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Synthesis and Antioxidant and Antibacterial Activities of Novel 4-({[3-Alkyl(aryl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}-methyl)phenyl 4-Nitrobenzoate Derivatives

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Abstract—It is known that acetyl derivatives, Schiff bases, and Mannich bases obtained from 1,2,4-triazol-5-ones have significant antibacterial and antioxidant activities. Herein, 27 new 1,2,4-triazole derivatives were synthesized, and their antioxidant and antibacterial properties were investigated. The metal chelating activity of the synthesized compounds and especially the effect against gram-positive bacteria were found to be significant. The effect against gram-negative strains was also important. Structure determination of the synthesized compounds was performed on the basis of their ^{1}H and ^{13}C NMR, IR, and high-resolution mass spectra.

Keywords: 1,2,4-triazole derivatives, antioxidant activity, antibacterial activity, Mannich bases, Schiff bases

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INTRODUCTION

The increase in epidemic diseases in recent years has stimulated studies to discover appropriate drugs for their treatment. With the emergence of antibiotic resistance as a common problem, there has been a trend toward the production of antibiotic equivalents [1]. On the other hand, interest in antioxidants has increased to strengthen the immune system [2]. Heterocyclic moieties are present in the structure of drugs that increase the life quality for people. Particularly, five-membered rings containing nitrogen atoms, such as the triazole ring, are included in the structure of many drugs; for example, 1,2,4-triazole ring is a structural fragment of alprazolam, triazolam, estazolam, trazodone, trapidil, terconazole, hexaconazole, etizolam, rilmazafone, and rizatriptan [3].

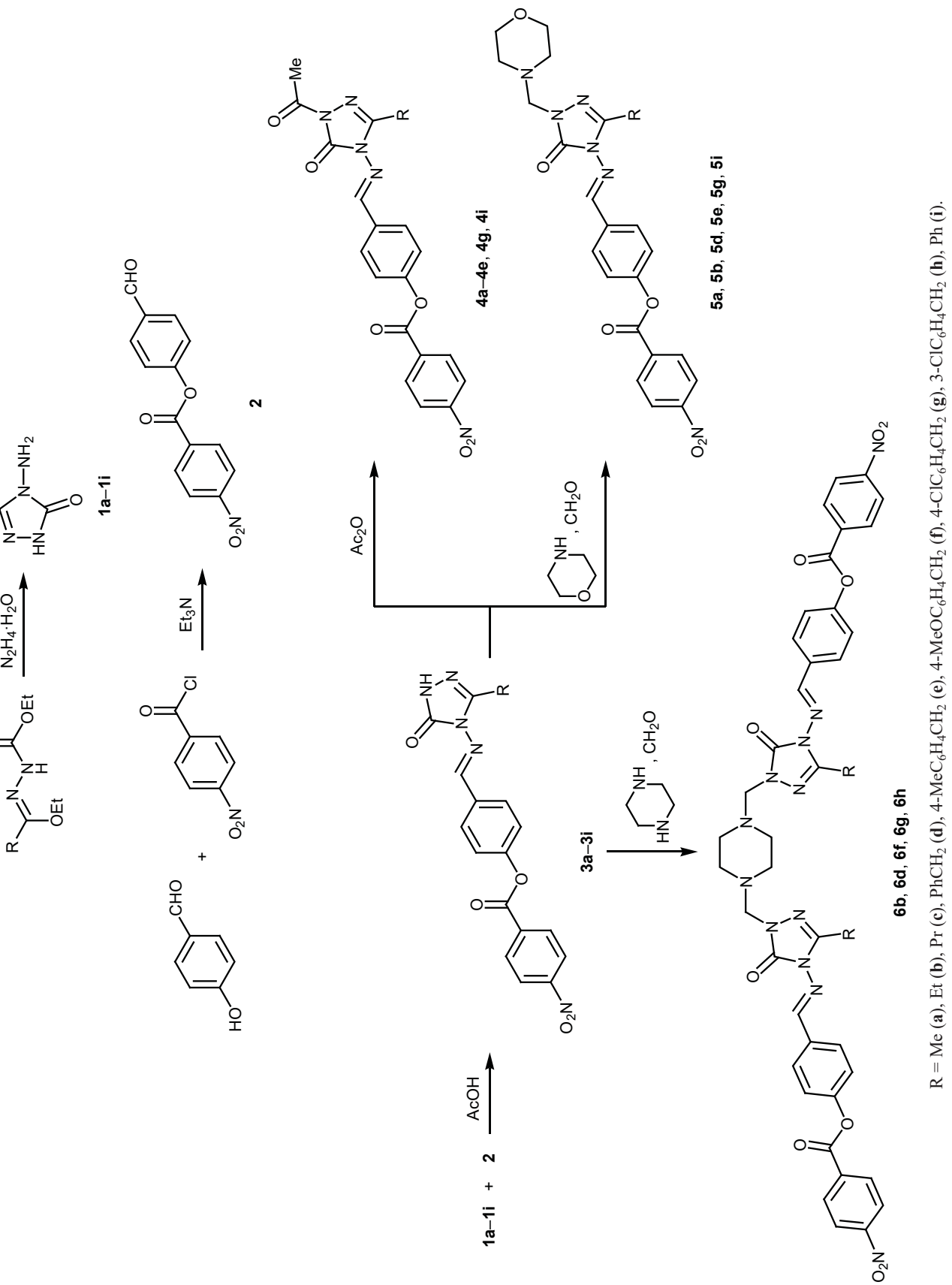
There are many studies revealing that compounds bearing a triazole ring have significant biological activity [4–9]. Mannich reactions provide an appropriate method to bring two different molecules together, and Mannich bases are used in the fields of cosmetics, paints, food industry, and pharmaceutics [10, 11]. The antioxidant, antibacterial, and antifungal effects of Mannich bases containing a triazole ring have been investigated in recent years, and important results have been found [12–17]. The purpose of this study was to synthesize four different groups of triazole derivatives and investigate their antioxidant and antibacterial activities.

RESULTS AND DISCUSSION

Nine new Schiff bases **3a**–**3i** were synthesized by the condensation of 5-substituted 4-amino-2,4-dihydro-3*H*-1,2,4-triazol-3-ones **1a**–**1i** with 4-formylphenyl 4-nitrobenzoate (**2**). Acetylation of **3** with acetic anhydride gave *N*-acetyl derivatives **4**, and Mannich bases **5** and **6** were obtained by reacting compounds **3** with morpholine and piperazine, respectively, in the presence of formaldehyde (Scheme 1).

Antioxidant Activity. The metal chelating activities of compounds **3**–**6** are given in Fig. 1. According to the antioxidant evaluation, the newly synthesized compounds showed neither scavenging effect nor reductive activities. As seen in Fig. 1, ferrous ion chelating activities of compounds **3** and **4** in comparison to BHT, BHA, and α-tocopherol and of **5** and **6** in comparison to EDTA and α-tocopherol demonstrated a marked capacity of the newly synthesized compounds for iron

Scheme 1.



R = Me (**a**), Et (**b**), Pr (**c**), $PhCH_2$ (**d**), 4-$MeC_6H_4CH_2$ (**e**), 4-$MeOC_6H_4CH_2$ (**f**), 4-$ClC_6H_4CH_2$ (**g**), 3-$ClC_6H_4CH_2$ (**h**), Ph (**i**).

