, G., Colak, F., Alemdar, N., Aliyazıcıoğlu, Y., "An investigation of the protective effect of Rhododendron luteum extract on cisplatin-induced DNA damage and nephrotoxicity and biochemical parameters in rats", *Pakistan Veterinary Journal*, 43(3), 2023. https://doi.org/10.29261/pakvetj/2023.047

[8] Küçükgüzel, Ş. G., Çıkla-Süzgün, P., "Recent advances bioactive 1, 2, 4-triazole-3-thiones", *European journal of medicinal chemistry*, 97, 830-870, 2015. https://doi.org/10.1016/j.ejmech.2014.11.033

[9] Turan, I., Canbolat, D., Demir, S., Kerimoglu, G., Colak, F., Alemdar, N. T., Aliyazicioglu, Y., "The ameliorative effect of Primula vulgaris on cisplatin-induced nephrotoxicity in rats and quantification of its phenolic components using LC-ESI-MS/MS", Saudi Pharmaceutical Journal, 31(9), 101730, 2023. https://doi.org/10.1016/j.jsps.2023.101730

[10] Pham-Huy, L. A., He, H., Pham-Huy, C., "Free radicals, antioxidants in disease and health", *International journal of biomedical science: IJBS*, 4(2), 89, 2008. https://doi.org/10.59566/ijbs.2008.4089

[11] Lü, J. M., Lin, P. H., Yao, Q., Chen, C., "Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems", *Journal of cellular and molecular medicine*, 14(4), 840-860, 2010. https://doi.org/10.1111/j.1582-4934.2009.00897.x

[12] Kumar, P., Kumar, A., Makrandi, JK., "Synthesis and Evaluation of Bioactivity of Thiazolo [3, 2-b]-[1, 2, 4]-triazoles and Isomeric Thiazolo [2, 3-c]-[1, 2, 4]-triazoles", *Journal of Heterocyclic Chemistry*, 50(5), 1223-1229, 2013. https://doi.org/10.1002/jhet.1600

[13] Popiołek, Ł., Rzymowska, J., Kosikowska, U., Hordyjewska, A., Wujec, M., & Malm, A., "Synthesis, antiproliferative and antimicrobial activity of new Mannich bases bearing 1, 2, 4-triazole moiety", *Journal of Enzyme Inhibition and Medicinal Chemistry*, 29(6), 786-795, 2014. https://doi.org/10.3109/14756366.2013.855926

[14] Sonawane, AD., Rode, ND., Nawale, L., Joshi, RR., Joshi, RA., Likhite, AP., Sarkar, D., "Synthesis and biological evaluation of 1, 2, 4-triazole-3-thione and 1, 3, 4-oxadiazole-2-thione as antimycobacterial agents", *Chemical Biology & Drug Design*, 90(2), 200-209, 2017. https://doi.org/10.1111/cbdd.12939

[15] Gonnet, L., Baron, M., Baltas, M., "Synthesis of biologically relevant 1, 2, 3-and 1, 3, 4-triazoles: from classical pathway to green chemistry", *Molecules*, 26(18), 5667, 2021. https://doi.org/10.3390/molecules26185667

[16] Dai, J., Tian, S., Yang, X., & Liu, Z., "Synthesis methods of 1, 2, 3-/1, 2, 4-triazoles: A review", *Frontiers in Chemistry*, 10, 891484, 2022. https://doi.org/10.3389/fchem.2022.891484

[17] Zhou, CH., and Wang, Y., "Recent researches in triazole compounds as medicinal drugs", *Current Medicinal Chemistry*, 19(2), 239-280, 2012. https://doi.org/10.2174/092986712803414213

[18] Peyton, LR., Gallagher, S., Hashemzadeh, M., "Triazole antifungals: a review", *Drugs Today*, 51(12), 705-718, 2015. https://doi.org/10.1358/dot.2015.51.12.2421058