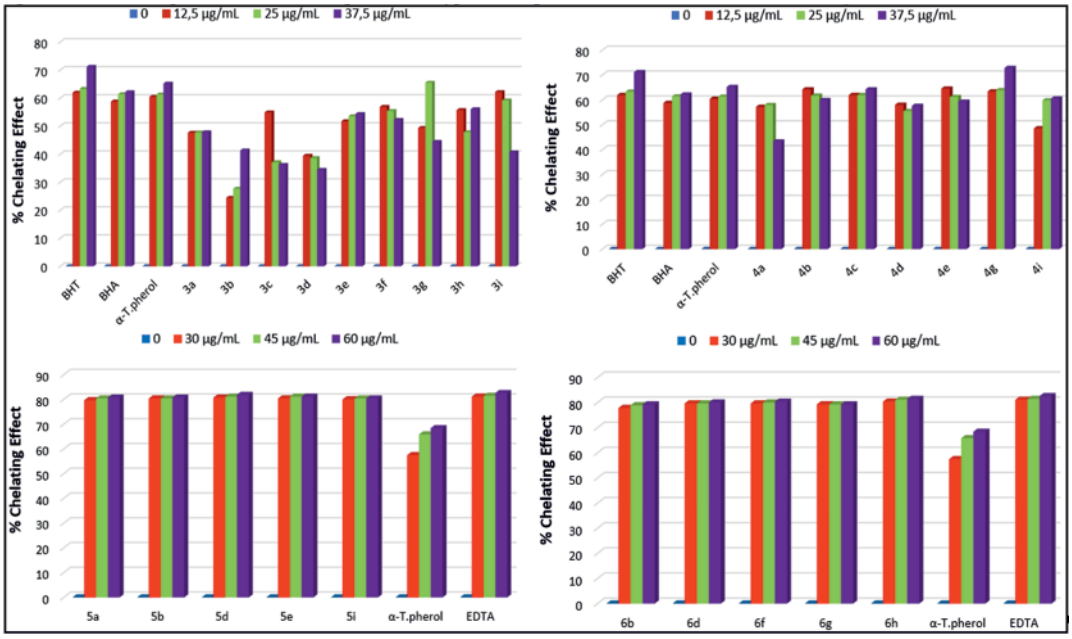


Fig. 1. Metal chelating activities of compounds **3**–**6**, α-tocopherol, BHT, and BHA for ferrous ions.

binding, which suggests that they could act as peroxidation protectors. It was also revealed that the metal chelating effects of the newly synthesized Schiff bases were not concentration-dependent, except **3a**, **3b**, **3e**, **4c**, **4g**, and **4i**. In addition, the antioxidant capacity of the newly synthesized Mannich bases was higher than that of the Schiff bases.

In vitro antibacterial activity. The results of antimicrobial evaluation of compounds **3**–**6** are given in Table 1. The activities of compounds **3** against gram-positive bacteria were higher than their activities against gram-negative bacteria. Compounds **3b** and **3i** showed low activity against *B. subtilis*, while the effects of **3d** and **3g** were moderate. Only low activities were observed for compounds **3c**, **3e**, and **3g** against *B. cereus*. Moderate effects of **3a**, **3b**, and **3d** were detected against *S. aureus* which is a common infectious agent. Type **3** compounds had no activity against *E. coli* and *P. aeruginosa*. Moderate effects of **3b**, **3d**, and **3i** against *K. pneumoniae* were revealed. Acetyl derivatives **4** showed no antibacterial effects, except compound **4a** which exhibited a low activity against *B. cereus*.

Compounds **5** had moderate effects on all Gram-positive bacteria studied. Although there was no activity against Gram-negative *E. coli*, compounds **5** were moderately active against *P. aeruginosa*. Compounds **5e** and **5g** showed low activities against *K. pneumoniae*, while the activity of **5i** was high. The other compounds **5** were moderately active against *K. pneumoniae*.

Compound **6g** had a low effect against *B. subtilis* and no effect on the other bacterial strains. Moderate activities against *B. cereus* were mostly exhibited by compounds **6**. Similarly, moderate activities of **6** were observed against *S. aureus* from **6** type compounds. Type **6** compounds had moderate effects on *P. aeruginosa*, but they were inactive against *E. coli*. Compounds **6b** and **6d** showed no activity against *K. pneumoniae*. A low level of effect was determined only for **6h**, and moderate effects were seen for **6f** and **6g**.

EXPERIMENTAL

The required chemicals were purchased from Fluka, Merck AG, and Aldrich. The melting points were measured with an electrothermal melting point apparatus. The IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ultrashield spectrometer at 400 and 100 MHz, respectively, using DMSO-d_6 as solvent and tetramethylsilane as internal standard. The high-resolution mass spectra were recorded on a Thermo Scientific DFS instrument. Initial 1,2,4-triazoles **1a**–**1i** were synthesized according to literature procedures [19, 20].

4-({[3-Alkyl(aryl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoates 3a–3i (*general procedure*). 4-Nitrobenzoyl chloride (0.01 mol) was added to a solution of 4-hydroxybenzaldehyde (0.01 mol) in 100 mL of ethyl acetate, the

Table 1. In vitro antimicrobial activity of compounds **3–6**

Compound no.	Diameter of inhibition zone (mm) and level of the antibacterial effect[a]					
	B. subtilis	*B. cereus*	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
3a	–	–	12 (++)	–	–	–
3b	10 (+)	–	11 (++)	–	–	11 (++)
3c	–	10 (+)	–	–	–	–
3d	12 (++)	–	13 (++)	–	–	12 (++)
3e	–	9 (+)	–	–	–	–
3f	–	–	–	–	–	–
3g	11 (++)	9 (+)	–	–	–	–
3h	–	–	–	–	–	–
3i	10 (+)	–	–	–	–	13 (++)
4a	–	10 (+)	–	–	–	–
4b	–	–	–	–	–	–
4c	–	–	–	–	–	–
4d	–	–	–	–	–	–
4e	–	–	–	–	–	–
4g	–	–	–	–	–	–
4i	–	–	–	–	–	–
5a	16 (++)	13 (++)	12 (++)	–	14 (++)	14 (++)
5b	15 (++)	13 (++)	14 (++)	–	14 (++)	14 (++)
5d	13 (++)	11 (++)	12 (++)	–	11 (++)	13 (++)
5e	12 (++)	11 (++)	11 (++)	–	11 (++)	9 (+)
5g	14 (++)	12 (++)	12 (++)	–	11 (++)	9 (+)
5i	15 (++)	13 (++)	11 (++)	–	13 (++)	19 (+++)
6b	–	13 (++)	11 (++)	–	11 (++)	–
6d	–	11 (++)	11 (++)	–	12 (++)	–
6f	–	9 (+)	11 (++)	–	12 (++)	13 (++)
6g	10 (+)	11 (++)	13 (++)	–	13 (++)	13 (++)
6h	–	10 (+)	11 (++)	–	13 (++)	9 (+)
Ampicillin	33 (+++)	36 (+++)	37 (+++)	34 (+++)	36 (+++)	35 (+++)
Neomycin	17 (+++)	17 (+++)	13 (++)	16 (++)	17 (+++)	16 (++)
Streptomycin	12 (++)	12 (++)	21 (+++)	10 (+)	12 (++)	11 (++)

[a] Evaluation of results according to the diameter of inhibition zone: <5.5 mm: no effect (–); 5.5–10 mm: low effect (+); 11–16 mm: moderate effect (++); ≥17 mm: high effect (+++) [18].

mixture was cooled to 0–5°C, and triethylamine (0.01 mol) was slowly added with stirring using a magnetic stirrer. The mixture was stirred for 2 h, refluxed for 3 h, and filtered. The filtrate was evaporated under reduced pressure, and the residue was washed with water and crystallized from ethyl acetate–petroleum ether (1:3). Yield 90.4%, mp 206°C [21]. IR spectrum, ν, cm^{-1}: 815 (1,4-C_6H_4), 1270 (C=O, ester), 1526/1349 (NO_2), 1742/1710 (C=O), 2846, 1760 (CHO). Compound **2** thus obtained (0.01 mol) was added to a solution of compound **1a**–**1i** (0.01 mol) in acetic acid (20 mL), and the mixture was refluxed for 1.5 h and evaporated at 50–55°C on a water bath. The residue was crystallized three times from a mixture of ethanol and water (1:3). Compounds **3a**–**3i** were isolated as colorless crystalline solids.