[19] Guan, Q., Gao, Z., Chen, Y., Guo, C., Chen, Y., Sun, H., "Structural modification strategies of triazoles in anticancer drug development", *European Journal of Medicinal Chemistry*, 275, 116578, 2024. https://doi.org/10.1016/j.ejmech.2024.116578

[20] Klingele, M. H., Brooker, S., "The coordination chemistry of 4-substituted 3, 5-di (2-pyridyl)-4H-1, 2, 4-triazoles and related ligands", *Coordination Chemistry Reviews*, 241(1-2), 119-132, 2003. https://doi.org/10.1016/S0010-8545(03)00049-3

[21] Bentley, R., "Role of sulfur chirality in the chemical processes of biology", *Chemical Society Reviews*, 34(7), 609-624, 2005. https://doi.org/10.1039/B418284G

[22] Hu, Y., Li, CY., Wang, XM., Yang, YH., & Zhu, HL., "1, 3, 4-Thiadiazole: synthesis, reactions, and applications in medicinal, agricultural, and materials chemistry", *Chemical reviews*, 114(10), 5572-5610, 2014. https://doi.org/10.1021/cr400131u

[23] Abdelli, A., Azzouni, S., Plais, R., Gaucher, A., Efrit, ML., & Prim, D., "Recent advances in the chemistry of 1, 2, 4-triazoles: Synthesis, reactivity and biological activities", *Tetrahedron Letters*, 86, 153518, 2021. https://doi.org/10.1016/j.tetlet.2021.153518

[24] Zhou, S., Zhang, L., Jin, J., Zhang, A., Lei, X., Lin, J., Zhang, H., "Synthesis and biological activities of some novel triazolothiadiazines and Schiff bases derived from 1, 2, 4-triazole", *Phosphorus, Sulfur, and Silicon and the Related Elements*, 182(2), 419-432, 2007. https://doi.org/10.1080/10426500600976884

[25] El Sayed, H., Awad, LF., Soliman, SM., Abd Al Moaty, MN., Ghabbour, HA., & Barakat, A., "Tautomerism aspect of thione-thiol combined with spectral investigation of some 4-amino-5-methyl-1, 2, 4-triazole-3-thione Schiff's bases", *Journal of Molecular Structure*, 1146, 432-440, 2017. https://doi.org/10.1016/j.molstruc.2017.06.002

[26] Patel, VM., Patel, NB., Chan-Bacab, MJ., Rivera, G., "Synthesis, biological evaluation and molecular dynamics studies of 1, 2, 4-triazole clubbed Mannich bases", *Computational biology and chemistry*, 76, 264-274, 2018. https://doi.org/10.1016/j.compbiolchem.2018.07.020

[27] Gültekin, E., Bekircan, O., Kara, Y., Güler, Hİ., Soylu, MS., Kolaylı, S., "1, 3, 4-Thiadiazole and 1, 2, 4-triazole-5-thione derivatives bearing 2-pentyl-5-phenyl-2, 4-dihydro-3H-1, 2, 4-triazole-3-one ring: Synthesis, molecular docking, urease inhibition, and crystal structure", *Archiv der Pharmazie*, 356(1), 2200355, 2023. https://doi.org/10.1002/ardp.202200355

[28] Jain, AK., Ravichandran, V., Sisodiya, M., & Agrawal, RK., "Synthesis and antibacterial evaluation of 2–substituted–4, 5–diphenyl–N–alkyl imidazole derivatives", *Asian Pacific Journal of Tropical Medicine*, 3(6), 471-474, 2010. https://doi.org/10.1016/S1995-7645(10)60113-7

[29] Pakravan, P., Kashanian, S., Khodaei, MM., Harding, FJ., "Biochemical and pharmacological characterization of isatin and its derivatives: from structure to activity", *Pharmacological Reports*, 65(2), 313-335, 2013.

[30] Kahriman, N., Serdaroğlu, V., Peker, K., Aydın, A., Usta, A., Fandaklı, S., Yaylı, N., "Synthesis and biological evaluation of new 2, 4, 6-trisubstituted pyrimidines and their N-alkyl derivatives", *Bioorganic Chemistry*, 83, 580-594, 2019. https://doi.org/10.1016/j.bioorg.2018.10.068

[31] Creaven, B. S., Czeglédi, E., Devereux, M., Enyedy, EA., Kia, AFA., Karcz, D., Walsh, M., "Biological activity and coordination modes of copper (II) complexes of Schiff base-derived coumarin ligands", *Dalton Transactions*, 39(45), 10854-10865, 2010. https://doi.org/10.1039/c0dt00068j

[32] Furman, R., Biswas, T., Danhart, E., Foster, MP., Tsodikov, OV., & Artsimovitch, I., "Dksa2, a zinc-independent structural analog of the transcription factor dksa", FEBS Letters, 587(6), 614-619, 2013. https://doi.org/10.1016/j.febslet.2013.01.073

[33] Zhang, S., Thompson, JP., Xia, J., Bogetti, AT., York, F., Skillman, AG., . LeBard, DN., "Mechanistic insights into passive membrane permeability of drug-like molecules from a weighted ensemble of trajectories", *Journal of Chemical Information and Modeling*, 62(8), 1891-1904, 2022. https://doi.org/10.26434/chemrxiv-2021-mmv2k

[34] Erdoğan, H., Yılmaz, Ö., Çevik, PK., Doğan, M., Özen, R., "Synthesis of schiff bases and secondary amines with indane skeleton; evaluation of their antioxidant, antibiotic, and antifungal activities", *Chemistry & Biodiversity*, 20(9), e202300684, 2023. https://doi.org/10.1002/cbdv.202300684

[35] Balouiri, M., Sadiki, M., Ibnsouda, SK., "Methods for in vitro evaluating antimicrobial activity: A review", *Journal of pharmaceutical analysis*, 6(2), 71-79, 2016. https://doi.org/10.1016/j.jpha.2015.11.005

[36] CLSI (Clinical and Laboratory Standards), 2007, Performance standards for antimicrobial susceptibility testing, 17th Informational Supplement, M100-S17, 27: 1.

[37] Oyaizu, M., "Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine", *The Japanese journal of nutrition and dietetics*, 44(6), 307-315, 1986.

[38] Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., Qian, M., "Free radical scavenging properties of wheat extracts", *Journal of agricultural and food chemistry*, 50(6), 1619-1624, 2002. https://doi.org/10.1021/jf010964p

[39] Munguia, J., LaRock, DL., Tsunemoto, H., Olson, J., Cornax, I., Pogliano, J., Nizet, V., "The Mla pathway is critical for Pseudomonas aeruginosa resistance to outer membrane permeabilization and host innate immune clearance", *Journal of molecular medicine*, 95(10), 1127-1136, 2017. https://doi.org/10.1007/s00109-017-1579-4

[40] Da Silva, CM., da Silva, DL., Modolo, LV., Alves, RB., de Resende, MA., Martins, C. V., de Fátima, Â., "Schiff bases: A short review of their antimicrobial activities", *Journal of Advanced research*, 2(1), 1-8, 2011. https://doi.org/10.1016/j.jare.2010.05.004

[41] Heimark, L., Shipkova, P., Greene, J., Munayyer, H., Yarosh-Tomaine, T., DiDomenico, B., Pramanik, BN., "Mechanism of azole antifungal activity as determined by liquid chromatographic/mass spectrometric monitoring of ergosterol biosynthesis", *Journal of mass spectrometry*, 37(3), 265-269, 2002. https://doi.org/10.1002/jms.280

[42] François, IE., Bink, A., Vandercappellen, J., Ayscough, KR., Toulmay, A., Schneiter, R., Thevissen, K., "Membrane rafts are involved in intracellular miconazole accumulation in yeast cells", *Journal of Biological Chemistry*, 284(47), 32680-32685, 2009. https://doi.org/10.1074/jbc.m109.014571