4-{[(3-Methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (3a). Yield: 97.3%, mp 253–254°C. IR spectrum, ν, cm^{-1}: 811 (1,4-C_6H_4), 1267 (COO), 1528, 1347 (NO_2), 1601 (C=N), 1741, 1707 (C=O), 3186 (NH). ^{1}H NMR spectrum, δ, ppm: 2.30 s (3H, CH_3), 7.50 d (2H, H_{arom}, J = 8.30 Hz), 7.97 d (2H, H_{arom}, J = 8.30 Hz), 8.39–8.44 m (4H, H_{arom}), 9.78 s (1H, HC=N), 11.85 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 11.65 (CH_3); 123.76 (2C), 125.23 (2C), 130.32 (2C), 132.45 (2C), 132.70, 135.39, 152.01, 153.89 (C_{arom}); 145.66 (C^3), 152.61 (C^5), 153.94 (HC=N), 164.20 (C=O). Mass spectrum: m/z 390.08084 $[M + Na]^+$; calculated for $C_{17}H_{13}N_5NaO_5$: 390.08089.

4-{[(3-Ethyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (3b). Yield 94.5%, mp 195–196°C. IR spectrum, ν, cm^{-1}: 828 (1,4-C_6H_4), 1257 (COO), 1524, 1347 (NO_2), 1594 (C=N), 1738, 1710 (C=O), 3174 NH). ^{1}H NMR spectrum, δ, ppm: 1.09 t (3H, $CH_2C\mathbf{H}_3$, J = 7.60 Hz), 2.71 q (2H, $C\mathbf{H}_2CH_3$, J = 7.60 Hz), 7.51 d (2H, H_{arom}, J = 8.50 Hz), 7.96 d (2H, H_{arom}, J = 8.50 Hz), 8.38–8.45 m (4H, H_{arom}), 9.78 s (1H, HC=N), 11.83 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 10.52 (CH_3), 18.99 (CH_2); 123.14 (2C), 124.44 (2C), 129.47 (2C), 131.82 (2C), 132.22, 134.69, 150.13, 152.88 (C_{arom}); 149.41 (C^3), 153.86 (C^5), 153.92 (HC=N), 164.37 (COO). Mass spectrum: m/z 404.09658 $[M + Na]^+$; calculated for $C_{18}H_{15}N_5NaO_5$: 404.09654.

4-{[(5-Oxo-3-propyl-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (3c). Yield 97.6%, mp 185–187°C. IR spectrum, ν, cm^{-1}: 813 (1,4-C_6H_4), 1262 (COO), 1522, 1347 (NO_2), 1593 (C=N), 1741, 1694 (C=O), 3176 (NH). ^{1}H NMR spectrum, δ, ppm: 1.05 t (3H, $CH_2C\mathbf{H}_3$, J = 7.60 Hz), 1.81 sext (2H, $CH_2C\mathbf{H}_2CH_3$, J = 7.60 Hz), 2.76 t (2H, $C\mathbf{H}_2CH_2CH_3$, J = 7.60 Hz), 7.45 d (2H, H_{arom}, J = 8.40 Hz), 8.01 d (2H, H_{arom}, J = 8.40 Hz), 8.37–8.39 m (4H, H_{arom},), 9.85 s (1H, HC=N), 11.80 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 13.68 ($CH_2\mathbf{C}H_3$), 19.44 ($CH_2\mathbf{C}H_2CH_3$), 27.41 ($\mathbf{C}H_2CH_2CH_3$); 122.03 (2C), 123.85 (2C), 129.30 (2C), 131.37 (2C), 131.41, 134.62, 151.11, 152.76 (C_{arom}); 148.79 (C^3), 151.87 (C^5), 153.05 (HC=N), 162.68 (COO). Mass spectrum: m/z 418.11224 $[M + Na]^+$; calculated for $C_{19}H_{17}N_5NaO_5$: 418.11219.

4-{[(3-Benzyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (3d). Yield 97.1%, mp 220–222°C. IR spectrum: 772, 710 (δC–H_{arom}), 806 (1,4-C_6H_4), 1260 (COO), 1525, 1347 (NO_2), 1593 (C=N), 1743, 1699 (C=O), 3168 (NH). ^{1}H NMR spectrum, δ, ppm: 4.09 s (2H, $C\mathbf{H}_2Ph$), 7.50 d (2H, H_{arom}, J = 8.60 Hz), 7.93 d (2H, H_{arom}, J = 8.60 Hz), 8.38–8.44 m (4H, H_{arom}), 9.74 s (1H, HC=N), 11.96 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 31.53 ($\mathbf{C}H_2Ph$); 123.02 (2C), 124.15 (2C), 129.28 (2C), 131.13 (2C), 131.84, 134.71, 151.09, 152.80 (C_{arom}); 127.21, 128.89 (2C), 128.93 (2C), 136.27 (C_6H_5); 146.72 (C^3), 151.67 (C^5), 153.02 (HC=N), 163.40 (COO). Mass spectrum: m/z 466.11224 $[M + Na]^+$; calculated for $C_{23}H_{17}N_5NaO_5$: 466.11219.

4-({[3-(4-Methylbenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (3e). Yield 96.8%, mp 224–225°C. IR spectrum, ν, cm^{-1}: 810 (1,4-C_6H_4), 1261 (COO), 1524, 1347 (NO_2), 1589 (C=N), 1742, 1696 (C=O), 3172 (NH). ^{1}H NMR spectrum, δ, ppm: 2.32 s (3H, $C_6H_4C\mathbf{H}_3$), 4.07 s (2H, $C_6H_4C\mathbf{H}_2$), 7.14 d (2H, H_{arom}, J = 7.60 Hz), 7.25 d (2H, H_{arom}, J = 8.00 Hz), 7.34 d (2H, H_{arom}, J = 8.00 Hz), 7.86 d (2H, H_{arom}, J = 8.00 Hz), 8.37–8.39 m (4H, H_{arom}), 9.85 s (1H, HC=N), 11.90 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 21.05 ($C_6H_4\mathbf{C}H_3$), 31.52 ($\mathbf{C}H_2C_6H_4$); 122.02 (2C), 123.86 (2C), 129.40 (2C), 131.41 (2C), 131.78, 134.61, 151.12, 152.76 (C_{arom}); 128.89 (2C), 129.34 (2C), 129.40, 132.10 ($\mathbf{C}_6H_4CH_3$); 147.99 (C^3), 151.71 (C^5), 153.04 (HC=N), 162.92 (COO). Mass spectrum: m/z 480.12787 $[M + Na]^+$; calculated for $C_{24}H_{19}N_5NaO_5$: 480.12784.

4-({[3-(4-Methoxybenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (3f). Yield 94.5%, mp 236–238°C. IR spectrum, ν, cm^{-1}: 850 (1,4-C_6H_4), 1244 (COO), 1530, 1347 (NO_2), 1607, 1589 (C=N), 1743, 1698 (C=O), 3175 (NH). ^{1}H NMR spectrum, δ, ppm: 3.78 s (3H, OCH_3),

4.05 s (2H, $C_6H_4C\mathbf{H}_2$), 6.87 d (2H, H_{arom}, J = 8.80 Hz), 7.35 d (2H, H_{arom}, J = 8.40 Hz), 7.44 d (2H, H_{arom}, J = 8.80 Hz), 8.01 d (2H, H_{arom}, J = 8.40 Hz), 8.39 s (4H, H_{arom}), 9.82 s (1H, HC=N), 11.85 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 31.50 ($\mathbf{C}H_2C_6H_4$), 55.40 (OCH_3); 122.80 (2C), 123.82 (2C), 129.33 (2C), 130.06, 131.37 (2C), 134.60, 151.00, 152.50 (C_{arom}); 114.18 (2C), 127.50, 131.37 (2C), 158.60 ($\mathbf{C}_6H_4OCH_3$); 148.00 (C^3), 152.10 (C^5), 152.90 (HC=N), 163.00 (COO). Mass spectrum: m/z 496.12286 $[M + Na]^+$; calculated for $C_{24}H_{19}N_5NaO_6$: 496.12275.

4-({[3-(4-Chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (3g). Yield 79.5%, mp 234–235°C. IR spectrum, ν, cm^{-1}: 845 (1,4-C_6H_4), 1260 (COO), 1527, 1346 (NO_2), 1604, 1585 (C=N), 1739, 1711 C=O), 3177 (NH). ^{1}H NMR spectrum, δ, ppm: 3.92 s (2H, $C\mathbf{H}_2C_6H_4$), 7.33–7.41 m (4H, H_{arom}), 7.50 d (2H, H_{arom}, J = 8.60 Hz), 7.93 d (2H, H_{arom}, J = 8.60 Hz), 8.39–8.43 m (4H, H_{arom}), 9.75 s (1H, HC=N), 11.98 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 30.85 ($\mathbf{C}H_2C_6H_4$); 123.06 (2C), 124.19 (2C), 129.59 (2C), 131.87 (2C), 131.90, 134.75, 151.13, 152.94 (C_{arom}); 128.83 (2C), 130.17 (2C), 131.24, 135.26 (ClC_6H_4); 146.41 (C^3), 151.66 (C^5), 153.07 (HC=N), 163.42 (COO). Mass spectrum: m/z 500.07327 $[M + Na]^+$; calculated for $C_{23}H_{16}ClN_5NaO_5$: 500.07322.

4-({[3-(3-Chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (3h). Yield 96.8%, mp 220–222°C. IR spectrum, ν, cm^{-1}: 816 (1,4-C_6H_4), 874, 788 (1,3-C_6H_4), 1267 (COO), 1524, 1351 (NO_2), 1602, 1583 C=N), 1741, 1701 (C=O), 3190 (NH). ^{1}H NMR spectrum, δ, ppm: 4.09 s (2H, $C_6H_4C\mathbf{H}_2$), 7.34–7.38 m (2H, H_{arom}), 7.44 d (2H, H_{arom}, J = 8.40 Hz), 7.82–7.84 m (2H, H_{arom}), 8.01d (2H, H_{arom}, J = 8.80 Hz), 8.39 m (4H, H_{arom}), 9.82 s (1H, HC=N), 12.00 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 31.30 ($\mathbf{C}H_2C_6H_4$); 122.30 (2C), 123.81 (2C), 129.35 (2C), 131.37 (2C), 131.41, 134.60, 151.10, 152.00 (C_{arom}); 127.09, 127.49, 128.80, 130.30, 133.00, 138.10 (ClC_6H_4); 147.11 (C^3), 151.37 (C^5), 152.50 (HC=N), 163.00 (COO). Mass spectrum: m/z 500.07330 $[M + Na]^+$; calculated for $C_{23}H_{16}ClN_5NaO_5$: 500.07322.

4-{[(5-Oxo-3-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (3i). Yield 99.3%, mp 215–216°C. IR spectrum, ν, cm^{-1}: 853, 804 (1,4-C_6H_4), 769, 694 (C_6H_5), 1259 (COO), 1526, 1348 (NO_2), 1602, 1582 (C=N), 1746, 1691 (C=O), 3160 (NH). ^{1}H NMR spectrum, δ, ppm: 7.43–7.58 m (5H, H_{arom}), 7.83–7.96 m (4H, H_{arom}), 8.36–8.46 m (4H, H_{arom}), 9.72 s (1H, HC=N), 12.37 s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 123.09 (2C), 124.41 (2C), 129.32 (2C), 131.11 (2C), 131.79, 134.63, 151.03, 153.18 (C_{arom}); 127.07, 128.31 (2C), 128.99 (2C), 130.56 (C_6H_5); 145.02 (C^3), 151.80 (C^5), 155.82 (HC=N), 163.34 (COO). Mass spectrum: m/z 452.09647 $[M + Na]^+$; calculated for $C_{22}H_{15}N_5NaO_5$: 452.09654.

4-({[1-Acetyl-3-alkyl(aryl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoates 4a–4e, 4g, and 4i (*general procedure*). A mixture of compound **3a–3e**, **3g**, or **3i** (0.01 mol) and acetic anhydride (15 mL) was refluxed for 30 min. Anhydrous ethanol (50 mL) was then added, the mixture was refluxed for 1 h and evaporated at 40–45°C, and the residue was purified by crystallization from an appropriate solvent.

4-{[(1-Acetyl-3-methyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (4a). Yield 93.0%, mp 240–241°C. IR spectrum, ν, cm^{-1}: 814 (1,4-C_6H_4), 1268 (COO), 1532, 1366 (NO_2), 1604 (C=N), 1737, 1713 (C=O). ^{1}H NMR spectrum, δ, ppm: 3.47 s (3H, CH_3), 2.64 s (3H, $COCH_3$), 7.37 d (2H, H_{arom}, J = 8.00 Hz), 7.90 d (2H, H_{arom}, J = 8.40 Hz), 8.40 m (4H, H_{arom}), 9.80 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 11.62 (CH_3), 23.52 ($CO\mathbf{C}H_3$); 122.17 (2C), 123.82 (2C), 129.53 (2C), 131.38 (2C), 131.54, 134.52, 148.64, 153.14 (C_{arom}); 147.45 (C^3), 153.15 (C^5), 154.22 (HC=N), 162.90 (COO), 166.24 ($\mathbf{C}OCH_3$). Mass spectrum: m/z 432.09143 $[M + Na]^+$; calculated for $C_{19}H_{15}N_5NaO_6$: 432.09145.

4-{[(1-Acetyl-3-ethyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (4b). Yield 74.1%, mp 224–225°C. IR spectrum, ν, cm^{-1}: 813 (1,4-C_6H_4), 1269 (COO), 1527, 1347 (NO_2), 1603 (C=N), 1735, 1713 (C=O). ^{1}H NMR spectrum, δ, ppm: 1.38 t (3H, $CH_2C\mathbf{H}_3$, J = 7.60 Hz), 2.64 s (3H, $COCH_3$), 2.85 q (2H, $C\mathbf{H}_2CH_3$, J = 7.60 Hz), 7.37 d (2H, H_{arom}, J = 8.40 Hz), 7.90 d (2H, H_{arom}, J = 8.40 Hz), 8.39 m (4H, H_{arom}), 9.80 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 10.08 ($CH_2\mathbf{C}H_3$), 19.36 ($\mathbf{C}H_2CH_3$), 23.55 ($CO\mathbf{C}H_3$); 122.18 (2C), 123.82 (2C), 129.49 (2C), 131.38 (2C), 131.63, 134.52, 151.14, 154.14 (C_{arom}); 148.80 (C^3), 153.11 (C^5), 154.14 (HC=N), 162.92 (COO), 166.43 ($\mathbf{C}OCH_3$). Mass spectrum: m/z 446.10718 $[M + Na]^+$; calculated for $C_{20}H_{17}N_5NaO_6$: 446.10710.