[43] Sadeghpour, H., Khabnadideh, S., Zomorodian, K., Pakshir, K., Hoseinpour, K., Javid, N., Rezaei, Z., "Design, synthesis, and biological activity of new triazole and nitro-triazole derivatives as antifungal agents", *Molecules*, 22(7), 1150, 2017. https://doi.org/10.3390/molecules22071150

[44] Ding, M., Wan, S., Wu, N., Yan, Y., Li, J., & Bao, X., "Synthesis, structural characterization, and antibacterial and antifungal activities of novel 1, 2, 4-triazole thioether and thiazolo [3, 2-b]-1, 2, 4-triazole derivatives bearing the 6-fluoroquinazolinyl moiety", *Journal of Agricultural and Food Chemistry,* 69(50), 15084-15096, 2021. https://doi.org/10.1021/acs.jafc.1c02144

[45] Shao, WB., Wang, PY., Fang, ZM., Wang, JJ., Guo, DX., Ji, J., Yang, S., "Synthesis and biological evaluation of 1, 2, 4-triazole thioethers as both potential virulence factor inhibitors against plant bacterial diseases and agricultural antiviral agents against tobacco mosaic virus infections", *Journal of Agricultural and Food Chemistry*, 69(50), 15108-15122, 2021. https://doi.org/10.1021/acs.jafc.1c05202

[46] Mohamed, HA., Ammar, YA., Elhagali, GA., Eyada, HA., Aboul-Magd, DS., & Ragab, A., "In vitro antimicrobial evaluation, single-point resistance study, and radiosterilization of novel pyrazole incorporating thiazol-4-one/thiophene derivatives as dual DNA gyrase and DHFR inhibitors against MDR pathogens", *ACS omega*, 7(6), 4970, 2022. https://doi.org/10.1021/acsomega.1c05801

[47] Gürsoy Kol, Ö. Alkan, M., Antioxidant Properties of 1,2,4-Triazoles, *Chemistry of 1,2,4-Triazoles in Current Science*, ISRES Publishing, s. 24-45, 2022.

[48] Rana, MS., Rayhan, NMA., Emon, MSH., Islam, MT., Rathry, K., Hasan, MM., Asraf, MA., "Antioxidant activity of Schiff base ligands using the DPPH scavenging assay: an updated review", *RSC advances*, 14(45), 33094-33123, 2024. https://doi.org/10.1039/D4RA04375H

[49] Borgohain, R., Handique, JG., Guha, AK., Pratihar, S., "A theoretical study of antioxidant activity of some Schiff bases derived from biologically important phenolic aldehydes and phenylenediamines", *Journal of Physical Organic Chemistry*, 31(2), e3757, 2018. https://doi.org/10.1002/poc.3757

[50] Aytac, S., Gundogdu, O., Bingol, Z., & Gulcin, İ., "Synthesis of Schiff bases containing phenol rings and investigation of their antioxidant capacity, anticholinesterase, butyrylcholinesterase, and carbonic anhydrase inhibition properties", *Pharmaceutics*, 15(3), 779, 2023. https://doi.org/10.3390/pharmaceutics15030779

[51] Temuz, M. M., Çankaya, N., Korcan, S. E., Yalçin Azarkan, S., & Kahraman, T. (2024). First In Vitro–In Silico Analysis for the Determination of Antimicrobial and Antioxidant Properties of 2-(4-Methoxyphenylamino)-2-oxoethyl Methacrylate and p-Acetamide. *ACS omega*, *9*(7), 7910-7922. https://doi.org/10.1021/acsomega.3c07836

[52] Quy Huong, D., Nam, PC., Quang, DT., Ngo, ST., Duong, T., Tam, NM., "Evaluation of free radical scavenging ability of triazole-3-thiol: a combination of experimental and theoretical approaches", *ACS omega*, *9*(22), 24071-24081, 2024. https://doi.org/10.1021/acsomega.4c02931