4-{[(1-Acetyl-5-oxo-3-propyl-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (4c). Yield 92.3%, mp 204–205°C. IR spectrum, ν, cm^{-1}: 813 (1,4-C_6H_4), 1269 (COO), 1529, 1347 (NO_2), 1603 (C=N), 1736, 1715 (C=O). ^1H NMR spectrum, δ, ppm: 1.06 t (3H, CH_2CH_3, J = 7.60 Hz), 1.84 sext (2H, $CH_2CH_2CH_3$, J = 7.60 Hz), 2.64 s (3H, $COCH_3$), 2.80 t (2H, $CH_2CH_2CH_3$, J = 7.60 Hz), 7.37 d (2H, H_{arom}, J = 8.40 Hz), 7.89 d (2H, H_{arom}, J = 8.40 Hz), 8.39–8.42 m (4H, H_{arom}), 9.79 s (1H, CH=N). ^{13}C NMR spectrum, δ_C, ppm: 13.73 (CH_2CH_3), 19.43 ($CH_2CH_2CH_3$), 23.55 ($COCH_3$), 27.47 ($CH_2CH_2CH_3$); 122.19 (2C), 123.82 (2C), 129.49 (2C), 131.39 (2C), 131.64, 134.52, 151.14, 152.91 (C_{arom}); 148.77 (C^3), 153.10 (C^5), 154.13 (CH=N), 162.92 (COO), 166.42 ($COCH_3$). Mass spectrum: m/z 460.12280 $[M + Na]^+$; calculated for $C_{21}H_{19}N_5NaO_6$: 460.12275.

4-{[(1-Acetyl-3-benzyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (4d). Yield 84.9%, mp 208–209°C. IR spectrum, ν, cm^{-1}: 774, 705 (C_6H_5), 832 (1,4-C_6H_4), 1262 (COO), 1524, 1356 (NO_2), 1601 (C=N), 1774, 1734, 1699 (C=O). ^1H NMR spectrum, δ, ppm: 2.64 s (3H, $COCH_3$), 4.16 s (2H, CH_2Ph), 7.27–7.40 m (7H, H_{arom}), 7.82 d (2H, H_{arom}, J = 8.80 Hz), 8.39 m (4H, H_{arom}), 9.71 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 23.58 ($COCH_3$), 31.98 (CH_2Ph); 122.18 (2C), 123.83 (2C), 129.51 (2C), 131.38 (2C), 131.55, 134.16, 151.14, 153.10 (C_{arom}); 127.45, 128.79 (2C), 128.98 (2C), 134.50 (C_6H_5); 148.99 (C^3), 154.12 (C^5), 154.42 (CH=N), 162.92 (COO), 166.36 ($COCH_3$). Mass spectrum: m/z 508.12299 $[M + Na]^+$; calculated for $C_{25}H_{19}N_5NaO_6$: 508.12275.

4-({[1-Acetyl-3-(4-methylbenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (4e). Yield 71.4%, mp 206–208°C. IR spectrum, ν, cm^{-1}: 829 (1,4-C_6H_4), 1254 (COO), 1521, 1347 (NO_2), 1601 (C=N), 1732 (C=O). ^1H NMR spectrum, δ, ppm: 2.31 s (3H, $C_6H_4CH_3$), 2.64 s (3H, $COCH_3$), 4.12 s (2H, $CH_2C_6H_4$), 7.12 d (2H, H_{arom}, J = 8.00 Hz), 7.28 d (2H, H_{arom}, J = 8.00 Hz), 7.36 d (2H, H_{arom}, J = 8.80 Hz), 7.84 d (2H, H_{arom}, J = 8.80 Hz), 8.39 s (4H, H_{arom}), 9.70 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 21.03 ($C_6H_4CH_3$), 23.58 ($COCH_3$), 31.55 ($CH_2C_6H_4$); 122.17 (2C), 123.83 (2C), 129.52 (2C), 131.05 (2C), 131.38, 134.51, 151.14, 153.08 (C_{arom}); 128.86 (2C), 129.34 (2C), 131.59, 137.16 ($C_6H_4CH_3$); 149.19 (C^3), 154.07 (C^5), 154.37 (HC=N), 162.93 (COO), 166.37 ($COCH_3$). Mass spectrum: m/z 522.13873 $[M + Na]^+$; calculated for $C_{26}H_{21}N_5NaO_6$: 522.13840.

4-({[1-Acetyl-3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (4g). Yield 72.5%, mp 213–215°C. IR spectrum, ν, cm^{-1}: 845 (1,4-C_6H_4), 1258 (COO), 1523, 1346 (NO_2), 1602 (C=N), 1737, 1720 (C=O). ^1H NMR spectrum, δ, ppm: 2.64 s (3H, $COCH_3$), 4.13 s (2H, $CH_2C_6H_4$), 7.30–7.38 m (6H, H_{arom}), 7.82 d (2H, H_{arom}, J = 8.40 Hz), 8.39 m (4H, H_{arom}), 9.72 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 23.58 ($COCH_3$), 31.36 ($CH_2C_6H_4$); 122.25 (2C), 123.84 (2C), 129.32 (2C), 129.50, 131.39 (2C), 133.50, 151.16, 153.20 (C_{arom}); 128.98 (2C), 130.33 (2C), 132.55, 134.47 (C_6H_4Cl); 148.60 (C^3), 154.35 (C^5), 154.64 (HC=N), 162.91 (COO), 166.26 ($COCH_3$). Mass spectrum: m/z 542.08417 $[M + Na]^+$; calculated for $C_{25}H_{18}ClN_5NaO_6$: 542.08378.

4-{[(1-Acetyl-5-oxo-3-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-4-yl)imino]methyl}phenyl 4-nitrobenzoate (4i). Yield 95.1%, mp 233–234°C. IR spectrum, ν, cm^{-1}: 765, 682 (C_6H_5), 820 (1,4-C_6H_4), 1274 (COO), 1529, 1347 (NO_2), 1603 (C=N), 1731 (C=O). ^1H NMR spectrum, δ, ppm: 2.71 s (3H, $COCH_3$), 7.36 d (2H, H_{arom}, J = 8.40 Hz), 7.50–7.57 m (3H, H_{arom}), 7.88 d (2H, H_{arom}, J = 8.80 Hz), 8.02–8.04 m (2H, H_{arom}), 8.39–8.41 m (4H, H_{arom}), 9.77 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 23.65 ($COCH_3$); 122.24 (2C), 123.82 (2C), 129.75 (2C), 131.39 (2C), 131.53, 134.49, 148.83, 151.14 (C_{arom}); 125.27, 128.52 (2C), 129.13 (2C), 131.43 (C_6H_5); 147.12 (C^3), 153.22 (C^5), 155.87 (HC=N), 162.92 (COO), 166.67 ($COCH_3$). Mass spectrum: m/z 494.10724 $[M + Na]^+$; calculated for $C_{24}H_{17}N_5NaO_6$: 494.10710.

4-({[3-Alkyl(aryl)-1-(morpholin-4-ylmethyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoates 5a, 5b, 5d, 5e, 5g, and 5i (*general procedure*). Compound **3a**, **3b**, **3d**, **3e**, **3g**, or **3i** (5 mmol) was dissolved in anhydrous ethanol, 10 mmol of 37% formaldehyde and 6 mmol of morpholine were added, and the mixture was refluxed for 4 h. The mixture was filtered, left at room temperature overnight, and then cooled to –18°C in a refrigerator. The precipitate was filtered off, washed with cold ethanol, and purified by recrystallization from ethanol several times.

4-({[3-Methyl-1-(morpholin-4-ylmethyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (5a). Yield 70.3%, mp 167–168°C. IR spectrum, ν, cm^{-1}: 829 (1,4-C_6H_4), 1251 (COO), 1524, 1349 (NO_2), 1599 (C=N), 1744, 1688 (C=O). ^1H NMR spectrum, δ, ppm: 2.41 s (3H, CH_3),

2.73 t (4H, CH_2NCH_2, J = 4.40 Hz), 3.72 t (4H, CH_2OCH_2, J = 4.40 Hz), 4.63 s (2H, NCH_2N), 7.34 d (2H, H_{arom}, J = 8.80 Hz), 7.89 d (2H, H_{arom}, J = 8.80 Hz), 8.36–8.39 m (4H, H_{arom}), 9.89 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 11.39 (CH_3), 50.49 (CH_2NCH_2), 66.62 (NCH_2N), 66.83 (CH_2OCH_2); 122.01 (2C), 123.80 (2C), 129.28 (2C), 131.36 (2C), 132.11, 134.62, 151.50, 152.75 (C_{arom}); 143.91 (C^3), 151.10 (C^5), 152.90 (HC=N), 162.94 (COO). Mass spectrum: m/z 489.14963 $[M + Na]^+$; calculated for $C_{22}H_{22}N_6NaO_6$: 489.14930.

4-({[3-Ethyl-1-(morpholin-4-ylmethyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (5b). Yield 71.2%, mp 156–157°C. IR spectrum, ν, cm^{-1}: 828 (1,4-C_6H_4), 1264 (COO), 1527, 1344 (NO_2), 1605, 1586 (C=N), 1739, 1706 (C=O). ^1H NMR spectrum, δ, ppm: 1.34 t (3H, $CH_2C\mathbf{H}_3$, J = 7.60 Hz), 2.74 t (4H, CH_2NCH_2, J = 4.40 Hz), 2.80 q (2H, $C\mathbf{H}_2CH_3$, J = 7.60 Hz), 3.72 t (4H, CH_2OCH_2, J = 4.40 Hz), 4.65 s (2H, NCH_2N), 7.34 d (2H, H_{arom}, J = 8.40 Hz), 7.88 d (2H, H_{arom}, J = 8.80 Hz), 8.36–8.41 m (4H, H_{arom}), 9.89 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 10.48 ($CH_2\mathbf{C}H_3$), 19.13 ($\mathbf{C}H_2CH_3$), 50.48 (CH_2NCH_2), 66.60 (NCH_2N), 66.85 (CH_2OCH_2); 122.01 (2C), 123.79 (2C), 129.24 (2C), 131.36 (2C), 132.20, 134.62, 151.11, 152.63 (C_{arom}); 147.82 (C^3), 151.26 (C^5), 152.72 (HC=N), 162.95 (COO). Mass spectrum: m/z 503.16519 $[M + Na]^+$; calculated for $C_{23}H_{24}N_6NaO_6$: 503.16495.

4-({[3-Benzyl-1-(morpholin-4-ylmethyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)-phenyl 4-nitrobenzoate (5d). Yield 72.9%, mp 160–161°C. IR spectrum, ν, cm^{-1}: 774, 711 (C_6H_5), 839, 810 (1,4-C_6H_4), 1258 (COO), 1522, 1347 (NO_2), 1603, 1583 (C=N), 1738, 1706 (C=O). ^1H NMR spectrum, δ, ppm: 2.74 t (4H, CH_2NCH_2, J = 4.40 Hz), 3.73 t (4H, CH_2OCH_2, J = 4.40 Hz), 4.11 s (2H, $C\mathbf{H}_2Ph$), 4.67 s (2H, NCH_2N), 7.23–7.27 m (2H, H_{arom}), 7.30–7.37 m (5H, H_{arom}), 7.80–7.82 m (2H, H_{arom}), 8.36–8.37 m (4H, H_{arom}), 9.83 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 31.82 ($\mathbf{C}H_2Ph$), 50.49 (CH_2NCH_2), 66.73 (NCH_2N), 66.86 (CH_2OCH_2); 122.01 (2C), 123.81 (2C), 129.28 (2C), 131.36 (2C), 132.11, 134.60, 151.08, 152.67 (C_{arom}); 125.13, 128.80 (2C), 129.28 (2C), 135.28 ($\mathbf{C}_6H_5CH_2$); 145.64 (C^3), 151.13 (C^5), 152.72 (HC=N), 162.96 (COO). Mass spectrum: m/z 565.18085 $[M + Na]^+$; calculated for $C_{28}H_{26}N_6NaO_6$: 565.18060.

4-({[3-(4-Methylbenzyl)-1-(morpholin-4-ylmethyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (5e). Yield 71.1%, mp 124–125°C. IR spectrum, ν, cm^{-1}: 852, 811 (1,4-C_6H_4), 1262 (COO), 1526, 1346 (NO_2), 1602 (C=N), 1740, 1699 (C=O). ^1H NMR spectrum, δ, ppm: 2.31 s (3H, $C_6H_4C\mathbf{H}_3$), 2.74 t (4H, CH_2NCH_2, J = 4.40), 3.72 t (4H, CH_2OCH_2, J = 4.40 Hz), 4.07 s (2H, $C\mathbf{H}_2C_6H_4$), 4.66 s (2H, NCH_2N), 7.12 d (2H, H_{arom}, J = 8.00 Hz), 7.23–7.26 m (2H, H_{arom}), 7.32–7.35 m (2H, H_{arom}), 7.81–7.84 m (2H, H_{arom}), 8.39 m (4H, H_{arom}), 9.80 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 21.03 ($C_6H_4\mathbf{C}H_3$), 31.39 ($\mathbf{C}H_2C_6H_4$), 50.48 (CH_2NCH_2), 66.70 (NCH_2N), 66.86 (CH_2OCH_2); 122.00 (2C), 123.81 (2C), 129.38 (2C), 131.36 (2C), 132.16, 134.62, 151.10, 152.63 (C_{arom}); 128.70 (2C), 129.28 (2C), 132.16, 136.77 ($\mathbf{C}_6H_4CH_3$); 145.86 (C^3), 151.14 (C^5), 152.70 (HC=N), 162.97 (COO).

4-({[3-(4-Chlorobenzyl)-1-(morpholin-4-ylmethyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)phenyl 4-nitrobenzoate (5g). Yield 70.6%, mp 161–163. IR spectrum, ν, cm^{-1}: 844 (1,4-C_6H_4), 1263 (COO), 1529, 1345 (NO_2), 1605, 1581 (C=N), 1740, 1706 (C=O). ^1H NMR spectrum, δ, ppm: 2.73 t (4H, CH_2NCH_2, J = 4.80 Hz), 3.72 t (4H, CH_2OCH_2, J = 4.80 Hz), 4.08 s (2H, $C\mathbf{H}_2Ph$), 4.67 s (2H, NCH_2N), 7.27–7.30 m (4H, H_{arom}), 7.34 d (2H, H_{arom}, J = 8.80 Hz), 7.81 d (2H, H_{arom}, J = 8.40 Hz), 8.39 m (4H, H_{arom}), 9.82 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 31.12 ($\mathbf{C}H_2Ph$), 50.47 (CH_2NCH_2), 66.75 (NCH_2N), 66.83 (CH_2OCH_2); 122.08 (2C), 123.81 (2C), 129.26 (2C), 131.36 (2C), 133.12, 134.57, 151.06, 152.82 (C_{arom}); 128.87 (2C), 130.32 (2C), 131.96, 133.67 (C_6H_4Cl); 145.17 (C^3), 151.12 (C^5), 152.91 (HC=N), 162.94 (COO). Mass spectrum: m/z 599.14221 $[M + Na]^+$; calculated for $C_{28}H_2ClN_6NaO_6$: 599.14163.

4-({[1-(Morpholin-4-ylmethyl)-5-oxo-3-phenyl-4,5-dihydro-1*H*-1,2,4-triazol-4-yl]imino}methyl)-phenyl 4-nitrobenzoate (5i). Yield 71.4%, mp 150–151°C. IR spectrum, ν, cm^{-1}: 767, 691 (C_6H_5), 812 (1,4-C_6H_4), 1261 (COO), 1531, 1346 (NO_2), 1602 (C=N), 1744, 1700 (C=O). ^1H NMR spectrum, δ, ppm: 2.81 t (4H, CH_2NCH_2, J = 4.40 Hz), 3.74 t (4H, CH_2OCH_2, J = 4.40 Hz), 4.79 s (2H, NCH_2N), 7.26–7.35 m (2H, H_{arom}), 7.48–7.51 m (3H, H_{arom}), 7.86–7.89 m (2H, H_{arom}), 7.96–7.98 m (2H, H_{arom}), 8.36–8.41 m (4H, H_{arom}), 9.89 s (1H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 50.50 (CH_2NCH_2), 66.88 (NCH_2N), 67.06 (CH_2OCH_2); 122.09 (2C), 123.80 (2C), 129.52 (2C), 131.37 (2C), 132.07, 134.59, 151.12, 152.85 (C_{arom}); 126.38, 128.44 (2C), 128.60

(2C), 130.37 (C_6H_5); 144.41 (C^3), 151.38 (C^5), 152.85 (HC=N), 162.96 (COO). Mass spectrum: *m*/*z* 551.16523 [*M* + Na]$^+$; calculated for $C_{27}H_{24}N_6O_6Na$: 551.16495.

[({[Piperazine-1,4-diylbis(methylene)]bis(3-alkyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazole-1,4-diyl)}bis(azanylylidene))bis(methanylylidene)]bis(4,1-phenylene) bis(4-nitrobenzoates) 6b, 6d, 6f, 6g, and 6h (*general procedure*). Compound **3b**, **3d**, **3f**, **3g**, or **3h** (5 mmol) was dissolved in anhydrous ethanol, 37% formaldehyde (10 mmol) and piperazine (6 mmol) were added, and the mixture was refluxed for 4 h. The mixture was filtered, the filtrate was left at room temperature overnight and cooled at –18°C in a refrigerator, and the precipitate was filtered off, washed with cold ethanol, and recrystallized from ethanol several times.

[({[Piperazine-1,4-diylbis(methylene)]bis(3-ethyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazole-1,4-diyl)}bis(azanylylidene))bis(methanylylidene)]bis(4,1-phenylene) bis(4-nitrobenzoate) (6b). Yield 74.4%, mp 242–243°C. IR spectrum, ν, cm^{-1}: 810 (1,4-C_6H_4), 1269 (COO), 1530, 1345 (NO_2), 1606, 1587 (C=N), 1738, 1702 (C=O). ^{1}H NMR spectrum, δ, ppm: 1.30 t (6H, $CH_2C\mathbf{H}_3$, *J* = 7.60 Hz), 2.78 q (4H, $C\mathbf{H}_2CH_3$, *J* = 7.60 Hz), 2.80 s (8H, CH_2), 4.67 s (4H, NCH_2N), 7.34 d (4H, H_{arom}, *J* = 8.40 Hz), 7.88 d (4H, H_{arom}, *J* = 8.80 Hz), 8.36–8.41 m (8H, H_{arom}), 9.89 s (2H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 10.51 (2C, $CH_2\mathbf{C}H_3$), 19.12 (2C, $\mathbf{C}H_2CH_3$), 49.98 (4C, CH_2), 66.35 (2C, NCH_2N); 122.00 (4C), 123.79 (4C), 129.75 (4C), 131.37 (4C), 132.21 (2C), 134.63 (2C), 151.19 (2C), 152.60 (2C) (C_{arom}); 147.69 (2C, C^3), 151.10 (2C, C^5), 152.70 (2C, HC=N), 162.96 (2C, COO). Mass spectrum: *m*/*z* 895.28882 [*M* + Na]$^+$; calculated for $C_{42}H_{40}N_{12}NaO_{10}$: 895.28826.

[({[Piperazine-1,4-diylbis(methylene)]bis(3-benzyl-5-oxo-4,5-dihydro-1*H*-1,2,4-triazole-1,4-diyl)}bis(azanylylidene))bis(methanylylidene)]bis(4,1-phenylene) bis(4-nitrobenzoate) (6d). Yield 75.6%, mp 237–239°C. IR spectrum, ν, cm^{-1}: 760, 702 (C_6H_5), 828 (1,4-C_6H_4), 1246 (COO), 1524, 1345 (NO_2), 1600, 1581 (C=N), 1739, 1696 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.80 s (8H, CH_2), 4.10 s (4H, $C\mathbf{H}_2Ph$), 4.69 s (4H, NCH_2N), 7.26–7.32 m (10H, H_{arom}), 7.35 d (4H, H_{arom}, *J* = 8.00 Hz), 7.81 d (4H, H_{arom}, *J* = 8.40 Hz), 8.39 m (8H, H_{arom}), 9.81 s (2H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 31.81 (2C, $\mathbf{C}H_2Ph$), 49.99 (4C, CH_2), 66.54 (2C, NCH_2N); 122.00 (4C), 123.81 (4C), 129.29 (4C), 131.37 (4C), 132.13 (2C), 134.61 (2C), 151.09 (2C), 152.64 (2C) (C_{arom}); 127.11 (2C), 128.68 (4C), 128.91 (4C), 135.27 (2C) ($\mathbf{C}_6H_5CH_2$); 145.52 (2C, C^3), 152.64 (2C, C^5), 152.70 (2C, HC=N), 162.97 (2C, COO). Mass spectrum: *m*/*z* 1019.32043 [*M* + Na]$^+$; calculated for $C_{52}H_{44}N_{12}NaO_{10}$: 1019.31956.

[({[Piperazine-1,4-diylbis(methylene)]bis[3-(4-methoxybenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazole-1,4-diyl]}bis(azanylylidene))bis(methanylylidene)]bis(4,1-phenylene) bis(4-nitrobenzoate) (6f). Yield 73.1%, mp 246–247°C. IR spectrum, ν, cm^{-1}: 815 (1,4-C_6H_4), 1262 (COO), 1513, 1348 (NO_2), 1595 (C=N), 1739, 1694 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.79 s (8H, CH_2), 3.78 s (6H, OCH_3), 4.03 s (4H, $C\mathbf{H}_2Ph$), 4.68 s (4H, NCH_2N), 6.85 d (4H, H_{arom}, *J* = 8.80 Hz), 7.26 d (4H, H_{arom}, *J* = 8.40 Hz), 7.33 d (4H, H_{arom}, *J* = 8.40 Hz), 7.83 d (4H, H_{arom}, *J* = 8.80 Hz), 8.39 m (8H, H_{arom}), 9.81 s (2H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 30.95 (2C, $\mathbf{C}H_2Ph$), 49.99 (4C, CH_2), 55.27 (2C, OCH_3), 66.51 (2C, NCH_2N); 122.02 (4C), 123.81 (4C), 129.29 (4C), 131.37 (4C), 132.17 (2C), 134.62 (2C), 151.10 (2C), 152.63 (2C) (C_{arom}); 114.15 (4C), 127.23 (2C), 129.89 (4C), 158.52 (2C) ($\mathbf{C}_6H_4OCH_3$); 145.82 (2C, C^3), 152.63 (2C, C^5), 152.70 (2C, HC=N), 162.98 (2C, COO). Mass spectrum: *m*/*z* 1079.34204 [*M* + Na]$^+$; calculated for $C_{54}H_{48}N_{12}O_{12}Na$: 1079.34069.

[({[Piperazine-1,4-diylbis(methylene)]bis[3-(4-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazole-1,4-diyl]}bis(azanylylidene))bis(methanylylidene)]bis(4,1-phenylene) bis(4-nitrobenzoate) (6g). Yield 71.4%, mp 229–230°C. IR spectrum, ν, cm^{-1}: 845 (1,4-C_6H_4), 1253 (COO), 1537, 1348 (NO_2), 1603, 1581 (C=N), 1743, 1710 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.79 s (8H, CH_2), 4.06 s (4H, $C\mathbf{H}_2C_6H_4$), 4.68 s (4H, NCH_2N), 7.26–7.31 m (8H, H_{arom}), 7.34 d (4H, H_{arom}, *J* = 8.80 Hz), 7.80 d (4H, H_{arom}, *J* = 8.80 Hz), 8.39 m (8H, H_{arom}), 9.82 s (2H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 31.19 (2C, $\mathbf{C}H_2Ph$), 49.98 (4C, CH_2), 66.46 (2C, NCH_2N); 122.08 (4C), 123.81 (4C), 129.27 (4C), 131.37 (4C), 132.20 (2C), 134.60 (2C), 151.10 (2C), 152.20 (2C) (C_{arom}); 128.86 (4C), 130.15 (4C), 131.50 (2C), 134.80 (2C) (C_6H_4Cl); 146.00 (2C, C^3), 152.10 (2C, C^5), 152.87 (2C, HC=N), 162.95(2C, COO). Mass spectrum: *m*/*z* 1087.24402 [*M* + Na]$^+$; calculated for $C_{52}H_{42}Cl_2N_{12}NaO_{10}$: 1087.24161.

[({[Piperazine-1,4-diylbis(methylene)]bis[3-(3-chlorobenzyl)-5-oxo-4,5-dihydro-1*H*-1,2,4-triazole-1,4-diyl]}bis(azanylylidene))bis(methanylylidene)]bis(4,1-phenylene) bis(4-nitrobenzoate) (6h). Yield

74.2%, mp 234–235°C. IR spectrum, ν, cm^{-1}: 826 (1,4-C_6H_4), 882, 781 (1,3-C_6H_4), 1259 (COO), 1524, 1343 (NO_2), 1598, 1587 (C=N), 1746, 1694 (C=O). ^1H NMR spectrum, δ, ppm: 2.80 s (8H, CH_2), 4.07 s (4H, C**H**$_2C_6H_4$), 4.69 s (4H, NCH_2N), 7.22–7.26 m (6H, H_{arom}), 7.33–7.39 m (6H, H_{arom}), 7.80 d (4H, H_{arom}, J = 8.80 Hz), 8.39 m (8H, H_{arom}), 9.82 s (2H, HC=N). ^{13}C NMR spectrum, δ_C, ppm: 31.56 (2C, **C**$H_2C_6H_4$), 50.00 (4C, CH_2), 66.58 (2C, NCH_2N); 122.07 (4C), 123.81 (4C), 129.30 (4C), 131.37 (4C), 131.99 (2C), 134.60 (2C), 151.02 (2C), 152.78 (2C) (C_{arom}); 126.94 (2C), 127.39 (2C), 129.30 (2C), 129.94 (2C), 134.35 (2C), 137.15 (2C) (C_6H_4Cl); 144.78 (2C, C^3), 151.02 (2C, C^5), 152.78 (2C, HC=N), 162.95 (2C, COO). Mass spectrum: m/z 1087.24390 $[M + Na]^+$; calculated for $C_{52}H_{42}Cl_2N_{12}NaO_{10}$: 1087.24161.

Antioxidant activity. The antioxidant activities of the newly synthesized compounds were evaluated by the chelating activity, reducing power, and radical scavenging methods according to [22–24].

Antibacterial activity. The bacterial strains used in this study were obtained from the Microbiological Environmental Protection Laboratories (France). They included three gram-positive bacterial strains (*Bacillus Substilis* ATCC 11774, *Bacillus Cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 6538) and three gram-negative bacterial strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 4352) were selected. Agar well diffusion method was selected for the evaluation of antibacterial effect [25, 26]. The standard antibiotics streptomycin, ampicillin, and neomycin were used to compare the effect levels of the synthesized compounds.

CONCLUSIONS

The in vitro antioxidant capacities of the newly synthesized 4,5-dihydro-1*H*-1,2,4-triazol-5-one derivatives were investigated under laboratory conditions. All compounds demonstrated promising activities in terms of the chelating effect, but Mannich bases were found to be more active than Schiff bases. The antibacterial and antioxidant investigations of 1,2,4-triazole derivatives have recently been studied. Important results were obtained in experiments on gram-negative and gram-positive bacteria, and antioxidant properties in the form of free radical scavenging activity were also determined for these compounds [27]. In the study of radical scavenging activities of Schiff Bases, gallic acid was used as a standard antioxidant. The effects of the synthesized Schiff Bases were determined to be considerably lower than that of gallic acid [28]. Furthermore, in the study using ascorbic acid, gallic acid, BHA, and BHT as standard antioxidants, the radical scavenging activities were not found at the desired level [29].

The antioxidant capacities of Mannich bases were found to be higher than those of Schiff bases [30]. Also, in the same study, the radical scavenging activities were examined, and the antioxidant values that competed with the standards were reached. However, the values obtained as the reducing capacity were lower [31].

The antibacterial investigation of the synthesized 1,2,4-triazol-5-one compounds and their acetyl and Mannich base derivatives revealed that the effects against spore-forming and Gram-positive bacteria were higher. Almost no antibacterial effect was observed for acetyl derivatives like **4**. However, Mannich bases derived from 1,2,4-triazol-5-ones showed a remarkable antibacterial effect comparable with the activity of neomycin and streptomycin antibiotics. Different studies have shown that compounds containing a 1,2,4-triazole ring exhibit antibacterial actvity [32, 33]. According to [34], Schiff Bases displayed moderate effects against *E. coli*, while low effects were observed against *S. typhi*, indicating that the antibacterial effects of Schiff Bases were not high. On the other hand, Mannich bases had antibacterial and antifungal effects even at low concentrations. Thus, there is a close relationship between the literature data and the data obtained from our study for biological activities of 1,2,4-triazole derivatives and Schiff and Mannich bases derived threfrom.

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CONFLICT OF INTEREST

There is no financial or personal interest relationship between the authors of this paper.

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